

***JP XVIII***

**THE JAPANESE PHARMACOPOEIA**

***EIGHTEENTH EDITION***

*Official from June 7, 2021*

English Version

THE MINISTRY OF HEALTH, LABOUR AND WELFARE

Notice: This *English Version* of the Japanese Pharmacopoeia is published for the convenience of users unfamiliar with the Japanese language. When and if any discrepancy arises between the Japanese original and its English translation, the former is authentic.

# The Ministry of Health, Labour and Welfare Ministerial Notification No. 220

Pursuant to Paragraph 1, Article 41 of Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Law No. 145, 1960), this notification stated that the Japanese Pharmacopoeia was revised as follows\*.

**TAMURA Norihisa**

The Minister of Health, Labour and Welfare

June 7, 2021

## Japanese Pharmacopoeia

Pursuant to Paragraph 1, Article 41 of Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Law No. 145, 1960), the Japanese Pharmacopoeia was revised as follows.

(The text referred to by the term “as follows” are omitted here. All of the revised Japanese Pharmacopoeia in accordance with this notification are made available for public exhibition at the Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare, at each Regional Bureau of Health and Welfare, and at each Prefectural Office in Japan).

## Supplementary Provisions

### (Effective Date)

Article 1 This Notification is applied from the date of the notification. (referred to as the “notification date” in the next and third articles)

### (Transitional measures)

Article 2 In the case of drugs which are listed in the Japanese Pharmacopoeia (hereinafter referred to as “previous Pharmacopoeia”) and have been approved as of the notification date as prescribed under Paragraph 1, Article 14 of Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices [including drugs the Minister of Health, Labour and Welfare specifies (the Ministry of Health and Welfare Ministerial Notification No. 104, 1994) as of the day before the notification date as those exempted from marketing approval pursuant to Paragraph 1, Article 14 of the same law (hereinafter referred to as “drugs exempted from approval”)], the Name and Standards established in the previous Pharmacopoeia (limited to part of the Name and Standards for the drugs concerned) may be accepted to conform to the Name and Standards established in the revised Japanese Pharmacopoeia (hereinafter referred to as “new Pharmacopoeia”) (limited to part of the Name and Standards for the drugs concerned) in accordance with this notification before and on December 31, 2022.

Article 3 In the case of drugs which are listed in the new Pharmacopoeia (excluding those listed in the previous Pharmacopoeia) and have been approved as of the notification date as prescribed under the Paragraph 1, Article 14 of the same law (including those exempted from approval), they may be accepted as those being not listed in the new Pharmacopoeia before and on December 31, 2022.

Article 4 In the case of drugs which are listed in the new Pharmacopoeia, their commonly used names established in the previous Pharmacopoeia may be accepted to conform to the Name established in the new Pharmacopoeia before and on June 30, 2024.

Article 5 In the case of drugs which are listed in the new Pharmacopoeia, the previous provisions are applied before and on June 30, 2024, regardless of the Paragraph 34 of General Notices.

\*The term “as follows” here indicates the contents of the Japanese Pharmacopoeia Eighteenth Edition from General Notice to Ultraviolet-Visible Reference Spectra (pp. 1 – 2587).

# CONTENTS

<b>Preface.....</b>	i
<b>The Japanese Pharmacopoeia, Eighteenth Edition ...1</b>	
<b>General Notices .....</b>	1
<b>General Rules for Crude Drugs.....</b>	5
<b>General Rules for Preparations.....</b>	7
<b>General Tests, Processes and Apparatus.....</b>	25
1. Chemical Methods	
1.01 Alcohol Number Determination .....	25
1.02 Ammonium Limit Test .....	26
1.03 Chloride Limit Test.....	27
1.04 Flame Coloration Test .....	27
1.05 Mineral Oil Test .....	28
1.06 Oxygen Flask Combustion Method.....	28
1.07 Heavy Metals Limit Test .....	29
1.08 Nitrogen Determination (Semimicro-Kjeldahl Method).....	30
1.09 Qualitative Tests.....	31
1.10 Iron Limit Test .....	36
1.11 Arsenic Limit Test .....	36
1.12 Methanol Test.....	38
1.13 Fats and Fatty Oils Test .....	38
1.14 Sulfate Limit Test .....	40
1.15 Readily Carbonizable Substances Test .....	40
2. Physical Methods	
Chromatography	
2.01 Liquid Chromatography.....	41
2.02 Gas Chromatography .....	44
2.03 Thin-layer Chromatography .....	45
2.04 Amino Acid Analysis of Proteins .....	46
2.05 Size-Exclusion Chromatography.....	47
Spectroscopic Methods	
2.21 Nuclear Magnetic Resonance Spectroscopy.....	48
2.22 Fluorometry .....	50
2.23 Atomic Absorption Spectrophotometry.....	50
2.24 Ultraviolet-visible Spectrophotometry.....	51
2.25 Infrared Spectrophotometry .....	53
2.26 Raman Spectroscopy .....	54
Other Physical Methods	
2.41 Loss on Drying Test .....	56
2.42 Congealing Point Determination .....	56
2.43 Loss on Ignition Test.....	57
2.44 Residue on Ignition Test .....	57
2.45 Refractive Index Determination .....	58
2.46 Residual Solvents.....	58
2.47 Osmolarity Determination .....	64
2.48 Water Determination (Karl Fischer Method).....	65
2.49 Optical Rotation Determination .....	68

2.50 Endpoint Detection Methods in Titrimetry .....	69
2.51 Conductivity Measurement .....	70
2.52 Thermal Analysis.....	72
2.53 Viscosity Determination .....	75
2.54 pH Determination.....	77
2.55 Vitamin A Assay .....	79
2.56 Determination of Specific Gravity and Density .....	80
2.57 Boiling Point and Distilling Range Test .....	81
2.58 X-Ray Powder Diffraction Method .....	82
2.59 Test for Total Organic Carbon .....	86
2.60 Melting Point Determination .....	87
2.61 Turbidity Measurement .....	89
2.62 Mass Spectrometry.....	90
2.63 Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry .....	94
2.64 Glycosylation Analysis of Glycoprotein .....	98
2.65 Methods for Color Matching .....	100
2.66 Elemental Impurities .....	102
3. Powder Property Determinations	
3.01 Determination of Bulk and Tapped Densities.....	108
3.02 Specific Surface Area by Gas Adsorption .....	110
3.03 Powder Particle Density Determination .....	113
3.04 Particle Size Determination .....	114
3.05 Water-Solid Interactions: Determination of Sorption-Desorption Isotherms and of Water Activity .....	118
3.06 Laser Diffraction Measurement of Particle Size .....	120
4. Biological Tests/Biochemical Tests/Microbial Tests	
4.01 Bacterial Endotoxins Test .....	124
4.02 Microbial Assay for Antibiotics.....	127
4.03 Digestion Test .....	131
4.04 Pyrogen Test .....	133
4.05 Microbiological Examination of Non-sterile Products .....	134
4.06 Sterility Test.....	144
5. Tests for Crude Drugs	
5.01 Crude Drugs Test .....	147
5.02 Microbial Limit Test for Crude Drugs and Preparations containing Crude Drugs as Main Ingredient .....	152
6. Tests for Preparations	
6.01 Test for Metal Particles in Ophthalmic Ointments .....	161
6.02 Uniformity of Dosage Units .....	161
6.03 Particle Size Distribution Test for Preparations .....	163
6.04 Test for Acid-neutralizing Capacity of	

Gastrointestinal Medicines.....	164	Ultraviolet-visible Reference Spectra .....	<b>2399–2587</b>
6.05 Test for Extractable Volume of Parenteral Preparations .....	164	<b>General Information</b>	
6.06 Foreign Insoluble Matter Test for Injections .....	165	G0 Basic Concepts on Pharmaceutical Quality Basic Concepts for Quality Assurance of Drug Substances and Drug Products <G0-1-172> .....	2591
6.07 Insoluble Particulate Matter Test for Injections .....	165	Basic Concept of Quality Risk Management <G0-2-170> .....	2593
6.08 Insoluble Particulate Matter Test for Ophthalmic Solutions.....	168	Concept on Impurities in Chemically synthesized Drug Substances and Drug Products <G0-3-172> .....	2596
6.09 Disintegration Test .....	169	Stability Testing of Drug Substances and Drug Products <G0-4-171> .....	2598
6.10 Dissolution Test .....	170	Basic Requirements and Terms for the Packaging of Pharmaceutical Products <G0-5-170> ....	2601
6.11 Foreign Insoluble Matter Test for Ophthalmic Liquids and Solutions .....	174	Glossary for Quality by Design (QbD), Quality Risk Management (QRM) and Pharmaceutical Quality System (PQS) <G0-6-172> .....	2604
6.12 Methods of Adhesion Testing .....	175	<b>G1 Physics and Chemistry</b>	
6.13 Release Test for Preparations for Cutaneous Application .....	177	Validation of Analytical Procedures <G1-1-130> .....	2607
6.14 Uniformity of Delivered Dose for Inhalations .....	180	System Suitability <G1-2-152> .....	2609
6.15 Aerodynamic Particle Size Measurement for Inhalations.....	183	Near Infrared Spectrometry <G1-3-161> .....	2611
6.16 Rheological Measurements for Semi-solid Preparations.....	191	<b>G2 Solid-state Properties</b>	
6.17 Insoluble Particulate Matter Test for Therapeutic Protein Injections .....	194	Solid and Particle Densities <G2-1-171>.....	2614
7. Tests for Containers and Packing Materials		Powder Fineness <G2-2-171>.....	2615
7.01 Test for Glass Containers for Injections ..	195	Powder Flow <G2-3-171> .....	2616
7.02 Test Methods for Plastic Containers.....	196	Measurement of the Diameter of Particles Dispersed in Liquid by Dynamic Light Scattering <G2-4-161> .....	2618
7.03 Test for Rubber Closure for Aqueous Infusions .....	201	<b>G3 Biotechnological/Biological Products</b>	
9. Reference Standards; Standard Solutions; Reagents, Test Solutions; Measuring Instruments, Appliances, etc.		Basic Concept of the Quality Control on Biotechnological Products (Biopharmaceuticals) <G3-1-180> .....	2621
Reference Standards		Amino Acid Analysis <G3-2-171> .....	2625
9.01 Reference Standards .....	203	Peptide Mapping <G3-3-142> .....	2632
Standard Solutions		Mass Spectrometry of Peptides and Proteins <G3-4-161> .....	2635
9.21 Standard Solutions for Volumetric Analysis .....	207	Monosaccharide Analysis and Oligosaccharide Analysis/Oligosaccharide Profiling <G3-5-170> .....	2637
9.22 Standard Solutions .....	218	Isoelectric Focusing <G3-6-142> .....	2641
9.23 Matching Fluids for Color .....	221	Capillary Electrophoresis <G3-7-180> .....	2643
Reagents, Test Solutions, etc.		SDS-Polyacrylamide Gel Electrophoresis <G3-8-170> .....	2648
9.41 Reagents, Test Solutions .....	221	Host Cell Protein Assay <G3-9-172> .....	2653
9.42 Solid Supports/Column Packings for Chromatography .....	391	Surface Plasmon Resonance <G3-10-170> ....	2656
9.43 Filter Papers, Filters for filtration, Test Papers, Crucibles, etc. ....	394	Enzyme-linked Immunosorbent Assay (ELISA) <G3-11-171> .....	2659
9.44 Standard Particles, etc.....	394	Total Protein Assay <G3-12-172> .....	2662
Measuring Instruments and Appliances, Thermometers, etc.		Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia <G3-13-141> .....	2666
9.61 Optical Filters for Wavelength and Transmission Rate Calibration .....	395	Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products <G3-14-170> .....	2678
9.62 Measuring Instruments, Appliances .....	395		
9.63 Thermometers .....	396		
<b>Official Monographs .....</b>	<b>399</b>		
<b>Crude Drugs and Related Drugs .....</b>	<b>1939</b>		
<b>Infrared Reference Spectra .....</b>	<b>2179–2395</b>		

Qualification of Animals as Origin of Animal-derived Medicinal Products provided in the General Notices of Japanese Pharmacopoeia and Other Standards <i>&lt;G3-15-141&gt;</i> .....	2682
<b>G4 Microorganisms</b>	
Microbial Attributes of Non-sterile Pharmaceutical Products <i>&lt;G4-1-170&gt;</i> .....	2684
Control of Culture Media and Strains of Microorganisms Used for Microbial Tests <i>&lt;G4-2-180&gt;</i> .....	2686
Preservatives-Effectiveness Tests <i>&lt;G4-3-170&gt;</i> .....	2688
Bacterial Endotoxins Test and Alternative Methods using Recombinant Protein-reagents for Endotoxin Assay <i>&lt;G4-4-180&gt;</i> .....	2691
Decision of Limit for Bacterial Endotoxins <i>&lt;G4-5-131&gt;</i> .....	2693
Rapid Microbial Methods <i>&lt;G4-6-170&gt;</i> .....	2693
Rapid Identification of Microorganisms Based on Molecular Biological Method <i>&lt;G4-7-160&gt;</i> .....	2695
Rapid Counting of Microbes using Fluorescent Staining <i>&lt;G4-8-152&gt;</i> .....	2696
Disinfection and Decontamination Methods <i>&lt;G4-9-170&gt;</i> .....	2698
Sterilization and Sterilization Indicators <i>&lt;G4-10-162&gt;</i> .....	2701
<b>G5 Crude Drugs</b>	
On the Scientific Names of Crude Drugs listed in the JP <i>&lt;G5-1-180&gt;</i> .....	2707
Notification for the Quantitative Marker Constituents of Crude Drugs and Crude Drug Preparations <i>&lt;G5-2-170&gt;</i> .....	2721
Thin-layer Chromatography for Crude Drugs and Crude Drug Preparations <i>&lt;G5-3-170&gt;</i> .....	2722
Aristolochic Acid <i>&lt;G5-4-141&gt;</i> .....	2724
Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy and its Application to Reagents in the Japanese Pharmacopoeia <i>&lt;G5-5-170&gt;</i> .....	2724
Purity Tests on Crude Drugs using Genetic Information <i>&lt;G5-6-172&gt;</i> .....	2726
Analytical Methods for Aflatoxins in Crude Drug and Crude Drug Preparations <i>&lt;G5-7-170&gt;</i> .....	2730
Radioactivity Measurements Method for Crude Drugs <i>&lt;G5-8-180&gt;</i> .....	2731
<b>G6 Drug Formulation</b>	
Criteria for Content Uniformity in Real Time Release Testing by Process Analytical Technology <i>&lt;G6-1-171&gt;</i> .....	2737
Standard Procedure for Mechanical Calibration of Dissolution Apparatus <i>&lt;G6-2-170&gt;</i> .....	2739
Aerodynamic Particle Size Measurement for Inhalations by Glass Impingers <i>&lt;G6-3-171&gt;</i> .....	2741
Tablet Hardness Determinations <i>&lt;G6-4-180&gt;</i> .....	2743
Tablet Friability Test <i>&lt;G6-5-150&gt;</i> .....	2744
pH Test for Gastrointestinal Medicine <i>&lt;G6-6-131&gt;</i> .....	2744
Test for Trace Amounts of Aluminum in Total Parenteral Nutrition (TPN) Solutions <i>&lt;G6-7-160&gt;</i> .....	2744
<b>G7 Containers and Package</b>	
Glass Containers for Pharmaceutical Products <i>&lt;G7-1-171&gt;</i> .....	2746
Basic Requirements for Plastic Containers for Pharmaceutical Use and Rubber Closures for Containers for Aqueous Infusions <i>&lt;G7-2-162&gt;</i> .....	2748
Moisture Permeability Test for Blister Packaging of Solid Preparations <i>&lt;G7-3-171&gt;</i> .....	2749
Packaging Integrity Evaluation of Sterile Products <i>&lt;G7-4-180&gt;</i> .....	2750
Leak Tests for Packaging of Sterile Products <i>&lt;G7-5-180&gt;</i> .....	2753
<b>G8 Reference Standards</b>	
Reference Standards and Reference Materials Specified in the Japanese Pharmacopoeia <i>&lt;G8-1-170&gt;</i> .....	2756
<b>GZ Others</b>	
Water to be used in the Tests of Drugs <i>&lt;GZ-1-161&gt;</i> .....	2758
Quality Control of Water for Pharmaceutical Use <i>&lt;GZ-2-172&gt;</i> .....	2759
International Harmonization Implemented in the Japanese Pharmacopoeia Eighteenth Edition <i>&lt;GZ-3-180&gt;</i> .....	2765
<b>Appendix</b>	
Atomic Weight Table (2017) .....	2769
Standard Atomic Weights 2017 .....	2771
Standard Atomic Weights 2010 .....	2773
<b>Index</b> .....	2775
<b>Index in Latin name</b> .....	2793
<b>Index in Japanese</b> .....	2795

# PREFACE

The Japanese Pharmacopoeia (JP) is an official document that defines the specifications, criteria and standard test methods necessary to properly assure the quality of medicines in Japan.

Paragraph 2, Article 41 of the Law on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices stipulates that full-fledged JP revisions shall be presented at least every 10 years. Since the JP 9th edition, full-fledged revisions have been made every 5 years. In addition to the full-fledged revisions, a supplement has been promulgated twice in every 5 years since the JP 12th edition as well as partial revisions have been made as necessary to take account of recent progress of science and in the interests of international harmonization.

The 17th Edition of the JP was promulgated by Ministerial Notification No. 64 of the Ministry of Health, Labour and Welfare (MHLW) on March 7, 2016.

In July 2016, the Committee on JP established the basic principles for the preparation of the JP 18th Edition, setting out the roles and characteristics of the JP, the definite measures for the revision, and the date of the revision.

At the Committee, the five basic principles of JP, which we refer to as the “five pillars”, were established as follows: 1) Including all drugs which are important from the viewpoint of health care and medical treatment; 2) Making qualitative improvement by introducing the latest science and technology; 3) Promoting further internationalization in response to globalization of drug market; 4) Making prompt partial revision as necessary and facilitating smooth administrative operation; and 5) Ensuring transparency regarding the revision, and disseminating the JP to the public. It was agreed that the Committee on JP should make efforts, on the basis of these principles, to ensure that the JP is used more effectively in the fields of health care and medical treatment by taking appropriate measurements, including getting the understanding and cooperation of other parties concerned.

It was agreed that the JP should provide an official standard, being required to assure the quality of medicines in Japan in response to the progress of science and technology and medical demands at the time. It should define the standards for specifications, as well as the methods of testing to assure overall quality of all drugs in principle, and it should have a role in clarifying the criteria for quality assurance of drugs that are recognized to be essential for public health

and medical treatment. The JP has been prepared with the aid of the knowledge and experience of many professionals in the pharmaceutical field. Therefore, the JP should have the characteristics of an official standard, which might be widely used by all parties concerned, and it should play an appropriate role of providing information and understanding about the quality of drugs to the public. Moreover, as a pharmaceutical quality standard, it should contribute promoting and maintaining of advancedness as well as international consistency and harmonization of technical requirements in the international community. It was also agreed that JP articles should cover drugs, which are important from the viewpoint of health care and medical treatment, clinical performance or merits and frequency of use, as soon as possible after they reach the market.

The target date for the publication of the JP 18th Edition (the Japanese edition) was set as April 2021.

JP drafts are discussed in the following committees that were established in the Pharmaceuticals and Medical Devices Agency: Expert Committee; Sub-expert Committee; Sub-committee on Manufacturing Process-related Matters; Committee on Chemicals; Committee on Antibiotics; Committee on Biologicals; Committee on Crude Drugs; Committee on Pharmaceutical Excipients; Committee on Physico-Chemical Methods; Committee on Drug Formulation; Committee on Physical Methods; Committee on Biological Methods; Committee on Nomenclature for Pharmaceuticals; Committee on International Harmonization; and Committee on Reference Standards.

In the Committee on JP, Mitsuru Hashida took the role of chairman from April 2016 to June 2021.

In accordance with the above principles, the committees initiated deliberations on selection of articles and on revisions for General Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests, Monographs and so on.

Draft revisions covering subjects in General Rules for Preparations, General Tests and Monographs, for which discussions were finished between August 2015 and March 2017, were prepared for a supplement to the JP 17. The draft revisions were examined by the Committee on JP in April 2017, followed by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in June 2017, and then submitted to the Minister of Health, Labour and Welfare.

The supplement was named “Supplement I to the JP 17th Edition”, promulgated on December 1, 2017

by Ministerial Notification No. 348 of MHLW, and became effective.

Numbers of discussions in the committees to prepare the supplement drafts were as follows: Expert Committee (8); Sub-committee on Manufacturing Process-related Matters (9); Committee on Chemicals (20); Committee on Antibiotics (5); Committee on Biologicals (8); Committee on Crude Drugs (17); Committee on Pharmaceutical Excipients (10); Committee on Physico-Chemical Methods (14, including working group); Committee on Drug Formulation (27, including working group); Committee on Physical Methods (8); Committee on Biological Methods (6); Committee on Nomenclature for Pharmaceuticals (7); Committee on International Harmonization (6); and Committee on Reference Standards (4).

It should be noted that in the preparation of the drafts for the supplement, generous cooperation was given by the Pharmaceutical Technology Committee of the Osaka Pharmaceutical Manufacturers Association, the Pharmacopeia and CMC Committee of the Pharmaceutical Manufacturers' Association of Tokyo, the Tokyo Crude Drugs Association, the International Pharmaceutical Excipients Council Japan, the Home Medicine Association of Japan, the Japan Kampo Medicines Manufacturers Association, the Japan Flavor and Fragrance Materials Association, the Japan Medicinal Plant Federation, the Japan Pharmaceutical Manufacturers Association, the Federation of Pharmaceutical Manufacturers' Association of Japan, the Parenteral Drug Association Japan Chapter, the Japan Reagent Association, the Japan Oilseed Processors Association, the Japan Analytical Instruments Manufacturers' Association, and the Asian Society of Innovative Packaging Technology.

In consequence of this revision, the JP 17th Edition carries 1977 articles, owing to the addition of 32 articles and the deletion of 17 articles.

Draft revisions covering subjects in General Notices, General Rules for Preparations, General Tests and Monographs, for which discussions were finished between April 2017 and November 2018, were prepared for a supplement to the JP 17. The draft revisions were examined by the Committee on JP in January 2019, followed by the PAFSC in March 2019, and then submitted to the Minister of Health, Labour and Welfare.

The supplement was named "Supplement II to the JP 17th Edition", promulgated on June 28, 2019 by Ministerial Notification No. 49 of MHLW, and became effective.

Numbers of discussions in the committees to prepare the supplement drafts were as follows: Expert Committee (11, including working group); Subcommittee on Manufacturing Process-related Matters (6); Committee on Chemicals (18); Committee on An-

tibiotics (3); Committee on Biologicals (7); Committee on Crude Drugs (15); Committee on Pharmaceutical Excipients (10); Committee on Physico-Chemical Methods (11, including working group); Committee on Drug Formulation (19, including working group); Committee on Physical Methods (7); Committee on Biological Methods (6); Committee on Nomenclature for Pharmaceuticals (5); and Committee on International Harmonization (4).

It should be noted that in the preparation of the drafts for the supplement, generous cooperation was given by the Pharmaceutical Technology Committee of the Kansai Pharmaceutical Industries Association, the Pharmacopeia and CMC Committee of the Pharmaceutical Manufacturers' Association of Tokyo, the Tokyo Crude Drugs Association, the International Pharmaceutical Excipients Council Japan, the Home Medicine Association of Japan, the Japan Kampo Medicines Manufacturers Association, the Japan Flavor and Fragrance Materials Association, the Japan Medicinal Plant Federation, the Japan Pharmaceutical Manufacturers Association, the Federation of Pharmaceutical Manufacturers' Association of Japan, the Parenteral Drug Association Japan Chapter, the Japan Reagent Association, the Japan Oilseed Processors Association, the Japan Analytical Instruments Manufacturers' Association, and the Asian Society of Innovative Packaging Technology.

In consequence of this revision, the JP 17th Edition carries 2008 articles, owing to the addition of 34 articles and the deletion of 3 articles.

Draft revisions covering subjects in General Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests and Monographs, for which discussions were finished between December 2018 and August 2020, were prepared for a supplement to the JP 18. The draft revisions were examined by the Committee on JP in October 2020, followed by the PAFSC in December 2020, and then submitted to the Minister of Health, Labour and Welfare.

Numbers of discussions in the committees to prepare the revision drafts were as follows: Expert Committee (12, including working group); Committee on Chemicals (19); Committee on Antibiotics (3); Committee on Biologicals (8); Committee on Crude Drugs (14); Committee on Pharmaceutical Excipients (10, including working group); Committee on Physico-Chemical Methods (7); Committee on Drug Formulation (20, including working group); Committee on Physical Methods (7); Committee on Biological Methods (7); Committee on Nomenclature for Pharmaceuticals (6); Committee on International Harmonization (7); and Committee on Reference Standards (7, including working group).

It should be noted that in the preparation of the revision drafts, generous cooperation was given by the

Pharmaceutical Technology Committee of the Kansai Pharmaceutical Industries Association, the Pharmacopeia and CMC Committee of the Pharmaceutical Manufacturers' Association of Tokyo, the Tokyo Crude Drugs Association, the International Pharmaceutical Excipients Council Japan, the Home Medicine Association of Japan, the Japan Kampo Medicines Manufacturers Association, the Japan Flavor and Fragrance Materials Association, the Japan Medicinal Plant Federation, the Japan Pharmaceutical Manufacturers Association, the Federation of Pharmaceutical Manufacturers' Association of Japan, the Parenteral Drug Association Japan Chapter, the Japan Reagent Association, the Japan Oilseed Processors Association, the Japan Analytical Instruments Manufacturers' Association, and the Asian Society of Innovative Packaging Technology.

In consequence of this revision, the JP 18th Edition carries 2033 articles, owing to the addition of 33 articles and the deletion of 8 articles.

The principles of description and the salient points of the revision in this volume are as follows:

1. The JP 18th Edition comprises the following items, in order: Notification of MHLW; Contents; Preface; General Notices; General Rules for Crude Drugs; General Rules for Preparations; General Tests, Processes and Apparatus; Official Monographs; then followed by Infrared Reference Spectra and Ultraviolet-visible Reference Spectra; General Information; Table of Standard Atomic Weights as an appendix; and a Cumulative Index.

2. The articles in Official Monographs, Infrared Reference Spectra and Ultraviolet-visible Reference Spectra are respectively placed in alphabetical order in principle.

3. The following items in each monograph are put in the order shown below, except that unnecessary items are omitted depending on the nature of the drug:

- (1) English title
- (2) Commonly used name(s)
- (3) Latin title (only for crude drugs)
- (4) Title in Japanese
- (5) Structural formula or empirical formula
- (6) Molecular formula and molecular mass
- (7) Chemical name
- (8) Chemical Abstracts Service (CAS) Registry Number
- (9) Origin
- (10) Limits of the content of the ingredient(s) and/or the unit of potency
- (11) Labeling requirements
- (12) Method of preparation
- (13) Manufacture
- (14) Description
- (15) Identification tests

- (16) Specific physical and/or chemical values
- (17) Purity tests
- (18) Potential adulteration
- (19) Loss on drying or Ignition, or Water
- (20) Residue on ignition, Total ash or Acid-insoluble ash
- (21) Tests being required for pharmaceutical preparations
- (22) Other special tests
- (23) Assay
- (24) Containers and storage
- (25) Shelf life
- (26) Others

4. In each monograph, the following physical and chemical values representing the properties and quality of the drug are given in the order indicated below, except that unnecessary items are omitted depending on the nature of drug:

- (1) Alcohol number
- (2) Absorbance
- (3) Congealing point
- (4) Refractive index
- (5) Osmolar ratio
- (6) Optical rotation
- (7) Constituent amino acids
- (8) Viscosity
- (9) pH
- (10) Content ratio of the active ingredients
- (11) Specific gravity
- (12) Boiling point
- (13) Melting point
- (14) Acid value
- (15) Saponification value
- (16) Ester value
- (17) Hydroxyl value
- (18) Iodine value

5. Identification tests comprise the following items, which are generally put in the order given below:

- (1) Coloration reactions
- (2) Precipitation reactions
- (3) Decomposition reactions
- (4) Derivatives
- (5) Infrared and/or ultraviolet-visible absorption spectrometry
- (6) Nuclear magnetic resonance spectrometry
- (7) Chromatography
- (8) Special reactions
- (9) Cations
- (10) Anions

6. Purity tests comprise the following items, which are generally put in the order given below, except that unnecessary items are omitted depending on the nature of drug:

- (1) Color

- (2) Odor
- (3) Clarity and/or color of solution
- (4) Acidity or alkalinity
- (5) Acidity
- (6) Alkalinity
- (7) Chloride
- (8) Sulfate
- (9) Sulfite
- (10) Nitrate
- (11) Nitrite
- (12) Carbonate
- (13) Bromide
- (14) Iodide
- (15) Soluble halide
- (16) Thiocyanate
- (17) Selenium
- (18) Cationic salts
- (19) Ammonium
- (20) Heavy metals
- (21) Iron
- (22) Manganese
- (23) Chromium
- (24) Bismuth
- (25) Tin
- (26) Aluminum
- (27) Zinc
- (28) Cadmium
- (29) Mercury
- (30) Copper
- (31) Lead
- (32) Silver
- (33) Alkaline earth metals
- (34) Arsenic
- (35) Free phosphoric acid
- (36) Foreign matters
- (37) Related substances
- (38) Isomer
- (39) Enantiomer
- (40) Diastereomer
- (41) Polymer
- (42) Residual solvent
- (43) Other impurities
- (44) Residue on evaporation
- (45) Readily carbonizable substances

7. The following paragraph was newly added to General Notices:

- (1) Paragraph 34: The provision for elemental impurities based on ICH Q3D “Guideline for Elemental Impurities” was added.

8. The following paragraph of General Notices was revised:

- (1) Paragraph 8: Atomic masses adopted in the Japanese Pharmacopoeia were determined to conform to the table of “Atomic Weights of the Elements 2015” (IUPAC), however, the atomic

masses of the elements whose atomic weight is indicated with an interval in the 2015 table were determined to conform to the table of “Atomic Weights of the Elements 2007” (IUPAC).

9. The following item was newly added to General Tests, Processes and Apparatus:

- (1) 2.05 Size-Exclusion Chromatography

10. The following items in General Tests, Processes and Apparatus were revised:

- (1) Preamble
- (2) 2.46 Residual Solvents
- (3) 2.48 Water Determination
- (4) 2.51 Conductivity Measurement
- (5) 2.52 Thermal Analysis
- (6) 2.66 Elemental Impurities
- (7) 4.06 Sterility Test
- (8) 5.01 Crude Drugs Test
- (9) 9.01 Reference Standards
- (10) 9.41 Reagents, Test Solutions
- (11) 9.62 Measuring Instruments, Appliances

11. The following Reference Standards were added:

- Bicalutamide RS
- Cabergoline RS
- Celecoxib RS
- Eribulin Mesilate RS
- Eribulin Mesilate Related Substance C for System Suitability RS
- Ethyl Loflazepate RS
- Fenofibrate RS
- Gefitinib RS
- Glucagon RS
- Rilmazafone Hydrochloride RS
- Rosuvastatin Calcium RS
- Saccharin RS
- Timolol Maleate RS
- Triazolam RS

12. The following Reference Standard was revised in Japanese title:

- Saccharin Sodium RS

13. The following Reference Standards were deleted from the list of “9.01 (2) The reference standards which are prepared by National Institute of Infectious Diseases” and added to the list of “9.01 (1) The reference standards which are prepared by those who have been registered to prepare them by the Minister of Health, Labour and Welfare, according to the Ministerial ordinance established by the Minister separately”:

- Cefepime Dihydrochloride RS
- Cefotiam Hydrochloride RS
- Ceftriaxone Sodium RS
- Clarithromycin RS
- Epirubicin Hydrochloride RS

Minocycline Hydrochloride RS  
Piperacillin RS  
Roxithromycin RS  
Sulbactam RS  
Tazobactam RS  
Vancomycin Hydrochloride RS

**14.** The following substances were newly added to the Official Monographs:

Bicalutamide  
Cabergoline  
Celecoxib  
Cloperastine Fendzoate  
Cloperastine Fendzoate Tablets  
Copovidone  
Dorzolamide Hydrochloride and Timolol Maleate  
Ophthalmic Solution  
Eribulin Mesilate  
Ethyl Loflazepate  
Ethyl Loflazepate Tablets  
Fenofibrate  
Fenofibrate Tablets  
Fludiazepam Tablets  
Gefitinib  
Glucagon (Genetical Recombination)  
Heparin Sodium Lock Solution  
Heparin Sodium Solution for Dialysis  
Irinotecan Hydrochloride Injection  
Methotrexate for Injection  
Miglyrol Tablets  
Phenobarbital Tablets  
Pitavastatin Calcium Orally Disintegrating Tablets  
Rilmazafone Hydrochloride Hydrate  
Rilmazafone Hydrochloride Tablets  
Rosuvastatin Calcium  
Rosuvastatin Calcium Tablets  
Silodosin Orally Disintegrating Tablets  
Telmisartan and Amlodipine Besilate Tablets  
Triazolam  
Zopiclone  
Zopiclone Tablets  
Byakkokaninjinto Extract  
Unseiin Extract

**15.** The following monographs were revised:

Aprindine Hydrochloride  
Azathioprine Tablets  
Bepotastine Besilate  
Bleomycin Hydrochloride  
Bleomycin Sulfate  
Calcitonin Salmon  
Calcium Folinate Hydrate  
Calcium Paraaminosalicylate Hydrate  
Carmellose Calcium  
Carmellose Sodium  
Crocarmellose Sodium  
Cefoperazone Sodium

Celmoleukin (Genetical Recombination)  
Clinofibrate  
Clopidogrel Sulfate  
Colistin Sodium Methanesulfonate  
Dihydroergotoxine Mesilate  
Dimorpholamine  
Diphenhydramine  
Diphenhydramine Hydrochloride  
Distigmine Bromide  
Dorzolamide Hydrochloride  
Enviomycin Sulfate  
Epoetin Alfa (Genetical Recombination)  
Epoetin Beta (Genetical Recombination)  
Ergometrine Maleate  
Ethambutol Hydrochloride  
Ethyl Aminobenzoate  
Filgrastim (Genetical Recombination)  
Fradiomycin Sulfate  
Gelatin  
Purified Gelatin  
Gentamicin Sulfate  
Glucose Injection  
Human Menopausal Gonadotrophin  
Heparin Calcium  
Heparin Sodium  
Low Substituted Hydroxypropylcellulose  
Hypromellose  
Insulin Human (Genetical Recombination)  
Insulin Aspart (Genetical Recombination)  
Insulin Glargine (Genetical Recombination)  
Kallidinogenase  
Kitasamycin Acetate  
Kitasamycin Tartrate  
Anhydrous Lactose  
Lactose Hydrate  
Lauromacrogol  
Lenograstim (Genetical Recombination)  
Levofloxacin Hydrate  
Levofloxacin Fine Granules  
Levofloxacin Injection  
Levofloxacin Tablets  
Lysozyme Hydrochloride  
Methylcellulose  
Methylergometrine Maleate  
Mexiletine Hydrochloride  
Montelukast Sodium  
Nafamostat Mesilate  
Nartograstim (Genetical Recombination)  
Norgestrel and Ethinylestradiol Tablets  
Olmesartan Medoxomil  
Oxybuprocaine Hydrochloride  
Oxygen  
Oxytocin  
Paroxetine Hydrochloride Hydrate  
Pitavastatin Calcium Hydrate  
Pitavastatin Calcium Tablets

Polymixin B Sulfate	Jujube Seed
Povidone	Kamikihito Extract
Povidone-Iodine	Kamishoyosan Extract
Protamine Sulfate	Keishibukuryogan Extract
Pullulan	Lilium Bulb
Saccharin	Lycium Fruit
Saccharin Sodium Hydrate	Magnolia Bark
Scopolamine Butylbromide	Powdered Magnolia Bark
Scopolamine Hydrobromide Hydrate	Mentha Oil
Purified Shellac	Moutan Bark
White Shellac	Powdered Moutan Bark
Silodosin	Nelumbo Seed
Silodosin Tablets	Notopterygium
Sodium Lauryl Sulfate	Nuphar Rhizome
Wheat Starch	Nutmeg
Sucrose	Nux Vomica
Teceleukin (Genetical Recombination)	Nux Vomica Extract Powder
Tetracaine Hydrochloride	Orengedokuto Extract
Valaciclovir Hydrochloride	Perilla Herb
Valsartan	Peucedanum Root
Vasopressin Injection	Phellodendron Bark
Voriconazole	Powdered Phellodendron Bark
Voriconazole for Injection	Plantago Herb
Alpinia Officinarum Rhizome	Plantago Seed
Asiasarum Root	Pogostemon Herb
Asparagus Root	Polyporus Sclerotium
Atractylodes Lancea Rhizome	Powdered Polyporus Sclerotium
Bearberry Leaf	Poria Sclerotium
Belladonna Root	Powdered Poria Sclerotium
Bupleurum Root	Pueraria Root
Cardamon	Quercus Bark
Cinnamon Bark	Ryokejutsukanto Extract
Cinnamon Oil	Saffron
Clove	Salvia Miltiorrhiza Root
Powdered Clove	Sappan Wood
Cnidium Rhizome	Scopolia Rhizome
Powdered Cnidium Rhizome	Scopolia Extract Powder
Coptis Rhizome	Scopolia Extract and Ethyl Aminobenzoate Powder
Powdered Coptis Rhizome	Sophora Root
Cornus Fruit	Powdered Sophora Root
Curcuma Rhizome	Toad Cake
Dioscorea Rhizome	Tokakujokito Extract
Ephedra Herb	Trichosanthes Root
Euodia Fruit	Uncaria Hook
Gardenia Fruit	
Powdered Gardenia Fruit	<b>16.</b> A part or all of the commonly used names
Glycyrrhiza	were deleted from the following monographs (chemical drugs, etc):
Goreisan Extract	Acebutolol Hydrochloride
Goshuyuto Extract	Acetazolamide
Hangekobokuto Extract	Acetylcholine Chloride for Injection
Houttuynia Herb	Acetylcysteine
Imperata Rhizome	Aclarubicin Hydrochloride
Ipecac Syrup	Acrinol Hydrate
Powdered Japanese Gentian	Adrenaline Injection
Japanese Zanthoxylum Peel	Adrenaline Solution
Powdered Japanese Zanthoxylum Peel	Afloqualone

Aldioxa	Betamethasone Dipropionate
Aldioxa Granules	Betamethasone Sodium Phosphate
Aldioxa Tablets	Betamethasone Valerate
Alimemazine Tartrate	Betamethasone Valerate and Gentamicin Sulfate Cream
Alprenolol Hydrochloride	Betamethasone Valerate and Gentamicin Sulfate Ointment
Alprostadiol	Betaxolol Hydrochloride
Alprostadiol Alfadex	Bethanechol Chloride
Aluminum Potassium Sulfate Hydrate	Bifonazole
Amantadine Hydrochloride	Biperiden Hydrochloride
Ambenonium Chloride	Bismuth Subgallate
Amikacin Sulfate	Bisoprolol Fumarate
Amikacin Sulfate for Injection	Bisoprolol Fumarate Tablets
Amikacin Sulfate Injection	Bleomycin Hydrochloride
Aminophylline Hydrate	Bleomycin Sulfate
Amiodarone Hydrochloride	Freeze-dried Botulism Antitoxin, Equine
Amiodarone Hydrochloride Tablets	Bromhexine Hydrochloride
Amitriptyline Hydrochloride	Bromocriptine Mesilate
Amitriptyline Hydrochloride Tablets	Bufetolol Hydrochloride
Amlodipine Besilate	Buformin Hydrochloride
Amlodipine Besilate Tablets	Buformin Hydrochloride Delayed-release Tablets
Amosulalol Hydrochloride	Buformin Hydrochloride Tablets
Amosulalol Hydrochloride Tablets	Bunazosin Hydrochloride
Amoxicillin Hydrate	Bupivacaine Hydrochloride Hydrate
Anhydrous Ampicillin	Buprenorphine Hydrochloride
Ampicillin Hydrate	Butenafine Hydrochloride
Aprindine Hydrochloride	Butenafine Hydrochloride Cream
Aprindine Hydrochloride Capsules	Butenafine Hydrochloride Solution
Arbekacin Sulfate	Butenafine Hydrochloride Spray
Arbekacin Sulfate Injection	Butropium Bromide
Argatroban Hydrate	Caffeine and Sodium Benzoate
L-Lysine Hydrochloride	Caffeine Hydrate
L-Lysine Hydrochloride Injection	Calcitonin Salmon
Arotinolol Hydrochloride	Precipitated Calcium Carbonate Fine Granules
Arsenic Trioxide	Precipitated Calcium Carbonate Tablets
Aspoxicillin Hydrate	Calcium Chloride Hydrate
Atropine Sulfate Hydrate	Calcium Folinate Hydrate
Atropine Sulfate Injection	Calcium Gluconate Hydrate
Azelastine Hydrochloride	Calcium Hydroxide
Azelastine Hydrochloride Granules	Calcium Lactate Hydrate
Bacampicillin Hydrochloride	Calcium Levofolinate Hydrate
Beclometasone Dipropionate	Calcium Oxide
Bekanamycin Sulfate	Calcium Paraaminosalicylate Hydrate
Benidipine Hydrochloride	Dibasic Calcium Phosphate Hydrate
Benidipine Hydrochloride Tablets	Monobasic Calcium Phosphate Hydrate
Benserazide Hydrochloride	Calcium Sodium Edetate Hydrate
Benzalkonium Chloride	Camostat Mesilate
Benzalkonium Chloride Concentrated Solution 50	Carbazochrome Sodium Sulfonate Hydrate
Benzalkonium Chloride Solution	Carbidopa Hydrate
Benzethonium Chloride	Carbon Dioxide
Benzethonium Chloride Solution	Carteolol Hydrochloride
Benzylpenicillin Benzathine Hydrate	Cefadroxil for Syrup
Benzylpenicillin Potassium	Cefalexin for Syrup
Berberine Chloride Hydrate	Cefatrizine Propylene Glycolate for Syrup
Betahistine Mesilate	Cefcapene Pivoxil Hydrochloride Hydrate
Betahistine Mesilate Tablets	

Cefcapene Pivoxil Hydrochloride Fine Granules	Clobetasol Propionate
Cefcapene Pivoxil Hydrochloride Tablets	Clocapramine Hydrochloride Hydrate
Cefditoren Pivoxil	Clofedanol Hydrochloride
Cefditoren Pivoxil Fine Granules	Clomifene Citrate
Cefditoren Pivoxil Tablets	Clomifene Citrate Tablets
Cefepime Dihydrochloride Hydrate	Clomipramine Hydrochloride
Cefepime Dihydrochloride for Injection	Clonidine Hydrochloride
Cefixime Hydrate	Cloperastine Hydrochloride
Cefmenoxime Hydrochloride	Cloxacillin Sodium Hydrate
Cefminox Sodium Hydrate	Cocaine Hydrochloride
Cefotiam Hydrochloride	Codeine Phosphate Hydrate
Cefotiam Hydrochloride for Injection	1% Codeine Phosphate Powder
Cefozopran Hydrochloride	10% Codeine Phosphate Powder
Cefozopran Hydrochloride for Injection	Codeine Phosphate Tablets
Cefpirome Sulfate	Colestimide
Cefpodoxime Proxetil	Colistin Sulfate
Cefpodoxime Proxetil for Syrup	Cortisone Acetate
Cefroxadine Hydrate	Croconazole Hydrochloride
Cefroxadine for Syrup	Cyclopentolate Hydrochloride
Ceftazidime Hydrate	Cyclophosphamide Hydrate
Cefteram Pivoxil	Cyproheptadine Hydrochloride Hydrate
Cefteram Pivoxil Fine Granules	Dantrolene Sodium Hydrate
Cefteram Pivoxil Tablets	Daunorubicin Hydrochloride
Ceftibuten Hydrate	Deferoxamine Mesilate
Ceftriaxone Sodium Hydrate	Dehydrocholic Acid Injection
Cefuroxime Axetil	Demethylchlortetracycline Hydrochloride
Cetirizine Hydrochloride	Dextran Sulfate Sodium Sulfur 5
Cetirizine Hydrochloride Tablets	Dextran Sulfate Sodium Sulfur 18
Cetotiamine Hydrochloride Hydrate	Dextromethorphan Hydrobromide Hydrate
Cetraxate Hydrochloride	Dibekacin Sulfate
Chloramphenicol Sodium Succinate	Dibekacin Sulfate Ophthalmic Solution
Chlorhexidine Gluconate Solution	Dibucaine Hydrochloride
Chlorhexidine Hydrochloride	Dicloxacillin Sodium Hydrate
Chlormadinone Acetate	Diethylcarbamazine Citrate
Chlorphenesin Carbamate	Diethylcarbamazine Citrate Tablets
Chlorphenesin Carbamate Tablets	Difenidol Hydrochloride
Chlorpheniramine Maleate	Diflorasone Diacetate
Chlorpheniramine Maleate Injection	Diflucortolone Valerate
Chlorpheniramine Maleate Powder	Dihydrocodeine Phosphate
Chlorpheniramine Maleate Tablets	1% Dihydrocodeine Phosphate Powder
d-Chlorpheniramine Maleate	10% Dihydrocodeine Phosphate Powder
Chlorpromazine Hydrochloride	Dihydroergotamine Mesilate
Chlorpromazine Hydrochloride Injection	Dihydroergotoxin Mesilate
Chlorpromazine Hydrochloride Tablets	Dilazep Hydrochloride Hydrate
Cibenzoline Succinate	Diltiazem Hydrochloride
Cibenzoline Succinate Tablets	Dimemorfan Phosphate
Ciclosporin	Dinoprost
Cilazapril Hydrate	Diphenhydramine Hydrochloride
Ciprofloxacin Hydrochloride Hydrate	Diphenhydramine and Bromovalerylurea Powder
Citric Acid Hydrate	Freeze-dried Diphtheria Antitoxin, Equine
Clebopride Malate	Distigmine Bromide
Clemastine Fumarate	Distigmine Bromide Tablets
Clindamycin Hydrochloride	Dobutamine Hydrochloride
Clindamycin Hydrochloride Capsules	Donepezil Hydrochloride
Clindamycin Phosphate	Donepezil Hydrochloride Fine Granules
Clindamycin Phosphate Injection	Donepezil Hydrochloride Tablets

Dopamine Hydrochloride	Gentamicin Sulfate Ophthalmic Solution
Dopamine Hydrochloride Injection	Glutathione
Doxapram Hydrochloride Hydrate	Glyceryl Monostearate
Doxazosin Mesilate	Glycine
Doxazosin Mesilate Tablets	Gonadorelin Acetate
Doxorubicin Hydrochloride	Guaiifenesin
Doxorubicin Hydrochloride for Injection	Guanabenz Acetate
Doxycycline Hydrochloride Hydrate	Freeze-dried Habu Antivenom, Equine
Doxycycline Hydrochloride Tablets	L-Histidine Hydrochloride Hydrate
Ecabet Sodium Hydrate	Homatropine Hydrobromide
Emedastine Fumarate	Homochlorcyclizine Hydrochloride
Enalapril Maleate	Hydralazine Hydrochloride
Enalapril Maleate Tablets	Hydralazine Hydrochloride for Injection
Enviomycin Sulfate	Hydralazine Hydrochloride Powder
Eperisone Hydrochloride	Hydralazine Hydrochloride Tablets
Ephedrine Hydrochloride	Hydrocortisone Acetate
Ephedrine Hydrochloride Injection	Hydrocortisone Butyrate
10% Ephedrine Hydrochloride Powder	Hydrocortisone Sodium Phosphate
Ephedrine Hydrochloride Tablets	Hydrocortisone Sodium Succinate
Epirizole	Hydrocortisone Succinate
Epirubicin Hydrochloride	Hydrocotarnine Hydrochloride Hydrate
Ergocalciferol	Hydroxocobalamin Acetate
Ergometrine Maleate	Hydroxyzine Hydrochloride
Ergometrine Maleate Injection	Hydroxyzine Pamoate
Ergometrine Maleate Tablets	Hypromellose
Ergotamine Tartrate	Hypromellose Acetate Succinate
Erythromycin Ethylsuccinate	Hypromellose Phthalate
Erythromycin Lactobionate	Idarubicin Hydrochloride
Erythromycin Stearate	Idarubicin Hydrochloride for Injection
Estradiol Benzoate	Ifenprodil Tartrate
Estradiol Benzoate Injection (Aqueous Suspension)	Ifenprodil Tartrate Fine Granules
Ethambutol Hydrochloride	Ifenprodil Tartrate Tablets
Ethenzamide	Imidapril Hydrochloride
Ethyl L-Cysteine Hydrochloride	Imidapril Hydrochloride Tablets
Ethylmorphine Hydrochloride Hydrate	Imipenem Hydrate
Etilefrine Hydrochloride	Imipramine Hydrochloride
Etilefrine Hydrochloride Tablets	Imipramine Hydrochloride Tablets
Faropenem Sodium Hydrate	Insulin Human (Genetical Recombination)
Fentanyl Citrate	Insulin Human (Genetical Recombination) Injection
Ferrous Sulfate Hydrate	Ipratropium Bromide Hydrate
Fexofenadine Hydrochloride	Irsogladine Maleate
Flavoxate Hydrochloride	Irsogladine Maleate Fine Granules
Flecainide Acetate	Irsogladine Maleate Tablets
Flecainide Acetate Tablets	Isepamicin Sulfate
Fludrocortisone Acetate	Isepamicin Sulfate Injection
Flurazepam Hydrochloride	<i>l</i> -Isoprenaline Hydrochloride
Fluvoxamine Maleate	Isosorbide Dinitrate
Fluvoxamine Maleate Tablets	Isosorbide Dinitrate Tablets
Formoterol Fumarate Hydrate	Isosorbide Mononitrate 70%/Lactose 30%
Fosfomycin Calcium Hydrate	Isoxsuprine Hydrochloride
Fradiomycin Sulfate	Isoxsuprine Hydrochloride Tablets
Fursultiamine Hydrochloride	Josamycin Propionate
Gabexate Mesilate	Kainic Acid Hydrate
$\beta$ -Galactosidase (Aspergillus)	Kanamycin Monosulfate
$\beta$ -Galactosidase (Penicillium)	Kanamycin Sulfate
Gentamicin Sulfate	Ketamine Hydrochloride

Ketoconazole Solution	Midecamycin Acetate
Ketotifen Fumarate	Minocycline Hydrochloride
Kitasamycin	Minocycline Hydrochloride for Injection
Kitasamycin Acetate	Minocycline Hydrochloride Tablets
Kitasamycin Tartrate	Morphine Hydrochloride Hydrate
Labetalol Hydrochloride	Morphine Hydrochloride Injection
Labetalol Hydrochloride Tablets	Morphine Hydrochloride Tablets
Lactose Hydrate	Morphine and Atropine Injection
Lauromacrogol	Morphine Sulfate Hydrate
Lenampicillin Hydrochloride	Mosapride Citrate Hydrate
Levallorphan Tartrate	Mosapride Citrate Powder
Levallorphan Tartrate Injection	Mosapride Citrate Tablets
Levofloxacin Hydrate	Mupirocin Calcium Hydrate
Levomepromazine Maleate	Nafamostat Mesilate
Levothyroxine Sodium Hydrate	Naloxone Hydrochloride
Lidocaine Injection	Naphazoline Hydrochloride
Limaprost Alfadex	Naphazoline Nitrate
Lincomycin Hydrochloride Hydrate	Neostigmine Methylsulfate
Lincomycin Hydrochloride Injection	Neostigmine Methylsulfate Injection
Lisinopril Hydrate	Nicardipine Hydrochloride
Lobenzarit Sodium	Nicardipine Hydrochloride Injection
Loxoprofen Sodium Hydrate	Noradrenaline Injection
L-Lysine Acetate	Noscapine
L-Lysine Hydrochloride	Noscapine Hydrochloride Hydrate
Lysozyme Hydrochloride	Olopatadine Hydrochloride
Magnesium Sulfate Hydrate	Olopatadine Hydrochloride Tablets
Maltose Hydrate	Opium Alkaloids Hydrochlorides
Freeze-dried Mamushi Antivenom, Equine	Opium Alkaloids Hydrochlorides Injection
Manidipine Hydrochloride	Opium Alkaloids and Atropine Injection
Manidipine Hydrochloride Tablets	Opium Alkaloids and Scopolamine Injection
D-Mannitol	Weak Opium Alkaloids and Scopolamine Injection
D-Mannitol Injection	Orciprenaline Sulfate
Maprotiline Hydrochloride	Oxapium Iodide
Meclofenoxate Hydrochloride	Oxeprenolol Hydrochloride
Mefloquine Hydrochloride	Oxybuprocaine Hydrochloride
Mepenzolate Bromide	Oxycodone Hydrochloride Hydrate
Mepivacaine Hydrochloride	Compound Oxycodone Injection
Mepivacaine Hydrochloride Injection	Compound Oxycodone and Atropine Injection
Mercaptopurine Hydrate	Oxytetracycline Hydrochloride
Meropenem Hydrate	Pancuronium Bromide
Metenolone Acetate	Papaverine Hydrochloride
Metenolone Enanthate	Papaverine Hydrochloride Injection
Metenolone Enanthate Injection	Paroxetine Hydrochloride Hydrate
Metformin Hydrochloride	Pentoxyverine Citrate
Metformin Hydrochloride Tablets	Peplomycin Sulfate
Methylbenacyzium Bromide	Peplomycin Sulfate for Injection
Methyldopa Hydrate	Perphenazine Maleate
dl-Methylephedrine Hydrochloride	Perphenazine Maleate Tablets
10% dl-Methylephedrine Hydrochloride Powder	Pethidine Hydrochloride
Methylergometrine Maleate	Pethidine Hydrochloride Injection
Methylergometrine Maleate Tablets	10% Phenobarbital Powder
Metoprolol Tartrate	Phenol
Metoprolol Tartrate Tablets	Phenol and Zinc Oxide Liniment
Mexiletine Hydrochloride	Phenylephrine Hydrochloride
Miconazole Nitrate	Phenytoin
Micronomicin Sulfate	Phenytoin Powder

Phenytoin Tablets	Riboflavin Butyrate
Phenytoin Sodium for Injection	Riboflavin Sodium Phosphate
Phytomedine	Riboflavin Sodium Phosphate Injection
Pilocarpine Hydrochloride	Ribostamycin Sulfate
Pilocarpine Hydrochloride Tablets	Ritodrine Hydrochloride
Pioglitazone Hydrochloride	Ritodrine Hydrochloride Tablets
Pioglitazone Hydrochloride Tablets	Roxatidine Acetate Hydrochloride
Pipemidic Acid Hydrate	Roxatidine Acetate Hydrochloride Extended-release Capsules
Piperazine Phosphate Hydrate	Roxatidine Acetate Hydrochloride Extended-release Tablets
Piperazine Phosphate Tablets	Roxatidine Acetate Hydrochloride for Injection
Pirenzepine Hydrochloride Hydrate	Saccharin Sodium Hydrate
Pitavastatin Calcium Hydrate	Salbutamol Sulfate
Pivmecillinam Hydrochloride	Sarpogrelate Hydrochloride
Pivmecillinam Hydrochloride Tablets	Sarpogrelate Hydrochloride Fine Granules
Polymixin B Sulfate	Sarpogrelate Hydrochloride Tablets
Polyoxyl 40 Stearate	Scopolamine Butylbromide
Povidone	Scopolamine Hydrobromide Hydrate
Prasterone Sodium Sulfate Hydrate	Freeze-dried Smallpox Vaccine
Prazosin Hydrochloride	Sodium Acetate Hydrate
Prednisolone Acetate	Sodium Bisulfite
Prednisolone Sodium Phosphate	Sodium Carbonate Hydrate
Prednisolone Succinate	Isotonic Sodium Chloride Solution
Prednisolone Sodium Succinate for Injection	Sodium Citrate Hydrate
Procainamide Hydrochloride	Disodium Eddetate Hydrate
Procainamide Hydrochloride Injection	Dibasic Sodium Phosphate Hydrate
Procainamide Hydrochloride Tablets	Sodium Picosulfate Hydrate
Procaine Hydrochloride	Sodium Starch Glycolate
Procaine Hydrochloride Injection	Dried Sodium Sulfite
Procarbazine Hydrochloride	Sodium Thiosulfate Hydrate
Procaterol Hydrochloride Hydrate	Sorbitan Sesquioleate
Prochlorperazine Maleate	D-Sorbitol
Prochlorperazine Maleate Tablets	D-Sorbitol Solution
Promethazine Hydrochloride	Spectinomycin Hydrochloride Hydrate
Propafenone Hydrochloride	Spiramycin Acetate
Propafenone Hydrochloride Tablets	Corn Starch
Propantheline Bromide	Potato Starch
Propiverine Hydrochloride	Rice Starch
Propiverine Hydrochloride Tablets	Wheat Starch
Propranolol Hydrochloride	Streptomycin Sulfate
Propranolol Hydrochloride Tablets	Streptomycin Sulfate for Injection
Protamine Sulfate	Sucralfate Hydrate
Protamine Sulfate Injection	Sulfamethoxazole
Protirelin Tartrate Hydrate	Sulfamonomethoxine Hydrate
Pyridostigmine Bromide	Sulpyrine Hydrate
Pyridoxal Phosphate Hydrate	Sultamicillin Tosilate Hydrate
Pyridoxine Hydrochloride	Sultamicillin Tosilate Tablets
Pyridoxine Hydrochloride Injection	Suxamethonium Chloride Hydrate
Quinapril Hydrochloride	Suxamethonium Chloride Injection
Quinapril Hydrochloride Tablets	Suxamethonium Chloride for Injection
Quinidine Sulfate Hydrate	Tacalcitol Hydrate
Quinine Hydrochloride Hydrate	Talampicillin Hydrochloride
Quinine Sulfate Hydrate	Taltirelin Hydrate
Ranitidine Hydrochloride	Tamoxifen Citrate
0.1% Reserpine Powder	Tamsulosin Hydrochloride
Retinol Acetate	
Retinol Palmitate	

Tamsulosin Hydrochloride Extended-release Tablets  
 Temocapril Hydrochloride  
 Temocapril Hydrochloride Tablets  
 Terbinafine Hydrochloride  
 Terbinafine Hydrochloride Cream  
 Terbinafine Hydrochloride Solution  
 Terbinafine Hydrochloride Spray  
 Terbinafine Hydrochloride Tablets  
 Terbutaline Sulfate  
 Testosterone Enanthate  
 Testosterone Enanthate Injection  
 Testosterone Propionate  
 Testosterone Propionate Injection  
 Tetracaine Hydrochloride  
 Tetracycline Hydrochloride  
 Thiamine Chloride Hydrochloride  
 Thiamine Chloride Hydrochloride Injection  
 Thiamine Chloride Hydrochloride Powder  
 Thiamine Nitrate  
 L-Threonine  
 Tiapride Hydrochloride  
 Tiapride Hydrochloride Tablets  
 Tiaramide Hydrochloride  
 Tiaramide Hydrochloride Tablets  
 Ticlopidine Hydrochloride  
 Timepidium Bromide Hydrate  
 Timolol Maleate  
 Tipepidine Hibenzate  
 Tipepidine Hibenzate Tablets  
 Tizanidine Hydrochloride  
 Tocopherol  
 Tocopherol Acetate  
 Tocopherol Calcium Succinate  
 Tocopherol Nicotinate  
 Todralazine Hydrochloride Hydrate  
 Tolnaftate  
 Tolnaftate Solution  
 Tolperisone Hydrochloride  
 Tosufloxacin Tosilate Hydrate  
 Tosufloxacin Tosilate Tablets  
 Trehalose Hydrate  
 Trichomycin  
 Triclofos Sodium  
 Triclofos Sodium Syrup  
 Trihexyphenidyl Hydrochloride  
 Trihexyphenidyl Hydrochloride Tablets  
 Trimebutine Maleate  
 Trimetazidine Hydrochloride  
 Trimetazidine Hydrochloride Tablets  
 Trimetoquinol Hydrochloride Hydrate  
 Tulobuterol Hydrochloride  
 L-Tyrosine  
 Ursodeoxycholic Acid  
 Ursodeoxycholic Acid Granules  
 Ursodeoxycholic Acid Tablets  
 Valaciclovir Hydrochloride Tablets

Vancomycin Hydrochloride  
 Vancomycin Hydrochloride for Injection  
 Verapamil Hydrochloride  
 Verapamil Hydrochloride Tablets  
 Vinblastine Sulfate  
 Vinblastine Sulfate for Injection  
 Vincristine Sulfate  
 Zinc Sulfate Hydrate  
 Zolpidem Tartrate  
 Zolpidem Tartrate Tablets

**17.** The following monographs were deleted:  
 Cholera Vaccine  
 Diphtheria-Tetanus Combined Toxoid  
 Gas Gangrene Antitoxin, Equine  
 Japanese Encephalitis Vaccine  
 Freeze-dried Japanese Encephalitis Vaccine  
 Methylrosanilinium Chloride  
 Live Oral Poliomyelitis Vaccine  
 Weil's Disease and Akiyami Combined Vaccine

**18.** The following articles were newly added to Ultraviolet-visible Reference Spectra:

Bicalutamide  
 Cabergoline  
 Celecoxib  
 Cloperastine Fendzoate  
 Ethyl Loflazepate  
 Fenofibrate  
 Gefitinib  
 Rilmazafone Hydrochloride Hydrate  
 Rosuvastatin Calcium  
 Triazolam  
 Zopiclone

**19.** The following articles were newly added to Infrared Reference Spectra:

Bicalutamide  
 Cabergoline  
 Celecoxib  
 Cloperastine Fendzoate  
 Copovidone  
 Ethyl Loflazepate  
 Fenofibrate  
 Gefitinib  
 Rilmazafone Hydrochloride Hydrate  
 Rosuvastatin Calcium  
 Sodium Lauryl Sulfate  
 Triazolam  
 Zopiclone

Those who were engaged in the preparation of JP 18 are as follows:

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 ABE Yasuhiro  
 AITA Youhei  
 AKAO Kenichi  
 ARATO Teruyo

ARIMOTO Keiko  
ARUGA Naoki  
ASAI Yumi  
ASAMA Hiroshi  
ASHIZAWA Kazuhide  
ASO Shinichiro  
ASO Yukio  
DEMIZU Yosuke  
EMURA Makoto  
FUCHINO Hiroyuki  
FUJII Hirosato  
FUJII Makiko  
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FUJIMOTO Yuzo  
FUKAMI Toshiro  
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HYUGA Masashi  
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IGOSHI Nobukazu  
IIMORI Jumpei  
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IKEDO Shingo  
IKEGAMI Kazuhiko  
IMAMOTO Tsuyoshi  
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ISHII Akiko  
ITAI Shigeru  
ITO Michiho  
ITO Ryoichi  
ITO Yuji  
IZUTANI Yusuke  
IZUTSU Kenichi  
KAIDA Naoki  
KAMMOTO Toshihiro  
KANEBAKO Makoto  
KATAYAMA Hirohito  
KATO Kumiko  
KATORI Noriko  
KAWAHARA Nobuo  
KAWAI Tamotsu  
KAWAMATA Tomomi  
KAWANISHI Toru  
KAWANO Noriaki  
KAWARASAKI Yoshihiko  
KAWASAKI Nana  
KIJIMA Keiji  
KIKUCHI Yutaka  
KIKUCHI Yuichi  
KIMURA Noritaka  
KISHIMOTO Yasuhiro  
KITADA Mitsukazu  
KITAJIMA Akihito  
KITTAKA Atsushi  
KIUCHI Fumiyuki  
KOBAYASHI Takashi  
KOCHI Rika  
KOHAMA Ai  
KOHITA Hideki  
KOIDE Tatsuo  
KOJIMA Takashi  
KOKUBO Hiroyasu  
KOMATSU Katsuko  
KONDA Toshifumi  
KONDO Seizo  
KUBOSAKI Atsutaka  
KUBOTA Kiyoshi  
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KUSUNOKI Hideki  
MAEKAWA Keiko  
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MASADA Sayaka  
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MATSUMOTO Makoto  
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MIKAMI Eiichi  
MISAWA Takashi  
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MIYATA Naoki  
MIYAZAKI Takashi  
MIYAZAKI Tamaki

MIZUNO Takeshi  
MORI Mitsuo  
MORIBE Kunikazu  
MORIMOTO Seiki  
MORIMOTO Takashi  
MORISAKI Takahito  
MORITA Osamu  
MORIYASU Takako  
MURABAYASHI Mika  
MURAI Toshimi  
MUROI Masashi  
NAKA Nobuyuki  
NAKAGAWA Hidehiko  
NAKAGAWA Shinsaku  
NAKAGAWA Tsutomu  
NAKAGAWA Yukari  
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NAKANO Tatsuya  
NAKASHIMA Tatsumi  
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NASU Masao  
NISHIHARA Yutaka  
NISHIKAWA Noriaki  
NISHIMURA Masahiro  
NOGUCHI Shuji  
OBA Sumiaki  
OBARA Sakae  
OCHIAI Masaki  
OGAWA Toru  
OGURA Yasumitsu  
OGURI Kazuki  
OHGAMI Yasutaka  
OHTA Shigeru  
OKUBO Tsuneo  
OKUDA Akihiro  
OKUDA Haruhiro  
OMURA Koichi  
ONO Makoto  
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SASAKI Satoshi  
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USHIRODA Osamu  
WADA Yoshio  
WATANABE Takumi  
YAMADA Rumiko  
YAMADA Yuko  
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YAMAJI Hiroki  
YAMAMOTO Eiichi  
YAMAMOTO Keiji  
YAMAMOTO Tosuke  
YAMAMOTO Yutaka  
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# The Japanese Pharmacopoeia

*EIGHTEENTH EDITION*

# GENERAL NOTICES

1. The official name of this pharmacopoeia is 第十八改正日本薬局方, and may be abbreviated as 日局十八, 日局18, JP XVIII or JP 18.
2. The English name of this pharmacopoeia is The Japanese Pharmacopoeia, Eighteenth Edition.
3. Among drugs, the Japanese Pharmacopoeia Drugs (the JP Drugs) are those specified in the monographs. The title names and the commonly used names adopted in the monograph should be used as official names. In the drug monograph, in addition to English name, chemical name or Latin name can be mentioned in the title, as appropriate.
4. Crude Drugs and their related products are placed together in “Crude Drugs and Related Drugs” in the posterior part of the Official Monographs. These include: Extracts, Powders, Tinctures, Syrups, Spirits, Fluidextracts or Suppositories containing Crude Drugs as the active ingredient, and combination preparations containing Crude Drugs as the principal active ingredient.
5. The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. However, the headings of “Description” and in addition “Containers and storage” and “Shelf life” in the monographs on preparations are given for information, and should not be taken as indicating standards for conformity. Nevertheless, Containers under “Containers and storage” in the monograph on preparations containing crude drugs as main active ingredients are the standards for conformity.
6. In principle, unless otherwise specified, animals used for preparing the JP Drugs or their source materials must be healthy.
7. In this English version, the JP Drugs described in the monographs begin with a capital letter.
8. The molecular formulas or constitution formulas in parentheses ( ) after the name of drugs or chemicals designate chemically pure substances. Atomic masses adopted in the Japanese Pharmacopoeia conform to the table of “Atomic Weights of the Elements 2015” (IUPAC)-Standard Atomic Weights 2017 (Atomic Weights Subcommittee of the Chemical Society of Japan). However, the atomic masses of the elements whose atomic weight is indicated with an interval in the 2015 table conform to the table of “Atomic Weights of the Elements 2007” (IUPAC)-Standard Atomic Weights 2010 (Atomic Weights Subcommittee

of the Chemical Society of Japan).

Molecular masses are indicated to two decimal places rounded from three decimals.

9. The following abbreviations are used for the principal units.

meter	m
centimeter	cm
millimeter	mm
micrometer	μm
nanometer	nm
kilogram	kg
gram	g
milligram	mg
microgram	μg
nanogram	ng
picogram	pg
Celsius degree	°C
mole	mol
millimole	mmol
square centimeter	cm <sup>2</sup>
liter	L
milliliter	mL
microliter	μL
megahertz	MHz
per centimeter	cm <sup>-1</sup>
newton	N
kilopascal	kPa
pascal	Pa
pascal second	Pa·s
millipascal second	mPa·s
square millimeter per second	mm <sup>2</sup> /s
lux	lx
mole per liter	mol/L
millimole per liter	mmol/L
mass per cent	%
mass parts per million	ppm
mass parts per billion	ppb
volume per cent	vol%
volume parts per million	vol ppm
mass per volume per cent	w/v%
microsiemens per centimeter	μS·cm <sup>-1</sup>
endotoxin unit	EU
colony forming unit	CFU

Note: “ppm” used in the Nuclear Magnetic Resonance Spectroscopy indicates the chemical shift, and “w/v%” is used in the formula or composition of preparations.

10. The unit used for expressing the potency of the JP Drugs is recognized as the quantity of drug.

Usually it is expressed by a definite quantity of a definite standard substance which shows a definite biological activity, and differs according to each drug. The units are determined, in principle, by comparison with each reference standard by means of biological methods. The term "Unit" used for the JP articles indicates the unit defined in the Japanese Pharmacopoeia.

**11.** The statement "Being specified separately." in the monographs means that the tests are to be specified when the drugs are granted approval based on the Law on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices.

**12.** From the point of view of quality assurance, requirements that should be noted on manufacturing processes, if appropriate in addition to the specifications, are shown in the heading "Manufacture" in monograph. It may contain requirements regarding control of materials, manufacturing processes and intermediates, and requirements regarding tests in process and omission of tests for the release. The fulfilment of requirements mentioned in this heading are confirmed based on the information obtained during the establishment of manufacturing method at the development stage, the control of manufacturing processes, or the tests for the release. Also even in the case of absence of the heading "Manufacture" in monograph, it is important to note appropriate controls of materials, manufacturing processes and intermediates in individual drugs.

**13.** When an assurance that a product is of the JP Drug quality is obtained consistently from data derived from the manufacturing process validation studies, and from the records of appropriate manufacturing process control and of the test results of the quality control, the performance of some test items in the monograph at release on a product may be omitted as occasion demands. Moreover, the quality evaluation of final products (drug substances and drug products) based on in-process data including in-process testing results and monitoring data on process parameters can replace specifications and test methods in the monograph or performing the test methods, if appropriate.

**14.** The test methods specified in the Japanese Pharmacopoeia can be replaced by alternative methods which give better accuracy and precision. However, where a difference in test results is suspected, only the result obtained by the procedure given in the Pharmacopoeia is effective for the final judgment.

**15.** The details of the biological test methods may be changed insofar as they do not affect the essential qualities of the test.

**16.** The temperature for the tests or storage is described, in principle, in specific figures. However, the following expressions may be used instead.

Standard temperature, ordinary temperature, room temperature, and lukewarm are defined as 20°C, 15 – 25°C, 1 – 30°C, and 30 – 40°C, respectively. A cold place, unless otherwise specified, shall be a place having a temperature of 1 – 15°C.

The temperature of cold water, lukewarm water, warm water, and hot water are defined as not exceeding 10°C, 30 – 40°C, 60 – 70°C, and about 100°C, respectively.

The term "heated solvent" or "hot solvent" means a solvent heated almost to the boiling point of the solvent, and the term "warmed solvent" or "warm solvent" usually means a solvent heated to a temperature between 60°C and 70°C. The term "heat on or in a water bath" indicates, unless otherwise specified, heating with a boiling water bath or a steam bath at about 100°C.

Cold extraction and warm extraction are usually performed at temperatures of 15 – 25°C and 35 – 45°C, respectively.

**17.** To measure the number of drops, a dropping device which delivers 20 drops of water weighing 0.90 – 1.10 g at 20°C shall be used.

**18.** The term "in vacuum" indicates, unless otherwise specified, a pressure not exceeding 2.0 kPa.

**19.** The acidity or alkalinity of a solution, unless otherwise specified, is determined by blue or red litmus papers. To indicate these properties more precisely, pH values are used.

**20.** The terms in Table 1 are used to express the degree of cutting of Crude Drugs or fineness of powder Drugs.

**Table 1**

Sieve No.	4	6.5	8.6	18	50	100	200
Nominal Designation of sieve	4750 µm	2800 µm	2000 µm	850 µm	300 µm	150 µm	75 µm
Names of the drugs which pass through the respective sieves	Coarse cutting	Moderately fine cutting	Fine cutting	Coarse powder	Moderately fine powder	Fine powder	Very fine powder

**21.** The water to be used in the tests of drugs shall be the water suitable for performing the relevant test, such as the water not containing any substance that would interfere with the test.

**22.** As for wording "solution of a solute", where the name of the solvent is not stated, the term "solution" indicates a solution in water.

**23.** For solution an expression such as "(1 in 3)", "(1 in 10)", or "(1 in 100)" means that 1 g of a solid is dissolved in, or 1 mL of a liquid is diluted with the solvent to make the total volume of 3 mL, 10 mL or 100 mL, respectively. For the liquid mixture an expression such as "(10:1)" or "(5:3:1)" means that

the respective numbers of parts, by volume, of the designated liquids are to be mixed.

**24.** The term "weigh accurately" means to weigh down to the degree of 0.1 mg, 10 µg, 1 µg or 0.1 µg by taking into account the purpose of the test and using a relevant weighing device. The term "weigh exactly" means to weigh to the given decimal places.

**25.** A value of "n" figures in a test of the JP Drugs shall be obtained by rounding off a value of "n + 1" figures.

**26.** Unless otherwise specified, all tests of the JP Drugs shall be performed at the ordinary temperature and observations of the results shall follow immediately after the operations. However, the judgment for a test which is affected by temperature should be based on the conditions at the standard temperature.

**27.** The terms "immediately"/"at once" used in the test of the JP Drugs mean that the procedure is to be performed within 30 seconds after the preceding procedure.

**28.** In the section under the heading Description, the term "white" is used to indicate white or practically white, and "colorless" is colorless or practically colorless. Unless otherwise specified, the test of color is carried out by placing 1 g of a solid drug on a sheet of white paper or in a watch glass placed on white paper. A liquid drug is put into a colorless test tube of 15-mm internal diameter and is observed in front of a white background through a layer of 30 mm. For the test of clarity of liquid drugs the same procedure is applied with either a black or white background. For the observation of fluorescence of a liquid drug, only a black background shall be used.

**29.** In the section under the heading Description, the term "odorless" is used to indicate odorless or practically odorless. Unless otherwise specified, the test of odor shall be carried out by placing 1 g of a solid drug or 1 mL of a liquid drug in a beaker.

**30.** In the section under the heading Description, solubilities are expressed by the terms in Table 2. Unless otherwise specified, solubility means the degree

**Table 2**

Descriptive term	Volume of solvent required for dissolving 1 g or 1 mL of solute
Very soluble	Less than 1 mL
Freely soluble	From 1 mL to less than 10 mL
Soluble	From 10 mL to less than 30 mL
Sparingly soluble	From 30 mL to less than 100 mL
Slightly soluble	From 100 mL to less than 1000 mL
Very slightly soluble	From 1000 mL to less than 10000 mL
Practically insoluble, or insoluble	10000 mL and over

of dissolution of the JP Drugs, previously powdered in the case of a solid drug, within 30 minutes in a solvent at  $20 \pm 5^\circ\text{C}$ , by vigorous shaking for 30 seconds each time at 5-minute intervals.

**31.** In the test of a drug, the term "dissolve" or "miscible" indicates that it dissolves in, or mixes in arbitrary proportion with the solvent to form a clear solution or mixture. Insoluble materials other than the drug including fibers should not be detected or practically invisible, if any.

**32.** Identification is the test to identify the active ingredient(s) of the drug based upon its specific property.

**33.** Purity is the test to detect impurities/contaminants in drugs, and it, as well as other requirements in each monograph, specifies the purity of the drug usually by limiting the kind/nature and quantity of the impurities/contaminants. The impurities/contaminants subject to the purity test are those supposed to generate/contaminate during the manufacturing process or storage, including hazardous agents such as heavy metals, arsenic, etc. If any foreign substances are used or supposed to be added, it is necessary to perform tests to detect or limit the presence of such substances.

**34.** In principle, the JP Drug Products are controlled appropriately according to the direction under Elemental Impurities of the General Tests. When elemental impurities in the drug products are appropriately controlled in accordance with the direction, it is not necessary to perform the tests on elemental impurities such as heavy metals and arsenic specified in the monographs including, but not limited to, those of drug products, drug substances and excipients.

**35.** In principle, unless specified in the monograph, the JP Drugs are controlled appropriately according to the direction under Residual Solvents of the general tests.

**36.** Concerning harmful substances reported as intentionally contaminated to drugs, the control requirement for the presence or absence of contamination is described in the heading "Potential adulteration" in the monograph, as necessary. These substances are controlled by tests on materials, manufacturing processes, intermediates, or final products. The necessity and frequency of the tests are specified separately on individual drugs depending on the control strategy established as part of quality risk management.

**37.** The term "constant mass" in drying or ignition, unless otherwise specified, means that the mass difference after an additional 1 hour of drying or ignition is not more than 0.10% of the preceding mass of the dried substance or ignited residue. For Crude Drugs, the difference is not more than 0.25%. However, when the difference does not exceed 0.5 mg in a chemical balance, 50 µg in a semi-microbalance, or 5 µg in a

microbalance, the constant mass has been attained.

**38.** Assay is the test to determine the composition, the content of the active ingredients, and the potency unit of medicine by physical, chemical or biological procedures.

**39.** In stating the appropriate quantities to be taken for assay, the use of the word "about" indicates a quantity within 10% of the specified mass. The word "dry" in respect of the sample indicates drying under the same conditions, as described in Loss on drying in the monograph.

**40.** For the content of an ingredient determined by Assay in the monographs, if it is expressed simply as "not less than a certain percentage" without indicating its upper limit, 101.0% is understood as the upper limit.

**41.** Sterility means a condition when no target microorganism is detected by the specified method. Sterilization means a process whereby killing or removal of all living microorganisms in an object to be sterilized is accomplished. Aseptic technique is controlled technique to maintain the aseptic condition.

**42.** The container is the device which holds the JP Drugs. The stopper or cap, etc., is considered as part of the container. The containers have no physical and chemical reactivity affecting the specified description and quality of the contents.

**43.** A well-closed container protects the contents from extraneous solids and from loss of the drug under ordinary or customary conditions of handling, shipment, and storage.

Where a well-closed container is specified, it may be

replaced by a tight container.

**44.** A tight container protects the contents from extraneous solids or liquids, from loss of the contents, and from efflorescence, deliquesce, or evaporation under ordinary or customary conditions of handling, shipment, and storage.

Where a tight container is specified, it may be replaced by a hermetic container.

**45.** A hermetic container is impervious to air or any other gas under ordinary or customary conditions of handling, shipment, and storage.

**46.** The term "light-resistant" means that it can prevent transmittance of light affecting in the specified properties and quality of the contents and protect the contained medicament from the light under ordinary or customary conditions of handling, shipment, and storage.

**47.** For the JP Drugs, the contents or potency in terms of units of the active ingredient(s) in the monographs have to be shown on the immediate container or wrapping of them.

**48.** The origin, numerical value or physical properties of the JP Drugs, being stipulated by the special labeling requirements in the monographs, have to be shown on the immediate container or wrapping of them.

**49.** The harmonized General Tests and Monographs among the Japanese Pharmacopoeia, the European Pharmacopoeia and the United States Pharmacopeia are preceded by the statement as such.

The parts of the text, being not harmonized, are surrounded by the symbols (♦ ◆ or ◇ ◇).

**—Abbreviations—**

CS: Colorimetric Stock Solution

RS: Reference Standard

TS: Test Solution

VS: Refer to a solution listed in Standard Solutions for Volumetric Analysis <9.21>.

# GENERAL RULES FOR CRUDE DRUGS

1. Crude drugs in the monographs include medicinal parts obtained from plants or animals, cell inclusions and secretes separated from the origins, their extracts, and minerals. General Rules for Crude Drugs and Crude Drugs Test are applicable to the following:

Acacia, Achyranthes Root, Agar, Akebia Stem, Alisma Tuber, Aloe, Alpinia Officinarum Rhizome, Aluminum Silicate Hydrate with Silicon Dioxide, Amomum Seed, Anemarrhena Rhizome, Angelica Duhurica Root, Apricot Kernel, Aralia Rhizome, Areca, Artemisia Capillaris Flower, Artemisia Leaf, Asiasarum Root, Asparagus Root, Astragalus Root, Atractylodes Lancea Rhizome, Atractylodes Rhizome, Bear Bile, Bearberry Leaf, Belladonna Root, Benincasa Seed, Benzoin, Bitter Cardamon, Bitter Orange Peel, Brown Rice, Bupleurum Root, Burdock Fruit, Calumba, Capsicum, Cardamon, Cassia Seed, Catalpa Fruit, Cherry Bark, Chrysanthemum Flower, Cimicifuga Rhizome, Cinnamon Bark, Cistanche Herb, Citrus Unshiu Peel, Clematis Root, Clove, Cnidium Monnierii Fruit, Cnidium Rhizome, Codonopsis Root, Coix Seed, Condurango, Coptis Rhizome, Cornus Fruit, Corydalis Tuber, Crataegus Fruit, Curcuma Rhizome, Cyperus Rhizome, Digenea, Dioscorea Rhizome, Dolichos Seed, Eleutherococcus Senticosus Rhizome, Ephedra Herb, Epimedium Herb, Eucommia Bark, Euodia Fruit, Fennel, Forsythia Fruit, Fritillaria Bulb, Gambir, Gardenia Fruit, Gastrodia Tuber, Gentian, Geranium Herb, Ginger, Ginseng, Glehnia Root and Rhizome, Glycyrrhiza, Gypsum, Hedysarum Root, Hemp Fruit, Honey, Houttuynia Herb, Immature Orange, Imperata Rhizome, Ipecac, Japanese Angelica Root, Japanese Gentian, Japanese Valerian, Japanese Zanthoxylum Peal, Jujube, Jujube Seed, Koi, Leonurus Herb, Lilium Bulb, Lindera Root, Lithospermum Root, Longan Aril, Longgu, Lonicera Leaf and Stem, Loquat Leaf, Lycium Bark, Lycium Fruit, Magnolia Bark, Magnolia Flower, Mallotus Bark, Malt, Mentha Herb, Moutan Bark, Mulberry Bark, Nelumbo Seed, Notopterygium, Nuphar Rhizome, Nutmeg, Nux Vomica, Ophiopogon Root, Oriental Bezoar, Oyster Shell, Panax Japonicus Rhizome, Peach Kernel, Peony Root, Perilla Herb, Peucedanum Root, Pharbitis Seed, Phellodendron Bark, Picrasma Wood, Pinellia Tuber, Plantago Herb, Plantago Seed, Platycodon Root, Pogostemon Herb, Polygala Root, Polygonatum Rhizome, Polygonum Root, Polyporus Sclero-

tium, Poria Sclerotium, Powdered Acacia, Powdered Agar, Powdered Alisma Tuber, Powdered Aloe, Powdered Amomum Seed, Powdered Atractylodes Lancea Rhizome, Powdered Atractylodes Rhizome, Powdered Calumba, Powdered Capsicum, Powdered Cinnamon Bark, Powdered Clove, Powdered Cnidium Rhizome, Powdered Coix Seed, Powdered Coptis Rhizome, Powdered Corydalis Tuber, Powdered Cyperus Rhizome, Powdered Dioscorea Rhizome, Powdered Fennel, Powdered Gambir, Powdered Gardenia Fruit, Powdered Gentian, Powdered Geranium Herb, Powdered Ginger, Powdered Ginseng, Powdered Glycyrrhiza, Powdered Ipecac, Powdered Japanese Angelica Root, Powdered Japanese Gentian, Powdered Japanese Valerian, Powdered Longgu, Powdered Magnolia Bark, Powdered Moutan Bark, Powdered Oyster Shell, Powdered Panax Japonicus Rhizome, Powdered Peach Kernel, Powdered Peony Root, Powdered Phellodendron Bark, Powdered Picrasma Wood, Powdered Platycodon Root, Powdered Polygala Root, Powdered Polyporus Sclerotium, Powdered Poria Sclerotium, Powdered Processed Aconite Root, Powdered Rhubarb, Powdered Rose Fruit, Powdered Scutellaria Root, Powdered Senega, Powdered Senna Leaf, Powdered Smilax Rhizome, Powdered Sophora Root, Powdered Sweet Hydrangea Leaf, Powdered Swertia Herb, Powdered Tragacanth, Powdered Turmeric, Powdered Zanthoxylum Fruit, Prepared Glycyrrhiza, Processed Aconite Root, Processed Ginger, Prunella Spike, Pueraria Root, Quercus Bark, Red Ginseng, Rehmannia Root, Rhubarb, Rose Fruit, Rosin, Royal Jelly, Safflower, Saffron, Salvia Miltorrhiza Root, Saposhnikovia Root and Rhizome, Sappan Wood, Saussurea Root, Schisandra Fruit, Schizonepeta Spike, Scopolia Rhizome, Scutellaria Root, Senega, Senna Leaf, Sesame, Sinomenium Stem and Rhizome, Smilax Rhizome, Sophora Root, Sweet Hydrangea Leaf, Swertia Herb, Toad Cake, Tragacanth, Tribulus Fruit, Trichosanthes Root, Turmeric, Uncaria Hook.

2. Crude drugs are usually used in the forms of whole crude drugs, cut crude drugs or powdered crude drugs.

Whole crude drugs are the medicinal parts or their ingredients prepared by drying and/or simple processes, as specified in the monographs.

Cut crude drugs are small pieces or small blocks prepared by cutting or crushing of the whole crude drugs,

and also coarse, moderately fine or fine cutting of the crude drugs in whole, and, unless otherwise specified, are required to conform to the specifications of the whole crude drugs used as original materials.

Powdered crude drugs are coarse, moderately fine, fine or very fine powder prepared from the whole crude drugs or the cut crude drugs; usually powdered crude drugs as fine powder are specified in the monographs.

**3.** Unless otherwise specified, crude drugs are used in dried form. The drying is usually carried out at a temperature not exceeding 60°C.

**4.** The origin of crude drugs is to serve as the acceptance criteria. Such statements as 'other species of the same genus' and 'allied plants' or 'allied animals' appearing in the origin of crude drugs usually indicate plants or animals which may be used as materials for crude drugs containing the same effective constituents.

**5.** Description in each monograph for crude drugs covers the crude drug derived from its typical original plant or animal and includes statements of characteristic properties of the crude drug, which are all to serve as the evaluation criteria as well as the aspects obtained by microscopic observation. As for the color, odor and solubility, apply correspondingly to the

prescription of the General Notices, except the odor which is to serve as the acceptance criteria. The taste is to serve as the acceptance criteria.

**6.** Powdered crude drugs, otherwise specified, may be mixed with diluents so as to attain proper content and potency.

**7.** Powdered crude drugs do not contain fragments of tissues, cells, cell inclusions or other foreign matter alien to the original crude drugs or cut crude drugs.

**8.** Crude drugs are as free as possible from contaminants and other impurities due to molds, insects and other animals and from other foreign matters, and are required to be kept in a clean and hygienic state.

**9.** Crude drugs are preserved under protection from moisture and insect damage, unless otherwise specified. In order to avoid insect damage, suitable fumigants may be used to preserve crude drugs, provided that the fumigants are so readily volatilized as to be harmless at the usual dosage of the crude drugs, and such fumigants that may affect the therapeutic efficacy of the crude drugs or interfere with the testing are precluded.

**10.** Crude drugs are preserved in well-closed containers unless otherwise specified.

# GENERAL RULES FOR PREPARATIONS

## [1] General Notices for Preparations

(1) General Notices for Preparations present general rules for pharmaceutical dosage forms.

(2) In [3] Monographs for Preparations, dosage forms are classified mainly by administration routes and application sites, and furthermore are subdivided according to their forms, functions and characteristics.

Those preparations containing mainly crude drugs as active raw materials are described under [4] Monographs for Preparations Related to Crude Drugs.

(3) In Monographs for Preparations and Monographs for Preparations Related to Crude Drugs, dosage forms, which are generally or widely used, are described. However, any other appropriate dosage forms may be used where appropriate. For example, a dosage form suitable for a particular application may be designated by combining an administration route and a name of a dosage form listed in these chapters.

(4) In these monographs, preparation characteristics are specified for the dosage forms. The preparation characteristics are confirmed by appropriate tests.

(5) In the case of preparations, functions that control the release rate of active substance(s) may be added for the purpose of controlling the onset and duration of therapeutic effects and/or decreasing adverse or side effects. The preparations modified in release rate must have an appropriate function of controlled release for the intended use. The added functional modification must generally be displayed on the pack insert and on the direct container or packaging of these preparations.

(6) Pharmaceutical excipients are substances other than active substances contained in preparations, and they are used to increase the utility of the active substance(s) and preparation, to make formulation process easier, to keep the product quality, to improve the usability, and so forth. Suitable excipients may be added for these purposes. The excipients to be used, however, must be pharmacologically inactive and harmless in the administered amount and must not interfere with the therapeutic efficacy of the preparations.

(7) Purified water to be used for preparations is Purified Water or Purified Water in Containers, and water for injection is Water for Injection or Water for

Injection in Containers.

Vegetable oils to be used for preparations are usually edible oils listed in the Pharmacopoeia. When starch is called for, unless otherwise specified, any kind of starch listed in the Pharmacopoeia may be used.

In addition, ethanol specified in vol% is prepared by adding Purified Water or Water for Injection to ethanol at the specified vol%.

(8) Sterile preparations are preparations verified to be aseptic. There are terminal sterilization and aseptic technique as basic manufacturing process of sterile preparations.

Terminal sterilization is a process to sterilize preparations after filling in a container. In this process microbial lethality after sterilization is quantitatively measured or evaluated, and this process is performed under the condition where the sterility assurance level of  $10^{-6}$  or less is ensured by using suitable biological indicators.

Aseptic technique is a process for appropriate control of a risk of microbial contamination, and is a manufacturing process of preparations using a series of aseptic processes with sterile raw materials or after filtration sterilization.

This technique generally requires the presterilization of all equipments and materials used, and this process is performed under the condition to give a defined sterility assurance level in the clean areas where microbial and particulate levels are adequately maintained by using appropriate techniques.

(9) Even non-sterile preparations should be prepared with precautions to prevent contamination and growth of microorganisms, and the test of Microbiological Examination of Non-sterile Products <4.05> or Microbial Limit Test for Crude Drugs and Preparations containing Crude Drugs as Main Ingredient <5.02> is applied to them, if necessary.

(10) The test for Content Uniformity under the Uniformity of Dosage Units and the Dissolution Test are not intended to apply to the crude drug component of preparations which are prepared using crude drugs or preparations related to crude drugs as raw materials.

(11) Unless otherwise specified, preserve preparations at room temperature. Store them in light-resistant containers or packaging, if light affects the quality of the preparation.

## [2] General Notices for Packaging of Preparations

(1) General notices for packaging of preparations describe the basic items on a principle and the packaging suitability for packaging of preparations using container and wrapper.

### (2) Principle of packaging of preparations

In the development phase of preparations, it is important for the packaging of preparations to fully evaluate its suitability for maintaining the specified quality of preparations over the shelf life. Based on the evaluation of the packaging suitability depending on the characteristics of the preparation, items such as specification and test methods of finished products, in-process tests and evaluation of the materials used for packaging and the like to control the quality appropriately are established. The properness of the established requirements can be verified conclusively by the stability studies of finished products.

For the change of the packaging, it is necessary to examine the items described above.

It is necessary to perform the appropriate test to confirm that the unintended changes of the packaging exert no influence to the quality of finished product.

### (3) Packaging suitability

Packaging suitability includes the components of Protection of preparation, Compatibility of preparation and package, Safety of the materials used for package, and additional Performance at the time of administration.

Depending on the characteristics of preparation, the package should have functions such as of moisture-proofness, light-resistance, barrier property for gases and microorganisms, and protection against the shock that might occur at the time of transportation, and the like (Protection).

The package should be composed of the shape and material that do not cause physical and chemical interaction with the preparation (Compatibility).

It should be composed of the materials which leaching and migrating quantity of the constituents and impurities to preparations are sufficiently low from the point of view of safety (Safety).

The packaging performance shall include not merely the protection of preparations but also the improvement of patient compliance, ease of use, etc. Functions of ensuring safety of patients such as a prevention of accidental ingestion and improvement of safety of medical staffs should also be included (Performance).

The packaging suitability is examined based on the test methods listed in the General Tests and appropriate techniques depending on the dosage form and the characteristics of the preparation. Items for appropri-

ate quality control are established based on the test methods and the like used for packaging suitability.

For designing of the packaging of injections, the packaging suitability is examined by appropriate selection from Test for Glass Containers for Injections <7.01>, Test Methods for Plastic Containers <7.02>, Test for Rubber Closure for Aqueous Infusions <7.03>, a container integrity test, a light stability test, the descriptions in Monographs, and the like. Items for appropriate quality control are established based on the adopted techniques for the packaging suitability.

## [3] Monographs for Preparations

(1) In the Monographs for Preparations, the definitions of dosage forms, manufacturing methods, test methods, containers and packaging, and storage are described.

(2) The descriptions of the test methods in these monographs are fundamental requirements, and the manufacturing methods represent commonly used methods.

(3) Preparation in single-dose package means a preparation packaged for single-dose use.

### 1. Preparations for Oral Administration

(1) Immediate-release dosage forms are preparations showing a release pattern of active substance(s) that is not intentionally modified and is generally dependent on the intrinsic solubility of the active substance.

(2) Modified-release dosage forms are preparations showing a release pattern of active substance(s) that is suitably modified for the desired purpose by means of a specific formulation design and/or manufacturing method. Modified-release dosage forms include delayed-release and extended-release preparations.

#### (i) Delayed-release preparations

Delayed-release preparations are designed to release the bulk of the active substance(s) not in stomach but mainly in small intestine, in order to prevent degradation or decomposition of the active substance(s) in stomach or to decrease the irritation of the active substance(s) on stomach. Delayed-release preparations are generally coated with an acid-insoluble enteric film. Delayed-release preparations are included in a group of modified-release dosage forms that delay the start to release active substance(s).

#### (ii) Extended-release preparations

Extended-release preparations are designed to control the release rate and release period of active substance(s) and to restrict the release to appropri-

ate sites in the gastrointestinal tracts in order to decrease the dosing frequency and/or to reduce adverse or side effects. Extended-release preparations are generally prepared by using suitable agents that prolong the release of the active substance(s).

(3) Oral dosage forms such as capsules, granules and tablets can be coated with appropriate coating agents, such as sugars, sugar alcohols, or polymers, for the purpose of enabling the ingestion easy or of preventing degradation of the active substance(s).

#### 1-1. Tablets

(1) Tablets are solid preparations having a desired shape and size, intended for oral administration. Orally Disintegrating Tablets, Chewable Tablets, Effervescent Tablets, Dispersible Tablets and Soluble Tablets are included in this category.

(2) Tablets are usually prepared by the following procedures. Delayed-release or extended-release tablets can be prepared by appropriate methods.

(i) Mix homogeneously active substance(s) and excipients such as diluents, binders and disintegrators, granulate with water or a binder solution by a suitable method, mix with a lubricant, and then compress into a desired shape and size.

(ii) Mix homogeneously active substance(s) and excipients such as diluents, binders, and disintegrators, and then directly compress with a lubricant, or compress after adding active substance(s) and a lubricant to granules previously prepared from excipients and then mixing homogeneously.

(iii) Mix homogeneously active substance(s) and excipients such as diluents and binders, moisten with a solvent, form into a certain shape and size or mold the mixed mass into a certain shape and size, and then dry by a suitable method.

(iv) Plain Tablets are usually prepared according to (i), (ii) or (iii).

(v) Film-coated Tablets can be prepared, usually, by coating Plain Tablets with thin films using suitable film coating agents such as polymers.

(vi) Sugar-coated Tablets can be prepared, usually, by coating Plain Tablets using suitable coating agents including sugars or sugar alcohols.

(vii) Multiple-layer Tablets can be prepared by compressing granules of different compositions to form layered tablets by a suitable method.

(viii) Pressure-coated Tablets can be prepared by compressing granules to cover inner core tablets with different compositions.

(3) Unless otherwise specified, Tablets meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Unless otherwise specified, Tablets meet the requirements of Dissolution Test <6.10> or Disintegration Test <6.09>. For Effervescent tablets from which active substance(s) are dissolved before use and Solu-

ble tablets, these tests are not required.

(5) Well-closed containers are usually used for the preparations. For preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

##### 1-1-1. Orally Disintegrating Tablets/Orodispersible Tablets

(1) Orally Disintegrating Tablets are tablets which are quickly dissolved or disintegrated in the oral cavity.

(2) Orally Disintegrating Tablets shows an appropriate disintegration.

##### 1-1-2. Chewable Tablets

(1) Chewable Tablets are tablets which are administered by chewing.

(2) Chewable Tablets must be in shape and size avoiding danger of suffocation.

##### 1-1-3. Effervescent Tablets

(1) Effervescent Tablets are tablets which are quickly dissolved or dispersed with bubbles in water.

(2) Effervescent tablets are usually prepared using suitable acidic substances and carbonates or hydrogen carbonates.

##### 1-1-4. Dispersible Tablets

(1) Dispersible Tablets are tablets which are administered after having been dispersed in water.

##### 1-1-5. Soluble Tablets

(1) Soluble Tablets are tablets which are administered after having been dissolved in water.

#### 1-2. Capsules

(1) Capsules are preparations enclosed in capsules or wrapped with capsule bases, intended for oral administration. Capsules are classified into Hard Capsules and Soft Capsules.

(2) Capsules are usually prepared by the following methods. Delayed-release or extended-release capsules can be prepared by a suitable method. Coloring agents, preservatives, etc. may be added to the capsule bases.

(i) Hard Capsules: A homogeneous mixture of active substance(s) with diluents and other suitable excipients, or granules or formed masses prepared by a suitable method, are filled into capsule shells as they are or after slight compression.

(ii) Soft Capsules: Active substance(s) and suitable excipients (including solvents) are mixed, enclosed by a suitable capsule base such as gelatin plasticized by addition of glycerin, D-sorbitol, etc. and molded in a suitable shape and size.

(3) Unless otherwise specified, Capsules meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Unless otherwise specified, Capsules meet the requirements of Dissolution Test <6.10> or Disintegration Test <6.09>.

(5) Well-closed containers are usually used for

Capsules. For Capsules susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

### 1-3. Granules

(1) Granules are preparations prepared by granulation, intended for oral administration. Effervescent Granules are included in this category.

(2) Granules are usually prepared by the following methods. Granules can be coated using suitable coating agents if necessary. Extended-release or delayed-release granules can also be prepared by a suitable method.

(i) To powdery active substance(s) add diluents, binders, disintegrators, or other suitable excipients, mix to homogenize, and granulate by a suitable method.

(ii) To previously granulated active substance(s) add excipients such as diluents, and mix to homogenize.

(iii) To previously granulated active substance(s) add excipients such as diluents, and granulate by a suitable method.

(3) Among Granules, the preparations may be referred to as "Fine Granules" if, when Particle Size Distribution Test for Preparations <6.03> is performed, all granules pass through a No. 18 (850 µm) sieve, and not more than 10% of which remain on a No. 30 (500 µm) sieve.

(4) Unless otherwise specified, the Granules in single-dose packages meet the requirements of Uniformity of Dosage Units <6.02>.

(5) Unless otherwise specified, Granules comply with Dissolution Test <6.10> or Disintegration Test <6.09>. However, this provision is not to be applied to Effervescent granules, which are dissolved before use, and Disintegration Test <6.09> is not required for the Granules not more than 10% of which remain on a No. 30 (500 µm) sieve when the test is performed as directed under Particle Size Distribution Test for Preparations <6.03>.

(6) Among Granules, the particulate preparations may be referred to as "Powders" if, when the Particle Size Distribution Test for Preparations <6.03> is performed, all granules pass through a No. 18 (850 µm) sieve, and not more than 5% remain on a No. 30 (500 µm) sieve.

(7) Well-closed containers are usually used for Granules. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

#### 1-3-1. Effervescent Granules

(1) Effervescent granules are granules which are quickly dissolved or dispersed with bubbles in water.

(2) Effervescent granules are usually prepared using suitable acidic substances and carbonates or

hydrogen carbonates.

### 1-4. Powders

(1) Powders are preparations in powder form, intended for oral administration.

(2) Powders are usually prepared by homogeneously mixing active substance(s) with diluents or other suitable excipients.

(3) Unless otherwise specified, the Powders in single-dose packages meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Unless otherwise specified, Powders meet the requirements of Dissolution Test <6.10>.

(5) Well-closed containers are usually used for Powders. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

### 1-5. Liquids and Solutions for Oral Administration

(1) Liquids and Solutions for Oral Administration are preparations in liquid form or flowable and viscous gelatinous state, intended for oral administration. Elixirs, Suspensions, Emulsions and Lemonades are included in this category.

(2) Liquids and Solutions for Oral Administration are usually prepared by dissolving, emulsifying or suspending active substance(s) in Purified Water together with excipients, and by filtering if necessary.

(3) For Liquids and Solutions for Oral Administration which are apt to deteriorate, prepare before use.

(4) Unless otherwise specified, the preparations in single-dose packages meet the requirement of Uniformity of Dosage Units <6.02>.

(5) Tight containers are usually used for Liquids and Solutions for Oral Administration. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

#### 1-5-1. Elixirs

(1) Elixirs are clear, sweetened and aromatic liquid preparations, containing ethanol, intended for oral administration.

(2) Elixirs are usually prepared by dissolving solid active substance(s) or their extractives in ethanol and Purified Water, adding aromatic agents and sucrose, other sugars or sweetening agents, and clarifying by filtration or other procedure.

#### 1-5-2. Suspensions

(1) Suspensions are liquid preparations of active substance(s) suspended finely and homogeneously in a vehicle, intended for oral administration.

(2) Suspensions are usually prepared by adding suspending agent or other suitable excipients and Purified Water or oil to solid active substance(s), and suspending homogeneously as the whole by a suitable

method.

- (3) Mix homogeneously before use, if necessary.
- (4) Unless otherwise specified, Suspensions meet the requirements of Dissolution Test <6.10>.

#### 1-5-3. Emulsions

(1) Emulsions are liquid preparations of active substance(s) emulsified finely and homogeneously in a liquid vehicle, intended for oral administration.

(2) Emulsions are usually prepared by adding emulsifying agents and Purified Water to liquid active substance(s), and emulsifying finely and homogeneously by a suitable method.

(3) Mix homogeneously before use, where necessary.

#### 1-5-4. Lemonades

(1) Lemonades are sweet and sour, clear liquid preparations, intended for oral administration.

### 1-6. Syrups

(1) Syrups are viscous liquid or solid preparations containing sugars or sweetening agents, intended for oral administration. Preparations for Syrups are included in this category.

(2) Syrups are usually prepared by dissolving, mixing, suspending or emulsifying active substance(s) in a solution of sucrose, other sugars or sweetening agents, or in Simple Syrup. Where necessary, the mixture is boiled, and filtered while hot.

(3) For Syrups which are apt to deteriorate, prepare before use.

(4) Unless otherwise specified, Syrups in single-dose packages meet the requirements of Uniformity of Dosage Units <6.02>.

(5) Unless otherwise specified, Syrups in which active substance(s) is suspended meet the requirements of Dissolution Test <6.10>.

(6) Tight containers are usually used for Syrups. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

#### 1-6-1. Preparations for Syrups

(1) Preparations for Syrups are preparations in form of granules or powders, which become syrups by adding water. They may be termed "Dry Syrups".

(2) Preparations for Syrup are usually prepared with sugars or sweetening agents as directed under 1-3. Granules or 1-4. Powders.

(3) Preparations for Syrups are usually to be used after having been dissolved or suspended in water.

(4) Unless otherwise specified, the Preparations for Syrups other than preparations which are to be used after having been dissolved meet the requirements of Dissolution Test <6.10> or Disintegration Test <6.09>. However, Disintegration Test <6.09> is not required for the Preparations, if, when the Particle Size Distribution Test for Preparations <6.03> is

performed, not more than 10% of the total amount remains on a No. 30 (500 µm) sieve.

(5) Well-closed containers are usually used for Preparations for Syrups. For the Preparations for Syrups susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

### 1-7. Jellies for Oral Administration

(1) Jellies for Oral Administration are non-flowable gelatinous preparations having a certain shape and size, intended for oral administration.

(2) Jellies for oral application are usually prepared by mixing active substance(s) with suitable excipients and polymer gel base, gelatinizing and forming into a certain shape and size by a suitable method.

(3) Unless otherwise specified, Jellies for Oral Administration meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Unless otherwise specified, Jellies for Oral Administration meet the requirements of Dissolution Test <6.10> or show an appropriate disintegration.

(5) Tight containers are usually used for Jellies for Oral Administration. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

### 1-8. Films for Oral Administration

(1) Films for Oral Administration are preparations in film form, intended for oral administration.

(2) Films for Oral Administration are usually prepared by spreading to dry a solution, composed of active substance(s) and a mixture of water-soluble polymer and other additives as a base, or by melting the mixture of active substances(s) and the base to form. Layers different in additive compositions may be stacked in appropriate manner to form the films.

(3) Unless otherwise specified, Films for Oral Administration meet the requirement of Uniformity of Dosage Units <6.02>.

(4) Unless otherwise specified, Films for Oral Administration meet the requirement of Dissolution Test <6.10> or show an appropriate disintegration.

(5) Well-closed containers are usually used for Films for Oral Administration. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

#### 1-8-1. Orally Disintegrating Films

(1) Orally Disintegrating Films are films which are quickly dissolved or disintegrated in the oral cavity.

(2) Orally Disintegrating Films show an appropriate disintegration.

## 2. Preparations for Oro-mucosal Application

### 2-1. Tablets for Oro-mucosal Application

(1) Tablets for Oro-mucosal Application are solid

preparations having a certain form, intended for oral cavity application.

Troches/Lozenges, Sublingual Tablets, Buccal Tablets, Mucoadhesive Tablets and Medicated Chewing Gums are included in this category.

(2) Tablets for Oro-mucosal Application are prepared as directed under 1-1. Tablets.

(3) Unless otherwise specified, Tablets for Oro-mucosal Application meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Tablets for Oro-mucosal Application have an appropriate dissolution or disintegration.

(5) Well-closed containers are usually used for Tablets for Oro-mucosal Application. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

#### 2-1-1. Troches/Lozenges

(1) Troches/Lozenges are tablets for oro-mucosal application, which are gradually dissolved or disintegrated in the mouth, and are intended for application locally to the oral cavity or the throat.

(2) Troches/Lozenges must be in shape and size avoiding danger of suffocation.

#### 2-1-2. Sublingual Tablets

(1) Sublingual Tablets are tablets for oro-mucosal application, from which active substance(s) are quickly dissolved sublingually and absorbed via the oral mucosa.

#### 2-1-3. Buccal Tablets

(1) Buccal Tablets are tablets for oro-mucosal application, from which the active substance(s) are dissolved gradually between the cheek and teeth, and absorbed via the oral mucosa.

#### 2-1-4. Mucoadhesive Tablets

(1) Mucoadhesive Tablets are tablets for oro-mucosal application that are applied by adhesion to the oral mucosa.

(2) Mucoadhesive Tablets are usually prepared by using hydrophilic polymers to form hydrogel.

#### 2-1-5. Medicated Chewing Gums

(1) Medicated Chewing Gums are tablets for oro-mucosal application, releasing active substance(s) by chewing.

(2) Medicated Chewing Gums are usually prepared using suitable gum bases such as vegetable resin, thermoplastic resin and elastomer.

### 2-2. Liquids and Solutions for Oro-mucosal Application

(1) Liquids and Solutions for Oro-mucosal Application are preparations in liquid form or flowable and viscous gelatinous state, intended for oral cavity application.

(2) Liquids and Solutions for Oro-mucosal Application are usually prepared by mixing active substance(s) with suitable excipients and Purified Water or

suitable vehicles to dissolve homogenously or to emulsify or suspend, and by filtering if necessary.

(3) For Liquids and Solutions for Oro-mucosal Application which are apt to deteriorate, prepare before use.

(4) Unless otherwise specified, the preparations in single-dose packages meet the requirement of the Uniformity of Dosage Units <6.02>.

(5) Tight containers are usually used for Liquids and Solutions for Oro-mucosal Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

#### 2-2-1. Preparations for Gargles

(1) Preparations for Gargles are liquid preparations intended to apply locally to the oral and throat cavities. Solid type preparations to be dissolved in water before use are also included in this category.

(2) Solid type preparations to be dissolved in water before use are prepared as directed under 1-1. Tablets or 1-3. Granules.

#### 2-3. Sprays for Oro-mucosal Application

(1) Sprays for Oro-mucosal Application are preparations that are applied active substance(s) by spraying into the oral cavity in mist, powder, foam or paste forms.

(2) Sprays for Oro-mucosal Application are usually prepared by the following methods:

(i) Dissolve or suspend active substance(s) and suitable excipients in a solvent, filter, where necessary, and fill into a container together with liquefied or compressed gas.

(ii) Dissolve or suspend active substance(s) and suitable excipients in a solvent, fill into a container, and fit with a pump for spraying.

(3) Unless otherwise specified, metered-dose types among Sprays for Oro-mucosal Application have an appropriate uniformity of delivered dose.

(4) Tight containers or pressure-resistant containers are usually used for Sprays for Oro-mucosal Application.

#### 2-4. Semi-solid Preparations for Oro-mucosal Application

(1) Semi-solid Preparations for Oro-mucosal Application are preparations in cream, gel or ointment forms, intended for application to the oral mucosa.

(2) Semi-solid Preparations for Oro-mucosal Application are usually prepared by emulsifying active substance(s) together with excipients using "Purified Water" and oil component such as petrolatum, or by homogenizing active substance(s) together with suitable excipients using polymer gel or oil and fats as the base.

(i) Creams for oro-mucosal application are pre-

pared as directed under 11-5. Creams.

(ii) Gels for oro-mucosal application are prepared as directed under 11-6. Gels.

(iii) Ointments for oro-mucosal application are prepared as directed under 11-4. Ointments.

For Semi-solid Preparations for Oro-mucosal Application which are apt to deteriorate, prepare before use.

(3) Sufficient amounts of suitable preservatives to prevent the growth of microorganisms may be added for Semi-solid Preparations for Oro-mucosal Application filled in multiple-dose containers.

(4) Semi-solid Preparations for Oro-mucosal Application have a suitable viscosity to apply to the oral mucosa.

(5) Tight containers are usually used for Semi-solid Preparations for Oro-mucosal Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

### 3. Preparations for Injection

#### 3-1. Injections

(1) Injections are sterile preparations to be administered directly into the body through skin, muscle or blood vessel, usually in form of a solution, a suspension or an emulsion of active substance(s), or of a solid that contains active substance(s) to be dissolved or suspended before use.

Parenteral Infusions, Implants/Pellets, Prolonged-Release Injections and Liposome Injections are included in this category.

(2) Injections in solution, suspension or emulsion form are usually prepared by the following methods.

(i) Dissolve, suspend or emulsify active substance(s) with or without excipients in Water for Injection or an aqueous or nonaqueous vehicle homogeneously, fill into containers for injection, seal, and sterilize.

(ii) Dissolve, suspend or emulsify active substance(s) with or without excipients in Water for Injection or an aqueous or nonaqueous vehicle, and filtrate aseptically, or prepare aseptically a homogeneous liquid, fill into containers for injection, and seal.

Every care should be taken to prevent contamination with microorganisms. The overall processes of preparing injections, from the preparation of active solution to the sterilization, should be completed as rapidly as possible, taking into consideration the composition of the injection and the storage conditions. The concentration of active substance(s) expressed in % represents w/v%.

Injections that are to be dissolved or suspended before use and are designated in the name as "for injection" may be accompanied by a suitable vehicle to dis-

solve or suspend the supplied preparation (hereinafter referred to as "vehicle attached to preparation").

(3) Injections may be prepared as Freeze-dried Injections or Powders for Injections to prevent degradation or deactivation of the active substance(s) in solution.

#### (i) Freeze-dried Injections

Freeze-dried Injections are usually prepared by dissolving active substance(s) with or without excipients such as diluents in Water for Injection, sterilizing the solution by aseptic filtration, filling the filtrate directly into individual containers for injection and being freeze-dried, or dividing the filtrate in special containers, being freeze-dried and transferred into individual containers for injection.

#### (ii) Powders for Injections

Powders for injections are usually prepared by filtrating aseptically a solution of active substance(s), obtaining powders by crystallization from the solution or mixing additionally the powders with sterilized excipients, and filling the powders into individual containers for injections.

(4) To prevent errors in the preparation with vehicles attached or administration of injections, or bacterial or foreign matter contamination, or for the purpose of urgent use, prefilled syringes or cartridges may be prepared.

#### (i) Prefilled Syringes for Injections

Prefilled Syringes for injections are usually prepared by dissolving, suspending or emulsifying active substance(s) with or without excipients in a vehicle, and filling into syringes.

#### (ii) Cartridges for Injections

Cartridges for Injections are usually prepared by dissolving, suspending or emulsifying active substance(s) with or without excipients in a vehicle, and filling into cartridges.

The cartridges are used by fixing in an injection device for exclusive use.

(5) Vehicles used in Injections or attached to preparations must be harmless in the amounts usually administered and must not interfere with the therapeutic efficacy of the active substance(s).

The vehicles are classified into the following two groups. They should meet each requirement.

(i) Aqueous vehicles: As the vehicle of aqueous injections, Water for Injection is usually used. Isotonic Sodium Chloride Solution, Ringer's Solution, or other suitable aqueous solutions may be used instead.

Unless otherwise specified, these aqueous vehicles, other than those exclusively for intracutaneous, subcutaneous or intramuscular administration, meet the requirements of Bacterial Endotoxins Test <4.01>.

When the Bacterial Endotoxins Test <4.01> is not

applicable to aqueous vehicles, the Pyrogen Test <4.04> may be applied instead.

(ii) Non-aqueous vehicles: Vegetable oils are usually used as vehicles for oily injections. These oils, unless otherwise specified, are clear at 10°C, the acid value is not more than 0.56, the saponification value is between 185 and 200, and the iodine value falls between 79 and 137. They meet the requirements of Mineral Oil Test <1.05>.

Organic vehicles miscible with water, such as ethanol, are usually used as vehicles for hydrophilic injections.

(6) Unless otherwise specified, any coloring agent must not be added solely for the purpose of coloring the preparations.

(7) Sodium chloride or other excipients may be added to aqueous injections to adjust them isotonic to blood or other body fluids. Acids or alkalis may be added to adjust the pH.

(8) Injections supplied in multiple-dose containers may be added sufficient amounts of suitable preservatives to prevent the growth of microorganisms.

(9) Unless otherwise specified, Injections and vehicles attached to preparations other than those used exclusively for intracutaneous, subcutaneous or intramuscular administration meet the requirements of Bacterial Endotoxins Test <4.01>. In the case where the Bacterial Endotoxins Test <4.01> is not applicable, Pyrogen Test <4.04> may be applied instead.

(10) Unless otherwise specified, Injections and vehicles attached to preparations meet the requirements of Sterility Test <4.06>.

(11) Containers of Injections are colorless and meet the requirements of Test for Glass Containers for Injections <7.01>. Where specified in individual monographs, these containers may be replaced by colored containers meeting the requirements of Test for Glass Containers for Injections <7.01> or by plastic containers for aqueous injections meeting the requirements of Test Methods for Plastic Containers <7.02>.

(12) Unless otherwise specified, rubber closures used for glass containers of 100 mL or more of aqueous infusions meet the requirements of Test for Rubber Closure for Aqueous Infusions <7.03>.

(13) Unless otherwise specified, Injections and vehicles attached to preparations meet the requirements of Foreign Insoluble Matter Test for Injections <6.06>.

(14) Unless otherwise specified, Injections and vehicles attached to preparations meet the requirements of Insoluble Particulate Matter Test for Injections <6.07> or Insoluble Particulate Matter Test for Therapeutic Protein Injections <6.17>.

(15) Unless otherwise specified, the actual volume of Injections meets the requirements of Test for Extractable Volume of Parenteral Preparations <6.05>.

(16) Unless otherwise specified, Injections to be

dissolved or suspended before use meet the requirements of Uniformity of Dosage Units <6.02>.

(17) Among the suspensions for injection in unit-dose containers, the preparations that could impair the uniform dispersion upon standing have an appropriate uniformity.

(18) Suspensions for injection are usually not to be injected into the blood vessels or spinal cord, and emulsions for injection are not to be injected into the spinal cord.

(19) The maximum size of particles observed in suspensions for injection is usually not larger than 150 µm, and that of particles in emulsions for injection is usually not larger than 7 µm.

(20) The following information, unless otherwise specified, must be written on the package leaflet, or the container or wrapper.

(i) In cases where the vehicle is not specified, the name of the employed vehicle, with the exception of Water for Injection, sodium chloride solution not exceeding 0.9 w/v% and those vehicles in which acids or alkalis are used in order to adjust the pH.

(ii) In case of vehicle attached to preparation, the name of the vehicle, content volume, ingredients and quantities or ratios, and a statement of the presence of the vehicle on the outer container or outer wrapper.

(iii) Name and quantity of stabilizers, preservatives, and diluents if added. In the case where nitrogen or carbon dioxide is filled in the container to replace the air inside, a statement of this replacement is not required.

(21) For ampoules or other containers of 2 mL or less, the designations "injection", "for injection" and "aqueous suspension for injection" may be replaced by "inj.", "for inj." and "aq. susp. for inj.", respectively.

For ampoules or other containers of more than 2 mL and not exceeding 10 mL, made of glass or similar materials, the designations "injection", "for injection" and "aqueous suspension for injection" may be abbreviated in the same way as above, when the information is printed directly on the surface of ampoules or containers.

(22) Hermetic containers or tight containers which are able to prevent microbial contamination are usually used for the preparations. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

### 3-1-1. Parenteral Infusions

(1) Parenteral Infusions are usually injections of not less than 100 mL, intended for intravenous administration.

(2) Parenteral Infusions are mainly administered for the purpose of water supply, correction of electro-

lyte abnormality and nutritional support, and they are also used by mixing with other injections for treatments by continual infusion.

### 3-1-2. Implants/Pellets

(1) Implants/Pellets are solid or gel-like form injections, intended for subcutaneous or intramuscular administration by means of an implant device or operative treatment, for the purpose of releasing active substance(s) for a long period of time.

(2) Implants/Pellets are usually prepared in a form of pellet, microsphere or gel using biodegradable polymers.

(3) Unless otherwise specified, Implants/Pellets meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Implants/Pellets have an appropriate function of controlled release.

(5) Foreign Insoluble Matter Test for Injections, Insoluble Particulate Matter for Injections and Test for Extractable Volume of Parenteral Preparations are not required for Implants/Pellets.

### 3-1-3. Prolonged Release Injections

(1) Prolonged Release Injections are injections to be used for intramuscular administration, for the purpose of releasing active substance(s) for a long period of time.

(2) Prolonged Release Injections are usually prepared by dissolving or suspending active substance(s) in a non-aqueous vehicle such as vegetable oil, or by suspending microspheres prepared with biodegradable polymers.

(3) Prolonged Release Injections have an appropriate function of controlled release.

### 3-1-4. Liposome Injections

(1) Liposome Injections are injections to be used for intravenous administration, which are intended for improvement of in vivo stability, delivery to a target region and control of release, of active substance(s).

(2) Liposome Injections are usually prepared by using amphipathic lipid, etc. to make aqueous injections or freeze-dried injections in which closed microvesicles composed of a lipid bilayer membrane are dispersed.

(3) Liposome Injections have an appropriate function of controlled release.

(4) Liposome Injections have an appropriate particle size.

## 4. Preparations for Dialysis

### 4-1. Dialysis Agents

(1) Dialysis Agents are preparations in liquid, or in solid which are to be dissolved before use, intended for peritoneal dialysis or hemodialysis.

They are classified into Peritoneal dialysis agents and Hemodialysis agents.

(2) Unless otherwise specified, Dialysis Agents

meet the requirements of Bacterial Endotoxins Test <4.01>.

(3) The solid preparations which are to be dissolved before use among Dialysis agents have an appropriate uniformity of dosage units.

### 4-1-1. Peritoneal Dialysis Agents

(1) Peritoneal Dialysis Agents are sterile dialysis agents, intended to be used for peritoneal dialysis.

(2) Peritoneal Dialysis Agents are usually prepared by dissolving active substance(s) with suitable excipients in a vehicle to make a certain volume, or by filling active substance(s) combined with suitable excipients in a container, and sealing it. Sterilize if necessary. Every care should be taken to prevent microbial contamination. The overall processes from preparation to sterilization for preparing the agents should be completed as rapidly as possible, taking into consideration the composition of the agents and the storage conditions. The concentration of Peritoneal dialysis agents expressed in % represents w/v%. In the case of solid preparations which are dissolved before use, prepare as directed under 1-1. Tablets or 1-3. Granules.

(3) If necessary, pH adjusting agents, isotonic agents or other excipients may be added.

(4) Unless otherwise specified, the vehicle used for Peritoneal dialysis agents is Water for Injection.

(5) Unless otherwise specified, Peritoneal Dialysis Agents meet the requirements of Sterility Test <4.06>.

(6) Unless otherwise specified, Peritoneal Dialysis Agents meet the requirements of 4. Parenteral infusions under Test for Extractable Volume of Parenteral Preparations <6.05>. The mass (g) of content may convert to the volume (mL) by dividing by the density.

(7) Unless otherwise specified, Peritoneal Dialysis Agents meet the requirements of Foreign Insoluble Matter Test for Injections <6.06>.

(8) Unless otherwise specified, Peritoneal Dialysis Agents meet the requirements of Insoluble Particulate Matter Test for Injections <6.07>.

(9) Colorless containers meeting the requirements of Test for Glass Containers for Injections <7.01> are used for Peritoneal Dialysis Agents. Where specified otherwise, the colored containers meeting the requirements of Test for Glass Containers for Injections <7.01> or the plastic containers for aqueous injections meeting the requirements of Test Methods for Plastic Containers <7.02> may be used.

(10) Unless otherwise specified, the rubber closures of the containers meet the requirements of Test for Rubber Closure for Aqueous Infusions <7.03>.

(11) Hermetic containers, or tight containers which are able to prevent microbial contamination are usually used for Peritoneal Dialysis Agents. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container

or packaging may be used.

#### 4-1-2. Hemodialysis Agents

(1) Hemodialysis agents are dialysis agents to be used for hemodialysis.

(2) Hemodialysis Agents are usually prepared by dissolving active substance(s) with excipients in a vehicle to make a certain volume, or by filling active substance(s) with excipient(s) in a container. In the case of the solid preparations to be dissolved before use, prepare as directed under 1-1. Tablets or 1-3. Granules.

(3) If necessary, pH adjusting agents, isotonic agents or other excipients may be added.

(4) Unless otherwise specified, the vehicle used for Hemodialysis agents is Water for Injection or water suitable for dialysis.

(5) Tight containers which are able to prevent microbial contamination are usually used for Hemodialysis Agents. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

### 5. Preparations for Inhalation

#### 5-1. Inhalations

(1) Inhalations are preparations intended for administration as aerosols to the bronchial tubes or lung.

Inhalations are classified into Dry Powder Inhalers, Inhalation Liquid Preparations and Metered-dose Inhalers.

(2) For administration of Inhalations, suitable devices or apparatus are used, or they are placed in containers which have an appropriate function of inhalation device.

##### 5-1-1. Dry Powder Inhalers

(1) Dry Powder Inhalers are preparations which deliver a constant respiratory intake, intended for administration as solid particle aerosols.

(2) Dry Powder Inhalers are usually prepared by pulverizing active substance(s) into fine particles. Where necessary, lactose or other suitable excipients are added to make homogenous mixture.

(3) Metered-dose types among Dry Powder Inhalers meet the requirements of Uniformity of Delivered Dose for Inhalations <6.14>, unless otherwise specified.

(4) Dry Powder Inhalers meet the requirements of Aerodynamic Particle Size Measurement for Inhalations <6.15>, unless otherwise specified.

(5) Well-closed containers are usually used for Dry Powder Inhalers. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

##### 5-1-2. Inhalation Liquids and Solutions

(1) Inhalation Liquid Preparations are liquid inhalations which are administered by an inhalation device such as operating nebulizers.

(2) Inhalation Liquid Preparations are usually pre-

pared by mixing active substance(s) with a vehicle and suitable isotonic agents and/or pH adjusting agents to make a solution or suspension, and by filtering where necessary.

(3) Sufficient amounts of suitable preservatives may be added to Inhalation Liquid Preparations to prevent the growth of microorganisms.

(4) Tight containers are usually used for Inhalation Liquid Preparations. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

##### 5-1-3. Metered-dose Inhalers

(1) Metered-dose Inhalers are preparations which deliver a constant dose of active substance(s) from the container together with propellant filled in.

(2) Metered-dose Inhalers are usually prepared by dissolving active substance(s) with a suitable dispersing agents and stabilizers in a vehicle to make a solution or suspension, and by filling in pressure-resistant containers together with liquid propellant, and setting metering valves.

(3) Metered-dose Inhalers meet the requirements of Uniformity of Delivered Dose for Inhalations <6.14>, unless otherwise specified.

(4) Metered-dose Inhalers meet the requirements of Aerodynamic Particle Size Measurement for Inhalations <6.15>, unless otherwise specified.

(5) Pressure-resistant and hermetic containers are usually used for Metered-dose Inhalers.

### 6. Preparations for Ophthalmic Application

#### 6-1. Ophthalmic Liquids and Solutions

(1) Ophthalmic Liquids and Solutions are sterile preparations of liquid, or solid to be dissolved or suspended before use, intended for application to the conjunctival sac or other ocular tissues.

(2) Ophthalmic Liquids and Solutions are usually prepared by dissolving, suspending active substance(s) in a vehicle after adding excipients to make a constant volume, or mixing active substance(s) and excipients, and filling into containers. The overall processes, from preparation to sterilization, should be completed with sufficient care to prevent microbial contamination as rapidly as possible, taking into consideration the composition of the preparations and the storage conditions. The concentration of active substance expressed in % represents w/v%.

Ophthalmic Liquids and Solutions to be dissolved or suspended before use and designated in the name as “for ophthalmic application” may be accompanied by a vehicle for dissolving or suspending the preparation (hereinafter referred to as “vehicle attached to preparation”).

(3) Vehicles to prepare Ophthalmic Liquids and Solutions or vehicle attached to the preparations must

be harmless in the amounts usually administered and must not interfere with the therapeutic efficacy of the active substance(s).

Vehicles for Ophthalmic Liquids and Solutions are classified into the following two groups.

(i) Aqueous vehicles: As the vehicles for the aqueous preparations Purified Water or suitable aqueous vehicles are used. For vehicles attached to the preparations sterilized Purified Water or sterilized aqueous vehicles are used.

(ii) Non-aqueous vehicles: As the vehicles for the non-aqueous preparations vegetable oils are usually used. Suitable organic solvents may be also used as the non-aqueous vehicles.

(4) Unless otherwise specified, any coloring agents must not be added solely for the purpose of coloring Ophthalmic Liquids and Solutions or vehicles attached to the preparations.

(5) Sodium chloride or other excipients may be added to Ophthalmic Liquids and Solutions to adjust them isotonic to lacrimal fluid. Acids or alkalis may be also added to adjust the pH.

(6) Unless otherwise specified, Ophthalmic Liquids and Solutions and vehicles attached to the preparations meet the requirements of Sterility Test <4.06>.

(7) Sufficient amounts of appropriate preservatives to prevent the growth of microorganisms may be added to the preparations filled in multiple dose containers.

(8) Unless otherwise specified, Ophthalmic Liquids and Solutions prepared in aqueous solutions or the vehicles attached to the preparations meet the requirements of Foreign Insoluble Matter Test for Ophthalmic Solutions <6.11>.

(9) Unless otherwise specified, Ophthalmic Liquids and Solutions and the vehicles attached to the preparations meet the requirements of Insoluble Particulate Matter Test for Ophthalmic Solutions <6.08>.

(10) The maximum particle size observed in Ophthalmic suspensions is usually not larger than 75  $\mu\text{m}$ .

(11) Transparent tight containers, which do not disturb the test of Foreign Insoluble Matter Test for Ophthalmic Solutions <6.11>, are usually used for Ophthalmic Liquids and Solutions. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

## 6-2. Ophthalmic Ointments

(1) Ophthalmic Ointments are sterile preparations of semi-solid, intended for application to the conjunctival sac or other ocular tissues.

(2) Ophthalmic Ointments are usually prepared by mixing homogeneously solution of or finely powdered active substance(s) with petrolatum or other bases, and filling into containers. The overall processes, from

preparation to sterilization, should be completed with sufficient care to prevent microbial contamination as rapidly as possible, taking into consideration the composition of the preparations and the storage conditions.

(3) Sufficient amounts of suitable preservatives may be added to Ophthalmic Ointments filled in multiple dose containers to prevent the growth of microorganisms.

(4) Unless otherwise specified, Ophthalmic Ointments meet the requirements of Sterility Test <4.06>, and unless otherwise specified, the test is carried out by the Membrane filtration method.

(5) Unless otherwise specified, Ophthalmic Ointments meet the requirements of Test for Metal Particles in Ophthalmic Ointments <6.01>.

(6) The maximum particle size of active substance(s) in Ophthalmic Ointments is usually not larger than 75  $\mu\text{m}$ .

(7) Ophthalmic Ointments have a suitable viscosity for applying to the ocular tissues.

(8) Tight containers which are able to prevent microbial contamination are usually used for Ophthalmic Ointments. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

## 7. Preparations for Otic Application

### 7-1. Ear Preparations

(1) Ear Preparations are liquid, semi-solid, or solid preparations which are to be dissolved or suspended before use, intended for application to the external or internal ear.

(2) Ear Preparations are usually prepared by filling in containers with liquids in which active substance(s) and excipients are dissolved or suspended in a vehicle to make a constant volume, or with powders in which active substance(s) and excipients are mixed. The overall processes, from preparation to sterilization, should be completed with sufficient care to prevent microbial contamination as rapidly as possible, taking into consideration the composition of the preparations and the storage conditions. The concentration of active substance of Ear Preparations expressed in % represents w/v%.

In the case where the sterile preparations are prepared, proceed as directed under 6-1. Ophthalmic Liquids and Solutions.

Ear Preparations which are to be dissolved or suspended before use and designated in the name as "for otic preparation" may be accompanied by a vehicle to dissolve or suspend (hereinafter referred to as "vehicle attached to preparation").

(3) Vehicles used for Ear Preparations or the vehicle attached to the preparation are classified into the following two groups.

(i) Aqueous vehicles: As the vehicles for the aqueous preparations or the vehicles attached to the preparations, Purified Water or suitable aqueous vehicles are used. For the sterile preparations, Sterilized Purified Water or suitable sterilized aqueous vehicles are used as the vehicle attached to the preparations.

(ii) Non-aqueous vehicles: As the vehicles for the non-aqueous preparations vegetable oils are usually used. Suitable organic solvents may be also used as non-aqueous vehicles.

(4) Unless otherwise specified, any coloring agents must not be added solely for the purpose of coloring Ear Preparations or vehicle attached to the preparations.

(5) Sufficient amounts of suitable preservatives to prevent the growth of microorganisms may be added to the preparations filled in multiple dose containers.

(6) Unless otherwise specified, sterile Ear preparations and the vehicles attached to the sterile preparations meet the requirements of Sterility Test <4.06>.

(7) Tight containers are usually used for Ear Preparations. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

## **8. Preparations for Nasal Application**

### **8-1. Nasal Preparations**

(1) Nasal Preparations are preparations intended for application to the nasal cavities or nasal mucous membrane.

Nasal preparations are classified into Nasal dry powder inhalers and Nasal Liquid Preparations.

(2) Where necessary, Nasal Preparations are sprayed for inhalation by using a suitable atomizing device such as spray-pump.

(3) Unless otherwise specified, metered-dose type preparations among Nasal Preparations show the appropriate uniformity of delivered dose.

#### **8-1-1. Nasal Dry Powder Inhalers**

(1) Nasal Dry Powder Inhalers are fine powdered preparations, intended for application to the nasal cavities.

(2) Nasal Dry Powder Inhalers are usually prepared by pulverizing active substance(s) into moderately fine particles, or by mixing homogeneously with excipients where necessary.

(3) Well-closed containers are usually used for Nasal Dry Powder Inhalers. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

#### **8-1-2. Nasal Liquids and Solutions**

(1) Nasal Liquids and Solutions are liquid preparations, or solid preparations to be dissolved or suspended before use, intended for application to the nasal cavities.

(2) Nasal Liquids and Solutions are usually prepared by dissolving or suspending active substance(s) in a vehicle together with excipients, and filtering where necessary. Isotonic agents and/or pH adjusting agents may be used.

(3) Nasal Liquids and Solutions, which are to be dissolved or suspended before use and designated in the name as "for nasal application", may be accompanied by a vehicle to dissolve or suspend.

(4) Sufficient amounts of suitable preservatives to prevent the growth of microorganisms may be added to the preparations filled in multiple dose containers.

(5) Tight containers are usually used for Nasal Liquids and Solutions. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

## **9. Preparations for Rectal Application**

### **9-1. Suppositories for Rectal Application**

(1) Suppositories for Rectal Application are semi-solid preparations of a desired shape and size, intended for intrarectal application, which release active substance(s) by melting at body temperature or dissolving or dispersing gradually in the secretions.

(2) Suppositories for Rectal Application are usually prepared by mixing homogenously active substance(s) and excipients such as dispersing agents and emulsifying agents, dissolving or suspending uniformly in a base which is liquefied by warming, filling a constant volume of the resultant material into containers, and molding it into a shape and size. Lipophilic bases or hydrophilic bases are usually used.

(3) Suppositories for Rectal Application are usually a conical- or spindle-shaped.

(4) Unless otherwise specified, Suppositories for Rectal Application meet the requirements of Uniformity of Dosage Units <6.02>.

(5) Suppositories for Rectal Application show an appropriate release. Release of Suppositories for Rectal Application prepared using a lipophilic base can be evaluated by melting behavior of suppositories in place of release of active substances. When the melting behavior of Suppositories for Rectal Application is measured according to Method 2 under Melting Point Determination <2.60> unless otherwise specified, it shows an appropriate melting temperature.

(6) Well-closed containers are usually used for Suppositories for Rectal Application. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

### **9-2. Semi-solid Preparations for Rectal Application**

(1) Semi-solid Preparations for Rectal Application are preparations which are in a form of cream, gel or ointment intended for application to around or inside

of the anus.

(2) Semi-solid Preparations for Rectal Application are usually prepared by emulsifying active substance(s) with excipients in Purified Water and oil component such as vaseline, or by homogenously mixing active substance(s) and excipients in a base of polymer gel or grease.

(i) Creams for rectal application: Prepare as directed under 11-5. Creams.

(ii) Gels for rectal application: Prepare as directed under 11-6. Gels.

(iii) Ointments for rectal application: Prepare as directed under 11-4. Ointments.

For the preparations which are apt to deteriorate, prepare before use.

(3) Sufficient amounts of suitable preservatives to prevent the growth of microorganisms may be added to the Preparations filled in multiple dose containers.

(4) Semi-solid Preparations for Rectal Application have a suitable viscosity for applying to the rectum.

(5) Tight containers are usually used for Semi-solid Preparations for Rectal Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

### 9-3. Enemas for Rectal Application

(1) Enemas for Rectal Application are preparations in liquid form or viscous and gelatinous state, intended for application via the anus.

(2) Enemas for Rectal Application are usually prepared by dissolving or suspending active substance(s) in Purified Water or a suitable aqueous vehicle to make a given volume, and filling in containers. Dispersing agents, stabilizers and/or pH adjusting agents may be used.

(3) Tight containers are usually used for Enemas for Rectal Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

## 10. Preparations for Vaginal Application

### 10-1. Tablets for Vaginal Use

(1) Tablets for Vaginal Use are solid preparations of a desired shape and size, intended for application to the vagina, which release active substance(s) by dissolving or dispersing gradually in the secretions.

(2) Tablets for Vaginal Use are usually prepared as directed under 1-1. Tablets.

(3) Unless otherwise specified, Tablets for Vaginal Use meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Tablets for Vaginal Use show an appropriate release.

(5) Well-closed containers are usually used for

Tablets for Vaginal Use. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

### 10-2. Suppositories for Vaginal Use

(1) Suppositories for Vaginal Use are semi-solid preparations of a desired shape and size, intended for application to the vagina, which release active substance(s) by melting at body temperature or by dissolving or dispersing gradually in the secretions.

(2) Suppositories for Vaginal Use are prepared according to 9-1. Suppositories for Rectal Application.

(3) Suppositories for Vaginal Use are usually spherical or ovoid shaped.

(4) Unless otherwise specified, Suppositories for Vaginal Use meet the requirements of Uniformity of Dosage Units <6.02>.

(5) Suppositories for Vaginal Use show an appropriate release. Release of Suppositories for Vaginal Use prepared using a lipophilic base can be evaluated by melting behavior of suppositories in place of release of active substances. When the melting behavior of Suppositories for Vaginal Use is measured according to Method 2 under Melting Point Determination <2.60> unless otherwise specified, it shows an appropriate melting temperature.

(6) Well-closed containers are usually used for Suppositories for Vaginal Use. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

## 11. Preparations for Cutaneous Application

(1) Preparations for Cutaneous Application also include Transdermal Systems which are intended for percutaneous absorption to deliver active substance(s) to the systemic circulation through the skin. The release rate of active substance(s) from Transdermal Systems is generally appropriately controlled.

### 11-1. Solid Preparations for Cutaneous Application

(1) Solid Preparations for Cutaneous Application are solid preparations intended for application to the skin (including scalp) or nails. Powders for Cutaneous Application are included in this category.

(2) Unless otherwise specified, Solid Preparations for Cutaneous Application in single-dose packages meet the requirements of Uniformity of Dosage Units <6.02>.

(3) Well-closed containers are usually used for Solid Preparations for Cutaneous Application. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

#### 11-1-1. Powders for Cutaneous Application

(1) Powders for Cutaneous Application are powdery solid preparations intended for external application.

(2) Powders for Cutaneous Application are usually prepared by mixing homogeneously active substance(s) and excipients such as diluents and pulverizing the mixture.

#### 11-2. Liquids and Solutions for Cutaneous Application

(1) Liquids and Solutions for Cutaneous Application are liquid preparations intended for application to the skin (including scalp) or nails. Liniments and Lotions are included in this category.

(2) Liquids and Solutions for Cutaneous Application are usually prepared by mixing active substance(s) and excipients in a vehicle, and filtering if necessary.

For the preparations which are apt to deteriorate, prepare before use.

(3) Unless otherwise specified, Liquids and Solutions for Cutaneous Application in single-dose packages such as Transdermal Systems meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Tight containers are usually used for Liquids and Solutions for Cutaneous Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

##### 11-2-1. Liniments

(1) Liniments are liquid or muddy preparations intended for external application to the skin by rubbing.

##### 11-2-2. Lotions

(1) Lotions are external liquids in which active substance(s) are dissolved, emulsified or finely dispersed in an aqueous vehicle.

(2) Lotions are usually prepared by dissolving, suspending or emulsifying active substance(s) in Purified Water with excipients and making homogeneous as a whole.

(3) Lotions in which the components have separated out during storage may be used after mixing to re-homogenize them, provided that the active substance(s) has not deteriorated.

#### 11-3. Sprays for Cutaneous Application

(1) Sprays for Cutaneous Application are preparations intended for spraying active substance(s) onto the skin in mists, powders, foams or paste state.

Sprays for Cutaneous Application are classified into Aerosols for Cutaneous Application and Pump Sprays for Cutaneous Application.

(2) Sprays for Cutaneous Application are usually prepared by dissolving or suspending active substance(s) in a vehicle, filtering where necessary, and filling in containers.

(3) Unless otherwise specified, metered-dose type sprays show an appropriate uniformity of delivered dose.

##### 11-3-1. Aerosols for Cutaneous Application

(1) Aerosols for Cutaneous Application are sprays which atomize active substance(s) together with liquefied or compressed gas filled in containers.

(2) Aerosols for Cutaneous Application are usually prepared by dissolving or suspending active substance(s) in a vehicle, filling with liquefied propellants in pressure-resistant containers, and setting a continuous spray valve. If necessary, dispersing agents and stabilizers may be used.

(3) Pressure-resistant containers are usually used for Aerosols for Cutaneous Application.

##### 11-3-2. Pump Sprays for Cutaneous Application

(1) Pump Sprays for Cutaneous Application are sprays which atomize active substance(s) in containers by pumping.

(2) Pump Sprays for Cutaneous Application are usually prepared by dissolving or suspending active substance(s) with excipients in a vehicle, filling in containers and setting pumps to the containers.

(3) Tight containers are usually used for Pump Sprays for Cutaneous Application. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

#### 11-4. Ointments

(1) Ointments are semi-solid preparations to be applied to the skin, which dissolve or disperse active substance(s) in a base. There are two types, hydrophobic ointments and hydrophilic ointments.

(2) Hydrophobic ointments are usually prepared by warming to melt hydrophobic bases such as fatty oils, waxes or paraffin, adding and mixing active substance(s) in the bases to be dissolved or dispersed, and kneading the whole to make homogeneous.

Hydrophilic ointments are usually prepared by warming to melt hydrophilic bases such as macrogol, adding and mixing active substance(s) in the bases, and kneading the whole to make homogeneous.

For Ointments which are apt to deteriorate, prepare before use.

(3) Ointments have a suitable viscosity for application to the skin.

(4) Tight containers are usually used for Ointments. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

#### 11-5. Creams

(1) Creams are semi-solid preparations to be applied to the skin, which are in the form of oil-in-water or water-in-oil emulsions. Hydrophobic preparations in the form of water-in-oil emulsions may be termed "Oily creams".

(2) Creams are usually prepared by mixing homogenously and emulsifying an oil-phase compo-

nent and a water-phase component, both warmed, of which either one contains the active substance(s). These components have the following constituents.

**Oil-phase component:** Vaseline, fatty alcohols, etc., with or without emulsifying agent(s) or other suitable excipients.

**Water-phase component:** Purified Water with or without emulsifying agent(s) or other suitable excipients.

For Creams which are apt to deteriorate, prepare before use.

(3) Creams have a suitable viscosity for applying to the skin.

(4) Tight containers are usually used for Creams. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

#### 11-6. Gels

(1) Gels are gelatinous preparations intended for application to the skin.

There are Aqueous Gels and Oily Gels.

(2) Gels are usually prepared by the following methods.

(i) **Aqueous Gels:** To active substance(s) add polymers, other excipients and Purified Water, dissolve or suspend, and gelatinize by warming and cooling or by adding a gelatinizing agents.

(ii) **Oily Gels:** To active substance(s) add liquid oily bases such as glycols, fatty alcohols and other excipients, and mix.

(3) Gels have a suitable viscosity for application to the skin.

(4) Tight containers are usually used for Gels. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

#### 11-7. Patches

(1) Patches are preparations intended to be attached on the skin.

Patches are classified into Tapes/Plasters and Cataplasms/Gel Patches.

(2) Patches are usually prepared by mixing active substance(s) homogeneously with a base such as a polymer or a mixture of polymers, spreading on a backing layer or liner, and cutting into a given size. Percutaneous absorption type preparations may be prepared by using a release rate-controlling membrane. Where necessary, adhesive agents or penetration enhancers may be used.

(3) Unless otherwise specified, Patches of Transdermal Systems meet the requirements of Uniformity of Dosage Units <6.02>.

(4) Unless otherwise specified, Patches meet the requirement of Methods of Adhesion Testing <6.12>.

(5) Unless otherwise specified, Patches meet the requirement of Release Test for Preparations for Cutaneous Application <6.13>.

##### 11-7-1. Tapes

(1) Tapes are patches which are prepared with bases containing practically no water.

Plasters are included in this category.

(2) Tapes are usually prepared by mixing homogeneously active substance(s) with or without excipients and a base of non water-soluble natural or synthetic polymers such as resins, plastics or rubber, and spreading on a cloth or spreading and sealing on a cloth or plastic film, cutting into a given size. The preparations may be also prepared by filling a mixture of active substance(s) and a base with or without other excipients in releasers composed with a release-controlling film, supporter and liner.

(3) Well-closed containers are usually used for Tapes. For the preparations susceptible to degradation by moisture, a moisture-proof container or packaging may be used.

##### 11-7-2. Cataplasms/Gel Patches

(1) Cataplasms/Gel Patches are patches using water containing bases.

(2) Cataplasms/Gel patches are usually prepared by mixing active substance(s), Purified Water, and Glycerin or other liquid materials, or by mixing and kneading natural or synthetic polymers, which are soluble in water or absorbent of water, with Purified Water, adding active substance(s), mixing the whole homogeneously, spreading on a cloth or film, and cutting into a given size.

(3) Tight containers are usually used for Cataplasms/Gel Patches. For the preparations susceptible to degradation by evaporation of water, a low-moisture-permeability container or packaging may be used.

## [4] Monographs for Preparations Related to Crude Drugs

### Preparations Related to Crude Drugs

(1) Preparations related to crude drugs are preparations mainly derived from crude drugs. Extracts, Pills, Spirits, Infusions and Decoctions, Teabags, Tinctures, Aromatic Waters, and Fluidextracts are included in this category.

Definitions, methods of preparations, test methods, containers and packaging, and storage of these preparations are described in this chapter.

(2) The descriptions of the test methods and the containers and packaging in this chapter are fundamental requirements, and the preparation methods represent commonly used methods.

## 1. Extracts

(1) Extracts are preparations, prepared by concentrating extractives of crude drugs. There are following two kinds of extracts.

- (i) Viscous extracts
- (ii) Dry extracts

(2) Unless otherwise specified, Extracts are usually prepared as follows.

(i) Crude drugs, pulverized to suitable sizes, are extracted for a certain period of time with suitable solvents by means of cold extraction or warm extraction, or by percolation as directed in (ii) of (2) under 6. Tinctures. The extractive is filtered, and the filtrate is concentrated or dried by a suitable method to make a millet jelly-like consistency for the viscous extracts, or to make crushable solid masses, granules or powder for the dry extracts.

Extracts, which are specified the content of active substance(s), are prepared by assaying active substance(s) in a portion of sample and adjusting, if necessary, to specified strength with suitable diluents.

(ii) Weigh crude drugs, pulverized to suitable sizes, according to the prescription and heat for a certain period of time after adding 10 – 20 times amount of water. After separating the solid and liquid by centrifugation, the extractive is concentrated or dried by a suitable method to make a millet jelly-like consistency for the viscous extracts, or to make crushable solid masses, granules or powder for the dry extracts.

(3) Extracts have order and taste derived from the crude drugs used.

(4) Unless otherwise specified, Extracts meet the requirements of Heavy Metals Limit Test <1.07> when the test solution and the control solution are prepared as follows.

Test solution: Ignite 0.30 g of Extracts to ash, add 3 mL of dilute hydrochloric acid, warm, and filter. Wash the residue with two 5-mL portions of water. Neutralize the combined filtrate and washings (indicator: a drop of phenolphthalein TS) by adding ammonia TS until the color of the solution changes to pale red, filter where necessary, and add 2 mL of dilute acetic acid and water to make 50 mL.

Control solution: Proceed with 3 mL of dilute hydrochloric acid in the same manner as directed in the preparation of the test solution, and add 3.0 mL of Standard Lead Solution and water to make 50 mL.

(5) Tight containers are used for these preparations.

## 2. Pills

(1) Pills are spherical preparations, intended for oral administration.

(2) Pills are usually prepared by mixing drug substance(s) uniformly with diluents, binders, disintegrators or other suitable excipient(s) and rolling into

spherical form by a suitable method. They may be coated with a coating agent by a suitable method.

(3) Unless otherwise specified, Pills comply with Disintegration Test <6.09>.

(4) Well-closed or tight containers are usually used for these preparations.

## 3. Spirits

(1) Spirits are fluid preparations, usually prepared by dissolving volatile drug substance(s) in ethanol or in a mixture of ethanol and water.

(2) Spirits should be stored remote from fire.

(3) Tight containers are used for these preparations.

## 4. Infusions and Decoctions

(1) Infusions and Decoctions are fluid preparations, usually obtained by macerating crude drugs in water.

(2) Infusions and Decoctions are usually prepared by the following method.

Cut crude drugs into a size as directed below, and transfer suitable amounts to an infusion or decoction apparatus.

Leaves, flowers and whole plants: Coarse cutting

Woods, stems, barks, roots and rhizomes

: Moderately fine cutting

Seeds and fruits: Fine cutting

(i) Infusions: Usually, damp 50 g of crude drugs with 50 mL of water for about 15 minutes, pour 900 mL of hot water to them, and heat for 5 minutes with several stirrings. Filter through a cloth after cooling.

(ii) Decoctions: Usually, heat one-day dose of crude drugs with 400 – 600 mL of water until to lose about a half amount of added water spending more than 30 minutes, and filter through a cloth while warm.

Prepare Infusions or Decoctions when used.

(3) These preparations have odor and taste derived from the crude drugs used.

(4) Tight containers are usually used for these preparations.

## 5. Teabags

(1) Teabags are preparations, usually packed one-day dose or one dose of crude drugs cut into a size of between coarse powder and coarse cutting in paper or cloth bags.

(2) Teabags are usually used according to the preparation method as directed under 4. Infusions and Decoctions.

(3) Well-closed or tight containers are usually used for these preparations.

## 6. Tinctures

(1) Tinctures are liquid preparations, usually prepared by extracting crude drugs with ethanol or with a mixture of ethanol and purified water.

(2) Unless otherwise specified, Tinctures are usually prepared from coarse powder or fine cuttings of crude drugs by means of either maceration or percolation as described below.

(i) Maceration: Place crude drugs in a suitable container, and add an amount of a solvent, equivalent to the same volume or about three-fourths of the volume of the crude drugs. Stopper container, and allow the container to stand for about 5 days or until the soluble constituents have satisfactorily dissolved at room temperature with occasional stirring. Separate the solid and liquid by centrifugation or other suitable methods. In the case where about three-fourths volume of the solvent is added, wash the residue with a suitable amount of the solvent, and squeeze the residue, if necessary. Combine the extract and washings, and add sufficient solvent to make up the volume. In the case where the total volume of the solvent is added, sufficient amounts of the solvent may be added to make up for reduced amount, if necessary. Allow the mixture to stand for about 2 days, and obtain a clear liquid by decantation or filtration.

(ii) Percolation: Pour solvent in small portions to crude drugs placed in a container, and mix well to moisten the crude drugs. Stopper container, and allow it to stand for about 2 hours at room temperature. Pack the contents as tightly as possible in an appropriate percolator, open the lower opening, and slowly pour sufficient solvent to cover the crude drugs. When the percolate begins to drip, close the opening, and allow the mixture to stand for 2 to 3 days at room temperature. Then, open the opening, and allow the percolate to drip at a rate of 1 to 3 mL per minute. Add an appropriate quantity of the solvent to the percolator, and continue to percolate until the desired volume has passed. Mix thoroughly, allow standing for 2 days, and obtain a clear liquid by decantation or filtration. The time of standing and the flow rate may be varied depending on the kind and amount of crude drugs to be percolated.

Tinctures, prepared by either of the above methods and specified the content of marker constituent or ethanol, are prepared by assaying the content using a portion of the sample and adjusting the content with a sufficient amount of the percolate or solvent as required on the basis of the result of the assay.

(3) Tinctures should be stored remote from fire.

(4) Tight containers are used for these preparations.

## 7. Aromatic Waters

(1) Aromatic Waters are clear liquid preparations,

saturated essential oils or other volatile substances in water.

(2) Unless otherwise specified, Aromatic Waters are usually prepared by the following process.

Shake thoroughly for 15 minutes 2 mL of an essential oil or 2 g of a volatile substance with 1000 mL of lukewarm purified water, set the mixture aside for 12 hours or longer, filter through moistened filter paper, and add purified water to make 1000 mL. Alternatively, incorporate thoroughly 2 mL of an essential oil or 2 g of a volatile substance with sufficient talc, refined siliceous earth or pulped filter-paper, add 1000 mL of purified water, agitate thoroughly for 10 minutes, and then filter the mixture. To obtain a clear filtrate repeat the filtration if necessary, and add sufficient purified water passed through the filter paper to make 1000 mL.

(3) Aromatic Waters have odor and taste derived from the essential oils or volatile substances used.

(4) Tight containers are used for these preparations.

## 8. Fluidextracts

(1) Fluidextracts are liquid percolates of crude drugs, usually prepared so that each mL contains soluble constituents from 1 g of the crude drugs. Where the content is specified, it takes precedence.

(2) Unless otherwise specified, Fluidextracts are usually prepared from coarse powder or fine cutting of crude drugs by either of following maceration or percolation.

(i) Maceration: Place a certain amount of crude drugs in a suitable vessel, add a solvent to cover the crude drugs, close the vessel, and allow the vessel to stand at room temperature with occasional stirring for about 5 days or until the soluble constituents have satisfactorily dissolved. Separate the solid and liquid by centrifugation or other suitable method. Usually, reserve a volume of the liquid equivalent to about three-fourths of the total volume, and use it as the first liquid. Wash the residue with appropriate amount of the solvent, combine the washings and the remaining of the first liquid, concentrate if necessary, mix with the first liquid, and use it as solution (A). To the solution (A) add the solvent, if necessary, to make equal amount of the mass of the crude drugs. Allow the mixture to stand for about 2 days, and collect a clear liquid by decantation or filtration.

(ii) Percolation: Mix well 1000 g of the crude drugs with the first solvent to moisten them, close the container, and allow it to stand for about 2 hours at room temperature. Transfer the content to a suitable percolator, stuff it as tightly as possible, open the lower opening of the percolator, and slowly pour the second solvent to cover the crude drugs. Close the lower opening when the solvent begins to drop, and allow the

mixture to stand for 2 to 3 days at room temperature. Open the lower opening, and allow the percolate to run out at the rate of 0.5 to 1.0 mL per minute.

Set aside the first 850 mL of the percolate as the first percolate. Add the second solvent to the percolator, then drip the percolate, and use it as the second percolate.

The period of standing and the flow rate during percolation may be varied depending on the kind and the amount of crude drugs used. The flow rate is usually regulated as follows, depending on the using amount of crude drugs.

Mass of crude drug	Volume of solution running per minute
Not more than 1000 g	0.5 – 1.0 mL
Not more than 3000 g	1.0 – 2.0 mL
Not more than 10000 g	2.0 – 4.0 mL

Concentrate the second percolate, taking care not to lose the volatile substances of the crude drug, mix with the first percolate, and use it as solution (A). To the solution (A) add the second solvent to make 1000 mL, and allow the mixture to stand for about 2 days. Decant the supernatant liquid or filter the liquid to obtain a clear solution.

Fluidextracts for which the content of marker constituent or ethanol is specified are obtained by adjusting the content with a sufficient amount of the second solvent as required on the basis of the result of the assay made with a portion of the solution (A).

(3) Fluidextracts have odor and taste derived from the crude drugs used.

(4) Unless otherwise specified, Fluidextracts meet the requirements of Heavy Metals Limit Test <1.07> when the test solution and the control solution are prepared as follows.

Test solution: Ignite 1.0 g of Fluidextracts to ash, add 3 mL of dilute hydrochloric acid, warm, and filter. Wash the residue with two 5-mL portions of water. Neutralize the combined filtrate and washings (indicator: a drop of phenolphthalein TS) by adding ammonia TS until the color of the solution changes to pale red, filter if necessary, and add 2 mL of the dilute acetic acid and water to make 50 mL.

Control solution: Proceed with 3 mL of dilute hydrochloric acid in the same manner as directed in the preparation of the test solution, and add 3.0 mL of Standard Lead Solution and water to make 50 mL.

(5) Tight containers are used for these preparations.

# GENERAL TESTS, PROCESSES AND APPARATUS

General Tests, Processes and Apparatus includes common methods for tests, useful test methods for quality recognition of drugs and other articles related to them.

The number of each test method is a category number given individually according to its content.

1. Chemical Methods
2. Physical Methods
3. Powder Property Determinations
4. Biological Tests/Biochemical Tests/Microbial Tests
5. Tests for Crude Drugs
6. Tests for Preparations
7. Tests for Containers and Packaging Materials
8. Others\*
9. Reference Standards; Standard Solutions; Reagents, Test Solutions; Measuring Instruments, Appliances, etc.

\* At present there are no methods in this section.

The number in brackets (< >) appeared in monograph indicates the number corresponding to the general test method.

## 1. Chemical Methods

### 1.01 Alcohol Number Determination

Alcohol Number Determination represents the number of milliliters of ethanol at 15°C obtained from 10 mL of tincture or other preparations containing ethanol by the following procedures.

#### 1. Method 1 Distilling method

This is a method to determine the Alcohol Number by reading the number of milliliters of ethanol distillate at 15°C obtained from 10 mL of a sample measured at 15°C by the following procedures.

##### 1.1. Apparatus

Use hard glass apparatus as illustrated in Fig. 1.01-1. Ground glass may be used for the joints.

##### 1.2. Reagent

Alkaline phenolphthalein solution: To 1 g of phenolphthalein add 7 mL of sodium hydroxide TS and water to

Table 1.01-1

Ethanol content in the sample (vol%)	Distillate to be collected (mL)
more than 80	13
80 - 70	12
70 - 60	11
60 - 50	10
50 - 40	9
40 - 30	8
less than 30	7

make 100 mL.

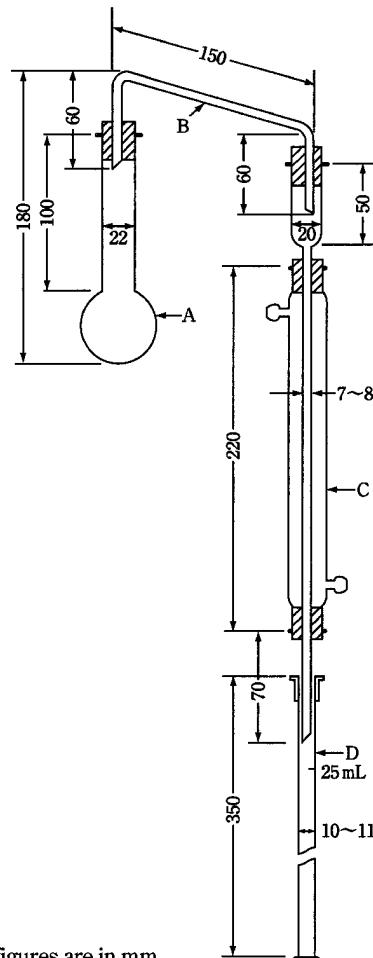
#### 1.3. Procedure

Transfer 10 mL of the sample preparation, accurately measured at 15 ± 2°C, to the distilling flask A, add 5 mL of water and boiling chips. Distil ethanol carefully into the glass-stoppered, volumetric cylinder D.

By reference to Table 1.01-1, a suitable volume of distillate (mL) should be collected, according to the content of ethanol in the sample preparation.

Prevent bumping during distillation by rendering the sample strongly acidic with phosphoric acid or sulfuric acid, or by adding a small amount of paraffin, beeswax or silicone resin before starting the distillation.

When the samples contain the following substances, carry out pretreatment as follows before distillation.



The figures are in mm.

A: Distilling flask (50 mL)

B: Delivery tube

C: Condenser

D: Glass-stoppered volumetric cylinder  
(25 mL, graduated in 0.1 mL)

Fig. 1.01-1

(i) Glycerin: Add sufficient water to the sample so that the residue in the distilling flask, after distillation, contains at least 50% of water.

(ii) Iodine: Decolorize the sample with zinc powder.

(iii) Volatile substances: Preparations containing appreciable proportions of essential oil, chloroform, diethyl ether or camphor require treatment as follows. Mix 10 mL of the sample, accurately measured, with 10 mL of saturated sodium chloride solution in a separator, add 10 mL of petroleum benzin, and shake. Collect the separated aqueous layer. The petroleum benzin layer was extracted with two 5 mL portions of saturated sodium chloride solution. Combine the aqueous layers, and distill. According to the ethanol content in the sample, collect a volume of distillate 2 to 3 mL more than that shown in the above Table.

(iv) Other substances: Render preparations containing free ammonia slightly acidic with dilute sulfuric acid. If volatile acids are present, render the preparation slightly alkaline with sodium hydroxide TS, and if the preparations contain soap along with volatile substances, decompose the soap with an excess of dilute sulfuric acid before the extraction with petroleum benzin in the treatment described in (iii).

To the distillate add 4 to 6 g of potassium carbonate and 1 to 2 drops of alkaline phenolphthalein solution, and shake vigorously. If the aqueous layer shows no white turbidity, agitate the distillate with additional potassium carbonate. After allowing to stand in water at  $15 \pm 2^\circ\text{C}$  for 30 minutes, read the volume of the upper reddish ethanol layer in mL, and regard it as the Alcohol Number. If there is no clear boundary surface between these two layers, shake vigorously after addition of a few drops of water, then observe in the same manner.

## 2. Method 2 Gas chromatography

This is a method to determine the alcohol number by determining ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) content (vol%) from a sample measured at  $15^\circ\text{C}$  by the following procedures.

### 2.1. Reagent

Ethanol for alcohol number: Ethanol (99.5) with determined ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) content. The relation between specific gravity  $d_{15}^{15}$  of ethanol and content of ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) is 0.797:99.46 vol%, 0.796:99.66 vol%, and 0.795:99.86 vol%.

### 2.2. Preparation of sample solution and standard solution

Sample solution: Measure accurately a volume of sample at  $15 \pm 2^\circ\text{C}$  equivalent to about 5 mL of ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ), and add water to make exactly 50 mL. Measure accurately 25 mL of this solution, add exactly 10 mL of the internal standard solution, and add water to make 100 mL.

Standard solution: Measure accurately 5 mL of ethanol for alcohol number at the same temperature as the sample, and add water to make exactly 50 mL. Measure accurately 25 mL of this solution, add exactly 10 mL of the internal standard solution, and add water to make 100 mL.

### 2.3. Procedure

Place 25 mL each of the sample solution and the standard solution in a 100-mL, narrow-mouthed, cylindrical glass bottle sealed tightly with a rubber closure and aluminum band, immerse the bottle up to the neck in water, allowed to stand at room temperature for more than 1 hour in a room with little change in temperature, shake gently so as not to splash the solution on the closure, and allow to stand for 30 minutes. Perform the test with 1 mL each of the gas in the bottle with a syringe according to the Gas Chromatography <2.02> under the following conditions, and calculate the ratios,  $Q_T$  and  $Q_S$ , of the peak height of ethanol to that of

the internal standard.

$$\text{Alcohol number} = \frac{Q_T}{Q_S} \times \frac{5 \text{ (mL)}}{\text{a volume (mL) of sample}} \times \frac{\text{ethanol } (\text{C}_2\text{H}_5\text{OH}) \text{ content (vol\%) of ethanol for alcohol number}}{9.406}$$

*Internal standard solution*—A solution of acetonitrile (3 in 50).

### Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass tube about 3 mm in inside diameter and about 1.5 m in length, packed with 150- to 180- $\mu\text{m}$  porous ethylvinylbenzene-divinylbenzene copolymer (mean pore size: 0.0075  $\mu\text{m}$ , 500 – 600  $\text{m}^2/\text{g}$ ) for gas chromatography.

Column temperature: A constant temperature between  $105^\circ\text{C}$  and  $115^\circ\text{C}$ .

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of ethanol is 5 to 10 minutes.

Selection of column: Proceed with 1 mL of the gas obtained from the standard solution in the bottle under the above operating conditions, and calculate the resolution. Use a column giving elution of ethanol and the internal standard in this order with the resolution between these peaks being not less than 2.0.

## 1.02 Ammonium Limit Test

Ammonium Limit Test is a limit test for ammonium salt contained in drugs.

In each monograph, the permissible limit for ammonium (as  $\text{NH}_4^+$ ) is described in terms of percentage (%) in parentheses.

### 1. Apparatus

Use a distilling apparatus for ammonium limit test as illustrated in Fig. 1.02-1. For the distillation under reduced pressure, use the apparatus shown in Fig. 1.02-2. Either apparatus are composed of hard glass, and ground-glass joints may be used. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

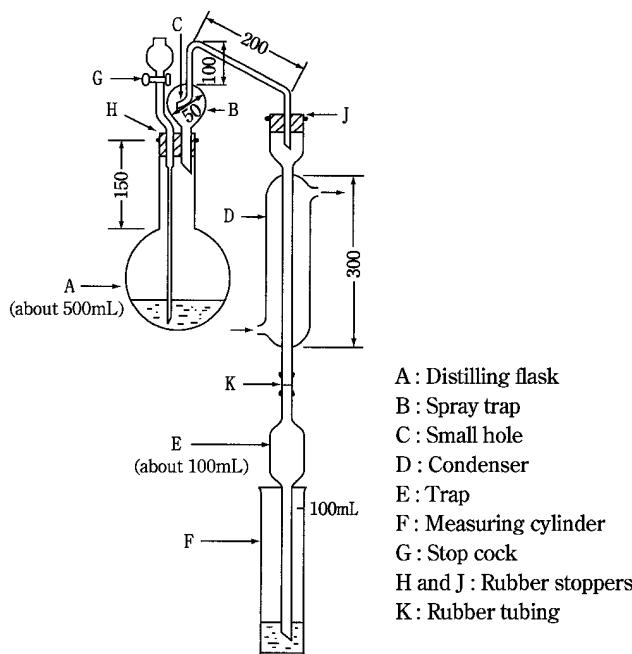
### 2. Procedure

#### 2.1. Preparation of test solution and control solution

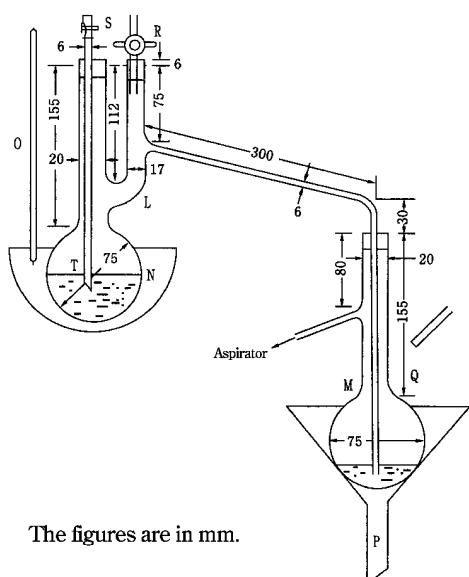
Unless otherwise specified, test solutions and control solution are prepared as directed in the following.

Place an amount of the sample, directed in the monograph, in the distilling flask A. Add 140 mL of water and 2 g of magnesium oxide, and connect the distillation apparatus. To the receiver (measuring cylinder) F add 20 mL of boric acid solution (1 in 200) as an absorbing solution, and immerse the lower end of the condenser. Adjust the heating to give a rate of 5 to 7 mL per minute of distillate, and distill until the distillate measures 60 mL. Remove the receiver from the lower end of the condenser, rinsing the end part with a small quantity of water, add sufficient water to make 100 mL and designate it as the test solution.

For the distillation under reduced pressure, take the amount of sample specified in the monograph to the vacuum distillation flask L, add 70 mL of water and 1 g of magnesium oxide, and connect to the apparatus (Fig. 1.02-2). To the receiver M add 20 mL of a solution of boric acid (1 in 200) as absorbing liquid, put the end of the branch tube of



**Fig. 1.02-1** Distilling apparatus for ammonium limit test



- |  |                                |
|--|--------------------------------|
| L: Vacuum distillation flask<br>(200-mL) | Q: Cooling water               |
| M: Receiver (a 200-mL flask)             | R: Glass cock                  |
| N: Water bath                            | S: Rubber tube with screw cock |
| O: Thermometer                           | T: Glass tube for anti-bumping |
| P: Funnel                                |                                |

**Fig. 1.02-2** Vacuum distilling apparatus for ammonium limit test

the distillation flask L in the absorbing liquid, and keep at 60°C using a water bath or alternative equipment. Adjust the reduced pressure to get the distillate at a rate of 1 to 2 mL per minute, and continue the distillation until to get 30 mL of the distillate. Cool the receiver M with running water during the distillation. Get off the end of the branch tube from surface of the absorbing liquid, rinse in the end with a small amount of water, then add water to the liquid to make

100 mL, and perform the test using this solution as the test solution.

Place a volume of Standard Ammonium Solution, directed in the monograph, in the distilling flask A or the vacuum distillation flask L, proceed as for the preparation of the test solution, and designate it as the control solution.

## 2.2. Test of the test solution and the control solution

Unless otherwise specified, proceed as directed in the following.

Place 30 mL each of the test solution and the control solution in Nessler tubes, add 6.0 mL of phenol-sodium pentacyanonitrosylferrate (III) TS to each solution, and mix. Then add 4 mL of sodium hypochlorite-sodium hydroxide TS and water to make 50 mL, mix, and allow to stand for 60 minutes. Compare the color of both solutions against a white background by viewing downward or transversely: the color developed in the test solution is not more intense than that of the control solution.

## 1.03 Chloride Limit Test

Chloride Limit Test is a limit test for chloride contained in drugs.

In each monograph, the permissible limit for chloride (as Cl) is described in terms of percentage (%) in parentheses.

### 1. Procedure

Unless otherwise specified, transfer the quantity of the sample, directed in the monograph, to a Nessler tube, and dissolve it in a proper volume of water to make 40 mL. Add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.01 mol/L hydrochloric acid VS, directed in the monograph, to another Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions by using the same procedure.

Add 1 mL of silver nitrate TS to the test solution and to the control solution, mix well, and allow to stand for 5 minutes protecting from light. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely.

The opalescence developed in the test solution is not more than that of the control solution.

## 1.04 Flame Coloration Test

Flame Coloration Test is a method to detect an element, by means of the property that the element changes the colorless flame of a Bunsen burner to its characteristic color.

**(1) Salt of metal**—The platinum wire used for this test is about 0.8 mm in diameter, and the end part of it is straight. In the case of a solid sample, make the sample into a gruel by adding a small quantity of hydrochloric acid, apply a little of the gruel to the 5-mm end of the platinum wire, and test by putting the end part in a colorless flame, keeping the platinum wire horizontal. In the case of a liquid sample, immerse the end of the platinum wire into the sample to about 5 mm in length, remove from the sample gently, and perform the test in the same manner as for the solid sample.

**(2) Halide**—Cut a copper net, 0.25 mm in opening and 0.174 mm in wire diameter, into a strip 1.5 cm in width and 5 cm in length, and wind in round one end of a copper wire. Heat the copper net strongly in the colorless flame of Bunsen

burner until the flame no longer shows a green or blue color, and then cool it. Repeat this procedure several times, and coat the net completely with cupric oxide. After cooling, unless otherwise specified, apply about 1 mg of the sample to the copper net, ignite, and burn it. Repeat this procedure three times, and then test by putting the copper net in the colorless flame.

The description, "Flame coloration persists", in a monograph, indicates that the reaction persists for 4 seconds.

## 1.05 Mineral Oil Test

Mineral Oil Test is a method to test mineral oil in non-aqueous solvents for injections and for eye drops.

### 1. Procedure

Pour 10 mL of the sample into a 100-mL flask, and add 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of ethanol (95). Put a short-stemmed, small funnel on the neck of the flask, and heat on a water bath to make clear, with frequent shaking. Then transfer the solution to a shallow porcelain dish, evaporate the ethanol on a water bath, add 100 mL of water to the residue, and heat on a water bath: no turbidity is produced in the solution.

## 1.06 Oxygen Flask Combustion Method

Oxygen Flask Combustion Method is a method for the identification or the determination of halogens or sulfur produced by combusting organic compounds, which contain chlorine, bromine, iodine, fluorine or sulfur, in a flask filled with oxygen.

### 1. Apparatus

Use the apparatus shown in Fig. 1.06-1.

### 2. Preparation of test solution and blank solution

Unless otherwise specified, prepare them by the following method.

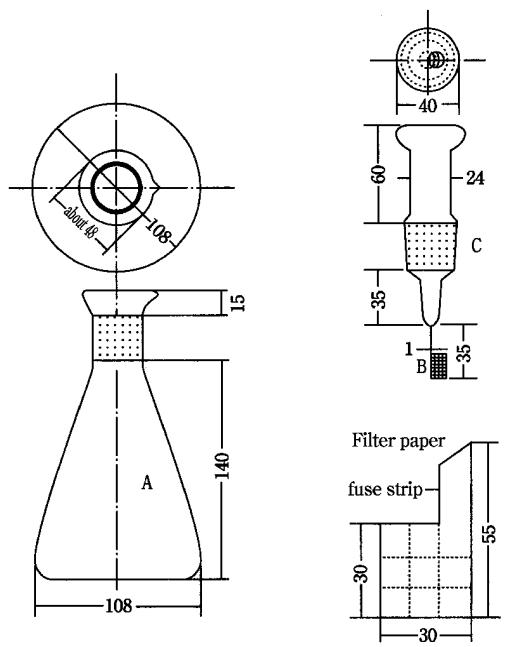
#### 2.1. Preparation of sample

(i) For solid samples: Place the quantity of the sample specified in the monograph on the center of the filter illustrated in the figure, weigh accurately, wrap the sample carefully along the dotted line without scattering, and place the parcel in a platinum basket or cylinder B, leaving its fuse-strip on the outside.

(ii) For liquid samples: Roll a suitable amount of absorbent cotton with filter paper, 50 mm in length and 5 mm in width, so that the end part of the paper is left to a length of about 20 mm as a fuse-strip, and place the parcel in a platinum basket or cylinder B. Place the sample in a suitable glass tube, weigh accurately, and moisten the cotton with the quantity of the sample specified in the monograph, bringing the edge of the sample in contact with the cotton.

#### 2.2. Method of combustion

Place the absorbing liquid specified in the monograph in flask A, fill it with oxygen, moisten the ground part of the stopper C with water, then ignite the fuse-strip, immediately transfer it to the flask, and keep the flask airtight until the combustion is completed. Shake the flask occasionally until the white smoke in A vanishes completely, allow to stand for 15 to 30 minutes, and designate the resulting solution as the test solution. Prepare the blank solution in the same manner, without sample.



The figures are in mm.

..... line to be folded

A: Colorless, thick-walled (about 2 mm), 500-mL hard glass flask, the upper part of which is made like a saucer. A flask made of quartz should be used for the determination of fluorine.

B: Platinum basket or cylinder made of platinum woven gauge.  
(It is hung at the end of the stopper C with platinum wire).

C: Ground stopper made of hard glass. A stopper made of quartz should be used for the determination of fluorine.

Fig. 1.06-1

### 3. Procedure of determination

Unless otherwise specified in the monograph, perform the test as follows.

#### 3.1. Chlorine and bromine

Apply a small amount of water to the upper part of A, pull out C carefully, and transfer the test solution to a beaker. Wash C, B and the inner side of A with 15 mL of 2-propanol, and combine the washings with the test solution. To this solution add 1 drop of bromophenol blue TS, add dilute nitric acid dropwise until a yellow color develops, then add 25 mL of 2-propanol, and titrate <2.50> with 0.005 mol/L silver nitrate VS according to the potentiometric titration. Perform the test with the blank solution in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L silver nitrate VS  
= 0.1773 mg of Cl

Each mL of 0.005 mol/L silver nitrate VS  
= 0.3995 mg of Br

#### 3.2. Iodine

Apply a small amount of water to the upper part of A, pull out C carefully, add 2 drops of hydrazine monohydrate to the test solution, put C on A, and decolorize the solution by vigorous shaking. Transfer the content of A to a beaker, wash C, B and the inner side of A with 25 mL of 2-propanol, and transfer the washings to the above beaker. To this solution add 1 drop of bromophenol blue TS, then add dilute nitric acid dropwise until a yellow color develops, and titrate <2.50> with 0.005 mol/L silver nitrate VS according to the

potentiometric titration. Perform the test with the blank solution in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.005 \text{ mol/L silver nitrate VS} \\ = 0.6345 \text{ mg of I} \end{aligned}$$

### 3.3. Fluorine

Apply a small amount of water to the upper part of A, pull out C carefully, transfer the test solution and the blank solution to 50 mL volumetric flasks separately, wash C, B and the inner side of A with water, add the washings and water to make 50 mL, and use these solutions as the test solution and the correction solution. Pipet the test solution ( $V$  mL) equivalent to about 30  $\mu\text{g}$  of fluorine,  $V$  mL of the correction solution and 5 mL of standard fluorine solution, transfer to 50-mL volumetric flasks separately, add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, and allow to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a blank prepared with 5 mL of water in the same manner. Determine the absorbances,  $A_T$ ,  $A_C$  and  $A_S$ , of the subsequent solutions of the test solution, the correction solution and the standard solution at 600 nm.

$$\begin{aligned} \text{Amount (mg) of fluorine (F) in the test solution} \\ = \text{amount (mg) of fluorine in 5 mL of} \end{aligned}$$

$$\text{the standard solution} \times \frac{A_T - A_C}{A_S} \times \frac{50}{V}$$

**Standard Fluorine Solution:** Dry sodium fluoride (standard reagent) in a platinum crucible between 500°C and 550°C for 1 hour, cool it in a desiccator (silica gel), weigh accurately about 66.3 mg of it, and dissolve in water to make exactly 500 mL. Pipet 10 mL of this solution, and dilute with sufficient water to make exactly 100 mL.

### 3.4. Sulfur

Apply a small amount of water to the upper part of A, pull out C carefully, and wash C, B and the inner side of A with 15 mL of methanol. To this solution add 40 mL of methanol, then add exactly 25 mL of 0.005 mol/L barium perchlorate VS, allow to stand for 10 minutes, add 0.15 mL of arsenazo III TS with a measuring pipet, and titrate <2.50> with 0.005 mol/L sulfuric acid VS. Perform the test with the blank solution in the same manner.

$$\begin{aligned} \text{Each mL of } 0.005 \text{ mol/L barium perchlorate VS} \\ = 0.1604 \text{ mg of S} \end{aligned}$$

## 1.07 Heavy Metals Limit Test

Heavy Metals Limit Test is a limit test of the quantity of heavy metals contained as impurities in drugs. The heavy metals are the metallic inclusions that are darkened with sodium sulfide TS in acidic solution, as their quantity is expressed in terms of the quantity of lead (Pb).

In each monograph, the permissible limit for heavy metals (as Pb) is described in terms of ppm in parentheses.

### 1. Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as directed in the following:

#### 1.1. Method 1

Place an amount of the sample, directed in the monograph, in a Nessler tube. Dissolve in water to make 40 mL. Add 2 mL of dilute acetic acid and water to make 50 mL,

and designate it as the test solution.

The control solution is prepared by placing the volume of Standard Lead Solution directed in the monograph in a Nessler tube, and adding 2 mL of dilute acetic acid and water to make 50 mL.

#### 1.2. Method 2

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat cautiously until white fumes are no longer evolved, and incinerate by ignition between 500°C and 600°C. Cool, add 2 mL of hydrochloric acid, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Then add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate a mixture of 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid on a water bath, further evaporate to dryness on a sand bath, and moisten the residue with 3 drops of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

#### 1.3. Method 3

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, heat cautiously, gently at first, and then incinerate by ignition between 500°C and 600°C. After cooling, add 1 mL of aqua regia, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate 1 mL of aqua regia to dryness on a water bath. Hereinafter, proceed as directed for the test solution, and add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

#### 1.4. Method 4

Place an amount of the sample, directed in the monograph, in a platinum or porcelain crucible, mix with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and carbonize by gradual heating. Cool, add 1 mL of sulfuric acid, heat carefully, and incinerate by ignition between 500°C and 600°C. If a carbonized substance remains, moisten with a small amount of sulfuric acid, and incinerate by ignition. Cool, dissolve the residue in 3 mL of hydrochloric acid, evaporate on a water bath to dryness, wet the residue with 3 drops of hydrochloric acid, add 10 mL of water, and dissolve by warming. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until a pale red color develops, then add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and the washing to a Nessler tube, add water to make 50 mL, and use this solution as the test solution.

The control solution is prepared as follows: Take 10 mL of a solution of magnesium nitrate hexahydrate in ethanol

(95) (1 in 10), and fire the ethanol to burn. Cool, add 1 mL of sulfuric acid, heat carefully, and ignite between 500°C and 600°C. Cool, and add 3 mL of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

## 2. Procedure

Add 1 drop of sodium sulfide TS to each of the test solution and the control solution, mix thoroughly, and allow to stand for 5 minutes. Then compare the colors of both solutions by viewing the tubes downward or transversely against a white background. The test solution has no more color than the control solution.

# 1.08 Nitrogen Determination (Semimicro-Kjeldahl Method)

Nitrogen Determination is a method to determine nitrogen in an organic substance in which the nitrogen is converted into ammonia nitrogen by thermal decomposition of the organic substance with sulfuric acid, and the ammonia liberated by alkali and trapped by distillation with steam is determined by titration.

## 1. Apparatus

Use the apparatus illustrated in Fig. 1.08-1. It is thoroughly constructed of hard glass, and ground glass surfaces may be used for joints. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

Alternatively, apparatus can be used in which some of the procedures, such as digestion of organic substances, distillation of the liberated ammonia, and endpoint detection methods in titrimetry (e.g., potentiometric titration or titration by colorimeter) are automated.

## 2. System suitability

If an automated apparatus is used, it is necessary to confirm periodically the suitability of the apparatus according to the following method:

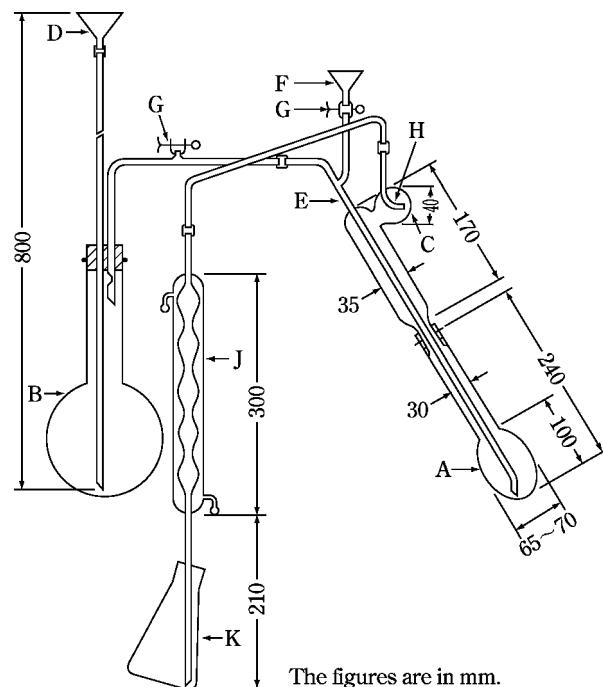
Weigh accurately about 1.7 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours, dissolve in water to make exactly 200 mL. Pipet 2 mL of this solution, and transfer to a digestion flask. When the test is performed as directed in the instrumental manual the nitrogen content (%) in amidosulfuric acid should be determined between 14.2% and 14.6%.

## 3. Reagents, Test Solutions

Decomposition accelerator: Unless otherwise specified, use 1 g of a powdered mixture of 10 g of potassium sulfate and 1 g of copper (II) sulfate pentahydrate. The composition and amount of the digestion accelerator may be modified if it is confirmed that the modified one give almost the same results using the sample as those obtained from the conventional catalyst.

## 4. Procedure

Unless otherwise specified, proceed by the following method. Weigh accurately or pipet a quantity of the sample corresponding to 2 to 3 mg of nitrogen (N:14.01), and place in the Kjeldahl flask A. Add the decomposition accelerator and wash down any adhering sample from the neck of the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to flow down the inside wall of the flask.



The figures are in mm.

A: Kjeldahl flask

B: Steam generator, containing water, to which 2 to 3 drops of sulfuric acid and fragments of boiling tips for preventing bumping have been added

C: Spray trap

D: Water supply funnel

E: Steam tube

F: Funnel for addition of alkali solution to flask A

G: Rubber tubing with a clamp

H: A small hole having a diameter approximately equal to that of the delivery tube

J: Condenser, the lower end of which is beveled

K: Absorption flask

Fig. 1.08-1

Then, while shaking the flask, add cautiously 1 mL of hydrogen peroxide (30) drop by drop along the inside wall of the flask. Heat the flask gradually, then heat so strong that the vapor of sulfuric acid is condensed at the neck of the flask, until the solution changes through a blue and clear to a vivid green and clear, and the inside wall of the flask is free from a carbonaceous material. If necessary, add a small quantity of hydrogen peroxide (30) after cooling, and heat again. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus (Fig. 1.08-1) washed beforehand by passing steam through it. To the absorption flask K add 15 mL of boric acid solution (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube J. Add 30 mL of sodium hydroxide solution (2 in 5) through the funnel F, rinse cautiously the funnel with 10 mL of water, close the clamp attached to the rubber tubing G, then begin the distillation with stream, and continue until the distillate measures 80 to 100 mL. Remove the absorption flask from the lower end of the condenser tube J, rinsing the end part with a small quantity of water, and titrate <2.50> the distillate with 0.005 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L sulfuric acid VS  
= 0.1401 mg of N

If an automated apparatus is used, proceed as directed in the instrumental procedure.

## 1.09 Qualitative Tests

Qualitative Tests are applied to the identification of drugs and are done generally with quantities of 2 to 5 mL of the test solution.

### Acetate

- (1) When warmed with diluted sulfuric acid (1 in 2), acetates evolve the odor of acetic acid.
- (2) When an acetate is warmed with sulfuric acid and a small quantity of ethanol (95), the odor of ethyl acetate is evolved.
- (3) Neutral solutions of acetates produce a red-brown color with iron (III) chloride TS, and a red-brown precipitate when boiled. The precipitate dissolves and the color of the solution changes to yellow upon addition of hydrochloric acid.

### Aluminum salt

- (1) Solutions of aluminum salts, when treated with ammonium chloride TS and ammonia TS, yield a gelatinous, white precipitate which does not dissolve in an excess of ammonia TS.
- (2) Solutions of aluminum salts, when treated with sodium hydroxide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.
- (3) Solutions of aluminum salts, when treated with sodium sulfide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.
- (4) Add ammonia TS to solutions of aluminum salts until a gelatinous, white precipitate is produced. The color of the precipitate changes to red upon addition of 5 drops of alizarin red S TS.

### Ammonium salt

When heated with an excess of sodium hydroxide TS, ammonium salts evolve the odor of ammonia. This gas changes moistened red litmus paper to blue.

### Antimony salt, primary

- (1) When primary antimony salts are dissolved in a slight excess of hydrochloric acid for the test and then diluted with water, a white turbidity is produced. The mixture produces an orange precipitate upon addition of 1 to 2 drops of sodium sulfide TS. When the precipitate is separated, and sodium sulfide TS is added to one portion of the precipitate and sodium hydroxide TS is added to another portion, it dissolves in either of these reagents.

- (2) Add water to acidic solutions of primary antimony salts in hydrochloric acid until a small quantity of precipitate is produced, and then add sodium thiosulfate TS: the precipitate dissolves. A red precipitate is reproduced when the solution is heated.

### Aromatic amines, primary

Acidic solutions of primary aromatic amines, when cooled in ice, mixed with 3 drops of sodium nitrite TS under agitation, allowed to stand for 2 minutes, mixed well with 1 mL of ammonium amidosulfate TS, allowed to stand for 1 minute, and then mixed with 1 mL of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS, exhibit a red-purple color.

### Arsenate

(1) Neutral solutions of arsenates produce no precipitate with 1 to 2 drops of sodium sulfide TS, but produce a yellow precipitate with hydrochloric acid subsequently added. The separated precipitate dissolves in ammonium carbonate TS.

(2) Neutral solutions of arsenates produce a dark red-brown precipitate with silver nitrate TS. When dilute nitric acid is added to one portion of the suspension, and ammonia TS is added to another portion, the precipitate dissolves in either of these reagents.

(3) Neutral or ammonia alkaline solutions of arsenates produce with magnesia TS a white, crystalline precipitate, which dissolves by addition of dilute hydrochloric acid.

### Arsenite

(1) Acidic solutions of arsenites in hydrochloric acid produce a yellow precipitate with 1 to 2 drops of sodium sulfide TS. When hydrochloric acid is added to one portion of the separated precipitate, it does not dissolve. When ammonium carbonate TS is added to another portion, the precipitate dissolves.

(2) Slightly alkaline solutions of arsenites produce a yellow-white precipitate with silver nitrate TS. When ammonia TS is added to one portion of the suspension, and dilute nitric acid is added to another portion, the precipitate dissolves in either of these reagents.

(3) Slightly alkaline solutions of arsenites produce a green precipitate with copper (II) sulfate TS. When the separated precipitate is boiled with sodium hydroxide TS, it changes to red-brown.

### Barium salt

- (1) When Flame Coloration Test (1) <1.04> is applied to barium salts, a persistent yellow-green color develops.
- (2) Solutions of barium salts produce with dilute sulfuric acid a white precipitate, which does not dissolve upon addition of dilute nitric acid.
- (3) Acidic solutions of barium salts in acetic acid produce a yellow precipitate with potassium chromate TS. The precipitate dissolves by addition of dilute nitric acid.

### Benzoate

(1) Concentrated solutions of benzoates produce a white, crystalline precipitate with dilute hydrochloric acid. The separated precipitate, washed with cold water and dried, melts between 120°C and 124°C <2.60>.

(2) Neutral solutions of benzoates produce a light yellow-red precipitate upon dropwise addition of iron (III) chloride TS. The precipitate changes to white on subsequent addition of dilute hydrochloric acid.

### Bicarbonate

(1) Bicarbonates effervesce upon addition of dilute hydrochloric acid, generating a gas, which produces a white precipitate immediately, when passed into calcium hydroxide TS (common with carbonates).

(2) Solutions of bicarbonates produce no precipitate with magnesium sulfate TS, but produce a white precipitate when boiled subsequently.

(3) Cold solutions of bicarbonates remain unchanged or exhibits only a slightly red color upon addition of 1 drop of phenolphthalein TS (discrimination from carbonates).

### Bismuth salt

(1) Bismuth salts, dissolved in a slight excess of hydrochloric acid, yield a white turbidity upon dilution with water. A dark brown precipitate is produced with 1 to 2 drops of sodium sulfide TS subsequently added.

(2) Acidic solutions of bismuth salts in hydrochloric acid

exhibit a yellow color upon addition of thiourea TS.

(3) Solution of bismuth salts in dilute nitric acid or in dilute sulfuric acid yield with potassium iodide TS a black precipitate, which dissolves in an excess of the reagent to give an orange-colored solution.

#### Borate

(1) When ignite a mixture of a borate with sulfuric acid and methanol, it burns with a green flame.

(2) Turmeric paper, when moistened with acidic solutions of borates in hydrochloric acid and dried by warming, exhibits a red color, which changes to blue with ammonia TS added dropwise.

#### Bromate

(1) Acidic solutions of bromates in nitric acid yield with 2 to 3 drops of silver nitrate TS a white, crystalline precipitate, which dissolves upon heating. When 1 drop of sodium nitrite TS is added to this solution, a light yellow precipitate is produced.

(2) Acidic solutions of bromates in nitric acid exhibit a yellow to red-brown color upon addition of 5 to 6 drops of sodium nitrite TS. When 1 mL of chloroform is added to the mixture and shaken, the chloroform layer exhibits a yellow to red-brown color.

#### Bromide

(1) Solutions of bromides yield a light yellow precipitate with silver nitrate TS. Upon addition of dilute nitric acid to a portion of the separated precipitate, it does not dissolve. When ammonia solution (28) is added to another portion and shaken, the separated solution yields a white turbidity upon acidifying with dilute nitric acid.

(2) Solutions of bromides exhibit a yellow-brown color with chlorine TS. The mixture is separated into 2 portions. When one portion is shaken with chloroform, the chloroform layer exhibits a yellow-brown to red-brown color. When phenol is added to the other portion, a white precipitate is produced.

#### Calcium salt

(1) When Flame Coloration Test (1) <1.04> is applied to calcium salts, a yellow-red color develops.

(2) Solutions of calcium salts yield a white precipitate with ammonium carbonate TS.

(3) Solutions of calcium salts yield a white precipitate with ammonium oxalate TS. The separated precipitate does not dissolve in dilute acetic acid, but dissolves in dilute hydrochloric acid.

(4) Neutral solutions of calcium salts produce no precipitate, when mixed with 10 drops of potassium chromate TS and heated (discrimination from strontium salts).

#### Carbonate

(1) Carbonates effervesce upon addition of dilute hydrochloric acid, generating a gas, which produces a white precipitate immediately, when passed into calcium hydroxide TS (common with bicarbonates).

(2) Solutions of carbonates yield with magnesium sulfate TS a white precipitate, which dissolves by addition of dilute acetic acid.

(3) Cold solutions of carbonates exhibit a red color with 1 drop of phenolphthalein TS (discrimination from bicarbonates).

#### Ceric salt

(1) When a cerous salt is mixed with 2.5 times its mass of lead (IV) oxide, nitric acid is added and the solution is boiled, it exhibits a yellow color.

(2) Solutions of cerous salts yield a yellow to red-brown

precipitate upon addition of hydrogen peroxide TS and ammonia TS.

#### Chlorate

(1) Solutions of chlorates yield no precipitate with silver nitrate TS. When 2 to 3 drops of sodium nitrite TS and dilute nitric acid are added to the mixture, a white precipitate is produced gradually, which dissolves by addition of ammonia TS.

(2) When indigocarmine TS is added dropwise to neutral solutions of chlorates until a light blue color appears, and the mixture is acidified with dilute sulfuric acid, the blue color vanishes promptly upon subsequent dropwise addition of sodium hydrogensulfite TS.

#### Chloride

(1) Solutions of chlorides evolve an odor of chlorine, when mixed with sulfuric acid and potassium permanganate, and heated. The gas evolved turns moistened potassium iodide starch paper blue.

(2) Solutions of chlorides yield a white precipitate with silver nitrate TS. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When an excess of ammonia TS is added to another portion, the precipitate dissolves.

#### Chromate

(1) Solutions of chromates exhibit a yellow color.

(2) Solutions of chromates produce a yellow precipitate with lead (II) acetate TS. When acetic acid (31) is added to a portion of the suspension, the precipitate does not dissolve. When dilute nitric acid is added to another portion, the precipitate dissolves.

(3) When acidic solutions of chromates in sulfuric acid are mixed with an equal volume of ethyl acetate and 1 to 2 drops of hydrogen peroxide TS, shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.

#### Citrate

(1) When 20 mL of a mixture of pyridine and acetic anhydride (3:1) is added to 1 or 2 drops of a solution of citrate, and the solution is allowed to stand for 2 to 3 minutes, a red-brown color develops.

(2) Neutral solutions of citrates, when mixed with an equal volume of dilute sulfuric acid and two-thirds volume of potassium permanganate TS, heated until the color of permanganate is discharged, and then treated dropwise with bromine TS to one-tenth of total volume, yield a white precipitate.

(3) Neutral solutions of citrates, when boiled with an excess of calcium chloride TS, yield a white crystalline precipitate. When sodium hydroxide TS is added to a portion of the separated precipitate, it does not dissolve. When dilute hydrochloric acid is added to another portion, the precipitate dissolves.

#### Cupric salt

(1) When a well polished iron plate is immersed in acidic solutions of cupric salts in hydrochloric acid, a red metallic film appears on its surface.

(2) Solutions of cupric salts produce a light blue precipitate with a small quantity of ammonia TS. The precipitate dissolves in an excess of the reagent, yielding a deep blue-colored solution.

(3) Solutions of cupric salts yield a red-brown precipitate with potassium hexacyanoferrate (II) TS. When dilute nitric acid is added to a portion of the suspension, the precipitate does not dissolve. When ammonia TS is added to another portion, the precipitate dissolves, yielding a deep blue-

colored solution.

(4) Solutions of cupric salts produce a black precipitate with sodium sulfide TS. When dilute hydrochloric acid, dilute sulfuric acid or sodium hydroxide TS is added to a portion of the separated precipitate, it does not dissolve. When hot dilute nitric acid is added to another portion, the precipitate dissolves.

#### Cyanide

(1) Solutions of cyanides yield a white precipitate with an excess of silver nitrate TS. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When ammonia TS is added to another portion, the precipitate dissolves.

(2) Solutions of cyanides yield a blue precipitate, when mixed by shaking with 2 to 3 drops of iron (II) sulfate TS, 2 to 3 drops of dilute iron (III) chloride TS and 1 mL of sodium hydroxide TS, and then acidified with dilute sulfuric acid.

#### Dichromate

(1) Solutions of dichromates exhibit a yellow-red color.

(2) Solutions of dichromates produce a yellow precipitate with lead (II) acetate TS. When acetic acid (31) is added to one portion of the suspension, the precipitate dose not dissolve. When dilute nitric acid is added to another portion, the precipitate dissolves.

(3) When acidic solutions of dichromates in sulfuric acid are mixed with an equal volume of ethyl acetate and with 1 to 2 drops of hydrogen peroxide TS, shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.

#### Ferric salt

(1) Slightly acidic solutions of ferric salts yield with potassium hexacyanoferrate (II) TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferric salts yield with sodium hydroxide TS a gelatinous, red-brown precipitate, which changes to black upon addition of sodium sulfide TS. The separated precipitate dissolves in dilute hydrochloric acid, yielding a white turbidity.

(3) Slightly acidic solutions of ferric salts exhibit a purple color with 5-sulfosalicylic acid TS.

#### Ferricyanide

(1) Solutions of ferricyanides exhibit a yellow color.

(2) Solutions of ferricyanides yield with iron (II) sulfate TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

#### Ferrocyanide

(1) Solutions of ferrocyanides yield with iron (III) chloride TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrocyanides yield with copper (II) sulfate TS a red-brown precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

#### Ferrous salt

(1) Slightly acidic solutions of ferrous salts yield with potassium hexacyanoferrate (III) TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrous salts yield with sodium hydroxide TS a grayish green, gelatinous precipitate, which changes to black with sodium sulfide TS. The separated precipitate dissolves in dilute hydrochloric acid.

(3) Neutral or slightly acidic solutions of ferrous salts ex-

hibit an deep red color upon dropwise addition of a solution of 1,10-phenanthroline monohydrate in ethanol (95) (1 in 50).

#### Fluoride

(1) When solutions of fluorides are heated with chromic acid-sulfuric acid TS, the inside of the test tube is not moistened uniformly.

(2) Neutral or slightly acidic solutions of fluorides exhibit a blue-purple color after standing with 1.5 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1:1:1).

#### Glycerophosphate

(1) Solutions of glycerophosphates remain unaffected by addition of calcium chloride TS, but yield a precipitate when boiled.

(2) Solutions of glycerophosphates yield no precipitate with hexaammonium heptamolybdate TS in the cold, but yield a yellow precipitate when boiled for a long time.

(3) When glycerophosphates are mixed with an equal mass of powdered potassium hydrogen sulfate and heated gently over a free flame, the pungent odor of acrolein is evolved.

#### Iodide

(1) Solutions of iodides yield a yellow precipitate with silver nitrate TS. When dilute nitric acid is added to one portion of the suspension, and ammonia solution (28) to another portion, the precipitates do not dissolve in either of these reagents.

(2) Acidic solutions of iodides exhibit a yellow-brown color with 1 to 2 drops of sodium nitrite TS and then yield a black-purple precipitate. The solutions exhibit a deep blue color with starch TS subsequently added.

#### Lactate

Acidic solutions of lactates in sulfuric acid, when heated with potassium permanganate TS, evolve the odor of acetaldehyde.

#### Lead salt

(1) Solutions of lead salts yield a white precipitate with dilute sulfuric acid. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When sodium hydroxide TS is added to another portion and warmed, or when ammonium acetate TS is added to another portion, the precipitate dissolves.

(2) Solutions of lead salts yield with sodium hydroxide TS a white precipitate, which dissolves in an excess of sodium hydroxide TS, and yields a black precipitate upon subsequent addition of sodium sulfide TS.

(3) Acidic solutions of lead salts in dilute acetic acid yield with potassium chromate TS a yellow precipitate, which does not dissolve in ammonia TS but dissolves in sodium hydroxide TS subsequently added.

#### Lithium salt

(1) When Flame Coloratution Test (1) <1.04> is applied to lithium salts, a persistent red color develops.

(2) Solutions of lithium salts yield with disodium hydrogenphosphate TS a white precipitate, which dissolves upon subsequent addition of dilute hydrochloric acid.

(3) Solutions of lithium salts yield no precipitate with dilute sulfuric acid (discrimination from strontium salts).

#### Magnesium salt

(1) Solutions of magnesium salts yield upon warming with ammonium carbonate TS a white precipitate, which dis-

solves in ammonium chloride TS. A white, crystalline precipitate is reproduced by subsequent addition of disodium hydrogenphosphate TS.

(2) Solutions of magnesium salts yield with sodium hydroxide TS a white, gelatinous precipitate. When iodine TS is added to one portion of the suspension, the precipitate develops a dark-brown color. When excess sodium hydroxide TS is added to another portion, the precipitate does not dissolve.

#### Manganese salt

(1) Solutions of manganese salts yield a white precipitate with ammonia TS. When silver nitrate TS is added to a portion of the suspension, the precipitate changes to black. When another portion is allowed to stand, the upper part of the precipitate exhibits a brownish color.

(2) Acidic solutions of manganese salts in dilute nitric acid exhibit a purple-red color with a small quantity of powdered bismuth sodium trioxide.

#### Mercuric salt

(1) A copper plate is immersed in solutions of mercuric salts, allowed to stand, taken out, and then washed with water. The plate becomes bright and silvery white in appearance, when rubbed with paper or cloth (common with mercurous salts).

(2) Solutions of mercuric salts yield with a small quantity of sodium sulfide TS a black precipitate, which dissolves in an excess of the reagent. The black precipitate is reproduced by subsequent addition of ammonium chloride TS.

(3) When potassium iodide TS is added dropwise to neutral solutions of mercuric salts, a red precipitate is produced. The precipitate dissolves in an excess of the reagent.

(4) Acidic solutions of mercuric salts in hydrochloric acid yield with a small quantity of tin (II) chloride TS a white precipitate, which changes to grayish black upon addition of an excess of the reagent.

#### Mercurous salt

(1) A copper plate is immersed in solutions of mercurous salts, allowed to stand, taken out, and then washed with water. The plate becomes bright and silvery white in appearance, when rubbed with paper or cloth (common with mercuric salts).

(2) Mercurous salts or their solutions exhibit a black color with sodium hydroxide TS.

(3) Solutions of mercurous salts yield a white precipitate with dilute hydrochloric acid. The separated precipitate changes to black upon addition of ammonia TS.

(4) Solutions of mercurous salts yield with potassium iodide TS a yellow precipitate, which changes to green, when allowed to stand, and changes again to black upon subsequent addition of an excess of the reagent.

#### Mesilate

(1) To mesilates add twice its mass of sodium hydroxide, heat gently to melt, and continue heating for 20 to 30 seconds. After cooling, add a little amount of water, then add dilute hydrochloric acid, and warm: the gas evolved changes moistened potassium iodate-starch paper to blue.

(2) To mesilates add threefold its mass of sodium nitrate and anhydrous sodium carbonate, mix, and heat gradually. After cooling, dissolve the residue in diluted hydrochloric acid (1 in 5), and filter if necessary. The filtrate yields a white precipitate upon addition of barium chloride TS.

#### Nitrate

(1) Solutions of nitrates, when mixed with an equal volume of sulfuric acid, the mixture is cooled, and iron (II)

sulfate TS is superimposed, a dark brown ring is produced at the junction of the two liquids.

(2) Solutions of nitrates exhibit a blue color with diphenylamine TS.

(3) When potassium permanganate TS is added to acidic solutions of nitrates in sulfuric acid, the red-purple color of the reagent does not fade (discrimination from nitrites).

#### Nitrite

(1) Solutions of nitrites, when acidified with dilute sulfuric acid, evolve a yellow-brown gas with a characteristic odor. The solutions exhibit a dark brown color upon addition of a small quantity of iron (II) sulfate crystals.

(2) Solutions of nitrites, when 2 to 3 drops of potassium iodide TS and dilute sulfuric acid are added dropwise, exhibit a yellow-brown color, and then yield a black-purple precipitate. When the mixture is shaken with 2 mL of chloroform, the chloroform layer exhibits a purple color.

(3) Solutions of nitrites, when mixed with thiourea TS and acidified with dilute sulfuric acid, and iron (III) chloride TS is added dropwise, exhibit a dark red color. When the mixture is shaken with 2 mL of diethyl ether, the diethyl ether layer exhibits a red color.

#### Oxalate

(1) When potassium permanganate TS is added dropwise to warm acidic solutions of oxalates in sulfuric acid, the reagent is decolorized.

(2) Solutions of oxalates yield a white precipitate with calcium chloride TS. The separated precipitate does not dissolve in dilute acetic acid but dissolves upon subsequent addition of dilute hydrochloric acid.

#### Permanganate

(1) Solutions of permanganates exhibit a red-purple color.

(2) When an excess of hydrogen peroxide TS is added to acidic solutions of permanganates in sulfuric acid, the solutions effervesce and decolorize permanganates.

(3) Acidic solutions of permanganates in sulfuric acid are decolorized, when an excess of oxalic acid TS is added and heated.

#### Peroxide

(1) Solutions of peroxides are mixed with an equal volume of ethyl acetate and 1 to 2 drops of potassium dichromate TS, and then acidified with dilute sulfuric acid. When the mixture is shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.

(2) Acidic solutions of peroxides in sulfuric acid decolorize dropwise added potassium permanganate TS, and effervesce to evolve a gas.

#### Phosphate (Orthophosphate)

(1) Neutral solutions of phosphates yield with silver nitrate TS a yellow precipitate, which dissolves upon addition of dilute nitric acid or ammonia TS.

(2) Acidic solutions in dilute nitric acid of phosphates yield a yellow precipitate with hexaammonium heptamolybdate TS on warming. The precipitate dissolves upon subsequent addition of sodium hydroxide TS or ammonia TS.

(3) Neutral or ammonia-alkaline solutions of phosphates yield with magnesia TS a white, crystalline precipitate, which dissolves upon subsequent addition of dilute hydrochloric acid.

#### Potassium salt

(1) When Flame Coloration Test (1) <1.04> is applied to potassium salts, a pale purple color develops. When it gives a yellow color, a red-purple color can be seen through cobalt

glass.

(2) Neutral solutions of potassium salts yield a white, crystalline precipitate with sodium hydrogen tartrate TS. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod. The separated precipitate dissolves upon addition of any of ammonia TS, sodium hydroxide TS or sodium carbonate TS.

(3) Acidic solutions of potassium salts in acetic acid (31) yield a yellow precipitate with sodium hexanitrocobaltate (III) TS.

(4) Potassium salts do not evolve the odor of ammonia, when an excess of sodium hydroxide TS is added and warmed (discrimination from ammonium salts).

#### Salicylate

(1) Salicylates evolve the odor of phenol, when an excess of soda-lime is added and heated.

(2) Concentrated solutions of salicylates yield a white, crystalline precipitate with dilute hydrochloric acid. The separated precipitate, washed well with cold water and dried, melts <2.60> at about 159°C.

(3) Neutral solutions of salicylates exhibit with 5 to 6 drops of dilute iron (III) chloride TS a red color, which changes to purple and then fades when dilute hydrochloric acid is added dropwise.

#### Silver salt

(1) Solutions of silver salts yield a white precipitate with dilute hydrochloric acid. When dilute nitric acid is added subsequently to a portion of the suspension, the precipitate does not dissolve. When an excess of ammonia TS is added to another portion, the precipitate dissolves.

(2) Solutions of silver salts yield with potassium chromate TS a red precipitate, which dissolves upon addition of dilute nitric acid.

(3) Solutions of silver salts yield a grayish brown precipitate with ammonia TS added dropwise. When ammonia TS is added dropwise until the precipitate dissolves, then 1 to 2 drops of formaldehyde solution are added and warmed, a mirror of metallic silver is deposited on the inside wall of the container.

#### Sodium salt

(1) When Flame Coloration Test (1) <1.04> is applied to sodium salts, a yellow color develops.

(2) Concentrated, neutral or slightly alkaline solutions of sodium salts yield a white, crystalline precipitate with potassium hexahydroxoantimonate (V) TS. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod.

#### Stannic salt

(1) When the outside bottom of a test tube containing water is moistened with acidic solutions of stannic salts in hydrochloric acid and is placed in a nonluminous flame of a Bunsen burner, a blue flame mantle is seen around the bottom of the test tube (common with stannous salts).

(2) When granular zinc is immersed in acidic solutions of stannic salts in hydrochloric acid, a spongy, gray substance is deposited on the surface of the granules (common with stannous salts).

(3) Add iron powder to acidic solutions of stannic salts in hydrochloric acid, allow to stand, and then filter. When iodine-starch TS is added dropwise to the filtrate, the color of the test solution disappears.

(4) Acidic solutions of stannic salts in hydrochloric acid, to which ammonia TS is added dropwise until a small quantity of precipitate is produced, yield a light yellow precipitate with 2 to 3 drops of sodium sulfide TS. The separated pre-

cipitate dissolves upon addition of sodium sulfide TS and light yellow precipitate is reproduced by subsequent addition of hydrochloric acid.

#### Stannous salt

(1) When the outside bottom of a test tube containing water is moistened with acidic solutions of stannous salts in hydrochloric acid and is placed in a nonluminous flame of a Bunsen burner, a blue flame mantle is seen around the bottom of the test tube (common with stannic salts).

(2) When granular zinc is immersed in acidic solutions of stannous salts in hydrochloric acid, a spongy, gray substance is deposited on the surface of the granules (common with stannic salts).

(3) When iodine-starch TS is added dropwise to solutions of stannous salts, the color of the test solution disappears.

(4) Acidic solutions of stannous salts in hydrochloric acid, to which ammonia TS is added dropwise until a small quantity of precipitate is produced, yield a dark brown precipitate with 2 to 3 drops of sodium sulfide TS. When sodium sulfide TS is added to a portion of the separated precipitate, it does not dissolve. When ammonium polysulfide TS is added to another portion, the precipitate dissolves.

#### Sulfate

(1) Solutions of sulfates yield with barium chloride TS a white precipitate, which does not dissolve upon addition of dilute nitric acid.

(2) Neutral solutions of sulfates yield with lead (II) acetate TS a white precipitate, which dissolves upon subsequent addition of ammonium acetate TS.

(3) When an equal volume of dilute hydrochloric acid is added, solutions of sulfates yield no white turbidity (discrimination from thiosulfates), and do not evolve the odor of sulfur dioxide (discrimination from sulfites).

#### Sulfide

(1) Most kinds of sulfides evolve the odor of hydrogen sulfide with dilute hydrochloric acid. This gas blackens lead (II) acetate paper moistened with water.

#### Sulfite and Bisulfite

(1) When iodine TS is added dropwise to acidic solutions of sulfites or bisulfites in acetic acid (31), the color of the reagent fades.

(2) When an equal volume of dilute hydrochloric acid is added, solutions of sulfites or bisulfites evolve the odor of sulfur dioxide but yield no turbidity (discrimination from thiosulfates). The solutions yield immediately with 1 drop of sodium sulfide TS a white turbidity, which changes gradually to a pale yellow precipitate.

#### Tartrate

(1) Neutral tartrate solutions yield a white precipitate with silver nitrate TS. When nitric acid is added to a portion of the separated precipitate, it dissolves. When ammonia TS is added to another portion and warmed, the precipitate dissolves and metallic silver is deposited gradually on the inside wall of the test tube, forming a mirror.

(2) Solutions of tartrates exhibit a red-purple to purple color, when 2 drops of acetic acid (31), 1 drop of iron (II) sulfate TS, 2 to 3 drops of hydrogen peroxide TS and an excess of sodium hydroxide TS are added.

(3) When a solution, prepared by mixing 2 to 3 drops of a solution of resorcinol (1 in 50) and 2 to 3 drops of a solution of potassium bromide (1 in 10) with 5 mL of sulfuric acid, is added to 2 to 3 drops of solutions of tartrates, and then heated for 5 to 10 minutes on a water bath, a deep blue

color is produced. The solution exhibits a red to red-orange color when poured to 3 mL of water after cooling.

#### Thiocyanate

(1) Solutions of thiocyanates yield a white precipitate with an excess of silver nitrate TS. When dilute nitric acid is added to a portion of the suspension, the precipitate does not dissolve. When ammonia solution (28) is added to another portion, the precipitate dissolves.

(2) Solutions of thiocyanates produce with iron (III) chloride TS a red color, which is not decolorized by addition of hydrochloric acid.

#### Thiosulfate

(1) When iodine TS is added dropwise to acidic solutions of thiosulfates in acetic acid (31), the color of the reagent fades.

(2) When an equal volume of dilute hydrochloric acid is added, solutions of thiosulfates evolve the odor of sulfur dioxide, and yield gradually a white turbidity, which changes to yellow on standing.

(3) Solutions of thiosulfates yield with an excess of silver nitrate TS a white precipitate, which changes to black on standing.

#### Zinc salt

(1) Neutral to alkaline solutions of zinc salts yield a whitish precipitate with ammonium sulfide TS or sodium sulfide TS. The separated precipitate does not dissolve in dilute acetic acid but dissolves upon subsequent addition of dilute hydrochloric acid.

(2) Solutions of zinc salts yield a white precipitate with potassium hexacyanoferrate (II) TS. When dilute hydrochloric acid is added to a portion of the suspension, the precipitate does not dissolve. When sodium hydroxide TS is added to another portion, the precipitate dissolves.

(3) Neutral to weakly acidic solutions of zinc salts yield a white precipitate, when 1 or 2 drops of pyridine and 1 mL of potassium thiocyanate TS are added.

## 1.10 Iron Limit Test

Iron Limit Test is a limit test for iron contained in drugs. The limit is expressed in term of iron (Fe).

In each monograph, the permissible limit for iron (as Fe) is described in terms of ppm in parentheses.

### 1. Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as follows:

#### 1.1. Method 1

Weigh the amount of sample specified in individual monograph, add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test (pH 4.5), dissolve by warming if necessary, and designate this solution as the test solution.

Prepare the control solution as follows: To the amount of Standard Iron Solution specified in individual monograph add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test (pH 4.5).

#### 1.2. Method 2

Weigh the amount of sample specified in individual monograph, add 10 mL of dilute hydrochloric acid, and dissolve by warming if necessary. Dissolve 0.5 g of L-tartaric acid, and add one drop of phenolphthalein TS. Add ammonia TS dropwise until the solution develops a pale red color. Add 20 mL of acetic acid-sodium acetate buffer solution for iron limit test (pH 4.5) and designate this solution as the test solu-

tion.

Prepare the control solution as follows: To the amount of Standard Iron Solution specified in individual monograph add 10 mL of dilute hydrochloric acid, and proceed as directed for the test solution.

#### 1.3. Method 3

Place the amount of sample specified in individual monograph in a crucible, moisten with a small amount of sulfuric acid, heat cautiously and gently at first, and then incinerate by ignition. After cooling, add 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and to the residue add 0.5 mL of diluted hydrochloric acid (2 in 3) and 10 mL of water. After dissolving by warming, add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test (pH 4.5), and designate this solution as the test solution.

Prepare the control solution as follows: Transfer the amount of Standard Iron Solution specified in individual monograph to a crucible, and add 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and proceed as directed for the test solution.

In this procedure, use a quartz or porcelain crucible, which is immersed in boiling dilute hydrochloric acid for 1 hour and washed thoroughly with water and dried.

### 2. Procedure

Unless otherwise specified, proceed as follows:

#### 2.1. Method A

Transfer the test solution and the control solution to separate Nessler tubes, to each add 2 mL of a solution of L-ascorbic acid (1 in 100), mix well, and allow to stand for 30 minutes. Add 1 mL of a solution of  $\alpha$ ,  $\alpha'$ -dipyridyl in ethanol (95) (1 in 200), add water to make 50 mL, and allow to stand for 30 minutes. Then compare the colors developed in both solutions against a white background. The test solution has no more color than the control solution.

#### 2.2. Method B

Dissolve 0.2 g of L-ascorbic acid in the test solution and the control solution, and allow to stand for 30 minutes. Add 1 mL of a solution of  $\alpha$ ,  $\alpha'$ -dipyridyl in ethanol (95) (1 in 200), and allow to stand for 30 minutes. Then add 2 mL of a solution of 2,4,6-trinitrophenol (3 in 1000) and 20 mL of 1,2-dichloroethane, shake vigorously, collect the 1,2-dichloroethane layer, and filter through a pledget of absorbent cotton in a funnel on which 5 g of anhydrous sodium sulfate is placed if necessary. Then compare the colors developed in both solutions against a white background. The test solution has no more color than the control solution.

## 1.11 Arsenic Limit Test

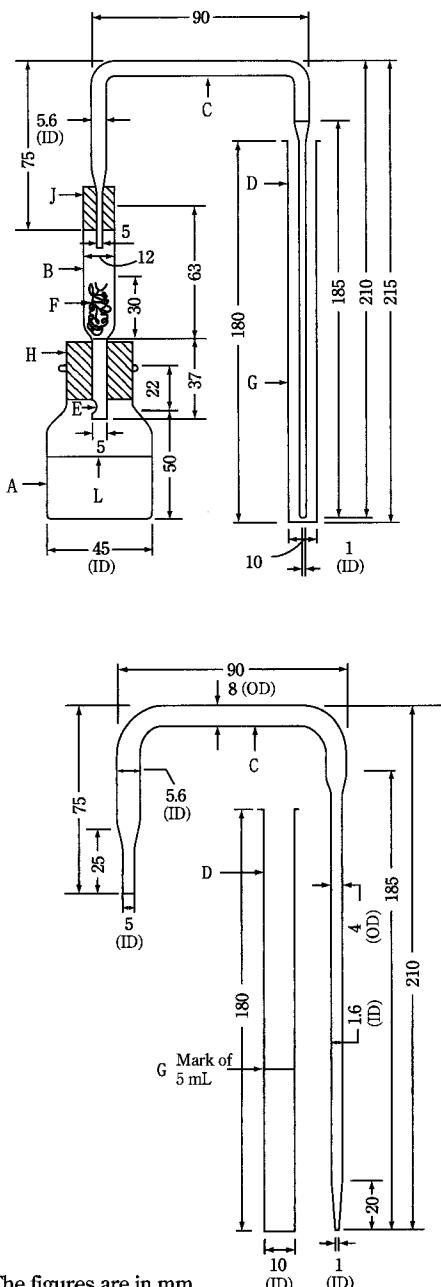
Arsenic Limit Test is a limit test for arsenic contained in drugs. The limit is expressed in terms of arsenic trioxide ( $As_2O_3$ ).

In each monograph, the permissible limit for arsenic (as  $As_2O_3$ ) is described in terms of ppm in parentheses.

### 1. Apparatus

Use the apparatus illustrated in Fig. 1.11-1.

Place glass wool F in the exit tube B up to about 30 mm in height, moisten the glass wool uniformly with a mixture of an equal volume of lead (II) acetate TS and water, and apply gentle suction to the lower end to remove the excess of the mixture. Insert the tube vertically into the center of the rubber stopper H, and attach the tube to the generator bottle A



The figures are in mm.

- A: Generator bottle (capacity up to the shoulder: approximately 70 mL)
- B: Exit tube
- C: Glass tube (inside diameter: 5.6 mm, the tip of the part to be inserted in the absorber tube D is drawn out to 1 mm in diameter)
- D: Absorber tube (inside diameter: 10 mm)
- E: Small perforation
- F: Glass wool (about 0.2 g)
- G: Mark of 5 mL
- H and J: Rubber stoppers
- L: Mark of 40 mL

**Fig. 1.11-1** Arsenic limit test apparatus

so that the small perforation E in the lower end of B extends slightly below. At the upper end of B, attach the rubber stopper J to hold the tube C vertically. Make the lower end to the exit tube of C level with that of the rubber stopper J.

## 2. Preparation of the test solution

Unless otherwise specified, proceed as directed in the following.

### 2.1. Method 1

Weigh the amount of the sample directed in the monograph, add 5 mL of water, dissolve by heating if necessary, and designate the solution as the test solution.

### 2.2. Method 2

Weigh the amount of the sample directed in the monograph, add 5 mL of water, and add 1 mL of sulfuric acid except in the cases that the samples are inorganic acids. Add 10 mL of sulfurous acid solution, transfer to a small beaker, and evaporate the mixture on a water bath until it is free from sulfurous acid and is reduced to about 2 mL in volume. Dilute with water to make 5 mL, and designate it as the test solution.

### 2.3. Method 3

Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), ignite the ethanol, and heat gradually to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate. After cooling, add 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.

### 2.4. Method 4

Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, heat gradually, and ignite to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate in the same manner. After cooling, add 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.

### 2.5. Method 5

Weigh the amount of the sample directed in the monograph, add 10 mL of *N,N*-dimethylformamide, dissolve by heating if necessary, and designate the solution as the test solution.

## 3. Test solutions

(i) Absorbing solution for hydrogen arsenide: Dissolve 0.50 g of silver *N,N*-diethyldithiocarbamate in pyridine to make 100 mL. Preserve this solution in a glass-stoppered bottle protected from light, in a cold place.

(ii) Standard Arsenic Stock Solution: Weigh exactly 0.100 g of finely powdered arsenic trioxide dried at 105°C for 4 hours, and add 5 mL of sodium hydroxide solution (1 in 5) to dissolve. Add dilute sulfuric acid to neutralize, add further 10 mL of dilute sulfuric acid, add freshly boiled and cooled water to make exactly 1000 mL, and preserve in a glass-stoppered bottle.

(iii) Standard Arsenic Solution: Pipet 10 mL of Standard Arsenic Stock Solution, add 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL. Each mL of the solution contains 1 µg of arsenic trioxide ( $As_2O_3$ ). Prepare Standard Arsenic Solution just before use.

In the case where the preparation of Standard Arsenic Stock Solution is difficult, Certified Standard Arsenic Solution may be used to prepare Standard Arsenic Solution as follows: Pipet 15 mL of Certified Standard Arsenic Solution, add 1 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 100 mL. Pipet 5 mL of this

solution, add 1 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 100 mL. Prepare just before use.

(iv) Certified Standard Arsenic Solution: JCSS Arsenic Standard Solution (100 mg/L) Each mL of this solution contains 0.1 mg of arsenic ( $\text{As}_5$ ).

JCSS (Japan Calibration Service System) is a registration system of calibration service.

#### 4. Procedure

Unless otherwise specified, proceed using apparatus shown in Fig. 1.11-1. Carry out the preparation of the standard color at the same time.

Place the test solution in the generator bottle A and, if necessary, wash down the solution in the bottle with a small quantity of water. Add 1 drop of methyl orange TS, and after neutralizing with ammonia TS, ammonia solution (28) or dilute hydrochloric acid, add 5 mL of diluted hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic tin (II) chloride TS, and allow to stand for 10 minutes. Then add water to make 40 mL, add 2 g of zinc for arsenic analysis, and immediately connect the rubber stopper H fitted with B and C with the generator bottle A. Transfer 5 mL of the absorbing solution for hydrogen arsenide to the absorber tube D, insert the tip of C to the bottom of the absorber tube D, then immerse the generator bottle A up to the shoulder in water maintained at 25°C, and allow to stand for 1 hour. Disconnect the absorber tube, add pyridine to make 5 mL, if necessary, and observe the color of the absorbing solution: the color produced is not more intense than the standard color.

Preparation of standard color: Measure accurately 2 mL of Standard Arsenic Solution in the generator bottle A. Add 5 mL of diluted hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic tin (II) chloride TS, allow to stand at room temperature for 10 minutes, and then proceed as directed above. The color produced corresponds to 2  $\mu\text{g}$  of arsenic trioxide ( $\text{As}_2\text{O}_3$ ) and is used as the standard.

#### 5. Note

Apparatus, reagents and test solutions used in the test should contain little or no arsenic. If necessary, perform a blank determination.

## 1.12 Methanol Test

Methanol Test is a method to determine methanol adhering in ethanol.

#### 1. Reagents

(i) Standard Methanol Solution—To 1.0 g of methanol, accurately measured, add water to make exactly 1000 mL. To 5 mL of this solution, exactly measured, add 2.5 mL of methanol-free ethanol and water to make exactly 50 mL.

(ii) Solution A—To 75 mL of phosphoric acid add water to make 500 mL, then dissolve 15 g of potassium permanganate in this solution.

(iii) Solution B—Add sulfuric acid carefully to an equal volume of water, cool, and dissolve 25 g of oxalic acid dihydrate in 500 mL of this dilute sulfuric acid.

#### 2. Procedure

Pipet 1 mL of the sample, and add water to make exactly 20 mL. Use this solution as the sample solution. Transfer 5 mL each of the sample solution and the Standard Methanol Solution, accurately measured, to test tubes, add 2 mL of

Solution A to each solution, and allow to stand for 15 minutes. Decolorize these solutions by adding 2 mL of Solution B, and mix with 5 mL of fuchsin-sulfurous acid TS. Allow to stand for 30 minutes at ordinary temperature. The sample solution has no more color than the Standard Methanol Solution.

## 1.13 Fats and Fatty Oils Test

Fats and Fatty Oils Test is a method applied to fats, fatty oils, waxes, fatty acids, higher alcohols, and related substances.

#### 1. Preparation of test sample

For a solid sample, melt with care, and, if necessary, filter the melted sample with a dry filter paper by warming. For a turbid liquid sample, heat at about 50°C. If it is still turbid, filter it with a dry filter paper while warm. In either case, mix the sample to make it homogeneous.

#### 2. Melting point

Proceed by the method described in Method 2 of Melting Point Determination <2.60>.

#### 3. Congealing point of fatty acids

##### 3.1. Preparation of fatty acids

Dissolve 25 g of potassium hydroxide in 100 g of glycerin. Transfer 75 g of this solution into a 1-L beaker, and heat at 150°C. Add 50 g of the sample to this solution, and heat at a temperature not higher than 150°C for 15 minutes under frequent stirring to saponify completely. Cool the solution to 100°C, dissolve by addition of 500 mL of hot water, and add slowly 50 mL of diluted sulfuric acid (1 in 4). Heat the solution under frequent stirring until the clear layer of fatty acid is separated distinctly. Separate the fatty acid layer, and wash the fatty acid with hot water until the washing shows no acidity to methyl orange TS. Transfer the fatty acid layer to a small beaker, and heat on a water bath until the fatty acid becomes clear owing to the separation of water. Filter the warm solution, and complete the evaporation of water by carefully heating the filtered solution to 130°C.

##### 3.2. Measurement of congealing point

Proceed by the method described in Congealing Point Determination <2.42>.

#### 4. Specific gravity

##### 4.1. Liquid sample at ordinary temperature

Proceed by the method described in Determination of Specific Gravity and Density <2.56>.

##### 4.2. Solid sample at ordinary temperature

Unless otherwise specified, fill a pycnometer with water at 20°C. Weigh accurately the pycnometer, and, after discarding the water and drying, weigh accurately the empty pycnometer. Then, fill the pycnometer with the melted sample to about three-fourths of the depth, and allow to stand at a temperature a little higher than the melting temperature of the sample for 1 hour to drive off the air in the sample. After keeping at the specified temperature, weigh accurately the pycnometer. Fill up the pycnometer with water over the sample at 20°C, and weigh accurately again.

The other procedure is the same as described in Method 1 of Determination of Specific Gravity and Density <2.56>.

$$d = \frac{M_1 - M}{(M_2 - M) - (M_3 - M_1)}$$

$M$ : Mass (g) of the empty pycnometer

$M_1$ : Mass (g) of the pycnometer filled with the sample

**Table 1.13-1**

Acid value	Amount (g) of sample
Less than 5	20
5 to 15	10
15 to 30	5
30 to 100	2.5
More than 100	1.0

$M_2$ : Mass (g) of the pycnometer filled with water

$M_3$ : Mass (g) of the pycnometer filled with the sample and water

### 5. Acid value

The acid value is the number of milligrams of potassium hydroxide (KOH) required to neutralize the free acids in 1 g of sample.

#### 5.1. Procedure

Unless otherwise specified, weigh accurately the amount of sample shown in Table 1.13-1, according to the expected acid value of the sample, in a glass-stoppered, 250-mL flask, add 100 mL of a mixture of diethyl ether and ethanol (95:1 or 2:1) as the solvent, and dissolve the sample by warming, if necessary. Then, add a few drops of phenolphthalein TS, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color which persists for 30 seconds. If the sample solutions are turbid at lower temperature, titration should be done while warm. To the solvent used add phenolphthalein TS as an indicator, and add 0.1 mol/L potassium hydroxide-ethanol VS before use, until the solvent remains light red for 30 seconds.

$$\text{Acid value} = \frac{\text{consumed volume (mL) of } 0.1 \text{ mol/L potassium hydroxide-ethanol VS}}{\text{amount (g) of sample}} \times 5.611$$

### 6. Saponification value

The saponification value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters and to neutralize the free acids in 1 g of the sample.

#### 6.1. Procedure

Unless otherwise specified, weigh accurately 1 to 2 g of the sample, transfer to a 200-mL flask, and add exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS. Attach a short reflux condenser or an air condenser 750 mm in length and 6 mm in diameter to the neck of the flask, and heat gently in a water bath for 1 hour with frequent shaking. Cool the solution, add 1 mL of phenolphthalein TS, and titrate <2.50> immediately the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS. If the sample solution is turbid at lower temperature, titration should be done while warm. Perform a blank determination in the same manner.

$$\text{Saponification value} = \frac{(a - b) \times 28.05}{\text{amount (g) of sample}}$$

a: Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed in the blank determination

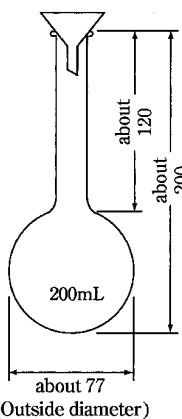
b: Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed for titration of the sample

### 7. Ester value

The ester value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters in 1 g of sample.

#### 7.1. Procedure

Unless otherwise specified, designate the difference between the saponification value and the acid value determined



The figures are in mm.

**Fig. 1.13-1** Hydroxyl value determination flask

as the ester value.

### 8. Hydroxyl value

The hydroxyl value is the number of milligrams of potassium hydroxide (KOH) required to neutralize acetic acid combined with hydroxyl groups, when 1 g of the sample is acetylated by the following procedure.

#### 8.1. Procedure

Place about 1 g of the sample, weighed accurately, in a 200-mL round-bottom flask (shown in Fig. 1.13-1), and add exactly 5 mL of pyridine-acetic anhydride TS. Place a small funnel on the neck of the flask, and heat by immersing the flask up to 1 cm from the bottom in an oil bath between 95°C and 100°C. Put a thick, round paper with a round hole on the joint of the neck of the flask to protect the neck from the heat of the oil bath. After heating for 1 hour, take the flask from the oil bath, and cool by standing. Add 1 mL of water to the flask, and shake to decompose acetic anhydride. Heat the flask in the oil bath for 10 minutes again. After cooling, wash the funnel and neck with 5 mL of neutralized ethanol down into the flask, and titrate <2.50> with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 1 mL of phenolphthalein TS). Perform a blank determination in the same manner.

$$\text{Hydroxyl value} = \frac{(a - b) \times 28.05}{\text{amount (g) of sample}} + \text{acid value}$$

a: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS consumed in the blank determination

b: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS consumed for titration of the sample

### 9. Unsaponifiable matter

Unsaponifiable matter is calculated as the difference between the amount of materials, which are unsaponifiable by the procedure described below, soluble in diethyl ether and insoluble in water, and the amount of fatty acids expressed in terms of the amount of oleic acid. Its limit is expressed as a percentage in the monograph.

#### 9.1. Procedure

Transfer about 5 g of the sample, accurately weighed, to a 250-mL flask. Add 50 mL of potassium hydroxide-ethanol TS, attach a reflux condenser to the flask, boil gently on a water bath for 1 hour with frequent shaking, and then transfer to the first separator. Wash the flask with 100 mL of warm water, and transfer the washing to the separator. Further, add 50 mL of water to the separator, and cool to room

**Table 1.13-2**

Iodine value	Amount (g) of sample
Less than 30	1.0
30 to 50	0.6
50 to 100	0.3
More than 100	0.2

temperature. Wash the flask with 100 mL of diethyl ether, add the washing to the separator, extract by vigorous shaking for 1 minute, and allow to stand until both layers are separated clearly. Transfer the water layer to the second separator, add 50 mL of diethyl ether, shake, and allow to stand in the same manner. Transfer the water layer in the second separator to the third separator, add 50 mL of diethyl ether, and extract by shaking again in the same manner. Combine the diethyl ether extracts in the second and third separators into the first separator, wash each separator with a small amount of diethyl ether, and combine the washings into the first separator. Wash the combined extracts in the first separator with 30 mL portions of water successively, until the washing does not develop a light red color with 2 drops of phenolphthalein TS. Add a small amount of anhydrous sodium sulfate to the diethyl ether extracts, and allow to stand for 1 hour. Filter the diethyl ether extracts with dry filter paper, and collect the filtrates into a tared flask. Wash well the first separator with diethyl ether, and add the washing to the flask through the above filter paper. After evaporation of the filtrate and washing almost to dryness on a water bath, add 3 mL of acetone, and evaporate again to dryness on a water bath. Complete the drying between 70°C and 80°C under reduced pressure (about 2.67 kPa) for 30 minutes, allow to stand for cooling in a desiccator (reduced pressure, silica gel) for 30 minutes, and then weigh. After weighing, add 2 mL of diethyl ether and 10 mL of neutralized ethanol, and dissolve the residue by shaking well. Add a few drops of phenolphthalein TS, and titrate <2.50> the remaining fatty acids in the residue with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color which persists for 30 seconds.

$$\text{Unsaponifiable matter (\%)} = \frac{a - (b \times 0.0282)}{\text{amount (g) of sample}} \times 100$$

a: Amount (g) of the extracts

b: Volume (mL) of 0.1 mol/L potassium hydroxide-ethanol VS consumed for titration

#### 10. Iodine value

The iodine value, when measured under the following conditions, is the number of grams of iodine (I), representing the corresponding amount of halogen, which combines with 100 g of sample.

##### 10.1. Procedure

Unless otherwise specified, weigh accurately the amount of sample shown in Table 1.13-2, according to the expected iodine value of the sample, in a small glass container. In a 500-mL glass-stoppered flask place the container containing the sample, add 20 mL of cyclohexane to dissolve the sample, then add exactly 25 mL of Wijs' TS, and mix well. Stopper the flask, and allow to stand, protecting against light, between 20°C and 30°C for 30 minutes (when the expected iodine value is more than 100, for 1 hour) with occasional shaking. Add 20 mL of potassium iodide solution (1 in 10) and 100 mL of water, and shake. Then, titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indica-

tor: 1 mL of starch TS). Perform a blank determination in the same manner.

$$\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{amount (g) of sample}}$$

a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination

b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed for titration of the sample

## 1.14 Sulfate Limit Test

Sulfate Limit Test is a limit test for sulfate contained in drugs.

In each monograph, the permissible limit for sulfate (as SO<sub>4</sub>) is described in terms of percentage (%) in parentheses.

#### 1. Procedure

Unless otherwise specified, transfer the quantity of the sample, directed in the monograph, to a Nessler tube, dissolve it in sufficient water, and add water to make 40 mL. Add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.005 mol/L sulfuric acid VS, directed in the monograph, to another Nessler tube, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions according to the same procedure.

Add 2 mL of barium chloride TS to the test solution and to the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely.

The turbidity produced in the test solution is not thicker than that of the control solution.

## 1.15 Readily Carbonizable Substances Test

Readily Carbonizable Substances Test is a method to examine the minute impurities contained in drugs, which are readily colored by addition of sulfuric acid.

#### 1. Procedure

Before use, wash the Nessler tubes thoroughly with sulfuric acid for readily carbonizable substances. Unless otherwise specified, proceed as follows. When the sample is solid, place 5 mL of sulfuric acid for readily carbonizable substances in a Nessler tube, to which add a quantity of the finely powdered sample, little by little, as directed in the monograph, and dissolve it completely by stirring with a glass rod. When the sample is liquid, transfer a volume of the sample, as directed in the monograph, to a Nessler tube, add 5 mL of sulfuric acid for readily carbonizable substances, and mix by shaking. If the temperature of the content of the tube rises, cool the content; maintain it at the standard temperature, if the reaction may be affected by the temperature. Allow to stand for 15 minutes, and compare the color of the liquid with that of the matching fluid in the Nessler tube specified in the monograph, by viewing transversely against a white background.

## 2. Physical Methods

### Chromatography

#### 2.01 Liquid Chromatography

Liquid Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a liquid as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a liquid or soluble sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio ( $k$ ) for each component.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

The ratio  $k$  represents the mass distribution ratio in liquid chromatography.

Since the relation given below exists among the ratio ( $k$ ), the time for which the mobile phase is passed through the column ( $t_0$ : time measured from the time of injection of a compound with  $k = 0$  to the time of elution at the peak maximum), and the retention time ( $t_R$ : time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

$$t_R = (1 + k) t_0$$

##### 1. Apparatus

Basically, the apparatus required for the liquid chromatographic procedure consists of a pumping system for the mobile phase, a sample injection port, a column, a detector and a recorder. A mobile phase component regulator, a thermostat for the column, a pumping system for reaction reagents and a chemical reaction chamber are also used, if necessary. The pumping system serves to deliver the mobile phase and the reagents into the column and connecting tube at a constant flow rate. The sample injection port is used to deliver a quantity of the sample to the apparatus with high reproducibility. The column is a tube with a smooth interior, made of inert metal, etc., in which a packing material for liquid chromatography is uniformly packed. A column with a stationary phase chemically bound on the inside wall instead of the column packed with the packing material may be used. The detector is used to detect a property of the samples which is different from that of the mobile phase, and may be an ultraviolet or visible spectrophotometer, fluorometric detector, differential refractometer, electrochemical detector, chemiluminescence detector, electric conductivity detector, mass spectrophotometer, etc. The output signal is usually proportional to the concentration of samples at amounts of less than a few  $\mu\text{g}$ . The recorder is used to record the output signals of the detector. As required, a data processor may be used as the recorder to record or output the chromatogram, retention times or amounts of the components. The mobile phase component regulator is used to vary the ratio of the mobile phase components in a stepwise

or gradient fashion.

##### 2. Procedure

Fix the detector, column and mobile phase to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram. If the components to be analyzed have no readily detectable physical properties such as absorbance or fluorescence, the detection is achieved by changing the components to suitable derivatives. Usually, the derivatization is performed as a pre- or post-column labeling.

##### 3. Identification and purity test

When Liquid Chromatography is used for identification of a component of a sample, it is performed by confirming identity of the retention time of the component and that of an authentic specimen, or by confirming that the peak shape of the component is unchanged after mixing the sample with an authentic specimen. If a detector which is able to obtain chemical structural information of the component at the same time is used, highly specific identification can be achieved by confirming identity of the chemical structure of the component and that of an authentic specimen, in addition to the identity of their retention times.

When Liquid Chromatography is used for purity test, it is generally performed by comparing the peak area of target impurity from the sample solution with that of the main component from a standard solution, which is prepared by diluting the sample solution to a concentration corresponding to the specified limit of the impurity, or by calculating target impurity content using the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on its correction factor to the principal component.

##### 4. Assay

###### 4.1. Internal standard method

In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the

preparation of the calibration curve according to the method specified in the individual monograph, perform the liquid chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

#### 4.2. Absolute calibration curve method

Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the liquid chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

### 5. Method for peak measuring

Generally, the following methods are used.

#### 5.1. Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on both sides of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing system.

#### 5.2. Peak area measuring method

(i) Width at half-height method: Multiply the peak width at the half-height by the peak height.

(ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

### 6. System suitability

System suitability testing is an integral part of test methods using chromatography, and is used to ensure that the performance of the chromatographic systems used is as suitable for the analysis of the drug as was at the time when the verification of the test method was performed using the system. System suitability testing should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability testing must be prescribed in the test method of drugs. The results of drug analyses are not acceptable unless the requirements of system suitability have been met.

In system suitability testing of the chromatographic sys-

tems, the evaluation of "System performance" and "System repeatability" is usually required. For quantitative purity tests, the evaluation of "Test for required detectability" may also be required.

#### 6.1. Test for required detectability

For purity tests, when it is confirmed that the target impurity is distinctly detected at the concentration of its specification limit, it is considered verified that the system used has adequate performance to achieve its intended use.

For quantitative purity tests, "Test for required detectability" is usually required, and in order to confirm, in some degree, the linearity of response near its specification limit, the range of expected response to the injection of a certain volume of target impurity solution at the concentration of its specification limit should be prescribed. For limit test, "Test for required detectability" is not required, if the test is performed by comparing the response from sample solution with that from standard solution at the concentration of its specification limit. "Test for required detectability" is also not required, if it is confirmed that the impurity can be detected at its specification limit by the evaluation of "System repeatability" or some other procedure.

#### 6.2. System performance

When it is confirmed that the specificity for determining the test ingredient is ensured, it is considered verified that the system used has adequate performance to achieve its intended use.

In assay, "System performance" should be defined by the resolution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable), and when appropriate, by their order of elution. In purity tests, both the resolution and the order of elution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable) should be prescribed. In addition, if necessary, the symmetry factor of the test ingredient should be prescribed together with them. However, if there is no suitable target substance to be separated, it is acceptable to define "System performance" using the number of theoretical plates and the symmetry factor of the test ingredient.

#### 6.3. System repeatability

When it is confirmed that the degree of variation (precision) of the response of the test ingredient is at a level that meets the requirement of "System repeatability", it is considered verified that the system used has adequate performance to achieve its intended use.

The allowable limit of "System repeatability" is normally defined as the relative standard deviation (*RSD*) of the response of the test ingredient in replicate injections of standard solution. It is acceptable to confirm the repeatability of the system not only by replicate injections of standard solution before sample injections, but also by divided injections of standard solution before and after sample injections, or by interspersed injections of standard solution among sample injections.

In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopting new allowable limit of "System repeatability" which can guarantee a level of "System repeatability" equivalent to that at 6 replicate injections.

The allowable limit of "System repeatability" should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test.

### 7. Point to consider on changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the packing material (pore size in the case of monolithic columns), column temperature, composition ratio of the mobile phase, composition of buffer solutions in the mobile phase, pH of the mobile phase, concentration of ion-pair forming agents in the mobile phase, ionic strength of the mobile phase, flow rate of the mobile phase, number and timing of mobile phase composition changes in gradient program, flow rate of mobile phase in gradient program, composition and flow rate of derivatizing reagents, and reaction time and chamber temperature in chemical reaction may be modified within the ranges in which the liquid chromatographic system used conforms to the requirements of system suitability.

### 8. Terminology

(i) **SN ratio:** It is defined by the following formula.

$$S/N = \frac{2H}{h}$$

*H:* Peak height of the target ingredient peak from the baseline

*h:* Width of background noise of the chromatogram of sample solution or solvent blank around the peak of the target ingredient

The baseline and background noise are measured over a range 20 times of peak width at the center point of peak height of the target ingredient. When a solvent blank is used, measure over almost the same range as mentioned above around the point where the target ingredient elutes.

(ii) **Symmetry factor:** It shows the degree of symmetry of a peak in the chromatogram, and is defined as *S* in the following equation.

$$S = \frac{W_{0.05h}}{2f}$$

*W<sub>0.05h</sub>:* Width of the peak at one-twentieth of the peak height

*f:* Distance between the perpendicular from the peak maximum and the leading edge of the peak at one-twentieth of the peak height

Where *W<sub>0.05h</sub>* and *f* have the same unit.

(iii) **Relative standard deviation:** Generally, it is defined as RSD (%) in the following equation.

$$RSD (\%) = \frac{100}{\bar{X}} \times \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{X})^2}{n - 1}}$$

*x<sub>i</sub>:* Observed value

*X̄:* Mean of observed values

*n:* Number of replicate measurements

(iv) **Complete separation of peak:** It means that the resolution between two peaks is not less than 1.5. It is also called as "baseline separation".

(v) **Peak-valley ratio:** It indicates the degree of separation between 2 peaks on a chromatogram when baseline separation cannot be attained, and is defined as *p/v* by the following formula.

$$p/v = \frac{H_p}{H_v}$$

*H<sub>p</sub>:* peak height from the baseline of the minor peak

*H<sub>v</sub>:* height from the baseline of the lowest point (peak valley) of the curve between major and minor peaks

(vi) **Separation factor:** It shows the relation between the retention times of peaks in the chromatogram, and is defined as *α* in the following equation.

$$\alpha = \frac{t_{R2} - t_0}{t_{R1} - t_0}$$

*t<sub>R1</sub>, t<sub>R2</sub>:* Retention times of two compounds used for the resolution measurement (*t<sub>R1</sub> < t<sub>R2</sub>*)

*t<sub>0</sub>:* Time of passage of the mobile phase through the column (time measured from the time of injection of a compound with *k* = 0 to the time of elution at the peak maximum)

The separation factor (*α*) indicates thermodynamic difference in partition of two compounds. It is basically the ratio of their partition equilibrium coefficients or of their mass-distribution ratios, and is obtained from the chromatogram as the ratio of the retention times of the two compounds.

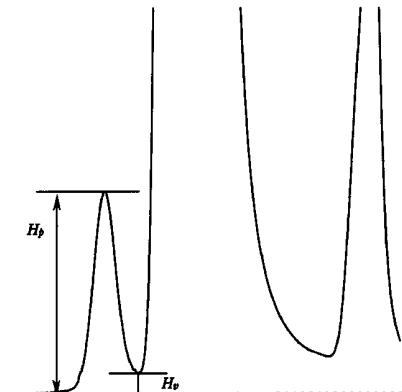
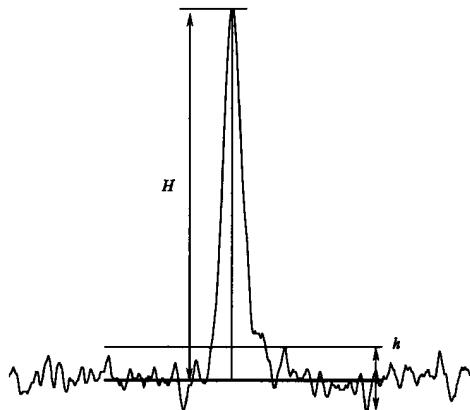
(vii) **Resolution:** It shows the relation between the retention time and the peak width of peaks in the chromatogram, and is defined as *R<sub>S</sub>* in the following equation.

$$R_S = 1.18 \times \frac{t_{R2} - t_{R1}}{W_{0.5h1} + W_{0.5h2}}$$

*t<sub>R1</sub>, t<sub>R2</sub>:* Retention times of two compounds used for the measurement of resolution (*t<sub>R1</sub> < t<sub>R2</sub>*)

*W<sub>0.5h1</sub>, W<sub>0.5h2</sub>:* Peak widths at half peak height

Where *t<sub>R1</sub>, t<sub>R2</sub>, W<sub>0.5h1</sub>* and *W<sub>0.5h2</sub>* have the same unit.



(viii) **Number of theoretical plates:** It indicates the extent of band broadening of a compound in the column, and is generally defined as  $N$  in the following equation.

$$N = 5.54 \times \frac{t_R^2}{W_{0.5 h}^2}$$

$t_R$ : Retention time of compound

$W_{0.5 h}$ : Width of the peak at half peak height

Where  $t_R$  and  $W_{0.5 h}$  have the same unit

#### 9. Note

Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

## 2.02 Gas Chromatography

Gas Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a gas (carrier gas) as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a gaseous or vaporizable sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio ( $k$ ) for each component.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

Since the relation given below exists among the ratio ( $k$ ), the time for which the mobile phase is passed through the column ( $t_0$ : time measured from the time of injection of a compound with  $k = 0$  to the time of elution at the peak maximum), and the retention time ( $t_R$ : time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

$$t_R = (1 + k) t_0$$

#### 1. Apparatus

Basically, the apparatus required for the gas chromatographic procedure consists of a carrier gas-introducing port and flow regulator, a sample injection port, a column, a column oven, a detector and a recorder. Gas introducing port and flow regulator for a combustion gas, a burning supporting gas and an accessory gas and sample injection port for headspace are also used, if necessary. The carrier gas-introducing port and flow regulator serves to deliver the carrier gas into the column at a constant flow rate, and usually consist of a pressure regulation valve, a flow rate regulation valve and a pressure gauge. The sample injection port is used to deliver a quantity of the sample to the flow line of carrier gas with high reproducibility. There are sample injection ports for packed column and for capillary column. There are both divided injection mode and non-divided injection mode to sample injection port for capillary column. The columns are usually classified as packed column or capillary column. The packed column is a tube made of inert metal, glass or synthetic resin, in which a packing material for gas chromatography is uniformly packed. The packed column with not more than 1 mm in inside diameter is also called a packed

capillary column (micro packed column). A capillary column is a tube made of inert metal, glass, quartz or synthetic resin, whose inside wall is bound chemically with stationary phase for gas chromatography. The column oven has the setting capacity for a column with required length and the temperature regulation system for keeping the constant column temperature. The detector is used to detect a component separated on the column, and may be an alkaline thermal ionization detector, a flame photometry detector, mass spectrophotometer, hydrogen flame-ionization detector, an electron capture detector, a thermal conductivity detector, etc. The recorder is used to record the output signals of the detector.

#### 2. Procedure

Unless otherwise specified, proceed by the following method. Fix the detector, column and carrier gas to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column system through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram.

#### 3. Identification and purity test

Identification of a component of a sample is performed by confirming identity of the retention time of the component and that of an authentic specimen, or by confirming that the peak shape of the component is unchanged after mixing the sample with an authentic specimen.

In general, the purity of the sample is determined by comparing the peak area of target impurity from the sample solution with that of the main component from a standard solution, which is prepared by diluting the sample solution to a concentration corresponding to the specified limit of the impurity, or by calculating target impurity content using the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on its response factor to the principal component.

#### 4. Assay

In general, perform the assay by using the internal standard method. The absolute calibration curve method is used when a suitable internal standard is not available. Perform the assay by using the standard addition method when the effect of the component other than the compound to be assayed on the quantitative determination is not negligible against a result of the determination.

##### 4.1. Internal standard method

In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, cal-

culate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the gas chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

#### 4.2. Absolute calibration curve method

Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the gas chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

#### 4.3. Standard addition method

Pipet a fixed volume of more than 4 sample solutions, add exactly the standard solution so that stepwise increasing amounts of the object compound are contained in the solutions except 1 sample solution, diluted exactly each solution with and without standard solution to a definite volume, and use each solution as the sample solution. Based on the chromatogram obtained by exact injection of a fixed volume of individual sample solutions, measure the peak area or peak height of individual sample solutions. Calculate the concentration of standard objective compound added into each sample solution, plot the amounts (concentration) of added standard object compound on the abscissa and the peak area or peak height on the ordinate on the graph, extend the calibration curve obtained by linking the plots, and determine the amount of object compound to be assayed from the distance between the origin and the intersecting point of the calibration curve with the abscissa. This method is available only in the case that the calibration curve is a straight line, and passes through the origin when the absolute calibration curve method is employed. In this method, all procedures must be carried out under a strictly constant condition.

### 5. Method for peak measuring

Generally, the following methods are used.

#### 5.1. Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on either side of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing system.

#### 5.2. Peak area measuring method

(i) Width at half-height method: Multiply the peak width at the half-height by the peak height.

(ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

### 6. System suitability

Refer to "System suitability" described under 2.01 Liquid Chromatography.

### 7. Point to consider in changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of column, particle size of packing material, concentration or thickness of stationary phase, column temperature, temperature-rising rate, kind and flow rate of carrier gas, and split ratio may be modified within the ranges in which the gas chromatographic system used conforms to the requirements of system suitability. Headspace sample injection device and its operating conditions may be also modified, provided that they give equivalent or more accuracy and precision.

### 8. Terminology

The definition of terms described under 2.01 Liquid Chromatography shall apply in 2.02 Gas Chromatography.

### 9. Note

Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

## 2.03 Thin-layer Chromatography

Thin-layer Chromatography is a method to separate each ingredient by developing a mixture in a mobile phase, using a thin-layer made of a suitable stationary phase, and is applied for identification, purity test, etc. of substances.

### 1. Preparation of thin-layer plate

Generally, proceed by the following method.

A smooth and uniformly thick glass plate having a size of 50 mm × 200 mm or 200 mm × 200 mm is used for preparing a thin-layer plate. Using a suitable apparatus, apply a water suspension of powdered solid substance for the stationary phase, directed in the monograph, on one side of the glass plate to make a uniform layer of 0.2 to 0.3 mm in thickness. After air-drying, dry further by heating at a fixed temperature between 105°C and 120°C for 30 to 60 minutes. A suitable plastic plate may be used instead of the glass plate. Preserve the dried plate with protection from moisture.

### 2. Procedure

Unless otherwise specified, proceed by the following method.

Designate a line about 20 mm distant from the bottom of

the thin-layer plate as the starting line, spot 2 to 6 mm in diameter the directed volumes of the sample solution or the standard solution in the monograph using micropipets at points on this line, separated by more than 10 mm, and air-dry. Unless otherwise specified, attach the filter paper along with the inside wall of the container, and wet the filter paper with the developing solvent. In the container, the developing solvent is placed up to about 10 mm in height from the bottom beforehand, seal the container closely, and allow it to stand for 1 hour at ordinary temperature. Place the plate in the container, avoiding contact with the inside wall, and seal the container. Develop it at ordinary temperature.

When the solvent front has ascended from the starting line to the distance directed in the monograph, remove the plate from the container. Immediately put a mark at the solvent front. After air-drying, observe the location, color, etc., of each spot by the method specified in the monograph. Calculate the *Rf* value by using the following equation:

$$R_f = \frac{\text{distance from the starting line to the center of the spot}}{\text{distance from the starting line to the solvent front}}$$

## 2.04 Amino Acid Analysis of Proteins

Amino acid analysis of proteins refers to the methodology used to determine the amino acid composition or content of proteins, peptides, and other pharmaceutical preparations. Amino acid analysis can be used to quantify protein and peptides, to determine the identity of proteins or peptides based on their amino acid composition, to support protein and peptide structure analysis, to evaluate fragmentation strategies for peptide mapping, and to detect atypical amino acids that might be present in a protein or peptide. It is necessary to hydrolyze a protein/peptide to its individual amino acid constituents before amino acid analysis. Following protein/peptide hydrolysis, the amino acid analysis procedure can be the same as that practiced for free amino acids in other pharmaceutical preparations. The amino acid constituents of the test sample are typically derivatized for analysis.

### 1. Hydrolysis of Protein and Peptide

Acid hydrolysis at 110°C for 24 hours using 6 mol/L hydrochloric acid containing phenol (Method 1) is the most common method for hydrolyzing protein and samples. The result should be analyzed carefully because several amino acids are chemically modified during the acid hydrolysis and thus not recovered quantitatively. Tryptophan is destroyed; serine and threonine are partially destroyed; methionine might undergo oxidation; and cysteine is typically recovered as cystine (but cystine recovery is usually poor because of partial destruction or reduction to cysteine). Peptide bonds involving isoleucine and valine are partially cleaved; and asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively.

The hydrolysis techniques, Methods 2 to 11, are used to address these concerns. Some of the hydrolysis techniques, Methods 4 to 11, may cause modifications of cysteine, methionine, asparagines and glutamine to other amino acids. Therefore, the benefits of using a given hydrolysis technique are weighed against the concerns with the technique and are tested adequately before employing a method other than acid hydrolysis by Method 1.

(i) Method 1: Hydrolysis using hydrochloric acid containing phenol (liquid phase hydrolysis, vapor phase hy-

drolysis)

Prevention of tryptophan oxidation

(ii) Method 2: Mercaptoethanesulfonic acid hydrolysis (vapor phase hydrolysis)

(iii) Method 3: Hydrolysis using hydrochloric acid containing thioglycolic acid (vapor phase hydrolysis)

Cysteine-cystine and methionine oxidation

(iv) Method 4: Hydrolysis by Method 1 or Method 2 after oxidation with performic acid

Cysteine-cystine oxidation

(v) Method 5: Hydrolysis using hydrochloric acid containing sodium azide (liquid phase hydrolysis)

(vi) Method 6: Hydrolysis using hydrochloric acid containing dimethylsulfoxide (vapor phase hydrolysis)

Cysteine-cystine reduction and alkylation

(vii) Method 7: Hydrochloric acid hydrolysis after a vapor phase pyridylethylation reaction

(viii) Method 8: Hydrochloric acid hydrolysis after a liquid phase pyridylethylation reaction

(ix) Method 9: Hydrochloric acid hydrolysis after a liquid phase carboxymethylation reaction

Conversion of cysteine-cystine to mixed disulfide

(x) Method 10: Hydrochloric acid hydrolysis after a reaction with dithiodiglycolic acid or dithiodipropionic acid

Derivatization of asparagine and glutamine

(xi) Method 11: Hydrochloric acid hydrolysis after reaction with bis(1,1-trifluoroacetoxy) iodobenzene

A time-course study is often employed to analyze the starting concentration of amino acids that are partially destroyed or slow to cleave. An acceptable alternative to the time-course study is to subject an amino acid calibration standard to the same hydrolysis conditions as the test sample. This technique will allow the analyst to account for some residue destruction.

Microwave acid hydrolysis has been used and is rapid but requires special equipment as well as special precautions. Complete proteolysis, using a mixture of proteases, has been used but can be complicated, requires the proper controls, and is typically more applicable to peptides than proteins.

### 2. Methodologies of Amino Acid Analysis

The amino acid analysis techniques include the postcolumn derivatization for detection (Methods 1 to 2) after the separation of the free amino acids by ion-exchange chromatography and the precolumn derivatization of the free amino acids (Methods 2 to 7) followed by reversed-phase HPLC.

(i) Method 1: Ninhydrin

(ii) Method 2: *o*-Phthalaldehyde (OPA)

(iii) Method 3: Phenylisothiocyanate (PITC)

(iv) Method 4: 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)

(v) Method 5: (Dimethylamino)azobenzenesulfonyl chloride (DABS-Cl)

(vi) Method 6: 9-Fluorenylmethyl chloroformate (FMOC-Cl)

(vii) Method 7: 7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F)

Among these methods, ion-exchange chromatography with postcolumn ninhydrin derivatization is one of the most common methods employed for quantitative amino acid analysis. The choice of any one technique often depends on the sensitivity required from the assay. Instruments and reagents for these procedures are available commercially. Furthermore, many modifications of these methodologies exist with different reagent preparations, reaction procedures, chromatographic systems, etc. Specific parameters may vary

according to the exact equipment and procedure used.

## 2.05 Size-Exclusion Chromatography

Size-exclusion chromatography is a separation technique of liquid chromatography, which separates molecules in solution based on their size. It is used for the determination of the molecular mass of high-molecular mass compounds such as polysaccharides, nucleic acids, proteins and chemically synthesized polymers, determination of the molecular mass distribution, purity tests and so on. A method using an aqueous solvent as the mobile phase for water-soluble high-molecular compounds is also called gel filtration chromatography. A method using an organic solvent as the mobile phase is also called gel permeation chromatography. This chapter describes procedures using an aqueous solvent as the mobile phase. The principle of the separation is the same when using an organic solvent as the mobile phase.

### 1. Principle of separation

Components to be tested are separated according to the easiness of entry into the pores of the packing material of the column. Molecules larger than the pores move rapidly through the spaces between the particles of the packing material without entering the pores, and elute at the position of the retention volume of an unretained compound ( $V_0$ ) of the column. Molecules smaller than the pores enter into the pores depending on their sizes, and smaller molecules elute later because they enter deeper into the pores. All molecules smaller than a certain size elute at the position of a completely permeated compound, or the total mobile phase volume ( $V_t$ ). The elution position of a molecule will be influenced not only by molecular mass but also by the structure of the molecule, solvent, interaction between the molecule and the packing material, etc.

### 2. Apparatus and measurement conditions

Usually follow Liquid Chromatography <2.01>. A porous packing material is used for a column. As packing materials, silica particles whose surface are coated with hydrophilic modification, or cross-linked hydrophilic polymers are used. Since measurable molecular mass range differs depending on the pore size of a packing material and its distribution, select an appropriate column. In order to expand the measurable molecular mass range, a column may be connected with another column having the different target molecular mass range. For a mobile phase, a buffer solution etc. is used. It is important to select appropriately a mobile phase to suppress interaction other than the principle of size exclusion between a packing material and a component to be tested. Adjustment of pH, addition of salt, etc. may be useful to suppress electrostatic interaction between a packing material and a component to be tested, and addition of an organic solvent (methanol, acetonitrile, etc.) may be useful to suppress hydrophobic interaction. The flow rate of a mobile phase, column temperature, sample injection volume, and the concentration of a sample solution should be set appropriately because they affect the separation. As a detector, an ultraviolet-visible spectrophotometer, a differential refractometer, a static light scattering detector, an evaporative light scattering detector, etc. are used. Usually, the molecular mass of a component to be tested is determined by comparing the elution position with molecular mass standards, but when using a static light scattering detector, the molecular mass of molecules in an eluate can be obtained directly.

### 3. Procedure

Follow Liquid Chromatography <2.01>.

### 4. Measurement of molecular mass

When determining molecular mass, average molecular mass or molecular mass distribution by size exclusion chromatography, measure a sample solution and molecular mass standard solutions prepared using appropriate molecular mass standards under the same test conditions, and determine as follows, unless otherwise specified. The molecular mass standards should have the similar physical properties as a component to be tested. The obtained molecular mass value depends on the molecular mass standards used and analysis conditions.

#### 4.1. Molecular mass of a monodisperse component

A molecular mass calibration curve is prepared by plotting the relationship of retention volume (or retention time) and the logarithmic value of molecular mass labelled on molecular mass standards. The molecular mass will be determined from the molecular mass calibration curve for the retention volume (or retention time) obtained from the chromatogram of a sample solution. Usually, the molecular mass of a component to be tested should be within the range of the molecular mass calibration curve.

#### 4.2. Average molecular mass of a polydisperse component

A molecular mass calibration curve is prepared from chromatograms obtained from molecular mass standard solutions. A chromatogram obtained from a sample solution is divided, and the molecular mass of each eluted fraction is obtained from the molecular mass calibration curve. The concentration or amount of the component in each fraction is determined, and the number-average molecular mass ( $M_n$ ), mass-average molecular mass ( $M_w$ ) and dispersity ( $d$ ) of the sample are calculated by the following equations.

The dispersity may be an indicator of the width of molecular mass distribution.

$$M_n = \frac{\sum M_i N_i}{\sum N_i} = \frac{\sum C_i}{\sum \frac{C_i}{M_i}} = \frac{1}{\sum \frac{w_i}{M_i}}$$

$$M_w = \frac{\sum M_i^2 N_i}{\sum M_i N_i} = \frac{\sum C_i M_i}{\sum C_i} = \sum w_i M_i,$$

$$d = \frac{M_w}{M_n}$$

$M_i$ : Molecular mass of i-th fraction

$C_i$ : Concentration of i-th fraction

$N_i$ : Number of molecules in i-th fraction

$w_i$ : Mass fraction of i-th fraction  $\left( w_i = \frac{M_i N_i}{\sum M_i N_i} = \frac{C_i}{\sum C_i} \right)$

### 4.3. Molecular mass distribution

As a distribution curve showing molecular mass distribution, an integral molecular mass distribution curve plotting the logarithmic value of molecular mass on the abscissa and the integral value of mass fraction on the ordinate, and a differential molecular mass distribution curve plotting the logarithmic value of molecular mass on the abscissa and the slope of the integral molecular mass distribution curve determined at each molecular mass on the ordinate, are used.

Specifications for molecular mass distribution can be shown in a form depending on the purpose, such as mass average molecular mass, dispersity, mass fractions of molecules in a specific molecular mass range.

## 5. Points to consider on system suitability and changes in operating conditions

Liquid Chromatography <2.01> is applied to points to consider on specifications of system suitability and changes in operating conditions.

# Spectroscopic Methods

## 2.21 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy is based on the phenomenon that specific radio frequency radiation is absorbed by magnetic nuclei in a sample placed in a magnetic field; target nuclei are  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$ , etc. These nuclei have intrinsic spin angular momentum, of which the magnitude is given by  $I(I+1)/h/2\pi$ , where  $I$  is the spin quantum number and is integral or half-integral ( $I = 1/2$  for  $^1\text{H}$  and  $^{13}\text{C}$ ). When the magnetic nuclei are placed in a magnetic field, they are oriented in  $2I + 1$  possible orientations corresponding to  $2I + 1$  equally spaced energy levels (two energy levels for  $^1\text{H}$  and  $^{13}\text{C}$ ). The transition between two successive quantized energy levels corresponding to adjacent orientations can be induced by electromagnetic radiation with a suitable frequency. The precise relation between the field strength and the resonant frequency  $\nu$  is given by

$$\nu = \gamma \cdot \frac{H_0}{2\pi}$$

where  $H_0$  is the strength of the applied external magnetic field and  $\gamma$  is the gyromagnetic ratio, a constant characterizing a particular isotope. The absorption of radiation (NMR signal) can occur only when the irradiating radio frequency satisfies the resonance condition. Since the absorption coefficient (the transition probability) does not depend on the environment in which the nuclei are located, the intensity is basically proportional to the number of nuclei. The excess spins shifted to the higher energy levels by the transition process return to the thermal equilibrium state at various rates determined by a characteristic time constant (known as the relaxation time).

A nucleus is shielded from the applied magnetic field by the electrons belonging to its own atom and to the molecule. Therefore nuclei in different environments are shielded to different extents and resonate at different frequencies. The difference in resonance frequencies is defined as chemical shift ( $\delta$ ), which is independent of the strength of the magnetic field, and is given by

$$\delta = \frac{\nu_s - \nu_r}{\nu_r} + \delta_r$$

where,

- $\nu_s$ : The resonance frequency of the observed signal,
- $\nu_r$ : The resonance frequency of the reference signal,
- $\delta_r$ : The chemical shift of the reference signal (in the case of the value not being 0).

The chemical shifts are normally expressed in ppm, a dimensionless unit, by assuming the chemical shift of the reference compound as 0 ppm. When the chemical shift of the reference compound is not assumed to be 0 ppm, chemical shifts of samples are corrected accordingly.

In addition to the shielding due to electrons, the nucleus is subjected to effects due to the spin orientations of other

magnetic nuclei through chemical bonds, resulting in an additional splitting of the signal. The spacing between two adjacent components of the signal is known as the spin-spin coupling constant ( $J$ ). Coupling constants are measured in hertz and are independent of the strength of the external magnetic field. The increased number of interacting nuclei will make the multiplet pattern more complex.

From the NMR spectrum the following four parameters can be obtained: chemical shift, spin-spin coupling constant, resonance intensity (intensities of  $^1\text{H}$  are proportional to the number of nuclei and those of  $^{13}\text{C}$  and others are susceptible to the nuclear Overhauser effect (NOE) and relaxation) and relaxation time. These parameters are useful for structural determination, identification and quantitative analysis of molecules. Spin decoupling, NOE, and two-dimensional NMR techniques are also available for structural analysis.

### 1. Spectrometer

There are two types of spectrometers.

#### 1.1. Fourier transform NMR (FT-NMR) spectrometers (Fig. 2.21-1)

Target nuclei are simultaneously excited in all frequency range of the nuclei by means of an intense radio frequency pulse. The FID (free induction decay) after the pulse is detected, which is a time domain signal, is converted to a frequency domain spectrum by Fourier transformation. Number of data points suitable for the spectral range, flip angle, acquisition time, delay time and number of scans should be set appropriately.

Recently FT-NMR is commonly used because of its high sensitivity and various advanced applications.

#### 1.2. Continuous wave NMR (CW-NMR) spectrometers (Fig. 2.21-2)

In the case of the CW method, a spectrum is obtained by sweeping the radio frequency or magnetic field continuously over the frequency range of the nuclei being observed.

### 2. Measurement

For NMR measurements, the sensitivity and resolution of the instrument must be adjusted to the optimum levels. The excitation and observation of magnetization are provided by using coils. The coils need the optimization to the Larmor frequency of the targeted nuclear spin called as "Tuning" and sensitivity setting called as "Matching". An additional operation is to apply an electronic current to plural shim coils wrapping the sample and/or a correction current for optimization of resolution. This is necessary for adjusting unevenness of spatial strength in the static magnetic field around the sample. After optimizing the sensitivity and resolution of NMR instrument using ethylbenzene, 1,2-

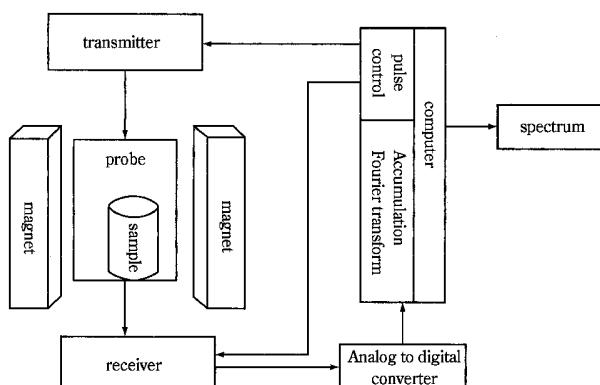
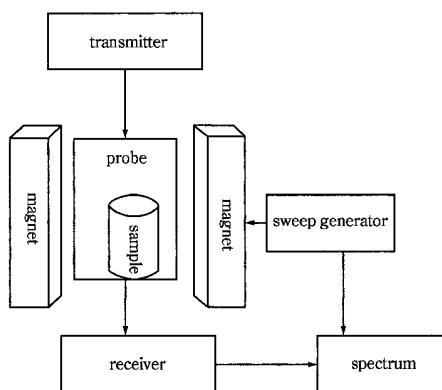


Fig. 2.21-1 FT-NMR spectrometer



**Fig. 2.21-2 CW-NMR spectrometer**

dichlorobenzene, etc. dissolved in an appropriate deuterated NMR solvent, spectrum is usually measured by the following method.

An NMR tube should be prepared for internal reference method (a solution which is the sample and a drop of the reference compound dissolved in a suitable solvent is directly transferred into the NMR tube) or external reference method (a sealed capillary tube containing the reference compound is inserted into the NMR tube with the sample solution). The measurement should be conducted by setting the NMR tube into the NMR probe. The sample solutions should be completely homogeneous. In particular, solid contaminants should be removed in order to obtain good spectra.

Various deuterated NMR solvents are commonly used for NMR measurement and the following points should be considered in selecting an appropriate solvent: (i) The solvent signals do not overlap with the sample signals. (ii) The sample must be soluble in the solvent selected. (iii) The solvent does not react with the sample. Furthermore, it should be noted that chemical shifts can depend upon the solvent employed, sample concentration and deuterium ion concentration, and that viscous solutions usually give rather broad, poorly resolved spectra.

For the reference standards use the reagents for nuclear magnetic resonance spectroscopy. For  $^1\text{H}$  and  $^{13}\text{C}$  spectra, tetramethylsilane (TMS) is usually used as the reference compound for samples dissolved in organic solvents. For samples dissolved in deuterium oxide, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or sodium 3-(trimethylsilyl)propionate (TSP) is used. For other nuclei, nitromethane, trichlorofluoromethane and phosphoric acid are used as reference compounds for  $^{15}\text{N}$ ,  $^{19}\text{F}$  and  $^{31}\text{P}$ , respectively. Furthermore, chemical shifts of residual protons in deuterated solvents and  $^{13}\text{C}$  in the solvent instead of a reference compound can be used for  $^1\text{H}$  and  $^{13}\text{C}$  NMR.

### 3. Record of apparatus and measurement conditions

Type of instrument, frequency, solvent, temperature, sample concentration, reference compound, experimental technique, etc. should be recorded to allow appropriate comparison of spectra, because NMR spectra depend on the measurement conditions.

### 4. Identification

The sample solution is prepared and tested by the method directed in each monograph. Usually in the case of  $^1\text{H}$  NMR, the sample is identified by the following method.

#### 4.1. Identification by the use of chemical shift, signal multiplicity and signal relative intensity

When chemical shifts, multiplicities and relative intensities

of signals are defined, the sample can be identified as being the same substance when all chemical shifts, multiplicities and relative intensities are the same as those prescribed in principle. However, when NMR spectra of the same sample are measured in the different magnitude of magnetic field, it should be noted that the multiplicities of signals sometimes are not identical. This is due to the difference in resolution ability among instruments and the relative relation between the size of spin-spin coupling and the difference in resonance frequency of spin-spin coupled nuclei. Therefore, the multiplicities of signals should be evaluated, considering the magnitude of magnetic field of NMR instrument.

#### 4.2. Identification by the use of a Reference Standard

Measurement conditions should be the same as those used in the case of the Reference Standard. When the spectra of a sample and the Reference Standard exhibit the same multiplicities and relative intensities of signal at the same chemical shifts, the sample can be identified as being the same substance as the Reference Standard.

### 5. Experimental techniques of $^1\text{H}$ and $^{13}\text{C}$ NMR spectroscopy

NMR spectroscopy includes one-, two- and multi-dimensional techniques, which are used for various purposes.

Spin decoupling, and NOE are available in one-dimensional  $^1\text{H}$  NMR spectroscopy. Spin decoupling can assign coupling correlations. NOE (nuclear Overhauser effect) can show correlations among spatially proximal protons, and provide information about configuration or conformation.

In one-dimensional  $^1\text{H}$  NMR spectroscopy, the peak intensity in a fully relaxed spectrum of a compound is directly proportional to the number of  $^1\text{H}$  nuclei, when NMR instrument and experimental conditions are optimized for quantitative analysis. Accordingly, high-reliable purity, content etc. based on molar amount (mol) can be determined by using the internal reference materials having traceability to International System of Units (SI). This measurement method is called quantitative  $^1\text{H}$  NMR.

Broadband decoupling, INEPT and DEPT are usually applied in one-dimensional  $^{13}\text{C}$  spectroscopy. The broadband decoupling technique simplifies a spectrum and achieves enhancement of sensitivity. INEPT (insensitive nuclei enhanced by polarization transfer) and DEPT (distortionless enhancement of polarization transfer) enhance the sensitivity of  $^{13}\text{C}$  by means of polarization transfer from directly bonded  $^1\text{H}$  with a large magnetic moment. They can be applied to identify primary, secondary, tertiary or quaternary carbon.

Two-dimensional spectroscopy can observe all correlation peaks between nuclei through spin-spin coupling or NOE in a single experiment, and there are many techniques for homonuclear and heteronuclear measurements. Representative techniques are described below.

(i) COSY (2D correlation spectroscopy), TOCSY (total correlation spectroscopy), HOHAHA (homonuclear Hartmann-Hahn spectroscopy): Correlation between protons through scalar spin-spin coupling is obtained and intramolecular connectivities of hydrogen atoms are revealed.

(ii) NOESY (2D nuclear Overhauser enhancement and exchange spectroscopy): NOE is measured by a two-dimensional technique. Approximate distances between spatially proximate hydrogen atoms are obtained to analyze the three-dimensional structure.

(iii) INADEQUATE (incredible natural abundance double quantum transfer experiment): Although this technique is insensitive because it involves double quantum transfer by  $^{13}\text{C}$ - $^{13}\text{C}$  scalar coupling in a sample with natural isotopic abundance, the connectivities of all neighboring  $^{13}\text{C}$  nuclei

can be obtained to analyze the carbon skeleton.

(iv) HMQC (heteronuclear multiple quantum coherence): This technique observes correlations between  $^1\text{H}$  and  $^{13}\text{C}$  with direct spin-spin coupling using  $^1\text{H}$  detection and reveals intramolecular chemical bonds between hydrogen and carbon atoms.

(v) HMBC (heteronuclear multiple bond connectivity): This technique observes correlations between  $^1\text{H}$  and  $^{13}\text{C}$  with long range spin-spin coupling using  $^1\text{H}$  detection and reveals intramolecular connectivities of hydrogen and carbon atoms.

There are many other techniques such as 2D J-resolved spectroscopy, DQF-COSY (double quantum filtered COSY), HSQC (heteronuclear single quantum coherence) and DOSY (diffusion ordered spectroscopy). Furthermore, multidimensional NMR techniques are used to analyze macromolecules.

## 2.22 Fluorometry

Fluorometry is a method to measure the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. This method is also applied to the phosphorescent substances.

Fluorescence intensity  $F$  in a dilute solution is proportional to the concentration  $c$  in mol per liter of the solution and the pathlength  $l$  of light through the solution in centimeter.

$$F = kI_0\phi ecl$$

$k$ : Constant

$I_0$ : Intensity of exciting light

$\phi$ : Quantum yield of fluorescence or phosphorescence

Quantum yield of fluorescence or phosphorescence  
 $= \frac{\text{number of quanta as fluorescence or phosphorescence}}{\text{number of quanta absorbed}}$

$\epsilon$ : Molar extinction coefficient of the substance at the excitation wavelength

### 1. Apparatus

Spectrofluorometer is usually used. Generally, a xenon lamp, laser, an alkaline halide lamp, etc. which provide stable exciting light are used as the light source. Usually, a non-fluorescent quartz cell ( $1\text{ cm} \times 1\text{ cm}$ ) with four transparent sides is used as the container for sample solution.

### 2. Procedure

Excitation spectrum is obtained by measuring fluorescence intensities of sample solution with varying excitation wavelengths at a fixed emission wavelength (in the vicinity of the fluorescence maximum) and drawing a curve showing the relationship between the excitation wavelength and the fluorescence intensity. Fluorescence spectrum is obtained by measuring fluorescence intensities of sample solution with varying emission wavelengths at a fixed excitation wavelength (in the vicinity of the excitation maximum) and drawing the same curve as described for the excitation spectrum. If necessary, the spectra are corrected with regard to the optical characteristics of the apparatus.

The fluorescence intensity is usually measured at the excitation and the emission wavelengths in the vicinity of excitation and emission maxima of the fluorescent substance. The fluorescence intensity is expressed as a value relative to that of a standard solution, because it is readily affected even by a slight change in the condition for the measurement.

Unless otherwise specified, the instrument is operated as follows with standard, sample, and reference solutions prepared as directed in the monograph: Fix the excitation and fluorescence wavelength scales at the designated positions, adjust the dark current to zero, put the quartz cell containing the standard solution in the light path, and adjust the instrument so that the standard solution shows the fluorescence intensity of 60% to 80% of full scale. Then perform the measurements with the cells containing the sample solution and the control solution, and read the fluorescence intensity as % under the same condition. Set the width of the wavelength properly unless otherwise specified.

### 3. Note

The fluorescence intensity is readily affected by the concentration, temperature and pH of the solution, and nature and purity of solvents or reagents used.

## 2.23 Atomic Absorption Spectrophotometry

Atomic Absorption Spectrophotometry is a method to determine the amount or the concentration of an element in a sample specimen being examined, by utilizing the phenomenon that atoms being in the ground state absorb the light of specific wavelength, characteristic of the respective atom, when the light passes through an atomic vapor layer of the element to be determined.

### 1. Apparatus

Usually, the apparatus consists of a light source, a sample atomizer, a spectroscope, a photometer and a recording system. Some are equipped with a background compensation system. As a light source, usually a hollow cathode lamp specified for each element is used and sometimes a discharge lamp is also used. There are three types of sample atomizer: the flame type, the electrothermal type, and the cold-vapor type. The first one is composed of a burner and a gas-flow regulator, the second one is composed of an electric furnace and a power source, and the third one is composed of a mercury generator and an absorption cell. The third one is further classified into two subtypes, which differ in the atomizing method for mercury containing-compounds: one utilizes chemical reduction-vaporization and the other utilizes a thermal reduction-vaporization method.

For the selection of an appropriate analytical wavelength in a spectroscope, a grating for light diffraction or an interference filter can be used. A recording system is composed of a display and a recording device. A background compensation system is employed for the correction of atmospheric effects on the measuring system. Several principles can be utilized for background compensation, using continuous spectrum sources, the Zeeman splitted spectrum, the non-resonance spectrum, or self-inversion phenomena.

Another special options such as a hydride generator and a heating cell, can also be used for analyzing such as selenium. As a hydride generator, a batch method and/or a continuous flow method can be applied. While as a heating cell, there are two kinds of cell: one for heating by flame and the other for heating by electric furnace.

### 2. Procedure

Unless otherwise specified, proceed by any of the following methods.

#### 2.1. Flame type

Fit the specific light source to the lamp housing and switch

on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and slit-width. Next, a mixture of a combustible gas and a supporting gas is ignited and the gas flow rate and/or pressure should be adjusted to optimum conditions. The zero adjustment of the detecting system must be done through nebulizing the blank solvent into the flame. After setting up the measuring system, the sample solution prepared by the specified procedure is introduced into the flame and the light absorption at the characteristic wavelength of the element to be determined is measured.

### 2.2. Electrothermal type

Fit the specific light source to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and slit-width. Further, set an electric furnace to the appropriate temperature, electric current, and heating program, as directed separately in the monograph. When a suitable amount of sample is injected into the heated furnace with an appropriate stream of inert gas, the sample is dried and ashed, simultaneously with atomization of the metallic compound included in the specimen. The atomic absorption specified is observed and the intensity of absorption is measured. Details of the sample preparation method are provided separately in the monograph.

### 2.3. Cold vapor type

Fit the mercury lamp to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and a slit-width. In the chemical atomization-vaporization method, a mercury containing compound in the sample solution, prepared by the specified procedure, is chemically reduced to metallic mercury by adding a proper reducing reagent to the closed vessel and the generated mercury is vaporized and introduced into the absorption cell with a flow of inert gas. In the thermal atomization-vaporization method, the sample specimen on a quartz dish is heated electrically and the generated atomic mercury is vaporized and introduced into the absorption cell with a flow of inert gas. Thus, in both methods, the generated atomic mercury is carried into the absorption cell as cold vapor and the intensity of the characteristic atomic absorption of mercury is measured.

## 3. Determination

Usually, proceed by any of the following methods. In the determination, the possibility of interference for various reasons and the background effect must be considered and avoided if possible.

### 3.1. Calibration curve method

Prepare standard solutions at more than 3 concentration levels, measure the specific absorption due to these standard solutions, and prepare the calibration curve of the atomic absorption against the concentration. Then measure the atomic absorption due to the sample specimen, in which the concentration of the element to be determined should be adjusted to be within the concentration range of the standard solutions, and determine the amount or the concentration of the element to be examined using the calibration curve.

### 3.2. Standard addition method

To equal volumes of more than 3 sample solutions, prepared as directed in the monograph, add a measured quantity of the standard solutions to produce a series of solutions containing increasing amounts of the element to be examined, and further add a solvent to make up a constant volume. Measure the atomic absorption for the respective

solutions, and plot the obtained values on a graph with the added amount or the concentration on the abscissa and the absorbance on the ordinate. Extrapolate the linear plot obtained by linking the data points, and determine the amount or the concentration of the element to be examined from the distance between the origin and the point where the plot intersects with the abscissa. This method is available only when the calibration curve obtained by Method (1) is confirmed to be linear and to pass through the origin.

### 3.3. Internal standard method

Prepare a series of standard solutions of the element to be determined, each containing a definite amount of the internal standard element directed in the monograph. For these standard solutions, measure the atomic absorption due to the standard element and the internal standard element separately at the respective wavelengths under the same operating conditions, and obtain the ratio of absorbance by the standard element to that by the internal standard element. Prepare a calibration curve for the element to be determined, with the amount or the concentration of the standard element on the abscissa and the above-mentioned ratio of the absorbance on the ordinate. Then prepare sample solutions, adding the same amount of the internal standard element as contained in the standard solutions. Measure the ratio of the absorbance due to the element to be determined to that due to the internal standard element under the same conditions as employed for preparing the calibration curve, and determine the amount or the concentration of the element being examined by using the calibration curve.

## 4. Note

Reagents, test solutions, and gases used in this test should not interfere in any process of the measurement.

## 2.24 Ultraviolet-visible Spectrophotometry

Ultraviolet-visible Spectrophotometry is a method to measure the degree of absorption of light between the wavelengths of 200 nm and 800 nm by substances for the tests of their identity and purity, and for assay. When an atomic absorption spectrophotometer is used for these purposes, proceed as directed under Atomic Absorption Spectrophotometry <2.23>. When monochromatic light passes through a substance in the solution, the ratio of transmitted light intensity  $I$  to incident light intensity  $I_0$  is called transmittance  $t$ ; transmittance expressed in the percentage is called percent transmission  $T$ , and common logarithm of the reciprocal of transmittance is called absorbance  $A$ .

$$t = \frac{I}{I_0} \quad T = \frac{I}{I_0} \times 100 = 100t \quad A = \log \frac{I_0}{I}$$

The absorbance  $A$  is proportional to the concentration  $c$  of a substance in the solution and the length  $l$  of the layer of the solution through which light passes.

$$A = kcl \quad (k: \text{constant})$$

The constant, calculated on the basis that  $l$  is 1 cm and  $c$  is 1 mol/L, is called molar absorption coefficient  $\varepsilon$ . The molar absorption coefficient at the wavelength of maximum absorption is expressed as  $\varepsilon_{\max}$ .

When a light beam passes through a substance in the solution, the absorbance by the sample differs depending on the wavelength of the light. So, an absorption spectrum is obtained by determining the absorbances of a light beam at

various wavelengths and by graphically plotting the relation between absorbance and wavelength. From the absorption spectrum, it is possible to determine the wavelength of maximum absorption  $\lambda_{\max}$  and that of minimum absorption  $\lambda_{\min}$ .

The absorption spectrum of a substance in the solution is characteristic, depending on its chemical structure. Therefore, it is possible to identify a substance by comparing the spectrum of a sample within the specified wavelength range with the Reference Spectrum or the spectrum of Reference Standard, by determining the wavelengths of maximum absorption, or by measuring the ratio of absorbances at two specified wavelengths. For the purpose of assay, the absorbance by a sample solution with a certain concentration is measured at the wavelength of the maximum absorption  $\lambda_{\max}$  and compared it with the absorbance of a standard solution with a certain concentration.

### 1. Apparatus and adjustment

A spectrophotometer or a photoelectric photometer is used for the measurement of absorbance.

After adjusting the spectrophotometer or photoelectric photometer based on the operation manual of the apparatus, it should be confirmed that the wavelength and the transmission rate meet the specifications of the tests described below.

The calibration of wavelength should be carried out as follows. Using an optical filter for wavelength calibration, measure the transmission rate in the vicinity of the standard wavelength value shown in the test results form, under the test conditions given in the test results form attached to each of the filters. When performing a test to determine the wavelength which shows minimal transmission rate, the difference between the measured wavelength and the standard wavelength value should be within  $\pm 0.5$  nm. When the measurement is repeated three times, each value obtained should be within the mean  $\pm 0.2$  nm. It is also possible to carry out the test using a deuterium discharge lamp at bright line wavelengths of 486.00 nm and 656.10 nm. In the case of these tests, the difference between the measured wavelength and the wavelength of the bright line should be within  $\pm 0.3$  nm. When the measurement is repeated three times, each value obtained should be within the mean  $\pm 0.2$  nm.

The calibration of transmission rate or absorbance should be carried out as follows. Using an optical filter for transmission rate calibration, determine the transmission rate at the standard wavelength value under the test conditions given in the test results form attached to each of the filters. The difference between the measured transmission rate and the standard transmission rate value should be within the range of from 1% larger of the upper limit to 1% smaller of the lower limit for the relative accuracy shown in the test results form. When the measurement is repeated three times, each absorbance obtained (or calculated from the transmission rate) should be within the mean  $\pm 0.002$  when the absorbance is not more than 0.500, and within the mean  $\pm 0.004$  when the absorbance is more than 0.500. In addition, it will be desirable to confirm the linearity of transmission rate at the same wavelength using several optical filters for calibration of transmission rate with different transmission rates.

### 2. Procedure

After adjusting the apparatus as directed in the Apparatus and adjustment, select and set the light source, detector, mode of measurement, measuring wavelength or wavelength range, spectrum width and scanning speed.

Subsequently, allow the apparatus to stand for a certain time to confirm its stability. Then, usually adjust the apparatus so that the transmittance is 0% at measuring wave-

length or over measuring wavelength range after shutting the sample side of light path. Then open the shutter and adjust the transmittance to 100% (the absorbance is zero). Adjusting the transmittance to 100% is usually done by putting cells containing the control solution in both light paths. For the control solution, unless otherwise specified, blank solvent is used.

Then perform the measurement with the cell containing the sample solution, and read the absorbance at measuring wavelength, or measure the spectrum over measuring wavelength range. Unless otherwise specified, a cell with a path length of 1 cm, made of quartz for ultraviolet range and of quartz or glass for visible range, is used. Special consideration is needed with the absorption of solvents in the ultraviolet range; use a solvent which does not disturb accurate measurement.

### 3. Specific absorbance

In the Japanese Pharmacopoeia, the absorbance, calculated on the basis that  $l$  is 1 cm and  $c$  (concentration of a medicament) is 1 w/v%, is called specific absorbance, and is expressed as  $E_{1\text{cm}}^{1\%}$ .

$$E_{1\text{cm}}^{1\%} = \frac{A}{c \times l}$$

$l$ : Length of the layer of the solution (cm)

$A$ : Absorbance value

$c$ : Concentration of the sample in the solution (w/v%)

The description of, for example, “ $E_{1\text{cm}}^{1\%}$  (241 nm): 500 – 530 (after drying, 2 mg, methanol, 200 mL)” in the monograph, indicates that observed  $E_{1\text{cm}}^{1\%}$  value is between 500 and 530, when the test is performed in the following manner: The sample is dried under the conditions specified in the Test for Loss on Drying, and about 2 mg of the sample is weighed accurately with a microbalance, and dissolved in methanol to make exactly 200 mL, then the absorbance of the solution is measured as directed in the Procedure at a wavelength of 241 nm using a cell with a path length of 1 cm.

### 4. Identification

Prepare the sample solution as directed in the monograph, and test as directed in the Procedure. Usually, the test is performed by a single method or in a combination of a few methods in the following methods using the absorbance or absorption spectrum obtained from the sample solution. Subtle differences in the absorption spectrum arising from differences in the apparatus used may be neglected.

#### 4.1. Identification using Reference Spectrum

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the Reference Spectrum, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

Reference spectrum: Reference spectra are specified under the Ultraviolet-visible Reference Spectra, which are used as the reference for the test of identification specified in the monograph.

#### 4.2. Identification using Reference Standard

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the spectrum obtained from the Reference Standard, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum. When the relevant Reference Spectrum is not available, the range is that specified in the monograph.

#### 4.3. Identification using absorption wavelength

When maximum absorption wavelengths of the spectrum obtained from the sample solution match the wavelengths specified in the monograph, the identity of the substance may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

#### 4.4. Identification using the ratio of the absorbances obtained at two or more wavelengths

When the ratios of absorbances at the specified wavelengths in the spectrum obtained from the sample solution meet the specifications in the monograph, the identity of the substance may be confirmed.

#### 5. Assay

Prepare the control solution, the sample solution and the standard solution as directed in the monograph, measure the absorbances of the sample solution and the standard solution according to the method described in the Procedure, and determine the amount of the substance to be assayed in the sample by comparing the absorbances.

## 2.25 Infrared Spectrophotometry

Infrared Spectrophotometry is a method of measurement of the extent, at various wave numbers, of absorption of infrared radiation when it passes through a layer of a substance. In the graphic representation of infrared spectra, the plot usually shows units of wave numbers as the abscissa and units of transmittance or absorbance as the ordinate. Wave number and transmittance or absorbance at each absorption maximum may be read graphically on an absorption spectrum and/or obtained by a data-processor. Since the wave number and the respective intensity of an absorption maximum depend on the chemical structure of a substance, this measurement can be used to identify or determine a substance.

#### 1. Instrument and adjustment

Several models of dispersive infrared spectrophotometers or Fourier-transform infrared spectrophotometers are available.

The instruments, adjusted according to the instruction manual of each individual instrument, should comply with the following test for resolving power, transmittance reproducibility and wave number reproducibility. When the spectrum of a polystyrene film about 0.04 mm thick is recorded, the depth of the trough from the maximum absorption at about  $2850\text{ cm}^{-1}$  to the minimum at about  $2870\text{ cm}^{-1}$  should be not less than 18% transmittance and that from the maximum at about  $1583\text{ cm}^{-1}$  to the minimum at about  $1589\text{ cm}^{-1}$  should be not less than 12% transmittance.

The wave number ( $\text{cm}^{-1}$ ) scale is usually calibrated by the use of several characteristic absorption wave numbers ( $\text{cm}^{-1}$ ) of a polystyrene film shown below. The number in parentheses indicates the permissible range.

3060.0 ( $\pm 1.5$ )	2849.5 ( $\pm 1.5$ )	1942.9 ( $\pm 1.5$ )
1601.2 ( $\pm 1.0$ )	1583.0 ( $\pm 1.0$ )	1154.5 ( $\pm 1.0$ )
1028.3 ( $\pm 1.0$ )		

When the dispersive infrared spectrophotometer is used, the permissible range of the absorption wave numbers at  $1601.2\text{ cm}^{-1}$  and at  $1028.3\text{ cm}^{-1}$  should be both within  $\pm 2.0\text{ cm}^{-1}$ .

As the repeatability of transmittance and wave number, the difference of transmittance should be within 0.5% when

the spectrum of a polystyrene film is measured twice at several wave numbers from  $3000$  to  $1000\text{ cm}^{-1}$ , and the difference of wave number should be within  $5\text{ cm}^{-1}$  at about  $3000\text{ cm}^{-1}$  and within  $1\text{ cm}^{-1}$  at about  $1000\text{ cm}^{-1}$ .

#### 2. Preparation of samples and measurement

Unless otherwise specified, when it is directed to perform the test "after drying the sample", use a sample dried under the conditions specified in the monograph. Prepare the specimen for the measurement according to one of the following procedures. Because the amount of specimen or mixture described is as an example and that depends on the measurement conditions, prepare it so that the transmittance of most of the absorption bands is in the range of 5% to 80%. If the sample is a salt it should be noted that the salt exchange can be occurred between added potassium bromide or potassium chloride. As a general rule in the disk method or the diffuse reflectance method, potassium chloride is used for a hydrochloride sample. For other salts, correspondence such as to try the paste method is needed.

Single crystals of sodium chloride, potassium bromide, etc. are available for the optical plate.

Generally, the reference cell or material is placed in the reference beam for double-beam instruments, while for single-beam instruments, it is placed in the same optical path in place of the specimen and measured separately under the same operating conditions. The composition and preparation of the reference depend on the sample preparation methods, and sometimes the background absorption of the atmosphere can be utilized.

Unless otherwise specified in the monograph, the spectrum is usually recorded between  $4000\text{ cm}^{-1}$  and  $400\text{ cm}^{-1}$ . The spectrum should be scanned using the same instrumental conditions as were used to ensure compliance with the requirements for the resolving power and for the precision of wave number scale and of wave numbers.

#### 2.1. Potassium bromide disk or potassium chloride disk method

Powder 1 to 2 mg of a solid sample in an agate mortar, triturate rapidly with 0.10 to 0.20 g of potassium bromide for infrared spectrophotometry or potassium chloride for infrared spectrophotometry with precautions against moisture absorption, and compress the mixture with a press in a suitable die (disk-forming container) to make the sample disk. Adjust the amount of sample, potassium bromide or potassium chloride according to the size of the disk. Prepare a potassium bromide reference disk or a potassium chloride reference disk in the same manner as the sample disk. If necessary to obtain a transparent disk, press the mixture under reduced pressure not exceeding 0.67 kPa in a die with pressure applied to the die of 50 to 100 kN (5000 – 10,000 kg) per  $\text{cm}^2$  for 5 to 8 minutes.

#### 2.2. Solution method

Place the sample solution prepared by the method directed in each monograph in a fixed cell for liquid, and usually measure the spectrum against the reference solvent used for preparing the sample solution. The solvent used in this method should not show any interaction or chemical reaction with the specimen to be examined and should not damage the optical plate. The thickness of the fixed cell is usually 0.1 mm or 0.5 mm.

#### 2.3. Paste method

Powder 5 to 10 mg of a solid specimen in an agate mortar, and, unless otherwise specified, triturate the specimen with 1 to 2 drops of liquid paraffin to give a homogeneous paste. After spreading the paste to make a thin film in the center of an optical plate, place the plate upon another optical plate

with precautions against intrusion of air, bubbles in the film, and examine its absorption spectrum.

#### 2.4. Liquid film method

Examine 1 to 2 drops of a liquid specimen as a thin film held between two optical plates. When the absorption intensity is not sufficient, place spacers of aluminum foil, etc., between the two optical plates to make a thicker liquid film.

#### 2.5. Film method

Examine a thin film just as it is or a prepared thin film as directed in each monograph.

#### 2.6. Gas sampling method

Put a sample gas in a gas cell previously evacuated under the pressure directed in the monograph, and examine its absorption spectrum. The path length of the gas cell is usually 5 cm or 10 cm, but, if necessary, may exceed 1 m.

#### 2.7. ATR method

Place a specimen in close contact with an attenuated total reflectance (ATR) prism, and examine its reflectance spectrum.

#### 2.8. Diffuse reflectance method

Powder 1 to 3 mg of a solid specimen into a fine powder of not more than about 50  $\mu\text{m}$  particle size in an agate mortar, and triturate rapidly with 0.05 to 0.10 g of potassium bromide for infrared spectrophotometry or potassium chloride for infrared spectrophotometry with precautions against moisture absorption. Place the mixture in a sample cup, and examine its reflectance spectrum.

### 3. Identification

When the spectrum of a specimen and the Reference Spectrum of the substance expected to be found or the spectrum of the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the substance expected to be found. Furthermore, when several specific absorption wave numbers are specified in the monograph, the identification of a specimen with the substance expected to be found can be confirmed by the appearance of absorption bands at the specified wave numbers.

#### 3.1. Identification by the use of a Reference Standard

When the spectra of a specimen and the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance as the Reference Standard. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with that of the Reference Standard, treat the specimen being examined and the Reference Standard in the same manner as directed in the monograph, then repeat the measurement.

#### 3.2. Identification by the use of a Reference Spectrum

When the spectra of a specimen and the Reference Spectrum exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance associated with the Reference Spectrum. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with the Reference Spectrum, treat the specimen being examined as directed in the monograph, then repeat the measurement. Infrared Reference Spectra, in the range between 4000  $\text{cm}^{-1}$  and 400  $\text{cm}^{-1}$ , are shown in the section "Infrared Reference Spectra" for the monographs requiring the identification test by Infrared Spectrophotometry, except for monographs in which "Identification by absorption wave number" is specified.

#### 3.3. Identification by the use of absorption wave number

When several specific absorption wave numbers of the substance being examined are specified in the monograph, a

specimen can be identified as being the same substance as the expected substance by confirmation of clear appearance of the absorption bands at all the specified wave numbers.

## 2.26 Raman Spectroscopy

Raman spectroscopy is a vibrational spectroscopic technique, which evaluates a sample to be examined qualitatively or quantitatively by analyzing a spectrum obtained by dispersing very weak scattered light, having different wavelengths from irradiation light, generated when the sample is irradiated with the light. Raman scattering is observed when the polarizability of molecules changes with the vibration of chemical bonds of molecules in a sample.

Raman spectroscopy generally uses monochromatic laser light as excitation light. When the laser light is irradiated to the sample to be examined, the molecules in the sample are excited and the light with the same wavelength of the irradiation light, known as Rayleigh scattering, is scattered. The scattered light detected in the shorter wavelength side than the Rayleigh scattering is referred to as anti-Stokes scattering. The scattered light detected in the longer wavelength side than the Rayleigh scattering is referred to as Stokes scattering. Generally Stokes scattering with strong Raman scattering intensity is used for analysis. Raman spectra are usually indicated by Raman shift on the horizontal axis and Raman scattering intensity on the vertical axis.

Raman spectroscopy is capable of measuring samples (solid, semi-solid, liquid, gas, etc.) rapidly and non-destructively without pre-treatment. Application of Raman spectroscopy in the pharmaceutical field includes qualitative or quantitative evaluation of the active pharmaceutical ingredients and additives in drug substances or drug products. Raman spectroscopy can also be used for the evaluation of the physical conditions of substances, such as crystal form and crystallinity. Raman micro-spectroscopy can also be used for the evaluation of the distributions of active pharmaceutical ingredients and additives in the drug products. Furthermore, using an optical fiber probe enables it to measure the spectra of samples at a location remote from the equipment body without sampling, so that it can be used to perform pharmaceutical manufacturing process control online (or in-line).

#### 1. Apparatus

Raman spectrometers are composed of a light source unit, a sample unit, a spectrometry unit, a detector unit, a signal processing unit, a data processing unit and a display-record-output unit. Raman spectrometers are classified into dispersive Raman spectrometers and Fourier transform Raman spectrometers according to their spectroscopic systems.

##### 1.1. Light source

The laser which stably emits monochromatic light as excitation light to samples is used for the light source. The lasers include gas lasers such as a He-Ne laser and solid state lasers, and select a laser with wavelengths and output power according to the purpose. Pay attention to safety standards relating to a laser, when this test is performed.

##### 1.2. Sample unit

The sample unit is composed of an optical system for collecting Raman scattering light generated by irradiation of excitation light and a sample cell. Combination of these take the form of a sample chamber, while there are apparatuses with no sample chamber, such as optical fiber probes and portable Raman spectrometers that can be carried. Representative sample chambers are macroscopic sample

chambers and microscopic sample chambers. The components of these optical systems are different, respectively.

### 1.3. Monochromator and detector

Many dispersive Raman spectrometers use an optical filter to eliminate excitation light and use a single monochromator combined with a multichannel detector, since the configuration is simple and high sensitivity can be obtained. Detectors include multiple elements detectors and single element detectors, and general dispersive Raman spectrometers use a multiple elements detector such as a CCD detector.

Fourier transform (FT) Raman spectrometers obtain spectra by Fourier transformation of interference waveforms using an interferometer. FT-Raman spectrometers are mainly used for near infrared Raman measurement.

## 2. Methods Used for Measurement

The Raman spectroscopy is applicable to solid samples having a complicated shape in addition to gas/solution samples inside a glass sample cell being transparent in the visible region, using mainly light in the visible region as excitation light. In the view of the size of a measurement region and Raman scattering efficiency, an optimum optical system is selected according to the sample. The excitation wavelength, the measurement mode of the apparatus, etc. are selected and set.

### 2.1. Macroscopic measurement

Since the macroscopic sample chamber has a high degree of freedom in the scattering configuration, samples can be measured irrespective of solid, liquid, gas, size and shape. It is also applicable to Raman measurement under low temperature, high temperature and high pressure which require the setting of a large sample cell. Usually, in the macroscopic sample chamber three configurations: forward scattering (transmission), 90° scattering and back scattering configurations, can be usable and an appropriate scattering configuration can be selected depending on a sample.

### 2.2. Microscopic measurement

The microscopic sample chamber is based on an optical microscope and applicable to local analysis. In the optical system of the microscopic sample chamber a microscope objective lens works simultaneously as an excitation light converging lens and a Raman scattering light condensing lens.

Mapping measurement repeats local measurements by moving a sample or laser light position to generate a Raman image showing the two or three dimensional distribution of Raman scattering intensity. Raman images are made by using various spectral information such as a ratio of the intensity of two bands.

### 2.3. Probe measurement

The optical fiber probe is the collective term of the apparatus of which sample section is separated from a Raman spectrometer body by using an optical fiber and is applicable to *in situ* measurement and on-line (or in-line) measurement.

### 2.4. Measurement by portable apparatus

The portable Raman spectrometer is possible to carry and perform analysis using Raman spectroscopy outside of laboratory. Main application of this apparatus is judgement on acceptance of pharmaceutical materials. It is used for rather simple measurement.

### 2.5. Points to note in measurement

Note the following points for solid, liquid and suspended samples.

(i) Measurement of solid sample: There is a possibility that the filling status, the difference in the particle diameter and the roughness of the surface of the sample could affect the scattering intensity. When measuring a crystalline sample, be careful about the effect of crystal shape. There is also

a possibility that the light transparency of the sample affect the spectrum intensity. When a sample is physically and chemically inhomogeneous, it might be recommended to enlarge the spot size of laser irradiation, measure plural samples, measure the plural points of the same sample or crush the sample to homogenize.

(ii) Measurement of liquid sample: It is possible to subtract the spectrum of the solvent if there is no interaction between the solvent and the sample. When there are insoluble matters in solution, remove the matters using a filter before measurement not to obtain the Raman scattering of the matters. When a sample shows high reactivity by laser irradiation in solution, measure the sample by stirring carefully not to irradiate the same place.

(iii) Measurement of suspended sample: A suspended sample may settle, so be careful about the positioning of laser irradiation. For samples that are prone to settle, devising measurement such as optimizing the irradiation time and stirring might be helpful. When the Raman scattering of a suspended sample is weak, it is also possible to subtract the spectrum of the solvent likewise the case of measurement of a liquid sample.

## 3. Factors that affect spectrum

When Raman spectroscopy is applied, note the following items as factors affecting spectra.

### 3.1. Temperature of sample

Sample heating by laser irradiation can cause a variety of effects, such as physical form change (melting and burning) and polymorph transform. Since the chance of sample heating is increased when the spot size of laser irradiation at a sample is squeezed, be careful not to damage the sample when microscopic measurement is carried out. To prevent the sample overheating, a variety of methods can be employed such as suppressing laser output, irradiating a laser without focusing and cooling a sample.

### 3.2. Sample characteristics

Since Raman signals are very weak, the fluorescence of a sample itself and minute impurities may interfere with Raman scattering light. Fluorescence can be reduced by choosing an excitation light source with a longer wavelength, however it should be noted that it generally decreases the intensity of the Raman scattering. Photobleaching resulted by laser irradiation before measurement, appropriate irradiation time and accumulation count may mitigate the fluorescence.

When measuring a colored sample, select the wavelength of an excitation laser depending on the absorption characteristics of the sample. When measuring a sample in a container such as, a cell for measurement, a bag or a bottle, take careful note of the spectral characteristics derived from the container in addition to the sample.

## 4. Control of apparatus performance

Estimate the accuracy of the wave number of Raman shift after adjusting a Raman spectrometer. Measure Raman spectra using an excitation laser utilized for actual measurement and an appropriate standard substance. Polystyrene is an example.

In the cases of 2.1., 2.2. and 2.3., make correction using at least three wave numbers among the below peak wave numbers ( $\text{cm}^{-1}$ ) obtained from the spectrum of polystyrene. The number in parentheses indicates the permissible range.

620.9 ( $\pm 1.5$ )

1001.4 ( $\pm 1.5$ )

1031.8 ( $\pm 1.5$ )

1602.3 ( $\pm 1.5$ )

3054.3 ( $\pm 3.0$ ) (Note: 3054.3  $\text{cm}^{-1}$  cannot be measured)

depending on an excitation wavelength.)

In the case of 2.4., make correction in the same manner using at least three wave numbers among the below peak wave numbers ( $\text{cm}^{-1}$ ).

620.9 ( $\pm$  2.5)

1001.4 ( $\pm$  2.0)

1031.8 ( $\pm$  2.0)

1602.3 ( $\pm$  3.0)

Other substance such as cyclohexane can be used as a standard substance, if it is validated.

## 5. Qualitative and quantitative analysis

### 5.1. Qualitative analysis

As Raman spectroscopy observe the vibrational energy of a molecule and can obtain a characteristic spectrum depending on the structure of a substance to be analyzed, qualitative analysis based on chemical structural information can be performed.

When the Raman spectra of a sample and the Reference Standard of a substance to be identified are compared and both spectra exhibit similar scattering intensities at the same Raman shifts, the identity of those can be confirmed.

When a sample treatment method for a solid sample is indicated in the monograph in the case of nonconformity of the scattering spectrum with that of the Reference Standard, treat the sample and the Reference Standard under the condition as directed in the monograph, then repeat the measurement.

When the characteristic scattering wave numbers of a substance to be identified are specified in the monograph, the clear appearance of the scattering of a sample at all the specified scattering wave numbers can confirm the identity of the sample with the substance to be identified.

Raman spectroscopy is also applicable to the process control of drug substances or drug products by using a score obtained from a Raman spectrum by a chemometric methodology such as principal component analysis, and characteristic peak wave numbers of the substance to be examined, as indices. Chemometrics usually means mathematical technique and statistic technique for quantization and informatization of chemical data.

### 5.2. Quantitative analysis

The concentrations of components of a sample to be analyzed can be calculated by calibration curves plotting the relationship between scattering intensity at a specified wave number and concentration.

In the case where the composition of components of a sample is complicated, the concentrations of components in the sample can also be calculated by developing a calibration model about a spectrum measured using an existing standard sample by a chemometric methodology and applying the model to the spectrum of the sample to be examined. Chemometric methodologies for obtaining a calibration model include multiple regression analysis method and PLS (Partial least squares) regression analysis method.

The variation of peak intensity at around the reference values of wave numbers using a standard sample, polystyrene etc. used in 4., is preferable to be within  $\pm$  10% compared to that obtained in the last measurement.

## Other Physical Methods

### 2.41 Loss on Drying Test

Loss on Drying Test is a method to measure the loss in mass of the sample, when dried under the conditions specified in each monograph. This method is applied to determine the amount of water, all or a part of water of crystallization, or volatile matter in the sample, which is removed during the drying.

The description, for example, “not more than 1.0% (1 g, 105°C, 4 hours)” in a monograph, indicates that the loss in mass is not more than 10 mg per 1 g of the substance in the test in which about 1 g of the substance is accurately weighed and dried at 105°C for 4 hours, and “not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours),” indicates that the loss in mass is not more than 5 mg per 1 g of the substance in the test in which about 1 g of the substance is accurately weighed, transferred into a desiccator (phosphorus (V) oxide), and dried in vacuum for 4 hours.

#### 1. Procedure

Weigh accurately a weighing bottle that has been dried for 30 minutes according to the method specified in the monograph. Take the sample within the range of  $\pm$  10% of the amount directed in the monograph, transfer into the weighing bottle, and, unless otherwise specified, spread the sample so that the layer is not thicker than 5 mm, then weigh it accurately. Place the loaded bottle in a drying chamber, and dry under the conditions specified in the monograph. When the size of the sample is large, convert it to small particles having a size not larger than 2 mm in diameter by quick crushing, and use the crushed sample for the test. After drying, remove from the drying chamber, and reweigh accurately. When the sample is dried by heating, the temperature is within the range of  $\pm$  2°C of that directed in the monograph, and, after drying the bottle, the sample is allowed to cool in a desiccator (silica gel) before weighing.

If the sample melts at a temperature lower than that specified in the monograph, expose the sample for 1 to 2 hours to a temperature between 5°C and 10°C below the melting temperature, dry under the conditions specified in the monograph. Use a desiccant specified in the monograph, and renew frequently.

### 2.42 Congealing Point Determination

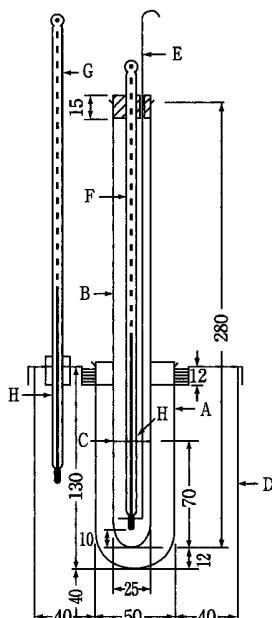
The congealing point is the temperature measured by the following method.

#### 1. Apparatus

Use the apparatus illustrated in Fig. 2.42-1.

#### 2. Procedure

Transfer the sample into sample container B up to the marked line C. When the sample is solid, melt the sample by heating to a temperature not higher than 20°C above the expected congealing point, and transfer to B. Fill the glass or plastic bath D with water at a temperature about 5°C below the expected congealing point. When the sample is liquid at room temperature, fill bath D with water at a temperature between 10°C and 15°C lower than the expected congealing point.



The figures are in mm.

- A: Cylinder made of glass (the tube is painted with silicone oil on both sides of the wall to prevent clouding).
- B: Sample container (a hard glass test tube, which is painted with silicone oil to prevent clouding, except at the region of the wall in contact with the sample; insert it into cylinder A, and fix with cork stopper).
- C: A marked line.
- D: Bath made of glass or plastics.
- E: Stirring rod made of glass or stainless steel (3 mm in diameter, the lower end part of it is bent to make a loop, about 18 mm in diameter).
- F: Thermometer with an immersion line.
- G: Thermometer with an immersion line or a total immersion thermometer.
- H: Immersion line

Fig. 2.42-1

Insert the sample container B containing the sample into cylinder A. Adjust the immersion line H of thermometer F to the same level of the meniscus of the sample. After cooling the sample to about 5°C above the expected congealing point, move vertically the stirrer E at the rate of about 60 to 80 strokes per minute, and observe the thermometer readings at 30-second intervals. The temperature falls gradually. Discontinue stirring, when an appreciable amount of crystals has formed and the temperature is constant or has begun to rise. Usually, read the maximum temperature (reading of F), that is constant for a while after a rise of temperature. If no rise of temperature occurs, read the temperature that is constant for a while. The average of not less than four consecutive readings that lie within a range of 0.2°C constitutes the congealing point.

### 3. Note

If a state of super cooling is anticipated, rub the inner wall of bath B or put a small fragment of the solid sample into bath B for promoting the congealment, when the temperature approaches near the expected congealing point.

## 2.43 Loss on Ignition Test

Loss on Ignition Test is a method to measure the loss in mass when the sample is ignited under the conditions specified in each monograph. This method is usually applied to inorganic drugs which lose a part of the components or impurities during ignition.

The description, for example, “40.0 – 52.0% (1 g, 450 – 550°C, 3 hours)” in a monograph, indicates that the loss in mass is 400 to 520 mg per g of the substance in the test in which about 1 g of the substance is weighed accurately and ignited between 450°C and 550°C for 3 hours.

### 1. Procedure

Previously ignite a crucible or a dish of platinum, quartz or porcelain to constant mass, at the temperature directed in the monograph, and weigh accurately after cooling.

Take the sample within the range of  $\pm 10\%$  of the amount directed in the monograph, transfer into the above ignited container, and weigh it accurately. Ignite under the conditions directed in the monograph, and, after cooling, reweigh accurately. Use a desiccator (silica gel) for the cooling.

## 2.44 Residue on Ignition Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia.

The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

♦The Residue on Ignition Test is a method to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid according to the procedure described below. This test is usually used for determining the content of inorganic impurities in an organic substance.

The description, for example, “not more than 0.1% (1 g)”, in a monograph, indicates that the mass of the residue is not more than 1 mg per 1 g of the substance in the test in which about 1 g of the substance is weighed accurately and ignited by the procedure described below, and “after drying” indicates that the sample is tested after being dried under the conditions specified in the test for Loss on drying..◆

### 1. Procedure

Ignite a suitable crucible (for example, silica, platinum, quartz or porcelain) at  $600 \pm 50^\circ\text{C}$  for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant) and weigh it accurately.

Take the amount of test sample specified in the individual monograph in the crucible and weigh the crucible accurately.

Moisten the sample with a small amount (usually 1 mL) of sulfuric acid, then heat gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount (usually 1 mL) of sulfuric acid, heat gently until white fumes are no longer evolved, and ignite at  $600 \pm 50^\circ\text{C}$  until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Cool the crucible in a desiccator (silica gel or other suitable desiccant), weigh accurately and calculate the percentage of residue.

Unless otherwise specified, if the amount of the residue so obtained exceeds the limit specified in the individual monograph, repeat the moistening with sulfuric acid, heating and ignition as before, using a 30-minute ignition period, until two consecutive weighings of the residue do not differ by more than 0.5 mg or until the percentage of residue complies with the limit in the individual monograph.

## 2.45 Refractive Index Determination

Refractive Index Determination is a method to measure the refractive index of the sample to air. Generally, when light proceeds from one medium into another, the direction is changed at the boundary surface. This phenomenon is called refraction. When light passes from the first isotropic medium into the second, the ratio of the sine of the angle of incidence,  $i$ , to that of the angle of refraction,  $r$ , is constant with regard to these two media and has no relation to the angle of incidence. This ratio is called the refractive index of the second medium with respect to the first, or the relative refractive index,  $n$ .

$$n = \frac{\sin i}{\sin r}$$

The refractive index obtained when the first medium is a vacuum is called the absolute refractive index,  $N$ , of the second medium.

In isotropic substances, the refractive index is a characteristic constant at a definite wavelength, temperature, and pressure. Therefore, this measurement is applied to purity test of substances, or to determination of the composition of homogeneous mixtures of two substances.

The measurement is usually carried out at 20°C, and the D line of the sodium spectrum is used for irradiation. This value is expressed as  $n_D^{20}$ .

### 1. Procedure

For the measurement of refractive index, usually the Abbé refractometer is used at a temperature in the range of  $\pm 0.2^\circ\text{C}$  of that directed in the monograph. Use of the Abbé refractometer permits direct reading of  $n_D$  under incandescent light, with a measurable range from 1.3 to 1.7, and an attainable precision of 0.0002.

## 2.46 Residual Solvents

The chapter of residual solvents describes the control, identification and quantification of organic solvents remaining in drug substances, excipients and drug products.

### I. Control of residual solvents

#### 1. Introduction

Residual solvents in pharmaceuticals (except for crude drugs and their preparations) are defined here as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of drug substance may enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical parameter in the synthetic process. The test method described in this chapter does not address

solvents deliberately used as excipients nor does it address solvates. However, the content of solvents in such products should be evaluated and justified.

Since there is no therapeutic benefit from residual solvents, all residual solvents should be reduced to the extent possible to meet product specifications, good manufacturing practices, or other quality-based requirements. Drug products should contain no higher levels of residual solvents than can be supported by safety data. Some solvents that are known to cause unacceptable toxicities (Class 1, Table 2.46-1) should be avoided in the production of drug substances, excipients, or drug products unless their use can be strongly justified in a risk-benefit assessment. Some solvents associated with less severe toxicity (Class 2, Table 2.46-2) should be limited in order to protect patients from potential adverse effects. Ideally, less toxic solvents (Class 3, Table 2.46-3) should be used where practical.

Testing should be performed for residual solvents when production or purification processes are known to result in the presence of such solvents. It is only necessary to test for solvents that are used or produced in the manufacture or purification of drug substances, excipients, or drug products. Although manufacturers may choose to test the drug product, a cumulative method may be used to calculate the residual solvent levels in the drug product from the levels in the ingredients used to produce the drug product. If the calculation results in a level equal to or below that recommended in this chapter, no testing of the drug product for residual solvents needs to be considered. If, however, the calculated level is above the recommended level, the drug product should be tested to ascertain whether the formulation process has reduced the relevant solvent level to within the acceptable amount. Drug product should also be tested if a solvent is used during its manufacture.

The limit applies to all dosage forms and routes of administration. Higher levels of residual solvents may be acceptable in certain cases such as short term (30 days or less) or topical application. Justification for these levels should be made on a case-by-case basis.

### 2. General principles

#### 2.1. Classification of residual solvents by risk assessment

The term "PDE" (Permitted Daily Exposure) is defined in this chapter as a pharmaceutically acceptable daily intake of residual solvents. Residual solvents regulated in this chapter were evaluated for their possible risk to human health and placed into one of three classes as follows:

(i) Class 1 solvents: Solvents to be avoided in the manufacture of pharmaceuticals

Known human carcinogens, strongly suspected human carcinogens, and environmental hazards. Class 1 solvents are listed in Table 2.46-1.

(ii) Class 2 solvents: Solvents to be limited in pharmaceuticals

Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. Solvents suspected of other significant but reversible toxicities. Class 2 solvents are listed in Table 2.46-2.

(iii) Class 3 solvents: Solvents with low toxic potential

Solvents with low toxic potential to human; no health-based exposure limit is needed. Class 3 solvents are listed in Table 2.46-3 and have PDEs of 50 mg or more per day.

#### 2.2. Option for describing limits of Class 2 solvents

Two options are available when setting limits for Class 2 solvents.

### 2.2.1. Option 1

The concentration limits in ppm can be calculated using equation (1) below by assuming a product mass of 10 g administered daily.

$$\text{Concentration limit (ppm)} = \frac{1000 \times \text{PDE}}{\text{dose}} \quad (1)$$

Here, PDE is given in terms of mg per day and dose is given in g per day.

These limits are considered acceptable for all drug substances, excipients, or drug products. Therefore, this option may be applied if the daily dose is not known or fixed. If all excipients and drug substances in a formulation meet the limits given in Option 1, then these components may be used in any proportion. No further calculation is necessary provided the daily dose does not exceed 10 g. Products that are administered in doses greater than 10 g per day should be considered under Option 2.

### 2.2.2. Option 2

It is not considered necessary for each component of the drug product to comply with the limits given in Option 1. The PDE in terms of mg per day as stated in Table 2.46-2 can be used with the known maximum daily dose and equation (1) above to determine the concentration of residual solvent allowed in drug product. Such limits are considered acceptable provided that it has been demonstrated that the residual solvent has been reduced to the practical minimum. The limits should be realistic in relation to analytical precision, manufacturing capability, reasonable variation in the manufacturing process, and the limits should reflect contemporary manufacturing standards.

Option 2 may be applied by adding the amounts of a residual solvent present in each of the components of the drug product. The sum of the amounts of solvent per day should be less than that given by the PDE.

### 3. Analytical procedures

Residual solvents are typically determined using chromatographic techniques such as gas chromatography. If only Class 3 solvents are present, a nonspecific method such as loss on drying may be used. The analytical method should be validated adequately.

### 4. Reporting levels of residual solvents

Manufacturers of drug products need certain information about the content of residual solvents in excipients or drug substances. The following statements are given as examples of the required information.

(i) Only Class 3 solvents are likely to be present. Loss on drying is not more than 0.5%.

(ii) Only Class 2 solvents are likely to be present. Name the Class 2 solvents that are present. All are not more than the Option 1 limit.

(iii) Only Class 2 solvents and Class 3 solvents are likely to be present. Residual Class 2 solvents are not more than the Option 1 limit and residual Class 3 solvents are not more than 0.5%.

If Class 1 solvents are likely to be present, they should be identified and quantified. "Likely to be present" refers to the solvents that were used in the final manufacturing step and to the solvents that were used in earlier manufacturing steps and not always possible to be excluded even in a validated process.

If solvents of Class 2 or Class 3 are present at greater than their Option 1 limits or 0.5%, respectively, they should be identified and quantified.

### 5. Limits of residual solvents

#### 5.1. Solvents to be avoided in manufacture of pharmaceuticals

Solvents in Class 1 should not be employed in the manufacture of drug substances, excipients, and drug products because of their unacceptable toxicity or their deleterious environmental effect. However, if their use is unavoidable in order to produce a drug product with a significant therapeutic advance, then their levels should be restricted as shown in Table 2.46-1, unless otherwise justified. 1,1,1-Trichloroethane is included in Table 2.46-1 because it is an environmental hazard. The stated limit of 1500 ppm shown in Table 2.46-1 is based on the assessment of the safety data.

**Table 2.46-1** Class 1 solvents (solvents that should be avoided in the manufacture of pharmaceuticals)

Solvent	Concentration Limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

**Table 2.46-2** Class 2 solvents (residual amount should be limited in pharmaceuticals)

Solvent	PDE (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cumene	0.7	70
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
Dichloromethane	6.0	600
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methyl butyl ketone	0.5	50
Methylcyclohexane	11.8	1180
Methyl isobutyl ketone	45	4500
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetrahydrofuran	7.2	720
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloroethene	0.8	80
Xylene*	21.7	2170

\* Usually 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethylbenzene

## 5.2. Solvents to be limited in pharmaceuticals

Solvents in Table 2.46-2 should be limited in drug products because of their inherent toxicities.

PDEs are given to the nearest 0.1 mg per day, and concentrations are given to the nearest 10 ppm. The stated values do not reflect the necessary analytical precision of determination. Precision should be determined as part of the validation of the method.

## 5.3. Solvents with low toxic potential

Solvents in Class 3 shown in Table 2.46-3 may be regarded as less toxic and of lower risk to human health. Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. The amounts of these residual solvents of 50 mg per day or less (corresponding to 5000 ppm or 0.5% under Option 1) would be acceptable without justification. Higher amounts may also be acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice.

## 5.4 Solvents for which no adequate toxicological data was found

The following solvents (Table 2.46-4) may also be related to the manufacturer of drug substances, excipients, or drug products. However, no adequate toxicological data on which to base a PDE was found. Manufacturers should supply justification for residual levels of these solvents in drug products.

## II. Identification and quantification of residual solvents

Whenever possible, the substance under test needs to be dissolved to release the residual solvent. Because drug products, as well as active ingredients and excipients are treated, it may be acceptable that in some cases, some of the components of formulations will not dissolve completely. In those cases, the drug product may first need to be pulverized into a fine powder so that any residual solvent that may be present can be released. This operation should be performed as fast as possible to prevent the loss of volatile solvents during the procedure.

**Table 2.46-3** Class 3 solvents (limited by GMP or other quality-based requirements in pharmaceuticals)

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
<i>n</i> -Butyl acetate	Methyl ethyl ketone
<i>tert</i> -Butyl methyl ether	2-Methyl-1-propanol
Dimethylsulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Diethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	Triethylamine

**Table 2.46-4** Solvents for which no adequate toxicological data were found

1,1-Diethoxypropane	Methyl isopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

In the operating conditions of gas chromatography and headspace described below, parameters to be set and their description may be different depending on the apparatus. When setting these conditions, it is necessary to change them according to the apparatus used, if it is confirmed that they meet the system suitability.

In addition to the reagents specified to be used for the test, those that meet the purpose of the test can be used.

### 1. Class 1 and Class 2 residual solvents

The following procedures are useful to identify and quantify residual solvents when the information regarding which solvents are likely to be present in the material is not available. When the information about the presence of specific residual solvents is available, it is not necessary to perform Procedure A and Procedure B, and only Procedure C or other appropriate procedure is needed to quantify the amount of residual solvents.

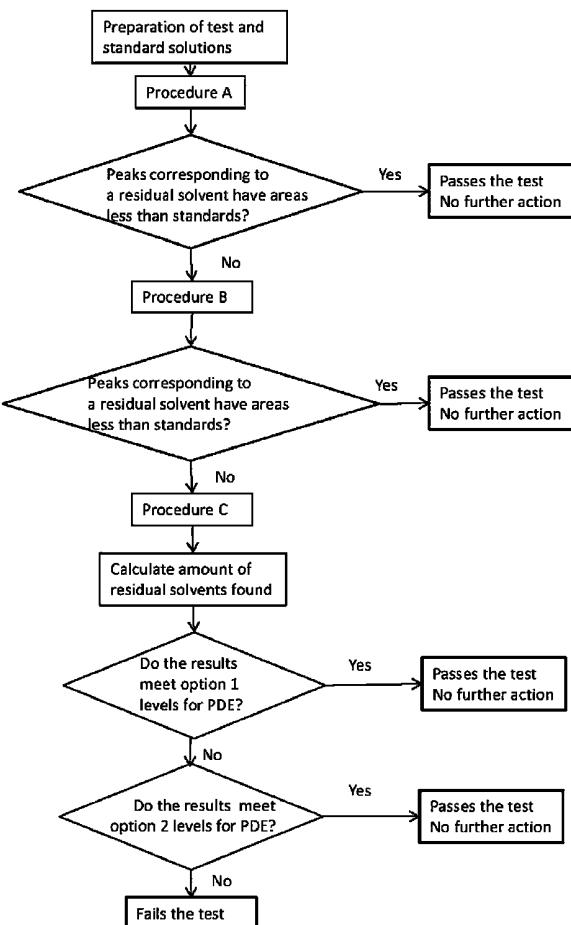
A flow chart for the identification of residual solvents and the application of limit and quantitative tests is shown in Fig. 2.46-1.

#### 1.1. Water-soluble articles

##### 1.1.1. Procedure A

The test is performed by gas chromatography <2.02> according to the following conditions.

Class 1 standard stock solution: To about 9 mL of dimethylsulfoxide add exactly 1 mL of Residual Solvents Class 1 RS, and add water to make exactly 100 mL. Pipet 1 mL of this solution in a volumetric flask, previously filled with about 50 mL of water and add water to make exactly 100 mL. Pipet 10 mL of this solution in a volumetric flask,



**Fig. 2.46-1** Flow chart for the identification of residual solvents and the application of limit and qualification tests

previously filled with about 50 mL of water and add water to make exactly 100 mL.

Class 1 standard solution: Pipet 1 mL of Class 1 standard stock solution in an appropriate head space vial containing exactly 5 mL of water, stopper, cap, and shake.

Class 2 standard stock solution A: Pipet 1 mL of Residual Solvents Class 2A RS, add water to make exactly 100 mL.

Class 2 standard stock solution B: Pipet 1 mL of Residual Solvents Class 2B RS, add water to make exactly 100 mL.

Class 2 standard stock solution C: Pipet 1 mL of Residual Solvents Class 2C RS, add water to make exactly 100 mL.

Class 2 standard solution A: Pipet 1 mL of Class 2 standard stock solution A in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

Class 2 standard solution B: Pipet 5 mL of Class 2 standard stock solution B in an appropriate headspace vial, add exactly 1 mL of water, stopper, cap, and shake.

Class 2 standard solution C: Pipet 1 mL of Class 2 standard stock solution C in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

Test stock solution: Dissolve 0.25 g of the article under test in water, and add water to make exactly 25 mL.

Test solution: Pipet 5 mL of the test stock solution in an appropriate headspace vial, add exactly 1 mL of water, and stopper, cap, and shake.

Class 1 system suitability solution: Pipet 1 mL of Class 1 standard stock solution in an appropriate headspace vial, add exactly 5 mL of the test stock solution, and stopper, cap, and shake.

#### *Operating conditions—*

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column (or a wide-bore column) 0.32 mm (or 0.53 mm) in inside diameter and 30 m in length, coated with 6% cyanopropylphenyl-94% dimethyl silicon polymer for gas chromatography in 1.8  $\mu\text{m}$  (or 3.0  $\mu\text{m}$ ) thickness.

Column temperature: Maintain the temperature at 40°C for 20 minutes after injection, raise to 240°C at a rate of 10°C per minute, and maintain at 240°C for 20 minutes.

Injection port temperature: 140°C.

Detector temperature: 250°C.

Carrier gas: Nitrogen or Helium.

Flow rate: About 35 cm per second.

Split ratio: 1:5. (Note: The split ratio can be modified in order to optimize sensitivity.)

#### *System suitability—*

Test for required detectability: When the procedure is run with Class 1 standard solution and Class 1 system suitability solution under the above operating conditions, the SN ratio of the peak of 1,1,1-trichloroethane obtained with Class 1 standard solution is not less than 5, and the SN ratio of each peak obtained with Class 1 system suitability solution is not less than 3, respectively.

System performance: When the procedure is run with Class 2 standard solution A or the solution for system suitability under the above operating conditions, the resolution between acetonitrile and dichloromethane is not less than 1.0. Pipet 1 mL of a solution of Residual Solvents for System Suitability RS (1 in 100) in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, mix, and use this solution as the solution for system suitability.

System repeatability: When the test is repeated 6 times with Class 1 standard solution under the above operating conditions, the relative standard deviation of each peak area is not more than 15%.

Separately inject (following one of the headspace operating parameter sets described in Table 2.46-5) equal volumes

of headspace (about 1.0 mL) of Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C and the test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If a peak response of any peak, other than a peak for 1,1,1-trichloroethane, in the test solution is greater than or equal to a corresponding peak in either Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B or Class 2 standard solution C, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in Class 1 standard solution, proceed to Procedure B to verify the identity of the peak; otherwise the article meets the requirements of this test.

#### **1.1.2. Procedure B**

The test is performed by gas chromatography <2.02> according to the following conditions.

Class 1 standard stock solution, Class 1 standard solution, Class 1 system suitability solution, Class 2 standard stock solutions A, B and C, Class 2 standard solutions A, B and C, test stock solution and test solution: Prepare as directed for Procedure A.

#### *Operating conditions—*

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column (or a wide-bore column) 0.32 mm (or 0.53 mm) in inside diameter and 30 m in length, coated with polyethylene glycol for gas chromatography in 0.25  $\mu\text{m}$  thickness.

Column temperature: Maintain the temperature at 50°C for 20 minutes after injection, raise to 165°C at a rate of 6°C per minute, and maintain at 165°C for 20 minutes.

Injection port temperature: 140°C.

Detector temperature: 250°C.

Carrier gas: Nitrogen or Helium.

Flow rate: About 35 cm per second.

Split ratio: 1:5. (Note: The split ratio can be modified in order to optimize sensitivity.)

#### *System suitability—*

Test for required detectability: When the procedure is run with Class 1 standard solution and Class 1 system suitability solution under the above operating conditions, the SN ratio of the peak of benzene obtained with Class 1 standard solution is not less than 5, and the SN ratio of each peak obtained with Class 1 system suitability solution is not less than 3, respectively.

System performance: When the procedure is run with Class 2 standard solution A or the solution for system suitability under the above operating conditions, the resolution between acetonitrile and *cis*-1,2-dichloroethene is not less than 1.0. Pipet 1 mL of a solution of Residual Solvents for System Suitability RS (1 in 100) in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, mix, and use this solution as the solution for system suitability.

System repeatability: When the test is repeated 6 times with Class 1 standard solution under the above operating conditions, the relative standard deviation of each peak area is not more than 15%.

Separately inject (following one of the headspace operating parameter sets described in Table 2.46-5) equal volumes of headspace (about 1.0 mL) of Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C and the test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) of the peak(s) in the test solution is/are greater than or equal to a corresponding peak(s) in either Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B or

Class 2 standard solution C, proceed to Procedure C to quantify the peak(s); otherwise, the article meets the requirements of this test.

### 1.1.3. Procedure C

The test is performed by gas chromatography <2.02> according to the following conditions.

Class 1 standard stock solution, Class 1 standard solution, Class 2 standard stock solution A, Class 2 standard solution A, Class 2 standard stock solution C, Class 2 standard solution C and Class 1 system suitability solution: Prepare as directed for Procedure A.

Standard stock solution (Note: Prepare a separate standard stock solution for each peak identified and verified by Procedures A and B. For Class 1 solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under Class 1 standard stock solution in Procedure A.): Transfer an accurately measured volume of each individual solvent corresponding to each residual solvent peak identified and verified by Procedures A and B to a suitable container, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a final concentration of 1/20 of the value stated in Table 2.46-1 or Table 2.46-2.

Standard solution: Pipet 1 mL of the standard stock solution in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

Test stock solution: Weigh accurately about 0.25 g of the article under test, dissolve in water, and add water to make exactly 25 mL.

Test solution: Pipet 5 mL of the test stock solution in an appropriate headspace vial, add exactly 1 mL of water, stopper, cap, and shake.

Spiked test solution (Note: prepare a separate spiked test solution for each peak identified and verified by Procedure A and B): Pipet 5 mL of the test stock solution in an appropriate headspace vial, add exactly 1 mL of the standard stock solution, stopper, cap, and shake.

Operating conditions and system suitability fundamentally follow the procedure A. Test for required detectability is unnecessary, and use Standard solution instead of Class 1 standard solution for system repeatability. If the results of the chromatography from Procedure A are found to be inferior to those found with Procedure B, the operating conditions from Procedure B may be substituted.

Perform the test (following one of the headspace operating parameters described in Table 2.46-5) with equal volumes of about 1.0 mL each of the standard solution, test solution, and spiked test solution, and measure the peak areas for the major peaks. Calculate the amount of each residual solvent found in the article under test by the formula:

$$\text{Amount of residual solvent (ppm)} = 5 (C/M) \{A_T/(A_S - A_T)\}$$

C: Concentration ( $\mu\text{g/mL}$ ) of the appropriate Reference Standard in the standard stock solution

M: Amount (g) of the article under test taken to prepare the test stock solution

$A_T$ : Peak responses of each residual solvent obtained from the test solution

$A_S$ : Peak responses of each residual solvent obtained from the spiked test solution

## 1.2. Water-insoluble articles

### 1.2.1. Procedure A

The test is performed by gas chromatography <2.02> according to the following conditions. Dimethylsulfoxide may

be substituted as an alternative solvent to *N,N*-dimethylformamide.

Class 1 standard stock solution: To about 80 mL of *N,N*-dimethylformamide add 1 mL of Residual Solvents Class 1 RS, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution in a volumetric flask, previously filled with about 80 mL of *N,N*-dimethylformamide and add *N,N*-dimethylformamide to make exactly 100 mL (this solution is the intermediate diluent prepared from Residual Solvents Class 1 RS and use it for preparation of Class 1 system suitability solution). Pipet 1 mL of this solution, and add *N,N*-dimethylformamide to make exactly 10 mL.

Class 1 standard solution: Pipet 1 mL of Class 1 standard stock solution in an appropriate head space vial containing exactly 5 mL of water, stopper, cap, and shake.

Class 2 standard stock solution A: Pipet 1 mL of Residual Solvents Class 2A RS, dissolve in about 80 mL of *N,N*-dimethylformamide, and add *N,N*-dimethylformamide to make exactly 100 mL.

Class 2 standard stock solution B: Pipet 0.5 mL of Residual Solvents Class 2B RS, add *N,N*-dimethylformamide to make exactly 10 mL.

Class 2 standard stock solution C: Pipet 1 mL of Residual Solvents Class 2C RS, dissolve in about 80 mL of *N,N*-dimethylformamide, and add *N,N*-dimethylformamide to make exactly 100 mL.

Class 2 standard solution A: Pipet 1 mL of Class 2 standard stock solution A in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

Class 2 standard solution B: Pipet 1 mL of Class 2 standard stock solution B in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

Class 2 standard solution C: Pipet 1 mL of Class 2 standard stock solution C in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

Test stock solution: Dissolve 0.5 g of the article under test in *N,N*-dimethylformamide, and add *N,N*-dimethylformamide to make exactly 10 mL.

Test solution: Pipet 1 mL of the test stock solution in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

Class 1 system suitability solution: Pipet 5 mL of the test stock solution and 0.5 mL of the intermediate dilution prepared from Residual Solvents Class 1 RS, and mix. Pipet 1 mL of this solution in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

#### Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A wide-bore column 0.53 mm in inside diameter and 30 m in length, coated with 6% cyanopropylphenyl-94% dimethyl silicon polymer for gas chromatography in 3.0  $\mu\text{m}$  thickness.

Column temperature: Maintain the temperature at 40°C for 20 minutes after injection, raise to 240°C at a rate of 10°C per minute, and maintain at 240°C for 20 minutes.

Injection port temperature: 140°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: About 35 cm per second.

Split ratio: 1:3. (Note: The split ratio can be modified in order to optimize sensitivity.)

#### System suitability—

Test for required detectability: When the procedure is run with Class 1 standard solution and Class 1 system suitability solution under the above operating conditions, the SN ratio of the peak of 1,1,1-trichloroethane obtained with Class 1

standard solution is not less than 5, and the SN ratio of each peak obtained with Class 1 system suitability solution is not less than 3, respectively.

**System performance:** When the procedure is run with Class 2 standard solution A or the solution for system suitability under the above operating conditions, the resolution between acetonitrile and dichloromethane is not less than 1.0. Pipet 1 mL of the *N,N*-dimethylformamide solution of Residual Solvents for System Suitability RS (1 in 100) in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake, and use this solution as the solution for system suitability.

**System repeatability:** When the test is repeated 6 times with Class 1 standard solution under the above operating conditions, the relative standard deviation of each peak areas is not more than 15%.

Separately inject (use headspace operating parameters in column 3 of Table 2.46-5) equal volumes of headspace (about 1.0 mL) of Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C, and the test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If a peak response of any peak, other than a peak for 1,1,1-trichloroethane, in the test solution is greater than or equal to a corresponding peak in either Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B or Class 2 standard solution C, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in Class 1 standard solution, proceed to Procedure B to verify the identity of the peak; otherwise, the article meets the requirements of this test.

### 1.2.2. Procedure B

The test is performed by gas chromatography <2.02> according to the following conditions.

Class 1 standard stock solution, Class 1 standard solution, Class 1 system suitability solution, Class 2 standard stock solutions A, B and C, Class 2 standard solutions A, B and C, test stock solution, and test solution: Proceed as directed for Procedure A.

Proceed as directed for Procedure B under Water-soluble articles with a split ratio of 1:3. (Note: The split ratio can be modified in order to optimize sensitivity.) The solution for system suitability: Proceed as directed for Procedure A.

Separately inject (use headspace operating parameters in Table 2.46-5) equal volumes of headspace (about 1.0 mL) of Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C and the test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) of the peak(s) in the test solution is/are greater than or equal to a corresponding peak(s) in either Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B or Class 2 standard solution C, proceed to Procedure C to quantify the peak; otherwise, the article meets the requirements of this test.

### 1.2.3. Procedure C

The test is performed by gas chromatography <2.02> according to the following conditions.

Class 1 standard stock solution, Class 1 standard solution, Class 1 system suitability solution, Class 2 standard stock solution A, Class 2 standard solution A, Class 2 standard stock solution C and Class 2 standard solution C: Proceed as directed for Procedure A.

Standard stock solution (Note: Prepare a separate standard stock solution for each peak identified and verified by Procedures A and B. For Class 1 solvents other than 1,1,1-

**Table 2.46-5** Headspace operating parameters

	Headspace Operating Parameter Sets		
	1	2	3
Equilibration temperature (°C)	80	105	80
Equilibration time (min.)	60	45	45
Transfer-line temperature (°C)	85	110	105
Syringe temperature (°C)	80 – 90	105 – 115	80 – 90
Carrier gas: nitrogen or helium at an appropriate pressure			
Pressurization time (s)	≥ 60	≥ 60	≥ 60
Injection volume (mL)*	1	1	1

\* Or follow the instrument manufacturer's recommendations, as long as the method criteria are met. Injecting less than 1 mL is allowed as long as adequate sensitivity is achieved.

trichloroethane, prepare the first dilution as directed for the first dilution under Class 1 standard stock solution in Procedure A.): Transfer an accurately measured volume of each individual solvent corresponding to each residual solvent peak identified and verified by Procedures A and B to a suitable container, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a final concentration of 1/20 of the value stated in Table 2.46-1 or Table 2.46-2.

**Standard solution:** Pipet 1 mL of the standard stock solution in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

**Test stock solution:** Weigh accurately about 0.5 g of the article under test, and add *N,N*-dimethylformamide to make exactly 10 mL.

**Test solution:** Pipet 1 mL of test stock solution in an appropriate headspace vial, add exactly 5 mL of water, stopper, cap, and shake.

**Spiked test solution (Note: prepare a separate spiked test solution for each peak identified and verified by Procedure A and B):** Pipet 1 mL of test stock solution in an appropriate headspace vial, add exactly 4 mL of water, stopper, cap, and shake.

Operating conditions and system suitability fundamentally follow the procedure A. Test for required detectability is unnecessary, and use Standard solution instead of Class 1 standard solution for system repeatability. If the results of the chromatography from Procedure A are found to be inferior to those found with Procedure B, the operating conditions from Procedure B may be substituted.

Perform the test (use headspace operating parameters in Table 2.46-5) with about 1.0 mL each of the standard solution, test solution, and spiked test solution, and measure the responses for the major peaks. Calculate the amount of each residual solvent found in the article under test by the formula:

$$\text{Amount of residual solvent (ppm)} = 10 (C/M) \{A_T/(A_S - A_T)\}$$

**C:** Concentration ( $\mu\text{g/mL}$ ) of the appropriate Reference Standard in the standard stock solution

**M:** Amount (g) of the article under test taken to prepare the test stock solution

**A<sub>T</sub>:** Peak responses of each residual solvent obtained from the test solution

**A<sub>S</sub>:** Peak responses of each residual solvent obtained from

the spiked test solution

### 1.3. Headspace operating parameters and other considerations

Examples of headspace operating parameters are shown in Table 2.46-5.

These test methods describe the analytical methods using the headspace gas chromatography. The following Class 2 residual solvents are not readily detected by the headspace injection conditions because of the low sensitivity: 2-ethoxyethanol, ethylene glycol, formamide, 2-methoxyethanol, *N*-methylpyrrolidone, and sulfolane. Other appropriate validated procedures are to be employed for the quantification of these residual solvents. In the headspace methods, *N,N*-dimethylformamide and *N,N*-dimethylacetamide are often used as solvents. As not only 6 solvents described above but these two solvents are not included in Residual Solvents Class 2A RS, Residual Solvents Class 2B RS and/or Residual Solvents Class 2C RS, appropriate validated procedures are to be employed for these residual solvents as necessary.

### 2. Class 3 residual solvents

Perform the test according to 1. Otherwise, an appropriate validated procedure is to be employed. Prepare appropriate standard solutions, etc. according to the residual solvent under test.

If only Class 3 solvents are present, the level of residual solvents may be determined by Loss on Drying <2.41>. However, when the value of the loss on drying is more than 0.5%, or other solvents exist, the individual Class 3 residual solvent or solvents present in the article under test should be identified using the procedures as described above or other appropriate procedure, and quantified as necessary.

### 3. Reference Standards

(i) Residual Solvents Class 1 RS (A mixture of benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene and 1,1,1-trichloroethane)

(ii) Residual Solvents Class 2A RS (A mixture of acetonitrile, chlorobenzene, cumene, cyclohexane, 1,2-dichloroethene (*cis*-1,2-dichloroethene, *trans*-1,2-dichloroethene), dichloromethane, 1,4-dioxane, methanol, methylcyclohexane, tetrahydrofuran, toluene and xylene (*m*-xylene, *p*-xylene, *o*-xylene, ethylbenzene))

(iii) Residual Solvents Class 2B RS (A mixture of chloroform, 1,2-dimethoxyethane, hexane, methyl butyl ketone, nitromethane, pyridine, tetralin and 1,1,2-trichloroethene)

(iv) Residual Solvents Class 2C RS (Methyl isobutyl ketone)

(v) Residual Solvents for System Suitability RS (A mixture of acetonitrile, *cis*-1,2-dichloroethene and dichloromethane)

## 2.47 Osmolarity Determination

Osmolarity Determination is a method for measuring the osmotic concentration of the sample solution from the extent of the freezing-point depression.

When a solution and a pure solvent are separated by a semipermeable membrane, through which the solvent can pass freely, but the solute cannot, a part of the solvent passes into the solution compartment through the membrane. The pressure difference produced between the two compartments concomitantly with the solvent migration through the membrane, is defined as the osmotic pressure  $\Pi$  (Pa). The osmotic pressure is a physical quantity depending on the total of the molecular species present, including neu-

tral molecules and ions, and does not depend on the kind of solute. A solution property, such as osmotic pressure, freezing-point depression, boiling-point elevation etc., which depends not on the kind of solute, but on the total number of all molecular species, is called a colligative property of a solution.

The osmotic pressure of a polymer solution can be measured directly as the hydrostatic pressure difference between two compartments separated by a semipermeable membrane, such as a cellulose membrane. However, this is not applicable to a solution containing low molecular species, which can pass through a semipermeable membrane. Though the osmotic pressure of such a solution cannot be measured directly, the direction and extent of solvent migration through biological membranes can be predicted from the total number of all molecular species present when the solution is placed under physiological conditions. Other colligative properties of a solution such as freezing-point depression, boiling-point elevation, vapor-pressure depression, etc. can be directly obtained by observing changes of temperature and/or pressure, etc. These solution properties depend on the total number of ionic and neutral species in the solution in the same way as the osmotic pressure, and the molecular particle concentration is defined as the osmotic concentration. The osmotic concentration can be defined in two ways, one being mass-based concentration (osmolality, mol/kg) and the other, volume-based concentration (osmolarity, mol/L). In practice, the latter is more convenient.

Unless otherwise specified, the freezing-point depression method is used for measuring the osmotic concentration. The method is based on the linear dependency of the freezing-point depression  $\Delta T$  ( $^{\circ}\text{C}$ ) upon the osmolality  $m$  (mol/kg), as expressed in the following equation,

$$\Delta T = K \cdot m$$

In this equation,  $K$  is the molal freezing-point depression constant, and it is known to be  $1.86^{\circ}\text{C}$  kg/mol for water. Since the constant  $K$  is defined on the basis of molarity, the molar osmotic concentration can be obtained from the above equation. In the dilute osmotic concentration range, osmolality  $m$  (mol/kg) can be assumed to be numerically equal to osmolarity  $c$  (mol/L). Thus, the conventional osmolarity (mol/L) and the unit of osmole (Osm) are adopted in this test method. One Osm means that the Avogadro number ( $6.022 \times 10^{23}/\text{mol}$ ) of species is contained in 1 L of solution. Usually the osmotic concentration is expressed as the submultiple milliosmole (mOsm, mosmol/L) in the Pharmacopoeia.

### 1. Apparatus

Usually, the osmotic concentration of a solution can be obtained by measuring the extent of the freezing-point depression. The apparatus (osmometer) is composed of a sample cell for a fixed volume of sample solution and a cell holder, a cooling unit and bath with a temperature regulator, and a thermistor for detecting temperature.

### 2. Procedure

A fixed volume of the test solution is introduced into the sample cell, as indicated for the individual apparatus.

The apparatus must first be calibrated by the two-point calibration method by using osmolal standard solutions. For the calibration, select two different standard solutions just covering the expected osmolar concentration of a sample solution. Other than the indicated osmolal standard solutions in the Table 2.47-1, water can also be used as a standard solution (0 mOsm) for measuring low osmolar sample solutions (0 – 100 mOsm). Next, after washing the sample cell

**Table 2.47-1**

Standard solution for osmometer calibration (milliosmoles)	Amount of sodium chloride (g)
100	0.309
200	0.626
300	0.946
400	1.270
500	1.593
700	2.238
1000	3.223

and the thermistor as indicated for the individual apparatus, measure the degree of the freezing-point depression caused by a sample solution. Using the above-mentioned relation of osmolar concentration  $m$  and  $\Delta T$ , the osmolarity of a sample solution can be obtained, and it is assumed to be numerically equal to the osmolarity.

In the case of higher osmolar solutions over 1000 mOsm, dilute the sample with water and prepare  $n'/n$  times diluted sample solution ( $n$  in  $n'$ ). Measure the osmolarity of the diluted solution, as described above. In this case, it is necessary to state that the calculated osmolarity for the sample (see below) is an apparent osmolarity obtained by the dilution method. When the determination is performed using  $n'/n$  times diluted solution, the dilution number should be selected so that the osmolar concentration is near but not exceeding 1000 mOsm, and dilute in one step.

In the case of solid samples, such as freeze-dried medicines, prepare a sample solution by dissolving the solid using the indicated solution for dissolution.

### 3. Suitability of the apparatus

After the calibration of the apparatus, a suitability test must be done by repeating the measurement of osmolarity for one of the standard solutions not less than 6 times. In performing the test, it is advisable that the osmolarity of a sample solution and the selected standard solution are similar to each other. In this test, the repeatability of measured values and the deviation of the average from the indicated value should be less than 2.0% and 3.0%, respectively. When the requirement is not met, calibrate the apparatus again by the two-point calibration method, and repeat the test.

### 4. Preparation of the osmolar standard solutions

Weigh exactly an amount indicated in Table 2.47-1 of sodium chloride (standard reagent), previously dried between 500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve the weighed sodium chloride in exactly 100 g of water to make the corresponding osmolar standard solution.

### 5. Osmolar ratio

In this test method the osmolar ratio is defined as the ratio of osmolarity of a sample solution to that of the isotonic sodium chloride solution. The ratio can be used as a measure of isotonicity of sample solution. Since the osmolarity of the isotonic sodium chloride solution (NaCl 0.900 g/100 mL)  $c_S$  (mOsm) is assumed to be constant (286 mOsm), the osmolar ratio of a sample solution, of which the osmolarity is  $c_T$  (mOsm), can be calculated by means of the following equation,

$$\text{Osmolar ratio} = c_T/c_S$$

$c_S$ : 286 mOsm

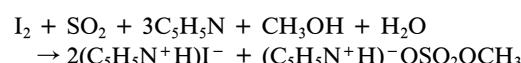
When the measurement is done by the dilution method,

### Water Determination (Karl Fischer Method)

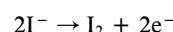
because the sample has an osmolarity over 1000 mOsm, the apparent osmolarity of the sample solution  $c_T$  can be calculated as  $n'/n \cdot c'_T = c_T$ , in which  $n'/n$  is the dilution number and  $c'_T$  is the measured osmolarity for the diluted solution. In this calculation, a linear relation between osmolarity and solute concentration is assumed. The dilution must be made in one step. Thus when the dilution measurement is performed, the dilution number is stated as ( $n$  in  $n'$ ).

## 2.48 Water Determination (Karl Fischer Method)

Water Determination determines water content in sample materials, utilizing the fact that water reacts with iodine and sulfur dioxide quantitatively in the presence of a lower alcohol such as methanol, and an organic base such as pyridine (Karl Fischer reaction). The reaction proceeds in the manner shown in the following equation:



In this measurement there are two methods different in iodine-providing principle (the volumetric titration method and the coulometric titration method). In the volumetric titration method, iodine is previously dissolved in a reagent for water determination, and water content is determined by measuring the amount of iodine consumed as a result of reaction with water. In the coulometric titration method, iodine is produced by electrolysis of iodide ion in an anolyte solution for water determination. Based on the quantitative reaction of the generated iodine with water, the water content in a sample specimen can be indirectly determined by measuring the quantity of electricity which is required for the production of iodine during the titration.



### 1. Volumetric titration

#### 1.1. Apparatus

Generally, the apparatus consists of automatic burettes, a titration flask, a stirrer, and equipment for amperometric titration at constant voltage or potentiometric titration at constant current. Karl Fischer TS for water determination is extremely hygroscopic, so the overall equipment for titration should be designed to be protected from the entry of water. Desiccants such as silica gel or calcium chloride for water determination can be used.

#### 1.2. Reagents

##### 1.2.1. Solvents for water determination

As a solvent for water determination, use principally methanol for water determination. Other solvents, including ethylene glycol for water determination, chloroform for water determination, diethylene glycol monoethyl ether for water determination, propylene carbonate for water determination, and formamide for water determination, or a mixture of these solvents can be used, taking solubility of a sample specimen and interference of a sample specimen with the Karl Fischer reaction into account.

##### 1.2.2. Base for water determination

Use pyridine for water determination, imidazole for water determination, and 2-methylaminopyridine for water determination, for example.

##### 1.2.3. Preparation and standardization of Karl Fischer TS for water determination

###### (1) Preparation

Use an appropriately prepared Karl Fischer TS for water

determination. For example, dissolve 63 g of iodine in 100 mL of pyridine for water determination, and cool the solution in an ice bath. Pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 32 g. Then make up to 500 mL by adding methanol for water determination, and use this solution as Karl Fischer TS for water determination. Karl Fischer TS for water determination can be prepared with other appropriate solvents for water determination and bases for water determination.

Store Karl Fischer TS for water determination in tightly stoppered bottles in a cold place, protecting it from light and moisture, and allow to stand for more than 24 hours before use.

#### (2) Standardization

When water is titrated with Karl Fischer TS for water determination, the equivalent amount of water per unit volume of Karl Fischer TS for water determination, factor  $f$  (mg/mL), change over time with slight moisture. Standardize before use.

According to the procedure described in section 1.3. Procedure, take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the solvent with a Karl Fischer TS for water determination to make the inside of the flask anhydrous. Then, weigh 5 to 30 mg of water accurately and put it in the titration flask quickly, and titrate the water dissolved in the solvent with a Karl Fischer TS for water determination to the end point, under vigorous stirring. When the quantity of titrant is  $V$  (mL), calculate the factor,  $f$  (mg/mL), of the Karl Fischer TS for water determination by using the following equation:

$$f(\text{mg/mL}) = M/V$$

$M$ : Amount (mg) of water taken

$V$ : Volume (mL) of Karl Fischer TS for water determination consumed for titration of water

#### 1.2.4. Preparation and standardization of standard water-methanol solution

Prepare and standardize the standard water-methanol solution as follows, beforehand.

##### (1) Preparation

Take 500 mL of methanol for water determination in a dried 1000-mL volumetric flask, add 2.0 mL of water, and adjust with the methanol for water determination to make 1000 mL. The standard water-methanol solution is preserved in a cold place, protecting it from light and moisture.

##### (2) Standardization

Take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS for water determination to make the content of the flask anhydrous. Then, add exactly 10 mL of Karl Fischer TS for water determination to this solution in the flask, titrate it with the prepared standard water-methanol solution to the end point, and measure the quantity of titrant,  $V$  (mL). Calculate the factor of the standard water-methanol solution,  $f'$  (mg/mL), by using the following equation:

$$f'(\text{mg/mL}) = (f \times 10)/V$$

$f$ : Factor (mg/mL) of Karl Fischer TS for water determination

10: Volume (mL) of Karl Fischer TS for water determination taken

$V$ : Volume (mL) of titrant of the standard water-methanol solution

#### 1.3. Procedure

As a rule, the titration of water with a Karl Fischer TS for

water determination should be performed at the same temperature as that at which the standardization was done, with protection from moisture.

Immerse a pair of platinum electrodes (or double platinum electrode) in a solution to be titrated, add a Karl Fischer TS for water determination while applying a constant small voltage between the two electrodes, and measure the changed small current ( $\mu\text{A}$ ) (Amperometric titration at constant voltage). When the current stops changing and persists for a certain time (usually, longer than 30 seconds), this electric state is designated as the end point of the titration.

Otherwise, apply a definite current between two platinum electrodes, and add Karl Fischer TS for water determination. The variable potential (mV) is measured (Potentiometric titration at constant current).

Unless otherwise specified, the titration of water with Karl Fischer TS for water determination should be performed by either direct titration or back titration.

##### 1.3.1. Direct titration

Unless otherwise specified, proceed by the following method.

Take a suitable quantity of the solvent for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS for water determination to the end point to make the content of the flask anhydrous. Weigh accurately  $M$  (mg) of sample specimen containing 5 to 30 mg of water, transfer it quickly into the titration flask, dissolve by stirring, titrate the solution to be examined with Karl Fischer TS for water determination to the end point under vigorous stirring, and measure the quantity of titrant,  $V$  (mL). In the case of an insoluble sample specimen, powder the sample quickly, weigh accurately  $M$  (mg) of the sample containing 5 to 30 mg of water, and transfer it quickly into the titration vessel, stir the mixture, protecting it from moisture, and perform the titration under vigorous stirring.

Though the titration procedure should be performed under atmospheric conditions at low humidity, if the effect of atmospheric moisture cannot be avoided, for instance, if a long time is required for extraction and titration of water, a blank test must be done and the data must be corrected, as necessary.

$$\text{Water content (\%)} = \{(V \times f)/M\} \times 100$$

$M$ : Amount (mg) of the sample taken

$V$ : Volume (mL) of titrant of Karl Fischer TS for water determination consumed for titration

$f$ : Factor (mg/mL) of Karl Fischer TS for water determination

##### 1.3.2. Back titration

Unless otherwise specified, proceed by the following method.

Take a suitable quantity of the solvent for water determination in the dried titration vessel, and titrate the water contaminated with Karl Fischer TS for water determination to the end point to make the content of the flask anhydrous. Weigh accurately  $M$  (mg) of sample specimen having 5 to 30 mg of water, transfer the sample quickly into the titration vessel, dissolve it in the solution by stirring, add an excessive and definite volume,  $V'$  (mL), of Karl Fischer TS for water determination, and then titrate the solution with the standard water-methanol solution to the end point under vigorous stirring, and measure the quantity of titrant,  $V$  (mL). In the case of an insoluble sample specimen, powder the sample quickly, weigh accurately the mass,  $M$  (mg), transfer it quickly into the titration vessel, and add an excessive and definite volume,  $V'$  (mL), of Karl Fischer TS for water de-

termination. After stirring, with protection from moisture, perform the titration under vigorous stirring. Calculate the water content (%) in the sample by using the following equation:

$$\text{Water content (\%)} = \left[ \frac{(V' \times f) - (V \times f')}{M} \right] \times 100$$

*M*: Amount (mg) of the sample taken

*V*: Volume (mL) of titrant of the standard water-methanol solution

*V'*: Excessive and definite volume (mL) of Karl Fischer TS for water determination

*f*: Factor (mg/mL) of Karl Fischer TS for water determination

*f'*: Factor (mg/mL) of the standard water-methanol solution

#### 1.4. Suitability of determination

Perform an appropriate suitability test for determination to verify the validity of devices and reagent systems for the volumetric titration method, before changing operating conditions, or periodically, as necessary. These conditions include device components such as electrodes and types of solvents and test solutions for water determination.

Perform a suitability test for determination, for example, for a sample containing 5 to 30 mg of water, as shown in the following procedure.

First, determine the water content in the sample, using the established devices and reagent systems. Then, add water or a solution containing a known quantity of water, equivalent to 50 to 100% of the water content determined in the sample, into the same titration flask, and determine the water. A solution containing a known quantity of water can be obtained by purchasing a certified, traceable, commercially available standard solution. Repeat these procedures 5 times and calculate each recovery, *r* (%), for each addition by using the following equation:

$$r (\%) = \frac{(M_2/M_1) \times 100}{}$$

*M*<sub>1</sub>: Amount (mg) of water added

*M*<sub>2</sub>: Amount (mg) of water determined

Plot the cumulative amount of water added on the *x*-axis, and the sum of the amount of water that is initially determined, *M*, and the cumulative amount of water that is determined after adding water or a solution containing a known amount of water on the *y*-axis. Determine the gradient, *b*, and the intercept on the *y*-axis, *a*, of the regression line obtained, and the intercept on the *x*-axis of the extrapolated regression line, *d*, and calculate the percentage errors, *e*<sub>1</sub> (%) and *e*<sub>2</sub> (%), by using the following equation:

$$e_1 (\%) = \frac{|(a - M)/M| \times 100}{}$$

$$e_2 (\%) = \frac{|(|d| - M)/M| \times 100}{}$$

*a*: Intercept on the *y*-axis of the regression line (mg H<sub>2</sub>O)

*d*: Intercept on the *x*-axis of the regression line (mg H<sub>2</sub>O)

*M*: Water content measured in the sample (mg H<sub>2</sub>O)

When the following criteria are met, the devices and reagent systems evaluated are appropriate water determination systems for the sample.

- When the mean water recovery, *R* (%), is calculated based on the water recovery, *r* (%), the *R* is between 97.5% and 102.5%.
- |*e*<sub>1</sub>| and |*e*<sub>2</sub>| are not more than 2.5%.
- *b* is between 0.975 and 1.025.

#### 2. Coulometric titration

##### 2.1. Apparatus

Usually, the apparatus used for coulometric titration is

#### Water Determination (Karl Fischer Method)

comprised of a titration flask equipped with an electrolytic cell for iodine production, a stirrer, and a potentiometric titration system at constant current. The electrolytic cell for iodine production is composed of an anode and a cathode, separated by a diaphragm. The anode is immersed in the anolyte solution for water determination and the cathode is immersed in the catholyte solution for water determination. Both electrodes are usually made of platinum-mesh.

Because both the anolyte and the catholyte solutions for water determination are strongly hygroscopic, the titration system should be designed to be protected from water. Desiccants such as silica gel or calcium chloride for water determination can be used.

##### 2.2. Preparation of anolyte and catholyte solutions for water determination

Unless otherwise specified, an anolyte solution for water determination and a catholyte solution for water determination are prepared using solutions having composition described below.

Anolyte solution for water determination—A mixture of iodine, sulfur dioxide, or imidazole for water determination [or 1,3-di(4-pyridyl)propane, diethanolamine, or alternative bases], and an organic solvent, such as methanol for water determination

Catholyte solution for water determination—A mixture of an inorganic or organic salt, such as lithium chloride, choline chloride, or diethanolamine hydrochloride, and an organic solvent, such as methanol for water determination

##### 2.3. Procedure

Take a suitable volume of an anolyte solution for water determination in a titration vessel, immerse in this solution a pair of platinum electrodes (or a double platinum electrode). Separately, immerse an electrolytic cell for iodine production filled with a catholyte solution for water determination in the anolyte. Switch on the electrolytic system and make the content of the titration vessel anhydrous. Next weigh accurately *M* (mg) of a sample specimen containing 0.2 to 5 mg of water, add it quickly to the vessel, dissolve by stirring, perform the titration to the end point under vigorous stirring, and calculate the quantity of electricity, *C* (C) {electric current (A) × time (s)} required for production of iodine during the titration.

Calculate the water content (%) in the sample based on the quantity of electricity, *C* (C), required for production of iodine and the amount, *M* (mg), of the sample taken, by using the following equation:

$$\text{Water content (\%)} = \frac{C/(10.71 \times M)}{100} \times 100$$

*M*: Amount (mg) of the sample taken

*C*: Quantity of electricity (C) required for production of iodine

10.71: Quantity of electricity (C/mg) corresponding to 1 mg of water (H<sub>2</sub>O)

When a sample specimen cannot be dissolved in the anolyte, powder it quickly, with protection from atmospheric moisture, and add an accurately weighed amount, *M* (mg), of the sample estimated to contain 0.2 to 5 mg of water to the vessel. After stirring the mixture, with protection from atmospheric moisture, perform the titration under vigorous stirring, and proceed in the same manner.

Though the titration procedure should be performed under atmospheric conditions at low humidity, if the effect of atmospheric moisture cannot be avoided, for instance, if a long time is required for extraction and titration of water, a blank test must be done and the data must be corrected, as necessary.

#### 2.4. Suitability of determination

Perform a suitability test for appropriate determination and verify the validity of devices and reagents systems for the coulometric titration method, before changing operating conditions, or periodically, as necessary. These conditions include device components such as electrodes and types of solvents and test solutions for water determination.

Perform a water recovery test, for example, using water or a solution containing known quantity of water before and after determination of water content in the sample. A solution containing a known quantity of water can be obtained by purchasing a certified, traceable, commercially available standard solution. Add water or a solution containing a known quantity of water, containing about 1000 µg or about 100 µg of water, which is closer to the expected quantity of water in the sample, perform the coulometric titration for water content, and calculate the recovery.

The devices and reagent systems are assessed as appropriate for the sample if the added water is 1000 µg and the recovery is between 97.5% and 102.5% or if the added water is 100 µg and the recovery is between 90.0% and 110.0%.

#### 3. Utilization of water vaporizer and suitability of determination

In the case of a sample which is insoluble in a solvent or interferes with the Karl Fischer reaction, water in the sample can be heated by a water vaporizer, and introduced into the titration vessel, by using a stream of nitrogen gas as a carrier.

Suitability of determination using a water vaporizer can be assessed by, for example, the quantity of water determined in another method as the theoretical quantity of water for an appropriate substance or a stable hydrate. An appropriate quantity close to the theoretical quantity of water of the substance needs to be detected under appropriate conditions (including temperature, time, and amount of sample) based on the instructions of the devices.

## 2.49 Optical Rotation Determination

#### 1. Principle

Generally, the vibrations of light take place on planes perpendicular to the direction of the beam. In case of the ordinary light, the directions of the planes are unrestricted, while in case of the plane polarized light, commonly called as polarized light, the vibrations take place on only one plane that includes the advancing direction of the beam. And it is called that these beams have plane of polarization. Some drugs in the liquid state or in solution have a property of rotating the plane of the polarized light either to the right or to the left. This property is referred to as optical activity or optical rotation, and is inherently related to the chemical constitution of the substance.

The optical rotation is a degree of rotation of polarized plane, caused by the optically active substance or its solution, and it is measured by the polarimeter. The optical rotation is proportional to the length of the polarimeter tube, and is also related to the solution concentration, the temperature and the measurement wavelength. The character of the rotation is indicated by the direction of the rotation, when facing to the advancing direction of the polarized light. Thus in case of rotation to the right, it is called dextrorotatory and expressed by placing plus sign (+), while in case of rotation to the left, it is called levorotatory and expressed by placing minus sign (-) before the figure of the angular rotation. For example, +20° means 20° of rotation to the right, while

-20° means 20° of rotation to the left.

The optical rotation  $\alpha_x^t$  (°) means degree of rotation of the plate of polarization, when it is measured at  $t^\circ\text{C}$  by using specific monochromatic light  $x$  (expressed by wavelength of light source or the specific beam name).

#### 2. Apparatus and measurement

Polarimeter consists of a light source, a polarizer, a polarimeter tube and an analyzer. The measurement is generally performed at 20°C or 25°C, using a 100-mm tube and the D line of sodium lamp as the light source. The bright line spectrum of mercury lamp can be used as the monochromatic light source.

If a light beam close to the sodium D line can be obtained by the use of an appropriate interference filter, a continuous beam such as of xenon lamp may be used alternatively.

#### 2.1. Verification for accuracy of apparatus

Accuracy of the scale of the apparatus is verified by measuring the optical rotation of a solution of sucrose for optical rotation if the reading indicates the value of its known specific optical rotation. For daily verification an optical rotation known quartz plate may be used for this purpose.

#### 3. Characteristic evaluation by optical rotation

Generally, when the optical rotation is settled as the specification to express the quality characteristic of a drug, Specific optical rotation  $[\alpha]_x^t$  (°), i.e., the optical rotation equivalent of 1 g/mL in sample concentration and 1 mm in path length of sample solution, is used. For the evaluation of the quality characteristic of drugs such as crude drugs, when it is not possible to determine the concentration of an optically active drug, the optical rotation  $\alpha_x^t$  (°) is used as the specification or to specify the amount of optically active impurities.

The specific optical rotation and the optical rotation can also be used for the description, purity and assay of drugs.

The specific optical rotation,  $[\alpha]_x^t$ , is calculated from the measured rotation angle of the polarization plane,  $\alpha_x^t$ , by the following equation. Though, the degree (°) is expediently used as the unit of the specific optical rotation in the pharmaceutical monographs, accurately it is stated as  $(^\circ \cdot \text{mm}^{-1} \cdot (\text{g/mL})^{-1})$ .

$$[\alpha]_x^t = \frac{\alpha}{lc} \times 100$$

$t$ : The temperature ( $^\circ\text{C}$ ) of measurement.

$x$ : The wavelength (nm) of the specific monochromatic light. In the case of the sodium D line, it is described as D.

$\alpha$ : The angle, in degrees, of rotation of the plane of the polarized light.

$l$ : The thickness of the layer of sample solution, i.e., the length of the polarimeter tube (mm).

$c$ : Drug concentration in g/mL. When an intact liquid drug is used for the direct measurement without dilution by an appropriate solvent,  $c$  equals to its density (g/mL). However, unless otherwise specified, the specific gravity may be used instead of the density.

The description in the monograph, for example, “[ $\alpha]_{\text{D}}^{20}$ : -33.0 - -36.0° (after drying, 1 g, water, 20 mL, 100 mm),” means the measured specific optical rotation  $[\alpha]_{\text{D}}^{20}$  should be in the range of -33.0° and -36.0°, when 1 g of accurately weighed sample dried under the conditions, specified in the test item of Loss on drying, is taken, and dissolved in water to make exactly 20 mL, then put in the polarimeter tube of 100 mm length, of which temperature is kept at 20°C. And the description “ $\alpha_{\text{D}}^{20}$ : -33.0 - -36.0°

(100 mm)" means the measured optical rotation,  $\alpha_D^{20}$ , should be in the range of  $-33.0^\circ$  and  $-36.0^\circ$ , when sample or a solution of sample is put in the tube of 100 mm length, at  $20^\circ\text{C}$ .

## 2.50 Endpoint Detection Methods in Titrimetry

Titrimetry is a method or a procedure for volumetric analysis, which is usually classified into acid-base titration (neutralization titration or pH titration), precipitation titration, complexation titration, oxidation-reduction titration, etc., according to the kind of reaction or the nature of the phenomenon occurring between the titrate and the titrant (standard solution for volumetric analysis). Furthermore, titration performed in a nonaqueous solvent is generally called nonaqueous titration, which is frequently used for volumetric analysis of weak acids, weak bases, and their salts. The endpoint in titrimetry can be detected by color changes of indicators and/or by changes of electrical signals such as electrical potential or electrical current.

The indicator method is one of the endpoint detection methods in titrimetry. In this method the color of an indicator dye, dissolved in the titrate, changes dramatically in the vicinity of the equivalence point due to its physico-chemical character, and this property is used for visual endpoint detection. Selection of an indicator and specification of the color change induced in the respective titration system, should be described in the individual monograph. An appropriate indicator should change color clearly, in response to a slight change in physico-chemical properties of the titrate, such as pH, etc., in the vicinity of the equivalence point.

Regarding the electrical endpoint detection methods, there are an electrical potential method and an electrical current method, which are called potentiometric and amperometric titration methods, respectively. They are generically named electrometric titration. In the potentiometric titration method, the endpoint of a titration is usually determined to be the point at which the differential potential change becomes maximum or minimum as a function of the quantity of titrant added. In the amperometric titration method, unless otherwise specified, a bi-amperometric titration method is used, and the endpoint is determined by following the change of microcurrent during the course of a titration. Furthermore, the quantity of electricity (electrical current  $\times$  time) is often used as another electrochemical signal to follow a chemical reaction, as described in Coulometric Titration under Water Determination <2.48>.

The composition of a titration system, such as amount of specimen, solvent, standard solution for volumetric analysis, endpoint detection method, equivalent amount of substance to be examined (mg)/standard solution (mL), should be specified in the individual monograph. Standardization of the standard solution and titration of a specimen are recommended to be done at the same temperature. When there is a marked difference in the temperatures at which the former and the latter are performed, it is necessary to make an appropriate correction for the volume change of the standard solution due to the temperature difference.

### 1. Indicator Method

Weigh an amount of a specimen in a flask or a suitable vessel as directed in the monograph or in "Standard Solutions for Volumetric Analysis", and add a specified quantity

of solvent to dissolve the specimen. After adding a defined indicator to the solution to prepare the titrate, titrate by adding a standard solution for volumetric analysis by using a buret. In the vicinity of the endpoint, observe the color change induced by the cautious addition of 0.1 mL or less of the titrant. Calculate the quantity of titrant added from the readings on the scale of the buret used for the titration at the starting point and at the endpoint at which the specified color change appears, as directed in the individual monograph or in the "Standard Solutions for Volumetric Analysis". Although addition of the volumetric standard solution by buret is usually done manually, an automatic buret can also be used.

Unless otherwise specified, perform a blank determination according to the following method, and make any necessary correction.

Measure a specified quantity of solvent, as directed in the monograph or in the "Standard Solutions for Volumetric Analysis", and titrate as directed. The required quantity of the standard solution added to reach a specified color change, is assumed to be the blank quantity for the titration system. However, when the blank quantity is too small to evaluate accurately, the quantity can be assumed to be zero.

### 2. Electrical Endpoint Detection Methods

#### 2.1. Potentiometric titration

##### 2.1.1. Apparatus

The apparatus consists of a beaker to contain the specimen, a buret for adding a standard solution, an indicator electrode and a reference electrode, a potentiometer for measuring potential difference between the electrodes or an adequate pH meter, a recorder, and a stirrer for gentle stirring of the solution to be examined. Separately, an automatic titration apparatus assembled from suitable units and/or parts, including a data processing system, can also be used.

In this titration method, unless otherwise specified, indicator electrodes designated in Table 2.50-1 are used according to the kind of titration. As a reference electrode, usually a silver-silver chloride electrode is used. Besides the single indicator electrodes as seen in Table 2.50-1, a combined refer-

**Table 2.50-1** Kind of titration and indicator electrode

Kind of titration	Indicator electrode
Acid-base titration (Neutralization titration, pH titration)	Glass electrode
Precipitation titration (Titration of halogen ion by silver nitrate)	Silver electrode. A silver-silver chloride electrode is used as a reference electrode, which is connected with the titrate by a salt bridge of saturated potassium nitrate solution.
Oxidation-reduction titration (Diazo titration, etc.)	Platinum electrode
Complexation titration (Chelometric titration)	Mercury-mercury chloride (II) electrode
Nonaqueous titration (Perchloric acid titration, Tetramethylammonium hydroxide titration)	Glass electrode

ence electrode and indicator electrode can also be used.

When the potentiometric titration is carried out by the pH measurement method, the pH meter should be adjusted according to the pH Determination <2.54>.

### 2.1.2. Procedure

Weigh a defined amount of a specimen in a beaker, and add an indicated quantity of solvent to dissolve the specimen, as directed in the monograph. After the potential difference  $E$  (mV) or the pH value of the solvent to be used for titration has reached a stable value, immerse both reference and indicator electrodes, which have previously been washed with the solvent being used, in the solution to be examined, and titrate with a standard solution for volumetric analysis with gentle stirring of the solution. During the titration, the tip of the buret should be dipped into the solution, to be examined. The endpoint of titration is determined by following the variation of the potential difference between two electrodes as a function of the quantity of titrant added. In the vicinity of the endpoint, the amounts of a titrant added should be 0.1 mL or less for adequate titrimetry. Plot the obtained potential values along the ordinate and the quantity of a titrant added  $V$  (mL) along the abscissa to draw a titration curve, and obtain the endpoint from the maximum or the minimum value of  $\Delta E/\Delta V$  or from the value of electromotive force or pH corresponding to the equivalence point.

Unless otherwise specified, the decision of the endpoint in this method is usually made by either of the following methods.

#### (i) Drawing method

Usually, draw two parallel tangent lines with a slope of about  $45^\circ$  to the obtained titration curve. Next, draw a 3rd parallel line at the same distance from the previously drawn two parallel lines, and decide the intersection point of this line with the titration curve. Further, from the intersection point, draw a vertical line to the abscissa, and read the quantity of titrant added as the endpoint of the titration.

Separately, the endpoint of the titration can also be obtained from the maximum or the minimum of the differential titration curve ( $\Delta E/\Delta V$  vs.  $V$ ).

#### (ii) Automatic detection method

In the case of potentiometric titration using an automatic titration system, the endpoint can be determined by following the respective instrumental indications. The endpoint is decided either by following the variation of the differential potential change or the absolute potential difference as a function of the quantity of titrant added: in the former case the quantity given by the maximum or the minimum of the differential values, and in the latter the quantity given by the indicator reaching the endpoint potential previously set for the individual titration system, are assumed to be the endpoint volumes, respectively.

## 2.2. Amperometric titration

### 2.2.1. Apparatus

The apparatus consists of a beaker for holding a specimen, a buret for adding a standard solution for volumetric analysis, two small platinum plates or wires of the same shape as the indicator electrode, a device to load direct current microvoltage between two electrodes, a microammeter to measure the indicator current between the two electrodes, a recorder, and a stirrer which can gently stir the solution in a beaker. Separately, an automatic titration apparatus assembled from suitable units and/or parts, including a data processing system, can also be used.

#### 2.2.2. Procedure

Weigh a defined amount of a specimen in a beaker, and add an indicated quantity of solvent to dissolve the speci-

men, as directed in the individual monograph. Next, after washing the two indicator electrodes with water, immerse both electrodes in the solution to be examined, apply a constant voltage suitable for measurement across two electrodes by using an appropriate device, and titrate the solution with a standard solution for volumetric analysis. During the titration, the tip of the buret should be dipped into the solution to be examined. The endpoint of titration is determined by following the changes of microcurrent between the two electrodes as a function of the quantity of titrant added. In the vicinity of the endpoint, the amounts of the titrant added should be 0.1 mL or less for adequate titrimetry. Plot the obtained current values along the ordinate and the quantity of the titrant added  $V$  (mL) along the abscissa to draw a titration curve, and usually take the inflection point of the titration curve (the point of intersection given by the extrapolation of two straight lines before and after the inflection) as the endpoint in amperometric titration.

The blank test in this titration is usually performed as follows: Take a volume of the solvent specified in the individual monograph or in the "Standard Solution for Volumetric Analysis", and use this as the sample solution. Determine the amount of the volumetric standard solution needed for giving the endpoint, and use this volume as the blank. If this volume is too small to determine accurately, the blank may be considered as 0 (mL).

Unless otherwise specified, the endpoint in this titration is decided by either of the following methods.

#### (i) Drawing method

Usually, extrapolate the two straight lines before and after the inflection, and obtain the inflection point of the titration curve. Next, read the quantity of titrant added at the inflection point, and assume this point to be the endpoint.

#### (ii) Automatic detection method

In the case of amperometric titration using an automatic titration system, the endpoint can be determined by following the instrumental indications. The endpoint is decided by following the variation of the indicator current during the course of a titration, and the quantity of titrant added is assumed to be that at which the current has reached the endpoint current set previously for the individual titration system.

When atmospheric carbon dioxide or oxygen is expected to influence the titration, a beaker with a lid should be used, and the procedure should be carried out in a stream of an inert gas, such as nitrogen gas. Further, when a specimen is expected to be influenced by light, use a light-resistant container to avoid exposure of the specimen to direct sunlight.

## 2.51 Conductivity Measurement

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia.

The parts of the text that are not harmonized among the targeted texts for the harmonization are marked with symbols (♦ ♦), and the texts that are uniquely specified by the JP other than the targeted texts for the harmonization are marked with symbols (◊ ◊).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

This chapter provides information on how to apply electrical conductivity measurements (hereafter referred to as "conductivity") of fluid solutions, including pure fluids.

This chapter is intended for fluid applications when conductivity is used to measure, monitor, or control chemical dispensing, chemical purity, ionic concentration, and other applications where the ionic character of the fluid needs to be known or controlled.

Applications include, but are not limited to, solutions that may be used in clean-in-place, chromatography detection, ionic solution preparations, end point detection, dosing, fermentation, and buffer production.

In some cases, conductivity measurements can be extended to pure organic fluids such as alcohols and glycols where a weak conductivity signal exists, and the signal can be significantly increased if the organics become contaminated with water or salts.

Conductivity is the measurement of the ability of a fluid to conduct electricity via its chemical ions. The ability of any ion to electrically conduct is directly related to its ion mobility. Conductivity is directly proportional to the concentrations of ions in the fluid, according to Equation (1):

$$\kappa = 1000 \sum_i^{all \ ions} C_i \cdot \lambda_i \quad (1)$$

$\kappa$  = conductivity (S/cm)

$C_i$  = concentration of chemical ion  $i$  (mol/L)

$\lambda_i$  = specific molar conductance of ion  $i$  (S·cm<sup>2</sup>/mol)

Although the SI unit S/m is the appropriate SI unit for conductivity, historically the unit S/cm has been selected by industry as the accepted unit.

On the basis of Equation (1), conductivity is not ion selective because it responds to all ions. Furthermore, the specific molar conductance of each ion is different. As a result, unless the percentage composition of ions of the solution is limited and known, the precise concentrations of ionic species cannot be determined from conductivity measurements.

However, for examples such as a solution of a single salt or acid or base, such as a caustic solution used in cleaning, the precise concentration can be directly determined. Despite the lack of ionic specificity, conductivity is a valuable laboratory and process tool for measurement and control of total ionic content because it is proportional to the sum of the concentrations of all ionic species (anions and cations) for diluted solutions as described in Equation (1). At higher concentrations, conductivity measurements are not perfectly linear with concentration. Conductivity measurements cannot be applied to solids or gases, but they can be applied to the condensate of gases.

Another variable that influences conductivity measurements is the fluid temperature. As the fluid temperature increases, the ion conductance increases, making this physicochemical phenomenon the predominant reason for the temperature-compensation requirement when testing conductive fluids.

The conductivity,  $\kappa$ , is proportional to the conductance,  $G$  (S), of a fluid between two electrodes (Equation (2)):

$$\kappa = G \times (d/A) = G \times K \quad (2)$$

$\kappa$  = conductivity (S/cm)

$G$  = conductance (S)

$d$  = distance between the electrodes (cm)

$A$  = area of the conducting electrodes (cm<sup>2</sup>)

$K$  = cell constant (cm<sup>-1</sup>), which also equals the ratio of  $d/A$

The resistivity  $\rho$  (Ω·cm) of the fluid is, by definition, the reciprocal of the conductivity (Equation (3)):

$$\rho = 1/\kappa = 1/(G \times K) = R/K \quad (3)$$

$\rho$  = resistivity (Ω·cm)

$\kappa$  = conductivity (S/cm)

$G$  = conductance (S)

$K$  = cell constant (cm<sup>-1</sup>)

$R$  = resistance (Ω), which is the reciprocal of the conductance,  $G$

## 1. Apparatus

An electrical conductivity measurement consists of the determination of resistance of the fluid between and around the electrodes of the conductivity sensor. To achieve this measurement, the primary instrumentation is the resistance-measuring circuit and the conductivity sensor, and they are usually connected by a cable when the sensor and the user interface are separated.

The resistance measurement is made by applying an alternating current (AC, meaning the flow of electric charge periodically reverses direction) voltage (or current) to the electrodes, measuring the current (or voltage), and calculating the resistance according to Ohm's Law. The alternating source is used to prevent the polarization (collection of ions) at the electrodes. Depending on the instrument, the measuring frequency of the measuring system adjusts automatically according to the measuring conditions of the instrument, and there may be multiple resistance-measuring circuits embedded in the measuring system. The resistance-measurement circuit may be embedded in the transmitter or in the sensor.

The conductivity sensor consists of at least two electrical conductors of a fixed size and geometry, separated by an electrical insulator. The electrodes, insulator, and any other wetted materials should be constructed of materials that are unreactive to fluids with which they may come into contact. Also, the sensor construction should withstand the environmental conditions (process or ambient temperature, pressure, cleaning applications) that it would be subjected to.

Most conductivity sensors have temperature devices such as a platinum resistance temperature device (RTD) or negative temperature coefficient (NTC) thermistor embedded inside the sensor, although external temperature measurement is possible. The purpose of the temperature measurement is for temperature compensation of the conductivity measurement.

## 2. Cell Constant Determination

The purpose of the sensor's cell constant is to normalize the conductance (or resistance) measurement for the geometrical construction of the two electrodes.

The cell constant is determined by immersing the conductivity sensor in a solution of known conductivity.

Solutions of known conductivity can be obtained by preparation of specific mixtures according to national authoritative sources or procurement of commercially available certified and traceable standard solutions.

These recipes or certified solutions can range from 5 to 200,000 μS/cm, depending on the level of accuracy desired. Alternatively the cell constant is determined by comparison to other reference conductivity measuring systems (also available as an accredited calibration service). [Note—Conductivity measurements are not perfectly linear with concentration.]

The measured cell constant of the conductivity sensor must be within 5% of the nominal value indicated by the sensor certificate, unless otherwise prescribed.

Modern conductivity sensors normally do not change their cell constant over their lifetime. If a change of the cell constant is detected during calibration, a cleaning of the sensor is appropriate according to the manufacturer's recommenda-

tions. Following that, the calibration procedure should be repeated. Sometimes “memory effects” appear, particularly when changing from high to low concentrations if the sensor is not well flushed.

### 3. Calibration of Temperature

In addition to verifying the sensor's cell constant, the embedded temperature device (or external temperature device) should be appropriately calibrated for the application to apply the temperature compensation algorithm accurately. The temperature accuracy that is required depends on the criticality of the temperature to the application. An accuracy of  $\pm 1^\circ\text{C}$  typically suffices.

### 4. Calibration of Measurement Electronics

The measurement circuit of the system is fundamentally an AC resistance measuring device. Appropriate verification and/or calibration of the measuring circuit is required for measurement systems with signal transfer via analog cable. This is accomplished by disconnecting the measuring circuit from the sensor's electrodes, attaching traceable resistors of known value with the cable of the measurement system to the measuring circuit, and verifying that the measured resistance agrees with the resistor value to an acceptable level. A typical acceptance criterion for the resistance accuracy is  $<2\%$  of the reading at resistances  $>100\ \Omega$ , and increasing to  $5\%$  at lower resistances. However, the application criticality should ultimately determine the desired accuracy.

For conductivity systems that cannot have the resistance-measuring circuit disconnected from the electrodes (e.g., measurement circuit and electrodes in one mutual housing), it may be difficult to directly adjust or verify the circuit accuracy, depending on the sensor design. An alternative method of verifying the measurement system integrity is a system calibration according to the procedures for the cell constant determination for each measuring circuit that is intended to be used.

If verification/calibration of the sensor's cell constant, temperature device, and measuring circuit are done at the same service interval, the measuring circuit should be verified first, the temperature device next, and the cell constant last. Because all of these parameters are typically very stable due to modern electronics and stable sensor construction, frequent calibration (such as daily) is not usually required. Comparison to qualified reference systems is also a proper means of calibration. Calibration is performed at appropriate intervals as defined in the quality management system.

### 5. Temperature Compensation

Because the conductivity of a fluid is temperature dependent, temperature compensation of the conductivity measurement is necessary unless otherwise prescribed. An appropriate temperature compensation algorithm will ensure that changes in the conductivity measurement can be ascribed to concentration changes and not temperature changes. Conductivity measurements are normally referenced to  $25^\circ\text{C}$ . A common form of linear temperature compensation uses Equation (4):

$$\kappa_{25} = \frac{\kappa_T}{[1 + \alpha(T - 25)]} \quad (4)$$

$\kappa_{25}$  = conductivity compensated to  $25^\circ\text{C}$

$\kappa_T$  = conductivity at  $T$

$\alpha$  = temperature coefficient of the conductivity

$T$  = measured temperature

A temperature coefficient of  $2.1\%$  per  $1^\circ\text{C}$  is commonly used for many salt solutions. Most salt-based solutions have linear compensation factors ranging from  $1.9\%$  to  $2.2\%$  per

$1^\circ\text{C}$ . Depending on the fluid samples, other forms of temperature compensation may be appropriate. ◇ Non-linear temperature compensation will carry out temperature compensation using preprogrammed data in the instrument. Non-linear temperature compensation data for a variety of solutions is widely available, e.g. for natural waters, and for ultrapure water with traces of ammonia. ◇

In cases of very low conductivity ( $<10\ \mu\text{S}/\text{cm}$ ), such as purified water for cleaning/rinsing purpose, two compensations need to be made. One is for the intrinsic conductivity of water, and the other is for the other ionic species in water. These compensations are normally combined and embedded in the microprocessor-controlled conductivity measurement systems. This is not supplied in all conductivity measurement technologies.

### 6. Conductivity Measurement of Fluids

For off-line batch measurements, rinse the cleaned sensor with the fluid to be measured. Then immerse the sensor in the fluid to be measured, and record the temperature and the temperature-compensated conductivity as required. Be sure that the position of the sensor in the container does not affect the conductivity measurement, because the container walls can affect the measurement for some electrode designs.

For continuous on-line or in-line measurements, install the cleaned sensor into the pipe, tank, or other containment vessel, and flush, if necessary. Make sure proper installation procedures are applied to prevent bubbles or particles from collecting between the electrodes. Be sure that the position of the sensor in the pipe or tank does not affect the conductivity measurement, because the nearby surfaces can affect the measurement for some electrode designs.

Record the temperature and the temperature-compensated conductivity as required.

For all batch or continuous measurements, ensure that the wetted components of the sensor are compatible with the fluid and the temperature to be measured.

## 2.52 Thermal Analysis

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (♦ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Thermal analysis is a group of techniques in which the variation of a physical property of a substance is measured as a function of temperature. The most commonly used techniques are those which measure changes of mass or changes in energy of a sample of a substance.

These techniques have different applications:

- determination of phase changes,
- determination of changes in chemical composition,
- determination of purity.

◇ Among the below methods, Thermogravimetry can be used as an alternative method for “Loss on Drying <2.41>” or “Water Determination <2.48>”. However, it must be confirmed beforehand that no volatile component except for

water is included in the test specimen when Thermogravimetry is used as an alternative method for "Water Determination". ◇

### 1. Thermogravimetry

Thermogravimetry (TG) or Thermogravimetric Analysis (TGA) is a technique in which the mass of a sample of a substance is recorded as a function of temperature according to a controlled temperature programme.

#### 1.1. Instrument

The essential components of a thermobalance are a device for heating or cooling the substance according to a given temperature program, a sample holder in a controlled atmosphere, an electrobalance and an electronic output of the signal to a recorder or a computer.

#### 1.2. Temperature calibration

The temperature sensor close to or in contact with the sample is calibrated using the Curie temperature of a ferromagnetic substance such as nickel. In the case of an instrument capable of simultaneously conducting TG/TGA and Differential Thermal Analysis (DTA), the same certified reference materials as those for Differential Scanning Calorimetry (DSC) and DTA may be used, such as indium for thermal analysis, tin for thermal analysis and/or zinc (standard reagent).

#### 1.3. Calibration of the electrobalance

Place an appropriate quantity of ♦Calcium Oxalate Monohydrate for Calibration of Apparatus RS or ♦a suitable certified reference material in the sample holder and record the mass. Set the heating rate according to the manufacturer's instructions (e.g. 5°C/min) and start the temperature increase. Record the thermogravimetric curve as a graph with temperature, or time, on the abscissa, increasing from left to right, and mass on the ordinate, decreasing downwards. Stop the temperature increase at about 250°C. Measure the difference on the graph between the initial and final mass-temperature plateaux, or mass-time plateaux, which corresponds to the loss of mass. The declared loss of mass for the certified reference material is stated on the label.

#### 1.4. Method

Apply the same procedure to the substance to be examined, using the conditions prescribed in the monograph. Calculate the loss of mass of the substance to be examined from the difference measured in the graph obtained. Express the loss of mass as  $\Delta m/m$  (%). If the instrument is in frequent use, carry out temperature calibration regularly. Otherwise, carry out such checks before each measurement.

Since the conditions are critical, the following parameters are noted for each measurement: pressure or flow rate, composition of the gas, mass of the sample, heating rate, temperature range, sample pre-treatment including any isothermal period.

### 2. Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a technique that can be used to demonstrate the energy phenomena produced during heating (or cooling) of a substance (or a mixture of substances) and to determine the changes in enthalpy and specific heat and the temperatures at which these occur.

The technique is used to determine the difference in heat flow (with reference to the temperature) evolved or absorbed by the test sample compared with the reference cell, as a function of the temperature. Two types of DSC instruments are available, those using power compensation to maintain a null temperature difference between sample and reference and those that apply a constant rate of heating and detect temperature differential as a difference in heat flow between sample and reference.

#### 2.1. Instrument

The instrument for the power compensation DSC consists of a furnace containing a sample holder with a reference cell and a test cell. The instrument for the heat flow DSC consists of a furnace containing a single cell with a sample holder for the reference crucible and the test crucible.

A temperature-programming device, thermal detector(s) and a recording system which can be connected to a computer are attached. The measurements are carried out under a controlled atmosphere.

#### 2.2. Calibration of the instrument

Calibrate the instrument for temperature and enthalpy change, using suitable certified materials or reference standards.

##### 2.2.1. Temperature calibration

It can be performed using certified reference materials having an intrinsic thermal property, such as melting point of pure metals or organic substances, or phase transition point of crystalline inorganic salts or oxides. Melting points of indium for thermal analysis, tin for thermal analysis and/or zinc (standard reagent) are usually employed for calibration.

##### 2.2.2. Heat-quantity calibration

For accurate estimation of a quantity of heat change (enthalpic change) of a test sample, caused by a certain physical change accompanying a temperature change, it is necessary to calibrate the instrument using suitable certified reference materials. Similarly to temperature calibration, heat-quantity calibration may be performed using suitable certified reference materials showing a known definite enthalpic change caused by physical changes, such as melting of pure metals and/or organic substances, or phase transition of crystalline inorganic salts. Heats of fusion of indium for thermal analysis, tin for thermal analysis and/or zinc (standard reagent) are usually employed for calibration.

#### 2.3. Operating procedure

Weigh in a suitable crucible an appropriate quantity of the substance to be examined; place it in the sample holder. Place an empty crucible in the reference holder. Set the initial and final temperatures, and the heating rate according to the operating conditions prescribed in the monograph.

Begin the analysis and record the differential scanning calorimetric curve, with the temperature or time on the abscissa (values increasing from left to right) and the energy change on the ordinate (specify whether the change is endothermic or exothermic).

The temperature at which the phenomenon occurs (the onset temperature) corresponds to the intersection (A) of the extension of the baseline with the tangent at the point of greatest slope (inflection point) of the curve (see Fig. 2.52-1). The end of the thermal phenomenon is indicated by the peak of the curve.

The enthalpy of the phenomenon is proportional to the

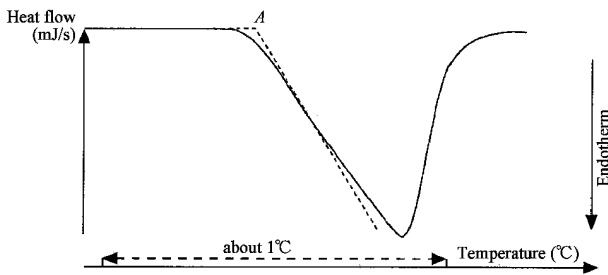


Fig. 2.52-1 Thermogram

**Table 2.52-1**

solid-solid transition	Allotropy-polymorphism desolvation amorphous-crystalline
solid-liquid transition	Melting glass transition
solid-gas transition	sublimation
liquid-solid transition	freezing recrystallisation glass transition
liquid-gas transition	evaporation

area under the curve limited by the baseline; the proportionality factor is determined from the measurement of the heat of fusion of a known substance (e.g., indium for thermal analysis) under the same operating conditions.

Each thermogram may be accompanied by the following data: conditions employed, record of last calibration, mass of the sample and identification (including thermal history), container, atmosphere (identity, flow rate, pressure), direction and rate of temperature change, instrument and recorder sensitivity.

## 2.4. Applications

### 2.4.1. Phase changes

Determination of the temperature, heat capacity change and enthalpy of phase changes undergone by a substance as a function of temperature. The transitions that may be observed include those shown in Table 2.52-1.

### 2.4.2. Changes in chemical composition

Measurement of heat and temperatures of reaction under given experimental conditions, so that, for example, the kinetics of decomposition or of desolvation can be determined.

### 2.4.3. Application to phase diagrams

Establishment of phase diagrams for solid mixtures. The establishment of a phase diagram may be an important step in the preformulation and optimization of the freeze-drying process.

### 2.4.4. Determination of purity

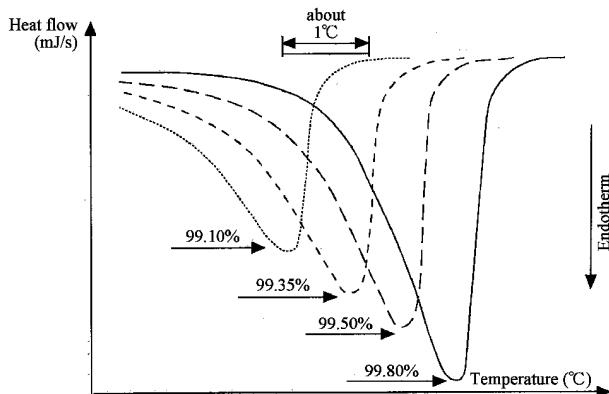
The measurements of the fraction of substance melted at a temperature and the heat of fusion by DSC enable the impurity content of a substance to be determined from a single thermal diagram, requiring the use of only a few milligrams of sample with no need for repeated accurate measurements of the true temperature.

In theory, the melting of an entirely crystalline, pure substance at constant pressure is characterized by a heat of fusion  $\Delta H_f$  in an infinitely narrow range, corresponding to the melting point  $T_0$ . A broadening of this range is a sensitive indicator of impurities. Hence, samples of the same substance, whose impurity contents vary by a few tenths of a per cent, give thermal diagrams that are visually distinct (see Fig. 2.52-2).

The determination of the molar purity by DSC is based on the use of a mathematical approximation of the integrated form of the van't Hoff equation applied to the concentrations (not the activities) in a binary system.

$$[\ln(1 - x_2) \approx -x_2 \text{ and } T \times T_0 \approx T_0^2]$$

For low amounts of impurities  $x_2 \ll 1$  and for temperatures close to the melting point  $T_0$  the equation can be written as follows, in which  $T$  and  $x_2$  are variables:

**Fig. 2.52-2** Thermal diagrams according to purity

$$T = T_0 - \frac{RT_0^2}{\Delta H_f} \times x_2 \quad (1)$$

$T$ : temperature of the sample, in kelvins,

$T_0$ : melting point of the chemically pure substance, in kelvins,

$R$ : gas constant for ideal gases, in joules-kelvin<sup>-1</sup>·mole<sup>-1</sup>,

$\Delta H_f$ : molar heat of fusion of the pure substance, in joules·mole<sup>-1</sup>,

$x_2$ : mole fraction of the impurity i.e. the number of molecules of the impurity divided by the total number of molecules in the liquid phase (or molten phase) at temperature  $T$  (expressed in kelvins),

Hence, the determination of purity by DSC is limited to the detection of impurities forming a eutectic mixture with the principal compound and present at a mole fraction of typically less than 2 per cent in the substance to be examined.

This method cannot be applied to:

- amorphous substances,
- solvates or polymorphic compounds that are unstable within the experimental temperature range,
- impurities forming solid solutions with the principal substance,
- impurities that are insoluble in the liquid phase or in the melt of the principal substance.

During the heating of the substance to be examined, the impurity melts completely at the eutectic temperature. Above this temperature, the solid phase contains only the pure substance. As the temperature increases progressively from the eutectic temperature to the melting point of the pure substance, the mole fraction of impurity in the liquid decreases, since the quantity of liquefied pure substance increases. For all temperatures above the eutectic point:

$$x_2 = \frac{1}{F} \times x_2^* \quad (2)$$

$F$ : molten fraction of the analyzed sample,

$x_2^*$ : mole fraction of the impurity in the analyzed sample.

When the entire sample has melted,  $F = 1$  and  $x_2 = x_2^*$ .

If equation (2) is combined with equation (1), the following equation is obtained:

$$T = T_0 - \frac{RT_0^2}{\Delta H_f} \times \frac{1}{F} \times x_2^*$$

The value of the heat of fusion of the pure substance is obtained by integrating the melting peak. The melting point  $T_0$  of the pure substance is extrapolated from the plot of temperature  $T$ , expressed in kelvins versus  $1/F$ . The slope  $\alpha$  of

the curve, obtained after linearization, if necessary, corresponding to  $RT_0^2x_2^*/\Delta H_f$  allows  $x_2^*$  to be evaluated. The fraction  $x_2^*$  multiplied by 100 gives the mole fraction in per cent for the total eutectic impurities.

## 2.53 Viscosity Determination

Viscosity Determination is a method to determine the viscosity of liquid samples using a viscometer.

When a liquid moves in a definite direction, and the liquid velocity has a gradient with respect to the direction rectangular to that of flow, a force of internal friction is generated along both sides of a hypothetical plane parallel to the movement. This flow property of a liquid is expressed in terms of viscosity. The internal friction per unit area on the parallel plane is called slip stress or shear stress, and the velocity gradient with respect to the direction rectangular to that of flow is called slip velocity or shear velocity. A liquid of which the slip velocity is proportional to its slip stress is called a Newtonian liquid. The proportionality constant,  $\eta$ , is a characteristic of a liquid at a certain temperature and is called viscosity. The viscosity is expressed in the unit of Pascal second (Pa·s), and usually milli-Pascal second (mPa·s).

A liquid whose slip velocity is not proportional to its slip stress is called a non-Newtonian liquid. Since the viscosity for a sample of a non-Newtonian liquid changes with its slip velocity, the viscosity measured at a certain slip velocity is called an apparent viscosity. In that case, the value of slip stress divided by the corresponding slip velocity is called an apparent viscosity. Thus, the relationship between apparent viscosity and slip velocity will permit characterization of the flow properties of a given non-Newtonian liquid.

The value of the viscosity,  $\eta$ , divided by the density,  $\rho$ , at the same temperature is defined as a kinematic viscosity,  $v$ , which is expressed in the unit of meters squared per second ( $m^2/s$ ), and usually millimeters squared per second ( $mm^2/s$ ).

The viscosity of a liquid is determined either by the following *Method I* or *Method II*.

### 1. Method I Viscosity measurement by capillary tube viscometer

For measuring the viscosity of a Newtonian liquid, a capillary tube viscometer is usually used, in which the downflowing time of a liquid,  $t$  (s), required for a definite volume of the liquid to flow through a capillary tube is measured and the kinematic viscosity,  $v$ , is calculated according to the following equation.

$$v = Kt$$

Further, the viscosity,  $\eta$ , is calculated from the next equation:

$$\eta = vp = Ktp$$

where  $\rho$  (g/mL) is the density of the liquid measured at the same temperature,  $t$  ( $^{\circ}C$ ).

The parameter  $K$  ( $mm^2/s^2$ ) represents the viscometer constant and is previously determined by using the *Standard Liquids for Calibrating Viscometers* with known kinematic viscosity. In the case of a liquid having a similar viscosity to water, water itself can be used as a reference standard liquid for the calibration. The kinematic viscosity of water is  $1.0038 \text{ mm}^2/\text{s}$  at  $20^{\circ}\text{C}$ . In the cases of liquids having a slightly higher viscosity than water, the *Standard Liquids for Calibrating Viscometers* should be used for the calibration.

The intrinsic viscosity,  $[\eta]$  (dL/g), of a polymer solution is

obtained by plotting the relation of viscosity versus concentration and extrapolating the obtained straight line to zero concentration. Intrinsic viscosity shows the degree of molecular expansion of a polymer substance in a given solvent (sample solution) and is also a measure of the average molecular mass of the polymer substance.

The downflowing time  $t$  (s) for a polymer solution, whose concentration is  $c$  (g/dL), and  $t_0$  (s) for the solvent used for dissolving the polymer, are measured by using the same viscometer, and then the intrinsic viscosity of a given polymer substance,  $[\eta]$ , is calculated according to the following equation:

$$[\eta] = \lim_{c \rightarrow 0} \frac{\left( \frac{t}{t_0} - 1 \right)}{c} \quad \text{or} \quad [\eta] = \lim_{c \rightarrow 0} \frac{\ln \frac{t}{t_0}}{c}$$

When the concentration dependency of  $\{(t/t_0) - 1\}/c$  is not large, the value of  $\{(t/t_0) - 1\}/c$  at a concentration directed in the respective monograph can be assumed to be the intrinsic viscosity for a given substance.

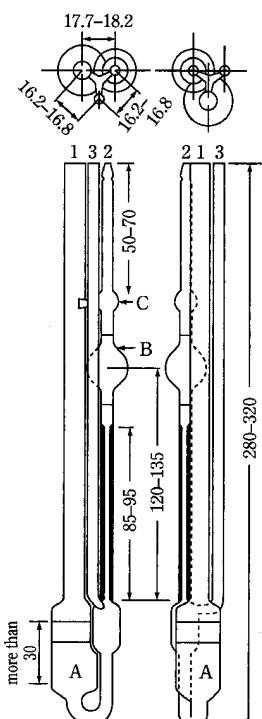
Unless otherwise specified, the viscosity of a sample solution is measured with the following apparatus and procedure.

### 1.1. Apparatus

For measurement of the kinematic viscosity in the range of 1 to 100,000  $\text{mm}^2/\text{s}$ , the Ubbelohde-type viscometer illustrated in Fig. 2.53-1 can be used. The approximate relations between kinematic viscosity range and inside diameter of the capillary tube suitable for the measurement of various liquids with different viscosity, are given in Table 2.53-1. Although a capillary tube viscometer other than the Ubbelohde-type one specified in Table 2.53-1 can also be used, a viscometer should be selected in which the downflowing time,  $t$  (s), of a sample solution to be determined would be between 200 s and 1000 s.

### 1.2. Procedure

Place a sample solution in a viscometer from the upper



The figures are in mm.

Fig. 2.53-1 Ubbelohde-type viscometer

**Table 2.53-1** Specifications of the Ubbelohde-type viscometer

Viscometer constant $K$ (mm $^2$ /s $^2$ )	Inner diameter of capillary tube (mm) Permissible tolerance $\pm 10\%$	Volume of bulb B (mL) Permissible tolerance $\pm 10\%$	Measuring range of kinematic viscosity (mm $^2$ /s)	
0.005	0.46	3.0	1-	5
0.01	0.58	4.0	2-	10
0.03	0.73	4.0	6-	30
0.05	0.88	4.0	10-	50
0.1	1.03	4.0	20-	100
0.3	1.36	4.0	60-	300
0.5	1.55	4.0	100-	500
1.0	1.83	4.0	200-	1,000
3.0	2.43	4.0	600-	3,000
5.0	2.75	4.0	1,000-	5,000
10.0	3.27	4.0	2,000-	10,000
30.0	4.32	4.0	6,000-	30,000
50.0	5.20	5.0	10,000-	50,000
100	6.25	5.0	20,000-	100,000

end of *tube 1*, so that the meniscus of the solution is at a level between the two marked lines of *bulb A*. Place the viscometer vertically in a thermostatted bath maintained at a specified temperature within 0.1°C, until *bulb C* is fully immersed, and let it stand for about 20 minutes to attain the specified temperature. Close *tube 3* with a finger and pull the sample solution up to the middle part of *bulb C* by gentle suction from the top of *tube 2*, taking care not to introduce any bubbles into *tube 2*, and stop the suction. Open the end of *tube 3*, and immediately close the end of *tube 2*. After confirming that the liquid column is cut off at the lowest end of the capillary tube, open the end of *tube 2* to make the sample solution flow down through the capillary tube. Record the time,  $t$  (s), required for the meniscus of the sample solution to fall from the upper to the lower marked line of *bulb B*.

Determine the viscometer constant  $K$  previously, using the *Standard Liquids for Calibrating Viscometers* under the same conditions. The temperature at which the calibration is conducted must be identical with that specified in the monograph.

## 2. Method II Viscosity measurement by rotational viscometer

A rotational viscometer is usually used for measuring the viscosity of Newtonian or non-Newtonian liquids. The measuring principle of a rotational viscometer generally consists in the detection and determination of the force acting on a rotor (torque), when it rotates at a constant angular velocity in a liquid. The extent of torque generated by the rotation can be detected in terms of the torsion of a spring and the liquid viscosity is calculated from the scale-indicated value corresponding to the degree of torsion.

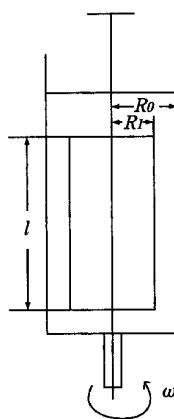
The viscosity of a sample solution is measured with the following apparatus and procedure.

### 2.1. Apparatus

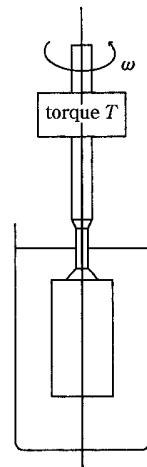
Viscosity measurement is performed by using any one of the following three types of rotational viscometers.

#### 2.1.1. Coaxial double cylinder-type rotational viscometer (Couette type viscometer)

In the coaxial double cylinder-type rotational viscometer, viscosity is determined by placing a liquid in the gap between the inner and the outer cylinders, which share the same cen-



**Fig. 2.53-2a** Coaxial double cylinder-type rotational viscometer



**Fig. 2.53-2b** Single cylinder-type rotational viscometer

tral axis and rotate separately, and the generated torque acting on one cylinder surface when the other cylinder is rotated, and the corresponding angular velocity, are measured.

As shown in Fig. 2.53-2a, the inner cylinder is hung by a wire whose twist constant is designated as  $k$ . In Fig. 2.53-2a, half the outer diameter of the inner cylinder and inner diameter of the outer cylinder are designated as  $R_i$  and  $R_o$ , respectively, and the length of the inner cylinder immersed in a liquid is designated as  $l$ . When a liquid is introduced into the gap between the two cylinders, and the outer cylinder is made to rotate at a constant angular velocity,  $\omega$ , the inner cylinder is also forced to rotate due to the viscosity of the liquid. Consequently, torque,  $T$ , is generated by the forced rotation in a viscous liquid, and in the steady state the torque is balanced by the torsion of the wire, as indicated by the degree of rotation  $\theta$ . Then, the relationship can be expressed by  $T = k\theta$  and the viscosity of a liquid,  $\eta$ , is determined from the following equation by measuring the relationship between  $\omega$  and  $\theta$ . Conversely, viscosity measurement can also be performed by rotating the inner cylinder, and the same relationship holds.

$$\eta = \frac{100T}{4\pi l\omega} \left[ \frac{1}{R_i^2} - \frac{1}{R_o^2} \right]$$

$\eta$ : Viscosity of a liquid (mPa·s)

$\pi$ : Circumference/diameter ratio

$l$ : Length of the inner cylinder (cm)

$\omega$ : Angular velocity (rad/s)

$T$ : Torque acting on cylinder surface ( $10^{-7}$  N·m)  
 $R_i$ : 1/2 of outer diameter of the inner cylinder (cm)  
 $R_o$ : 1/2 of inner diameter of the outer cylinder (cm)

### 2.1.2. Single cylinder-type rotational viscometer (Brookfield type viscometer)

In the single cylinder-type rotational viscometer, viscosity is determined by measuring the torque acting on the cylinder surface when the cylinder immersed in a liquid is rotated at a given angular velocity. Use an apparatus of the type illustrated in Fig. 2.53-2b. If the apparatus constant  $K_B$  is previously determined experimentally by using the *Standard Liquids for Calibrating Viscometers*, the viscosity of a liquid,  $\eta$ , can be obtained from the following equation.

$$\eta = K_B \frac{T}{\omega}$$

where,  $\eta$ : viscosity of a liquid (mPa·s)

$K_B$ : Apparatus constant of viscometer (rad/cm<sup>3</sup>)

$\omega$ : Angular velocity (rad/s)

$T$ : Torque acting on cylinder surface ( $10^{-7}$  N·m)

### 2.1.3. Cone-flat plate-type rotational viscometer (Cone-plate type viscometer)

In the cone-flat plate-type rotational viscometer, viscosity is determined by placing a liquid in the gap between a flat disc and a cone with a large vertical angle sharing the same rotational axis, and the torque and the corresponding angular velocity are measured, when either the disc or the cone is rotated in a viscous liquid.

As shown in Fig. 2.53-2c, a liquid is introduced to fill the gap between a flat disc and a cone forming an angle  $\alpha$ (rad). When either the flat disc or the cone is rotated at a constant angular velocity or a constant torque, the torque acting on the disc or cone surface rotated by the viscous flow and the corresponding angular velocity in the steady state, are measured. The viscosity of the liquid,  $\eta$ , can be calculated from the following equation.

$$\eta = \frac{3\alpha}{2\pi R^3} \cdot \frac{100T}{\omega}$$

$\eta$ : Viscosity of a liquid (mPa·s)

$\pi$ : Circumference/diameter ratio

$R$ : Radius of cone (cm)

$\alpha$ : Angle between flat disc and cone (rad)

$\omega$ : Angular velocity (rad/s)

$T$ : Torque acting on flat disc or cone surface ( $10^{-7}$  N·m)

## 2.2. Procedure

Set up the viscometer so that its rotational axis is perpendicular to the horizontal plane. Place a sufficient quantity of a sample solution in the viscometer, and allow the measuring system to stand until a specified temperature is attained, as

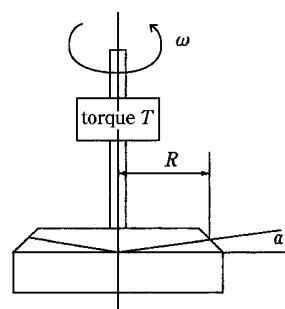


Fig. 2.53-2c Cone-flat plate-type rotational viscometer

directed in the monograph. Where it is desired to measure the viscosity within a precision of 1%, measuring temperature should be controlled within 0.1°C. Next, after confirming that the sample solution is at the designated temperature, start operating the rotational viscometer. After the forced rotation induced by the viscous flow has reached a steady state and the indicated value on the scale, which corresponds to the rotational frequency or the torque, has become constant, read the value on the scale. Then, calculate the viscosity  $\eta$  by using the respective equation appropriate to the type of viscometer being used. Determination or confirmation of the apparatus constant should be conducted beforehand by using the *Standard Liquids for Calibrating Viscometers*, and the validation of the apparatus and operating procedure should also be performed by using those standard liquids.

In the case of a non-Newtonian liquid, repeat the procedure for measuring the viscosity of the liquid with variation of the rotation velocity or torque from one measurement to another. From a series of such viscosity measurements, the relationship between the slip velocity and the slip stress of a non-Newtonian liquid, i.e., the flow characteristics of a non-Newtonian liquid, can be obtained.

Calibration of a rotational viscometer is conducted by using water and the *Standard Liquids for Calibrating Viscometers*. These standard liquids are used for the determination or confirmation of the apparatus constant of the rotational viscometer. They are also used for periodic recalibration of the viscometer to confirm maintenance of a specified precision.

## 2.54 pH Determination

pH is defined as the reciprocal of the common logarithm of hydrogen ion activity, which is the product of hydrogen ion concentration and the activity coefficient. Conventionally it is used as a scale of hydrogen ion concentration of a sample solution.

pH of a sample solution is expressed by the following equation in relation to the pH of a standard solution (pHs), and can be measured by a pH meter using a glass electrode.

$$\text{pH} = \text{pHs} + \frac{E - E_s}{2.3026 RT/F}$$

pHs: pH value of a pH standard solution.

E: Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a sample solution:

Glass electrode | sample solution | reference electrode

$E_s$ : Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a pH standard solution:

Glass electrode | standard pH solution | reference electrode

R: Gas constant

T: Absolute temperature

F: Faraday's constant

The value of 2.3026  $RT/F$  (V) in the above equation means the degree of electromotive force (V) per one pH unit and it is dependent on the temperature as shown in Table 2.54-1:

### 1. pH Standard solution

The pH standard solutions are used as a standard of pH,

**Table 2.54-1** Temperature dependency of the electromotive force (V)

Temperature of solution (°C)	2.3026 RT/F (V)	Temperature of solution (°C)	2.3026 RT/F (V)
5	0.05519	35	0.06114
10	0.05618	40	0.06213
15	0.05717	45	0.06313
20	0.05817	50	0.06412
25	0.05916	55	0.06511
30	0.06015	60	0.06610

**Table 2.54-2** pH values of six pH standard solutions

Temper-ature (°C)	Oxalate pH standard solution	Phthalate pH standard solution	Phosphate pH standard solution	Borate pH standard solution	Carbonate pH standard solution	Calcium hydroxide pH standard solution
0	1.67	4.01	6.98	9.46	10.32	13.43
5	1.67	4.01	6.95	9.39	10.25	13.21
10	1.67	4.00	6.92	9.33	10.18	13.00
15	1.67	4.00	6.90	9.27	10.12	12.81
20	1.68	4.00	6.88	9.22	10.07	12.63
25	1.68	4.01	6.86	9.18	10.02	12.45
30	1.69	4.01	6.85	9.14	9.97	12.30
35	1.69	4.02	6.84	9.10	9.93	12.14
40	1.70	4.03	6.84	9.07		11.99
50	1.71	4.06	6.83	9.01		11.70
60	1.73	4.10	6.84	8.96		11.45

for standardization of a pH meter. To prepare the pH standard solutions, use distilled water or water with a conductivity not more than  $2 \mu\text{S} \cdot \text{cm}^{-1}$  ( $25^\circ\text{C}$ ) and an organic carbon not more than  $0.50 \text{ mg/L}$ , boiled for not less than 15 minutes and cooled in a container fitted with a carbon dioxide-absorbing tube (soda lime). Next, prepare individually 6 kinds of pH standard solutions shown in Table 2.54-2.

Store the pH standard solutions in hard glass or polyethylene bottles. For storage of alkaline pH standard solutions, it is preferable to use a bottle fitted with a carbon dioxide-absorbing tube. Since the pH may change gradually during storage over a long period, it is necessary to ascertain whether the expected pH value is held or not by comparison with newly prepared standard, when the solution is used after long storage.

(i) Oxalate pH standard solution—Pulverize potassium trihydrogen dioxalate dihydrate for pH determination, and dry in a desiccator (silica gel). Weigh  $12.71 \text{ g}$  (0.05 mole) of it accurately, and dissolve in water to make exactly  $1000 \text{ mL}$ .

(ii) Phthalate pH standard solution—Pulverize potassium hydrogen phthalate for pH determination, and dry at  $110^\circ\text{C}$  to constant mass. Weigh  $10.21 \text{ g}$  (0.05 mole) of it accurately, and dissolve in water to make exactly  $1000 \text{ mL}$ .

(iii) Phosphate pH standard solution—Pulverize potassium dihydrogen phosphate for pH determination and disodium hydrogen phosphate for pH determination, and dry at  $110^\circ\text{C}$  to constant mass. Weigh  $3.40 \text{ g}$  (0.025 mole) of potassium dihydrogen phosphate and  $3.55 \text{ g}$  (0.025 mole) of disodium hydrogen phosphate accurately, and dissolve in water to make exactly  $1000 \text{ mL}$ .

(iv) Borate pH standard solution—Allow sodium tetraborate for pH determination to stand in a desiccator (saturated sodium bromide aqueous solution) until it reaches constant mass. Weigh  $3.81 \text{ g}$  (0.01 mole) of it accurately, and dissolve in water to make exactly  $1000 \text{ mL}$ .

(v) Carbonate pH standard solution—Dry sodium hydrogen carbonate for pH determination in a desiccator (silica gel) to constant mass, and weigh  $2.10 \text{ g}$  (0.025 mole) of it accurately. Dry sodium carbonate for pH determination between  $300^\circ\text{C}$  and  $500^\circ\text{C}$  to constant mass, and weigh  $2.65 \text{ g}$  (0.025 mole) of it accurately. Dissolve both reagents in water to make exactly  $1000 \text{ mL}$ .

(vi) Calcium hydroxide pH standard solution—Reduce calcium hydroxide for pH determination to a fine powder, transfer  $5 \text{ g}$  to a flask, add  $1000 \text{ mL}$  of water, shake well, and allow the solution to become saturated at a temperature between  $23^\circ\text{C}$  and  $27^\circ\text{C}$ . Then filter the supernatant at the same temperature and use the clear filtrate (about  $0.02 \text{ mol/L}$ ).

The pH values of these pH standard solutions at various temperatures are shown in the Table 2.54-2. pH values at an arbitrary temperature not indicated in Table 2.54-2 can be calculated by the interpolation method.

## 2. Apparatus

A pH meter generally consists of an electrode system of a glass electrode and a reference electrode, an amplifier and an indicating unit for controlling the apparatus and for displaying the measured value of electromotive force. The indicating unit is usually fitted with dials for zero and span (sensitivity) adjustment. Sometimes a temperature compensation dial is included.

The reproducibility of a pH meter should be within  $0.05 \text{ pH unit}$ , when measurements for an arbitrary pH standard solution are repeated five times, following the procedure described below. After each measurement it is necessary to wash the detecting unit well with water.

## 3. Procedure

Immerse the glass electrode previously in water for more than several hours. Start the measurement after confirming stable running of the apparatus. Rinse well the detecting unit with water, and remove the remaining water gently with a piece of filter paper.

To standardize the pH meter, two pH standard solutions are usually used as follows. Immerse the detection unit in the phosphate pH standard solution and adjust the indicated pH to the pH value shown in the Table. Next, immerse the detection system in the second pH standard solution, which should be selected so that the expected pH of the sample solution to be determined is between the pH values of the two pH standard solutions, and measure the pH under the same conditions as used for the first pH standard solution. Adjust the indicated pH to the defined pH value using the span adjustment dial, when the observed pH is not identical with that tabulated. Repeat the above standardization procedure until both pH standard solutions give observed pH values within  $0.05 \text{ pH unit}$  of the tabulated value without further adjustments. When a pH meter is fitted with a temperature compensation dial, the standardization procedure is done after the setting of the temperature to that of the pH standard solution to be measured.

In the case of using an apparatus having an auto-calibration function, it is necessary to confirm periodically that the pH values of two pH standard solutions are identical with the tabulated values within  $0.05 \text{ pH unit}$ .

After finishing the standardization procedure described above, rinse well the electrodes with water, remove the attached water using a filter paper, immerse the electrode system in the sample solution, and read the indicated pH value after confirming the value is stable. If necessary, a sample solution can be agitated gently.

In the pH determination, the temperature of a sample so-

lution must be controlled to be the same as that of the pH standard solutions with which the pH meter was standardized (within 2°C). When a sample solution is alkaline, the measurement should be done in a vessel with a cover and if necessary, in a stream of inert gas such as nitrogen. Furthermore for a strongly alkaline solution above pH 11 containing alkali metal ions, an alkali error may be induced in the pH measurement. Thus, in such a case, an electrode with less alkali error should be used and an appropriate correction should be applied to the measured value.

#### 4. Note

Construction and treatment in detail are different for different pH meters.

## 2.55 Vitamin A Assay

Vitamin A Assay is a method to determine vitamin A in Retinol Acetate, Retinol Palmitate, Vitamin A Oil, Cod Liver Oil and other preparations. Method 1 is for the assay of synthetic vitamin A esters, using the ultraviolet-visible spectrophotometry (Method 1-1) or the liquid chromatography (Method 1-2). Method 2 is for the assay of vitamin A of natural origin, containing many geometrical isomers, using the ultraviolet-visible spectrophotometry to determine vitamin A as vitamin A alcohol obtained by saponification in an alkaline solution and extraction.

One Vitamin A Unit (equal to 1 vitamin A I.U.) is equivalent to 0.300 µg of vitamin A (all-trans vitamin A alcohol).

#### 1. Procedure

All procedures should be carried out quickly and care should be taken as far as possible to avoid exposure to light, air, oxidants, oxidizing catalysts (e.g. copper, iron), acids and heat. If necessary, light-resistant vessels may be used.

Generally, for synthetic vitamin A esters apply Method 1-1 or Method 1-2, but if the assay conditions required for Method 1-1 are not suitable, apply Method 2.

#### 1.1. Method 1-1

Weigh accurately about 0.1 g of the sample, and dissolve in 2-propanol for vitamin A assay to make exactly 50 mL. Dilute this solution with 2-propanol for vitamin A assay to make a solution so that each mL contains 10 to 15 vitamin A Units, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution between 220 nm and 400 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> to obtain the wavelength of the maximum absorption and the absorbances at 300 nm, 310 nm, 320 nm, 326 nm, 330 nm, 340 nm and 350 nm. When the maximum absorption lies between 325 nm and 328 nm, and the ratios,  $A_{\lambda_i}/A_{326}$ , of each absorbance,  $A_{\lambda_i}$ , at 300 nm, 310 nm, 320 nm, 330 nm, 340 nm and 350 nm to the absorbance,  $A_{326}$ , at 326 nm are within the range of  $\pm 0.030$  of the values in the Table 2.55-1, the potency of vitamin A in Units per g of the sample is calculated from the following equation.

$$\text{Units of vitamin A in 1 g} = \frac{A_{326}}{M} \times \frac{V}{100} \times 1900$$

$A_{326}$ : Absorbance at 326 nm

$V$ : Total volume (mL) of the sample solution

$M$ : Amount (g) of sample in  $V$  mL of the sample solution

1900: Conversion factor from specific absorbance of retinol ester to IU (Unit/g)

This method is applied to drugs or preparations containing vitamin A esters (retinol acetate or retinol palmitate) as the

**Table 2.55-1** Absorbance ratio,  $A_{\lambda_i}/A_{326}$ , of retinol acetate and retinol palmitate

$\lambda_i$ (nm)	$A_{\lambda_i}/A_{326}$	
	Retinol acetate	Retinol palmitate
300	0.578	0.590
310	0.815	0.825
320	0.948	0.950
330	0.972	0.981
340	0.786	0.795
350	0.523	0.527

main component. However, when the wavelength of maximum absorption does not lie between 325 nm and 328 nm, or when the absorbance ratio  $A_{\lambda_i}/A_{326}$  is not within the range of  $\pm 0.030$  of the values in Table 2.55-1, apply Method 2.

#### 1.2. Method 1-2

Proceed with an appropriate amount of sample as directed under Liquid Chromatography <2.01>.

For the assay of retinol acetate and retinol palmitate use Retinol Acetate Reference Standard and Retinol Palmitate Reference Standard, respectively, and fix appropriately the operating procedure, the operating conditions and the system suitability based on the characteristics of the substance to be tested and the species and amount of coexisting substances.

#### 1.3. Method 2

Unless otherwise specified, weigh accurately a sample containing not less than 500 Units of vitamin A, and not more than 1 g of fat, transfer to a flask, and add 30 mL of aldehyde-free ethanol and 1 mL of a solution of pyrogallol in ethanol (95) (1 in 10). Then add 3 mL of a solution of potassium hydroxide (9 in 10), attach a reflux condenser, and heat on a water bath for 30 minutes to saponify. Cool quickly to ordinary temperature, add 30 mL of water, transfer to a separator A, wash the flask with 10 mL of water and then 40 mL of diethyl ether, transfer the washings to the separator A, shake well, and allow to stand. Transfer the water layer so obtained to a separator B, wash the flask with 30 mL of diethyl ether, add the washing to the separator B, and extract by shaking. Transfer the water layer to a flask, add the diethyl ether layer to the separator A, transfer the water layer in the flask to the separator B, add 30 mL of diethyl ether, and extract by shaking. Transfer the diethyl ether layer so obtained to the separator A, add 10 mL of water, allow the separator A to stand after gentle turning upside-down 2 or 3 times, and remove the water layer. Wash the content of the separator A with three 50-mL portions of water with increasingly vigorous shaking as the washing proceeds. Further wash with 50-mL portions of water until the washing no longer shows a pink color with phenolphthalein TS, and allow to stand for 10 minutes. Remove remaining water as far as possible, transfer the diethyl ether to an Erlenmeyer flask, wash the separator with two 10-mL portions of diethyl ether, add the washings to the flask, add 5 g of anhydrous sodium sulfate to the flask, mix by shaking, and transfer the diethyl ether to a round-bottomed flask by decantation. Wash the remaining sodium sulfate in the flask with two or more 10-mL portions of diethyl ether, and transfer the washings to the flask. Evaporate the diethyl ether in a water bath at 45°C while swirling the flask, using an aspirator, to about 1 mL, immediately add an exactly measured amount of 2-propanol for vitamin A assay to make a solution containing 6 to 10 vitamin A Units per mL, and desig-

nate the solution as the sample solution. Determine the absorbances,  $A_{310}$  at 310 nm,  $A_{325}$  at 325 nm, and  $A_{334}$  at 334 nm, of the sample solution as directed under Ultraviolet-visible Spectrophotometry.

Units of vitamin A in 1 g of the sample

$$= \frac{A_{325}}{M} \times \frac{V}{100} \times f \times 1830$$

$$f = 6.815 - 2.555 \times \frac{A_{310}}{A_{325}} - 4.260 \times \frac{A_{334}}{A_{325}}$$

$A_{325}$ : Absorbance at 325 nm

$V$ : Total volume (mL) of the sample solution

$M$ : Amount (g) of sample in  $V$  mL of the sample solution

$f$ : Correction factor

1830: Conversion factor from specific absorbance of retinol alcohol to IU (Unit/g)

## 2.56 Determination of Specific Gravity and Density

The density  $\rho$  (g/mL or g/cm<sup>3</sup>) means the mass per unit volume, and the relative density means the ratio of the mass of a sample specimen to that of an equal volume of a standard substance. The relative density is also called the specific gravity.

The specific gravity,  $d'_t$ , means the ratio of the mass of the sample specimen at  $t^{\circ}\text{C}$  to that of an equal volume of water ( $\text{H}_2\text{O}$ ) at  $t^{\circ}\text{C}$ . Unless otherwise specified, the measurement is to be performed by Method 1, Method 2 or Method 4. When the specified value is accompanied with the term "about" in the monograph, Method 3 is also available.

### 1. Method 1. Measurement using a pycnometer

A pycnometer is a glass vessel with a capacity of usually 10 mL to 100 mL, having a ground-glass stopper fitted with a thermometer, and a side inlet-tube with a marked line and a ground-glass cap.

Weigh a pycnometer, previously cleaned and dried, to determine its mass  $M$ . Remove the stopper and the cap. Fill the pycnometer with the sample solution, keeping them at a slightly lower temperature by  $1^{\circ}\text{C}$  to  $3^{\circ}\text{C}$  than the specified temperature  $t^{\circ}\text{C}$ , and stopper them, taking care not to leave bubbles. Raise the temperature gradually, and when the thermometer shows the specified temperature, remove the portion of the sample solution above the marked line through the side tube, cap the side tube, and wipe the outside surface thoroughly. Measure the mass  $M_1$  of the pycnometer filled with the sample solution. Perform the same procedure, using the same pycnometer containing water, and note the mass  $M_2$  at the specified temperature  $t^{\circ}\text{C}$ . The specific gravity  $d'_t$  can be calculated by use of the following equation.

$$d'_t = \frac{M_1 - M}{M_2 - M}$$

Further, when measurements for a sample solution and water are performed at the same temperature ( $t^{\circ}\text{C} = t'^{\circ}\text{C}$ ), the density of the sample solution at the temperature  $t'^{\circ}\text{C}$  ( $\rho'_t$ ) can be calculated from the measured specific gravity  $d'_t$  and the density of water at the temperature  $t'^{\circ}\text{C}$  ( $\rho'_{\text{SI}}$ ) indicated in Table 2.56-1 by using the following equation.

$$\rho'_t = \rho'_{\text{SI}} d'_t$$

Table 2.56-1 Density of water

Temp. °C	Density g/mL	Temp. °C	Density g/mL	Temp. °C	Density g/mL	Temp. °C	Density g/mL
0	0.999 84	11	0.999 61	21	0.997 99	31	0.995 34
1	0.999 90	12	0.999 50	22	0.997 77	32	0.995 03
2	0.999 94	13	0.999 38	23	0.997 54	33	0.994 70
3	0.999 96	14	0.999 24	24	0.997 30	34	0.994 37
4	0.999 97	15	0.999 10	25	0.997 04	35	0.994 03
5	0.999 96	16	0.998 94	26	0.996 78	36	0.993 68
6	0.999 94	17	0.998 77	27	0.996 51	37	0.993 33
7	0.999 90	18	0.998 60	28	0.996 23	38	0.992 97
8	0.999 85	19	0.998 41	29	0.995 94	39	0.992 59
9	0.999 78	20	0.998 20	30	0.995 65	40	0.992 22

\* In this Table, although the unit of density is represented by g/mL in order to harmonize with the unit expression in the text, it should be expressed in g/cm<sup>3</sup> seriously.

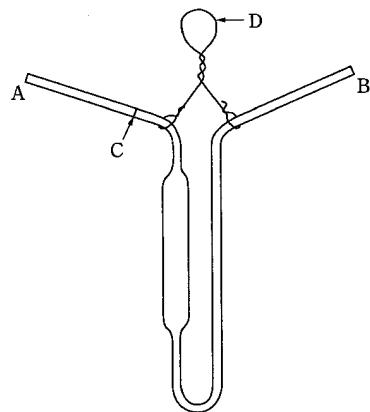


Fig. 2.56-1 Sprengel-Ostwald pycnometer

### 2. Method 2. Measurement using a Sprengel-Ostwald pycnometer

A Sprengel-Ostwald pycnometer is a glass vessel with a capacity of usually 1 mL to 10 mL. As shown in Fig. 2.56-1, both ends are thick-walled fine tubes (inside diameter: 1 – 1.5 mm, outside diameter: 3 – 4 mm), one of which, tube A, has a line C marked on it. Determine the mass of a pycnometer,  $M$ , previously cleaned and dried, by hanging it on the arm of a chemical balance with a platinum or aluminum wire D. Immerse the fine tube B in the sample solution, which is at a lower temperature by  $3^{\circ}\text{C}$  to  $5^{\circ}\text{C}$  than the specified temperature  $t^{\circ}\text{C}$ . Attach rubber tubing or a ground-glass tube to the end of A, and suck up the sample solution until the meniscus is above the marked line C, taking care to prevent bubble formation. Immerse the pycnometer in a water bath kept at the specified temperature  $t^{\circ}\text{C}$  for about 15 minutes, and then, by attaching a piece of filter paper to the end of B, adjust the level of the sample solution to the marked line C. Take the pycnometer out of the water bath, wipe thoroughly the outside surface and determine the mass  $M_1$ . By use of the same pycnometer, perform the same procedure for the standard solution of water. Weigh the pycnometer containing water at the specified temperature  $t^{\circ}\text{C}$ , and note the mass  $M_2$ . Calculate the specific gravity  $d'_t$ , according to the equation described in Method 1.

Further, when measurements of specific gravity for a sample solution and water are performed at the same temperature ( $t^{\circ}\text{C} = t'^{\circ}\text{C}$ ), the density of sample solution at tempera-

ture  $t^{\circ}\text{C}$  can be calculated by using the equation described in Method 1.

### 3. Method 3. Measurement using a hydrometer

Clean a hydrometer with ethanol (95) or diethyl ether. Stir the sample well with a glass rod, and float the hydrometer in the well. When the temperature is adjusted to the specified temperature  $t^{\circ}\text{C}$  and the hydrometer comes to a standstill, read the specific gravity  $d_t'$  or the density  $\rho_T'$  at the upper brim of the meniscus. Here the temperature  $t^{\circ}\text{C}$  indicates the temperature at which the hydrometer is calibrated. If specific instructions for reading the meniscus are supplied with the hydrometer, the reading must be in accordance with the instructions.

Further, when measurement of the specific gravity for a sample solution is performed at the same temperature ( $t^{\circ}\text{C} = t^{\circ}\text{C}$ ), at which the hydrometer is calibrated, the density of a sample solution at  $t^{\circ}\text{C}$ ,  $\rho_T'$ , can be calculated by using the specific gravity  $d_t'$  and the equation shown in Method 1.

### 4. Method 4. Measurement using an oscillator-type density meter

Density measurement with an oscillator-type density meter is a method for obtaining the density of liquid or gas by measuring the intrinsic vibration period  $T(\text{s})$  of a glass tube cell filled with sample specimen. When a glass tube containing a sample is vibrated, it undergoes a vibration with an intrinsic vibration period  $T$  in proportion to the mass of the sample specimen. If the volume of the vibrating part of the sample cell is fixed, the relation of the square of intrinsic oscillation period and density of the sample specimen shall be linear.

Before measuring a sample density, the respective intrinsic oscillation periods  $T_{S1}$  and  $T_{S2}$  for two reference substances (density:  $\rho_{S1}$ ,  $\rho_{S2}$ ) must be measured at a specified temperature  $t^{\circ}\text{C}$ , and the cell constant  $K_t'$  ( $\text{g} \cdot \text{cm}^{-3} \text{s}^{-2}$ ) must be determined by using the following equation.

$$K_t' = \frac{\rho_{S1}' - \rho_{S2}'}{T_{S1}^2 - T_{S2}^2}$$

Usually, water and dried air are chosen as reference substances. Here the density of water at  $t^{\circ}\text{C}$ ,  $\rho_{S1}'$ , is taken from Table 2.56-1, and that of dried air  $\rho_{S2}'$  is calculated by using the following equation, where the pressure of dried air is at  $p$  kPa.

$$\rho_{S2}' = 0.0012932 \times \{273.15/(273.15 + t')\} \times (p/101.325)$$

Next, introduce a sample specimen into a sample cell having a cell constant  $K_t'$ , the intrinsic vibration period,  $T_t$ , for the sample under the same operation conditions as employed for the reference substances. The density of a sample specimen at  $t^{\circ}\text{C}$ ,  $\rho_T'$ , is calculated by use of the following equation, by introducing the intrinsic oscillation period  $T_{S1}$  and the density of water at a specified temperature  $t^{\circ}\text{C}$ ,  $\rho_{S1}'$ , into the equation.

$$\rho_T' = \rho_{S1}' + K_t' (T_t^2 - T_{S1}^2)$$

Further, the specific gravity of a sample specimen  $d_t'$  against water at a temperature  $t^{\circ}\text{C}$  can be obtained by using the equation below, by introducing the density of water at a temperature  $t^{\circ}\text{C}$ ,  $\rho_{S1}'$ , indicated in Table 2.56-1.

$$d_t' = \frac{\rho_T'}{\rho_{S1}'}$$

#### 4.1. Apparatus

An oscillator-type density meter is usually composed of a glass tube cell of about 1 mL capacity, the curved end of which is fixed to the vibration plate, an oscillator which ap-

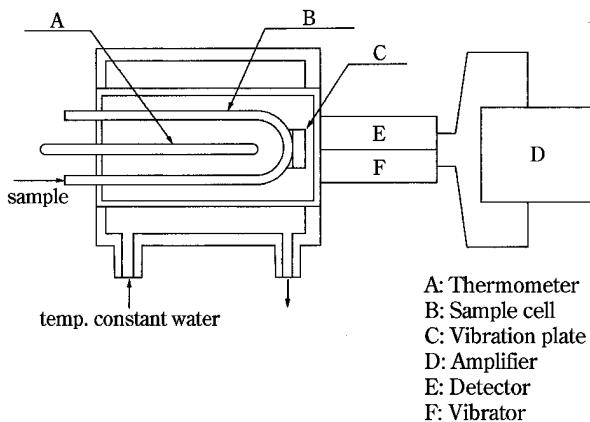


Fig. 2.56-2 Oscillator-type density meter

plies an initial vibration to the cell, a detector for measuring the intrinsic vibration period, and a temperature controlling system.

A schematic illustration of the apparatus is depicted in Fig. 2.56-2.

#### 4.2. Procedure

A sample cell, water, and a sample specimen are previously adjusted to a specified temperature  $t^{\circ}\text{C}$ . Wash the sample cell with water or an appropriate solvent, and dry it thoroughly with a flow of dried air. Stop the flow of dried air, confirm that the temperature is at the specified value, and then measure the intrinsic oscillation period  $T_{S2}$  given by the dried air. Separately, the atmospheric pressure  $p$  (kPa) must be measured at the time and place of the examination. Next, introduce water into the sample cell and measure the intrinsic oscillation period  $T_{S1}$  given by water. Using these values of the intrinsic oscillation period and the atmospheric pressure, the sample cell constant  $K_t'$  can be determined by use of the above-mentioned equation.

Next, introduce a sample specimen into the glass cell, confirm the specified temperature, and measure the intrinsic oscillation period  $T_t$  given by the sample specimen. Using the intrinsic oscillation periods for water and the sample specimen, the density of water  $\rho_{S1}'$ , and the cell constant  $K_t'$ , the density of the sample specimen  $\rho_T'$  can be obtained by use of the above equation. If necessary, the specific gravity of the sample specimen  $d_t'$  against water at a temperature  $t^{\circ}\text{C}$ , can be calculated by using the density of water  $\rho_{S1}'$  shown in Table 2.56-1.

In this measurement, avoid the occurrence of bubble formation in the sample cell, when a sample specimen or water is introduced into the cell.

## 2.57 Boiling Point and Distilling Range Test

The boiling point and distilling range are determined by Method 1 or Method 2 as described herein, unless otherwise specified. Boiling point is the temperature shown between when the first 5 drops of distillate leave the tip of the condenser and when the last liquid evaporates from the bottom of the flask. Distilling range test is done to determine the volume of the distillate which has been collected in the range of temperature directed in the monograph.

**1. Method 1** This method is applied to a sample for which the permissible range of boiling temperature is smaller than 5°C.

### 1.1. Apparatus

Use the apparatus illustrated in Fig. 2.57-1.

### 1.2. Procedure

Measure 25 mL of the sample, whose temperature is previously noted, using a volumetric cylinder G graduated in 0.1 mL, and transfer it to a distilling flask A of 50- to 60-mL capacity. Use this cylinder as the receiver for the distillate without rinsing out any of the adhering liquid. Put boiling chips into the distilling flask A, insert a thermometer B with an immersion line C is on a level with the lower end of cork stopper D and the upper end of its mercury bulb is located in the center of the delivery tube, and connect condenser E with the distilling flask A and adapter F with the condenser E. Insert the open end of F into the mouth of cylinder G (receiver) so that air can pass through slightly. Use a hood with a height sufficient to shield A, and heat A with a suitable heat source. When direct flame is applied as the heat source, put A on a hole of a fire-resistant, heat-insulating board [a board consisting of a fire-resistant, heat-insulating material, 150 mm square and about 6 mm thick (or a wire gauge of 150 mm square bonded to fire-resistant, heat-insulation materials in about 6 mm thickness), having an its center a round hole 30 mm in diameter].

Unless otherwise specified, distil the liquid sample by the application of heat, at a rate of 4 to 5 mL per minute of distillate in the case of liquids whose boiling temperature to be determined is lower than 200°C and at a rate of 3 to 4 mL per minute in the case of liquids whose boiling temperature is 200°C or over, and read the boiling point. For the distilling range test, bring the temperature of distillate to the temperature at which the volume was originally measured, and measure the volume of distillate.

Liquids that begin to distil below 80°C are cooled to between 10°C and 15°C before measuring the volume, and the receiving cylinder is kept immersed in ice up to a point 25 mm from the top during the distillation.

Correct the observed temperature for any variation in the barometric pressure from the normal (101.3 kPa), by allowing 0.1 degree for each 0.36 kPa of variation, adding if

the pressure is lower, or subtracting if higher than 101.3 kPa.

**2. Method 2** This method is applied to the sample for which the permissible range of boiling temperature is 5°C or more.

### 2.1. Apparatus

The same apparatus as described in Method 1 is used. However, use a 200-mL distilling flask A with a neck 18 to 24 mm in inside diameter having a delivery tube 5 to 6 mm in inside diameter. The fire-resistant, heat-insulating board used for direct flame heating should have in its center a round hole 50 mm in diameter.

### 2.2. Procedure

Measure 100 mL of the sample, whose temperature is previously noted, using a volumetric cylinder graduated in 1 mL, and carry out the distillation in the same manner as in Method 1.

## 2.58 X-Ray Powder Diffraction Method

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

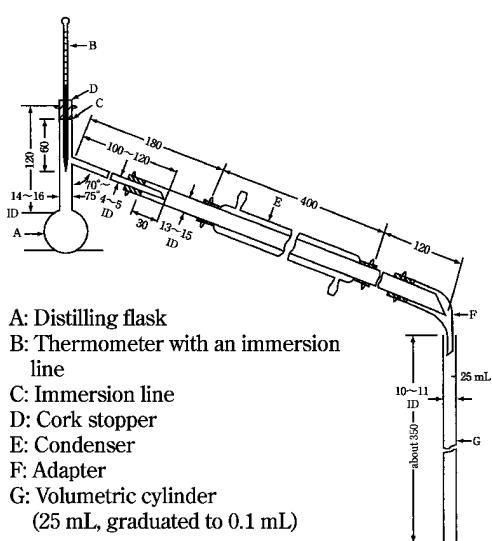
♦X-Ray Powder Diffraction Method is a method for measuring characteristic X-ray diffraction angles and intensities from randomly oriented powder crystallites irradiated by a monochromated X-ray beam.♦

Every crystalline phase of a given substance produces a characteristic X-ray diffraction pattern. Diffraction patterns can be obtained from a randomly oriented crystalline powder composed of crystallites or crystal fragments of finite size. Essentially 3 types of information can be derived from a powder diffraction pattern: angular position of diffraction lines (depending on geometry and size of the unit cell); intensities of diffraction lines (depending mainly on atom type and arrangement, and particle orientation within the sample); and diffraction line profiles (depending on instrumental resolution, crystallite size, strain and specimen thickness).

Experiments giving angular positions and intensities of lines can be used for applications such as qualitative phase analysis (for example, identification of crystalline phases) and quantitative phase analysis of crystalline materials. An estimate of the amorphous and crystalline fractions<sup>(1)</sup> can also be made. The X-ray powder diffraction (XRPD) method provides an advantage over other means of analysis in that it is usually non-destructive in nature (specimen preparation is usually limited to grinding to ensure a randomly oriented sample). XRPD investigations can also be carried out under *in situ* conditions on specimens exposed to non-ambient conditions, such as low or high temperature and humidity.

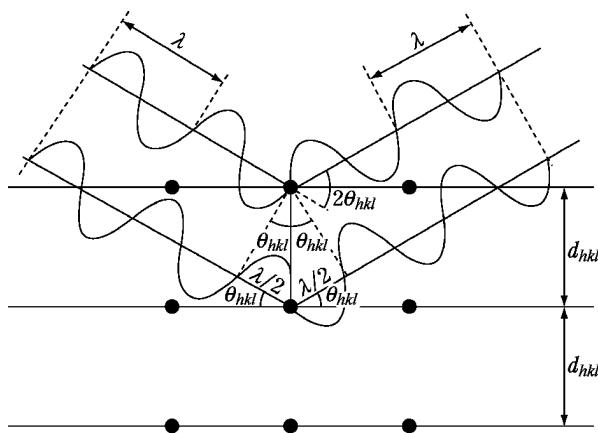
### 1. Principle

X-ray diffraction results from the interaction between X-rays and electron clouds of atoms. Depending on the atomic arrangement, interferences arise from the scattered X-rays. These interferences are constructive when the path difference between 2 diffracted X-ray waves differs by an integral num-



The figures are in mm.

Fig. 2.57-1



**Fig. 2.58-1** Diffraction of X-rays by a crystal according to Bragg's law

ber of wavelengths. This selective condition is described by the Bragg equation, also called Bragg's law (see Fig. 2.58-1)

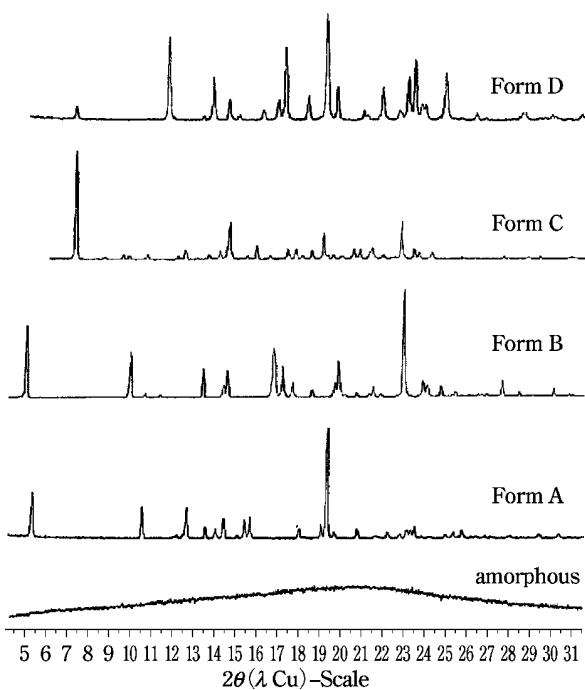
$$2d_{hkl} \sin\theta_{hkl} = n\lambda$$

The wavelength  $\lambda$  of the X-rays is of the same order of magnitude as the distance between successive crystal lattice planes, or  $d_{hkl}$  (also called 'd-spacings').  $\theta_{hkl}$  is the angle between the incident ray and the family of lattice planes, and  $\sin\theta_{hkl}$  is inversely proportional to the distance between successive crystal planes or d-spacings.

The direction and spacing of the planes with reference to the unit cell axes are defined by the Miller indices  $\{hkl\}$ . These indices are the reciprocals, reduced to the next-lower integer, of the intercepts that a plane makes with the unit cell axes. The unit cell dimensions are given by the spacings,  $a$ ,  $b$  and  $c$  and the angles between them,  $\alpha$ ,  $\beta$  and  $\gamma$ . The interplanar spacing for a specified set of parallel  $hkl$  planes is denoted by  $d_{hkl}$ . Each such family of planes may show higher orders of diffraction where the  $d$  values for the related families of planes,  $nh$ ,  $nk$ ,  $nl$  are diminished by the factor  $1/n$  ( $n$  being an integer: 2, 3, 4, etc.). Every set of planes throughout a crystal has a corresponding Bragg diffraction angle,  $\theta_{hkl}$ , associated with it (for a specific wavelength  $\lambda$ ).

A powder specimen is assumed to be polycrystalline so that at any angle  $\theta_{hkl}$  there are always crystallites in an orientation allowing diffraction according to Bragg's law<sup>(2)</sup>. For a given X-ray wavelength, the positions of the diffraction peaks (also referred to as 'lines', 'reflections' or 'Bragg reflections') are characteristic of the crystal lattice (d-spacings), their theoretical intensities depend on the crystallographic unit cell content (nature and positions of atoms), and the line profiles on the perfection and extent of the crystal lattice. Under these conditions the diffraction peak has a finite intensity arising from atomic arrangement, type of atoms, thermal motion and structural imperfections, as well as from instrument characteristics. The intensity is dependent upon many factors such as structure factor, temperature factor, crystallinity, polarization factor, multiplicity and Lorentz factor. The main characteristics of diffraction line profiles are  $2\theta$  position, peak height, peak area and shape (characterized by, for example, peak width or asymmetry, analytical function, empirical representation). An example of the type of powder patterns obtained for 5 different solid phases of a substance are shown in Fig. 2.58-2.

In addition to the diffraction peaks, an X-ray diffraction experiment also generates a more-or-less uniform background, upon which the peaks are superimposed. Besides



**Fig. 2.58-2** X-ray powder diffraction patterns collected for 5 different solid phases of a substance (the intensities are normalized)

specimen preparation, other factors contribute to the background, for instance the sample holder, diffuse scattering from air and equipment, other instrumental parameters such as detector noise, general radiation from the X-ray tube, etc. The peak to background ratio can be increased by minimizing background and by choosing prolonged exposure times.

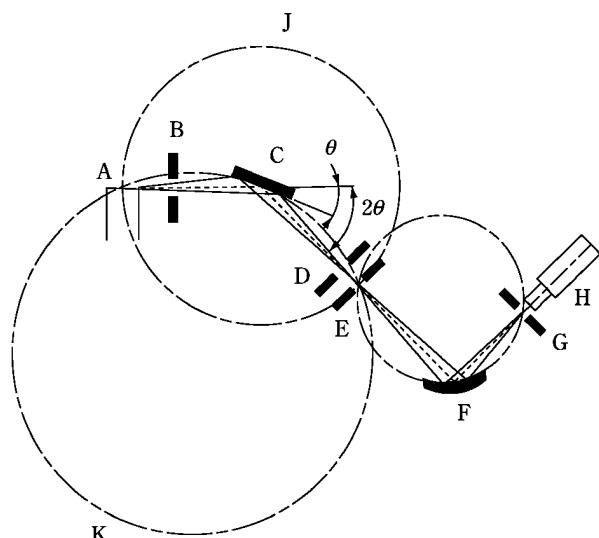
## 2. Instrument

### 2.1. Instrument set-up

X-ray diffraction experiments are usually performed using powder diffractometers or powder cameras. A powder diffractometer generally comprises 5 main parts: an X-ray source; incident beam optics, which may perform monochromatization, filtering, collimation and/or focusing of the beam; a goniometer; diffraction beam optics, which may perform monochromatization, filtering, collimation and focusing or parallelising of the beam; and a detector. Data collection and data processing systems are also required and are generally included in current diffraction measurement equipment.

Depending on the type of analysis to be performed (phase identification, quantitative analysis, lattice parameters determination, etc.), different XRPD instrument configurations and performance levels are required. The simplest instruments used to measure powder patterns are powder cameras. Replacement of photographic film as the detection method by photon detectors has led to the design of diffractometers in which the geometric arrangement of the optics is not truly focusing but parafocusing, such as in the Bragg-Brentano geometry. The Bragg-Brentano parafocusing configuration is currently the most widely used and is therefore briefly described here.

A given instrument may provide a horizontal or vertical  $\theta/2\theta$  geometry or a vertical  $\theta/\theta$  geometry. For both geometries, the incident X-ray beam forms an angle  $\theta$  with the specimen surface plane and the diffracted X-ray beam forms an angle  $2\theta$  with the direction of the incident X-ray beam (an



- A. X-ray tube
- B. Divergence slit
- C. Sample
- D. Anti-diffusion slit
- E. Receiving slit
- F. Monochromator
- G. Detector receiving slit
- H. Detector
- J. Diffractometer circle
- K. Focusing circle

**Fig. 2.58-3** Geometric arrangement of the Bragg-Brentano parafocusing geometry

angle  $\theta$  with the specimen surface plane). The basic geometric arrangement represented in Fig. 2.58-3. The divergent beam of radiation from the X-ray tube (the so-called ‘primary beam’) passes through the parallel plate collimators and a divergence slit assembly and illuminates the flat surface of the specimen. All the rays diffracted by suitably oriented crystallites in the specimen at an angle  $2\theta$  converge to a line at the receiving slit. A second set of parallel plate collimators and a scatter slit may be placed either behind or before the receiving slit. The axes of the line focus and of the receiving slit are at equal distances from the axis of the goniometer. The X-ray quanta are counted by a radiation detector, usually a scintillation counter, a sealed-gas proportional counter, or a position-sensitive solid-state detector such as imaging plate or CCD detector. The receiving slit assembly and the detector are coupled together and move tangentially to the focusing circle. For  $\theta/2\theta$  scans the goniometer rotates the specimen about the same axis as that of the detector, but at half the rotational speed, in  $\theta/2\theta$  motion. The surface of the specimen thus remains tangential to the focusing circle. The parallel plate collimator limits the axial divergence of the beam and hence partially controls the shape of the diffracted line profile.

A diffractometer may also be used in transmission mode. The advantage with this technology is to lessen the effects due to preferred orientation. A capillary of about 0.5–2 mm thickness can also be used for small sample amounts.

## 2.2. X-ray radiation

In the laboratory, X-rays are obtained by bombarding a metal anode with electrons emitted by the thermionic effect and accelerated in a strong electric field (using a high-voltage generator). Most of the kinetic energy of the electrons is

converted to heat, which limits the power of the tubes and requires efficient anode cooling. A 20- to 30-fold increase in brilliance can be obtained using rotating anodes and by using X-ray optics. Alternatively, X-ray photons may be produced in a large-scale facility (synchrotron).

The spectrum emitted by an X-ray tube operating at sufficient voltage consists of a continuous background of polychromatic radiation and additional characteristic radiation that depends on the type of anode. Only this characteristic radiation is used in X-ray diffraction experiments. The principal radiation sources utilized for X-ray diffraction are vacuum tubes utilizing copper, molybdenum, iron, cobalt or chromium as anodes; copper, molybdenum or cobalt X-rays are employed most commonly for organic substances (the use of cobalt anodes can be especially preferred to separate distinct X-ray lines). The choice of radiation to be used depends on the absorption characteristics of the specimen and possible fluorescence by atoms present in the specimen. The wavelengths used in powder diffraction generally correspond to the  $K_\alpha$  radiation from the anode. Consequently, it is advantageous to make the X-ray beam ‘monochromatic’ by eliminating all the other components of the emission spectrum. This can be partly obtained using  $K_\beta$  filters, i.e. metal filters selected as having an absorption edge between the  $K_\alpha$  and  $K_\beta$  wavelengths emitted by the tube.

Such a filter is usually inserted between the X-ray tube and the specimen. Another, more-and-more-commonly used way to obtain a monochromatic X-ray beam is via a large monochromator crystal (usually referred to as a ‘monochromator’). This crystal is placed before or behind the specimen and diffracts the different characteristic peaks of the X-ray beam (i.e.  $K_\alpha$  and  $K_\beta$ ) at different angles, so that only one of them may be selected to enter into the detector. It is even possible to separate  $K_{\alpha 1}$  and  $K_{\alpha 2}$  radiations by using a specialized monochromator. Unfortunately, the gain in getting a monochromatic beam by using a filter or a monochromator is counteracted by a loss in intensity. Another way of separating  $K_\alpha$  and  $K_\beta$  wavelengths is by using curved X-rays mirrors that can simultaneously monochromate and focus or parallelize the X-ray beam.

## 2.3. Radiation protection

Exposure of any part of the human body to X-rays can be injurious to health. It is therefore essential that whenever X-ray equipment is used, adequate precautions are taken to protect the operator and any other person in the vicinity. Recommended practice for radiation protection as well as limits for the levels of X-radiation exposure are those established by national legislation in each country. If there are no official regulations or recommendations in a country, the latest recommendations of the International Commission on Radiological Protection should be applied.

## 3. Specimen preparation and mounting

The preparation of the powdered material and mounting of the specimen in a suitable holder are critical steps in many analytical methods, and are particularly so for X-ray powder diffraction analysis, since they can greatly affect the quality of the data to be collected<sup>(3)</sup>. The main sources of error due to specimen preparation and mounting are briefly discussed here for instruments in Bragg-Brentano parafocusing geometry.

### 3.1. Specimen preparation

In general, the morphology of many crystalline particles tends to give a specimen that exhibits some degree of preferred orientation in the specimen holder. This is particularly evident for needle-like or plate-like crystals when size reduction yields finer needles or platelets. Preferred orienta-

tion in the specimen influences the intensities of various reflections, so that some are more intense and others are less intense, compared to what would be expected from a completely random specimen. Several techniques can be employed to improve randomness in the orientation of crystallites (and therefore to minimize preferred orientation), but further reduction of particle size is often the best and simplest approach. The optimum number of crystallites depends on the diffractometer geometry, the required resolution and the specimen attenuation of the X-ray beam. In some cases, particle sizes as large as 50 µm will provide satisfactory results in phase identification. However, excessive milling (crystallite sizes less than approximately 0.5 µm) may cause line broadening and significant changes to the sample itself such as:

- (i) specimen contamination by particles abraded from the milling instruments (mortar, pestle, balls, etc.);
- (ii) reduced degree of crystallinity;
- (iii) solid-state transition to another polymorph;
- (iv) chemical decomposition;
- (v) introduction of internal stress;
- (vi) solid-state reactions.

Therefore, it is advisable to compare the diffraction pattern of the non-ground specimen with that corresponding to a specimen of smaller particle size (e.g. a milled specimen). If the X-ray powder diffraction pattern obtained is of adequate quality considering its intended use, then grinding may not be required. It should be noted that if a sample contains more than one phase and if sieving is used to isolate particles to a specific size, the initial composition may be altered.

#### 4. Control of the instrument performance

Goniometers and the corresponding incident and diffracted X-ray beam optics have many mechanical parts that need adjustment. The degree of alignment or misalignment directly influences the quality of the results of an XRPD investigation. Therefore, the different components of the diffractometer must be carefully adjusted (optical and mechanical systems, etc.) to adequately minimize systematic errors, while optimizing the intensities received by the detector. The search for maximum intensity and maximum resolution is always antagonistic when aligning a diffractometer. Hence, the best compromise must be sought whilst performing the alignment procedure. There are many different configurations and each supplier's equipment requires specific alignment procedures.

The overall diffractometer performance must be tested and monitored periodically using suitable certified reference materials. Depending on the type of analysis, other well-defined reference materials may also be employed, although the use of certified reference materials is preferred.

#### 5. Qualitative phase analysis (Identification of phases)

The identification of the phase composition of an unknown sample by XRPD is usually based on the visual or computer-assisted comparison of a portion of its X-ray diffraction powder pattern to the experimental or calculated pattern of a reference material. Ideally, these reference patterns are collected on well-characterized single-phase specimens. This approach makes it possible in most cases to identify a crystalline substance by its 2θ diffraction angles or d-spacings and by its relative intensities. The computer-aided comparison of the diffraction pattern of the unknown sample to the comparison data can be based either on a more-or-less extended 2θ-range of the whole diffraction pattern or on a set of reduced data derived from the pattern. For example, the list of d-spacings and normalized intensities  $I_{\text{norm}}$ , a so-called ( $d, I_{\text{norm}}$ )-list extracted from the pattern, is the crystal-

lographic fingerprint of the material, and can be compared to ( $d, I_{\text{norm}}$ )-lists of single-phase samples complied in databases.

For most organic crystals, when using CuK $\alpha$  radiation, it is appropriate to record the diffraction pattern in a 2θ-range from as near 0° as possible to at least 40°. The agreement in the 2θ-diffraction angles between specimen and reference is within 0.2° for the same crystal form, while relative intensities between specimen and reference may vary considerably due to preferred orientation effects. By their very nature, variable hydrates and solvates are recognized to have varying unit cell dimensions and as such shifting occurs in peak positions of the measured XRPD patterns for these materials. In these unique materials, variance in 2-θ positions of greater than 0.2° is not unexpected. As such, peak position variances such as 0.2° are not applicable to these materials. For other types of samples (e.g. inorganic salts), it may be necessary to extend the 2θ-region scanned to well beyond 40°. It is generally sufficient to scan past the 10 strongest reflections identified in single phase X-ray powder diffraction database files.

It is sometimes difficult or even impossible to identify phases in the following cases:

- (i) non-crystallized or amorphous substances;
- (ii) the components to be identified are present in low mass fractions of the analyte amounts (generally less than 10 per cent  $m/m$ );
- (iii) pronounced preferred orientation effects;
- (iv) the phase has not been filed in the database used;
- (v) formation of solid solutions;
- (vi) presence of disordered structures that alter the unit cell;
- (vii) the specimen comprises too many phases;
- (viii) presence of lattice deformations;
- (ix) structural similarity of different phases.

#### 6. Quantitative phase analysis

If the sample under investigation is a mixture of 2 or more known phases, of which not more than 1 is amorphous, the percentage (by volume or by mass) of each crystalline phase and of the amorphous phase can, in many cases, be determined. Quantitative phase analysis can be based on the integrated intensities, on the peak heights of several individual diffraction lines<sup>(4)</sup>, or on the full pattern. These integrated intensities, peak heights or full-pattern data points are compared to the corresponding values of reference materials. These reference materials shall be single-phase or a mixture of known phases. The difficulties encountered during quantitative analysis are due to specimen preparation (the accuracy and precision of the results require in particular homogeneity of all phases and a suitable particle size distribution in each phase) and to matrix effects. In favorable cases, amounts of crystalline phases as small as 10 per cent may be determined in solid matrices.

##### 6.1. Polymorphic samples

For a sample composed of 2 polymorphic phases a and b, the following expression may be used to quantify the fraction  $F_a$  of phase a:

$$F_a = \frac{1}{1 + K (I_b/I_a)}$$

The fraction is derived by measuring the intensity ratio between the 2 phases, knowing the value of the constant  $K$ .  $K$  is the ratio of the absolute intensities of the 2 pure polymorphic phases  $I_{\text{oa}}/I_{\text{ob}}$ . Its value can be determined by measuring standard samples.

## 6.2. Methods using a standard

The most commonly used methods for quantitative analysis are:

- the ‘external standard method’;
- the ‘internal standard method’;
- the ‘spiking method’ (often also called the ‘standard addition method’).

The ‘external standard method’ is the most general method and consists of comparing the X-ray diffraction pattern of the mixture, or the respective line intensities, with those measured in a reference mixture or with the theoretical intensities of a structural model, if it is fully known.

To limit errors due to matrix effects, an internal reference material with crystallite size and X-ray absorption coefficient comparable to those of the components of the sample, and with a diffraction pattern that does not overlap at all that of the sample to be analyzed, can be used. A known quantity of this reference material is added to the sample to be analyzed and to each of the reference mixtures. Under these conditions, a linear relationship between line intensity and concentration exists. This application, called the ‘internal standard method’, requires a precise measurement of diffraction intensities.

In the ‘spiking method’ (or ‘standard addition method’), some of the pure phase *a* is added to the mixture containing the unknown concentration of *a*. Multiple additions are made to prepare an intensity-versus-concentration plot in which the negative *x* intercept is the concentration of the phase *a* in the original sample.

## 7. Estimate of the amorphous and crystalline fractions

In a mixture of crystalline and amorphous phases, the crystalline and amorphous fractions can be estimated in several ways. The choice of the method used depends on the nature of the sample:

(i) if the sample consists of crystalline fractions and an amorphous fraction of different chemical compositions, the amounts of each of the individual crystalline phases may be estimated using appropriate standard substances as described above; the amorphous fraction is then deduced indirectly by subtraction;

(ii) if the sample consists of one amorphous and one crystalline fraction, either as a 1-phase or a 2-phase mixture, with the same elemental composition, the amount of the crystalline phase (‘the degree of crystallinity’) can be estimated by measuring 3 areas of the diffractogram:

*A* = total area of the peaks arising from diffraction from the crystalline fraction of the sample;

*B* = total area below area *A*;

*C* = background area (due to air scattering, fluorescence, equipment, etc.)

When these areas have been measured, the degree of crystallinity can be roughly estimated using the following formula:

$$\% \text{ crystallinity} = 100A/(A + B - C)$$

It is noteworthy that this method does not yield absolute degree-of-crystallinity values and hence is generally used for comparative purposes only. More sophisticated methods are also available, such as the Ruland method.

## 8. Single crystal structure

In general, the determination of crystal structures is performed from X-ray diffraction data obtained using single crystals. However, crystal structure analysis of organic crystals is a challenging task, since the lattice parameters are comparatively large, the symmetry is low and the scattering

properties are normally very low. For any given crystalline form of a substance, knowledge of the crystal structure allows the calculation of the corresponding XRPD pattern, thereby providing a ‘preferred-orientation-free’ reference XRPD pattern, which may be used for phase identification.

- (<sup>1</sup>) There are many other applications of the X-ray powder diffraction technique that can be applied to crystalline pharmaceutical substances such as: determination of crystal structures, refinement of crystal structures, determination of crystallographic purity of crystalline phases, characterization of crystallographic texture, etc. These applications are not described in this chapter.
- (<sup>2</sup>) An ‘ideal’ powder for diffraction experiments consists of a large number of small, randomly oriented spherical crystallites (coherently diffracting crystalline domains). If this number is sufficiently large, there are always enough crystallites in any diffracting orientation to give reproducible diffraction patterns.
- (<sup>3</sup>) Similarly, changes in the specimen can occur during data collection in the case of a non-equilibrium specimen (temperature, humidity).
- (<sup>4</sup>) If the crystal structures of all components are known, the Rietveld method can be used to quantify them with good accuracy. If the crystal structures of the components care not known, the Pawley or least squares methods can be used.

## 2.59 Test for Total Organic Carbon

Test for Total Organic Carbon is a method for measuring the amount of organic carbon, which forms organic compounds, in water. Normally, organic carbon can be oxidized to carbon dioxide by a dry decomposition method, where organic compounds are oxidized by combustion, or by a wet decomposition method, where organic compounds are oxidized by applying ultraviolet rays or by adding oxidizing agent. The amount of carbon dioxide generated in the decomposition process is measured using an appropriate method such as infrared gas analysis, electric conductivity measurement, or resistivity measurement. The amount of organic carbon in water can be calculated from the amount of carbon dioxide measured in one of the above methods.

There are two types of carbon in water: organic carbon and inorganic carbon. For measuring the amount of organic carbon, two approaches can be taken. One method is to measure the amount of total carbon in water, then to subtract the amount of inorganic carbon from that of total carbon. The other method is to remove inorganic carbon from the test water, then to measure the amount of remaining organic carbon.

### 1. Instrument

The instrument consists of a sample injection port, a decomposition device, a carbon dioxide separation block, a detector, and a data processor or a recorder. The instrument should be capable of measuring the amount of organic carbon down to 0.050 mg/L.

The sample injection port is designed to be able to accept a specific amount of sample injected by a microsyringe or other appropriate sampling devices. The decomposition device for the dry decomposition method consists of a combustion tube and an electric furnace to heat the sample. Both devices are adjusted to operate at specified temperatures. The decomposition device for the wet decomposition method consists of an oxidizing reaction box, an ultraviolet ray lamp, a decomposition aid injector, and a heater. The de-

composition device for either method should be capable of generating not less than 0.450 mg/L of organic carbon when using a solution of 0.806 mg/L sodium dodecylbenzenesulfonate as the sample. The carbon dioxide separation block removes water from carbon dioxide formed in the decomposition process or separates carbon dioxide from the decomposed gas. An infrared gas analyzer, electric conductivity meter or specific resistance meter is used as the detector which converts the concentration of carbon dioxide into electric signal. The data processor calculates the concentration of the total organic carbon in the sample based on the electric signal converted by the detector. The recorder records the electric signal intensity converted by the detector.

## 2. Reagents and standard solutions

(i) Water used for measuring organic carbon (water for measurement): This water is used for preparing standard solutions or decomposition aid or for rinsing the instrument. The amount of organic carbon in this water, when collected into a sample container, should be not more than 0.250 mg/L.

(ii) Standard potassium hydrogen phthalate solution: The concentration of this standard solution is determined as specified for the instrument. Dry potassium hydrogen phthalate (standard reagent) at 105°C for 4 hours, and allow it to cool in a desiccator (silica gel). Weigh accurately a prescribed amount of dried potassium hydrogen phthalate, and dissolve it in the water for measurement to prepare the standard solution.

(iii) Standard solution for measuring inorganic carbon: The concentration of this standard solution is determined as specified for the instrument. Dry sodium hydrogen carbonate in a desiccator (sulfuric acid) for not less than 18 hours. Dry sodium carbonate decahydrate separately between 500°C and 600°C for 30 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately prescribed amounts of these compounds so that the ratio of their carbon content is 1:1, and dissolve them in the water for measurement to prepare the standard solution.

(iv) Decomposition aid: Dissolve a prescribed amount of potassium peroxodisulfate or other substances that can be used for the same purpose, in the water for measurement up to the concentration as specified for the instrument.

(v) Gas for removing inorganic carbon or carrier gas: Nitrogen, oxygen, or other gases that can be used for the same purpose.

(vi) Acid for removing inorganic carbon: Dilute hydrochloric acid, phosphoric acid or other acids that can be used for the same purpose, with the water for measurement down to the concentration as specified for the instrument.

## 3. Apparatus

(i) Sample container and reagent container: Use a container made of the material which does not release organic carbon from its surface, such as hard glass. Soak the container before use in a mixture of diluted hydrogen peroxide solution (1 in 3) and dilute nitric acid (1:1), and wash well with the water for measurement.

(ii) Microsyringe: Wash a microsyringe with a mixture of a solution of sodium hydroxide (1 in 20) and ethanol (99.5) (1:1), or diluted hydrochloric acid (1 in 4), and rinse well with the water for measurement.

## 4. Procedure

Employ an analytical method suitable for the instrument used. Calibrate the instruments using the standard potassium hydrogen phthalate solution with the test procedure specified for the instrument.

It is recommended that this instrument be incorporated into the manufacturing line of the water to be tested.

Otherwise, this test should be performed in a clean circumstance where the use of organic solvents or other substances that may affect the result of this test is prohibited, using a large sample container to collect a large volume of the water to be tested. The measurement should be done immediately after the sample collection.

### 4.1. Measurement of organic carbon by subtracting inorganic carbon from total carbon

According to the test procedure specified for the instrument used, inject a suitable volume of the sample for measuring the expected amount of total carbon into the instrument from sample injection port, and decompose organic and inorganic carbon in the sample. Detect the generated carbon dioxide with the detector, and calculate the amount of total carbon in the sample using a data processor or a recorder. Change the setting of the instrument for measuring inorganic carbon exclusively, and measure the amount of inorganic carbon in the same manner as total carbon. The amount of organic carbon can be obtained by subtracting the amount of inorganic carbon from that of total carbon.

### 4.2. Measurement of organic carbon after removing inorganic carbon

Remove inorganic carbon by adding the acid for removing inorganic carbon to the sample, followed by bubbling the gas for removing inorganic carbon (e.g. nitrogen) into the sample. According to the test procedure specified for the instrument used, inject a suitable volume of the sample for measuring the expected amount of organic carbon into the instrument from sample injection port, and decompose the sample. Detect the generated carbon dioxide with the detector, and calculate the amount of organic carbon in the sample using a data processor or a recorder.

For the instrument where the removal of inorganic carbon is performed in the instrument, first inject a suitable volume of the sample for measuring the expected amount of organic carbon into the instrument from sample injection port, according to the test procedure specified for the instrument used. Then, remove inorganic carbon by adding the acid for removing inorganic carbon to the sample in the decomposition device, followed by bubbling the gas for removing inorganic carbon into the sample. Decompose organic carbon, detect the generated carbon dioxide with the detector, and calculate the amount of organic carbon using a data processor or a recorder.

## 2.60 Melting Point Determination

The melting point is defined to be the temperature at which a crystalline substance melts during heating, when the solid phase and the liquid phase are in an equilibrium. However, in this Pharmacopoeia it is conventionally defined to be the temperature at which the remaining solid sample melts completely when it is subjected to continuous heating and the change of the sample state that accompanies heating is accurately observed. Since a pure substance has an intrinsic melting point, it is used for the identification and/or confirmation of a substance and also as an indicator of the purity of a substance.

The melting point is determined by either of the following methods: Method 1 is applied to those substances of which the purity is comparably high and which can be pulverized, Method 2 to those substances which are insoluble in water and can not be readily pulverized, and Method 3 to petro-

tums.

Unless otherwise specified, measurement is performed by Method 1.

### 1. Method 1

This method is applied to those substances of which the purity is comparably high and which can be pulverized.

#### 1.1. Apparatus

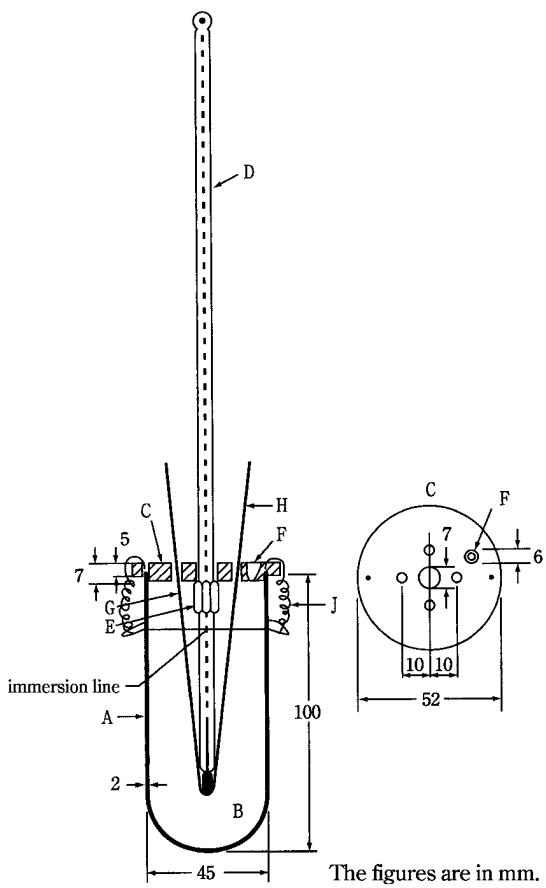
Use the apparatus illustrated in the Fig. 2.60-1.

Alternatively, apparatus in which some of the procedures, such as stirring, heating, and cooling are automated, can be used.

(i) Bath fluid: Usually use clear silicone oil having a viscosity of 50 to 100 mm<sup>2</sup>/s at an ordinary temperature.

(ii) Thermometer with an immersion line: There are six types of thermometers, Type 1—Type 6, which are specified by an appropriate measuring temperature range. For melting points lower than 50°C, use a thermometer Type 1; for 40°C to 100°C, Type 2; for 90°C to 150°C, Type 3; for 140°C to 200°C, Type 4; for 190°C to 250°C, Type 5; for 240°C to 320°C, Type 6.

(iii) Capillary tube: Use a hard glass capillary tube 120 mm long, 0.8 to 1.2 mm in inner diameter and 0.2 to 0.3 mm thick, with one end closed.



- A: Heating vessel of hard glass
- B: Bath fluid
- C: Polytetrafluoroethylene stopper
- D: Thermometer with an immersion line
- E: Thermometer-fastening spring
- F: Vent for adjustment of the bath fluid volume
- G: Coil spring
- H: Capillary tube
- J: Spring for fastening Polytetrafluoroethylene stopper

**Fig. 2.60-1** Melting point determination apparatus

### 1.2. Procedure

Pulverize the sample to a fine powder, and, unless otherwise specified, dry in a desiccator (silica gel) for 24 hours. When it is specified to do the test after drying, dry the sample under the conditions specified in the monograph before measurement. Place the sample in a dried capillary tube H, and pack it tightly so as to form a layer about 2.5 – 3.5 mm high by dropping the capillary repeatedly, with the closed end of H down, through a glass tube, about 70 cm long, held vertically on a glass or porous plate.

Heat the bath fluid B until the temperature rises to about 10°C below the expected melting point, place the thermometer D in the bath with the immersion line at the same level as the meniscus of the bath fluid, and insert capillary tube H into a coil spring G so that the packed sample is placed in a position corresponding to the center of the mercury bulb of the thermometer D. Continue heating to raise the temperature at a rate of approximately 3°C per minute until the temperature rises to 5°C below the expected melting point, then carefully regulate the rate of temperature increase to 1°C per minute.

Read the thermometer indication of the instantaneous temperature at which the sample liquefies completely and no solid is detectable in the capillary, and designate the indicated temperature as the melting point of the sample specimen.

#### 1.2.1. System suitability test

Confirmation of the system suitability of the apparatus should be done periodically by using Reference Standards for Apparatus Suitability. The Reference Standard is prepared for the suitability test of the apparatus when it is used with Type 2—Type 5 thermometers, and consists of 6 highly purified substances: acetanilide, acetophenetidine, caffeine, sulfanilamide, sulfapyridine, and vanillin. The label shows the certified melting points of the respective substances (the end point of the melting change),  $MP_f$ .

After selecting one of the thermometers and the appropriate Melting Point Standard based upon the expected melting point of a sample specimen, perform a melting point measurement of the selected Reference Standard, according to the above procedure. When the value of the obtained melting point of the Reference Standard is within  $MP_f \pm 0.5^\circ\text{C}$  in the case of vanillin and acetanilide, within  $MP_f \pm 0.8^\circ\text{C}$  in the case of acetophenetidine and sulfanilamide, and within  $MP_f \pm 1.0^\circ\text{C}$  in the case of sulfapyridine and caffeine, the apparatus is assumed to be suitable.

The above-mentioned measurement is repeated 3 times and the average is determined to be the melting point of the Reference Standard tested. When the above suitability test criteria are not met in a certain melting point measurement system of an apparatus and a Reference Standard, do the test again, after checking the packing of the sample specimen into the capillary tube, the locations and positioning of the thermometer and the capillary tube, the heating and stirring of the bath fluid, and the control of the temperature increasing rate. When a melting point measurement system does not meet the suitability test criteria again after checking these measuring conditions, the thermometer with an immersion line should be calibrated again or replaced with a new one.

### 2. Method 2

This method is applied to substances such as fats, fatty acids, paraffins or waxes.

#### 2.1. Apparatus

Instead of the apparatus specified in Method 1, use a water-containing beaker as a bath fluid and a heating vessel. In this measurement, total immersion mercury-filled ther-

mometers can also be used in place of the thermometer with an immersion line. Furthermore, the capillary tube should be the same as specified in Method 1, except that both ends of the tube are open.

## 2.2. Procedure

Carefully melt the sample at as low a temperature as possible, and, taking care to prevent bubbles, introduce it into a capillary tube to a height of about 10 mm. Allow the capillary containing the sample to stand for 24 hours at below 10°C, or for at least 1 hour in contact with ice, holding the capillary so that the sample can not flow out. Then attach the capillary to the thermometer by means of a rubber band so that the absorbed sample is located at a position corresponding to the center of the mercury bulb. Adjust the capillary tube in a water-containing beaker to such a position that the lower edge of the sample is located 30 mm below the water surface. Heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of temperature increase to 1°C per minute. The temperature at which the sample begins floating in the capillary is taken as the melting point of the sample specimen.

## 3. Method 3

This method is applied to petrolatum.

### 3.1. Apparatus

Instead of the apparatus specified in Method 1, use a water-containing beaker as a bath fluid and a heating vessel. In this measurement, total immersion mercury-filled thermometers can also be used in place of the thermometer with an immersion line.

### 3.2. Procedure

Melt the sample slowly by heating, with thorough stirring, until the temperature reaches 90–92°C. Discontinue the heating, and allow the sample to cool to 8–10°C above the expected melting point. Chill the bulb of the thermometer to 5°C, wipe and dry, and, while still cold, stick half of the thermometer bulb into the melted sample. Withdraw it immediately, hold vertically, cool until the attached sample becomes turbid, then dip the sample-bearing bulb for 5 minutes in water having a temperature below 16°C. Next, fix the thermometer securely in a test tube by means of a cork stopper so that the lower end is located 15 mm above the bottom. Suspend the test tube in a water-containing beaker held at a temperature about 16°C, and raise the temperature of the water bath to 30°C at a rate of 2°C per minute, then continue heating carefully at a rate of 1°C per minute until it reaches the melting point. Read the thermometer indication of the instantaneous temperature at which the first drop of the sample leaves the thermometer. If the variations between three repeated determinations are not more than 1°C, take the average of the three as the melting point. If any variation is greater than 1°C, make two additional measurements, and take the average of the five as the melting point.

## 2.61 Turbidity Measurement

Turbidity measurement is used to determine the turbidity (degree of opalescence) for the decision whether the article to be examined complies with the clarity requirement stated in the Purity.

As a rule, the visual method is specified for the requirement in individual monograph.

### 1. Visual method

This is used to determine the degree of opalescence with

white (or faintly-colored) fine particles. So the degree of opalescence of a colored sample is liable to be determined lower than it is difficult to compare the degree correctly without using similarly colored reference suspension.

### 1.1. Reference suspensions

Pipet 5 mL, 10 mL, 30 mL and 50 mL of formazin opalescence standard solution, dilute them separately to exactly 100 mL with water, and use these solutions so obtained as Reference suspensions I, II, III and IV, respectively. Shake before use. Degrees of opalescence of Reference suspensions I, II, III and IV are equivalent to 3 NTU, 6 NTU, 18 NTU and 30 NTU, respectively.

### 1.2. Procedure

Place sufficient of the test solution, water or the solvent to prepare the test solution and, where necessary, newly prepared Reference suspensions in separate flat-bottomed test tubes, 15–25 mm in inside diameter and of colorless and transparent, to a depth of 40 mm, and compare the contents of the tubes against a black background by viewing in diffused light down the vertical axes of the tubes. The diffused light must be such that Reference suspension I can be readily distinguished from water, and that Reference suspension II can readily be distinguished from Reference suspension I.

In this test Reference suspensions are used when the clarity of the test solution is obscurely and it is not easy to determine that its degree of opalescence is similar or not similar to water or to the solvent used to prepare the test solution.

### 1.3. Interpretation

A liquid is considered "clear" when its clarity is the same as that of water or of the solvent used to prepare the liquid or its turbidity is not more pronounced than that of Reference suspension I. If the turbidity of the liquid is more than that of Reference suspension I, consider as follows: When the turbidity is more than that of Reference suspension I but not more than that of Reference suspension II, express "it is not more than Reference suspension II". In the same way, when the turbidity is more than that of Reference suspension II but not more than that of Reference suspension III, express "it is not more than Reference suspension III", and when the turbidity is more than that of Reference suspension III but not more than that of Reference suspension IV, express "it is not more than Reference suspension IV". When the turbidity is more than that of Reference suspension IV, express "it is more than Reference suspension IV".

### 1.4. Reagent solutions

Formazin opalescence standard solution: To exactly 3 mL of formazin stock suspension add water to make exactly 200 mL. Use within 24 hours after preparation. Shake thoroughly before use. Degrees of opalescence of this standard solution is equivalent to 60 NTU.

## 2. Photoelectric photometry

The turbidity can also be estimated by instrumental measurement of the light absorbed or scattered on account of submicroscopic optical density inhomogeneities of opalescent solutions and suspensions. The photoelectric photometry is able to provide more objective determination than the visual method. Though they can determine the turbidity by measuring the scattered or transmitted light, the measuring system and light source must be specified in individual test method, and for the comparison of observed data, the same measuring system and light source should be used.

In each case, the linear relationship between turbidity and concentration must be demonstrated by constructing a calibration curve using at least 4 concentrations. For colored samples, the turbidity value is liable to be estimated lower

because of attenuating both incident and scattered lights due to the absorption by the color, and the transmission-dispersed method is principally used.

### 2.1. Turbidimetry

When a light passes through a turbid liquid the transmitted light is decreased by scattering with the particles dispersed in the liquid. A linear relationship is observed between turbidity and concentration when the particles with a constant size are uniformly dispersed, the size is small and the suspension is not higher concentration. The turbidity can be measured by Ultraviolet-visual Spectrophotometry <2.24> using spectrophotometer or photoelectric photometer. The turbidity of the sample in higher concentration can also be measured, however, it is susceptible to the color of the sample, and the measurement is usually performed at around 660 nm to avoid possible disturbance occurred from the absorption by the color.

### 2.2. Nephelometry

When a suspension is viewed at right angles to the direction of the incident light, it appears opalescent due to the refraction of light from the particles of the suspension (Tyndall effect). A certain portion of the light entering a turbid liquid is transmitted, another portion is absorbed and the remaining portion is scattered by the suspended particles. The scattered light measuring method shows the linear relationship between the nephelometric turbidity units (NTU) values and relative detector signals in a low turbidity range. As the degree of turbidity increases, not all the particles are exposed to the incident light and the scattered radiation of other particles is hindered on its way to the detector.

### 2.3. Ratio Turbidimetry

This method measures both scattered and transmitted light values at the same time, and the turbidity is determined from the ratio of the scattered light value to the transmitted light value. This procedure compensates for the light that is diminished by the color of the sample and eliminates the influence of the color. When the measurement is performed by using an integrating sphere, it is particularly called the integrating sphere method, which measures the total transmitted light value as well as the scattered light value occurred with the suspended particles, and the turbidity can be determined from the ratio of them.

### 2.4. Application of photoelectric photometry for monograph requirements

The turbidity of the test solution, determined by the photoelectric photometry, can be used as an indicating standard for the conformity to the clarity requirements by converting into NTU by using turbidity known reference solutions such as Reference suspensions I - IV, if needed, and water or the solvent used. In an automatically compensable apparatus being calibrated with turbidity known reference solutions, the measuring result is given in NTU and it can be compared directly with required specified value.

NTU is often used as the unit in the turbidity determinations. It is the unit used in the case when the turbidity is estimated by the instrument which measures the  $90 \pm 30^\circ$  scattered light against the incident light intensity, using tungsten lamp, and in the case the estimation is performed by the instrument which measures the  $90 \pm 2.5^\circ$  scattered light against the incident light intensity using 860 nm infrared light, FNU is used as the unit. FNU is equivalent to NTU at a range of smaller measurements (less than 40 NTU). For the unit of formazin concentration, FTU is also used, which is defined as a suspension of 1 mg formazin in 1L of purified water is 1 FTU.

## 2.62 Mass Spectrometry

Mass spectrometry (MS) is a method to separate and detect the ions generated from the ionization of molecules according to their  $m/z$  values, and it is used for the identification and purity test of the substances. The  $m/z$  value is the dimensionless parameter obtained from dividing the relative mass ( $m$ ) of the ion (the ratio of the mass of the ion to the unified atomic mass unit) by the charge number ( $z$ ) of the ion. The unified atomic mass unit is defined as one-twelfth of the mass of a  $^{12}\text{C}$  atom in its ground state, and it is used to express masses of atoms, molecules and ions. The result of measurement is shown as the mass spectrum in which the  $m/z$  values of the ions and the relative intensities of the signals corresponding to the ions are shown on the x-axis and the y-axis, respectively.

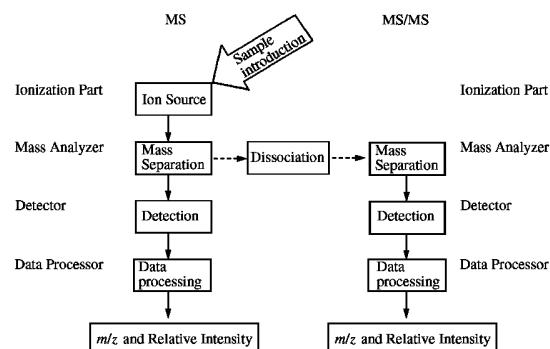
The precise mass of a molecule or an ion consisting of only a single isotope (usually, the isotope with the greatest natural abundance) of each element comprising a sample molecule is referred to as the "monoisotopic mass." Usually not only a monoisotopic ion but also its isotopic ions are seen in the mass spectrum. The molecular mass of the sample substance can be determined from the  $m/z$  value of the molecular ion. When the fragment ions are observed, the molecular structure of the sample substance can be estimated and confirmed based on the masses of the fragment ions and the mass differences among the molecular ion and the fragment ions. In tandem mass spectrometry (MS/MS), the product ions, generated by the dissociation of the selected precursor ion with  $m/z$  value, are used for the mass spectrometry. The structural estimation and confirmation of the precursor ion can be performed using the  $m/z$  value of the product ion observed in the measurement. The schematic diagram of the MS and the MS/MS is shown in Fig. 2.62-1.

### 1. Mass Spectrometer

A mass spectrometer usually consists of a sample introduction unit, an ionization unit (ion source), a mass analyzer, a detector and data processor, and an exhaust system to maintain the mass analyzer under high vacuum (Fig. 2.62-1).

#### 1.1. Sample Introduction

For the introduction of the sample into the ion source, the following methods are used; Direct infusion method, in which solution samples are injected into the ion source by using a syringe pump or capillary tip, for example; Direct inlet method, in which a liquid or solid sample is placed in a glass tube or other appropriate vessel and introduced into



**Fig. 2.62-1** Schematic diagram of mass spectrometry (MS) and tandem mass spectrometry (MS/MS)

the vicinity of the electron beams or reactant ion atmosphere of the ion source. In addition, the method, in which each component separated by the chromatographic technique such as gas chromatography or liquid chromatography and capillary electrophoresis is introduced into the ion source successively, is also used.

### 1.2. Ion Source

When the sample substances are introduced into the mass spectrometer, ions with a positive or negative charge are generated from the substance in the ion source. There are various ionization methods in mass spectrometry, and it is important to select the most suitable ionization method according to the polarity and molecular mass of the sample substance to be measured and the purpose of the measurement. Typical ionization methods are as follows.

#### 1.2.1. Electron Ionization (EI) Method

In the EI method, the vaporized sample molecule ( $M$ ) is ionized by receiving the energy of thermal electrons (usually, 70 eV), and the molecular ion ( $M^{+·}$ ) and fragment ions with the structural information of the sample molecule are generated. This method is suitable for ionizing nonpolar molecules such as volatile or gaseous samples with low molecular mass up to approximately 1000. It is used for the identification of substances using a data library or other source, because mass spectra with reproducible fragmentation patterns can be obtained by this method.

#### 1.2.2. Chemical Ionization (CI) Method

In the CI method, the vaporized sample molecules are ionized through ion/molecule reactant with reaction ions generated from reagent gases such as methane, isobutane and ammonia. When a reagent gas is introduced into the ionization chamber, protonated ions of the molecules  $[M + H]^+$ , deprotonated ions of the molecules  $[M - H]^-$  or reactant ion adducts of the molecules are generated. Since the ions generated by the CI method have internal energy values that are much lower than those obtained by the EI method, the fragmentation of sample molecules hardly occurs.

#### 1.2.3. Electrospray Ionization (ESI) Method

When the sample solution is sprayed through a capillary with a tip to which high voltage is applied, atomized charged droplets are produced. Subsequently, the sample molecules will be ionized when the charge density of the droplets increases, accompanied by the evaporation of the solvent;  $[M + H]^+$ ,  $[M - H]^-$ , or alkali metal ion adduct of the molecules is thus generated. This method is used for the ionization of sample substances from those with low molecular mass and relatively high polarity to those with high molecular mass. The ESI method can also be applied for the measurement of biopolymers such as peptides, proteins and polysaccharides, because the method makes it easy to generate multiply-charged ions such as  $[M + nH]^{n+}$  and  $[M - nH]^{n-}$ .

#### 1.2.4. Atmospheric Pressure Chemical Ionization (APCI) Method

In the APCI method, the sample solution is sprayed and vaporized by passing through a heated capillary using nitrogen as the carrier, and the corona discharge is induced at the time with a high-voltage needle electrode, and the solvent molecules are thus ionized. The sample molecules will be ionized through the ion/molecule reaction with the solvent ions, and  $[M + H]^+$ ,  $[M - H]^-$ , or alkali metal ion adduct of the molecules will be generated. This method is suitable for ionizing nonpolar to highly polar compounds with a molecular mass up to approximately 1500.

### 1.2.5. Matrix-assisted Laser Desorption/Ionization (MALDI) Method

When a mixture of the sample and a matrix such as  $\alpha$ -cyano-4-hydroxycinnamic acid or sinapinic acid is irradiated with a pulsed laser, the sample molecules will be vaporized quickly and ionized, accompanied by the electronic excitation of the matrix. At that time, the proton transfer occurs between the matrix and the sample molecules, and  $[M + H]^+$ ,  $[M - H]^-$ , or alkali metal ion adduct of the molecules is generated. With the MALDI method, it is possible to ionize the compounds from low molecular mass of several hundreds to high molecular mass of several hundred thousand by selecting the appropriate matrix. Since the amount of the sample required for the measurement is very small, this method is used for the ionization of samples of biological origin such as peptides and proteins.

#### 1.2.6. Other Ionization Methods

Various other ionization methods have been developed, including the field ionization (FI) method, the field desorption (FD) method, the fast atom bombardment (FAB) method, the secondary ion mass spectrometry (SIMS) method, the atmospheric pressure photoionization (APPI) method, and an ionization method in which the volatile substances on the material surface can be directly ionized using the ionization by the collision reaction with helium in the excited state in the open space.

#### 1.2.7. Sample Introduction Method and Ionization Method

Each ionization technique is closely related to the sample introduction methods. In the case of the gas chromatography mass spectrometry (GC-MS), vaporized substances separated by a capillary column are directly introduced into a high-vacuum ion source and ionized by the EI method or CI method, for example. In the case of liquid chromatography mass spectrometry (LC-MS), the liquid phase containing sample substances separated by the LC column is sprayed under atmospheric pressure, and the sample substances are ionized by an ionization method described above at the interface to introduce the ions to the high-vacuum mass analyzer. At that time, it is necessary to ensure that the mobile phase to be used has an appropriate composition for both the column separation and the ionization. In the case of capillary electrophoresis mass spectrometry, the flow rate is usually adjusted by adding an appropriate solution to the electrolyte at the end of the capillary, and the sample substance is ionized by the ESI method or other ionization method.

### 1.3. Mass Analyzers

In a mass analyzer, the ions generated in the ion source are separated according to their  $m/z$  values. As a result, the mass and the relative abundance of the ions derived from the samples to be analyzed can be measured. The following mass analyzers are commonly used for MS.

#### 1.3.1. Quadrupole (Q) Analyzer

The quadrupole (Q) analyzer has four rod electrodes set parallel to each other, to which high-frequency alternating current voltage is applied and on which direct current voltage is superimposed. The ions that enter this space oscillate according to their  $m/z$  values, and only ions with a specific  $m/z$  value have a stable trajectory and will be able to pass through the space. The ions with different  $m/z$  values can also become able to pass through the analyzer with a change in the applied voltage, and thereby the mass spectrum can be obtained. The mass resolving power of a Q analyzer is generally low, but Q analyzers are widely used for the qualitative and quantitative analyses as general-purpose equipment, since they have a relatively wide dynamic range and simple composition that can be downsized.

### 1.3.2. Ion-trap (IT) Analyzers

An ion-trap (IT) analyzer is made of an electric field or magnetic field or a combination of field, and is used to trap the ions in a space. The three most commonly used IT analyzers are as follows.

#### 1.3.2.1. Paul Ion-trap

The Paul ion-trap is a synonym for quadrupole ion-trap (QIT). Although it is similar to the quadrupole analyzer in principle, it is able to trap ions stably by using ring electrodes and end-capped electrodes instead of rod electrodes. The trapped ions are discharged into the detector according to their  $m/z$  values by a scan of the high-frequency voltage, and thereby the mass spectrum can be obtained. This method is frequently used for qualitative analyses such as structure analysis because multiple-stage mass spectrometry (MS<sup>n</sup>) can be achieved by using only one analyzer. The instrument with the sensitivity and dynamic range improved by using four electrodes with a hyperbolic surface is referred to as a linear ion-trap (LIT) analyzer.

#### 1.3.2.2. Kingdon Trap

In the Kingdon trap analyzer, ions are trapped while rotating around a spindle-shaped electrode. The image current induced by the ions oscillating according to their  $m/z$  values is measured. The mass spectrum is obtained by Fourier-transforming the measured waveform data on the time axis to those on the frequency axis. This analyzer is used for qualitative analyses such as structure analyses because it has extremely high mass resolving power and mass accuracy.

#### 1.3.2.3. Penning Ion-trap

The Penning ion-trap is used for Fourier transform-ion cyclotron resonance (FT-ICR). The ions that enter into the strong magnetic field formed by a superconducting magnet show cyclotron movement due to the effect of the Lorenz force. Here, the angular frequency ( $\omega$ ) can be expressed by the following equation.

$$\omega = qB/m$$

where  $m$  is the mass in atomic mass units of the ion,  $q$  is the electric charge of the ion, and  $B$  is the magnetic flux density. When the high-frequency electric field with this frequency is applied to the magnetic field, the ions move along the spiral orbital. These rotating ion groups induce the electric current, which changes periodically according to its respective  $m/z$  value in the detecting electrode. The mass spectrum can be obtained by Fourier-transforming the signals measured above and further converting the frequencies to the  $m/z$  values. The Penning ion-trap is used for precise structural studies in combination with various dissociation techniques for precursor ions, since an FT-ICR analyzer has extremely high mass resolving power and mass accuracy.

#### 1.3.3. Time-of-flight (TOF) Analyzer

In the time-of-flight (TOF) analyzer, the ions are separated based on the difference of the flight times necessary for reaching the detector. For the ions with the mass  $m$  accelerated by the constant voltage  $V$ , the time  $t$  necessary for the ions to fly a distance  $L$  and reach the detector can be expressed by the following equation.

$$t = \sqrt{m/z} \times \frac{L}{\sqrt{2eV}}$$

The time of flight  $t$  is proportional to the square root of the  $m/z$  value, and consequently, the ions with smaller mass reach the detector faster. In the reflector mode in which the ions are reflected by the reflectron with the electrodes arranged side by side, high mass resolving power can be obtained by bringing the distribution of the kinetic energy of the ions into focus and doubling the flight distance of the

ions. TOF analyzers are used for the analyses of high-molecular-mass compounds such as proteins in combination with the MALDI method and other techniques, since the mass range measurable by this method does not have a margin, theoretically. It is also frequently used for the qualitative analyses of low-molecular-mass substances, since it has high mass resolving power.

#### 1.3.4. Magnetic Sector Analyzer

The ions that enter a magnetic sector analyzer are deflected by the Lorenz force of the magnetic field perpendicular to the ion current. At that time, ions with different  $m/z$  values (with the velocity  $v$ ) fly into the magnetic field with different radii of curvature  $r$  according to the following equation.

$$r = \frac{mv}{qB}$$

Only ions with a specific  $m/z$  value are able to pass through the slit placed on the path of the ions. The mass spectrum can be obtained by scanning the magnetic flux density  $B$ , and introducing the ions with different  $m/z$  values passed through the slit into the detector in order. A magnetic sector analyzer is usually used as a double-focusing-type instrument in which the electric sector is combined with the magnetic sector, and the analyzer is used for both qualitative and quantitative analyses, since it has high mass resolving power and is also highly quantitative.

### 1.4. Detectors

Ions that have passed through a mass analyzer are usually transduced to the electric signal by releasing the electrons at the detector. The following detectors are in current use. In Fourier-transform-type instruments, the electric current induced by the movement of the ions at the detector is identified with a detection electrode.

#### 1.4.1. Secondary Electron Multiplier (SEM)

A secondary electron multiplier (SEM) has a multistage arrangement of electrodes called dynodes. The secondary electrons emitted by the collision of the ions that enter the multiplier to the first dynode are sequentially multiplied, and finally transduced to the electric signal and recorded. This multiplying effect of the secondary electrons enables the detection of small amounts of ions.

#### 1.4.2. Channel Electron Multiplier (CEM)

A channel electron multiplier (CEM) has a pipe-shaped channel configuration, and the secondary electrons are emitted by the collision of the ions that entered the multiplier to the inner wall of the channel. Multiple amplification is achieved by repeating this process at every opposite side of the inner wall. A CEM is simpler compared to SEMs, and with a CEM it is possible to downsize.

#### 1.4.3. Microchannel Plate (MCP)

A microchannel plate (MCP) has a configuration in which many very small CEMs are accumulated to form a detector. It is used for the detector of TOF-type instruments, since an MCP has a wide ion-receiving surface, and the time dispersion of the secondary electrons is small because of the very thin structure of the MCP.

#### 1.4.4. Faraday Cup (FC)

A Faraday cup (FC) is a simple detector that receives the charge of the ions that have entered an ion detector, and it transduces the charge to the electric current. It has a cup-shaped configuration so that the secondary electrons emitted from the ions can be captured.

### 2. Tandem Mass Spectrometers

Tandem mass spectrometry (TMS) is a technique in which precursor ions are selected from the fragment ions of the sample substance at the first-stage mass analyzer, and the

product ions generated by dissociating the precursor ions are separated and detected at the second-stage mass analyzer. TMS is used for (1) the structural estimation and confirmation of fragment ions, and (2) specific and high sensitive analyses. There are two categories of TMS: TMA in space, and TMS in time.

With TMS in space, the selection of the precursor ions, the dissociation of the precursor ions, and the separation of the product ions are conducted at the first-stage mass analyzer, the intermediate region, and the second stage mass analyzer, respectively. With TMS in time, the selection/dissociation/separation of the ions are conducted at the different time zones in the same mass analyzer. TMS in space includes the triple quadrupole-type, quadrupole/time-of-flight-type, and time-of-flight/time of flight-type mass analyzers. The latter includes the ion-trap type mass analyzer, with which multiple-stage mass spectrometry ( $MS^n$ ) can be performed by repeating the selection and dissociation of the precursor ions and the separation of the product ions multiple times.

### 2.1. Dissociation of Precursor Ions

#### 2.1.1. Collision-induced Dissociation (CID)

In this dissociation method, a part or all of the collision energy is converted to the internal energy of the ions by the collision of the accelerated ions with the neutral collision gases (He, Ar, N<sub>2</sub>, etc.), and subsequently the ions obtaining excess internal energy are excited and dissociated.

#### 2.1.2. Post-source Decay (PSD)

In the MALDI method, the ions generated at the ionization source are dissociated during the interval between leaving the accelerating region and reaching the detector, due to the excess internal energy of the ions themselves or the collision with the residual gas. PSD is used for MS/MS using a reflectron time-of-flight mass spectrometer.

#### 2.1.3. Others

Other dissociation methods are electron capture dissociation, electron transfer dissociation, infrared multiphoton dissociation, and surface-induced dissociation.

### 2.2. Constitutions of Principal Tandem Mass Spectrometers

#### 2.2.1. Triple Quadrupole Mass Spectrometer (Q-q-Q)

A triple quadrupole mass spectrometer (Q-q-Q) has a configuration in which three quadrupoles are tandemly connected so that the first quadrupole is used for the selection of the precursor ions, the second quadrupole is used as the collision chamber for the dissociation of the precursor ions, and the third quadrupole is used for the mass separation of the product ions. Various scanning methods can be employed, and this type of spectrometer is frequently used for quantitative analyses in particular.

#### 2.2.2. Quadrupole Time-of-flight Mass Spectrometer (Q-TOF)

A quadrupole time-of-flight mass spectrometer (Q-TOF) has a configuration in which the third quadrupole in the Q-q-Q is replaced with a TOF mass analyzer. The precursor ions are selected at the first quadrupole, and the separation of generated ions is conducted by the orthogonal-type TOF. Measurement with high sensitivity and high resolution is possible.

#### 2.2.3. Time-of-flight Time-of-flight Mass Spectrometer (TOF-TOF)

A time-of-flight time-of-flight mass spectrometer (TOF-TOF) consists of a TOF analyzer in which the precursor ions are selected, the collision chamber, and a TOF analyzer in which the mass separation of the product ions is performed. It is used for MALDI-TOF-TOF mass spectrometry.

#### 2.2.4. Other Mass Spectrometers

The mass spectrometers other than those described above are the four-sector mass spectrometer with the configuration in which two double-focusing instruments are connected, and the LIT-Kingdon trap and QIT-TOF, in which an in-time-type mass analyzer is used.

### 3. Methods Used for Measurement

#### 3.1. Mass Spectrometry

The following measurement methods are used with mass spectrometry. An outline of the data obtained by each method is also described.

##### 3.1.1. Total Ion Monitoring (TIM)

Total ion monitoring (TIM) is also known as the full-scan mode. It is the technique in which the mass spectrometer is operated so that all ions within the selected  $m/z$  range are detected and recorded, and the integrated value of the amounts of ions observed in each scanning is called the total ion current (TIC).

The chromatogram in which the total ion current obtained from the mass spectrum measured in LC-MS and GC-MS is plotted against the retention time is called the total ion current chromatogram (TICC), and the chromatogram in which the relative intensity at the specific  $m/z$  value is expressed as the function of time is called the extracted ion chromatogram (EIC).

##### 3.1.2. Selected Ion Monitoring (SIM)

In selected ion monitoring (SIM), the mass spectrometer is operated so that only the ions with a specific  $m/z$  value are continuously detected and recorded instead of measuring the mass spectrum. SIM is used for the assay and high-sensitivity detection of sample substances in LC-MS and GC-MS.

#### 3.2. Tandem Mass Spectrometry (TMS)

The following methods are used for measurements using TMS. An outline of the data obtained by each method is also described.

##### 3.2.1. Product Ion Analysis

Product ion analysis is used to detect the product ions generated from the precursor ions with a selected  $m/z$  value, and with this method the sample's qualitative information can be obtained.

##### 3.2.2. Precursor Ion Scan

Precursor ion scan is a method for scanning the precursor ions from which the product ions with a specific  $m/z$  value are generated by dissociation, and it is used for the specific detection of a substance with a specified substructure in the sample.

##### 3.2.3. Constant Neutral Loss Scan

In constant neutral loss scan, the precursor ions that undergo the loss of specified mass (desorption of neutral species) due to dissociation are scanned. This method is used for the specific detection of substances with a specified substructure in the sample.

##### 3.2.4. Selected Reaction Monitoring (SRM)

Selected reaction monitoring (SRM) detects product ions with a specific  $m/z$  value generated by the dissociation of the precursor ions with a specified  $m/z$  value, and it is used for the quantitative detection of trace amounts of substances present in a complex matrix. Although this method is similar to SIM, the specificity is improved by using the product ions generated from the precursor ions for the detection.

### 4. Application to Various Tests

In pharmaceutical analyses, mass spectrometry is used for the identification and purity tests of molecules as a specific detection method based on the mass and the structural information of the molecules.

#### 4.1. Optimization of Instruments

In mass spectrometry, in order to obtain a good shape, sensitivity, and mass accuracy of the ion peak it is necessary to pre-optimize the measurement parameters of each component unit of the instrument by using an appropriate standard material in accord with the ionization method and mass range.

##### 4.1.1. Tuning

The shape, sensitivity, and relative intensity of the ion peak detected are optimized by adjusting the parameters such as the gas pressure, temperature, and voltage of the ion source, mass analyzer, and detector. The various parameters of the ion source affect the generation of ion species, the species transported to the mass analyzer, and the relative intensity. The parameters related to the mass analyzer influence the peak width, mass accuracy, resolving power, and sensitivity, and the detector parameters affect the signal intensity and system sensitivity.

##### 4.1.2. Calibration

The mass calibration of a mass spectrometer is carried out based on the mass of standard material. The reproducibility of the measurement mass values is affected by the electrical variation of the instrument, the surface cleanliness of each component unit such as the ion source, and the room temperature. There are the external and internal standard techniques for mass calibration. The number of points for the calibration differs according to the type of mass spectrometer.

##### 4.1.3. Mass Resolving Power

The ability to separate two adjacent ion peaks from each other is referred to as the mass resolving power. Higher mass resolving power facilitates to separate and detect the ion peaks with a small mass difference. In magnetic-sector mass spectrometry, the mass resolving power  $R$  is calculated by the following equation when two peaks with the mass of  $M$  and  $M + \Delta M$  overlap each other to 10% of either peak height.

$$R = M/\Delta M$$

When an instrument other than a magnetic-sector mass spectrometer is used, such as a quadrupole mass spectrometer or a time-of-flight mass spectrometer, the mass resolving power can usually be calculated by the method using peak width at half-height. When the width of the ion peak with the mass of  $m$  is  $\Delta m$ , the mass resolving power is calculated by  $R = m/\Delta m$ , and is discriminated from that of the magnetic-sector mass spectrometer.

#### 4.2. Test for Identification

The identification of a test substance using mass spectrometry is usually performed by the confirmation of the mass of the test substance molecule. The test should be performed after confirming in advance that the measurement value is within the range specified in the monograph using the standard solutions defined in the monograph, or the specified ion can be detected. According to the mass resolving power of the instrument and the mass of the test substance molecule, the mass of the test substance molecule obtained from mass spectrometry can be adjusted to the monoisotopic mass or the average mass.

In general, the mass of the molecule consisting of only principle isotopes should be obtained from the monoisotopic peak. However, when the monoisotopic peak cannot be identified because, for example, the molecular mass is high or the resolution is not sufficient, the average mass should be calculated from the weighted average of the peak. When samples with high molecular mass such as proteins are analyzed by ESI/MS, the average mass should be calculated by

the deconvolution technique, because the ESI mass spectra would show a series of multiply-charged ions with different charge states. It may be combined with the detection of the fragment ions or the product ions generated from the test substance molecule, which includes characteristic partial structural information.

#### 4.3. Purity test

The purity test of a test substance using mass spectrometry is usually performed in combination with a separation technique such as chromatography using a standard solution with a concentration corresponding to the specified limit of the impurity in the sample. The peak responses of the molecular ions or the characteristic fragment ions and product ions generated from the specified impurity in the sample solution should be compared with those of the ions generated from the substance in the standard solution. To obtain more precise values, the method in which the stable isotope-labeled compound of the analyte is added to the sample solution as the internal standard is also important. When the test is performed using mass spectrometry in combination with, for example, chromatography, a system suitability test should also be required in accord with the chromatography.

## 2.63 Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) are elemental analysis methods in which inductively coupled plasma (ICP) is used as the excitation source or the ion source.

ICP is an excitation source composed of high-temperature argon plasma with intense thermal energy, which is formed by the inductive coupling method. The atoms contained in the sample solution are excited when the solution is sprayed into the plasma. ICP-AES is the method used to measure the atomic emission spectrum of the light emitted from the plasma at the time and to identify and analyze the contents of elements contained in the sample by determining the wavelength and intensity of its spectral lines. Since ICP is also a good ionization source, the atoms in the sample solution are ionized when the solution is sprayed into the plasma. ICP-MS is the method used to measure the mass spectrum of the element ions generated by ICP at the time by separating the element ions into those with each  $m/z$  value and counting the intensities of ion peaks using a mass spectrometer as the detector.

When intense energy is added to an atom from the outside, the peripheral electrons of the atom would transit to an excited state by absorbing certain energy. The electron in the excited state would release the absorbed energy as the light when it returns to its ground state. The light released at the time has a frequency  $v$  (or wavelength  $\lambda$ ) characteristic of each element. When  $h$  is Planck's constant and  $c$  is the velocity of light, the energy  $\Delta E$  of the released light is expressed by the following equation.

$$\Delta E = hv = hc/\lambda$$

Since there are many excited states with various energy levels to which peripheral electrons might transit, many emission lines with various levels of energy can generate from one element, although some lines are strong and others

are weak. However, there is only a limited number of emission lines observed in the ultraviolet/visible region and with enough sensitivity for the qualitative and quantitative analyses of each element. Since each element exhibits its own spectral line with a characteristic frequency (or wavelength) in the atomic emission spectrum, the elements contained in the sample solution can be identified by determining the wavelengths of spectral lines in the spectrum. Quantitative analyses of the elements in the sample solution can also be performed by determining the intensity of the spectral line characteristic of each element. The elemental analysis method using this principle is ICP-AES.

ICP-MS is the elemental analysis method alternative to optical analysis methods such as atomic absorption spectrometry and ICP-AES. In ICP-MS, the element ions generated by the ICP are separated into those with each *m/z* value and the intensities of the separated ions are counted with a mass spectrometer. Compared to ICP-AES, ICP-MS is higher sensitive, and with it an isotope analysis can be performed.

ICP-AES and ICP-MS are both excellent trace analysis methods specific for the inorganic impurities or coexisting elements in drug substances and drug products. Therefore, using these methods, qualitative and quantitative analyses can be performed not only for alkaline/alkaline-earth metals and heavy metals, but also for many elements for which adequate control is required to ensure the safety of pharmaceutical products. It would be useful for the quality assurance of drug substances to apply these methods to the profile analyses of inorganic elements contained in the substances, because these methods enable the simultaneous analysis of many elements.

## 1. Instruments

### 1.1. Instrument Configuration of ICP-AES

An ICP-AES is composed of an excitation source, a sample introduction system, a light emission unit, a spectroscope, a photometer and a data processor.

The excitation source consists of a high-frequency power generator, a control circuit to supply and control the electric energy to the light emission unit, and a gas source. The sample introduction system, the main components of which are a nebulizer and a spray chamber, is used for introducing sample solutions to the light emission unit after nebulizing the solutions.

The main components of the light emission unit, in which the elements containing in the sample solution are atomized and excited to induce light emission, are a torch and a high-frequency induction coil. The torch has a triple tube structure, and the sample solution is introduced through the central tube. Argon gas is used to form the plasma and to transport the sample solution. For the observation method of the light emitted from the light emission unit, there are two viewing modes: the lateral viewing mode in which the radial light of the plasma is observed, and the axial viewing mode in which the central light of the plasma is observed.

The spectroscope separates the light from the light emission unit to the spectral lines, and is composed of optical devices such as a light-converging system and diffraction grating. There are two types of spectrometers: wavelength-scanning spectrometers (monochromators) and simultaneously measuring spectrometers (polychromators) of the wavelength-fixed type. In addition, it is necessary to form a vacuum or to substitute the air in the chamber of the photometer with argon or nitrogen gas, when it is required to measure the spectral lines of the vacuum ultraviolet region (190 nm or shorter).

The photometer, which consists of a detector and a signal

processing system, transduces the light energy of incident light to the electric signal proportional to the intensity of the light. For the detector, a photomultiplier or a semiconductor detector is used.

The data processor is used to process the data obtained by the measurements, and it displays the calibration curves and measurement results.

### 1.2. Instrument Configuration of ICP-MS

An ICP-MS system is composed of an excitation source, a sample introduction system, an ionization port, an interface, an ion lens, a mass analyzer, an ion detector and a data processor.

The excitation source, sample introduction system and ionization port have the same configuration as their counterparts in an ICP-AES system.

The interface is the boundary component for introducing the ions generated by the plasma under atmospheric pressure into a high-vacuum mass analyzer, and is composed of the sampling cone and skimmer cone.

The ion lens brings the ions introduced via the interface into focus and helps introduce the focused ions into the mass analyzer efficiently.

For the mass analyzer, a common choice is a quadrupole mass analyzer. The interference caused by the polyatomic ions described later can be suppressed by placing a collision/reaction cell within the vacuum region before the mass analyzer, and introducing a gas such as hydrogen, helium, ammonia or methane into the cell.

The ion detector transduces the energy of the ions that reached the detector to an electric signal which is amplified by the multiplier. The data processor is used to process the data of the electric signal from the ion detector, and to display the calibration curves and measurement results, etc.

## 2. Pretreatment of Sample

When the samples to be analyzed are organic compounds such as pharmaceutical drug substances, they are usually digested and ashed by the dry ash method or the wet digestion method, and the sample solutions for ICP-AES or ICP-MS are prepared by dissolving the residues in small quantities of nitric acid or hydrochloric acid. When a sample is difficult to digest in the usual manner, the sample can be sealed in a closed, pressurized container and digested using microwave digestion equipment. Although liquid samples containing small amounts of organic solvents can be introduced directly into an ICP-AES or ICP-MS instrument without pretreatment, another alternative is introducing oxygen as the option gas to prevent the build-up of carbon generated from the solvent onto the torch and the interface by contributing to the incineration of organic solvents.

## 3. Operation of ICP-AES

The operation of an ICP-AES system is as follows. The argon plasma is formed by setting the argon gas flow at the specified rate and turning the high-frequency power source on. After confirmation that the state of the plasma is stable, a quantity of the sample solution or the standard solution prepared by the method prescribed in the monograph is introduced into the instrument via the sample injection port, and the emission intensity of the analytical line specified for the element is measured. When it is necessary to perform a test for the confirmation or identification of some elements, the emission spectrum in the wavelength range in which analytical lines specified for the elements that appear is measured.

### 3.1. Performance Evaluations of Spectrometers

Since each spectrometer requires its own calibration method that accords with its properties, a wavelength

calibration must be performed according to the procedure indicated by the manufacturer.

For expressing the wavelength-resolving power of a spectrometer, the half height width of the analytical line in the emission spectrum of a specified element is usually defined in the form of "not more than xxx nm (a constant value)." The following emission lines, from the line with a low wavelength to that with a high wavelength, are usually selected for the above purpose: arsenic (As: 193.696 nm), manganese (Mn: 257.610 nm), copper (Cu: 324.754 nm) and barium (Ba: 455.403 nm).

### 3.2. Optimization of Operating Conditions

The operating conditions usually adopted are as follows. The operating conditions of the instrument should be optimized after stabilizing the state of the plasma by warming up the instrument for 15 – 30 min. The operating parameters should usually be set as follows: high frequency power, 0.8 – 1.4 kW; argon gas flow rate, 10 – 18 L/min for the coolant gas (plasma gas), 0 – 2 L/min for the auxiliary gas, and 0.5 – 2 L/min for the carrier gas. In the lateral viewing mode, the point for measuring the light emitted from the plasma should be set within the range of 10 – 25 mm from the top edge of the induction coil, and the aspiration rate of the sample solution should be set at 0.5 – 2 mL/min. In the axial viewing mode, the optical axis should be adjusted so that the maximum value can be obtained for the intensity of emission line measured. The integration time should be set within the range of one to several tens of seconds, taking the stability of the intensity of the emission line measured into account. When a test using an ICP-AES system is defined in a JP monograph, the operating conditions such as the analytical line (nm), high-frequency power (kW), and argon gas flow rate (L/min) should be prescribed in the monograph. However, it is necessary to optimize the operating conditions individually for each instrument and for each viewing mode used for the measurement.

### 3.3. Interference and Its Suppression or Correction

In the term ICP-AES, the word "interference" is used as a general term that indicates the influence of the coexisting components or matrix on the measurement results. Various interferences are roughly classified as either non-spectral interference (such as physical interference and ionization interference) or spectral interference. Their effects can be eliminated or reduced by applying the appropriate suppression or correction methods for the measurement.

Physical interference means that the measurement results are influenced by the difference between the spray efficiencies of the sample solution and the standard solution used for its calibration in the light emission unit, when the physical properties (such as viscosity, density and surface tension) of the solutions differ. The effective methods for eliminating or reducing this type of physical influence are as follows. The sample solution should be diluted to the level at which such interference will not occur; the properties between the sample solution and the standard solution used for its calibration should be matched as much as possible (matrix-matching method); and the internal standard method (intensity ratio method) or the standard addition method should be used.

Ionization interference indicates the influence due to the change in the ionization rate caused by the increase of electron density in the plasma, which is induced by a large number of electrons generated from the elements coexisting in the sample solution at a high concentration. The suppression or correction method against the ionization interference is essentially the same as the method used in the case of physical interference. The measurement conditions with low ioniza-

zation interference can also be set by the selection and adjustment of the observation method of emitted light, the height for viewing, high-frequency power and carrier gas flow rate, and so on.

Spectral interference is the phenomenon which influences the analytical results of the sample by overlapping the various emission lines and/or the light with a continuous spectrum with the analytical line of the analyte element. To avoid this type of interference, it is necessary to select another analytical line which will not suffer from the spectral interference. However, when no suitable analytical lines can be found, it is necessary to carry out the correction of the spectral interference. In addition, when the pretreatment of the organic samples is not sufficient, the molecular band spectra (NO, OH, NH, CH, etc.) derived from nitrogen, oxygen, hydrogen and carbon remaining in the sample solution might appear at the wavelength close to the analytical line of the analyte element, and could interfere with the analysis.

## 4. Operation of ICP-MS

In the operation of an ICP-MS system, after the confirmation that the state of the plasma is stable, the optimization of the instrument is performed and the system's suitability is confirmed. A quantity of the sample solution or the standard solution prepared by the method prescribed in the monograph is introduced, and the ion count numbers of the signal at the  $m/z$  value specified for the analyte element are determined. When it is necessary to perform a test for the confirmation or identification of some elements, the mass spectrum in the  $m/z$  value range specified for the analyte elements is measured.

### 4.1. Performance Evaluation of Mass Spectrometer

The performance evaluation items for mass spectrometers are the mass accuracy and the mass resolving power. The mass accuracy should be adjusted by matching the  $m/z$  value of the mass axis of the mass analyzer to that of the standard element in the standard solution for the optimization specified in the operating conditions section of the monograph. With quadrupole mass spectrometers, it is preferable that the mass accuracy be within  $\pm 0.2$ . For the mass resolving power, it is preferable that the peak width at 10% of the peak height in the observed ion peak is not more than 0.9.

### 4.2. Optimization of Operating Conditions

When a limit test or a quantitative test is performed, the sensitivity, background and generation ratio of oxide ions and doubly charged ions defined below should be optimized previously to assure that the performance of the instrument is suitable. For the optimization of operating conditions, the solutions of the elements which represent the low mass number elements, intermediate mass number elements and high mass number elements and are unlikely to be contaminated from the environment (e.g.,  $^7\text{Li}$ ,  $^9\text{Be}$ ,  $^{59}\text{Co}$ ,  $^{89}\text{Y}$ ,  $^{115}\text{In}$ ,  $^{140}\text{Ce}$ ,  $^{205}\text{Tl}$  and  $^{209}\text{Bi}$ ) are usually used as the standard solutions after adjusting to adequate concentrations.

The sensitivity is evaluated by the ion count numbers per second of integration time (cps). When a limit test or quantitative test is performed, it is preferable to have the sensitivity of several tens of thousands cps per  $1 \mu\text{g/L}$  (ppb) for each element with a low mass number, intermediate mass number or high mass number.

For the background, it is preferable to be not more than 10 cps, when the measurement is performed at the  $m/z$  value at which no elements exist naturally (e.g.,  $m/z$  value of 4, 8 or 220).

For the generation ratio of oxide ions and doubly charged ions, the count numbers of oxide ions (e.g.,  $^{140}\text{Ce}$ :  $^{140}\text{Ce}^{16}\text{O}^+$ ,  $m/z$  156), doubly charged ions ( $^{140}\text{Ce}^{2+}$ ,  $m/z$  70) and

monovalent ions ( ${}^{140}\text{Ce}^+$ ,  $m/z$  140) should be measured, and the generation ratios are calculated by dividing the ion count number of the oxide ions and doubly charged ions by that of the monovalent ions. It is preferable that the generation ratio of oxide ions (i.e.,  ${}^{140}\text{Ce}^{16}\text{O}^+ / {}^{140}\text{Ce}^+$ ) is not more than 0.03 and that of doubly charged ions (i.e.,  ${}^{140}\text{Ce}^{2+} / {}^{140}\text{Ce}^+$ ) is not more than 0.05.

#### 4.3. Interferences and their Suppression or Correction

In measurements using ICP-MS, attention must be paid to spectral interference and non-spectral interference.

Spectral interference includes isobaric interference and the interference caused by overlapping the mass spectrum of the analyte element with those of polyatomic ions or doubly charged ions. Isobaric interference is the interference by the isobaric element with the atomic mass adjacent to that of the analyte element, for example, the overlap of  ${}^{40}\text{Ar}$  with  ${}^{40}\text{Ca}$  and  ${}^{204}\text{Hg}$  with  ${}^{204}\text{Pb}$ . Since argon plasma is used as the ionization source, the polyatomic ions such as  ${}^{40}\text{Ar}^{16}\text{O}$ ,  ${}^{40}\text{Ar}^{16}\text{O}^1\text{H}$ ,  ${}^{40}\text{Ar}_2$  might be generated, and they would interfere with the measurements of  ${}^{56}\text{Fe}$ ,  ${}^{57}\text{Fe}$  and  ${}^{80}\text{Se}$ , respectively. When an instrument equipped with a collision/reaction cell is used, these polyatomic ions can be decreased in the cell. Doubly charged ions are the ions exhibiting their ion peaks at 1/2 the  $m/z$  value of the corresponding monovalent ions, and the interference might occur when the element with an isotope with the mass number twice that of the analyte element might be present in the sample solution.

Non-spectral interference includes not only the physical interference and the ionization interference as in the case of the ICP-AES, but also the matrix interference unique to ICP-MS. Matrix interference is the phenomenon in which the ion count numbers of every analyte element generally decrease when large amounts of other elements might co-exist in the sample solution. This tendency becomes more significant when the mass number of a co-existing element is larger and its concentration is higher, and when the mass number of the analyte element is smaller. The extent of non-spectral interference can be estimated based on the recovery rate obtained by adding a known amount of the analyte element to the unknown sample. When it is found that the recovery rate is low and the reliability of the analysis is not assured, the correction should be carried out by using the internal standard method or the standard addition method. For ICP-MS in particular, the influence of non-spectral interference can be reduced by using the isotope dilution method.

#### 5. System Suitability

When a limit test or quantitative test is performed using these methods, it is necessary to confirm that the performance of the instrument is suitable by carrying out a system suitability test as defined below in advance of the limit test or quantitative test.

##### 5.1. Evaluation for Required Detectability and Linearity

In an evaluation of an ICP-MS system for the required detectability and linearity, a solution is prepared in which the analyte element is not contained and the standard solution with the concentration of the specification limit of the analyte element, and these solutions are used as the blank solution and the solution for the system suitability test, respectively. The spectra obtained with these solutions are measured according to the test conditions optimized individually for each instrument, and it must be confirmed whether the emission line (or ion peak) of the analyte element is clearly observed at the specified wavelength (or  $m/z$  value) in the solution for system suitability test when compared with the blank solution. In this regard, the limit of the analyte ele-

ment should be specified at the concentration of more than the quantitation limit ( $10\sigma$ ). The test for required detectability is not required in the assay.

For the evaluation of linearity, it should be confirmed that the correlation coefficient of the calibration curve prepared by the procedure described in the section below, “6.2. Quantitative Analysis” is not less than 0.99. The confirmation of linearity is not required in quantitative analyses in the section 6.1. or when isotope dilution in the section 6.2. is performed.

#### 5.2. Evaluation for System Repeatability

Unless otherwise specified, when the test is repeated six times using the solution with the lowest concentration among those used for plotting the calibration curve according to the test conditions optimized individually for each instrument, it should be confirmed that the relative standard deviation of the observed values for the emission intensity (or ion count number) of the analyte element is not more than the specified value (e.g., not more than 3% for an assay, and not more than 5% for a purity test).

#### 6. Qualitative and Quantitative Analyses

##### 6.1. Qualitative Analyses

In ICP-AES, when the wavelengths and relative emission intensities of multiple emission lines from the sample solution conform to those of the emission lines from the elements contained in the standard solution, the presence of the elements can be confirmed. In addition, the library of ICP-emission spectra attached to each instrument or the wavelength table of the spectra can also be used instead of the standard solution. Since the mass number region covering all the elements can be scanned in a short time in ICP-MS, the elements contained in the sample solution can be analyzed qualitatively based on the  $m/z$  value of the ion peak in the mass spectrum obtained from the sample solution.

It would be feasible to list the metal catalysts and inorganic elements that might be contained in the sample as impurities, and for some elements (such as arsenic and lead) it might be necessary to monitor them in a routine manner from the point of view of safety, and to carry out the profile of these inorganic impurities as a part of the manufacturing controls for a drug substance. In addition, the standard solution of each element should be prepared at an appropriate concentration considering the acceptance limit of each element to be specified separately.

##### 6.2. Quantitative Analyses

The quantitative analysis of an inorganic element in the sample solution is usually performed by one of the following methods based on the emission intensity or ion count numbers obtained by the integration of measurement data in a specified time.

(i) Calibration curve method: Prepare standard solutions for plotting a calibration curve with different concentrations (four or more) of the analyte element. Using these standard solutions, the emission intensities at the analytical line specified for the analyte element by ICP-AES or the ion count numbers at the  $m/z$  value specified for the analysis of the element by ICP-MS are measured. The data obtained are then plotted against the concentrations, and this plot is used as the calibration curve. The concentration of the analyte element in the sample solution is determined by using this calibration curve.

(ii) Internal standard method: Prepare standard solutions for plotting a calibration curve with a fixed concentration of the internal standard element and different concentrations (four or more) of the analyte element. Using these standard solutions, the ratios of the emission intensities (or

ion count numbers) of the analyte element to those of the internal standard element are determined. The data obtained are plotted against the concentrations, and this plot is used as the calibration curve. The internal standard element is also added to the sample solution, so that the concentration of internal standard element in the solution becomes the same as that in the standard solution. The concentration of the analyte element in the sample solution is determined by using the calibration curve plotted above.

Before this method is applied, it is necessary to verify that the internal standard element to be added is not contained in the sample solution. If the internal standard element to be added is present in the sample solution, it is necessary to verify that the contaminated amount of standard element is negligible compared to the amount to be added. In addition, in ICP-AES, the following requirements are to be met for the internal standard element: the changes in the emission intensity due to the measurement conditions and properties of the solution should be similar to those of the analyte element, and the emission line which does not cause spectral interference to the analytical line of the analyte element should be selected for the analysis. In contrast, in ICP-MS, it is preferable to select an internal standard element which does not cause spectral interference to the analyte element and has the ionization efficiency and mass number equivalent to the analyte element.

(iii) Standard addition method: Take 4 portions or more of the sample solution with the same volume, and prepare the following solutions; the solution in which the analyte element is not added; the standard solutions for plotting calibration curve in which the analyte element is added at different concentrations (3 or more). Measure the emission intensities at the specified analytical line or the ion count numbers at the specified  $m/z$  value for these solutions. Plot the obtained data against the concentrations calculated from the added amount of the analyte element. Calibrate the concentration of the analyte element in the sample solution from the absolute value of the horizontal axis (concentration)-intercept of the regression line.

In ICP-AES, this method is useful for the correction of non-spectral interference caused by coexisting substances in the sample solution, and it is applicable only to the cases in which spectral interference does not exist, or the background and the spectral interference are exactly corrected and the relationship between the emission intensity and the concentration shows good linearity. In ICP-MS, this method is useful for the correction of non-spectral interference caused by coexisting substances in the sample solution, and it is applicable only to the cases in which the spectral interference is exactly corrected and the relationship between the ion count number and the concentration shows good linearity down to the low concentration region.

(iv) Isotope dilution method: Isotope dilution method is applicable only to the ICP-MS. The concentration of the analyte element is determined from the change of the isotope composition ratio of the element by adding a substance containing a concentrated isotope with a known isotope composition that is different from the natural composition to the sample solution. It is applicable only to the element which has two or more stable isotopes naturally and is able to perform the isotope analysis. It is the feature of this method that the analytical precision is high and is not influenced by non-spectral interference, because the quantitation can be performed only by adding an adequate amount of a substance containing a concentrated isotope and measuring the isotope composition ratio of the sample solution.

## 7. Note

Water and reagents and the standard solutions used in this test are as follows.

(i) For water, water for an ICP analysis should be used. It should be verified prior to the test that the impurities contained in the water do not interfere with the analysis of the analyte element. Here, the water for an ICP analysis has the electric conductivity of  $1 \mu\text{S}\cdot\text{cm}^{-1}$  or less ( $25^\circ\text{C}$ ).

(ii) Reagents that are suitable for ICP analyses and are of high quality should be used.

(iii) For argon gas, either liquefied argon or compressed argon gas with the purity of 99.99 vol% or higher should be used.

(iv) For the standard solutions, they should be prepared by diluting the Standard Solution (e.g., the Standard Solution defined in the JP, or a standard solution with a concentration certified by a public institution or scientific organization) to the specified concentration using the water for ICP analysis. However, in cases in which interference with the analysis might occur, it is preferable to match the properties of the standard solution to those of the sample solution.

(v) When a standard solution containing multiple elements is prepared, a combination of the test solutions and elements should be selected so that precipitation and/or mutual interference does not occur.

## 2.64 Glycosylation Analysis of Glycoprotein

Glycosylation analysis is a method to confirm the consistency of the oligosaccharides attached to glycoprotein drug. The main types of oligosaccharides observed in glycoprotein drug are N-linked oligosaccharides, which are attached to asparagine residue, and O-linked oligosaccharides, which are attached to serine or threonine residues. Oligosaccharides have a diverse variety in structure, and attached oligosaccharides may be heterogeneous even in same glycoprotein and at same attachment site. Glycoprotein generally consists of a mixture of heterogeneous molecules (glycoforms), which differ only in glycosylation. Some oligosaccharides of glycoprotein may involve in stabilization of glycoprotein structure, prevention of enzymatic degradation, modulation of the biological activities, clearance from the bloodstream, intake into the cell, and immunogenicity. Since the oligosaccharide structures and their heterogeneity in the recombinant glycoproteins may change depending on the cell lines, culture conditions, etc., it is important to ensure consistency of the glycosylation for ensurment of the efficacy and safety of glycoprotein drug. Methods to evaluate the glycosylation of glycoprotein are classified into 1) analysis of released monosaccharides obtained after degradation of glycoprotein (monosaccharide analysis), 2) analysis of released oligosaccharides obtained from the glycoprotein (oligosaccharide analysis/oligosaccharide profiling), 3) analysis of glycopeptides obtained after proteolytic treatment of the glycoprotein (glycopeptide analysis), and 4) analysis of intact glycoprotein (glycoform analysis). In the setting specification for glycosylation analysis, methods should be properly selected and used alone or in combination, in consideration of the structural characteristics in the oligosaccharides, which affect efficacy and safety of the substance being tested.

### 1. Monosaccharide analysis

Monosaccharide analysis is a method to provide the infor-

mation of the identity and contents of monosaccharides, which constitute the oligosaccharides attached to the glycoprotein drugs, etc. The kind of the monosaccharides that constitute oligosaccharide is limited. Typically, amino sugars, such as *N*-acetyl glucosamine and *N*-acetyl galactosamine, neutral sugars, such as galactose, mannose, glucose and fucose, and sialic acids, such as *N*-acetyl neuraminic acid and *N*-glycolyl neuraminic acid, may be analyzed. Monosaccharide analysis consist of releasing monosaccharides from the glycoprotein and quantitative analysis of released monosaccharides. Monosaccharide analysis is generally performed after isolation and purification of the glycoprotein using appropriate methods, because excipients and salts can interfere the analysis.

### 1.1. Release of monosaccharides

#### 1.1.1. Neutral and amino sugars

Neutral and amino sugars are generally released by acid hydrolysis. The hydrolysis rate is dependent on the identity of monosaccharide and linkage, and the degradation rate of released monosaccharide is different between the individual monosaccharides. Thus, the conditions of acid hydrolysis should be optimized in order to release and recover the monosaccharides with high efficiency. Standard materials of neutral and amino sugars should be treated as tested samples.

#### 1.1.2. Sialic acids

Sialic acids are released by mild acid hydrolysis or sialidase digestion, because they are labile. In general, sialidases with broad substrate specificity, such as those from *Arthrobacter ureafaciens* or *Clostridium perfringens* may be used.

### 1.2. Quantitative analysis of monosaccharides

Released monosaccharides are quantitatively analyzed by several methods, including high-pH anion-exchange chromatography/pulsed amperometric detection as underivatized monosaccharides, and liquid chromatography with fluorometric or UV detection after derivatization. Content of each monosaccharide is determined by internal standard method or absolute calibration method. For derivatization of neutral and amino sugar, 2-aminobenzoic acid, 2-aminopyridine, ethyl 4-aminobenzoate, 3-methyl-1-phenyl-5-pyrazolone may be used. For derivatization of sialic acid, 1,2-diamino-4,5-methylenedioxybenzene, or 1,2 phenylenediamine can be used. Analysis of derivatized monosaccharides can be performed by reversed-phase chromatography or anion-exchange chromatography with formation of borate complexes, etc. The analytical results are typically expressed as molar ratio of individual monosaccharide to glycoprotein, and then confirmed to be within the specified criteria.

### 2. Oligosaccharide analysis/Oligosaccharide profiling

Oligosaccharide analysis is a method to confirm consistency of the oligosaccharide identities, structures and distribution. Oligosaccharides attached to glycoprotein are released by enzymatic or chemical treatments, and then the released oligosaccharides were analyzed using liquid chromatography <2.01>, capillary electrophoresis, mass spectrometry <2.62> and in combination of them as underivatized or derivatized for the purpose of improvement in sensitivity and separation. The analytical results were obtained as chromatogram, electropherogram or mass spectrum, respectively, and they are referred to as oligosaccharide profile which provides oligosaccharide identities and distribution. If oligosaccharide profiling is performed after decreasing heterogeneity by exoglycosidase digestions for insufficient resolutions due to high oligosaccharide heterogeneity, the relationship of oligosaccharide structures to the efficacy and safety of the

therapeutic glycoprotein are considered, and enzymes should be selected so that structures to be evaluated are not lost.

#### 2.1. Release and purification of oligosaccharides

Enzymatic or chemical treatment are used for releasing oligosaccharides from the glycoprotein. N-linked oligosaccharides are released by peptide N-glycosidase digestion or hydrazinolysis, and O-linked oligosaccharides are released by alkaline  $\beta$ -elimination, hydrazine degradation, and endo O-glycanase. Because releasing efficacy may be affected by identity of protein, glycosylation sites and oligosaccharide structure, releasing conditions should be optimized for each glycoprotein. Careful attention should be taken to the possibility of structural changes in oligosaccharides, such as loss of sialic acids, anomeration at reducing end residues, and successive degradation from reducing end (peeling reaction).

There are several methods to recover the oligosaccharides from the reaction mixtures after releasing, including depletion of protein by ice-cold ethanol precipitation and then extraction from the supernatant, and solid phase extraction using a media, which oligosaccharides have adsorption to, etc. Reproducibility of oligosaccharide recovery should be evaluated, and it should be confirmed that recovery does not differ between oligosaccharides.

#### 2.2. Analysis of released oligosaccharides

Derivatization of released oligosaccharides is usually carried out by reacting aldehyde group at reducing end with derivatizing agent. Given that oligosaccharide react with derivatizing agent stoichiometrically, relative peak responses may suggest molar ratios of the oligosaccharides attached to the glycoprotein. It should be confirmed that derivatizing method has sufficient reaction yield and reproducibility, and that structural changes during derivatization, such as loss of sialic acid residues, are at minimum. If needed, excessive reagents are removed or derivatized oligosaccharides are purified not to affect the test results. It should be confirmed that oligosaccharide profile does not change due to different recovery rates. Test methods should be appropriately selected considering structures and distribution of oligosaccharides which affect efficacy and safety.

Oligosaccharide profile obtained from the product under test is compared with that of reference material, treated in the same conditions, and then it is confirmed that peaks of oligosaccharides that is considered important for efficacy and safety are comparable from the view point of peak position and peak response by visual inspection. Otherwise, relative abundance of each oligosaccharide is calculated as a percentage of the total peak response or as a relative peak response, and then it is confirmed to be within the specified range.

##### 2.2.1. Liquid chromatography <2.01>

Oligosaccharides derivatized with a tag, such as 2-aminobenzamide, 2-aminobenzoic acid, 2-aminopyridine, or others, may be separated by chromatography based on hydrophilic interaction, reversed-phase, ion-exchange, or mix mode of them, and then detected using fluorometric detector. Underivatized oligosaccharides may be analyzed using high-pH anion-exchange chromatography/pulsed amperometric detection. Analytical methods should be selected and optimized according to the characteristics of the oligosaccharides to be tested.

##### 2.2.2. Capillary electrophoresis

Derivatizing agents with multiple negative charges, such as 8-aminopyrene-1,3,6-trisulfonic acid, are often used for analysis of lower sialylated oligosaccharides, to reduce the time required for analysis. Derivatizing agents with low negative charge, such as 2-aminobenzoic acid may be used for analysis of highly sialylated oligosaccharides in order to

achieve the separation based on the number of sialylation. Electrolyte buffer containing borate may be used for adding negative charge and increasing resolution by forming oligosaccharide-borate complex. Derivatized oligosaccharides are separated by capillary zone electrophoresis using appropriate electrolyte buffer, and then detected using laser-induced fluorometric detector, etc. In general, capillary is used with the inner wall surface modified using neutral polymers covalently or dynamically in order to prevent electroosmotic flow. pH and compositions of the electrolyte are selected so that good separation is achieved.

### 2.2.3. Mass spectrometry <2.62>

Derivatized or underivatized oligosaccharides may be analyzed by mass spectrometer, where they are ionized by soft ionization techniques, such as electrospray ionization or matrix-assisted laser desorption/ionization, then separated based on *m/z* values, and detected. Both of positive and negative ion mode are available. Because ionization efficiency of oligosaccharides depends on their structures, ionization polarity should be selected according to the characteristics of the oligosaccharides. Mass spectrometry coupled with liquid chromatography or capillary electrophoresis provides not only elution time or migration time but also information of molecular mass, and allow us more specific oligosaccharide profiling. It is noted that the reproducibility of oligosaccharide profile obtained by mass spectrometry is lower than liquid chromatography and capillary electrophoresis, and that sialic acid residues in the sialo-oligosaccharides tend to be lost in positive ion mode. Mass spectrometry should be used with consideration of the characteristics of oligosaccharides responsible for efficacy and safety.

### 3. Glycopeptide analysis

Glycopeptide analysis is a method to provide the information about site-specific glycosylation properties, such as the degree of occupancy, oligosaccharide structures and heterogeneity. If particular oligosaccharides at specific sites affect biological activity or pharmacokinetics, glycopeptide analysis should be performed. Glycoprotein is digested by specific protease, and resultant mixture of glycopeptides and peptides are subjected to liquid chromatography coupled with mass spectrometer, and mass spectra of the glycopeptides are obtained. Glycopeptide ion is assigned based on masses of peptides and information of product ions obtained by tandem mass spectrometry or multiple-stage mass spectrometry. Glycopeptides may be fractionated by liquid chromatography, and then glycopeptides are subjected to mass spectrometry by offline coupling, or oligosaccharides released from glycopeptides were subjected to oligosaccharide analysis/oligosaccharide profiling using liquid chromatography or capillary electrophoresis.

### 4. Glycoform analysis of glycoprotein

Glycoform analysis is a method to confirm overall glycosylation characteristics and their consistency as glycoprotein. It is desirable to obtain the glycoform profiles to reflect oligosaccharide structures that play a role in efficacy and safety of the glycoprotein. If the degree of sialylation significantly contributes to the efficacy of the glycoprotein, isoelectric focusing, capillary isoelectric focusing, capillary zone electrophoresis, or ion-exchange chromatography, etc, may be performed to provide the charge-based glycoform profiles. Mass spectrometry provides the glycoform profiles based on molecular mass. Size-exclusion chromatography, capillary gel electrophoresis and SDS-PAGE may be useful for confirmation of the glycosylation status of a protein. Glycoform profile of the product under test is confirmed that peak position and response are com-

parable with that of similarly treated standard material, or that the distribution of glycoform is within the specified ranges. If glycoprotein has high molecular mass and/or many glycosylation sites, it may be difficult to separate each glycoform peak adequately. Separation and reproducibility of glycoform profile should be evaluated during method development.

## 2.65 Methods for Color Matching

Methods for Color Matching are applied to the purity test where the color of a test solution is examined by comparing with a matching fluid for color.

### 1. Matching fluids for color

Matching fluids for color A to T are prepared by measuring exactly the volume of three colorimetric stock solutions and water as directed in Table 2.65-1 with a buret or a pipet graduated to less than 0.1 mL, and mixing. Store the solutions in glass-stoppered bottles.

For each of the matching fluids of B-series (B1 to B9), BY-series (BY1 to BY7), Y-series (Y1 to Y7), GY-series (GY1 to GY7) and R-series (R1 to R7), the primary matching solutions for individual color are prepared first by mixing the three colorimetric stock solutions as directed in Table 2.65-2, then mix the primary matching solution for corresponding color as directed in Table 2.65-3 to prepare desired matching fluid for color.

### 2. Procedure

Compare a test solution with a matching fluid for color specified in monograph according to the following manners, and confirm that the test solution has no more color than the specified matching fluid for color.

When the matching fluids for color A to T are used, unless otherwise specified, place the test solution and the matching fluid for color in Nessler tubes, and view transversely against a white background.

When the matching fluids for color of B-series, BY-series, Y-series, GY-series or R-series are used, compare the color by the following two methods, and state the used method number in the monograph. A solution is *colourless* if it has the appearance of water or the solvent or is not more intensely coloured than matching solution B9.

**Method 1:** Place separately 2.0 mL each of a test solution and a reference liquid such as water, solvent or the matching fluid for color specified in the monograph in clear and colorless glass test tubes, 12 mm in outside diameter, and compare the color by viewing transversely against a white background under scattering light.

**Method 2:** Place separately a test solution and a reference liquid such as water, solvent or the matching fluid for color specified in the monograph in clear and colorless flat-bottom test tubes, 15 – 25 mm in internal diameter, so that the depth of the layer is 40 mm, and compare the color by viewing vertically against a white background under scattering light.

### 3. Colorimetric stock solutions

**Cobalt (II) Chloride CS:** Dissolve 65 g of cobalt (II) chloride hexahydrate in 25 mL of hydrochloric acid and water to make 1000 mL. Pipet 10 mL of this solution, add water to make exactly 250 mL. Pipet 25 mL of the solution, add 75 mL of water and 50 mg of murexide-sodium chloride indicator, and add dropwise diluted ammonia solution (28) (1 in 10) until the color of the solution changes from red-purple to orange-yellow. Titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of

**Table 2.65-1** Composition of matching fluids for color A to T

Matching fluid for color	Cobalt (II) Chloride CS (mL)	Iron (III) Chloride CS (mL)	Copper (II) Sulfate CS (mL)	Water (mL)
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	3.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0.4	1.2	0.3	3.1
F	0.3	1.2	—	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	—	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	—	—
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	—	4.9	0.1	—
O	0.1	4.8	0.1	—
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	—	4.7
T	0.5	0.5	0.4	3.6

**Table 2.65-2** Primary matching solutions for color used for preparation of a series of matching fluids for color (B-series, BY-series, Y-series, GY-series, R-series)

Primary matching solutions for individual color	Volumes to mix (mL)			
	Iron (III) Chloride CS	Cobalt (II) Chloride CS	Copper (II) Sulfate CS	Diluted hydrochloric acid 1 in 10
Brown primary matching solution	3.0	3.0	2.4	1.6
Brownish yellow primary matching solution	2.4	1.0	0.4	6.2
Yellow primary matching solution	2.4	0.6	0.0	7.0
Greenish yellow primary matching solution	9.6	0.2	0.2	0.0
Red primary matching solution	1.0	2.0	0.0	7.0

the solution changes from yellow to red-purple, after the addition of 0.2 mL of diluted ammonia solution (28) (1 in 10) near the end-point.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 2.379 mg of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 59.5 mg of

**Table 2.65-3** Compositions of a series of matching fluids for color (B-series, BY-series, Y-series, GY-series, R-series)

Matching fluid for color	Volumes to mix (mL)	
	Primary matching solutions for individual color	Diluted dilute hydrochloric acid 1 in 10
Brown primary matching solution		
B1	75.0	25.0
B2	50.0	50.0
B3	37.5	62.5
B4	25.0	75.0
B5	12.5	87.5
B6	5.0	95.0
B7	2.5	97.5
B8	1.5	98.5
B9	1.0	99.0
Brownish yellow primary matching solution		
BY1	100.0	0.0
BY2	75.0	25.0
BY3	50.0	50.0
BY4	25.0	75.0
BY5	12.5	87.5
BY6	5.0	95.0
BY7	2.5	97.5
Yellow primary matching solution		
Y1	100.0	0.0
Y2	75.0	25.0
Y3	50.0	50.0
Y4	25.0	75.0
Y5	12.5	87.5
Y6	5.0	95.0
Y7	2.5	97.5
Greenish yellow primary matching solution		
GY1	25.0	75.0
GY2	15.0	85.0
GY3	8.5	91.5
GY4	5.0	95.0
GY5	3.0	97.0
GY6	1.5	98.5
GY7	0.75	99.25
Red primary matching solution		
R1	100.0	0.0
R2	75.0	25.0
R3	50.0	50.0
R4	37.5	62.5
R5	25.0	75.0
R6	12.5	87.5
R7	5.0	95.0

cobalt (II) chloride hexahydrate ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ : 237.93) in each mL, and use. Store the solution in a glass-stoppered bottle.

**Copper (II) Sulfate CS:** Dissolve 65 g of copper (II) sulfate pentahydrate in 25 mL of hydrochloric acid and water to make 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 250 mL. Pipet 25 mL of this solution, add 75 mL of water, 10 mL of a solution of ammonium chloride (3 in 50), 2 mL of diluted ammonia solution (28) (1 in 10) and 50 mg of murexide-sodium chloride indicator. Titrate

<2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from green to purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 2.497 mg of CuSO<sub>4</sub>.5H<sub>2</sub>O

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 62.4 mg of copper (II) sulfate pentahydrate (CuSO<sub>4</sub>.5H<sub>2</sub>O: 249.69) in each mL, and use. Store the solution in a glass-stoppered bottle.

**Iron (III) Chloride CS:** Dissolve 55 g of iron (III) chloride hexahydrate in 25 mL of hydrochloric acid and water to make 1000 mL. Pipet 10 mL of this solution in an iodine flask, add 15 mL of water and 3 g of potassium iodide, stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 mL of water to the mixture, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 27.03 mg of FeCl<sub>3</sub>.6H<sub>2</sub>O

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 45.0 mg of iron (III) chloride hexahydrate (FeCl<sub>3</sub>.6H<sub>2</sub>O: 270.30) in each mL, and use. Store the solution in a glass-stoppered bottle.

## 2.66 Elemental Impurities

### I. Control of Elemental Impurities in Drug Products

#### 1. Introduction

Elemental impurities in drug products may arise from several sources; they may be residues intentionally added such as catalysts in the synthetic process of drug substances, impurities from natural products contained in drug substances and excipients, etc., which are components of the drug product, and contaminants from manufacturing equipment and container/closure systems. The amounts of these impurities in drug products should be controlled within acceptable limits, except when they are stipulated in monographs.

The permitted daily exposures (PDEs) of elemental impurities are established to protect the health of all patients based on the evaluation of the toxic data of elemental impurities, and more strict limits are not needed if elemental impurities in drug products do not exceed the PDEs. In some cases, lower level of elemental impurities may be warranted when it is known that elemental impurities have been shown to have an impact on the quality attributes of the drug product (e.g., element catalyzed degradation of drug substances).

Elemental impurities in drug products are assessed and controlled based on a risk management approach.

#### 2. Scope

The control of elemental impurities applies to drug products. It also applies to drug products containing purified proteins and peptides (including proteins and peptides produced from genetic recombinant or non-recombinant origins), their derivatives, and drug products which they are components (e.g., conjugates) are within the scope of this chapter, as are drug products containing synthetic peptides, polynucleotides, and oligosaccharides.

It does not apply to crude drugs, radiopharmaceuticals,

vaccines, cell metabolites, DNA products, allergenic extracts, cells, whole blood, cellular blood components, plasma, blood plasma protein fraction preparations, blood preparations, dialysate solutions not intended for systematic circulation, and drug products based on genes (gene therapy), cells (cell therapy) and tissues (tissue engineering). Also, it does not apply to elements that are intentionally included in the drug product for therapeutic benefit.

#### 3. The PDEs for Elemental Impurities for Oral, Parenteral and Inhalation Routes of Administration, and Element Classification

The PDEs of elemental impurities established for preparations for oral, parenteral and inhalation routes of administration are shown in Table 2.66-1. If the PDEs for the other administration route are necessary, generally consider the oral PDE as a starting point in the establishment, and assess if the elemental impurity is expected to have local effects when administered by the intended route of administration.

Parenteral drug products with maximum daily volumes up to 2 L may use the maximum daily volume to calculate permissible concentrations from PDEs. For products whose daily volumes or general clinical practice may exceed 2 L (e.g., saline, dextrose, total parenteral nutrition, solutions for irrigation), a 2-L volume may be used to calculate permissible concentrations from PDEs.

As shown in Table 2.66-1, elemental impurities are divided into three classes based on their toxicity (PDE) and likelihood of occurrence in the drug product. The likelihood of occurrence is derived from several factors, such as probability of use in pharmaceutical processes, elemental impurities in materials used in pharmaceutical processes, the observed natural abundance and environmental distribution of the

Table 2.66-1 PDEs for Elemental Impurities

Element	Class	Oral PDE ( $\mu\text{g}/\text{day}$ )	Parenteral PDE ( $\mu\text{g}/\text{day}$ )	Inhalation PDE ( $\mu\text{g}/\text{day}$ )
Cd	1	5	2	3
Pb	1	5	5	5
As	1	15	15	2
Hg	1	30	3	1
Co	2A	50	5	3
V	2A	100	10	1
Ni	2A	200	20	5
Tl	2B	8	8	8
Au	2B	100	100	1
Pd	2B	100	10	1
Ir	2B	100	10	1
Os	2B	100	10	1
Rh	2B	100	10	1
Ru	2B	100	10	1
Se	2B	150	80	130
Ag	2B	150	10	7
Pt	2B	100	10	1
Li	3	550	250	25
Sb	3	1200	90	20
Ba	3	1400	700	300
Mo	3	3000	1500	10
Cu	3	3000	300	30
Sn	3	6000	600	60
Cr	3	11000	1100	3

element.

Class 1: The elements, As, Cd, Hg, and Pb, are classified as this category and are human toxicant elements. As these elements are limited in the manufacture of pharmaceuticals, they are rarely used. Their presence in drug products usually comes from used materials such as mined excipients. These four elements require evaluation during the risk assessment, across all sources and routes of administration having possibility of contamination. Testing may be applied when the risk assessment identifies further control necessary to ensure that the PDE will be met, however it is not necessary for all components to determine for Class 1 elemental impurities.

Class 2: Elemental impurities classified as Class 2 have lower toxicity than the elements in Class 1, and are route-dependent human toxicants. These elements are further divided in 2A and 2B based on their relative likelihood of occurrence in the drug product. The class 2A elements are Co, Ni and V, which are known to exist naturally. These elements have relatively high probability of occurrence in drug products, and thus require evaluation during the risk assessment, across all potential sources and routes of administration. Because the Class 2B elements have the low probability of their existence in natural, they may be excluded from the risk assessment unless they are intentionally added during the manufacture of drug substances, excipients or other components of the drug product. The elemental impurities in Class 2B include Ag, Au, Ir, Os, Pd, Pt, Rh, Ru, Se and Tl.

Class 3: The elements in this class have relatively low toxicities by the oral route of administration, and their oral PDEs are more than 500 µg/day. For oral routes of administration, unless these elements are intentionally added, they do not need to be considered during the risk assessment. For parenteral and inhalation products, the potential for inclusion of these elemental impurities should be evaluated even in the case where they are not intentionally added, unless the route specific PDE is above 500 µg/day. The elements in this class include Ba, Cr, Cu, Li, Mo, Sb and Sn.

#### 4. Risk Assessment and Control of Elemental Impurities

The technique of quality risk management should be considered in controls for elemental impurities in drug products, and the risk assessment should be based on scientific knowledge and principles. The risk assessment would be focused on assessing the levels of elemental impurities in a drug product in relation to the PDEs. Useful information for this risk assessment includes measured data of drug products and components, measured data and the risk assessment result supplied by drug substance and/or excipient manufacturers, and/or data available in published literature, but is not limited to them.

The risk assessment should be performed depending on the level of risk, and do not always require a formal risk management process. The use of informal risk management processes may also be considered acceptable.

##### 4.1. General Principles

The risk assessment process consists of the following three steps.

- 1) Identify known and potential sources of elemental impurities that may find their way into the drug product.
- 2) Evaluate the presence of a particular elemental impurity in the drug product by determining the observed or predicted level of the impurity and comparing with the established PDE.
- 3) Summarize the risk assessment, and identify if con-

trols built into the process are sufficient. Identify additional controls to be considered to limit elemental impurities in the drug product.

In many cases, the steps are considered simultaneously. The risk assessment may be iterated to develop a final approach to ensure the elemental impurities do not exceed the PDE certainly.

##### 4.2. Sources of Elemental Impurities

In considering the production of a drug product, there are broad categories of potential sources of elemental impurities.

- Residual impurities resulting from elements intentionally added (e.g., metal catalysts) in the formation of the drug substance, excipients or other components. The risk assessment of the drug substance should be studied about the potential for inclusion of elemental impurities in the drug product.
- Elemental impurities that are not intentionally added and are potentially present in the drug substance, water or excipients used in the preparation of the drug product.
- Elemental impurities that are potentially introduced into the drug substance and/or drug product from manufacturing equipment.
- Elemental impurities that have the potential to be leached into the drug substance and drug product from container closure systems.

During the risk assessment, the potential contributions from each of these sources should be considered to determine the overall contribution of elemental impurities to the drug product.

##### 4.3. Identification of Potential Elemental Impurities

Potential elemental impurities derived from intentionally added catalysts and inorganic reagents: If any element is intentionally added, it should be considered in the risk assessment.

Potential elemental impurities that may be present in drug substances and/or excipients: While not intentionally added, some elemental impurities may be present in some drug substances and/or excipients. The possibility for inclusion of these elements in the drug product should be reflected in the risk assessment.

Potential elemental impurities derived from manufacturing equipment: The contribution of elemental impurities from this source may be limited and the subset of elemental impurities that should be considered in the risk assessment will depend on the manufacturing equipment used in the production of the drug product. The specific elemental impurities of concern should be assessed based on the knowledge of the composition of the components of the manufacturing equipment that come in contact with components of the drug product. The risk assessment of this source of elemental impurities is one that can potentially be utilized for many drug products using similar process trains or processes.

In general, the processes used to prepare a given drug substance are considerably more aggressive than processes used in preparing the drug product when assessed relative to the potential to leach or remove elemental impurities from manufacturing equipment. Contributions of elemental impurities from drug product processing equipment would be expected to be lower than contributions observed for the drug substance. However, when this is not the case based on process knowledge or understanding, the potential for incorporation of elemental impurities from the drug product manufacturing equipment in the risk assessment (e.g., hot melt extrusion) should be considered.

Elemental impurities leached from container closure sys-

tems: The identification of potential elemental impurities that may be introduced from container closure systems should be based on a scientific understanding of likely interactions between a particular drug product type and its packaging. When a review of the materials of construction demonstrates that the container closure system does not contain elemental impurities, no additional risk assessment needs to be performed. It is recognized that the probability of elemental leaching into solid dosage forms is minimal and does not require further consideration in the risk assessment. For liquid and semi-solid dosage forms there is a higher probability that elemental impurities could leach from the container closure system during the shelf-life of the drug product. Studies to understand potential leachables from the container closure system (after washing, sterilization, irradiation, etc.) should be performed.

Factors that should be considered (for liquid and semi-solid dosage forms) are shown as follows, but are not limited.

- Hydrophilicity/hydrophobicity, Ionic content, pH, Temperature (cold chain vs room temperature and processing conditions), Contact surface area, Container/material composition, Terminal sterilization, Packaging process, Material sterilization, Duration of storage

Table 2.66-2 provides recommendations for inclusion of elemental impurities in the risk assessment. This table can be applied to all sources of elemental impurities in the drug product.

#### 4.4. Evaluation

As the potential elemental impurity identification process is concluded, there are following two possible outcomes.

**Table 2.66-2** Elements to be Considered in the Risk Assessment

Element	Class	If intentionally added (all routes)			If not intentionally added		
		Oral	Parenteral	Inhalation			
Cd	1	○	○	○	○		
Pb	1	○	○	○	○		
As	1	○	○	○	○		
Hg	1	○	○	○	○		
Co	2A	○	○	○	○		
V	2A	○	○	○	○		
Ni	2A	○	○	○	○		
TI	2B	○	×	×	×		
Au	2B	○	×	×	×		
Pd	2B	○	×	×	×		
Ir	2B	○	×	×	×		
Os	2B	○	×	×	×		
Rh	2B	○	×	×	×		
Ru	2B	○	×	×	×		
Se	2B	○	×	×	×		
Ag	2B	○	×	×	×		
Pt	2B	○	×	×	×		
Li	3	○	×	○	○		
Sb	3	○	×	○	○		
Ba	3	○	×	×	○		
Mo	3	○	×	×	○		
Cu	3	○	×	○	○		
Sn	3	○	×	×	○		
Cr	3	○	×	×	○		

○: necessary    ×: unnecessary

1) The risk assessment process does not identify any potential elemental impurities.

2) The risk assessment process identifies one or more potential elemental impurities. For any elemental impurities identified in the process, the risk assessment should consider if there are multiple sources of the identified elemental impurity or impurities.

During the risk assessment, a number of factors that can influence the level of the potential elemental impurity in the drug product should be considered.

#### 4.5. Summary of Risk Assessment Process

The risk assessment is summarized by reviewing relevant product or component specific data combined with information and knowledge gained across products or processes to identify the significant probable elemental impurities that may be observed in the drug product.

The significance of the observed or predicted level of the elemental impurity should be considered in relation to the PDE of the elemental impurity. As a measure of the significance of the observed elemental impurity level, a control threshold is defined as a level that is 30% of the established PDE in the drug product. The control threshold may be used to determine if additional controls may be required.

If the total elemental impurity level from all sources in the drug product is expected to be consistently less than 30% of the PDE, then additional controls are not required, provided adequate controls on elemental impurities are demonstrated by the appropriate assessment of the data.

If the risk assessment fails to demonstrate that an elemental impurity level is consistently less than the control threshold, controls should be established to ensure that the elemental impurity level does not exceed the PDE in the drug product.

The variability of the level of an elemental impurity should be factored into the application of the control threshold to drug products. Sources of variability may include the following.

- Variability of the analytical method
- Variability of the elemental impurity level in the specific sources
- Variability of the elemental impurity level in the drug product

For some components that have inherent variability (e.g., mined excipients), more data may be needed to apply the control threshold.

#### 5. Converting between PDEs and Concentration Limits

The PDEs reported in  $\mu\text{g}$  per day ( $\mu\text{g}/\text{day}$ ) give the maximum permitted quantity of each element that may be contained in the maximum daily dose of a drug product. Because the PDE reflects total exposure from the drug product, it is useful to convert the PDE into concentrations as a tool in evaluating elemental impurities in drug products or their components. Any of the following options may be selectable as long as the resulting permitted concentrations assure that the drug product does not exceed the PDEs. In the choice of a specific option the daily dose of the drug product needs to be determined or assumed.

Option 1: Common permitted concentration limits of elements across drug product components for drug products with daily doses of not more than 10 g: This option is not intended to imply that all elements are present at the same concentration, but rather provides a simplified approach to the calculations. The option assumes the daily dose of the drug product is 10 g or less, and that elemental impurities identified in the risk assessment (the target elements) are present in all components of the drug product. Using Equation (1)

below and a daily dose of 10 g of drug product, this option calculates a common permissible target elemental concentration for each component in the drug product.

$$\text{Concentration } (\mu\text{g/g}) = \frac{\text{PDE } (\mu\text{g/day})}{\text{daily dose of drug product } (\text{g/day})} \quad (1)$$

This approach, for each target element, allows determination of a fixed common maximum concentration in  $\mu\text{g}$  per g in each component.

The permitted concentrations are provided in Table 2.66-3.

If all the components in a drug product do not exceed the Option 1 permitted concentrations for all target elements identified in the risk assessment, then all these components may be used in any proportion in the drug product. If the permitted concentrations in Table 2.66-3 are not applied, Options 2a, 2b, or 3 should be followed.

Option 2a: Common permitted concentration limits of elements across drug product components for a drug product with a specified daily dose: This option is similar to Option 1, except that the drug daily dose is not assumed to be 10 g. The common permitted concentration of each element is determined using Equation (1) and the actual maximum daily dose. This approach, for each target element, allows determination of a fixed common maximum concentration in  $\mu\text{g}$  per g in each component based on the actual daily dose provided. If all components in a drug product do not exceed the Option 2a permitted concentrations for all target elements identified in the risk assessment, then all these components may be used in any proportion in the drug product.

Option 2b: Permitted concentration limits of elements in

**Table 2.66-3** Permitted Concentrations of Elemental Impurities for Option 1

Element	Class	Oral Concentration ( $\mu\text{g/g}$ )	Parenteral Concentration ( $\mu\text{g/g}$ )	Inhalation Concentration ( $\mu\text{g/g}$ )
Cd	1	0.5	0.2	0.3
Pb	1	0.5	0.5	0.5
As	1	1.5	1.5	0.2
Hg	1	3	0.3	0.1
Co	2A	5	0.5	0.3
V	2A	10	1	0.1
Ni	2A	20	2	0.5
Tl	2B	0.8	0.8	0.8
Au	2B	10	10	0.1
Pd	2B	10	1	0.1
Ir	2B	10	1	0.1
Os	2B	10	1	0.1
Rh	2B	10	1	0.1
Ru	2B	10	1	0.1
Se	2B	15	8	13
Ag	2B	15	1	0.7
Pt	2B	10	1	0.1
Li	3	55	25	2.5
Sb	3	120	9	2
Ba	3	140	70	30
Mo	3	300	150	1
Cu	3	300	30	3
Sn	3	600	60	6
Cr	3	1100	110	0.3

individual components of a drug product with a specified daily dose: Permitted concentrations based on the distribution of elements in the components (e.g., higher concentrations in components with the presence of an element in question) may be set. For each element identified as potentially present in the components of the drug product, the maximum expected mass of the elemental impurity in the final drug product can be calculated by multiplying the mass of each component material times the permitted concentration pre-established in each material and summing over all components in the drug product, as described in Equation (2). The total mass of the elemental impurity in the drug product should comply with the PDEs unless justified according to other relevant sections of this general information. If the risk assessment has determined that a specific element is not a potential impurity in a specific component, there is no need to establish a quantitative result for that element in that component. This approach allows that the maximum permitted concentration of an element in certain components of the drug product may be higher than the Option 1 or Option 2a limit, but this should then be compensated by lower allowable concentrations in the other components of the drug product. Equation (2) may be used to demonstrate that component-specific limits for each element in each component of a drug product assure that the PDE will be met.

$$\text{PDE } (\mu\text{g/day}) \geq \sum_{k=1}^N C_k \cdot M_k \quad (2)$$

$k$  = an index for each of  $N$  components in the drug product

$C_k$  = permitted concentration of the elemental impurity in component  $k$  ( $\mu\text{g/g}$ )

$M_k$  = mass of component  $k$  in the maximum daily dose of the drug product (g)

**Option 3: Finished Product Analysis:** The concentration of each element may be measured in the final drug product. Equation (1) may be used with the maximum total daily dose of the drug product to calculate a maximum permitted concentration of the elemental impurity.

## 6. Speciation and Other Considerations

Speciation is defined as the distribution of elements among chemical species based on the difference of molecular structure including ionic element, molecules, or complexes, reflecting isotopic composition, electronic or oxidation state. When the toxicities of different species of the same element are known to be different, the PDE has been established using the toxicity information on the species expected to be in the drug product.

When elemental impurity measurements are used in the risk assessment, total elemental impurity levels in drug products may be used to assess compliance with the PDEs. The identification of speciation is not particularly expected, however such information could be used to justify lower or higher levels when the identified species is more or less toxic, respectively, than the species used for the calculation of the PDEs.

When total elemental impurity levels in components are used in the risk assessment, providing information on release of an elemental impurity from the component in which it is found is not expected. However, such information could be used to justify levels higher than those based on the total elemental impurity content of the drug product.

## 7. Analytical Procedures

The determination of elemental impurities should be conducted using appropriate procedures suitable for their in-

tended purposes. Unless otherwise justified, the test should be specific for each elemental impurity identified for control during the risk assessment. The following II. Elemental Impurities-Procedures or suitable alternative procedures (analytical procedures) for determining levels of elemental impurities should be used.

## 8. Lifecycle Management

If changes to the drug product or components have the potential to change the elemental impurity content of the drug product, the risk assessment, including established controls for elemental impurities, should be re-evaluated. Such changes could include changes in synthetic routes, excipient suppliers, raw materials, processes, equipment, container closure systems or facilities.

## II. Elemental Impurities—Procedures

Procedures of Elemental Impurities are methods to control elemental impurities contained in drug products and their components, etc. This chapter describes two analytical procedures (Procedures 1 and 2) and validation criteria for the evaluation of the levels of elemental impurities. The chapter permits the use of any procedure that meets the validation criteria specified in this chapter. As the chemical composition of the considered substances and the specification limits for the element(s) of interest vary considerably, it is difficult to describe all suitable sample preparation and measurement methods. By means of validation studies, analysts will confirm that the analytical procedure is suitable for use on specified material. It is not necessary to cross validate against either procedure 1 or 2 provided that requirements for procedure validation are met. As elemental impurities may be ubiquitous and have the potential to be present in trace amounts therefore special precautions may be necessary to avoid sample contamination. (Note: Methods such as atomic absorption spectrometry other than methods described in this chapter, if validated, can also be used without cross validation against analytical procedure 1 or 2.)

### 1. Sample Preparation

Forms of sample preparation include Neat, Direct aqueous solution, Direct organic solution, and Indirect solution. The selection of the appropriate sample preparation depends on the material under test and is the responsibility of the analyst. When a sample preparation is not indicated in the monograph, an analyst may use any appropriately validated sample preparation procedure, including but not limited to procedures described below. In cases where spiking of a material under test is necessary to provide an acceptable signal intensity, the blank should be spiked with the same *Target elements*, and where possible, using the same spiking solution. The material or mixture under test must be spiked before any sample preparation steps are performed. Standard solutions may contain multiple *Target elements*. (Note: If intended for a quantitative test, appropriate material handling procedures should be followed e.g. volatile liquids should be pipetted, viscous liquids should be weighed.)

**Neat:** Used for liquids or analytical procedures that allow the examination of unsolvated samples.

**Direct aqueous solution:** Used when the sample is soluble in an aqueous solvent.

**Direct organic solution:** Used when the sample is soluble in an organic solvent.

**Indirect solution:** Generally, an indirect solution is obtained when a material is not directly soluble in aqueous or organic solvents. Total metal extraction is the preferred sample preparation approach to obtain an *indirect solution*.

Digest the sample using the *Closed vessel digestion* procedure provided below or one similar to it.

**Closed vessel digestion:** This sample preparation procedure is designed for samples that must be digested in a *Concentrated acid* using a closed vessel digestion apparatus. *Closed vessel digestion* minimizes the loss of volatile impurities. The choice of a *Concentrated acid* depends on the sample matrix. The use of any of the *Concentrated acids* may be appropriate, but each introduces inherent safety risks. Therefore, appropriate safety precautions should be used at all times. (Note: Weights and volumes provided may be adjusted to meet the requirements of the digestion apparatus used.)

An example procedure that has been shown to have broad applicability is the following. Dehydrate and predigest 0.5 g of material under test in 5 mL of freshly prepared *Concentrated acid*. Allow to sit loosely covered for 30 min in a fume hood. Add an additional 10 mL of *Concentrated acid*, and digest, using a closed vessel technique, until digestion or extraction results in a clear solution. Repeat, if necessary, by adding an additional 5 mL of *Concentrated acid*. (Note: Where *closed vessel digestion* is necessary, follow the manufacturer's recommended procedures to ensure safe use.)

Clear solutions are expected in the validation. In those cases where a clear solution cannot be obtained, appropriate studies should ensure that the recovery is suitable for the intended use.

**Reagents:** All reagents used for the preparation of sample and standard solutions should be sufficiently pure for the intended purpose.

### 2. Analytical Procedures 1 and 2

System standardization and suitability evaluation using applicable reference materials should be performed for each analytical sequence.

#### 2.1. Procedure and Detection Technique

**Procedure 1** can be used for elemental impurities generally amenable to detection by inductively coupled plasma-optical (optical) emission spectroscopy (ICP-AES or ICP-OES). **Procedure 2** can be used for elemental impurities generally amenable to detection by inductively coupled plasma-mass spectrometry (ICP-MS). Before initial use, the analyst should verify that the procedure is appropriate for the instrument and sample used (procedural verification) by meeting the procedure validation requirements below.

#### 2.2. Procedure 1: ICP-OES

**Standard solution 1:** 1.5J of the *Target element(s)* in a *Matrix matched solution*.

**Standard solution 2:** 0.5J of the *Target element(s)* in a *Matrix matched solution*.

**Sample stock solution:** Proceed as directed in 1. *Sample Preparation* above. Allow the sample to cool, if necessary. For mercury determination, add an appropriate stabilizer, if necessary.

**Sample solution:** Dilute the *Sample stock solution* with an appropriate solvent to obtain a final concentration of the *Target element(s)* within the calibrated range.

**Blank:** *Matrix matched solution*.

**Elemental spectrometric system**

**Mode:** ICP.

**Detector:** Optical detection system.

**Rinse:** Diluent used.

**Standardization:** *Standard solution 1*, *Standard solution 2*, and *Blank*.

**System suitability Sample:** Standard solution of the *Target element(s)* in a *Matrix matched solution* at a concentration within the calibrated range.

### Suitability requirements

**Short term Instrumental Stability:** Compare results obtained from *System suitability sample* before and after the analysis of the *Sample solution*.

**Suitability criteria:** NMT 20% deviation between both samples for each *Target element*. (Note: If samples are high in mineral content, rinse the system well in order to minimize carryover and check it by measuring a blank solution before introducing the *System Suitability Sample*.)

**Analysis:** Analyze according to manufacturer's suggestion for programs and wavelength. Calculate and report results on the basis of the original sample size. [Note: Appropriate measures must be taken to correct for matrix-induced interferences (e.g., wavelength overlaps).]

### 2.3. Procedure 2: ICP-MS

**Standard solution 1:** 1.5J of the *Target element(s)* in a *Matrix matched solution*.

**Standard solution 2:** 0.5J of the *Target element(s)* in a *Matrix matched solution*.

**Sample stock solution:** Proceed as directed in 1. *Sample Preparation* above. Allow the sample to cool, if necessary. For mercury determination, add an appropriate stabilizer, if necessary.

**Sample solution:** Dilute the *Sample stock solution* with an appropriate solvent to obtain a final concentration of the *Target element(s)* within the calibrated range.

**Blank:** *Matrix matched solution*.

### Elemental spectrometric system

**Mode:** ICP. [Note: An instrument with a cooled spray chamber is recommended. (A collision cell or reaction cell may also be beneficial.)]

**Detector:** Mass spectrometer.

**Rinse:** Diluent used.

**Standardization:** *Standard solution 1*, *Standard solution 2*, and *Blank*.

**System suitability Sample:** Standard solution of the *Target element(s)* in a *Matrix matched solution* at a concentration within the calibrated range.

### Suitability requirements

**Short term Instrumental Stability:** Compare results obtained from *system suitability sample* before and after the analysis of the *Sample solution*.

**Suitability criteria:** NMT 20% deviation between both samples for each *Target element*. (Note: If samples are high in mineral content, rinse the system well in order to minimize carryover and check it by measuring a blank before introducing the *System suitability sample*.)

**Analysis:** Analyze according to the manufacturer's suggestions for program and *m/z*. Calculate and report results based on the original sample size. [Note: Appropriate measures must be taken to correct for matrix-induced interferences (e.g., argon chloride interference with arsenic determinations).]

## 3. Requirements for Procedure Validation

All procedures must be validated and shown to be acceptable, in accordance with the validation requirements described below. The level of validation necessary to ensure that a procedure is acceptable depends on whether a limit test or a quantitative determination is used. Any procedure that has been validated and meets the acceptance criteria that follow is considered to be suitable for use. If appropriate, the validation method and criteria may be changed according to the purpose of evaluating the levels of the content of elemental impurities. They may differ from the requirements to meet the system suitability criteria described in Inductively Coupled Plasma-Atomic Emission Spectrometry and Induc-

tively Coupled Plasma-Mass Spectrometry <2.63>.

### 3.1. Procedures for Limits Tests

The following section defines the validation parameters for the acceptability of limit tests. Meeting these requirements must be demonstrated experimentally using an appropriate system suitability test and reference materials. The suitability of the method must be determined by conducting studies with the material or mixture under test spiked with known concentrations of each *Target element* of interest at the appropriate *Target concentration*.

#### 3.1.1. Detectability

**Standard solution:** A preparation of reference materials for the *Target element(s)* at 1.0J in a *Matrix matched solution*.

**Spiked sample solution 1:** Prepare a solution of the sample under test, spiked with appropriate reference materials for the *Target element(s)* at the *Target concentration*, solubilized or digested as described in *Sample Preparation*.

**Spiked sample solution 2:** Prepare a solution of the sample under test, spiked with appropriate reference materials for the *Target element(s)* at 80% of the *Target concentration*, solubilized or digested as described in *Sample Preparation*.

**Unspiked sample solution:** A sample of material under test, solubilized or digested in the same manner as the spiked *Sample solutions*.

#### Acceptance criteria

**Non-instrumental procedures:** *Spiked sample solution 1* provides a signal or intensity equivalent to or greater than that of the *Standard solution*. *Spiked sample solution 2* must provide a signal or intensity less than that of *Spiked sample solution 1*. (Note: The signal from each *Spiked sample solution* is NLT the *Unspiked sample solution* determination.)

**Instrumental procedures:** The average value of the three replicate measurements of *Spiked sample solution 1* is within  $\pm 15\%$  of the average value obtained for the replicate measurements of the *Standard solution*. The average value of the replicate measurements of *Spiked sample solution 2* must provide a signal intensity or value less than that of the *Standard solution*. (Note: Correct the values obtained for each of the spiked solutions using the *Unspiked sample solution*.)

#### 3.1.2. Specificity

The procedure must be able to unequivocally assess each *Target element* in the presence of components that may be expected to be present, including other *Target elements*, and matrix components.

#### 3.1.3. Precision, only for Instrumental Methods (Repeatability)

**Sample solutions:** Six independent samples of the material under test, spiked with appropriate reference materials for the *Target elements* at the *Target concentration*.

#### Acceptance criteria

**Relative standard deviation:** NMT 20% for each *Target element*

## 3.2. Procedures for Quantitative Tests

The following section defines the validation parameters for the acceptability of procedures for quantitative tests. Meeting these requirements must be demonstrated experimentally, using an appropriate system suitability test and reference materials.

#### 3.2.1. Accuracy

**Standard solutions:** Prepare solutions containing the *Target element(s)* at three concentrations ranging from 0.5 to 1.5 of J, using appropriate reference materials, in a *Matrix matched solution and blank*.

**Test samples:** Prepare samples of the material under test spiked with appropriate reference materials for the *Target element(s)* before any sample preparation steps (digestion or solubilization) at 3 concentrations ranging from 50% to

150% of the *Target concentration*. The concentrations of the added reference materials after the preparation of the samples range from 0.5 to 1.5 of *J*, and should contain at least three different concentrations.

#### Acceptance criteria

**Spike recovery:** 70%–150% for the mean of three replicate preparations at each concentration

#### 3.2.2. Precision

##### Repeatability

**Test samples:** Six independent samples of material under test (taken from the same lot) spiked with appropriate reference materials for the *Target element(s)* at the *Target concentration*. Or at least 9 determinations (e.g., 3 replicates of 3 concentrations) covering the specified range.

##### Acceptance criteria

**Relative standard deviation:** NMT 20% ( $n = 6$ ) for each *Target element*

##### Intermediate precision (ruggedness)

Perform the *Repeatability* analysis again at least once either on a different day, with a different instrumentation, with a different analyst, or a combination thereof. Combine the results of this analysis with the *Repeatability* analysis so the total number of samples is at least 12.

##### Acceptance criteria

**Relative standard deviation:** NMT 25% ( $n = 12$ ) for each *Target element*

#### 3.2.3. Specificity

The procedure must be able to unequivocally assess each *Target element* in the presence of components that may be expected to be present, including other *Target elements*, and matrix components.

#### 3.2.4. Range and Linearity

Demonstrated by meeting the *Accuracy* requirement.

#### 3.2.5. Limit of Quantification

LOQ of 50% of *J* is confirmed when the accuracy acceptance criteria for the corresponding spiked solution is met. Acceptance criterion: the LOQ is less than or equal to 50% of *J*.

#### 4. Glossary

(i) **Concentrated acid:** Concentrated ultra-pure nitric, sulfuric, hydrochloric, or hydrofluoric acids or any other acid or mixture of acids that is demonstrated suitable.

(ii) **Matrix matched solution:** Solutions having the same solvent composition as the *Sample solution*. In the case of an aqueous solution, *Matrix matched solution* would indicate that the same acids, acid concentrations and mercury stabilizer are used in both preparations.

(iii) **Target elements:** Elements whose levels in the drug product must be controlled within acceptable limits.

(iv) **Target limit or Target concentration:** The acceptance value for the elemental impurity being evaluated. Exceeding the *Target limit* indicates that a material under test exceeds the acceptable value. *Target limits* in the final drug product can be approximated by dividing the *PDEs* by the maximum daily dose. When evaluating the significance of elemental impurity levels, it is possible to set the *Target limits* to the values obtained by dividing 30% of *PDEs* by the maximum daily dose. Furthermore, when the permitted concentration limit of each element in the individual components of the drug product is set, it can be set as the *Target concentration*.

(v) **J:** The concentration (w/v) of the *Target element(s)* at the *Target limit*, appropriately diluted to the working range of the instrument. If a dilution is not necessary, *J* is equal to the *Target concentration*. For example, if the target elements are lead and arsenic for an analysis of an oral solid drug product with a daily dose of 10 g/day using inductively

coupled plasma-mass spectrometry (ICP-MS), the target limit for these elements would be 0.5 µg/g and 1.5 µg/g. However, in both cases, the linear dynamic range of the ICP-MS is known to extend from 0.01 ng/mL to 0.1 µg/mL for these elements. Therefore, a dilution factor of at least 1:100 is required to ensure that the analysis occurs in the linear dynamic range of the instrument. *J* would thus equal 5 ng/mL and 15 ng/mL for lead and arsenic, respectively.

(vi) **Appropriate reference materials:** In principle, where *appropriate reference materials* are specified in the chapter, certified reference materials (CRM) from a national metrology institute (NMI), or reference materials that are traceable to the CRM of an NMI should be used.

(vii) **Cross validate:** Verification whether or not the same result can be obtained from the corresponding analyses for the same sample.

## 3. Powder Property Determinations

### 3.01 Determination of Bulk and Tapped Densities

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

♦Determination of Bulk and Tapped Densities is a method to determine the bulk densities of powdered drugs under loose and tapped packing conditions respectively. Loose packing is defined as the state obtained by pouring a powder sample into a vessel without any consolidation, and tapped packing is defined as the state obtained when the vessel containing the powder sample is to be repeatedly dropped a specified distance at a constant drop rate until the apparent volume of sample in the vessel becomes almost constant.◆

#### 1. Bulk density

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per milliliter (g/mL) although the international unit is kilogram per cubic meter (1 g/mL = 1000 kg/m<sup>3</sup>) because the measurements are made using cylinders. It may also be expressed in grams per cubic centimeter (g/cm<sup>3</sup>).

The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it was handled. The particles can be packed to have a range of bulk densities and, moreover, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made.

The bulk density of a powder is determined by measuring the volume of a known mass of powder sample, that may

have been passed through a sieve into a graduated cylinder (Method 1), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (Method 2) or a measuring vessel (Method 3). Method 1 and Method 3 are favoured.

### 1.1. Method 1: Measurement in a graduated cylinder

#### 1.1.1. Procedure

Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated cylinder of 250 mL (readable to 2 mL), gently introduce, without compacting, approximately 100 g of the test sample ( $m$ ) weighed with 0.1 per cent accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume ( $V_0$ ) to the nearest graduated unit. Calculate the bulk density in g per mL by the formula  $m/V_0$ . Generally, replicate determinations are desirable for the determination of this property.

If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as test sample, such that its untapped apparent volume is 150 mL to 250 mL (apparent volume greater than or equal to 60 per cent of the total volume of the cylinder); the mass of the test sample is specified in the expression of results.

For test samples having an apparent volume between 50 mL and 100 mL, a 100 mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results.

### 1.2. Method 2: Measurement in a volumeter

#### 1.2.1. Apparatus

The apparatus (Fig. 3.01-1) consists of a top funnel fitted with a 1.0 mm sieve. The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup mounted directly below it. The cup may be cylindrical ( $25.00 \pm 0.05$  mL volume with an inside diameter of  $30.00 \pm 2.00$  mm) or cubical ( $16.39 \pm 0.20$  mL volume with inside dimensions of  $25.400 \pm 0.076$  mm).

#### 1.2.2. Procedure

Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of  $25 \text{ cm}^3$  of powder with the cubical cup and  $35 \text{ cm}^3$  of powder with the cylindrical cup. Carefully, scrape excess

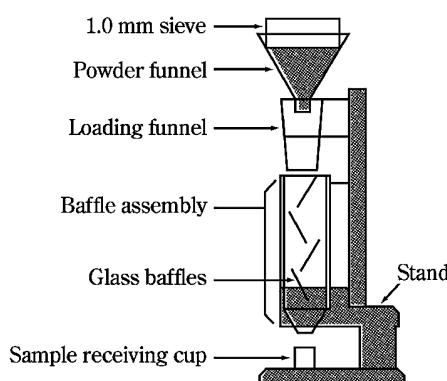


Fig. 3.01-1 Volumeter

powder from the top of the cup by smoothly moving the edge of the blade of a spatula perpendicular to and in contact with the top surface of the cup, taking care to keep the spatula perpendicular to prevent packing or removal of powder from the cup. Remove any material from the side of the cup and determine the mass ( $m$ ) of the powder to the nearest 0.1 per cent. Calculate the bulk density in g per mL by the formula  $m/V_0$  in which  $V_0$  is the volume of the cup and record the average of 3 determinations using 3 different powder samples.

### 1.3. Method 3: Measurement in a vessel

#### 1.3.1. Apparatus

The apparatus consists of a 100 mL cylindrical vessel of stainless steel with dimensions as specified in Fig. 3.01-2.

#### 1.3.2. Procedure

Pass a quantity of powder sufficient to complete the test through a 1.0 mm sieve, if necessary, to break up agglomerates that may have formed during storage and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for Method 2. Determine the mass ( $m_0$ ) of the powder to the nearest 0.1 per cent by subtraction of the previously determined mass of the empty measuring vessel. Calculate the bulk density (g/mL) by the formula  $m_0/100$  and record the average of 3 determinations using 3 different powder samples.

## 2. Tapped density

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample.

The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume or mass readings are taken until little further volume or mass change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop, under its own mass, a specified distance by either of 3 methods as described below. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down.

### 2.1. Method 1

#### 2.1.1. Apparatus

The apparatus (Fig. 3.01-3) consists of the following:

- (i) a 250 mL graduated cylinder (readable to 2 mL) with a mass of  $220 \pm 44$  g,
- (ii) a settling apparatus capable of producing, in 1 min, either nominally  $250 \pm 15$  taps from a height of  $3 \pm 0.2$  mm, or nominally  $300 \pm 15$  taps from a height of  $14 \pm 2$  mm. The support for the graduated cylinder, with its holder, has a mass of  $450 \pm 10$  g.

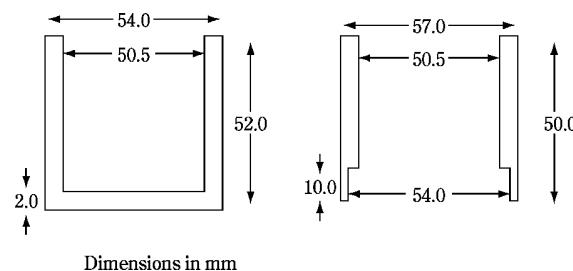
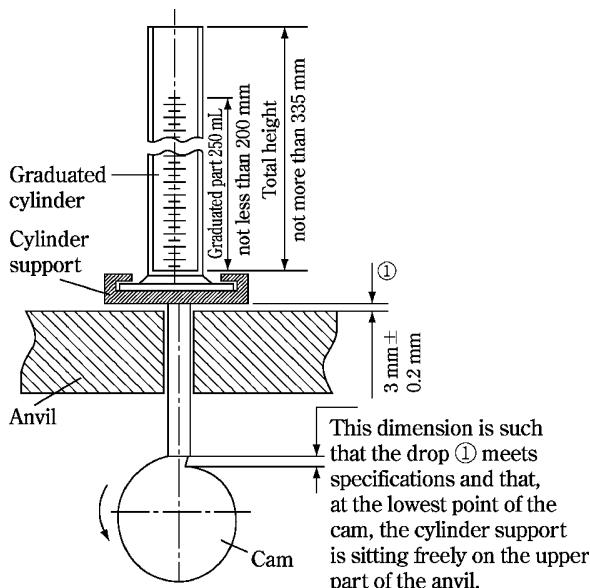


Fig. 3.01-2 Measuring vessel (left) and cap (right)



**Fig. 3.01-3** Tapping apparatus

### 2.1.2. Procedure

Proceed as described above for the determination of the bulk volume ( $V_0$ ).

Secure the cylinder in the holder. Carry out 10, 500 and 1250 taps on the same powder sample and read the corresponding volumes  $V_{10}$ ,  $V_{500}$  and  $V_{1250}$  to the nearest graduated unit. If the difference between  $V_{500}$  and  $V_{1250}$  is less than or equal to 2 mL,  $V_{1250}$  is the tapped volume. If the difference between  $V_{500}$  and  $V_{1250}$  exceeds 2 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula  $m/V_f$  in which  $V_f$  is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results.

If it is not possible to use a 100 g test sample, use a reduced amount and a suitable 100 mL graduated cylinder (readable to 1 mL) weighing  $130 \pm 16$  g and mounted on a holder weighing  $240 \pm 12$  g. If the difference between  $V_{500}$  and  $V_{1250}$  is less than or equal to 1 mL,  $V_{1250}$  is the tapped volume. If the difference between  $V_{500}$  and  $V_{1250}$  exceeds 1 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 1 mL. The modified test conditions are specified in the expression of the results.

### 2.2. Method 2

#### 2.2.1. Procedure

Proceed as directed under Method 1 except that the mechanical tester provides a fixed drop of  $3 \pm 0.2$  mm at a nominal rate of 250 taps per minute.

### 2.3. Method 3

#### 2.3.1. Procedure

Proceed as described in the method for measuring the bulk density using the measuring vessel equipped with the cap shown in Fig. 3.01-2. The measuring vessel with the cap is lifted 50-60 times per minute by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap and carefully scrape excess powder from the top of the measuring vessel as described in Method 3 for measuring the bulk density. Repeat the procedure using 400 taps. If the difference between the 2 masses obtained after 200 and 400 taps exceeds 2 per cent, carry out a test using 200 additional taps until the

difference between succeeding measurements is less than 2 per cent. Calculate the tapped density (g/mL) using the formula  $m_f/100$  where  $m_f$  is the mass of powder in the measuring vessel. Record the average of 3 determinations using 3 different powder samples. The test conditions including tapping height are specified in the expression of the results.

### 3. Measures of powder compressibility

Because the interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, for example the Compressibility Index or the Hausner Ratio.

The Compressibility Index and Hausner Ratio are measures of the propensity of a powder to be compressed as described above. As such, they are measures of the powder ability to settle and they permit an assessment of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticulate interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Compressibility Index and the Hausner Ratio.

Compressibility Index:

$$100 (V_0 - V_f)/V_0$$

$V_0$ : Unsettled apparent volume

$V_f$ : Final tapped volume

Hausner Ratio:

$$V_0/V_f$$

Depending on the material, the compressibility index can be determined using  $V_{10}$  instead of  $V_0$ . If  $V_{10}$  is used, it is clearly stated in the results.

## 3.02 Specific Surface Area by Gas Adsorption

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia.

The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

♦The specific surface area determination method is a method to determine specific surface area (the total surface area of powder per unit mass) of a pharmaceutical powder sample by using gas adsorption method.◆ The specific surface area of a powder is determined by physical adsorption of a gas on the surface of the solid and by calculating the amount of adsorbate gas corresponding to a monomolecular layer on the surface. Physical adsorption results from relatively weak forces (van der Waals forces) between the adsorbate gas molecules and the adsorbent surface of the test powder. The determination is usually carried out at the temperature of liquid nitrogen. The amount of gas adsorbed can be measured by a volumetric or continuous flow procedure.

## 1. Measurements

### 1.1. Multi-point measurement

When the gas is physically adsorbed by the powder sample, the following relationship (Brunauer, Emmett and Teller (BET) adsorption isotherm) holds when the relative pressure ( $P/P_0$ ) is in the range of 0.05 to 0.30 for pressure  $P$  of the adsorbate gas in equilibrium for the volume of gas adsorbed,  $V_a$ .

$$\frac{1}{[V_a\{(P_0/P) - 1\}]} = \{(C - 1)/V_m C\} \times (P/P_0) + (1/V_m C) \quad (1)$$

$P$ : Partial vapour pressure of adsorbate gas in equilibrium with the surface at  $-195.8^\circ\text{C}$  (b.p. of liquid nitrogen), in pascals

$P_0$ : Saturated pressure of adsorbate gas, in pascals

$V_a$ : Volume of gas adsorbed at standard temperature and pressure (STP) [ $0^\circ\text{C}$  and atmospheric pressure ( $1.013 \times 10^5 \text{ Pa}$ )], in milliliters

$V_m$ : Volume of gas adsorbed at STP to produce an apparent monolayer on the sample surface, in milliliters

$C$ : Dimensionless constant that is related to the enthalpy of adsorption of adsorbate gas on the powder sample

A value of  $V_a$  is measured at each of not less than 3 values of  $P/P_0$ . Then the BET value,  $1/[V_a\{(P_0/P) - 1\}]$ , is plotted against  $P/P_0$  according to equation (1). This plot should yield a straight line usually in the approximate relative pressure range 0.05 to 0.3. The data are considered acceptable if the correlation coefficient,  $r$ , of the linear regression is not less than 0.9975; that is,  $r^2$  is not less than 0.995. From the resulting linear plot, the slope, which is equal to  $(C - 1)/V_m C$ , and the intercept, which is equal to  $1/(V_m C)$ , are evaluated by linear regression analysis. From these values,  $V_m$  is calculated as  $1/(slope + intercept)$ , while  $C$  is calculated as  $(slope/intercept) + 1$ . From the value of  $V_m$  so determined, the specific surface area,  $S$ , in  $\text{m}^2\text{g}^{-1}$ , is calculated by the equation:

$$S = (V_m Na)/(m \times 22,400) \quad (2)$$

$N$ : Avogadro constant ( $6.022 \times 10^{23} \text{ mol}^{-1}$ ),

$a$ : Effective cross-sectional area of one adsorbate molecule, in square meters ( $0.162 \times 10^{-18} \text{ m}^2$  for nitrogen and  $0.195 \times 10^{-18} \text{ m}^2$  for krypton)

$m$ : Mass of test powder, in grams

22,400: Volume, in milliliters, occupied by one mole of the adsorbate gas at STP allowing for minor departures from the ideal

A minimum of 3 data points is required. Additional measurements may be carried out, especially when non-linearity is obtained at a  $P/P_0$  value close to 0.3. Because non-linearity is often obtained at a  $P/P_0$  value below 0.05, values in this region are not recommended. The test for linearity, the treatment of the data, and the calculation of the specific surface area of the sample are described above.

### 1.2. Single-point measurement

Normally, at least 3 measurements of  $V_a$  each at different values of  $P/P_0$  are required for the determination of specific surface area by the dynamic flow gas adsorption technique (*Method I*) or by volumetric gas adsorption (*Method II*). However, under certain circumstances described below, it may be acceptable to determine the specific surface area of a powder from a single value of  $V_a$  measured at a single value of  $P/P_0$  such as 0.300 (corresponding to 0.300 mole of nitrogen or 0.001038 mole fraction of krypton), using the following equation for calculating  $V_m$ :

$$V_m = V_a\{1 - (P/P_0)\} \quad (3)$$

The single-point method may be employed directly for a series of powder samples of a given material for which the material constant  $C$  is much greater than unity. These circumstances may be verified by comparing values of specific surface area determined by the single-point method with that determined by the multiple-point method for the series of powder samples. Close similarity between the single-point values and multiple-point values suggests that  $1/C$  approaches zero. The single-point method may be employed indirectly for a series of very similar powder samples of a given material for which the material constant  $C$  is not infinite but may be assumed to be invariant. Under these circumstances, the error associated with the single-point method can be reduced or eliminated by using the multiple-point method to evaluate  $C$  for one of the samples of the series from the BET plot, from which  $C$  is calculated as  $(1 + slope/intercept)$ . Then  $V_m$  is calculated from the single value of  $V_a$  measured at a single value of  $P/P_0$  by the equation:

$$V_m = V_a\{(P_0/P) - 1\}[(1/C) + \{(C - 1)/C\} \times (P/P_0)] \quad (4)$$

## 2. Sample preparation

Before the specific surface area of the sample can be determined, it is necessary to remove gases and vapors that may have become physically adsorbed onto the surface during storage and handling. If outgassing is not achieved, the specific surface area may be reduced or may be variable because some parts of surface area are covered with molecules of the previously adsorbed gases or vapors. The outgassing conditions are critical for obtaining the required precision and accuracy of specific surface area measurements on pharmaceuticals because of the sensitivity of the surface of the materials. The outgassing conditions must be demonstrated to yield reproducible BET plots, a constant weight of test powder, and no detectable physical or chemical changes in the test powder.

The outgassing conditions defined by the temperature, pressure and time should be so chosen that the original surface of the solid is reproduced as closely as possible.

Outgassing of many substances is often achieved by applying a vacuum, by purging the sample in a flowing stream of a non-reactive, dry gas, or by applying a desorption-adsorption cycling method. In either case, elevated temperatures are sometimes applied to increase the rate at which the contaminants leave the surface. Caution should be exercised when outgassing powder samples using elevated temperatures to avoid affecting the nature of the surface and the integrity of the sample.

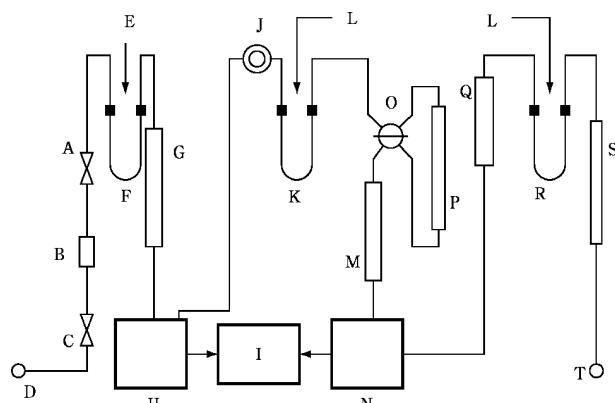
If heating is employed, the recommended temperature and time of outgassing are as low as possible to achieve reproducible measurement of specific surface area in an acceptable time. For outgassing sensitive samples, other outgassing methods such as the desorption-adsorption cycling method may be employed.

The standard technique is the adsorption of nitrogen at liquid nitrogen temperature.

For powders of low specific surface area ( $<0.2 \text{ m}^2\text{g}^{-1}$ ) the proportion adsorbed is low. In such cases the use of krypton at liquid nitrogen temperature is preferred because the low vapor pressure exerted by this gas greatly reduces error. All gases used must be free from moisture.

Accurately weigh a quantity of the test powder such that the total surface of the sample is at least  $1 \text{ m}^2$  when the adsorbate is nitrogen and  $0.5 \text{ m}^2$  when the adsorbate is krypton. Lower quantities of sample may be used after appropriate validation.

Because the amount of gas adsorbed under a given pressure tends to increase on decreasing the temperature, adsorp-



A: Flow control valve  
B: Differential flow controller  
C: On-off valve  
D: Gas inlet  
E: O ring seals  
F: Cold trap  
G: Thermal equilibration tube  
H: Detector  
I: Digital display  
J: Calibrating septum  
K: Sample cell  
L: Self seals quick connection  
M: Short path ballast  
N: Detector  
O: Path selection valve  
P: Long path ballast  
Q: Flow meter  
R: Outgassing station  
S: Diffusion baffle  
T: Vent

**Fig. 3.02-1** Schematic diagram of the dynamic flow method apparatus

tion measurements are usually made at a low temperature. Measurement is performed at  $-195.8^{\circ}\text{C}$ , the boiling point of liquid nitrogen.

Adsorption of gas should be measured either by Method I or Method II.

### 3. Methods

#### 3.1. Method 1: The dynamic flow method

In the dynamic flow method (see Fig. 3.02-1), the recommended adsorbate gas is dry nitrogen or krypton, while helium is employed as a diluent gas, which is not adsorbed under the recommended conditions. A minimum of 3 mixtures of the appropriate adsorbate gas with helium are required within the  $P/P_0$  range 0.05 to 0.30.

The gas detector-integrator should provide a signal that is approximately proportional to the volume of the gas passing through it under defined conditions of temperature and pressure. For this purpose, a thermal conductivity detector with an electronic integrator is one among various suitable types. A minimum of 3 data points within the recommended range of 0.05 to 0.30 for  $P/P_0$  is to be determined.

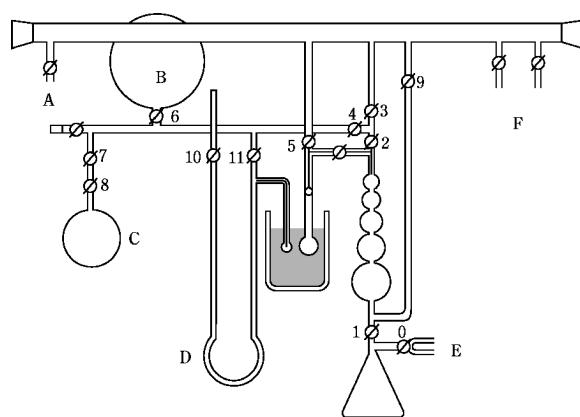
A known mixture of the gases, usually nitrogen and helium, is passed through a thermal conductivity cell, through the sample again through the thermal conductivity cell and then to a recording potentiometer. Immerse the sample cell in liquid nitrogen, then the sample adsorbs nitrogen from the mobile phase. This unbalances the thermal conductivity cell, and a pulse is generated on a recorder chart.

Remove from the coolant; this gives a desorption peak equal in area and in the opposite direction to the adsorption peak.

Since this is better defined than the adsorption peak, it is the one used for the determination.

To effect the calibration, inject a known quantity of adsorbate into the system, sufficient to give a peak of similar magnitude to the desorption peak and obtain the proportion of gas volume per unit peak area.

Use a nitrogen/helium mixture for a single-point determination and several such mixtures or premixing 2 streams of



A: Vacuum gage  
B: Nitrogen reservoir  
C: Helium reservoir  
D: Vapour pressure manometer  
E: Vacuum air  
F: To cold traps and vacuum pumps

**Fig. 3.02-2** Schematic diagram of the volumetric method apparatus

gas for a multiple-point determination. Calculation is essentially the same as for the volumetric method.

#### 3.2. Method 2: The volumetric method

In the volumetric method (see Fig. 3.02-2), the recommended adsorbate gas is nitrogen is admitted into the evacuated space above the previously outgassed powder sample to give a defined equilibrium pressure,  $P$ , of the gas. The use of a diluent gas, such as helium, is therefore unnecessary, although helium may be employed for other purposes, such as to measure the dead volume.

Since only pure adsorbate gas, instead of a gas mixture, is employed, interfering effects of thermal diffusion are avoided in this method.

Admit a small amount of dry nitrogen into the sample tube to prevent contamination of the clean surface, remove the sample tube, insert the stopper, and weigh it. Calculate the weight of the sample. Attach the sample tube to the volumetric apparatus. Cautiously evacuate the sample down to the specified pressure (e.g. between 2 Pa and 10 Pa). Alternatively, some instruments operate by evacuating to a defined rate of pressure change (e.g. less than 13 Pa/30 s) and holding for a defined period of time before commencing the next step.

If the principle of operation of the instrument requires the determination of the dead volume in the sample tube, for example, by the admission of a non-adsorbed gas, such as helium, this procedure is carried out at this point, followed by evacuation of the sample. The determination of dead volume may be avoided using difference measurements, that is, by means of reference and sample tubes connected by a differential transducer.

Raise a Dewar vessel containing liquid nitrogen at  $-195.8^{\circ}\text{C}$  up to a defined point on the sample cell. Admit a sufficient volume of adsorbate gas to give the lowest desired relative pressure. Measure the volume adsorbed,  $V_a$ . For multipoint measurements, repeat the measurement of  $V_a$  at successively higher  $P/P_0$  values. When nitrogen is used as the adsorbate gas,  $P/P_0$  values of 0.10, 0.20, and 0.30 are often suitable.

### 4. Reference materials

Periodically verify the functioning of the apparatus using appropriate reference materials of known surface area, such as  $\alpha$ -alumina for specific surface area determination, which

should have a specific surface area similar to that of the sample to be examined.

### 3.03 Powder Particle Density Determination

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

The parts of the test that are not harmonized are marked with symbols (♦ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Powder Particle Density Determination is ♦ a method to determine particle density of powdered pharmaceutical drugs or raw materials of drugs◆, and generally performed using a gas displacement pycnometer. The gas pycnometric density is determined by measuring the volume occupied by a known mass of powder which is equivalent to the volume of gas displaced by the powder using a gas displacement pycnometer. In gas pycnometric density measurements, the volume determined excludes the volume occupied by open pores; however, it includes the volume occupied by sealed pores or pores inaccessible to the gas. Usually, helium is used as a test gas due to its high diffusivity into small open pores. If gases other than helium are used, different values would be obtained, since the penetration of the gas is dependent on the size of the pore as well as the cross-sectional area of the gas.

The measured density is a volume weighted average of the densities of individual powder particles. It is called the particle density, distinct from the true density of solid or the bulk density of powder. The density of solids is expressed in grams per cubic centimeter ( $\text{g}/\text{cm}^3$ ), although the international unit is the kilogram per cubic meter ( $1 \text{ g}/\text{cm}^3 = 1000 \text{ kg}/\text{m}^3$ ).

#### 1. Apparatus

The schematic diagram of particle density apparatus for gas displacement pycnometric measurement is shown in Fig. 3.03-1. The apparatus consists of a test cell in which the sample is placed, an expansion cell and a manometer (M). The test cell, with an empty cell volume ( $V_c$ ), is connected through a valve (A) to an expansion cell, with a volume ( $V_r$ ).

Generally, helium is used as the measurement gas. The apparatus has to be equipped with a system capable of pressuring the test cell to the defined pressure ( $P$ ) through the

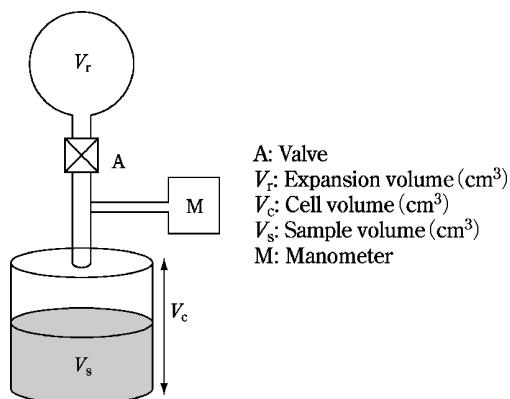


Fig. 3.03-1 Schematic diagram of a gas pycnometer

manometer.

#### 2. Calibration of apparatus

The volumes of the test cell ( $V_c$ ) and the expansion cell ( $V_r$ ) must be accurately determined to the nearest  $0.001 \text{ cm}^3$ , and to assure accuracy of the results of volume obtained, calibration of the apparatus is carried out as follows using a calibration ball of known volume for particle density measurement.

The final pressures ( $P_f$ ) are determined for the initial empty test cell followed by the test cell placed with the calibration ball for particle density measurement in accordance with the procedures, and  $V_c$  and  $V_r$  are calculated using the equation described in the section of Procedure. Calculation can be made taking into account that the sample volume ( $V_s$ ) is zero in the first run.

#### 3. Procedure

The gas pycnometric density measurement is performed at a temperature between  $15^\circ\text{C}$  and  $30^\circ\text{C}$  and must not vary by more than  $2^\circ\text{C}$  during the course of measurement.

Volatile contaminants in the powder are removed by degassing the powder under a constant purge of helium prior to the measurement. Occasionally, powders may have to be degassed under vacuum. Because volatiles may be evolved during the measurement, weighing of the sample is done after the pycnometric measurement of volume.

Weigh the mass of the test cell and record it. After weighing out the amount of the sample as described in the individual monograph and placing it in the test cell, seal the cell in the pycnometer.

Open the valve (A) which connects the expansion cell with the test cell, confirm with the manometer (M) that the pressure inside the system is stable, and then read the system reference pressure ( $P_r$ ). Secondly, close the valve that connects to the two cells, and introduce the measurement gas into the test cell to achieve positive pressure. Confirm with the manometer that the pressure inside the system is stable, and then read the initial pressure ( $P_i$ ). Open the valve to connect the test cell with the expansion cell. After confirming that the indicator of the manometer is stable, read the final pressure ( $P_f$ ), and calculate the sample volume ( $V_s$ ) with the following equation.

$$V_s = V_c - \frac{V_r}{\frac{P_i - P_r}{P_f - P_r} - 1}$$

$V_r$ : Expansion volume ( $\text{cm}^3$ )

$V_c$ : Cell volume ( $\text{cm}^3$ )

$V_s$ : Sample volume ( $\text{cm}^3$ )

$P_i$ : Initial pressure (kPa)

$P_f$ : Final pressure (kPa)

$P_r$ : System reference pressure (kPa)

Repeat the measurement sequence for the same powder sample until consecutive measurements of the sample volume agree to within 0.2%, and calculate the mean of sample volumes ( $V_s$ ). Finally, unload the test cell, weigh the mass of the test cell, and calculate the final sample mass ( $m$ ) by deducting the empty cell mass from the test cell mass. The powder particle density  $\rho$  is calculated by the following equation:

$$\rho = m/V_s$$

$\rho$ : Powder particle density in  $\text{g}/\text{cm}^3$ ,

$m$ : Final sample mass in g,

$V_s$ : Sample volume in  $\text{cm}^3$

If the pycnometer differs in operation or construction

from the one shown in Fig. 3.03-1, follow the instructions of the manufacturer of the pycnometer. The sample conditioning is indicated with the results. For example, indicate whether the sample was tested as is or dried under specific conditions such as those described for loss on drying.

## 3.04 Particle Size Determination

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

◆Particle Size Determination is a method to determine directly or indirectly morphological appearance, shape, size and its distribution of powdered pharmaceutical drugs and excipients to examine their micromeritic properties. Optical microscopy and analytical sieving method may be used depending on the measuring purpose and the properties of test specimen.◆

### 1. Method 1. Optical Microscopy

◆The optical microscopy is used to observe the morphological appearance and shape of individual particle either directly with the naked eye or by using a microscopic photograph, in order to measure the particle size. The particle size distribution can also be determined by this method. It is also possible with this method to measure the size of the individual particle even when different kinds of particles mingle if they are optically distinguishable. Data processing techniques, such as image analysis, can be useful for determining the particle size distribution.◆

This method for particle characterization can generally be applied to particles 1  $\mu\text{m}$  and greater. The lower limit is imposed by the resolving power of the microscope. The upper limit is less definite and is determined by the increased difficulty associated with the characterization of larger particles. Various alternative techniques are available for particle characterization outside the applicable range of optical microscopy. Optical microscopy is particularly useful for characterizing particles that are not spherical. This method may also serve as a base for the calibration of faster and more routine methods that may be developed.

#### 1.1. Apparatus

Use a microscope that is stable and protected from vibration. The microscope magnification (product of the objective magnification, ocular magnification, and additional magnifying components) must be sufficient to allow adequate characterization of the smallest particles to be classified in the test specimen. The greatest numerical aperture of the objective should be sought for each magnification range. Polarizing filters may be used in conjunction with suitable analyzers and retardation plates. Color filters of relatively narrow spectral transmission should be used with achromatic objectives and are preferable with apochromats and are required for appropriate color rendition in photomicrography. Condensers corrected for at least spherical aberration should be used in the microscope substage and with the lamp. The numerical aperture of the substage condenser should match that of the objective under the condition of use; this is affected by the actual aperture of the condenser diaphragm and the presence of immersion oils.

#### 1.1.1. Adjustment

The precise alignment of all elements of the optical system and proper focusing are essential. The focusing of the elements should be done in accordance with the recommendations of the microscope manufacturer. Critical axial alignment is recommended.

##### 1.1.1.1. Illumination

A requirement for good illumination is a uniform and adjustable intensity of light over the entire field of view; Kohler illumination is preferred. With colored particles, choose the color of the filters used so as to control the contrast and detail of the image.

##### 1.1.1.2. Visual Characterization

The magnification and numerical aperture should be sufficiently high to allow adequate resolution of the images of the particles to be characterized. Determine the actual magnification using a calibrated stage micrometer to calibrate an ocular micrometer. Errors can be minimized if the magnification is sufficient that the image of the particle is at least 10 ocular divisions. Each objective must be calibrated separately. To calibrate the ocular scale, the stage micrometer scale and the ocular scale should be aligned. In this way, a precise determination of the distance between ocular stage divisions can be made.

◆When the particle size is measured, an ocular micrometer is inserted at the position of the ocular diaphragm, and a calibrated stage micrometer is placed at the center of the microscope stage and fixed in place. The ocular is attached to the lens barrel and adjusted to the focus point of the stage micrometer scale. Then, the distance between the scales of the two micrometers is determined, and the sample size equivalent 1 division of the ocular scale is calculated using the following formula:

The particle size equivalent 1 division on the ocular scale ( $\mu\text{m}$ ) = Length on the stage micrometer ( $\mu\text{m}$ )/Number of scale divisions on the ocular micrometer

The stage micrometer is removed and the test specimen is placed on the microscope stage. After adjusting the focus, the particle sizes are determined from the number of scale divisions read through the ocular.◆

Several different magnifications may be necessary to characterize materials having a wide particle size distribution.

#### 1.1.1.3. Photographic Characterization

If particle size is to be determined by photographic methods, take care to ensure that the object is sharply focused at the plane of the photographic emulsion. Determine the actual magnification by photographing a calibrated stage micrometer, using photographic film of sufficient speed, resolving power, and contrast. Exposure and processing should be identical for photographs of both the test specimen and the determination of magnification. The apparent size of a photographic image is influenced by the exposure, development, and printing processes as well as by the resolving power of the microscope.

#### 1.2. Preparation of the Mount

The mounting medium will vary according to the physical properties of the test specimen. Sufficient, but not excessive, contrast between the specimen and the mounting medium is required to ensure adequate detail of the specimen edge. The particles should rest in one plane and be adequately dispersed to distinguish individual particles of interest. Furthermore, the particles must be representative of the distribution of sizes in the material and must not be altered during preparation of the mount. Care should be taken to ensure that this important requirement is met. Selection of the mounting me-

dium must include a consideration of the analyte solubility.

### 1.3. Characterization

#### 1.3.1. Crystallinity Characterization

The crystallinity of a material may be characterized to determine compliance with the crystallinity requirement where stated in the individual monograph of a drug substance. Unless otherwise specified in the individual monograph, mount a few particles of the specimen in mineral oil on a clean glass slide. Examine the mixture using a polarizing microscope: the particles show birefringence (interference colors) and extinction positions when the microscope stage is revolved.

#### 1.3.2. Limit Test of Particle Size by Microscopy

Weigh a suitable quantity of the powder to be examined (for example, 10 to 100 mg), and suspend it in 10 mL of a suitable medium in which the powder does not dissolve, adding, if necessary, a wetting agent. A homogeneous suspension of particles can be maintained by suspending the particles in a medium of similar or matching density and by providing adequate agitation. Introduce a portion of the homogeneous suspension into a suitable counting cell, and scan under a microscope an area corresponding to not less than 10  $\mu\text{g}$  of the powder to be examined. Count all the particles having a maximum dimension greater than the prescribed size limit. The size limit and the permitted number of particles exceeding the limit are defined for each substance.

#### 1.3.3. Particle Size Characterization

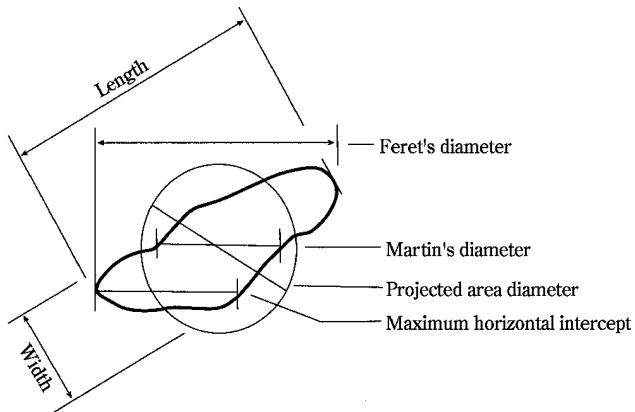
The measurement of particle size varies in complexity depending on the shape of the particle and the number of particles characterized must be sufficient to insure an acceptable level of uncertainty in the measured parameters<sup>1)</sup>. For spherical particles, size is defined by the diameter. For irregular particles, a variety of definitions of particle size exist. In general, for irregularly shaped particles, characterization of particle size must also include information on the type of diameter measured as well as information on particle shape. Several commonly used measurements of particle size are defined below (see Fig. 3.04-1).

- (i) Feret's Diameter: The distance between imaginary parallel lines tangent to a randomly oriented particle and perpendicular to the ocular scale.
- (ii) Martin's Diameter: The diameter of the particle at the point that divides a randomly oriented particle into two equal projected areas.
- (iii) Projected area Diameter: The diameter of a circle that has the same projected area as the particle.
- (iv) Length: The longest dimension from edge to edge of a particle oriented parallel to the ocular scale.
- (v) Width: The longest dimension of the particle measured at right angles to the length.

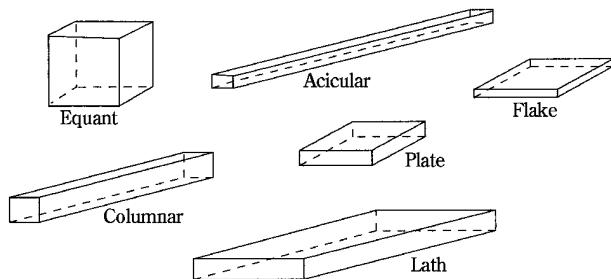
#### 1.3.4. Particle Shape Characterization

For irregularly shaped particles, characterization of particle size must also include information on particle shape. The homogeneity of the powder should be checked using appropriate magnification. The following defines some commonly used descriptors of particle shape (see Fig. 3.04-2).

- (i) Acicular: Slender, needle-like particle of similar width and thickness.
- (ii) Columnar: Long, thin particle with a width and thickness that are greater than those of an acicular particle.
- (iii) Flake: Thin, flat particle of similar length and width.
- (iv) Plate: Flat particles of similar length and width but with greater thickness than flakes.
- (v) Lath: Long, thin, and blade-like particle.
- (vi) Equant: Particles of similar length, width, and thickness; both cubical and spherical particles are included.



**Fig. 3.04-1** Commonly used measurements of particle size



**Fig. 3.04-2** Commonly used descriptions of particle shape

#### 1.3.5. General Observations

A particle is generally considered to be the smallest discrete unit. A particle may be a liquid or semisolid droplet; a single crystal or polycrystalline; amorphous or an agglomerate. Particles may be associated.

This degree of association may be described by the following terms.

- (i) Lamellar: Stacked plates.
- (ii) Aggregate: Mass of adhered particles.
- (iii) Agglomerate: Fused or cemented particles.
- (iv) Conglomerate: Mixture of two or more types of particles.
- (v) Spherulite: Radial cluster.
- (vi) Drusy: Particle covered with tiny particles.

Particle condition may be described by the following terms.

- (i) Edges: Angular, rounded, smooth, sharp, fractured.
- (ii) Optical: Color (using proper color balancing filters), transparent, translucent, opaque.
- (iii) Defects: Occlusions, inclusions.

Surface characteristics may be described by the following terms.

- (i) Cracked: Partial split, break, or fissure.
- (ii) Smooth: Free of irregularities, roughness, or projections.
- (iii) Porous: Having openings or passageways.
- (iv) Rough: Bumpy, uneven, not smooth.
- (v) Pitted: Small indentations.

#### 2. Method 2. Analytical Sieving Method

◆The analytical sieving method is a method to estimate the particle size distribution of powdered pharmaceutical drugs by sieving. The particle size determined by this method is shown as the size of a minimum sieve opening through which the particle passes. “Powder” here means a gathering of

numerous solid particles.♦

Sieving is one of the oldest methods of classifying powders and granules by particle size distribution. When using a woven sieve cloth, the sieving will essentially sort the particles by their intermediate size dimension (i.e., breadth or width). Mechanical sieving is most suitable where the majority of the particles are larger than about 75  $\mu\text{m}$ . For smaller particles, the light weight provides insufficient force during sieving to overcome the surface forces of cohesion and adhesion that cause the particles to stick to each other and to the sieve, and thus cause particles that would be expected to pass through the sieve to be retained. For such materials other means of agitation such as air-jet sieving or sonic sifting may be more appropriate. Nevertheless, sieving can sometimes be used for some powders or granules having median particle sizes smaller than 75  $\mu\text{m}$  where the method can be validated. In pharmaceutical terms, sieving is usually the method of choice for classification of the coarser grades of single powders or granules. It is a particularly attractive method in that powders and granules are classified only on the basis of particle size, and in most cases the analysis can be carried out in the dry state.

Among the limitations of sieving method are the need for an appreciable amount of sample (normally at least 25 g, depending on the density of the powder or granule, and the diameter of test sieves) and difficulty in sieving oily or other cohesive powders or granules that tend to clog the sieve openings. The method is essentially a two-dimensional estimate of size because passage through the sieve aperture is frequently more dependent on maximum width and thickness than on length.

This method is intended for estimation of the total particle size distribution of a single material. It is not intended for determination of the proportion of particles passing or retained on one or two sieves.

Estimate the particle size distribution as described under *Dry Sieving Method*, unless otherwise specified in the individual monograph. Where difficulty is experienced in reaching the endpoint (i.e., material does not readily pass through the sieves) or when it is necessary to use the finer end of the sieving range (below 75  $\mu\text{m}$ ), serious consideration should be given to the use of an alternative particle-sizing method.

Sieving should be carried out under conditions that do not cause the test sample to gain or lose moisture. The relative humidity of the environment in which the sieving is carried out should be controlled to prevent moisture uptake or loss by the sample. In the absence of evidence to the contrary, analytical test sieving is normally carried at ambient humidity. Any special conditions that apply to a particular material should be detailed in the individual monograph.

**Principles of Analytical Sieving:** Analytical test sieves are constructed from a woven-wire mesh, which is of simple weave that is assumed to give nearly square apertures and is sealed into the base of an open cylindrical container. The basic analytical method involves stacking the sieves on top of one another in ascending degrees of coarseness, and then placing the test powder on the top sieve.

The nest of sieves is subjected to a standardized period of agitation, and then the weight of material retained on each sieve is accurately determined. The test gives the weight percentage of powder in each sieve size range.

This sieving process for estimating the particle size distribution of a single pharmaceutical powder is generally intended for use where at least 80% of the particles are larger than 75  $\mu\text{m}$ . The size parameter involved in determining particle size distribution by analytical sieving is the length of the

size of the minimum square aperture through which the particle will pass.

## 2.1. Procedure

### 2.1.1. Test Sieves

Test sieves suitable for pharmacopoeial tests conform to the most current edition of International Organisation for Standardization (ISO) Specification ISO 3310-1; Test sieves—Technical requirements and testing (see Table 3.04-1).

Unless otherwise specified in the monograph, use those ISO sieves listed in the Table 3.04-1 as recommended in the particular region.

Sieves are selected to cover the entire range of particle sizes present in the test specimen. A nest of sieves having a  $\sqrt{2}$  progression of the area of the sieve openings is recommended. The nest of sieves is assembled with the coarsest screen at the top and the finest at the bottom. Use micrometers or millimeters in denoting test sieve openings. [Note—Mesh numbers are provided in the table for conversion purposes only.] Test sieves are made from stainless steel or, less preferably, from brass or other suitable non-reactive wire.

#### 2.1.1.1. Calibration of test sieves

Calibration and recalibration of test sieves is in accordance with the most current edition of ISO 3310-1<sup>2)</sup>. Sieves should be carefully examined for gross distortions and fractures, especially at their screen frame joints, before use. Sieves may be calibrated optically to estimate the average opening size, and opening variability, of the sieve mesh. Alternatively, for the evaluation of the effective opening of test sieves in the size range of 212 to 850  $\mu\text{m}$ , Standard Glass Spheres are available. Unless otherwise specified in the individual monograph, perform the sieve analysis at controlled room temperature and at ambient relative humidity.

#### 2.1.1.2. Cleaning Test Sieves

Ideally, test sieves should be cleaned using only an air jet or a liquid stream. If some apertures remain blocked by test particles, careful gentle brushing may be used as a last resort.

#### 2.1.2. Test Specimen

If the test specimen weight is not given in the monograph for a particular material, use a test specimen having a weight between 25 and 100 g, depending on the bulk density of the material, and test sieves having a 200 mm diameter. For 76 mm sieves the amount of material that can be accommodated is approximately 1/7<sup>th</sup> that which can be accommodated on a 200 mm sieve. Determine the most appropriate weight for a given material by test sieving accurately weighed specimens of different weights, such as 25, 50, and 100 g, for the same time period on a mechanical shaker. [Note—if the test results are similar for the 25-g and 50-g specimens, but the 100-g specimen shows a lower percentage through the finest sieve, the 100-g specimen size is too large.] Where only a specimen of 10 to 25 g is available, smaller diameter test sieves conforming to the same mesh specifications may be substituted, but the endpoint must be re-determined. The use of test samples having a smaller mass (e.g. down to 5 g) may be needed. For materials with low apparent particle density, or for materials mainly comprising particles with a highly iso-diametrical shape, specimen weights below 5 g for a 200 mm screen may be necessary to avoid excessive blocking of the sieve. During validation of a particular sieve analysis method, it is expected that the problem of sieve blocking will have been addressed.

If the test material is prone to picking up or losing significant amounts of water with varying humidity, the test must be carried out in an appropriately controlled environ-

**Table 3.04-1.** Sizes of standard sieve series in range of interest

ISO Nominal Aperture			US Sieve No.	Recommended USP Sieves (microns)	European Sieve No.	Japan Sieve No.
Principal sizes	Supplementary sizes	R 40/3				
R 20/3	R 20					
11.20 mm	11.20 mm 10.00 mm	11.20 mm			11200	
		9.50 mm				
8.00 mm	9.00 mm 8.00 mm 7.10 mm	8.00 mm				
		6.70 mm				
5.60 mm	6.30 mm 5.60 mm 5.00 mm	5.60 mm			5600	3.5
		4.75 mm				4
4.00 mm	4.50 mm 4.00 mm 3.55 mm	4.00 mm	5	4000	4000	4.7
		3.35 mm	6			5.5
2.80 mm	3.15 mm 2.80 mm 2.50 mm	2.80 mm	7	2800	2800	6.5
		2.36 mm	8			7.5
2.00 mm	2.24 mm 2.00 mm 1.80 mm	2.00 mm	10	2000	2000	8.6
		1.70 mm	12			10
1.40 mm	1.60 mm 1.40 mm 1.25 mm	1.40 mm	14	1400	1400	12
		1.18 mm	16			14
1.00 mm	1.12 mm 1.00 mm 900 µm	1.00 mm	18	1000	1000	16
		850 µm	20			18
710 µm	800 µm 710 µm 630 µm	710 µm	25	710	710	22
		600 µm	30			26
500 µm	560 µm 500 µm 450 µm	500 µm	35	500	500	30
		425 µm	40			36
355 µm	400 µm 355 µm 315 µm	355 µm	45	355	355	42
		300 µm	50			50
250 µm	280 µm 250 µm 224 µm	250 µm	60	250	250	60
		212 µm	70			70
180 µm	200 µm 180 µm 160 µm	180 µm	80	180	180	83
		150 µm	100			100
125 µm	140 µm 125 µm 112 µm	125 µm	120	125	125	119
		106 µm	140			140
90 µm	100 µm 90 µm 80 µm	90 µm	170	90	90	166
		75 µm	200			200
63 µm	71 µm 63 µm 56 µm	63 µm	230	63	63	235
		53 µm	270			282
45 µm	50 µm 45 µm 40 µm	45 µm	325	45	45	330
		38 µm			38	391

ment. Similarly, if the test material is known to develop an electrostatic charge, careful observation must be made to ensure that such charging is not influencing the analysis. An antistatic agent, such as colloidal silicon dioxide and/or aluminum oxide, may be added at a 0.5 percent (m/m) level to minimize this effect. If both of the above effects cannot be eliminated, an alternative particle-sizing technique must be selected.

### 2.1.3. Agitation Methods

Several different sieve and powder agitation devices are commercially available, all of which may be used to perform sieve analyses. However, the different methods of agitation may give different results for sieve analyses and endpoint determinations because of the different types and magnitude of the forces acting on the individual particles under test. Methods using mechanical agitation or electromagnetic agitation, and that can induce either a vertical oscillation or a horizontal circular motion, or tapping or a combination of both tapping and horizontal circular motion are available. Entrainment of the particles in an air stream may also be used. The results must indicate which agitation method was used and the agitation parameters used (if they can be varied), since changes in the agitation conditions will give different results for the sieve analysis and endpoint determinations, and may be sufficiently different to give a failing result under some circumstances.

### 2.1.4. Endpoint Determination

The test sieving analysis is complete when the weight on any of the test sieves does not change by more than 5% or 0.1 g (10% in the case of 76 mm sieves) of the previous weight on that sieve. If less than 5% of the total specimen weight is present on a given sieve, the endpoint for that sieve is increased to a weight change of not more than 20% of the previous weight on that sieve.

If more than 50% of the total specimen weight is found on any one sieve, unless this is indicated in the monograph, the test should be repeated, but with the addition to the sieve nest of a coarser sieve intermediate between that carrying the excessive weight and the next coarsest sieve in the original nest, i.e., addition of the ISO series sieve omitted from the nest of sieves.

## 2.2. Sieving Methods

### 2.2.1. Mechanical Agitation (Dry Sieving Method)

Tare each test sieve to the nearest 0.1 g. Place an accurately weighed quantity of test specimen on the top (coarsest) sieve, and place the lid. Agitate the nest of sieves for 5 minutes. Then carefully remove each from the nest without loss of material. Reweigh each sieve, and determine the weight of material on each sieve. Determine the weight of material in the collecting pan in a similar manner. Reassemble the nest of sieves, and agitate for 5 minutes. Remove and weigh each sieve as previously described. Repeat these steps until the endpoint criteria are met (see *Endpoint Determination* under *Test Sieves*). Upon completion of the analysis, reconcile the weights of material. Total losses must not exceed 5% of the weight of the original test specimen.

Repeat the analysis with a fresh specimen, but using a single sieving time equal to that of the combined times used above. Confirm that this sieving time conforms to the requirements for endpoint determination. When this endpoint has been validated for a specific material, then a single fixed time of sieving may be used for future analyses, providing the particle size distribution falls within normal variation.

If there is evidence that the particles retained on any sieve are aggregates rather than single particles, the use of mechanical dry sieving is unlikely to give good reproducibility, a different particle size analysis method should be used.

### 2.2.2. Air Entrainment Methods (Air Jet and Sonic Shifter Sieving)

Different types of commercial equipment that use a moving air current are available for sieving. A system that uses a single sieve at a time is referred to as *air jet* sieving. It uses the same general sieving methodology as that described under the *Dry Sieving Method*, but with a standardized air jet replacing the normal agitation mechanism. It requires sequential analyses on individual sieves starting with the finest sieve to obtain a particle size distribution. Air jet sieving often includes the use of finer test sieves than used in ordinary dry sieving. This technique is more suitable where only oversize or undersize fractions are needed.

In the *sonic sifting* method, a nest of sieves is used, and the test specimen is carried in a vertically oscillating column of air that lifts the specimen and then carries it back against the mesh openings at a given number of pulses per minute. It may be necessary to lower the sample amount to 5 g, when sonic shifting is employed.

The air jet sieving and sonic sieving methods may be useful for powders or granules when mechanical sieving techniques are incapable of giving a meaningful analysis.

These methods are highly dependent upon proper dispersion of the powder in the air current. This requirement may be hard to achieve if the method is used at the lower end of the sieving range (i.e., below 75 µm), when the particles tend to be more cohesive, and especially if there is any tendency for the material to develop an electrostatic charge. For the above reasons endpoint determination is particularly critical, and it is very important to confirm that the oversize material comprises single particles and is not composed of aggregates.

## 2.3. Interpretation

The raw data must include the weight of test specimen, the total sieving time, and the precise sieving methodology and the set values for any variable parameters, in addition to the weights retained on the individual sieves and in the pan. It may be convenient to convert the raw data into a cumulative weight distribution, and if it is desired to express the distribution in terms of a cumulative weight undersize, the range of sieves used should include a sieve through which all the material passes. If there is evidence on any of the test sieves that the material remaining on it is composed of aggregates formed during the sieving process, the analysis is invalid.

<sup>1)</sup>Additional information on particle size measurement, sample size, and data analysis is available, for example, in ISO 9276.

<sup>2)</sup>International Organization for Standardization (ISO) Specification ISO 3310-1; Test sieves—Technical requirements and testing

## 3.05 Water-Solid Interactions: Determination of Sorption-Desorption Isotherms and of Water Activity

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

♦The powder of drug as drug substances or preparations

often contacts with water during the production process or storage. For the assessment of the water-solid interactions, determinations of sorption-desorption isotherms and water activity are used. Water is interacted physically with solid in two ways, by an adsorption onto the surface of solid or an absorption permeating into the solid. In the case where both the adsorption and absorption are occurred, the term "sorption" is usually used.♦

## 1. Determination of Sorption-Desorption Isotherms

### 1.1 Principle

The tendency to take up water vapour is best assessed by measuring sorption or desorption as a function of relative humidity, at constant temperature, and under conditions where sorption or desorption is essentially occurring independently of time, i.e. equilibrium. Relative humidity, RH, is defined by the following equation:

$$RH = (P_c/P_0) \times 100$$

$P_c$ : pressure of water vapour in the system;

$P_0$ : saturation pressure of water vapour under the same conditions.

The ratio  $P_c/P_0$  is referred to as the relative pressure. Sorption or water uptake is best assessed by starting with dried samples and subjecting them to a known relative humidity. Desorption is studied by beginning with a system already containing sorbed water and reducing the relative humidity. As the name indicates, the sorption-desorption isotherm is valid only for the reference temperature, hence a special isotherm exists for each temperature. Ordinarily, at equilibrium, moisture content at a particular relative humidity must be the same, whether determined from sorption or desorption measurements. However, it is common to see sorption-desorption hysteresis.

### 1.2 Methods

Samples may be stored in chambers at various relative humidities. The mass gained or lost for each sample is then measured. The major advantage of this method is convenience, while the major disadvantages are the slow rate of reaching constant mass, particularly at high relative humidities, and the error introduced in opening and closing the chamber for weighing. Dynamic gravimetric water sorption

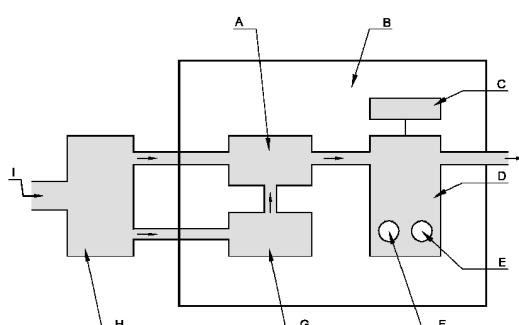
systems allow the on-line weighing of a sample in a controlled system to assess the interaction of the material with moisture at various programmable levels of relative humidity at a constant temperature. The major benefit of a controlled system is that isothermal conditions can be more reliably established and that the dynamic response of the sample to changing conditions can be monitored. Data points for the determination of the sorption isotherm (e.g. from 0% to approximately 95% RH, non condensing) are only taken after a sufficiently constant signal indicates that the sample has reached equilibrium at a given level of humidity. In some cases (e.g. deliquescence), the maximum time may be restricted although the equilibrium level is not reached. The apparatus must adequately control the temperature to ensure a good baseline stability as well as accurate control of the relative humidity generation. The required relative humidities can be generated, e.g. by accurately mixing dry and saturated vapour gas with flow controllers. The electrostatic behaviour of the powder must also be considered. The verification of the temperature and the relative humidity (controlled with, for example, a certified hygrometer, certified salt solutions or deliquescence points of certified salts over an adequate range), must be consistent with the instrument specification. The balance must provide a sufficient mass resolution and long term stability.

It is also possible to measure amounts of water uptake not detectable gravimetrically using volumetric techniques. In the case of adsorption, to improve sensitivity, one can increase the specific surface area of the sample by reducing particle size or by using larger samples to increase the total area. It is important, however, that such comminution of the solid does not alter the surface structure of the solid or render it more amorphous or otherwise less ordered in crystallinity. For absorption, where water uptake is independent of specific surface area, only increasing sample size will help. Increasing sample size, however, will increase the time to establish some type of equilibrium. To establish accurate values, it is important to get desolvation of the sample as thoroughly as possible. Higher temperatures and lower pressures (vacuum) facilitate this process; however, one must be aware of any adverse effects this might have on the solid such as dehydration, chemical degradation or sublimation. Using higher temperatures to induce desorption, as in a thermogravimetric apparatus, likewise must be carefully carried out because of these possible pitfalls.

### 1.3 Report and interpretation of the data

Sorption data are usually reported as a graph of the apparent mass change in per cent of the mass of the dry sample as a function of relative humidity or time. Sorption isotherms are reported both in tabular form and as a graph. The measurement method must be traceable with the data.

Adsorption-desorption hysteresis can be interpreted, for example, in terms of the porosity of the sample, its state of agglomeration (capillary condensation), the formation of hydrates, polymorphic change, or liquefying of the sample. Certain types of systems, particularly those with microporous solids and amorphous solids, are capable of sorbing large amounts of water vapour. Here, the amount of water associated with the solid as relative humidity is decreased, is greater than the amount that originally sorbed as the relative humidity was increased. For microporous solids, vapour adsorption-desorption hysteresis is an equilibrium phenomenon associated with the process of capillary condensation. This takes place because of the high degree of irregular curvature of the micropores and the fact that they "fill" (adsorption) and "empty" (desorption) under different equilibrium conditions. For non-porous solids capable of



- A. Humidity controller
- B. Temperature controlled chamber
- C. Balance module
- D. Humidity regulated module
- E. Reference
- F. Sample
- G. Vapour humidifier
- H. Flow control module
- I. Dry gas

Fig. 3.05-1 Example of an apparatus for the determination of the water sorption (other designs are possible)

absorbing water, hysteresis occurs because of a change in the degree of vapour-solid interaction due to a change in the equilibrium state of the solid, e.g. conformation of polymer chains, or because the time scale for structural equilibrium is longer than the time scale for water desorption. In measuring sorption-desorption isotherms, it is therefore important to establish that something close to an equilibrium state has been reached. Particularly with hydrophilic polymers at high relative humidities, the establishment of water sorption or desorption values independent of time is quite difficult, since one is usually dealing with a polymer plasticised into its "fluid" state, where the solid is undergoing significant change.

In the case of crystal hydrate formation, the plot of water uptake versus pressure or relative humidity will in these cases exhibit a sharp increase in uptake at a particular pressure and the amount of water taken up will usually exhibit a stoichiometric mole: mole ratio of water to solid. In some cases, however, crystal hydrates will not appear to undergo a phase change or the anhydrous form will appear amorphous. Consequently, water sorption or desorption may appear more like that seen with adsorption processes. X-ray crystallographic analysis and thermal analysis are particularly useful for the study of such systems.

For situations where water vapour adsorption occurs predominantly, it is very helpful to measure the specific surface area of the solid by an independent method and to express adsorption as mass of water sorbed per unit area of solid surface. This can be very useful in assessing the possible importance of water sorption in affecting solid properties. For example, 0.5% m/m uptake of water could hardly cover the bare surface of 100 m<sup>2</sup>/g, while for 1.0 m<sup>2</sup>/g this amounts to 100 times more surface coverage. In the case of pharmaceutical solids which have a specific surface area in the range of 0.01 m<sup>2</sup>/g to 10 m<sup>2</sup>/g, what appears to be low water content could represent a significant amount of water for the available surface. Since the "dry surface area" is not a factor in absorption, sorption of water with amorphous or partially amorphous solids can be expressed on the basis of unit mass corrected for crystallinity, when the crystal form does not sorb significant amounts of water relative to the amorphous regions.

## 2. Determination of the Water Activity

### 2.1. Principle

Water activity,  $A_w$ , is the ratio of vapour pressure of water in the product ( $P$ ) to saturation pressure of water vapour ( $P_0$ ) at the same temperature. It is numerically equal to 1/100 of the relative humidity (RH) generated by the product in a closed system. RH can be calculated from direct measurements of partial vapour pressure or dew point, or from indirect measurement by sensors whose physical or electric characteristics are altered by the RH to which they are exposed. Ignoring activity coefficients, the relationship between  $A_w$  and equilibrium relative humidity (ERH) are represented by the following equations:

$$A_w = P/P_0$$

$$\text{ERH (\%)} = A_w \times 100$$

### 2.2. Method

The water activity is determined by placing the sample in a small airtight cup inside which the equilibrium between the water in the solid and the headspace can be established. The volume of the headspace must be small in relation to the sample volume in order not to change the sorption state of sample during the test. The equilibration as a thermodynamic process takes time but may be accelerated by forced

circulation within the cell. The acquired water activity value is only valid for the simultaneously determined temperature. This requires a precise temperature-measuring device as part of the equipment. Furthermore, the probe must be thermally insulated to guarantee a constant temperature during the test. The sensor measuring the humidity of the headspace air above the sample is a key component. Theoretically, all types of hygrometers can be used, but for analytical purposes miniaturization and robustness are a precondition. The  $A_w$  measurement may be conducted using the dew point/chilled mirror method<sup>1)</sup>. A polished, chilled mirror is used as a condensing surface. The cooling system is electronically linked to a photoelectric cell into which light is reflected from the condensing mirror. An air stream, in equilibrium with the test sample, is directed at the mirror which cools until condensation occurs on the mirror. The temperature at which this condensation begins is the dew point from which the ERH is determined. Commercially available instruments using the dew point/chilled mirror method or other technologies need to be evaluated for suitability, validated, and calibrated when used to make water activity determinations.

These instruments are typically calibrated over an adequate range, for example, using some saturated salt solutions at 25°C such as those listed in Table 3.05-1.

**Table 3.05-1** Standard saturated salt solutions

Saturated salt solutions at 25°C	ERH (%)	$A_w$
Potassium sulphate (K <sub>2</sub> SO <sub>4</sub> )	97.3	0.973
Barium chloride (BaCl <sub>2</sub> )	90.2	0.902
Sodium chloride (NaCl)	75.3	0.753
Magnesium nitrate (Mg(NO <sub>3</sub> ) <sub>2</sub> )	52.9	0.529
Magnesium chloride (MgCl <sub>2</sub> )	32.8	0.328
Lithium chloride (LiCl)	11.2	0.112

1) AOAC International Official Method 978.18.

## 3.06 Laser Diffraction Measurement of Particle Size

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopoeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

The laser light diffraction technique used for the determination of particle-size distribution is based on the analysis of the diffraction pattern produced when particles are exposed to a beam of monochromatic light. Historically, the early laser diffraction instruments only used scattering at small angles. However, the technique has since been broadened to include laser light scattering in a wider range and application of the Mie theory, in addition to the Fraunhofer approximation and anomalous diffraction.

The technique cannot distinguish between scattering by primary particles and scattering by clusters of primary particles, i.e. by agglomerates or aggregates. As most particulate samples contain agglomerates or aggregates and as the focus of interest is generally on the size distribution of primary particles, the clusters are usually dispersed into primary par-

ticles before measurement.

For non-spherical particles, an equivalent sphere-size distribution is obtained because the technique assumes spherical particles in its optical model. The resulting particle-size distribution may differ from those obtained by methods based on other physical principles (e.g. sedimentation, sieving).

This chapter provides guidance for the measurement of size distributions of particles in different dispersed systems, for example, powders, sprays, aerosols, suspensions, emulsions, and gas bubbles in liquids, through analysis of their angular light-scattering patterns. It does not address specific requirements of particle-size measurement of specific products. This technique complies with ISO13320-1 (1999) and 9276-1 (1998).

## 1. Instrument

The instrument is located in an environment where it is not affected by electrical noise, mechanical vibrations, temperature fluctuations, humidity or direct bright light. An example of a set-up of a laser light diffraction instrument is given in Fig. 3.06-1. Other equipment may be used.

The instrument comprises a laser light source, beam processing optics, a sample measurement region (or cell), a Fourier lens, and a multi-element detector for measuring the scattered light pattern. A data system is also required for deconvolution of the scattering data into a volumetric size distribution and associated data analysis and reporting.

The particles can enter the laser beam in 2 positions. In the conventional case the particles enter the parallel beam before the collecting lens and within its working distance. In so-called reversed Fourier optics the particles enter behind the collecting lens and thus, in a converging beam. The advantage of the conventional set-up is that a reasonable path length for the sample is allowed within the working distance of the lens. The second set-up allows only small path lengths but enables measurement of scattered light at larger angles, which is useful when submicron particles are present.

The interaction of the incident light beam and the ensemble of dispersed particles results in a scattering pattern with different light intensities at various angles. The total angular intensity distribution, consisting of both direct and scattered light, is then focused onto a multi-element detector by a lens

or a series of lenses. These lenses create a scattering pattern that, within limits, does not depend on the location of the particles in the light beam. Hence, the continuous angular intensity distribution is converted into a discrete spatial intensity distribution on a set of detector elements.

It is assumed that the measured scattering pattern of the particle ensemble is identical to the sum of the patterns from all individual single scattering particles presented in random relative positions. Note that only a limited angular range of scattered light is collected by the lens(es) and, therefore, by the detector.

## 2. Development of the method

The measurement of particle size by laser diffraction can give reproducible data, even in the sub-micron region, provided the instrument used and the sample tested are carefully controlled to limit variability of the test conditions (e.g. dispersion medium, method of preparation of the sample dispersion).

Traditionally, the measurement of particle size using laser diffraction has been limited to particles in the range of approximately  $0.1\text{ }\mu\text{m}$  to  $3\text{ mm}$ . Because of recent advances in lens and equipment design, newer instruments are capable of exceeding this range routinely. With the validation report the user demonstrates the applicability of the method for its intended use.

### 2.1. Sampling

The sampling technique must be adequate to obtain a representative sample of a suitable volume for the particle size measurement. Sample splitting techniques such as rotating riffler or the cone and quartering method may be applied.

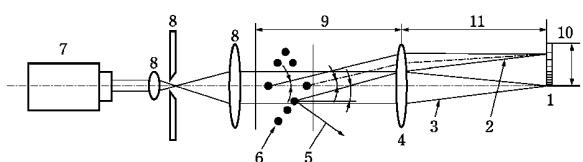
### 2.2. Evaluation of the dispersion procedure

Inspect the sample to be analyzed, visually or with the aid of a microscope, to estimate its size range and particle shape. The dispersion procedure must be adjusted to the purpose of the measurement. The purpose may be such that it is preferable to deagglomerate clusters into primary particles as far as possible, or it may be desirable to retain clusters as intact as possible. In this sense, the particles of interest may be either primary particles or clusters.

For the development of a method it is highly advisable to check that comminution of the particles does not occur, and conversely, that dispersion of particles or clusters is satisfactory. This can usually be done by changing the dispersing energy and monitoring the change of the particle-size distribution. The measured size distribution must not change significantly when the sample is well dispersed and the particles are neither fragile nor soluble. Moreover, if the manufacturing process (e.g. crystallization, milling) of the material has changed, the applicability of the method must be verified (e.g. by microscopic comparison).

Sprays, aerosols and gas bubbles in a liquid should be measured directly, provided that their concentration is adequate, because sampling or dilution generally alters the particle-size distribution.

In other cases (such as emulsions, pastes and powders), representative samples may be dispersed in suitable liquids. Dispersing aids (wetting agents, stabilizers) and/or mechanical forces (e.g. agitation, sonication) are often applied for deagglomeration or disaggregation of clusters and stabilization of the dispersion. For these liquid dispersions, a recirculating system is most commonly used, consisting of an optical measuring cell, a dispersion bath usually equipped with stirrer and ultrasonic elements, a pump, and tubing. Non-recirculating, stirred cells are useful when only small amounts of a sample are available or when special dispersion



1. Obscuration detector
2. Scattered beam
3. Direct beam
4. Fourier lens
5. Scattered light not collected by lens (4)
6. Particle ensemble
7. Light source laser
8. Beam processing unit
9. Working distance of lens (4)
10. Multi-element detector
11. Focal distance of lens (4)

**Fig. 3.06-1** Example of a set-up of a laser light diffraction instrument

liquids are used.

Dry powders can also be converted into aerosols through the use of suitable dry powder dispersers, which apply mechanical force for deagglomeration or deaggregation. Generally, the dispersers use the energy of compressed gas or the differential pressure of a vacuum to disperse the particles to an aerosol, which is blown through the measuring zone, usually into the inlet of a vacuum unit that collects the particles. However, for free flowing, coarser particles or granules the effect of gravity may be sufficient to disperse the particles adequately.

If the maximum particle size of the sample exceeds the measuring range of the instrument, the material that is too coarse can be removed by sieving and the mass and percentage of removed material are reported. However, after presieving, note that the sample is no longer representative, unless otherwise proven.

### 2.3. Optimization of the liquid dispersion

Liquids, surfactants, and dispersing aids used to disperse powders must:

- (i) be transparent at the laser wavelength and practically free from air bubbles or particles;
- (ii) have a refractive index that differs from that of the test material;
- (iii) be non-solvent of the test material (pure liquid or pre-filtered, saturated solution);
- (iv) not alter the size of the test materials (e.g. by solubility, solubility enhancement, or recrystallization effects);
- (v) favor easy formation and stability of the dispersion;
- (vi) be compatible with the materials used in the instrument (such as O-rings, gaskets, tubing, etc.);
- (vii) possess a suitable viscosity to facilitate recirculation, stirring and filtration.

Surfactants and/or dispersing aids are often used to wet the particles and to stabilize the dispersion. For weak acids and weak bases, buffering of the dispersing medium at low or high pH respectively can assist in identifying a suitable dispersant.

A preliminary check of the dispersion quality can be performed by visual or microscopic inspection. It is also possible to take fractional samples out of a well-mixed stock dispersion. Such stock dispersions are formed by adding a liquid to the sample while mixing it with, for example, a glass rod, a spatula or a vortex mixer. Care must be taken to ensure the transfer of a representative sample and that settling of larger particles does not occur. Therefore a sample paste is prepared or sampling is carried out quickly from a suspension maintained under agitation.

### 2.4. Optimization of the gas dispersion

For sprays and dry powder dispersions, a compressed gas free from oil, water and particles may be used. To remove such materials from the compressed gas, a dryer with a filter can be used. Any vacuum unit should be located away from the measurement zone, so that its output does not disturb the measurement.

### 2.5. Determination of the concentration range

In order to produce an acceptable SN ratio in the detector, the particle concentration in the dispersion must exceed a minimum level. Likewise, it must be below a maximum level in order to avoid multiple scattering. The concentration range is influenced by the width of the laser beam, the path length of the measurement zone, the optical properties of the particles, and the sensitivity of the detector elements.

In view of the above, measurements must be performed at different particle concentrations to determine the appropriate concentration range for any typical sample of material. (Note: in different instruments, particle concentrations are

usually represented by differently scaled and differently named numbers, e.g. obscuration, optical concentration, proportional number of total mass).

### 2.6. Determination of the measuring time

The time of measurement, the reading time of the detector and the acquisition frequency is determined experimentally in accordance with the required precision. Generally, the time for measurement permits a large number of detector scans or sweeps at short time intervals.

### 2.7. Selection of an appropriate optical model

Most instruments use either the Fraunhofer or the Mie theory, though other approximation theories are sometimes applied for calculation of the scattering matrix. The choice of the theoretical model depends on the intended application and the different assumptions (size, absorbance, refractive index, roughness, crystal orientation, mixture, etc.) made for the test material. If the refractive index values (real and imaginary parts for the used wavelength) are not exactly known, then the Fraunhofer approximation or the Mie theory with a realistic estimate of the refractive index can be used. The former has the advantages that it is simple and it does not need refractive index values; the latter usually provides less-biased particle-size distributions for small particles. For instance, if the Fraunhofer model is used for samples containing an appreciable amount of small, transparent particles, a significantly large amount of small particles may be calculated. In order to obtain traceable results, it is essential to document the refractive index values used, since small differences in the values assumed for the real and imaginary part of the complex refractive index may cause significant differences in the resulting particle-size distributions. Small values of the imaginary part of the refractive index (about  $0.01 - 0.1i$ ) are often applied to allow the correction of the absorbance for the surface roughness of the particles. It should be noted, in general, that the optical properties of the substance to be tested, as well as the structure (e.g. shape, surface roughness and porosity) bear upon the final result.

### 2.8. Validation

Typically, the validity of a procedure may be assessed by the evaluation of its specificity, linearity, range, accuracy, precision and robustness. In particle-size analysis by laser light diffraction, specificity as defined by ICH (Validation of Analytical Procedures) is not applicable as it is not possible to discriminate different components into a sample, as is neither possible to discriminate between agglomerates from dispersed particles unless properly complemented by microscopic techniques. Exploring a linear relationship between concentration and response, or a mathematical model for interpolation, is not applicable to this procedure. Rather than evaluating linearity, this method requires the definition of a concentration range within which the result of the measurements does not vary significantly. Concentrations below that range produce an error due to a poor SN ratio, while concentrations above that range produce an error due to multiple scattering. The range depends mostly in the instrument hardware. Accuracy should be confirmed through an appropriate instrument qualification and comparison with microscopy, while precision may be assessed by means of a repeatability determination.

The attainable repeatability of the method mainly depends on the characteristics of the material (milled/not milled, robust/fragile, width of its size distribution, etc.), whereas the required repeatability depends on the purpose of the measurement. Mandatory limits cannot be specified in this chapter, as repeatabilities (different sample preparations) may vary appreciably from one substance to another. However, it is good practice to aim at acceptance criteria for

repeatability such as RSD (%)  $\leq 10\%$  [ $n=6$ ] for any central value of the distribution (e.g. for  $x_{50}$ ). Values at the sides of the distribution (e.g.  $x_{10}$  and  $x_{90}$ ) are oriented towards less stringent acceptance criteria such as RSD  $\leq 15\%$  [ $n=6$ ]. Below  $10\text{ }\mu\text{m}$ , these values must be doubled. Robustness may be tested during the selection and optimization of the dispersion media and forces. The change of the dispersing energy may be monitored by the change in the particle-size distribution.

### 3. Measurement

A representative sample, dispersed at an adequate concentration in a suitable liquid or gas, is passed through a beam of monochromatic light, usually a laser. The light scattered by the particles at various angles is measured by a multi-element detector. Numerical values representing the scattering pattern are then recorded for analysis. These scattering pattern values are then transformed, using an appropriate optical model and mathematical procedure, to yield the proportion of total volume to a discrete number of size classes, forming a volumetric particle-size distribution.

#### 3.1. Precautions

- (i) never look into the direct path of the laser beam or its reflections;
- (ii) earth all instrument components to prevent ignition of solvents or dust explosions;
- (iii) check the instrument set-up (e.g. warm-up, required measuring range and lens, appropriate working distance, position of the detector, no direct bright daylight);
- (iv) in the case of wet dispersions, avoid air bubbles, evaporation of liquid, schlieren or other inhomogeneities in the dispersion; similarly, avoid improper mass-flow from the disperser or turbulent air-flow in the case of dry dispersions; such effects can cause erroneous particle-size distributions.

#### 3.2. Measurement of the light scattering of dispersed sample(s)

After proper alignment of the optical part of the instrument, a blank measurement of the particle-free dispersion medium must be performed using the same method as that used for the measurement of the sample. The background signal must be below an appropriate threshold. The detector data are saved in order to subtract them later from the data obtained with the sample. The sample dispersion is measured according to the developed method.

For each detector element, an average signal is calculated, sometimes together with its standard deviation. The magnitude of the signal from each detector element depends upon the detection area, the light intensity and the quantum efficiency. The co-ordinates (size and position) of the detector elements together with the focal distance of the lens determine the range of scattering angles for each element. Most instruments also measure the intensity of the central (unscattered) laser beam. The ratio of the intensity of a dispersed sample to that in its absence (the blank measurement) indicates the proportion of scattered light and hence the particle concentration.

#### 3.3. Conversion of scattering pattern into particle-size distribution

This deconvolution step is the inverse of the calculation of a scattering pattern for a given particle-size distribution. The assumption of spherical particle shape is particularly important as most algorithms use the mathematical solution for scattering from spherical particles. Furthermore, the measured data always contain some random and systematic errors, which may vitiate the size distributions. Several mathematical procedures have been developed for use in the available instruments. They contain some weighting of deviations

between measured and calculated scattering patterns (e.g. least squares), some constraints (e.g. non-negativity for amounts of particles), and/or some smoothing of the size distribution curve.

The algorithms used are specific to each maker and model of equipment, and are proprietary. The differences in the algorithms between different instruments may give rise to differences in the calculated particle-size distributions.

#### 3.4. Replicates

The number of replicate measurements (with individual sample preparations) to be performed, depends on the required measurement precision. It is recommended to set this number in a substance-specific method.

### 4. Reporting of results

The particle-size distribution data are usually reported as cumulative undersize distribution and/or as density distribution by volume. The symbol  $x$  is used to denote the particle size, which in turn is defined as the diameter of a volume-equivalent sphere.  $Q_3(x)$  denotes the volume fraction undersize at the particle size  $x$ . In a graphical representation,  $x$  is plotted on the abscissa and the dependent variable  $Q_3(x)$  on the ordinate. Most common characteristic values are calculated from the particle-size distribution by interpolation. The particle sizes at the undersize values of 10%, 50%, and 90% (denoted as  $x_{10}$ ,  $x_{50}$ , and  $x_{90}$  respectively) are frequently used.  $x_{50}$  is also known as the median particle size. It is recognized that the symbol  $d$  is also widely used to designate the particle size, thus the symbol  $x$  may be replaced by  $d$ .

Moreover, sufficient information must be documented about the sample, the sample preparation, the dispersion conditions, and the cell type. As the results depend on the particular instrument, data analysis program, and optical model used, these details must also be documented.

### 5. Control of the instrument performance

Use the instrument according to the manufacturer's instructions and carry out the prescribed qualifications at an appropriate frequency, according to the use of the instrument and substances to be tested.

#### 5.1. Calibration

Laser diffraction systems, although assuming idealized properties of the particles, are based on first principles of laser light scattering. Thus, calibration in the strict sense is not required. However, it is still necessary to confirm that the instrument is operating correctly. This can be undertaken using any certified reference material that is acceptable in industrial practice. The entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, measurement, and the deconvolution procedure. It is essential that the total operational procedure is fully described.

The preferred certified reference materials consist of spherical particles of a known distribution. They must be certified as to the mass-percentage size distribution by an absolute technique, if available, and used in conjunction with an agreed, detailed operation procedure. It is essential that the real and imaginary parts of the complex refractive index of the material are indicated if the Mie theory is applied in data analysis. The representation of the particle-size distribution by volume will equal that of the distribution by mass, provided that the density of the particles is the same for all size fractions.

The response of a laser diffraction instrument is considered to meet the requirements if the mean value of  $x_{50}$  from at least 3 independent measurements does not deviate by more than 3% from the certified range of values of the certified reference material. The mean values for  $x_{10}$  and  $x_{90}$  must

not deviate by more than 5% from the certified range of values. Below 10 µm, these values must be doubled.

Although the use of materials consisting of spherical particles is preferable, non-spherical particles may also be employed. Preferably, these particles have certified or typical values from laser diffraction analysis performed according to an agreed, detailed operating procedure. The use of reference values from methods other than laser diffraction may cause a significant bias. The reason for this bias is that the different principles inherent in the various methods may lead to different sphere-equivalent diameters for the same non-spherical particle.

Although the use of certified reference materials is preferred, other well-defined reference materials may also be employed. They consist of substances of typical composition and particle-size distribution for a specified class of substances. Their particle-size distribution has proven to be stable over time. The results must comply with previously determined data, with the same precision and bias as for the certified reference material.

### 5.2. Qualification of the system

In addition to the calibration, the performance of the instrument must be qualified at regular time intervals or as frequently as appropriate. This can be undertaken using any suitable reference material as mentioned in the previous paragraph.

The qualification of the system is based on the concept that the equipment, electronics, software and analytical operations constitute an integral system, which can be evaluated as an entity. Thus the entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, and the measurement and deconvolution procedure. It is essential that the total operational procedure is fully described.

In general, unless otherwise specified in the individual monograph, the response of a laser diffraction instrument is considered to meet the requirements if the  $x_{50}$  value does not deviate by more than 10% from the range of values of the reference material. If optionally the values at the sides of the distribution are evaluated (e.g.  $x_{10}$  and  $x_{90}$ ), then these values must not deviate by more than 15% from the certified range of values. Below 10 µm, these values must be doubled. For calibration of the instrument stricter requirements are laid down in 5.1. Calibration.

## 4. Biological Tests/Biochemical Tests/Microbial Tests

### 4.01 Bacterial Endotoxins Test

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Bacterial Endotoxins Test is a test to detect or quantify bacterial endotoxins of gram-negative bacterial origin using an amoebocyte lysate prepared from blood corpuscle extracts of horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). There are two types of techniques for this test: the gel-clot techniques, which are based on gel formation by the reaction of the lysate TS with endotoxins, and the photo-

metric techniques, which are based on endotoxin-induced optical changes of the lysate TS. The latter include turbidimetric techniques, which are based on the change in lysate TS turbidity during gel formation, and chromogenic techniques, which are based on the development of color after cleavage of a synthetic peptide-chromogen complex.

Proceed by any one of these techniques for the test. In the event of doubt or dispute, the final decision is made based on the limit test of the gel-clot techniques, unless otherwise indicated.

The test is carried out in a manner that avoids endotoxin contamination.

#### 1. Apparatus

Depyrogenate all glassware and other heat-stable materials in a hot-air oven using a validated process. Commonly used minimum time and temperature settings are 30 minutes at 250°C. If employing plastic apparatus, such as multi-well plates and tips for micropipettes, use only that which has been shown to be free of detectable endotoxin and which does not interfere with the test.

#### 2. Preparation of Solutions

##### 2.1. Standard Endotoxin Stock Solution

Prepare Standard Endotoxin Stock Solution by dissolving Japanese Pharmacopoeia Endotoxin Reference Standard that has been calibrated to the current WHO International Standard for Endotoxin, using water for bacterial endotoxins test (BET). Endotoxin is expressed in Endotoxin Units (EU). One EU is equal to one International Unit (IU) of endotoxin.

##### 2.2. Standard Endotoxin Solution

After mixing Standard Endotoxin Stock Solution thoroughly, prepare appropriate serial dilutions of Standard Endotoxin Solution, using water for BET. Use dilutions as soon as possible to avoid loss of activity by adsorption.

##### 2.3. Sample Solutions

Unless otherwise specified, prepare sample solutions by dissolving or diluting drugs, using water for BET. By the sample, an aqueous solution other than water for BET may be used to dissolve or dilute. If necessary, adjust the pH of the sample solution so that the pH of the mixture of the lysate TS and sample solution falls within the specified pH range for the lysate to be used. The pH of the sample solution may be in the range of 6.0 to 8.0. For adjustment of pH, acid, base or a suitable buffer solution may be used. The acid and base are prepared from their concentrated solutions or solids using water for BET, and then stored in containers free of detectable endotoxin. The buffer solutions must be validated to be free of detectable endotoxin and interfering factors.

#### 3. Determination of Maximum Valid Dilution

The Maximum Valid Dilution (MVD) is the maximum allowable dilution of a sample solution at which the endotoxin limit can be determined.

Determine the MVD from the following equation:

$$\text{MVD}$$

$$= (\text{Endotoxin limit} \times \text{Concentration of sample solution})/\lambda$$

Endotoxin limit:

The endotoxin limit for injections, defined on the basis of dose, equals  $K/M$ , where  $K$  is a threshold pyrogenic dose of endotoxin per kg body mass (EU/kg), and  $M$  is equal to the maximum bolus dose of product per kg body mass. When the product is to be injected at frequent intervals or infused continuously,  $M$  is the

maximum total dose administered in a single hour period.

Concentration of sample solution:

mg/mL in the case of endotoxin limit specified by mass (EU/mg)

mEq/mL in the case of endotoxin limit specified by equivalent (EU/mEq)

Units/mL in the case of endotoxin limit specified by biological unit (EU/Unit)

mL/mL in the case of endotoxin limit specified by volume (EU/mL)

$\lambda$ : the labeled lysate reagent sensitivity in the gel-clot techniques (EU/mL) or the lowest point used (EU/mL) in the standard regression curve of the turbidimetric or chromogenic techniques

#### 4. Gel-clot techniques

The gel-clot techniques detect or quantify endotoxins based on clotting of the lysate TS in the presence of endotoxin.

To ensure both the precision and validity of the test, perform the tests for confirming the labeled lysate reagent sensitivity (4.1.1.) and for interfering factors (4.1.2.) as described under Preparatory testing (4.1.).

##### 4.1. Preparatory testing

###### 4.1.1. Test for confirmation of labeled lysate reagent sensitivity

The labeled sensitivity of lysate is defined as the lowest concentration of endotoxin that is needed to cause the lysate TS to clot under the conditions specified for the lysate to be used.

The test for confirmation of the labeled lysate sensitivity is to be carried out when each new lot of lysate is used or when there is any change in the experimental conditions which may affect the outcome of the test.

Prepare standard solutions having four concentrations equivalent to  $2\lambda$ ,  $\lambda$ ,  $0.5\lambda$  and  $0.25\lambda$  by diluting the Standard Endotoxin Stock Solution with water for BET. Mix a volume of the lysate TS with an equal volume of one of the standard solutions (usually, 0.1 mL aliquots) in each test tube. When single test vials or ampoules containing lyophilized lysate are used, add solutions directly to the vial or ampoule.

Keep the tubes (or containers such as vials or ampoules) containing the reaction mixture usually at  $37 \pm 1^\circ\text{C}$  for  $60 \pm 2$  minutes, avoiding vibration. To test the integrity of the gel after incubation, invert each tube or container through approximately  $180^\circ$  in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if either a firm gel is not formed, or if a fragile gel has formed but flows out upon inversion.

Making the standard solutions of four concentrations one set, test four replicates of the set.

The test is valid when  $0.25\lambda$  of the standard solution shows a negative result in each set of tests. If the test is not valid, repeat the test after verifying the test conditions.

The endpoint is the last positive test in the series of decreasing concentrations of endotoxin. Calculate the geometric mean endpoint concentration of the four replicate series using the following formula:

$$\text{Geometric Mean Endpoint Concentration} = \text{antilog} (\Sigma e/f)$$

$\Sigma e$ : The sum of the log endpoint concentrations of the dilution series used

$f$ : The number of replicates

If the geometric mean endpoint concentration is not less

Table 4.01-1

Solution	Endotoxin Concentration/ Solution to which endotoxin is added	Diluent	Dilution factor	Endotoxin concentration	Number of replicates
A* <sup>1</sup>	0/Sample solution	—	—	—	4
B* <sup>2</sup>	$2\lambda$ /Sample solution	Sample solution	1	$2\lambda$	4
			2	$1\lambda$	
			4	$0.5\lambda$	
			8	$0.25\lambda$	
C* <sup>3</sup>	$2\lambda$ /Water for BET	Water for BET	1	$2\lambda$	2
			2	$1\lambda$	
			4	$0.5\lambda$	
			8	$0.25\lambda$	
D* <sup>4</sup>	0/Water for BET	—	—	—	2

\*<sup>1</sup> Negative control. Sample solution only.

\*<sup>2</sup> Sample solutions added with standard endotoxin (for testing interfering factors).

\*<sup>3</sup> Standard endotoxin solutions for confirmation of the labeled lysate reagent sensitivity.

\*<sup>4</sup> Negative control. Water for BET only.

than  $0.5\lambda$  and not more than  $2.0\lambda$ , the labeled sensitivity is confirmed, and is used in tests performed with this lysate.

###### 4.1.2. Test for interfering factors

This test is performed to check for the presence of enhancing or inhibiting factors for the reaction in sample solutions.

Prepare the solutions A, B, C and D according to Table 4.01-1, and test solutions A and B and solutions C and D in quadruplicate and in duplicate, respectively. Concerning the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure described in 4.1.1.

The geometric mean endpoint concentrations of B and C solutions are determined by using the formula described in 4.1.1.

This test must be repeated when there is any change in the experimental conditions which may affect the outcome of the test.

The test is valid if solutions A and D show no reaction and the result for solution C confirms the labeled sensitivity.

If the geometric mean endpoint concentration of solution B is not less than  $0.5\lambda$  and not greater than  $2.0\lambda$ , the sample solution being examined does not contain interfering factors and complies with the test for interfering factors. Otherwise the sample solution interferes with the test.

If the sample under test does not comply with the test at a dilution less than the MVD, repeat the test using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a greater dilution of the sample to be examined. Furthermore, interference of the sample solution or diluted sample solution may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment. To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen treatment.

##### 4.2. Limit test

This method tests whether or not a sample contains endotoxins greater than the endotoxin limit specified in the individual monograph based on the gel formation in the presence of endotoxins at a concentration of more than the labeled lysate sensitivity.

###### 4.2.1. Procedure

Prepare solutions A, B, C and D according to Table

**Table 4.01-2**

Solution	Endotoxin concentration/Solution to which endotoxin is added	Number of replicates
A* <sup>1</sup>	0/Sample solution	2
B* <sup>2</sup>	2λ/Sample solution	2
C* <sup>3</sup>	2λ/Water for BET	2
D* <sup>4</sup>	0/Water for BET	2

\*1 Sample solution for the limit test. The solution may be diluted not to exceed the MVD.

\*2 Positive control. Sample solution at the same dilution as solution A, containing standard endotoxin at a concentration of 2λ.

\*3 Positive control. Standard endotoxin solution containing standard endotoxin concentration of 2λ.

\*4 Negative control. Water for BET only.

**Table 4.01-3**

Solution	Endotoxin concentration/Solution to which endotoxin is added	Diluent	Dilution factor	Endotoxin concentration	Number of replicates
A* <sup>1</sup>	0/Sample solution	Water for BET	1	—	2
			2	—	
			4	—	
			8	—	
B* <sup>2</sup>	2λ/Sample solution	—	1	2λ	2
C* <sup>3</sup>	2λ/Water for BET	Water for BET	1	2λ	2
			2	1λ	
			4	0.5λ	
			8	0.25λ	
D* <sup>4</sup>	0/Water for BET	—	—	—	2

\*1 Sample solutions for the Quantitative test. The dilution range of the dilution series may be changed as appropriate, but not exceeding the MVD.

\*2 Positive control. Sample solution at the same dilution as the solution A diluted at the lowest dilution factor, containing standard endotoxin at a concentration of 2λ.

\*3 Standard endotoxin solutions for confirmation of the labeled lysate sensitivity.

\*4 Negative control. Water for BET only.

4.01-2. Making these four solutions one set, test two replicates of the set. In preparing solutions A and B, use the sample solutions complying with 4.1.2.

Concerning the test conditions including the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure under 4.1.1.

#### 4.2.2. Interpretation

The test is valid when both replicates of solutions B and C are positive and those of solution D are negative.

When a negative result is found for both replicates of solution A, the sample complies with the Bacterial Endotoxins Test.

When a positive result is found for both replicates of solution A, the sample does not comply with the test.

When a positive result is found for one replicate of solution A and a negative result is found for the other, repeat the test. In the repeat test, the sample complies with the test if a negative result is found for both replicates of solution A. The sample does not comply with the test if a positive result is found for one or both replicates of solution A.

However, if the sample does not comply with the test at a dilution less than the MVD, the test may be repeated using a greater dilution, not exceeding the MVD.

#### 4.3. Quantitative Test

This method measures endotoxin concentrations of sam-

ples by determining an endpoint of gel formation.

##### 4.3.1. Procedure

Prepare solutions A, B, C and D according to Table 4.01-3. Making these four solutions one set, test two replicates of the set. When preparing solutions A and B, use sample solutions complying with 4.1.2. Concerning the test conditions, follow the procedure described in 4.1.1.

##### 4.3.2. Calculation and interpretation

The test is valid when the following three conditions are met: (a) both replicates of the negative control solution D are negative, (b) both replicates of the positive product control solution B are positive and (c) the geometric mean endpoint concentration of solution C is in the range of 0.5 λ to 2 λ.

The endpoint is defined as the maximum dilution showing the last positive test in the dilution series of solution A, and the endotoxin concentration of the sample solution is calculated by multiplying the endpoint dilution factor by λ.

If none of the dilutions of solution A is positive, report the endotoxin concentration of the sample solution as less than λ × the lowest dilution factor of the sample solution.

If all dilutions are positive, the endotoxin concentration of the sample solution is reported as equal to or greater than the greatest dilution factor of solution A multiplied by λ.

Calculate the endotoxin concentration (in EU/mL, EU/mg, EU/mEq or EU/Unit) of the sample based on the endotoxin concentration of the sample solution. The sample complies with the Bacterial Endotoxins Test if the endotoxin concentration of the sample in both replicates meets the requirement for the endotoxin limit (in EU/mL, EU/mg, EU/mEq or EU/Unit) specified in the individual monograph.

## 5. Photometric quantitative techniques

### 5.1. Turbidimetric technique

This technique measures the endotoxin concentrations of samples based on the measurement of turbidity change accompanying gel formation of the lysate TS. This technique is classified as either endpoint-turbidimetric or kinetic-turbidimetric.

The endpoint-turbidimetric technique is based on the quantitative relationship between the concentration of endotoxins and the turbidity of the reaction mixture at a specified reaction time.

The kinetic-turbidimetric technique is based on the quantitative relationship between the concentration of endotoxins and either the time needed to reach a predetermined turbidity of the reaction mixture or the rate of turbidity development.

The test is usually carried out at 37 ± 1°C, and turbidity is expressed in terms of either absorbance or transmission.

### 5.2. Chromogenic technique

This technique measures the endotoxin concentrations of sample solutions based on the measurement of chromophore released from a synthetic chromogenic substrate by the reaction of endotoxins with the lysate TS. This technique is classified as either endpoint-chromogenic or kinetic-chromogenic.

The endpoint-chromogenic technique is based on the quantitative relationship between the concentration of endotoxins and the release of chromophore at the end of an incubation period.

The kinetic-chromogenic technique is based on the quantitative relationship between the concentration of endotoxins and either the time needed to reach a predetermined absorbance (or transmittance) of the reaction mixture or the rate of color development.

The test is usually carried out at 37 ± 1°C.

### 5.3. Preparatory testing

To assure the precision and validity of the turbidimetric or chromogenic techniques, perform both Test for assurance of criteria for the standard curve (5.3.1.) and Test for interfering factors (5.3.2.), as indicated below.

#### 5.3.1. Test for assurance of criteria for the standard curve

The test is to be carried out when each new lot of lysate reagent is used or when there is any change in the experimental conditions which may affect the outcome of the test.

Using the Standard Endotoxin Solution, prepare at least three endotoxin concentrations to generate the standard curve within the range of endotoxin concentrations indicated by the instructions for the lysate reagent used. Perform the test using at least three replicates of each standard endotoxin concentration according to the optimal conditions for the lysate reagent used (with regard to volume ratios, incubation time, temperature, pH, etc.).

If the desired range is greater than two logs, additional standards should be included to bracket each log increase in the range of the standard curve.

If the absolute value of the correlation coefficient,  $|r|$ , is greater than or equal to 0.980 for the range of endotoxin concentrations set up, the criteria for the standard curve are valid and the curve complies with the test.

If the standard curve does not comply with the test, repeat the test after verifying the test conditions.

#### 5.3.2. Test for interfering factors

Prepare solutions A, B, C and D according to Table 4.01-4. Perform the test on these solutions following the optimal conditions for the lysate reagent used (with regard to volume of sample solution and lysate TS, volume ratio of sample solution to lysate TS, incubation time, etc.).

The test for interfering factors must be repeated when any condition changes, which is likely to influence the result of the test.

The test is valid when the following conditions are met.

- 1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.
- 2: The result with solution D does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate employed.

Calculate the recovery of the endotoxin added to solution B from the concentration found in solution B after subtracting the endotoxin concentration found in solution A. When the recovery of the endotoxin added to solution B is within 50% to 200%, the sample solution under test is considered to be free of interfering factors and the solution complies with the test.

When the endotoxin recovery is out of the specified range, the sample solution under test is considered to contain interfering factors. If the sample under test does not comply with the test, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the sample solution or diluted sample solution not to exceed the MVD may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment. To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen treatment.

### 5.4. Quantitative test

#### 5.4.1. Procedure

Prepare solutions A, B, C and D according to Table 4.01-4, and follow the procedure described in 5.3.2.

**Table 4.01-4**

Solution	Endotoxin concentration	Solution to which endotoxin is added	Number of test tubes or wells
A <sup>*1</sup>	0	Sample solution	Not less than 2
B <sup>*2</sup>	Middle concentration of the standard curve	Sample solution	Not less than 2
C <sup>*3</sup>	At least 3 concentrations	Water for BET	Each not less than 2
D <sup>*4</sup>	0	Water for BET	Not less than 2

\*1 Sample solution only (for assaying endotoxin concentration in the sample solution). The sample solution may be diluted not to exceed the MVD.

\*2 Sample solution at the same dilution as solution A, containing added standard endotoxin at a concentration equal to or near the middle of the standard curve.

\*3 Standard endotoxin solutions at the concentrations used in 5.3.1. (for the standard curve).

\*4 Negative control. Water for BET only.

#### 5.4.2. Calculation of endotoxin concentration

Calculate the mean endotoxin concentration of solution A using the standard curve generated with solution C. The test is valid when all the following requirements are met.

- 1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.
- 2: The endotoxin recovery, calculated from the concentration found in solution B after subtracting the concentration of endotoxin found in solution A, is within the range of 50% to 200%.
- 3: The result with solution D does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate employed.

#### 5.4.3. Interpretation

The sample complies with the Bacterial Endotoxins Test if the endotoxin concentration of the sample calculated from the mean endotoxin concentration of solution A meets the requirement of the endotoxin limit (in EU/mL, EU/mg, EU/mEq or EU/Unit) specified in the individual monograph.

## 4.02 Microbial Assay for Antibiotics

Microbial Assay for Antibiotics is a method to determine the antimicrobial potency of antibiotics based on their antimicrobial activities. There are three methods for this test: the cylinder-plate, perforated plate, and turbidimetric methods. The former two are based on the measurement of the size of the zones of microbial growth inhibition in a nutrient agar medium, and the turbidimetric method is based on the measurement of the inhibition of turbidity development in a fluid medium with microbial growth. Unless otherwise specified in the individual monograph, tests specified to be carried out by the cylinder-plate method may be conducted under the same test conditions using the perforated plate method instead. If necessary, first sterilize water, isotonic sodium chloride solution, buffer solutions, reagents, test solutions and essential parts of measuring instruments and appliances to be used for the test. In performing the test, precautions must be taken to prevent biohazard.

#### 1. Cylinder-plate method

The cylinder-plate method is a method to determine the

antimicrobial potency of the antibiotic to be tested, and is based on the measurement of the size of the zone of growth inhibition of a test organism by the use of cylinder-agar plates.

### 1.1. Test organisms

Use the test organism specified in the individual monograph.

### 1.2. Culture media

Unless otherwise specified, use media with the following compositions. When 'peptone' is indicated as an ingredient of a medium, either meat peptone or casein peptone is applicable. Use sodium hydroxide TS or 1 mol/L hydrochloric acid TS to adjust the pH of the medium to obtain the specified value after sterilization. In the case of the medium for *Bacillus subtilis* ATCC 6633, adjust the pH using ammonia TS, potassium hydroxide TS or 1 mol/L hydrochloric acid TS. A different medium to the one specified for each test organism may be used if it has both a similar composition and an equal or better growth efficiency of the test organism in comparison with the specified medium. Unless otherwise specified, sterilize the media to be used in an autoclave.

#### (1) Agar media for seed and base layer

##### 1) Media for test organism *Bacillus subtilis* ATCC 6633

i. Peptone	5.0 g
Meat extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

ii. Peptone	5.0 g
Meat extract	3.0 g
Trisodium citrate dihydrate	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

##### 2) Medium for test organism *Saccharomyces cerevisiae* ATCC 9763

Glucose	10.0 g
Peptone	9.4 g
Meat extract	2.4 g
Yeast extract	4.7 g
Sodium chloride	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

##### 3) Media for other organisms

i. Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

ii. Glucose	1.0 g
Meat peptone	6.0 g
Casein peptone	4.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

iii. Peptone	10.0 g
Meat extract	5.0 g

Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

#### (2) Agar media for transferring test organisms

##### 1) Medium for test organism *Saccharomyces cerevisiae* ATCC 9763

Glucose	15.0 g
Peptone	5.0 g
Yeast extract	2.0 g
Magnesium sulfate heptahydrate	0.5 g
Potassium dihydrogen phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

##### 2) Media for other organisms

i. Glucose	1.0 g
Meat peptone	6.0 g
Casein peptone	4.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

ii. Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

### 1.3. Preparation of agar slant or plate media

Unless otherwise specified, dispense approximately 9 mL of melted agar medium in each test tube (approximately 16 mm in inside diameter), and make them as slant media, or dispense approximately 20 mL of melted agar medium in each Petri dish (approximately 90 mm in inside diameter), and make them as plate media.

### 1.4. Preparation of stock suspensions of test spores or organisms

Unless otherwise specified, prepare stock suspensions of test spore or organism cultures as follows. Check the aspects of the test spores or organisms as occasion demands.

#### (i) Preparation of a stock spore suspension of test organism *Bacillus subtilis* ATCC 6633

Inoculate the test organism onto the slant or plate of the agar medium which was prepared for transferring the test organisms specified in 1.2. (2) 2 i. Incubate at 32 to 37°C for 16 to 24 hours. Inoculate the subcultured test organism onto a suitable volume of slant or plate of the agar medium (described above), which was prepared for transferring the test organisms specified in 1.2. (2) 2 ii. Then incubate at 32 to 37°C for not less than 1 week to prepare spores. Suspend the spores in isotonic sodium chloride solution, heat at 65°C for 30 minutes, and then centrifuge. Wash the spore sediment three times with isotonic sodium chloride solution by means of centrifugation. Re-suspend the spore sediment in water or isotonic sodium chloride solution, and heat again at 65°C for 30 minutes to prepare the stock spore suspension. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock spore suspension at a temperature not exceeding 5°C, and use within 6 months. If the stock spore suspension shows a clear and definite zone of growth inhibition in an

antibiotics potency test using adequate antibiotics, it may be used for further 6 months.

(ii) Preparation of a stock suspension of the test organism *Saccharomyces cerevisiae* ATCC 9763

Inoculate test organism onto the slant or plate agar medium which has been prepared for transferring test organism specified in 1.2. (2) 1). Incubate at 25 to 26°C for 40 to 48 hours. The subculture should be performed at least three times. Inoculate the subcultured test organism onto another slant or plate of the agar medium (described above), and incubate at 25 to 26°C for 40 to 48 hours. Scrape away and suspend the resulting growth from the agar surface in isotonic sodium chloride solution, and use this as a stock suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock suspensions of the test organisms at a temperature not exceeding 5°C, and use within 30 days.

(iii) Preparation of a stock suspension of other test organisms

Inoculate the test organism onto the slant or the plate of the agar medium which has been prepared for transferring the test organisms specified in 1.2. (2) 2) i. Incubate the inoculated slant at 32 to 37°C for 16 to 24 hours. The subculture should be performed at least three times. Inoculate the subcultured test organism onto another slant or plate agar medium (described above), and incubate the slant at 32 to 37 °C for 16 to 24 hours. Scrape away and suspend the resulting growth from the agar surface in isotonic sodium chloride solution, and use this as a stock suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock suspensions of the test organisms at a temperature not exceeding 5°C, and use within 5 days.

### 1.5. Preparation of agar base layer plates

Unless otherwise specified, dispense 20 mL of the melted agar medium for the base layer into each Petri dish, and in the case of a large dish, dispense a quantity of the agar medium to form a uniform layer 2 to 3 mm thick. Distribute the agar evenly in each dish on a flat, level surface, and allow it to harden.

### 1.6. Preparation of seeded agar layers

Unless otherwise specified, determine the volume of the stock suspension of the spore or the test organism with which the employed standard solution shows a clear and definite zone of growth inhibition. Prepare the seeded agar layer by mixing thoroughly the previously determined volume of stock suspension of spore or test organism with agar medium for the seed layer kept at 48 to 51°C. Usually, the rate of a stock spore suspension and a stock suspension of the test organism to add to the agar medium for the seed layer are 0.1 to 1.0 vol% and 0.5 to 2.0 vol%, respectively.

### 1.7. Preparation of cylinder-agar plates

Dispense 4 to 6 mL of the seeded agar layer, which is specified in the individual monograph, on an agar base layer plate in a Petri dish. In the case of large dishes, dispense a quantity of the agar medium to form a uniform layer 1.5 to 2.5 mm thick, and spread evenly over the surface before hardening. After coagulating the agar, allow the plate to stand under a clean atmosphere to exhale moisture vapor of the inside of Petri or large dishes and water on the agar surface. Place 4 cylinders on an agar plate in a Petri dish so that the individual cylinders are equidistant from the center of the plate and equally spaced from one another (the cylinders are set on the circumference of a circle of 25 to 28 mm radius). When large dish plates are used, place cylinders on each plate according to the method of preparation for Petri

dish agar plates. A set of 4 cylinders on each large dish plate is considered to be equivalent to one Petri dish plate. Use stainless steel cylinders with the following dimensions: outside diameter 7.9 to 8.1 mm; inside diameter 5.9 to 6.1 mm; length 9.9 to 10.1 mm. The cylinders should not interfere with the test. Prepare the cylinder-agar plates before use.

### 1.8. Standard solutions

Use both a standard solution of high concentration and one of low concentration, as specified in the individual monograph. Unless otherwise specified, prepare the standard solutions before use.

### 1.9. Sample solutions

Use both a sample solution of high concentration and one of low concentration, as specified in the individual monograph. Unless otherwise specified, prepare the sample solutions before use.

### 1.10. Procedure

Unless otherwise specified, use 5 cylinder-agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cylinders for one assay set should be equal to that defined when using Petri dishes. Apply the standard solution of high concentration and that of low concentration to a pair of cylinders set opposite each other on each plate. Apply the high and low concentration sample solutions to the remaining 2 cylinders. The same volume of these solutions must be added to each cylinder. Incubate the plates at 32 to 37°C for 16 to 20 hours. Using a suitable measuring tool, measure the diameters of circular inhibition zones with a precision that can discriminate differences of at least 0.25 mm. Each procedure should be performed quickly under clean laboratory conditions.

### 1.11. Estimation of potency

The following correlation between the potency ( $P$ ) of solution in a cylinder and the diameter ( $d$ ) of zone of inhibition is established.

$$d = \alpha \log P + \beta$$

where,  $\alpha$  and  $\beta$  are constants.

If necessary, ascertain the values in the above equation.

Based on this equation, estimate the potency of the sample solutions by application of the following equation:

Amount (potency) of sample

$$= A \times \text{Potency of } S_H \text{ per mL} \times \text{Dilution factor of } U_H$$

where:

$$\log A = \frac{IV}{W}$$

$$I = \log (\text{potency of } S_H / \text{potency of } S_L)$$

$$V = \Sigma U_H + \Sigma U_L - \Sigma S_H - \Sigma S_L$$

$$W = \Sigma U_H + \Sigma S_H - \Sigma U_L - \Sigma S_L$$

The sum of the diameter (mm) of the inhibitory zone measured in each plate is designated as follows:

for standard solution of high concentration ( $S_H$ ) =  $\Sigma S_H$

for standard solution of low concentration ( $S_L$ ) =  $\Sigma S_L$

for sample solution of high concentration ( $U_H$ ) =  $\Sigma U_H$

for sample solution of low concentration ( $U_L$ ) =  $\Sigma U_L$

### 2. Perforated plate method

The perforated plate method is a method to determine the antimicrobial potency of an antibiotic, based on the measurement of the size of the zone of growth inhibition of a test organism by the use of perforated agar plates.

This method is carried out by the use of perforated agar plates in lieu of cylinder-agar plates used in Cylinder-plate method.

Proceed as directed below, but comply with the require-

ments of Cylinder-plate method, such as test organisms, media, preparation of agar slant or plate media, preparation of stock suspensions of spores or test organisms, preparation of agar base layer plates, preparation of seeded agar layers, standard solutions, sample solutions, and estimation of potency.

### 2.1. Preparation of perforated agar plates

Dispense 4 to 6 mL of the seeded agar layer specified in the individual monograph on each agar base layer plate of the Petri dish. In the case of large dishes, dispense a quantity of the agar medium to form a uniform layer 1.5 to 2.5 mm thick, and spread evenly over the surface before hardening. After coagulating the agar, allow the plate to stand under a clean atmosphere to exhale moisture vapor of the inside of Petri or large dishes and water on the agar surface. Using a suitable tool, prepare 4 circular cavities having a diameter of 7.9 to 8.1 mm on a Petri dish agar plate so that the individual cavities are equidistant from the center of the plate. The cavities spaced equally from one another on the circumference of a circle with radius 25 to 28 mm, and are deep enough to reach the bottom of dish. When large dish plates are used, prepare the circular cavities on each plate according to the method of preparation for Petri dish agar plates. A set of 4 cavities on each large dish plate is considered to be equivalent to one Petri dish plate. Prepare the perforated agar plates before use.

### 2.2. Procedure

Unless otherwise specified, use 5 perforated agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cavities for one assay set should be equal to that defined when using Petri dishes. Apply the high and low concentration standard solutions to a pair of cavities prepared opposite each other on each plate, and apply the high and low concentration sample solutions to the remaining 2 cavities. The same volume of these solutions must be added to each cavity. Incubate the plates at 32 to 37°C for 16 to 20 hours. Using a suitable measuring tool, measure the diameters of the circular inhibition zones with a precision that can discriminate differences of at least 0.25 mm. Each procedure should be performed quickly under clean laboratory conditions.

### 3. Turbidimetric method

The turbidimetric method is a method to determine the antimicrobial potency of an antibiotic, based on the measurement of the inhibition of growth of a microbial culture in a fluid medium. The inhibition of growth of a test organism is photometrically measured as changes in turbidity of the microbial culture.

#### 3.1. Test organisms

Use the test organism specified in the individual monograph.

#### 3.2. Culture media

Unless otherwise specified, use media with the following compositions. When peptone is indicated as an ingredient of a medium, either meat peptone or casein peptone is applicable. Use sodium hydroxide TS or 1 mol/L hydrochloric acid TS to adjust the pH of the medium to obtain the specified value after sterilization. A different medium to the one specified for each test organism may be used if it has both a similar composition and an equal or better growth efficiency of the test organism in comparison with the specified medium. Unless otherwise specified, sterilize the media to be used in an autoclave.

##### (1) Agar media for transferring test organisms

Glucose	1.0 g
Peptone	6.0 g

Meat extract	1.5 g
Yeast extract	3.0 g
Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

##### (2) Liquid media for suspending test organisms

Glucose	1.0 g
Peptone	5.0 g
Meat extract	1.5 g
Yeast extract	1.5 g
Sodium chloride	3.5 g
Potassium dihydrogen phosphate	1.32 g
Disodium hydrogen phosphate*	3.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.0 to 7.1 after sterilization.

\*Dipotassium hydrogen phosphate (3.68 g) may be used in lieu of disodium hydrogen phosphate (3.0 g).

### 3.3. Preparation of agar slant or plate media

Unless otherwise specified, proceed as directed in Preparation of agar slant or plate media under Cylinder-plate method.

### 3.4. Preparation of stock suspensions of test organisms

Unless otherwise specified, inoculate the test organism onto the slant or plate of the agar medium which was prepared for transferring the specified test organism. Incubate the inoculated medium at 32 to 37°C for 16 to 24 hours. The subculture should be performed at least three times. Check the aspects of the test spores or organisms as occasion demands. Inoculate the subcultured test organism onto another slant or plate of the agar medium (described above), and incubate the slant at 32 to 37°C for 16 to 24 hours. After incubation, suspend the test organism in the liquid medium for suspending the test organism, and use as the suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands.

### 3.5. Standard solutions

Use the standard solutions specified in the individual monograph. Unless otherwise specified, prepare the standard solutions before use.

### 3.6. Sample solutions

Use the sample solutions specified in the individual monograph. Unless otherwise specified, prepare the sample solutions before use.

### 3.7. Procedure

Unless otherwise specified, proceed as follows:

Distribute 1.0 mL of each concentration of the standard solution, the sample solution, and water used as a control, into each set composed of 3 test tubes (about 14 mm in inside diameter and about 13 cm in length). Add 9.0 mL of the suspension of the test organism to each tube, and then incubate in a water bath maintained at 35 to 37°C for 3 to 4 hours. After incubation, add 0.5 mL of dilute formaldehyde (1 in 3) to each tube, and read each transmittance or absorbance at a wavelength of 530 nm.

### 3.8. Estimation of potency

Average the transmittance or absorbance values of each concentration of the standard solution, the sample solution and water used as a control, respectively. Generate the standard curve based on the average values of transmittance or absorbance of each concentration of the standard solution, and estimate the potency of the sample solution from its average value of transmittance or absorbance using the obtained standard curve.

If the standard dilutions of five concentrations in geometric progression are used, calculate the  $L$  and  $H$  values from the following equations. Plot point  $L$  and point  $H$  on graph paper and construct a straight line for the standard curve.

$$L = \frac{3a + 2b + c - e}{5}$$

$$H = \frac{3e + 2d + c - a}{5}$$

where:

$L$ : Calculated value of transmittance or absorbance for the lowest concentration of the standard curve.

$H$ : Calculated value of transmittance or absorbance for the highest concentration of the standard curve.

$a, b, c, d, e$ : Average transmittance or absorbance values for each standard dilution, where  $a$  is the value from the lowest concentration standard solution,  $b, c$  and  $d$  are the values from each geometrically increased concentration standard solution, respectively, and  $e$  is the value from the highest concentration standard solution.

## 4.03 Digestion Test

Digestion Test is a test to measure the activity of digestive enzymes, as crude materials or preparations, on starch, protein and fat.

### 1. Assay for Starch Digestive Activity

The assay for starch digestive activity is performed through the measurement of starch saccharifying activity, dextrinizing activity, and liquefying activity.

#### 1.1. Measurement of starch saccharifying activity

The starch saccharifying activity can be obtained by measuring an increase of reducing activity owing to the hydrolysis of the glucoside linkages when amylase acts on the starch. Under the conditions described in Procedure, one starch saccharifying activity unit is the amount of enzyme that catalyzes the increase of reducing activity equivalent to 1 mg of glucose per minute.

##### 1.1.1. Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the reducing activity increases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.4 to 0.8 starch saccharifying activity unit/mL. Filter if necessary.

##### 1.1.2. Preparation of Substrate Solution

Use potato starch TS for measuring the starch digestive activity. If necessary, add 10 mL of buffer or salts solution specified in the monograph, instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution (pH 5.0).

##### 1.1.3. Procedure

Pipet 10 mL of the substrate solution, stand at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at  $37 \pm 0.5^\circ\text{C}$  for exactly 10 minutes, add exactly 2 mL of alkaline tartrate solution of the Fehling's TS for amyloytic activity test, and shake immediately. Then, add exactly 2 mL of copper solution of the Fehling's TS for amyloytic activity test, shake gently, heat the solution in a water bath for exactly 15 minutes, and then immediately cool to below  $25^\circ\text{C}$ . Then, add exactly 2 mL of concentrated potassium iodide TS and 2 mL of diluted sulfuric acid (1 in 6), and titrate <2.50>

the released iodine with 0.05 mol/L sodium thiosulfate VS to the disappearance of the blue color produced by addition of 1 to 2 drops of soluble starch TS ( $a$  mL). Separately, pipet 10 mL of water instead of the substrate solution and titrate <2.50> in the same manner ( $b$  mL).

Starch saccharifying activity (unit/g)

$$= \text{amount (mg) of glucose} \times \frac{1}{10} \times \frac{1}{M}$$

$$\text{Amount (mg) of glucose} = (b - a) \times 1.6$$

$M$ : Amount (g) of sample in 1 mL of sample solution

### 1.2. Measurement of starch dextrinizing activity

The starch dextrinizing activity can be obtained by measuring a decrease in starch coloration by iodine resulting from hydrolysis of the straight chain component (amylose) in starch when amylase acts on the starch. Under the conditions described in Procedure, one starch dextrinizing activity unit is the amount of enzyme required to reduce the coloration of potato starch by iodine by 10% per minute.

#### 1.2.1. Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water or a buffer or salts solution specified in the monograph so that the coloration of starch by iodine decreases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.2 to 0.5 starch dextrinizing activity unit/mL. Filter if necessary.

#### 1.2.2. Preparation of Substrate Solution

Prepare the substrate solution in the same manner as the substrate solution in the measurement of starch saccharifying activity.

#### 1.2.3. Procedure

Pipet 10 mL of the substrate solution, stand at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at  $37 \pm 0.5^\circ\text{C}$  for exactly 10 minutes. Pipet 1 mL of this solution, add it to 10 mL of 0.1 mol/L hydrochloric acid TS, and shake immediately. Pipet 0.5 mL of this solution, add exactly 10 mL of 0.0002 mol/L iodine TS, and shake. Determine the absorbance  $A_T$  of this solution at the wavelength of 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. Separately, using 1 mL of water instead of the sample solution, determine the absorbance  $A_B$  in the same manner.

Starch dextrinizing activity (unit/g)

$$= \frac{(A_B - A_T)}{A_B} \times \frac{1}{M}$$

$M$ : Amount (g) of sample in 1 mL of sample solution

### 1.3. Measurement of starch liquefying activity

The starch liquefying activity can be obtained by measuring a decrease in the viscosity of starch solution resulting from the hydrolysis of molecules when amylase acts on the starch. Under the conditions described in Procedure, one starch liquefying activity unit is the amount of enzyme required to reduce the viscosity of the substrate solution equivalent to 1 g of potato starch from 200% to 100% of that of the 50% sucrose standard solution.

#### 1.3.1. Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the viscosity decreases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.15

to 0.25 starch liquefying activity unit/mL. Filter if necessary.

### 1.3.2. Preparation of Substrate Solution

Weigh accurately about 1 g of potato starch, and measure the loss on drying at 105°C for 2 hours. Weigh exactly potato starch equivalent to 15.00 g calculated on the dried basis, add 300 mL of water, then add gradually 25 mL of 2 mol/L sodium hydroxide TS under thorough shaking, until the mixture forms a paste. Heat the mixture in a water bath for 10 minutes, shaking it occasionally. After cooling, neutralize the mixture with 2 mol/L hydrochloric acid TS, and add 50 mL of the buffer solution specified in the monograph and water to make exactly 500 g. Prepare before use.

### 1.3.3. Preparation of 50% Standard Sucrose Solution

Dissolve 50.0 g of sucrose in 50.0 mL of water.

### 1.3.4. Procedure

Put 50 mL of the 50% standard sucrose solution in a 100-mL conical flask, and allow it to stand in a thermostat at 37 ± 0.5°C for 15 minutes. Fix a viscometer shown in Fig. 4.03-1 so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. After slowly pulling up the 50% standard sucrose solution by suction to the middle of the upper bulb of the viscometer, let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators ( $t_1$  seconds). Take exactly 50 g of the substrate solution in another 100-mL conical flask, and stand it in another thermostat at 37 ± 0.5°C for 20 minutes. Add exactly 1 mL of the sample solution to it, and shake the flask immediately. Fix a viscometer vertically so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. Occasionally pull the reaction solution up by suction to the middle of the upper bulb slowly, then let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators ( $t$  seconds). Repeat this operation until  $t$  becomes shorter than  $t_1$ . At each measurement, record the time ( $T'$  seconds) from the moment that the sample solution is added to the moment that the solution surface in the flask passes the upper indicator. ( $T' + t/2$ ) is the reaction time ( $T$ ) corresponding to  $t$ . Draw a curve for both  $t$  and  $T$ . Obtain  $T_1$  and  $T_2$  that correspond to  $t_1$  and  $(2 \times t_1)$  by interpolation.

$$\text{Starch liquefying activity (unit/g)} = \frac{60}{(T_1 - T_2)} \times \frac{1.5}{M}$$

$M$ : Amount (g) of sample in 1 mL of sample solution

## 2. Assay for Protein Digestive Activity

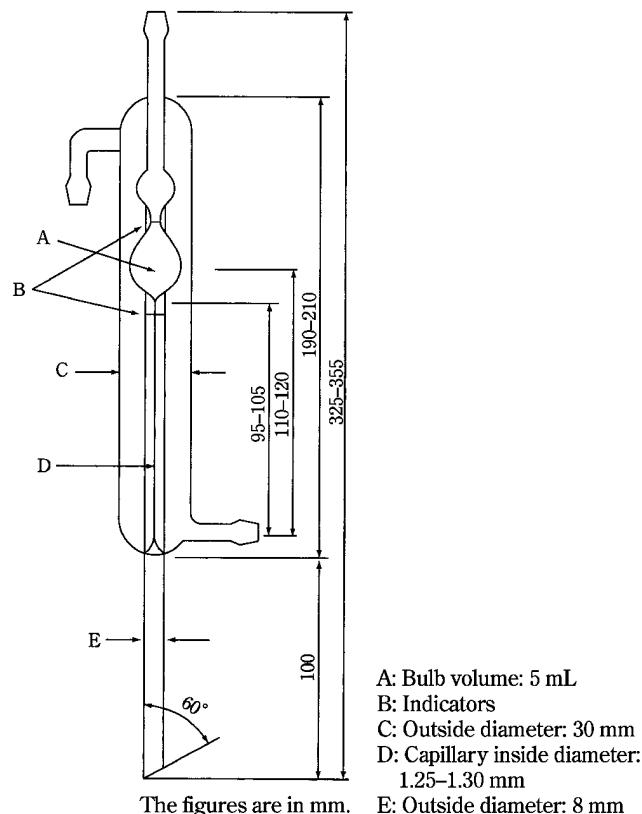
The protein digestive activity can be obtained by the colorimetric measurement, making use of Folin's reaction, of the amount of acid-soluble low-molecular products, which is increased owing to the hydrolysis of the peptide linkages when protease acts on casein. One protein digestive activity unit is the amount of enzymes that produces Folin's TS-colorable substance equivalent to 1 µg of tyrosine per minute under the conditions described in Procedure.

### 2.1. Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the amount of non-protein, Folin's TS-colorable substances increase in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 15 to 30 protein digestive activity unit/mL.

### 2.2. Tyrosine Calibration Curve

Weigh exactly 50 mg of Tyrosine for Digestion Test RS,



A: Bulb volume: 5 mL  
B: Indicators  
C: Outside diameter: 30 mm  
D: Capillary inside diameter: 1.25-1.30 mm  
E: Outside diameter: 8 mm  
The figures are in mm.

Fig. 4.03-1

previously dried at 105°C for 3 hours, and dissolve in 0.2 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 1 mL, 2 mL, 3 mL and 4 mL of this solution separately, and add 0.2 mol/L hydrochloric acid TS to each solution to make exactly 100 mL. Pipet 2 mL of each solution, and add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to each solution, shake immediately, then stand them at 37 ± 0.5°C for 30 minutes. Determine the absorbances,  $A_1$ ,  $A_2$ ,  $A_3$  and  $A_4$ , of these solutions at 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with exactly 2 mL of 0.2 mol/L hydrochloric acid TS in the same manner as the blank. Then, draw a calibration curve with the absorbances,  $A_1$ ,  $A_2$ ,  $A_3$  and  $A_4$  as the ordinate, and with the amount (µg) of tyrosine in 2 mL of each solution as the abscissa. Obtain the amount (µg) of tyrosine for the absorbance difference of 1.

### 2.3. Preparation of Substrate Solution

(i) Substrate solution 1: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105°C for 2 hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 12 mL of lactic acid TS and 150 mL of water, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200 mL. Prepare before use.

(ii) Substrate solution 2: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105°C for 2 hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 160 mL of 0.05 mol/L disodium hydrogenphosphate TS, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with the 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water

to make exactly 200 mL. Prepare before use.

#### 2.4. Preparation of Precipitation Reagent

(i) Trichloroacetic acid TS A: Dissolve 7.20 g of trichloroacetic acid in water to make 100 mL.

(ii) Trichloroacetic acid TS B: Dissolve 1.80 g of trichloroacetic acid, 1.80 g of anhydrous sodium acetate and 5.5 mL of 6 mol/L acetic acid TS in water to make 100 mL.

#### 2.5. Procedure

Pipet 5 mL of the substrate solution specified in the monograph, stand at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. After standing this solution at  $37 \pm 0.5^\circ\text{C}$  for exactly 10 minutes, add exactly 5 mL of trichloroacetic acid TS A or B as specified in the monograph, shake, stand it at  $37 \pm 0.5^\circ\text{C}$  for 30 minutes, and then filter. Discard the first 3 mL of the filtrate, exactly measure the subsequent 2 mL of the filtrate, add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to the solution, shake well, and stand it at  $37 \pm 0.5^\circ\text{C}$  for 30 minutes. Determine the absorbance  $A_T$  of this solution at 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS A or B to the solution as specified in the monograph, and shake. To this solution add exactly 5 mL of the substrate solution specified in the monograph, shake immediately, and stand it at  $37 \pm 0.5^\circ\text{C}$  for 30 minutes. Follow the same procedure for the sample solution, and determine the absorbance  $A_B$  at 660 nm.

Protein digestive activity (unit/g)

$$= (A_T - A_B) \times F \times \frac{11}{2} \times \frac{1}{10} \times \frac{1}{M}$$

M: Amount (g) of sample in 1 mL of sample solution

F: Amount ( $\mu\text{g}$ ) of tyrosine for absorbance 1 determined from Tyrosine Calibration Curve

#### 3. Assay for Fat Digestive Activity

The fat digestive activity can be obtained by back titration of the amount of fatty acid produced from the hydrolysis of the ester linkage, when lipase acts on olive oil. One fat digestive activity unit is the amount of enzymes that produces 1  $\mu\text{mole}$  of fatty acid per minute under the conditions described in Procedure.

##### 3.1. Preparation of Sample Solution

Dissolve or suspend the sample in an appropriate amount of cold water, or a buffer or salts solution specified in the monograph so that the amount of fatty acid increases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 1 to 5 fat digestive activity unit/mL.

##### 3.2. Preparation of Substrate Solution

Take 200 to 300 mL of a mixture of emulsifier and olive oil (3:1) in a blender (see Fig. 4.03-2), and emulsify it at 12,000 to 16,000 revolutions per minute for 10 minutes, while cooling the solution to a temperature below  $10^\circ\text{C}$ . Stand this solution in a cool place for 1 hour, and make sure before use that the oil does not separate.

##### 3.3. Preparation of Emulsifier

Dissolve 20 g of polyvinyl alcohol specified in the monograph in 800 mL of water by heating between  $75^\circ\text{C}$  and  $80^\circ\text{C}$  for 1 hour while stirring. After cooling, filter the solution if necessary, and add water to make exactly 1000 mL.

##### 3.4. Procedure

Pipet 5 mL of the substrate solution and 4 mL of the buffer solution specified in the monograph, transfer them to

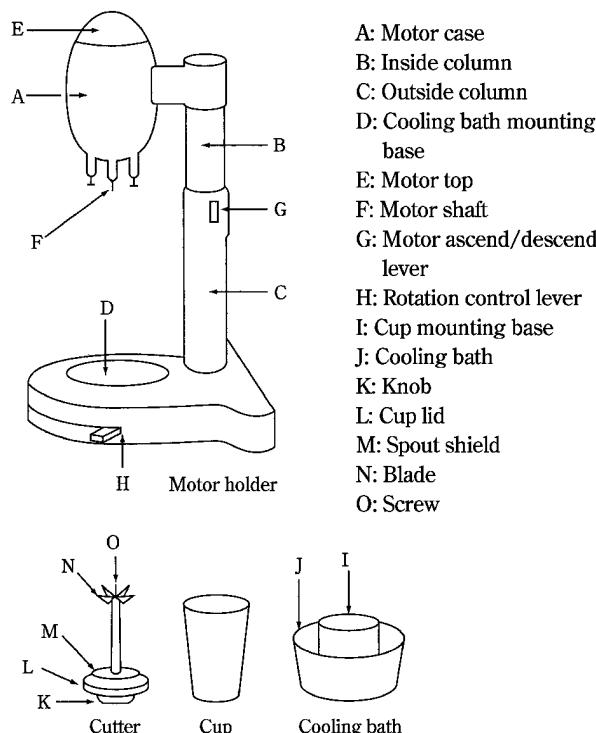


Fig. 4.03-2 Blender

a conical flask, and shake. After standing the mixture at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Stand this solution at  $37 \pm 0.5^\circ\text{C}$  for exactly 20 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Then add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Titrate <2.50> the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS ( $b$  mL) (indicator: 2 to 3 drops of phenolphthalein TS). Separately, pipet 5 mL of the substrate solution and 4 mL of buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing it at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), then add exactly 1 mL of the sample solution, and shake. Add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, and titrate <2.50> in the same manner ( $a$  mL).

Fat digestive activity (unit/g)

$$= 50 \times (a - b) \times \frac{1}{20} \times \frac{1}{M}$$

M: Amount (g) of sample in 1 mL of sample solution

## 4.04 Pyrogen Test

Pyrogen Test is a method to test the existence of pyrogens by using rabbits.

#### 1. Test animals

Use healthy mature rabbits, each weighing not less than 1.5 kg, which have not lost body mass when kept on a constant diet for not less than one week. House the rabbits individually in an area free from disturbances likely to excite them. Keep the temperature of the area constant between  $20^\circ\text{C}$  and  $27^\circ\text{C}$  for at least 48 hours before and throughout the test. Before using a rabbit that has not previously been

used for a pyrogen test, condition it 1 to 3 days prior to the test by conducting a sham test omitting the injection. Do not use a rabbit for pyrogen tests more frequently than once every 48 hours, or after it has been given a test sample that was adjudged pyrogen-positive or that contained an antigen present commonly in the test sample to be examined.

## 2. Apparatus, instruments

(i) Thermometer—Use a rectal thermometer or temperature-measuring apparatus with an accuracy of  $\pm 0.1^\circ\text{C}$  or less.

(ii) Syringe and injection needle—Depyrogenate the syringes and needles in a hot-air oven using a validated process, usually by heating at  $250^\circ\text{C}$  for not less than 30 minutes. Sterilized syringes with needles are also available provided that they have been validated to assure that they are free of detectable pyrogens and do not interfere with the test.

## 3. Test procedures

### 3.1. Quantity of injection

Unless otherwise specified, inject 10 mL of the sample per kg of body mass of each rabbit.

### 3.2. Procedure

Perform the test in a separate area at an environmental temperature similar to that of the room wherein the animals were housed and free from disturbances likely to excite them. Withhold food from the rabbits for several hours before the first record of the temperature and throughout the testing period. The test animals are usually restrained with loosely fitting neck stocks that allow the rabbits to assume a natural resting posture. Determine the temperature of each rabbit by inserting the thermometer or temperature-measuring probe into the rectum of the test animal to a constant depth within the range of 60 mm to 90 mm. The “control temperature” of each rabbit is the mean of two temperature readings recorded for that rabbit at an interval of 30 min in the 40 min immediately preceding the injection of the sample to be examined. Rabbits showing a temperature variation greater than  $0.2^\circ\text{C}$  between the two successive temperature readings or rabbits having an initial temperature higher than  $39.8^\circ\text{C}$  are withdrawn from the test.

Warm the test solution to a temperature of  $37 \pm 2^\circ\text{C}$  before injection, and inject the solution slowly into the marginal vein of the ear of each rabbit over a period not exceeding 10 min. Hypotonic test sample may be made isotonic by the addition of pyrogen-free sodium chloride. Record the temperature of each rabbit during a period of 3 hours after the injection, taking the measurements at intervals of not more than 30 min. The difference between the control temperature and the maximum temperature of each rabbit is taken to be the rise in body temperature. Consider any temperature decreases as zero rise.

## 4. Interpretation of results

The test is carried out on a group of three rabbits and the result is judged on the basis of the sum of the three temperature rises. Repeat if necessary on further groups of three rabbits to a total of three groups, depending on the results obtained. If the summed response of the first group does not exceed  $1.3^\circ\text{C}$ , the sample is judged to be pyrogen-negative. If the summed response exceeds  $2.5^\circ\text{C}$ , the sample is judged to be pyrogen-positive. If the summed response exceeds  $1.3^\circ\text{C}$  but does not exceed  $2.5^\circ\text{C}$ , repeat the test on another group of three rabbits. If the summed response of the first and second group does not exceed  $3.0^\circ\text{C}$ , the sample is judged to be pyrogen-negative. If the summed response of the 6 rabbits exceeds  $4.2^\circ\text{C}$ , the sample is judged to be

pyrogen-positive. If the summed response exceeds  $3.0^\circ\text{C}$  but does not exceed  $4.2^\circ\text{C}$ , repeat the test on one more group of three rabbits. If the summed response of the 9 rabbits does not exceed  $5.0^\circ\text{C}$ , the sample is judged to be pyrogen-negative. If the summed response exceeds  $5.0^\circ\text{C}$ , the sample is judged to be pyrogen-positive.

When the test sample is judged to be pyrogen-negative, the sample passes the pyrogen test.

## 4.05 Microbiological Examination of Non-sterile Products

This chapter includes microbial enumeration tests and tests for specified micro-organisms. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

### I. Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests

These tests are harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

The tests described hereafter will allow quantitative enumeration of mesophilic bacteria and fungi which may grow under aerobic conditions.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

The methods are not applicable to products containing viable micro-organisms as active ingredients.

Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

### 1. General Procedures

Carry out the determination under conditions designed to avoid extrinsic microbial contamination of the product to be examined. The precautions taken to avoid contamination must be such that they do not affect any micro-organisms which are to be revealed in the test.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized. If inactivators are used for this purpose their efficacy and their absence of toxicity for micro-organisms must be demonstrated.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated.

### 2. Enumeration Methods

Use the membrane filtration method, or the plate-count methods, as prescribed. The most probable number (MPN) method is generally the least accurate method for microbial counts, however, for certain product groups with very low bioburden, it may be the most appropriate method.

The choice of a method is based on factors such as the nature of the product and the required limit of micro-organisms. The method chosen must allow testing of a sufficient

Table 4.05-I-1 Preparation and use of test micro-organisms

Micro-organism	Preparation of test strain	Growth promotion		Suitability of counting method in the presence of the product	
		Total aerobic microbial count	Total yeasts and moulds count	Total aerobic microbial count	Total yeasts and moulds count
<i>Staphylococcus aureus</i> such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276	<i>Casein soya bean digest agar</i> or <i>casein soya bean digest broth</i> 30 – 35°C 18 – 24 hours	<i>Casein soya bean digest agar</i> and <i>casein soya bean digest broth</i> ≤ 100 CFU 30 – 35°C ≤ 3 days		<i>Casein soya bean digest agar/MPN</i> <i>casein soya bean digest broth</i> ≤ 100 CFU 30 – 35°C ≤ 3 days	
<i>Pseudomonas aeruginosa</i> such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	<i>Casein soya bean digest agar</i> or <i>casein soya bean digest broth</i> 30 – 35°C 18 – 24 hours	<i>Casein soya bean digest agar</i> and <i>casein soya bean digest broth</i> ≤ 100 CFU 30 – 35°C ≤ 3 days		<i>Casein soya bean digest agar/MPN</i> <i>casein soya bean digest broth</i> ≤ 100 CFU 30 – 35°C ≤ 3 days	
<i>Bacillus subtilis</i> such as ATCC 6633, NCIMB 8054, CIP 52.62 or NBRC 3134	<i>Casein soya bean digest agar</i> or <i>casein soya bean digest broth</i> 30 – 35°C 18 – 24 hours	<i>Casein soya bean digest agar</i> and <i>casein soya bean digest broth</i> ≤ 100 CFU 30 – 35°C ≤ 3 days		<i>Casein soya bean digest agar/MPN</i> <i>casein soya bean digest broth</i> ≤ 100 CFU 30 – 35°C ≤ 3 days	
<i>Candida albicans</i> such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594	<i>Sabouraud-dextrose agar</i> or <i>Sabouraud-dextrose broth</i> 20 – 25°C 2 – 3 days	<i>Casein soya bean digest agar</i> ≤ 100 CFU 30 – 35°C ≤ 5 days	<i>Sabouraud-dextrose agar</i> ≤ 100 CFU 20 – 25°C ≤ 5 days	<i>Casein soya bean digest agar</i> ≤ 100 CFU 30 – 35°C ≤ 5 days MPN: not applicable	<i>Sabouraud-dextrose agar</i> ≤ 100 CFU 20 – 25°C ≤ 5 days
<i>Aspergillus brasiliensis</i> such as ATCC 16404, IMI 149007, IP 1431.83 or NBRC 9455	<i>Sabouraud-dextrose agar</i> or <i>potato-dextrose agar</i> 20 – 25°C 5 – 7 days, or until good sporulation is achieved	<i>Casein soya bean digest agar</i> ≤ 100 CFU 30 – 35°C ≤ 5 days	<i>Sabouraud-dextrose agar</i> ≤ 100 CFU 20 – 25°C ≤ 5 days	<i>Casein soya bean digest agar</i> ≤ 100 CFU 30 – 35°C ≤ 5 days MPN: not applicable	<i>Sabouraud-dextrose agar</i> ≤ 100 CFU 20 – 25°C ≤ 5 days

sample size to judge compliance with the specification. The suitability of the chosen method must be established.

### 3. Growth Promotion Test, Suitability of the Counting Method and Negative Controls

The ability of the test to detect micro-organisms in the presence of product to be tested must be established.

Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

#### 3.1. Preparation of test strains

Use standardised stable suspensions of test strains or prepare as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in Table 4.05-I-1.

Use buffered sodium chloride-peptone solution pH 7.0 or phosphate buffer solution pH 7.2 to make test suspensions;

to suspend *Aspergillus brasiliensis* spores, 0.05 per cent of polysorbate 80 may be added to the buffer. Use the suspensions within 2 hours or within 24 hours if stored at 2 – 8°C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Aspergillus brasiliensis* or *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 – 8°C for a validated period of time.

#### 3.2. Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described under 4. Testing of Products. A failed negative control requires an investigation.

#### 3.3. Growth promotion of the media

Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described.

**Table 4.05-I-2** Common neutralizing agents for interfering substances

Interfering substance	Potential neutralizing method
Glutaraldehyde, Mercurials	Sodium hydrogen sulfite (Sodium bisulfite)
Phenolics, Alcohol, Aldehydes, Sorbate	Dilution
Aldehydes	Glycine
Quaternary Ammonium Compounds (QACs), Parahydroxybenzoates (Parabens), Bis-biguanides	Lecithin
QAC, Iodine, Parabens	Polysorbate
Mercurials	Thioglycollate
Mercurials, Halogens, Aldehydes	Thiosulfate
EDTA (edetate)	Mg or Ca ions

Inoculate portions/plates of *casein soya bean digest broth* and *casein soya bean digest agar* with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 4.05-I-1, using a separate portion/plate of medium for each. Inoculate plates of *Sabouraud-dextrose agar* with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 4.05-I-1, using a separate plate of medium for each. Incubate in the conditions described in Table 4.05-I-1.

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs. Liquid media are suitable if clearly visible growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

### 3.4. Suitability of the counting method in the presence of product

#### 3.4.1. Preparation of the sample

The method for sample preparation depends on the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, an alternative procedure must be developed.

(i) Water-soluble products: Dissolve or dilute (usually a 1 in 10 dilution is prepared) the product to be examined in *buffered sodium chloride-peptone solution pH 7.0*, *phosphate buffer solution pH 7.2* or *casein soya bean digest broth*. If necessary adjust to pH 6–8. Further dilutions, where necessary, are prepared with the same diluent.

(ii) Non-fatty products insoluble in water: Suspend the product to be examined (usually a 1 in 10 dilution is prepared) in *buffered sodium chloride-peptone solution pH 7.0*, *phosphate buffer solution pH 7.2* or *casein soya bean digest broth*. A surface-active agent such as 1 g/L of polysorbate 80 may be added to assist the suspension of poorly wettable substances. If necessary adjust to pH 6–8. Further dilutions, where necessary, are prepared with the same diluent.

(iii) Fatty products: Dissolve in isopropyl myristate, sterilised by filtration or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent, heated if necessary to not more than 40°C, or in exceptional cases to not more than 45°C. Mix carefully and if necessary maintain the temperature in a water-bath. Add sufficient of the pre-warmed chosen diluent to make a 1 in 10 dilution of

the original product. Mix carefully whilst maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial tenfold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent.

(iv) Fluids or solids in aerosol form: Aseptically transfer the product into a membrane filter apparatus or a sterile container for further sampling. Use either the total contents or a defined number of metered doses from each of the containers tested.

(v) Transdermal patches: Remove the protective cover sheets (“release liner”) of the transdermal patches and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive surface with sterile porous material, for example sterile gauze, to prevent the patches from sticking together, and transfer the patches to a suitable volume of the chosen diluent containing inactivators such as polysorbate 80 and/or lecithin. Shake the preparation vigorously for at least 30 minutes.

#### 3.4.2. Inoculation and dilution

Add to the sample prepared as described above (3.4.1.) and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the suspension of the inoculum should not exceed 1 per cent of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed. If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralization, dilution or filtration.

#### 3.4.3. Neutralization/removal of antimicrobial activity

The number of micro-organisms recovered from the prepared sample diluted as described in 3.4.2. and incubated following the procedure described in 3.4.4., is compared to the number of micro-organisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example, (1) an increase in the volume of the diluent or culture medium, (2) incorporation of a specific or general neutralizing agents into the diluent,

(3) membrane filtration or (4) a combination of the above measures.

**Neutralizing agents.** Neutralizing agents may be used to neutralize the activity of antimicrobial agents (Table 4.05-I-2). They may be added to the chosen diluent or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for micro-organisms must be demonstrated by carrying out a blank with neutralizer and without product.

If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the article is not likely to be contaminated with the given species of the micro-organism. However, it is possible that the product only inhibits some of the micro-organisms specified herein, but does not inhibit others not included amongst the test strains or for which the latter are not representative. Then, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion.

#### 3.4.4. Recovery of micro-organism in the presence of product

For each of the micro-organisms listed in Table 4.05-I-1, separate tests are performed. Only micro-organisms of the added test strain are counted.

##### 3.4.4.1. Membrane filtration

Use membrane filters having a nominal pore size not greater than  $0.45\text{ }\mu\text{m}$ . The type of filter material is chosen in such a way that the bacteria-retaining efficiency is not affected by the components of the sample to be investigated. For each of the micro-organisms listed in Table 4.05-I-1, one membrane filter is used.

Transfer a suitable amount of the sample prepared as described under 3.4.1. to 3.4.3. (preferably representing 1 g of the product, or less if large numbers of CFU are expected) to the membrane filter, filter immediately and rinse the membrane filter with an appropriate volume of diluent.

For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of *casein soya bean digest agar*. For the determination of total combined yeasts/moulds count (TYMC) transfer the membrane to the surface of *Sabouraud-dextrose agar*. Incubate the plates as indicated in Table 4.05-I-1. Perform the counting.

##### 3.4.4.2. Plate-count methods

Perform plate-count methods at least in duplicate for each medium and use the mean count of the result.

(i) Pour-plate method: For Petri dishes 9 cm in diameter, add to the dish 1 mL of the sample prepared as described under 3.4.1. to 3.4.3. and 15 – 20 mL of *casein soya bean digest agar* or *Sabouraud-dextrose agar*, both media being at not more than  $45^\circ\text{C}$ . If larger Petri dishes are used, the amount of agar medium is increased accordingly. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used.

Incubate the plates as indicated in Table 4.05-I-1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

(ii) Surface-spread method: For Petri dishes 9 cm in diameter, add 15 – 20 mL of *casein soya bean digest agar* or *Sabouraud-dextrose agar* at about  $45^\circ\text{C}$  to each Petri dish and allow to solidify. If larger Petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example in a laminar-air-flow cabinet or in an incubator. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used. Spread a measured volume of not less than 0.1 mL of the sample prepared as described

under 3.4.1. to 3.4.3. over the surface of the medium. Incubate and count as prescribed under 3.4.4.2. (i).

##### 3.4.4.3. Most-probable-number (MPN) method

The precision and accuracy of the MPN method is less than that of the membrane filtration method or the plate-count method. Unreliable results are obtained particularly for the enumeration of moulds. For these reasons the MPN method is reserved for the enumeration of TAMC in situations where no other method is available. If the use of the method is justified, proceed as follows.

Prepare a series of at least 3 serial tenfold dilutions of the product as described under 3.4.1. to 3.4.3.. From each level of dilution, 3 aliquots of 1 g or 1 mL are used to inoculate 3 tubes with 9 – 10 mL of *casein soya bean digest broth*. If necessary a surface-active agent such as polysorbate 80, or an inactivator of antimicrobial agents may be added to the medium. Thus, if 3 levels of dilution are prepared 9 tubes are inoculated.

Incubate all tubes at  $30 - 35^\circ\text{C}$  for not more than 3 days. If reading of the results is difficult or uncertain owing to the nature of the product to be examined, subculture in the same broth, or *casein soya bean digest agar*, for 1 – 2 days at the same temperature and use these results. Determine the most probable number of micro-organisms per gram or millilitre of the product to be examined from Table 4.05-I-3.

#### 3.5. Results and interpretation

When verifying the suitability of the membrane filtration method or the plate-count method, a mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control defined in 3.4.2. in the absence of the product must be obtained. When verifying the suitability of the MPN method the calculated value from the inoculum must be within 95 per cent confidence limits of the results obtained with the control.

If the above criteria cannot be met for one or more of the organisms tested with any of the described methods, the method and test conditions that come closest to the criteria are used to test the product.

#### 4. Testing of Products

##### 4.1. Amount used for the test

Unless otherwise prescribed, use 10 g or 10 mL of the product to be examined taken with the precautions referred to above. For fluids or solids in aerosol form, sample 10 containers. For transdermal patches, sample 10 patches.

The amount to be tested may be reduced for active substances that will be formulated in the following conditions: the amount per dosage unit (e.g. tablet, capsule, injection) is less than or equal to 1 mg or the amount per gram or millilitre (for preparations not presented in dose units) is less than 1 mg. In these cases, the amount of sample to be tested is not less than the amount present in 10 dosage units or 10 g or 10 mL of the product.

For materials used as active substances where sample quantity is limited or batch size is extremely small (i.e. less than 1000 mL or 1000 g), the amount tested shall be 1 per cent of the batch unless a lesser amount is prescribed or justified and authorised.

For products where the total number of entities in a batch is less than 200 (e.g. samples used in clinical trials), the sample size may be reduced to 2 units, or 1 unit if the size is less than 100.

Select the sample(s) at random from the bulk material or from the available containers of the preparation. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

Table 4.05-I-3 Most-probable-number values of micro-organisms

Observed combinations of numbers of tubes showing growth in each set			MPN per g or per mL of product	95 per cent confidence limits		
Number of g or mL of product per tube						
0.1	0.01	0.001				
0	0	0	Less than 3	0 - 9.4		
0	0	1	3	0.1 - 9.5		
0	1	0	3	0.1 - 10		
0	1	1	6.1	1.2 - 17		
0	2	0	6.2	1.2 - 17		
0	3	0	9.4	3.5 - 35		
1	0	0	3.6	0.2 - 17		
1	0	1	7.2	1.2 - 17		
1	0	2	11	4 - 35		
1	1	0	7.4	1.3 - 20		
1	1	1	11	4 - 35		
1	2	0	11	4 - 35		
1	2	1	15	5 - 38		
1	3	0	16	5 - 38		
2	0	0	9.2	1.5 - 35		
2	0	1	14	4 - 35		
2	0	2	20	5 - 38		
2	1	0	15	4 - 38		
2	1	1	20	5 - 38		
2	1	2	27	9 - 94		
2	2	0	21	5 - 40		
2	2	1	28	9 - 94		
2	2	2	35	9 - 94		
2	3	0	29	9 - 94		
2	3	1	36	9 - 94		
3	0	0	23	5 - 94		
3	0	1	38	9 - 104		
3	0	2	64	16 - 181		
3	1	0	43	9 - 181		
3	1	1	75	17 - 199		
3	1	2	120	30 - 360		
3	1	3	160	30 - 380		
3	2	0	93	18 - 360		
3	2	1	150	30 - 380		
3	2	2	210	30 - 400		
3	2	3	290	90 - 990		
3	3	0	240	40 - 990		
3	3	1	460	90 - 1980		
3	3	2	1100	200 - 4000		
3	3	3	More than 1100			

## 4.2. Examination of the product

### 4.2.1. Membrane filtration

Use a filtration apparatus designed to allow the transfer of the filter to the medium. Prepare the sample using a method that has been shown suitable as described in section 3 and transfer the appropriate amount to each of 2 membrane filters and filter immediately. Wash each filter following the

procedure shown to be suitable.

For the determination of TAMC, transfer one of the membrane filters to the surface of *casein soya bean digest agar*. For the determination of TYMC, transfer the other membrane to the surface of *Sabouraud-dextrose agar*. Incubate the plate of *casein soya bean digest agar* at 30 - 35°C for 3 - 5 days and the plate of *Sabouraud-dextrose agar* at

20 – 25°C for 5 – 7 days. Calculate the number of CFU per gram or per millilitre of product.

When examining transdermal patches, filter 10 per cent of the volume of the preparation described under 3.4.1. separately through each of 2 sterile filter membranes. Transfer one membrane to *casein soya bean digest agar* for TAMC and the other membrane to *Sabouraud-dextrose agar* for TYMC.

#### 4.2.2. Plate-count methods

(i) Pour-plate method: Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare for each medium at least 2 Petri dishes for each level of dilution. Incubate the plates of *casein soya bean digest agar* at 30 – 35°C for 3 – 5 days and the plates of *Sabouraud-dextrose agar* at 20 – 25°C for 5 – 7 days. Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of the counts and calculate the number of CFU per gram or per millilitre of product.

(ii) Surface-spread method: Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare at least 2 Petri dishes for each medium and each level of dilution. For incubation and calculation of the number of CFU proceed as described for the pour-plate method.

#### 4.2.3. Most-probable-number method

Prepare and dilute the sample using a method that has been shown to be suitable as described in section 3. Incubate all tubes for 3 – 5 days at 30 – 35°C. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of micro-organisms per gram or millilitre of the product to be examined from Table 4.05-I-3.

#### 4.3. Interpretation of the results

The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found using *casein soya bean digest agar*; if colonies of fungi are detected on this medium, they are counted as part of TAMC. The total combined yeasts/mould count (TYMC) is considered to be equal to the number of CFU found using *Sabouraud-dextrose agar*; if colonies of bacteria are detected on this medium, they are counted as part of TYMC. When the TYMC is expected to exceed the acceptance criterion due to the bacterial growth, *Sabouraud-dextrose agar* containing antibiotics may be used. If the count is carried out by the MPN method the calculated value is the TAMC.

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

- 10<sup>1</sup> CFU: maximum acceptable count = 20,
- 10<sup>2</sup> CFU: maximum acceptable count = 200,
- 10<sup>3</sup> CFU: maximum acceptable count = 2000,
- and so forth.

The recommended solutions and media are described in II. *Tests for specified micro-organisms*.

## II. Microbiological Examination of Non-sterile Products: Tests for Specified Micro-organisms

These tests are harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopoeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

The tests described hereafter will allow determination of

the absence or limited occurrence of specified micro-organisms which may be detected under the conditions described.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

Alternative microbiological procedures, including automated methods may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

#### 1. General Procedures

The preparation of samples is carried out as described in I. *Microbial enumeration tests*.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized as described in I. *Microbial enumeration tests*.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated as described in I. *Microbial enumeration tests*.

#### 2. Growth Promoting and Inhibitory Properties of the Media, Suitability of the Test and Negative Controls

The ability of the test to detect micro-organisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

##### 2.1. Preparation of test strains

Use standardized stable suspensions of test strains or prepare as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot.

##### 2.1.1. Aerobic micro-organisms

Grow each of the bacterial test strains separately in containers containing *casein soya bean digest broth* or on *casein soya bean digest agar* at 30 – 35°C for 18 – 24 hours. Grow the test strain for *Candida albicans* separately on *Sabouraud-dextrose agar* or in *Sabouraud-dextrose broth* at 20 – 25°C for 2–3 days.

*Staphylococcus aureus* such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276,

*Pseudomonas aeruginosa* such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275,

*Escherichia coli* such as ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972,

*Salmonella enterica* subsp. *enterica* serovar Typhimurium such as ATCC 14028

or, as an alternative,

*Salmonella enterica* subsp. *enterica* serovar Abony such as NBRC 100797, NCTC 6017 or CIP 80.39,

*Candida albicans* such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594.

Use buffered sodium chloride-peptone solution pH 7.0 or phosphate buffer solution pH 7.2 to make test suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2 – 8°C.

##### 2.1.2. Clostridia

Use *Clostridium sporogenes* such as ATCC 11437 (NBRC 14293, NCIMB 12343, CIP 100651) or ATCC 19404 (NCTC 532 or CIP 79.3). Grow the clostridial test strain under anaerobic conditions in reinforced medium for *Clostridia* at 30 – 35°C for 24 – 48 hours. As an alternative to preparing and then diluting down a fresh suspension of vegetative cells

of *Cl. sporogenes*, a stable spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 – 8°C for a validated period.

## 2.2. Negative control

To verify testing conditions a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described under 3. A failed negative control required an investigation.

## 2.3. Growth promotion and inhibitory properties of the media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients.

Verify suitable properties of relevant media as described in Table 4.05-II-1.

(i) Test for growth promoting properties, liquid media: inoculate a portion of the appropriate medium with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(ii) Test for growth promoting properties, solid media: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(iii) Test for inhibitory properties, liquid or solid media: inoculate the appropriate medium with at least 100 CFU of the appropriate micro-organism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test micro-organism occurs.

(iv) Test for indicative properties: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

## 2.4. Suitability of the test method

For each product to be tested perform sample preparation as described in the relevant paragraph in section 3. Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use a number of micro-organisms equivalent to not more than 100 CFU in the inoculated test preparation.

Perform the test as described in the relevant paragraph in section 3 using the shortest incubation period prescribed.

The specified micro-organisms must be detected with the indication reactions as described in section 3.

Any antimicrobial activity of the product necessitates a modification of the test procedure (see 3.4.3. of I. *Microbial enumeration tests*).

If for a given product the antimicrobial activity with respect to a micro-organism for which testing is prescribed cannot be neutralized, then it is to be assumed that the inhibited micro-organism will not be present in the product.

## 3. Testing of Products

### 3.1. Bile-tolerant gram-negative bacteria

#### 3.1.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. *Microbial enumeration tests*, but using *casein soya bean digest broth* as the chosen diluent, mix and incubate at 20 – 25°C for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours but not more than 5 hours).

#### 3.1.2. Test for absence

Unless otherwise prescribed use the volume corresponding to 1 g of the product, as prepared in 3.1.1. to inoculate *enterobacteria enrichment broth-Mossel*. Incubate at 30 – 35°C for 24 – 48 hours. Subculture on plates of *violet red bile glucose agar*. Incubate at 30 – 35°C for 18 – 24 hours.

The product complies with the test if there is no growth of colonies.

#### 3.1.3. Quantitative test

##### 3.1.3.1. Selection and subculture

Inoculate suitable quantities of *enterobacteria enrichment broth-Mossel* with the preparation as described under 3.1.1. and/or dilutions of it containing respectively 0.1 g, 0.01 g and 0.001 g (or 0.1 mL, 0.01 mL and 0.001 mL) of the product to be examined. Incubate at 30 – 35°C for 24 – 48 hours. Subculture each of the cultures on a plate of *violet red bile glucose agar*. Incubate at 30 – 35°C for 18 – 24 hours.

##### 3.1.3.2. Interpretation

Growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine from Table 4.05-II-2 the probable number of bacteria.

## 3.2. *Escherichia coli*

### 3.2.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. *Microbial enumeration tests* and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of *casein soya bean digest broth*, mix and incubate at 30 – 35°C for 18 – 24 hours.

#### 3.2.2. Selection and subculture

Shake the container, transfer 1 mL of *casein soya bean digest broth* to 100 mL of *MacConkey broth* and incubate at 42 – 44°C for 24 – 48 hours. Subculture on a plate of *MacConkey agar* at 30 – 35°C for 18 – 72 hours.

#### 3.2.3. Interpretation

Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification tests.

The product complies with the test if no colonies are present or if the identification tests are negative.

## 3.3. *Salmonella*

### 3.3.1. Sample preparation and pre-incubation

Prepare the product to be examined as described in I. *Microbial enumeration tests* and use the quantity corresponding to not less than 10 g or 10 mL to inoculate a suitable amount (determined as described under 2.4.) of *casein soya bean digest broth*, mix and incubate at 30 – 35°C for 18 – 24 hours.

#### 3.3.2. Selection and subculture

Transfer 0.1 mL of *casein soya bean digest broth* to 10 mL of *Rappaport Vassiliadis Salmonella enrichment broth* and incubate at 30 – 35°C for 18 – 24 hours. Subculture on plates of *xylose, lysine, deoxycholate agar*. Incubate at 30 – 35°C for 18 – 48 hours.

**Table 4.05-II-1** Growth promoting, inhibitory and indicative properties of media

Medium	Property	Test strains
<b>Test for bile-tolerant gram-negative bacteria</b>		
<i>Enterobacteria enrichment broth-Mossel</i>	Growth promoting	<i>E. coli</i> <i>P. aeruginosa</i>
	Inhibitory	<i>S. aureus</i>
<i>Violet red bile glucose agar</i>	Growth promoting + Indicative	<i>E. coli</i> <i>P. aeruginosa</i>
<b>Test for <i>Escherichia coli</i></b>		
<i>MacConkey broth</i>	Growth promoting	<i>E. coli</i>
	Inhibitory	<i>S. aureus</i>
<i>MacConkey agar</i>	Growth promoting + Indicative	<i>E. coli</i>
<b>Test for <i>Salmonella</i></b>		
<i>Rappaport Vassiliadis Salmonella enrichment broth</i>	Growth promoting	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium or <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony
	Inhibitory	<i>S. aureus</i>
<i>Xylose, lysine, deoxycholate agar</i>	Growth promoting + Indicative	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium or <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony
<b>Test for <i>Pseudomonas aeruginosa</i></b>		
<i>Cetrimide agar</i>	Growth promoting	<i>P. aeruginosa</i>
	Inhibitory	<i>E. coli</i>
<b>Test for <i>Staphylococcus aureus</i></b>		
<i>Mannitol salt agar</i>	Growth promoting + Indicative	<i>S. aureus</i>
	Inhibitory	<i>E. coli</i>
<b>Test for Clostridia</b>		
<i>Reinforced medium for Clostridia</i>	Growth promoting	<i>Cl. sporogenes</i>
<i>Columbia agar</i>	Growth promoting	<i>Cl. sporogenes</i>
<b>Test for <i>Candida albicans</i></b>		
<i>Sabouraud-dextrose broth</i>	Growth promoting	<i>C. albicans</i>
<i>Sabouraud-dextrose agar</i>	Growth promoting + Indicative	<i>C. albicans</i>

**3.3.3. Interpretation**

The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centres. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

**3.4. *Pseudomonas aeruginosa*****3.4.1. Sample preparation and pre-incubation**

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. *Micro-*

*bial enumeration tests* and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of *casein soya bean digest broth* and mix. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in I. *Microbial enumeration tests* (3.4.1.) through a sterile filter membrane and place in 100 mL of *casein soya bean digest broth*. Incubate at 30–35°C for 18–24 hours.

**3.4.2. Selection and subculture**

Subculture on a plate of *cetrimide agar* and incubate at

Table 4.05-II-2 Interpretation of results

Results for each quantity of product			Probable number of bacteria per gram or mL of product
0.1 g or 0.1 mL	0.01 g or 0.01 mL	0.001 g or 0.001 mL	
+	+	+	more than $10^3$
+	+	-	less than $10^3$ and more than $10^2$
+	-	-	less than $10^2$ and more than 10
-	-	-	less than 10

30 – 35°C for 18 – 72 hours.

### 3.4.3. Interpretation

Growth of colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification tests.

The product complies with the test if colonies are not present or if the confirmatory identification tests are negative.

### 3.5. *Staphylococcus aureus*

#### 3.5.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. *Microbial enumeration tests* and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of *casein soya bean digest broth* and homogenise. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in I. *Microbial enumeration tests* (3.4.1.) through a sterile filter membrane and place in 100 mL of *casein soya bean digest broth*. Incubate at 30 – 35°C for 18 – 24 hours.

#### 3.5.2. Selection and subculture

Subculture on a plate of *mannitol salt agar* and incubate at 30 – 35°C for 18 – 72 hours.

#### 3.5.3. Interpretation

The possible presence of *S. aureus* is indicated by the growth of yellow/white colonies surrounded by a yellow zone. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

### 3.6. *Clostridia*

#### 3.6.1. Sample preparation and heat treatment

Prepare a sample using a 1 in 10 dilution (with a minimum total volume of 20 mL) of not less than 2 g or 2 mL of the product to be examined as described in I. *Microbial enumeration tests*. Divide the sample into two portions of at least 10 mL. Heat 1 portion at 80°C for 10 minutes and cool rapidly. Do not heat the other portion.

#### 3.6.2. Selection and subculture

Use 10 mL or the quantity corresponding to 1 g or 1 mL of the product to be examined of both portions to inoculate suitable amounts (determined as described under 2.4.) of *reinforced medium for Clostridia*. Incubate under anaerobic conditions at 30 – 35°C for 48 hours. After incubation, make subcultures from each tube on *Columbia agar* and incubate under anaerobic conditions at 30 – 35°C for 48 – 72 hours.

#### 3.6.3. Interpretation

The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of Clostridia. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identifica-

tion tests are negative.

### 3.7. *Candida albicans*

#### 3.7.1. Sample preparation and pre-incubation

Prepare the product to be examined as described in I. *Microbial enumeration tests* and use 10 mL or the quantity corresponding to not less than 1 g or 1 mL to inoculate 100 mL of *Sabouraud-dextrose broth* and mix. Incubate at 30 – 35°C for 3-5 days.

#### 3.7.2. Selection and subculture

Subculture on a plate of *Sabouraud-dextrose agar* and incubate at 30 – 35°C for 24 – 48 hours.

#### 3.7.3. Interpretation

Growth of white colonies may indicate the presence of *C. albicans*. This is confirmed by identification tests.

The product complies with the test if such colonies are not present or if the confirmatory identification tests are negative.

*The following section is given for information.*

## 4. Recommended Solutions and Culture Media

The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the test for microbial contamination in the Pharmacopoeia. Other media may be used provided that their suitability can be demonstrated.

#### (i) Phosphate buffer solution pH 7.2

Prepare a mixture of water and stock buffer solution (800:1 V/V) and sterilize.

*Stock buffer solution.* Transfer 34 g of potassium dihydrogen phosphate to a 1000 mL volumetric flask, dissolve in 500 mL of purified water, adjust to pH 7.2 to  $\pm 0.2$  with sodium hydroxide, add purified water to volume and mix. Dispense in containers and sterilize. Store at a temperature of 2 – 8°C.

#### (ii) Buffered sodium chloride-peptone solution pH 7.0

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate	7.2 g
(equivalent to 0.067 mol phosphate)	

Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Water	1000 mL

Sterilize in an autoclave using a validated cycle.

#### (iii) Casein soya bean digest broth

Pancreatic digest of casein	17.0 g
Papaic digest of soya bean	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate	2.5 g
Purified water	1000 mL

Adjust the pH so that after sterilization it is  $7.3 \pm 0.2$  at 25°C. Sterilize in an autoclave using a validated cycle.

(iv) <i>Casein soya bean digest agar</i>	Pancreatic digest of casein	15.0 g	(xi) <i>MacConkey agar</i>	Pancreatic digest of gelatin	17.0 g
Papaic digest of soya bean	5.0 g		Peptones (meat and casein)	3.0 g	
Sodium chloride	5.0 g		Lactose monohydrate	10.0 g	
Agar	15.0 g		Sodium chloride	5.0 g	
Purified water	1000 mL		Bile salts	1.5 g	
Adjust the pH so that after sterilization it is $7.3 \pm 0.2$ at $25^{\circ}\text{C}$ . Sterilize in an autoclave using a validated cycle.			Agar	13.5 g	
(v) <i>Sabouraud-dextrose agar</i>	Dextrose	40.0 g	Neutral red	30 mg	
Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1)	10.0 g		Crystal violet	1 mg	
Agar	15.0 g		Purified water	1000 mL	
Purified water	1000 mL		Adjust the pH so that after sterilization it is $7.1 \pm 0.2$ at $25^{\circ}\text{C}$ . Boil for 1 minute with constant shaking then sterilize in an autoclave using a validated cycle.		
Adjust the pH so that after sterilization it is $5.6 \pm 0.2$ at $25^{\circ}\text{C}$ . Sterilize in an autoclave using a validated cycle.					
(vi) <i>Potato dextrose agar</i>	Infusion from potatoes	200 g	(xii) <i>Rappaport Vassiliadis Salmonella Enrichment broth</i>	Soya peptone	4.5 g
Dextrose	20.0 g		Magnesium chloride hexahydrate	29.0 g	
Agar	15.0 g		Sodium chloride	8.0 g	
Purified water	1000 mL		Dipotassium hydrogen phosphate	0.4 g	
Adjust the pH so that after sterilization it is $5.6 \pm 0.2$ at $25^{\circ}\text{C}$ . Sterilize in an autoclave using a validated cycle.			Potassium dihydrogen phosphate	0.6 g	
(vii) <i>Sabouraud-dextrose broth</i>	Dextrose	20.0 g	Malachite green	36 mg	
Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1)	10.0 g		Purified water	1000 mL	
Purified water	1000 mL		Dissolve, warming slightly. Sterilize in an autoclave using a validated cycle, at a temperature not exceeding $115^{\circ}\text{C}$ . The pH is to be $5.2 \pm 0.2$ at $25^{\circ}\text{C}$ after heating and autoclaving.		
Adjust the pH so that after sterilization it is $5.6 \pm 0.2$ at $25^{\circ}\text{C}$ . Sterilize in an autoclave using a validated cycle.					
(viii) <i>Enterobacteria enrichment broth-Mossel</i>	Pancreatic digest of gelatin	10.0 g	(xiii) <i>Xylose, lysine, deoxycholate agar</i>	Xylose	3.5 g
Glucose monohydrate	5.0 g		L-Lysine	5.0 g	
Dehydrated ox bile	20.0 g		Lactose monohydrate	7.5 g	
Potassium dihydrogen phosphate	2.0 g		Sucrose	7.5 g	
Disodium hydrogen phosphate dihydrate	8.0 g		Sodium chloride	5.0 g	
Brilliant green	15 mg		Yeast extract	3.0 g	
Purified water	1000 mL		Phenol red	80 mg	
Adjust the pH so that after heating it is $7.2 \pm 0.2$ at $25^{\circ}\text{C}$ . Heat at $100^{\circ}\text{C}$ for 30 minutes and cool immediately.			Agar	13.5 g	
(ix) <i>Violet red bile glucose agar</i>	Yeast extract	3.0 g	Sodium desoxycholate	2.5 g	
Pancreatic digest of gelatin	7.0 g		Sodium thiosulfate	6.8 g	
Bile salts	1.5 g		Ammonium iron (III) citrate	0.8 g	
Sodium chloride	5.0 g		Purified water	1000 mL	
Glucose monohydrate	10.0 g		Adjust the pH so that after heating it is $7.4 \pm 0.2$ at $25^{\circ}\text{C}$ . Heat to boiling, cool to $50^{\circ}\text{C}$ and pour into Petri dishes. Do not heat in an autoclave.		
Agar	15.0 g				
Neutral red	30 mg				
Crystal violet	2 mg				
Purified water	1000 mL				
Adjust the pH so that after heating it is $7.4 \pm 0.2$ at $25^{\circ}\text{C}$ . Heat to boiling; do not heat in an autoclave.					
(x) <i>MacConkey broth</i>	Pancreatic digest of gelatin	20.0 g	(xiv) <i>Cetrimide agar</i>	Pancreatic digest of gelatin	20.0 g
Lactose monohydrate	10.0 g		Magnesium chloride	1.4 g	
Dehydrated ox bile	5.0 g		Dipotassium sulfate	10.0 g	
Bromocresol purple	10 mg		Cetrimide	0.3 g	
Purified water	1000 mL		Agar	13.6 g	
Adjust the pH so that after sterilization it is $7.3 \pm 0.2$ at $25^{\circ}\text{C}$ . Sterilize in an autoclave using a validated cycle.			Purified water	1000 mL	
			Glycerol	10.0 mL	
			Heat to boiling for 1 minute with shaking. Adjust the pH so that after sterilization it is $7.2 \pm 0.2$ at $25^{\circ}\text{C}$ . Sterilize in an autoclave using a validated cycle.		
(xv) <i>Mannitol salt agar</i>	Pancreatic digest of casein	5.0 g			
Peptic digest of animal tissue	5.0 g				
Beef extract	1.0 g				
D-Mannitol	10.0 g				
Sodium chloride	75.0 g				
Agar	15.0 g				
Phenol red	25 mg				
Purified water	1000 mL				
Heat to boiling for 1 minute with shaking. Adjust the pH so that after sterilization it is $7.4 \pm 0.2$ at $25^{\circ}\text{C}$ . Sterilize in an autoclave using a validated cycle.					

(xvi) *Reinforced medium for Clostridia*

Beef extract	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Soluble starch	1.0 g
Glucose monohydrate	5.0 g
Cysteine hydrochloride	0.5 g
Sodium chloride	5.0 g
Sodium acetate	3.0 g
Agar	0.5 g
Purified water	1000 mL

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilization it is about  $6.8 \pm 0.2$  at  $25^\circ\text{C}$ . Sterilize in an autoclave using a validated cycle.

(xvii) *Columbia agar*

Pancreatic digest of casein	10.0 g
Meat peptic digest	5.0 g
Heart pancreatic digest	3.0 g
Yeast extract	5.0 g
Maize starch	1.0 g
Sodium chloride	5.0 g
Agar, according to gelling power	10.0 g to 15.0 g
Purified water	1000 mL

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilization it is  $7.3 \pm 0.2$  at  $25^\circ\text{C}$ . Sterilize in an autoclave using a validated cycle. Allow to cool to  $45 - 50^\circ\text{C}$ ; add, where necessary, gentamicin sulfate corresponding to 20 mg of gentamicin base and pour into Petri dishes.

## 4.06 Sterility Test

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols ( $\spadesuit$   $\clubsuit$ ), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols ( $\diamondsuit$   $\diamond$ ).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

The test is applied to substances, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating micro-organism has been found in the sample examined in the conditions of the test.

### 1. Precautions against microbial contamination

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any micro-organisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

### 2. Culture media and incubation temperatures

Media for the test may be prepared as described below, or equivalent commercial media may be used provided that they

comply with the growth promotion test.

The following culture media have been found to be suitable for the test for sterility. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soya-bean casein digest medium is suitable for the culture of both fungi and aerobic bacteria.

(i) *Fluid thioglycollate medium*

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose monohydrate/anhydrous	5.5/5.0 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or Thioglycolic acid	0.5 g
Resazurin sodium solution (1 in 1000), freshly prepared	1.0 mL
Water	1000 mL

(pH after sterilization  $7.1 \pm 0.2$ )

Mix the L-cystine, agar, sodium chloride, glucose, water-soluble yeast extract and pancreatic digest of casein with water, and heat until solution is effected. Dissolve the sodium thioglycollate or thioglycolic acid in the solution and, if necessary, add sodium hydroxide TS so that, after sterilization, the solution will have a pH of  $7.1 \pm 0.2$ . If filtration is necessary, heat the solution again without boiling and filter while hot through moistened filter paper. Add the resazurin sodium solution (1 in 1000), mix and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a color change indicative of oxygen uptake at the end of the incubation period. Sterilize using a validated process. If the medium is stored, store at a temperature between  $2^\circ\text{C}$  and  $25^\circ\text{C}$  in a sterile, tight container. If more than the upper one-third of the medium has acquired a pink color, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink color disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid thioglycollate medium is to be incubated at  $30 - 35^\circ\text{C}$ . For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at  $20 - 25^\circ\text{C}$  may be used instead of soya-bean casein digest medium provided that it has been validated as described in growth promotion test.

Where prescribed or justified and authorized, the following alternative thioglycollate medium might be used. Prepare a mixture having the same composition as that of the fluid thioglycollate medium, but omitting the agar and the resazurin sodium solution (1 in 1000), sterilize as directed above. The pH after sterilization is  $7.1 \pm 0.2$ . Heat in a water bath prior to use and incubate at  $30 - 35^\circ\text{C}$  under anaerobic conditions.

(ii) *Soya-bean casein digest medium*

Pancreatic digest of casein	17.0 g
Papaic digest of soya-bean meal	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate/anhydrous	2.5/2.3 g
Water	1000 mL

(pH after sterilization  $7.3 \pm 0.2$ )

Dissolve the solids in water, warming slightly to effect solution. Cool the solution to room temperature. Add sodium

**Table 4.06-1.** Strains of the test micro-organisms suitable for use in the Growth Promotion Test and the Method suitability Test

Aerobic bacteria <i>Staphylococcus aureus</i>	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276
<i>Bacillus subtilis</i>	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134
<i>Pseudomonas aeruginosa</i>	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275
Anaerobic bacterium <i>Clostridium sporogenes</i>	ATCC 19404, CIP 79.3, NCTC 532 or ATCC 11437, NBRC 14293
Fungi <i>Candida albicans</i>	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594
<i>Aspergillus brasiliensis</i>	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455

hydroxide TS, if necessary, so that after sterilization the solution will have a pH of  $7.3 \pm 0.2$ . Filter, if necessary, to clarify, distribute into suitable vessels and sterilize using a validated process. Store at a temperature between 2°C and 25°C in a sterile tight container, unless it is intended for immediate use. Do not use the medium for a longer storage period than has been validated.

Soya-bean casein digest medium is to be incubated at 20 – 25°C.

### 3. Suitability of the culture medium

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined.

#### 3.1. Sterility

Incubate portions of the media for 14 days. No growth of micro-organisms occurs.

#### 3.2. Growth promotion test of aerobes, anaerobes and fungi

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Suitable strains of micro-organisms are indicated in Table 4.06-1.

Inoculate portions of fluid thioglycollate medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of micro-organism:

*Clostridium sporogenes*,  
*Pseudomonas aeruginosa*,  
*Staphylococcus aureus*.

Inoculate portions of soya-bean casein digest medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of micro-organism:

*Aspergillus brasiliensis*,  
*Bacillus subtilis*,  
*Candida albicans*.

Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi.

Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than five passages removed from the original master seed-lot.

The media are suitable if a clearly visible growth of the micro-organisms occurs.

### 4. Method suitability test

Carry out a test as described below under 5. Test for sterility of the product to be examined using exactly the same methods except for the following modifications.

(i) Membrane filtration: After transferring the content of the container or containers to be tested to the membrane add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the final portion of sterile diluent used to rinse the filter.

(ii) Direct inoculation: After transferring the contents of the container or containers to be tested to the culture medium add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the medium.

In both cases use the same micro-organisms as those described above under 3.2. Growth promotion test of aerobes, anaerobes and fungi. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days.

If clearly visible growth of micro-organisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification.

If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control vessels without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated under the conditions of the test. Modify the conditions in order to eliminate the antimicrobial activity and repeat the method suitability test.

This method suitability is performed:

- a) when the test for sterility has to be carried out on a new product;
- b) whenever there is a change in the experimental conditions of the test.

The method suitability may be performed simultaneously with the Test for sterility of the product to be examined.

### 5. Test for sterility of the product to be examined

The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily preparations and for preparations miscible with or soluble in aqueous or oily solvents provided these solvents do not have an antimicrobial effect in the conditions of the test.

#### 5.1. Membrane filtration

Use membrane filters having a nominal pore size not greater than  $0.45 \mu\text{m}$  whose effectiveness to retain micro-organisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters, for example, for strongly alcoholic solutions. Specially adapted filters may be needed for certain products, e.g. for antibiotics.

The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane are sterilized by appropriate means. The apparatus is designed so that the solution to be examined can be introduced and filtered under aseptic conditions; it permits the aseptic removal of the membrane for transfer to the

**Table 4.06-2.** Minimum quantity to be used for each medium

Quantity per container	Minimum quantity to be used for each medium unless otherwise justified and authorized
Liquids	
—Less than 1 mL:	The whole contents of each container
—1 – 40 mL:	Half the contents of each container but not less than 1 mL 20 mL
—Greater than 40 mL and not greater than 100 mL	10% of the contents of the container but not less than 20 mL
—Greater than 100 mL:	1 mL
Antibiotic liquids	1 mL
Insoluble preparations, creams and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200 mg
Solids	
—Less than 50 mg	The whole contents of each container
—50 mg or more but less than 300 mg	Half the contents of each container but not less than 50 mg 150 mg
—300 mg – 5 g	500 mg
—Greater than 5 g	

medium or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

(i) Aqueous solutions: If appropriate, transfer a small quantity of a suitable, sterile diluent such as a 1 g/L neutral solution of meat or casein peptone pH 7.1 ± 0.2 onto the membrane in the apparatus and filter. The diluent may contain suitable neutralizing substances and/or appropriate inactivating substances for example in the case of antibiotics.

Transfer the contents of the container or containers to be tested to the membrane or membranes, if necessary after diluting to the volume used in the method suitability test with the chosen sterile diluent but in any case using not less than the quantities of the product to be examined prescribed in Table 4.06-2. Filter immediately. If the product has antimicrobial properties, wash the membrane not less than three times by filtering through it each time the volume of the chosen sterile diluent used in the method suitability test. Do not exceed a washing cycle of 5 times 100 mL per filter, even if during method suitability it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity. Transfer the whole membrane to the culture medium or cut it aseptically into two equal parts and transfer one half to each of two suitable media. Use the same volume of each medium as in the method suitability test. Alternatively, transfer the medium onto the membrane in the apparatus. Incubate the media for not less than 14 days.

(ii) Soluble solids: Use for each medium not less than the quantity prescribed in Table 4.06-2 of the product dissolved in a suitable solvent such as the solvent provided with the preparation, water for injection, saline or a 1 g/L neutral solution of meat or casein peptone and proceed with the test as described above for aqueous solutions using a membrane appropriate to the chosen solvent.

(iii) Oils and oily solutions: Use for each medium not less than the quantity of the product prescribed in Table

4.06-2. Oils and oily solutions of sufficiently low viscosity may be filtered without dilution through a dry membrane. Viscous oils may be diluted as necessary with a suitable sterile diluent such as isopropyl myristate shown not to have antimicrobial activity in the conditions of the test. Allow the oil to penetrate the membrane by its own weight then filter, applying the pressure or suction gradually. Wash the membrane at least three times by filtering through it each time about 100 mL of a suitable sterile solution such as 1 g/L neutral meat or casein peptone containing a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g/L. Transfer the membrane or membranes to the culture medium or media or vice versa as described above for aqueous solutions, and incubate at the same temperatures and for the same times.

(iv) Ointments and creams: Use for each medium not less than the quantities of the product prescribed in Table 4.06-2. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1 per cent in isopropyl myristate as described above, by heating, if necessary, to not more than 40°C. In exceptional cases it may be necessary to heat to not more than 44°C. Filter as rapidly as possible and proceed as described above for oils and oily solutions.

## 5.2. Direct inoculation of the culture medium

Transfer the quantity of the preparation to be examined prescribed in Table 4.06-2 directly into the culture medium so that the volume of the product is not more than 10 per cent of the volume of the medium, unless otherwise prescribed. If the product to be examined has antimicrobial activity, carry out the test after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate the concentrated medium may be added directly to the product in its container.

(i) Oily liquids: Use media to which have been added a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g/L.

(ii) Ointments and creams: Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluent such as a 1 g/L neutral solution of meat or casein peptone. Transfer the diluted product to a medium not containing an emulsifying agent.

Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Shake cultures containing oily products gently each day. However when fluid thioglycollate medium is used for the detection of anaerobic micro-organisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

## 6. Observation and interpretation of results

At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1 mL) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days.

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evi-

**Table 4.06-3.** Minimum number of items to be tested

Number of items in the batch*	Minimum number of items to be tested for each medium, unless otherwise justified and authorized**
Parenteral preparations —Not more than 100 containers —More than 100 but not more than 500 containers —More than 500 containers	10% or 4 containers whichever is the greater 10 containers 2% or 20 containers ◇(10 containers for parenterals with a nominal volume of 100 mL or more)◇ whichever is the less
Ophthalmic and other non-injectable preparations —Not more than 200 containers —More than 200 containers —If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral use	5% or 2 containers whichever is the greater 10 containers
Bulk solid products —Up to 4 containers —More than 4 containers but not more than 50 containers —More than 50 containers	Each container 20% or 4 containers whichever is the greater 2% or 10 containers whichever is the greater

\* If the batch size is not known, use the maximum number of items prescribed.

\*\* If the contents of one container are enough to inoculate the two media, this column gives the number of containers needed for both the media together.

dence of microbial growth is found the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test may be considered invalid only if one or more of the following conditions are fulfilled:

- (i) the data of the microbiological monitoring of the sterility testing facility show a fault;
- (ii) a review of the testing procedure used during the test in question reveals a fault;
- (iii) microbial growth is found in the negative controls;
- (iv) after determination of the identity of the microorganisms isolated from the test, the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

If the test is declared to be invalid it is repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test the product examined complies with the test for sterility. If microbial growth is found in the repeat test the product examined does not comply with the test for sterility.

## 7. Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility

When using the technique of membrane filtration, use, whenever possible, the whole contents of the container, but not less than the quantities indicated in Table 4.06-2, diluting where necessary to about 100 mL with a suitable sterile solution, such as 1 g/L neutral meat or casein peptone.

When using the technique of direct inoculation of media, use the quantities shown in Table 4.06-2, unless otherwise justified and authorized. The tests for bacterial and fungal sterility are carried out on the same sample of the product to be examined. When the volume or the quantity in a single container is insufficient to carry out the tests, the contents of two or more containers are used to inoculate the different media.

### 8. Minimum number of items to be tested

The minimum number of items to be tested in relation to the size of the batch is given in Table 4.06-3.

## 5. Tests for Crude Drugs

### 5.01 Crude Drugs Test

Crude Drugs Test is applied to the crude drugs mentioned in the General Rules for Crude Drugs.

#### 1. Sampling

Unless otherwise specified, sample should be taken by the following methods. If necessary, preserve the samples in tight containers.

(i) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.

(ii) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly.

(iii) When the mass of each single piece of the crude drugs is not less than 100 g, not less than 5 pieces should be taken for a sample, or not less than 500 g of the sample should be taken after cutting to a suitable size and mixing thoroughly.

#### 2. Preparation of the test sample for analysis

Preparations are to be made by mixing the sample well. Powdered drugs should be used as they are, and in the case of unpowdered drugs, unless otherwise specified, grind the sample into powder. If the sample cannot be ground into powder, reduce it as finely as possible, spread it out in a thin layer, and withdraw a typical portion for analysis. If necessary, preserve the test sample in a tight container.

#### 3. Microscopic examination

##### 3.1. Apparatus

Use an optical microscope with objectives of 10 and 40 magnifications, and an ocular of 10 magnifications.

##### 3.2. Preparation for microscopic examination

(i) Section: To a section on a slide glass add 1 to 2 drops of a mounting agent, and put a cover glass on it, taking precaution against inclusion of bubbles. Usually use a section 10 to 20  $\mu\text{m}$  in thickness.

(ii) Powder: Place about 1 mg of powdered sample on a slide glass, apply 1 to 2 drops of a swelling agent, stir well with a small rod preventing inclusion of bubbles, and allow to stand for a while to swell the sample. Apply 1 drop of the mounting agent, and put a cover glass on it so that the tissue

sections spread evenly without overlapping each other, taking precaution against inclusion of bubbles. In the case where the tissue sections are opaque, place about 1 mg of powdered sample on a slide glass, apply 1 to 2 drops of chloral hydrate TS, heat to make the tissues clear while stirring with a small glass rod to prevent boiling. After cooling, apply 1 drop of mounting agent, and put a cover glass on it in the same manner as above.

Unless otherwise specified, use a mixture of water and glycerin (1:1) or a mixture of water, ethanol (95) and glycerin (1:1:1) as the mounting agent and swelling agent.

### 3.3. Observation of components in the Description

In each monograph, description is usually given of the outer portion and the inner portion of a section in this order, followed by a specification of cell contents. Observation should be made in the same order. In the case of a powdered sample, description is given of a characteristic component or a matter present in large amount, rarely existing matter, and cell contents in this order. Observation should be made in the same order.

## 4. Purity

### 4.1 Heavy metals

There are two ways to specify the heavy metals, one is to specify with the total amount of heavy metals and the other is with individual amount of particular metal(s). Heavy metals for crude drugs are usually specified with the total amount of heavy metals according to Heavy Metals Limit Test <1.07> described in monographs. However, rarely there is the case where the test cannot be carried out due to getting turbid or such of the test solution. In these cases, the acceptance can be evaluated by determining individual amount of particular metal(s) using Atomic Absorption Spectrophotometry <2.23> or Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry <2.63>.

### 4.2. Foreign matter

Unless otherwise specified, weigh 25 to 500 g of the sample, spread out in a thin layer, and separate the foreign matter by inspecting with the naked eye or with the use of a magnifying glass of 10 magnifications. Weigh, and determine the percentage of foreign matter.

### 4.3. Total BHC's and total DDT's

Sodium chloride, anhydrous sodium sulfate and synthetic magnesium silicate for column chromatography used in this procedure are used after drying by heating at about 130°C for more than 12 hours and cooling in a desiccator (silica gel). Chromatographic column is prepared as follows: Place 20 g of synthetic magnesium silicate for column chromatography in a 200-mL flask, add 50 mL of hexane for Purity of crude drug, shake vigorously, and immediately pour the mixture into a chromatographic tube about 2 cm in inside diameter and about 30 cm in length. Drip until the depth of hexane layer at the upper part is about 5 cm, introduce 8 g of anhydrous sodium sulfate from the top, and further drip until a small quantity of hexane is left at the upper part.

Weigh accurately about 5 g of pulverized sample, place in a glass-stoppered centrifuge tube, add 30 mL of a mixture of acetone for Purity of crude drug and water (5:2), stopper tightly, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the same procedure twice with the residue using two 30-mL portions of the mixture of acetone for Purity of crude drug and water (5:2). Combine all the supernatant liquids, and concentrate under low pressure (in vacuo) at a temperature not higher than 40°C until the order of acetone is faint. Transfer the concentrated solution to a separator containing 100 mL of sodium chloride TS,

and shake twice with two 50-mL portions of hexane for Purity of crude drug for 5 minutes each. Combine the hexane layers, transfer to a separator containing 50 mL of sodium chloride TS, and shake for 5 minutes. Take the hexane layer, dry with 30 g of anhydrous sodium sulfate, and filter. Wash the residue on the filter paper with 20 mL of hexane for Purity of crude drug. Combine the filtrate and the washings, and concentrate under low pressure (in vacuo) at a temperature not higher than 40°C to about 5 mL. Transfer this solution to the chromatographic column and allow to pass with 300 mL of a mixture of hexane for Purity of crude drug and diethyl ether for Purity of crude drug (17:3) at a rate of not more than 5 mL per minute. After concentrating the eluate under low pressure (vacuo) at a temperature not higher than 40°C, add hexane for Purity of crude drug to make exactly 5 mL. Transfer this solution to a glass-stoppered test tube, add 1 mL of sulfuric acid, and shake carefully. Take 4 mL of the upper layer, transfer to a separate glass-stoppered test tube, add 2 mL of water, and shake gently. Take 3 mL of the upper layer so obtained, transfer to a glass-stoppered centrifuge tube, dry with 1 g of anhydrous sodium sulfate, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg each of  $\alpha$ -BHC,  $\beta$ -BHC,  $\gamma$ -BHC,  $\delta$ -BHC,  $o,p'$ -DDT,  $p,p'$ -DDT,  $p,p'$ -DDD and  $p,p'$ -DDE, dissolve in 5 mL of acetone for Purity of crude drug, and add hexane for Purity of crude drug to make exactly 100 mL. Pipet 10 mL of this solution, and add hexane for Purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, add hexane for Purity of crude drug to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 1  $\mu$ L each of the sample solution and the standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas corresponding to  $\alpha$ -BHC,  $\beta$ -BHC,  $\gamma$ -BHC,  $\delta$ -BHC,  $o,p'$ -DDT,  $p,p'$ -DDT,  $p,p'$ -DDD and  $p,p'$ -DDE,  $A_{TA}$  and  $A_{SA}$ ;  $A_{TB}$  and  $A_{SB}$ ;  $A_{TC}$  and  $A_{SC}$ ;  $A_{TD}$  and  $A_{SD}$ ;  $A_{TE}$  and  $A_{SE}$ ;  $A_{TF}$  and  $A_{SF}$ ;  $A_{TG}$  and  $A_{SG}$ ;  $A_{TH}$  and  $A_{SH}$ . Calculate the content of each of  $\alpha$ -BHC,  $\beta$ -BHC,  $\gamma$ -BHC,  $\delta$ -BHC,  $o,p'$ -DDT,  $p,p'$ -DDT,  $p,p'$ -DDD and  $p,p'$ -DDE by means of the following equations.

Content (ppm) of  $\alpha$ -BHC

$$= \frac{\text{amount (g) of } \alpha - \text{BHC}}{M} \times \frac{A_{TA}}{A_{SA}} \times 50$$

Content (ppm) of  $\beta$ -BHC

$$= \frac{\text{amount (g) of } \beta - \text{BHC}}{M} \times \frac{A_{TB}}{A_{SB}} \times 50$$

Content (ppm) of  $\gamma$ -BHC

$$= \frac{\text{amount (g) of } \gamma - \text{BHC}}{M} \times \frac{A_{TC}}{A_{SC}} \times 50$$

Content (ppm) of  $\delta$ -BHC

$$= \frac{\text{amount (g) of } \delta - \text{BHC}}{M} \times \frac{A_{TD}}{A_{SD}} \times 50$$

Content (ppm) of  $o,p'$ -DDT

$$= \frac{\text{amount (g) of } o,p' - \text{DDT}}{M} \times \frac{A_{TE}}{A_{SE}} \times 50$$

Content (ppm) of  $p,p'$ -DDT

$$= \frac{\text{amount (g) of } p,p' - \text{DDT}}{M} \times \frac{A_{TF}}{A_{SF}} \times 50$$

Content (ppm) of *p,p'*-DDD

$$= \frac{\text{amount (g) of } p,p' - \text{DDD}}{M} \times \frac{A_{TG}}{A_{SG}} \times 50$$

Content (ppm) of *p,p'*-DDE

$$= \frac{\text{amount (g) of } p,p' - \text{DDE}}{M} \times \frac{A_{TH}}{A_{SH}} \times 50$$

*M*: Amount (g) of pulverized sample

Content (ppm) of total BHC's

$$= \text{content (ppm) of } \alpha\text{-BHC} + \text{content (ppm) of } \beta\text{-BHC} + \text{content (ppm) of } \gamma\text{-BHC} + \text{content (ppm) of } \delta\text{-BHC}$$

Content (ppm) of total DDT's

$$= \text{content (ppm) of } o,p' - \text{DDT} + \text{content (ppm) of } p,p' - \text{DDT} + \text{content (ppm) of } p,p' - \text{DDD} + \text{content (ppm) of } p,p' - \text{DDE}$$

#### Operating conditions—

Detector: An electron capture detector.

Sample injection system: A splitless injection system.

Column: A fused silica capillary column about 0.3 mm in inside diameter and about 30 m in length, coated the inside wall with 7% cyanopropyl-7% phenylmethylsilicone polymer for gas chromatography in a thickness of 0.25 to 1.0  $\mu\text{m}$ .

Column temperature: Maintain the temperature at 60°C for 2 minutes after injection, program to increase the temperature at a rate of 10°C per minute to 200°C, and then program to increase the temperature at a rate of 2°C per minute to 260°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention times of the objective compounds are between 10 and 30 minutes.

#### System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add hexane for Purity of crude drug to make exactly 10 mL. Confirm that the peak area of each objective compound obtained with 1  $\mu\text{L}$  of this solution is equivalent to 5 to 15% of that of corresponding compound with 1  $\mu\text{L}$  of the standard solution.

System performance: When the procedure is run with 1  $\mu\text{L}$  of the standard solution under the above operating conditions, the peaks of the object compounds separate completely each other.

System repeatability: Repeat the test 6 times with 1  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of each object compound is not more than 10%.

#### 5. Loss on drying

Unless otherwise specified, transfer 2 to 6 g of the test sample for analysis to a tared weighing bottle, and weigh accurately. Dry at 105°C for 5 hours, allow to cool in a desiccator (silica gel), and weigh accurately. Continue the drying at 105°C, and weigh accurately at 1-hour intervals. When the mass of the sample becomes constant, the loss of mass represents the percentage of loss on drying (%). When the period of time for drying is specified, weigh accurately after drying for the period of time specified, and determine the loss on drying (%).

#### 6. Total ash

Ignite previously a crucible of platinum, quartz or porcelain between 500°C and 550°C for 1 hour. Cool, and weigh accurately the crucible. Unless otherwise specified, weigh accurately 2 to 4 g of the test sample for analysis in

this crucible, take off the lid or keep it open a little if necessary, heat the crucible at a low temperature at first, then gradually heat to a temperature between 500°C and 550°C, ignite to incinerate the residue for more than 4 hours until no carbonized substance remains in the ash, cool, and weigh accurately the ash. Incinerate repeatedly to constant mass, cool, weigh accurately, and determine the amount (%) of total ash. If a carbonized substance remains and a constant mass cannot be obtained in the above-mentioned method, extract the charred mass with hot water, collect the insoluble residue on filter paper for quantitative analysis, and incinerate the residue and filter paper until no carbonized substance remains in the ash. Then add the filtrate, evaporate it to dryness, and incinerate. Cool, weigh accurately, and determine the mass (%) of the total ash. If a carbon-free ash cannot be obtained even in this way, moisten the ash with a small amount of ethanol (95), break up the ash with a glass rod, wash the rod with a small amount of ethanol (95), evaporate carefully, and determine the mass of the total ash as described above. A desiccator (silica gel) is used for cooling.

#### 7. Acid-insoluble ash

Add carefully 25 mL of dilute hydrochloric acid to the total ash, boil gently for 5 minutes, collect the insoluble matter on filter paper for quantitative analysis, and wash thoroughly with hot water. Dry the residue together with the filter paper, and ignite to incinerate in a tared crucible of platinum, quartz or porcelain for 3 hours. Cool in a desiccator (silica gel), weigh, and determine the amount (%) of acid-insoluble ash. When the amount determined exceeds the limit specified, incinerate repeatedly to a constant mass.

#### 8. Extract content

The test for the extract content in crude drugs is performed as directed in the following methods:

##### 8.1. Dilute ethanol-soluble extract

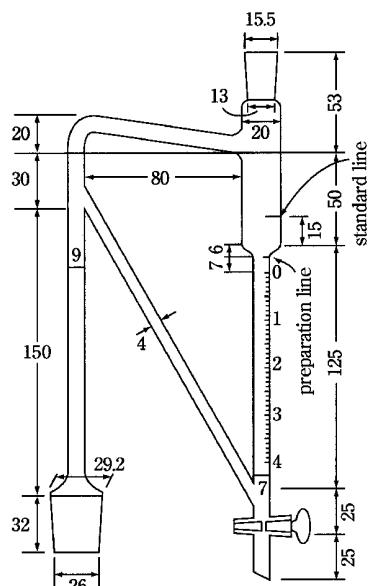
Unless otherwise specified, weigh accurately about 2.3 g of the sample for analysis, extract with 70 mL of dilute ethanol in a suitable flask with occasional shaking for 5 hours, and allow to stand for 16 to 20 hours. Filter, and wash the flask and residue with small portions of dilute ethanol until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness, dry at 105°C for 4 hours, and cool in a desiccator (silica gel). Weigh accurately the amount, multiply it by 2, and determine the amount of dilute ethanol-soluble extract. Calculate the extract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

##### 8.2. Water-soluble extract

Proceed as directed in 8.1., using water instead of dilute ethanol, weigh accurately the amount, multiply by 2, and determine the amount of water-soluble extract. Calculate the extract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

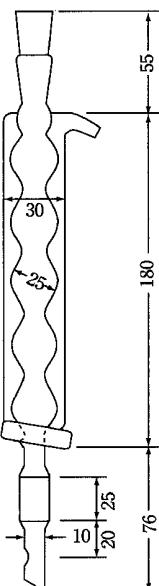
##### 8.3. Diethyl ether-soluble extract

Unless otherwise specified, dry the test sample for analysis in a desiccator (silica gel) for 48 hours, weigh accurately about 2 g of it, and place in a suitable flask. Add 70 mL of diethyl ether, attach a reflux condenser to the flask, and boil gently on a water bath for 4 hours. Cool, filter, and wash the flask and the residue with small portions of diethyl ether until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness on a water bath, dry in a desiccator (silica gel) for 24 hours, weigh accurately the amount, multiply it by 2, determine the amount of diethyl ether-soluble extract, and calculate the extract content (%).



The figures are in mm.

**Fig. 5.01-1**



**Fig. 5.01-2**

## **9. Essential oil content**

The test of essential oil content in crude drugs is performed as directed in the following method.

### **9.1. Essential oil determination**

Weigh the quantity of the test sample for analysis directed in the monograph in a 1-L hard glass-stoppered flask, and add from 5 to 10 times as much water as the drug. Set up an apparatus for essential oil determination (Fig. 5.01-1), inserting a reflux condenser (Fig. 5.01-2) in the upper mouth of it, and heat the content of the flask in an oil bath between 130°C and 150°C to boiling. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2.0 mL of xylene is added to the graduated tube. Unless otherwise specified, continue boiling for 5 hours, allow to stand for some time, and open the stopper of the apparatus. Draw off the water slowly until the surface of the oil layer corresponds to the preparation line, and allow it to stand for more than 1 hour at ordinary temperature. Then lower the surface of the oil layer to the zero line, and read the volume (mL) of the oil at ordinary temperature. Subtract the volume (mL) of xylene from the volume of the total oil.

## **10. Assay of Marker Compounds for the Assay of Crude Drugs and Extracts of Kampo Formulations Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy**

### **10.1. Principle of Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy**

The spectra obtained by proton nuclear magnetic resonance ( $^1\text{H-NMR}$ ) spectroscopy after dissolving the substance to be measured in a solvent, are frequently used as a powerful analytical method for determining the chemical structure of the substance from the following reasons: the resonance signals appear at different chemical shifts depending on the chemical structure of the substance measured; the signals are split by spin-spin interactions through chemical bonds mainly depending on the number of  $^1\text{H}$  bonded to adjacent carbon atoms; the signal intensities (areas) are proportional to the number of  $^1\text{H}$  resonating at the same frequency; etc.

In the  $^1\text{H}$ -NMR spectra, the proton nuclei ( $^1\text{Hs}$ ) in differ-

ent chemical environments within the same molecule are observed as the separate signals having different chemical shifts depending on their resonance frequencies. Accordingly, we can compare the intensities of 2 signals having different chemical shifts each other. The intensity of the signal  $S_i$  would be given by the following equation (1);

$$S_i \propto N_i \frac{m}{VM} p \sin \beta \frac{1 - e^{-T_r/T_{li}}}{1 - e^{-T_r/T_{li}} \cos \beta} M_0 \quad (1)$$

where  $N_i$  is the number of resonating  $^1\text{H}$  which gives the signal,  $V$  is the volume of the sample solution,  $m$  is the mass of the sample,  $M$  is the molecular mass of the substance measured,  $p$  is the purity of the sample,  $\beta$  is the excitation pulse angle,  $T_{1i}$  is the spin-lattice relaxation time of  $^1\text{H}$  which gives the signal,  $T_r$  is the repetition time,  $M_0$  is the equilibrium magnetization and the subscript  $i$  indicates the independent signal. The relaxation time of a  $^1\text{H}$  is different depending on the environments of the  $^1\text{H}$ s. Since the sensitivity of NMR is not so good, the signal-to-noise ratio (SN ratio) of signals should generally be improved by measuring it repeatedly and averaging noises. When the NMR measurement is performed under the condition with the repetition time  $T_r$  sufficiently longer than the longest  $T_1$  among the  $T_1$ s of the signals observed for the analyte compound, the condition of  $1 - e^{-T_r/T_1} \approx 1$  for all of the signals of the analyte compounds would be satisfied and quantitative analysis utilizing NMR (quantitative NMR) can be performed. On the other hand, when NMR is used for the structural determination, priority is given to improve detection sensitivity, and the condition for increasing the SN ratio of signals by using repeated measurements is usually used. Under this condition, since the repetition time is not long enough to ensure quantitative NMR, the proportion of signal intensity to the number of each equivalent  $^1\text{H}$  nuclei in the measured molecule is not obtained precisely. However, when NMR is measured under the conditions which ensure quantitative performance, the signal intensity ratio proportional to each number of equivalent molecule is obtained.

When the intensity of two signals having different chemical shifts in the same molecule are compared under the quantitative conditions which ensure quantitative performance, the following equation (2) is obtained and the signal intensities  $S_i$  and  $S_j$  are found to be proportional to the number of resonating  $^1\text{H}$ s.

$$\frac{S_i}{S_j} = \frac{N_i}{N_j} \quad (2)$$

This proportionality between the signal area and number of resonating  $^1\text{H}$  can be applied to the signals from 2 different molecules. In this case, since it is considered that the excitation pulse angle and the volume of the sample solution used for the measurement can be kept constant independent of the substance measured, the following equation (3), in which the observed signal area  $S$  is proportional only to the purity, molecular mass and mass used for the measurement of analyte compound, can be obtained. (a and s indicate the signals of the analyte compound and a reference substance (internal standard), respectively.)

$$P_a = \frac{S_a}{S_s} \frac{N_s}{N_a} \frac{M_a}{M_s} \frac{m_s}{m_a} P_s \quad (3)$$

Although there are some prerequisites to be met, such that each molecule should not interact (such as react) with other molecules in the solution and the molecule should have separate signals at different chemical shifts from others, we will be able to evaluate the purity of the analyte compound by measuring its  $^1\text{H-NMR}$  under the conditions which ensure

quantitative performance, if we have a standard material with known purity and use it as an internal standard for the measurement. In other words, if a standard material whose molecular mass and accurate purity are known would be provided as the superior standard, we can evaluate the purity of the substances coexisting in the solution of the standard material by measuring  $^1\text{H-NMR}$  of the solution. In this case, when traceability of the measurement to the International System of Units (SI) is guaranteed for the standard material, purity of the analyte compound can be calculated indirectly as the SI traceable value by using the standard material as the superior standard. In such a measurement, it is necessary to dissolve the sample and the standard material in a solution. Thus, it is practically important for precise evaluation of the purity of analyte compound that both of the sample and the standard material should be weighed accurately, and dissolved in a solvent for NMR measurement.

#### 10.2. Supply of Reference Standard and Software for Quantitative NMR

Quantitative determination based on the aforementioned principle is called quantitative NMR (qNMR). From among certified reference materials supplied from public institutions (NMIJ CRM), those with SI traceable pricing have been marketed as reference standard for qNMR. Easy-to-use solid-state compounds include 1,4-bis(trimethylsilyl)benzene- $d_4$  (1,4-BTMSB- $d_4$ ), methanol, and dimethylsulfoxide for organic solvent use and 3-(trimethylsilyl)-1-propanesulfonic acid- $d_6$ -sodium salt (DSS- $d_6$ ), maleic acid and dimethyl sulfone for aquatic use which both exhibit a sharp peak for specific chemical shifts in  $^1\text{H-NMR}$ . In addition, such measurement software capable of performing qNMR easier is also supplied by NMR manufacturers.

#### 10.3. Marker Compounds for Assay and Reference Substances for Quantitative Analysis of Crude Drugs and Extracts of Kampo Formulations in the JP

If it is possible to price a reagent used as a quantitative index component in a crude medicine with a correct content using qNMR based on the above-mentioned principle, it also becomes possible to use the reagent as a reference substance for analysis with assured metrological traceability. According to a result of a validation experiment, in case of a compound with molecular mass of around 300 to be measured, it is possible to perform pricing at an ordinary laboratory level by using about 10 mg of the compound for the measurement while ensuring two significant figures even if including errors between used devices. As content of quantitative index component in a crude medicine is just a few percent at most in general and the minimum unit of regulation values is 0.1%, two significant figures is believed to be enough to ensure the accuracy of the content of reference substances for quantitative analysis in consideration of variation for each crude medicine as a natural substance.

Such reagents priced with SI traceable quantitative value (degree of purity) by qNMR that have been defined in a paragraph for reagents and test solutions are available as Japanese Pharmacopoeia reagents for quantitative analysis. Further, in cases where a reagent priced by qNMR is used as a reference substance for quantitative analysis such as HPLC and involved in a calculation of quantitative value of subject compound after converting degree of purity (%) of the priced reagent, it becomes possible to use the resulting quantitative value as a SI traceable value. In addition, in cases where a reagent priced by qNMR is used as a reference material for a quantitative analysis based on HPLC, condition of the quantitative analysis is based on an assumption that no impurity is recognized at any peak of a component of the reagent to be quantified, which is required to be confirmed

separately by a device such as photodiode array detector or mass spectrometer.

#### 10.4. Precautions for Performing qNMR

In order to perform qNMR, such device is required that is capable of gated decoupling for  $^{13}\text{C-NMR}$  with higher accuracy in a magnetic field with a resonance frequency of at least 400 MHz or higher for  $^1\text{H-NMR}$  in consideration of resolution required for separation of impurities from peaks and even detection sensitivity as well. Further, it is also required to perform measurement under the condition that receiving sensitivity of the receiver is appropriate with optimally adjusted probe tuning and shim.

In terms of reagents for quantification for which qNMR is performed, amounts of reagents and reference standard for qNMR to be taken are defined in paragraph 9.41 Reagents, Test Solutions. As high accuracy is required for weighing both of them, it is required to take the amounts by the minimum weight of the balance or higher. Defined amounts to be taken for both of them are those described as validated realistic minimum amounts using an ultramicrobalance. Therefore, in cases where both of them are completely dissolved, SN ratio of spectrum is improved when measured after increasing the amounts while keeping the quantitative ratio, resulting in measurements with higher accuracy in most cases. Even though SN ratio is even more improved when a measurement is performed by integrating as many times as possible resulting in a measurement result with higher accuracy, it is required to consider stability of the magnetic field and the devices if the measurement lasts more than a few hours. Sensitivity is also improved, albeit a little, by using deuterated solvent with higher deuteration rate. In some cases, impurity signals may be detected on a spectrum which have not been observed before, by further improving SN ratio. In cases where any existence of signal derived from such impurities has been made clear, the range of chemical shift where such signal exists should not be integrated. In addition, as signals of small amount of impurities have been observed also in deuterated solvent for NMR measurement or 1,4-BTMSB- $d_4$  or DSS- $d_6$  as reference standard for qNMR, it is important to recognize the range of these impurities signals before qNMR measurement. Moreover, qNMR measurement should be performed immediately after sample preparation, since impurity signals have been confirmed to increase albeit a little by little if samples are kept in the solvent for measurement for long hours. Even though it is not necessary to measure NMR under qNMR condition for confirming impurity signals, it is easier to distinguish them from satellite signals by performing a measurement under the condition of decoupling of  $^{13}\text{C}$  nuclei without spinning. While 1,4-BTMSB- $d_4$  and DSS- $d_6$ , which are used for qNMR as reference standard for qNMR, have chemical shift values at around 0.2 ppm and 0.1 ppm respectively when tetramethylsilane (in organic solvent) or DSS (in deuterated water) is used as the reference for chemical shifts ( $\delta$ ), chemical shifts of other signals are indicated by regarding the chemical shifts of these reference standard for qNMR as 0 ppm for convenience when measuring qNMR.

The SN ratio of a signal used in NMR is calculated using the formula of signal intensity/(2 × noise intensity), and this noise intensity is the root-mean-square value of individual noise intensities in the noise area.

## 5.02 Microbial Limit Test for Crude Drugs and Preparations containing Crude Drugs as Main Ingredient

Microbial limit test for crude drugs and preparations containing crude drugs as main ingredient (crude drug preparations) includes microbial enumeration tests and tests for specified micro-organisms. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

### I. Total Viable Aerobic Count

The tests described hereafter will allow quantitative enumeration of mesophilic bacteria and fungi which may grow under aerobic conditions.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

Alternative microbiological procedures, including rapid methods, may be used, provided that they give a result equal to or better than that of the Pharmacopoeial methods.

#### 1. General Procedures

Carry out the determination under conditions designed to avoid extrinsic microbial contamination of the product to be examined. The precautions taken to avoid contamination must be such that they do not affect any micro-organisms which are to be revealed in the test.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralised. If inactivators are used for this purpose their efficacy and their absence of toxicity for micro-organisms must be demonstrated.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated.

#### 2. Enumeration Methods

The choice of a method is based on factors such as the nature of the product and the required limit of micro-organisms. The method chosen must allow testing of a sufficient sample size to judge compliance with the specification. The suitability of the chosen method must be established.

#### 3. Growth Promotion Test, Suitability of the Counting Method and Negative Controls

The ability of the test to detect micro-organisms in the presence of product to be tested must be established.

Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

##### 3.1. Preparation of test strains

Use standardised stable suspensions of test strains or prepare as stated below.

Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in Table 5.02-I-1.

Use Buffered Sodium Chloride-Peptone Solution (pH 7.0)

or Phosphate Buffer (pH 7.2) to make test suspensions; to suspend *Aspergillus brasiliensis* spores, 0.05% of polysorbate 80 may be added to the buffer. Use the suspensions within 2 h or within 24 h if stored at 2 – 8°C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Aspergillus brasiliensis* or *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 – 8°C for a validated period of time.

##### 3.2. Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described under 4. Testing of Products. A failed negative control requires an investigation.

##### 3.3. Growth promotion of the media

Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described.

Inoculate portions/plates of Fluid Soybean-Casein Digest Medium and Soybean-Casein Digest Agar Medium with a small number (not more than 100 CFU) of the microorganisms indicated in Table 5.02-I-1, using a separate portion/plate of medium for each. Inoculate plates of Sabouraud Glucose Agar Medium with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 5.02-I-1, using a separate plate of medium for each. Incubate in the conditions described in Table 5.02-I-1.

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Liquid media are suitable if clearly visible growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

##### 3.4. Suitability of the counting method in the presence of product

###### 3.4.1. Preparation of the sample

The method for sample preparation depends on the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, an alternative procedure must be developed.

Buffered Sodium Chloride-Peptone Solution (pH 7.0), Phosphate Buffer (pH 7.2) or Fluid Soybean-Casein Digest Medium is used to suspend or dilute the test specimen. Unless otherwise specified, usually take 10 g or 10 mL of the test specimen, and suspend or dissolve it in 90 mL of the buffer or fluid medium specified. A test specimen as a suspension must be shaken for 10 minutes. If necessary, for the product to be tested which recovery rate of microorganisms is low, repeat the same method and use this as the test fluid. A different quantity or volume may be used if the nature of the test specimen requires it. A surface-active agent such as 1 g/L of polysorbate 80 may be added to assist the suspension of poorly wettable substances. If necessary adjust to pH 6 – 8. Further dilutions, where necessary, are prepared with the same diluent.

###### 3.4.2. Inoculation and dilution

Add to the sample prepared as described above (3.4.1.) and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the suspension of

**Table 5.02-I-1** Preparation and use of test micro-organisms

Micro-organism	Preparation of test strain	Growth promotion		Suitability of counting method in the presence of the product	
		Total aerobic microbial count	Total yeasts and moulds count	Total aerobic microbial count	Total yeasts and moulds count
<i>Staphylococcus aureus</i> such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276	Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h	Soybean-Casein Digest Agar Medium* and Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 – 35°C ≤ 3 days		Soybean-Casein Digest Agar Medium/MPN Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 – 35°C ≤ 5 days	
<i>Pseudomonas aeruginosa</i> such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h	Soybean-Casein Digest Agar Medium* and Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 – 35°C ≤ 3 days		Soybean-Casein Digest Agar Medium/MPN Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 – 35°C ≤ 5 days	
<i>Bacillus subtilis</i> such as ATCC 6633, NCIMB 8054, CIP 52.62 or NBRC 3134	Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h	Soybean-Casein Digest Agar Medium* and Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 – 35°C ≤ 3 days		Soybean-Casein Digest Agar Medium/MPN Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 – 35°C ≤ 5 days	
<i>Candida albicans</i> such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594	Sabouraud Glucose Agar Medium or Fluid Sabouraud Glucose Medium 20 – 25°C 2 – 3 days	Soybean-Casein Digest Agar Medium* ≤ 100 CFU 30 – 35°C ≤ 5 days	Sabouraud Glucose Agar Medium with antibiotics ≤ 100 CFU 20 – 25°C ≤ 5 days	Soybean-Casein Digest Agar Medium ≤ 100 CFU 30 – 35°C ≤ 5 days MPN: not applicable	Sabouraud Glucose Agar Medium with antibiotics ≤ 100 CFU 20 – 25°C ≤ 5 days
<i>Aspergillus brasiliensis</i> such as ATCC 16404, IMI 149007, IP 1431.83 or NBRC 9455	Sabouraud Glucose Agar Medium or Potato Dextrose Agar Medium 20 – 25°C 5 – 7 days, or until good sporulation is achieved	Soybean-Casein Digest Agar Medium* ≤ 100 CFU 30 – 35°C ≤ 5 days	Sabouraud Glucose Agar Medium with antibiotics ≤ 100 CFU 20 – 25°C ≤ 5 days	Soybean-Casein Digest Agar Medium ≤ 100 CFU 30 – 35°C ≤ 5 days MPN: not applicable	Sabouraud Glucose Agar Medium with antibiotics ≤ 100 CFU 20 – 25°C ≤ 5 days

\* In the case where TTC TS or amphotericin B TS is added, check with the media with these additives. When amphotericin B TS is added, the check for *C. albicans* and *A. brasiliensis* is not required.

the inoculum should not exceed 1 per cent of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed.

If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralization, dilution or filtration.

### 3.4.3. Neutralization/removal of antimicrobial activity

The number of micro-organisms recovered from the prepared sample diluted as described in 3.4.2. and incubated following the procedure described in 3.4.4., is compared to the number of micro-organisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example, (1) an increase in the

volume of the diluent or culture medium, (2) incorporation of a specific or general neutralizing agents into the diluent, (3) membrane filtration or (4) a combination of the above measures.

**Neutralizing agents**—Neutralizing agents may be used to neutralize the activity of antimicrobial agents. They may be added to the chosen diluent or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for micro-organisms must be demonstrated by carrying out a blank with neutralizing agents, without product.

If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the article is not likely to be contaminated with the given species of the micro-organism. However, it is possible that the product only inhibits some of the micro-organisms specified herein, but does not inhibit others not included amongst the test strains or for which the latter are not representative. Then, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion.

#### 3.4.4. Recovery of micro-organism in the presence of product

For each of the micro-organisms listed in Table 5.02-I-1, separate tests are performed. Only micro-organisms of the added test strain are counted.

##### 3.4.4.1. Membrane filtration method

Use membrane filters having a nominal pore size not greater than  $0.45\text{ }\mu\text{m}$ . The type of filter material is chosen in such a way that the bacteria-retaining efficiency is not affected by the components of the sample to be investigated. For each of the micro-organisms listed in Table 5.02-I-1, one membrane filter is used.

Transfer a suitable amount of the sample prepared as described under 3.4.1. to 3.4.3. (preferably representing 1 g of the product, or less if large numbers of CFU are expected) to the membrane filter, filter immediately and rinse the membrane filter with an appropriate volume of diluent.

For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of Soybean-Casein Digest Agar Medium. For the determination of total combined yeasts/moulds count (TYMC) transfer the membrane to the surface of Sabouraud Glucose Agar Medium with antibiotics. For Soybean-Casein Digest Agar Medium that is suffused with fungi or when the TAMC is expected to exceed the acceptance criterion due to the fungus growth, amphotericin B TS as an antimycotic may be added to the agar. Incubate the plates as indicated in Table 5.02-I-1. Perform the counting.

##### 3.4.4.2. Plate-count methods

Perform plate-count methods at least in duplicate for each medium and use the mean count of the result.

(i) Pour-plate method: For Petri dishes 9 cm in diameter, add to the dish 1 mL of the sample prepared as described under 3.4.1. to 3.4.3. and 15 – 20 mL of Soybean-Casein Digest Agar Medium or Sabouraud Glucose Agar Medium with antibiotics, both media being warmed at not more than  $45^\circ\text{C}$ . For Soybean-Casein Digest Agar Medium, TTC TS may be added to distinct the colonies from the fragments of crude drugs existed in specimens, and when the agar medium is suffused with fungi or the TAMC is expected to exceed the acceptance criterion due to the fungus growth, amphotericin B TS as an antimycotic may be added to the medium. In the case of Sabouraud Glucose Agar Medium with antibiotics that is suffused with fungi, Rose Bengal TS may be added. If larger Petri dishes are used, the amount of agar medium is increased accordingly. For each of the micro-organisms list-

ed in Table 5.02-I-1, at least 2 Petri dishes are used.

Incubate the plates as indicated in Table 5.02-I-1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

(ii) Surface-spread method: For Petri dishes 9 cm in diameter, add 15 – 20 mL of Soybean-Casein Digest Agar Medium or Sabouraud Glucose Agar Medium with antibiotics at about  $45^\circ\text{C}$  to each Petri dish and allow to solidify. Dry the plates, for example in a laminar-air-flow cabinet or in an incubator. The test solutions to be added to the agar medium are the same as described in (i) Pour-plate method. If larger Petri dishes are used, the volume of the agar is increased accordingly. For each of the micro-organisms listed in Table 5.02-I-1, at least 2 Petri dishes are used. Spread a measured volume of not less than 0.1 mL of the sample prepared as described under 3.4.1. to 3.4.3. over the surface of the medium. Incubate and count as prescribed under 3.4.4.2. (i).

##### 3.4.4.3. Most-probable-number (MPN) method

The precision and accuracy of the MPN method is less than that of the membrane filtration method or the plate-count method. Unreliable results are obtained particularly for the enumeration of moulds. For these reasons the MPN method is reserved for the enumeration of TAMC in situations where no other method is available. If the use of the method is justified, proceed as follows.

Prepare a series of at least 3 serial tenfold dilutions of the product as described under 3.4.1. to 3.4.3. From each level of dilution, 3 aliquots of 1 g or 1 mL are used to inoculate 3 tubes with 9 – 10 mL of Fluid Soybean-Casein Digest Medium. If necessary a surface-active agent such as polysorbate 80, or an inactivator of antimicrobial agents may be added to the medium. Thus, if 3 levels of dilution are prepared 9 tubes are inoculated.

Incubate all tubes at  $30$  –  $35^\circ\text{C}$  for not more than 3 days. If reading of the results is difficult or uncertain owing to the nature of the product to be examined, subculture in the same broth, or Soybean-Casein Digest Agar Medium, for 1 – 2 days at the same temperature and use these results. Determine the most probable number of micro-organisms per gram or millilitre of the product to be examined from Table 5.02-I-2.

#### 3.5. Results and interpretation

When verifying the suitability of the membrane filtration method or the plate-count method, a mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control defined in 3.4.2. in the absence of the product must be obtained. When verifying the suitability of the MPN method the calculated value from the inoculum must be within 95 per cent confidence limits of the results obtained with the control.

If the above criteria cannot be met for one or more of the organisms tested with any of the described methods, the method and test conditions that come closest to the criteria are used to test the product.

#### 4. Testing of Products

##### 4.1. Sampling and preparation of the test specimens

Select the sample(s) at random from the bulk material or from the available containers of the preparation. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

Unless otherwise specified, samples should be taken by the following methods.

(i) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.

(ii) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly and cutting.

(iii) When the mass of each single piece of the crude drug is not less than 100 g, not less than 5 pieces should be taken for a sample, or not less than 500 g of the sample should be taken after cutting to a suitable size and mixing thoroughly. If necessary, cut more for use.

(iv) When crude drugs to be sampled are in the form of a solution or a preparation, the sample should be taken after mixing thoroughly.

#### 4.2. Examination of the product

##### 4.2.1. Membrane filtration method

Use a filtration apparatus designed to allow the transfer of the filter to the medium.

Prepare the sample using a method that has been shown suitable as described in section 3 and transfer the appropriate amount to each of 2 membrane filters and filter immediately. Wash each filter following the procedure shown to be suitable.

For the determination of TAMC, transfer one of the membrane filters to the surface of Soybean-Casein Digest Agar Medium. For the determination of TYMC, transfer the other membrane to the surface of Sabouraud Glucose Agar Medium with antibiotics. Incubate the plate of Soybean-Casein Digest Agar Medium at 30–35°C for 5–7 days and the plate of Sabouraud Glucose Agar Medium with antibiotics at 20–25°C for 5–7 days.

Calculate the number of CFU per gram or per millilitre of product.

##### 4.2.2. Plate-count methods

(i) Pour-plate method: Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare for each medium at least 2 Petri dishes for each level of dilution. Incubate the plates of Soybean-Casein Digest Agar Medium at 30–35°C for 5–7 days and the plates of Sabouraud Glucose Agar Medium with antibiotics at 20–25°C for 5–7 days. Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of the counts and calculate the number of CFU per gram or per millilitre of product.

(ii) Surface-spread method: Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare at least 2 Petri dishes for each medium and each level of dilution. For incubation and calculation of the number of CFU proceed as described for the pour-plate method.

##### 4.2.3. Most-probable-number method

Prepare and dilute the sample using a method that has been shown to be suitable as described in section 3. Incubate all tubes for 3–5 days at 30–35°C. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth.

Determine the most probable number of micro-organisms per gram or millilitre of the product to be examined from Table 5.02-I-2.

#### 4.3. Interpretation of the results

The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found using Soybean-Casein Digest Agar Medium; if colonies of fungi are detected on this medium, they are counted as part of TAMC. The total combined yeasts/mould count (TYMC) is considered to be equal to the number of CFU found using Sabouraud Glucose Agar Medium with antibiotics; if colonies of bacteria

**Table 5.02-I-2** Most-probable-number values of micro-organisms

Observed combinations of numbers of tubes showing growth in each set			MPN per g or mL of product	95 percent confidence limits
Number of g or mL of product per tube	0.1	0.01		
0	0	0	< 3	0–9.4
0	0	1	3	0.1–9.5
0	1	0	3	0.1–10
0	1	1	6.1	1.2–17
0	2	0	6.2	1.2–17
0	3	0	9.4	3.5–35
1	0	0	3.6	0.2–17
1	0	1	7.2	1.2–17
1	0	2	11	4–35
1	1	0	7.4	1.3–20
1	1	1	11	4–35
1	2	0	11	4–35
1	2	1	15	5–38
1	3	0	16	5–38
2	0	0	9.2	1.5–35
2	0	1	14	4–35
2	0	2	20	5–38
2	1	0	15	4–38
2	1	1	20	5–38
2	1	2	27	9–94
2	2	0	21	5–40
2	2	1	28	9–94
2	2	2	35	9–94
2	3	0	29	9–94
2	3	1	36	9–94
3	0	0	23	5–94
3	0	1	38	9–104
3	0	2	64	16–181
3	1	0	43	9–181
3	1	1	75	17–199
3	1	2	120	30–360
3	1	3	160	30–380
3	2	0	93	18–360
3	2	1	150	30–380
3	2	2	210	30–400
3	2	3	290	90–990
3	3	0	240	40–990
3	3	1	460	90–1980
3	3	2	1100	200–4000
3	3	3	> 1100	

are detected on this medium, they are counted as part of TYMC. In the case here the bacterial growth is expected not to give any affection to the interpretation of the result, Sabouraud Glucose Agar Medium containing no antibiotics may be used. If the count is carried out by the MPN method the calculated value is the TAMC.

The recommended solutions and media are described in "II. Tests for Specified Micro-organisms".

## II. Tests for Specified Micro-organisms

The tests described hereafter will allow determination of the absence of, or limited occurrence of specified micro-organisms which may be detected under the conditions described.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

Alternative microbiological procedures, including rapid methods, may be used, provided that they give a result equal to or better than that of the Pharmacopoeial methods.

### 1. General Procedures

The preparation of samples is carried out as described in I. Total Viable Aerobic Count.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized as described in I. Total viable aerobic count.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated as described in I. Total Viable Aerobic Count.

For the scarce crude drugs and those products, the quantity of sample or the volume of medium may be adjusted accordingly based on a risk estimation.

### 2. Growth Promoting and Inhibitory Properties of the Media, Suitability of the Test and Negative Controls

The ability of the test to detect micro-organisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

#### 2.1. Preparation of test strains

Use standardised stable suspensions of test strains or prepare as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot.

Grow each of the bacterial test strains separately in containers containing Fluid Soybean-Casein Digest Medium or on Soybean-Casein Digest Agar Medium at 30 – 35°C for 18 – 24 hours.

*Staphylococcus aureus* such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276,

*Pseudomonas aeruginosa* such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275,

*Escherichia coli* such as ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972,

*Salmonella enterica* subsp. *enterica* serovar Typhimurium such as ATCC 14028  
or, as an alternative,

*Salmonella enterica* subsp. *enterica* serovar Abony such as NBRC 100797, NCTC 6017 or CIP 80.39,

Use Buffered Sodium Chloride-Peptone Solution (pH 7.0) or Phosphate Buffer (pH 7.2) to make test suspensions. Use

**Table 5.02-II-1** Growth promoting, inhibitory and indicative properties of media

Medium	Property	Test strains
<b>Test for bile-tolerant gram-negative bacteria</b>		
Enterobacteria enrichment broth-Mossel	Growth promoting	<i>E. coli</i> <i>P. aeruginosa</i>
	Inhibitory	<i>S. aureus</i>
VRB (Violet/Red/Bile) Agar with glucose	Growth promoting + Indicative	<i>E. coli</i> <i>P. aeruginosa</i>
<b>Test for Escherichia coli</b>		
Fluid MacConkey Broth Medium	Growth promoting	<i>E. coli</i>
	Inhibitory	<i>S. aureus</i>
MacConkey Agar Medium	Growth promoting + Indicative	<i>E. coli</i>
Enzyme substrate medium for <i>E. coli</i>	Growth promoting + Indicative	<i>E. coli</i>
<b>Test for Salmonella</b>		
Fluid Rappaport Vassiliadis Salmonella Enrichment Broth Medium	Growth promoting	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium or <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony
	Inhibitory	<i>S. aureus</i>
XLD (Xylose-Lysine-Deoxycholate) Agar Medium	Growth promoting + Indicative	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium or <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony
Enzyme substrate medium for <i>Salmonella</i>	Growth promoting + Indicative	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium or <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony
<b>Test for Staphylococcus aureus</b>		
Fluid Soybean-Casein Digest Medium with 7.5% Sodium Chloride	Growth promoting	<i>S. aureus</i>
	Inhibitory	<i>E. coli</i>
Vogel-Johnson Agar Medium	Growth promoting + Indicative	<i>S. aureus</i>
	Inhibitory	<i>E. coli</i>
Baird-Parker Agar Medium	Growth promoting + Indicative	<i>S. aureus</i>
	Inhibitory	<i>E. coli</i>
Mannitol Salt Agar Medium	Growth promoting + Indicative	<i>S. aureus</i>
	Inhibitory	<i>E. coli</i>

the suspensions within 2 hours or within 24 hours if stored at 2 – 8°C.

#### 2.2. Negative control

To verify testing conditions a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A failed negative control required an investigation. A negative con-

trol is also performed when testing the products as described under 3. Testing of Products.

### 2.3. Growth promotion and inhibitory properties of the media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients.

Verify suitable properties of relevant media as described in Table 5.02-II-1.

(i) Test for growth promoting properties, liquid media: inoculate a portion of the appropriate medium with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(ii) Test for growth promoting properties, solid media: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(iii) Test for inhibitory properties, liquid or solid media: inoculate the appropriate medium with at least 100 CFU of the appropriate micro-organism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test micro-organism occurs.

(iv) Test for indicative properties, solid media: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in indication reactions to those previously obtained with a previously tested and approved batch of medium.

(v) Test for indicative properties, liquid media: inoculate a portion of the appropriate medium with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in indication reactions to those previously obtained with a previously tested and approved batch of medium.

### 2.4. Suitability of the test method

For each product to be tested perform sample preparation as described in the relevant paragraph in section 3. Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use a number of micro-organisms equivalent to not more than 100 CFU in the inoculated test preparation.

Perform the test as described in the relevant paragraph in section 3 using the shortest incubation period prescribed.

The specified micro-organisms must be detected with the indication reactions as described in section 3.

Any antimicrobial activity of the product necessitates a modification of the test procedure (see 3.4.3. of I. Total Viable Aerobic Count).

If for a given product the antimicrobial activity with respect to a micro-organism for which testing is prescribed cannot be neutralised, then it is to be assumed that the inhibited micro-organism will not be present in the product.

**Table 5.02-II-2 Interpretation of results**

<b>Results for each quantity of product</b>				<b>Probable number of bacteria per gram or mL of product</b>
<b>0.1 g or 0.1 mL</b>	<b>0.01 g or 0.01 mL</b>	<b>0.001 g or 0.001 mL</b>	<b>0.0001 g or 0.0001 mL</b>	
+	+	+	+	more than $10^4$
+	+	+	-	less than $10^4$ and more than $10^3$
+	+	-	-	less than $10^3$ and more than $10^2$
+	-	-	-	less than $10^2$ and more than 10
-	-	-	-	less than 10

### 3. Testing of Products

#### 3.1. Bile-tolerant gram-negative bacteria

##### 3.1.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. Total viable aerobic count, but using Fluid Soybean-Casein Digest Medium as the chosen diluent, mix and incubate at 20 – 25°C for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours but not more than 5 hours).

##### 3.1.2. Selection and subculture

Inoculate to a suitable amount of Fluid Enterobacteria Enrichment Broth Mossel Medium successive three dilutions which are chosen according to an objective acceptable limit from four dilutions of the sample preparation described under 3.1.1. and/or the dilutions of it, containing respectively 0.1 g, 0.01 g, 0.001 g and 0.0001 g (or 0.1 mL, 0.01 mL, 0.001 mL and 0.0001 mL) of the product to be examined. Incubate at 30 – 35°C for 24 – 48 hours. Subculture each of the cultures on a plate of VRB (Violet/Red/Bile) Agar with glucose. Incubate at 30 – 35°C for 18 – 24 hours.

##### 3.1.3. Interpretation

Generally, growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine from Table 5.02-II-2 the probable number of bile-tolerant gram-negative bacteria.

#### 3.2. Escherichia coli

##### 3.2.1. Qualitative test

###### 3.2.1.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in "I. Total viable aerobic count" and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium, mix and incubate at 30 – 35°C for 18 – 24 hours.

###### 3.2.1.2. Selection and subculture

Shake the container, transfer 1 mL of Fluid Soybean-Casein Digest Medium to 10 mL of Fluid MacConkey Broth Medium and incubate at 44 ± 0.5°C for 24 – 48 hours. Subculture on a plate of MacConkey Agar Medium at 30 – 35°C for 18 – 72 hours. An appropriate enzyme substrate medium for *E. coli* such as CHE Agar Medium or ESC Medium may be used instead of MacConkey Agar Medium. When such enzyme substrate media are used, incubate under the conditions indicated for each medium.

### 3.2.1.3. Interpretation

Growth of brick-red colonies surrounded by a reddish precipitation line on MacConkey Agar Medium or of colonies showing aspects or responses corresponding to *E. coli* on an enzyme substrate medium for *E. coli* indicates the possible presence of *E. coli*. This is confirmed by identification tests.

The product complies with the test if no colonies showing such aspects or responses are present or if the identification tests are negative.

### 3.2.2. Quantitative test

#### 3.2.2.1. Sample preparation and pre-incubation

Inoculate to a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium three amounts of a 1 in 10 dilution, prepared as directed in I. Total Viable Aerobic Count, respectively equivalent to 0.1 g, 0.01 g and 0.001 g (or 0.1 mL, 0.01 mL and 0.001 mL) of the product to be examined, mix, and incubate at 30 – 35°C for 18 – 24 hours.

#### 3.2.2.2. Selection and subculture

Shake the container, transfer 1 mL of Fluid Soybean-Casein Digest Medium to 10 mL of Fluid MacConkey Broth Medium and incubate at 44 ± 0.5°C for 24 – 48 hours. Subculture on a plate of MacConkey Agar Medium at 30 – 35°C for 18 – 72 hours. An appropriate enzyme substrate medium for *E. coli* such as CHE Agar Medium or ESC Medium may be used instead of MacConkey Agar Medium. When such enzyme substrate media are used, incubate under the conditions indicated for each medium.

#### 3.2.2.3. Interpretation

Growth of brick-red colonies surrounded by a reddish precipitation line on MacConkey Agar Medium or of colonies showing aspects or responses corresponding to *E. coli* on an enzyme substrate medium for *E. coli* indicates the possible presence of *E. coli*. This is confirmed by identification tests.

Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result, and determine the probable number of *E. coli* from Table 5.02-II-3.

### 3.3. Salmonella

#### 3.3.1. Sample preparation and pre-incubation

Inoculate 10 g or 10 mL of the product to be examined to a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium, mix and incubate at 30 – 35°C for 18 – 24 hours.

#### 3.3.2. Selection and subculture

Inoculate 0.1 mL of Fluid Soybean-Casein Digest Medium to 10 mL of Fluid Rappaport Vassiliadis Salmonella Enrichment Broth Medium and incubate at 42 ± 0.5°C for 18 – 24 hours. Transfer on plates of XLD (Xylose-Lysine-Desoxycholate) Agar Medium, and incubate at 30 – 35°C for 18 – 48 hours. An appropriate enzyme substrate medium such as CHS Agar Medium or ES II Agar Medium may be used instead of XLD Agar Medium. When such enzyme substrate media are used, incubate under the conditions indicated for each medium.

#### 3.3.3. Interpretation

The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centers or of colonies showing aspects or responses corresponding to *Salmonella*. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

**Table 5.02-II-3** Interpretation of results

Results for each quantity of product			Probable number of microorganisms per gram or mL
0.1 g or 0.1 mL	0.01 g or 0.01 mL	0.001 g or 0.001 mL	
+	+	+	more than 10 <sup>3</sup>
+	+	-	less than 10 <sup>3</sup> and more than 10 <sup>2</sup>
+	-	-	less than 10 <sup>2</sup> and more than 10
-	-	-	less than 10

**Table 5.02-II-4** Morphologic characteristics of *Staphylococcus aureus* on selective agar media

Medium	Colonial characteristic
Vogel-Johnson Agar Medium	Black surrounded by a yellow zone
Baird-Parker Agar Medium	Black, shiny, surrounded by a clear zone
Mannitol-Salt Agar Medium	Yellow colonies surrounded by a yellow zone

The following section is given for information.

### 3.4. *Staphylococcus aureus*

#### 3.4.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. Total Viable Aerobic Count and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium and homogenise.

Incubate at 30 – 35°C for 24 – 48 hours.

#### 3.4.2. Selection and enrichment subculture

To 9 mL of Fluid Soybean-Casein Digest Medium with 7.5% Sodium Chloride add 1 mL of Fluid Soybean-Casein Digest Medium and incubate at 30 – 35°C for 24 – 48 hours.

#### 3.4.3. Selection and subculture

If the growth is apparent, take a portion of the culture fluid by means of an inoculating loop and streak it on the surface of either Vogel-Johnson Agar Medium, Baird-Parker Agar Medium or Mannitol-Salt Agar Medium, and incubate at 30 – 35°C for 24 – 48 hours.

#### 3.4.4. Interpretation

The product complies with the test if colonies of the characteristics described in Table 5.02-II-4 are not present or if the confirmatory identification tests are negative.

### 4. Recommended Solutions, Culture Media and Test Solutions

The following solutions, culture media and test solutions have been found satisfactory for the purposes for which they are prescribed in the test for microbial contamination in the Pharmacopoeia. Other media may be used provided that their suitability can be demonstrated.

#### (i) Phosphate Buffer (pH 7.2)

Prepare a mixture of water and stock buffer solution (800:1 V/V) and sterilize.

*Stock buffer solution:* Transfer 34 g of potassium dihydrogen phosphate to a 1000 mL volumetric flask, dissolve in 500

mL of water, adjust to pH 7.0 to 7.4 with sodium hydroxide TS, add water to volume and mix. Dispense in containers and sterilize. Store at a temperature of 2 – 8°C.

(ii) Buffered Sodium Chloride-Peptone Solution (pH 7.0)

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate (equivalent to 0.067 mol phosphate)	7.2 g
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Water	1000 mL

Sterilize in an autoclave using a validated cycle.

(iii) Fluid Soybean-Casein Digest Medium

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate	2.5 g
Water	1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Sterilize in an autoclave using a validated cycle.

(iv) Soybean-Casein Digest Agar Medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Sterilize in an autoclave using a validated cycle.

(v) Sabouraud Glucose Agar Medium

Glucose	40.0 g
Peptones (animal tissue and casein 1:1)	10.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25°C. Sterilize in an autoclave using a validated cycle. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

(vi) Potato Dextrose Agar Medium

Infusion from potatoes	200 g
Glucose	20.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25°C. Sterilize in an autoclave using a validated cycle.

(vii) Fluid Sabouraud Glucose Medium

Glucose	20.0 g
Peptones (animal tissue and casein 1:1)	10.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25°C. Sterilize in an autoclave using a validated cycle.

(viii) Fluid Enterobacteria Enrichment Broth Mossel Medium

Gelatin peptone	10.0 g
Glucose monohydrate	5.0 g
Bile salts	20.0 g
Potassium dihydrogen phosphate	2.0 g
Disodium hydrogen phosphate dihydrate	8.0 g
Brilliant green	15 mg
Water	1000 mL

Adjust the pH so that after heating it is 7.0 – 7.4 at 25°C.

Heat at 100°C for 30 min and cool immediately.

(ix) VRB (Violet/Red/Bile) Agar with glucose

Yeast extract	3.0 g
Gelatin peptone	7.0 g
Bile salts	1.5 g
Sodium chloride	5.0 g
Glucose monohydrate	10.0 g
Agar	15.0 g
Neutral red	30 mg
Crystal violet	2 mg
Water	1000 mL

Adjust the pH so that after heating it is 7.2 – 7.6 at 25°C. Heat to boiling; do not heat in autoclave.

(x) Fluid MacConkey Broth Medium

Gelatin peptone	20.0 g
Lactose monohydrate	10.0 g
Dehydrated ox bile	5.0 g
Bromocresol purple	10 mg
Water	1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Sterilize in an autoclave using a validated cycle.

(xi) MacConkey Agar Medium

Gelatin peptone	17.0 g
Peptones (meat and casein)	3.0 g
Lactose monohydrate	10.0 g
Sodium chloride	5.0 g
Bile salts	1.5 g
Agar	13.5 g
Neutral red	30 mg
Crystal violet	1 mg
Water	1000 mL

Adjust the pH so that after sterilization it is 6.9 – 7.3 at 25°C. Boil for 1 min with constant shaking then sterilize in an autoclave using a validated cycle.

(xii) Fluid Rappaport Vassiliadis Salmonella Enrichment Medium

Soya peptone	4.5 g
Magnesium chloride hexahydrate	29.0 g
Sodium chloride	8.0 g
Dipotassium hydrogen phosphate	0.4 g
Potassium dihydrogen phosphate	0.6 g
Malachite green	36 mg
Water	1000 mL

Dissolve, warming slightly. Sterilize in an autoclave using a validated cycle, at a temperature not exceeding 115°C. The pH is to be 5.0 – 5.4 at 25°C after heating and autoclaving.

(xiii) XLD (Xylose-Lysine-Desoxycholate) Agar Medium

Xylose	3.5 g
L-Lysine	5.0 g
Lactose monohydrate	7.5 g
Sucrose	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red	80 mg
Agar	13.5 g
Sodium desoxycholate	2.5 g
Sodium thiosulfate	6.8 g
Ammonium iron (III) citrate	0.8 g
Water	1000 mL

Adjust the pH so that after heating it is 7.2 – 7.6 at 25°C. Heat to boiling, cool to 50°C and pour into Petri dishes. Do not heat in autoclave.

(xiv) Fluid Soybean-Casein Digest Medium with 7.5% Sodium Chloride

Pancreatic digest of casein	17.0 g	Water	1000 mL
Soybean peptone	3.0 g	Adjust the pH so that after heating it is 5.8 – 6.2 at 25°C.	
Sodium chloride	75.0 g	Sterilize in an autoclave using a validated cycle, or heat to boiling, cool to 50°C and pour into Petri dishes.	
Dipotassium hydrogen phosphate	2.5 g		
Glucose monohydrate	2.5 g		
Water	1000 mL		
To (iii) Fluid Soybean-Casein Digest Medium (containing 5.0 g of sodium chloride) add 70.0 g of sodium chloride, mix, and adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Sterilize in an autoclave using a validated cycle.			
(xv) Vogel-Johnson Agar Medium			
Casein peptone	10.0 g	Peptone	5.0 g
Yeast extract	5.0 g	Potassium nitrate	1.0 g
D-Mannitol	10.0 g	Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	5.0 g	Sodium lauryl sulfate	0.1 g
Lithium chloride	5.0 g	Sodium pyruvate	1.0 g
Glycine	10.0 g	Isopropyl-β-thiogalactopyranoside	0.1 g
Phenol red	25 mg	Potassium dihydrogen phosphate	1.0 g
Agar	16.0 g	Dipotassium hydrogen phosphate	4.0 g
Water	1000 mL	5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside	0.1 g
Mix all the components and boil for 1 min to resolve. Adjust the pH so that after sterilization it is 7.0 – 7.4. After sterilization in an autoclave using a validated cycle, cool to 45 – 50°C, add 20 mL of a sterile solution of potassium tellurite (1 in 100), and mix.		4-Methylumbelliferyl-β-D-glucuronide	0.1 g
(xvi) Baird-Parker Agar Medium		Water	1000 mL
Pancreatic digest of casein	10.0 g	Adjust the pH so that after sterilization it is 6.9 – 7.3 at 25°C. Sterilize in an autoclave using a validated cycle.	
Meat extract	5.0 g		
Yeast extract	1.0 g	Enzyme substrate media for <i>Salmonella</i>	
Lithium chloride	5.0 g	Use the media shown below as examples and that have been validated their capabilities.	
Glycine	12.0 g		
Sodium pyruvate	10.0 g	(xx) CHS Agar Medium	
Agar	20.0 g	Peptone	5.0 g
Water	950 mL	Yeast extract	2.0 g
Mix all the components and boil for 1 min with frequent agitation. Adjust the pH so that after sterilization it is 6.6 – 7.0. After sterilization in an autoclave using a validated cycle, cool to 45 – 50°C, add 10 mL of a sterile solution of potassium tellurite (1 in 100) and 50 mL of egg-yolk emulsion, mix gently, and pour into Petri dishes. The egg-yolk emulsion is prepared by mixing egg-yolk and sterile saline in a ratio of about 30% to 70%.		Sodium chloride	0.8 g
(xvii) Mannitol Salt Agar Medium		Other salts	7.2 g
Casein peptone	5.0 g	A mixture of selecting agent and particular enzyme substrate	
Animal tissue peptone	5.0 g	4.9 g	
Beef extract	1.0 g	Ager	15.0 g
D-Mannitol	10.0 g	Water	1000 mL
Sodium chloride	75.0 g	Adjust the pH so that after heating it is 7.4 – 7.8 at 25°C. Heat to boiling, cool to 50°C and pour into Petri dishes. Do not heat in autoclave.	
Agar	15.0 g		
Phenol red	25 mg	(xxi) ESII Agar Medium	
Water	1000 mL	Peptone	10.0 g
Heat to boiling for 1 min with shaking. Adjust the pH so that after sterilization it is 7.2 – 7.6 at 25°C. Sterilize in an autoclave using a validated cycle.		Yeast extract	1.0 g
Enzyme substrate media for <i>Escherichia coli</i>		Sodium chloride	5.0 g
Use the media shown below as examples and that have been validated their capabilities.		Disodium hydrogen phosphate	1.0 g
(xviii) CHE Ager Medium		Sodium thiosulfate	1.0 g
Casein peptone	5.0 g	Sodium deoxycholate	1.0 g
A mixture of yeast extract and meat extract	3.3 g	D-Mannitol	15.0 g
A mixture of selecting agent and particular enzyme substrate	9.0 g	Neutral red	30 mg
Sodium chloride	5.0 g	Synthetic enzyme substract	0.45 g
Agar	15.0 g	Novobiocin	0.02 g
Phenol red	25 mg	Ager	15.0 g
Water	1000 mL	Water	1000 mL
Heat to boiling for 1 min with shaking. Adjust the pH so that after sterilization it is 7.2 – 7.6 at 25°C. Sterilize in an autoclave using a validated cycle.		Adjust the pH so that after sterilization it is 6.9 – 7.3 at 25°C. Sterilize in an autoclave using a validated cycle. Cool to 50°C and pour into Petri dishes.	
(xxii) Amphotericin B TS: Dissolve 22.5 mg of amphotericin B powder in 9 mL of sterile purified water.			
Amphotericin B powder: Amphotericin B that is added sodium deoxycholate, sterilized by γ-ray.			
(xxiii) Rose bengal TS: Dissolve 1 g of rose bengal in water to make 100 mL.			
(xxiv) TTC TS: Dissolve 0.8 g of 2,3,5-triphenyl-2H-tetrazolium chloride in water to make 100 mL, divide into small tubes, and sterilize in an autoclave using a validated cycle. Store protected from light.			
<b>Preparation method</b>			
(i) Ager medium with TTC: Just prior to use, add 2.5 to 5 mL of TTC TS per liter (20 to 40 mg/L) of sterile ager medium and mix.			

(ii) Ager medium with amphotericin B: Just prior to use, add 2 mL of amphotericin B TS per liter (5 mg/L) of agar medium previously sterilized in an autoclave using a validated cycle, and mix.

(iii) Ager medium with rose bengal: Add 5 mL of rose bengal TS per liter (50 mg/L) of agar medium, mix, and sterilize in an autoclave using a validated cycle.

## 6. Tests for Preparations

### 6.01 Test for Metal Particles in Ophthalmic Ointments

Test of Metal Particles in Ophthalmic Ointments is a method to test the existence of foreign metal particles in the ophthalmic ointments described in General Rules for Preparations.

#### 1. Preparation of test sample

The test should be carried out in a clean place. Take 10 ophthalmic ointments to be tested, and extrude 5 g each of their contents into separate flat-bottomed petri dishes 60 mm in diameter. Cover the dishes, and heat between 85°C and 110°C for 2 hours to dissolve bases completely. Allow the samples to cool to room temperature without agitation to solidify the contents. When the amount of the content is less than 5 g, extrude the contents as completely as practicable, and proceed in the same manner as described above.

#### 2. Procedure

Invert each dish on the stage of a suitable microscope previously adjusted to provide not less than 40 times magnifications and equipped with an eyepiece micrometer disk. Each dish is illuminated from above 45° relative to the plane of the dish. Examine the entire bottom of each dish for metal particles, and record the total number of particles, measuring 50 µm or more in any dimension.

Note: Use petri dishes with a clean bottom and free from foams and scratches, and if possible, the walls are at right angles with the bottom.

#### 3. Evaluation

The preparation complies with the test if the total number of metal particles of a size equal to or greater than 50 µm found in 10 units tested, is not more than 50, and also the number of dishes containing more than 8 particles is not more than 1. If this requirement is not met, repeat the test with a further 20 units in the same manner, and if the total number of the particles found in the total of 30 units is not more than 150, and also the number of dishes containing more than 8 particles is not more than 3, the preparation complies with the test.

## 6.02 Uniformity of Dosage Units

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (♦ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◊ ◇).

Information on the harmonization with the European

Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

The term "Uniformity of dosage unit" is defined as the degree of uniformity in the amount of the drug substance among dosage units. Therefore, the requirements of this chapter apply to each drug substance being comprised in dosage units containing one or more drug substances, unless otherwise specified elsewhere in this Pharmacopoeia.

To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage forms such as tablets, capsules, packets of powders or granules, ampoules, contain a single dose or a part of a dose of a drug substance in each dosage unit. The Uniformity of Dosage Units specification is not intended to apply to solutions, suspensions, emulsions, or gels in unit-dose containers intended for external, cutaneous administration.

The uniformity of dosage units can be demonstrated by either of two methods, *Content uniformity* or *Mass variation* (see Table 6.02-1). The test for *Content Uniformity* of preparations presented in dosage units is based on the assay of the individual contents of drug substance(s) of a number of dosage units to determine whether the individual contents are within the limits set. The *Content Uniformity* method may be applied in all cases.

The test for *Mass Variation* is applicable for the following dosage forms:

(i) solutions enclosed in unit-dose containers and into soft capsules ◇ in which all components are perfectly dissolved ◇;

(ii) solids (including powders, granules and sterile solids) that are packaged in single-dose packages and contain no active or inactive added substances;

(iii) solids (including sterile solids) that are packaged in single-dose packages, with or without active or inactive added substances, that have been prepared from true solutions ◇ in which all components are perfectly dissolved ◇ and freeze-dried in the final packages and are labeled to indicate this method of preparation; and

(iv) hard capsules, uncoated tablets, or film-coated tablets, containing 25 mg or more of a drug substance comprising 25% or more, by weight, of the dosage unit or, in the case of hard capsules, the capsule contents, ◇ or in the case of film-coated tablets, the pre-coated tablets, ◇ except that uniformity of other drug substances present in lesser proportions is demonstrated by meeting *Content Uniformity* requirements.

The test for *Content Uniformity* is required for all dosage forms not meeting the above conditions for the *Mass Variation* test. Alternatively, products listed in item (iv) above that do not meet the 25 mg/25% threshold limit may be tested for uniformity of dosage units by *Mass Variation* instead of the *Content Uniformity* test if the concentration relative standard deviation (RSD) of the drug substance in the final dosage units is not more than 2%, based on process validation data and development data, and if there has been regulatory approval of such a change. The concentration RSD is the RSD of the concentration per dosage unit (w/w or w/v), where concentration per dosage unit equals the assay result per dosage unit divided by the individual dosage unit weight. See the RSD formula in Table 6.02-2.

#### 1. Content Uniformity

Select not less than 30 units, and proceed as follows for the dosage form designated.

Where different procedures are used for assay of the

**Table 6.02-1** Application of Content Uniformity (CU) and Mass Variation (MV) Test for dosage forms

Dosage Forms	Type	Sub-type	Dose and ratio of drug substance	
			≥ 25 mg & ≥ 25%	< 25 mg or < 25%
Tablets	uncoated		MV	CU
	coated	Film	MV	CU
		Others	CU	CU
Capsules	hard		MV	CU
	soft	Sus., eml., gel	CU	CU
		Solutions	MV	MV
Solids in single-dose packages ◇(divided forms, lyophilized forms, et al.)◇	Single component		MV	MV
	Multiple components	Freeze-dried from solutions in final container	MV	MV
		Others	CU	CU
Solutions ◇(perfectly dissolved)◇ enclosed in unit-dose containers			MV	MV
Others—Among the preparations not classified as the above dosage forms in this table, suppositories, percutaneous absorption type preparations (patches), semi-solid dosage forms intended for application of active pharmaceutical ingredients to the skin for the purpose of systemic action, and the like.			CU	CU

Sus.: suspension; eml.: emulsion;

preparation and for the content uniformity test, it may be necessary to establish a correction factor to be applied to the results of the latter.

(i) Solid dosage forms: Assay 10 units individually using an appropriate analytical method. Calculate the acceptance value (see Table 6.02-2).

(ii) Liquid or Semi-Solid dosage forms: Assay 10 units individually using an appropriate analytical method. Carry out the assay on the amount of well-mixed material that is removed from an individual container in conditions of normal use and express the results as delivered dose. Calculate the acceptance value (see Table 6.02-2).

### 1.1. Calculation of Acceptance Value

Calculate the acceptance value by the formula:

$$|M - \bar{X}| + ks,$$

in which the terms are as defined in Table 6.02-2.

### 2. Mass Variation

◇*Mass Variation* is carried out based on the assumption that the concentration (mass of drug substance per mass of dosage unit) is uniform in a lot.◇

Carry out an assay for the drug substance(s) on a representative sample of the batch using an appropriate analytical method. This value is result A, expressed as % of label claim (see *Calculation of the Acceptance Value*). Select not less than 30 dosage units, and proceed as follows for the dosage form designated.

(i) Uncoated or Film-coated Tablets: Accurately weigh 10 tablets individually. Calculate the content, expressed as % of label claim, of each tablet from the mass of the individual tablets and the result of the assay. Calculate the acceptance value.

(ii) Hard Capsules: Accurately weigh 10 capsules indi-

vidually, taking care to preserve the identity of each capsule. Remove the contents of each capsule by suitable means. Accurately weigh the emptied shells individually, and calculate for each capsule the net mass of its contents by subtracting the mass of the shell from the respective gross mass. Calculate the drug substance content of each capsule from the mass of the individual capsules and the result of the assay. Calculate the acceptance value.

(iii) Soft Capsules: Accurately weigh the 10 intact capsules individually to obtain their gross masses, taking care to preserve the identity of each capsule. Then cut open the capsules by means of a suitable clean, dry cutting instrument such as scissors or a sharp open blade, and remove the contents by washing with a suitable solvent. Allow the occluded solvent to evaporate from the shells at room temperature over a period of about 30 minutes, taking precautions to avoid uptake or loss of moisture. Weigh the individual shells, and calculate the net contents. Calculate the drug substance content in each capsule from the mass of product removed from the individual capsules and the result of the assay. Calculate the acceptance value.

(iv) Solid dosage forms other than tablets and capsules: Proceed as directed for *Hard Capsules*, treating each dosage unit as described therein. Calculate the acceptance value.

(v) Liquid dosage forms: Accurately weigh the amount of liquid that is removed from each of 10 individual containers in conditions of normal use. If necessary, compute the equivalent volume after determining the density. Calculate the drug substance content in each container from the mass of product removed from the individual containers and the result of the assay. Calculate the acceptance value.

### 2.1. Calculation of Acceptance Value

Calculate the acceptance value as shown in *Content Uniformity*, except that ◇the value of  $\bar{X}$  is replaced with A,

Table 6.02-2

Variable	Definition	Conditions	Value
$\bar{X}$	Mean of individual contents ( $x_1, x_2, \dots, x_n$ ) expressed as a percentage of the label claim		
$x_1, x_2, \dots, x_n$	Individual contents of the dosage units tested, expressed as a percentage of the label claim		
$n$	Sample size (number of dosage units in a sample)		
$k$	Acceptability constant	If $n = 10$ , then If $n = 30$ , then	2.4 2.0
$s$	Sample standard deviation		$\sqrt{\frac{\sum_{i=1}^n (x_i - \bar{X})^2}{n - 1}}$
RSD	Relative standard deviation (the sample standard deviation expressed as a percentage of the mean)		$\frac{100s}{\bar{X}}$
$M$ (case 1)	Reference value	If $98.5\% \leq \bar{X} \leq 101.5\%$ , then	$M = \bar{X}$ ( $AV = ks$ )
To be applied when $T \leq 101.5$		If $\bar{X} < 98.5\%$ , then	$M = 98.5\%$ ( $AV = 98.5 - \bar{X} + ks$ )
		If $\bar{X} > 101.5\%$ , then	$M = 101.5\%$ ( $AV = \bar{X} - 101.5 + ks$ )
$M$ (case 2)	Reference value	If $98.5\% \leq \bar{X} \leq T$ , then	$M = \bar{X}$ ( $AV = ks$ )
To be applied when $T > 101.5$		If $\bar{X} < 98.5\%$ , then	$M = 98.5\%$ ( $AV = 98.5 - \bar{X} + ks$ )
		If $\bar{X} > T$ , then	$M = T\%$ ( $AV = \bar{X} - T + ks$ )
Acceptance Value ( $AV$ )			General formula: $ M - \bar{X}  + ks$ [Calculations are specified above for the different cases.]
$L1$	Maximum allowed acceptance value		$L1 = 15.0$ unless otherwise specified.
$L2$	Maximum allowed range for deviation of each dosage unit tested from the calculated value of $M$	On the low side, no dosage unit result can be less than $0.75M$ while on the high side, no dosage unit result can be greater than $1.25M$ (This is based on an $L2$ value of 25.0.)	$L2 = 25.0$ unless otherwise specified.
$T$	Target content per dosage unit at time of manufacture, expressed as the percentage of the label claim. Unless otherwise stated, $T$ is 100.0%, or $T$ is the manufacturer's approved target content per dosage unit.		

and that  $\diamond$  the individual contents of the dosage units are replaced with the individual estimated contents defined below.

$x_1, x_2 \dots x_n$ : individual estimated contents of the dosage units tested

$$x_i = w_i \times \frac{A}{\bar{W}}$$

$w_1, w_2 \dots w_n$ : Individual masses of the dosage units tested  
 $A$ : Content of drug substance (% of label claim) obtained using an appropriate analytical method

$\bar{W}$ : Mean of individual masses ( $w_1, w_2 \dots w_n$ )

### 3. Criteria

Apply the following criteria, unless otherwise specified.

(i) Solid, Semi-Solid and Liquid dosage forms: The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to

$L1\%$ . If the acceptance value is greater than  $L1\%$ , test the next 20 dosage units and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage units is less than or equal to  $L1\%$  and no individual content of the dosage unit is less than  $(1 - L2 \times 0.01)M$  nor more than  $(1 + L2 \times 0.01)M$  in *Calculation of Acceptance Value* under *Content Uniformity* or under *Mass Variation*. Unless otherwise specified,  $L1$  is 15.0 and  $L2$  is 25.0.

## 6.03 Particle Size Distribution Test for Preparations

Particle Size Distribution Test for Preparations is a method to determine the particle size distribution of preparations described in General Rules for Preparations.

### 1. Procedure

The test is performed employing No. 18 ( $850\text{ }\mu\text{m}$ ) and No. 30 ( $500\text{ }\mu\text{m}$ ) sieves with the inside diameter of 75 mm.

Weigh accurately 10.0 g of sample to be tested, and place on the uppermost sieve which is placed on the other sieves described above and a close-fitting receiving pan and is covered with a lid. Shake the sieves in a horizontal direction for 3 minutes, and tap slightly at intervals. Weigh the amount remaining on each sieve and in the receiving pan.

## 6.04 Test for Acid-neutralizing Capacity of Gastrointestinal Medicines

Test for Acid-neutralizing Capacity of Gastrointestinal Medicines is a test to determine the acid-neutralizing capacity of a medicine, as a crude material or preparation, which reacts with the stomach acid and exercises an acid control action in the stomach. When performing the test according to the following procedure, the acid-neutralizing capacity of a crude material is expressed in terms of the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per g of the material, and that of a preparation is expressed by the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per dose per day (when the daily dose varies, the minimum dose is used).

### 1. Preparation of sample

A crude material and a solid preparation which conforms to Powders in the General Rules for Preparations: may be used, without any treatment, as the sample. Preparations in dose-unit packages: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, mix uniformly, and use the mixture as the sample. Granules in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, powder it, and use as the sample. Granules not in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: take not less than 20 doses, powder it, and use as the sample. Capsules and tablets: take not less than 20 doses, weigh accurately, calculate the average mass for a daily dose, powder it, and use as the sample. Liquid preparations: shake well, and use as the sample.

### 2. Procedure

Take an amount of the sample so that 'a' in the equation falls between 20 mL and 30 mL, and perform the test.

Accurately weigh the sample of the crude material or preparation, and place it in a glass-stoppered 200-mL flask. Add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper tightly, shake at  $37 \pm 2^\circ\text{C}$  for 1 hour, and filter. Take precaution against gas to be generated on the addition of 0.1 mol/L hydrochloric acid VS, and stopper tightly. After cooling, filter the solution again, if necessary. Pipet 50 mL of the filtrate, and titrate <2.50> the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination <2.54>, end point: pH 3.5). Perform a blank determination in the same manner.

For liquid preparations, pipet the sample in a 100-mL volumetric flask, add water to make 45 mL, then add exactly 50 mL of 0.2 mol/L hydrochloric acid VS while shaking. Add water again to make the solution 100 mL. Transfer the

solution to a glass-stoppered 200-mL flask, wash the residue with 20.0 mL of water, stopper tightly, shake at  $37 \pm 2^\circ\text{C}$  for 1 hour, and filter. Pipet 60 mL of the filtrate, and titrate <2.50> the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination <2.54>, end point: pH 3.5). Perform a blank determination in the same manner.

Acid-neutralizing capacity (amount of 0.1 mol/L hydrochloric acid VS consumed per g or daily dose) (mL)

$$= (b - a) \times 2 \times (t/s)$$

a: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

b: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank determination

t: 1000 mg of crude material, or daily dose of preparation (in mg of solid preparation, mL of liquid preparation)

s: Amount of the sample taken (in mg of crude material and solid preparation, mL of liquid preparation)

## 6.05 Test for Extractable Volume of Parenteral Preparations

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

♦Test for Extractable Volume of Parenteral Preparations is performed to confirm that a slightly excess volume is filled for the nominal volume to be withdrawn. Injections may be supplied in single-dose containers such as ampoules or plastic bags, or in multi-dose containers filled with a volume of injection which is sufficient to permit administration of the nominal volume declared on the label. The excess volume is determined by the characteristics of the product.◆

Suspensions and emulsions must be shaken before withdrawal of the contents and before the determination of the density. Oily and viscous preparations may be warmed according to the instructions on the label, if necessary, and thoroughly shaken immediately before removing the contents. The contents are then cooled to  $20 - 25^\circ\text{C}$  before measuring the volume.

### 1. Single-dose containers

Select one container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. Take up individually the total contents of each container selected into a dry syringe of a capacity not exceeding three times the volume to be measured, and fitted with a 21-gage needle not less than 2.5 cm in length. Expel any air bubbles from the syringe and needle, then discharge the contents of the syringe without emptying the needle into a standardised dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40% of its graduated volume. Alternatively, the volume of the contents in milliliters may be calculated as the mass in grams divided by the density.

For containers with a nominal volume of 2 mL or less the contents of a sufficient number of containers may be pooled to obtain the volume required for the measurement provided

that a separate, dry syringe assembly is used for each container. The contents of containers holding 10 mL or more may be determined by opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is not less than the nominal volume in case of containers examined individually, or, in case of containers with a nominal volume of 2 mL or less, is not less than the sum of the nominal volumes of the containers taken collectively.

## 2. Multi-dose containers

For injections in multidose containers labeled to yield a specific number of doses of a stated volume, select one container and proceed as directed for single-dose containers using the same number of separate syringe assemblies as the number of doses specified. The volume is such that each syringe delivers not less than the stated dose.

## 3. Cartridges and pre-filled syringes

Select one container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. If necessary, fit the containers with the accessories required for their use (needle, piston, syringe) and transfer the entire contents of each container without emptying the needle into a dry tared beaker by slowly and constantly depressing the piston. Determine the volume in milliliters calculated as the mass in grams divided by the density.

The volume measured for each of the containers is not less than the nominal volume.

## 4. Parenteral infusions

Select one container. Transfer the contents into a dry measuring cylinder of such a capacity that the volume to be determined occupies at least 40% of the nominal volume of the cylinder. Measure the volume transferred.

The volume is not less than the nominal volume.

constituted must be free from foreign insoluble matters that is clearly detectable when inspected against both a white and a black background for 5 seconds each time with the unaided eyes at a position of light intensity of 2000 to 3750 lx under a white light source. The inspection time should be extended accordingly if the inspection is not easy.

# 6.07 Insoluble Particulate Matter Test for Injections

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Insoluble particulate matters in injections and parenteral infusions consist of extraneous, mobile undissolved particles, other than gas bubbles, that are unintentionally present in the solutions.

For the determination of particulate contamination, 2 procedures, Method 1 (Light Obscuration Particle Count Test) and Method 2 (Microscopic Particle Count Test), are specified hereinafter. When examining injections and parenteral infusions for sub-visible particles, Method 1 is preferably applied. However, it may be necessary to test some preparations by Method 1 followed by Method 2 to reach a conclusion on conformance to the requirements.

Not all parenteral preparations can be examined for sub-visible particles by one or both of these methods. When Method 1 is not applicable, e.g. in case of preparations having reduced clarity or increased viscosity, the test should be carried out according to Method 2. Emulsions, colloids, and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor may also require microscopic particle count testing. If the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

The results obtained in examining a discrete unit or group of units for particulate contamination cannot be extrapolated with certainty to other units that remain untested. Thus, statistically sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterise the level of particulate contamination in a large group of units.

## 1. Method 1. Light Obscuration Particle Count Test

### 1.1. Apparatus

Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size. ♦It is necessary to perform calibration, as well as to demonstrate the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and counting accuracy, at least once a year.◆

#### ♦1.1.1. Calibration

Particles to be used for calibration should be subject to particle size sensitivity measurement, using spherical polystyrene particles having at least 5, 10 and 25  $\mu\text{m}$  in diameter (PSL particles) in mono-dispersed suspension. The PSL par-

ticles should have either a domestic or international traceability in terms of length, with a level of uncertainty at not greater than 3%. The particles to be used for calibration should be dispersed in *particle-free water*.

#### 1.1.1.1. Manual method

The particle size response of the system to be applied should be determined using at least 3 channels for threshold-voltage setting, according to the half counting method of window moving type. The threshold-voltage window should be  $\pm 20\%$  of the measuring particle size. After measuring the sensitivity of response for the designated particle size, the size response curve is prepared by the method indicated by the manufacturer from particle-response measuring point, and threshold-voltage of 5, 10 and 25  $\mu\text{m}$  of the apparatus is obtained.

#### 1.1.1.2. Electronic method

In the use of multichannel peak height analyzer, the particle size response is measured by half-count method of moving window system same as the manual method, and the particle size response curve is prepared by the method designated by the instrument manufacturer, then, the threshold-voltage of 5, 10 and 25  $\mu\text{m}$  of the apparatus is obtained. In this case, the instrument manufacturer or the user should validate the obtainability of the same result as that of the manual method.

#### 1.1.1.3. Automated method

The particle size response curve of the apparatus may be obtained by using the software developed by the user or supplied by the instrument manufacturer, whereas the manufacturer or the user should validate the obtainability of the same result as that of the manual method.

#### 1.1.2. Sample volume accuracy

Sample volume accuracy should fall within 5% of the measuring value in case the decrease of test solution is measured by the mass method after measuring the test solution of 10 mL.

#### 1.1.3. Sample flow rate

The flow rate of the sample indicated into the sensor should be calculated from the observed sample volume and time, and should be conformed within the range of the manufacturer's specification for sensor used.

#### 1.1.4. Sensor

There is a possibility of changes of particle size resolution and counting rate of particle-detecting sensor in each sensor by assembling accuracy and parts accuracy even in the same type sensor. The threshold accuracy also needs to be confirmed. Testing should accordingly be performed for each of particle size resolution, accuracy in counting and in threshold setting, using Particle Count Reference Standard Suspension (PSL) spheres having mean diameter of approximately 10  $\mu\text{m}$ , of a concentration at 1000 particles/mL  $\pm 10\%$ , not more than 5% of CV value).

During measurement, stirring should be made for assuring the uniformity in sample concentration.

#### 1.1.4.1. Sensor resolution (Particle size resolution of apparatus)

Measurement should be made by either one of the following methods. The difference between the threshold of particle size counting 16% and 84% of the total counts and the test-particle size should be within 10%, whereas, electronic method and automated method should be both validated for obtaining the same result as that of the manual method.

(i) Manual method to obtain the spread of histogram prepared from the counting value of the apparatus.

(ii) Electronic method to obtain the spread of histogram of the classification of system-responding signal by using the multichannel peak height analyzer.

(iii) Automated method to obtain the spread of histogram of responsive signal of the test-particle by using the software prepared by the manufacturer or the user.

#### 1.1.4.2. Particle counting accuracy

Data obtained by counting particles of 5  $\mu\text{m}$  and greater should be 763 to 1155 particles per mL.

#### 1.1.4.3. Threshold accuracy

Particle size calculated from a threshold corresponding to 50% counts for particles of 5  $\mu\text{m}$  and greater should fall within  $\pm 5\%$  of the mean diameter of the test particles.◆

### 1.2. General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with *particle-free water*.

Take care not to introduce air bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out.

In order to check that the environment is suitable for the test, that the glassware is properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of 5 samples of *particle-free water*, each of 5 mL, according to the method described below. If the number of particles of 10  $\mu\text{m}$  or greater size exceeds 25 for the combined 25 mL, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware and water are suitable for the test.

#### 1.3. Method

Mix the contents of the sample by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of *particle-free water* and remove the closure, avoiding any contamination of the contents. Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 minutes or sonicating.

For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container to obtain a volume of not less than 25 mL; where justified and authorised, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with *particle-free water* or with an appropriate solvent without contamination of particles when *particle-free water* is not suitable.

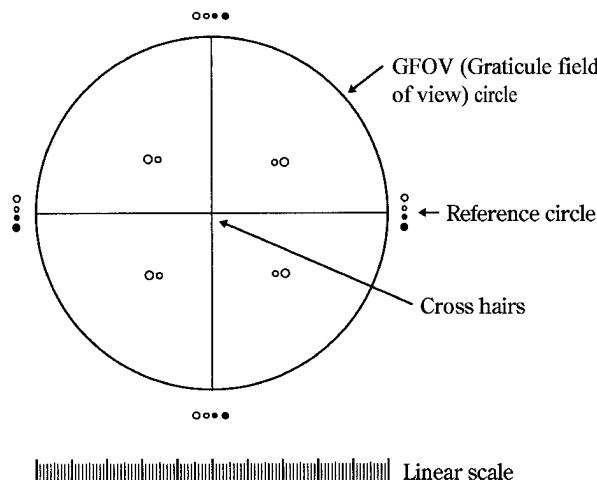
Powders for parenteral use are reconstituted with *particle-free water* or with an appropriate solvent without contamination of particles when *particle-free water* is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Remove 4 portions, each of not less than 5 mL, and count the number of particles equal to or greater than 10  $\mu\text{m}$  and 25  $\mu\text{m}$ . Disregard the result obtained for the first portion, and calculate the mean number of particles for the preparation to be examined.

#### 1.4. Evaluation

If the average number of particles exceeds the limits, test the preparation by Method 2 (Microscopic Particle Count



**Fig. 6.07-1 Circular diameter graticule**

Test).

**Test 1.A—Solutions for injection supplied in containers with a nominal content of equal to or more than 100 mL**  
The preparation complies with the test if the average number of particles present in the units tested does not exceed 25 per milliliter equal to or greater than 10  $\mu\text{m}$  and does not exceed 3 per milliliter equal to or greater than 25  $\mu\text{m}$ .

**Test 1.B—Solutions for injection supplied in containers with a nominal content of less than 100 mL**  
The preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than 10  $\mu\text{m}$  and does not exceed 600 per container equal to or greater than 25  $\mu\text{m}$ .

## 2. Method 2. Microscopic Particle Count Test

### 2.1. Apparatus

Use a suitable binocular microscope, filter assembly for retaining particulate contamination and membrane filter for examination.

The microscope is equipped with an ocular micrometer calibrated with an objective micrometer, a mechanical stage capable of holding and traversing the entire filtration area of the membrane filter, two suitable illuminators to provide episcopic illumination in addition to oblique illumination, and is adjusted to  $100 \pm 10$  magnifications. The ocular micrometer is a circular diameter graticule (see Fig. 6.07-1) and consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles 10  $\mu\text{m}$  and 25  $\mu\text{m}$  in diameter at 100 magnifications, and a linear scale graduated in 10  $\mu\text{m}$  increments. It is calibrated using a stage micrometer that is certified by either a domestic or international standard institution. A relative error of the linear scale of the graticule within  $\pm 2\%$  is acceptable. The large circle is designated the graticule field of view (GFOV).

Two illuminators are required. One is an episcopic bright-field illuminator internal to the microscope, the other is an external, focussable auxiliary illuminator adjustable to give reflected oblique illumination at an angle of 10° to 20°.

The filter assembly for retaining particulate contamination consists of a filter holder made of glass or other suitable material, and is equipped with a vacuum source and a suitable membrane filter.

The membrane filter is of suitable size, black or dark gray in color, non-gridded or gridded, and 1.0  $\mu\text{m}$  or finer in nominal pore size.

### 2.2. General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse both sides of the membrane filter and the equipment from top to bottom, outside and then inside, with *particle-free water*.

In order to check that the environment is suitable for the test, that the glassware and the membrane filter are properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of a 50 mL volume of *particle-free water* according to the method described below. If more than 20 particles 10  $\mu\text{m}$  or larger in size or if more than 5 particles 25  $\mu\text{m}$  or larger in size are present within the filtration area, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, membrane filter and water are suitable for the test.

### 2.3. Method

Mix the contents of the samples by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of *particle-free water* and remove the closure, avoiding any contamination of the contents.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units is combined in a cleaned container; where justified and authorised, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with *particle-free water* or with an appropriate solvent without contamination of particles when *particle-free water* is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are constituted with *particle-free water* or with an appropriate solvent without contamination of particles when *particle-free water* is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several milliliter of *particle-free water*. Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply vacuum. If needed add stepwise a portion of the solution until the entire volume is filtered. After the last addition of solution, begin rinsing the inner walls of the filter holder by using a jet of *particle-free water*. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the filter in a petri dish and allow the filter to air-dry with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light from the illuminating device, and count the number of particles that are equal to or greater than 10  $\mu\text{m}$  and the number of particles that are equal to or greater than 25  $\mu\text{m}$ . Alternatively, partial filter count and determination of the total filter count by calculation is allowed. Calculate the mean number of particles for the preparation to be examined.

The particle sizing process with the use of the circular diameter graticule is carried out by transforming mentally the

image of each particle into a circle and then comparing it to the 10  $\mu\text{m}$  and 25  $\mu\text{m}$  graticule reference circles. Thereby the particles are not moved from their initial locations within the graticule field of view and are not superimposed on the reference circles for comparison. The inner diameter of the transparent graticule reference circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles.

In performing the microscopic particle count test (Method 2) do not attempt to size or enumerate amorphous, semi-liquid, or otherwise morphologically indistinct materials that have the appearance of a stain or discoloration on the membrane filter. These materials show little or no surface relief and present a gelatinous or film-like appearance. In such cases the interpretation of enumeration may be aided by testing a sample of the solution by Method 1.

#### 2.4. Evaluation

*Test 2.A—Solutions for injection supplied in containers with a nominal content of ♦equal to or♦ more than 100 mL*

The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per milliliter equal to or greater than 10  $\mu\text{m}$  and does not exceed 2 per milliliter equal to or greater than 25  $\mu\text{m}$ .

*Test 2.B—Solutions for injection supplied in containers with a nominal content of less than 100 mL*

The preparation complies with the test if the average number of particles present in the units tested does not exceed 3000 per container equal to or greater than 10  $\mu\text{m}$  and does not exceed 300 per container equal to or greater than 25  $\mu\text{m}$ .

#### ♦3. Reagents

*Particle-free water:* The filtered water through a membrane filter with a pore size not exceeding 0.45  $\mu\text{m}$ , containing not more than 5 particles of 10  $\mu\text{m}$  or greater size, and not more than 2 particles of 25  $\mu\text{m}$  or greater size in 10 mL of the insoluble particle number measured by the light obscuration particle counter.♦

with water for particulate matter test.

#### 2. Reagents

(i) Water for particulate matter test: Water prepared before use by filtering through a membrane filter with a pore size not exceeding 0.45  $\mu\text{m}$ . It contains not more than 10 particles of 10  $\mu\text{m}$  or greater size in 100 mL.

#### 3. Procedure

##### 3.1. Aqueous ophthalmic solutions

Carry out all operations carefully in clean equipment and facilities which are low in dust. Fit the membrane filter onto the membrane filter holder, and fix them with the clip. Thoroughly rinse the holder inside with water for particulate matter test, and filter under reduced pressure with 200 mL of water for particulate matter test at a rate of 20 to 30 mL per minute. Apply the vacuum until the surface of the membrane filter is free from water, and remove the membrane filter. Place the filter in a flat-bottomed petri dish with the cover slightly ajar, and dry the filter fully at a temperature not exceeding 50°C. After the filter has been dried, place the petri dish on the stage of the microscope. Under a down-light from an illuminating device, adjust the grid of the membrane filter to the coordinate axes of the microscope, adjust the microscope so as to get the best view of the insoluble particulate matter, then count the number of particles that are equal to or greater than 150  $\mu\text{m}$  within the effective filtering area of the filter, moving the mobile stage, and ascertain that the number is not more than 1. In this case the particle is sized on the longest axis.

Fit another membrane filter to the filtration device, and fix them with the clip, then wet the inside of the filter holder with several mL of water for particulate matter test. Clean the outer surface of the container, and mix the sample solution gently by inverting the container several times. Remove the cap, clean the outer surface of the nozzle, and pour the sample solution into a measuring cylinder which has been rinsed well with water for particulate matter test. Repeat the process to prepare 25 mL of the test solution. Pour the test solution into the filter holder along the inner wall of the holder. Apply the vacuum and filter mildly so as to keep the solution always on the filter. As for viscous sample solution, dilute suitably with water for particulate matter test or suitable diluent and then filter as described above. When the amount of the solution on the filter becomes small, add 30 mL of water for particulate matter test or suitable diluent in such manner as to wash the inner wall of the filter holder. Repeat the process 3 times with 30 mL of the water for particulate matter test. Apply the vacuum gently until the surface of the membrane filter is free from water. Place the filter in a petri dish, and dry the filter at a temperature below 50°C with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, and count the number of particles which are equal to or larger than 300  $\mu\text{m}$  within the effective filtering area of the filter according to the same procedure of the microscope as described above. In this case the particle is sized on the longest axis.

##### 3.2. Ophthalmic solutions which are dissolved before use

Proceed as directed in Aqueous Ophthalmic Solutions after dissolving the sample with the constituted solution.

##### 3.3. Suspension type ophthalmic solutions

Proceed as directed in Aqueous Ophthalmic Solutions. Take 25 mL of the sample in a vessel, which has been rinsed well with water for particulate matter test, add a suitable amount of a suspension-solubilizing solvent or an adequate solvent, shake to dissolve the suspending particles, and use this solution as the sample solution.

Use a membrane filter which is not affected by the solvent to be used.

#### 3.4. Ophthalmic solutions contained in a single-dose container

Proceed as directed in Aqueous Ophthalmic Solutions, using 10 samples for the test. A 13-mm diameter membrane filter and a 4-mm diameter filter holder for retaining insoluble particulate matter are used.

#### 4. Evaluation

The preparation complies with the test if the calculated number per mL of insoluble particles of a size equal to or greater than 300  $\mu\text{m}$  is not more than 1.

## 6.09 Disintegration Test

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

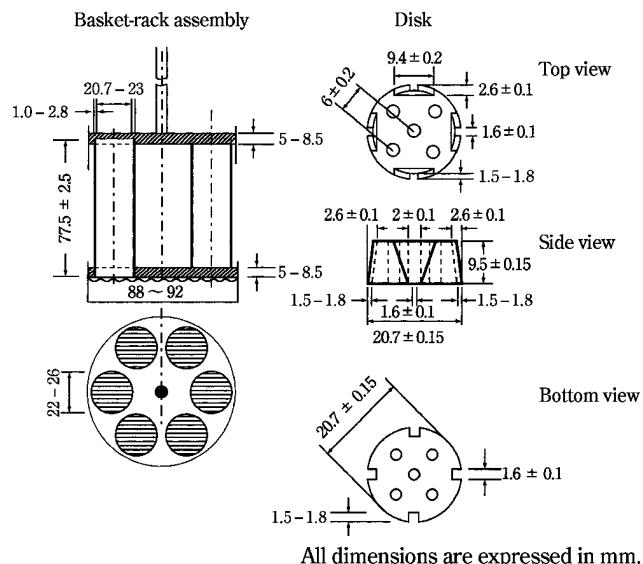
Disintegration Test is provided to determine whether tablets, capsules, ♦granules, dry syrups or pills◆ disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions presented below.

For the purposes of this test, disintegration does not imply complete solution of the unit or even of its active constituent.

#### 1. Apparatus

The apparatus consists of a basket-rack assembly, a 1000-mL, low-form beaker, 138 to 160 mm in height and having an inside diameter of 97 to 115 mm for the immersion fluid, a thermostatic arrangement for heating the fluid between 35° and 39°, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute through a distance of not less than 53 mm and not more than 57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke the wire mesh remains at least 15 mm below the surface of the fluid and descends to not less than 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction is a smooth transition, rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

(i) Basket-rack assembly: The basket-rack assembly consists of six open-ended transparent tubes, each  $77.5 \pm 2.5$  mm long and having an inside diameter of 20.7 to 23 mm and a wall 1.0 to 2.8 mm thick; the tubes are held in a vertical position by two plates, each 88 to 92 mm in diameter and 5 to 8.5 mm in thickness, with six holes, each 22 to 26 mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached to the under surface of the lower plate is a woven stainless steel wire cloth, which has a plain square weave with 1.8- to 2.2-mm apertures and with a wire diameter of 0.57 to 0.66 mm. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plates. A suitable means

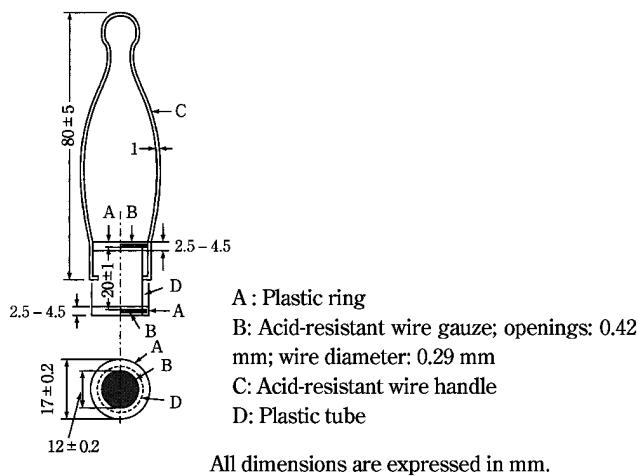


**Fig. 6.09-1** Disintegration apparatus

is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis. The basket-rack assembly conforms to the dimensions found in Fig. 6.09-1. The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained: ♦for example, in order to secure the glass tubes and the upper and the lower plastic plates in position at the top or the bottom, an acid-resistant metal plate, 88 – 92 mm in diameter and 0.5 – 1 mm in thickness, having 6 perforations, each about 22 to 26 mm in diameter, may be used which coincide with those of the upper plastic plate and upper open ends of the glass tubes.◆

(ii) Disks: The use of disks is permitted only where specified or allowed. Each tube is provided with a cylindrical disk  $9.5 \pm 0.15$  mm thick and  $20.7 \pm 0.15$  mm in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Five parallel  $2 \pm 0.1$  mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are centered  $6 \pm 0.2$  mm from the axis on imaginary lines perpendicular to the axis and parallel to each other. Four identical trapezoidal-shaped planes are cut into the wall of the cylinder, nearly perpendicular to the ends of the cylinder. The trapezoidal shape is symmetrical; its parallel sides coincide with the ends of the cylinder and are parallel to an imaginary line connecting the centers of two adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of  $1.6 \pm 0.1$  mm, and its bottom edges lie at a depth of  $1.5 - 1.8$  mm from the cylinder's circumference. The parallel side of the trapezoid on the top of the cylinder has a length of  $9.4 \pm 0.2$  mm, and its center lies at a depth of  $2.6 \pm 0.1$  mm from the cylinder's circumference. All surfaces of the disk are smooth. If the use of disks is specified, add a disk to each tube, and operate the apparatus as directed under Procedure. The disks conform to dimensions found in Fig. 6.09-1. The use of automatic detection employing modified disks is permitted where the use of disks is specified or allowed. Such disks must comply with the requirements for density and dimension given in this chapter.

♦(iii) Auxiliary tube: The auxiliary tube, as illustrated in Fig. 6.09-2, consists of a plastic tube D,  $12 \pm 0.2$  mm in in-



◆Fig. 6.09-2 Auxiliary tube◆

side diameter,  $17 \pm 0.2$  mm in outside diameter,  $20 \pm 1$  mm in length, having both outside ends screw-cut, and two plastic rings A, each  $12 \pm 0.2$  mm in inside diameter,  $17 \pm 0.2$  mm in outside diameter,  $2.5 - 4.5$  mm in length, having one inside end screw-cut. Acid-resistant woven wire gauze having 0.42-mm openings and 0.29-mm wire diameter is placed in each plastic ring and the rings are attached by screws to each end of the plastic tube. The distance between two wire gauzes is  $20 \pm 1$  mm. A handle of an acid-resistant wire, 1 mm in diameter and  $80 \pm 5$  mm in length, is attached to the mid portion of the plastic tube. The auxiliary tube is used for the test of granules and capsules containing enteric coated granules.◆

## 2. Procedure

### 2.1. Immediate-release preparations

In case of tablets, capsules ◆and pills (except for pills containing crude drugs),◆ place 1 dosage unit in each of the six tubes of the basket, and if prescribed add a disk. ◆Unless otherwise specified, operate the apparatus, using water as the immersion fluid,◆ maintained at  $37 \pm 2^\circ\text{C}$  as the immersion fluid. ◆Unless otherwise specified, carry out the test for 20 minutes for capsules, 30 minutes for plain tablets, and 60 minutes for coated tablets and pills.◆ Lift the basket from the fluid, and observe the dosage units. ◆Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the disks, if used, is a soft mass having no palpably firm core.◆ The test is met if all of the dosage units have disintegrated completely. If 1 or 2 dosage units fail to disintegrate, repeat the test on 12 additional dosage units. The test is met if not less than 16 of the total of 18 dosage units tested are disintegrated.

◆For pills containing crude drugs, carry out the test for 60 minutes in the same manner, using 1st fluid for disintegration test as the immersion fluid. When any residue of the unit is observed, carry out the test successively for 60 minutes, using 2nd fluid for disintegration test.◆

◆In case of granules and dry syrups, shake preparations on a No. 30 (500  $\mu\text{m}$ ) sieve as directed in Particle Size Distribution Test for Preparations <6.03>, transfer 0.10 g of the residue on the sieve to each of the 6 auxiliary tubes, secure the 6 tubes to the bottom of the basket tightly, and operate the apparatus, using water as the immersion fluid, maintained at  $37 \pm 2^\circ\text{C}$  as the immersion fluid, unless otherwise specified. Observe the samples after 30 minutes of operation

for plain granules and after 60 minutes for coated granules, unless otherwise specified. Complete disintegration is defined as that state in which any residue of the granules, except fragments of insoluble coating in the auxiliary tube, is a soft mass having no palpably firm core. The test is met if all of 6 samples in the auxiliary tubes have disintegrated completely. If 1 or 2 samples fail to disintegrate, repeat the test on 12 additional samples. The test is met if not less than 16 of the total of 18 samples tested are disintegrated..◆

### ◆2.2. Enteric coated preparations

Unless otherwise specified, perform the following two tests, (a) the test with 1st fluid for disintegration test and (b) the test with the 2nd fluid for disintegration test, separately.

#### 2.2.1. Enteric coated tablets and capsules

(i) The test with 1st fluid for disintegration test: Carry out the test for 120 minutes, using 1st fluid for disintegration test according to the procedure described in immediate release preparations. Disintegration is defined as that state in which the tablet or capsule is broken or the enteric coating film is ruptured or broken. The test is met if none of six dosage units is disintegrated. If 1 or 2 dosage units are disintegrated, repeat the test on additional 12 dosage units. The test is met if not less than 16 of the total of 18 dosage units tested are not disintegrated.

(ii) The test with 2nd fluid for disintegration test: According to the procedure described in immediate-release preparations, carry out the test with new dosage units for 60 minutes, using 2nd fluid for disintegration test and determine if the test is met or not.

#### 2.2.2. Enteric coated granules and capsules containing the enteric coated granules

Shake granules or contents taken out from capsules on a No. 30 (500  $\mu\text{m}$ ) sieve as directed in Particle Size Distribution Test for Preparations <6.03>, transfer 0.10 g of the residue on the sieve to each of the 6 auxiliary tubes, secure the 6 tubes to the bottom of the basket tightly, and operate the apparatus, using the 1st and 2nd fluids for disintegration test.

(i) The test with 1st fluid for disintegration test: According to the procedure described in immediate-release preparations, carry out the test for 60 minutes, using 1st fluid for disintegration test. The test is met if particles fallen from the openings of the wire gauze number not more than 15.

(ii) The test with 2nd fluid for disintegration test: According to the procedure described in immediate-release preparations, carry out the test with new samples for 30 minutes, using 2nd fluid for disintegration test and determine if test is met or not..◆

## 6.10 Dissolution Test

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The parts of the text that are not harmonized are marked with symbols (◆ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopoeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Dissolution Test is provided to determine compliance with the dissolution requirements for dosage forms administered orally. ◆This test also aims at preventing significant bioequivalence..◆ In this test, a dosage unit is defined as 1 tablet or 1 capsule or the amount specified equivalent to minimum dose.

## 1. Apparatus

### 1.1. Apparatus for Basket Method (Apparatus 1)

The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material<sup>\*1</sup>; a motor; a drive shaft; and a cylindrical basket. The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device such as a heating jacket. The water bath or heating device permits holding the temperature inside the vessel at  $37 \pm 0.5^\circ\text{C}$  during the test and keeping the bath fluid in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smoothly rotating stirring element. Make the apparatus to permit observation of the specimen and stirring element during the test. The vessel is cylindrical, with a hemispherical bottom and a capacity of 1 liter. Its height is 160 mm to 210 mm and its inside diameter is 98 mm to 106 mm. Its sides are flanged at the top. Use a fitted cover to retard evaporation.<sup>\*2</sup> The shaft is positioned so that its axis is not more than 2 mm at any point from the vertical axis of the vessel and rotates smoothly and without significant wobble that could affect the results. Adjust a speed-regulating device to maintain the shaft rotation speed at a specified rate, within  $\pm 4\%$ .

Shaft and basket components of the stirring element shown in Fig. 6.10-1 are fabricated of stainless steel (SUS316) or other inert material. A basket having a gold coating of about 0.0001 inch (2.5  $\mu\text{m}$ ) thick may be used. The dosage unit is placed in a dry basket at the beginning of each test. The distance between the inside bottom of the vessel and the bottom of the basket is maintained at  $25 \pm 2$  mm during the test.

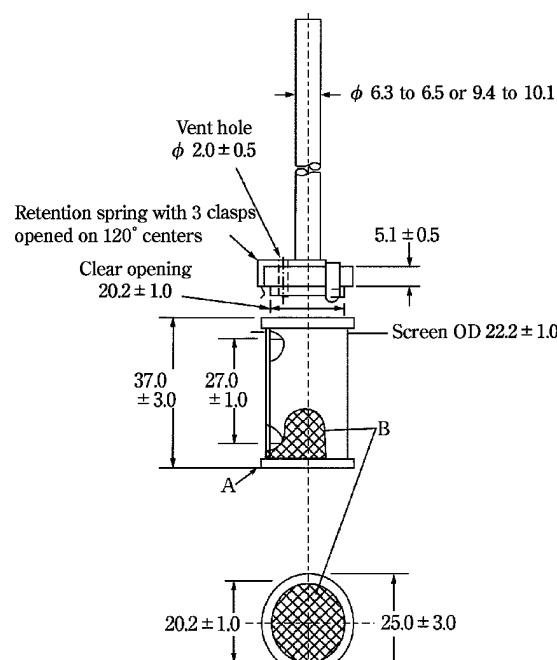
### 1.2. Apparatus for Paddle Method (Apparatus 2)

Use the assembly from Apparatus 1, except that a paddle formed from a blade and a shaft is used as the stirring element. The shaft is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel, at any point, and rotates smoothly without significant wobble that could affect the results. The vertical center line of the blade passes through the axis of the shaft so that the bottom of the blade is flush with the bottom of the shaft. The paddle conforms to the specifications shown in Fig. 6.10-2. The distance of  $25 \pm 2$  mm between the bottom of the blade and the inside bottom of the vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-part detachable design may be used provided the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make them inert. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of non-reactive material, such as not more than a few turns of wire helix or such one shown in Fig. 6.10-2a, may be attached to the dosage unit that would otherwise float. Other validated sinker devices may also be used. If the use of sinker is specified, unless otherwise specified, use the sinker device shown in Fig. 6.10-2a.

### 1.3. Apparatus for Flow-Through Cell Method (Apparatus 3)

The assembly consists of a reservoir and a pump for the dissolution medium; a flow-through cell; a water bath that maintains the dissolution medium at  $37 \pm 0.5^\circ\text{C}$ . Use the cell size specified in the individual monograph.

The pump forces the dissolution medium upwards through the flow-through cell. The pump has a delivery range between 4 and 16 mL per minute, with standard flow rates of



All dimensions are expressed in mm.  
φ=diameter

A: Note—Maximum allowable runout at "A" is  $\pm 1.0$  mm when the part is rotated on center line axis with basket mounted.

B: Screen with welded seam, 0.22–0.31 mm wire diameter with wire openings of 0.36–0.44 mm [Note—After welding, the screen may be slightly altered.]

**Fig. 6.10-1** Apparatus 1, Basket stirring element

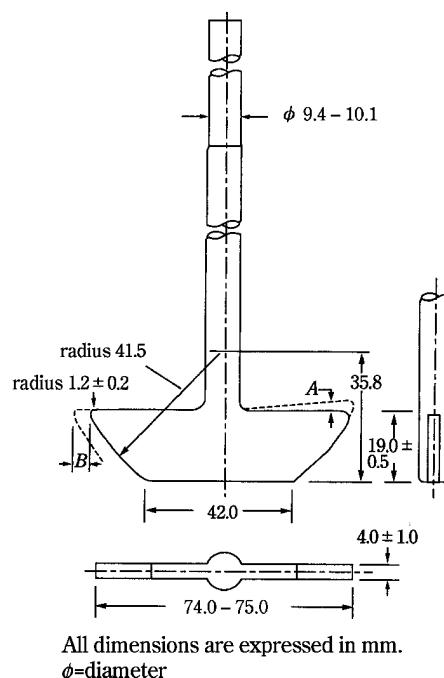
4, 8, and 16 mL per minute. It must deliver a constant flow ( $\pm 5\%$  of the nominal flow rate); the flow profile should be sinusoidal with a pulsation of  $120 \pm 10$  pulses per minute. A pump without the pulsation may also be used. Dissolution test procedure using the flow-through cell must be characterized with respect to rate and any pulsation.

The flow-through cell (see Figures 6.10-3 and 6.10-4), of transparent and inert material, is mounted vertically with a filter system (specified in the individual monograph) that prevents escape of undissolved particles from the top of the cell; standard cell diameters are 12 and 22.6 mm; the bottom cone is usually filled with small glass beads of about 1-mm diameter with one bead of about 5 mm positioned at the apex to protect the fluid entry tube; a tablet holder (see Figures 6.10-3 and 6.10-4) is available for positioning of special dosage forms. The cell is immersed in a water bath, and the temperature is maintained at  $37 \pm 0.5^\circ\text{C}$ .

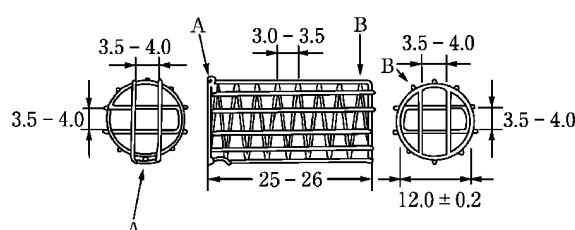
The apparatus uses a clamp mechanism of two O-rings to assemble the cell. The pump is separated from the dissolution unit in order to shield the latter against any vibrations originating from the pump. The position of the pump should not be on a level higher than the reservoir flasks. Tube connections are as short as possible. Use suitably inert tubing, such as polytef, with about 1.6-mm inner diameter and inert flanged-end connections.

## 2. Apparatus Suitability

The determination of suitability of a test assembly to perform dissolution testing must include conformance to the dimensions and tolerances of the apparatus as given above. In addition, critical test parameters that have to be monitored periodically during use include volume and tempera-

**Notes:**

- (1) A and B dimensions are not to vary more than 0.5 mm when part is rotated on centering axis.
- (2) Tolerances are  $\pm 1.0$  mm unless otherwise stated.

**Fig. 6.10-2** Apparatus 2, Paddle stirring element

A: Acid-resistant wire clasp  
B: Acid-resistant wire support

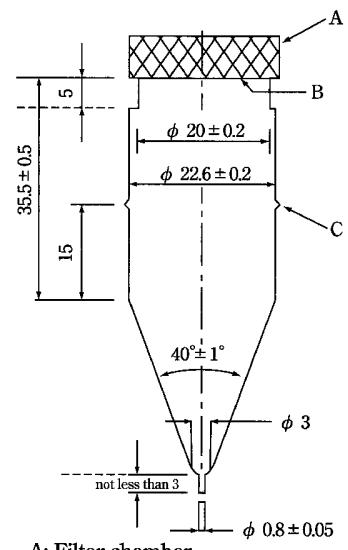
**Fig. 6.10-2a** Alternative sinker

ture of the dissolution medium, rotation speed (Basket Method and Paddle Method), and flow rate of medium (Flow-Through Cell Method).

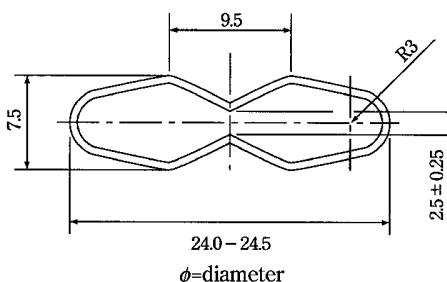
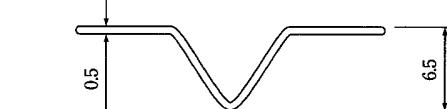
Determine the acceptable performance of the dissolution test assembly periodically.

**3. Procedure****3.1. Basket Method or Paddle Method****3.1.1. Immediate-release Dosage Forms**

(i) Procedure: Place the stated volume of the dissolution medium ( $\pm 1\%$ ) in the vessel of the specified apparatus, assemble the apparatus, equilibrate the dissolution medium to  $37 \pm 0.5^\circ\text{C}$ , and remove the thermometer. Place 1 dosage unit in the apparatus, taking care to exclude air bubbles from the surface of the dosage unit, and immediately operate the apparatus at the specified rate. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the Dissolution Medium and the top of the rotating basket or



A: Filter chamber  
B: No. 36 sieve (0.425 mm) or wire gauze  
(d: 0.2 mm, w: 0.45 mm)  
C: Score for the sample holder

**Fig. 6.10-3** Apparatus 3

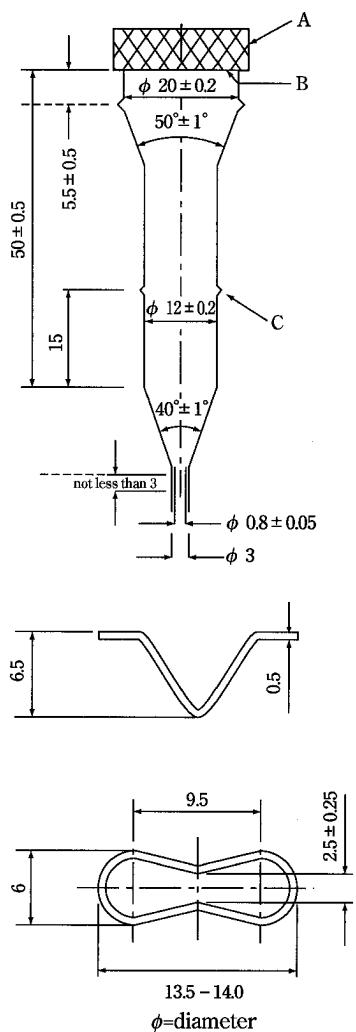
Large cell for tablets and capsules (top); tablet holder for the large cell (bottom)

(All dimensions are expressed in mm unless otherwise noted.)

blade, not less than 10 mm from the vessel wall. [NOTE—Where multiple sampling times are specified, replace the aliquots withdrawn for analysis with equal volumes of fresh Dissolution Medium at  $37^\circ\text{C}$  or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test, and verify the temperature of the mixture under test at suitable times.] Perform the analysis using an indicated assay method.\*<sup>3</sup> Repeat the test with additional dosage units.

If automated equipment is used for sampling or the apparatus is otherwise modified, verification that the modified apparatus will produce results equivalent to those obtained with the standard apparatus described in this chapter, is necessary.

(ii) Dissolution Medium: An appropriate dissolution medium is used. The volume specified refers to measurements made between  $20^\circ\text{C}$  and  $25^\circ\text{C}$ . If the dissolution medium is a buffered solution, adjust the solution so that its pH is within 0.05 unit of the specified pH. [NOTE—Dissolved gases can cause bubbles to form, which may change the results of the test. If dissolved gases influence the dissolution

**Fig. 6.10-4** Apparatus 3

Small cell for tablets and capsules (top); tablet holder for the small cell (bottom)

(All dimensions are expressed in mm unless otherwise noted.)

results, remove dissolved gases prior testing.\*<sup>4]</sup>

(iii) Time: Where a single time specification is given, the test may be concluded in a shorter period if the requirement for minimum amount dissolved is met. Specimens are to be withdrawn only at the stated times, within a tolerance of ± 2%.

### 3.1.2. Extended-release Dosage Forms

(i) Procedure: Proceed as described for Immediate-Release Dosage Forms.

(ii) Dissolution Medium: Proceed as directed under Immediate-Release Dosage Forms.

(iii) Time: The test-time points, generally three, are expressed in hours.

### 3.1.3. Delayed-release Dosage Forms

(i) ♦Procedure: Unless otherwise specified, proceed the acid stage test and buffer stage test separately as described for Immediate-Release Dosage Forms.♦

(ii) ♦Dissolution Medium: Acid stage: Unless 1st fluid for dissolution test is used, proceed as directed under Immediate-Release Dosage Forms. Buffer stage: Unless 2nd fluid for dissolution test is used, proceed as directed under Immediate-Release Dosage Forms.♦

(iii) ♦Time: Acid stage: Generally, test time is 2 hours for tablets and capsules, and 1 hour for granules. Buffer

stage: The same as directed under Immediate-Release Dosage Forms.♦ All test times stated are to be observed within a tolerance of ± 2%, unless otherwise specified.

### 3.2. Flow-Through Cell Method

#### 3.2.1. Immediate-release Dosage Forms

(i) Procedure: Place the glass beads into the cell specified in the individual monograph. Place 1 dosage unit on top of the beads or, if specified, on a wire carrier. Assemble the filter head and fix the parts together by means of a suitable clamping device. Introduce by the pump the dissolution medium warmed to  $37 \pm 0.5^\circ\text{C}$  through the bottom of the cell to obtain the flow rate specified and measured with an accuracy of 5%. Collect the eluate by fractions at each of the times stated. Perform the analysis as directed. Repeat the test with additional dosage units.

(ii) Dissolution Medium: Proceed as directed under Immediate-Release Dosage Forms under Basket Method and Paddle Method.

(iii) Time: Proceed as directed under Immediate-Release Dosage Forms under Basket Method and Paddle Method.

#### 3.2.2. Extended-release Dosage Forms

(i) Procedure: Proceed as described for Immediate-Release Dosage Forms under Flow-Through Cell Method.

(ii) Dissolution Medium: Proceed as described for Immediate-Release under Flow-Through Cell Method.

(iii) Time: The test-time points, generally three, are expressed in hours.

### 4. Interpretation

#### 4.1. Immediate-release Dosage Forms

♦Follow Interpretation 1 when the value  $Q$  is specified in the individual monograph, otherwise follow Interpretation 2.♦

##### 4.1.1. Interpretation 1

Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to Acceptance Table 6.10-1. Continue testing through the three stages unless the results conform at either S1 or S2. The quantity,  $Q$ , ♦is the specified amount of dissolved active ingredient,♦ expressed as a percentage of the labeled content of the dosage unit; the 5%, 15%, and 25% values in the Acceptance Tables are percentage of the labeled content so that three values and  $Q$  are in the same terms.

##### 4.1.2. ♦Interpretation 2

Unless otherwise specified, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on another 6 dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test.♦

#### 4.2. Extended-release Dosage Forms

##### 4.2.1. ♦Interpretation 1♦

Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to Acceptance Table 6.10-2. Continue testing through the three levels unless the results conform at either L1 or L2. Limits on the amounts of active ingredient dissolved are expressed in terms of the percentage of labeled content. The limits embrace each value of  $Q_i$ , the amount dissolved at each specified fractional dosing interval. Where more than one range is specified, the acceptance criteria apply individually to each range.

##### 4.2.2. ♦Interpretation 2

Unless otherwise specified, perform the test on 6 dosage forms: if the individual dissolution rate meet the require-

**Acceptance Table 6.10-1**

Stage	Number Tested	Acceptance Criteria
S1	6	Each value is not less than $Q + 5\%$ .
S2	6	Average value of the 12 dosage units ( $S1 + S2$ ) is equal to or greater than $Q$ , and no value is less than $Q - 15\%$ .
S3	12	Average value of the 24 dosage units ( $S1 + S2 + S3$ ) is equal to or greater than $Q$ , not more than 2 values are less than $Q - 15\%$ , and no value is less than $Q - 25\%$ .

**Acceptance Table 6.10-2**

Level	Number Tested	Criteria
L1	6	No individual value lies outside each of the stated ranges and no individual value is less than the stated amount at the final test time.
L2	6	The average value of the 12 dosage units ( $L1 + L2$ ) lies within each of the stated ranges and is not less than the stated amount at the final test time; no value is more than 10% of labeled content outside each of the stated ranges; and no value is more than 10% of labeled content below the stated amount at the final test time.
L3	12	The average value of the 24 dosage units ( $L1 + L2 + L3$ ) lies within each of the stated ranges, and is not less than the stated amount at the final test time; not more than 2 of the 24 values are more than 10% of labeled content outside each of the stated ranges; not more than 2 of the 24 values are more than 10% of labeled content below the stated amount at the final test time; and no value is more than 20% of labeled content outside each of the stated ranges or more than 20% of labeled content below the stated amount at the final test time.

ments specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on another 6 dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test. Where more than one range is specified, the acceptance criteria apply individually to each range.♦

#### 4.3. Delayed-release Dosage Forms

♦Follow Interpretation 1 when the value  $Q$  is specified in the test using 2nd fluid for dissolution test in the individual monograph, otherwise follow Interpretation 2.

##### 4.3.1. Interpretation 1

(i) Test using 1st fluid for dissolution test: Unless otherwise specified, the requirements of this portion of the test are met if the quantities, based on the percentage of the labeled content, of active ingredient dissolved from the units tested conform to Acceptance Table 6.10-3. Continue testing through the three levels unless the result conforms at A2.♦

(ii) ♦Test using 2nd fluid for dissolution test♦: Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the units tested conform to Acceptance Table 6.10-4. Continue testing through the three levels unless the results of both stages conform at an earlier level. The value of  $Q$  in Acceptance Table 6.10-4 is the amount ♦specified in monograph♦ of active ingredient dissolved, expressed as a percentage of the labeled content.

**Acceptance Table 6.10-3**

Level	Number Tested	Criteria
A1	6	No individual value exceeds 10% dissolved.
A2	6	The average value of the 12 dosage units ( $A1 + A2$ ) is not more than 10% dissolved, and no value is greater than 25% dissolved.
A3	12	The average value of the 24 dosage units ( $A1 + A2 + A3$ ) is not more than 10% dissolved, and no value is greater than 25% dissolved.

**Acceptance Table 6.10-4**

Level	Number Tested	Criteria
B1	6	No value is less than $Q + 5\%$ .
B2	6	The average value of the 12 dosage units ( $B1 + B2$ ) is equal to or greater than $Q$ , and no value is less than $Q - 15\%$ .
B3	12	The average value of the 24 dosage units ( $B1 + B2 + B3$ ) is equal to or greater than $Q$ , not more than 2 values are less than $Q - 15\%$ , and no value is less than $Q - 25\%$ .

The 5% and 15% and 25% values in Acceptance Table 6.10-4 are percentages of the labeled content so that these values and  $Q$  are in the same terms.

#### 4.3.2. ♦Interpretation 2

Unless otherwise specified, both the tests using 1st fluid for dissolution test and 2nd fluid for dissolution test in acid and buffer stages, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on another 6 dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test.♦

\*<sup>1</sup>The materials should not sorb, react, or interfere with the specimen being tested.

\*<sup>2</sup>If a cover is used, it provides sufficient openings to allow ready insertion of the thermometer and withdrawal of specimens.

\*<sup>3</sup>Test specimens are filtered immediately upon sampling unless filtration is demonstrated to be unnecessary. Use an inert filter that does not cause adsorption of the ingredient or contain extractable substances that would interfere with the analysis.

\*<sup>4</sup>One method of deaeration is as follows: Heat the medium, while stirring gently, to about 41°C, immediately filter under vacuum using a filter having a porosity of 0.45 µm or less, with vigorous stirring, and continue stirring under vacuum for about 5 minutes. Other validated deaeration techniques for removal of dissolved gases may be used.

## 6.11 Foreign Insoluble Matter Test for Ophthalmic Liquids and Solutions

Foreign Insoluble Matter Test for Ophthalmic Liquids and Solutions is a test method to examine foreign insoluble matters in ophthalmic liquids and solutions.

When inspect with the unaided eyes at a position of luminous intensity of 3000 – 5000 lx under a white light source

after cleaning the exterior of containers, Ophthalmic Solutions must be clear and free from readily detectable foreign insoluble matters.

## 6.12 Methods of Adhesion Testing

These are testing methods to measure the adhesion of patches. The methods include peel adhesion testing, inclined ball tack testing, rolling ball tack testing, and probe tack testing.

The tests are conducted at  $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$  unless otherwise specified. However, if the acceptable range of  $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$  temperature cannot be maintained, set a range as close to that as possible.

### 1. Preparation of samples

The following method will be followed to prepare samples unless otherwise specified. Use a package, such as an aluminum material that is not affected by humidity for the sample, and allow it to stand at  $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for over 12 hours. The sample can be cut to an appropriate size as needed. Furthermore, visually confirm that dust is not adhered to the adhesive side of the sample, take care not to touch the sample with bare hands, and prevent foreign matter from adhering to it.

### 2. Cleaning method for testing instruments

To clean the testing plates, balls, and probe for adhesion testing, use cleaning solvents such as acetone, 2-butanone, ethanol (99.5), ethyl acetate, heptane, water, and methanol. Utilize soft cloth, such as gauze, absorbent cotton, or waste cloth, that does not generate lint or dust during use, is absorbent, and free from additives that dissolve in cleaning solvents. Apply a cleaning solvent on a clean cloth, wipe the surfaces of the instruments, and repeat wiping with a new cloth until dry. Repeat this step until the instruments are determined clean by visual observation. Lastly, apply acetone, 2-butanone, or another appropriate solvent on a cloth, wipe the surfaces of the instruments, and repeat wiping with a new cloth until dry. Use the cleaned instruments for testing within 10 hours. Take care not to touch the surfaces with fingers, and preserve them without damaging or contaminating. Do not use any instrument if it is dirty, discolored, or has a number of scratches. With respect to new testing plates, balls, and probe, wipe well using cloth soaked with a cleaning solvent, and additionally, clean with the method described above before use.

### 3. Measurement methods

#### 3.1. Peel adhesion testing

The peel adhesion test measures the force required to remove (peel away) the sample adhered to the testing plate at a 180 or 90-degree angle.

##### 3.1.1. Equipment

The equipment consists of an application device and a tensile tester. The application device (Figures of equipment 6.12-1a and 6.12-1b) is structured in such a way that only the mass of the roller is applied to the sample as pressure when the sample is crimped. The roller should be made of steel or equivalent covered with the rubber for crimping rollers whose material is prescribed in the Japanese Industrial Standard Z 0237:2009 (a diameter of  $85 \pm 2.5$  mm, a width of  $45 \pm 1.5$  mm, a thickness of about 6 mm). In addition, the shape must be exactly cylindrical, and without irregularities on the surface. Set the mass of the roller to  $2000 \pm 100$  g or  $1000 \pm 50$  g.

With regard to the testing plate for adhesion testing, the

plate prescribed in the Japanese Industrial Standard Z 0237:2009 or equivalent should be used unless otherwise specified.

Use a tensile tester with a relative pointing error of  $\pm 1.0\%$ . The representation of measurements may be any one of the analog type, digital type, digital recording system, and chart recording system.

#### 3.1.2. Operation procedures

Prepare the sample in such a way that it has a chucking allowance on one end, and adhere it to the testing plate using the application device after exposing the adhesive side within 5 minutes. Loosen the sample in holding the chucking allowance on the testing plate so that the testing plate will not come into contact with the sample prior to adhesion. Adhere the sample to the testing plate while crimping along the long side direction with the roller. This will prevent air from entering between the sample and the testing plate. If air enters, do not use the sample. Crimping should be done under constant load either at the rate of approximately 10 mm per second back and forth two times or at the rate of approximately 5 mm per second back and forth once. After crimping the sample with the roller, perform a peel adhesion test at the prescribed time (for example,  $30 \pm 10$  minutes). Use a 2 kg crimping roller for a sample with a width of 17 mm or larger, and a 1 kg crimping roller for a sample with a width under 17 mm.

##### 3.1.2.1. 180-degree peel test

Prepare an upper chuck and a lower chuck at the top and the bottom of the tensile tester as parts to secure the testing plate and the sample. Figure 6.12-2a shows an example of measuring instruments for 180-degree peel tests. When peeling the sample, hold the chucking allowance and fold back to a 180-degree angle so that it overlaps the backside of the sample. Secure one end of the testing plate to the lower chuck and the chucking allowance to the upper chuck of the tensile tester. Next, run the tensile tester at the tension rate of  $5.0 \pm 0.2$  mm per second and start measuring. Ignore the measurements for the first 25% of the sample length. After that, average the measurements of adhesion for the 50% of the length peeled from the testing plate, which will be the value of the peel adhesion test. Express the unit in N/cm.

##### 3.1.2.2. 90-degree peel test

Figure 6.12-2b provides an example of measuring instruments for 90-degree peel tests. Except for securing the

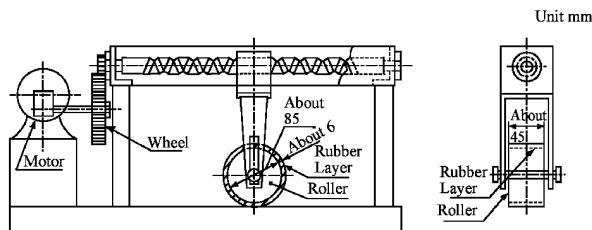


Fig. 6.12-1a Example of the automatic application device

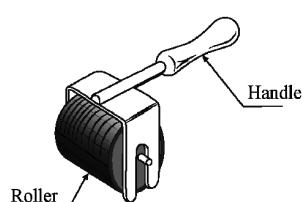


Fig. 6.12-1b Example of the manual application device

chucking allowance to the upper chuck and folding back the sample to a 90-degree angle, perform the test in the same manner as the 180-degree peel test.

### 3.2. Inclined ball tack testing

In the inclined ball tack test, balls are rolled down a ramp, and the largest size of the ball that stops is determined.

#### 3.2.1. Equipment

##### 3.2.1.1. Ball rolling device

Use a ramp with an inclined plane of 300 mm or longer having an inclination angle of 30 degrees. An example is given in Figure 6.12-3.

##### 3.2.1.2. Balls

Use No. 2 to No. 32 balls for adhesion testing. SUJ2, a high carbon content chromium bearing steel material specified in Japanese Industrial Standard G 4805:2008, should be used for the material of the balls for adhesion testing. As for precision, use hard balls for rolling bearing in Grade 40 or above prescribed in Japanese Industrial Standard B 1501:2009. Table 6.12-1 shows the numbers and sizes of balls.

##### 3.2.2. Operation procedures

Secure the ball-rolling device horizontally on the stand using a level. The sample must be larger than 10 mm in width and 70 mm in length unless otherwise specified. Fix the sample in the prescribed position on the ramp with the adhesive side up, and attach paper for the runway to the upper end of the sample. Set the length of the runway to 100 mm. When fixing the sample, take care that it will not be off the plate, wrinkled, or bent; if the edge of the sample is curved and off the plate, stick the part to the plate with another adhesive tape. Secure the ball-rolling device horizontally on the stand with the sample using a level. Roll the ball down from the start position.

Roll the balls down from the top of the ramp, and the number (No.) of the largest ball that stops on the adhesive face will be the value of the inclined ball tack test.

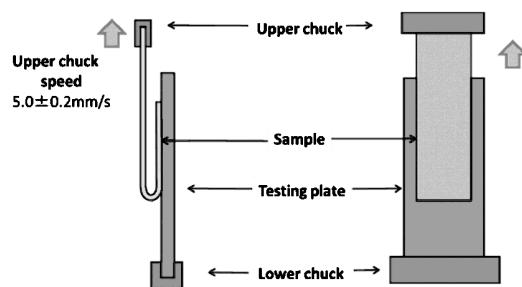


Fig. 6.12-2a Example of 180-degree peeling tester

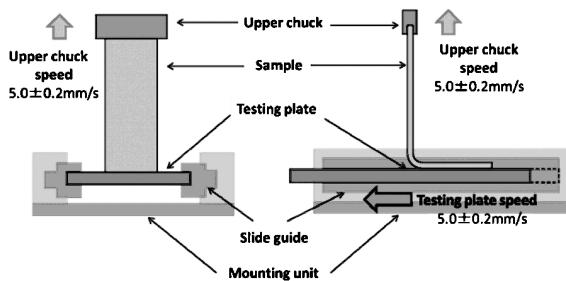


Fig. 6.12-2b Example of 90-degree peeling tester

### 3.3. Rolling ball tack testing

In the rolling ball tack test, a ball of specified size is rolled down a ramp from the start position, and the distance to the position at which the ball stops is measured.

#### 3.3.1. Equipment

##### 3.3.1.1. Ball-rolling device

The ball-rolling device has an inclination angle of 21.5 degrees, and Figure 6.12-4 shows an example.

##### 3.3.1.2. Balls

Use No. 14 balls (a diameter of 7/16 inch) for adhesion testing as noted in 3.2.1.2 unless otherwise specified.

##### 3.3.2. Operation procedures

Secure the sample on the smooth, hard, and flat plate with another adhesive tape, etc. When fixing the sample, take care that it will not be off the plate, wrinkled, or bent; if the edge of the sample is curved and off the plate, stick the part to the plate with another adhesive tape. Secure the ball-rolling device horizontally on the stand with the sample using a level. Roll the ball down from the start position.

Measure the distance when the ball stopped on the adhesive face. Measure the length between the end of the inclined plane and the midpoint at which the adhesive is in contact with the ball, which will be the value of the rolling ball tack test. Express the unit in mm.

Table 6.12-1 Types of steel ball  
Diameter (mm) is a reference level

No.	Diameter (inch)	Diameter (mm)	No.	Diameter (inch)	Diameter (mm)
1	1/32	0.8	17	17/32	13.5
2	1/16	1.6	18	9/16	14.3
3	3/32	2.4	19	19/32	15.1
4	1/8	3.2	20	5/8	15.9
5	5/32	4.0	21	21/32	16.7
6	3/16	4.8	22	11/16	17.5
7	7/32	5.6	23	23/32	18.3
8	1/4	6.4	24	3/4	19.1
9	9/32	7.1	25	25/32	19.8
10	5/16	7.9	26	13/16	20.6
11	11/32	8.7	27	27/32	21.4
12	3/8	9.5	28	7/8	22.2
13	13/32	10.3	29	29/32	23.0
14	7/16	11.1	30	15/16	23.8
15	15/32	11.9	31	31/32	24.6
16	1/2	12.7	32	1	25.4

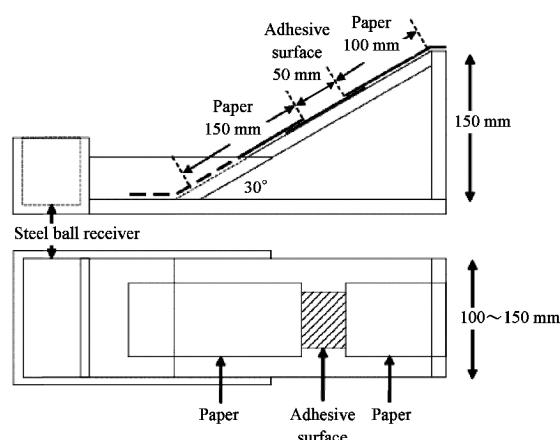
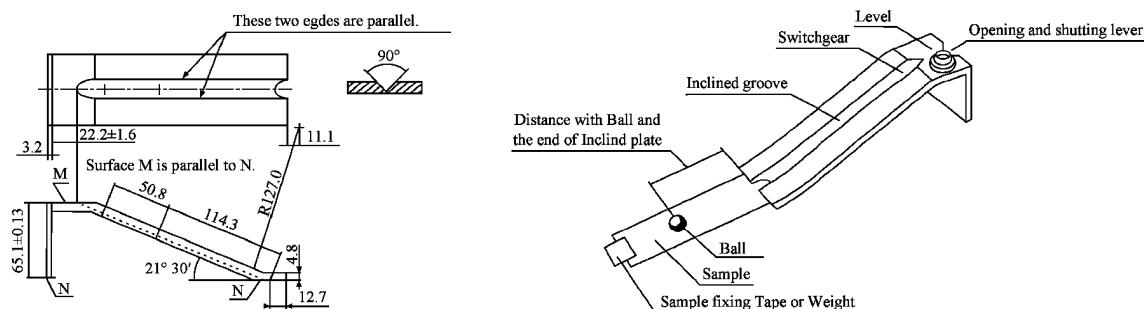
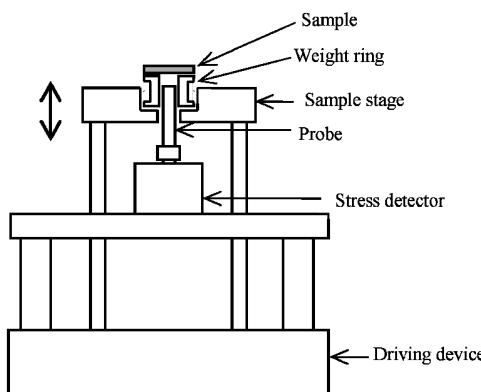


Fig. 6.12-3 Example of inclined ball tack tester



**Fig. 6.12-4** Example of rolling ball tack tester  
(All dimensions are expressed in mm unless otherwise noted.)



**Fig. 6.12-5** Example of probe tack tester

### 3.4. Probe tack testing

The probe tack test measures the force required for peeling a prescribed cylindrical probe after bringing the probe into contact with the adhesive side of a patch briefly.

#### 3.4.1. Equipment

The equipment consists of a probe, a sample stage, and a stress detector, and has a mechanism to give constant load for a certain period of time by a weight ring. As for the material of the probe for adhesion testing, use SUS304 with the root-mean-square value ( $Rq$ ) of 250 to 500 nm for surface roughness, and a diameter of 5 mm unless otherwise specified. Furthermore, the equipment has a feature that can control the speed so that the probe's contact with the adhesive side of the patch and peeling will be done at a constant rate. An example of device that applies a load by weight rings is provided in Figure 6.12-5. A device without weight rings may also be used.

#### 3.4.2. Operation procedures

Adhere the sample to the weight ring ensuring that there is no slack and place on the sample stage. Next, bring the probe into contact with the adhesive side of the sample at the rate of  $10 \pm 0.01$  mm per second, and maintain for  $1.0 \pm 0.1$  seconds with the contact load of  $0.98 \pm 0.01$  N/cm<sup>2</sup> unless otherwise specified. Immediately after that, peel the probe vertically from the adhesive face at the rate of  $10 \pm 0.01$  mm per second. Measure the maximum load required for peeling, which will be the value of the probe tack test. Express the unit in N/cm<sup>2</sup>.

## 6.13 Release Test for Preparations for Cutaneous Application

This chapter describes the method to measure release profiles of active ingredients from preparations for cutaneous application and is provided to determine compliance of the preparations with drug-release requirements. Since the relation between efficacy and release profile depends on each characteristic of these preparations, this release test is an effective method for a quality control of each preparation. Particularly, for transdermal absorption type pharmaceutical preparations, it is necessary to maintain an appropriate control on the release profiles of active ingredients.

### 1. Paddle over disk method

#### 1.1. Apparatus

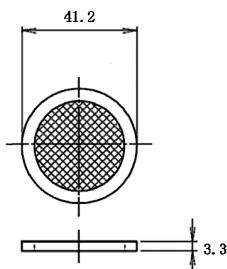
Use the assembly for the paddle method (Apparatus 2) described under Dissolution Test <6.10> and, addition to the paddle and vessel, a stainless steel (SUS316) disk consisting of a screen with a wire opening of 125 µm is used to sink samples on the bottom of the vessel. If necessary, other device which is similar to Fig. 6.13-1 with a different size or shape may be used. Other appropriate devices instead of the disk may be used as long as they are chemically inert and do not interfere with the analysis. The disk attached with a sample is installed parallel to the bottom of the paddle blade. The distance between the bottom of the paddle blade and the surface of the disk is  $25 \pm 2$  mm, unless otherwise specified in individual monograph (Fig. 6.13-2).

Additionally, apparatus suitability and handling of dissolution medium, etc., are in principle proceed as directed under Dissolution Test <6.10>.

#### 1.2. Procedure

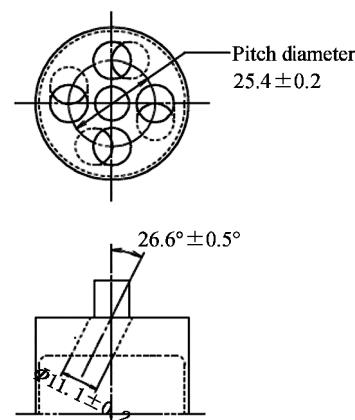
Prior to set a disk, place the prescribed volume of the dissolution medium in the vessel and maintain the temperature of the medium at  $32 \pm 0.5^\circ\text{C}$ . Fix the sample on a disk as the release surface facing up in a suitable manner by using a double-sided adhesive tape or the like. When a function of the sample is not compromised by cutting, an appropriate and exactly measured piece of the sample may be cut and used for the test. If necessary, a porous membrane may be attached to the release surface for suppressing a shape change of the preparation. The characteristics of the membrane used such as hydrophobicity or hydrophilicity and the pore size must be stated in the test method. When a membrane is used, it is applied so that no air bubbles occur between the membrane and the release surface.

Install the disk at the bottom of the vessel as that the release surface of the sample is set upwards, and paralleled

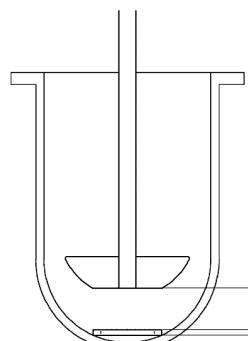


All dimensions are expressed in mm.

**Fig. 6.13-1** Paddle over disk



All dimensions are expressed in mm.



All dimensions are expressed in mm.

**Fig. 6.13-2** Paddle and vessel

to the bottom of the paddle blade and to the surface of the dissolution medium. Immediately after the installation, rotate the paddle at the specified rate, and then at the specified interval or time, withdraw a specimen from the zone midway between the surface of the dissolution medium and the top of the paddle blade, not less than 10 mm from the vessel wall. (Note: Where multiple sampling times are specified, replace the aliquots withdrawn for analysis with equal volumes of fresh dissolution medium of 32°C or, where replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test, and verify the temperature of the mixture under test at suitable intervals.) Perform the assay of the released active ingredients using the specified analytical method.

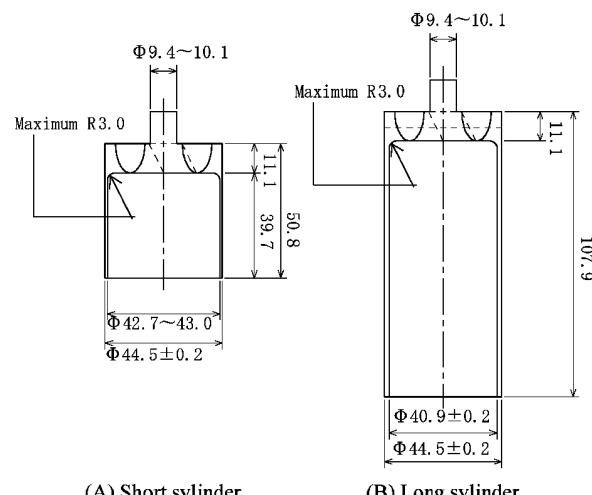
In the case where a different device in shape and material from that in Fig. 6.13-2 is used for sinking a sample with almost the same procedure stated, the method can be considered as the paddle over disk method, however, it needs to state the information about the used device.

## 2. Cylinder method

### 2.1. Apparatus

Use the vessel of the apparatus for the Paddle Method (Apparatus 2) described in Dissolution Test <6.10>, and use a stainless steel cylinder stirring element shown in Fig. 6.13-3-1 and Fig. 6.13-3-2 instead of a paddle. The cylinder is fabricated of chemically inert material such as stainless steel (SUS316), and the surface of the cylinder is electrolytically polished. The cylinder whose size is adjusted to the same size as Fig. 6.13-3-2 (B) by equipping with an additional cylindrical device to the cylinder of Fig. 6.13-3-2 (A) can be used. The distance between the inside bottom of the vessel and the lower side of the cylinder is maintained at 25 ± 2 mm. Additionally, apparatus suitability and handling of dissolution

**Fig. 6.13-3-1** Upper structure of cylinder stirring element



(A) Short cylinder

(B) Long cylinder

All dimensions are expressed in mm.

**Fig. 6.13-3-2** Cylinder stirring element

medium, etc., are performed as directed under Dissolution Test <6.10>.

### 2.2. Procedure

Place the prescribed volume of the dissolution medium in the vessel and maintain the temperature of the medium to 32 ± 0.5°C. Remove the protective liner from the sample and fix the sample on the cylinder as the release surface being outside by a suitable method using a double-sided adhesive tape or the like. If necessary, a porous membrane may be attached to the release surface. The characteristics of the membrane used such as hydrophobicity or hydrophilicity and the pore size must be stated in the test method.

Install the cylinder in the dissolution test apparatus, and immediately after the installation, rotate the cylinder at the specified rate. At the specified interval or time, withdraw a specimen from the zone midway between the surface of the dissolution medium and the bottom of the cylinder, not less than 10 mm from the vessel wall. (Note: Where multiple sampling times are specified, replace the aliquots withdrawn for analysis with equal volumes of fresh dissolution medium of 32°C or, where replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test, and verify the

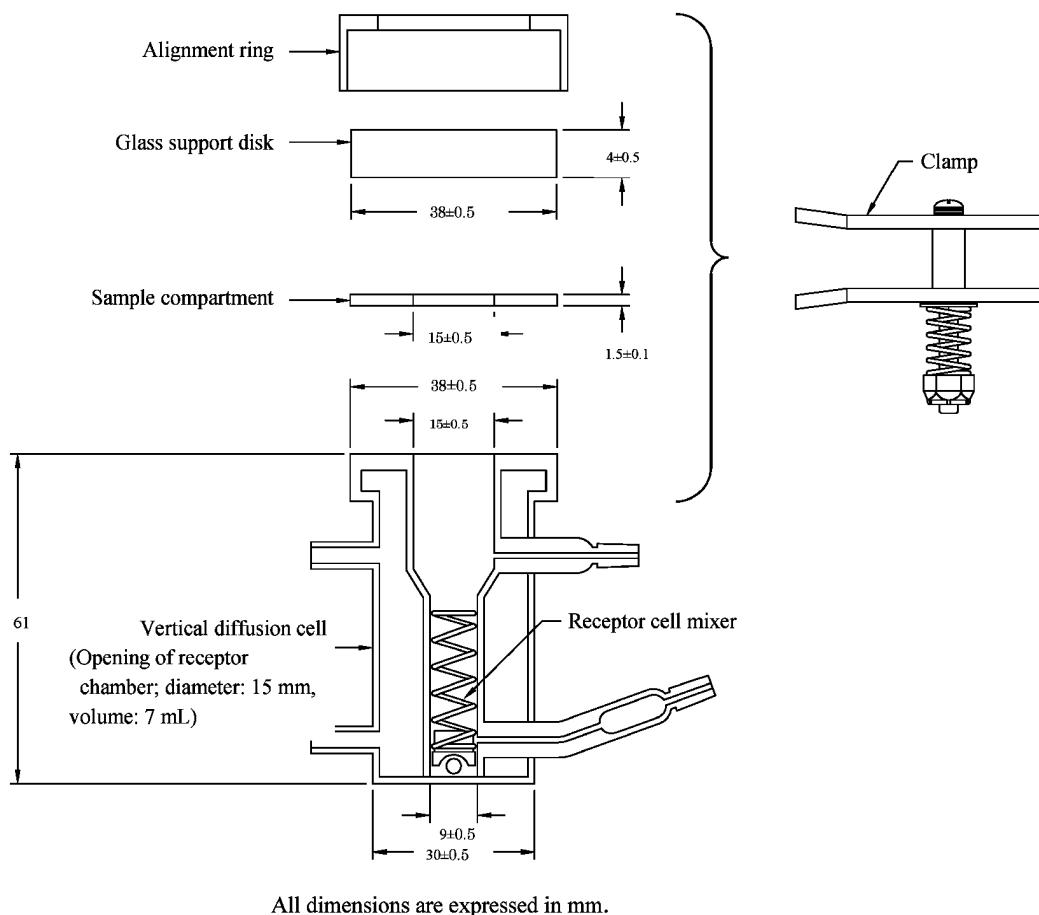


Fig. 6.13-4 Vertical diffusion cell

temperature of the mixture under test at appropriate intervals.) Perform the assay of the released active ingredients on each sample using the specified analytical method.

### 3. Vertical diffusion cell method

#### 3.1. Apparatus

The assembly is composed of a vertical diffusion cell separated to two chambers and the chambers are fixed by a clamp. An example of the vertical diffusion cell is shown in Fig. 6.13-4. These cells are made of chemically inert materials such as glass or plastic, which do not interfere with the analysis.

#### 3.2. Procedure

Place the prescribed volume of the dissolution medium in the receptor chamber with a rotator and maintain the medium temperature at  $32 \pm 1.0^\circ\text{C}$ . If necessary, a porous membrane may be attached to the release surface. The characteristics of the membrane used such as hydrophobicity or hydrophilicity and the pore size must be stated. Place the sample evenly in the donor side and immediately rotate the rotator at a constant rate by a magnetic stirrer. At the specified intervals or time, withdraw a specimen. Take care not to enter bubble in the dissolution medium at the sampling. Perform the assay of the released active ingredients using the specified analytical method. Repeat the test with additional sample in the same manner.

#### 4. Dissolution medium

Usually an arbitrary buffer in the range of pH 5 to 7 (ion strength is about 0.05) may be used as a dissolution medium. If necessary, addition of surfactant, change of the pH or ion strength may be allowed. Water, a mixture of water and alcohol, organic solvents, etc., may be used where they do

Table 6.13-1 Acceptance criteria

Level	Number tested	Acceptance criteria
L <sub>1</sub>	6	No individual value lies outside each of the stated ranges (including the limit values).
L <sub>2</sub>	6	The average value of the 12 samples (L <sub>1</sub> + L <sub>2</sub> ) lies within each of the stated ranges (including the limit values) and no individual value is more than 10% of labeled content outside each of the stated ranges.
L <sub>3</sub>	12	The average value of the 24 samples (L <sub>1</sub> + L <sub>2</sub> + L <sub>3</sub> ) lies within each of the stated ranges (including the limit values), and not more than 2 of the 24 values are more than 10% of labeled content outside each of the stated ranges and no value is more than 20% of labeled content outside each of the stated ranges.

not affect the shape of the samples. The volume of the dissolution medium used in the test is 200 mL, 500 mL or 900 mL, though in the case of 200 mL, a special vessel and mini-paddle should be used.

#### 5. Interpretation

The specified range of quantities released from a sample at each sampling time is described in the individual monograph.

Unless otherwise specified, the requirements are met if the quantities of active ingredients released from the samples tested conform to the acceptance criteria in Table 6.13-1. Continue testing through the three levels unless the results conform at either  $L_1$  or  $L_2$ . Limits on the amounts of active ingredients released at each time are expressed in terms of the percentage of labeled content. The limit values are release ratios at each specified sampling time. Where more than one range is specified, the acceptance criteria apply individually to each range.

## **6.14 Uniformity of Delivered Dose for Inhalations**

This test is used to quantitatively evaluate the uniformity of the amount of active substances sprayed or discharged from inhalations (metered-dose inhalers and dry powder inhalers). Uniformity of the amount of active substances which are administered to patients from these preparations is necessary, and is confirmed by this test. Examples for the evaluation is shown as follows. Select a suitable test method from the following, according to the characteristic of preparations. Original methods are able to be set, including the test that can evaluate intra and inter-inhalers dose uniformity simultaneously.

## 1. Test methods for metered-dose inhalers

Metered-dose inhalers usually operate in a valve-down position. For inhalers that operate in a valve-up position, an equivalent test is applied using methods that ensure the complete collection of the delivered dose.

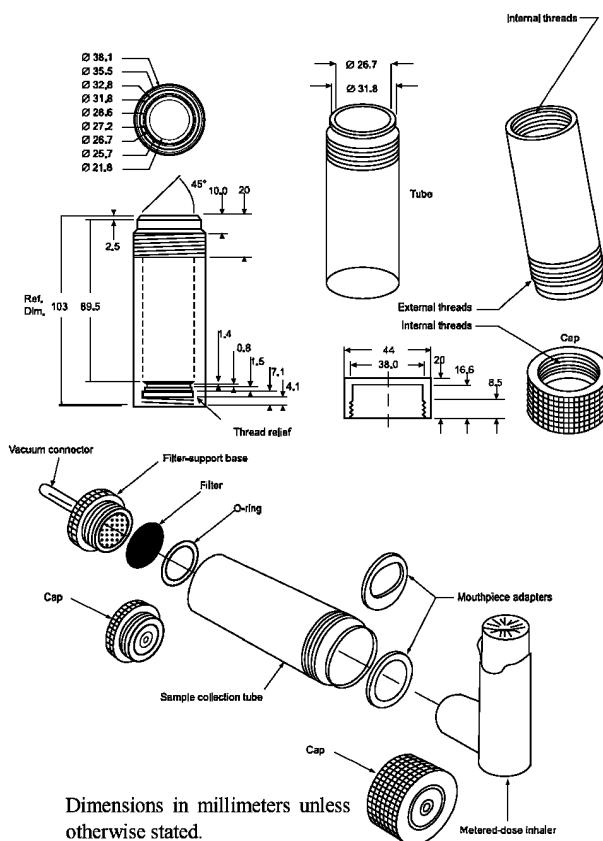
The dose collection apparatus must be capable of quantitatively capturing the delivered dose.

The following apparatus (Fig. 6.14-1) and procedure may be used.

The apparatus consists of a filter-support base with an open-mesh filter-support, such as a stainless steel screen, a collection tube that is clamped or screwed to the filter-support base, and a mouthpiece adapter to ensure an airtight seal between the collection tube and the mouthpiece. Use a mouthpiece adapter that ensures that the front face of the inhaler's mouthpiece is flush with the front face or the 2.5 mm indented shoulder of the sample collection tube, as appropriate. The vacuum connector is connected to a system comprising a vacuum source and a flow regulator. The source is adjusted to draw air through the complete assembly, including the filter and the inhaler to be tested, at 28.3 L per minute ( $\pm 5\%$ ). Air should be drawn continuously through the apparatus to avoid loss of the active substance into the atmosphere. The filter-support base is designed to accommodate 25 mm diameter filter disks. The filter disk and other materials used in the construction of the apparatus must be compatible with the active substance and solvents that are used to extract the active substance from the filter. One end of the collection tube is designed to hold the filter disk tightly against the filter-support base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection tube passes through the inhaler.

### **1.1. Test method 1: evaluation of intra-inhaler dose uniformity**

Take one inhaler, and perform the test. Unless otherwise prescribed in the instructions to the patient, shake the inhaler for 5 seconds and discharge one delivery to waste. Discharge the inverted inhaler into the apparatus, depressing the



**Fig. 6.14-1** Dose collection apparatus for metered-dose inhalers

valve for a sufficient time to ensure complete discharge. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste, waiting not less than 5 seconds between actuations, until  $(n/2) + 1$  deliveries remain, where  $n$  is the number of deliveries stated on the label. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste, waiting not less than 5 seconds between actuations, until 3 doses remain. Collect these 3 doses using the procedure described above. Determine 10 delivered doses per one inhaler, i.e., 3 doses at the beginning, 4 doses at the middle and 3 doses at the end by the above process.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the test for 2 more inhalers, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

**Table 6.14-1** Specifications of the apparatus described in Fig. 6.14-2

Code	Component	Description
A	Sample collection tube	Dimensions of 34.85 mm internal diameter × 12 cm length
B	Filter	47 mm glass fiber filter
C	Connector	Internal diameter ≥ 8 mm (e.g., short metal coupling, with low-diameter branch to P3)
D	Vacuum tubing	A length of suitable tubing having an internal diameter ≥ 8 mm and an internal volume of $25 \pm 5$ mL.
E	Two-way solenoid valve	A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with internal diameter ≥ 8 mm and an opening time ≤ 100 ms
F	Vacuum pump	Pump must be capable of drawing the required flow rate through the assembled apparatus with the dry powder inhaler in the mouthpiece adapter. Connect the pump to the 2-way solenoid valve using short and/or wide (≥ 10 mm internal diameter) vacuum tubing and connectors to minimize pump capacity requirements.
G	Timer	Timer capable of driving the solenoid valve for the required time period.
P1	Pressure tap	2.2 mm internal diameter, 3.1 mm outer diameter, flush with internal surface of the sample collection tube, centered and burr-free, 59 mm from its inlet. The pressure tap P1 must never be open to the atmosphere. Differential pressure to the atmosphere is measured at P1.
P2, P3	Pressure measurements	Absolute pressure
H	Flow control valve	Adjustable regulating valve with maximum $Cv \geq 1$ .

## 1.2. Test method 2: evaluation of inter-inhaler dose uniformity

Take one inhaler, and perform the test. Unless otherwise prescribed in the instructions to the patient, shake the inhaler for 5 seconds and discharge one delivery to waste. Discharge the inverted inhaler into the apparatus, depressing the valve for a sufficient time to ensure complete discharge. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 9 inhalers. Determine 10 delivered doses, each 1 dose at the beginning for 10 inhalers, by the above process.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

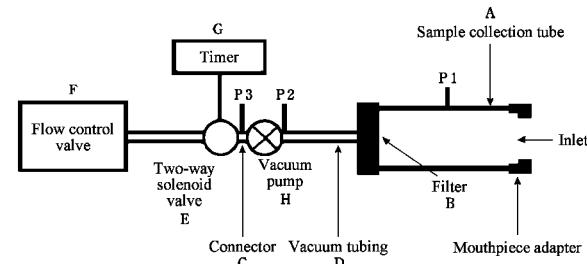
The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the above procedure for 20 more inhalers, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

## 2. Test method for dry powder inhalers

The dose collection apparatus must be capable of quantitatively capturing the delivered dose. A dose collection apparatus similar to that described for the valuation of metered-dose inhalers may be used provided that the dimensions of the tube and the filter can accommodate the measured flow rate. A suitable tube is defined in Table 6.14-1. Connect the tube to a flow system according to the scheme specified in Table 6.14-1 and Fig. 6.14-2.

**Fig. 6.14-2** Apparatus suitable for measuring the uniformity of delivered dose for dry powder inhalers

sions of the tube and the filter can accommodate the measured flow rate. A suitable tube is defined in Table 6.14-1. Connect the tube to a flow system according to the scheme specified in Table 6.14-1 and Fig. 6.14-2.

Unless otherwise specified, determine the test flow rate and duration using the dose collection tube, the associated flow system, a suitable differential pressure meter and a suitable volumetric flowmeter, calibrated for the flow leaving the meter, according to the following procedure.

Prepare the inhaler for use according to the instructions to the patient and connect it to the inlet of the apparatus using a mouthpiece adapter to ensure an airtight seal. Use a mouthpiece adapter that ensures that the front face of the inhaler mouthpiece is flush with the front face of the sample collection tube. Connect one port of a differential pressure meter to the pressure reading point P1 in Fig. 6.14-2, and let the other be open to the atmosphere. Switch on the pump, open the 2-way solenoid valve and adjust the flow control valve until the pressure drop across the inhaler is 4.0 kPa (40.8 cm H<sub>2</sub>O) as indicated by the differential pressure meter. Remove the inhaler from the mouthpiece adapter and, without touching the flow control valve, connect a

flowmeter to the inlet of the sampling apparatus. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter ( $Q_{\text{out}}$ ) using the ideal gas law. For a meter calibrated for the entering volumetric flow ( $Q_{\text{in}}$ ), use the following expression.

$$Q_{\text{out}} = \frac{Q_{\text{in}} \times P_0}{P_0 - \Delta P}$$

$P_0$ : atmospheric pressure

$\Delta P$ : pressure drop over the meter

If the flow rate is above 100 L per minute, adjust the flow control valve to obtain a flow rate of 100 L per minute ( $\pm 5\%$ ). Note the volumetric airflow rate exiting the meter and define this as the test flow rate,  $Q_{\text{out}^*}$ , in L per minute. Define the test flow duration,  $T$ , in seconds so that a volume of 4 L of air is drawn from the mouthpiece of the inhaler at the test flow rate,  $Q_{\text{out}^*}$ . Ensure that critical flow occurs in the flow control valve by the following procedure: with the inhaler in place and the test flow rate  $Q_{\text{out}^*}$ , measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Fig. 6.14-2); a ratio P3/P2 of less than or equal to 0.5 indicates critical flow; switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

Dry powder inhalers contain two types of inhalers, pre-metered inhalers where powders for one emission are pre-dispensed in capsules or other suitable dosage forms and device-metered inhalers where powders for one emission are metered within the inhalers. Perform the test by the following methods depending on each function of the pre-metered inhalers or device-metered inhalers.

## 2.1. Pre-metered inhalers

Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 9 doses. The sampling procedure to obtain 10 values of delivered doses is prescribed individually in considering the discharge mechanism of each preparation.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the above procedure for 20 more delivered doses, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

## 2.2. Device-metered inhalers

### 2.2.1. Test method 1: evaluation of intra-inhaler dose uniformity

Take one inhaler, and perform the test. Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler under the predetermined condi-

tions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste until  $(n/2) + 1$  deliveries remain, where  $n$  is the number of deliveries stated on the label. If necessary, store the inhaler to discharge electrostatic charges. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste until 3 doses remain. If necessary, store the inhaler to discharge electrostatic charges. Collect 3 doses using the procedure described above. Determine 10 delivered doses per one inhaler, i.e., 3 doses at the beginning, 4 doses at the middle and 3 doses at the end by the above process.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the test for 2 more inhalers, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

### 2.2.2. Test method 2: evaluation of inter-inhaler dose uniformity

Take one inhaler, and perform the test. Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 9 inhalers. Determine 10 delivered doses, each 1 dose at the beginning for 10 inhalers, by the above process.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the above procedure for 20 more inhalers, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

## 6.15 Aerodynamic Particle Size Measurement for Inhalations

This test is used to evaluate the fine particle characteristics of the aerosol clouds generated by preparations for inhalation, and is performed using one of the following apparatuses and test procedures. If justified, modified equipment or test procedure may be used.

### 1. Stage mensuration

The most reliable calibration for the separation characteristic of each impaction stage is performed in terms of the relationship between the stage collection efficiency and the aerodynamic diameter of particles and droplets passing through it as an aerosol.

Calibration is usually performed by examination of the jet nozzle dimensions, the spatial arrangement of the jet nozzle and its collection part, and the airflow rate passing through it.

Because jet nozzles can corrode and wear over time, the critical dimensions of each stage must be measured on a regular basis to confirm them being within required ranges.

Only apparatuses that conform to specifications are used for aerodynamic particle size measurement for inhalations. An alternate validated and justified method of mensuration may be used.

### 2. Re-entrainment

When using the apparatuses 2 and 3, the selected technique should seek to minimize particle re-entrainment (from an upper to a lower impaction stage) where this may affect the amounts of drug collected. For example, minimizing the number of sampled doses and coating the particle collection surfaces are used to minimize particle re-entrainment. Glycerol, silicone oil or similar high viscosity liquid are used to coat particle collection surfaces. Plate coating must be part of method validation and may be omitted where it is demonstrated that the aerodynamic particle size is not influenced by the coating.

### 3. Inter-stage drug losses (wall losses)

Wall losses should be considered in method development and validation. If the wall losses affect the recovery rate (mass balance) of drugs, they should be controlled. Wall losses will be dependent upon a number of factors including the impactor type, operating conditions, formulation type and discharged amount to an impactor. How the wall loss is reflected within the calculation of the aerodynamic diameter of particles should be judged based up on the level and variability of the wall loss. For example, in cases where wall losses are low or have a low level of variability, the aerodynamic particle size is calculated by the assay of the drug recovered from the collection plate. In cases where wall losses are high or variable, it may be necessary to collect the wall loss drug separately and take it into account for calculation of the aerodynamic particle size.

### 4. Recovery rate of drugs (mass balance)

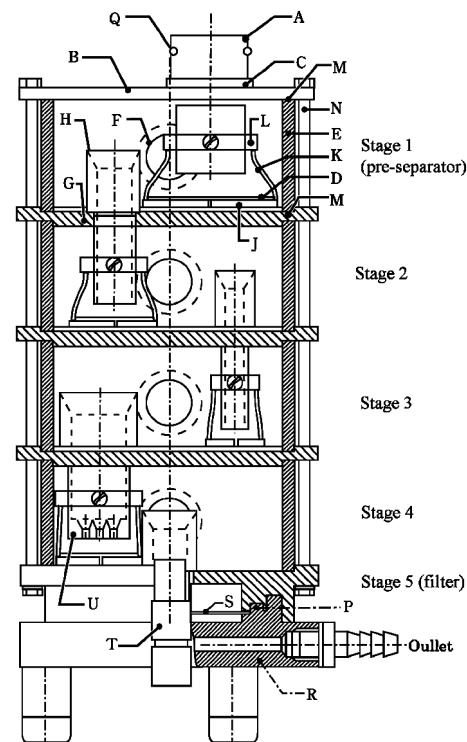
In addition to the size distribution, good analytical practice dictates that a mass balance be performed in order to confirm that the amount of the drug discharged from the inhaler, which is collected in the mouthpiece adapter and the apparatus, is within an acceptable range around the expected value. The total mass of drug collected in all of the components of the mouthpiece adapter and the apparatus divided by the minimum recommended dose described in the dosage and administration is not less than 75% and not more than 125% of the average delivered dose determined under

Uniformity of Delivered Dose for Inhalations <6.14>. This mass balance is necessary to ensure that the test results of particle size distributions are valid.

### 5. Measurement of fine particle dose and particle size distribution

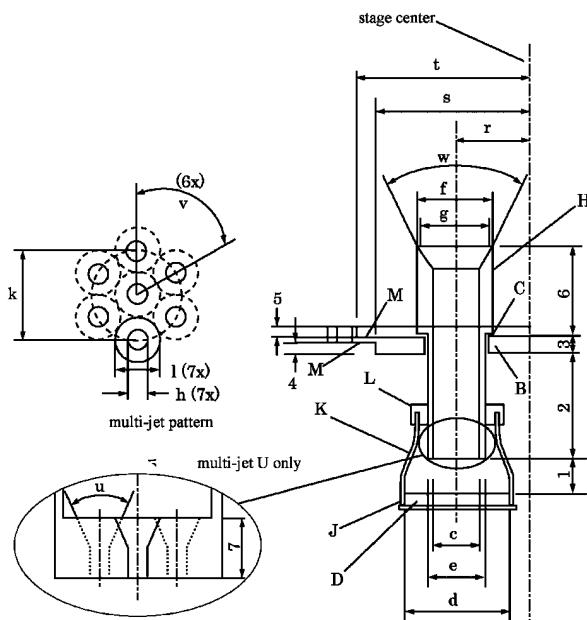
#### 5.1. Multi-stage liquid impinger method (Apparatus 1)

The apparatus used for the multi-stage liquid impinger method (apparatus 1) is shown in Fig. 6.15-1. The apparatus 1 consists of impaction stages 1 (pre-separator), 2, 3 and 4 and an integral filter stage (stage 5), see Figures 6.15-1 to 6.15-3. An impaction stage comprises an upper horizontal metal partition wall (B) through which a metal inlet jet tube (A) with its collection plate (D) is protruding. A glass cylinder (E) with sampling port (F) forms the vertical wall of the stage, and the stage is connected to the next lower stage by the tube (H) through a lower horizontal metal partition wall (G). The tube into stage 4 (U) ends in a multi-jet arrangement. The collection plate (D) is secured in a metal frame (J) which is fastened by two wires (K) to a sleeve (L) secured on the jet tube. The horizontal face of the collection plate is perpendicular to the axis of the jet tube and centrally aligned. The upper surface of the collection plate is slightly raised above the edge of the metal frame. A recess around the perimeter of the horizontal partition wall guides the position of the glass cylinder. The glass cylinders are sealed against the horizontal partition walls with gaskets (M) and clamped together by six bolts (N). The sampling ports are sealed by stoppers. The bottom-side (back) of the lower partition wall of stage 4 has a concentrical protrusion fitted with a rubber O-ring (P) which seals against the edge of a filter placed in the filter holder. The filter holder (R) is constructed as a basin with a concentrical recess in which a perforated filter support (S) is flush fitted. The filter holder is dimensioned for 76 mm diameter filters. The assembly of impac-



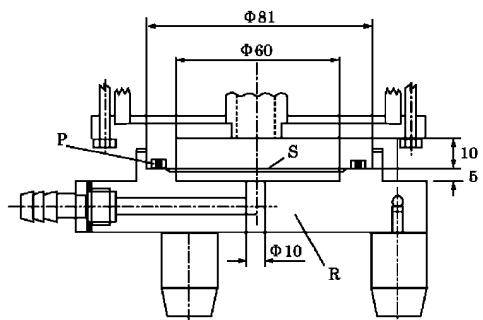
Capital letters refer to Table 6.15-1.

**Fig. 6.15-1** Multi-stage liquid impinger (Apparatus 1)



Inserts show end of multi-jet tube U leading to stage 4.  
Numbers and lowercase letters refer to Table 6.15-2 and capital letters refer to Table 6.15-1.

**Fig. 6.15-2** Apparatus 1: Details of jet tube and collection plate



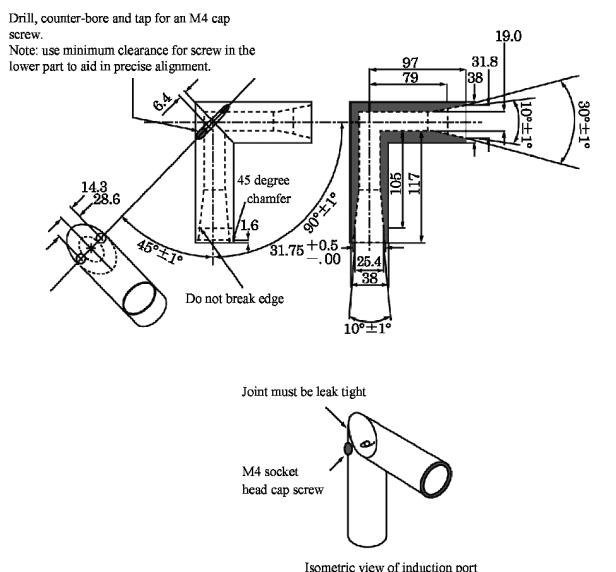
Numbers refer to dimensions ( $\phi$ : diameter). Capital letters refer to Table 6.15-1. Dimensions in mm.

**Fig. 6.15-3** Apparatus 1: Details of the filter stage (stage 5)

tion stages is clamped onto the filter holder by two snap-locks (T). Connect an induction port (see Fig. 6.15-4) onto the stage 1 inlet jet tube of the impinger. A rubber O-ring on the jet tube provides an airtight connection to the induction port. A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port.

### 5.1.1. Procedure for metered-dose inhalers

Dispense 20 mL of a solvent, capable of dissolving the active substance, into each of stages 1 to 4 and replace the stoppers. Tilt the apparatus to wet the stoppers, thereby neutralizing electrostatic charge. Place a suitable filter capable of quantitatively collecting the active substance in stage 5 and assemble the apparatus. Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use. Con-



Dimensions in mm unless otherwise stated.

### Note

- (1) Material may be aluminium, stainless steel or other suitable material.
- (2) Machine from 38 mm bar stock.
- (3) Bore 19 mm hole through bar.
- (4) Cut tube to exact 45° as shown.
- (5) The inner bores and tapers should be smooth – surface roughness Ra approx. 0.4 µm.
- (6) Mill joining cads of stock to provide a liquid tight leak-free seal.
- (7) Set up a holding fixture for aligning the inner 19 mm bore and for drilling and tapping M4 × 0.7 threads. There must be virtually no mismatch of the inner bores in the miter joint.

**Fig. 6.15-4** Induction port

nected a suitable vacuum pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 30 L per minute ( $\pm 5\%$ ). Switch off the pump.

Unless otherwise prescribed in the patient instructions, shake the inhaler for 5 seconds and discharge one delivery to waste. Switch on the pump to the apparatus, locate the mouthpiece end of the inhaler in the adapter and discharge the inhaler into the apparatus, actuating the inhaler for a sufficient time to ensure complete discharge. Wait for 5 seconds before removing the assembled inhaler from the adapter. Repeat the procedure. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of the fine particle dose. After the final discharge, wait for 5 seconds and then switch off the pump.

Dismantle the filter stage of the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. If necessary, rinse the inside of the inlet jet tube to stage 1 with the solvent, allowing the solvent to flow into the stage. Extract the active substance from the inner walls and the collection plate of each of the 4 upper stages of the apparatus into the solution in the respective stage by carefully tilting and rotating the apparatus, observing that no liquid transfer occurs be-

**Table 6.15-1** Component specification for apparatus 1 in Figures 6.15-1, 2 and 3

Code*	Item	Description	Dimensions**
A, H	Jet tube	Metal tube screwed onto partition wall sealed by gasket (C), polished inner surface	see Fig. 6.15-2
B, G	Partition wall	Circular metal plate	
		—diameter	120
		—thickness	see Fig. 6.15-2
C	Gasket	e.g. polytetrafluoroethylene	to fit jet tube
D	Collection plate	Porosity 0 sintered-glass disk	
		—diameter	see Fig. 6.15-2
E	Glass cylinder	Plane polished cut glass tube	
		—height, including gaskets	46
		—outer diameter	100
		—wall thickness	3.5
		—sampling port (F) diameter	18
		—stopper in sampling port	ISO24/25
J	Metal frame	L-profiled circular frame with slit	
		—inner diameter	to fit collection plate
		—height	4
		—thickness of horizontal section	0.5
		—thickness of vertical section	2
K	Wire	Steel wire interconnecting metal frame and sleeve (2 for each frame)	
		—diameter	1
L	Sleeve	Metal sleeve secured on jet tube by screw	
		—inner diameter	to fit jet tube
		—height	6
		—thickness	5
M	Gasket	e.g. silicone	to fit glass cylinder
N	Bolt	Metal bolt with nut (6 pairs)	
		—length	205
		—diameter	4
P	O-ring	Rubber O-ring	
		—diameter × thickness	66.34 × 2.62
Q	O-ring	Rubber O-ring	
		—diameter × thickness	29.1 × 1.6
R	Filter holder	Metal housing with stand and outlet	see Fig. 6.15-3
S	Filter support	Perforated sheet metal	
		—diameter	65
		—hole diameter	3
		—distance between holes (center-points)	4
T	Snap-locks		
U	Multi-jet tube	Jet tube (H) ending in multi-jet arrangement	see Fig. 6.15-2

\* Refers to Fig. 6.15-1.

\*\* Measures in mm with tolerances according to JIS B 0405 unless otherwise stated.

**Table 6.15-2** Dimensions<sup>(1)</sup> of jet tube with collection plate of apparatus 1

Type	Code <sup>(2)</sup>	Stage 1	Stage 2	Stage 3	Stage 4	Filter (Stage 5)
Distance (Length)	1	9.5 (- .0 + .5)	5.5 (- .0 + .5)	4.0 (- .0 + .5)	6.0 (- .0 + .5)	n.a.
Distance (Length)	2	26	31	33	30.5	0
Distance (Length)	3	8	5	5	5	5
Distance (Length)	4	3	3	3	3	n.a.
Distance (Length)	5	0	3	3	3	3
Distance (Length)	6 <sup>(3)</sup>	20	25	25	25	25
Distance (Length)	7	n.a.	n.a.	n.a.	8.5	n.a.
Diameter	c	25	14	8.0 (± .1)	21	14
Diameter	d	50	30	20	30	n.a.
Diameter	e	27.9	16.5	10.5	23.9	n.a.
Diameter	f	31.75 (- .0 + .5)	22	14	31	22
Diameter	g	25.4	21	13	30	21
Diameter	h	n.a.	n.a.	n.a.	2.70 (± .5)	n.a.
Diameter	i	n.a.	n.a.	n.a.	6.3	n.a.
Diameter	k	n.a.	n.a.	n.a.	12.6	n.a.
Radius <sup>(4)</sup>	r	16	22	27	28.5	0
Radius	s	46	46	46	46	n.a.
Radius	t	n.a.	50	50	50	50
Angle	w	10°	53°	53°	53°	53°
Angle	u	n.a.	n.a.	n.a.	45°	n.a.
Angle	v	n.a.	n.a.	n.a.	60°	n.a.

(1) Measures in mm with tolerances according to JIS B 0405 unless otherwise stated.

(2) Refer to Fig. 6.15-2

(3) Including gasket

(4) Relative centerline of stage compartment

n.a.: not applicable

tween the stages.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

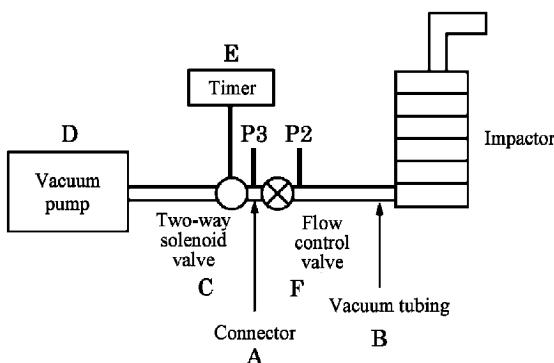
**5.1.2. Procedure for dry powder inhalers**

Place a suitable low resistance filter capable of quantitatively collecting the active substance in stage 5 and assemble the apparatus. Connect the apparatus to a flow system according to the scheme specified in Fig. 6.15-5 and Table 6.15-3. Unless otherwise prescribed, conduct the test at the flow rate,  $Q_{out}$ , used in Uniformity of Delivered Dose for Inhalations <6.14>, drawing 4 L of air from the mouthpiece of the inhaler and through the apparatus.

Connect a flowmeter to the induction port. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter ( $Q_{out}$ ) using the ideal gas law. For a meter calibrated for the entering volumetric flow ( $Q_{in}$ ), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

 $P_0$ : atmospheric pressure $\Delta P$ : pressure drop over the meter



Capital letters refer to Table 6.15-3.

**Fig. 6.15-5** Experimental set-up for testing dry powder inhalers

Adjust the flow control valve to achieve steady flow through the system at the required rate,  $Q_{out}$  ( $\pm 5\%$ ). Ensure that critical flow occurs in the flow control valve by the following procedure. Switch off the pump.

With the inhaler in place and the test flow rate established, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Fig. 6.15-5). A ratio  $P3/P2$  of less than or equal to 0.5 indicates critical flow. Switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

Dispense 20 mL of a solvent, capable of dissolving the active substance, into each of the 4 upper stages of the apparatus and replace the stoppers. Tilt the apparatus to wet the stoppers, thereby neutralizing electrostatic charge. Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use.

Prepare the dry powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time,  $T$  ( $\pm 5\%$ ). Repeat the discharge procedure. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the filter stage of the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. If necessary, rinse the inside of the inlet jet tube to stage 1 with the solvent, allowing the solvent to flow into the stage. Extract the active substance from the inner walls and the collection plate of each of the 4 upper stages of the apparatus into the solution in the respective stage by carefully tilting and rotating the apparatus, observing that no liquid transfer occurs between the stages.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

**Table 6.15-3** Component specification for Fig. 6.15-5

Code*	Item	Description
A	Connector	ID $\geq 8$ mm, e.g., short metal coupling, with low-diameter branch to P3.
B	Vacuum tubing	A length of suitable tubing having an ID $\geq 8$ mm and an internal volume of $25 \pm 5$ mL.
C	Two-way solenoid valve	A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with ID $\geq 8$ mm and an opening time $\leq 100$ ms.
D	Vacuum pump	Pump must be capable of drawing the required flow rate through the assembled apparatus with the inhaler in the mouthpiece adapter, or equivalent. Connect the pump to the 2-way solenoid valve using short and/or wide (ID $\geq 10$ mm) vacuum tubing and connectors to minimize pump capacity requirements.
E	Timer	Timer capable to drive the 2-way solenoid valve for the required duration, or equivalent.
P2, P3	Pressure measurements	Determine under steady-state flow condition with an absolute pressure transducer.
F	Flow control valve	Adjustable regulating valve with maximum $C_v \geq 1$ .

\* Refer to Fig. 6.15-5.

ID: inner diameter

## 5.2. Andersen cascade impactor method (Apparatus 2)

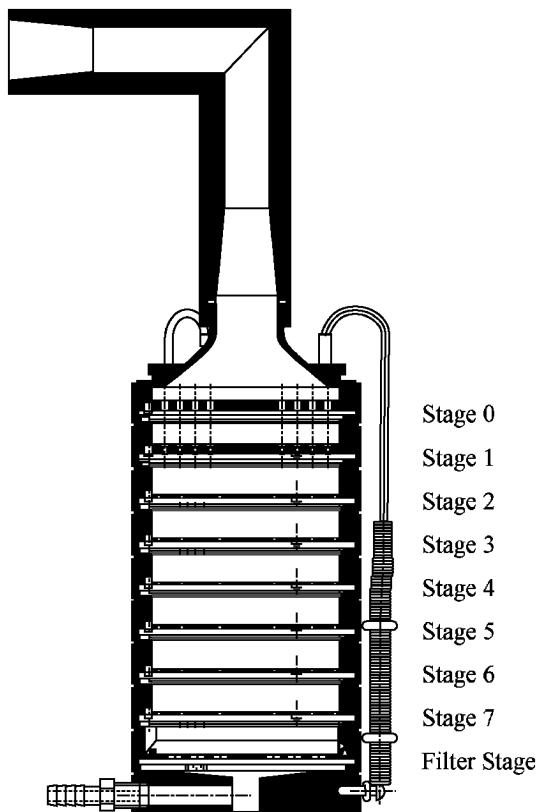
The apparatus used for Andersen cascade impactor method (apparatus 2) is shown in Fig. 6.15-6. The apparatus 2 consists of 8 stages together with a final filter. Material of construction may be aluminium, stainless steel or other suitable material. The stages are clamped together and sealed with O-rings. Critical dimensions of apparatus 2 are provided in Table 6.15-4. In use, some occlusions and wear of nozzles will occur. In-use mensuration tolerances need to be justified.

The configuration used for metered-dose inhalers is shown in Fig. 6.15-6. The entry cone (see Fig. 6.15-7b) of the impactor is connected to an induction port (see Fig. 6.15-4). A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port.

In the configuration for dry powder inhalers, a pre-separator is placed above the top stage to collect large masses of non-respirable powder. The top of the pre-separator shown in Fig. 6.15-7a is used to adapt the pre-separator to the induction port. To accommodate high flow rates through the impactor, the outlet nipple, used to connect the impactor to the vacuum system, is enlarged to have an internal diameter greater than or equal to 8 mm.

### 5.2.1. Procedure for metered-dose inhalers

Assemble the Andersen cascade impactor with a suitable filter in place. Ensure that the system is airtight by a suitable method. Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler unit is positioned in the same orientation as the intended use. Connect a suitable pump to the outlet of the apparatus and adjust the air flow through the apparatus,



**Fig. 6.15-6** Andersen cascade impactor used for metered-dose inhalers (Apparatus 2)

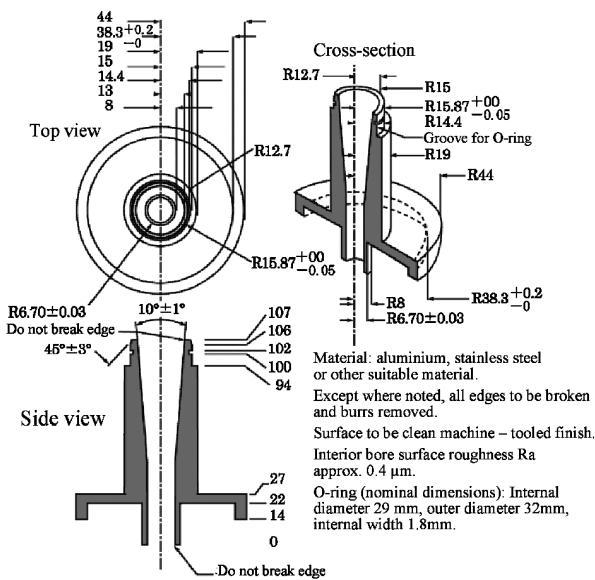
**Table 6.15-4** Critical dimensions for apparatus 2

Description	Number of nozzle	Dimension (mm)
Stage 0 nozzle diameter	96	$2.55 \pm 0.025$
Stage 1 nozzle diameter	96	$1.89 \pm 0.025$
Stage 2 nozzle diameter	400	$0.914 \pm 0.0127$
Stage 3 nozzle diameter	400	$0.711 \pm 0.0127$
Stage 4 nozzle diameter	400	$0.533 \pm 0.0127$
Stage 5 nozzle diameter	400	$0.343 \pm 0.0127$
Stage 6 nozzle diameter	400	$0.254 \pm 0.0127$
Stage 7 nozzle diameter	201	$0.254 \pm 0.0127$

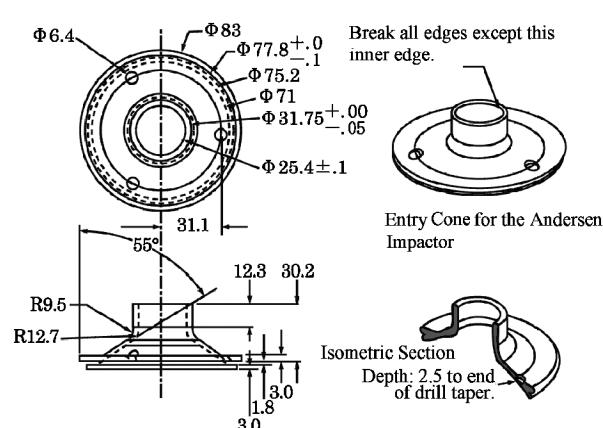
as measured at the inlet to the induction port, to 28.3 L per minute ( $\pm 5\%$ ). Switch off the pump.

Unless otherwise prescribed in the patient instructions, shake the inhaler for 5 seconds and discharge one delivery to waste. Switch on the pump to the apparatus, locate the mouthpiece end of the inhaler in the adapter and discharge the inhaler into the apparatus, actuating the inhaler for a sufficient time to ensure complete discharge. Wait for 5 seconds before removing the assembled inhaler from the adapter. Repeat the procedure. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of the fine particle dose. After the final discharge, wait for 5 seconds and then switch off the pump.

Dismantle the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the induction port and mouthpiece adapter from the



**Fig. 6.15-7a** Expanded view of top for the Andersen pre-separator adapted to the induction port



Material may be aluminum, stainless steel, or other suitable material. Surface roughness (Ra) should be approximately 0.4  $\mu\text{m}$ . Dimensions in mm unless otherwise stated.

**Fig. 6.15-7b** Expanded view of the entry cone for mounting induction port on the Andersen cascade impactor without pre-separator.

apparatus and extract the active substance into an aliquot of the solvent. Extract the active substance from the inner walls and the collection plate of each of the stages of the apparatus into aliquots of the solvent.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

#### 5.2.2. Procedure for dry powder inhalers

The aerodynamic cut-off diameters of the individual stages of this apparatus are currently not well-established at flow rates other than 28.3 L per minute. Users must justify and validate the use of the impactor in the chosen conditions, when flow rates different from 28.3 L per minute are selected.

Assemble the Andersen cascade impactor with the pre-separator and a suitable filter in place and ensure that the system is airtight. Depending on the product characteristics,

the pre-separator may be omitted, where justified. Stages 6 and 7 may also be omitted at high flow rates, if justified. The pre-separator may be coated in the same way as the collection plates or may contain 10 mL of a suitable solvent. Connect the apparatus to a flow system according to the scheme specified in Fig. 6.15-5 and Table 6.15-3.

Unless otherwise prescribed, conduct the test at the flow rate,  $Q_{\text{out}}$ , used in Uniformity of Delivered Dose for Inhalers <6.14> drawing 4 L of air from the mouthpiece of the inhaler and through the apparatus.

Connect a flowmeter to the induction port. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter ( $Q_{\text{out}}$ ) using the ideal gas law. For a meter calibrated for the entering volumetric flow ( $Q_{\text{in}}$ ), use the following expression:

$$Q_{\text{out}} = \frac{Q_{\text{in}} \times P_0}{P_0 - \Delta P}$$

$P_0$ : atmospheric pressure

$\Delta P$ : pressure drop over the meter

Adjust the flow control valve to achieve steady flow through the system at the required rate,  $Q_{\text{out}}$  ( $\pm 5\%$ ). Ensure that critical flow occurs in the flow control valve by the procedure described in 5.1.2. Procedure for dry powder inhalers. Switch off the pump.

Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use.

Prepare the dry powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time,  $T$  ( $\pm 5\%$ ). Repeat the discharge sequence. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the pre-separator, induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. Extract the active substance from the inner walls and the collection plate of each of the stages of the apparatus into the solvent.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

### 5.3. Next generation impactor method (Apparatus 3)

The apparatus used for next generation impactor method (apparatus 3) is shown in Fig. 6.15-8. The apparatus 3 is a cascade impactor with 7 stages and a micro-orifice collector (MOC). Over the flow rate range of 30 to 100 L per minute the 50%-collection efficiency cut-off diameters ( $D_{50}$  values) range between 0.24  $\mu\text{m}$  and 11.7  $\mu\text{m}$ , evenly spaced on a logarithmic scale. In this flow range, there are always at least 5 stages with  $D_{50}$  values between 0.5  $\mu\text{m}$  and 6.5  $\mu\text{m}$ . The collection efficiency curves for each stage are sharp and minimize overlap between stages.

Material of construction may be aluminium, stainless steel or other suitable material.

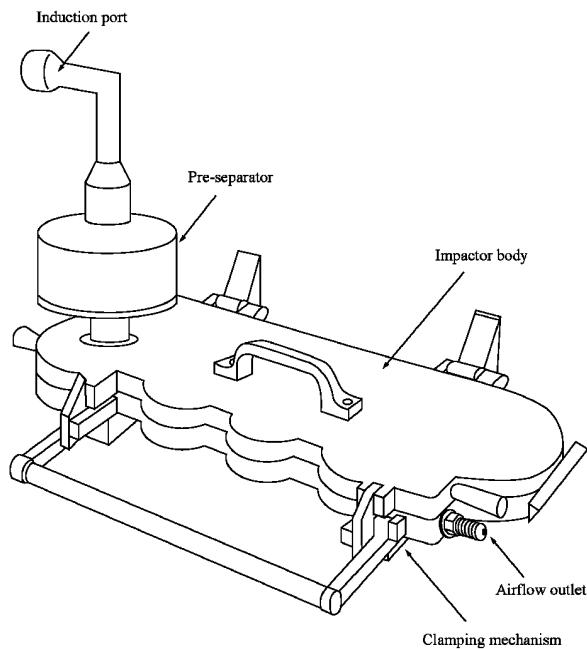


Fig. 6.15-8 Next generation impactor (shown with the pre-separator in place) (Apparatus 3)

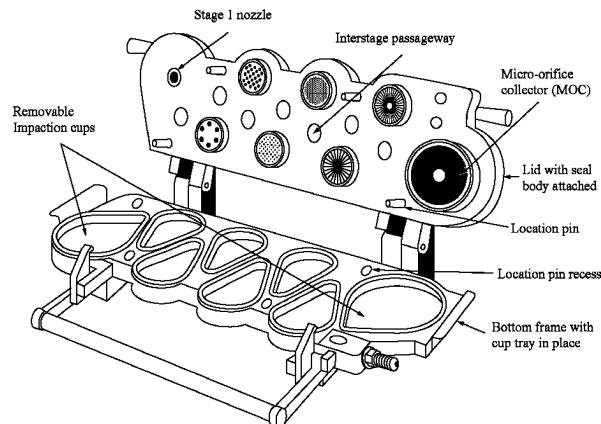


Fig. 6.15-9 Apparatus 3 showing component parts

The impactor configuration has removable impaction cups with all the cups in one plane (Figures 6.15-8 to 6.15-11). There are three main sections to the impactor; the bottom frame that holds the impaction cups, the seal body that holds the jets and the lid that contains the interstage passageways (Figures 6.15-8 and 6.15-9). Multiple nozzles are used at all but the first stage (Fig. 6.15-10). The flow passes through the impactor in a saw-tooth pattern.

Critical dimensions are provided in Table 6.15-5.

In routine operation, the seal body and lid are held together as a single assembly. The impaction cups are accessible when this assembly is opened at the end of an inhaler test. The cups are held in a support tray, so that all cups can be removed from the impactor simultaneously by lifting out the tray.

An induction port with internal dimensions defined in Fig. 6.15-4 connects to the impactor inlet. A pre-separator can be added when required, typically with dry powder inhalers, and connects between the induction port and the impactor. A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port.

**Table 6.15-5** Critical dimensions for apparatus 3

Description	Dimension (mm)
Pre-separator (dimension a—see Fig. 6.15-12)	12.8 ± 0.05
Stage 1* nozzle diameter	14.3 ± 0.05
Stage 2* nozzle diameter	4.88 ± 0.04
Stage 3* nozzle diameter	2.185 ± 0.02
Stage 4* nozzle diameter	1.207 ± 0.01
Stage 5* nozzle diameter	0.608 ± 0.01
Stage 6* nozzle diameter	0.323 ± 0.01
Stage 7* nozzle diameter	0.206 ± 0.01
MOC*	aprox. 0.070
Cup depth (dimension b—see Fig. 6.15-11)	14.625 ± 0.10
Collection cup surface roughness (Ra)	0.5 to 2 µm
Stage 1 nozzle to seal body distance** —dimension c	0 ± 1.18
Stage 2 nozzle to seal body distance** —dimension c	5.236 ± 0.736
Stage 3 nozzle to seal body distance** —dimension c	8.445 ± 0.410
Stage 4 nozzle to seal body distance** —dimension c	11.379 ± 0.237
Stage 5 nozzle to seal body distance** —dimension c	13.176 ± 0.341
Stage 6 nozzle to seal body distance** —dimension c	13.999 ± 0.071
Stage 7 nozzle to seal body distance** —dimension c	14.000 ± 0.071
MOC nozzle to seal body distance** —dimension c	14.429 to 14.571

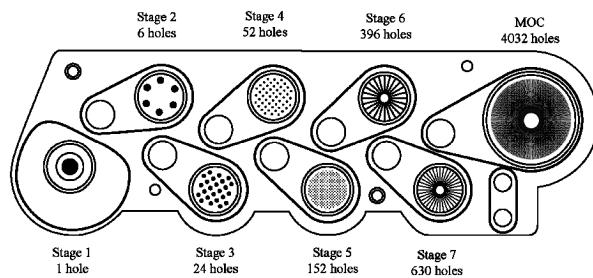
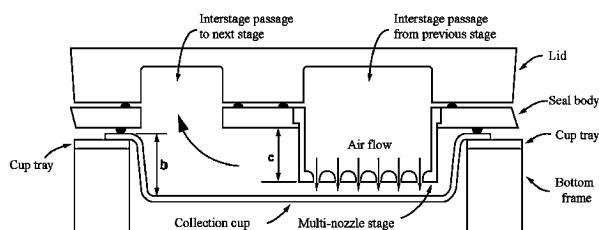
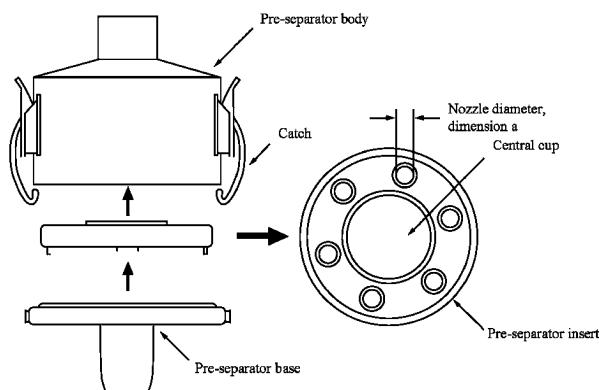
\* See Fig. 6.15-10

\*\* See Fig. 6.15-11

Apparatus 3 contains a terminal MOC that for most preparations will eliminate the need for a final filter as determined by method validation. The MOC is a collection plate with nominally 4032 holes, each approximately 70 µm in diameter. Most particles not captured on stage 7 of the impactor will be captured on the cup surface below the MOC. For impactors operated at 60 L per minute, the MOC is capable of collecting 80% of 0.14 µm particles. For preparations with a significant fraction of particles not captured by the MOC, there is an optional filter holder that can replace the MOC or be placed downstream of the MOC (a glass fiber filter is suitable).

### 5.3.1. Procedure for metered-dose inhalers

Place cups into the apertures in the cup tray. Insert the cup tray into the bottom frame, and lower into place. Close the impactor lid with the seal body attached and operate the

**Fig. 6.15-10** Apparatus 3: nozzle configuration**Fig. 6.15-11** Apparatus 3: configuration of interstage passageways**Fig. 6.15-12** Apparatus 3: pre-separator configuration

handle to lock the impactor together so that the system is airtight.

Connect an induction port with internal dimensions defined in Fig. 6.15-4 to the impactor inlet. Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler to the mouthpiece adapter, when inserted, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use. Connect a suitable pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 30 L per minute ( $\pm 5\%$ ). Switch off the pump.

Unless otherwise prescribed in the patient instructions, shake the inhaler for 5 seconds and discharge one delivery to waste. Switch on the pump to the apparatus, locate the mouthpiece end of the inhaler in the adapter and discharge the inhaler into the apparatus, actuating the inhaler for a sufficient time to ensure a complete discharge.

Wait for 5 seconds before removing the assembled inhaler

from the adapter. Repeat the procedure. The number of discharges should be minimized, and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of the fine particle dose. After the final discharge, wait for 5 seconds and then switch off the pump.

Dismantle the apparatus and recover the active substance as follows. Remove the induction port and mouthpiece adapter from the apparatus and extract the deposited active substance into an aliquot of the solvent. Open the impactor by releasing the handle and lifting the lid. Remove the cup tray, with the collection cups, and extract the active substance in each cup into an aliquot of the solvent.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

### 5.3.2. Procedure for dry powder inhalers

Assemble the apparatus with the pre-separator (Fig. 6.15-12). Depending on the product characteristics, the pre-separator may be omitted, where justified.

Place cups into the apertures in the cup tray. Insert the cup tray into the bottom frame, and lower into place. Close the impactor lid with the seal body attached and operate the handle to lock the impactor together so that the system is airtight.

When used, the pre-separator should be assembled as follows.

Assemble the pre-separator insert into the pre-separator base. Fit the pre-separator base to the impactor inlet. Add 15 mL of the solvent used for active substance recovery to the central cup of the pre-separator insert. Place the pre-separator body on top of this assembly and close the two catches.

Connect an induction port with internal dimensions defined in Fig. 6.15-4 to the impactor inlet or pre-separator inlet. Connect the apparatus to a flow system according to the scheme specified in Fig. 6.15-5 and Table 6.15-3.

Unless otherwise prescribed, conduct the test at the flow rate,  $Q_{\text{out}}$ , used in Uniformity of Delivered Dose for Inhalations <6.14> drawing 4 L of air from the mouthpiece of the inhaler and through the apparatus. Connect a flowmeter to the induction port. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter ( $Q_{\text{out}}$ ) using the ideal gas law. For a meter calibrated for the entering volumetric flow ( $Q_{\text{in}}$ ), use the following expression:

$$Q_{\text{out}} = \frac{Q_{\text{in}} \times P_0}{P_0 - \Delta P}$$

$P_0$ : atmospheric pressure

$\Delta P$ : pressure drop over the meter

Adjust the flow control valve to achieve steady flow through the system at the required rate,  $Q_{\text{out}}$  ( $\pm 5\%$ ). Ensure that critical flow occurs in the flow control valve by the procedure described in 5.1.2. Procedure for dry powder inhalers. Switch off the pump.

Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use.

Prepare the dry powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in

**Table 6.15-6** Calculations for Apparatus 1

Cut-off diameter ( $\mu\text{m}$ )	Mass of active substance deposited on stage per discharge	Cumulative mass per discharge	Cumulative fraction (%)
$d_4 = 1.7 \times q$	Mass from filter stage ( $m_5^*$ )	$c_4 = m_5$	$f_4 = (c_4/c) \times 100$
$d_3 = 3.1 \times q$	Mass from stage 4 ( $m_4$ )	$c_3 = c_4 + m_4$	$f_3 = (c_3/c) \times 100$
$d_2 = 6.8 \times q$	Mass from stage 3 ( $m_3$ )	$c_2 = c_3 + m_3$	$f_2 = (c_2/c) \times 100$
	Mass from stage 2 ( $m_2$ )	$c = c_2 + m_2$	100

\* Stage 5 is the filter stage.

$q = \sqrt{(60/Q)}$ ,  $Q$ : the test flow rate in L per minute ( $Q_{\text{out}}$  for dry powder inhalers).

**Table 6.15-7** Calculations for Apparatus 2 when used at a flow rate of 28.3 L per minute

Cut-off diameter ( $\mu\text{m}$ )	Mass of active substance deposited on stage per discharge	Cumulative mass per discharge	Cumulative fraction (%)
$d_7 = 0.4$	Mass from filter stage ( $m_8$ )	$c_7 = m_8$	$f_7 = (c_7/c) \times 100$
$d_6 = 0.7$	Mass from stage 7 ( $m_7$ )	$c_6 = c_7 + m_7$	$f_6 = (c_6/c) \times 100$
$d_5 = 1.1$	Mass from stage 6 ( $m_6$ )	$c_5 = c_6 + m_6$	$f_5 = (c_5/c) \times 100$
$d_4 = 2.1$	Mass from stage 5 ( $m_5$ )	$c_4 = c_5 + m_5$	$f_4 = (c_4/c) \times 100$
$d_3 = 3.3$	Mass from stage 4 ( $m_4$ )	$c_3 = c_4 + m_4$	$f_3 = (c_3/c) \times 100$
$d_2 = 4.7$	Mass from stage 3 ( $m_3$ )	$c_2 = c_3 + m_3$	$f_2 = (c_2/c) \times 100$
$d_1 = 5.8$	Mass from stage 2 ( $m_2$ )	$c_1 = c_2 + m_2$	$f_1 = (c_1/c) \times 100$
$d_0 = 9.0$	Mass from stage 1 ( $m_1$ )	$c_0 = c_1 + m_1$	$f_0 = (c_0/c) \times 100$
	Mass from stage 0 ( $m_0$ )	$c = c_0 + m_0$	100

the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time,  $T$  ( $\pm 5\%$ ). Repeat the discharge sequence. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the apparatus and recover the active substance as follows.

Remove the induction port and mouthpiece adapter from the pre-separator, when used, and extract the deposited active substance into an aliquot of the solvent. When used, remove the pre-separator from the impactor, being careful to avoid spilling the cup liquid into the impactor. Recover the active substance from the pre-separator.

Open the impactor by releasing the handle and lifting the

**Table 6.15-8** Calculations for Apparatus 3

Cut-off diameter ( $\mu\text{m}$ )	$x$	Mass of active substance deposited on stage per discharge	Cumulative mass of active substance per discharge	Cumulative fraction of active substance (%)
$d_7 = 0.34 \times q$	0.67	Mass from MOC or terminal filter ( $m_8$ )	$c_7 = m_8$	$F_7 = (c_7/c) \times 100$
$d_6 = 0.55 \times q$	0.60	Mass from stage 7 ( $m_7$ )	$c_6 = c_7 + m_7$	$F_6 = (c_6/c) \times 100$
$d_5 = 0.94 \times q$	0.53	Mass from stage 6 ( $m_6$ )	$c_5 = c_6 + m_6$	$F_5 = (c_5/c) \times 100$
$d_4 = 1.66 \times q$	0.47	Mass from stage 5 ( $m_5$ )	$c_4 = c_5 + m_5$	$F_4 = (c_4/c) \times 100$
$d_3 = 2.82 \times q$	0.50	Mass from stage 4 ( $m_4$ )	$c_3 = c_4 + m_4$	$F_3 = (c_3/c) \times 100$
$d_2 = 4.46 \times q$	0.52	Mass from stage 3 ( $m_3$ )	$c_2 = c_3 + m_3$	$F_2 = (c_2/c) \times 100$
$d_1 = 8.06 \times q$	0.54	Mass from stage 2 ( $m_2$ )	$c_1 = c_2 + m_2$	$F_1 = (c_1/c) \times 100$
		Mass from stage 1 ( $m_1$ )	$c = c_1 + m_1$	100

$q = (60/Q)^x$ ,  $Q$ : the test flow rate in L per minute,  $x$ : listed in the table

lid. Remove the cup tray, with the collection cups, and extract the active substance in each cup into an aliquot of the solvent.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

## 6. Calculations

From the analysis of the solutions, calculate the mass of active substance deposited on each stage per discharge and the mass of active substance per discharge deposited in the induction port, mouthpiece adapter and when used, the pre-separator.

Starting at the collection site (filter or MOC) close to the airflow outlet of the apparatus, derive a table of cumulative mass versus cut-off diameter of the respective stage (see Table 6.15-6 for Apparatus 1, Table 6.15-7 for Apparatus 2, Table 6.15-8 for Apparatus 3). Calculate the Fine Particle Dose (FPD) by interpolation of the mass of the active substance less than or equal to  $5 \mu\text{m}$ . Or it is possible to calculate the FPD as the mass of the active substance deposited on the stages corresponding to the cut-off diameter of  $5 \mu\text{m}$  and less.

If necessary, and where appropriate (e.g., where there is a log-normal distribution), determine values for the Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) from the cumulative fraction of active substance versus cut-off diameter (see Tables 6.15-6 to 6.15-8). Appropriate computational methods may also be used.

## 6.16 Rheological Measurements for Semi-solid Preparations

Rheological measurements for semi-solid preparations are methods to measure fluidity and deformation by adding force to semi-solid preparations such as Semi-solid Preparations for Oro-mucosal Application, Ophthalmic Ointments, Ointments, Creams and Gels.

There are two methods: spreadability test and penetrometry.

These methods are more practical to determine rheological properties of semi-solid preparations, though Method II Viscosity measurement by rotational viscometer under Viscosity Determination <2.53> can evaluate precisely rheological properties of semi-solids.

### 1. Spreadability test

Spreadability test is a method for measuring flowability of semi-solid preparations using a spread meter (also known as a parallel plate viscometer).

A spread meter measures a spreading diameter etc. of a sample by observing the characteristic of the concentric spreading over time when the sample is sandwiched between two parallel plates [glass (or plastic) plate and fixed plate] placed horizontally and pushed outward with the own weight of the glass plate.

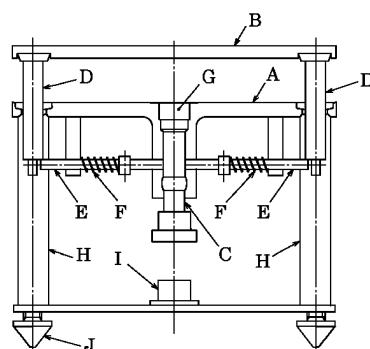
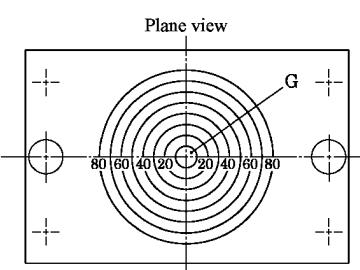
Generally there is a reciprocal relationship between fluidity as an index of flowability and viscosity, however flowability measured by this method does not necessarily correlate with apparent viscosity ( $\text{mPa}\cdot\text{s}$ ) measured by viscosity determination.

This method targets relatively soft preparations in particular among semi-solid preparations.

### 1.1. Apparatus

An example of spread meters is shown in Fig. 6.16-1.

There are two parallel plates placed horizontally. The fixed plate installed in the lower part has engraved scales at 1 mm intervals to measure the distance from the center and has a cylindrical hole (0.5 mL) at the center for inserting a sample.



- A: Fixed plate
- B: Glass plate
- C: Piston
- D: Support rod for glass plate
- E: Push rod for piston
- F: Spring
- G: Specimen hole
- H: Support
- I: Level
- J: Screw for horizontal control

**Fig. 6.16-1** Example of spread meter

The weight-loading glass plate positioned in the upper part is made of transparent glass or acrylate resin etc. and has a mass of 115 g. The glass plate is supported by rods. Pushing the piston up raise the bottom of the hole to push the sample above the fixed plate. In connection with it the glass plate falls (20 mm, commonly) and the sample spreads concentrically on the fixed plate with the own weight of the glass plate. Measuring the extent of the spread provides the flowability of the sample.

### 1.2. Procedure and measurement conditions

Before measurement remove a glass plate from an apparatus and clean the glass plate, a fixed plate and a specimen hole. Fill a sample in the specimen hole at the center of the fixed plate and flatten the sample by a spatula etc. so that the upper face of the sample is flush with the fixed plate and become flat. Wipe the sample protruded off. Make sure that the apparatus is horizontal by a level and install the glass plate to the support rod. Push the piston up and start time measurement simultaneously. Measure the spread of the sample on the fixed plate by the scale in mm unit over time and record them.

A constant temperature is needed during measurement and it is preferable to perform the measurement in the room controlled at  $25 \pm 2^\circ\text{C}$ .

Bring the temperature of samples equal to that of the measurement environment by allowing them to stand in the measurement environment.

### 1.3. Analysis

Characteristics of flowability obtained with the measurement using a spread meter are expressed as spread meter diameter  $D$  and spread meter yield value  $YV$  in the case of the single-point method and as spread meter slope  $S$  and spread meter intercept  $IC$  in the case of the multi-point method. They are calculated by the following methods, respectively.

#### 1.3.1. Single-point method

(i) Spread meter diameter  $D$ : Expressed as the spreading diameter (mm) after the specified time (usually, 60 seconds).

A larger spreading diameter indicates higher flowability.

(ii) Spread meter yield value  $YV$ : Calculate by the following equation.

$$YV = (4.8 \times W \times V \times g_n) / (\pi^2 \times D_\infty^5)$$

$YV$ : Yield value (Pa)

$W$ : Mass (kg) of glass plate

$V$ : Sample volume ( $\text{m}^3$ )

$g_n$ : Standard acceleration due to gravity ( $\text{m/s}^2$ )

$D_\infty$ : Maximum of spreading diameter (m)

The above equation is expressed in the International System of Units, however actual measurement is performed in the Centimeter-Gram-Second System of Units.

Many semi-solid preparations have no flowability when leave them as they are, however they flow by adding force. The limit value of the force is the yield value and a larger yield value indicates that a larger force is required for the flow of a sample.

#### 1.3.2. Multi-point method

(i) Spread meter slope  $S$ : Calculate by the following equation.

$$S = (D_2 - D_1) / \log_{10}(T_2/T_1)$$

$D_1$ : Spreading diameter after  $T_1$  seconds (mm)

$D_2$ : Spreading diameter after  $T_2$  seconds (mm)

$T_1, T_2$ : Measurement time (seconds)  $T_2 > T_1$ ,  $5 \leq T_1$  and

$$T_2 \leq 100, \Delta T = (T_2 - T_1) > 40$$

In general, plot the spreading diameter measured each

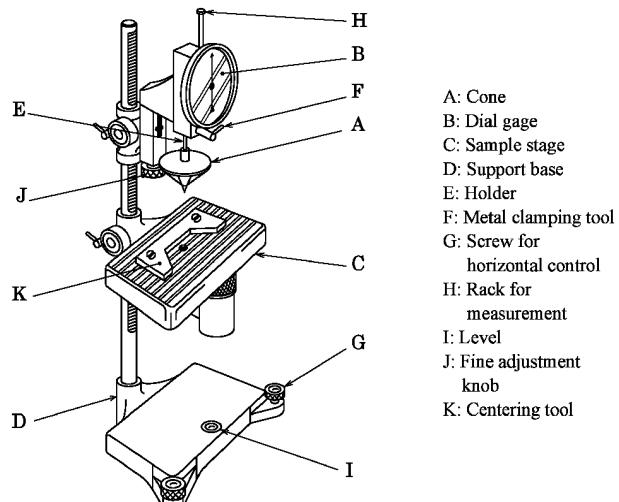


Fig. 6.16-2 Example of penetrometer

time on a semi-logarithmic graph to obtain an almost straight line connecting each point. Spread meter slope  $S$  corresponds to its slope.

A larger  $S$  indicates a larger flow of a sample.

(ii) Spread meter intercept  $IC$ : Plot logarithms of times ( $T_1, T_2$ ) as abscissa against spreading diameters ( $D_1, D_2$ ) as ordinate and draw a line connecting these two points. Obtain the intersection point ( $T = 1$ ) of this extension line and the ordinate axis as spread meter  $IC$ .

A larger  $IC$  indicates higher flowability of a sample.

## 2. Penetrometry

Penetrometry is a method for measuring hardness or softness of semi-solid preparations using a penetrometer.

A penetrometer is an apparatus for measuring the distance traveled by a cone penetrant inside a sample and the consistency is expressed as ten times of the value measured in units of 0.1 mm. A smaller value indicates a harder sample.

This method targets relatively hard preparations in particular among semi-solid preparations.

### 2.1. Apparatus

#### 2.1.1. Penetrometer

An example of penetrometers is shown in Fig. 6.16-2.

Adjust a penetrometer so that the position of the tip of a prescribed cone touches the surface of a sample filled in a sample container, penetrate the cone into the sample with its own weight for a certain time and calculate the consistency from the depth measured in units of 0.1 mm.

Adjust exactly the cone part or the sample stage of the penetrometer so that the position of the tip of the cone touches the horizontal surface of the sample keeping the reading of the dial gage zero. Adjust the cone in advance so that the cone falls more than 62 mm smoothly when released from the state fixed to the penetrometer and the top of the cone does not hit the bottom of the sample container after the fall. The penetrometer must be equipped with a screw for horizontal control and a level to maintain a cone holder being vertical.

#### 2.1.2. Cone

A standard cone is a conical body made of magnesium or other suitable metal, which has a removable hardened steel needle. Fig. 6.16-3 shows the specifications of the standard cone.

The upper end of a holder is equipped with a stopper and the lower end is devised to connect a cone. The inner struc-

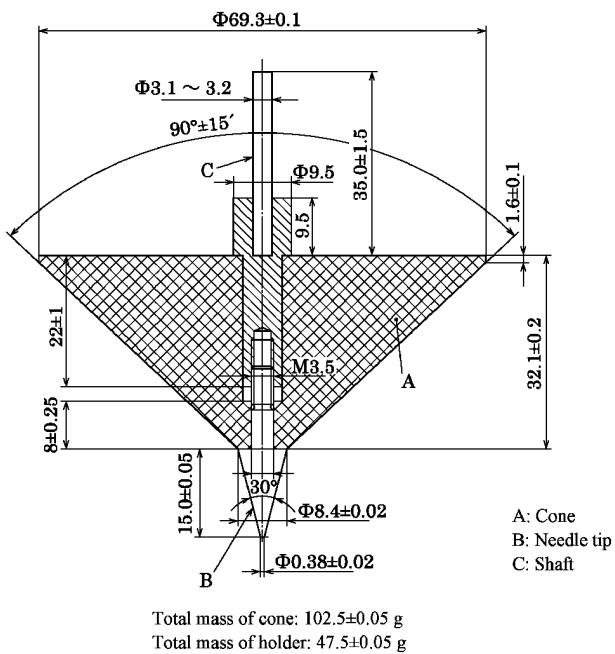


Fig. 6.16-3 Standard cone

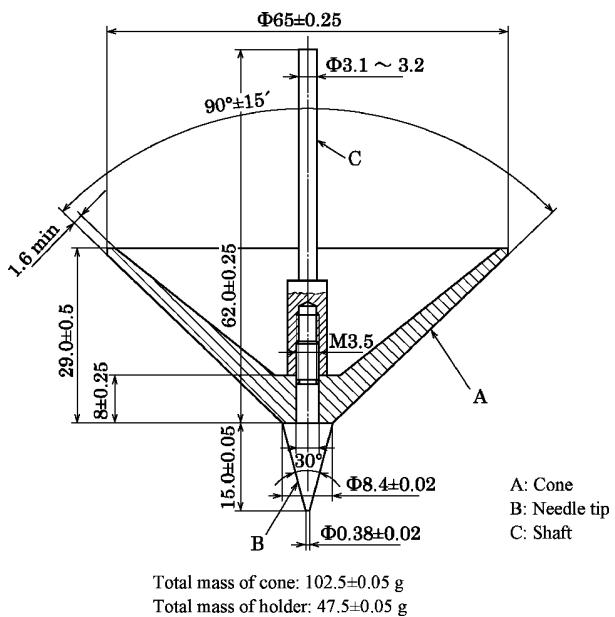


Fig. 6.16-4 Optional cone

ture of the cone may be changed in order to conform to the prescribed mass as far as there is no change in shape and mass distribution of the cone. The outer surface of the cone must be without scratches and smooth enough.

Fig. 6.16-4, 6.16-5 and 6.16-6 show the specifications of an optional cone, a half-scale cone and a quarter-scale cone. The half-scale and quarter-scale cones are the prescribed cones that are scaled down to a half and a quarter of the standard cone or the optional cone.

Select a suitable cone based on the sample volume and the consistency of a preparation to be tested and convert the measured consistency to the value equivalent to that measured using a standard cone.

An optional cone may be used to measure the sample having a consistency of not more than 400. Use a half-scale cone or a quarter-scale cone, when a sample has a consistency of

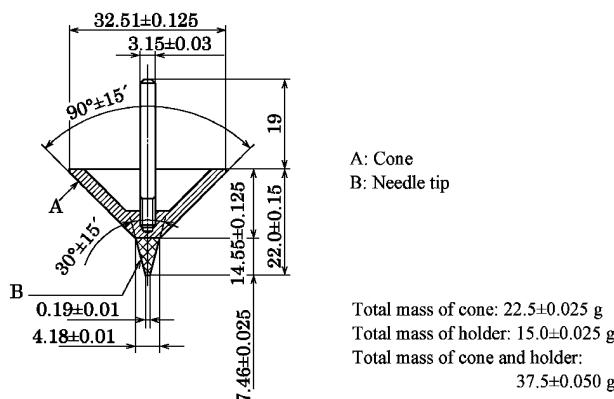


Fig. 6.16-5 Half-scale cone

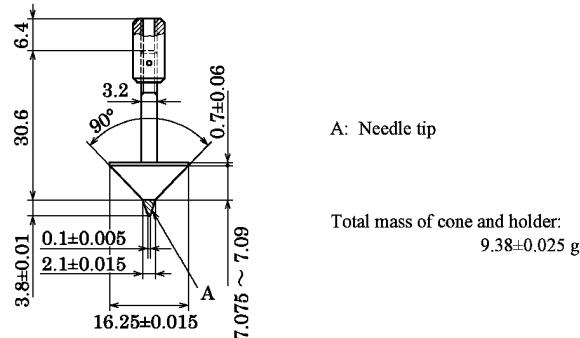


Fig. 6.16-6 Quarter-scale cone

175 to 385 and a standard cone is not available because of small sample amounts.

## 2.2. Procedure and measurement conditions

### 2.2.1. Sample preparation

Stand a prepared empty container and a container with a lid containing a necessary amount of a sample in a water bath at 25°C and bring the temperature of the sample at 25 ± 0.5°C. Transfer the sample in the container with a lid to the empty container at a time, if possible. Remove air bubbles mixed in the sample in the sample container by a suitable method and fill the container with an excessive amount of the sample using a spatula again. Be careful not to stir the sample and not to make vacant space inside the sample in this procedure. Flatten the surface of the sample by removal of the excessive sample by moving a spatula, of which surface is inclined about 45 degrees in the direction of the movement, along the upper edge of the container. Hereafter do not touch the surface of the sample before measurement. Perform the test promptly to keep the temperature of the sample evenly at 25 ± 0.5°C.

The consistency of soft samples is affected by the container diameter. With soft samples having a consistency of not less than 265, use a container with an inside diameter of 76.2 mm (a half-scale cone: 38.1 mm, a quarter-scale cone: 19.0 mm). The consistency of relatively hard samples having a consistency of not more than 265 is hardly affected by the container diameter when using a container with an inside diameter of not less than 76.2 mm.

The amount of sample required for the test depends on the sizes of the sample container and the cone, and the procedure specified according to the consistency.

## 2.2.2. Procedure

### 2.2.2.1. Standard cone

Stand a sample container gently on the sample stage of the penetrometer adjusted to horizontal position. After placing the position of the cone to the zero point of the dial gage adjust the cone so that the tip touches the surface of a sample at the position prescribed in (i), (ii) or (iii) by moving either the cone part or the sample stage up and down. Push the metal clamping tool immediately to penetrate the cone for  $5 \pm 0.1$  seconds. The holder must move smoothly in the drop unit. Push the rack for measurement down gently until it stops and read the dial gage in first place after the decimal point.

- (i) With a sample having a consistency of more than 400, only one test may be made in one container by placing the center of the sample container within 0.3 mm from the needle tip. Three tests may be made in three containers.
- (ii) With a sample having a consistency of more than 200 and less than 400, perform carefully the centering of the cone in the sample container. Only one test may be made with this sample. Three tests may be made in three containers.
- (iii) With a sample having a consistency of not more than 200, three tests may be made in one container. The measurement points are the midpoints between the center and the edge of the sample container at about 120 degrees intervals on a concentric circle.

### 2.2.2.2. Half-scale cone and quarter-scale cone

Place a cone at the center of a sample surface and perform a penetration pretest. The pretest may be omitted if an approximate value of consistency is known. Perform the measurement of consistency according to the procedure in 2.2.2.1. and place the position of the tip according to the following (i) or (ii).

- (i) With a sample having a consistency of more than 97 when using a half-scale cone or more than 47 when using a quarter-scale cone, only one test may be made in one container by placing carefully the needle tip at the center of the sample container. Three tests may be made in three containers.
- (ii) With a sample having a consistency of not more than 97 when using a half-scale cone or not more than 47 when using a quarter-scale cone, three tests may be made in one sample container. The measurement points are the midpoints between the center and the edge of the sample container at about 120 degrees intervals on a concentric circle so that the cone does not collide with the edge of the sample container and the measurement position of previous tests.

## 2.3. Analysis

### 2.3.1. Conversion of consistency measured using a half-scale or quarter-scale cone

Convert the consistency obtained using a half-scale or quarter-scale cone to the value equivalent to that obtained using a standard cone by the following equation.

### 2.3.1.1. Conversion of consistency obtained using a half-scale cone

$$P = 2p_{1/2} + 5$$

P: Consistency converted to the value equivalent to that measured using the standard cone

$p_{1/2}$ : Consistency measured using a half-scale cone

### 2.3.1.2. Conversion of consistency obtained using a quarter-scale cone

$$P = 3.75p_{1/4} + 24$$

P: Consistency converted to the value equivalent to that

measured using a standard cone

$p_{1/4}$ : Consistency measured using a quarter-scale cone

## 6.17 Insoluble Particulate Matter Test for Therapeutic Protein Injections

Insoluble particulate matters in injections consist of mobile undissolved particles other than gas bubbles in preparations. Extraneous substances, substances derived from manufacturing processes, protein aggregates and so on may be included in therapeutic protein injections. In this chapter, Method 1 (Light Obscuration Particle Count Test) under Insoluble Particulate Matter Test for Injections <6.07> is used for the determination of insoluble particulates in therapeutic protein injections. This test is applied to the injections whose active ingredients are peptides, proteins or their derivatives.

Since this test is a sampling test conducted on a part of samples, the test must be performed under a statistically sound sampling plan in order to estimate correctly the number of particles in the population.

### 1. Apparatus

Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size. Calibration, and verification of sample volume accuracy, sample flow rate accuracy and counting accuracy are performed according to Method 1 under Insoluble Particulate Matter Test for Injections <6.07>. When one measurement is performed with volume less than 1 mL, confirm the sample volume accuracy separately by an appropriate method.

### 2. General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet. Very carefully wash the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with *particle-free water*. Take care not to introduce air bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out. In order to check that the environment is suitable for the test, that the glassware is properly cleaned and that the number of particles in the *particle-free water* to be used is within specifications, the following test is carried out using 5 mL of the *particle-free water*. When one measurement is performed with volume less than 1 mL, 1 mL of *particle-free water* may be used. Determine the particulate contamination of 5 samples of *particle-free water*. If the number of particles of 10  $\mu\text{m}$  or greater size exceeds 1 per 1 mL, the precautions taken for the test are not sufficient. In this case, the preparatory steps must be repeated until the environment, filtration equipment glassware and *particle-free water* are suitable for the test.

### 3. Method

Treat a protein solution in an appropriate manner because of its tendency to generate air bubbles. In the case of an injection to be dissolved before use, use a specified solvent. When solvent is not specified, dissolve in *particle-free water* or use other suitable solvent comparable to *particle-free water*. Mix the contents of the sample gently and thoroughly by an appropriate procedure such as swirling the container slowly. If the container is sealed, cautiously remove the seal-

ing closure. Clean the outer surfaces of the container opening using a jet of *particle-free water* and remove the closure, avoiding any contamination at the contents. For elimination of air bubbles, it is recommended to allow a container to stand under ambient pressure or reduced pressure. Other procedures are applicable if confirmed to be appropriate. Sonicating is not appropriate because it may aggregate or denature proteins. If necessary, after degassing, mix the contents gently and thoroughly by swirling slowly the container so as not to introduce air bubbles, and use it for the test. The measurement volume is 1 to 5 mL. The measurement volume can be reduced to 0.2 mL when the validity of the reduction is confirmed in considering the property of the sample and the tare volume of the apparatus. Set the volume necessary for the test in consideration of counting 4 portions.

In the case of injections where the volume necessary for the test can be obtained from one container, individual containers are tested. For injections with insufficient volume, combine the contents of several containers in one clean container to obtain the necessary volume after mixing the contents of containers gently and thoroughly. Where justified, the volume necessary for the test may be prepared by diluting the test solution with *particle-free water* or an appropriate solvent comparable to *particle-free water*. The validity of the dilution procedure and the solvent used for the dilution is confirmed by, for example, demonstrating consistent result regardless of the dilution. The number of test specimens must be adequate to provide a statistically sound assessment.

Take 4 portions and count the number of particles equal to or greater than 10  $\mu\text{m}$  and 25  $\mu\text{m}$ . Disregard the result obtained for the first portion, and calculate the mean number of particles for the preparation to be examined.

#### 4. Evaluation

The preparation complies with the test if the average number of particles meets the following requirement.

A—Solutions for injection supplied in containers with a nominal content of equal to or more than 100 mL.

The average number of particles of equal to or greater than 10  $\mu\text{m}$  does not exceed 25 per mL and that of particles of equal to or greater than 25  $\mu\text{m}$  does not exceed 3 per mL.  
B—Solutions for injection supplied in containers with a nominal content of less than 100 mL.

The average number of particles of equal to or greater than 10  $\mu\text{m}$  does not exceed 6000 per container and that of particles of equal to or greater than 25  $\mu\text{m}$  does not exceed 600 per container.

(1) The containers are colorless or light brown and transparent, and have no bubbles which interfere the test of Foreign Insoluble Matter Test for Injections <6.06>.

(2) Multiple-dose containers are closed by rubber stoppers or any other suitable stoppers. The stoppers permit penetration of an injection needle without detachment of fragments, and upon withdrawal of the needle, they reclose the containers immediately to prevent external contamination, and also do not interact physically or chemically with the contained medicaments.

Containers intended for aqueous infusions are closed by rubber stoppers meeting the requirements of the test for Rubber Closure for Aqueous Infusions <7.03>.

(3) Soluble alkali test—The testing methods may be divided into the following two methods according to the type of container or the dosage form of the medicament.

(i) Method 1: This method is applied to containers to be fused, or containers not to be fused except containers for aqueous infusions with a capacity exceeding 100 mL.

Rinse thoroughly the inside and outside of the containers to be tested with water, dry, and roughly crush, if necessary. Transfer 30 to 40 g of the glass to a steel mortar, and crush. Sieve the crushed glass through a No. 12 (1400  $\mu\text{m}$ ) sieve. Transfer the portion retained on the sieve again to the steel mortar, and repeat this crushing procedure until 2/3 of the amount of powdered glass has passed through a No. 12 (1400  $\mu\text{m}$ ) sieve. Combine all portions of the glass powder passed through a No. 12 (1400  $\mu\text{m}$ ) sieve, shake the sieve in a horizontal direction for 5 minutes with slight tapping at intervals using No. 18 (850  $\mu\text{m}$ ) and No. 50 (300  $\mu\text{m}$ ) sieves. Transfer 7 g of the powder, which has passed through a No. 18 (850  $\mu\text{m}$ ) sieve but not through a No. 50 (300  $\mu\text{m}$ ) sieve to a No. 50 (300  $\mu\text{m}$ ) sieve, immerse it in a suitable container filled with water, and wash the contents with gentle shaking for 1 minute. Rinse again with ethanol (95) for 1 minute, dry the washed glass powder at 100°C for 30 minutes, and allow to cool in a desiccator (silica gel). Transfer exactly 5.0 g of the powder thus prepared to a 200-mL conical flask of hard glass, add 50 mL of water, and gently shake the flask so that the powder disperses on the bottom of the flask evenly. Cover the flask with a small beaker of hard glass or a watch glass of hard glass, then heat it in boiling water for 2 hours, and immediately cool to room temperature. Decant the water from the flask into a 250-mL conical flask of hard glass, wash well the residual powdered glass with three 20-mL portions of water, and add the washings to the decanted water. Add 5 drops of bromocresol green-methyl red TS and titrate <2.50> with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed the following quantity, according to the type of containers.

Containers to be fused	0.30 mL
Containers not to be fused (including injection syringes used as containers)	2.00 mL

(ii) Method 2: This method is applied to containers not to be fused for aqueous infusions with a capacity exceeding 100 mL.

Rinse thoroughly the inside and outside of the containers to be tested with water, and dry. Add a volume of water equivalent to 90% of the actual capacity of the container, cover it with a small beaker of hard glass or close tightly with a suitable stopper, heat in an autoclave at 121°C for 1 hour,

## 7. Tests for Containers and Packing Materials

### 7.01 Test for Glass Containers for Injections

The glass containers for injections do not interact physically or chemically with the contained medicament to alter any property or quality, can protect the contained medicament from the invasion of microbes by means of perfect sealing or other suitable process, and meet the following requirements. The surface-treated container for aqueous infusion is made from glass which meets the requirements for the soluble alkali test for a container not to be fused under method 1.

and allow to stand until the temperature falls to room temperature. Pipet 100 mL of this solution, and transfer to a 250-mL conical flask of hard glass. Add 5 drops of bromocresol green-methyl red TS, and titrate <2.50> with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Pipet 100 mL of water, transfer to a 250-mL conical flask of hard glass, perform a blank determination in the same manner, and make any necessary correction. The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed 0.10 mL.

(4) Soluble iron test for light-resistant containers—Rinse thoroughly five or more light-resistant containers to be tested with water, and dry at 105°C for 30 minutes. Pour a volume of 0.01 mol/L hydrochloric acid VS corresponding to the labeled volume of the container into individual containers, and fuse them. In the case of containers not to be fused, cover them with small beakers of hard glass or watch glasses of hard glass. Heat them at 105°C for 1 hour. After cooling, prepare the test solution with 40 mL of this solution according to Method 1 of Iron Limit Test <1.10>, and perform the test according to Method B. Prepare the control solution with 2.0 mL of the Standard Iron Solution.

(5) Light transmission test for light-resistant containers—Cut five light-resistant containers to be tested, prepare test pieces with surfaces as flat as possible, and clean the surfaces. Fix a test piece in a cell-holder of a spectrophotometer to allow the light pass through the center of the test piece perpendicularly to its surface. Measure the light transmittance of the test piece with reference to air between 290 nm and 450 nm and also between 590 nm and 610 nm at intervals of 20 nm each. The percent transmissions obtained between 290 nm and 450 nm are not more than 50% and that between 590 nm and 610 nm are not less than 60%. In the case of containers not to be fused having a wall thickness not less than 1.0 mm, the percent transmissions between 590 nm and 610 nm are not less than 45%.

## 7.02 Test Methods for Plastic Containers

Test methods for plastic containers may be used for designing and assuring quality of plastic containers. Not all tests described here will be necessary in any phases for any containers. On the other hand, the set does not include sufficient numbers and kinds of tests needed for any design verification and quality assurance of any containers. Additional tests may be considered if necessary.

It is not allowable for plastic containers for aqueous injections to interact with the pharmaceutical contained therein resulting in the deterioration of its efficacy, safety or stability, and to contaminate with microorganisms. They should meet the requirements prescribed in 2. Requirements for Plastic Containers for Aqueous Injections.

### 1. Test methods

#### 1.1. Combustion tests

##### 1.1.1. Residue on ignition

Weigh accurately about 5 g of cut pieces of the container and perform the test according to Residue on Ignition <2.44>.

##### 1.1.2. Heavy metals

Place an appropriate amount of cut pieces of the container in a porcelain crucible, and perform the test according to Method 2 of Heavy Metals Limit Test <1.07>. Prepare the

control solution with 2.0 mL of Standard Lead Solution.

#### 1.1.3. Lead

##### 1.1.3.1. Method 1

Place 2.0 g of cut pieces of a container in a crucible of platinum or quartz, moisten with 2 mL of sulfuric acid, heat slowly to dryness, then heat to combustion at between 450°C and 500°C. Repeat this procedure, if necessary. After cooling, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, evaporate to dryness on a water bath, then add 1 to 5 mL of hydrochloric acid, and warm to dissolve. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1), and add 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter (G3) if insoluble matter remains. To the obtained filtrate add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonia TS until the color of the solution changes from yellow to green. Then add 10 mL of a solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium N,N-diethylthiocarbamate trihydrate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter if necessary, and use the layer as the sample solution.

Separately, to 2.0 mL of Standard Lead Solution add water to make exactly 10 mL. To 1.0 mL of this solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the sample solution, and use the solution so obtained as the standard solution.

Perform the test with the sample solution and standard solution according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the concentration of lead in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen.

Supporting gas—Air.

Lamp: Lead hollow-cathode lamp.

Wavelength: 283.3 nm.

##### 1.1.3.2. Method 2

Cut a container into pieces smaller than 5-mm square, take 2.0 g of the pieces into a glass beaker, add 50 mL of 2-butanone and 0.1 mL of nitric acid, and warm to dissolve. To this solution add 96 mL of methanol gradually to precipitate a resinous substance, and filter by suction.

Wash the beaker and the resinous substance with 12 mL of methanol followed by 12 mL of water, combine the washings and the filtrate, and concentrate to about 10 mL under reduced pressure. Transfer into a separator, add 10 mL of ethyl acetate and 10 mL of water, shake vigorously, and allow to stand to separate the water layer. Evaporate the water layer to dryness, add 5 mL of hydrochloric acid to the residue, and warm to dissolve. Then add 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1), and add 1 mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter (G3) if insoluble matter remains. To the solution so obtained add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and then add ammonia TS until the color of the solution changes from yellow to green. Further add 10 mL of a solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium N,N-diethylthiocarbamate trihydrate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter the layer if necessary, and use the layer as the

sample solution.

Separately, pipet 5 mL of Standard Lead Solution, add water to make exactly 50 mL, and to 2.0 mL of this solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and standard solution according to Atomic Absorption Spectrophotometry <2.23> under the conditions described in Method 1, and determine the concentration of lead in the sample solution.

#### 1.1.4. Cadmium

##### 1.1.4.1. Method 1

To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in "1.1.3.1. Method 1", and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in "1.1.3.1. Method 1" and the standard solution according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the concentration of cadmium in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen.

Supporting gas—Air.

Lamp: Cadmium hollow-cathode lamp.

Wavelength: 228.8 nm.

##### 1.1.4.2. Method 2

To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in "1.1.3.2. Method 2", and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in "1.1.3.2. Method 2" and the standard solution according to Atomic Absorption Spectrophotometry <2.23> under the conditions described in "1.1.4.1. Method 1", and determine the concentration of cadmium in the sample solution.

#### 1.1.5. Tin

Cut a container into pieces smaller than 5-mm square, place 5.0 g of the pieces in a Kjeldahl flask, add 30 mL of a mixture of sulfuric acid and nitric acid (1:1), and decompose by gentle heating in a muffle furnace, occasionally adding dropwise a mixture of sulfuric acid and nitric acid (1:1) until the content changes to a clear, brown solution. Then heat until the color of the solution changes to a clear, light yellow, and heat to slowly concentrate to practical dryness. After cooling, dissolve the residue in 5 mL of hydrochloric acid by warming, and after cooling, add water to make exactly 10 mL. Pipet 5 mL of this solution into a 25-mL volumetric flask (A). Transfer the remaining solution to a 25-mL beaker (B) by washing out with 10 mL of water, add 2 drops of bromocresol green TS, neutralize with diluted ammonia solution (28) (1 in 2), and measure the volume consumed for neutralization as  $a$  mL. To the volumetric flask, A, add potassium permanganate TS dropwise until a slight pale red color develops, and add a small amount of L-ascorbic acid to decolorize. Add 1.5 mL of 1 mol/L hydrochloric acid TS, 5 mL of a solution of citric acid monohydrate (1 in 10),  $a$  mL of diluted ammonia solution (28) (1 in 2), 2.5 mL of polyvinyl alcohol TS, 5.0 mL of phenylfluorone-ethanol TS and water to make 25 mL. Shake well, then allow to stand for about 20 minutes, and use this solution as the sample solution.

Separately, pipet 1.0 mL of Standard Tin Solution, add 5 mL of water, add potassium permanganate TS dropwise until a slight pale red color develops, proceed in the same

manner as for the sample solution, and use this solution as the standard solution.

Determine the absorbances of the sample solution and the standard solution according to Ultraviolet-visible Spectrophotometry <2.24> at 510 nm, using water as the blank.

#### 1.2. Extractable substances

Cut the container at homogeneous regions of low curvature and preferably the same thickness, gather pieces to make a total surface area of about 1200 cm<sup>2</sup> when the thickness is 0.5 mm or less, or about 600 cm<sup>2</sup> when the thickness is greater than 0.5 mm, and subdivide in general into strips approximately 0.5 cm in width and 5 cm in length. Wash them with water, and dry at room temperature. Place these strips in a 300-mL hard glass vessel, add exactly 200 mL of water, and seal the opening with a suitable stopper. After heating the vessel in an autoclave at 121°C for 1 hour, take out the vessel, allow to stand until the temperature falls to room temperature, and use the content as the test solution.

For containers made of composite plastics, the extraction may be performed by filling a labeled volume of water in the container. In this case, it is necessary to record the ratio of the volume of water used and the inside area of the container.

When containers are deformed at 121°C, the extraction may be performed at the highest temperature which does not cause deformation among the following conditions: at 100 ± 2°C for 2 ± 0.2 hours, at 70 ± 2°C for 24 ± 2 hours, at 50 ± 2°C for 72 ± 2 hours or at 37 ± 1°C for 72 ± 2 hours.

Prepare the blank solution with water in the same manner. For containers made of composite plastics, water is used as the blank solution.

Perform the following tests with the test solution and the blank solution:

(i) Foaming test: Place 5 mL of the test solution in a glass-stoppered test tube about 15 mm in inside diameter and about 200 mm in length, shake vigorously for 3 minutes, and measure the time needed for almost complete disappearance of the foam thus generated.

(ii) pH <2.54>: To 20 mL each of the test solution and the blank solution add 1.0 mL of a solution of potassium chloride (1 in 1000), and obtain the difference in the reading of pH between these solutions.

(iii) Potassium permanganate-reducing substances: Place 20.0 mL of the test solution in a glass-stoppered conical flask, add 20.0 mL of 0.002 mol/L potassium permanganate VS and 1 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper tightly, shake, then allow to stand for 10 minutes, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the test in the same manner, using 20.0 mL of the blank solution, and obtain the difference of the consumption of 0.002 mol/L potassium permanganate VS between these solutions.

(iv) UV spectrum: Read the maximum absorbances between 220 nm and 240 nm and between 241 nm and 350 nm of the test solution against the blank solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

(v) Residue on evaporation: Evaporate 20 mL of the test solution on a water bath to dryness, and weigh the residue after drying at 105°C for 1 hour.

#### 1.3. Test for fine particles

##### 1.3.1. Test procedure

Rinse thoroughly the inside and outside of containers to be tested with water for particle matter test, fill the container with the labeled volume of water for particulate matter test or 0.9 w/v% sodium chloride solution, adjust the amount of

air in the container to about 50 mL per 500 mL of the labeled volume, put tight stopper to the container, and heat it at 121°C for 25 minutes in an autoclave. After allowing to cool for 2 hours, take out the container from the autoclave, and then allow to stand at ordinary temperature for about 24 hours. If the containers are deformed at 121°C, employ a suitable temperature-time combination as directed under 1.2. Extractable substances. Clean the outside of the container, mix by turning upside-down 5 or 6 times, insert immediately a clean needle of filterless infusion set into the container through the rubber closure of the container, take the effluent from the container while mixing gently in a clean container for measurement, and use it as the test solution.

Counting of the fine particles must be performed in dustless, clean facilities or apparatus, using a light-shielded automatic fine particle counter. The sensor of the counter to be used must be able to count fine particles of 1.5 µm or more in diameter. The volume to be measured is 10 mL. Adjust the counter before measurement. For calibration of the diameter and number of particles, the standard particles for calibration of the light-shielded automatic fine particle counter should be used in suspension in water for particulate matter test or 0.9 w/v% sodium chloride solution.

Count five times the numbers of particles with diameters of 5 – 10 µm, 10 – 25 µm and more than 25 µm while stirring the test solution, and calculate the average particle numbers of four counts, excluding the first, as the number of particles in 1.0 mL of the test solution.

### 1.3.2. Reagent

Water for particulate matter test and 0.9 w/v% sodium chloride solution to be used for the tests should not contain more than 0.5 particles of 5 – 10 µm in size per 1.0 mL.

## 1.4. Transparency test

### 1.4.1. Method 1

This method can only be applied to containers which have a smooth and not embossed surface and rather low curvature.

Cut the container at homogeneous regions of low curvature and preferably the same thickness to make 5 pieces of about 0.9 × 4 cm in size, immerse each piece in a cell for determination of the ultraviolet spectrum filled with water, and determine the transmittance at 450 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a cell filled with water as a blank.

### 1.4.2. Method 2

Sensory test—This method can be applied to containers which have a rough or embossed surface. It can also be applied to testing of the transparency of containers in case where the turbidity of their pharmaceutical contents must be checked.

#### 1.4.2.1. Test solutions

(i) Formazin standard suspension: To 15 mL of formazin stock suspension add water to make 1000 mL. Use within 24 hours of preparation. Shake thoroughly before use.

(ii) Reference suspension: To 50 mL of Formazin standard suspension add water to make 100 mL.

#### 1.4.2.2. Test procedures

(i) with control: Take two of containers to be tested, and fill one of them with the labeled volume of the reference suspension and the other with the same volume of water. Show these two containers to five subjects, separately, ask which one seems to be more turbid, and calculate the rate of correct answers.

(ii) without control: Take six of containers to be tested, put number to each of them, and fill three of them with the labeled volume of the reference suspension and the others

with the same volume of water. Show each one of these containers at random order to five subjects, separately, ask if it is turbid or not, and calculate the percentage of the answer judged as “turbid” ( $100 X/15$ , X: number of containers judged as “turbid”) for reference suspension-filled containers group and water-filled containers group, respectively.

## 1.5. Water vapor permeability test

### 1.5.1. Method 1

This test method is applicable to containers for aqueous injection. Fill the container with the labeled volume of water. After closing it hermetically, accurately weigh the container and record the value. Store the container at  $65 \pm 5\%$  relative humidity and a temperature of  $20 \pm 2^\circ\text{C}$  for 14 days, and then accurately weigh the container again and record the value. Calculate the mass loss during storage.

### 1.5.2. Method 2

This test method is provided for evaluating moisture permeability of containers for hygroscopic drugs. Unless otherwise specified, perform the test according to the following procedure.

#### 1.5.2.1. Desiccant

Place a quantity of calcium chloride for water determination in a shallow container, taking care to exclude any fine powder, then dry at  $110^\circ\text{C}$  for 1 hour, and cool in a desiccator.

#### 1.5.2.2. Procedure

Select 12 containers, clean their surfaces with a dry cloth, and close and open each container 30 times in the same manner. Ten among the 12 containers are used as “test containers” and the remaining two, as “control containers”. A torque for closing screw-capped containers is specified in Table 7.02-1. Add desiccant to 10 of the containers, designated test containers, filling each to within 13 mm of the closure if the container volume is 20 mL or more, or filling each to two-thirds of capacity if the container volume is less than 20 mL. If the interior of the container is more than 63 mm in depth, an inert filler or spacer may be placed in the bottom to minimize the total mass of the container and

**Table 7.02-1** Torque applicable to screw-type container

Closure Diameter (mm)	Torque (N·cm)
8	59
10	60
13	88
15	59 – 98
18	78 – 118
20	88 – 137
22	98 – 157
24	118 – 206
28	137 – 235
30	147 – 265
33	167 – 284
38	196 – 294
43	196 – 304
48	216 – 343
53	235 – 402
58	265 – 451
63	284 – 490
66	294 – 510
70	314 – 569
83	363 – 735
86	451 – 735
89	451 – 794
100	510 – 794
110	510 – 794
120	618 – 1069
132	677 – 1069

desiccant; the layer of desiccant in such a container shall be not less than 5 cm in depth. Close each container immediately after adding desiccant, applying the torque designated in the table. To each of the control containers, add a sufficient number of glass beads to attain a mass approximately equal to that of each of the test containers, and close, applying the torque designated in the table. Record the mass of the individual containers so prepared to the nearest 0.1 mg if the container volume is less than 20 mL, to the nearest 1 mg if the container volume is 20 mL or more but less than 200 mL, or to the nearest 10 mg if the container volume is 200 mL or more, and store the containers at  $75 \pm 3\%$  relative humidity and a temperature of  $20 \pm 2^\circ\text{C}$ .

After 14 days, record the mass of the individual containers in the same manner. Completely fill 5 empty containers with water or a non-compressible, free-flowing solid such as fine glass beads, to the level indicated by the closure surface when in place. Transfer the contents of each to a graduated cylinder, and determine the average container volume, in mL. Calculate the rate of moisture permeability, in mg per day per liter, by use of the formula:

$$(1000/14V) [(T_f - T_i) - (C_f - C_i)]$$

*V*: Average volume (mL)

*T<sub>f</sub>* - *T<sub>i</sub>*: Difference between the final and initial masses of each test container (mg)

*C<sub>f</sub>* - *C<sub>i</sub>*: Average of the differences between the final and initial masses of the two controls (mg)

#### 1.6. Leakage test

Fill a container with a solution of fluorescein sodium (1 in 1000), stopper tightly, place filter papers on and under the container, and apply a pressure of 6.9 N (0.7 kg/cm<sup>2</sup>) at  $20^\circ\text{C}$  for 10 minutes. Judge the leakiness by observing the color of the paper.

#### 1.7. Cytotoxicity test

The following test methods are designed to detect cytotoxic substances in plastic materials by evaluating the cytotoxicity of the culture medium extracts from plastic containers for pharmaceutical products. Other appropriate standard methods of cytotoxicity testing may be used for the evaluation, if appropriate. However, the final decision shall be made based upon the test methods given here, if the test results obtained according to the other methods are questionable. Other than those of the culture medium, reagents and test solutions being specified for the test may be used if they meet for the purpose of the test.

##### 1.7.1. Cell lines

The recommended cell lines are L929 cells (ATCC. CCL1) and V79 cells (JCRB0603). In addition, other established cell lines may be used when it is confirmed that they form well-defined colonies reproducibly, with characteristics comparable to those of L929 cells and V79 cells.

##### 1.7.2. Culture medium

(i) Medium for L929 cells: To Eagle's minimum essential medium add fetal calf serum (FCS) to make 10 vol% FCS.

(ii) Medium for V79 cells: M05 medium prepared by adding 10 mL each of nonessential amino acid TS and 100 mmol/L sodium pyruvate TS to 1000 mL of Eagle's minimum essential medium, then adding fetal calf serum (FCS) to make 5 vol% FCS. Medium for L929 cells may be used instead if it gives equivalent sensitivity.

##### 1.7.3. Reference materials and control substances

(i) Negative reference material: high-density polyethylene film

(ii) Positive reference material (A): polyurethane film containing 0.1% zinc diethyldithiocarbamate

(iii) Positive reference material (B): polyurethane film

containing 0.25% zinc dibutyldithiocarbamate

(iv) Control substances: zinc diethyldithiocarbamate or zinc dibutyldithiocarbamate

##### 1.7.4. Test procedure

(i) Sample preparation: When the material of the container consists of a single homogeneous layer, subdivide the cut pieces of a container into pieces of the size of approximately  $2 \times 15$  mm and subject the pieces to the test. When the material of the container has multiple layers, prepare cut pieces with a surface area of one side of  $2.5 \text{ cm}^2$  and subject the pieces to the test without subdividing them into smaller pieces.

(ii) Preparation of sample solutions: Transfer an appropriate amount of the sample to a screw-capped glass bottle or a sterile disposable centrifuge tube. Cap the bottle or tube loosely and cover the cap with clean aluminum foil. Sterilize the bottle or tube by autoclaving at  $121^\circ\text{C}$  for 15 minutes. When the material of the sample is not resistant to heat during autoclaving, gas sterilization with ethylene oxide (EO) may be used. In the case of EO sterilization, sufficient aeration should be achieved to avoid an additional toxic effect of residual EO in the test results. To the bottle or tube add the culture medium in a proportion of 1 mL per  $2.5 \text{ cm}^2$  (one side) or 10 mL per 1 g of the sample, loosely cap the bottle or tube, and allow to stand in an incubator maintaining 5% carbon dioxide at  $37^\circ\text{C}$  for 24 hours. Transfer the culture medium extract, which is designated 100% sample solution, to a sterilized screw-capped glass bottle or a sterile disposable centrifuge tube. Dilute the 100% sample solution with fresh culture medium using a dilution factor of two to prepare serial dilutions having extract concentrations of 50%, 25%, 12.5%, 6.25%, 3.13% and so on.

(iii) Preparation of cell suspension: Remove the culture medium from the maintained cell culture vessel (flask or dish), and add gently a suitable volume of phosphate buffer solution for cytotoxicity test. Rinse the cells by gentle rotation of the slanted cell culture vessel two or three times, and discard the phosphate buffer solution. Add a sufficient volume of trypsin solution to cover the cell layer. Cap the vessel and place in an incubator maintaining 5% carbon dioxide at  $37^\circ\text{C}$  for 1 to 2 minutes. After confirming detachment of the cell layer from the bottom surface of the vessel by using a microscope and by gently tapping of the vessel, add an appropriate volume of the fresh culture medium and gently pipet the cells completely out of the vessel. Transfer the pipetted cell suspension into a sterile disposable centrifuge tube and centrifuge. Discard the supernatant liquid, resuspend the cells in an appropriate volume of flesh phosphate buffer solution for cytotoxicity test by pipetting, and centrifuge the tube again. Discard the supernatant liquid, and add an appropriate volume of fresh culture medium to the tube. Resuspend the cells by gentle pipetting and make a homogeneous cell suspension. Determine the cell concentration using a hemocytometer.

(iv) Cytotoxicity test: Dilute the cell suspension prepared according to procedure (iii) with culture medium to adjust the cell concentration to 100 cells/mL. Place a 0.5 mL aliquot of the diluted cell suspension on each well of a sterile disposable multiple well plate (24 wells). Incubate the plate in the incubator maintaining 5% carbon dioxide at  $37^\circ\text{C}$  for 4 - 24 hours to attach the cells to the bottom surface of the well. Discard the medium from each well, and add a 0.5 mL aliquot of the prepared sample solutions having various concentrations or fresh medium to at least 3 wells each. Place the plate immediately in the incubator and incubate the plate for the appropriate period: 7 - 9 days for L929 cells; 6 - 7 days for V79 cells. After the incubation, discard the medium

from the plate, add an appropriate volume of methanol or dilute formaldehyde TS to each well and allow the plate to stand for about 30 minutes to fix the cells. Discard the methanol or dilute formaldehyde TS from each well and add an appropriate volume of dilute Giemsa's TS to each well. After ensuring good staining of the colonies, discard the stain solution from the wells, wash with water, dry, and count the number of colonies in each well. Calculate a mean number of colonies for each concentration of the sample solution, and divide the mean by the mean number of colonies for the fresh medium to obtain the relative plating efficiency (%) for each extract concentration of the sample solution. Plot the extract concentration (%) of the sample solution on a logarithmic scale and the relative plating efficiency on an ordinary scale on semilogarithmic graph paper to obtain a colony formation inhibition curve of the container. Read the 50% inhibition concentration,  $IC_{50}$  (%), at which the colony number is half that in the control group, from the inhibition curve.

It is recommended to check the sensitivity and the reproducibility of the test system by the use of suitable reference materials or control substances in the test system, if necessary.

## 2. Requirements for plastic containers for aqueous injections

### 2.1. Polyethylene or polypropylene containers for aqueous injections

The containers are made of polyethylene or polypropylene and free from any adhesive.

(1) Transparency—The containers have a transmittance of not less than 55%, when tested as directed in “1.4.1. Method 1”. When “1.4.1. Method 1” can not be applied, test according to “1.4.2.2. (ii) Method 2B”. In this case, the rate that the water-containing container is judged as “being turbid” is not more than 20%, and the rate that the reference suspension-containing container is judged as “being turbid” is not less than 80%.

(2) Appearance—The containers do not have strips, cracks, bubbles, or other faults which cause difficulties in practical use.

(3) Water vapor permeability—Proceed as directed in “1.5.1. Method 1”. The loss of mass is not more than 0.20%.

(4) Heavy metals <1.07>—The test solution has no more color than the control solution when the amount of the sample taken is 1.0 g.

(5) Lead—Perform the test as directed in “1.1.3.1. Method 1”. The absorbance of the sample solution is not more than that of the standard solution.

(6) Cadmium—Perform the test as directed in “1.1.4.1. Method 1”. The absorbance of the sample solution is not more than that of the standard solution.

(7) Residue on ignition <2.44>—Not more than 0.1% (5 g).

#### (8) Extractable substances—

(i) Foaming test: the foam formed almost disappears within 3 minutes.

(ii) pH: the difference in the reading of pH between the test solution and the blank solution is not more than 1.5.

(iii) Potassium permanganate-reducing substances: The difference in the consumption of 0.002 mol/L potassium permanganate VS between the test solution and the blank solution is not more than 1.0 mL.

(iv) UV spectrum: The maximum absorbance between 220 nm and 240 nm is not more than 0.08, and that between 241 nm and 350 nm is not more than 0.05.

(v) Residue on evaporation: Not more than 1.0 mg.

(9) Cytotoxicity— $IC_{50}$  (%) is not less than 90%. The result obtained by the other standard methods is negative.

### 2.2. Polyvinyl chloride containers for aqueous injections

The containers are composed of homopolymer of vinyl chloride, free from any adhesive, and the plasticizer added to the material should be only di(2-ethylhexyl)phthalate. The containers may be covered with easily removable material to prevent the permeation of water vapor. In this case, perform the water vapor permeability test on the covered containers.

(1) Thickness—Measure the thickness of a container at five different locations. The difference between the maximum and minimum values of thickness is 0.05 mm or less.

(2) Transparency—Proceed as directed in (1) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(3) Appearance—Proceed as directed in (2) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(4) Leakage—Proceed with the test according to “1.6. Leakage test”. The solution contained does not leak.

(5) Flexibility—Insert the spike needle for infusion through a rubber closure of the container used in (4) Leakage. The contained solution is almost completely discharged without displacement by air.

(6) Water vapor permeability—Proceed as directed in (3) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(7) Heavy metals <1.07>—The test solution has no more color than the control solution when the amount of the sample taken is 1.0 g.

(8) Lead—Perform the test as directed in “1.1.3.2. Method 2”. The absorbance of the sample solution is not more than that of the standard solution.

(9) Cadmium—Perform the test as directed in “1.1.4.2. Method 2”. The absorbance of the sample solution is not more than that of the standard solution.

(10) Tin—The absorbance of the sample solution is not more than that of the standard solution.

(11) Vinyl chloride—Wash cut pieces of a container with water, wipe them thoroughly with a filter paper, subdivide them into pieces smaller than 5 mm square, and put 0.5 g of them into a 20-mL vial. Add 2.5 mL of *N,N*-dimethylacetamide to the vial to dissolve the sample pieces, put a tight stopper on the vial, and use the solution in the vial as the sample solution. If the sample is hardly soluble, allow to stand the vial at room temperature for a night, put a tight stopper on the vial, and use the liquid part in the vial as the sample solution. Separately, to a 20-mL vial add 2.5 mL of *N,N*-dimethylacetamide, add 50  $\mu$ L of Standard Vinyl Chloride Solution, previously cooled with dry ice-methanol, put a tight stopper on the vial, and use the solution in the vial as the standard solution.

After heating the vials containing sample solution and standard solution at 90°C for 1 hour, perform the test with 0.5 mL each of vapor phases in these vials as directed under Gas Chromatography <2.02> according to the following conditions: the peak area of vinyl chloride obtained from the sample solution is not larger than that from the standard solution.

#### Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silicate column 0.25 mm in inside diameter and 25 m in length, coated the inside surface with porous styrene-divinylbenzene copolymer for gas chromatography the inside surface in 3  $\mu$ m thickness.

Column temperature: Maintain at 50°C for 2 minutes

after injection, then rise to 120°C at a rate of 10°C per minute, then rise to 250°C at a rate of 20°C per minute, and keep at 250°C for 10 minutes.

Injection port temperature: A constant temperature of about 200°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention time of vinyl chloride is about 7 minutes.

Split ratio: 1:5.

#### *System suitability—*

System performance: When the procedure is run with 0.5 mL of the vapor phase of the standard solution under the above operating conditions, vinyl chloride and ethanol are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times under the above operating conditions with 0.5 mL of the vapor phase of the standard solution heated at 90°C for 1 hour, the relative standard deviation of the peak area of vinyl chloride is not more than 5.0%.

(12) Fine particles—The number of fine particles in 1.0 mL of the test solution is not more than 100 of 5 to 10 µm, not more than 10 of 10 to 25 µm and not more than 1 of 25 µm or more.

(13) Residue on ignition <2.44>—Not more than 0.1% (5 g).

(14) Extractable substances—Proceed as directed in (8) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(15) Cytotoxicity—Proceed as directed in (9) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

#### **2.3. Plastic containers for aqueous injections being not described above**

The containers meet the following specifications and other necessary specifications for their materials with regard to heavy metals, residue on ignition and extractable substances, etc.

(1) Transparency—Proceed as directed in (1) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(2) Appearance—Proceed as directed in (2) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(3) Vapor permeability—Proceed as directed in (3) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(4) Cytotoxicity—Proceed as directed in (9) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

#### **1. Cadmium**

Wash the rubber closures with water, dry at room temperature, cut into minute pieces, mix well, place 2.0 g of them in a crucible of platinum or quartz, moisten them with 2 mL of sulfuric acid, heat gradually to dryness, and ignite between 450°C and 500°C until the residue is incinerated. When incineration was insufficient, moisten the residue with 1 mL of sulfuric acid, heat to dryness, and ignite again. Repeat the above-mentioned procedure if necessary. Cool the crucible, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, heat on a water bath to dryness, add 1 to 5 mL of hydrochloric acid, and dissolve by heating. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1) and 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). When any insoluble residue remains, filter through a glass filter. To the solution thus obtained add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonia TS until the color of the solution changes from yellow to green. Then add 10 mL of ammonium sulfate solution (2 in 5) and water to make 100 mL. Next, add 20 mL of a solution of sodium N,N-diethyldithiocarbamate trihydrate (1 in 20), mix, allow to stand for a few minutes, add 20 mL of 4-methyl-2-pentanone, and mix by vigorous shaking. Allow to stand to separate the 4-methyl-2-pentanone layer from the solution, filter if necessary, and use as the sample solution. On the other hand, to exactly 10 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Perform the tests according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, using the sample solution and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen.

Supporting gas—Air.

Lamp: Cadmium hollow-cathode lamp.

Wavelength: 228.8 nm.

#### **2. Lead**

To exactly 1 mL of the Standard Lead Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed as directed for the sample solution under 1, and use this solution as the standard solution. Perform the tests according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, using the sample solution and the standard solution obtained in 1. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen.

Supporting gas—Air.

Lamp: Lead hollow-cathode lamp.

Wavelength: 283.3 nm.

#### **3. Extractable substances**

Wash the rubber closures with water, and dry at room temperature. Place an amount of them, equivalent to about 150 cm<sup>2</sup> in surface area, in a glass vessel, add 2 mL of water per cm<sup>2</sup> of the sample, stopper adequately, heat at 121°C for 1 hour in an autoclave, take out the glass vessel, allow to cool to room temperature, then remove immediately the rubber closures, and use the remaining solution as the test solution. Prepare the blank solution with water in the same manner. Perform the following tests with the test solution and the blank solution.

## **7.03 Test for Rubber Closure for Aqueous Infusions**

The rubber closure for aqueous infusions means a rubber closure (containing material coated or laminated with the stuff like plastics) used for a container for aqueous infusion having a capacity of 100 mL or more. The rubber closure when in use does not interact physically or chemically with the contained medicament to alter any property or quality, does not permit the invasion of microbes, does not disturb the use of the contained infusion, and meets the following requirements.

### 3.1. Description

The test solution is clear and colorless. Read the transmittance of the test solution at 430 nm and 650 nm (10 mm), using the blank solution as the blank. Both of them are not less than 99.0%.

### 3.2. pH <2.54>

To 20 mL each of the test solution and the blank solution add 1 mL each of potassium chloride solution, prepared by dissolving 1.0 g of potassium chloride in water to make 1000 mL. The difference of pH between the two solutions is not more than 1.0.

### 3.3. Zinc

To exactly 10 mL of the test solution add diluted nitric acid (1 in 3) to make exactly 20 mL, and use this solution as the sample solution. Further, to exactly 1 mL of Standard Zinc Solution for atomic absorption spectrophotometry add diluted nitric acid (1 in 3) to make exactly 20 mL, and use this solution as the standard solution. Perform the tests according to Atomic Absorption Spectrophotometry <2.23>, using these solutions, under the following conditions. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: Zinc hollow-cathode lamp.

Wavelength: 213.9 nm.

### 3.4. Potassium Permanganate-reducing substances

Measure 100 mL of the test solution in a glass-stoppered conical flask, add 10 mL of 0.002 mol/L potassium permanganate VS, then add 5 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper, mix by shaking, then allow to stand for 10 minutes, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the blank test in the same manner, using 100 mL of the blank solution. The difference in mL of 0.002 mol/L potassium permanganate VS required between the tests is not more than 2.0 mL.

### 3.5. Residue on evaporation

Measure 100 mL of the test solution, evaporate on a water bath to dryness, and dry the residue at 105°C for 1 hour. The mass of the residue is not more than 2.0 mg.

### 3.6. UV spectrum

Read the absorbance of the test solution between 220 nm and 350 nm against the blank solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it is not more than 0.20.

## 4. Cytotoxicity test

The following test methods are designed to detect cytotoxic substances in rubber materials by evaluating the cytotoxicity of the culture medium extracts from rubber closure for aqueous infusion. Other appropriate standard methods of cytotoxicity testing may be used for the evaluation, if appropriate. However, the final decision shall be made based upon the test methods given here, if the test results obtained according to the other methods are questionable. Other than those of the culture medium, reagents and test solutions being specified for the test may be used if they meet for the purpose of the test.

### 4.1. Cell lines

The recommended cell lines are L929 cells (ATCC. CCL1) and V79 cells (JCRB0603). In addition, other established cell lines may be used when it is confirmed that they form well-defined colonies reproducibly, with characteristics comparable to those of L929 cells and V79 cells.

### 4.2. Culture medium

(i) Medium for L929 cells: To Eagle's minimum essential

medium add fetal calf serum (FCS) to make 10 vol% FCS.

(ii) Medium for V79 cells: M05 medium prepared by adding 10 mL each of nonessential amino acid TS and 100 mmol/L sodium pyruvate TS to 1000 mL of Eagle's minimum essential medium, then adding fetal calf serum (FCS) to make 5 vol% FCS. Medium for L929 cells may be used instead if it gives equivalent sensitivity.

### 4.3. Reference materials and control substances

(i) Negative reference material: Highdensity polyethylene film

(ii) Positive reference material (A): Polyurethane film containing 0.1% zinc diethyldithiocarbamate

(iii) Positive reference material (B): Polyurethane film containing 0.25% zinc dibutylidithiocarbamate

(iv) Control substances: Zinc diethyldithiocarbamate (reagent grade) or zinc dibutylidithiocarbamate

### 4.4. Test procedure

(i) Sample preparation: Rubber closure is subjected to the test without cutting into pieces. Reference material is divided into pieces of approximately 2 × 15 mm and subjected to the test.

(ii) Preparation of sample solutions: Transfer an appropriate amount of the sample to a screw-capped glass bottle or a sterile disposable centrifuge tube. Cap the bottle or tube loosely and cover the cap with clean aluminum foil. Sterilize the bottle or tube by autoclaving at 121°C for 15 minutes. When the material of the sample is not resistant to heat during autoclaving, gas sterilization with ethylene oxide (EO) may be used. In the case of EO sterilization, sufficient aeration should be achieved to avoid an additional toxic effect of residual EO in the test results. To the bottle or tube add the culture medium in a proportion of 60 cm<sup>2</sup> surface area or 10 mL per 1 g of the sample, loosely cap the bottle or tube, and allow to stand in an incubator maintaining 5% carbon dioxide at 37°C for 24 hours. To the reference material add 10 mL of the culture medium per 1 g and extract in the same manner. Transfer the culture medium extract, which is designated 100% sample solution, to a sterilized screw-capped glass bottle or a sterile disposable centrifuge tube. Dilute the 100% sample solution with fresh culture medium using a dilution factor of two to prepare serial dilutions having extract concentrations of 50%, 25%, 12.5%, 6.25%, 3.13% and so on.

(iii) Preparation of cell suspension: Remove the culture medium from the maintained cell culture vessel (flask or dish), and add gently a suitable volume of phosphate buffer solution for cytotoxicity test. Rinse the cells by gentle rotation of the slanted cell culture vessel two or three times, and discard the phosphate buffer solution. Add a sufficient volume of trypsin solution to cover the cell layer. Cap the vessel and place in an incubator maintaining 5% carbon dioxide at 37°C for 1 to 2 minutes. After confirming detachment of the cell layer from the bottom surface of the vessel by using a microscope and by gently tapping of the vessel, add an appropriate volume of the fresh culture medium and gently pipet the cells completely out of the vessel. Transfer the pipetted cell suspension into a sterile disposable centrifuge tube and centrifuge. Discard the supernatant liquid, resuspend the cells in an appropriate volume of flesh phosphate buffer solution for cytotoxicity test by pipetting, and centrifuge the tube again. Discard the supernatant liquid, and add an appropriate volume of fresh culture medium to the vessel. Resuspend the cells by gentle pipetting and make a homogeneous cell suspension. Determine the cell concentration using a hemocytometer.

(iv) Cytotoxicity test: Dilute the cell suspension prepared according to procedure (iii) with culture medium to adjust

the cell concentration to 100 cells/mL. Place a 0.5 mL aliquot of the diluted cell suspension on each well of a sterile disposable multiple well plate (24 wells). Incubate the plate in the incubator maintaining 5% carbon dioxide at 37°C for 4 – 24 hours to attach the cells to the bottom surface of the well. Discard the medium from each well, and add a 0.5 mL aliquot of the prepared sample solutions having various concentration or fresh medium to at least 3 wells each. Place the plate immediately in the incubator and incubate the plate for the appropriate period: 7 – 9 days for L929 cells; 6 – 7 days for V79 cells. After the incubation, discard the medium from the plate, add an appropriate volume of methanol or dilute formaldehyde TS to each well and allow the plate to stand for about 30 minutes to fix the cells. Discard the methanol or dilute formaldehyde TS from each well and add an appropriate volume of dilute Giemsa's TS to each well. After ensuring good staining of the colonies, discard the stain solution from the wells, wash with water, dry, and count the number of colonies in each well. Calculate a mean number of colonies for each concentration of the sample solution, and divide the mean by the mean number of colonies for the fresh medium to obtain the relative plating efficiency (%) for each extract concentration of the sample solution. Plot the extract concentration (%) of the sample solution on a logarithmic scale and the relative plating efficiency on an ordinary scale on semilogarithmic graph paper to obtain a colony formation inhibition curve of the rubber closure. Read the 50% inhibition concentration, IC<sub>50</sub> (%), at which the colony number is half that in the negative control group, from the inhibition curve.

It is recommended to check the sensitivity and the reproducibility of the test system by the use of suitable reference materials or control substances in the test system, if necessary.

#### 4.5. Interpretation

IC<sub>50</sub> (%) is not less than 90%.

#### 5. Acute systemic toxicity

This test is performed when the sample solution does not meet the requirements of the cytotoxicity test.

The sample solution meets the requirements, when examined under the following conditions against the blank solution.

#### 5.1. Preparation of the sample solution and the blank solution

Wash the rubber closures with water and Water for Injection successively, and dry under clean conditions at room temperature avoiding contamination. Transfer the rubber closures to a glass container. Add isotonic sodium chloride solution of 10 times the mass of the test material, stopper adequately, heat in an autoclave at 121°C for 1 hour, take out the glass container, and allow to cool to room temperature. The solution thus obtained is used as the sample solution. The blank solution is prepared in the same manner.

#### 5.2. Test procedures

(i) Test animals: Use healthy male or female mice of inbred strain or from a closed colony, weighing 17 to 25 g.

(ii) Procedure: Separate the animals into two groups of 5 mice, and inject intravenously 50 mL each of the solutions per kg body mass. From the viewpoint of animal rights, it is recommended to start the test with small size animal groups first, such as with 3 animals, and then add 2 animals to each group if the acceptable result was obtained.

#### 5.3. Interpretation

Observe the animals for 72 hours after injection: During the observation period, none of the animals treated with the sample solution show any weight loss, abnormality or death.

## 9. Reference Standards; Standard Solutions; Reagents, Test Solutions; Measuring Instruments, Appliances, etc.

### Reference Standards

#### 9.01 Reference Standards

Generally, reference standards are standard materials used for quality tests of pharmaceuticals, prepared to constant quality, assured its level of quality by official organization, and supplied officially. The Japanese Pharmacopoeia Reference Standards are reference standards used for the tests of drugs specified in the Japanese Pharmacopoeia and for the General Tests. Besides, standard materials are substances employed as the standard for measuring chemical, physical and biological characteristics in a quantitative and qualitative manner, and also used for calibration and checking accuracy of apparatus for the tests of pharmaceuticals.

The Japanese Pharmacopoeia Reference Standards are used for Assay, Identification, Purity, calibration of apparatus and system suitability in monographs and in the General Tests. The application and usage of The Japanese Pharmacopoeia Reference Standards are directed in monographs and in the General Tests.

The Japanese Pharmacopoeia Reference Standards are as follows:

(1) The reference standards which are prepared by those who have been registered to prepare them by the Minister of Health, Labour and Welfare, according to the Ministerial ordinance established by the Minister separately.

- Acetaminophen RS
- Acetanilide for Apparatus Suitability RS
- Acetophenetidine for Apparatus Suitability RS
- Aciclovir RS
- Adrenaline Bitartrate for Purity RS
- Alendronate Sodium RS
- Alprostadiol RS
- p-Aminobenzoyl Glutamic Acid for Purity RS
- Amitriptyline Hydrochloride RS
- Amlexanox RS
- Amlodipine Besilate RS
- Ampicillin RS
- Anhydrous Lactose for Identification RS
- Ascorbic Acid RS
- Aspirin RS
- Atorvastatin Calcium RS
- Atropine Sulfate RS
- Auranofin RS
- Azathioprine RS
- Azithromycin RS
- Baclofen RS
- Baicalin RS
- Beclometasone Dipropionate RS
- Berberine Chloride RS
- Betamethasone RS
- Betamethasone Sodium Phosphate RS
- Betamethasone Valerate RS
- Bicalutamide RS

Bisacodyl RS	Eplerenone RS
Bromfenac Sodium RS	Epoetin Alfa RS
Butyl Parahydroxybenzoate RS	Epoetin Beta RS
Cabergoline RS	Ergocalciferol RS
Caffeine RS	Ergometrine Maleate RS
Caffeine for Apparatus Suitability RS	Eribulin Mesilate RS
Calcitonin Salmon RS	Eribulin Mesilate Related Substance C for System Suitability RS
Calcium Folinate RS	Estradiol Benzoate RS
Calcium Oxalate Monohydrate for Calibration of Apparatus RS	Estriol RS
Calcium Pantothenate RS	Ethenzamide RS
Camostat Mesilate RS	Ethinylestradiol RS
d-Camphor RS	Ethyl Aminobenzoate RS
dl-Camphor RS	Ethyl Icosapentate RS
Carbidopa RS	Ethyl Loflazepate RS
Carboplatin RS	Ethyl Parahydroxybenzoate RS
L-Carnosine RS	Etoposide RS
Cefazolin RS	Fenofibrate RS
Cefepime Dihydrochloride RS	Fexofenadine Hydrochloride RS
Cefmetazole RS	Filgrastim RS
Cefotiam Hydrochloride RS	Fludrocortisone Acetate RS
Ceftriaxone Sodium RS	Fluocinolone Acetonide RS
Celecoxib RS	Fluocinonide RS
Cellacefate for Identification RS	Fluorometholone RS
Cetotiamine Hydrochloride RS	Flutamide RS
Chlordiazepoxide RS	Fluvoxamine Maleate RS
Chlormadinone Acetate RS	Folic Acid RS
Chlorpheniramine Maleate RS	Fradiomycin Sulfate RS
Cholecalciferol RS	Furosemide RS
Ciclosporin RS	Fursultiamine Hydrochloride RS
Cilnidipine RS	Gabexate Mesilate RS
Cilostazol RS	Gatifloxacin RS
Ciprofloxacin RS	Gefarnate RS
Cisplatin RS	Gefitinib RS
Citicoline RS	Ginsenoside Rb <sub>1</sub> RS
Clarithromycin RS	Ginsenoside Rg <sub>1</sub> RS
Clobetasol Propionate RS	Gitoxin for Purity RS
Clofibrate RS	Glimepiride RS
Clomifene Citrate RS	Glucagon RS
Clopidogrel Sulfate RS	Glucose RS
Cortisone Acetate RS	D-Glucuronolactone RS
Cyanocobalamin RS	Glycyrrhizic Acid RS
Danazol RS	Gonadorelin Acetate RS
Deferoxamine Mesilate RS	Guaifenesin RS
Deslanoside RS	Heparin Sodium RS
Dexamethasone RS	Heparin Sodium for Identification RS
Diethylcarbamazine Citrate RS	Low-molecular Mass Heparin RS
Diflorasone Diacetate RS	High-molecular Mass Urokinase RS
Diflucortolone Valerate RS	Human Chorionic Gonadotrophin RS
Digoxin RS	Human Menopausal Gonadotrophin RS
Dihydroergotoxine Mesilate RS	Hydrochlorothiazide RS
Dobutamine Hydrochloride RS	Hydrocortisone RS
Docetaxel RS	Hydrocortisone Acetate RS
Donepezil Hydrochloride RS	Hydrocortisone Sodium Phosphate RS
Doripenem RS	Hydrocortisone Succinate RS
Dorzolamide Hydrochloride RS	Hydroxyethylcellulose for Identification RS
Doxazosin Mesilate RS	Idoxuridine RS
Edrophonium Chloride RS	Imipramine Hydrochloride RS
Elcatonin RS	Indapamide RS
Enalapril Maleate RS	Indometacin RS
Endotoxin RS	Insulin Aspart RS
Entacapone RS	Insulin Glargine RS
Entacapone Related Substance A for System Suitability RS	Insulin Human RS
Epalrestat RS	Interleukin-2 RS
Epirubicin Hydrochloride RS	Ipriflavone RS
Epitiostanol RS	Isoflurane RS
	Isomalt RS

Kallidinogenase RS	Prednisolone RS
Lactose for Identification RS	Prednisolone Acetate RS
Lactulose RS	Prednisolone Succinate RS
Lanoconazole RS	Primidone RS
Lansoprazole RS	Probencid RS
Lenograstim RS	Probucol RS
Leuprorelin Acetate RS	Prochlorperazine Maleate RS
Limaprost RS	Progesterone RS
Losartan Potassium RS	Propiverine Hydrochloride RS
Loxoprofen RS	Propyl Parahydroxybenzoate RS
Lysozyme RS	Puerarin RS
Maltose RS	Pyridoxal Phosphate RS
Manidipine Hydrochloride RS	Pyridoxine Hydrochloride RS
D-Mannitol RS	Quetiapine Fumarate RS
Mecobalamin RS	Rabeprazole Sodium RS
Medroxyprogesterone Acetate RS	Ranitidine Hydrochloride RS
Menatetrenone RS	Reserpine RS
Meropenem RS	Residual Solvents Class 1 RS
Mestranol RS	Residual Solvents Class 2A RS
Methotrexate RS	Residual Solvents Class 2B RS
Methoxsalen RS	Residual Solvents Class 2C RS
Methyldopa RS	Residual Solvents for System Suitability RS
Methylergometrine Maleate RS	Retinol Acetate RS
Methyl Parahydroxybenzoate RS	Retinol Palmitate RS
Methylprednisolone Succinate RS	Ribavirin RS
Methyltestosterone RS	Riboflavin RS
Metildigoxin RS	Rilmazafone Hydrochloride RS
Mexiletine Hydrochloride RS	Risedronic Acid RS
Microcrystalline Cellulose for Identification RS	Ritodrine Hydrochloride RS
Miglitol RS	Rosuvastatin Calcium RS
Minocycline Hydrochloride RS	Roxatidine Acetate Hydrochloride RS
Mitiglinide Calcium RS	Roxithromycin RS
Mizoribine RS	Saccharated Pepsin RS
Montelukast Dicyclohexylamine RS	Saccharin RS
Montelukast Racemate for System Suitability RS	Saccharin Sodium RS
Montelukast for System Suitability RS	Sarpogrelate Hydrochloride RS
Montelukast Sodium for Identification RS	Scopolamine Hydrobromide RS
Nabumetone RS	Sennoside A RS
Nartogrestim RS	Sennoside B RS
Nateglinide RS	Sevoflurane RS
Neostigmine Methylsulfate RS	Silodosin RS
Nicotinamide RS	Simvastatin RS
Nicotinic Acid RS	Sitagliptin Phosphate RS
Nilvadipine RS	Sitagliptin Phosphate for System Suitability RS
Nizatidine RS	Sivelestat RS
Noradrenaline Bitartrate RS	Spironolactone RS
Norgestrel RS	Sulbactam RS
Olmesartan Medoxomil RS	Sulfadiazine Silver RS
Over-sulfated Chondroitin Sulfate for System Suitability RS	Sulfanilamide for Apparatus Suitability RS
Oxytocin RS	Sulfapyridine for Apparatus Suitability RS
Ozagrel Sodium RS	Swertiamarin RS
Paeoniflorin RS	Tacalcitol RS
Paroxetine Hydrochloride RS	Tacrolimus RS
Pazufloxacin Mesilate RS	Tazobactam RS
Pemirolast Potassium RS	Teprenone RS
Pentobarbital RS	Testosterone Propionate RS
Perphenazine RS	Thiamine Chloride Hydrochloride RS
Phytomedicine RS	Thiamylal RS
Pioglitazone Hydrochloride RS	Thrombin RS
Piperacillin RS	Timolol Maleate RS
Pitavastatin Methylbenzylamine RS	Tocopherol RS
Potassium Sucrose Octasulfate RS	Tocopherol Acetate RS
Povidone for Identification RS	Tocopherol Nicotinate RS
Pranlukast RS	Tocopherol Succinate RS
Pravastatin 1,1,3,3-Tetramethylbutylammonium RS	Tolbutamide RS
Prazosin Hydrochloride RS	Tolnaftate RS
	Tosufloxacin Tosilate RS

Tranexamic Acid RS  
 Trehalose RS  
 Triamcinolone RS  
 Triamcinolone Acetonide RS  
 Triazolam RS  
 Trichlormethiazide RS  
 Trihexyphenidyl Hydrochloride RS  
 Troxipide RS  
 Tyrosine for Digestion Test RS  
 Ubidecarenone RS  
 Ulinastatin RS  
 Valaciclovir Hydrochloride RS  
 Valsartan RS  
 Vancomycin Hydrochloride RS  
 Vanillin for Apparatus Suitability RS  
 Vasopressin RS  
 Vinblastine Sulfate RS  
 Vincristine Sulfate RS  
 Voriconazole RS  
 Warfarin Potassium RS  
 Zidovudine RS  
 Zonisamide RS

(2) The reference standards which are prepared by National Institute of Infectious Diseases.

Aclarubicin RS  
 Actinomycin D RS  
 Amikacin Sulfate RS  
 Amoxicillin RS  
 Amphotericin B RS  
 Arbekacin Sulfate RS  
 Aspoxicillin RS  
 Aztreonam RS  
 Bacampicillin Hydrochloride RS  
 Bacitracin RS  
 Bekanamycin Sulfate RS  
 Benzylpenicillin Potassium RS  
 Bleomycin A<sub>2</sub> Hydrochloride RS  
 Carumonam Sodium RS  
 Cefaclor RS  
 Cefadroxil RS  
 Cefalexin RS  
 Cefalotin Sodium RS  
 Cefatrizine Propylene Glycolate RS  
 Cefbuperazone RS  
 Cefcapene Pivoxil Hydrochloride RS  
 Cefdinir RS  
 Cefditoren Pivoxil RS  
 Cefixime RS  
 Cefmenoxime Hydrochloride RS  
 Cefminox Sodium RS  
 Cefodizime Sodium RS  
 Cefoperazone RS  
 Cefotaxime RS  
 Cefotetan RS  
 Cefotiam Hexetil Hydrochloride RS  
 Cefozopran Hydrochloride RS  
 Cefpiramide RS  
 Cefpirome Sulfate RS  
 Cefpodoxime Proxetil RS  
 Cefroxadine RS  
 Cefsulodin Sodium RS  
 Ceftazidime RS  
 Ceferam Pivoxil Mesitylene Sulfonate RS  
 Ceftibuten Hydrochloride RS  
 Ceftizoxime RS

Cefuroxime Axetil RS  
 Chloramphenicol RS  
 Chloramphenicol Palmitate RS  
 Chloramphenicol Succinate RS  
 Ciclacillin RS  
 Clindamycin Hydrochloride RS  
 Clindamycin Phosphate RS  
 Cloxacillin Sodium RS  
 Colistin Sodium Methanesulfonate RS  
 Colistin Sulfate RS  
 Cycloserine RS  
 Daunorubicin Hydrochloride RS  
 Demethylchlortetracycline Hydrochloride RS  
 Dibekacin Sulfate RS  
 Dicloxacillin Sodium RS  
 Diethanolammonium Fusidate RS  
 Doxorubicin Hydrochloride RS  
 Doxycycline Hydrochloride RS  
 Enviomycin Sulfate RS  
 Erythromycin RS  
 Faropenem Sodium RS  
 Flomoxef Triethylammonium RS  
 Fosfomycin Phenethylammonium RS  
 Gentamicin Sulfate RS  
 Idarubicin Hydrochloride RS  
 Imipenem RS  
 Interferon Alfa RS  
 Isepamicin Sulfate RS  
 Josamycin RS  
 Josamycin Propionate RS  
 Kanamycin Monosulfate RS  
 Latamoxef Ammonium RS  
 Lenampicillin Hydrochloride RS  
 Leucomycin A<sub>5</sub> RS  
 Lincomycin Hydrochloride RS  
 Lithium Clavulanate RS  
 Micronomicin Sulfate RS  
 Midecamycin RS  
 Midecamycin Acetate RS  
 Mitomycin C RS  
 Mupirocin Lithium RS  
 Nystatin RS  
 Oxytetracycline Hydrochloride RS  
 Panipenem RS  
 Peplomycin Sulfate RS  
 Phenethicillin Potassium RS  
 Pimaricin RS  
 Pirarubicin RS  
 Pivmecillinam Hydrochloride RS  
 Polymixin B Sulfate RS  
 Pyrrolnitrin RS  
 Ribostamycin Sulfate RS  
 Rifampicin RS  
 Spectinomycin Hydrochloride RS  
 Spiramycin II Acetate RS  
 Streptomycin Sulfate RS  
 Sulbenicillin Sodium RS  
 Sultamicillin Tosilate RS  
 Talampicillin Hydrochloride RS  
 Teicoplanin RS  
 Tetracycline Hydrochloride RS  
 Tobramycin RS  
 Trichomycin RS

## Standard Solutions

### 9.21 Standard Solutions for Volumetric Analysis

Standard Solutions for Volumetric Analysis are the solutions of reagent with an accurately known concentration, mainly used for the volumetric analysis.

They are prepared to a specified molar concentration. A 1 molar solution is a solution which contains exactly 1 mole of a specified substance in each 1000 mL of the solution and is designated as 1 mol/L.

If necessary, these solutions are diluted to other specified molar concentrations and the diluted solutions are also used as standard solutions. For example, 0.1 mol/L solution is obtained by diluting 1 mol/L solution 10 times by volume.

Unless otherwise directed, standard solutions for volumetric analysis should be stored in colorless or light-resistant, glass-stoppered bottles.

#### Preparation and Standardization

A volumetric standard solution is prepared according to one of the following methods. The degree of difference from a specified concentration  $n$  (mol/L) is expressed as a factor (molar concentration coefficient)  $f$ . Usually, standard solutions are prepared so that the factor is in the range of 0.970 – 1.030. The determination procedure of the factor is called standardization of the standard solution.

(1) Weigh accurately a quantity equivalent to about 1 mole or its multiple or a fractional mole number of the pure substance, and dissolve it in the specified solvent to make exactly 1000 mL to prepare a standard solution having a concentration close to the specified molarity  $n$  (mol/L). In this case, the factor  $f$  of the standard solution is obtained by dividing the mass of the pure substance taken (g) by the molecular mass of the substance (g) and the specified molarity number  $n$ .

When a pure substance is not obtainable, it is permissible to use a highly purified substance whose purity has been exactly determined and certified.

(2) In the case where a pure substance or a highly purified substance is not obtainable, weigh a quantity equivalent to about 1 mole or its multiple or a fractional mole number of the substance specified for each standard solution and dissolve it in the specified solvent to make about 1000 mL to prepare a standard solution having a concentration close to the specified molarity  $n$  (mol/L). The factor  $f$  of this solution is determined by applying the standardization procedure described for the respective standard solution. The procedure is classified into direct and indirect methods, as follows:

#### a) Direct method

Weigh accurately a standard reagent or an indicated substance specified for each standard solution, dissolve it in the specified solvent, then titrate with the prepared standard solution to be standardized, and determine the factor  $f$  by applying the following equation.

$$f = \frac{1000m}{VMn}$$

$M$ : Molecular mass equivalent to 1 mole of the standard reagent or the specified substance (g)

$m$ : Mass of the standard reagent or the specified substance taken (g)

$V$ : Volume of the prepared standard solution consumed for the titration (mL)

$n$ : Arithmetical mole number of the specified molar concentration of the standard solution to be standardized (e.g.  $n = 0.02$  for 0.02 mol/L standard solution)

#### b) Indirect method

When an appropriate standard reagent is not available, titrate a defined volume  $V_2$  (mL) of a standard solution to be standardized with the specified standard solution having a known factor ( $f_1$ ), and calculate the factor ( $f_2$ ) by applying the following equation.

$$f_2 = \frac{V_1 \times f_1}{V_2}$$

$f_1$ : Factor of the titrating standard solution having a known factor

$f_2$ : Factor of the prepared standard solution to be standardized

$V_1$ : Volume of the titrating standard solution consumed (mL)

$V_2$ : Volume of the prepared standard solution taken (mL)

(3) Standard solutions may be prepared by diluting exactly an accurately measured volume of a standard solution having a known factor, according to the specified dilution procedure. During this dilution procedure, the original factor of the standard solution is assumed to remain constant.

#### Ammonium Iron (II) Sulfate, 0.1 mol/L

1000 mL of this solution contains 39.214 g of ammonium iron (II) sulfate hexahydrate  $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ : 392.14].

*Preparation*—Dissolve 40 g of ammonium iron (II) sulfate hexahydrate in a cooled mixture of 30 mL of sulfuric acid and 300 mL of water, dilute with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared ammonium iron (II) sulfate solution, and add 25 mL of water and 5 mL of phosphoric acid. Titrate  $\langle 2.50 \rangle$  the solution with 0.02 mol/L potassium permanganate VS. Calculate the molarity factor.

Note: Prepare before use.

#### Ammonium Iron (II) Sulfate, 0.02 mol/L

1000 mL of this solution contains 7.843 g of ammonium iron (II) sulfate hexahydrate  $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ : 392.14].

*Preparation*—Before use, dilute 0.1 mol/L ammonium Iron (II) sulfate VS with diluted sulfuric acid (3 in 100) to make exactly 5 times the initial volume.

#### Ammonium Iron (III) Sulfate, 0.1 mol/L

1000 mL of this solution contains 48.22 g of ammonium iron (III) sulfate dodecahydrate  $[FeNH_4(SO_4)_2 \cdot 12H_2O]$ : 482.19].

*Preparation*—Dissolve 49 g of ammonium iron (III) sulfate dodecahydrate in a cooled mixture of 6 mL of sulfuric acid and 300 mL of water, add water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared ammonium iron (III) sulfate solution into an iodine flask, add 5 mL of hydrochloric acid, and shake the mixture. Dissolve 2 g of potassium iodide, and stopper the flask. After allowing the mixture to stand for 10 minutes, add 50 mL of water, and titrate  $\langle 2.50 \rangle$  the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a light yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination, make any neces-

sary correction, and calculate the molarity factor.

**Note:** Store protected from light. This solution, if stored for a long period of time, should be restandardized.

#### Ammonium Thiocyanate, 0.1 mol/L

1000 mL of this solution contains 7.612 g of ammonium thiocyanate ( $\text{NH}_4\text{SCN}$ : 76.12).

**Preparation**—Dissolve 8 g of ammonium thiocyanate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the 0.1 mol/L silver nitrate VS, and add 50 mL of water, 2 mL of nitric acid and 2 mL of ammonium iron (III) sulfate TS. Titrate  $\langle 2.50 \rangle$  the solution with the prepared ammonium thiocyanate solution to the first appearance of a persistent red-brown color with shaking. Calculate the molarity factor.

**Note:** Store protected from light.

#### Ammonium Thiocyanate, 0.02 mol/L

1000 mL of this solution contains 1.5224 g of ammonium thiocyanate ( $\text{NH}_4\text{SCN}$ : 76.12).

**Preparation**—Before use, dilute 0.1 mol/L ammonium thiocyanate VS with water to make exactly 5 times the initial volume.

#### Barium chloride, 0.1 mol/L

1000 mL of this solution contains 24.426 g of barium chloride dihydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ : 244.26).

**Preparation**—Dissolve 24.5 g of barium chloride dihydrate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 20 mL of the prepared solution, add 3 mL of hydrochloric acid, and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), previously warmed, heat the mixture on a water bath for 30 minutes, and allow it to stand overnight. Filter the mixture, wash the precipitate on the filter paper with water until the last washing shows no turbidity with silver nitrate TS, transfer the precipitate together with the filter paper to a tared crucible, and then heat strongly to ashes. After cooling, add 2 drops of sulfuric acid, and heat again at about 700°C for 2 hours. After cooling, weigh accurately the mass of the residue, and calculate the molarity factor as barium sulfate ( $\text{BaSO}_4$ ).

Each mL of 0.1 mol/L barium chloride VS  
= 23.34 mg of  $\text{BaSO}_4$

#### Barium Chloride, 0.02 mol/L

1000 mL of this solution contains 4.885 g of barium chloride dihydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ : 244.26).

**Preparation**—Dissolve 4.9 g of barium chloride dihydrate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 100 mL of the prepared barium chloride solution, add 3 mL of hydrochloric acid, and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), warmed previously, heat the mixture on a water bath for 30 minutes, and allow to stand overnight. Filter the mixture, wash the collected precipitate of filter paper with water until the last washing shows no turbidity with silver nitrate TS, transfer the precipitate together with the filter paper to a tared crucible, and then heat strongly to ashes. After cooling, add 2 drops of sulfuric acid, and heat strongly again at about 700°C for 2 hours. After cooling, weigh accurately the residue as barium sulfate ( $\text{BaSO}_4$ ), and calculate the molarity factor.

Each mL of 0.02 mol/L barium chloride VS  
= 4.668 mg of  $\text{BaSO}_4$

#### Barium Chloride, 0.01 mol/L

1000 mL of this solution contains 2.4426 g of barium chloride dihydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ : 244.26).

**Preparation**—Before use, dilute 0.02 mol/L barium chloride VS with water to make exactly twice the initial volume.

#### Barium Perchlorate, 0.005 mol/L

1000 mL of this solution contains 1.6812 g of barium perchlorate [ $\text{Ba}(\text{ClO}_4)_2$ : 336.23].

**Preparation**—Dissolve 1.7 g of barium perchlorate in 200 mL of water, dilute with 2-propanol to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 20 mL of the prepared barium perchlorate solution, add 55 mL of methanol and 0.15 mL of arsenazo III TS. Titrate  $\langle 2.50 \rangle$  the solution with 0.005 mol/L sulfuric acid VS until its purple color changes through red-purple to red. Calculate the molarity factor.

#### Benzethonium chloride, 0.004 mol/L

1000 mL of this solution contains 1.7923 g of benzethonium chloride ( $\text{C}_{27}\text{H}_{42}\text{ClNO}_2$ : 448.08).

**Preparation**—Dissolve 1.792 g of benzethonium chloride for assay, previously dried at 105°C for 4 hours, in water to make exactly 1000 mL, and standardize the solution as follows:

**Standardization**—Pipet 10 mL of the prepared benzethonium chloride solution, adjust to pH between 2.6 and 3.4 by adding dropwise diluted dilute hydrochloric acid (1 in 2), add 1 drop of methyl orange TS, and titrate  $\langle 2.50 \rangle$  with 0.02 mol/L sodium tetraphenylboron VS until the color of the solution becomes red. Calculate the molarity factor.

Each mL of 0.02 mol/L sodium tetraphenylboron VS  
= 8.962 mg of  $\text{C}_{27}\text{H}_{42}\text{ClNO}_2$

#### Bismuth Nitrate, 0.01 mol/L

1000 mL of this solution contains 4.851 g of bismuth nitrate pentahydrate [ $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ : 485.07].

**Preparation**—Dissolve 4.86 g of bismuth nitrate pentahydrate in 60 mL of dilute nitric acid, add water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the prepared bismuth nitrate solution, add 50 mL of water and 1 drop of xylenol orange TS, and titrate  $\langle 2.50 \rangle$  the solution with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red color changes to yellow. Calculate the molarity factor.

#### Bromine, 0.05 mol/L

1000 mL of this solution contains 7.990 g of bromine (Br: 79.90).

**Preparation**—Dissolve 2.8 g of potassium bromate and 15 g of potassium bromide in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the prepared solution into an iodine flask. Add 120 mL of water, quickly add 5 mL of hydrochloric acid, stopper the flask immediately, and shake it gently. Then add 5 mL of potassium iodide TS, re-stopper immediately, shake the mixture gently, and allow to stand for 5 minutes. Titrate  $\langle 2.50 \rangle$  the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a light yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determina-

tion, make any necessary correction, and calculate the molarity factor.

#### Ceric Ammonium Sulfate, 0.1 mol/L

See cerium (IV) tetraammonium sulfate, 0.1 mol/L.

#### Ceric Ammonium Sulfate, 0.01 mol/L

See cerium (IV) tetraammonium sulfate, 0.01 mol/L.

#### Cerium (IV) Sulfate, 0.1 mol/L

1000 mL of this solution contains 40.43 g of cerium (IV) sulfate tetrahydrate [Ce(SO<sub>4</sub>)<sub>2</sub>·4H<sub>2</sub>O: 404.30].

**Preparation**—Dissolve 40.43 g of cerium sulfate (IV) tetrahydrate in 550 mL of diluted sulfuric acid (1 in 11). After cooling, add water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.2 g of sodium oxalate (standard reagent), previously dried between 150°C and 200°C for 1 to 1.5 hours, and allow to cool in a desiccator (silica gel), and dissolve in 75 mL of water. Add a mixture of 5 mL of water and 2 mL of sulfuric acid with stirring, add 10 mL of hydrochloric acid, and warm to 70–75°C. Titrate <2.50> the solution with 0.1 mol/L cerium (IV) sulfate VS until the solution shows a persistent pale yellow color, and calculate the molarity factor.

$$\begin{aligned} \text{Each mL of 0.1 mol/L cerium sulfate (IV) VS} \\ = 6.700 \text{ mg of Na}_2\text{C}_2\text{O}_4 \end{aligned}$$

#### Cerium (IV) Tetraammonium Sulfate, 0.1 mol/L

1000 mL of this solution contains 63.26 g of cerium (IV) tetraammonium sulfate dihydrate [Ce(NH<sub>4</sub>)<sub>4</sub>(SO<sub>4</sub>)<sub>4</sub>·2H<sub>2</sub>O: 632.55].

**Preparation**—Dissolve 64 g of cerium (VI) tetraammonium sulfate dihydrate in 0.5 mol/L sulfuric acid VS to make 1000 mL, allow to stand for 24 hours, filter the solution through a glass filter (G3 or G4), if necessary, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the prepared cerium (IV) tetraammonium sulfate solution into an iodine flask. Add 20 mL of water and 20 mL of dilute sulfuric acid, then dissolve 1 g of potassium iodide in the mixture. Immediately titrate <2.50> the solution with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a light yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination, make any necessary correction, and calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period of time, should be restandardized.

#### Cerium (IV) Tetraammonium Sulfate, 0.01 mol/L

1000 mL of this solution contains 6.326 g of cerium (IV) tetraammonium sulfate dihydrate [Ce(NH<sub>4</sub>)<sub>4</sub>(SO<sub>4</sub>)<sub>4</sub>·2H<sub>2</sub>O: 632.55].

**Preparation**—Before use, dilute 0.1 mol/L cerium (IV) tetraammonium sulfate VS with 0.5 mol/L sulfuric acid VS to make exactly 10 times the initial volume.

#### Copper (II) Nitrate, 0.1 mol/L

1000 mL of this solution contains 24.16 g of copper (II) nitrate trihydrate [Cu(NO<sub>3</sub>)<sub>2</sub>·3H<sub>2</sub>O: 241.60].

**Preparation**—Dissolve 24.2 g of copper (II) nitrate trihydrate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 10 mL of the prepared 0.1 mol/L copper (II) nitrate, and add 1 mL of sodium ni-

trate solution (9 in 20), 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.8, and 70 mL of water. Titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS using a copper electrode as the indicator electrode, a complex type silver-silver chloride electrode as the reference electrode, and potassium chloride solution (1 in 4) as the inner solution.

#### Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.1 mol/L

1000 mL of this solution contains 37.224 g of disodium dihydrogen ethylenediamine tetraacetate dehydrate (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>·2H<sub>2</sub>O: 372.24).

**Preparation**—Dissolve 38 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Wash zinc (standard reagent) with dilute hydrochloric acid, water and then acetone, dry at 110°C for 5 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately about 1.3 g of this zinc, add 20 mL of dilute hydrochloric acid and 8 drops of bromine TS, and dissolve it by gentle warming. Expel any excess of bromine by boiling, and add water to make exactly 200 mL. Pipet 25 mL of this solution, and neutralize with sodium hydroxide solution (1 in 50). Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate <2.50> the solution with the prepared disodium dihydrogen ethylenediamine tetraacetate solution until the red-purple color changes to blue-purple. Calculate the molarity factor.

$$\begin{aligned} \text{Each mL of 0.1 mol/L disodium dihydrogen} \\ \text{ethylenediamine tetraacetate VS} \\ = 6.538 \text{ mg of Zn} \end{aligned}$$

Note: Store in polyethylene bottles.

#### Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.05 mol/L

1000 mL of this solution contains 18.612 g of disodium dihydrogen ethylenediamine tetraacetate dehydrate (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>·2H<sub>2</sub>O: 372.24).

**Preparation**—Dissolve 19 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Wash zinc (standard reagent) with dilute hydrochloric acid, water and then acetone, dry at 110°C for 5 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately about 0.8 g of this zinc, add 12 mL of dilute hydrochloric acid and 5 drops of bromine TS, and dissolve it by gentle warming. Expel any excess of bromine by boiling, and add water to make exactly 200 mL. Measure exactly 20 mL of this solution, and neutralize with sodium hydroxide solution (1 in 50). Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate <2.50> the solution with the prepared disodium dihydrogen ethylenediamine tetraacetate solution until the red-purple color changes to blue-purple. Calculate the molarity factor.

$$\begin{aligned} \text{Each mL of 0.05 mol/L disodium dihydrogen} \\ \text{ethylenediamine tetraacetate VS} \\ = 3.269 \text{ mg of Zn} \end{aligned}$$

Note: Store in polyethylene bottles.

#### Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.02 mol/L

1000 mL of this solution contains 7.445 g of disodium

dihydrogen ethylenediamine tetraacetate dihydrate ( $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ : 372.24).

*Preparation*—Dissolve 7.5 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, but weigh accurately 0.3 g of zinc (standard reagent), previously washed with dilute hydrochloric acid, with water and with acetone, and cooled in a desiccator (silica gel) after drying at 110°C for 5 minutes, and add 5 mL of dilute hydrochloric acid and 5 drops of bromine TS.

$$\begin{aligned} \text{Each mL of } 0.02 \text{ mol/L disodium dihydrogen ethylenediamine tetraacetate VS} \\ = 1.308 \text{ mg of Zn} \end{aligned}$$

Note: Store in polyethylene bottles.

#### Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.01 mol/L

1000 mL of this solution contains 3.7224 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate ( $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ : 372.24).

*Preparation*—Before use, dilute 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS with water to make exactly twice the initial volume.

#### Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.001 mol/L

1000 mL of this solution contains 0.37224 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate ( $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ : 372.24).

*Preparation*—Before use, dilute 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS with water to make exactly 10 times the initial volume.

#### Ferric Ammonium Sulfate, 0.1 mol/L

See Ammonium Iron (III) Sulfate, 0.1 mol/L.

#### Ferrous Ammonium Sulfate, 0.1 mol/L

See Ammonium Iron (II) Sulfate, 0.1 mol/L.

#### Ferrous Ammonium Sulfate, 0.02 mol/L

See Ammonium Iron (II) Sulfate, 0.02 mol/L.

#### Hydrochloric Acid, 2 mol/L

1000 mL of this solution contains 72.92 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Dilute 180 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh about 1.5 g of sodium carbonate (standard reagent) accurately, and dissolve in 100 mL of water.

$$\begin{aligned} \text{Each mL of } 2 \text{ mol/L hydrochloric acid VS} \\ = 106.0 \text{ mg of Na}_2\text{CO}_3 \end{aligned}$$

#### Hydrochloric Acid, 1 mol/L

1000 mL of this solution contains 36.461 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Dilute 90 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh accurately about 0.8 g of sodium carbonate (standard reagent), previously heated between

500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve it in 50 mL of water, and titrate <2.50> with the prepared hydrochloric acid to calculate the molarity factor (Indicator method: 3 drops of methyl red TS; or potentiometric titration). In the indicator method, when the end-point is approached, boil the content carefully, stopper the flask loosely, allow to cool, and continue the titration until the color of the solution changes to persistent orange to orange-red. In the potentiometric titration, titrate with vigorous stirring, without boiling.

$$\begin{aligned} \text{Each mL of } 1 \text{ mol/L hydrochloric acid VS} \\ = 53.00 \text{ mg of Na}_2\text{CO}_3 \end{aligned}$$

#### Hydrochloric Acid, 0.5 mol/L

1000 mL of this solution contains 18.230 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Dilute 45 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh accurately about 0.4 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

$$\begin{aligned} \text{Each mL of } 0.5 \text{ mol/L hydrochloric acid VS} \\ = 26.50 \text{ mg of Na}_2\text{CO}_3 \end{aligned}$$

#### Hydrochloric Acid, 0.2 mol/L

1000 mL of this solution contains 7.292 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Dilute 18 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh accurately about 0.15 g of sodium carbonate (standard reagent), and dissolve in 30 mL of water.

$$\begin{aligned} \text{Each mL of } 0.2 \text{ mol/L hydrochloric acid VS} \\ = 10.60 \text{ mg of Na}_2\text{CO}_3 \end{aligned}$$

#### Hydrochloric Acid, 0.1 mol/L

1000 mL of this solution contains 3.6461 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly twice the initial volume.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L hydrochloric acid VS} \\ = 5.300 \text{ mg of Na}_2\text{CO}_3 \end{aligned}$$

#### Hydrochloric Acid, 0.05 mol/L

1000 mL of this solution contains 1.8230 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 4 times the initial volume.

#### Hydrochloric Acid, 0.02 mol/L

1000 mL of this solution contains 0.7292 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 10 times the initial volume.

#### Hydrochloric Acid, 0.01 mol/L

1000 mL of this solution contains 0.36461 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric

acid VS with water to make exactly 20 times the initial volume.

#### Hydrochloric Acid, 0.001 mol/L

1000 mL of this solution contains 0.036461 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 200 times the initial volume.

#### Iodine, 0.05 mol/L

1000 mL of this solution contains 12.690 g of iodine (I: 126.90).

*Preparation*—Dissolve 13 g of iodine in 100 mL of a solution of potassium iodide (2 in 5), add 1 mL of dilute hydrochloric acid and water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 15 mL of the iodine solution, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (Indicator method: starch TS; or potentiometric titration: platinum electrode). In the indicator method, when the solution assumes a light yellow color as the end point is approached, add 3 mL of starch TS, and continue the titration until the blue color disappears. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period, should be restandardized before use.

#### Iodine, 0.025 mol/L

1000 mL of this solution contains 6.345 g of iodine (I: 126.90).

*Preparation*—Before use, dilute 0.05 mol/L iodine VS with water to make exactly twice the initial volume.

#### Iodine, 0.01 mol/L

1000 mL of this solution contains 2.5381 g of iodine (I: 126.90).

*Preparation*—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 5 times the initial volume.

#### Iodine, 0.005 mol/L

1000 mL of this solution contains 1.2690 g of iodine (I: 126.90).

*Preparation*—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 10 times the initial volume.

#### Iodine, 0.002 mol/L

1000 mL of this solution contains 0.5076 g of iodine (I: 126.90).

*Preparation*—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 25 times the initial volume.

#### Magnesium Chloride, 0.05 mol/L

1000 mL of this solution contains 10.165 g of magnesium chloride hexahydrate (MgCl<sub>2</sub>.6H<sub>2</sub>O: 203.30).

*Preparation*—Dissolve 10.2 g of magnesium chloride hexahydrate in freshly boiled and cooled water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared magnesium chloride solution. Add 50 mL of water, 3 mL of pH 10.7 ammonia-ammonium chloride buffer solution and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution changes to blue-purple. Calculate the molarity factor.

## Standard Solutions for Volumetric Analysis

#### Magnesium Chloride, 0.01 mol/L

1000 mL of this solution contains 2.0330 g of magnesium chloride hexahydrate (MgCl<sub>2</sub>.6H<sub>2</sub>O: 203.30).

*Preparation*—Before use, dilute 0.05 mol/L magnesium chloride VS with water to make exactly 5 times the initial volume.

#### Oxalic Acid, 0.05 mol/L

1000 mL of this solution contains 6.303 g of oxalic acid (C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>.2H<sub>2</sub>O: 126.07).

*Preparation*—Dissolve 6.3 g of oxalic acid dihydrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared oxalic acid solution in a 500-mL conical flask, and add 200 mL of diluted sulfuric acid (1 in 20), previously boiled for 10 to 15 minutes and then cooled to 27 ± 3°C. Transfer freshly standardized 0.02 mol/L potassium permanganate VS to a burette. Add quickly 22 mL of the 0.02 mol/L potassium permanganate VS to the oxalic acid solution from the burette under gentle stirring, and allow to stand until the red color of the mixture disappears. Heat the solution up to between 55°C and 60°C, and complete the titration <2.50> by adding 0.02 mol/L potassium permanganate VS until a light red color persists for 30 seconds. Add the last 0.5 to 1 mL dropwise, being particularly careful to allow the solution to become decolorized before the next drop is added. Calculate the molarity factor.

Note: Store protected from light.

#### Oxalic Acid, 0.005 mol/L

1000 mL of this solution contains 0.6303 g of oxalic acid dihydrate (C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>.2H<sub>2</sub>O: 126.07).

*Preparation*—Before use, dilute 0.05 mol/L oxalic acid VS with water to make exactly 10 times the initial volume.

#### Perchloric Acid, 0.1 mol/L

1000 mL of this solution contains 10.046 g of perchloric acid (HClO<sub>4</sub>: 100.46).

*Preparation*—Add slowly 8.7 mL of perchloric acid to 1000 mL of acetic acid (100) while keeping the temperature at about 20°C. Allow the mixture to stand for about 1 hour. Perform quickly the test as directed under Water Determination with 3.0 mL of the mixture, and designate the water content as A (g/dL). To the rest mixture add slowly [(A - 0.03) × 52.2] mL of acetic anhydride with shaking at about 20°C. Allow the solution to stand for 24 hours, and standardize it as follows:

*Standardization*—Weigh accurately about 0.3 g of potassium hydrogen phthalate (standard reagent), previously dried at 105°C for 4 hours and allowed to cool in a desiccator (silica gel). Dissolve it in 50 mL of acetic acid (100), and titrate <2.50> the solution with the prepared perchloric acid solution (Indicator method: 3 drops of crystal violet TS; or potentiometric titration). In the indicator method, titrate until the solution acquires a blue color. Perform a blank determination, make any necessary correction, and calculate the molarity factor.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 20.42 \text{ mg of KHC}_6\text{H}_4(\text{COO})_2 \end{aligned}$$

Note: Store protected from moisture.

#### Perchloric Acid, 0.05 mol/L

1000 mL of this solution contains 5.023 g of perchloric acid (HClO<sub>4</sub>: 100.46).

*Preparation*—Before use, dilute 0.1 mol/L perchloric acid

VS with acetic acid for nonaqueous titration to make exactly twice the initial volume. Perform quickly the test as directed under Water Determination with 8.0 mL of acetic acid for nonaqueous titration, and designate the water content as A (g/dL). If A is not less than 0.03, add  $[(A - 0.03) \times 52.2]$  mL of acetic anhydride to 1000 mL of acetic acid for nonaqueous titration, and use it for the preparation.

#### Perchloric Acid, 0.02 mol/L

1000 mL of this solution contains 2.0092 g of perchloric acid ( $\text{HClO}_4$ : 100.46).

**Preparation**—Before use, dilute 0.1 mol/L perchloric acid VS with acetic acid for nonaqueous titration to make exactly 5 times the initial volume. Perform quickly the test as directed under Water Determination with 8.0 mL of acetic acid for nonaqueous titration, and designate the water content as A (g/dL). If A is not less than 0.03, add  $[(A - 0.03) \times 52.2]$  mL of acetic anhydride to 1000 mL of acetic acid for nonaqueous titration, and use it for the preparation.

#### Perchloric Acid-1,4-Dioxane, 0.1 mol/L

1000 mL of this solution contains 10.046 g of perchloric acid ( $\text{HClO}_4$ : 100.46).

**Preparation**—Dilute 8.5 mL of perchloric acid with 1,4-dioxane to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.5 g of potassium hydrogen phthalate (standard reagent), previously dried at 105°C for 4 hours and allowed to cool in a desiccator (silica gel). Dissolve it in 80 mL of acetic acid for nonaqueous titration, and add 3 drops of crystal violet TS. Titrate <2.50> the solution with the prepared perchloric acid-1,4-dioxane solution until it acquires a blue color. Perform a blank determination, make any necessary correction, and calculate the molarity factor.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS  
= 20.42 mg of  $\text{KHC}_6\text{H}_4(\text{COO})_2$

Note: Store in a cold place, protected from moisture.

#### Perchloric Acid-1,4-Dioxane, 0.05 mol/L

1000 mL of this solution contains 5.023 g of perchloric acid ( $\text{HClO}_4$ : 100.46).

**Preparation**—Before use, dilute 0.1 mol/L perchloric acid-1,4-dioxane VS with 1,4-dioxane to make exactly twice the initial volume.

#### Perchloric Acid-1,4-Dioxane, 0.004 mol/L

1000 mL of this solution contains 0.4018 g of perchloric acid ( $\text{HClO}_4$ : 100.46).

**Preparation**—Before use, dilute 0.1 mol/L perchloric acid-1,4-dioxane VS with 1,4-dioxane to make exactly 25 times the initial volume.

#### Potassium Bichromate, 1/60 mol/L

See Potassium Dichromate, 1/60 mol/L.

#### Potassium Bromate, 1/60 mol/L

1000 mL of this solution contains 2.7833 g of potassium bromate ( $\text{KBrO}_3$ : 167.00).

**Preparation**—Dissolve 2.8 g of potassium bromate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the prepared potassium bromate solution into an iodine flask. Add 2 g of potassium iodide and 5 mL of dilute sulfuric acid, stopper the flask, and allow to stand for 5 minutes. Add

100 mL of water, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a light yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration until the blue color disappears. Perform a blank determination, make any necessary correction, and calculate the molarity factor.

#### Potassium Dichromate, 1/60 mol/L

1000 mL of this solution contains 4.903 g of potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ : 294.18).

**Preparation**—Weigh accurately about 4.903 g of potassium dichromate (standard reagent), previously powdered, dried between 100°C and 110°C for 3 to 4 hours and allowed to cool in a desiccator (silica gel), dissolve it in water to make exactly 1000 mL, and calculate the molarity factor.

#### Potassium Ferricyanide, 0.1 mol/L

See Potassium Hexacyanoferrate (III), 0.1 mol/L.

#### Potassium Ferricyanide, 0.05 mol/L

See Potassium Hexacyanoferrate (III), 0.05 mol/L.

#### Potassium Hexacyanoferrate (III), 0.1 mol/L

1000 mL of this solution contains 32.924 g of potassium hexacyanoferrate (III) [ $\text{K}_3\text{Fe}(\text{CN})_6$ : 329.24].

**Preparation**—Dissolve 33 g of potassium hexacyanoferrate (III) in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 25 mL of the prepared potassium hexacyanoferrate (III) solution into an iodine flask. Add 2 g of potassium iodide and 10 mL of dilute hydrochloric acid, stopper the flask, and allow to stand for 15 minutes. Add 15 mL of zinc sulfate TS, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a light yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination, make any necessary correction, and calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

#### Potassium Hexacyanoferrate (III), 0.05 mol/L

1000 mL of this solution contains 16.462 g of potassium hexacyanoferrate (III) [ $\text{K}_3\text{Fe}(\text{CN})_6$ : 329.24].

**Preparation**—Before use, dilute 0.1 mol/L potassium hexacyanoferrate (III) VS with water to make exactly twice the initial volume.

#### Potassium Hydroxide, 1 mol/L

1000 mL of this solution contains 56.11 g of potassium hydroxide ( $\text{KOH}$ : 56.11).

**Preparation**—Dissolve 65 g of potassium hydroxide in 950 mL of water. Add a freshly prepared, saturated solution of barium hydroxide octahydrate until no more precipitate is produced. Shake the mixture thoroughly, and allow it to stand for 24 hours in a tightly stoppered bottle. Decant the supernatant liquid or filter the solution through a glass filter (G3 or G4), and standardize the solution as follows:

**Standardization**—Weigh accurately about 2.5 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours. Dissolve it in 25 mL of freshly boiled and cooled water, and add 2 drops of bromothymol blue TS. Titrate <2.50> the solution with the prepared potassium hydroxide solution until it acquires a green color. Calculate the molarity factor.

Each mL of 1 mol/L potassium hydroxide VS  
= 97.09 mg of  $\text{HOSO}_2\text{NH}_2$

Note: Store in tightly stoppered bottles or in containers provided with a carbon dioxide-absorbing tube (soda-lime). This solution, if stored for a long period, should be restandardized.

#### Potassium Hydroxide, 0.5 mol/L

1000 mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

*Preparation*—Weigh 32 g of potassium hydroxide, proceed as directed for preparation under 1 mol/L potassium hydroxide VS, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L potassium hydroxide VS, but weigh accurately about 1.3 g of amidosulfuric acid (standard reagent).

Each mL of 0.5 mol/L potassium hydroxide VS  
= 48.55 mg of  $\text{HOSO}_2\text{NH}_2$

Note: Store as directed under 1 mol/L potassium hydroxide VS. This solution, if stored for a long period, should be restandardized.

#### Potassium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

*Preparation*—Weigh 6.5 g of potassium hydroxide, proceed as directed for preparation under 1 mol/L potassium hydroxide VS, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L potassium hydroxide VS, but weigh accurately about 0.25 g of amidosulfuric acid (standard reagent).

Each mL of 0.1 mol/L potassium hydroxide VS  
= 9.709 mg of  $\text{HOSO}_2\text{NH}_2$

Note: Store as directed under 1 mol/L potassium hydroxide VS. This solution, if stored for a long period, should be restandardized.

#### Potassium Hydroxide-Ethanol, 0.5 mol/L

1000 mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

*Preparation*—Dissolve 35 g of potassium hydroxide in 20 mL of water, and add aldehyde-free ethanol to make 1000 mL. Allow the solution to stand for 24 hours in a tightly stoppered bottle. Then quickly decant the supernatant liquid, and standardize the solution as follows:

*Standardization*—Measure exactly 15 mL of 0.25 mol/L sulfuric acid VS, add 50 mL of water, and titrate with the prepared potassium hydroxide-ethanol solution to calculate the molarity factor (Indicator method: 2 drops of phenolphthalein TS; or potentiometric titration). In the indicator method, titrate <2.50> until the solution acquires a light red color.

Note: Store in tightly stoppered bottles, protected from light. Standardize before use.

#### Potassium Hydroxide-Ethanol, 0.1 mol/L

1000 mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

*Preparation*—Weigh 7 g of potassium hydroxide, proceed as directed for preparation under 0.5 mol/L potassium hydroxide-ethanol VS, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 0.5 mol/L potassium hydroxide-ethanol VS, but measure exactly 15 mL of 0.05 mol/L sulfuric acid VS.

#### Standard Solutions for Volumetric Analysis

213

Note: Store as directed under 0.5 mol/L potassium hydroxide-ethanol VS. Standardize before use.

#### Potassium Iodate, 0.05 mol/L

1000 mL of this solution contains 10.700 g of potassium iodate ( $\text{KIO}_3$ : 214.00).

*Preparation*—Weigh accurately about 10.700 g of potassium iodate (standard reagent), previously dried between 120°C and 140°C for 1.5 to 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000 mL. Calculate the molarity factor.

#### Potassium Iodate, 1/60 mol/L

1000 mL of this solution contains 3.567 g of potassium iodate ( $\text{KIO}_3$ : 214.00).

*Preparation*—Weigh accurately about 3.567 g of potassium iodate (standard reagent), previously dried between 120°C and 140°C for 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000 mL. Calculate the molarity factor.

#### Potassium Iodate, 1/1200 mol/L

1000 mL of this solution contains 0.17833 g of potassium iodate ( $\text{KIO}_3$ : 214.00).

*Preparation*—Weigh accurately about 0.17833 g of potassium iodate, previously dried between 120°C and 140°C for 1.5 to 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000 mL. Calculate the molarity factor.

#### Potassium Permanganate, 0.02 mol/L

1000 mL of this solution contains 3.1607 g of potassium permanganate ( $\text{KMnO}_4$ : 158.03).

*Preparation*—Dissolve 3.2 g of potassium permanganate in water to make 1000 mL, and boil the solution for 15 minutes. Allow the solution to stand for at least 48 hours in a tightly stoppered flask, and filter it through a glass filter (G3 or G4). Standardize the solution as follows:

*Standardization*—Weigh accurately about 0.3 g of sodium oxalate (standard reagent), previously dried between 150°C and 200°C for 1 to 1.5 hours and allowed to cool in a desiccator (silica gel), transfer it to a 500 mL conical flask, dissolve in 30 mL of water, add 250 mL of diluted sulfuric acid (1 in 20), and warm the mixture between 30°C and 35°C. Transfer the prepared potassium permanganate solution to a buret, add quickly 40 mL of the solution under gentle stirring from the buret, and allow to stand until the red color of the mixture disappears. Warm the solution between 55°C and 60°C, and complete the titration <2.50> with the potassium permanganate solution until a light red color persists for 30 seconds. Add the last 0.5 to 1 mL dropwise before the end point, being particularly careful to allow the solution to be decolorized before the next drop is added. Calculate the molarity factor.

Each mL of 0.02 mol/L potassium permanganate VS  
= 6.700 mg of  $\text{Na}_2\text{C}_2\text{O}_4$

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

#### Potassium Permanganate, 0.002 mol/L

1000 mL of this solution contains 0.31607 g of potassium permanganate ( $\text{KMnO}_4$ : 158.03).

*Preparation*—Before use, dilute 0.02 mol/L potassium permanganate VS with water to make exactly 10 times the initial volume.

**Silver Nitrate, 0.1 mol/L**

1000 mL of this solution contains 16.987 g of silver nitrate ( $\text{AgNO}_3$ : 169.87).

*Preparation*—Dissolve 17.0 g of silver nitrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh accurately about 80 mg of sodium chloride (standard reagent), previously dried between 500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel), dissolve it in 50 mL of water, and titrate <2.50> under vigorous stirring with the prepared silver nitrate solution to calculate the molarity factor (Indicator method: 3 drops of fluorescein sodium TS; or potentiometric titration: silver electrode). In the indicator method, titrate until the color of the solution changes from yellow-green to orange through yellow.

$$\begin{aligned} \text{Each mL of 0.1 mol/L silver nitrate VS} \\ = 5.844 \text{ mg of NaCl} \end{aligned}$$

Note: Store protected from light.

**Silver Nitrate, 0.02 mol/L**

1000 mL of this solution contains 3.3974 g of silver nitrate ( $\text{AgNO}_3$ : 169.87).

*Preparation*—Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 5 times the initial volume.

**Silver Nitrate, 0.01 mol/L**

1000 mL of this solution contains 1.6987 g of silver nitrate ( $\text{AgNO}_3$ : 169.87).

*Preparation*—Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 10 times the initial volume.

**Silver Nitrate, 0.005 mol/L**

1000 mL of this solution contains 0.8494 g of silver nitrate ( $\text{AgNO}_3$ : 169.87).

*Preparation*—Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 20 times the initial volume.

**Silver Nitrate, 0.001 mol/L**

1000 mL of this solution contains 0.16987 g of silver nitrate ( $\text{AgNO}_3$ : 169.87).

*Preparation*—Dilute 0.1 mol/L silver nitrate VS with water to make exactly 100 times of the initial volume before use.

**Sodium Acetate, 0.1 mol/L**

1000 mL of this solution contains 8.203 g of sodium acetate ( $\text{CH}_3\text{COONa}$ : 82.03).

*Preparation*—Dissolve 8.20 g of anhydrous sodium acetate in acetic acid (100) to make 1000 mL, and standardize the solution as follows:

*Standardization*—Pipet 25 mL of the prepared sodium acetate solution, add 50 mL of acetic acid (100) and 1 mL of *p*-naphtholbenzeine TS, and titrate <2.50> with 0.1 mol/L perchloric acid VS until a yellow-brown color changes through yellow to green. Perform a blank determination, make any necessary correction, and calculate the molarity factor.

**Sodium Hydroxide, 1 mol/L**

1000 mL of this solution contains 39.997 g of sodium hydroxide ( $\text{NaOH}$ : 40.00).

*Preparation*—Dissolve 42 g of sodium hydroxide in 950 mL of water. Add a freshly prepared, saturated solution of barium hydroxide octahydrate until no more precipitate is produced. Mix well the mixture, and allow to stand for 24 hours in a tightly stoppered bottle. Decant the supernatant

liquid or filter the solution through a glass filter (G3 or G4), and standardize the solution as follows:

*Standardization*—Weigh accurately about 1.5 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours. Dissolve it in 25 mL of freshly boiled and cooled water, and titrate <2.50> the solution with the prepared sodium hydroxide solution to calculate the molarity factor (Indicator method: 2 drops of bromothymol blue TS; or potentiometric titration). In the indicator method, titrate until the solution acquires a green color.

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 97.09 \text{ mg of } \text{HOSO}_2\text{NH}_2 \end{aligned}$$

*Note*: Store in tightly stoppered bottles or in containers provided with a carbon dioxide-absorbing tube (soda lime). This solution, if stored for a long period, should be restandardized.

**Sodium Hydroxide, 0.5 mol/L**

1000 mL of this solution contains 19.999 g of sodium hydroxide ( $\text{NaOH}$ : 40.00).

*Preparation*—Weigh 22 g of sodium hydroxide, proceed as directed for preparation under 1 mol/L sodium hydroxide VS, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.7 g of amidosulfuric acid (standard reagent).

$$\begin{aligned} \text{Each mL of 0.5 mol/L sodium hydroxide VS} \\ = 48.55 \text{ mg of } \text{HOSO}_2\text{NH}_2 \end{aligned}$$

*Note*: Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

**Sodium Hydroxide, 0.2 mol/L**

1000 mL of this solution contains 7.999 g of sodium hydroxide ( $\text{NaOH}$ : 40.00).

*Preparation*—Weigh 9 g of sodium hydroxide, proceed as directed for preparation under 1 mol/L sodium hydroxide VS, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.3 g of amidosulfuric acid (standard reagent).

$$\begin{aligned} \text{Each mL of 0.2 mol/L sodium hydroxide VS} \\ = 19.42 \text{ mg of } \text{HOSO}_2\text{NH}_2 \end{aligned}$$

*Note*: Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

**Sodium Hydroxide, 0.1 mol/L**

1000 mL of this solution contains 3.9997 g of sodium hydroxide ( $\text{NaOH}$ : 40.00).

*Preparation*—Weigh 4.5 g of sodium hydroxide, proceed as directed for preparation under 1 mol/L sodium hydroxide VS, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.15 g of amidosulfuric acid (standard reagent).

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 9.709 \text{ mg of } \text{HOSO}_2\text{NH}_2 \end{aligned}$$

*Note*: Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

**Sodium Hydroxide, 0.05 mol/L**

1000 mL of this solution contains 1.9999 g of sodium hydroxide (NaOH: 40.00).

**Preparation**—Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly twice the initial volume.

**Sodium Hydroxide, 0.02 mol/L**

1000 mL of this solution contains 0.7999 g of sodium hydroxide (NaOH: 40.00).

**Preparation**—Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

**Sodium Hydroxide, 0.01 mol/L**

1000 mL of this solution contains 0.39997 g of sodium hydroxide (NaOH: 40.00).

**Preparation**—Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly 10 times the initial volume.

**Sodium Hydroxide-Ethanol, 0.025 mol/L**

1000 mL of this solution contains 1.000 g of sodium hydroxide (NaOH: 40.00).

**Preparation**—Dissolve 2.1 g of sodium hydroxide in 100 mL of ethanol (99.5), stopper tightly, and allow to stand for a night. To 50 mL of the supernatant liquid add 650 mL of ethanol (99.5) and freshly boiled and cooled water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 25 mg of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for 48 hours. Dissolve in 30 mL of diluted ethanol (99.5) with fleshly boiled and cooled water (7 in 10), and titrate <2.50> with the prepared sodium hydroxide-ethanol to calculate the molarity factor (potentiometric titration).

$$\begin{aligned} \text{Each mL of 0.025 mol/L sodium hydroxide-ethanol VS} \\ = 2.427 \text{ mg of HOSO}_2\text{NH}_2 \end{aligned}$$

Note: Store in light-resistant, well-stoppered bottles. The standardization should be performed before using.

**Sodium Lauryl Sulfate, 0.01 mol/L**

1000 mL of this solution contains 2.8838 g of sodium lauryl sulfate ( $\text{C}_{12}\text{H}_{25}\text{NaO}_4\text{S}$ : 288.38).

**Preparation**—Dissolve 2.9 g of sodium lauryl sulfate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.3 g of papaverine hydrochloride for assay, previously dried, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a glass-stoppered conical flask, add 5 mL each of water and dilute sulfuric acid and 60 mL of dichloromethane, then add 5 to 6 drops of a solution of methyl yellow in dichloromethane (1 in 500) as indicator, and titrate <2.50>, while vigorous shaking, with the sodium lauryl sulfate solution prepared above, using a buret with a minimum graduation of 0.02 mL. End point is reached when the color of the dichloromethane layer changes from yellow to orange-red after dropwise addition of the sodium lauryl sulfate solution, vigorous shaking and standing for a while.

$$\begin{aligned} \text{Each mL of 0.01 mol/L sodium lauryl sulfate VS} \\ = 3.759 \text{ mg of C}_{20}\text{H}_{21}\text{NO}_4\text{HCl} \end{aligned}$$

**Sodium Methoxide, 0.1 mol/L**

1000 mL of this solution contains 5.402 g of sodium

**Standard Solutions for Volumetric Analysis**

methoxide ( $\text{CH}_3\text{ONa}$ : 54.02).

**Preparation**—Add little by little 2.5 g of freshly cut sodium pieces to 150 mL of methanol cooled in ice-water. After the sodium has dissolved, add benzene to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.3 g of benzoic acid, previously dried for 24 hours in a desiccator (silica gel), dissolve it in 80 mL of *N,N*-dimethylformamide, and add 3 drops of thymol blue-*N,N*-dimethylformamide TS. Titrate <2.50> the solution with the prepared sodium methoxide solution until a blue color appears. Perform a blank determination, make any necessary correction, and calculate the molarity factor.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium methoxide VS} \\ = 12.21 \text{ mg of C}_6\text{H}_5\text{COOH} \end{aligned}$$

Note: Store in a cold place, protected from moisture. Standardize before use.

**Sodium Methoxide-Dioxane, 0.1 mol/L**

See Sodium Methoxide-1,4-Dioxane, 0.1 mol/L.

**Sodium Methoxide-1,4-Dioxane, 0.1 mol/L**

1000 mL of this solution contains 5.402 g of sodium methoxide ( $\text{CH}_3\text{ONa}$ : 54.02).

**Preparation**—Add little by little 2.5 g of freshly cut sodium pieces to 150 mL of methanol cooled in ice-water. After the sodium has dissolved, add 1,4-dioxane to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.3 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 80 mL of *N,N*-dimethylformamide, and add 3 drops of thymol blue-*N,N*-dimethylformamide TS. Titrate <2.50> the solution with the prepared sodium methoxide-1,4-dioxane solution until a blue color appears. Perform a blank determination, make any necessary correction, and calculate the molarity factor.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium methoxide-1,4-dioxane VS} \\ = 12.21 \text{ mg of C}_6\text{H}_5\text{COOH} \end{aligned}$$

Note: Store in a cold place, protected from moisture. Standardize before use.

**Sodium Nitrite, 0.1 mol/L**

1000 mL of this solution contains 6.900 g of sodium nitrite ( $\text{NaNO}_2$ : 69.00).

**Preparation**—Dissolve 7.2 g of sodium nitrite in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.44 g of sulfanilamide for titration of diazotization, previously dried at 105°C for 3 hours and allowed to cool in a desiccator (silica gel), dissolve in 10 mL of hydrochloric acid, 40 mL of water and 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate with the prepared sodium nitrite solution as directed in the potentiometric titration or amperometric titration under Endpoint Detection Methods in Titrimetry <2.50>. Calculate the molarity factor.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium nitrite VS} \\ = 17.22 \text{ mg of H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2 \end{aligned}$$

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

**Sodium Oxalate, 0.005 mol/L**

1000 mL of this solution contains 0.6700 g of sodium oxalate ( $\text{Na}_2\text{C}_2\text{O}_4$ : 134.00).

**Preparation**—Weigh accurately about 0.6700 g of sodium

oxalate (standard reagent), previously dried between 150°C and 200°C for 2 hours and allowed to cool in a desiccator (silica gel), dissolve it in water to make exactly 1000 mL, and calculate the molarity factor.

#### Sodium Tetraphenylborate, 0.02 mol/L

1000 mL of this solution contains 6.844 g of sodium tetraphenylborate [NaB(C<sub>6</sub>H<sub>5</sub>)<sub>4</sub>: 342.22].

*Preparation*—Dissolve 7.0 g of sodium tetraphenylborate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh 0.5 g of potassium hydrogen phthalate (standard reagent), dissolve it in 100 mL of water, add 2 mL of acetic acid (31), and warm to 50°C in a water bath. Add slowly 50 mL of the prepared sodium tetraphenylborate solution under stirring from a buret, then cool the mixture quickly, and allow to stand for 1 hour at room temperature. Transfer the precipitate to a tared glass filter (G4), wash with three 5 mL portions of potassium tetraphenylborate TS, dry at 105°C for 1 hour, and weigh accurately the glass filter. Calculate the molarity factor from the mass of potassium tetraphenylborate [KB(C<sub>6</sub>H<sub>5</sub>)<sub>4</sub>: 358.32].

Each mL of 0.02 mol/L sodium tetraphenylborate VS  
= 7.166 mg of KB(C<sub>6</sub>H<sub>5</sub>)<sub>4</sub>

Note: Prepare before use.

#### Sodium Tetraphenylboron, 0.02 mol/L

See Sodium Tetraphenylborate, 0.02 mol/L.

#### Sodium Thiosulfate, 0.1 mol/L

1000 mL of this solution contains 24.818 g of sodium thiosulfate pentahydrate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.5H<sub>2</sub>O: 248.18).

*Preparation*—Dissolve 25 g of sodium thiosulfate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000 mL, allow to stand for 24 hours, and standardize the solution as follows:

*Standardization*—Weigh accurately about 50 mg of potassium iodate (standard reagent), previously dried between 120°C and 140°C for 1.5 to 2 hours and allowed to cool in a desiccator (silica gel), and transfer to an iodine flask. Dissolve it in 25 mL of water, add 2 g of potassium iodide and 10 mL of dilute sulfuric acid, and stopper the flask. After allowing the mixture to stand for 10 minutes, add 100 mL of water, and titrate <2.50> the liberated iodine with the prepared sodium thiosulfate solution (Indicator method; or potentiometric titration: platinum electrode). In the indicator method, when the solution assumes a light yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination, make any necessary correction, and calculate the molarity factor.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 3.567 mg of KIO<sub>3</sub>

Note: This solution, if stored for a long period, should be restandardized.

#### Sodium Thiosulfate, 0.05 mol/L

1000 mL of this solution contains 12.409 g of sodium thiosulfate pentahydrate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.5H<sub>2</sub>O: 248.18).

*Preparation*—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 2 times the initial volume.

#### Sodium Thiosulfate, 0.02 mol/L

1000 mL of this solution contains 4.964 g of sodium thio-

sulfate pentahydrate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.5H<sub>2</sub>O: 248.18).

*Preparation*—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

#### Sodium Thiosulfate, 0.01 mol/L

1000 mL of this solution contains 2.4818 g of sodium thiosulfate pentahydrate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.5H<sub>2</sub>O: 248.18).

*Preparation*—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 10 times the initial volume.

#### Sodium Thiosulfate, 0.005 mol/L

1000 mL of this solution contains 1.2409 g of sodium thiosulfate pentahydrate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.5H<sub>2</sub>O: 248.18).

*Preparation*—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 20 times the initial volume.

#### Sodium Thiosulfate, 0.002 mol/L

1000 mL of this solution contains 0.4964 g of sodium thiosulfate pentahydrate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.5H<sub>2</sub>O: 248.18).

*Preparation*—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 50 times the initial volume.

#### Sulfuric Acid, 0.5 mol/L

1000 mL of this solution contains 49.04 g of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>: 98.08).

*Preparation*—Add slowly, under stirring, 30 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

*Standardization*—Weigh accurately about 0.8 g of sodium carbonate (standard reagent), previously heated between 500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve it in 50 mL of water, and titrate <2.50> the solution with the prepared sulfuric acid (Indicator method: 3 drops of methyl red TS; or potentiometric titration). In the indicator method, when the end point is approached, boil the solution carefully, stopper the flask loosely, allow to cool, and continue the titration, until the color of the solution changes to persistent orange to orange-red. Calculate the molarity factor. In the potentiometric titration, titrate with vigorous stirring without boiling.

Each mL of 0.5 mol/L sulfuric acid VS  
= 53.00 mg of Na<sub>2</sub>CO<sub>3</sub>

#### Sulfuric Acid, 0.25 mol/L

1000 mL of this solution contains 24.520 g of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>: 98.08).

*Preparation*—Add slowly, under stirring, 15 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 0.4 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

Each mL of 0.25 mol/L sulfuric acid VS  
= 26.50 mg of Na<sub>2</sub>CO<sub>3</sub>

#### Sulfuric Acid, 0.1 mol/L

1000 mL of this solution contains 9.808 g of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>: 98.08).

*Preparation*—Add slowly, under stirring, 6 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 0.15 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sulfuric acid VS} \\ = 10.60 \text{ mg of Na}_2\text{CO}_3 \end{aligned}$$

#### Sulfuric Acid, 0.05 mol/L

1000 mL of this solution contains 4.904 g of sulfuric acid ( $\text{H}_2\text{SO}_4$ : 98.08).

**Preparation**—Add slowly, under stirring, 3 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 80 mg of sodium carbonate (standard reagent), and dissolve in 30 mL of water.

$$\begin{aligned} \text{Each mL of 0.05 mol/L sulfuric acid VS} \\ = 5.300 \text{ mg of Na}_2\text{CO}_3 \end{aligned}$$

#### Sulfuric Acid, 0.025 mol/L

1000 mL of this solution contains 2.4520 g of sulfuric acid ( $\text{H}_2\text{SO}_4$ : 98.08).

**Preparation**—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly twice the initial volume.

#### Sulfuric Acid, 0.02 mol/L

1000 mL of this solution contains 1.9616 g of sulfuric acid ( $\text{H}_2\text{SO}_4$ : 98.08).

**Preparation**—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 2.5 times the initial volume.

#### Sulfuric Acid, 0.01 mol/L

1000 mL of this solution contains 0.9808 g of sulfuric acid ( $\text{H}_2\text{SO}_4$ : 98.08).

**Preparation**—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 5 times the initial volume.

#### Sulfuric Acid, 0.005 mol/L

1000 mL of this solution contains 0.4904 g of sulfuric acid ( $\text{H}_2\text{SO}_4$ : 98.08).

**Preparation**—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 10 times the initial volume.

#### Sulfuric Acid, 0.0005 mol/L

1000 mL of this solution contains 0.04904 g of sulfuric acid ( $\text{H}_2\text{SO}_4$ : 98.08).

**Preparation**—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 100 times the initial volume.

#### Tetrabutylammonium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 25.947 g of tetrabutylammonium hydroxide [ $(\text{C}_4\text{H}_9)_4\text{NOH}$ : 259.47].

**Preparation**—Before use, dilute a volume of 10% tetrabutylammonium hydroxide-methanol TS, equivalent to 26.0 g of tetrabutylammonium hydroxide, with 2-propanol to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.3 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 50 mL of acetone, and titrate <2.50> the solution with the prepared tetrabutylammonium hydroxide solution (potentiometric titration). Perform a blank determination in the same manner and make necessary corrections.

#### Standard Solutions for Volumetric Analysis

217

$$\begin{aligned} \text{Each mL of 0.1 mol/L tetrabutylammonium} \\ \text{hydroxide VS} \\ = 12.21 \text{ mg of C}_6\text{H}_5\text{COOH} \end{aligned}$$

**Note:** Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

#### Tetramethylammonium Hydroxide, 0.2 mol/L

1000 mL of this solution contains 18.231 g of tetramethylammonium hydroxide [ $(\text{CH}_3)_4\text{NOH}$ : 91.15].

**Preparation**—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 18.4 g of tetramethylammonium hydroxide, with water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Weigh accurately about 0.4 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 60 mL of *N,N*-dimethylformamide, and titrate <2.50> the solution with the prepared 0.2 mol/L tetramethylammonium hydroxide solution (Indicator method: 3 drops of thymol blue-*N,N*-dimethylformamide TS; or potentiometric titration). In the indicator method, titrate until a blue color is produced. Perform a blank determination in the same manner, make any necessary correction, and calculate the molarity factor.

$$\begin{aligned} \text{Each mL of 0.2 mol/L tetramethylammonium} \\ \text{hydroxide VS} \\ = 24.42 \text{ mg of C}_6\text{H}_5\text{COOH} \end{aligned}$$

**Note:** Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

#### Tetramethylammonium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 9.115 g of tetramethylammonium hydroxide [ $(\text{CH}_3)_4\text{NOH}$ : 91.15].

**Preparation**—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 9.2 g of tetramethylammonium hydroxide, with water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 0.2 mol/L tetramethylammonium hydroxide VS. Weigh accurately about 0.2 g of benzoic acid and titrate <2.50>.

$$\begin{aligned} \text{Each mL of 0.1 mol/L tetramethylammonium} \\ \text{hydroxide VS} \\ = 12.21 \text{ mg of C}_6\text{H}_5\text{COOH} \end{aligned}$$

**Note:** Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

#### Tetramethylammonium Hydroxide, 0.02 mol/L

1000 mL of this solution contains 1.8231 g of tetramethylammonium hydroxide [ $(\text{CH}_3)_4\text{NOH}$ : 91.15].

**Preparation**—Before use, dilute 0.1 mol/L tetramethylammonium hydroxide VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

#### Tetramethylammonium Hydroxide-Methanol, 0.1 mol/L

1000 mL of this solution contains 9.115 g of tetramethylammonium hydroxide [ $(\text{CH}_3)_4\text{NOH}$ : 91.15].

**Preparation**—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 9.2 g of tetramethylammonium hydroxide, with methanol to make 1000 mL, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 0.1 mol/L tetramethylammonium hydroxide VS.

**Note:** Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

**Titanium (III) Chloride, 0.1 mol/L**

1000 mL of this solution contains 15.423 g of titanium (III) chloride ( $\text{TiCl}_3$ : 154.23).

**Preparation**—Add 75 mL of hydrochloric acid to 75 mL of titanium (III) chloride (20), and dilute with freshly boiled and cooled water to make 1000 mL. Transfer the solution into a buret provided with a reservoir protected from light, replace the air with hydrogen, and allow to stand for 48 hours. Before use, standardize the solution as follows:

**Standardization**—Weigh 3 g of ammonium iron (II) sulfate hexahydrate in a wide-mouthed, 500 mL conical flask. Passing carbon dioxide through the flask, dissolve it in 50 mL of freshly boiled and cooled water, and add 25 mL of diluted sulfuric acid (27 in 100). Rapidly add exactly 40 mL of 0.02 mol/L potassium permanganate VS to the mixture, while passing carbon dioxide through the flask. Titrate <2.50> with the prepared titanium (III) chloride solution until the calculated end point is approached, then add 5 g of ammonium thiocyanate immediately, and continue the titration with the prepared titanium (III) chloride solution until the color of the solution disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store after the air has been displaced with hydrogen.

**Titanium Trichloride, 0.1 mol/L**

See Titanium (III) Chloride, 0.1 mol/L.

**Zinc, 0.1 mol/L**

1000 mL of this solution contains 6.538 g of zinc (Zn: 65.38).

**Preparation**—To 6.538 g of zinc (standard reagent), previously washed with dilute hydrochloric acid, with water and then acetone, and cooled in a desiccator (silica gel) after drying at 110°C for 5 minutes, add 80 mL of dilute hydrochloric acid and 2.5 mL of bromine TS, dissolve by gentle warming, evaporate excess bromine by boiling, and add water to make exactly 1000 mL.

**Zinc Acetate, 0.05 mol/L**

1000 mL of this solution contains 10.975 g of zinc acetate dihydrate [ $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ : 219.50].

**Preparation**—Dissolve 11.1 g of zinc acetate dihydrate in 40 mL of water and 4 mL of dilute acetic acid, add water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Measure exactly 20 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and add 50 mL of water, 3 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride reagent. Titrate <2.50> the solution with the prepared zinc acetate solution, until the blue color changes to blue-purple. Calculate the molarity factor.

**Zinc Acetate, 0.02 mol/L**

1000 mL of this solution contains 4.390 g of zinc acetate dihydrate [ $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ : 219.50].

**Preparation**—Dissolve 4.43 g of zinc acetate dihydrate in 20 mL of water and 2 mL of dilute acetic acid, add water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Proceed as directed for standardization under 0.05 mol/L zinc acetate VS, but measure exactly 20 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS.

**Zinc Sulfate, 0.1 mol/L**

1000 mL of this solution contains 28.755 g of zinc sulfate heptahydrate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ : 287.55).

**Preparation**—Dissolve 28.8 g of zinc sulfate heptahydrate in water to make 1000 mL, and standardize the solution as follows:

**Standardization**—Pipet 25 mL of the prepared zinc sulfate solution, add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <2.50> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue-purple. Calculate the molarity factor.

**Zinc sulfate, 0.05 mol/L**

1000 mL of this solution contains 14.378 g of zinc sulfate heptahydrate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ : 287.55).

**Preparation**—Before use, dilute 0.1 mol/L zinc sulfate VS with water to make exactly twice the initial volume.

**Zinc sulfate, 0.02 mol/L**

1000 mL of this solution contains 5.7510 g of zinc sulfate heptahydrate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ : 287.55).

**Preparation**—Before use, dilute 0.1 mol/L zinc sulfate VS with water to make exactly 5 times the initial volume.

## 9.22 Standard Solutions

Standard Solutions are used as the standard for the comparison in a text of the Pharmacopoeia.

**Borate pH Standard Solution** See pH Determination <2.54>.

**Calcium Hydroxide pH Standard Solution** See pH Determination <2.54>.

**Carbonate pH Standard Solution** See pH Determination <2.54>.

**Certified Standard Arsenic Solution** See Arsenic Limit Test <1.11>.

**Formazin stock suspension** To 25 mL of hexamethylenetetramine TS add 25 mL of hydrazinium sulfate TS, mix, and use after allowing to stand at room temperature for 24 hours. Store in a glass container free from surface defects. Use within 2 months. Shake thoroughly before use. The turbidity of this suspension is equivalent to 4000 NTU.

**Oxalate pH Standard Solution** See pH Determination <2.54>.

**pH Standard Solution, Borate** See pH Determination <2.54>.

**pH Standard Solution, Calcium Hydroxide** See pH Determination <2.54>.

**pH Standard Solution, Carbonate** See pH Determination <2.54>.

**pH Standard Solution, Oxalate** See pH Determination <2.54>.

**pH Standard Solution, Phosphate** See pH Determination <2.54>.

**pH Standard Solution, Phthalate** See pH Determination <2.54>.

**Phosphate pH Standard Solution** See pH Determination <2.54>.

**Phthalate pH Standard Solution** See pH Determination

&lt;2.54&gt;

**Standard Aluminum Solution for Atomic Absorption Spectrophotometry** To exactly 10 mL of Standard Aluminum Stock Solution add water to make exactly 100 mL. Prepare before use. Each mL of this solution contains 0.100 mg of aluminum (Al).

**Standard Aluminum Stock Solution** Weigh 1.0 g of aluminum, add 60 mL of diluted hydrochloric acid (1 in 2), dissolve by heating, cool, add water to make 1000 mL. Pipet 10 mL of this solution, add 30 mL of water and 5 mL of acetic acid-ammonium acetate buffer solution, pH 3.0, and adjust the pH to about 3 with ammonia TS added dropwise. Then, add 0.5 mL of Cu-PAN TS, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS while boiling until the color of the solution changes from red to yellow lasting for more than 1 minute. Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} \\ = 0.2698 \text{ mg of Al} \end{aligned}$$

**Standard Ammonium Solution** Dissolve 2.97 g of ammonium chloride, exactly weighed, in water for ammonium limit test to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add water for ammonium limit test to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of ammonium ( $\text{NH}_4^+$ ).

**Standard Arsenic Solution** See Arsenic Limit Test <1.11>.

**Standard Arsenic Stock Solution** See Arsenic Limit Test <1.11>.

**Standard Boron Solution** Weigh exactly 0.286 g of boric acid, previously dried in a desiccator (silica gel) to constant mass, and dissolve in water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.5  $\mu\text{g}$  of boron (B).

**Standard Cadmium Solution** Measure exactly 10 mL of Standard Cadmium Stock Solution, and add diluted nitric acid (1 in 3) to make exactly 1000 mL. Pipet 10 mL of this solution, and add diluted nitric acid (1 in 3) to make 100 mL. Each mL of this solution contains 0.001 mg of cadmium (Cd). Prepare before use.

**Standard Cadmium Stock Solution** Dissolve 1.000 g of cadmium ground metal, exactly weighed, in 100 mL of dilute nitric acid by gentle heating, cool, and add dilute nitric acid to make exactly 1000 mL.

**Standard Calcium Solution** Weigh exactly 0.250 g of calcium carbonate, add 5 mL of dilute hydrochloric acid and 25 mL of water, and dissolve by heating. After cooling, add water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of calcium (Ca).

**Standard Calcium Solution for Atomic Absorption Spectrophotometry** Weigh accurately 0.250 g of calcium carbonate, and add 1 mol/L hydrochloric acid TS to make exactly 100 mL. Each mL of this solution contains 1.00 mg of calcium (Ca).

**Standard Chloride Solution** Pipet 10 mL of Standard Chloride Stock Solution, add water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 5  $\mu\text{g}$  of chloride (Cl).

## General Tests / 9.22 Standard Solutions

219

**Standard Chloride Stock Solution** Weigh accurately 0.842 g of sodium chloride, previously dried at 130°C for 2 hours, and dissolve in water to make exactly 1000 mL.

**Standard Chromium Solution for Atomic Absorption Spectrophotometry** Weigh exactly 0.283 g of potassium dichromate (standard reagent), dissolve in water to make exactly 1000 mL. Each mL contains 0.10 mg of chromium (Cr).

**Standard Copper Solution** Pipet 10 mL of Standard Copper Stock Solution, and dilute with water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of copper (Cu). Prepare before use.

**Standard Copper Stock Solution** Weigh exactly 1.000 g of copper (standard reagent), add 100 mL of dilute nitric acid, and dissolve by heating. After cooling, add water to make exactly 1000 mL.

**Standard Cyanide Solution** Measure exactly a volume of Standard Cyanide Stock Solution, equivalent to 10 mg of cyanide (CN), add 100 mL of sodium hydroxide TS and water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of cyanide (CN). Prepare before use.

**Standard Cyanide Stock Solution** Dissolve 2.5 g of potassium cyanide in water to make exactly 1000 mL. Measure exactly 100 mL of this solution, add 0.5 mL of 4-dimethylaminobenzylidene rhodanine TS, and titrate <2.50> with 0.1 mol/L silver nitrate VS until the solution shows a red color.

$$\begin{aligned} \text{Each mL of 0.1 mol/L silver nitrate VS} \\ = 5.204 \text{ mg of CN} \end{aligned}$$

**Standard Fluorine Solution** See Oxygen Flask Combustion Method <1.06>.

**Standard Glyoxal Solution** Dilute Standard Glyoxal Stock Solution to 10 times with ethanol (99.5). Prepare before use. Each mL of this solution contains 2  $\mu\text{g}$  of glyoxal ( $\text{C}_2\text{H}_2\text{O}_2$ ).

**Standard Glyoxal Stock Solution** Transfer a quantity of 40% glyoxal TS, equivalent to 0.200 g of glyoxal, in a 100-mL volumetric flask, and dilute to 100 mL with ethanol (99.5). Dilute to 100-fold with ethanol (99.5) before use. Each mL of this solution contains 20  $\mu\text{g}$  of glyoxal ( $\text{C}_2\text{H}_2\text{O}_2$ ).

**Standard Gold Solution for Atomic Absorption Spectrophotometry** To 25 mL of Standard Gold Stock Solution, exactly measured, add water to make exactly 1000 mL. Each mL of this solution contains 0.025 mg of gold (Au).

**Standard Gold Stock Solution** Dissolve 0.209 g of hydrogen tetrachloroaurate (III) tetrahydrate, exactly weighed, in 2 mL of aqua regia, heat on a water bath for 10 minutes, and add 1 mol/L hydrochloric acid TS to make exactly 100 mL. Each mL of this solution contains 1.00 mg of gold (Au).

**Standard Hydrogen Peroxide Stock Solution** To an amount of hydrogen peroxide (30) add water to make a solution so that each mL contains 0.30 g of hydrogen peroxide ( $\text{H}_2\text{O}_2$ :34.01). Pipet 1 mL of this solution, add water to make exactly 10 mL, pipet 1 mL of this solution, transfer it to a flask containing 10 mL of water and 10 mL of dilute sulfuric acid, and titrate <2.50> with 0.02 mol/L potassium permanganate VS until the color of the solution changes to slightly red. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.02 mol/L potassium permanganate VS  
= 1.701 mg of H<sub>2</sub>O<sub>2</sub>

**Standard Hydrogen Peroxide Solution** To exactly 10 mL of Standard Hydrogen Peroxide Stock Solution add water to make exactly 100 mL. Prepare before use. Each mL contains 30 mg of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>:34.01).

**Standard Iron Solution** Weigh exactly 86.3 mg of ammonium iron (III) sulfate dodecahydrate, dissolve in 100 mL of water, and add 5 mL of dilute hydrochloric acid and water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of iron (Fe).

**Standard Iron Solution for Atomic Absorption Spectrophotometry** To exactly 5 mL of Standard Iron Stock Solution add water to make exactly 200 mL. Prepare before use. Each mL of this solution contains 0.250 mg of iron (Fe).

**Standard Iron Solution (2) for Atomic Absorption Spectrophotometry** To exactly 2 mL of Standard Iron Stock Solution add water to make exactly 250 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL. Prepare before use. Each mL contains 8 µg of iron (Fe).

**Standard Iron Stock Solution** Dissolve exactly 4.840 g of iron (III) chloride hexahydrate in diluted hydrochloric acid (9 in 25) to make exactly 100 mL.

**Standard Lead Solution** Measure exactly 10 mL of Standard Lead Stock Solution, and add water to make exactly 100 mL. Each mL of this solution contains 0.01 mg of lead (Pb). Prepare before use.

**Standard Lead Stock Solution** Weigh exactly 159.8 mg of lead (II) nitrate, dissolve in 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Prepare and store this solution using glass containers, free from soluble lead salts.

**Standard Liquids for Calibrating Viscosimeters [JIS, Standard Liquids for Calibrating Viscosimeters (Z 8809)]**

**Standard Magnesium Solution for Atomic Absorption Spectrophotometry** To exactly 1 mL of Standard Magnesium Stock Solution add water to make exactly 100 mL. Prepare before use. Each mL of this solution contains 0.0100 mg of magnesium (Mg).

**Standard Magnesium Stock Solution** Dissolve exactly 8.365 g of magnesium chloride hexahydrate in 2 mol/L hydrochloric acid TS to make exactly 1000 mL.

**Standard Mercury Solution** Weigh exactly 13.5 mg of mercury (II) chloride, previously dried for 6 hours in a desiccator (silica gel), dissolve in 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, and add 10 mL of dilute nitric acid and water to make exactly 1000 mL. Each mL of this solution contains 0.1 µg of mercury (Hg). Prepare before use.

**Standard Methanol Solution** See Methanol Test <1.12>.

**Standard Nickel Solution** Dissolve 6.73 g of ammonium nickel (II) sulfate hexahydrate, exactly weighed, in water to make exactly 1000 mL. Pipet 5 mL of this solution, add water to make exactly 1000 mL. Each mL of this solution contains 0.005 mg of nickel (Ni).

**Standard Nickel Solution for Atomic Absorption Spectrophotometry** To exactly 10 mL of Standard Nickel Stock Solution add water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 0.01 mg of nickel (Ni).

**Standard Nickel Stock Solution** Dissolve exactly 4.48 g of nickel (II) sulfate hexahydrate in water to make exactly 1000 mL.

**Standard Nitric Acid Solution** Weigh exactly 72.2 mg of potassium nitrate, dissolve in water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of nitrogen (N).

**Standard Palladium Solution for ICP Analysis** Standard solution specified by the Measurement Law. Each mL of this solution contains 1 mg of palladium (Pd).

**Standard Phosphoric Acid Solution** Weigh exactly 0.358 g of potassium dihydrogen phosphate, previously dried to constant mass in a desiccator (silica gel), and add 10 mL of diluted sulfuric acid (3 in 10) and water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Each mL of this solution contains 0.025 mg of phosphoric acid (as PO<sub>4</sub>).

**Standard Potassium Stock Solution** Weigh exactly 9.534 g of potassium chloride, previously dried at 130°C for 2 hours, and dissolve in water to make exactly 1000 mL. Each mL of this solution contains 5.00 mg of potassium (K).

**Standard Selenium Solution** To exactly 1 mL of Standard Selenium Stock Solution add water to make exactly 1000 mL. Prepare before use. It contains 1.0 µg of selenium (Se) per mL.

**Standard Selenium Stock Solution** Dissolve exactly 1.405 g of selenium dioxide in 0.1 mol/L nitric acid to make exactly 1000 mL.

**Standard Silver Solution for Atomic Absorption Spectrophotometry** Measure exactly 10 mL of Standard Silver Stock Solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of silver (Ag). Prepare before use.

**Standard Silver Stock Solution** Dissolve 1.575 g of silver nitrate, exactly weighed, in water to make exactly 1000 mL. Each mL of this solution contains 1.00 mg of silver (Ag).

**Standard Sodium Dodecylbenzene Sulfonate Solution** Weigh exactly 1.000 g of sodium dodecylbenzene sulfonate, and dissolve in water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of sodium dodecylbenzene sulfonate [CH<sub>3</sub>(CH<sub>2</sub>)<sub>11</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub>Na].

**Standard Sodium Stock Solution** Weigh exactly 2.542 g of sodium chloride (standard reagent), previously dried at 130°C for 2 hours, and dissolve in water to make exactly 1000 mL. Each mL of this solution contains 1.00 mg of sodium (Na).

**Standard Sulfite Solution** Dissolve exactly 3.150 g of anhydrous sodium sulfite in freshly prepared distilled water to make exactly 100 mL. Pipet 0.5 mL of this solution, add freshly prepared distilled water to make exactly 100 mL. Each mL of this solution contains 80 µg of sulfur dioxide (SO<sub>2</sub>). Prepare before use.

**Standard Tin Solution** Weigh exactly 0.250 g of tin, and dissolve in 10 mL of sulfuric acid by heating. After cooling, transfer this solution with 400 mL of diluted hydrochloric acid (1 in 5) to a 500-mL volumetric flask, and add diluted hydrochloric acid (1 in 5) to make 500 mL. Pipet 10 mL of this solution, and add diluted hydrochloric acid (1 in 5) to make exactly 1000 mL. Each mL of this solution contains 0.005 mg of tin (Sn). Prepare before use.

**Standard Vinyl Chloride Solution** Transfer about 190 mL of ethanol for gas chromatography into a 200-mL volumetric flask, and stopper with a silicone rubber stopper. Cooling this volumetric flask in a methanol-dry ice bath, inject 0.20 g of vinyl chloride, previously liquidized, through the silicone rubber stopper, and then inject ethanol for gas chromatography, previously cooled in a methanol-dry ice bath, through the silicone rubber stopper to make 200 mL. Pipet 1 mL of this solution, add ethanol for gas chromatography, cooled previously in a methanol-dry ice bath to make exactly 100 mL. Preserve in a hermetic container, at a temperature not exceeding  $-20^{\circ}\text{C}$ . This solution contains 10  $\mu\text{g}$  of vinyl chloride per mL.

**Standard Water-Methanol Solution** See Water Determination <2.48>.

**Standard Zinc Solution** Measure exactly 25 mL of Standard Zinc Stock Solution, and add water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 0.025 mg of zinc (Zn).

**Standard Zinc Solution for Atomic Absorption Spectrophotometry** To exactly 10 mL of Standard Zinc Stock Solution add water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 0.01 mg of zinc (Zn).

**Standard Zinc Stock Solution** Dissolve exactly 1.000 g of zinc (standard reagent), in 100 mL of water and 5 mL of hydrochloric acid with the aid of gentle heating, cool, and add water to make exactly 1000 mL.

## 9.23 Matching Fluids for Color

Refer to Color Comparison Tests <2.65>.

## Reagents, Test Solutions, etc.

### 9.41 Reagents, Test Solutions

Reagents are the substances used in the tests of the Pharmacopoeia. The reagents that are described as “Standard reagent for volumetric analysis”, “Special class”, “First class”, “For water determination”, etc. in square brackets meet the corresponding requirements of the Japan Industrial Standards (JIS). The tests for them are performed according to the test methods of JIS. The reagents that are described as “Certified reference material” are those noted a certificate on the basis of JIS Q 0030 and guaranteed the traceability of the international system of units. These reference materials are provided by the Metrology Management Center, National Institute of Advanced Industrial Science and Technology (AIST) and manufacturers of the certified reference materials. In the case where the reagent name in the Pharmacopoeia differs from that of JIS, the JIS name is given in the brackets. The reagents for which a monograph’s title is given in the brackets meet the requirements of the corresponding monograph. In the case of the reagents that are described merely as test items, the corresponding test method of the Pharmacopoeia is applied.

Test Solutions are the solutions prepared for use in the tests of the Pharmacopoeia.

**Acemetacin**  $\text{C}_{21}\text{H}_{18}\text{ClNO}_6$  [Same as the namesake monograph]

**Acemetacin for assay**  $\text{C}_{21}\text{H}_{18}\text{ClNO}_6$  [Same as the monograph Acemetacin. When dried, it contains not less than 99.5% of acemetacin ( $\text{C}_{21}\text{H}_{18}\text{ClNO}_6$ ) meeting the following additional specifications.]

**Purity** Related substances—Dissolve 40 mg of acemetacin for assay in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than acemetacin obtained from the sample solution is not larger than 1/2 times the peak area of acemetacin from the standard solution, and the total area of the peaks other than acemetacin from the sample solution is not larger than the peak area of acemetacin from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Acemetacin Tablets.

Time span of measurement: About 4 times as long as the retention time of Acemetacin.

#### System Suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of acemetacin obtained with 10  $\mu\text{L}$  of this solution is equivalent to 3 to 7% of that with 10  $\mu\text{L}$  of the standard solution.

System performance: Dissolve 75 mg of acemetacin and 75 mg of indometacin in 50 mL of methanol. To 4 mL of this solution add 1 mL of a solution of hexyl parahydroxybenzoate in methanol (1 in 250), and add methanol to make 50

mL. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, acemetacin, indometacin and hexyl parahydroxybenzoate are eluted in this order with the resolutions between the peaks of acemetacin and indometacin and between the peaks of indometacin and hexyl parahydroxybenzoate being not less than 3, respectively.

**System repeatability:** When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of acemetacin is not more than 1.5%.

**Acenaphthene** C<sub>12</sub>H<sub>10</sub> White to pale yellow-white, crystals or crystalline powder, having a characteristic aroma. Freely soluble in diethyl ether and in chloroform, soluble in acetonitrile, sparingly soluble in methanol, and practically insoluble in water.

**Identification**—Determine the infrared absorption spectrum of acenaphthene according to the paste method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1605 cm<sup>-1</sup>, 840 cm<sup>-1</sup>, 785 cm<sup>-1</sup> and 750 cm<sup>-1</sup>.

**Melting point** <2.60>: 93 – 96°C

**Purity**—Dissolve 0.1 g of acenaphthene in 5 mL of chloroform, and use this solution as the sample solution. Perform the test with 2  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of acenaphthene by the area percentage method: it is not less than 98.0 %.

#### Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with 150 to 180  $\mu$ m siliceous earth for gas chromatography coated with 10% of polyethylene glycol 20 M for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of acenaphthene is about 8 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of acenaphthene obtained with 2  $\mu$ L of the solution prepared by adding chloroform to 1.0 mL of the sample solution to make 100 mL is 5 to 15% of the full scale.

Time span of measurement: About 3 times as long as the retention time of acenaphthene, beginning after the solvent peak.

**Residue on ignition** <2.44>—Not more than 0.1% (1 g).

**Acetal** C<sub>6</sub>H<sub>14</sub>O<sub>2</sub> A clear and colorless, volatile liquid. Miscible with water and with ethanol (95).

*Refractive index* <2.45>  $n_D^{20}$ : about 1.382

*Specific gravity* <2.56>  $d_{20}^{20}$ : about 0.824

*Boiling point* <2.57>: about 103°C

**Acetaldehyde** CH<sub>3</sub>CHO [K 8030, First class]

**Acetaldehyde ammonia trimer trihydrate** (C<sub>2</sub>H<sub>5</sub>N)<sub>3</sub>.3H<sub>2</sub>O Colorless or white to pale yellow, crystals or powder.

**Content:** not less than 95.0%. Assay—Weigh accurately about 0.9 g of acetaldehyde ammonia trimer trihydrate, dissolve in 50 mL of water, and titrate <2.50> with 1 mol/L hydrochloric acid VS (potentiometric titration).

Each mL of 1 mol/L hydrochloric acid VS  
= 61.08 mg of (C<sub>2</sub>H<sub>5</sub>N)<sub>3</sub>.3H<sub>2</sub>O

**Acetaldehyde for assay** CH<sub>3</sub>CHO Distil 100 mL of

acetaldehyde under reduced pressure, discard the first 20 mL of the distillate, and use the subsequent distillate. Prepare before use.

**Acetaldehyde for gas chromatography** CH<sub>3</sub>CHO A clear and colorless, flammable liquid. Miscible with water and with ethanol (95).

*Refractive index* <2.45>  $n_D^{20}$ : about 1.332

*Specific gravity* <2.56>  $d_{20}^{20}$ : about 0.788

*Boiling point* <2.57>: about 21°C

**Acetaminophen** C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub> [Same as the namesake monograph]

**Acetanilide** C<sub>8</sub>H<sub>9</sub>NO White, crystals or crystalline powder.

*Melting point* <2.60>: 114 – 117°C

**o-Acetanisidine** C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> White to light brown, crystals or crystalline powder. Freely soluble in acetonitrile and in ethanol (99.5), and slightly soluble in water. Melting point: 86 – 89°C

**p-Acetanisidine** C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> White to purplish white, crystals or crystalline powder, having a characteristic odor.

It is freely soluble in acetonitrile and in ethanol (95), and very slightly soluble in water.

*Melting point* <2.60>: 126 – 132°C

**Content:** not less than 98.0%. Assay—Dissolve 0.1 g of *p*-acetanisidine in 5 mL of ethanol (95). Perform the test with 2  $\mu$ L of this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the area of each peak by the automatic integration method.

$$\text{Content (\%)} = \frac{\text{peak area of } p\text{-acetanisidine}}{\text{total of all peak areas}} \times 100$$

#### Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass tube 3 mm in inside diameter and 2 m in length, packed with acid-treated and silanized siliceous earth for gas chromatography coated with alkylene glycol phthalate ester for gas chromatography in 1% (177–250  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen.

Flow rate: Adjust to a constant flow rate of between 30 mL and 50 mL per minute and so that the retention time of *p*-acetanisidine is between 11 minutes and 14 minutes.

Time span of measurement: About 3 times as long as the retention time of *p*-acetanisidine, beginning after the solvent peak.

**Acetate buffer solution (pH 3.5)** Dissolve 50 g of ammonium acetate in 100 mL of 6 mol/L hydrochloric acid TS, adjust to pH 3.5 with ammonia TS or 6 mol/L hydrochloric acid TS, if necessary, and add water to make 200 mL.

**0.05 mol/L Acetate buffer solution (pH 4.0)** To 3.0 mL of acetic acid (100) add 900 mL of water, adjust to pH 4.0 with sodium hydroxide TS, and add water to make 1000 mL.

**Acetate buffer solution (pH 4.5)** Dissolve 63 g of anhydrous sodium acetate in a suitable amount of water, add 90 mL of acetic acid (100) and water to make 1000 mL.

**0.01 mol/L Acetate buffer solution (pH 5.0)** Dissolve 770 g of ammonium acetate in 900 mL of water, add acetic acid (31) to adjust the pH to 5.0, and then add water to make 1000 mL.

**Acetate buffer solution (pH 5.4)** To 5.78 mL of acetic acid (100) add water to make 1000 mL (solution A). Dissolve 8.2 g of anhydrous sodium acetate in water to make 1000 mL (solution B). Mix 176 mL of the solution A and 824 mL of the solution B, and adjust, if necessary, the pH to 5.4 with the solution A or the solution B.

**Acetate buffer solution (pH 5.5)** Dissolve 2.72 g of sodium acetate trihydrate in water to make 1000 mL, and adjust the pH to 5.5 with diluted acetic acid (100) (3 in 2500).

**0.02 mol/L Acetate buffer solution (pH 6.0)** Dissolve 1.76 g of sodium chloride in 4 mL of 1 mol/L acetic acid-sodium acetate buffer solution (pH 6.0), and add water to make 200 mL.

**Acetic acid** See acetic acid (31).

**Acetic acid (31)** Dilute 31.0 g of acetic acid (100) with water to make 100 mL (5 mol/L).

**Acetic acid (100)** CH<sub>3</sub>COOH [K 8355, Acetic Acid, Special class]

**Acetic acid-ammonium acetate buffer solution (pH 3.0)** Add acetic acid (31) to ammonium acetate TS, and adjust the pH to 3.0.

**Acetic acid-ammonium acetate buffer solution (pH 4.5)** Dissolve 77 g of ammonium acetate in 200 mL of water, adjust the pH to 4.5 by adding acetic acid (100), and add water to make 1000 mL.

**Acetic acid-ammonium acetate buffer solution (pH 4.8)** Dissolve 77 g of ammonium acetate in about 200 mL of water, and add 57 mL of acetic acid (100) and water to make 1000 mL.

**Acetic acid buffer solution containing 0.1% bovine serum albumin** Dissolve 0.1 g of bovine serum albumin in sodium acetate trihydrate solution (1 in 100) to make exactly 100 mL, and adjust the pH to 4.0 with 1 mol/L hydrochloric acid TS.

**Acetic acid, dilute** Dilute 6 g of acetic acid (100) with water to make 100 mL (1 mol/L).

**Acetic acid for nonaqueous titration** CH<sub>3</sub>COOH [K 8355, Special class. meeting with following requirement.]

**Purity** Acetic anhydride—Dissolve 1.0 g of aniline in acetic acid for nonaqueous titration to make 100 mL, and use this solution as the sample solution. Pipet 25 mL of the sample solution, titrate <2.50> with 0.1 mol/L perchloric acid VS, and designate the consumed volume as A (mL). A is not less than 26 mL. Pipet 25 mL of the sample solution, add 75 mL of acetic acid for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS, and designate the consumed volume as B (mL) (potentiometric titration). A - B is not more than 0.1 (mL) (not more than 0.001 g/dL).

**Acetic acid, glacial** See acetic acid (100).

**Acetic acid-potassium acetate buffer solution (pH 4.3)** Dissolve 14 g of potassium acetate in 20.5 mL of acetic acid (100), and add water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution (pH 4.0)** Dissolve 5.44 g of sodium acetate trihydrate in 900 mL of water, adjust the pH 4.0 with acetic acid (100), and add water to make 1000 mL.

**0.05 mol/L Acetic acid-sodium acetate buffer solution (pH 4.0)** To 3.0 g of acetic acid (100) add water to make 1000 mL. Adjust to pH 4.0 with a solution prepared by dis-

solving 3.4 g of sodium acetate trihydrate in water to make 500 mL.

**0.1 mol/L Acetic acid-sodium acetate buffer solution (pH 4.0)** Dissolve 13.61 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution (pH 4.5)** To 80 mL of sodium acetate TS add 120 mL of dilute acetic acid and water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution (pH 4.5), for iron limit test** Dissolve 75.4 mL of acetic acid (100) and 111 g of sodium acetate trihydrate in 1000 mL of water.

**0.05 mol/L Acetic acid-sodium acetate buffer solution (pH 4.6)** Dissolve 6.6 g of sodium acetate trihydrate in 900 mL of water, and add 3 mL of acetic acid and water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution (pH 4.7)** Dissolve 27.2 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 4.7 by adding acetic acid (100) dropwise, and add water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution (pH 5.0)** To 140 mL of sodium acetate TS add 60 mL of dilute acetic acid and water to make 1000 mL.

**1 mol/L Acetic acid-sodium acetate buffer solution (pH 5.0)** To sodium acetate TS add dilute acetic acid, and adjust the pH to 5.0.

**Acetic acid-sodium acetate buffer solution (pH 5.5)** Dissolve 20 g of sodium acetate trihydrate in 80 mL of water, adjust the pH to 5.5 by adding acetic acid (100) dropwise, and add water to make 100 mL.

**Acetic acid-sodium acetate buffer solution (pH 5.6)** Dissolve 12 g of sodium acetate trihydrate in 0.66 mL of acetic acid (100) and water to make 100 mL.

**1 mol/L Acetic acid-sodium acetate buffer solution (pH 6.0)** Adjust the pH of sodium acetate TS to 6.0 with dilute acetic acid.

**Acetic acid-sodium acetate TS** Mix 17 mL of 1 mol/L sodium hydroxide VS with 40 mL of dilute acetic acid, and add water to make 100 mL.

**Acetic acid-sodium acetate TS (pH 7.0)** Dissolve 4.53 g of sodium acetate trihydrate in water to make 100 mL, and adjust the pH to 7.0 with diluted acetic acid (100) (1 in 50).

**0.02 mol/L Acetic acid-sodium acetate TS** Dissolve 2.74 g of sodium acetate trihydrate in a suitable amount of water, and add 2 mL of acetic acid (100) and water to make 1000 mL.

**0.25 mol/L Acetic acid TS** To 3 g of acetic acid (100) add water to make 200 mL.

**2 mol/L Acetic acid TS** To 12 g of acetic acid (100) add water to make 100 mL.

**6 mol/L Acetic acid TS** Dilute 36 g of acetic acid (100) with water to make 100 mL.

**Acetic acid-sulfuric acid TS** To 5 mL of acetic acid (100) add cautiously 5 mL of sulfuric acid while cooling in an ice bath, and mix.

**Acetic anhydride** (CH<sub>3</sub>CO)<sub>2</sub>O [K 8886, Special class]

**Acetic anhydride-pyridine TS** Place 25 g of acetic anhy-

dride in a 100 mL volumetric flask, add pyridine to make 100 mL, and mix well. Preserve in light-resistant containers, protected from air. This solution may be used even if it becomes colored during storage.

**Acetone**  $\text{CH}_3\text{COCH}_3$  [K 8034, Special class]

**Acetone for nonaqueous titration** Add potassium permanganate to acetone in small portions, and shake. When the mixture keeps its purple color after standing for 2 to 3 days, distil, and dehydrate with freshly ignited anhydrous potassium carbonate. Distil by using a fractionating column under protection from moisture, and collect the fraction distilling at 56°C.

**Acetone for purity of crude drug**  $\text{CH}_3\text{COCH}_3$  [K 8034, Acetone, Special class] Use acetone meeting the following additional specification. Evaporate 300.0 mL of acetone to be tested in vacuum at a temperature not higher than 40°C, add the acetone to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of  $\gamma$ -BHC in hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane for purity of crude drug to make exactly 100 mL. Further pipet 2 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the solvent peak obtained from the sample solution is not larger than the peak area of  $\gamma$ -BHC from the standard solution (1).

#### Operating conditions

Proceed the operating conditions in 4.3. under Crude Drugs Test <5.01> except time span of measurement.

Time span of measurement: About three times as long as the retention time of  $\gamma$ -BHC, beginning after the solvent peak.

#### System suitability

Proceed the system suitability in 4.3. under Crude Drugs Test <5.01> except test for required detectability.

Test for required detectability: Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of  $\gamma$ -BHC obtained from 1  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method, and the peak height of  $\gamma$ -BHC from 1  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

**Acetonitrile**  $\text{CH}_3\text{CN}$  [K 8032, Special class]

**Acetonitrile for liquid chromatography**  $\text{CH}_3\text{CN}$  Colorless and clear liquid. Miscible with water.

**Purity** Ultraviolet light absorbing substances—Determine the absorbances of acetonitrile for liquid chromatography at the following wavelengths as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control: not more than 0.07 at 200 nm, not more than 0.046 at 210 nm, not more than 0.027 at 220 nm, not more than 0.014 at 230 nm and not more than 0.009 at 240 nm.

**Acetrizoic acid**  $\text{C}_9\text{H}_6\text{I}_3\text{NO}_3$  White powder.

**Purity** Related substances—Dissolve 60 mg of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL. To 10 mL of this solution add water to make 100 mL, and use this solution as the sample solution. Proceed the test with 5  $\mu\text{L}$  of the sample solution as directed in the Assay under

Meglumine Sodium Amidotrizoate Injection: any peaks other than the principal peak are not observed.

**Acetylacetone**  $\text{CH}_3\text{COCH}_2\text{COCH}_3$  [K 8027, Special class]

**Acetylacetone TS** Dissolve 150 g of ammonium acetate in a sufficient quantity of water, and add 3 mL of acetylacetone (100), 2 mL of acetylacetone and water to make 1000 mL. Prepare before use.

**Acetyl chloride**  $\text{CH}_3\text{COCl}$  A clear and colorless liquid.

**Acetylene** See dissolved acetylene.

**N-Acetylgalactosamine**  $\text{C}_8\text{H}_{15}\text{NO}_6$  White, crystals or crystalline powder.

**Content:** not less than 98.0%. Assay—Dissolve 36 mg of N-acetylgalactosamine in 1 mL of water. Perform the test with 15  $\mu\text{L}$  of this solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method. Operating conditions

Detector: A differential refractometer (Detector temperature: a constant temperature of about 40°C).

Column: A stainless steel column 8 mm in inside diameter and 30 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (7  $\mu\text{m}$  in particle diameter).

Column temperature: A constant temperature of about 80°C.

Mobile phase: Water.

Flow rate: 0.5 mL per minute.

Time span of measurement: About 3 times as long as the retention time of N-acetylgalactosamine.

**N-Acetylneuraminic acid**  $\text{C}_{11}\text{H}_{19}\text{NO}_9$  White, crystals or crystalline powder.

**Content:** not less than 98.0%. Assay—Dissolve 30 mg of N-acetylneuraminic acid in 1 mL of the mobile phase. Perform the test with 15  $\mu\text{L}$  of this solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method.

Operating conditions

Detector: A differential refractometer (detector temperature: a constant temperature of about 40°C).

Column: A stainless steel column 8 mm in inside diameter and 30 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (6  $\mu\text{m}$  in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: 10 mmol/L perchloric acid.

Flow rate: 0.5 mL per minute.

Time span of measurement: About 3 times as long as the retention time of N-acetylneuraminic acid.

**N-Acetylneuraminic acid for epoetin alfa**  $\text{C}_{11}\text{H}_{19}\text{NO}_9$  White needle crystalline powder.

**0.4 mmol/L N-Acetylneuraminic acid TS** Weigh accurately about 15.5 mg of N-acetylneuraminic acid for epoetin alfa, dissolve in water to make exactly 50 mL. To exactly V mL of this solution add water to make exactly 100 mL.

$$V(\text{mL}) = 309.3 \times 2/\text{amount (mg)} \\ \text{of } N\text{-acetylneuraminic acid taken}$$

**Achyranthes root for thin-layer chromatography** A heat-

dried, pulverized root of *Achyranthes fauriei* H. Léveillé et Vaniot (*Amaranthaceae*) meeting the following additional specifications.

**Identification** (1) To 2 g of pulverized achyranthes root for thin-layer chromatography add 10 mL of water, shake for 10 minutes, add 5 mL of 1-butanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: the standard solution shows a deep purplish red spot at around *Rf* value of 0.35, and the sample solution shows spots equivalent to those described below:

<i>Rf</i> value	Color and shape of the spot
Around 0	A weak spot, black
Around 0.1	A weak spot, strong purplish red
Around 0.2	A weak, tailing spot, strong purplish red
Around 0.25	A strong spot, deep purplish red
Around 0.35	A leading spot, deep purplish red
Around 0.45	A weak spot, dull yellow
Around 0.5	A weak spot, grayish purplish red
Around 0.75	A weak spot, grayish red
Around 0.9	A weak spot, dull red

(2) Perform the test with the sample solution and standard solution obtained under (1) as directed in the method under (1), except using a mixture of 1-propanol, ethyl acetate and water (4:4:3) as the developing solvent: the standard solution shows a deep purplish red spot at around *Rf* value of 0.45, and the sample solution shows spots equivalent to those described below:

<i>Rf</i> value	Color and shape of the spot
Around 0.25	A weak spot, strongly purplish red
Around 0.25 – 0.3	A leading spot or two strong spots, strongly purplish red
Around 0.35	A deep purplish red spot
Around 0.4	A weak spot, dull red
Around 0.42	A dark red spot
Around 0.45	A weak spot, grayish red
Around 0.65	A weak spot, dull greenish yellow
Around 0.7	A weak spot, grayish red
Around 0.85	A weak spot, grayish red
Around 0.95	A weak spot, dull yellow-red

**Acidic ferric chloride TS** See iron (III) chloride TS, acidic.

**Acidic potassium chloride TS** See potassium chloride TS, acidic.

**Acidic potassium permanganate TS** See potassium permanganate TS, acidic.

**Acidic stannous chloride TS** See tin (II) chloride TS, acidic.

**Acid-treated gelatin** See gelatin, acid-treated.

**Aconitine for purity** C<sub>34</sub>H<sub>47</sub>NO<sub>11</sub> White, crystals or crystalline powder. Sparingly soluble in acetonitrile and in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water. Melting point: about 185°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of aconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3500  $\text{cm}^{-1}$ , 1718  $\text{cm}^{-1}$ , 1278  $\text{cm}^{-1}$ , 1111  $\text{cm}^{-1}$ , 1097  $\text{cm}^{-1}$  and 717  $\text{cm}^{-1}$ .

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (230 nm): 211 – 243 [5 mg, ethanol (99.5), 200 mL].

**Purity** Related substances—(1) Dissolve 5.0 mg of aconitine for purity in 2 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

(2) Dissolve 5.0 mg of aconitine for purity in 5 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than aconitine obtained from the sample solution is not larger than the peak area of aconitine from the standard solution.

#### Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity (3) under Processed Aconitine Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust so that the retention time of aconitine is about 26 minutes.

Time span of measurement: About 3 times as long as the retention time of aconitine, beginning after the solvent peak. System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of aconitine obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

System performance: Dissolve 1 mg each of aconitine for purity, hypaconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aconitine is not more than 1.5%.

**Water** <2.48>: not more than 1.0% (5 mg, coulometric

titration).

**Aconitum diester alkaloids standard solution for purity**

It is a solution containing 10 mg of aconitine for purity, 10 mg of jesaconitine for purity, 30 mg of hypaconitine for purity and 20 mg of mesaconitine for purity in 1000 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). When proceed the test with 20  $\mu$ L of this solution as directed in the Purity (3) under Processed Aconite Root at the detection wavelength 231 nm, the peaks of aconitine, jesaconitine, hypaconitine and mesaconitine are observed, and the ratio of their peak heights is about 10:1:3:5:30. When proceed the test at the detection wavelength 254 nm, the peaks of aconitine, jesaconitine, hypaconitine and mesaconitine are observed, and the ratio of their peak heights is about 2:8:7:6.

**Aconitum monoester alkaloids standard TS for assay**

Weigh accurately about 20 mg of benzoylmesaconine hydrochloride for assay (separately, determine the water), about 10 mg of benzoylhypaconine hydrochloride for assay (separately, determine the water) and about 20 mg of 14-anisoylaconine hydrochloride for assay (separately, determine the water), dissolve in a mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17) to make exactly 1000 mL. Perform the test with 20  $\mu$ L of this solution as directed in the Purity under benzoylmesaconine hydrochloride for assay: the peaks of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine appear with a peak area ratio of about 2:1:2.

**Aconitum monoester alkaloids standard TS for component determination** See aconitum monoester alkaloids standard TS for assay.

**Acrinol** See acrinol hydrate.

**Acrinol hydrate**  $C_{15}H_{15}N_3O \cdot C_3H_6O_3 \cdot H_2O$  [Same as the namesake monograph]

**Acrylamide**  $CH_2CHCONH_2$  White or pale yellow crystalline powder.

*Melting point* <2.60>: 83 – 87°C

*Content:* not less than 97.0%.

**Acteoside for thin-layer chromatography** See Verbasco-side for thin-layer chromatography.

**Activated alumina** Aluminum oxide with specially strong adsorptive activity.

**Activated charcoal** [Same as the monograph Medicinal Carbon]

**Activated thromboplastin-time assay reagent** It is prepared by lyophilization of phospholipid (0.4 mg/mL) which is suspended in 1 mL of a solution of *N*-2-Hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (61 in 5000), mixed with both silica-gel (4.3 mg/mL) and dextran after the extraction and purification from rabbit brain. Activated thromboplastin-time: 25 – 45 seconds (as assayed with human normal plasma).

**Activated thromboplastin-time assay TS** Dissolve an aliquot of activated thromboplastin-time assay reagent equivalent to 0.4 mg of phospholipid in 1 mL of water.

**Adipic acid**  $C_4H_8(COOH)_2$  White, crystals or crystalline powder. Freely soluble in ethanol (95), and sparingly soluble in water.

*Melting point* <2.60>: 151 – 154°C

*Content:* not less than 98.0%. Assay—Weigh accurately about 1 g of adipic acid, and dissolve in 100 mL of water by

warming, cool, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 73.07 \text{ mg of } C_6H_{10}O_4 \end{aligned}$$

**Agar** [K 8263, Special class, or same as the monograph Agar or Agar Powder. Loss on drying is not more than 15%.]

**Agar medium, ordinary** See ordinary agar medium.

**Agar slant** Dispense portions of about 10 mL of ordinary agar medium into test tubes, and sterilize by autoclaving. Before the medium congeals, allow to stand in a slanting position, and solidify. When the coagulating water is lost, reprepare by dissolving with the aid of heat.

**Ajmaline for assay**  $C_{20}H_{26}N_2O_2$  [Same as the monograph Ajmaline. When dried, it contains not less than 99.0% of ajmaline ( $C_{20}H_{26}N_2O_2$ ).]

**Alacepril**  $C_{20}H_{26}N_2O_5S$  [Same as the namesake monograph]

**Alacepril for assay**  $C_{20}H_{26}N_2O_5S$  [Same as the monograph Alacepril. When dried, it contains not less than 99.0% of alacepril ( $C_{20}H_{26}N_2O_5S$ ).]

**$\beta$ -Alanine**  $C_3H_7NO_2$  Colorless crystals or a white crystalline powder. Freely soluble in water, very slightly soluble in methanol, and practically insoluble in ethanol (99.5) and in diethyl ether.

**Purity** Related substances—Dissolve 5.0 mg of  $\beta$ -Alanine in 10 mL of diluted methanol (4 in 5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (5:2:2) to a distance of about 8 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat the plate at 80°C for 5 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**L-Alanine**  $C_3H_7NO_2$  [K 9101, Special class]

**Albiflorin**  $C_{23}H_{28}O_{11}$  White powder having no odor. Freely soluble in water, in methanol and in ethanol (99.5).

**Identification**—Determine the absorption spectrum of a solution of albiflorin in diluted methanol (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 230 nm and 234 nm.

**Purity** (1) Related substances 1—Dissolve 1 mg of albiflorin in 1 mL of methanol, and perform the test with 10  $\mu$ L of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot with an *R*f value of about 0.2 does not appear.

(2) Related substances 2—Dissolve 1 mg of albiflorin in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed in the Assay under Peony Root: when measure the peak areas for 2 times the retention time of paeoniflorin, the total area of the peaks other than albiflorin obtained from the sample solution is not larger than 1/10 times the total area of the peaks other than the solvent peak.

**Albumin TS** Carefully separate the white from the yolk of a fresh hen's egg. Shake the white with 100 mL of water until the mixture is thoroughly mixed, and filter. Prepare before use.

**Alcian blue 8 GX**  $C_{56}H_{68}Cl_{14}CuN_{16}S_4$  Dark blue-purple powder.

**Alcian blue staining solution** Dissolve 0.5 g of alcian blue 8 GX in 100 mL of diluted acetic acid (100) (3 in 100).

**Aldehyde dehydrogenase** Each mg contains not less than 2 enzyme activity units. White powder.

**Assay**—Dissolve about 20 mg of aldehyde dehydrogenase, accurately weighed, in 1 mL of water, add ice-cold solution of bovine serum albumin (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. In a spectrophotometric cell, place 2.50 mL of pyrophosphate buffer solution (pH 9.0), 0.20 mL of a solution prepared by dissolving 20.0 mg of  $\beta$ -nicotinamide adenine dinucleotide ( $\beta$ -NAD) in water to make exactly 1 mL, 0.10 mL of a pyrazole solution (17 in 2500) and 0.10 mL of the sample solution, stir, stopper tightly, and allow to stand at  $25 \pm 1^\circ\text{C}$  for 2 minutes. To this solution add 0.01 mL of an acetaldehyde solution (3 in 1000), stir, stopper tightly, determine every 30 seconds the absorbance at 340 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, and calculate a change ( $\Delta A$ ) in absorbance per minute starting from the spot where the relation of time and absorbance is shown with a straight line. One enzyme activity unit means an amount of enzyme which oxidizes 1  $\mu\text{mol}$  of acetaldehyde per minute when the test is conducted under the conditions of the Procedure.

Enzyme activity unit (unit/mg) of aldehyde dehydrogenase

$$= \frac{2.91 \times \Delta A \times 200}{6.3 \times M \times 0.10 \times 1000}$$

M: Amount (g) of aldehyde dehydrogenase taken

**Aldehyde dehydrogenase TS** Dissolve an amount equivalent to 70 aldehyde dehydrogenase units in 10 mL of water. Prepare before use.

**Aldehyde-free ethanol** See ethanol, aldehyde-free.

**Aldioxa for assay**  $C_4H_7AlN_4O_5$  [Same as the monograph Aldioxa. When dried, it contains not less than 67.3% and not more than 71.0% of allantoin ( $C_4H_6N_4O_3$ ) and not less than 11.6% and not more than 12.5% of aluminum (Al).]

**Alendronate sodium hydrate**  $C_4H_{12}NNaO_7P_2.3H_2O$   
[Same as the namesake monograph]

**Alisma tuber triterpenes TS for identification** Dissolve 1 mg of alisol A for thin-layer chromatography, 1 mg of alisol B and 1 mg of alisol B monoacetate in 5 mL of methanol.

**Alisol A for thin-layer chromatography**  $C_{30}H_{50}O_5$  A white to pale yellow powder. Very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

**Optical rotation** <2.49>  $[\alpha]^{20}_D: +86 - +106^\circ$  (5 mg previously dried on silica gel for 24 hours, methanol, 1 mL, 50 mm).

**Purity** Related substances—Dissolve 1 mg of alisol A for thin-layer chromatography in 1 mL of methanol. Proceed the test with 5  $\mu\text{L}$  of this solution as directed in the Identification (6) under Saireito Extract: no spot appears other than the principal spot of around  $R_f$  value of 0.3.

**Alisol B**  $C_{30}H_{48}O_4$  White, crystals or crystalline powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** Determine the infrared absorption spectrum of alisol B as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $1704\text{ cm}^{-1}$ ,  $1458\text{ cm}^{-1}$  and  $1244\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 1 mg of alisol B in 1 mL of methanol, and use this solution as the sample solution. To exactly 0.5 mL of the sample solution add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat the plate at  $105^\circ\text{C}$  for 5 minutes: the spot other than the principal spot which appears at an  $R_f$  value of about 0.4 obtained from the sample solution is not more intense than the spot from the standard solution.

**Alisol B monoacetate**  $C_{32}H_{50}O_5$  White, crystals or crystalline powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** Determine the infrared absorption spectrum of alisol B monoacetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3480\text{ cm}^{-1}$ ,  $1743\text{ cm}^{-1}$ ,  $1704\text{ cm}^{-1}$  and  $1232\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 1 mg of alisol B monoacetate in 1 mL of methanol, and use this solution as the sample solution. To exactly 0.5 mL of the sample solution add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat the plate at  $105^\circ\text{C}$  for 5 minutes: the spot other than the principal spot which appears at an  $R_f$  value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

**Alizarin complexone**  $C_{19}H_{15}NO_8$  (1,2-Dihydroxyanthraquin-3-ylmethylamine-*N,N*-diacetate) A yellow-brown powder. Soluble in ammonia TS, and practically insoluble in water, in ethanol (95) and in diethyl ether.

**Sensitivity**—Dissolve 0.1 g of alizarin complexone by adding 2 drops of ammonia solution (28), 2 drops of ammonium acetate TS and 20 mL of water. To 10 mL of this solution add acetic acid-potassium acetate buffer solution (pH 4.3) to make 100 mL. Place 1 drop of this solution on a white spot plate, add 1 drop of a solution of sodium fluoride (1 in 100,000) and 1 drop of cerium (III) nitrate TS, stir, and observe under scattered light after 1 minute: a blue-purple color is produced, and the color of the control solution is red-purple. Use a solution prepared in the same manner, to which 1 drop of water is added in place of a solution of sodium fluoride, as the control solution.

**Alizarin complexone TS** Dissolve 0.390 g of alizarin complexone in 20 mL of a freshly prepared solution of so-

dium hydroxide (1 in 50), then add 800 mL of water and 0.2 g of sodium acetate trihydrate, and dissolve. Adjust the pH to 4 to 5 with 1 mol/L hydrochloric acid VS, and add water to make 1000 mL.

**Alizarin red S**  $C_{14}H_7NaO_7S$  [K 8057, Special class]

**Alizarin red S TS** Dissolve 0.1 g of alizarin red S in water to make 100 mL, and filter if necessary.

**Alizarin S** See alizarin red S.

**Alizarin S TS** See alizarin red S TS.

**Alizarin yellow GG**  $C_{13}H_8N_3NaO_5$  [K 8056, Special class]

**Alizarin yellow GG-thymolphthalein TS** Mix 10 mL of alizarin GG TS with 20 mL of thymolphthalein TS.

**Alizarin yellow GG TS** Dissolve 0.1 g of alizarin yellow GG in 100 mL of ethanol (95), and filter if necessary.

**Alkali copper TS** Dissolve 70.6 g of disodium hydrogen phosphate dodecahydrate, 40.0 g of potassium sodium tartrate tetrahydrate and 180.0 g of anhydrous sodium sulfate in 600 mL of water, and add 20 mL of a solution of sodium hydroxide (1 in 5). To this mixture add, with stirring, 100 mL of a solution of copper (II) sulfate pentahydrate (2 in 25), 33.3 mL of 0.05 mol/L potassium iodate VS and water to make 1000 mL.

**Alkaline blue tetrazolium TS** See blue tetrazolium TS, alkaline.

**Alkaline copper solution** See alkaline copper TS for protein content determination.

**Alkaline copper sulfate TS** See copper (II) sulfate TS, alkaline.

**Alkaline copper TS** Dissolve 2 g of anhydrous sodium carbonate in 100 mL of dilute sodium hydroxide TS. To 50 mL of this solution add 1 mL of a mixture of a solution of copper (II) sulfate pentahydrate (1 in 100) and a solution of potassium tartrate (1 in 50) (1:1), and mix.

**Alkaline copper TS for protein content determination**  
Dissolve 0.8 g of sodium hydroxide in water to make 100 mL. Dissolve 4 g of anhydrous sodium carbonate in this solution to make solution A. Combine 1 mL of copper (II) sulfate pentahydrate solution (1 in 50) and 1 mL of sodium tartrate dihydrate solution (1 in 25) to make solution B. Mix 50 mL of solution A and 1 mL of solution B. Prepare at the time of use.

**Alkaline copper TS (2)** Dissolve 20 g of anhydrous sodium carbonate in dilute sodium hydroxide TS to make 1000 mL, and use this solution as solution A. Dissolve 0.5 g of copper (II) sulfate pentahydrate in potassium sodium tartrate tetrahydrate solution (1 in 100) to make 100 mL, and use this solution as solution B. To 50 mL of solution A add 1 mL of solution B. Prepare before use.

**Alkaline 1,3-dinitrobenzen TS** See 1,3-dinitrobenzene TS, alkaline.

**Alkaline m-dinitrobenzene TS** See 1,3-dinitrobenzene TS, alkaline.

**Alkaline glycerin TS** To 200 g of glycerin add water to make 235 g, and add 142.5 mL of sodium hydroxide TS and 47.5 mL of water.

**Alkaline hydroxylamine TS** See hydroxylamine TS, alkaline.

**Alkaline phenolphthalein TS** See Alcohol Number Determination <1.01>.

**Alkaline phosphatase** See phosphatase, alkaline.

**Alkaline phosphatase TS** See phosphatase TS, alkaline.

**Alkaline picric acid TS** See 2,4,6-trinitrophenol TS, alkaline.

**Alkaline 1.6% potassium periodate-0.2% potassium permanganate TS** See 1.6% potassium periodate-0.2% potassium permanganate TS, alkaline.

**Alkaline 2,4,6-trinitrophenol TS** See 2,4,6-trinitrophenol TS, alkaline.

**Alkaline potassium ferricyanide TS** See potassium hexacyanoferrate (III) TS, alkaline.

**Alkylene glycol phthalate ester for gas chromatography** Prepared for gas chromatography.

**Allantoin for thin-layer chromatography**  $C_4H_6N_4O_3$  A white, crystalline powder or powder. Slightly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

**Identification** Determine the infrared absorption spectrum of the substance to be examined as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3440\text{ cm}^{-1}$ ,  $3340\text{ cm}^{-1}$ ,  $1721\text{ cm}^{-1}$ ,  $1532\text{ cm}^{-1}$  and  $1061\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 2 mg of the substance to be examined in 1 mL of water by warming, and add 2 mL of methanol. Perform the test with 5  $\mu\text{L}$  of this solution as directed in the Identification (3) under Dioscorea Rhizome: no spot is observed except the principal spot with an *Rf* value of about 0.5.

**Allopurinol**  $C_5H_4N_4O$  [Same as the namesake monograph]

**Allopurinol for assay**  $C_5H_4N_4O$  [Same as the monograph Allopurinol. When dried, it contains not less than 99.0% of allopurinol ( $C_5H_4N_4O$ ).]

**Alminoprofen for assay**  $C_{13}H_{17}NO_2$  [Same as the monograph Alminoprofen. When dried, it contains not less than 99.5% of alminoprofen ( $C_{13}H_{17}NO_2$ ).]

**Alternative thioglycolate medium** See Sterility Test <4.06>.

**Aluminon**  $C_{22}H_{23}N_3O_9$  [K 8011, Special class]

**Aluminon TS** Dissolve 0.1 g of aluminon in water to make 100 mL, and allow this solution to stand for 24 hours.

**Aluminum** Al [K 8069, Special class]

**Aluminum chloride** See aluminum (III) chloride hexahydrate.

**Aluminum chloride TS** See Aluminum (III) chloride TS.

**Aluminum (III) chloride TS** Dissolve 64.7 g of aluminum (III) chloride hexahydrate in 71 mL of water, add 0.5 g of activated charcoal, then shake for 10 minutes, and filter. Adjust the pH of the filtrate to 1.5 with a solution of sodium hydroxide (1 in 100) with stirring, and filter if necessary.

**Aluminum (III) chloride hexahydrate**  $AlCl_3 \cdot 6H_2O$  [K 8114, Special class]

**Aluminum oxide**  $Al_2O_3$  White crystals, crystalline powder, or powder. Boiling point: about 3000°C. Melting point:

about 2000°C.

**Aluminum potassium sulfate dodecahydrate**  
AlK(SO<sub>4</sub>)<sub>2</sub>.12H<sub>2</sub>O [K 8255, Special class]

**6-Amidino-2-naphthol methanesulfonate**

C<sub>11</sub>H<sub>10</sub>N<sub>2</sub>O.CH<sub>4</sub>O<sub>3</sub>S A white to pale yellow crystalline powder. Melting point: about 233°C (with decomposition).

*Purity*—A solution obtained by dissolving 0.5 g of 6-amidino-2-naphthol methanesulfonate in 10 mL of methanol is clear.

**Amidosulfuric acid (standard reagent)** HOSO<sub>2</sub>NH<sub>2</sub> In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

**Amidotrizoic acid for assay** C<sub>11</sub>H<sub>9</sub>I<sub>3</sub>N<sub>2</sub>O<sub>4</sub> [Same as the monograph Amidotrizoic Acid. It contains not less than 99.0% of amidotrizoic acid (C<sub>11</sub>H<sub>9</sub>I<sub>3</sub>N<sub>2</sub>O<sub>4</sub>), calculated on the dried basis.]

**p-Aminoacetophenone** See 4-aminoacetophenone.

**p-Aminoacetophenone TS** See 4-aminoacetophenone TS.

**4-Aminoacetophenone** H<sub>2</sub>NC<sub>6</sub>H<sub>4</sub>COCH<sub>3</sub> Light yellow, crystals or crystalline powder, having a characteristic odor.

*Melting point* <2.60>: 105 – 108°C

**4-Aminoacetophenone TS** Dissolve 0.100 g of 4-aminoacetophenone in methanol to make exactly 100 mL.

**4-Aminoantipyrine** C<sub>11</sub>H<sub>13</sub>N<sub>3</sub>O [K 8048, Special class]

**4-Aminoantipyrine hydrochloride** C<sub>11</sub>H<sub>13</sub>N<sub>3</sub>O.HCl

Light yellow crystalline powder. It dissolves in water. Melting point: 232 – 238°C (decomposition).

*Purity* Clarity of solution—Dissolve 1 g of 4-aminoantipyrine hydrochloride in 25 mL of water: the solution is almost clear.

*Content:* 100.6 – 108.5%. *Assay*—Weigh accurately about 0.5 g of 4-aminoantipyrine hydrochloride, dissolve in 50 mL of water, and, if necessary, neutralize with 0.1 mol/L sodium hydroxide VS (indicator: red litmus paper). Add 4 drops of dichlorofluorescein TS, and titrate <2.50> with 0.1 mol/L silver nitrate VS.

Each mL of 0.1 mol/L silver nitrate VS  
= 23.97 mg of C<sub>11</sub>H<sub>13</sub>N<sub>3</sub>O.HCl

**4-Aminoantipyrine hydrochloride TS** Dissolve 1 g of 4-aminoantipyrine hydrochloride in water to make 50 mL.

**4-Aminoantipyrine TS** Dissolve 0.1 g of 4-aminoantipyrine in 30 mL of water, add 10 mL of a solution of sodium carbonate decahydrate (1 in 5), 2 mL of sodium hydroxide TS and water to make 100 mL. Prepare before use.

**2-Aminobenzimidazole** C<sub>7</sub>H<sub>7</sub>N<sub>3</sub> White to light yellow, crystals or crystalline powder. Melting point: about 231°C (with decomposition).

**Aminobenzoate derivatization TS** To 0.28 g of ethyl aminobenzoate add 600 μL of methanol, warm at about 50°C to dissolve, and add 170 μL of acetic acid and 145 μL of borane-pyridine complex.

**p-Aminobenzoic acid** See 4-aminobenzoic acid.

**3-Aminobenzoic acid** C<sub>7</sub>H<sub>7</sub>NO<sub>2</sub> White crystals.

*Melting point* <2.60>: About 174°C

**4-Aminobenzoic acid** H<sub>2</sub>NC<sub>6</sub>H<sub>4</sub>COOH White to very pale yellow crystalline powder.

*Purity* Clarity of solution—Dissolve 0.1 g of 4-aminobenzoic acid in 10 mL of ethanol (95): the solution is clear.

**2-Amino-1-butanol** CH<sub>3</sub>CH<sub>2</sub>CH(NH<sub>2</sub>)CH<sub>2</sub>OH

Clear, colorless to light yellow liquid. Miscible with water and with methanol.

*Refractive index* <2.45> n<sub>D</sub><sup>20</sup>: 1.450 – 1.455

*Specific gravity* <2.56> d<sub>20</sub><sup>20</sup>: 0.944 – 0.950

*Purity* Related substances—Weigh 50 mg of 2-amino-1-butanol, and mix with exactly 10 mL of methanol. Perform the test with 2 μL of this solution as directed in the Purity (4) under Ethambutol Hydrochloride: any spot other than the principal spot at the R<sub>f</sub> value of about 0.3 does not appear.

**4-Aminobutylic acid** H<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>COOH White, crystals or crystalline powder. Melting point: about 200°C (with decomposition).

**ε-Aminocaproic acid** C<sub>6</sub>H<sub>13</sub>NO<sub>2</sub> White, crystals or crystalline powder, having no odor or slightly a characteristic odor. Freely soluble in water and in acetic acid (100), slightly soluble in methanol, and practically insoluble in ethanol. Melting point: about 200°C (with decomposition).

*Identification*—Determine the infrared absorption spectrum of ε-aminocaproic acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1564 cm<sup>-1</sup>, 1541 cm<sup>-1</sup>, 1391 cm<sup>-1</sup> and 1269 cm<sup>-1</sup>.

**4-Amino-6-chlorobenzene-1,3-disulfonamide**

C<sub>6</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub> White, crystals or crystalline powder.

*Identification*—Determine the infrared absorption spectrum of 4-amino-6-chlorobenzene-1,3-disulfonamide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3380 cm<sup>-1</sup>, 3250 cm<sup>-1</sup>, 1638 cm<sup>-1</sup>, 1597 cm<sup>-1</sup>, 1544 cm<sup>-1</sup> and 1324 cm<sup>-1</sup>.

*Storage*—Preserve in tight containers.

**2-Amino-5-chlorobenzophenone for thin-layer chromatography** C<sub>13</sub>H<sub>10</sub>ClNO Yellow crystalline powder.

*Melting point* <2.60>: 97 – 101°C

*Purity* Related substances—Dissolve 10 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and perform the test with this solution as directed in the purity (2) under Chlordiazepoxide: any spot other than the principal spot at the R<sub>f</sub> value about 0.7 does not appear.

**4-Amino-N,N-diethylaniline sulfate monohydrate**

H<sub>2</sub>NC<sub>6</sub>H<sub>4</sub>N(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>.H<sub>2</sub>SO<sub>4</sub>.H<sub>2</sub>O White to slightly colored powder. It dissolves in water.

*Melting point* <2.60>: 173 – 176°C

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

**4-Amino-N,N-diethylaniline sulfate TS** Dissolve 0.2 g of 4-amino-N,N-diethylaniline sulfate monohydrate in water to make 100 mL. Prepare before use, protected from light.

**2-Aminoethanethiol hydrochloride** H<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>SH.HCl White, crystal or granule.

*Melting point* <2.60>: 65 – 71°C

**2-Aminoethanol** H<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>OH [K 8109, Special class]

**3-(2-Aminoethyl)indole** C<sub>10</sub>H<sub>12</sub>N<sub>2</sub> Yellow-brown crystals.

*Melting point* <2.60>: about 118°C.

**N-Aminohexamethyleneimine** (CH<sub>2</sub>)<sub>6</sub>NNH<sub>2</sub> Clear, colorless to pale yellow liquid.

**Refraction index**  $\langle 2.45 \rangle n_{D}^{20}$ : 1.482 – 1.487

**Specific gravity**  $\langle 2.56 \rangle d_{20}^{20}$ : 0.936 – 0.942

**2-Amino-2-hydroxymethyl-1,3-propanediol**  $C_4H_{11}NO_3$   
[K 9704, Special class]

**2-Amino-2-hydroxymethyl-1,3-propanediol hydrochloride**  $C_4H_{11}NO_3 \cdot HCl$  White, crystals or crystalline powder

**4-(Aminomethyl)benzoic acid**  $C_8H_9NO_2$  A white powder.

**Purity**—Dissolve 10 mg of 4-(aminomethyl)benzoic acid in 100 mL of water, and use this as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu L$  each of the sample solution and standard solution as directed under Liquid Chromatography  $\langle 2.01 \rangle$  according to the operating conditions as directed in the Purity (5) under Tranexamic Acid, and determine each peak area by the automatic integration method: each area of the peak other than 4-(aminomethyl)benzoic acid obtained from the sample solution is not larger than the peak area of 4-(aminomethyl)benzoic acid from the standard solution.

**1-Amino-2-methylnaphthalene**  $C_{11}H_{11}N$  Pale yellow to pale brown, masses or liquid.

**2-Aminomethylpiperidine**  $C_6H_{14}N$  A colorless or light yellow, clear liquid, having an amine like characteristic odor.

**Identification**—Determine the infrared absorption spectrum as directed in the liquid film method under Infrared Spectrophotometry  $\langle 2.25 \rangle$ ; it exhibits absorption at the wave numbers of about  $3280\text{ cm}^{-1}$ ,  $1600\text{ cm}^{-1}$ ,  $1440\text{ cm}^{-1}$ ,  $1120\text{ cm}^{-1}$  and  $840\text{ cm}^{-1}$ .

**Purity** Related substances—Perform the test with 0.8  $\mu L$  of 2-aminomethylpiperidine as directed under Gas Chromatography  $\langle 2.02 \rangle$ . Determine each peak area by the automatic integration method, and calculate these amounts by the area percentage method: the total amount of the peaks other than 2-aminomethylpiperidine is not more than 1.5%.

#### Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (150 – 180  $\mu m$ ) coated with 10% of polyethylene glycol 20M for gas chromatography and 2% of potassium hydroxide.

Column temperature: 100°C at beginning, and raise to 200°C at a rate of 10°C per minute after injection.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of 2-aminomethylpiperidine is about 5 minutes.

Time span of measurement: About 2 times as long as the retention time of 2-aminomethylpiperidine.

**1-Amino-2-naphthol-4-sulfonic acid**  $C_{10}H_9NO_4S$   
[K 8050, Special class]

**1-Amino-2-naphthol-4-sulfonic acid TS** Mix thoroughly 5 g of anhydrous sodium sulfite, 94.3 g of sodium bisulfite and 0.7 g of 1-amino-2-naphthol-4-sulfonic acid. Before use, dissolve 1.5 g of this mixture in water to make 10 mL.

**m-Aminophenol** See 3-aminophenol.

**2-Aminophenol**  $C_6H_7NO$  Pale yellow-brown crystals. Soluble in ethanol (99.5), and sparingly soluble in water.

**Melting point**  $\langle 2.60 \rangle$ : About 172°C

**3-Aminophenol**  $H_2NC_6H_4OH$  White, crystals or crystalline powder.

**Melting point**  $\langle 2.60 \rangle$ : 121 – 125°C

**Content:** not less than 97.0%. **Assay**—Weigh accurately about 0.2 g, dissolve in 50 mL of acetic acid for nonaqueous titration, and titrate  $\langle 2.50 \rangle$  with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 10.91 mg of  $H_2NC_6H_4OH$

**4-Aminophenol**  $C_6H_7NO$  A white to yellowish white crystalline powder. Soluble in ethanol (99.5), and sparingly soluble in water.

**Melting point**  $\langle 2.60 \rangle$ : About 186°C

**p-Aminophenol hydrochloride** See 4-aminophenol hydrochloride.

**4-Aminophenol hydrochloride**  $HOC_6H_4NH_2 \cdot HCl$

White to pale colored crystals. Freely soluble in water and in ethanol (95). Melting point: about 306°C (with decomposition).

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 0.17 g of 4-aminophenol hydrochloride, dissolve in 50 mL of acetic acid for nonaqueous titration and 5 mL of mercury (II) acetate TS for nonaqueous titration, and titrate  $\langle 2.50 \rangle$  with 0.1 mol/L perchloric acid-1,4-dioxane VS (indicator: 1 mL of *p*-naphtholbenzene TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS  
= 14.56 mg of  $C_6H_8NOCl$

**Storage**—Preserve in tight, light-resistant containers.

**Aminopropylsilanized silica gel for pretreatment** Prepared for pretreatment.

**Aminopyrine**  $C_{13}H_{17}N_3O$  White to pale yellow, crystals or crystalline powder.

**Melting point**  $\langle 2.60 \rangle$ : 107 – 109°C

**6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate**

$C_{14}H_{11}N_3O_4$  Prepared for biochemistry or amino acid analysis.

**L-2-Aminosuberic acid**  $C_8H_{15}NO_4$  White, crystals or crystalline powder. Odorless.

**Optical rotation**  $\langle 2.49 \rangle [\alpha]_D^{20}$ : +19.1 – +20.1° (after drying, 0.1 g, 5 mol/L hydrochloric acid TS, 100 mm).

**Loss on drying**  $\langle 2.41 \rangle$ : not more than 0.3% (1 g, 105°C, 2 hours).

**Assay**—Weigh accurately about 0.3 g of L-2-aminosuberic acid, previously dried, add exactly 6 mL of formic acid to dissolve, then add exactly 50 mL of acetic acid (100), and titrate  $\langle 2.50 \rangle$  with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 18.92 mg of  $C_8H_{15}NO_4$

**Amiodarone hydrochloride for assay**  $C_{25}H_{29}I_2NO_3 \cdot HCl$  [Same as the monograph Amiodarone Hydrochloride. When dried, it contains not less than 99.5% of amiodarone hydrochloride ( $C_{25}H_{29}I_2NO_3 \cdot HCl$ ).]

**Ammonia-ammonium acetate buffer solution (pH 8.0)**

To ammonium acetate TS add ammonia TS dropwise to adjust the pH to 8.0.

**Ammonia-ammonium acetate buffer solution (pH 8.5)**

Dissolve 50 g of ammonium acetate in 800 mL of water and 200 mL of ethanol (95), and add ammonia solution (28) to

adjust the pH to 8.5.

**Ammonia-ammonium chloride buffer solution (pH 8.0)**

Dissolve 1.07 g of ammonium chloride in water to make 100 mL, and adjust the pH to 8.0 by adding diluted ammonia TS (1 in 30).

**Ammonia-ammonium chloride buffer solution (pH 10.0)**

Dissolve 70 g of ammonium chloride in water, add 100 mL of ammonia solution (28), dilute with water to make 1000 mL, and add ammonia solution (28) dropwise to adjust the pH to 10.0.

**Ammonia-ammonium chloride buffer solution (pH 10.7)**

Dissolve 67.5 g of ammonium chloride in water, add 570 mL of ammonia solution (28), and dilute with water to make 1000 mL.

**Ammonia-ammonium chloride buffer solution (pH 11.0)**

Dissolve 53.5 g of ammonium chloride in water, add 480 mL of ammonia solution (28), and dilute with water to make 1000 mL.

**Ammonia copper TS** To 0.5 g of cupric carbonate monohydrate add 10 mL of water, triturate, and add 10 mL of ammonia solution (28).

**Ammonia-ethanol TS** To 20 mL of ammonia solution (28) add 100 mL of ethanol (99.5).

**Ammonia gas NH<sub>3</sub>** Prepare by heating ammonia solution (28).

**Ammonia-saturated 1-butanol TS** To 100 mL of 1-butanol add 60 mL of diluted ammonia solution (28) (1 in 100), shake vigorously for 10 minutes, and allow to stand. Use the upper layer.

**Ammonia solution (28)** NH<sub>3</sub> [K 8085, Ammonia Water, Special class, Density: about 0.90 g/mL, Content: 28 – 30%]

**Ammonia TS** To 400 mL of ammonia solution (28) add water to make 1000 mL (10%).

**1 mol/L Ammonia TS** To 65 mL of ammonia solution (28) add water to make 1000 mL.

**13.5 mol/L Ammonia TS** To exactly 9 mL of water add ammonia solution (28) to make exactly 50 mL.

**Ammonia water** See ammonia TS.

**1 mol/L Ammonia water** See 1 mol/L ammonia TS.

**13.5 mol/L Ammonia water** See 13.5 mol/L ammonia TS.

**Ammonia water, strong** See ammonia solution (28).

**Ammonium acetate** CH<sub>3</sub>COONH<sub>4</sub> [K 8359, Special class]

**Ammonium acetate TS** Dissolve 10 g of ammonium acetate in water to make 100 mL.

**0.5 mol/L Ammonium acetate TS** Dissolve 38.5 g of ammonium acetate in water to make 1000 mL.

**Ammonium amidosulfate** NH<sub>4</sub>OSO<sub>2</sub>NH<sub>2</sub> [K 8588, Special class]

**Ammonium amidosulfate TS** Dissolve 1 g of ammonium amidosulfate in water to make 40 mL.

**Ammonium amminetrichloroplatinate for liquid chromatography** Cl<sub>3</sub>H<sub>7</sub>N<sub>2</sub>Pt To 20 g of cisplatin add 600 mL of 6 mol/L hydrochloric acid TS, and heat under a reflux con-

denser for 4 – 6 hours to boil while stirring. After cooling, evaporate the solvent, and dry the orange residue at room temperature under reduced pressure. To the residue so obtained add 300 mL of methanol, and heat at about 50°C to dissolve. Filter, separate insoluble yellow solids, and wash the solids with 10 mL of methanol. Combine the filtrate and the washing, heat at about 50°C, and add slowly 100 mL of ethyl acetate while stirring. Cool the mixture to room temperature avoiding exposure to light, and allow to stand at – 10°C for 1 hour. Filter the mixture to take off the formed crystals, wash the crystals with 100 mL of acetone, combine the washing to the filtrate, and evaporate to dryness to obtain orange crystals. If necessary, repeat the purification procedure described above to take off the insoluble crystals. To the orange crystals obtained add 300 to 500 mL of a mixture of acetone and methanol (5:1), and heat at about 50°C while stirring to dissolve. Filter while hot to take off the insoluble crystals, wash the crystals with the mixture, and combine the filtrate and washing. Repeat the procedure several times, and evaporate to dryness. Suspense the crystals so obtained in 50 mL of acetone, filter, wash the crystals with 20 mL of acetone, and dry the crystals at room temperature under reduced pressure. It is a yellow-brown crystalline powder.

**Identification**—Determine the infrared absorption spectrum of the substance to be examined, previously dried at 80°C for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3480 cm<sup>-1</sup>, 3220 cm<sup>-1</sup>, 1622 cm<sup>-1</sup>, 1408 cm<sup>-1</sup> and 1321 cm<sup>-1</sup>.

**Purity** Related substances—Cisplatin Conduct this procedure using light-resistant vessels. Dissolve 10 mg in N,N-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of Cisplatin in N,N-dimethylformamide to make exactly 50 mL. Pipet 5 mL of this solution, add N,N-dimethylformamide to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area of cisplatin by the automatic integration method: the peak area obtained from the sample solution is not larger than that from the standard solution.

**Operating conditions**

Proceed as directed in the operating conditions in the Assay under Cisplatin.

**System suitability**

**System performance:** When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cisplatin are not less than 2500 and not more than 2.0, respectively.

**System repeatability:** When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cisplatin is not more than 5.0%.

**Ammonium aurintricarboxylate** See aluminon.

**Ammonium carbonate** [K 8613, Special class]

**Ammonium carbonate TS** Dissolve 20 g of ammonium carbonate in 20 mL of ammonia TS and water to make 100 mL.

**Ammonium chloride** NH<sub>4</sub>Cl [K 8116, Special class]

**Ammonium chloride-ammonia TS** To ammonia solution

(28) add an equal volume of water, and saturate this solution with ammonium chloride.

**Ammonium chloride buffer solution (pH 10)** Dissolve 5.4 g of ammonium chloride in water, and add 21 mL of ammonia solution (28) and water to make 100 mL.

**Ammonium chloride TS** Dissolve 10.5 g of ammonium chloride in water to make 100 mL (2 mol/L).

**Ammonium citrate** See diammonium hydrogen citrate.

**Ammonium dihydrogenphosphate**  $\text{NH}_4\text{H}_2\text{PO}_4$  [K 9006, Special class]

**0.02 mol/L Ammonium dihydrogenphosphate TS**

Dissolve 2.30 g of ammonium dihydrogen phosphate in water to make 1000 mL.

**Ammonium formate**  $\text{HCOONH}_4$  Colorless crystals. Very soluble in water.

*Melting point* <2.60>: 116 – 119°C

**0.05 mol/L Ammonium formate buffer solution (pH 4.0)**

Dissolve 3.15 g of ammonium formate in 750 mL of water, adjust to pH 4.0 with formic acid, and add water to make 1000 mL.

**Ammonium hydrogen carbonate**  $\text{NH}_4\text{HCO}_3$  White or semi-transparency, crystals, crystalline powder or masses, having an ammonia odor.

**0.1 mol/L Ammonium hydrogen carbonate TS** Dissolve 7.9 g of ammonium hydrogen carbonate in 500 mL of water. Adjust to pH 10.3 with 5 mol/L sodium hydroxide TS, and add water to make 1000 mL.

**Ammonium iron (II) sulfate hexahydrate**

$\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$  [K 8979, Special class]

**Ammonium iron (III) citrate** [Same as the monograph Ferric Ammonium Citrate in the Japanese Standards of Food Additives]

**Ammonium iron (III) sulfate dodecahydrate**

$\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  [K 8982, Special class]

**Ammonium iron (III) sulfate TS** Dissolve 8 g of ammonium iron (III) sulfate dodecahydrate in water to make 100 mL.

**Ammonium iron (III) sulfate TS, acidic** Dissolve 20 g of ammonium iron (III) sulfate dodecahydrate in a suitable amount of water, add 9.4 mL of sulfuric acid, and add water to make 100 mL.

**Ammonium iron (III) sulfate TS, dilute** To 2 mL of ammonium iron (III) sulfate TS add 1 mL of 1 mol/L hydrochloric acid TS and water to make 100 mL.

**Ammonium molybdate** See hexaammonium heptamolybdate tetrahydrate.

**Ammonium molybdate-sulfuric acid TS** See hexaammonium heptamolybdate-sulfuric acid TS

**Ammonium molybdate TS** See hexaammonium heptamolybdate TS.

**Ammonium nickel (II) sulfate** See ammonium nickel (II) sulfate hexahydrate.

**Ammonium nickel (II) sulfate hexahydrate**

$(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  Green, crystals or crystalline powder.

*Identification*—(1) Dissolve 1 g of ammonium nickel (II) sulfate hexahydrate in 20 mL of water, and use this solution

as the sample solution. To 5 mL of the sample solution add 1 mL of barium chloride TS: a white precipitate is produced.

(2) To 5 mL of the sample solution obtained in (1) add 5 mL of 8 mol/L sodium hydroxide TS: a green precipitate is formed, and the liquid evolves ammonia on heating.

(3) To 5 mL of the sample solution obtained in (1) add 1 mL each of ammonia TS and dimethylglyoxime TS: a red precipitate is formed.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 1 g of ammonium nickel (II) sulfate hexahydrate, add 100 mL of water and 5 mL of ammonium chloride TS, then add exactly 20 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, warm to 50 – 60°C, add 10 mL of diluted ammonia solution (28) (1 in 2), and titrate with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution is changed from green to blue-purple (indicator: 50 mg of murexide-sodium chloride indicator).

Each mL of disodium dihydrogen ethylenediamine tetraacetate VS

$$= 39.50 \text{ mg of } (\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$$

**Ammonium nitrate**  $\text{NH}_4\text{NO}_3$  [K 8545, Special class]

**Ammonium oxalate** See ammonium oxalate monohydrate.

**Ammonium oxalate monohydrate**  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  [K 8521, Special class]

**Ammonium oxalate TS** Dissolve 3.5 g of ammonium oxalate monohydrate in water to make 100 mL (0.25 mol/L).

**Ammonium peroxodisulfate**  $(\text{NH}_4)_2\text{S}_2\text{O}_8$  [K 8252, Special class]

**10% Ammonium peroxodisulfate TS** Dissolve 1 g of ammonium peroxodisulfate in water to make 10 mL.

**Ammonium persulfate** See ammonium peroxodisulfate.

**Ammonium polysulfide TS**  $(\text{NH}_4)_2\text{S}_n$  [K 8943, Ammonium Sulfide Solution (yellow),  $(\text{NH}_4)_2\text{S}_x$ , First class]

**Ammonium pyrrolidinedithiocarbamate**  $\text{C}_5\text{H}_{12}\text{N}_2\text{S}_2$  A white or light yellow, crystalline powder. Sparingly soluble in water, and very slightly soluble in ethanol (95).

**Storage**—Preserve in a light-resistant glass container, at 2 – 10°C.

**Ammonium sodium hydrogenphosphate tetrahydrate**  $\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$  [K 9013, Special class]

**Ammonium sulfamate** See ammonium amidosulfate.

**Ammonium sulfamate TS** See ammonium amidosulfate TS.

**Ammonium sulfate**  $(\text{NH}_4)_2\text{SO}_4$  [K 8960, Special class]

**Ammonium sulfate TS** Dissolve 39.6 g of ammonium sulfate in 70 mL of water, adjust to pH 8.0 with sodium hydroxide TS, and add water to make 100 mL (3 mol/L).

**Ammonium sulfate buffer solution** Dissolve 264 g of ammonium sulfate in 1000 mL of water, add 1000 mL of 0.5 mol/L sulfuric acid TS, shake, and filter. The pH of this solution is about 1.

**Ammonium sulfide TS**  $(\text{NH}_4)_2\text{S}$  [K 8943, Ammonium Sulfide Solution, (colorless), First class] Store in small, well-filled containers, protected from light.

**Ammonium tartrate** See L-ammonium tartrate.

**L-Ammonium tartrate**  $C_4H_{12}N_2O_6$  [K 8534, (+) Ammonium tartrate, Special class]

**Ammonium thiocyanate**  $NH_4SCN$  [K 9000, Special class]

**Ammonium thiocyanate-cobalt (II) nitrate TS** Dissolve 17.4 g of ammonium thiocyanate and 2.8 g of cobalt (II) nitrate hexahydrate in water to make 100 mL.

**Ammonium thiocyanate TS** Dissolve 8 g of ammonium thiocyanate in water to make 100 mL (1 mol/L).

**Ammonium trifluoromethanesulfonate** White, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum of ammonium trifluoromethanesulfonate as directed in the ATR method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3190  $\text{cm}^{-1}$ , 3090  $\text{cm}^{-1}$ , 1227  $\text{cm}^{-1}$ , 1164  $\text{cm}^{-1}$ , and 1032  $\text{cm}^{-1}$ .

**Ammonium vanadate** See ammonium vanadate (V).

**Ammonium vanadate (V)**  $NH_4VO_3$  [K 8747, Special class]

**Amosulalol hydrochloride for assay**  $C_{18}H_{24}N_2O_5S \cdot HCl$  [Same as the monograph Amosulalol Hydrochloride. It contains not less than 99.0% of amosulalol hydrochloride ( $C_{18}H_{24}N_2O_5S \cdot HCl$ ), calculated on the anhydrous basis.]

**Amoxicillin** See amoxicillin hydrate.

**Amoxicillin hydrate**  $C_{16}H_{19}N_3O_5S \cdot 3H_2O$  [Same as the namesake monograph]

**Amphoteric electrolyte solution for pH 3 to 10** Extremely pale yellow liquid. Mixture consisting of multiple types of molecules, buffer capacity is 0.35 mmol/pH-mL. Forms a pH gradient over a pH range of 3 to 10 when mixed with polyacrylamide gel and placed in an electric field.

**Amphoteric electrolyte solution for pH 6 to 9** Forms a pH gradient over a pH range of 6 to 9 when mixed with polyacrylamide gel and placed in an electric field. Prepare by diluting a 0.35 mmol/pH-mL buffer capacity solution about 20-fold with water. Almost colorless.

**Amphoteric electrolyte solution for pH 8 to 10.5** Extremely pale yellow liquid. Mixture consisting of multiple types of molecules, buffer capacity is 0.35 mmol/pH-mL. Forms a pH gradient over a pH range of 8 to 10.5 when mixed with polyacrylamide gel and placed in an electric field.

**Ampiroxicam for assay**  $C_{20}H_{21}N_3O_7S$  [Same as the monograph Ampiroxicam]

**Amygdalin for assay**  $C_{20}H_{27}NO_{11}$  Amygdalin for thin-layer chromatography. However, it meets the following requirements:

*Absorbance* <2.24>  $E_{1\text{ cm}}^{1\%}$  (263 nm): 5.2 – 5.8 [20 mg, methanol, 20 mL; separately determine the water <2.48> (5 mg, coulometric titration) and calculate on the anhydrous basis].

**Purity** Related substances—Dissolve 5 mg of amygdalin for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than amygdalin obtained from the sample solution is not larger than the peak area of amygdalin

from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Keishibukuryogan Extract.

Time span of measurement: About 3 times as long as the retention time of amygdalin.

**System suitability**

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (3) under Keishibukuryogan Extract.

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of amygdalin obtained with 10  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu\text{L}$  of the standard solution.

**Amygdalin for component determination** See amygdalin for assay.

**Amygdalin for thin-layer chromatography**  $C_{20}H_{27}NO_{11}$

A white, odorless powder. Soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

**Identification**—Determine the absorption spectrum of a solution of amygdalin for thin-layer chromatography in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, between 261 nm and 265 nm, and between 267 nm and 271 nm.

**Purity** Related substances—Dissolve 5 mg of amygdalin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification under Peach Kernel: any spot other than the principal spot at the *Rf* value of about 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

**n-Amyl alcohol**  $CH_3(CH_2)_4OH$  Clear, colorless liquid, having a characteristic odor. Sparingly soluble in water, and miscible with ethanol (95) and with diethyl ether.

*Refractive index* <2.45>  $n_D^{20}$ : 1.409 – 1.411

*Specific gravity* <2.56>  $d_4^{20}$ : 0.810 – 0.820

*Distilling range* <2.57>: 135 – 140°C, not less than 95 vol%.

**t-Amyl alcohol**  $(CH_3)_2C(OH)CH_2CH_3$  Clear, colorless liquid, having a characteristic odor. Miscible with *t*-butyl alcohol and with 2-butanone, and freely soluble in water.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.808 – 0.815

**Purity** Acid and ester—To 20 mL of *t*-amyl alcohol add 20 mL of ethanol (95) and 5.0 mL of 0.1 mol/L sodium hydroxide VS, and heat gently under a reflux condenser in a water bath for 10 minutes. Cool, add 2 drops of phenolphthalein TS, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS. Perform a blank determination in the same manner: not more than 1.25 mL of 0.1 mol/L sodium hydroxide VS is consumed.

**Nonvolatile residue**—Evaporate 50 mL of *t*-amyl alcohol, and dry at 105°C for 1 hour: the residue is not more than 1.6 mg.

*Distilling range* <2.57>: 100 – 103°C, not less than 95 vol%.

**tert-Amyl alcohol** See *t*-amyl alcohol.

**Amyl alcohol, iso** See 3-methyl-1-butanol.

**Anemarrhena rhizome** [Same as the namesake monograph]

**Anesthetic ether** See ether, anesthetic.

**Anhydrous caffeine** See caffeine, anhydrous.

**Anhydrous cupric sulfate** See copper (II) sulfate.

**Anhydrous dibasic sodium phosphate** See disodium hydrogen phosphate, anhydrous.

**Anhydrous dibasic sodium phosphate for pH determination** See disodium hydrogen phosphate for pH determination.

**Anhydrous hydrazine for amino acid analysis** Prepared for amino acid analysis.

**Anhydrous lactose**  $C_{12}H_{22}O_{11}$  [Same as the monograph Anhydrous Lactose]

**Anhydrous potassium carbonate** See potassium carbonate.

**Anhydrous sodium acetate** See sodium acetate, anhydrous.

**Anhydrous sodium carbonate** See sodium carbonate, anhydrous.

**Anhydrous sodium sulfate** See sodium sulfate, anhydrous.

**Anhydrous sodium sulfite** See sodium sulfite, anhydrous.

**Aniline**  $C_6H_5NH_2$  [K 8042, Special class]

**Aniline sulfate**  $(C_6H_5NH_2)_2 \cdot H_2SO_4$  White to gray-white crystalline powder.

**Purity** Clarity and color of solution—Dissolve 1.0 g of aniline sulfate in 50 mL of water: the solution is clear and colorless.

**Animal tissue peptone** See peptone, animal tissue.

**p-Anisaldehyde** See 4-methoxybenzaldehyde.

**p-Anisaldehyde-acetic acid TS** See 4-methoxybenzaldehyde-acetic acid TS.

**p-Anisaldehyde-sulfuric acid TS** See 4-methoxybenzaldehyde-sulfuric acid TS.

**Anisole**  $C_7H_8O$  A colorless liquid. Boiling point: about 155°C.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.995 – 1.001

#### 14-Anisoylaconine hydrochloride for assay

$C_{33}H_{47}NO_{11} \cdot HCl$  White, crystalline powder or powder. Freely soluble in methanol, sparingly soluble in water and in ethanol (99.5). Melting point: about 210°C (with decomposition).

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (258 nm): 276 – 294 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

**Purity** (1) Related substances—To 1.0 mg of 14-anisoylaconine hydrochloride for assay add exactly 1 mL of ethanol (99.5). Perform the test with 5  $\mu$ L of this solution as directed in the Identification under Processed Aconite Root: any spot other than the principle spot with an  $R_f$  value of about 0.5 does not appear.

(2) Related substances—Dissolve 5.0 mg of 14-anisoylaconine hydrochloride for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution.

Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than 14-anisoylaconine obtained from the sample solution is not larger than the peak area of 14-anisoylaconine from the standard solution.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 4 times as long as the retention time of 14-anisoylaconine.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of 14-anisoylaconine obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

System performance: When the procedure is run with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

#### 14-Anisoylaconine hydrochloride for component determination

See 14-anisoylaconine hydrochloride for assay.

**Anode solution A for water determination** Dissolve 100 g of diethanolamine in 900 mL of a mixture of methanol for water determination and chloroform for water determination (1:1), pass dried sulfur dioxide gas through this solution while cooling until the mass increase of the solution reaches 64 g. Then add 20 g of iodine, and add water until the color of the solution changes from brown to yellow. To 600 mL of this solution add 400 mL of chloroform for water determination.

**Anthrone**  $C_{14}H_{10}O$  Light yellow, crystals or crystalline powder.

**Melting point** <2.60>: 154 – 160°C

**Storage**—Preserve in a light-resistant tight container.

**Anthrone TS** Dissolve 35 mg of anthrone in 100 mL of sulfuric acid.

**Anti-A type antibody for blood typing** Conforms to the requirements of antibody for blood typing.

**Anti-B type antibody for blood typing** Conforms to the requirements of antibody for blood typing.

**Anti-bradykinin antibody** A colorless to light brown, clear solution prepared by dissolving rabbit origin anti-bradykinin antibody in 0.04 mol/L phosphate buffer solution (pH 7.0) containing 1 mg/mL of bovine serum albumin.

**Performance test**—To a suitable amount of anti-bradykinin antibody to be tested add 0.04 mol/L phosphate buffer solution (pH 7.0) containing 1 mg/mL bovine serum albumin to make a 1 vol% solution. Perform the test with 0.1

mL of this solution as directed in the Purity (2) under Kal-lidinogenase, and determine the absorbances at 490–492 nm,  $A_1$  and  $A_2$ , of the standard solution (1) and the standard solution (7); the value,  $A_2 - A_1$ , is not less than 1.

**Anti-bradykinin antibody TS** To 0.15 mL of anti-bradykinin antibody, 15 mg of bovine serum albumin, 2.97 mg of sodium dihydrogen phosphate dihydrate, 13.5 mg of disodium hydrogen phosphate dodecahydrate and 13.5 mg of sodium chloride add water to make 15 mL, and lyophilize. Dissolve this in 15 mL of water. Prepare before use.

**Anti-interferon alfa antiserum** Antiserum prepared by immunizing rabbits with interferon alfa, which is capable of reacting specifically with interferon alfa to neutralize 10,000 Units or more of interferon alfa in 1 mL.

**Antimony (III) chloride**  $\text{SbCl}_3$  [K 8400, Special class]

**Antimony (III) chloride TS** Wash chloroform with an equal volume of water twice or three times, add freshly ignited and cooled potassium carbonate, and allow to stand overnight in a well-closed container protected from light. Separate the chloroform layer, and distil it, preferably with protection from light. With this chloroform, wash the surface of antimony (III) chloride until the rinsing solution becomes clear, add the chloroform to this antimony (III) chloride to make a saturated solution, and place in light-resistant, glass-stoppered bottles. Prepare before use.

**Antimony trichloride** See antimony (III) chloride.

**Antimony trichloride TS** See antimony (III) chloride TS.

**Antipyrine**  $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}$  [Same as the namesake monograph]

**Anti-thrombin III** A white powder.

**Water <2.48>**: not more than 5%.

**Content:** not less than 80% and not more than 130% of the labeled amount.

**Anti-thrombin III TS** Dissolve 10 unit of anti-thrombin III in 10 mL of water.

**Anti-ulinastatin rabbit serum** Antiserum obtained by immunizing rabbits with Ulinastatin, with an antibody titer of 16 times or more. Preserve at below  $-20^{\circ}\text{C}$ .

**Anti-urokinase serum** Take Urokinase containing not less than 140,000 Unit per mg of protein, dissolve in isotonic sodium chloride solution to make a solution containing 1 mg of protein per mL, and emulsify with an equal volume of Freund's complete adjuvant. Inject intracutaneously three 2-mL portions of the emulsion to a healthy rabbit weighed between 2.5 kg and 3.0 kg in a week interval. Collect the blood from the rabbit at 7 to 10 days after the last injection, and prepare the anti-serum.

**Performance test**—Dissolve 1.0 g of agar in 100 mL of boric acid-sodium hydroxide buffer solution (pH 8.4) by warming, and pour the solution into a Petri dish to make a depth of about 2 mm. After cooling, bore three of a pair-well 2.5 mm in diameter with a space of 6 mm each other. In one of the wells of each pair-well, place 10  $\mu\text{L}$  of anti-urokinase serum, and in each another well, place 10  $\mu\text{L}$  of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution, 10  $\mu\text{L}$  of human serum and 10  $\mu\text{L}$  of human urine, respectively, and allow to stand overnight: a precipitin line appears between anti-urokinase serum and urokinase, and not appears between anti-urokinase serum and human serum or human urine.

**$\alpha$ -Apooxytetracycline**  $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_8$  Yellow-brown to

green powder.

**Melting point <2.60>**: 200–205°C

**$\beta$ -Apooxytetracycline**  $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_8$  Yellow-brown to brown powder.

**Purity** Related substances—Dissolve 8 mg of  $\beta$ -apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as the sample solution. Proceed the test with 20  $\mu\text{L}$  of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than  $\beta$ -apooxytetracycline is not more than 10%.

**Aprindine hydrochloride for assay**  $\text{C}_{22}\text{H}_{30}\text{N}_2\text{HCl}$

[Same as the monograph Aprindine Hydrochloride. When dried, it contains not less than 99.5% of aprindine hydrochloride ( $\text{C}_{22}\text{H}_{30}\text{N}_2\text{HCl}$ ).]

**Aprotinin** A clear and colorless liquid containing aprotinin extracted from the lung or parotid gland of a healthy cattle. The pH is between 5.0 and 7.0.

**Content:** not less than 15,000 KIE Units and not more than 25,000 KIE Units of aprotinin per mL. Assay—(i) Trypsin solution: Weigh an amount of crystalline trypsin equivalent to about 250 FIP Units of trypsin according to the labeled FIP Units, and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 10 mL. Prepare before use, and preserve in ice.

(ii) Sample solution: To a suitable quantity of aprotinin add sodium tetraborate-calcium chloride buffer solution (pH 8.0) so that each mL contains 800 KIE Units of aprotinin, and use this solution as the sample solution.

(iii) Apparatus: Use a glass bottle as a reaction reservoir, 20 mm in inside diameter and 50 mm in height, stopper with a rubber stopper equipped with a glass/silver-silver chloride electrode for pH determination, a nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature of the bath at  $25 \pm 0.1^{\circ}\text{C}$  by means of a precise thermoregulator.

(iv) Procedure: To 5.0 mL of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester TS add 45.0 mL of sodium tetraborate-calcium chloride buffer solution (pH 8.0), and use this solution as the substrate solution. Pipet 1 mL of the trypsin solution, add sodium tetraborate-calcium chloride buffer solution (pH 8.0) to make exactly 10 mL, and use this solution as the test solution I. Transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00 by adding dropwise 0.1 mol/L sodium hydroxide VS while stirring and passing a current of nitrogen, add exactly 1 mL of the test solution I previously allowed to stand at  $25 \pm 0.1^{\circ}\text{C}$  for 10 minutes, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50- $\mu\text{L}$  micropipet (minimum graduation of 1  $\mu\text{L}$ ), while stirring, to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 6 minutes. Separately, pipet 2 mL of the trypsin solution and 1 mL of the sample solution, add sodium tetraborate-calcium chloride buffer solution (pH 8.0) to make exactly 10 mL, and use this solution as the test solution II. Transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00, while stirring and passing a current of nitrogen, add exactly 1 mL of the test solution II, previously allowed to stand at  $25 \pm 0.1^{\circ}\text{C}$  for 10 minutes, and proceed in the same manner. Separately, transfer 10.0 mL of the sub-

strate solution to the reaction reservoir, adjust the pH of the solution to 8.00, while stirring and passing a current of nitrogen, add 1.0 mL of sodium tetraborate-calcium chloride buffer solution (pH 8.0), previously allowed to stand at 25 ± 0.1°C for 10 minutes, and perform a blank determination in the same manner.

(v) Calculation: Plot the amount of consumption ( $\mu\text{L}$ ) of 0.1 mol/L sodium hydroxide VS against the reaction time (minutes), select linear reaction times,  $t_1$  and  $t_2$ , designate the corresponding consumption amount of 0.1 mol/L sodium hydroxide VS as  $v_1$  and  $v_2$ , respectively, and designate  $\mu\text{mol}$  of sodium hydroxide consumed per minute as  $D$ .

$$D \text{ } (\mu\text{mol NaOH/minute}) = \frac{v_2 - v_1}{t_2 - t_1} \times \frac{1}{10}$$

$$\text{KIE Units per mL of aprotinin} \\ = \frac{2(D_A - D_0) - (D_B - D_0)}{L} \times n \times 32.5$$

*L*: Amount (mL) of the sample solution added to the test solution II

*n*: Dilution coefficient of aprotinin

$D_A$ :  $\mu\text{mol}$  of sodium hydroxide consumed in 1 minute when the test solution I is used

$D_B$ :  $\mu\text{mol}$  of sodium hydroxide consumed in 1 minute when the test solution II is used

$D_0$ :  $\mu\text{mol}$  of sodium hydroxide consumed in 1 minute when the solution for blank determination is used

32.5: Equivalent coefficient for calculation of KIE Units from FIP Units

One KIE Unit means an amount of aprotinin making a reduction of 50% off the potency of 2 Units of kallidinogenase at pH 8.0 and room temperature for 2 hours.

**Storage**—Preserve in a light-resistant, hermetic containers and in a cold place.

**Aprotinin TS** Measure an appropriate amount of aprotinin, and dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to prepare a solution containing 50 KIE Units per mL.

**Aqua regia** Add 1 volume of nitric acid to 3 volumes of hydrochloric acid. Prepare before use.

**L-Arabinose**  $C_5H_{10}O_5$  A white crystalline powder.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +103.0 – +105.5° Weigh accurately about 5 g of L-arabinose, previously dried at 105°C for 2 hours, dissolve in 30 mL of water, add 0.4 mL of ammonia TS, and add water to make exactly 50 mL. Allow to stand for 1 hour, and determine using a 100-mm cell.

*Melting point* <2.60>: 155 – 160°C

**Arbutin for assay**  $C_{12}H_{16}O_7$  Use arbutin for thin-layer chromatography meeting the following additional specifications.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (280 nm): 70 – 76 [4 mg, previously dried in a desiccator (in vacuum, silica gel) for 12 hours, water, 100 mL].

**Purity** Related substances—Dissolve 40 mg of arbutin for assay in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of the both solutions by the automatic integration method: the total area of the peaks other than arbutin from the sample

solution is not larger than the peak area of arbutin from the standard solution (1).

#### Operating conditions

Proceed as directed in the operating conditions in the Assay under Bearberry Leaf except time span of measurement and test for required detectability.

**Time span of measurement:** About 3 times as long as the retention time of arbutin, beginning after the solvent peak.  
**System suitability**

**Test for required detectability:** Pipet 1 mL of the standard solution (1), add water to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of arbutin obtained with 10  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method and the peak height of arbutin with 10  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

**Arbutin for component determination** See arbutin for assay.

**Arbutin for thin-layer chromatography**  $C_{12}H_{16}O_7$

Colorless to white, crystals or crystalline powder, and odorless. Freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in ethyl acetate and in chloroform.

*Melting point* <2.60>: 199 – 201°C

**Purity** Related substances—Dissolve 1.0 mg of arbutin for thin-layer chromatography in exactly 1 mL of a mixture of ethanol (95) and water (7:3). Perform the test with 20  $\mu\text{L}$  of this solution as directed in the Identification (2) under Bearberry Leaf: any spot other than the principal spot with an *Rf* value of about 0.4 does not appear.

**Arecoline hydrobromide for thin-layer chromatography**

$C_8H_{13}NO_2 \cdot HBr$  White crystals. Freely soluble in water, soluble in methanol, and practically insoluble in diethyl ether.

*Melting point* <2.60>: 169 – 171°C

**Purity** Related substances—Dissolve 5 mg of arecoline hydrobromide for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 10  $\mu\text{L}$  of this solution as directed in the Identification under Areca: any spot other than the principal spot at the *Rf* value of about 0.6 does not appear.

**L-Arginine**  $C_6H_{14}N_4O_2$  White, crystals or crystalline powder. It has a characteristic odor.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +26.9 – +27.9° (After drying, 4 g, 6 mol/L hydrochloric acid TS, 50 mL, 200 mm).

*Loss on drying* <2.41>: not more than 0.50% (1 g, 105°C, 3 hours).

**Content:** not less than 98.0% and not more than 102.0%.

**Assay**—Weigh accurately about 0.15 g of L-arginine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes to green through yellow (indicator: 10 drops of *p*-naphtholbenzein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 8.710 mg of  $C_6H_{14}N_4O_2$

**L-Arginine hydrochloride**  $C_6H_{14}N_4O_2 \cdot HCl$  [Same as the namesake monograph]

**Argon** Ar [K 1105, First class]

**Aristolochic acid I for crude drugs purity test**

$C_{17}H_{11}NO_7$  Yellow crystalline powder. Melting point: about 280°C (with decomposition).

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (318 nm): 384 – 424 (1 mg, methanol, 100 mL).

**Purity** Related substances—Dissolve 1.0 mg of aristolochic acid I for crude drugs purity test in 100 mL of diluted methanol (3 in 4), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (3 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than aristolochic acid I obtained from the sample solution is not larger than the peak area of aristolochic acid I from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (5) under *Asiasarum Root*.

Time span of measurement: About 3 times as long as the retention time of aristolochic acid I, beginning after the solvent peak.

**System suitability**

Proceed as directed in the system suitability in the Purity (5) under *Asiasarum Root*.

**Arsenazo III**  $C_{22}H_{18}As_2N_4O_{14}S_2$  [K 9524, Special class]

**Arsenazo III TS** Dissolve 0.1 g of arsenazo III in water to make 50 mL.

**Arsenic-free zinc** See zinc for arsenic analysis.**Arsenic trioxide**  $As_2O_3$  [K 8044, Diarsenic trioxide, Special class]

**Arsenic trioxide TS** Add 1 g of arsenic trioxide to 30 mL of a solution of sodium hydroxide (1 in 40), dissolve by heating, cool, and add gently acetic acid (100) to make 100 mL.

**Arsenic (III) trioxide** See arsenic trioxide.**Arsenic (III) trioxide TS** See arsenic trioxide TS.**Artemisia argyi for purity test** Powder of the leaf and twig of *Artemisia argyi* H. Léveillé et Vaniot (*Compositae*).

**Identification**—To 0.5 g of artemisia argyi for purity test add 5 mL of a mixture of methanol and water (3:2), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu\text{L}$  of the sample solution on a plate of octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (4:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): two green fluorescent spots appear at  $R_f$  values of about 0.3 and about 0.4 (eupatilin and jaceosidin).

**Asarinin for thin-layer chromatography**  $C_{20}H_{18}O_6$ 

White, crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 118 – 122°C.

**Identification**—Determine the absorption spectrum of a solution in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 234 nm and 238 nm, and between 285 nm and 289 nm.

**Purity** Related substances—Dissolve 1 mg of asarinin for thin-layer chromatography in 1 mL of methanol, and perform the test with 1  $\mu\text{L}$  of this solution as directed in the Identification (7) under *Shoseiryuto Extract*: no spot other than the principal spot at an  $R_f$  value of about 0.4 appears.

**(E)-Asarone**  $C_{12}H_{16}O_3$  White powder. Freely soluble in methanol and in ethanol (99.5) and practically insoluble in water. Melting point: about 60°C.

**Identification**—Determine the infrared absorption spectrum of (E)-asarone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, it exhibits absorption at the wave numbers of about 2990  $\text{cm}^{-1}$ , 2940  $\text{cm}^{-1}$ , 2830  $\text{cm}^{-1}$ , 1609  $\text{cm}^{-1}$ , 1519  $\text{cm}^{-1}$ , 1469  $\text{cm}^{-1}$ , 1203  $\text{cm}^{-1}$ , 1030  $\text{cm}^{-1}$ , 970  $\text{cm}^{-1}$  and 860  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 2 mg of (E)-asarone in 10 mL of methanol, and use this solution as the sample solution. Perform the test with 10  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the area of each peak by the automatic integration method, and calculate the amount by the area percentage method: the total amount of the peaks other than (E)-asarone is not larger than 10%.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu\text{m}$  in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: 1.0 mL per minute.

Time span of measurement: About 3 times as long as the retention time of (E)-asarone, beginning after the solvent peak.

**System suitability**

System performance: Dissolve 1 mg of (E)-asarone and 1 mg of perillaldehyde for thin-layer chromatography in 50 mL of methanol. When the procedure is run with 10  $\mu\text{L}$  of this solution under the above operating conditions, perillaldehyde and (E)-asarone are eluted in this order with the resolutions between the peaks being not less than 1.5.

**Ascorbic acid** See L-ascorbic acid.

**L-Ascorbic acid**  $C_6H_8O_6$  [K 9502, L(+)-Ascorbic Acid, Special class]

**Ascorbic acid for iron limit test** See L-ascorbic acid.

**0.012 g/dL L-Ascorbic acid-hydrochloric acid TS** Dissolve 15 mg of L-ascorbic acid in 25 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

**0.02 g/dL L-Ascorbic acid-hydrochloric acid TS** Dissolve 25 mg of L-ascorbic acid in 25 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

**0.05 g/dL L-Ascorbic acid-hydrochloric acid TS** Dissolve 50 mg of L-ascorbic acid in 30 mL of methanol, add carefully hydrochloric acid to make 100 mL. Prepare before use.

**L-Asparagine monohydrate**  $C_4H_8N_2O_3 \cdot H_2O$  [K8021, Special class]

**DL-Aspartic acid**  $C_4H_7NO_4$  A white crystalline powder that is sparingly soluble in water. Melting point: 270 to

271°C.

**L-Aspartic acid** C<sub>4</sub>H<sub>7</sub>NO<sub>4</sub> [K 9045, Special class]

**Aspartic acid** See L-aspartic acid.

**Aspirin** C<sub>9</sub>H<sub>8</sub>O<sub>4</sub> [Same as the namesake monograph]

#### Astragaloside IV for thin-layer chromatography

C<sub>41</sub>H<sub>68</sub>O<sub>14</sub> A white powder. Sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water.

*Optical rotation* <2.49> [α]<sub>D</sub><sup>20</sup>: +19° – +26° (10 mg dried with silica gel for 24 hours, methanol, 2 mL, 50 mm).

*Purity* Related substances—Dissolve 1 mg of astragaloside IV for thin-layer chromatography in 1 mL of methanol. Proceed the test with 5 μL of this solution as directed in the Identification (4) under Hochuekkito Extract: no spot appears other than the principal spot of around *Rf* value of 0.4.

**Atractylenolide III for assay** C<sub>15</sub>H<sub>20</sub>O<sub>3</sub> Use atractylenolide III for thin-layer chromatography. It meets the following additional specifications.

*Absorbance* <2.24> E<sub>1</sub><sup>cm</sup> (219 nm): 446 – 481 (5 mg, methanol, 500 mL).

*Purity* Related substances—Dissolve 5 mg of atractylenolide III for assay in 50 mL of methanol, and use this solution as the sample solution. To exactly 1 mL of the sample solution add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than atractylenolide III obtained from the sample solution is not larger than the peak area of atractylenolide III from the standard solution.

#### Operating conditions

Column, column temperature and mobile phase: Proceed as directed in the operating conditions in the Assay (3) under Tokishakuyakusan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Flow rate: Adjust so that the retention time of atractylenolide III is about 11 minutes.

Time span of measurement: About 5 times as long as the retention time of atractylenolide III, beginning after the solvent peak.

#### System suitability

Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 20 mL. Confirm that the peak area of atractylenolide III obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atractylenolide III are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atractylenolide III is not more than 1.5%.

#### Atractylenolide III for thin-layer chromatography

C<sub>15</sub>H<sub>20</sub>O<sub>3</sub> White, crystals or crystalline powder. Freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 193 – 196°C.

*Identification*—(1) Determine the absorption spectrum of

a solution of atractylenolide III for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 217 nm and 221 nm.

(2) Determine the infrared absorption spectrum of atractylenolide III for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3350 cm<sup>-1</sup>, 1742 cm<sup>-1</sup>, 1641 cm<sup>-1</sup> and 1384 cm<sup>-1</sup>.

*Purity* Related substances—Dissolve 2 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Proceed the test with 5 μL each of the sample solution and standard solution as directed in the Identification (3) under Tokishakuyakusan Extract: the spot other than the principal spot with an *Rf* value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

**Atractylodin for assay** C<sub>13</sub>H<sub>10</sub>O White to pale yellow-crystals. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 54°C.

*Identification*—Conduct this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectrum of a solution of atractylodin for assay in methanol (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 256 nm and 260 nm, between 270 nm and 274 nm, between 332 nm and 336 nm and between 352 nm and 356 nm.

*Absorbance* <2.24> E<sub>1</sub><sup>cm</sup> (272 nm): 763 – 819 (2 mg, methanol, 250 mL). Conduct this procedure without exposure to light, using light-resistant vessels.

#### Purity Related substances

(i) Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 2 mg of atractylodin for assay in 2 mL of methanol, and use this solution as the sample solution. To exactly 1 mL of the sample solution add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography and immediately develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for splaying on the plate, and heat the plate at 105°C for 5 minutes: the spot other than the principle spot which appears at an *Rf* value of about 0.4 obtained from the sample solution is not more intense than the spot from the standard solution.

(ii) Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 5 mg of atractylodin for assay in 250 mL of methanol, and use this solution as the sample solution. To exactly 1 mL of the sample solution add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than atractylodin obtained from the sample solution is not larger than the peak area of atractylodin from the standard solution.

**Operating conditions**

Detector, column, column temperature and mobile phase: Proceed as directed in the operating conditions in the Assay (4) under Tokishakuyakusan Extract.

Flow rate: Adjust so that the retention time of atractylodin is about 13 minutes.

Time span of measurement: About 5 times as long as the retention time of atractylodin, beginning after the solvent peak.

**System suitability**

Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 20 mL. Confirm that the peak area of atractylodin obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

System performance: Put a suitable amount of the standard solution in a colorless vessel, and expose to ultraviolet light (main wavelength: 365 nm) for about 1 minute. When the procedure is run with 20  $\mu$ L of this solution under the above operating conditions, a peak of an isomer is found in addition to the peak of atractylodin, and the isomer and atractylodin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atractylodin is not more than 1.5%.

**Atractylodin TS for assay** Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 5 mg of atractylodin for assay, and dissolve in methanol to make exactly 1000 mL.

**Atropine sulfate** See atropine sulfate hydrate.

**Atropine sulfate for assay** See atropine sulfate hydrate for assay.

**Atropine sulfate for thin-layer chromatography** See atropine sulfate hydrate for thin-layer chromatography.

**Atropine sulfate hydrate** ( $C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$   
[Same as the namesake monograph]

**Atropine sulfate hydrate for assay** ( $C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$   
[Same as the monograph Atropine Sulfate Hydrate. When dried, it contains not less than 99.0% of atropine sulfate [ $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$ ].]

**Atropine sulfate hydrate for thin-layer chromatography** ( $C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$  Use atropine sulfate hydrate for assay meeting the following additional specification. To about 50 mg of the substance to be examined, dissolve in ethanol (95) to make 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 50  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of chloroform and diethylamine (9:1) to a distance of about 10 cm, air-dry the plate, and spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: any spot other than the principle spot at the *R<sub>f</sub>* value of about 0.4 does not appear.

**A-type erythrocyte suspension** Prepare a suspension containing 1 vol% of erythrocyte separated from human A-type blood in isotonic sodium chloride solution.

**Avidin-biotin TS** To 15 mL of phosphate-buffered sodium chloride TS add 2 drops each of avidin TS and biotinylated peroxidase TS, and mix.

**Azelastine hydrochloride for assay**  $C_{22}H_{24}ClN_3O \cdot HCl$

[Same as the monograph Azelastine Hydrochloride]

**Azelnidipine for assay**  $C_{33}H_{34}N_4O_6$  [Same as the monograph Azelnidipine. When dried, it contains not less than 99.5% of azelnidipine ( $C_{33}H_{34}N_4O_6$ ).]

**2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt**  $C_{18}H_{16}N_4O_6S_4 \cdot (NH_4)_2$  A bluish green crystalline powder.

*Melting point* <2.60>: about 330°C (with decomposition).

**2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt TS** Dissolve 5.3 g of citric acid monohydrate in water to make 500 mL. To this solution add a solution prepared by dissolving 7.1 g of anhydrous disodium hydrogen phosphate in water to make 500 mL to adjust to pH 4.3. To 20 mL of this solution add 15 mg of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt. To this solution add 14  $\mu$ L of hydrogen peroxide TS before use.

**Azosemide for assay**  $C_{12}H_{11}ClN_6O_2S_2$  [Same as the monograph Azosemide]

**Baicalein for resolution check**  $C_{15}H_{10}O_5$  Yellow, crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification**—Determine the absorption spectrum of a solution of baicalein for resolution check in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 213 nm and 217 nm, between 273 nm and 277 nm, and between 321 nm and 325 nm.

**Purity** Related substances—Dissolve 1 mg of baicalein for resolution check in 50 mL of methanol, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions: the total area of the peaks other than baicalein obtained from the sample solution is not larger than 1/10 times the total area of all peaks other than the solvent peak.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (4) i) under Saikokeishito Extract.

Time span of measurement: About 2 times as long as the retention time of baicalein.

**System Suitability**

System performance: When the procedure is run with 10  $\mu$ L of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalein are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the sample solution under the above operating conditions, the relative standard deviation of the peak area of baicalein is not more than 1.5%.

**Baicalin for thin-layer chromatography**  $C_{21}H_{18}O_{11}$

Light yellow, crystals or powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** Determine the infrared absorption spectrum of baicalin to be examined as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3390  $cm^{-1}$ , 1662  $cm^{-1}$ , 1492  $cm^{-1}$ , 1068  $cm^{-1}$  and 685  $cm^{-1}$ .

**Purity** Related substance—Dissolve 1 mg of baicalin to be examined in exactly 1 mL of methanol. Perform the test with 10  $\mu$ L of this solution as directed in the Identification

(2) under Scutellaria Root: any spot other than the principal spot with an *R<sub>f</sub>* value of about 0.4 does not appear.

**Baicalin hydrate for thin-layer chromatography** See baicalin for thin-layer chromatography.

**Balsam** Canada balsam for microscopy. Before use, dilute to a suitable concentration with xylene.

**Bamethan sulfate**  $(C_{12}H_{19}NO_2)_2 \cdot H_2SO_4$  [Same as the namesake monograph]

**Barbaloin for assay**  $C_{21}H_{22}O_9$  Use barbaloin for thin-layer chromatography meeting the following additional specifications.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (360 nm): 260 – 290 [10 mg dried in a desiccator (in vacuum, phosphorus (V) oxide) for not less than 24 hours, methanol, 500 mL].

**Purity** Related substances—Dissolve 10 mg of barbaloin for assay in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than barbaloin obtained from the sample solution is not larger than the peak area of barbaloin from the standard solution (1).

#### Operating conditions

Proceed the operating conditions in the Assay under Aloe except detector and time span of measurement.

**Detector:** An ultraviolet absorption photometer (wavelength: 300 nm).

**Time span of measurement:** About 3 times as long as the retention time of barbaloin, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of barbaloin obtained with 20  $\mu$ L of the standard solution (2) can be measured by the automatic integration method and the peak height of barbaloin with 20  $\mu$ L of the standard solution (1) is about 20 % of the full scale.

**Barbaloin for component determination** See barbaloin for assay.

**Barbaloin for thin-layer chromatography**  $C_{21}H_{22}O_9$  Light yellow crystalline powder. Freely soluble in methanol, practically insoluble in water.

**Melting point** <2.60>: 148°C

**Purity** Related substances—Dissolve 1.0 mg of barbaloin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 20  $\mu$ L of this solution as directed in the Identification (2) under Aloe: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.3 does not appear.

**Barbital**  $C_8H_{12}N_2O_3$  [Same as the namesake monograph]

**Barbital buffer solution** Dissolve 15 g of barbital sodium in 700 mL of water, adjust the pH to 7.6 with dilute hydrochloric acid, and filter.

**Barbital sodium**  $C_8H_{11}N_2NaO_3$  White, odorless, crystals or crystalline powder, having a bitter taste. Freely soluble in water, slightly soluble in ethanol (95), and practically

insoluble in diethyl ether.

**pH** <2.54>—The pH of a solution of 1.0 g of barbital sodium in 200 mL of water is between 9.9 and 10.3.

**Loss on drying** <2.41>: not more than 1.0% (1 g, 105°C, 4 hours).

**Content:** not less than 98.5%. **Assay**—Weigh accurately about 0.5 g of barbital sodium, previously dried, transfer to a separator, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform. Then extract with three 25-mL portions of chloroform, combine the total extract, wash with two 5-mL portions of water, and extract the washings with two 10-mL portions of chloroform. Combine the chloroform extracts, and filter into a conical flask. Wash the filter paper with three 5-mL portions of chloroform, combine the filtrate and the washings, add 10 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow to purple through light purple (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS  
= 20.62 mg of  $C_8H_{11}N_2NaO_3$

**Barium chloride** See barium chloride dihydrate.

**Barium chloride dihydrate**  $BaCl_2 \cdot 2H_2O$  [K 8155, Special class]

**Barium chloride TS** Dissolve 12 g of barium chloride dihydrate in water to make 100 mL (0.5 mol/L).

**Barium hydroxide** See barium hydroxide octahydrate.

**Barium hydroxide octahydrate**  $Ba(OH)_2 \cdot 8H_2O$  [K 8577, Special class] Store in tightly stoppered containers.

**Barium hydroxide TS** Saturate barium hydroxide octahydrate in freshly boiled and cooled water (0.25 mol/L). Prepare before use.

**Barium nitrate**  $Ba(NO_3)_2$  [K 8565, Special class]

**Barium nitrate TS** Dissolve 6.5 g of barium nitrate in water to make 100 mL (0.25 mol/L).

**Barium oxide**  $BaO$  A white to yellow-white or grayish white powder.

**Identification** (1) Dissolve 0.5 g of barium oxide in 15 mL of water and 5 mL of hydrochloric acid, and add 10 mL of dilute sulfuric acid: white precipitates appear.

(2) Perform the test with barium oxide as directed under Flame Coloration Test <1.04> (1): a green color appears.

**Barium perchlorate**  $Ba(ClO_4)_2$  [K 9551, Special class]

**Beclanamycin sulfate**  $C_{18}H_{37}N_5O_{10} \cdot xH_2SO_4$  [Same as the namesake monograph]

**Beclometasone dipropionate**  $C_{28}H_{37}ClO_7$  [Same as the namesake monograph]

**Benidipine hydrochloride**  $C_{28}H_{31}N_3O_6 \cdot HCl$  [Same as the namesake monograph]

**Benidipine hydrochloride for assay**  $C_{28}H_{31}N_3O_6 \cdot HCl$  [Same as the monograph Benidipine Hydrochloride. When dried, it contains not less than 99.5% of benidipine hydrochloride ( $C_{28}H_{31}N_3O_6 \cdot HCl$ )]

**Benzaldehyde**  $C_6H_5CHO$  [K 8857, First class]

**Benzalkonium chloride** [Same as the namesake mono-

graph]

**Benzalphthalide**  $C_{15}H_{10}O_2$  Yellow crystalline powder. Melting point: 99 – 102°C.

**Benz[a]anthracene**  $C_{18}H_{12}$  White to yellow, crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 158 – 163°C.

**Identification** Perform the test with benz[a]anthracene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak ( $m/z$  228) and a fragment ion peak ( $m/z$  114).

**Purity** Related substances—Dissolve 3.0 mg of benz[a]anthracene in methanol to make 100 mL, and use the sample solution as the sample solution. Perform the test with 1  $\mu$ L of this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than benz[a]anthracene is not more than 2.0%.

#### Operating conditions

Detector: A mass spectrophotometer (EI).

Mass scan range: 15.00 – 300.00.

Time of measurement: 12 – 30 minutes.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25 – 0.5  $\mu$ m.

Column temperature: Inject at a constant temperature of about 45°C, raise the temperature to 240°C at a rate of 40°C per minute, maintain at 240°C for 5 minutes, raise to 300°C at a rate of 4°C per minute, raise to 320°C at a rate of 10°C per minute, and maintain at 320°C for 3 minutes.

Injection port temperature: At a constant temperature of about 250°C.

Interface temperature: At a constant temperature of 300°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of benz[a]anthracene is about 15 minutes.

Splitless.

#### System suitability

Test for required detectability: Pipet 1 mL of the sample solution, and add methanol to make exactly 10 mL. Confirm that the peak area of benz[a]anthracene obtained with 1  $\mu$ L of this solution is equivalent to 5 to 15% of that with 1  $\mu$ L of the standard solution.

**Benzene**  $C_6H_6$  [K 8858, Special class]

**Benzethonium chloride for assay**  $C_{27}H_{42}ClNO_2$  [Same as the monograph Benzethonium Chloride. When dried, it contains not less than 99.0% of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).]

**Benzoic acid**  $C_6H_5COOH$  [K 8073, Special class]

**Benzoin**  $C_6H_5CH(OH)COC_6H_5$  White to pale yellow, crystals or powder.

Melting point <2.60>: 132 – 137°C

**Benzophenone**  $C_6H_5COC_6H_5$  Colorless crystals, having a characteristic odor.

Melting point <2.60>: 48 – 50°C

**Benzo[a]pyrene**  $C_{20}H_{12}$  Light yellow to green-yellow, crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 176 – 181°C.

**Identification**—Perform the test with benzo[a]pyrene as

directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak ( $m/z$  252) and a fragment ion peak ( $m/z$  125).

**Purity** Related substances—Dissolve 3.0 mg of benzo[a]pyrene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> under the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than benzo[a]pyrene is not more than 3.0%.

#### Operating conditions

Detector: A mass spectrophotometer (EI).

Mass scan range: 15.00 – 300.00.

Time of measurement: 12 – 30 minutes.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25 – 0.5  $\mu$ m.

Column temperature: Inject at a constant temperature of about 45°C, raise the temperature to 240°C at a rate of 40°C per minute, maintain at 240°C for 5 minutes, raise to 300°C at a rate of 4°C per minute, raise to 320°C at a rate of 10°C per minute, and maintain at 320°C for 3 minutes.

Injection port temperature: A constant temperature of about 250°C.

Interface temperature: A constant temperature of about 300°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of benzo[a]pyrene is about 22 minutes.

Splitless.

#### System suitability

Test for required detectability: Pipet 1 mL of the sample solution, add methanol to make exactly 10 mL. Confirm that the peak area of benzo[a]pyrene obtained with 1  $\mu$ L of this solution is equivalent to 5 to 15% of that with 1  $\mu$ L of the sample solution.

**p-Benzoquinone**  $C_6H_4O_2$  Yellow to yellow-brown, crystals or crystalline powder, having a pungent odor. Soluble in ethanol (95) and in diethyl ether, slightly soluble in water. It is gradually changed to a black-brown color by light.

Melting point <2.60>: 111 – 116°C

**Content:** not less than 98.0%. Assay—Weigh accurately about 0.1 g of *p*-benzoquinone, place in an iodine bottle, add exactly 25 mL of water and 25 mL of diluted sulfuric acid (1 in 15), dissolve 3 g of potassium iodide by shaking, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 5.405 mg of  $C_6H_4O_2$

**p-Benzoquinone TS** Dissolve 1 g of *p*-benzoquinone in 5 mL of acetic acid (100), and add ethanol (95) to make 100 mL.

**N-*α*-Benzoyl-L-arginine ethyl ester hydrochloride**

$C_{15}H_{22}N_4O_3 \cdot HCl$  White, crystals or crystalline powder. Freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : –15.5 – –17.0° (2.5 g, water, 50 mL, 100 mm).

Melting point <2.60>: 129 – 133°C

**Purity** (1) Clarity and color of solution—Dissolve 0.1 g

of *N*-α-benzoyl-L-arginine ethyl ester hydrochloride in 20 mL of water; the solution is clear and colorless.

(2) Related substances—Weigh 0.10 g of *N*-α-benzoyl-L-arginine ethyl ester hydrochloride, dissolve in 6 mL of water, add 4 mL of hydrochloric acid, heat in a boiling water bath for 5 minutes to decompose, and use this solution as the sample solution. Perform the test with the sample solution as directed under Paper Chromatography. Spot 5  $\mu$ L of the sample solution on a chromatographic filter paper. Develop with a mixture of water, acetic acid (100) and 1-butanol (5:4:1) to a distance of about 30 cm, and air-dry the paper. Spray evenly a solution of ninhydrin in acetone (1 in 50) upon the paper, and heat at 90°C for 10 minutes: only one purple spot appears.

**Content:** not less than 99.0%. **Assay:**—Weigh accurately about 0.6 g of *N*-α-benzoyl-L-arginine ethyl ester hydrochloride, dissolve in 50 mL of water, neutralize with 0.1 mol/L sodium hydroxide VS, if necessary, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: 4 drops of dichlorofluorescein TS).

$$\begin{aligned} \text{Each mL of 0.1 mol/L silver nitrate VS} \\ = 34.28 \text{ mg of C}_{15}\text{H}_{22}\text{N}_4\text{O}_3\text{.HCl} \end{aligned}$$

***N*-α-Benzoyl-L-arginine ethyl ester TS** Dissolve 70 mg of *N*-α-benzoyl-L-arginine ethyl ester hydrochloride in freshly boiled and cooled water to make exactly 10 mL.

***N*-α-Benzoyl-L-arginine-4-nitroanilide hydrochloride**  
C<sub>19</sub>H<sub>22</sub>N<sub>6</sub>O<sub>4</sub>.HCl Light yellow crystalline powder.

**Optical rotation** <2.49> [ $\alpha$ ]<sub>D</sub><sup>20</sup>: +45.5° – +48.0° (after drying, 0.5 g, *N,N*-dimethylformamide, 25 mL, 100 mm).

**Purity** Related substances—Dissolve 0.20 g of *N*-α-benzoyl-L-arginine-4-nitroanilide hydrochloride in 10 mL of *N,N*-dimethylformamide, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:1:1) to a distance of about 10 cm, and air-dry the plate. Exposure the plate to a vapor of iodine: only one spot appears.

***N*-α-Benzoyl-L-arginine-4-nitroanilide TS** Dissolve 0.1 g of *N*-α-benzoyl-L-arginine-4-nitroanilide hydrochloride in water to make 100 mL.

**Benzoyl chloride** C<sub>6</sub>H<sub>5</sub>COCl A clear and colorless fuming liquid. Specific gravity: about 1.2 g/mL.

**Identification**—Determine the infrared absorption spectrum of benzoyl chloride as directed in the liquid film method as directed under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1775 cm<sup>-1</sup>, 1596 cm<sup>-1</sup>, 1450 cm<sup>-1</sup>, 1307 cm<sup>-1</sup>, 1206 cm<sup>-1</sup>, 873 cm<sup>-1</sup>, 776 cm<sup>-1</sup> and 671 cm<sup>-1</sup>.

#### Benzoylhypaconine hydrochloride for assay

C<sub>31</sub>H<sub>43</sub>NO<sub>9</sub>.HCl White, crystals or crystalline powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5). Melting point: about 230°C (with decomposition).

**Absorbance** <2.24> E<sub>1cm</sub><sup>1%</sup> (230 nm): 225 – 240 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

**Purity** (1) Related substances—To 1.0 mg of benzoylhypaconine hydrochloride for assay add exactly 1 mL of ethanol (99.5). Perform the test with 5  $\mu$ L of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot with an R<sub>f</sub> value of about 0.5 appears.

(2) Related substance—Dissolve 5.0 mg of benzoyl-

hypaconine hydrochloride for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than benzoylhypaconine obtained from the sample solution is not larger than the peak area of benzoylhypaconine from the standard solution.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 5 times as long as the retention time of benzoylhypaconine.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of benzoylhypaconine obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

System performance: When the procedure is run with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisolaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisolaconine are not more than 1.5%, respectively.

#### Benzoylhypaconine hydrochloride for component determination

See benzoylhypaconine hydrochloride for assay.

***N*-Benzoyl-L-isoleucyl-L-glutamyl( $\gamma$ -OR)-glycyl-L-arginyl-p-nitroanilide hydrochloride** An equal amount mixture of two components, R = H and R = CH<sub>3</sub>. A white powder. Slightly soluble in water.

**Absorbance** <2.24> E<sub>1cm</sub><sup>1%</sup> (316 nm): 166 – 184 (10 mg, water, 300 mL).

#### Benzoylmesaconine hydrochloride for assay

C<sub>31</sub>H<sub>43</sub>NO<sub>10</sub>.HCl Benzoylmesaconine hydrochloride for thin-layer chromatography meeting the following additional specifications.

**Purity** Related substances—Dissolve 5.0 mg of benzoylmesaconine hydrochloride for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than benzoylmesaconine obtained from the sample solution is not larger than the peak area of benzoylmesaconine from the standard solution.

#### Operating conditions

Column, column temperature, mobile phase and flow

rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

**Detector:** An ultraviolet absorption photometer (wavelength: 245 nm).

**Time span of measurement:** About 6 times as long as the retention time of benzoylmesaconine.

#### System suitability

**Test for required detectability:** Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of benzoylmesaconine obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

**System performance:** When the procedure is run with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

**System repeatability:** When the test is repeated 6 times with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

**Benzoylmesaconine hydrochloride for component determination** See benzoylmesaconine hydrochloride for assay.

**Benzoylmesaconine hydrochloride for thin-layer chromatography**  $C_{31}H_{43}NO_{10}.HCl$  White, crystals or crystalline powder. Soluble in water and in ethanol (99.5) and sparingly soluble in methanol. Melting point: about 250°C (with decomposition).

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (230 nm): 217 – 231 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

**Purity** Related substances—Dissolve 1.0 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in exactly 1 mL of ethanol (99.5). Perform the test with 5  $\mu$ L of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot with an *Rf* value of about 0.4 appears.

**Benzoyl peroxide, 25% water containing** ( $C_6H_5CO_2O_2$ )<sub>0.25</sub> White, moist, crystals or powder. Soluble in chloroform and in diethyl ether, and very slightly soluble in water and in ethanol (95). Melting point: 103 – 106°C (dried substance) (with decomposition).

**Loss on drying** <2.41>: not more than 30% (0.1 g, in vacuum, silica gel, constant mass).

**Benzyl alcohol**  $C_6H_5CH_2OH$  Clear and colorless liquid, having a characteristic odor.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 1.045 – 1.050.

**Storage**—Preserve in a light-resistant tight container.

**Benzyl benzoate**  $C_6H_5COOCH_2C_6H_5$  A colorless oily liquid. Congealing point: about 18°C. Boiling point: about 323°C.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 1.118 – 1.123.

**Storage**—Preserve in a light-resistant tight container.

**Benzyl parahydroxybenzoate**  $C_{14}H_{12}O_3$  White, fine crystals or crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.

**Melting point** <2.60>: 109 – 112°C

**Residue on ignition** <2.44>: not more than 0.1%.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 1 g of benzyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the

excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 228.2 \text{ mg of } C_{14}H_{12}O_3 \end{aligned}$$

**Benzylpenicillin benzathine** See benzylpenicillin benzathine hydrate.

**Benzylpenicillin benzathine hydrate**  $(C_{16}H_{18}N_2O_4S)_2.C_{16}H_{20}N_2.4H_2O$  [Same as the namesake monograph]

**Benzylpenicillin potassium**  $C_{16}H_{17}KN_2O_4S$  [Same as the namesake monograph]

**Benzyl p-hydroxybenzoate** See benzyl parahydroxybenzoate.

**p-Benzylphenol**  $C_6H_5CH_2C_6H_4OH$  White to pale yellow-white, crystals or crystalline powder.

**Melting point** <2.60>: 80 – 85°C

**Bepotastine besilate for assay**  $C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$  [Same as the monograph Bepotastine Besilate. However, it contains not less than 99.5% of bepotastine besilate ( $C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$ ), calculated on the anhydrous and residual solvent-free basis.]

**Beraprost sodium**  $C_{24}H_{29}NaO_5$  [Same as the namesake monograph]

**Beraprost sodium for assay**  $C_{24}H_{29}NaO_5$  [Same as the monograph Beraprost Sodium. When dried it contains not less than 99.0% of beraprost sodium ( $C_{24}H_{29}NaO_5$ ).]

**Berberine chloride** See berberine chloride hydrate.

**Berberine chloride for thin-layer chromatography** See berberine chloride hydrate for thin-layer chromatography.

**Berberine chloride hydrate**  $C_{20}H_{18}ClNO_4.xH_2O$  [Same as the namesake monograph]

**Berberine chloride hydrate for thin-layer chromatography**  $C_{20}H_{18}ClNO_4.xH_2O$  [Same as the monograph Berberine Chloride Hydrate] or berberine chloride hydrate meeting the following requirements. Yellow, crystals or crystalline powder. Sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

**Identification** Determine the absorption spectrum of a solution of the substance to be examined (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 226 nm and 230 nm, between 261 nm and 265 nm, and between 342 nm and 346 nm.

**Purity** Related substances—Dissolve 10 mg of the substance to be examined in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification (2) under Phellodendron Bark: any spot other than the principal spot with an *Rf* value of about 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

**Bergenin for thin-layer chromatography**  $C_{14}H_{16}O_9$  White, crystals or crystalline powder. Freely soluble in methanol, slightly soluble in ethanol (99.5), very slightly soluble in water, and practically insoluble in diethyl ether.

**Identification**—Determine the absorption spectrum of a solution of bergenin for thin-layer chromatography in methanol (1 in 50,000) as directed under Ultraviolet-visible Spec-

trophotometry <2.24>; it exhibits maxima between 217 nm and 221 nm, and between 273 nm and 277 nm, and a minimum between 241 nm and 245 nm.

**Purity** Related substances—Dissolve 1.0 mg of bergenin for thin-layer chromatography in 1 mL of methanol. Perform the test with 20  $\mu$ L of this solution as directed in the Identification under *Mallotus Bark*: no spot other than the principal spot at the *R<sub>f</sub>* value of about 0.5 appears.

**Betahistine mesilate** C<sub>8</sub>H<sub>12</sub>N<sub>2</sub>.2CH<sub>4</sub>O<sub>3</sub>S [Same as the namesake monograph]

**Betahistine mesilate for assay** C<sub>8</sub>H<sub>12</sub>N<sub>2</sub>.2CH<sub>4</sub>O<sub>3</sub>S [Same as the monograph *Betahistine Mesilate*. When dried, it contains not less than 99.0% of betahistine mesilate (C<sub>8</sub>H<sub>12</sub>N<sub>2</sub>.2CH<sub>4</sub>O<sub>3</sub>S).]

**Betamipron** C<sub>10</sub>H<sub>11</sub>NO<sub>3</sub> [Same as the namesake monograph]

**Betamipron for assay** C<sub>10</sub>H<sub>11</sub>NO<sub>3</sub> [Same as the monograph *Betamipron*. It contains not less than 99.5% of betamipron (C<sub>10</sub>H<sub>11</sub>NO<sub>3</sub>), calculated on the anhydrous basis.]

**Bezafibrate for assay** C<sub>19</sub>H<sub>20</sub>ClNO<sub>4</sub> [Same as the monograph *Bezafibrate*. When dried it contains not less than 99.0% of bezafibrate (C<sub>19</sub>H<sub>20</sub>ClNO<sub>4</sub>).]

**BGLB** Dissolve 10 g of peptone and 10 g of lactose monohydrate in 500 mL of water, add 200 mL of fresh ox bile or a solution prepared by dissolving 20 g of dried ox bile powder in 200 mL of water and adjusted the pH to between 7.0 and 7.5, then add water to make 975 mL, and again adjust to pH 7.4. Then add 13.3 mL of a solution of brilliant green (1 in 1000) and water to make 1000 mL in total volume, and filter through absorbent cotton. Dispense 10 mL portions of the filtrate into tubes for fermentation, and sterilize by autoclaving at 121°C for not more than 20 minutes, then cool quickly, or sterilize fractionally on each of three successive days for 30 minutes at 100°C.

**$\alpha$ -BHC ( $\alpha$ -Hexachlorocyclohexane)** C<sub>6</sub>H<sub>6</sub>Cl<sub>6</sub>

*Melting point* <2.60>: 157 – 159°C

**Purity** Related substances—Dissolve 10 mg of  $\alpha$ -BHC in 5 mL of acetone for purity of crude drug, and add hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1  $\mu$ L each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total area of the peaks other than  $\alpha$ -BHC obtained from the sample solution is not larger than the peak area of  $\alpha$ -BHC from the standard solution (1).

#### Operating conditions

Proceed the operating conditions in 4. Purity 4.3. under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

**Detection sensitivity:** Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20 mL, and use this solution as standard solution (2). Adjust the detection sensitivity so that the peak area of  $\alpha$ -BHC obtained with 1  $\mu$ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of  $\alpha$ -BHC with 1  $\mu$ L of the standard solution (1) is about 20% of the full scale.

**Time span of measurement:** About twice as long as the retention time of  $\alpha$ -BHC, beginning after the solvent peak.

**$\beta$ -BHC ( $\beta$ -Hexachlorocyclohexane)** C<sub>6</sub>H<sub>6</sub>Cl<sub>6</sub>

*Melting point* <2.60>: 308 – 310°C

**Purity** Related substances—Proceed as directed in the Purity under  $\alpha$ -BHC using the following standard solution (1).

**Standard solution (1):** Pipet 2 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

**$\gamma$ -BHC ( $\gamma$ -Hexachlorocyclohexane)** C<sub>6</sub>H<sub>6</sub>Cl<sub>6</sub>

*Melting point* <2.60>: 112 – 114°C

**Purity** Related substances—Proceed as directed in the Purity under  $\alpha$ -BHC.

**$\delta$ -BHC ( $\delta$ -Hexachlorocyclohexane)** C<sub>6</sub>H<sub>6</sub>Cl<sub>6</sub>

*Melting point* <2.60>: 137 – 140°C

**Purity** Related substances—Proceed as directed in the Purity under  $\alpha$ -BHC using the following standard solution (1).

**Standard solution (1):** Pipet 5 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

**Bifonazole** C<sub>22</sub>H<sub>18</sub>N<sub>2</sub> [Same as the namesake monograph]

**Bile salts** See Microbial Limit Test for Crude Drugs <5.02>.

**Bilirubin for assay** C<sub>33</sub>H<sub>36</sub>N<sub>4</sub>O<sub>6</sub> A red-orange powder. Very slightly soluble in dimethyl sulfoxide, and practically insoluble in water and in ethanol (99.5%).

**Identification**—Determine the infrared absorption spectrum of bilirubin for assay as directed in the ATR method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3400 cm<sup>-1</sup>, 2910 cm<sup>-1</sup>, 1686 cm<sup>-1</sup> and 1643 cm<sup>-1</sup>.

**Absorbance** <2.24> E<sub>1 cm</sub><sup>%</sup> (453 nm): 970 – 1134 (1 mg, dimethyl sulfoxide, 200 mL). Conduct this procedure without exposure to light, using light-resistant vessels.

**Purity** Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. The following sample solution and standard solution should be prepared before use. Dissolve 5 mg of bilirubin for assay in 50 mL of a warmed mixture of dimethyl sulfoxide and acetic acid (100) (9:1), cool and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of dimethyl sulfoxide and acetic acid (100) (9:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than bilirubin obtained from the sample solution is not larger than the peak area of bilirubin from the standard solution.

#### Operating conditions

**Detector, column, column temperature, mobile phase and flow rate:** Proceed as directed in the operating conditions in the Assay under Oriental Bezoar.

**Time span of measurement:** About 3 times as long as the retention time of bilirubin, beginning after the solvent peak. System suitability

**System performance and system repeatability:** Proceed as directed in the system suitability in the Assay under Oriental Bezoar.

**Biotin-labeled elderberry lectin** A solution of elderberry lectin labeled with biotin, dissolved in appropriate buffer solution.

**2-(4-Biphenylyl)propionic acid** C<sub>15</sub>H<sub>14</sub>O<sub>2</sub> Light yellow-white powder.

*Melting point* <2.60>: 145 – 148°C

**Purity**—Dissolve 1 mg of 2-(4-biphenylyl) propionic acid in a mixture of water and acetonitrile (11:9) to make 50 mL. Perform the test with 20 μL of this solution as directed under Liquid Chromatography <2.01> according to the operating conditions of the Related substances in the Purity (3) under Flurbiprofen. Determine each peak area of the solution in about twice as long as the retention time of the main peak by the automatic integration method, and calculate the amount of 2-(4-biphenylyl)propionic acid by the area percentage method: it is not less than 98.0%.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.5 g of 2-(4-biphenylyl)propionic acid, previously dried in vacuum over silica gel for 4 hours, dissolve in 50 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 22.63 \text{ mg of C}_{15}\text{H}_{14}\text{O}_2 \end{aligned}$$

**2,2'-Bipyridyl** C<sub>10</sub>H<sub>8</sub>N<sub>2</sub> [K 8486, Special class]

**Bis(cis-3,3,5-trimethylcyclohexyl) phthalate** C<sub>6</sub>H<sub>4</sub>[COOC<sub>6</sub>H<sub>8</sub>(CH<sub>3</sub>)<sub>3</sub>]<sub>2</sub> White crystalline powder.

*Melting point* <2.60>: 91 – 94°C

**Bisdemethoxycurcumin** C<sub>19</sub>H<sub>16</sub>O<sub>4</sub> Yellow to orange crystalline powder. Sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water. **Melting point:** 213 – 217°C.

**Identification**—Determine the absorption spectrum of a solution of bisdemethoxycurcumin in methanol (1 in 400,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 413 nm and 417 nm.

**Purity Related substances**—(1) Dissolve 4 mg of bisdemethoxycurcumin in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at R<sub>f</sub> value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of bisdemethoxycurcumin in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the total area of the peaks other than bisdemethoxycurcumin obtained from the sample solution is not larger than the peak area of bisdemethoxycurcumin from the standard solution.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Turmeric.

**Detector:** A visible absorption photometer (wavelength: 422 nm).

**Time span of measurement:** About 4 times as long as the retention time of bisdemethoxycurcumin, beginning after the solvent peak.

#### System suitability

**System performance and system repeatability:** Proceed as directed in the system suitability in the Assay under Turmeric.

**Test for required detectability:** Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of bisdemethoxycurcumin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

**4,4'-Bis(diethylamino)benzophenone** [(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>NC<sub>6</sub>H<sub>4</sub>]<sub>2</sub>CO  
Light yellow crystals.

**Content:** not less than 98%. **Assay**—Weigh accurately 0.25 g of 4,4'-bis(diethylamino)benzophenone, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank titration in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 16.22 \text{ mg of C}_{21}\text{H}_{28}\text{N}_2\text{O} \end{aligned}$$

**N,N'-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetethylamino-2,4,6-triiodoisophthalamide** C<sub>16</sub>H<sub>20</sub>I<sub>3</sub>N<sub>3</sub>O<sub>8</sub>  
White crystalline powder.

**Identification** (1) Heat 0.1 g of N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetethylamino-2,4,6-triiodoisophthalamide over free flame: a purple colored gas evolves.

(2) Determine the infrared absorption spectrum of N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetethylamino-2,4,6-triiodoisophthalamide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3390 cm<sup>-1</sup>, 3230 cm<sup>-1</sup>, 2880 cm<sup>-1</sup>, 1637 cm<sup>-1</sup>, 1540 cm<sup>-1</sup>, 1356 cm<sup>-1</sup> and 1053 cm<sup>-1</sup>.

**Purity**—Dissolve 0.10 g of N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetethylamino-2,4,6-triiodoisophthalamide in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total area of the peaks other than N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetethylamino-2,4,6-triiodoisophthalamide obtained from the sample solution is not larger than 3 times of the peak area of N,N'-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetethylamino-2,4,6-triiodoisophthalamide from the standard solution.

#### Operating conditions

Proceed as directed in the operating conditions in the Purity (6) under Iopamidol.

#### System suitability

Proceed as directed in the system suitability in the Purity (6) under Iopamidol.

**Bismuth nitrate** See bismuth nitrate pentahydrate.

**Bismuth nitrate pentahydrate**  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  [K 8566, Special class]

**Bismuth nitrate-potassium iodide TS** Dissolve 0.35 g of bismuth nitrate pentahydrate in 4 mL of acetic acid (100) and 16 mL of water (solution A). Dissolve 8 g of potassium iodide in 20 mL of water (solution B). To 20 mL of a mixture of solution A and solution B (1:1) add 80 mL of dilute sulfuric acid and 0.2 mL of hydrogen peroxide (30). Prepare before use.

**Bismuth nitrate TS** Dissolve 5.0 g of bismuth nitrate pentahydrate in acetic acid (100) to make 100 mL.

**Bismuth potassium iodide TS** Dissolve 10 g of L-tartaric acid in 40 mL of water, add 0.85 g of bismuth subnitrate, shake for 1 hour, add 20 mL of a solution of potassium iodide (2 in 5), shake thoroughly, allow to stand for 24 hours, and filter (solution A). Separately, dissolve 10 g of L-tartaric acid in 50 mL of water, add 5 mL of solution A, and preserve in a light-resistant, glass-stoppered bottle.

**Bismuth sodium trioxide**  $\text{NaBiO}_3$  A yellow-brown powder.

**Identification**—(1) To 10 mg of bismuth sodium trioxide add 5 mL of a solution of manganese (II) nitrate hexahydrate (4 in 125) and 1 mL of diluted nitric acid (1 in 3), and shake vigorously for 10 seconds: a red-purple color is developed.

(2) Dissolve 10 mg of bismuth sodium trioxide in 2 mL of diluted hydrochloric acid (1 in 2): this solution responds to Qualitative Tests <1.09> (1) for sodium salt.

**Bismuth subnitrate** [Same as the namesake monograph]

**Bismuth subnitrate TS** Dissolve 10 g of L-tartaric acid in 40 mL of water, add 0.85 g of bismuth subnitrate, stir for 1 hour, then add 20 mL of a solution of potassium iodide (2 in 5), and shake well. After standing for 24 hours, filter, and preserve the filtrate in a light-resistant bottle.

**Bismuth sulfite indicator** Prepared for microbial test.

**Bisoprolol fumarate for assay**  $(\text{C}_{18}\text{H}_{31}\text{NO}_4)_2 \cdot \text{C}_4\text{H}_4\text{O}_4$  [Same as the monograph Bisoprolol Fumarate. However, when dried, it contains not less than 99.0% of bisoprolol fumarate  $[(\text{C}_{18}\text{H}_{31}\text{NO}_4)_2 \cdot \text{C}_4\text{H}_4\text{O}_4]$ . Also, when performing the Purity (2) under Bisoprolol Fumarate, the total area of the peaks other than bisoprolol obtained from the sample solution is not larger than 1/5 times the peak area of bisoprolol from the standard solution].

Purify as follows if needed.

**Purification method**—Dissolve, with heating, 2 g of Bisoprolol Fumarate in 200 mL of ethyl acetate, add 0.5 g of activated carbon, shake well, and filter using a glass filter (G4). Place the filtrate in ice water for 2 hours while occasional shaking. Collect the crystals that precipitate out using a glass filter (G3). Dry the crystals obtained in vacuum at 80°C for 5 hours using phosphorus (V) oxide as a desiccant.

**Bis-(1-phenyl-3-methyl-5-pyrazolone)**  $\text{C}_{20}\text{H}_{18}\text{N}_4\text{O}_2$

White to pale yellow, crystals or crystalline powder. It dissolves in mineral acids and in alkali hydroxides, and it does not dissolve in water, in ammonia TS, or in organic solvents. Melting point: not below 300°C.

*Residue on ignition* <2.44>: not more than 0.1%.

*Nitrogen content* <1.08>: 15.5 – 16.5%

**Bis(1,1-trifluoroacetoxy)iodobenzene**  $\text{C}_{10}\text{H}_5\text{F}_6\text{IO}_4$

Prepared for amino acid analysis or biochemistry.

**Bis-trimethyl silyl acetamide**  $\text{CH}_3\text{CON}[\text{Si}(\text{CH}_3)_3]_2$

Colorless liquid.

*Refractive index* <2.45>  $n_{\text{D}}^{20}$ : 1.414 – 1.418

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.825 – 0.835

*Boiling point* <2.57>: 71 – 73°C

**1,4-Bis(trimethylsilyl)benzene-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy** See 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy.

**Bitter orange peel** [Same as the namesake monograph]

**Block buffer solution** Dissolve 4 g of blocking agent in 100 mL of water, and add 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS (pH 7.4).

**Blocking agent** Powder whose main ingredient is bovine-derived lactoprotein. For immunological research purposes.

**Blocking TS for epoetin alfa** Used for Western blotting.

**Blocking TS for nartograstim test** Dissolve 1.0 g of bovine serum albumin in phosphate-buffered sodium chloride TS to make 100 mL.

**Blood agar medium** Sterilize 950 mL of heart infusion agar medium under increased pressure. Allow the media to cool to about 50°C, add 50 mL of horse or sheep defibrinated blood, dispense in sterilized Petri dishes, and make them as plate media.

**1% blood suspension** Wash a defibrinated animal blood in isotonic solution, and make it into suspension to contain 1 vol%. Prepare before use.

**Blotting TS** Dissolve 5.81 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 2.93 g of glycine and 0.38 g of sodium lauryl sulfate in a suitable amount of water, add 200 mL of methanol, and add water to make 1000 mL.

**Blue tetrazolium**  $\text{C}_{40}\text{H}_{32}\text{Cl}_2\text{N}_8\text{O}_2$  Light yellow crystals. Freely soluble in methanol, in ethanol (95) and in chloroform, slightly soluble in water, and practically insoluble in acetone and in ether. Melting point: about 245°C (with decomposition).

*Absorbance* <2.24>  $E_{1\text{ cm}}^{1\%}$  (252 nm): not less than 826 (methanol).

**Blue tetrazolium TS, alkaline** To 1 volume of a solution of blue tetrazolium in methanol (1 in 200) add 3 volumes of a solution of sodium hydroxide in methanol (3 in 25). Prepare before use.

**Borane-pyridine complex**  $\text{C}_5\text{H}_8\text{BN}$

**Content:** not less than 80%. **Assay**—Accurately weigh about 30 mg of borane-pyridine complex, dissolve in 40 mL of 0.05 mol/L iodide solution, add 10 mL of diluted sulfuric acid (1 in 6), and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 1.549 mg of  $\text{C}_5\text{H}_8\text{BN}$

**Borate-hydrochloric acid buffer solution (pH 9.0)** Dissolve 19.0 g of sodium tetraborate decahydrate in 900 mL of water, adjust the pH to exactly 9.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Borax** See sodium tetraborate decahydrate.

**Boric acid**  $\text{H}_3\text{BO}_3$  [K 8863, Special class]

**Boric acid-magnesium chloride buffer solution (pH 9.0)** Dissolve 3.1 g of boric acid in 210 mL of dilute sodium hy-

dioxide TS, and add 10 mL of a solution of magnesium chloride hexahydrate (1 in 50) and water to make 1000 mL. Adjust the pH to 9.0, if necessary.

**Boric acid-methanol buffer solution** Weigh exactly 2.1 g of boric acid, dissolve in 28 mL of sodium hydroxide TS, and dilute with water to exactly 100 mL. Mix equal volumes of this solution and methanol, and shake.

**Boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.0)** To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 21.30 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

**Boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.2)** To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 26.70 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

**Boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6)** To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 36.85 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

**Boric acid-potassium chloride-sodium hydroxide buffer solution (pH 10.0)** To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 43.90 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

**0.2 mol/L Boric acid-0.2 mol/L potassium chloride TS for buffer solution** Dissolve 12.376 g of boric acid and 14.911 g of potassium chloride in water to make 1000 mL.

**Boric acid-sodium hydroxide buffer solution (pH 8.4)** Dissolve 24.736 g of boric acid in 0.1 mol/L sodium hydroxide VS to make exactly 1000 mL.

**Borneol acetate**  $C_{12}H_{20}O_2$  A white to pale brown solid, or colorless to pale brown, clear liquid. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** Determine the infrared absorption spectrum of borneol acetate as directed in the liquid film method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $2950\text{ cm}^{-1}$ ,  $1736\text{ cm}^{-1}$ ,  $1454\text{ cm}^{-1}$  and  $1248\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 50 mg of borneol acetate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at  $105^\circ\text{C}$  for 10 minutes; the spot at an  $R_f$  value of about 0.7 obtained from the sample solution is not more intense than the spot from the standard solution.

**Boron trifluoride**  $\text{BF}_3$  Colorless gas, having an irritating odor.

*Boiling point* <2.57>:  $-100.3^\circ\text{C}$

*Melting point* <2.60>:  $-127.1^\circ\text{C}$

**Boron trifluoride-methanol TS** A solution containing 14 g/dL of boron trifluoride ( $\text{BF}_3$ ; 67.81) in methanol.

**Bovine activated blood coagulation factor X** A protein obtained from bovine plasma. It has an activity to decompose prothrombin specifically and limitedly and produce thrombin. It does not contain thrombin and plasmin. It contains not less than 500 Units per mg protein. One unit indicates an amount of the factor X which hydrolyzes 1  $\mu\text{mol}$  of *N*-benzoyl-L-isoleucyl-L-glutamyl( $\gamma$ -OR)-glycyl-L-arginyl-*p*-nitroanilide in 1 minute at  $25^\circ\text{C}$ .

**Bovine serum** Serum obtained from blood of bovine. Interleukin-2 dependent cell growth suppression substance is removed by heat at  $56^\circ\text{C}$  for 30 minutes before use

**Bovine serum albumin** Obtained from cattle serum as Cohn's fifth fraction. Contains not less than 95% of albumin.

**Bovine serum albumin for assay** White or pale yellow, crystals or crystalline powder.

Take about 50 mg of bovine serum albumin containing 99% or more albumin in glass ampoules and put them in the desiccator, whose humidity is adjusted to 31%RH at  $25^\circ\text{C}$  with calcium chloride-saturated solution, for 2 weeks, and then take out and seal them immediately.

**Protein content:** not less than 88%. Assay—Weigh accurately about 0.1 g of bovine serum albumin for assay, dissolve in water, and add water to make exactly 20 mL. Put exactly 3 mL of the solution in the Kjeldahl flask, and determine protein content following Nitrogen Determination <1.08>.

$$\begin{aligned} \text{Each mL of } 0.005\text{ mol/L sulfuric acid VS} \\ = 0.8754\text{ mg protein} \end{aligned}$$

**Storage**—Store at  $4^\circ\text{C}$  or lower.

**Bovine serum albumin for gel filtration molecular mass marker** Albumin obtained from bovine serum. For gel filtration chromatography.

**Bovine serum albumin for test of ulinastatin** White crystalline powder obtained from bovine serum by a purification method which does not denature albumin and other serum proteins. It contains not less than 99% of albumin.

**0.1% Bovine serum albumin-acetate buffer solution** Dissolve 0.1 g of bovine serum albumin in a solution of sodium acetate trihydrate (1 in 100) to make exactly 100 mL, and adjust to pH 4.0 with 1 mol/L hydrochloric acid TS.

**Bovine serum albumin-isotonic sodium chloride solution** Dissolve 0.1 g of bovine serum albumin in 100 mL of isotonic sodium chloride solution. Prepare before use.

**1 w/v% Bovine serum albumin-phosphate buffer-sodium chloride TS** Dissolve 1 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS (pH 7.4).

**0.1 w/v% Bovine serum albumin-sodium chloride-phosphate buffer solution** Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 1.15 g of anhydrous disodium hydrogen phosphate and 0.2 g of potassium dihydrogen phosphate in water to make 1000 mL. To this solution add a solution of bovine serum albumin dissolved 1.0 g in 10 mL of water.

**Bovine serum albumin-sodium chloride-phosphate buffer solution (pH 7.2)** Dissolve 10.75 g of disodium hydrogen phosphate dodecahydrate, 7.6 g of sodium chloride and 1.0 g of bovine serum albumin in water to make 1000 mL. Adjust to pH 7.2 with dilute sodium hydroxide TS or diluted phosphoric acid (1 in 10) before use.

**Bovine serum albumin TS for nartogastim test** Dissolve 0.5 g of bovine serum albumin and 0.5 mL of polysorbate 20 in phosphate-buffered sodium chloride TS to make 500 mL.

**Bovine serum albumin TS for secretin** Dissolve 0.1 g of bovine serum albumin, 0.1 g of L-cysteine hydrochloride monohydrate, 0.8 g of L-alanine, 0.01 g of citric acid monohydrate, 0.14 g of disodium hydrogen phosphate dodecahydrate and 0.45 g of sodium chloride in 100 mL of water for injection.

**Bovine serum albumin TS for Secretin RS** Dissolve 0.1 g of bovine serum albumin, 0.8 g of L-alanine, 0.01 g of citric acid monohydrate, 0.14 g of disodium hydrogen phosphate dodecahydrate and 0.45 g of sodium chloride in 100 mL of water for injection.

**Bradykinin**  $C_{50}H_{73}N_{15}O_{11}$  A white powder. Freely soluble in water and in acetic acid (31), and practically insoluble in diethyl ether.

*Optical rotation*  $<2.49> [\alpha]_D^{20}: -80 - -90^\circ$  (15 mg, water, 5 mL, 100 mm).

**Purity** Related substances—Dissolve 2.0 mg of bradykinin in 0.2 mL of water, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography  $<2.03>$ . Spot 5  $\mu$ L of the sample solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (31) (15:12:10:3) to a distance of about 10 cm, and dry the plate at 60°C. Spray evenly a solution of ninhydrin in 1-butanol (1 in 1000) on the plate, and heat at 60°C for 30 to 60 minutes: any spot other than the principal spot arisen from bradykinin does not appear.

**Brilliant green**  $C_{27}H_{34}N_2O_4S$  Fine, glistening, yellow crystals. It dissolves in water and in ethanol (95). The wavelength of absorption maximum: 623 nm.

**Bromine** Br [K 8529, Special class]

**Bromine-acetic acid TS** Dissolve 10 g of sodium acetate trihydrate in acetic acid (100) to make 100 mL, add 5 mL of bromine, and shake.

**Storage**—Preserve in light-resistant containers, preferably in a cold place.

**Bromine-carbon tetrachloride TS** To 0.1 g of bromine add carbon tetrachloride to make 100 mL, and to 2 mL of this solution add carbon tetrachloride to make 100 mL. Prepare before use.

**Bromine-cyclohexane TS** Dissolve 0.1 g of bromine in cyclohexane to make 100 mL. To 2 mL of this solution add cyclohexane to make 10 mL. Prepare before use.

**Bromine-sodium hydroxide TS** To 100 mL of a solution of sodium hydroxide (3 in 100) add 0.2 mL of bromine. Prepare before use.

**Bromine TS** Prepare by saturating water with bromine as follows: Transfer 2 to 3 mL of bromine to a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum, add 100 mL of cold water, insert the stopper, and shake.

**Storage**—Preserve in light-resistant containers, preferably in a cold place.

**Bromocresol green**  $C_{21}H_{14}Br_4O_5S$  [K 8840, Special class]

**Bromocresol green-crystal violet TS** Dissolve 0.3 g of bromocresol green and 75 mg of crystal violet in 2 mL of

ethanol (95), and dilute with acetone to make 100 mL.

**Bromocresol green-methyl red TS** Dissolve 0.15 g of bromocresol green and 0.1 g of methyl red in 180 mL of ethanol (99.5), and add water to make 200 mL.

**Bromocresol green-sodium hydroxide-acetic acid-sodium acetate TS** To 0.25 g of bromocresol green add 15 mL of water and 5 mL of dilute sodium hydroxide TS, then add a small quantity of acetic acid-sodium acetate buffer solution (pH 4.5), dissolve while shaking, and add acetic acid-sodium acetate buffer solution (pH 4.5) to make 500 mL. Wash 250 mL of this solution with two 100-mL portions of dichloromethane. Filter if necessary.

**Bromocresol green-sodium hydroxide-ethanol TS** Dissolve 50 mg of bromocresol green in 0.72 mL of 0.1 mol/L sodium hydroxide VS and 20 mL of ethanol (95), and add water to make 100 mL.

**Test for sensitivity**—To 0.2 mL of the bromocresol green-sodium hydroxide-ethanol TS add 100 mL of freshly boiled and cool water: the solution is blue, and not more than 0.2 mL of 0.02 mol/L hydrochloric acid VS is required to change the color of this solution to yellow.

**Color change**: pH 3.6 (yellow) to pH 5.2 (blue).

**Bromocresol green-sodium hydroxide TS** Triturate 0.2 g of bromocresol green with 2.8 mL of 0.1 mol/L sodium hydroxide VS in a mortar, add water to make 200 mL, and filter if necessary.

**Bromocresol green TS** Dissolve 50 mg of bromocresol green in 100 mL of ethanol (95), and filter if necessary.

**Bromocresol purple**  $C_{21}H_{16}Br_2O_5S$  [K 8841, Special class]

**Bromocresol purple-dipotassium hydrogenphosphate-citric acid TS** Mix 30 mL of bromocresol purple-sodium hydroxide TS and 30 mL of dipotassium hydrogen phosphate-citric acid buffer solution (pH 5.3), and wash with three 60-mL portions of chloroform.

**Bromocresol purple-sodium hydroxide TS** Triturate 0.4 g of bromocresol purple with 6.3 mL of dilute sodium hydroxide TS in a mortar, add water to make 250 mL, and filter if necessary.

**Bromocresol purple TS** Dissolve 50 mg of bromocresol purple in 100 mL of ethanol (95), and filter if necessary.

**Bromophenol blue**  $C_{19}H_{10}Br_4O_5S$  [K 8844, Special class]

**Bromophenol blue-potassium hydrogen phthalate TS** Dissolve 0.1 g of bromophenol blue in potassium hydrogen phthalate buffer solution (pH 4.6) to make 100 mL.

**Bromophenol blue TS** Dissolve 0.1 g of bromophenol blue in 100 mL of dilute ethanol, and filter if necessary.

**0.05% Bromophenol blue TS** Dissolve 10 mg of bromophenol blue in water to make 20 mL.

**Bromophenol blue TS, dilute** Dissolve 50 mg of bromophenol blue in 100 mL of ethanol (99.5). Prepare before use.

**Bromophenol blue TS (pH 7.0)** Mix 10 mL of bromophenol blue TS and 10 mL of ethanol (95), and adjust the pH to 7.0 with diluted dilute sodium hydroxide TS (1 in 10).

**N-Bromosuccinimide**  $C_4H_4BrNO_2$  [K 9553, Special class]

**N-Bromosuccinimide TS** Dissolve 1 g of N-bromosuc-

cinimide in 1000 mL of water.

**Bromothymol blue**  $C_{27}H_{28}Br_2O_5S$  [K 8842, Special class]

**Bromothymol blue TS** Dissolve 0.1 g of bromothymol blue in 100 mL of dilute ethanol, and filter if necessary.

**Bromothymol blue-sodium hydroxide TS** To 0.2 g of powdered bromothymol blue add 5 mL of dilute sodium hydroxide TS and a small quantity of water, dissolve by shaking in a water bath at 50°C, then add water to make 100 mL.

**Bromothymol blue-sodium hydroxide-ethanol TS** Dissolve 50 mg of bromothymol blue in 4 mL of diluted 0.2 mol/L sodium hydroxide TS (1 in 10) and 20 mL of ethanol (95), and add water to make 100 mL.

**Bromovalerylurea**  $C_6H_{11}BrN_2O_2$  [Same as the namesake monograph]

**Brotizolam for assay**  $C_{15}H_{10}BrClN_4S$  [Same as the monograph Brotizolam. When dried, it contains not less than 99.0% of brotizolam ( $C_{15}H_{10}BrClN_4S$ ).]

**Brucine** See brucine *n*-hydrate.

**Brucine dihydrate** See brucine *n*-hydrate.

**Brucine *n*-hydrate**  $C_{23}H_{26}N_2O_4.nH_2O$  [K 8832, Special class]

**1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy**  $C_{12}H_{18}D_4Si_2$  1,4-Bis(trimethylsilyl)benzene-*d*<sub>4</sub> that the traceability to the international unit system was secured.

**B-type erythrocyte suspension** Prepare a suspension containing 1 vol% of erythrocyte separated from human B-type blood in isotonic sodium chloride solution.

**Bucillamine**  $C_7H_{13}NO_3S_2$  [Same as the namesake monograph]

**Bucillamine for assay**  $C_7H_{13}NO_3S_2$  [Same as the monograph Bucillamine. However, when dried, it contains not less than 99.0% of bucillamine ( $C_7H_{13}NO_3S_2$ ). Furthermore, it conforms to the following test.]

**Purity** Related substances—Dissolve 60 mg of bucillamine for assay in 20 mL of a mixture of water and methanol (1:1) and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. When the test is performed according to the Purity (3) under Bucillamine, the total area of the peaks other than bucillamine obtained from the sample solution is not larger than the peak area of bucillamine from the standard solution.

**Bufalin for assay**  $C_{24}H_{34}O_4.xH_2O$  White, odorless crystalline powder.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (300 nm): 143 – 153 (10 mg, methanol, 250 mL). Use the sample dried in a desiccator (silica gel) for 24 hours for the test.

**Purity** Related substances—Dissolve 40 mg of bufalin for assay in 5 mL of chloroform and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and chloroform (4:3:3) to a distance of about 14 cm, and air-dry. Spray evenly sulfuric acid, and heat at 100°C for 2 to 3

minutes: any spot other than the principal spot obtained from the sample solution is not larger and not more intense than the spot from the standard solution.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 10 mg of bufalin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area by the automatic integration method, and calculate the amount of bufalin by the area percentage method.

#### Operating conditions

**Detector:** An ultraviolet absorption photometer (wavelength: 300 nm).

**Column:** A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10  $\mu$ m in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of water and acetonitrile (1:1).

**Flow rate:** Adjust so that the retention time of bufalin is about 6 minutes.

**Time span of measurement:** About twice as long as the retention time of bufalin, beginning after the solvent peak.

#### System suitability

**Test for required detectability:** Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of bufalin obtained with 20  $\mu$ L of the standard solution (2) can be measured by the automatic integration method and the peak height of bufalin with 20  $\mu$ L of the standard solution (1) is about 20% of the full scale.

**System performance:** Dissolve 10 mg each of bufalin for assay, cinobufagin for assay and resibufogenin for assay in methanol to make 200 mL. When the procedure is run with 20  $\mu$ L of this solution under the above operating conditions, bufalin, cinobufagin and resibufogenin are eluted in this order with the resolutions between these peaks being not less than 1.5, respectively.

**Bufalin for component determination** See bufalin for assay.

**Buffer solution for clemoleukin** Combine 12.5 mL of 0.5 mol/L tris buffer solution (pH 6.8), 10 mL of sodium lauryl sulfate solution (1→10), 10 mL of glycerin, and 17.5 mL of water, shake, then add 5 mg of bromophenol blue to dissolve.

**Storage**—Store in a cool place, shielded from light.

**Buffer solution for enzyme digestion** Dissolve 0.30 g of urea in a mixture of 100  $\mu$ L of 2-amino-2-hydroxymethyl-1,3-propanediol solution containing 6.06 g in 100 mL of water, 100  $\mu$ L of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride solution containing 7.88 g in 100 mL of water, 100  $\mu$ L of methylamine hydrochloride solution containing 2.70 g in 100 mL of water, 50  $\mu$ L of dithiothreitol in solution containing 30.9 mg in 1 mL of water and 420  $\mu$ L of water.

**Buffer solution for epoetin alfa sample** Dissolve 1.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 3.2 g of sodium lauryl sulfate in a suitable amount of water, adjust to pH 6.8 with 6 mol/L hydrochloric acid TS, 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS, add

32 mg of bromophenol blue and 16 mL of glycerin, and add water to make 40 mL. Before use, dissolve 50 mg of dithiothreitol in 10 mL of this solution.

**Buffer solution for filgrastim sample** Dissolve 1.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 3.2 g of sodium lauryl sulfate in a suitable amount of water, adjust to pH 6.8 with 6 mol/L hydrochloric acid TS, 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS, add 32 mg of bromophenol blue and 16 mL of glycerin, and add water to make 40 mL.

**Buffer solution for nartograstim sample** Mix 0.8 mL of sodium lauryl sulfate solution (1 in 10), 0.5 mL of 0.5 mol/L tris buffer solution (pH 6.8), 0.4 mL of glycerin and 0.1 mL of bromophenol blue solution (1 in 200). Prepare before use.

**Buffer solution for SDS polyacrylamide gel electrophoresis** Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 14.4 g of glycine and 1.0 g of sodium lauryl sulfate in water to make 1000 mL.

**Buformin hydrochloride for assay**  $C_6H_{15}N_5.HCl$  [Same as the monograph Buformin Hydrochloride. When dried, it contains not less than 99.5% of buformin hydrochloride ( $C_6H_{15}N_5.HCl$ ).]

**n-Butanol** See 1-butanol.

**sec-Butanol** See 2-butanol.

**1-Butanol**  $CH_3(CH_2)_2CH_2OH$  [K 8810, Special class]

**2-Butanol**  $CH_3CH_2CH(OH)CH_3$  [K 8812, Special class]

**2-Butanone**  $CH_3COC_2H_5$  [K 8900, Special class]

**Butenafine hydrochloride for assay**  $C_{22}H_{27}N.HCl$  [Same as the monograph Butenafine Hydrochloride]

**N-t-Butoxycarbonyl-L-glutamic acid- $\alpha$ -phenyl ester**  $C_{16}H_{21}NO_6$  White powder.

*Melting point* <2.60>: 95–104°C

**Purity** Related substances—Dissolve 10 mg of *N*-t-butoxycarbonyl-L-glutamic acid- $\alpha$ -phenyl ester in 5 mL of dilute ethanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L each of the sample solution and standard solution on three plates of silica gel with fluorescent indicator for thin-layer chromatography. Develop the first plate with a mixture of chloroform, ethyl acetate and acetic acid (100) (25:25:1), the second plate with a mixture of benzene, 1,4-dioxane and acetic acid (100) (95:25:4), and the third plate with a mixture of chloroform, methanol and acetic acid (100) (45:4:1) to a distance of about 12 cm, and air-dry these plates. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution in all plates.

**Butyl acetate**  $CH_3COOCH_2CH_2CH_2CH_3$  [K 8377, Special class]

**n-Butyl acetate** See butyl acetate

**t-Butyl alcohol**  $(CH_3)_3COH$  A crystalline solid, having a characteristic odor. A colorless liquid at above an ordinary temperature. Specific gravity  $d_{20}^{20}$ : about 0.78; Boiling point: about 83°C; Melting point: about 25°C.

**Identification**—Determine the infrared absorption spec-

trum as directed in the liquid film method as directed under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3370  $\text{cm}^{-1}$ , 2970  $\text{cm}^{-1}$ , 1471  $\text{cm}^{-1}$ , 1202  $\text{cm}^{-1}$ , 1022  $\text{cm}^{-1}$ , 913  $\text{cm}^{-1}$  and 749  $\text{cm}^{-1}$ .

**n-Butylamine**  $CH_3CH_2CH_2CH_2NH_2$  A colorless liquid, having an amine-like, characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether. The solution in water shows alkalinity and rapidly absorbs carbon dioxide from the air.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.740 – 0.747

*Distilling range* <2.57>: 76.5 – 79°C, not less than 96 vol%.

**Butyl benzoate**  $C_6H_5COOCH_2CH_2CH_2CH_3$  A clear and colorless liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.495 – 1.500

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.006 – 1.015

**n-Butylboronic acid**  $C_4H_{11}BO_2$  White flakes.

*Melting point* <2.60>: 90 – 92°C

**n-Butyl chloride** See 1-chlorobutane.

**n-Butyl formate**  $HCOO(CH_2)_3CH_3$  Clear and colorless liquid, having a characteristic odor.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.884 – 0.904

**tert-Butyl methyl ether**  $(CH_3)_3COCH_3$  Clear colorless liquid, having a specific odor.

*Refractive index* <2.45>  $n_D^{20}$ : 1.3689

*Specific gravity* <2.56>  $d_4^{20}$ : 0.7404

**Butyl parahydroxybenzoate**

$HOC_6H_4COOCH_2CH_2CH_2CH_3$  [Same as the namesake monograph]

**Butyl parahydroxybenzoate for resolution check**

$C_{11}H_{14}O_3$  Colorless crystals or a white crystalline powder. Very soluble in methanol, freely soluble in ethanol (95) and in acetone, and practically insoluble in water. Melting point: 68 – 71°C.

**Identification**—Determine the infrared absorption spectrum of butyl parahydroxybenzoate for resolution check as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum of Butyl Parahydroxybenzoate or the spectrum of Butyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** Related substances—Dissolve 50 mg of butyl parahydroxybenzoate for resolution check in 2.5 mL of methanol, and add the mobile phase to make 50 mL. To 10 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: total area of the peaks other than butyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of butyl parahydroxybenzoate from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Butyl Parahydroxybenzoate.

**Time span of measurement:** About 1.5 times as long as the retention time of butyl parahydroxybenzoate.

**System suitability**

**Test for required detectability:** Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of butyl parahydroxybenzoate obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

**System performance:** When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of butyl parahydroxybenzoate are not less than 2500 and not more than 2.0, respectively.

**System repeatability:** When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of butyl parahydroxybenzoate is not more than 5.0%.

**Butyrolactone**  $C_4H_6O_2$  Clear, colorless to practically colorless liquid.

*Specific gravity* <2.56>  $d_4^{25}$ : 1.128 – 1.135

*Boiling point* <2.57>: 198 – 208°C

**Cadmium acetate** See cadmium acetate dihydrate.

**Cadmium acetate dihydrate**  $Cd(CH_3COO)_2 \cdot 2H_2O$

White, crystals or crystalline powder.

**Identification—(1)** Dissolve 0.2 g of cadmium acetate dihydrate in 20 mL of water, and use this solution as the sample solution. To 10 mL of the sample solution add 2 mL of iron (III) chloride TS: a red-brown color is produced.

(2) To 10 mL of the sample solution obtained in (1) add 1 mL of sodium sulfide TS: a yellow precipitate is produced.

**Cadmium ground metal** Cd [H 2113, First class]

**Cadmium-ninhydrin TS** Dissolve 50 mg of cadmium acetate dihydrate in 5 mL of water and 1 mL of acetic acid (100), add 2-butanone to make 50 mL, and dissolve 0.1 g of ninhydrin in this solution. Prepare before use.

**Cadralazine for assay**  $C_{12}H_{21}N_5O_3$  [Same as the monograph Cadralazine. When dried, it contains not less than 99.0% of cadralazine ( $C_{12}H_{21}N_5O_3$ ).]

**Caffeine** See caffeine hydrate.

**Caffeine hydrate**  $C_8H_{10}N_4O_2 \cdot H_2O$  [Same as the namesake monograph]

**Caffeine, anhydrous**  $C_8H_{10}N_4O_2$  [Same as the monograph Anhydrous Caffeine]

**Calcium acetate monohydrate**  $(CH_3COO)_2Ca \cdot H_2O$  [K 8364, Special class]

**Calcium carbonate**  $CaCO_3$  [K 8617, Special class]

**Calcium carbonate for assay**  $CaCO_3$  [Same as the monograph Precipitated Calcium Carbonate. When dried, it contains not less than 99.0% of calcium carbonate ( $CaCO_3$ ).]

**Calcium chloride** See calcium chloride dihydrate.

**Calcium chloride dihydrate**  $CaCl_2 \cdot 2H_2O$  [K 8122, Special class]

**Calcium chloride dihydrate for assay** See calcium chloride hydrate for assay.

**Calcium chloride for drying**  $CaCl_2$  [K 8124, For drying]

**Calcium chloride hydrate for assay**  $CaCl_2 \cdot 2H_2O$  [Same as the monograph, Calcium Chloride Hydrate. It contains not less than 99.0% of calcium chloride hydrate ( $CaCl_2 \cdot 2H_2O$ ).]

**Calcium chloride for water determination**  $CaCl_2$  [K 8125, For water determination]

**Calcium chloride TS** Dissolve 7.5 g of calcium chloride dihydrate in water to make 100 mL (0.5 mol/L).

**Calcium gluconate for thin-layer chromatography** See calcium gluconate hydrate for thin-layer chromatography.

**Calcium gluconate hydrate for thin-layer chromatography** [Same as the monograph Calcium Gluconate Hydrate. When the test is performed as directed in the Identification (1) under Calcium Gluconate Hydrate, any spot other than the principal spot at the  $R_f$  value of about 0.4 does not appear.]

**Calcium hydroxide**  $Ca(OH)_2$  [K 8575, Special class]

**Calcium hydroxide for pH determination** Calcium hydroxide prepared for pH determination.

**Calcium hydroxide pH standard solution** See pH Determination <2.54>.

**Calcium hydroxide TS** To 3 g of calcium hydroxide add 1000 mL of cold distilled water, and occasionally shake the mixture vigorously for 1 hour. Allow to stand, and use the supernatant liquid (0.04 mol/L).

**Calcium nitrate** See calcium nitrate tetrahydrate.

**Calcium nitrate tetrahydrate**  $Ca(NO_3)_2 \cdot 4H_2O$  [K 8549, Special class]

**Calcium oxide**  $CaO$  [K 8410, Special class]

**Calcium pantothenate**  $C_{18}H_{32}CaN_2O_{10}$  [Same as the namesake monograph]

**Calcium paraaminosalicylate hydrate for assay**

$C_7H_5CaNO_3 \cdot 3\frac{1}{2}H_2O$  [Same as the monograph Calcium Paraaminosalicylate Hydrate. It contains not less than 99.0% of calcium paraaminosalicylate ( $C_7H_5CaNO_3$ ), calculated on the anhydrous basis.]

**Camphor**  $C_{10}H_{16}O$  [Same as the monograph *d*-Camphor or *dl*-Camphor]

**d-Camphorsulfonic acid**  $C_{10}H_{16}O_4S$  White, crystals or crystalline powder, having a characteristic odor. Very soluble in water, and soluble in chloroform.

**Purity** Clarity and color of solution—Dissolve 1.0 g of *d*-camphorsulfonic acid in 10 mL of water: the solution is clear and colorless or pale yellow.

*Loss on drying* <2.41>: not more than 2.0% (1 g, 105°C, 5 hours).

**Content:** not less than 99.0%, calculated on the dried basis. Assay—Weigh accurately about 4 g of *d*-camphorsulfonic acid, dissolve in 50 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS  
= 232.3 mg of  $C_{10}H_{16}O_4S$

**Candesartan cilexetil**  $C_{33}H_{34}N_6O_6$  [Same as the namesake monograph]

**Candesartan cilexetil for assay**  $C_{33}H_{34}N_6O_6$  [Same as the monograph Candesartan Cilexetil. It contains not less than 99.5% of candesartan cilexetil ( $C_{33}H_{34}N_6O_6$ ), calculated on the anhydrous basis, and when performed the test as directed in the Purity (2) under Candesartan Cilexetil, the total area of the peaks other than candesartan cilexetil obtained from the sample solution is not larger than 1/2 times the

peak area of candesartan cilexetil from the standard solution.]

**Caprylic acid**  $\text{CH}_3(\text{CH}_2)_6\text{COOH}$  A clear and colorless, oily liquid, having a slight unpleasant odor. Freely soluble in ethanol (95) and in chloroform, and very slightly soluble in water.

*Refractive index* <2.45>  $n_{\text{D}}^{20}$ : 1.426 – 1.430

*Specific gravity* <2.56>  $d_4^{20}$ : 0.908 – 0.912

*Distilling range* <2.57>: 238 – 242°C, not less than 95 vol%.

**(E)-Capsaicin for assay**  $\text{C}_{18}\text{H}_{27}\text{NO}_3$  Use (E)-capsaicin for thin-layer chromatography meeting the following additional specifications.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (281 nm): 97 – 105 (10 mg, methanol, 200 mL). Use the sample dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours for the test.

**Purity** Related substances—Dissolve 10 mg of (E)-capsaicin for assay in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area from these solutions by the automatic integration method: the total area of the peaks other than capsaicin obtained from the sample solution is not larger than the peak area of capsaicin from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Capsicum.

Time span of measurement: About 3 times as long as the retention time of capsaicin, beginning after the solvent peak.

#### System suitability

System performance and system repeatability: Proceed the system suitability in the Assay under Capsicum.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of capsaicin obtained with 20  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu\text{L}$  of the standard solution.

**(E)-Capsaicin for component determination** See (E)-capsaicin for assay.

**Capsaicin for thin-layer chromatography** See (E)-capsaicin for thin-layer chromatography.

**(E)-Capsaicin for thin-layer chromatography**  $\text{C}_{18}\text{H}_{27}\text{NO}_3$  White crystals, having a strong irritative odor. Very soluble in methanol, freely soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: 65 – 70°C

**Purity** Related substances—Dissolve 20 mg of (E)-capsaicin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification under Capsicum: any spot other than the principal spot at an *R<sub>f</sub>* value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

**Carbazochrome**  $\text{C}_{10}\text{H}_{12}\text{N}_4\text{O}_3$  Yellow-red to red, crystals or crystalline powder. Melting point: about 222°C (with decomposition).

**Content:** not less than 98.0%. **Assay**—Dissolve about 0.2 g of carbazochrome, weighed accurately, in 20 mL of acetic acid (100) by heating, add 80 mL of acetic anhydride, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 23.62 mg of  $\text{C}_{10}\text{H}_{12}\text{N}_4\text{O}_3$

**Carbazochrome sodium sulfonate for component determination** See carbazochrome sodium sulfonate trihydrate.

#### Carbazochrome sodium sulfonate trihydrate

$\text{C}_{10}\text{H}_{11}\text{N}_4\text{NaO}_5\text{S} \cdot 3\text{H}_2\text{O}$  [Same as the monograph Carbazochrome Sodium Sulfonate Hydrate. It contains not less than 99.0% of carbazochrome sodium sulfonate ( $\text{C}_{10}\text{H}_{11}\text{N}_4\text{NaO}_5\text{S}$ ), calculated on the anhydrous basis, and meets the following additional requirement.]

*Water* <2.48>: 14.0 – 15.0%.

**Carbazole**  $\text{C}_{12}\text{H}_9\text{N}$  White to nearly white foliaceous or plate-like crystals or crystalline powder. Freely soluble in pyridine and in acetone, slightly soluble in ethanol (99.5), and practically insoluble in water. It readily sublimes when heated.

*Melting point* <2.60>: 243 – 245°C

**Purity** Clarity and color of solution—To 0.5 g of carbazole add 20 mL of ethanol (99.5), and dissolve by warming: the solution is clear.

Residue on ignition: Not more than 0.1% (1 g).

**Carbazole TS** Dissolve 0.125 g of carbazole in ethanol (99.5) to make 100 mL.

**L-Carbocisteine for assay**  $\text{C}_5\text{H}_9\text{NO}_4\text{S}$  [Same as the monograph L-Carbocisteine. When dried, it contains not less than 99.0% of L-carbocisteine ( $\text{C}_5\text{H}_9\text{NO}_4\text{S}$ .)]

**0.1 mol/L Carbonate buffer solution (pH 9.6)** Dissolve 3.18 g of anhydrous sodium carbonate and 5.88 g of sodium hydrogen carbonate in water to make 1000 mL.

**Carbon dioxide**  $\text{CO}_2$  [Same as the namesake monograph]

**Carbon disulfide**  $\text{CS}_2$  [K 8732, Special class]

Preserve in tightly stoppered containers in a dark, cold place, remote from fire.

**Carbonic anhydrase** White powder. Derived from bovine RBC. Molecular weight about 29,000.

**Carbon monoxide**  $\text{CO}$  A toxic, colorless gas. Prepare by passing the gas generated by reacting formic acid with sulfuric acid through a layer of sodium hydroxide TS. Carbon monoxide from a metal cylinder may be used.

**Carbon tetrachloride**  $\text{CCl}_4$  [K 8459, Special class]

**Carboplatin**  $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{Pt}$  [Same as the namesake monograph]

**Carvedilol for assay**  $\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_4$  [Same as the monograph Carvedilol]

**Casein, milk** A white to light yellow, powder or grain.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1650  $\text{cm}^{-1}$ , 1540  $\text{cm}^{-1}$  and 1250  $\text{cm}^{-1}$ .

**Casein (milk origin)** See casein, milk.

**Casein peptone** See peptone, casein.

**Castor oil** [Same as the namesake monograph]

**Catechol**  $C_6H_4(OH)_2$  White crystals.

*Melting point* <2.60>: 104 – 107°C

*Storage*—Preserve in a light-resistant tight container.

**Cefadroxil**  $C_{16}H_{17}N_3O_5S$  [Same as the namesake monograph]

**Cefatrizine propylene glycolate**  $C_{18}H_{18}N_6O_5S_2.C_3H_8O_2$   
[Same as the namesake monograph]

**Cefcapene pivoxil hydrochloride hydrate**

$C_{23}H_{29}N_5O_8S_2.HCl.H_2O$  [Same as the namesake monograph]

**Cefdinir lactam ring-cleavage lactones**  $C_{14}H_{15}N_5O_6S_2$  A mixture of 4 diastereoisomers. A white to yellow powder.

*Identification*—Determine the infrared absorption spectrum of cefdinir lactam ring-cleavage lactones as directed in the paste method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1743  $\text{cm}^{-1}$ , 1330  $\text{cm}^{-1}$ , 1163  $\text{cm}^{-1}$  and 1047  $\text{cm}^{-1}$ .

*Content*: not less than 90%. *Assay*—Dissolve about 5 mg of cefdinir lactam ring-cleavage lactones in 5 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), and use this solution as the sample solution. Perform the test with 5  $\mu\text{L}$  of the sample solution as directed in the operating conditions of the Purity (2) under Cefdinir, and determine the areas of each peak by the automatic integration method. Calculate the percent of the total peak area of 4 cefdinir lactam ring-cleavage lactones to the total area of all peaks.

**Cell suspension solution for teceleukin** Centrifuge for 5 minutes at 1000 rpm culture medium of NK-7 cells that have been cultured statically for 2 to 4 hours. Remove the supernatant by aspiration, and add potency measuring medium for teceleukin to a cell concentration of 2 to 4  $\times 10^5$  cells/mL.

**Celmoleukin for liquid chromatography**

$C_{69}H_{118}N_{178}O_{203}S_7$  [Same as the monograph Celmoleukin (Genetical Recombination). However, contains 0.5 to 1.5 mg of protein per mL, polymers amount for 0.5% or less, and conforms to the following test].

*Identification* (1) When the amino acid sequence is investigated using the Edman technique and liquid chromatography, the amino acids are detected in the following sequence: alanine, proline, threonine, serine, serine, serine, threonine, lysine, lysine, threonine, glutamine, leucine, glutamine, leucine, and glutamic acid. Also, based on the results of the protein content determination test, place an amount of celmoleukin equivalent to about 0.3 mg in a hydrolysis tube, evaporate to dryness under vacuum, and then add 100  $\mu\text{L}$  of hydrazine anhydride for amino acid sequence analysis. Reduce the internal pressure of the hydrolysis tube by heating at about 100°C for 6 hours. After evaporating to dryness under vacuum, add 250  $\mu\text{L}$  of water to dissolve the residue. To this solution add 200  $\mu\text{L}$  of benzaldehyde, shake occasionally, leave for one hour, centrifuge, and remove the aqueous layer. Add 250  $\mu\text{L}$  of water to the benzaldehyde layer, shake, centrifuge, combine the aqueous layers, and evaporate to dryness under vacuum. Threonine is detected when amino acid analysis is conducted using the postcolumn technique with ninhydrin on a solution of the residue dissolved by adding 100  $\mu\text{L}$  of 0.02 mol/L hydrochloric acid TS.

(2) Add 1 mL of protein digestive enzyme TS to 1 mL of celmoleukin for liquid chromatography, shake, and leave at

37°C for 18 to 24 hours. Pipet 1 mL of this solution and add 25  $\mu\text{L}$  of trifluoroacetic acid (1 in 10). To another 1 mL, add 10  $\mu\text{L}$  of 2-mercaptoethanol, leave at 37°C for 30 minutes, and then add 25  $\mu\text{L}$  of trifluoroacetic acid (1 in 10). Perform the test with these two solutions separately as directed in the operating conditions of the Identification (1) under Celmoleukin (Genetical Recombination). Repeatedly pipet the celmoleukin derived peak fraction that elutes and when the test is performed according to Celmoleukin (Genetical Recombination), Constituent amino acids, except for the lysines in positions 9 and 49 from the amino terminal amino acid, a peptide estimated from the complete primary structure is detected.

**Cephaeline hydrobromate**  $C_{28}H_{38}N_2O_4.2HBr$  A white or light-yellow crystalline powder.

*Purity*—Dissolve 10 mg of cephaeline hydrobromate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Assay under Ipecac: when measure the peak areas for 2 times the retention time of emetine, the total area of the peaks other than cephaeline obtained from the sample solution is not larger than the peak area of cephaeline from the standard solution.

**Ceric ammonium sulfate** See cerium (IV) tetraammonium sulfate dihydrate.

**Ceric ammonium sulfate-phosphoric acid TS** See cerium (IV) tetraammonium sulfate-phosphoric acid TS.

**Ceric ammonium sulfate TS** See cerium (IV) tetraammonium sulfate TS.

**Cerium (III) nitrate hexahydrate**  $Ce(NO_3)_3.6H_2O$  A colorless or light yellow crystalline powder. It dissolves in water.

*Purity* (1) Chloride <1.03>: not more than 0.036%.

(2) Sulfate <1.14>: not more than 0.120%.

*Content*: not less than 98.0%. *Assay*—To about 1.5 g of cerium (III) nitrate hexahydrate, accurately weighed, add 5 mL of sulfuric acid, and heat it until white fumes are evolved vigorously. After cooling, add 200 mL of water, 0.5 mL of 0.1 mol/L silver nitrate VS, dissolve 5 g of ammonium peroxodisulfate, dissolve, and boil it for 15 minutes. After cooling, add 2 drops of 1,10-phenanthroline TS, and titrate <2.50> with 0.1 mol/L ammonium iron (II) sulfate VS until the pale blue color of the solution changes to red.

Each mL of 0.1 mol/L ammonium iron (II) sulfate VS  
= 43.42 mg of  $Ce(NO_3)_3.6H_2O$

**Cerium (III) nitrate TS** Dissolve 0.44 g of cerium (III) nitrate hexahydrate in water to make 1000 mL.

**Cerium (IV) diammonium nitrate**  $Ce(NH_4)_2(NO_3)_6$   
[K 8556, Diammonium cerium (IV) nitrate, Special class]

**Cerium (IV) diammonium nitrate TS** Dissolve 6.25 g of cerium (IV) diammonium nitrate in 160 mL of diluted dilute nitric acid (9 in 50). Use within 3 days.

**Cerium (IV) sulfate tetrahydrate**  $Ce(SO_4)_2 \cdot 4H_2O$   
[K 8976, Special class]

**Cerium (IV) tetraammonium sulfate dihydrate**  
 $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 2H_2O$  [K 8977, Tetraammonium cerium (IV) sulfate dihydrate, Special class]

**Cerium (IV) tetraammonium sulfate-phosphoric acid TS** Dissolve 0.1 g of cerium (IV) tetraammonium sulfate dihydrate in diluted phosphoric acid (4 in 5) to make 100 mL.

**Cerium (IV) tetraammonium sulfate TS** Dissolve 6.8 g of cerium (IV) tetraammonium sulfate dihydrate in diluted sulfuric acid (3 in 100) to make 100 mL.

**Cerous nitrate** See cerium (III) nitrate hexahydrate.

**Cerous nitrate TS** See cerium (III) nitrate TS.

**Cesium chloride** CsCl White, crystals or crystalline powder. Very soluble in water, and freely soluble in ethanol (99.5).

*Loss on drying* <2.41>: Not more than 1.0% (1 g, 110°C, 2 hours).

*Content:* not less than 99.0%. Assay—Weigh accurately about 0.5 g, previously dried, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution, add 30 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS  
= 16.84 mg of CsCl

**Cesium chloride TS** To 25.34 g of cesium chloride add water to make 1000 mL.

**Cetanol** [Same as the namesake monograph]

**Cetirizine hydrochloride for assay** C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub>.2HCl [Same as the monograph Cetirizine Hydrochloride. When dried, it contains not less than 99.5% of cetirizine hydrochloride (C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub>.2HCl).]

**Cetrimide** C<sub>17</sub>H<sub>38</sub>BrN White to pale yellow-white powder, having a faint, characteristic odor.

*Purity* Clarity of solution—Dissolve 1.0 g of cetrimide in 5 mL of water; the solution is clear.

*Content:* not less than 96.0%. Assay—Weigh accurately about 2 g of cetrimide, previously dried, and dissolve in water to make exactly 100 mL. Pipet 25 mL of this solution into a separator, add 25 mL of chloroform, 10 mL of 0.1 mol/L sodium hydroxide VS and 10 mL of a freshly prepared solution of potassium iodide (1 in 20), shake well, allow to stand, and remove the chloroform layer. Wash the solution with three 10-mL portions of chloroform, take the water layer, and add 40 mL of hydrochloric acid. After cooling, titrate with 0.05 mol/L potassium iodide VS until the deep brown color of the solution almost disappears, add 2 mL of chloroform, and titrate <2.50> again until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color of the chloroform layer no more reappears within 5 minutes after the chloroform layer is decolorized. Perform a blank determination with 20 mL of water, 10 mL of a solution of potassium iodide (1 in 20) and 40 mL of hydrochloric acid.

Each mL of 0.05 mol/L potassium iodate VS  
= 33.64 mg of C<sub>17</sub>H<sub>38</sub>BrN

**Cetylpyridinium chloride monohydrate** C<sub>21</sub>H<sub>38</sub>ClN.H<sub>2</sub>O White, powder or crystals. Odorless or having slightly a characteristic odor.

*Melting point* <2.60>: 80 – 84°C

*Water* <2.48>: 4.5 – 5.5%

*Residue on ignition* <2.44>: Not more than 0.2% (1 g).

*Content:* 99.0 – 102.0%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.2 g of cetylpyridinium chloride monohydrate, dissolve in 75 mL of water, add 10 mL of chloroform, 0.4 mL of bromophenol blue solution

(1 in 2000) and 5 mL of freshly prepared sodium hydrogen carbonate solution (21 in 5000), and titrate <2.50> with 0.02 mol/L sodium tetraphenylboron VS until the blue color in the chloroform layer disappears by vigorous shaking after adding 1 drop of the titrant.

Each mL of 0.02 mol/L sodium tetraphenylboron VS  
= 6.800 mg of C<sub>21</sub>H<sub>38</sub>ClN

#### Chenodeoxycholic acid for thin-layer chromatography

C<sub>24</sub>H<sub>40</sub>O<sub>4</sub> White, crystals or crystalline powder. Very soluble in methanol and in acetic acid (100), freely soluble in ethanol (95), soluble in acetone, sparingly soluble in ethyl acetate, slightly soluble in chloroform, and practically insoluble in water. Melting point: about 119°C (recrystallize from ethyl acetate).

*Purity* Related substances—Dissolve 25 mg of chenodeoxycholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 250 mL and use this solution as the sample solution. Perform the test with this solution as directed under the Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Dry the plate at 120°C for 30 minutes, spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 5) immediately, and heat at 120°C for 2 or 3 minutes: any spot other than the principal spot at the R<sub>f</sub> value of about 0.4 does not appear.

*Content:* not less than 98.0%. Assay—Weigh accurately about 0.5 g of chenodeoxycholic acid for thin-layer chromatography, previously dried under reduced pressure (phosphorus (V) oxide) at 80°C for 4 hours, and dissolve in 40 mL of neutralized ethanol and 20 mL of water. Add 2 drops of phenolphthalein TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS. Near the end point add 100 mL of freshly boiled and cooled water, and titrate again.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 39.26 mg of C<sub>24</sub>H<sub>40</sub>O<sub>4</sub>

**0.5 vol% Chicken erythrocyte suspension** Centrifuge the blood taken from healthy chicken, discard the supernatant liquid. To the residue add 0.01 mol/L phosphate buffer solution to make 45 mL, suspend the cells, and centrifuge. Discard the supernatant liquid, and repeat the same procedure 3 times more. Suspend 5 mL of the middle layer of the residue so obtained in 40 mL of 0.01 mol/L phosphate buffer solution, and centrifuge. Discard the supernatant liquid, suspend 3 mL of the middle layer of the residue in 10 mL of 0.01 mol/L phosphate buffer solution, and centrifuge. Discard the supernatant liquid, and suspend 2 mL of the middle layer of the residue in 18 mL of 0.01 mol/L phosphate buffer solution. To 10 mL of this solution add 190 mL of 0.01 mol/L phosphate buffer solution, and stir to suspend.

#### Chikusetsusaponin IV for thin-layer chromatography

C<sub>47</sub>H<sub>74</sub>O<sub>18</sub> White crystalline powder. Freely soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether. Melting point: about 215°C (with decomposition).

*Purity* Related substances—Dissolve 2 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and perform the test with 5 μL of this solution as directed in the Identification under Panax Japonicus Rhizome: any spot other than the principal spot with an R<sub>f</sub> value of about 0.4 does not appear.

**Chloral hydrate** C<sub>2</sub>H<sub>3</sub>Cl<sub>3</sub>O<sub>2</sub> [Same as the namesake

monograph]

**Chloral hydrate TS** Dissolve 5 g of chloral hydrate in 3 mL of water.

**Chloramine** See sodium toluensulfonchloramide trihydrate.

**Chloramine TS** See sodium toluensulfonchloramide TS.

**Chloramphenicol**  $C_{11}H_{12}Cl_2N_2O_5$  [Same as the monograph Chloramphenicol]

**Chlauric acid** See hydrogen tetrachloroaurate (III) tetrahydrate.

**Chlauric acid TS** See hydrogen tetrachloroaurate (III) tetrahydrate TS.

**Chlordiazepoxide**  $C_{16}H_{14}ClN_3O$  [Same as the namesake monograph]

**Chlordiazepoxide for assay**  $C_{16}H_{14}ClN_3O$  [Same as the monograph Chlordiazepoxide. When dried, it contains not less than 99.0% of  $C_{16}H_{14}ClN_3O$ .]

**Chlorhexidine hydrochloride**  $C_{22}H_{30}Cl_2N_{10}.2HCl$  [Same as the namesake monograph]

**Chlorinated lime** [Same as the namesake monograph]

**Chlorinated lime TS** Triturate 1 g of chlorinated lime with 9 mL of water, and filter. Prepare before use.

**Chlorine**  $Cl_2$  A yellow-green gas, having a suffocating odor. It is heavier than air, and dissolves in water. Prepare from chlorinated lime with hydrochloric acid. Chlorine from a metal cylinder may be used.

**Chlorine TS** Use a saturated solution of chlorine in water. Preserve this solution in fully filled, light-resistant, glass-stoppered bottles, preferably in a cold place. In the case where the preparation of the saturated solution of chlorine is difficult, a ready-to-use aqueous solution of chlorine may be used by considering adjustment of conditions such as amount of the solution used in a test tube while noting the difference from the saturated concentration.

**Chloroacetic acid**  $C_2H_3ClO_2$  [K 8899, Special class]

**p-Chloroaniline** See 4-chloroaniline

**4-Chloroaniline**  $H_2NC_6H_4Cl$  White, crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and soluble in hot water.

*Melting point* <2.60>: 70 – 72°C

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

**4-Chlorobenzenediazonium TS** Dissolve 0.5 g of 4-chloroaniline in 1.5 mL of hydrochloric acid, and add water to make 100 mL. To 10 mL of this solution add 10 mL of sodium nitrite TS and 5 mL of acetone. Prepare before use.

**p-Chlorobenzene sulfonamide** See 4-chlorobenzene sulfonamide.

**4-Chlorobenzene sulfonamide**  $ClC_6H_4SO_2NH_2$  White to pale yellow, odorless, crystalline powder. Dissolves in acetone.

**Purity** Related substances—Dissolve 0.60 g of 4-chlorobenzene sulfonamide in acetone to make exactly 300 mL, and perform the test with 5  $\mu$ L of this solution as directed in the Purity (5) under Chlorpropamide: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.5 does not appear.

**p-Chlorobenzoic acid** See 4-chlorobenzoic acid.

**4-Chlorobenzoic acid**  $ClC_6H_4COOH$  White, crystals or powder. Sparingly soluble in ethanol (95), slightly soluble in chloroform, and practically insoluble in water.

*Melting point* <2.60>: 238 – 242°C

**Content:** not less than 99.0%. Assay—Weigh accurately about 0.3 g of 4-chlorobenzoic acid, dissolve in 30 mL of neutralized ethanol, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 15.66 mg of  $C_7H_5ClO_2$

**4-Chlorobenzophenone**  $C_{13}H_9ClO$  A white, crystalline powder or powder.

**Identification**—Determine the absorption spectrum of a solution of 4-chlorobenzophenone in ethanol (99.5) (3 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 256 nm and 260 nm.

*Melting point* <2.60>: 73 – 78°C

**Content:** not less than 98.0%.

**Assay**—Dissolve 1 g of 4-chlorobenzophenone in acetone to make 10 mL, and use this solution as the sample solution. Perform the test with 1  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of 4-chlorobenzophenone by the area percentage method.

**Operating conditions**

**Detector:** A hydrogen flame-ionization detector.

**Column:** A fused silica column 0.25 mm in inside diameter and 30 m in length, coated with dimethylpolysiloxane for gas chromatography in thickness of 0.25  $\mu$ m.

**Column temperature:** A constant temperature of about 220°C.

**Injection port temperature:** 270°C.

**Detector temperature:** 250°C.

**Carrier gas:** Helium.

**Flow rate:** 1.33 mL per minute.

**Split ratio:** 1:100.

**Time span of measurement:** About 3 times as long as the retention time of 4-chlorobenzophenone.

**System suitability**

**System performance:** To 1 mL of the sample solution add acetone to make 10 mL. When the procedure is run with 1  $\mu$ L of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of 4-chlorobenzophenone are not less than 50,000 and not more than 1.2, respectively.

**System repeatability:** To 1 mL of the sample solution add acetone to make 10 mL. When the test is repeated 6 times with 1  $\mu$ L of this solution under the above operating conditions, the relative standard deviation of the peak area of 4-chlorobenzophenone is not more than 2.0%.

**1-Chlorobutane**  $CH_3(CH_2)_3Cl$  Clear and colorless liquid, miscible with ethanol (95) and with diethyl ether, practically insoluble in water.

*Refractive index* <2.45>  $n_D^{20}$ : 1.401 – 1.045

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.884 – 0.890

*Boiling point* <2.57>: about 78°C

**Chlorobutanol**  $C_4H_7Cl_3O$  [Same as the namesake monograph]

**1-Chloro-2,4-dinitrobenzene**  $C_6H_3(NO_2)_2Cl$  Light yellow, crystals or crystalline powder.

*Melting point* <2.60>: 50 – 54°C

**Storage**—Preserve in a light-resistant tight container.

**3'-Chloro-3'-deoxythymidine for liquid chromatography**

$C_{10}H_{13}N_2O_4Cl$  Occurs as a white powder.

**Purity**—Dissolve 10 mg of 3'-chloro-3'-deoxythymidine for liquid chromatography in the mobile phase to make 100 mL. Perform the test with 10  $\mu$ L of this solution as directed in the Purity (3) under Zidovudine: a peak is not observed at the retention time for zidovudine.

**(2-Chloroethyl) diethylamine hydrochloride**

$C_6H_{14}ClN.HCl$  White powder.

**Content:** not less than 95.0%. **Assay**—Weigh accurately about 0.2 g of (2-chloroethyl)diethylamine hydrochloride, previously dried at 45°C for 3 hours under reduced pressure, and dissolve in 15 mL of acetic acid (100). To this solution add 10 mL of a mixture of acetic acid (100) and mercury (II) acetate TS for nonaqueous titration (5:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 17.21 \text{ mg of } C_6H_{14}ClN.HCl \end{aligned}$$

**Chloroform**  $CHCl_3$  [K 8322, Special class]

**Chloroform, ethanol-free** Mix 20 mL of chloroform with 20 mL of water, gently shake for 3 minutes, separate the chloroform layer, wash the layer again with two 20-mL portions of water, and filter it through dry filter paper. To the filtrate add 5 g of anhydrous sodium sulfate, shake for 5 minutes, allow the mixture to stand for 2 hours, and filter through dry filter paper. Prepare before use.

**Chloroform for water determination** To 1000 mL of chloroform add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of chloroform. Preserve the chloroform, protecting it from moisture. The water content of this chloroform should not be more than 0.1 mg per mL.

**Chlorogenic acid for thin-layer chromatography** See (*E*)-chlorogenic acid for thin-layer chromatography.

**(E)-Chlorogenic acid for thin-layer chromatography**

$C_{16}H_{18}O_9$  A white powder. Freely soluble in methanol and in ethanol (99.5), and sparingly soluble in water. Melting point: about 205°C (with decomposition).

**Purity** Related substances—Dissolve 1.0 mg of (*E*)-chlorogenic acid for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): no spot other than the principal spot with an *Rf* value of about 0.5 appears.

**p-Chlorophenol** See 4-Chlorophenol.

**4-Chlorophenol**  $ClC_6H_4OH$  Colorless or pale red, crystals or crystalline mass, having a characteristic odor. Very soluble in ethanol (95), in chloroform, in diethyl ether and in glycerin, and sparingly soluble in water. Melting point: about 43°C.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 0.2 g of 4-chlorophenol, and dissolve in water to make 100 mL. Measure exactly 25 mL of this solution into an

iodine flask, add exactly 20 mL of 0.05 mol/L bromine VS and then 5 mL of hydrochloric acid, stopper immediately, shake occasionally for 30 minutes, and allow to stand for 15 minutes. Add 5 mL of a solution of potassium iodide (1 in 5), stopper immediately, shake well, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of 0.05 mol/L bromine VS} \\ = 3.214 \text{ mg of } C_6H_5ClO \end{aligned}$$

**Storage**—Preserve in tight, light-resistant containers.

**(2-Chlorophenyl)-diphenylmethanol for thin-layer chromatography**  $C_{19}H_{15}ClO$  To 5 g of clotrimazole add 300 mL of 0.2 mol/L hydrochloric acid TS, boil for 30 minutes, cool, and extract with 100 mL of diethyl ether. Wash the diethyl ether extract with two 10 mL portions of 0.2 mol/L hydrochloric acid TS, then with two 10-mL portions of water. Shake the diethyl ether extract with 5 g of anhydrous sodium sulfate, and filter. Evaporate the diethyl ether of the filtrate, dissolve the residue in 200 mL of methanol by warming, and filter. Warm the filtrate, and add gradually 100 mL of water by stirring. Cool in an ice bath, filter the separated crystals, and dry in a desiccator (phosphorus (V) oxide) for 24 hours. A white crystalline powder. Very soluble in dichloromethane, freely soluble in diethyl ether, soluble in methanol, and practically insoluble in water.

**Melting point** <2.60>: 92 – 95°C

**Purity** Related substances—Dissolve 10 mg of (2-chlorophenyl)-diphenylmethanol for thin-layer chromatography in dichloromethane to make exactly 20 mL, and perform the test with 10  $\mu$ L of this solution as directed in the Purity (7) under Clotrimazole: any spot other than the principal spot does not appear.

**Chloroplatinic acid** See hydrogen hexachloroplatinate (IV) hexahydrate.

**Chloroplatinic acid-potassium iodide TS** See hydrogen hexachloroplatinate (IV)-potassium iodide TS.

**Chloroplatinic acid TS** See hydrogen hexachloroplatinate (IV) TS.

**3-Chloro-1,2-propanediol**  $C_3H_7ClO_2$  A clear and colorless viscous liquid.

**Purity** Dissolve 0.20 g of 3-chloro-1,2-propanediol in 100 mL of diethyl ether, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diethyl ether to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5  $\mu$ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than 3-chloro-1,2-propanediol obtained from the sample solution is not larger than 2 times the peak area from the standard solution.

**Operating conditions**

Proceed as directed in the operating conditions in the Purity (6) under Iohexol except the time span of measurement.

**Time span of measurement:** About 5 times as long as the retention time of 3-chloro-1,2-propanediol, beginning after the solvent peak.

**System suitability**

**System performance and system repeatability:** Proceed as directed in the system suitability in the Purity (6) under Iohexol.

Test for required detectability: To exactly 5 mL of the standard solution add diethyl ether to make exactly 20 mL. Confirm that the peak area of 3-chloro-1,2-propanediol obtained with 5  $\mu$ L of this solution is equivalent to 20 to 30% of that with 5  $\mu$ L of the standard solution.

**Chlorotrimethylsilane** ( $\text{CH}_3)_3\text{SiCl}$  A colorless or practically colorless liquid, having a pungent odor. Evolves fumes in a damp atmosphere. Very soluble in diethyl ether, and reactable with water or with ethanol. Boiling point: about 58°C.

**Chlorphenesin carbamate for assay**  $\text{C}_{10}\text{H}_{12}\text{ClNO}_4$  [Same as the monograph Chlorphenesin Carbamate. When dried, it contains not less than 99.0% of chlorphenesin carbamate ( $\text{C}_{10}\text{H}_{12}\text{ClNO}_4$ ).]

**Chlorpheniramine maleate**  $\text{C}_{16}\text{H}_{19}\text{ClN}_2\cdot\text{C}_4\text{H}_4\text{O}_4$  [Same as the namesake monograph]

**Chlorpromazine hydrochloride for assay**  $\text{C}_{17}\text{H}_{19}\text{ClN}_2\text{S}\cdot\text{HCl}$  [Same as the monograph Chlorpromazine Hydrochloride]

**Chlorpropamide for assay**  $\text{C}_{10}\text{H}_{13}\text{ClN}_2\text{O}_3\text{S}$  [Same as the monograph Chlorpropamide. When dried, it contains not less than 99.0% of chlorpropamide ( $\text{C}_{10}\text{H}_{13}\text{ClN}_2\text{O}_3\text{S}$ ).]

**Cholesterol**  $\text{C}_{27}\text{H}_{46}\text{OH}$  [Same as the namesake monograph]

**Cholesterol benzoate**  $\text{C}_{34}\text{H}_{50}\text{O}_2$  White crystalline powder. Melting point: 145 – 152°C.

**Cholic acid for thin-layer chromatography**  $\text{C}_{24}\text{H}_{40}\text{O}_5$  White, crystals or crystalline powder. Soluble in acetic acid (100), sparingly soluble in acetone and in ethanol (95), and very slightly soluble in water. Melting point: about 198°C.

**Purity** Related substances—Dissolve 25 mg of cholic acid for thin-layer chromatography in acetone to make exactly 250 mL and use this solution as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Dry the plate at 120°C for 30 minutes, spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 5) immediately, and heat at 120°C for 2 or 3 minutes: any spot other than the principal spot, having *Rf* value of about 0.1, does not appear.

**Content:** not less than 98.0%. Assay—Weigh accurately about 0.5 g of cholic acid for thin-layer chromatography, previously dried at 80°C for 4 hours (in vacuum, phosphorous (V) oxide), dissolve in 40 mL of neutralized ethanol and 20 mL of water, add 2 drops of phenolphthalein TS, and titrate with 0.1 mol/L sodium hydroxide VS until immediately before the end-point has been reached. Then add 100 mL of freshly boiled and cooled water, and continue the titration <2.50>. Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L sodium hydroxide VS} \\ = 40.86 \text{ mg of } \text{C}_{24}\text{H}_{40}\text{O}_5 \end{aligned}$$

**Choline chloride**  $[(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OH}]\text{Cl}$  White crystalline powder.

*Melting point* <2.60>: 303 – 305°C (with decomposition).

*Water* <2.48>: less than 0.1%.

**Chromic acid-sulfuric acid TS** Saturate chromium (VI) oxide in sulfuric acid.

**Chromium trioxide** See chromium (VI) oxide.

**Chromium trioxide TS** See chromium (VI) oxide TS.

**Chromium (VI) oxide**  $\text{CrO}_3$  A dark red-purple thin needle-shaped or inner prism-like crystals, or light masses.

*Identification*—To 5 mL of a solution (1 in 50) add 0.2 mL of lead (II) acetate TS: yellow precipitates appear, which do not dissolve on the addition of acetic acid.

**Chromium (VI) oxide TS** Dissolve 3 g of chromium (VI) oxide in water to make 100 mL.

**Chromogenic synthetic substrate** Equal amount mixture of *N*-benzoyl-L-isoleucyl-L-glutamyl-glycyl-L-arginyl-*p*-nitroanilid hydrochloride and *N*-benzoyl-L-isoleucyl-*y*-methoxy glutamyl-glycyl-L-arginyl-*p*-nitroanilid hydrochloride. White to pale yellow, masses or powder. It is slightly soluble in water.

*Identification*—Perform the test with the solution of chromogenic synthetic substrate (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorption maximum at about 316 nm is observed.

**Purity** Free 4-nitroaniline: not more than 0.5%.

*Loss on drying* <2.41>: not more than 5% (0.2 g, reduced pressure (0.3 kPa), calcium chloride, 30 to 40°C, 18 hours).

**Content:** not less than 95% and not more than 105% of the labeled amount.

**Chromophore TS for teceleukin** Mix 0.1 mL of diluted hydrogen peroxide (30) (1 in 20) with 10 mL of 0.2 mol/L citric acid buffer (pH 3.8) containing 0.2 mmol/L 3,3',5,5'-tetramethylbenzidine dihydrochloride dihydrate, and use immediately.

**Chromotropic acid** See disodium chromotropic dihydrate.

**Chromotropic acid TS** Dissolve 50 mg of disodium chromotropic dihydrate in the solution prepared by cautiously adding 68 mL of sulfuric acid to 30 mL of water, cooling, then adding water to make 100 mL. Preserve in light-resistant containers.

**Chromotropic acid TS, concentrated** Suspend 0.5 g of disodium chromotropic dihydrate in 50 mL of sulfuric acid, centrifuge, and use the supernatant liquid. Prepare before use.

**$\alpha$ -Chymotrypsin** A slightly yellowish white lyophilized powder. It contains not less than 350 U per mg of  $\alpha$ -chymotrypsin.

**Chymotrypsinogen for gel filtration molecular mass marker** A chymotrypsinogen obtained from bovine spleen. For gel filtration chromatography.

**Cibenzoline succinate for assay**  $\text{C}_{18}\text{H}_{18}\text{N}_2\cdot\text{C}_4\text{H}_6\text{O}_4$

[Same as the monograph Cibenzoline Succinate. When dried, it contains not less than 99.0% of cibenzoline succinate ( $\text{C}_{18}\text{H}_{18}\text{N}_2\cdot\text{C}_4\text{H}_6\text{O}_4$ ) and meets the following requirement.]

**Purity** Related substances—Dissolve 0.10 g of cibenzoline succinate for assay in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. To exactly 1 mL of this solution add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate

with a mixture of ethyl acetate, methanol and ammonia solution (28) (20:3:2) to a distance of about 10 cm, air-dry the plate, and dry at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution. On standing the plate for 30 minutes in the tank saturated with iodine vapor, the spot other than the principal spot with the sample solution is not more intense than the spot with the standard solution.

**Cilastatin ammonium for assay**  $C_{16}H_{29}N_3O_5S$ : 375.48  
A white crystalline powder.

**Purity** Related substances—Dissolve 40 mg of the substance to be examined in 25 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the each peak area by the automatic integration method. Separately, perform the test with 20  $\mu$ L of water in the same manner to correct any variance of the peak area caused the variation of the baseline: the total area of the peaks other than cilastatin obtained from the sample solution is not larger than 1/6 times the peak area of cilastatin from the standard solution.

#### Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (7:3).

Mobile phase B: Diluted phosphoric acid (1 in 1000).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	15 → 100	85 → 0
30 – 40	100	100

Flow rate: 2.0 mL per minute.

Time span of measurement: 40 minutes.

#### System suitability

Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 30 mL. Confirm that the peak area of cilastatin obtained with 20  $\mu$ L of this solution is equivalent to 2.3 to 4.5% of that with 20  $\mu$ L of the standard solution.

System performance: When the procedure is run with 20  $\mu$ L of the standard solution under the above operating conditions: the retention time of cilastatin is about 20 minutes, and the number of theoretical plates and the symmetry factor of the peak of cilastatin are not less than 10,000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 3 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilastatin is not more than 3.0%.

**Residual solvent**—Weigh accurately about 1 g of cilastatin

ammonium for assay, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.10 g of ethanol (99.5), add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 1  $\mu$ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the peak areas,  $A_T$  and  $A_S$ , of ethanol in each solution, and calculate the amount of ethanol ( $C_2H_5OH$ ): not more than 0.5%.

$$\text{Amount (\%)} \text{ of ethanol } (C_2H_5OH) \\ = M_S/M_T \times A_T/A_S \times 100$$

$M_S$ : Amount (mg) of ethanol (99.5) taken

$M_T$ : Amount (mg) of cilastatin ammonium for assay taken

#### Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.5 mm in inside diameter and 30 m in length, coated the inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 5  $\mu$ m.

Column temperature: Inject the sample at a constant temperature of about 50°C, maintain for 150 seconds, then raise to 70°C at the rate of 8°C per minute, and maintain for 30 seconds.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of ethanol is about 1 minute.

Split ratio: 5:1.

#### System suitability

Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 10 mL, and designate this the solution for system suitability test. To exactly 1 mL of the solution for system suitability test add water to make exactly 10 mL. Confirm that the peak area of ethanol obtained with 1  $\mu$ L of this solution is equivalent to 7 to 13% of that with 1  $\mu$ L of the solution for system suitability test.

System performance: When the procedure is run with 1  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ethanol are not less than 1500 and not more than 3.0, respectively.

System repeatability: When the test is repeated 6 times with 1  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethanol is not more than 2.0%.

**Water** <2.48>: not more than 0.5% (0.5 g, volumetric titration, direct titration).

**Residue on ignition** <2.44>: not more than 0.5% (1 g).

**Content:** not less than 99.0% of cilastatin ammonium ( $C_{16}H_{29}N_3O_5S$ ), calculated on the anhydrous and ethanol-free basis. Assay—Weigh accurately about 0.5 g of cilastatin ammonium for assay, dissolve in 30 mL of methanol, and add 5 mL of water. Adjust to pH 3.0 with 0.1 mol/L hydrochloric acid TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS from the first equivalence point to the second equivalence point (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 37.55 mg of  $C_{16}H_{29}N_3O_5S$

**Cilazapril** See cilazapril hydrate.

**Cilazapril for assay** See cilazapril hydrate for assay.

**Cilazapril hydrate**  $C_{22}H_{31}N_3O_5 \cdot H_2O$  [Same as the

namesake monograph]

**Cilazapril hydrate for assay**  $\text{C}_{22}\text{H}_{31}\text{N}_3\text{O}_5 \cdot \text{H}_2\text{O}$  [Same as the monograph Cilazapril Hydrate. It contains not less than 99.0% of cilazapril ( $\text{C}_{22}\text{H}_{31}\text{N}_3\text{O}_5$ ), calculated on the anhydrous basis.]

**Cinchonidine**  $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}$  White, crystals or crystalline powder. Soluble in methanol, in ethanol (95) and in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water. A solution of cinchonidine in ethanol (95) (1 in 100) is levorotatory. Melting point: about 207°C.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.3 g of cinchonidine, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 14.72 \text{ mg of } \text{C}_{19}\text{H}_{22}\text{N}_2\text{O} \end{aligned}$$

**Cinchonine**  $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}$  White, crystals or powder.

**Identification**—Dissolve 1 g of cinochochine in 20 mL of diluted hydrochloric acid (1 in 4), and add 2 mL of potassium hexacyanoferrate (II) TS: yellow precipitates appear, which are dissolved by heating, and crystals are formed after allowing to cool.

**Purity** Cinchonidine and quinine—To 1 g of cinchonine add 30 mL of water, add diluted hydrochloric acid (2 in 3) dropwise until the substance to be tested dissolves, and neutralize with ammonia TS. To this solution add 10 mL of a solution of sodium tartrate dihydrate (1 in 2), boil, and allow to stand for 1 hour: no precipitates appear.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.3 g of cinchonine, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 14.72 \text{ mg of } \text{C}_{19}\text{H}_{22}\text{N}_2\text{O} \end{aligned}$$

**Cineol for assay**  $\text{C}_{10}\text{H}_{18}\text{O}$  Clear and colorless liquid, having a characteristic aroma.

**Refractive index** <2.45>  $n_{\text{D}}^{20}$ : 1.457 – 1.459

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.920 – 0.930

**Purity** Related substances—Dissolve 0.10 g of cineol for assay in 25 mL of hexane and use this solution as the sample solution. Perform the test with 2  $\mu\text{L}$  of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method and calculate the amount of them by the area percentage method: the total amount of the peaks other than cineol is not more than 1.0%.

**Operating conditions**

Proceed in the operating conditions in the Assay under Eucalyptus Oil except test for time span of measurement.

**Time span of measurement:** About 3 times as long as the retention time of cineol, beginning after the solvent peak.

**System suitability**

**Test for required detectability:** To 1 mL of the sample solution add hexane to make 100 mL. Adjust so that the peak height of cineol obtained with 2  $\mu\text{L}$  of this solution is 40 to 60% of the full scale.

**Cinnamaldehyde for thin-layer chromatography** See (E)-cinnamaldehyde for thin-layer chromatography.

**(E)-Cinnamaldehyde for thin-layer chromatography**  $\text{C}_9\text{H}_8\text{O}$  A colorless or light yellow liquid, having a charac-

teristic aromatic odor. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Absorbance** <2.24>  $E_{1\text{ cm}}^{1\%}$  (285 nm): 1679 – 1943 (5 mg, methanol, 2000 mL).

**Purity** Related substances—Dissolve 10 mg of (E)-cinnamaldehyde for thin-layer chromatography in 2 mL of methanol. Perform the test with 1  $\mu\text{L}$  of this solution as directed in the Identification (3) under Kakkonto Extract: no spot other than the principal spot at an  $R_f$  value of about 0.4 appears.

**Cinnamic acid**  $\text{C}_9\text{H}_8\text{O}_2$  White crystalline powder, having a characteristic odor.

**Melting point** <2.60>: 132 – 135°C

**(E)-Cinnamic acid for assay**  $\text{C}_9\text{H}_8\text{O}_2$  Use (E)-cinnamic acid for thin-layer chromatography meeting the following additional specifications. Its content is corrected based on the amount (%) of (E)-cinnamic acid obtained in the Assay.

**Unity of peak**—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 1 mg of (E)-cinnamic acid for assay in 50 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of (E)-cinnamic acid peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

**Operating conditions**

**Column, column temperature, mobile phase, and flow rate:** Proceed as directed in the operating conditions in the Assay (1) under Ryokejutsukanto Extract.

**Detector:** A photodiode array detector (wavelength: 273 nm, spectrum range of measurement: 220 – 400 nm).

**System suitability**

**System performance:** Proceed as directed in the system suitability in the Assay (1) under Ryokejutsukanto Extract.

**Assay**—Weigh accurately 5 mg of (E)-cinnamic acid for assay and 1 mg of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve both in 1 mL of deuterated chloroform for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure  $^1\text{H-NMR}$  as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity  $A$  (equivalent to 1 hydrogen) of the signal around  $\delta$  6.20 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\begin{aligned} \text{Amount (\%)} \text{ of (E)-cinnamic acid } (\text{C}_9\text{H}_8\text{O}_2) \\ = M_S \times I \times P / (M \times N) \times 0.6541 \end{aligned}$$

**M:** Amount (mg) of (E)-cinnamic acid for assay 2 taken

**M<sub>S</sub>:** Amount (mg) of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy taken

**I:** Signal resonance intensity  $A$  based on the signal resonance intensity of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy as 18.000

**N:** Number of the hydrogen derived from  $A$

**P:** Purity (%) of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy

**Operating conditions**

**Apparatus:** A nuclear magnetic resonance spectrometer having  $^1\text{H}$  resonance frequency of not less than 400 MHz.

Target nucleus:  $^1\text{H}$ .

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between -5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

$^{13}\text{C}$  decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal of around  $\delta$  6.20 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal of around  $\delta$  6.20 ppm is no overlapped with any obvious signal of foreign substance.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity  $A$  to that of the reference standard for qNMR is not more than 1.0%.

#### (E)-Cinnamic acid for thin-layer chromatography

$\text{C}_9\text{H}_8\text{O}_2$  White, crystals or crystalline powder, having a characteristic aromatic odor. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (273 nm): 1307 – 1547 (5 mg dried with silica gel for 24 hours, methanol, 1000 mL).

*Melting point* <2.60>: 132 – 136°C

*Purity* Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 10 mg of (E)-cinnamic acid for thin-layer chromatography in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed the test with 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification (1) under Ryokejutsukanto Extract: the spot other than the principal spot at an  $R_f$  value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

**(E)-Cinnamic acid for component determination** See (E)-cinnamic acid for assay.

**Cinobufagin for assay**  $\text{C}_{26}\text{H}_{34}\text{O}_6$  A white crystalline powder. It is odorless.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (295 nm): 125 – 137 (10 mg, methanol, 250 mL). Use the sample dried in a desiccator (silica gel) for 24 hours for the test.

*Purity* Related substances—Proceed with 40 mg of cinobufagin for assay as directed in the Purity under bufalin for assay.

*Content:* not less than 98.0%. *Assay*—Weigh accurately about 10 mg of cinobufagin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method and calculate the amount of cinobufagin by the area percentage method.

#### Operating conditions

*Detector:* An ultraviolet absorption photometer (wavelength: 295 nm).

*Column:* A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 to 10  $\mu\text{m}$  in particle diameter).

*Column temperature:* A constant temperature of about 40°C.

*Mobile phase:* A mixture of water and acetonitrile (1:1).

*Flow rate:* Adjust so that the retention time of cinobufagin is about 7 minutes.

*Time span of measurement:* About twice as long as the retention time of cinobufagin, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of cinobufagin obtained with 20  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method and the peak height of cinobufagin with 20  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

System performance: Dissolve 10 mg each of cinobufagin for assay, bufalin for assay and resibufogenin for assay in 200 mL of methanol. When the procedure is run with 20  $\mu\text{L}$  of this solution under the above operating conditions, bufalin, cinobufagin and resibufogenin are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

**Cinobufagin for component determination** See cinobufagin for assay.

**Cinoxacin for assay**  $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_5$  [Same as the monograph Cinoxacin. When dried, it contains not less than 99.0% of cinoxacin ( $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_5$ ).]

**Cisplatin**  $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$  [Same as the namesake monograph]

**0.05 mol/L Citrate buffer solution (pH 6.6)** Dissolve 147 g of trisodium citrate dihydrate in 2000 mL of water, add 3.6 g of citric acid monohydrate to dissolve, and add water to make 10 L. To this solution add 0.1 mol/L sodium citrate TS or 0.1 mol/L citric acid TS to adjust the pH to 6.6.

**Citric acid** See citric acid monohydrate.

**Citric acid-acetic acid TS** To 1 g of citric acid monohydrate add 90 mL of acetic anhydride and 10 mL of acetic acid (100), and dissolve under shaking.

**Citric acid-acetic anhydride TS** To 1 g of citric acid monohydrate add 50 mL of acetic anhydride, and dissolve by heating. Prepare before use.

**Citric acid monohydrate**  $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$  [K 8283, or same as the monograph Citric Acid Hydrate]

**Citric acid-phosphate-acetonitrile TS** Dissolve 2.1 g of citric acid monohydrate, 13.4 g of dipotassium hydrogen phosphate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1).

**0.01 mol/L Citric acid TS** Dissolve 2.1 g of citric acid monohydrate in water to make 1000 mL.

**0.1 mol/L Citric acid TS** Dissolve 21 g of citric acid monohydrate in water to make 1000 mL.

**1 mol/L Citric acid TS for buffer solution** Dissolve 210.14 g of citric acid monohydrate in water to make 1000 mL.

**Clofibrate**  $C_{12}H_{15}ClO_3$  [Same as the namesake monograph]

**Clomipramine hydrochloride for assay**  $C_{19}H_{23}ClN_2.HCl$  [Same as the monograph Clomipramine Hydrochloride. When dried, it contains not less than 99.0% of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2.HCl$ ).]

**Clonazepam for assay**  $C_{15}H_{10}ClN_3O_3$  [Same as the monograph Clonazepam]

**32D Clone3 cells** A cloned cell line established by culturing mouse bone marrow origin 32D cell line in the presence of G-CSF.

**Cloperastine fendizoate for assay**  $C_{20}H_{24}ClNO.C_{20}H_{14}O_4$  [Same as the monograph Cloperastine Fendizoate]

**Clorazepate dipotassium for assay**  $C_{16}H_{10}ClKN_2O_3.KOH$  [Same as the monograph Clorazepate Dipotassium. When dried it contains not less than 99.0% of clorazepate dipotassium ( $C_{16}H_{10}ClKN_2O_3.KOH$ ).]

**Clotiazepam for assay**  $C_{16}H_{15}ClN_2OS$  [Same as the monograph Clotiazepam. When dried, it contains not less than 99.0% of clotiazepam ( $C_{16}H_{15}ClN_2OS$ ).]

**Clotrimazole**  $C_{22}H_{17}ClN_2$  [Same as the namesake monograph]

**Cloxacizolam**  $C_{17}H_{14}Cl_2N_2O_2$  [Same as the namesake monograph]

**Cobalt (II) chloride-ethanol TS** Dissolve 0.5 g of cobalt (II) chloride hexahydrate, previously dried at 105°C for 2 hours, in ethanol (99.5) to make 100 mL.

**Cobalt (II) chloride hexahydrate**  $CoCl_2.6H_2O$  [K 8129, Special class]

**Cobalt (II) chloride TS** Dissolve 2 g of cobalt (II) chloride hexahydrate in 1 mL of hydrochloric acid and water to make 100 mL (0.08 mol/L).

**Cobalt (II) nitrate hexahydrate**  $Co(NO_3)_2.6H_2O$  [K 8552, Special class]

**Cobaltous chloride** See cobalt (II) chloride hexahydrate.

**Cobaltous nitrate** See cobalt (II) nitrate hexahydrate.

**Codeine phosphate for assay** See codeine phosphate hydrate for assay.

**Codeine phosphate hydrate for assay**  $C_{18}H_{21}NO_3.H_3PO_4.1/2H_2O$  [Same as the monograph Codeine Phosphate Hydrate. It contains not less than 99.0% of codeine phosphate ( $C_{18}H_{21}NO_3.H_3PO_4$ ), calculated on the anhydrous basis.]

**Collodion** Clear, colorless, viscous liquid, having a diethyl ether-like odor.

**pH** <2.54>: 5.0–8.0

Stir 5 g of collodion while warming, add 10 mL of water gradually, and dry at 110°C after evaporating to dryness: mass of the residue is 0.250–0.275 g.

**Concentrated chromotropic acid TS** See chromotropic acid, concentrated.

**Concentrated diazobenzenesulfonic acid TS** See diazobenzenesulfonic acid TS, concentrated.

**Concentrated potassium iodide TS** See potassium iodide TS, concentrated.

**Congo red**  $C_{32}H_{22}N_6Na_2O_6S_2$  [K 8352, Special class]

**Congo red TS** Dissolve 0.5 g of congo red in 100 mL of a mixture of water and ethanol (95) (9:1).

**Coomassie brilliant blue G-250**  $C_{47}H_{48}N_3NaO_7S_2$  A deep violet powder. A solution in ethanol (99.5) (1 in 100,000) exhibits an absorption maxima at a wavelength of 608 nm.

**Coomassie brilliant blue R-250**  $C_{45}H_{44}N_3NaO_7S_2$  Deep blue-purple powder. Odorless.

**Content:** not less than 50%.

**Coomassie brilliant blue TS for interferon alfa** Dissolve 20 mg of Coomassie brilliant blue G-250 in diluted perchloric acid (43 in 1000) to make 100 mL, and filter. Determine the absorbance of the filtrate at 465 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, and add Coomassie brilliant blue G-250 or diluted perchloric acid (43 in 1000) so that the absorbance is 1.3–1.5.

**Coomassie staining TS** Dissolve 125 mg of Coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

**Copper** Cu [K 8660, Special class]

**Copper (standard reagent)** Cu In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

**Copper (II) acetate monohydrate**  $Cu(CH_3COO)_2.H_2O$  Blue-green, crystals or crystalline powder.

**Identification**—(1) Dissolve 1 g of copper (II) acetate monohydrate in 10 mL of diluted sulfuric acid (1 in 2), and heat: the odor of acetic acid is perceptible.

(2) Dissolve 0.1 g of copper (II) acetate monohydrate in 20 mL of water, and add 3 mL of ammonia solution (28): a dark blue color is developed.

**Copper (II) acetate TS, strong** Dissolve 13.3 g of copper (II) acetate monohydrate in a mixture of 195 mL of water and 5 mL of acetic acid.

**Copper (II) chloride-acetone TS** Dissolve 0.3 g of copper (II) chloride dihydrate in acetone to make 10 mL.

**Copper (II) chloride dihydrate**  $CuCl_2.2H_2O$  [K 8145, Special class]

**Copper (II) citrate TS** Dissolve 25 g of copper (II) sulfate pentahydrate, 50 g of citric acid monohydrate and 144 g of anhydrous sodium carbonate in water to make 1000 mL.

**Copper (II) disodium ethylenediamine tetraacetate tetrahydrate**  $C_{10}H_{12}CuN_2Na_2O_8.4H_2O$  A blue powder.

**pH** <2.54>: 7.0–9.0

**Purity** Clarity and color of solution—Add 0.10 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate to 10 mL of freshly boiled and cooled water: the solution is blue in color and clear.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.45 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate, and add water to make exactly 100 mL. Pipet 10 mL of this solution, adjust the pH of the mixture to about 1.5 by adding 100 mL of dilute nitric acid,

then add 5 mL of a solution of 1,10-phenanthroline monohydrate in methanol (1 in 20), and titrate <2.50> with 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylanol orange TS).

Each mL of 0.01 mol/L bismuth nitrate VS  
= 4.698 mg of  $C_{10}H_{12}CuN_2Na_2O_8 \cdot 4H_2O$

**Copper (II) hydroxide**  $Cu(OH)_2$  Light blue powder. Practically insoluble in water.

**Content:** not less than 95.0% as  $Cu(OH)_2$ . Assay—Weigh accurately about 0.6 g of Copper (II) hydroxide, and dissolve in 3 mL of hydrochloric acid and water to make exactly 500 mL. Pipet 25 mL of this solution, add 75 mL of water, 10 mL of a solution of ammonium chloride (3 in 50), 3 mL of diluted ammonia solution (28) (1 in 10) and 0.05 g of murexide-sodium chloride indicator, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the liquid is changed from yellow-green to red-purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 0.9756 mg of  $Cu(OH)_2$

**Copper (II) nitrate trihydrate**  $Cu(NO_3)_2 \cdot 3H_2O$  Blue, crystals or crystalline powder. Very soluble in water, and freely soluble in ethanol (99.5).

**Identification** (1) A solution of copper (II) nitrate trihydrate (1 in 10) responds to Qualitative Tests <1.09> (2) for cupric salt.

(2) A solution of copper (II) nitrate trihydrate (1 in 10) responds to Qualitative Tests <1.09> (1) for nitrate.

**Purity** (1) Iron—Weigh accurately 5.0 g of copper (II) nitrate trihydrate, add 10 mL of a mixture of water and nitric acid (2:1), add water to make exactly 100 mL, and use this solution as the sample stock solution. Pipet 20 mL of the sample stock solution, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, pipet 20 mL of the sample stock solution, add exactly 3 mL of Standard Iron Solution add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the absorbances,  $A_T$  and  $A_S$ , of the sample solution and standard solution:  $A_T$  is not greater than ( $A_S - A_T$ ) (not more than 0.003%).

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: Iron hollow-cathode lamp.

Wavelength: 248.3 nm.

(2) Zinc—Use the sample solution in (1) as the sample solution. Separately, pipet 20 mL of the sample stock solution in (1), add exactly 5 mL of a solution, prepared by adding water to exactly 4 mL of Standard Zinc Solution to make exactly 10 mL, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the absorbances,  $A_T$  and  $A_S$ , of the sample solution and standard solution:  $A_T$  is not greater than ( $A_S - A_T$ ) (not more than 0.005%).

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: Zinc hollow-cathode lamp.

Wavelength: 213.9 nm.

(3) Calcium—Use the sample solution in (1) as the sam-

ple solution. Separately, pipet 20 mL of the sample stock solution in (1), add exactly 5 mL of a solution, prepared by adding water to exactly 1 mL of Standard Calcium Solution to make exactly 10 mL, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the absorbances,  $A_T$  and  $A_S$ , of the sample solution and standard solution:  $A_T$  is not greater than ( $A_S - A_T$ ) (not more than 0.005%).

Gas: Combustible gas—Acetylene.

Supporting gas—Air or nitrous oxide.

Lamp: Calcium hollow-cathode lamp.

Wavelength: 422.7 nm.

(4) Nickel—Use the sample solution in (1) as the sample solution. Separately, pipet 20 mL of the sample stock solution in (1), add exactly 4 mL of Standard Nickel Solution and water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the absorbances,  $A_T$  and  $A_S$ , of the sample solution and standard solution:  $A_T$  is not greater than ( $A_S - A_T$ ) (not more than 0.002%).

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: Nickel hollow-cathode lamp.

Wavelength: 232.0 nm.

**Content**—Not less than 77.0% and not more than 80.0% as  $Cu(NO_3)_2$ . Assay—Weigh accurately about 0.6 g of copper (II) nitrate trihydrate, dissolve in water to make exactly 250 mL. Pipet 25 mL of this solution, add 75 mL of water, 6 mL of ammonium chloride solution (1 in 10), and 1 mL of a mixture of water and ammonia solution (28) (10:1), and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution is changed from green to red-purple (indicator: 50 mg of murexide-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 1.876 mg of  $Cu(NO_3)_2$

**Copper (II) sulfate**  $CuSO_4$  [K 8984, First class]

**Copper (II) sulfate pentahydrate**  $CuSO_4 \cdot 5H_2O$   
[K 8983, Special class]

**Copper (II) sulfate-pyridine TS** Dissolve 4 g of copper (II) sulfate pentahydrate in 90 mL of water, then add 30 mL of pyridine. Prepare before use.

**Copper (II) sulfate TS** Dissolve 12.5 g of copper (II) sulfate pentahydrate in water to make 100 mL (0.5 mol/L).

**Copper (II) sulfate TS, alkaline** Dissolve 150 g of potassium bicarbonate, 101.4 g of potassium carbonate and 6.93 g of copper (II) sulfate pentahydrate in water to make 1000 mL.

#### Coptisine chloride for thin-layer chromatography

$C_{19}H_{14}NO_4Cl$  Orange-red, crystals or crystalline powder. Slightly soluble in methanol, and very slightly soluble in water and in ethanol (99.5). Melting point: about 260°C (with decomposition).

**Identification** Determine the absorption spectrum of a solution of coptisine chloride for thin-layer chromatography (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 237 nm and

241 nm, between 264 nm and 268 nm, between 354 nm and 358 nm, and between 452 nm and 462 nm.

**Purity** Related substances—Dissolve 1 mg of coptisine chloride for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of methanol, ammonium acetate solution (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spot other than the principal spot at an *R<sub>f</sub>* value of about 0.4 obtained from the sample solution is not more intense than the spot from the standard solution.

**Corn oil** [Same as the namesake monograph]

**Cortisone acetate** C<sub>23</sub>H<sub>30</sub>O<sub>6</sub> [Same as the namesake monograph]

**Cottonseed oil** A refined, nonvolatile fatty oil obtained from the seed of plants of *Gossypium hirsutum* Linné (*Gossypium*) or of other similar species. A pale yellow, odorless, oily liquid. Miscible with chloroform, with diethyl ether, and with hexane and with carbon disulfide. Slightly soluble in ethanol (95).

Refractive index <2.45>  $n_D^{20}$ : 1.472 – 1.474

Specific gravity <2.56>  $d_{25}^{25}$ : 0.915 – 0.921

Acid value <1.13>: not more than 0.5.

Saponification value <1.13>: 190 – 198

Iodine value <1.13>: 103 – 116

**Cresol** CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>(OH) [Same as the namesake monograph]

**m-Cresol** CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>(OH) [K 8305, Special class]

**p-Cresol** C<sub>7</sub>H<sub>8</sub>O [K 8306, Special class]

**Cresol red** C<sub>21</sub>H<sub>18</sub>O<sub>5</sub>S [K 8308, Special class]

**Cresol red TS** Dissolve 0.1 g of cresol red in 100 mL of ethanol (95), and filter if necessary.

**Crystalline trypsin** To trypsin obtained from bovine pancreas add an appropriate amount of trichloroacetic acid to precipitate the trypsin, and recrystallize in ethanol (95). White to yellow-white, crystals or powder. It is odorless. Freely soluble in water and in sodium tetraborate-calcium chloride buffer solution (pH 8.0).

**Content:** not less than 45 FIP Units of trypsin per mg.

**Assay**—(i) Sample solution: Weigh accurately an appropriate amount of crystalline trypsin, dissolve in 0.001 mol/L hydrochloric acid TS to prepare a solution containing 50 FIP Units per mL, and use this solution as the sample solution. Prepare before use, and preserve in ice.

(ii) Apparatus: Use a glass bottle as a reaction reservoir, 20 mm in inside diameter and 50 mm in height, stopper with a rubber stopper equipped with a glass/silver-silver chloride electrode for pH determination, a nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature of the bath at 25 ± 0.1°C by means of a precise thermoregulator.

(iii) Procedure: Pipet 1.0 mL of *N*-α-benzoyl-L-arginine ethyl ester TS, transfer to the reaction reservoir, and add 9.0 mL of sodium tetraborate-calcium chloride buffer solution (pH 8.0). Allow to stand in the thermostat for 10 minutes to make the temperature of the contents reach to 25 ± 0.1°C,

adjust the pH of the solution to 8.00 by adding dropwise 0.1 mol/L sodium hydroxide VS while stirring and passing a current of nitrogen, add 0.05 mL of the sample solution previously allowed to stand at 25 ± 0.1°C, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50  $\mu$ L-micropipet (minimum graduation of 1  $\mu$ L) while stirring, to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 8 minutes. Separately, transfer 10 mL of sodium tetraborate-calcium chloride buffer solution (pH 8.0), and perform a blank determination in the same manner.

(iv) Calculation: Plot the amount of consumption ( $\mu$ L) of 0.1 mol/L sodium hydroxide VS against the reaction time (minutes), select linear reaction times,  $t_1$  and  $t_2$ , designate the corresponding consumption amount of 0.1 mol/L sodium hydroxide VS as  $v_1$  and  $v_2$ , respectively, and designate  $\mu$ mol of sodium hydroxide consumed per minute as  $D$  (FIP Unit).

$$D \text{ } (\mu\text{mol NaOH/min}) = \frac{v_2 - v_1}{t_2 - t_1} \times \frac{1}{10}$$

FIP Units per mL of crystalline trypsin

$$= \frac{(D_1 - D_0) \times T}{L \times M}$$

$D_1$ :  $\mu$ mol of sodium hydroxide consumed in 1 minute when the sample solution is used

$D_0$ :  $\mu$ mol of sodium hydroxide consumed in 1 minute when the solution for blank determination is used

$M$ : Amount (mg) of crystalline trypsin taken

$L$ : Amount (mL) of the sample solution put in the reaction reservoir

$T$ : Total volume (mL) of the sample solution

One FIP Unit is an amount of enzyme which decomposes 1  $\mu$ mol of *N*-α-benzoyl-L-arginine ethyl ester per minute under the conditions described in the Assay.

**Storage**—Preserve in a cold place.

**Crystalline trypsin for ulinastatin assay** A proteolytic enzyme prepared from bovine pancreas. White to light yellow crystalline powder. Odorless. Sparingly soluble in water, and dissolves in 0.001 mol/L hydrochloric acid TS.

**Content:** not less than 3200 trypsin Units per mg. **Assay**—(i) Sample solution: Weigh accurately about 20 mg of crystalline trypsin for ulinastatin assay, and dissolve in 0.001 mol/L hydrochloric acid TS so that each mL of the solution contains about 3000 trypsin Units. Dilute this solution with 0.001 mol/L hydrochloric acid TS so that each mL of the solution contains about 40 trypsin Units, and use this solution as the sample solution.

(ii) Diluent: Dissolve 4.54 g of potassium dihydrogen phosphate in water to make exactly 500 mL (Solution I). Dissolve 4.73 g of anhydrous disodium hydrogen phosphate in water to make exactly 500 mL (Solution II). To 80 mL of Solution II add a suitable amount of Solution I to adjust to pH 7.6.

(iii) Substrate solution: Dissolve 85.7 mg of *N*-α-benzoyl-L-arginine ethyl ester hydrochloride in water to make exactly 100 mL, and use this solution as the substrate stock solution. Pipet 10 mL of the substrate stock solution, add the diluent to make exactly 100 mL, and use this solution as the substrate solution. The absorbance of the substrate solution determined at 253 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank is between 0.575 and 0.585. If the absorbance of the substrate solution is not in this range, adjust with the diluent or the substrate stock solution.

(iv) Procedure: Pipet 3 mL of the substrate solution, previ-

ously warmed at  $25 \pm 0.1^\circ\text{C}$ , into a 1-cm quartz cell, add exactly 0.2 mL of the sample solution, and start the determination of the absorbance change at 253 nm for 5 minutes at  $25 \pm 0.1^\circ\text{C}$  using a solution prepared by adding exactly 0.2 mL of 0.001 mol/L hydrochloric acid TS to exactly 3 mL of the substrate solution as the blank. Determine the difference of the absorbance change per minute,  $A$ , when the difference has been constant for at least 3 minutes.

(v) Calculation: Trypsin Units per mg is obtained by use of the following equation. One trypsin Unit is an amount of the enzyme which gives 0.003 change in absorbance per minute under the conditions described above.

$$\text{Trypsin Units per mg} = \frac{A}{0.003 \times M}$$

$M$ : Amount (mg) of the substance to be assayed in 0.2 mL of the sample solution

**Storage**—Preserve in a cold place.

**Crystal violet**  $\text{C}_{25}\text{H}_{30}\text{ClN}_3 \cdot 9\text{H}_2\text{O}$  [K 8294, Special class]

**Crystal violet TS** Dissolve 0.1 g of crystal violet 10 mL of acetic acid (100).

**Culture medium for celmoleukin** Take a specified amount of RPMI-1640 powdered medium, add water to dissolve, and add *N*-2-hydroxyethylpiperazine-*N'*-2-ethansulfonic acid as a buffering agent to a concentration of 0.025 mol/L. To 1000 mL of this solution add 0.1 g (potency) of streptomycin sulfate, 100,000 units of potassium benzylpenicillin, and 2 g of sodium hydrogen carbonate, adjust the pH to 7.1 to 7.2 with sodium hydroxide TS, and then sterilize by filtration. To this solution add fetal calf serum heated at  $56^\circ\text{C}$  for 30 minutes to 20 vol%.

**Cu-PAN** Prepare by mixing 1 g of 1-(2-pyridylazo)-2-naphthol (free acid) with 11.1 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate. A grayish orange-yellow, grayish red-brown or light grayish purple powder.

**Absorbance**—Dissolve 0.50 g of Cu-PAN in diluted 1,4-dioxane (1 in 2) to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 100 mL. Read the absorbance of this solution at 470 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank solution: the absorbance is not less than 0.48.

**Purity** Clarity and color of solution—Dissolve 0.50 g of Cu-PAN in 50 mL of diluted 1,4-dioxane (1 in 2): the solution is clear and yellow-brown.

**Cu-PAN TS** Dissolve 1 g of Cu-PAN in 100 mL of diluted 1,4-dioxane (1 in 2).

**Cupferron**  $\text{C}_6\text{H}_9\text{N}_3\text{O}_2$  [K 8289, Special class]

**Cupferron TS** Dissolve 6 g of cupferron in water to make 100 mL. Prepare before use.

**Cupric acetate** See copper (II) acetate monohydrate.

**Cupric acetate TS, strong** See copper (II) acetate monohydrate TS, strong.

**Cupric carbonate** See cupric carbonate monohydrate.

**Cupric carbonate monohydrate**  $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2 \cdot \text{H}_2\text{O}$  A blue to blue-green powder. It is insoluble in water, and dissolves foamingly in dilute acid. It dissolves in ammonia TS and shows a deep blue color.

**Purity** (1) Chloride <1.03>: not more than 0.036%.

(2) Sulfate <1.14>: not more than 0.120%.

(3) Iron—Dissolve 5.0 g of cupric carbonate monohy-

drate in excess ammonia TS and filter. Wash the residue with ammonia TS, dissolve in dilute hydrochloric acid, add excess ammonia TS and filter. Wash the residue with ammonia TS, and dry to constant mass: the residue is not more than 10 mg.

**Cupric chloride** See copper (II) chloride dihydrate.

**Cupric chloride-acetone TS** See copper (II) chloride-acetone TS.

**Cupric sulfate** See copper (II) sulfate pentahydrate.

**Cupric sulfate, anhydrous** See copper (II) sulfate (anhydrous).

**Cupric sulfate-pyridine TS** See copper (II) sulfate-pyridine TS.

**Cupric sulfate TS, alkaline** See copper (II) sulfate TS, alkaline.

**Cupric sulfate TS** See copper (II) sulfate TS.

**1 mol/L Cupriethylenediamine TS** Put 100 g of copper (II) hydroxide in a 1-L thick-walled bottle marked a 500-mL line, and add water to make 500 mL. Connect the bottle with a liquid introducing funnel, a nitrogen introducing glass tube and a gas removing glass tube. Adjust so that the lower end of the nitrogen introducing tube is located at about 1.3 cm above of the bottom of the bottle. Introduce the nitrogen for about 3 hours to replacing the inside gas by adjusting the pressure (about 14 kPa) to get a mild bubbling. Then add gradually 160 mL of ethylenediamine TS through the funnel while introducing the nitrogen and cooling the bottle with the running water, and replace the funnel with a glass rod to close tightly. After introducing the nitrogen for further 10 minutes, replace the gas removing tube with a glass rod to close tightly. Keep the inside pressure with the nitrogen to about 14 kPa. After allowing the bottle to stand for about 16 hours while occasional shaking, filter the content if necessary using a glass-filter under reducing pressure, and reserve under nitrogen atmosphere. The concentration of copper (II) ion of this solution is about 1.3 mol/L. Determine the concentration of ethylenediamine of this solution  $X$  (mol/L) and copper (II) ion  $Y$  (mol/L) by the following Assays, and adjust to that  $X$  is 1.96–2.04,  $Y$  is 0.98–1.02 and  $X/Y$  is 1.96–2.04 by adding water, copper (II) hydroxide or ethylenediamine TS, then determine  $X$  and  $Y$  again in the same manner, and use this solution as the test solution.

**Assay** (1) Ethylenediamine—Pipet 1 mL ( $V_1$ ) of the solution to be assayed, add 60 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (pH Determination <2.54>; End point is about pH 8.4).

$$X = \frac{N_1 a}{V_1}$$

$X$ : Concentration of ethylenediamine (mol/L)

$a$ : Volume of 0.1 mol/L hydrochloric acid VS consumed for the titration (mL)

$N_1$ : Concentration of 0.1 mol/L hydrochloric acid VS (mol/L)

(2) Copper (II) ion—Pipet 2 mL ( $V_2$ ) of the solution to be assayed, add 20 mL of water, about 3 g of potassium iodide and 50 mL of 2 mol/L sulfuric acid TS, shake for 5 minutes, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution turns light yellow at near the end point add 3 mL of starch TS and 10 mL of a solution of ammonium thiocyanate (1 in 5), and then titrate until the blue color disappears.

$$Y = \frac{N_2 b}{V_2}$$

*Y*: Concentration of copper (II) ion (mol/L)  
*b*: Volume of 0.1 mol/L sodium thiosulfate VS consumed for the titration (mL)  
*N<sub>2</sub>*: Concentration of 0.1 mol/L sodium thiosulfate VS (mol/L)

**Curcumin** C<sub>21</sub>H<sub>20</sub>O<sub>6</sub> A reddish yellow crystalline powder.

*Melting point* <2.60>: 180 – 183°C

*Storage*—Preserve in a light-resistant tight container.

**Curcumin TS** Dissolve 0.125 g of curcumin in acetic acid (100) to make 100 mL. Prepare before use.

**Curcumin for assay** C<sub>21</sub>H<sub>20</sub>O<sub>6</sub> Yellow to orange crystalline powder. Slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water.

*Absorbance* <2.24> E<sub>1 cm</sub><sup>1%</sup> (422 nm): 1460 – 1700 [dried in a desiccator (in vacuum, silica gel for 24 hours), 2.5 mg, methanol, 1000 mL.]

*Melting point* <2.60>: 180 – 184°C

**Purity** Related substances—(1) Dissolve 4 mg of curcumin for assay in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at the *Rf* value of about 0.5 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of curcumin for assay in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than curcumin obtained from the sample solution is not larger than the peak area of curcumin from the standard solution.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Turmeric.

Detector: A visible absorption photometer (wavelength: 422 nm).

Time span of measurement: About 4 times as long as the retention time of curcumin, beginning after the solvent peak. System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Turmeric.

Test for required detectability: Pipet 1 mL of the standard solution, add methanol to make exactly 20 mL. Confirm that the peak area of curcumin obtained with 10 µL of this solution is equivalent to 3.5 to 6.5% of that with 10 µL of the standard solution.

**Curcumin for component determination** See curcumin for assay.

**Cyanoacetic acid** C<sub>3</sub>H<sub>3</sub>NO<sub>2</sub> White to light yellow crystals. Very soluble in water.

**Content:** not less than 99%. Assay—Weigh accurately about 300 mg of cyanoacetic acid, add 25 mL of water and 25 mL of ethanol (95) to dissolve, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 85.06 \text{ mg of C}_3\text{H}_3\text{NO}_2 \end{aligned}$$

**Cyanocobalamin** C<sub>63</sub>H<sub>88</sub>CoN<sub>14</sub>O<sub>14</sub>P [Same as the namesake monograph]

**Cyanogen bromide TS** To 100 mL of ice-cold water add 1 mL of bromine, shake vigorously, and add ice-cold potassium cyanide TS dropwise until the color of bromine just disappears. Prepare this test solution in a draft chamber before use.

On handling this solution, be careful not to inhale its vapors, which are very toxic.

**1-Cyanoguanidine** NH<sub>2</sub>C(NH)NHCN A white crystalline powder. Freely soluble in water.

*Melting point* <2.60>: 209 – 212°C

*Loss on drying* <2.41>: not more than 0.1% (1 g, 105°C, 3 hours).

*Nitrogen content* <1.08>: 66.0 – 67.3% (after drying).

**Cyanopropylmethylphenylsilicone for gas chromatography** Prepared for gas chromatography.

**6% Cyanopropylphenyl-94% dimethyl silicone polymer for gas chromatography** Prepared for gas chromatography.

**6% Cyanopropyl-6% phenyl-methyl silicone polymer for gas chromatography** Prepared for gas chromatography.

**7% Cyanopropyl-7% phenyl-methyl silicone polymer for gas chromatography** Prepared for gas chromatography.

#### Cycloartenyl ferulate for thin-layer chromatography

C<sub>40</sub>H<sub>58</sub>O<sub>4</sub> A white to light brown, crystalline powder or powder. Soluble in acetone, slightly soluble in acetonitrile, and practically insoluble in water and in methanol. Melting point: about 155°C.

**Identification** (1) Determine the absorption spectrum of a solution of cycloartenyl ferulate for thin-layer chromatography in heptane (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 229 nm and 233 nm, between 289 nm and 293 nm, and between 313 nm and 317 nm.

(2) Determine the infrared absorption spectrum of cycloartenyl ferulate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 2940 cm<sup>-1</sup>, 1691 cm<sup>-1</sup>, 1511 cm<sup>-1</sup> and 1270 cm<sup>-1</sup>.

**Purity** Related substances—Dissolve 2.0 mg of cycloartenyl ferulate for thin-layer chromatography in 2 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 5 µL each of the sample solution and standard solution as directed in the Identification under Brown Rice: the spot other than the principle spot at an *Rf* value of about 0.4, obtained from the sample solution is not more intense than the spot from the standard solution.

**Cyclobutanecarboxylic acid**  $C_5H_8O_2$  A clear and colorless liquid. Congealing point:  $-7.5^{\circ}\text{C}$ .

**1,1-Cyclobutanedicarboxylic acid**  $C_6H_8O_4$  White crystals.

*Melting point* <2.60>: 159 – 163  $^{\circ}\text{C}$

**Purity** Related substances—Dissolve 20 mg of 1,1-cyclobutanedicarboxylic acid in 100 mL of the mobile phase used in the Purity (1) under Carboplatin, and use this solution as the sample solution. Perform the test with 25  $\mu\text{L}$  of the sample solution as directed in the Purity (1) under Carboplatin. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the total amount of the peaks other than 1,1-cyclobutanedicarboxylic acid is not more than 2%. However, the time span of measurement for this calculation is about 2 times as long as the retention time of 1,1-cyclobutanedicarboxylic acid, beginning after the solvent peak.

**Content:** not less than 99.0%. **Assay**—Dissolve about 30 mg of 1,1-cyclobutanedicarboxylic acid, accurately weighed, in 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 7.207 \text{ mg of } C_6H_8O_4 \end{aligned}$$

**Cyclohexane**  $C_6H_{12}$  [K 8464, Special class]

**Cyclohexylamine**  $C_6H_{11}NH_2$  A clear and colorless liquid, having a characteristic amine-like odor. Miscible with water, with *N,N*-dimethylformamide and with acetone.

**Purity** Related substances—Use cyclohexylamine as the sample solution. Separately, pipet 1 mL of cyclohexylamine, add hexane to make exactly 100 mL, and use this solution as the standard solution. Perform the test as directed in Thin-layer Chromatography <2.03>. Spot 5  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol, ammonia water (28) and cyclohexane (6:2:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Cyclohexylmethanol**  $C_7H_{14}O$  A liquid having slight camphor odor. Soluble in ethanol (99.5).

*Refractive index* <2.45>  $n_D^{20}$ : about 1.464

*Boling point* <2.57>: about 185  $^{\circ}\text{C}$ .

**Cyclophosphamide hydrate for assay**

$C_7H_{15}Cl_2N_2O_2P.H_2O$  [Same as the monograph Cyclophosphamide Hydrate. It contains not less than 99.0% of cyclophosphamide hydrate ( $C_7H_{15}Cl_2N_2O_2P.H_2O$ ).]

**Cyclosporine U**  $C_{81}H_{109}N_{11}O_{12}$  White powder.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : about  $-190^{\circ}\text{C}$  (0.1 g, methanol, 20 mL 100 mm).

**L-Cysteic acid**  $C_3H_7NO_5S$  White powder.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +7.5 – +9.0° (1.5 g, water, 20 mL, 100 mm).

*Melting point* <2.60>: about 260  $^{\circ}\text{C}$ .

**L-Cysteine hydrochloride** See L-cysteine hydrochloride monohydrate.

**L-Cysteine hydrochloride monohydrate**

$HSCH_2CH(NH_2)COOH.HCl.H_2O$  [K 8470, Special class]

**L-Cystine**  $HOOCCH(NH_2)CH_2SSCH_2CH(NH_2)COOH$  [K 9048, L(-)-Cystine, Special class]

**Cytochrome c** An oxidase (molecular weight: 8000 – 13,000) derived from bovine cardiac muscle.

**Cytosine**  $C_4H_5N_3O$  White, crystalline powder or powder.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (276 nm): not less than 800 (after drying, 40 mg, 10,000 mL of 0.1 mol/L hydrochloric acid TS).

**Dacuronium bromide for thin-layer chromatography**

$C_{33}H_{58}Br_2N_2O_3$  White crystalline powder. Very soluble in water, freely soluble in ethanol (95), and practically insoluble in acetic anhydride. Hygroscopic.

**Identification**—Determine the infrared absorption spectrum of dacuronium bromide for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorptions at the wave numbers at about  $2940\text{ cm}^{-1}$ ,  $1737\text{ cm}^{-1}$ ,  $1630\text{ cm}^{-1}$ ,  $1373\text{ cm}^{-1}$ ,  $1233\text{ cm}^{-1}$  and  $1031\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 10 mg of dacuronium bromide for thin-layer chromatography in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Purity (2) Related substances under Pancuronium Bromide: the spots other than the principal spot obtained from the sample solution do not show more intense color than the spot from the standard solution.

*Water* <2.48>: not more than 1.0% (1 g, volumetric titration, direct titration).

**Content:** not less than 98.0%, calculated on the anhydrous basis. **Assay**—Weigh accurately about 0.2 g of dacuronium bromide for thin-layer chromatography, dissolve in 50 mL of acetic anhydride by warming, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 34.53 \text{ mg of } C_{33}H_{58}Br_2N_2O_3 \end{aligned}$$

**p,p'-DDD (2,2-Bis(4-chlorophenyl)-1,1-dichloroethane)**

$C_{14}H_{10}Cl_4$

*Melting point* <2.60>: 108 – 110  $^{\circ}\text{C}$

**Purity** Related substances—Dissolve 10 mg of *p,p'*-DDD in hexane for purity of crude drug to make exactly 100 mL, pipet 1 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak area other than *p,p'*-DDD obtained from the sample solution is not larger than the peak area of *p,p'*-DDD from the standard solution (1).

**Operating conditions**

Proceed the operating conditions in the Purity 4.3. under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

**Detection sensitivity:** Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20

mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of *p,p'*-DDD obtained with 1  $\mu$ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of *p,p'*-DDD with 1  $\mu$ L of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of *p,p'*-DDD, beginning after the solvent peak.

***p,p'*-DDE (2,2-Bis(4-chlorophenyl)-1,1-dichloroethylene)**  $C_{14}H_8Cl_4$

*Melting point* <2.60>: 88 – 90°C

*Purity* Related substances—Proceed as directed in the *Purity* of *p,p'*-DDD using the following standard solution (1).

Standard solution (1): Pipet 1 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

***o,p'*-DDT (1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane)**  $C_{14}H_9Cl_5$

*Melting point* <2.60>: 73 – 75°C

*Purity* Related substances—Proceed as directed in the *Purity* of *p,p'*-DDD.

***p,p'*-DDT (1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane)**  $C_{14}H_9Cl_5$

*Melting point* <2.60>: 108 – 110°C

*Purity* Related substances—Proceed as directed in the *Purity* of *p,p'*-DDD using the following standard solution (1).

Standard solution (1): Pipet 1 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

**Decolorized fuchsin TS** Add 1 g of fuchsin in 100 mL of water, heat at about 50°C, then cool with occasional shaking. After standing for 48 hours, mix and filter. To 4 mL of the filtration add 6 mL of hydrochloric acid and water to make 100 mL. Use after standing for at least 1 hour. Prepare before use.

***n-Decyl trimethylammonium bromide***  $C_{13}H_{30}NBr$

White powder. Melting point: about 232°C (with decomposition).

**Content:** not less than 99%. **Assay**—Weigh accurately about 0.5 g of *n*-decyl trimethylammonium bromide, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: 1 mL of potassium chromate TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 28.03 mg of  $C_{13}H_{30}NBr$

**0.005 mol/L *n*-Decyl trimethylammonium bromide TS**

Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.40 g of *n*-decyl trimethylammonium bromide in water to make 1000 mL.

**Defibrinated blood of rabbit** Transfer 100 mL of blood obtained from rabbit to a flask, put in about 20 glass balls 8 mm in diameter, shake for 5 minutes gently, and filter through gauze. Prepare before use.

**Dehydrated ethanol** See ethanol (99.5).

**Dehydrated ether** See diethyl ether, dehydrated.

**Dehydrated pyridine** See pyridine, dehydrated.

**Dehydrocorydaline nitrate for assay**  $C_{22}H_{24}N_2O_7$  Yellow, crystals or crystalline powder. It is sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5). *Melting point*: about 240°C (with decomposition).

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (333 nm): 577 – 642 (3 mg, water, 500 mL). Use the sample dried in a desiccator (silica gel) for not less than 1 hour for the test.

**Purity** (1) Related substances 1—Dissolve 5.0 mg of dehydrocorydaline nitrate for assay in 1 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop immediately with a mixture of methanol, a solution of ammonium acetate (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Spray Dragendorff's TS for spraying on the plate, air-dry, and spray sodium nitrite TS: the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Related substances 2—Dissolve 5.0 mg of dehydrocorydaline nitrate for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area from these solutions by the automatic integration method: the total area of peaks other than dehydrocorydaline obtained from the sample solution is not larger than the peak area of dehydrocorydaline from the standard solution.

**Operating conditions**

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under *Corydalis Tuber*.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Time span of measurement: About 3 times as long as the retention time of dehydrocorydaline, beginning after the peak of nitric acid.

**System suitability**

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under *Corydalis Tuber*.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of dehydrocorydaline obtained with 5  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 5  $\mu$ L of the standard solution.

**Dehydrocorydaline nitrate for component determination**  
See dehydrocorydaline nitrate for assay.

**Dehydrocorydaline nitrate for thin-layer chromatography**  $C_{22}H_{24}N_2O_7$  Yellow, crystals or crystalline powder. Sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5). *Melting point*: about 240°C (with decomposition).

**Purity** Related substances—Dissolve 5.0 mg of dehydrocorydaline nitrate for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly

50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop immediately with a mixture of methanol, a solution of ammonium acetate (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm) and then spray Dragendorff's TS on the plate: the spot other than the principal spot obtained from the sample solution is not more intense than the spot obtained from the standard solution in either case.

**Demethoxycurcumin**  $C_{20}H_{18}O_5$  Yellow to orange, crystalline powder or powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 166 – 170°C.

**Identification** Determine the absorption spectrum of a solution of demethoxycurcumin in methanol (1 in 400,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 416 nm and 420 nm.

**Purity** Related substances—(1) Dissolve 4 mg of demethoxycurcumin in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at the  $R_f$  value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of demethoxycurcumin in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the total area of the peaks other than demethoxycurcumin obtained from the sample solution is not larger than the peak area of demethoxycurcumin from the standard solution.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Turmeric.

Detector: A visible absorption photometer (wavelength: 422 nm).

Time span of measurement: About 4 times as long as the retention time of demethoxycurcumin, beginning after the solvent peak.

#### System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Turmeric.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of demethoxycurcumin obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

**N-Demethylerythromycin**  $C_{36}H_{65}NO_{13}$  White to light yellow-white powder.

**N-Demethylroxithromycin**  $C_{40}H_{74}N_2O_{15}$  White powder.

**Identification**—Determine the infrared absorption spectrum of a solution of the substance to be tested in chloroform (1 in 20) as directed in the solution method under Infrared Spectrophotometry <2.25> using a 0.1-mm cell made of potassium bromide: it exhibits absorption at the wave numbers of about 3600  $\text{cm}^{-1}$ , 3520  $\text{cm}^{-1}$ , 3450  $\text{cm}^{-1}$ , 3340  $\text{cm}^{-1}$ , 1730  $\text{cm}^{-1}$  and 1627  $\text{cm}^{-1}$ .

#### Deoxycholic acid for thin-layer chromatography

$C_{24}H_{40}O_4$  A white powder. Soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 175°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of deoxycholic acid for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 2930  $\text{cm}^{-1}$ , 1716  $\text{cm}^{-1}$ , 1447  $\text{cm}^{-1}$  and 1042  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 20 mg of deoxycholic acid for thin-layer chromatography in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Perform the test with 5  $\mu$ L each of the sample solution and standard solution as directed in the Identification under Oriental Bezoar: the spots other than the principal spot at an  $R_f$  value of about 0.5 obtained from the sample solution are not more intense than the spot from the standard solution.

**2'-Deoxyuridine for liquid chromatography**  $C_9H_{12}N_2O_5$  White crystalline powder.

**Melting point** <2.60>: 162 – 166°C

**Purity**—Dissolve 3.0 mg of 2'-deoxyuridine for liquid chromatography in diluted methanol (1 in 25) to make 50 mL. Perform the test with 10  $\mu$ L of this solution as directed under Liquid Chromatography <2.01> according to the operating conditions in the Purity under Idoxuridine Ophthalmic Solution. Determine each peak area by the automatic integration method to the range about twice the retention time of 2'-deoxyuridine, and calculate the amount of 2'-deoxyuridine by the area percentage method: it shows a purity of not less than 98.5%.

**Content:** not less than 98.5%. **Assay**—Weigh accurately about 5 mg of 2'-deoxyuridine for liquid chromatography, previously dried in vacuum at 60°C for 3 hours, and dissolve in water to make exactly 250 mL. Pipet 10 mL of this solution, dilute with water to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine absorbance  $A$  at the maximum wavelength at about 262 nm.

Amount (mg) of deoxyuridine ( $C_9H_{12}N_2O_5$ )

$$= \frac{A}{447} \times 5000$$

**Dermatan sulfate** Dermatan sulfate is mucopolysaccharide purified from the skin and small intestines of pigs by alkaline extraction, followed by digestion with protease and fractionation by alcohol. When cellulose acetate membrane electrophoresis of dermatan sulfate is performed and the membrane is stained in a toluidine blue O solution (1 in 200), a single band appears.

Operation conditions of cellulose acetate membrane electrophoresis—

Cellulose acetate membrane: 6 cm in width and 10 cm in length.

Mobile phase: Dissolve 52.85 g of calcium acetate monohydrate in water to make 1000 mL.

Run time: 3 hours (1.0 mA/cm).

**Deuterated acetone for nuclear magnetic resonance spectroscopy**  $\text{CD}_3\text{COCD}_3$  Prepared for nuclear magnetic resonance spectroscopy.

**Deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy**  $(\text{CD}_3)_2\text{SO}$  Prepared for nuclear magnetic resonance spectroscopy.

**Deuterated formic acid for nuclear magnetic resonance spectroscopy** DCOOD Prepared for nuclear magnetic resonance spectroscopy.

**Deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy** DCl Prepared for nuclear magnetic resonance spectroscopy.

**Deuterated methanol for nuclear magnetic resonance spectroscopy**  $\text{CD}_3\text{OD}$  Prepared for nuclear magnetic resonance spectroscopy.

**Deuterated NMR solvents** Prepared for nuclear magnetic resonance spectroscopy. For example: deuterated dimethylsulfoxide  $[(\text{CD}_3)_2\text{SO}]$ , deuterated pyridine ( $\text{C}_5\text{D}_5\text{N}$ ), deuteriochloroform ( $\text{CDCl}_3$ ), heavy water ( $\text{D}_2\text{O}$ ), etc.

**Deuterated pyridine for nuclear magnetic resonance spectroscopy**  $\text{C}_5\text{D}_5\text{N}$  Prepared for nuclear magnetic resonance spectroscopy.

**Deuteriochloroform for nuclear magnetic resonance spectroscopy**  $\text{CDCl}_3$  Prepared for nuclear magnetic resonance spectroscopy.

**Devara's alloy** [K 8653, For Nitrogen analysis]

**Diacetyl**  $\text{CH}_3\text{COCOCH}_3$  A yellow to yellow-green, clear liquid, having a strong, pungent odor. Miscible with ethanol (95) and with diethyl ether, and freely soluble in water.

*Congealing point* <2.42>: -2.0 – -5.5°C

*Refractive index* <2.45>  $n_D^{20}$ : 1.390 – 1.398

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.98 – 1.00

*Boiling point* <2.57>: 85 – 91°C

**Purity** Clarity of solution—Dissolve 1.0 g of diacetyl in 10 mL of water: the solution is clear.

**Content:** not less than 95.0%. **Assay**—Weigh accurately about 0.4 g of diacetyl, add exactly 75 mL of hydroxylamine TS, and heat on a water bath for 1 hour under a reflux condenser. After cooling, titrate <2.50> the excess hydroxylamine with 0.5 mol/L hydrochloric acid VS until the color of the solution changes from blue to yellow-green through green (indicator: 3 drops of bromophenol blue TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.5 mol/L hydrochloric acid VS  
= 21.52 mg of  $\text{C}_4\text{H}_6\text{O}_2$

**Diacetyl TS** Dissolve 1 mL of diacetyl in water to make 100 mL, and dilute 5 mL of this solution with water to make 100 mL. Prepare before use.

**3,3'-Diaminobenzidine tetrahydrochloride**  $\text{C}_{12}\text{H}_{14}\text{N}_4 \cdot 4\text{HCl}$  Occurs as white to yellowish brown, needle-shaped crystals, and is soluble in water.

**2,3-Diaminonaphthalene**  $\text{C}_{10}\text{H}_{10}\text{N}_2$  Light yellow-brown, crystals or powder. Slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: 193 – 198°C

**Sensitivity**—Pipet separately 40 mL each of the selenium standard solution and diluted nitric acid (1 in 60) as the blank solution into beakers, and to these solutions add ammonia solution (28) to adjust the pH to between 1.8 and 2.2. Dissolve 0.2 g of hydroxylammonium chloride in each of these solutions under gentle shaking, add 5 mL of 2,3-diaminonaphthalene TS, mix by shaking, and allow to stand for 100 minutes. Transfer these solutions to separators separately, rinse the beakers with 10 mL of water, add these rinsings to the separators, extract each with 5.0 mL of cyclohexane by thorough shaking for 2 minutes, and centrifuge the cyclohexane layers to remove moisture. When the absorbance at 378 nm of cyclohexane extract obtained from selenium standard solution for sensitivity is determined using the solution obtained from the blank solution as the reference solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, it is not less than 0.08.

**Selenium standard solution for sensitivity**—Weigh accurately 40 mg of selenium, dissolve in 100 mL of diluted nitric acid (1 in 2), by heating on water bath if necessary, and add water to make exactly 1000 mL. Pipet 5 mL of this solution, and add water to make exactly 200 mL. Pipet 2 mL of this solution, and add diluted nitric acid (1 in 60) to make exactly 50 mL. Prepare before use. This solution contains 0.04 µg of selenium (Se) per mL.

**2,4-Diaminophenol dihydrochloride**  $\text{C}_6\text{H}_8\text{N}_2\text{O} \cdot 2\text{HCl}$

Pale yellow-brown to grayish yellow-green crystalline powder. Freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

**Purity** Clarity of solution—Dissolve 1.0 g of 2,4-diaminophenol dihydrochloride in 20 mL of water: the solution is clear or a slight turbidity is produced.

*Loss on drying* <2.41>: not more than 0.5% (1 g, 105°C, 3 hours).

*Residue on ignition* <2.44>: not more than 0.5% (1 g).

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.2 g of 2,4-diaminophenol dihydrochloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 9.853 mg of  $\text{C}_6\text{H}_8\text{N}_2\text{O} \cdot 2\text{HCl}$

**2,4-Diaminophenol dihydrochloride TS** Dissolve 1 g of 2,4-diaminophenol dihydrochloride and 20 g of sodium bisulfite in 100 mL of water, and filter, if necessary.

**2,4-Diaminophenol hydrochloride** See 2,4-diaminophenol dihydrochloride.

**2,4-Diaminophenol hydrochloride TS** See 2,4-diaminophenol dihydrochloride TS.

**Diammonium hydrogen citrate**  $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_7$  [K 8284, Special class]

**Diammonium hydrogen phosphate**  $(\text{NH}_4)_2\text{HPO}_4$  [K 9016, Special class]

**Diazepam for assay**  $\text{C}_{16}\text{H}_{13}\text{ClN}_2\text{O}$  [Same as the monograph, Diazepam. When dried, it contains not less than 99.0% of diazepam ( $\text{C}_{16}\text{H}_{13}\text{ClN}_2\text{O}$ ), and meets the additional following requirement.]

**Purity** Related substance—Dissolve 50 mg of diazepam

for assay in 10 mL of water, add methanol to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than diazepam obtained from the sample solution is not larger than the peak area of diazepam from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Diazepam Tablets.

Time span of measurement: About 4.5 times as long as the retention time of diazepam, beginning after the solvent peak.  
System suitability

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of diazepam are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diazepam is not more than 2.0%.

**Diazobenzenesulfonic acid TS** Weigh 0.9 g of sulfanilic acid, previously dried at 105°C for 3 hours, dissolve it in 10 mL of dilute hydrochloric acid by heating, and add water to make 100 mL. To 3.0 mL of this solution add 2.5 mL of sodium nitrite TS, and allow to stand for 5 minutes while cooling with ice. Then add 5 mL of sodium nitrite TS and water to make 100 mL, and allow to stand in ice water for 15 minutes. Prepare before use.

**Diazobenzenesulfonic acid TS, concentrated** Weigh 0.2 g of sulfanilic acid, previously dried at 105°C for 3 hours, dissolve it in 20 mL of 1 mol/L hydrochloric acid TS by warming. Cool this solution with ice, and add 2.2 mL of a solution of sodium nitrite (1 in 25) dropwise under stirring. Allow to stand in ice water for 10 minutes, and add 1 mL of a solution of sulfamic acid (1 in 20). Prepare before use.

**Diazo TS** Weigh accurately 0.9 g of sulfanilic acid, add 0.9 mL of hydrochloric acid and 20 mL of water, and dissolve by heating. After cooling, filter, and dilute the filtrate with water to make exactly 100 mL. Pipet 1.5 mL of this solution, cool in an ice bath, and add exactly 1 mL of sodium nitrite solution (1 in 20) dropwise, while shaking. Cool in an ice bath for 10 minutes, add cold water to make exactly 50 mL. Store in a cold place, and use within 8 hours.

**Dibasic ammonium phosphate** See diammonium hydrogen phosphate.

**Dibasic potassium phosphate** See dipotassium hydrogen phosphate.

**Dibasic potassium phosphate-citric acid buffer solution (pH 5.3)** See dipotassium hydrogen phosphate-citric acid buffer solution (pH 5.3).

**1 mol/L Dibasic potassium phosphate TS for buffer solution** See 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

**Dibasic sodium ammonium phosphate** See ammonium sodium hydrogen phosphate tetrahydrate.

**Dibasic sodium phosphate** See disodium hydrogen phosphate dodecahydrate.

**Dibasic sodium phosphate, anhydrous** See disodium hydrogen phosphate.

**Dibasic sodium phosphate, anhydrous, for pH determination** See disodium hydrogen phosphate for pH determination.

**Dibasic sodium phosphate-citric acid buffer solution (pH 4.5)** See disodium hydrogen phosphate-citric acid buffer solution (pH 4.5).

**Dibasic sodium phosphate-citric acid buffer solution (pH 5.4)** See disodium hydrogen phosphate-citric acid buffer solution (pH 5.4).

**Dibasic sodium phosphate-citric acid buffer solution (pH 6.0)** See disodium hydrogen phosphate-citric acid buffer solution (pH 6.0).

**Dibasic sodium phosphate TS** See disodium hydrogen phosphate TS.

**0.05 mol/L Dibasic sodium phosphate TS** See 0.05 mol/L disodium hydrogen phosphate TS.

**0.5 mol/L Dibasic sodium phosphate TS** See 0.5 mol/L disodium hydrogen phosphate TS.

**Dibekacin sulfate**  $C_{18}H_{37}N_5O_8.xH_2SO_4$  [Same as the namesake monograph]

**Dibenz[a,h]anthracene**  $C_{22}H_{14}$  Very pale yellow to green-yellow, crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 265 – 270°C.

**Identification** Perform the test with dibenz[a,h]anthracene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak ( $m/z$  278) and a fragment ion peak ( $m/z$  139).

**Purity** Related substances—Dissolve 3.0 mg of dibenz[a,h]anthracene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than dibenz[a,h]anthracene is not more than 7.0%.

#### Operating conditions

Detector: A mass spectrophotometer (EI).

Mass scan range: 15.00 – 300.00.

Time of measurement: 12 – 30 minutes.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25 – 0.5  $\mu$ m.

Column temperature: Inject at a constant temperature of about 45°C, raise the temperature to 240°C at a rate of 40°C per minute, maintain at 240°C for 5 minutes, raise to 300°C at a rate of 4°C per minute, raise to 320°C at a rate of 10°C per minute, and maintain at 320°C for 3 minutes.

Injection port temperature: A constant temperature of about 250°C.

Interface temperature: A constant temperature of about 300°C.

Carrier gas: Helium.

Flow rate: Adjust so that the reaction time of the peak of dibenz[a,h]anthracene is about 27 minutes.

Splitless.

### System suitability

Test for required detectability: Pipet 1 mL of the sample solution, and add methanol to make exactly 10 mL. Confirm that the peak area of dibenz[a,h]anthracene obtained with 1  $\mu$ L of this solution is equivalent to 5 to 15% of that with 1  $\mu$ L of the standard solution.

**Dibenzyl** C<sub>14</sub>H<sub>14</sub> White crystals, freely soluble in diethyl ether, soluble in methanol and in ethanol (95), and practically insoluble in water.

*Melting point* <2.60> 50 – 54°C

**Purity** Related substances—Dissolve 32 mg of dibenzyl in methanol to make exactly 50 mL, and use this solution as the sample solution. Perform the test with 20  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the operating conditions in the Assay under Vinblastine Sulfate for Injection: any peak other than dibenzyl does not appear. Adjust the detection sensitivity so that the peak height of dibenzyl obtained from 20  $\mu$ L of the solution prepared by adding methanol to 10 mL of the sample solution to make 20 mL, is 3 to 5 cm, and the time span of measurement is about 1.2 times as long as the retention time of dibenzyl after the solvent peak.

### N,N'-Dibenzylethylenediamine diacetate

C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>.2C<sub>2</sub>H<sub>4</sub>O<sub>2</sub> A white to slightly pale yellow crystalline powder.

**Identification**—Determine the infrared absorption spectrum of the substance to be examined as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1530 cm<sup>-1</sup>, 1490 cm<sup>-1</sup>, 1460 cm<sup>-1</sup>, 1400 cm<sup>-1</sup> and 1290 cm<sup>-1</sup>.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 25 mg of N,N'-dibenzylethylenediamine diacetate, dissolve in 25 mL of methanol, and add a solution containing 1.02 g of disodium hydrogen phosphate, anhydrous and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 8 mg of acetic acid (100), add 25 mL of methanol, and add the solution containing 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate, anhydrous and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the control solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and control solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. After making correction for the peak areas based on the variance of the base-line and the peak of acetic acid on the chromatogram obtained with the sample solution, calculate the amount of N,N'-dibenzylethylenediamine by the area percentage method.

### Operating conditions

**Detector:** An ultraviolet absorption photometer (wavelength: 220 nm).

**Column:** A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

**Column temperature:** A constant temperature of about

40°C.

**Mobile phase:** A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS (pH 3.5) (11:7:2).

**Flow rate:** Adjust so that the retention time of N,N'-dibenzylethylenediamine is about 4 minutes.

**Time span of measurement:** About 5 times as long as the retention time of N,N'-dibenzylethylenediamine.

### System suitability

**System performance:** Dissolve an amount of benzylpenicillin benzathine, equivalent to about 85,000 Units, in 25 mL of methanol, add a solution containing 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL. When the procedure is run with 20  $\mu$ L of this solution under the above operating conditions, N,N'-dibenzylethylenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 20.

**System repeatability:** When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of N,N'-dibenzylethylenediamine is not more than 2.0%.

### 2,6-Dibromo-N-chloro-1,4-benzoquinone monoimine

C<sub>6</sub>H<sub>2</sub>Br<sub>2</sub>ClNO [K 8491, Special class]

### 2,6-Dibromo-N-chloro-1,4-benzoquinone monoimine TS

Dissolve 0.5 g of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in methanol to make 100 mL.

**2,6-Dibromo-N-chloro-1,4-benzoquinone monoimine TS, dilute** Dissolve 0.2 g of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in methanol to make 100 mL.

**2,6-Dibromoquinone chlorimide** See 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine.

**2,6-Dibromoquinone chlorimide TS** See 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine TS.

**Dibucaine hydrochloride** C<sub>20</sub>H<sub>29</sub>N<sub>3</sub>O<sub>2</sub>.HCl [Same as the namesake monograph]

### Dimethylamine

C<sub>8</sub>H<sub>19</sub>N Colorless, clear liquid.

*Refractive index* <2.45>  $n_D^{20}$  1.415 – 1.419

*Density* <2.56> (20°C): 0.756 – 0.761 g/mL

**Di-n-butyl ether** (C<sub>4</sub>H<sub>9</sub>)<sub>2</sub>O Clear, colorless, water-non-miscible liquid.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.768 – 0.771

**Di-n-butyl phthalate** C<sub>6</sub>H<sub>4</sub>(COOC<sub>4</sub>H<sub>9</sub>)<sub>2</sub> Clear, colorless liquid.

**Purity** Related substances—Dissolve 0.5 g of di-n-butyl phthalate in 50 mL of methanol, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed in the Assay under Nicardipine Hydrochloride Injection, and determine the peak area by the automatic integration method. Calculate the amount of di-n-butyl phthalate by the area percentage method: the amount of di-n-butyl phthalate is not less than 98.0%, and no peak appears at the same position as nicardipine. Adjust the detection sensitivity so that the peak height of di-n-butyl phthalate obtained from 10  $\mu$ L of the sample solution is 50 to 100% of the full scale, and measure about 2 times as long

as the retention time of di-*n*-butyl phthalate, beginning after the solvent peak.

**3,4-Dichloroaniline** C<sub>6</sub>H<sub>5</sub>Cl<sub>2</sub>N A white to brown solid.  
Melting point <2.60>: 69 – 75°C

**1,2-Dichlorobenzene** C<sub>6</sub>H<sub>4</sub>Cl<sub>2</sub> A colorless liquid.  
Specific gravity <2.56> d<sub>4</sub><sup>20</sup>: 1.306  
Boiling point <2.57>: 180 – 181°C

**1,2-Dichloroethane** ClCH<sub>2</sub>CH<sub>2</sub>Cl [K 8465, Special class]

**Dichlorofluorescein** C<sub>20</sub>H<sub>10</sub>Cl<sub>2</sub>O<sub>5</sub> Orange to red-brown powder.

*Identification* (1) Dissolve 0.1 g in 10 mL of sodium hydroxide TS: the solution is an orange-red color, and red-orange precipitates appear by the addition of 10 mL of dilute hydrochloric acid.

(2) Dissolve 0.1 g in 10 mL of sodium hydroxide TS, and add 40 mL of water: a green-yellow fluorescence is exhibited.

**Dichlorofluorescein TS** Dissolve 0.1 g of dichlorofluorescein in 60 mL of ethanol (95), add 2.5 mL of 0.1 mol/L sodium hydroxide VS, and dilute with water to make 100 mL.

**2,6-Dichloroindophenol sodium dihydrate**  
C<sub>12</sub>H<sub>6</sub>Cl<sub>2</sub>NNaO<sub>2</sub>.2H<sub>2</sub>O [K 8469, Special class]

**2,6-Dichloroindophenol sodium TS** Add 0.1 g of 2,6-dichloroindophenol sodium dihydrate to 100 mL of water, warm, and filter. Use within 3 days.

**2,6-Dichloroindophenol sodium TS for titration** See the monograph Ascorbic Acid Powder.

**2,6-Dichloroindophenol sodium-sodium acetate TS** Mix equal volumes of 2,6-dichloroindophenol sodium dihydrate solution (1 in 20) and acetic acid-sodium acetate TS (pH 7.0). Prepare before use.

**Dichloromethane** CH<sub>2</sub>Cl<sub>2</sub> [K 8161, Special class]

**2,6-Dichlorophenol** C<sub>6</sub>H<sub>4</sub>Cl<sub>2</sub>O White to purplish white crystals.

Melting point <2.60>: 65 – 67°C

**2,6-Dichlorophenol-indophenol sodium** See 2,6-dichloroindophenol sodium dihydrate.

**2,6-Dichlorophenol-indophenol sodium TS** See 2,6-dichloroindophenol sodium TS.

**2,6-Dichlorophenol-indophenol sodium TS for titration** See 2,6-dichloroindophenol sodium TS for titration.

**Diclofenac sodium** C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>NNaO<sub>2</sub> [Same as the namesake monograph]

**Diclofenac sodium for assay** C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>NNaO<sub>2</sub> [Same as the monograph Diclofenac Sodium. When dried, it contains not less than 99.0% of diclofenac sodium (C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>NNaO<sub>2</sub>).]

**Dicyclohexyl** C<sub>12</sub>H<sub>22</sub>  
Specific gravity <2.56> d<sub>20</sub><sup>20</sup>: about 0.864  
Boiling point <2.57>: about 227°C  
Melting point <2.60>: about 4°C

**N,N'-Dicyclohexylcarbodiimide** C<sub>13</sub>H<sub>22</sub>N<sub>2</sub> Colorless or white, crystals or crystalline mass. Dissolves in ethanol (95), but decomposes in water to produce a white precipitate.

Melting point <2.60>: 35 – 36°C

**N,N'-Dicyclohexylcarbodiimide-dehydrated ethanol TS**  
See N,N'-dicyclohexylcarbodiimide-ethanol TS.

**N,N'-Dicyclohexylcarbodiimide-ethanol TS** Dissolve 6 g of N,N'-dicyclohexylcarbodiimide in ethanol (99.5) to make 100 mL.

*Storage*—Preserve in tight containers, in a cold place.

**Dicyclohexyl phthalate** C<sub>6</sub>H<sub>4</sub>(COOC<sub>6</sub>H<sub>11</sub>)<sub>2</sub> A white, crystalline powder.

Melting point <2.60>: 63 – 66°C

*Purity* Clarity and color of solution—Dissolve 1.0 g of dicyclohexyl phthalate in 20 mL of ethanol (95): the solution is clear and colorless.

**Dicyclohexylurea** C<sub>6</sub>H<sub>11</sub>NHCONHC<sub>6</sub>H<sub>11</sub> A white crystalline powder, having no odor.

*Purity* Related substances—Dissolve 50 mg of dicyclohexylurea in methanol to make 100 mL. Pipet 10 mL of this solution, and add methanol to make 100 mL. Pipet 20 mL of this solution, add 10 mL of 0.5 mol/L sodium hydroxide TS, shake, then add 5 mL of diluted hydrochloric acid (1 in 10), shake, and use this solution as the sample solution. Perform the test with 50 µL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the area of each peak by the automatic integration method, and calculate the amount by the area percentage method: the total amount of the peaks other than dicyclohexylurea is not more than 3.0%.

*Operating conditions*

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) (ii) under Acetohexamide.

Time span of measurement: About 5 times as long as the retention time of dicyclohexylurea, beginning after the solvent peak.

*System suitability*

System performance, and system repeatability: Proceed as directed in the system suitability in the Purity (4) (ii) under Acetohexamide.

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 200 mL. Confirm that the peak area of dicyclohexylurea obtained with 50 µL of this solution is equivalent to 1.8 to 3.3% of that with 50 µL of the standard solution.

**Diethanolamine** C<sub>4</sub>H<sub>11</sub>NO<sub>2</sub> Colorless viscous liquid.

Melting point <2.60>: 27 – 30°C

Water <2.48>: less than 0.1%.

**Diethanolamine hydrochloride** See 2,2'-iminodiethanol hydrochloride.

**Diethylamine** (C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>NH A clear, colorless liquid, having an amine-like odor. Miscible with water and with ethanol (95). The solution in water is alkaline, and readily absorbs carbon dioxide in air.

Specific gravity <2.56> d<sub>4</sub><sup>10</sup>: 0.702 – 0.708

Distilling range <2.57>: 54 – 58°C; not less than 96 vol%.

*Content*: not less than 99.0%. Assay—Weigh accurately about 1.5 g of diethylamine in a flask containing exactly 30 mL of 0.5 mol/L sulfuric acid VS, and titrate <2.50> the excess of sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank determination in the same manner.

Each mL of 0.5 mol/L sulfuric acid VS  
= 73.14 mg of (C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>NH

**Diethylene glycol** HO(CH<sub>2</sub>CH<sub>2</sub>O)<sub>2</sub>H Colorless and odorless liquid. Miscible with water and with ethanol (95).

**Specific gravity** <2.56>  $d_{20}^{20}$ : 1.118 – 1.120

**Diethylene glycol adipate for gas chromatography** Prepared for gas chromatography.

**Diethylene glycol dimethyl ether** ( $\text{CH}_3\text{OCH}_2\text{CH}_2\text{O}$ ) Clear and colorless liquid, miscible with water.

**Specific gravity** <2.56>  $d_4^{20}$ : 0.940 – 0.950

**Distilling range** <2.57>: 158 – 160°C, not less than 95 vol%.

**Diethylene glycol monoethyl ether** [2-(2-ethoxyethoxy) ethanol]  $\text{C}_2\text{H}_5(\text{OCH}_2\text{CH}_2)_2\text{OH}$  Clear, colorless liquid, of which boiling point is about 203°C. Miscible with water.

**Refractive index** <2.45>  $n_D^{20}$ : 1.425 – 1.429

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.990 – 0.995

**Acid (as  $\text{CH}_3\text{COOH}$ )**: less than 0.01%.

**Diethylene glycol monoethyl ether for water determination** To 1000 mL of diethylene glycol monoethyl ether add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of diethylene glycol monoethyl ether. Preserve this protecting it from moisture. The water content of this diethylene glycol monoethyl ether should not be more than 0.3 mg per mL.

**Diethylene glycol succinate ester for gas chromatography** Prepared for gas chromatography.

**Diethylene glycol succinate polyester for gas chromatography** Prepared for gas chromatography.

**Diethyl ether**  $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$  [K 8103, Special class]

**Diethyl ether, dehydrated**  $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$  [K 8103, Special class. The water content is not more than 0.01%.]

**Diethyl ether for purity of crude drug**  $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$  [K 8103, Special class] Use diethyl ether meeting the following additional specification. Evaporate 300.0 mL of diethyl ether for purity of crude drug in vacuum at a temperature not higher than 40°C, add the diethyl ether to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of  $\gamma$ -BHC in hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane for purity of crude drug to make exactly 100 mL. Pipet 2 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the solvent peak obtained from the sample solution is not larger than the peak area of  $\gamma$ -BHC from the standard solution (1).

#### Operating conditions

Proceed the operating conditions in the 4. Purity 4.3. under the Crude Drugs Test <5.01> except time span of measurement.

**Time span of measurement:** About three times as long as the retention time of  $\gamma$ -BHC, beginning after the peak of solvent.

**Test for required detectability:** Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of  $\gamma$ -BHC obtained from 1  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method, and the peak height of  $\gamma$ -BHC from 1  $\mu\text{L}$  of the standard solution (1)

is about 20% of the full scale.

***N,N-Diethyl-N'-1-naphthylethylenediamine oxalate***

$\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_4$  A white crystalline powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3340  $\text{cm}^{-1}$ , 2940  $\text{cm}^{-1}$ , 1581  $\text{cm}^{-1}$ , 1536  $\text{cm}^{-1}$ , 1412  $\text{cm}^{-1}$ , 789  $\text{cm}^{-1}$ , 774  $\text{cm}^{-1}$  and 721  $\text{cm}^{-1}$ .

**Purity** Clarity of solution—To 0.1 g of *N,N-diethyl-N'-1-naphthylethylenediamine oxalate* add 20 mL of water, and dissolve by warming; the solution is clear.

***N,N-Diethyl-N'-1-naphthylethylenediamine oxalate-acetone TS*** Dissolve 1 g of *N,N-diethyl-N'-1-naphthylethylenediamine oxalate* in 100 mL of a mixture of acetone and water (1:1). Prepare before use.

***N,N-Diethyl-N'-1-naphthylethylenediamine oxalate TS***

Dissolve 1 g of *N,N-diethyl-N'-1-naphthylethylenediamine oxalate* in water to make 1000 mL.

**Diethyl phthalate**  $\text{C}_6\text{H}_4(\text{COOC}_2\text{H}_5)_2$  A colorless, clear liquid.

**Refractive index** <2.45>  $n_D^{20}$ : 1.500 – 1.505

**Diethyl terephthalate**  $\text{C}_6\text{H}_4(\text{COOC}_2\text{H}_5)_2$  White to pale brownish white, crystalline or mass.

**Melting point** <2.60>: 44 – 46°C

**Content:** not less than 99%. **Assay**—Dissolve 0.1 g of diethyl terephthalate in 10 mL of methanol. Perform the test with 2  $\mu\text{L}$  of this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the area of each peak by the automatic integration method.

$$\text{Content (\%)} = \frac{\text{peak area of diethyl terephthalate}}{\text{total of all peak areas}} \times 100$$

#### Operating conditions

**Detector:** A hydrogen flame-ionization detector.

**Column:** A glass tube 4 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography, 177- to 250-  $\mu\text{m}$  in particle diameter, coated with methylsilicone polymer for gas chromatography at the ratio of 10%.

**Column temperature:** A constant temperature of about 200°C.

**Carrier gas:** Helium.

**Flow rate:** Adjust so that the retention time of diethyl terephthalate is between 6 and 7 minutes.

**Time span of measurement:** About 5 times as long as the retention time of diethyl terephthalate, beginning after the solvent peak.

**Difenidol hydrochloride**  $\text{C}_{21}\text{H}_{27}\text{NO.HCl}$  [Same as the namesake monograph]

**4,4'-Difluorobenzophenone**  $\text{C}_{13}\text{H}_8\text{F}_2\text{O}$  A white crystalline powder.

**Melting point** <2.60>: 106 – 109°C

**Digitonin**  $\text{C}_{56}\text{H}_{92}\text{O}_{29}$  White to whitish, crystals or crystalline powder.

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : -47 – -50° (2 g dried at 105°C for 2 hours, diluted acetic acid (100) (3 in 4), 50 mL, 100 mm).

**Sensitivity**—Dissolve 0.5 g of digitonin in 20 mL of ethanol (95) by warming, and add ethanol (95) to make 50 mL. To 0.5 mL of this solution add 10 mL of a solution of cholesterol in ethanol (95) (1 in 5000), cool to 10°C, and allow to stand for 30 minutes while vigorous shaking occa-

sionally: A precipitate is produced.

**Digoxin**  $C_{41}H_{64}O_{14}$  [Same as the namesake monograph]

**Dihydrocodeine phosphate for assay**  $C_{18}H_{23}NO_3.H_3PO_4$  [Same as the monograph Dihydrocodeine Phosphate. It contains not less than 99.0% of dihydrocodeine phosphate ( $C_{18}H_{23}NO_3.H_3PO_4$ ), calculated on the dried basis.]

**Dihydroergocristine mesilate for thin-layer chromatography**  $C_{35}H_{41}N_5O_5.CH_4O_3S$  A pale yellowish white powder. Freely soluble in methanol, in ethanol (95) and in chloroform, sparingly soluble in water. Melting point: about 190°C (with decomposition).

**Purity** Related substances—Dissolve 6 mg of dihydroergocristine mesilate for thin-layer chromatography in exact 100 mL of a mixture of chloroform and methanol (9:1), and perform the test with 5  $\mu$ L of this solution as directed in the Purity (3) under Dihydroergotoxine Mesilate: any spot other than the principal spot at the  $R_f$  value around 0.4 does not appear.

**1-[*(2R,5S)*-2,5-Dihydro-5-(hydroxymethyl)-2-furyl] thymine for thin-layer chromatography**  $C_{10}H_{12}N_2O_4$  Occurs as a white powder.

**Purity**—Dissolve 0.1 g of 1-[*(2R,5S)*-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine for thin-layer chromatography in 100 mL of methanol and perform the test as directed in the Purity (2) under Zidovudine: spots other than the principal spot with an  $R_f$  value of about 0.23 are not observed.

**3,4-Dihydro-6-hydroxy-2(1*H*)-quinolinone**  $C_9H_9NO_2$  A white to light brown, powder or granule. Melting point: about 240°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3210  $cm^{-1}$ , 1649  $cm^{-1}$ , 1502  $cm^{-1}$ , 1252  $cm^{-1}$  and 816  $cm^{-1}$ .

**2,4-Dihydroxybenzoic acid**  $C_7H_6O_4$  White to pale brown powder.

**Purity** Clarity of solution—Dissolve 1.0 g of 2,4-dihydroxybenzoic acid in 20 mL of ethanol (95); the solution is clear.

**Content:** not less than 95%. **Assay**—Weigh accurately about 1 g of 2,4-dihydroxybenzoic acid, dissolve in 50 mL of ethanol (95) and 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 15.41 mg of  $C_7H_6O_4$

**1,3-Dihydroxynaphthalene**  $C_{10}H_6(OH)_2$  Crystals or purple-brown powder. Freely soluble in water and in ethanol (95).

*Melting point* <2.60>: about 125°C

**2,7-Dihydroxynaphthalene**  $C_{10}H_6(OH)_2$

**Purity:** not less than 97.0%.

**2,7-Dihydroxynaphthalene TS** Dissolve 0.10 g of 2,7-dihydroxynaphthalene in 1000 mL of sulfuric acid, and allow to stand until the yellow color initially developed disappears. If the solution is blackened notably, prepare freshly.

**Diisopropylamine**  $[(CH_3)_2CH]_2NH$  Colorless, clear liquid, having an amine-like odor. Miscible with water and with ethanol (95). The solution in water is alkaline.

**Refractive index** <2.45>  $n_D^{20}$ : 1.391 – 1.394

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.715 – 0.722

**Diisopropyl 1,3-dithiolan-2-ylidenemalonate**  $C_{12}H_{18}O_4S_2$  White crystals.

**Identification**—Determine the absorption spectrum of a solution of diisopropyl 1,3-dithiolan-2-ylidenemalonate in methanol (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 304 nm and 308 nm.

*Melting point* <2.60>: 54 – 57°C

**Diltiazem hydrochloride**  $C_{22}H_{26}N_2O_4S.HCl$  [Same as the namesake monograph]

**Diltiazem hydrochloride for assay**  $C_{22}H_{26}N_2O_4S.HCl$  [Same as the monograph Diltiazem Hydrochloride. However, when dried, it contains not less than 99.0% of diltiazem hydrochloride ( $C_{22}H_{26}N_2O_4S.HCl$ ).]

**Dilute acetic acid** See acetic acid, dilute.

**Dilute ammonium iron (III) sulfate TS** See ammonium iron (III) sulfate TS, dilute.

**Dilute bismuth subnitrate-potassium iodide TS for spraying** Dissolve 10 g of L-tartaric acid in 50 mL of water, and add 5 mL of bismuth subnitrate TS.

**Dilute bromophenol blue TS** See bromophenol blue TS, dilute.

**Dilute 2,6-Dibromo-N-chloro-1,4-benzoquinone monoamine TS** See 2,6-dibromo-N-chloro-1,4-benzoquinone monoamine TS, dilute.

**Dilute p-dimethylaminobenzaldehyde-ferric chloride TS** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS, dilute.

**Dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS, dilute.

**Diluted ethanol** See ethanol, diluted.

**Dilute ethanol** See ethanol, dilute.

**Dilute ferric ammonium sulfate TS** See ammonium iron (III) sulfate TS, dilute.

**Dilute ferric chloride TS** See iron (III) chloride TS, dilute.

**Dilute Folin's TS** See folin's TS, dilute.

**Dilute formaldehyde TS** See formaldehyde TS, dilute.

**Dilute Giemsa's TS** See Giemsa's TS, dilute.

**Dilute hydrochloric acid** See hydrochloric acid, dilute.

**Dilute hydrogen peroxide TS** See hydrogen peroxide TS, dilute.

**Dilute iodine TS** See iodine TS, dilute.

**Dilute iron-phenol TS** See iron-phenol TS, dilute.

**Dilute lead subacetate TS** See lead subacetate TS, dilute.

**Dilute methyl red TS** See methyl red TS, dilute.

**Dilute nitric acid** See nitric acid, dilute.

**Dilute phenolphthalein TS** See phenolphthalein TS, dilute.

**Dilute phenol red TS** See phenol red TS, dilute.

**Dilute potassium hydroxide-ethanol TS** See potassium hydroxide-ethanol TS, dilute.

**Dilute sodium hydroxide TS** See sodium hydroxide TS, dilute.

**Dilute sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS** See sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS, dilute.

**Dilute sulfuric acid** See sulfuric acid, dilute.

**Dilute thymol blue TS** See thymol blue TS, dilute.

**Dilute vanadium pentoxyde TS** See vanadium (V) oxide TS, dilute.

**Dilution fluid for particle counter** A fluid used for blood dilution.

**Dimedon**  $C_8H_{12}O_2$  White to pale yellow crystalline powder.

*Melting point* <2.60>: 145 – 149°C

**Dimenhydrinate for assay**  $C_{17}H_{21}NO.C_7H_7ClN_4O_2$

[Same as the monograph Dimenhydrinate. When dried, it contains not less than 53.8% and not more than 54.9% of diphenhydramine ( $C_{17}H_{21}NO$ ) and not less than 45.2% and not more than 46.1% of 8-chlorotheophylline ( $C_7H_7ClN_4O_2$ ).]

**Dimethoxymethane**  $C_3H_8O_2$  Colorless, clear and volatile liquid. Miscible with methanol, with ethanol (95) and with diethyl ether.

**N,N-Dimethylacetamide**  $CH_3CON(CH_3)_2$  Clear and colorless liquid.

*Specific gravity* <2.56> *d*: 0.938 – 0.945 (Method 3).

*Boiling point* <2.57>: 163 – 165°C

**Purity**—Perform the test with 3  $\mu$ L of N,N-dimethylacetamide as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of N,N-dimethylacetamide by the area percentage method: not less than 98.0%.

**Operating conditions**

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated the inside surface 0.5  $\mu$ m in thickness with polyethylene glycol 20 M for gas chromatography.

Column temperature: The sample is injected at a constant temperature of about 70°C, maintain this temperature for 1 minute, then raise to 200°C at a rate of 10°C per minute, and maintain at 200°C for 3 minutes.

Carrier gas: Helium.

Flow rate (linear velocity): About 30 cm per second.

Time span of measurement: About 2 times as long as the retention time of N,N-dimethylacetamide.

**System suitability**

Test for required detectability: To exactly 1.0 g of N,N-dimethylacetamide add acetone to make exactly 100 mL. Pipet 5 mL of this solution, and add acetone to make exactly 50 mL. Confirm that the peak area of N,N-dimethylacetamide obtained with 3  $\mu$ L of this solution is equivalent to 40 to 60% of the full-scale.

System repeatability: When the test is repeated with 3  $\mu$ L of N,N-dimethylacetamide under the above operating conditions, the relative standard deviation of the peak area of N,N-dimethylacetamide is not more than 2.0%.

**Water** <2.48>: not more than 0.2% (0.1 g, Coulometric titration).

**Dimethylamine** ( $CH_3)_2NH$  Colorless, clear liquid, having amine-like, characteristic odor. It is miscible with water and with ethanol (99.5). It is alkaline.

*Specific gravity* <2.56> *d*<sub>20</sub><sup>20</sup>: 0.85 – 0.93

**Content:** 38.0 – 45.0%. Assay—Weigh accurately about 1 g of dimethylamine, transfer to a flask containing exactly 20 mL of 0.5 mol/L sulfuric acid VS, and titrate <2.50> the excess sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of } 0.5 \text{ mol/L sulfuric acid VS} \\ = 45.08 \text{ mg of } (CH_3)_2NH \end{aligned}$$

**2,6-Dimethylaniline**  $C_8H_{11}N$  A clear liquid. Soluble in ethanol (95), and sparingly soluble in water. Specific gravity *d*<sub>20</sub><sup>20</sup>: about 0.98.

**4-Dimethylaminoantipyrine**  $C_{13}H_{17}N_3O$  Colorless to white crystals, or a white crystalline powder.

**Purity** Related substances—Proceed the test with 5  $\mu$ L of a solution of 4-dimethylaminoantipyrine (1 in 2000) as directed in the Assay under Cefpiramide Sodium, determine each peak area in a range of about 2 times as long as the retention time of 4-dimethylaminoantipyrine, beginning after the solvent peak by the automatic integration method, and calculate the total amount of the peaks other than 4-dimethylaminoantipyrine by the area percentage method: not more than 1.0%.

**(Dimethylamino)azobenzenesulfonyl chloride**

$C_{14}H_{14}ClN_3O_2S$  Prepared for amino acid analysis or biochemistry.

**p-Dimethylaminobenzaldehyde** See 4-dimethylaminobenzaldehyde.

**4-Dimethylaminobenzaldehyde**  $(CH_3)_2NC_6H_4CHO$   
[K 8496, *p*-Dimethylaminobenzaldehyde, Special class]

**p-Dimethylaminobenzaldehyde-ferric chloride TS** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS.

**p-Dimethylaminobenzaldehyde-ferric chloride TS, dilute** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS, dilute.

**p-Dimethylaminobenzaldehyde-hydrochloric acid TS** See 4-dimethylaminobenzaldehyde-hydrochloric acid TS.

**4-Dimethylaminobenzaldehyde-hydrochloric acid TS**

Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 50 mL of hydrochloric acid while cooling, and add 50 mL of ethanol (95).

**4-Dimethylaminobenzaldehyde-hydrochloric acid-acetic acid TS** Dissolve 8 g of 4-dimethylaminobenzaldehyde in 50 mL of a mixture of acetic acid (100) and hydrochloric acid (19:1). Prepare before use.

**4-Dimethylaminobenzaldehyde-iron (III) chloride TS**

Dissolve 0.125 g of 4-dimethylaminobenzaldehyde in a cold mixture of 65 mL of sulfuric acid and 35 mL of water, then add 0.05 mL of iron (III) chloride TS. Use within 7 days.

**4-Dimethylaminobenzaldehyde-iron (III) chloride TS, dilute** To 80 mL of water add carefully 100 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS and 0.15 mL of iron (III) chloride TS, while cooling with ice.

**p-Dimethylaminobenzaldehyde TS** See 4-dimethylaminobenzaldehyde TS.

**4-Dimethylaminobenzaldehyde TS** Dissolve 10 g of 4-dimethylaminobenzaldehyde in a cold mixture of 90 mL of sulfuric acid and 10 mL of water. Prepare before use.

**p-Dimethylaminobenzaldehyde TS for spraying** See 4-dimethylaminobenzaldehyde TS for spraying.

**4-Dimethylaminobenzaldehyde TS for spraying**

Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 20 mL of dilute sulfuric acid. Prepare before use.

**p-Dimethylaminobenzylidene rhodanine** See 4-dimethylaminobenzylidene rhodanine.

**4-Dimethylaminobenzylidene rhodanine**  $C_{12}H_{12}N_2OS_2$  [K 8495, Special class]

**p-Dimethylaminobenzylidene rhodanine TS** See 4-dimethylaminobenzylidene rhodanine TS.

**4-Dimethylaminobenzylidene rhodanine TS** Dissolve 20 mg of 4-dimethylaminobenzylidene rhodanine in acetone to make 100 mL.

**p-Dimethylaminocinnamaldehyde** See 4-dimethylaminocinnamaldehyde.

**4-Dimethylaminocinnamaldehyde**  $C_{11}H_{13}NO$  Orange, crystals or crystalline powder, having a characteristic odor. Freely soluble in dilute hydrochloric acid, sparingly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: 140 – 142°C

*Purity* Clarity of solution—Dissolve 0.20 g of 4-dimethylaminocinnamaldehyde in 20 mL of ethanol (95); the solution is clear.

*Loss on drying* <2.41>: not more than 0.5% (1 g, 105°C, 2 hours).

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

*Nitrogen content* <1.08>: 7.8 – 8.1% (105°C, 2 hours, after drying).

**p-Dimethylaminocinnamaldehyde TS** See 4-dimethylaminocinnamaldehyde TS.

**4-Dimethylaminocinnamaldehyde TS** Before use, add 1 mL of acetic acid (100) to 10 mL of a solution of 4-dimethylaminocinnamaldehyde in ethanol (95) (1 in 2000).

**Dimethylaminophenol**  $(CH_3)_2NC_6H_4OH$  Dark purple, crystals or crystalline mass.

*Melting point* <2.60>: 85°C

**Dimethylaniline** See *N,N*-dimethylaniline.

**N,N-Dimethylaniline**  $C_6H_5N(CH_3)_2$  Colorless or light yellow liquid, having a characteristic odor.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.955 – 0.960

*Distilling range* <2.57>: 192 – 195°C, not less than 95 vol%.

**Dimethylformamide** See *N,N*-dimethylformamide.

**N,N-Dimethylformamide**  $HCON(CH_3)_2$  [K 8500, Special class]

**N,N-Dimethylformamide for liquid chromatography**  
HCON(CH<sub>3</sub>)<sub>2</sub> [K 8500, *N,N*-Dimethylformamide, Special class] Read absorbance as directed under Ultraviolet-visible Spectrophotometry <2.24> (in a 1-cm cell, using water as the blank): the absorbance is not more than 0.60 at 270 nm, not more than 0.15 at 280 nm, and not more than 0.05 at 300 nm.

**Dimethylglyoxime**  $C_4H_8N_2O_2$  [K 8498, Special class]

**Dimethylglyoxime-thiosemicarbazide TS** Solution A: Dissolve 0.5 g of dimethylglyoxime in hydrochloric acid to make 100 mL. Prepare before use. Solution B: Dissolve 0.1 g

of thiosemicarbazide in 50 mL of water with the aid of warming if necessary, and add diluted hydrochloric acid (1 in 2) to make 100 mL. Prepare before use.

Mix 10 mL each of solution A and solution B, add diluted hydrochloric acid (1 in 2) to make 100 mL, and allow the mixture to stand for 1 hour. Use within 24 hours.

**Dimethylglyoxime TS** Dissolve 1 g of dimethylglyoxime in ethanol (95) to make 100 mL.

**Dimethyl malonate**  $C_5H_8O_4$  Clear, colorless or pale yellow liquid.

*Specific gravity* <2.56>  $d_4^{20}$ : 1.152 – 1.162

*Water* <2.48>: not more than 0.3%.

*Residue on ignition* <2.44>: not more than 0.1%.

**N,N-Dimethyl-n-octylamine**  $C_{10}H_{23}N$  Colorless liquid.  
*Refractive index* <2.45>  $n_D^{20}$ : 1.424

**N,N-Dimethyl-p-phenylenediammonium dichloride**  
 $H_2NC_6H_4N(CH_3)_2\cdot 2HCl$  [K 8193, *N,N*-Dimethyl-p-phenylenediammonium dichloride, Special class]

**N,N-Dimethyl-p-phenylenediammonium hydrochloride**  
See *N,N*-dimethyl-p-phenylenediamine dichloride.

**Dimethyl phthalate**  $C_{10}H_{10}O_4$  A colorless, clear liquid, having a slight aroma.

*Refractive index* <2.45>  $n_D^{20}$ : 1.513 – 1.517

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.191 – 1.196

**Dimethylpolysiloxane for gas chromatography** Prepared for gas chromatography.

**Dimethylsulfoxide**  $(CH_3)_2SO$  [K 9702, Special class]

**Dimethylsulfoxide for ultraviolet-visible spectrophotometry**  $(CH_3)_2SO$  Colorless crystals or clear colorless liquid, having a characteristic odor. It is highly hygroscopic.

*Congealing point* <2.42>: not less than 18.3°C.

*Purity*—Read absorbance of dimethylsulfoxide for ultraviolet-visible spectrophotometry, immediately after saturating with nitrogen, using water as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>; its value is not more than 0.20 at 270 nm, not more than 0.09 at 275 nm, not more than 0.06 at 280 nm, and not more than 0.015 at 300 nm. It exhibits no characteristic absorption between 260 nm and 350 nm.

*Water* <2.48>: not more than 0.1%.

**2,6-Dimethyl-4-(2-nitrosophenyl)3,5-pyridinedicarboxylic acid dimethyl ester for thin-layer chromatography**

$C_{17}H_{16}N_2O_5$  Irradiate xenon light at 50,000 lx of illumination for 8 hours to a methanol solution of nifedipine (1 in 100), and evaporate the methanol on a water bath. Recrystallize the residue 4 times from 1-propanol, and dry in a desiccator (in vacuum, phosphorus pentoxide). Pale blue crystals. Very soluble in chloroform, freely soluble in acetone, and practically insoluble in water.

*Melting point* <2.60>: 93 – 95°C

*Content*: not less than 99.0%. Assay—Weigh accurately about 0.4 g of 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester for thin-layer chromatography, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 32.83 mg of  $C_{17}H_{16}N_2O_5$

**3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide**  $C_{18}H_{16}BrN_5S$  Yellow crystals. Melting point:

about 195°C (with decomposition).

**3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide TS** Dissolve 5 g of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide in phosphate-buffered sodium chloride TS to make 1000 mL.

**Dimidium bromide**  $C_{20}H_{18}BrN_3$  Red to dark brown, crystalline powder or powder.

**Identification**—(1) Determine the infrared absorption spectrum of dimidium bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3300  $\text{cm}^{-1}$ , 1619  $\text{cm}^{-1}$ , 1489  $\text{cm}^{-1}$ , 1470  $\text{cm}^{-1}$ , 1422  $\text{cm}^{-1}$  and 1316  $\text{cm}^{-1}$ .

(2) A solution of dimidium bromide (1 in 1000) responds to Qualitative Tests <1.09> (1) for bromide.

**Dimidium bromide-patent blue TS** Dissolve each 0.5 g of dimidium bromide and 0.25 g of patent blue in 30 mL of a warmed mixture of water and ethanol (99.5) (9:1), combine the solutions, and add a mixture of water and ethanol (99.5) (9:1) to make 250 mL. To 20 mL of this solution add 270 mL of diluted sulfuric acid (7 in 675) and water to make 500 mL.

**Storage**—Preserve in light-resistant containers.

**Dimorpholamine for assay**  $C_{20}H_{38}N_4O_4$  [Same as the monograph Dimorpholamine. When dried, it contains not less than 99.0% of dimorpholamine ( $C_{20}H_{38}N_4O_4$ ).]

**2,2'-dinaphthylether**  $C_{20}H_{14}O$  White crystals.

*Melting point* <2.60>: 102 – 107°C

**m-Dinitrobenzene** See 1,3-dinitrobenzene.

**1,2-Dinitrobenzene**  $C_6H_4(NO_2)_2$  Occurs as yellowish white to brownish yellow, crystals or a crystalline powder.

**Identification**—Determine the infrared absorption spectrum of 1,2-dinitrobenzene as directed in the paste method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3100  $\text{cm}^{-1}$ , 1585  $\text{cm}^{-1}$ , 1526  $\text{cm}^{-1}$ , 1352  $\text{cm}^{-1}$ , and 793  $\text{cm}^{-1}$ .

*Melting point* <2.60>: 116 – 119°C

**1,3-Dinitrobenzene**  $C_6H_4(NO_2)_2$  Light yellow to reddish-yellow, crystals or crystalline powder.

*Melting point* <2.60>: 88 – 92°C.

**Storage**—Preserve in a light-resistant tight container.

**m-Dinitrobenzene TS** See 1,3-dinitrobenzene TS.

**1,3-Dinitrobenzene TS** Dissolve 1 g of 1,3-dinitrobenzene in 100 mL of ethanol (95). Prepare before use.

**m-Dinitrobenzene TS, alkaline** See 1,3-dinitrobenzene TS, alkaline.

**1,3-Dinitrobenzene TS, alkaline** Mix 1 mL of tetramethylammonium hydroxide and 140 mL of ethanol (99.5), titrate a part of the mixture with 0.01 mol/L hydrochloric acid VS (indicator: methyl red TS), and dilute the remainder with ethanol (99.5) to give a 0.008 mol/L solution. Before use, mix 40 mL of this solution with 60 mL of a solution of 1,3-dinitrobenzene in benzene (1 in 20).

**2,4-Dinitrochlorobenzene** See 1-chloro-2, 4-dinitrobenzene.

**2,4-Dinitrofluorobenzene** See 1-fluoro-2, 4-dinitrobenzene.

**2,4-Dinitrophenol**  $C_6H_3OH(NO_2)_2$  Yellow, crystals or crystalline powder.

*Melting point* <2.60>: 110 – 114°C

**2,4-Dinitrophenol TS** Dissolve 0.5 g of 2,4-dinitrophenol in 100 mL of ethanol (95).

**2,4-Dinitrophenylhydrazine**  $(NO_2)_2C_6H_3NHNH_2$  [K 8480, Special class]

**2,4-Dinitrophenylhydrazine-diethylene glycol dimethyl ether TS** Dissolve 3 g of 2,4-dinitrophenylhydrazine in 100 mL of diethylene glycol dimethyl ether while heating, cool, and filter if necessary.

**2,4-Dinitrophenylhydrazine-ethanol TS** Dissolve 1.5 g of 2,4-dinitrophenylhydrazine in a cold mixture of 10 mL of sulfuric acid and 10 mL of water, then add a mixture of 1 volume of aldehyde-free ethanol and 3 volumes of water to make 100 mL, and filter if necessary.

**2,4-Dinitrophenylhydrazine TS** Dissolve 1.5 g of 2,4-dinitrophenylhydrazine in a cold mixture of 10 mL of sulfuric acid and 10 mL of water, then add water to make 100 mL, and filter if necessary.

**Dinonyl phthalate**  $C_6H_4(COOC_9H_{19})_2$  Colorless to pale yellow, clear liquid.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.967 – 0.987

*Acid value* <1.13>: not more than 2.

**Dioxane** See 1,4-dioxane.

**1,4-Dioxane**  $C_4H_8O_2$  [K 8461, Special class]

**Diphenhydramine**  $C_{17}H_{21}NO$  [Same as the namesake monograph]

**Diphenhydramine tannate** [Same as the namesake monograph]

**Diphenyl**  $C_{12}H_{10}$  White, crystals or crystalline powder, having a characteristic odor. Freely soluble in acetone and in diethyl ether, soluble in ethanol (95), and practically insoluble in water.

*Melting point* <2.60>: 68 – 72°C

**Purity**—Dissolve 0.10 g of diphenyl in 5 mL of acetone and use this solution as the sample solution. Perform the test with 2  $\mu\text{L}$  of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method and calculate the amount of diphenyl by the area percentage method: it shows the purity of not less than 98.0%.

**Operating conditions**

Detector: A hydrogen flame-ionization detector.

Column: A glass tube about 3 mm in inside diameter and about 2 m in length, packed with 150 to 180  $\mu\text{m}$  mesh siliceous earth for gas chromatography coated with 10% of polyethylene glycol 20 M for thin-layer chromatography.

Column temperature: A constant temperature of about 180°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of diphenyl is about 8 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of diphenyl obtained with 2  $\mu\text{L}$  of the solution prepared by adding acetone to 1.0 mL of the sample solution to make 100 mL, is 5 to 15% of the full scale.

Time span of measurement: About 3 times as long as the retention time of diphenyl, beginning after the solvent peak.

**Diphenylamine**  $(C_6H_5)_2NH$  [K 8487, Special class]

**Diphenylamine-acetic acid TS** Dissolve 1.5 g of diphenylamine in 1.5 mL of sulfuric acid and acetic acid (100) to make 100 mL.

**Diphenylamine-acetic acid (100) TS** See diphenylamine-acetic acid TS.

**Diphenylamine TS** Dissolve 1 g of diphenylamine in 100 mL of sulfuric acid. Use the colorless solution.

**9,10-Diphenylanthracene**  $C_{26}H_{18}$  Yellow crystalline powder. Soluble in diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: about 248°C

**1,4-Diphenylbenzene**  $C_{18}H_{14}$  White scaly crystals, having a slight aromatic odor. It is freely soluble in ethanol (99.5), and slightly soluble in water.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3050  $\text{cm}^{-1}$ , 3020  $\text{cm}^{-1}$ , 1585  $\text{cm}^{-1}$ , 1565  $\text{cm}^{-1}$ , 1476  $\text{cm}^{-1}$ , 1450  $\text{cm}^{-1}$ , 995  $\text{cm}^{-1}$ , 834  $\text{cm}^{-1}$ , 740  $\text{cm}^{-1}$  and 680  $\text{cm}^{-1}$ .

**Diphenylcarbazide** See 1,5-diphenylcarbonohydrazide.

**Diphenylcarbazide TS** See 1,5-diphenylcarbonohydrazide TS.

**Diphenylcarbazone**  $C_6H_5N_2CON_2H_2C_6H_5$  A yellowish-red crystalline powder.

**Identification**—Determine the infrared absorption spectrum of diphenylcarbazone as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1708  $\text{cm}^{-1}$ , 1602  $\text{cm}^{-1}$ , 1497  $\text{cm}^{-1}$ , 1124  $\text{cm}^{-1}$ , 986  $\text{cm}^{-1}$ , 748  $\text{cm}^{-1}$  and 692  $\text{cm}^{-1}$ .

**Storage**—Preserve in a light-resistant tight container.

**Diphenylcarbazone TS** Dissolve 1 g of diphenylcarbazone in ethanol (95) to make 1000 mL.

**1,5-Diphenylcarbonohydrazide**  $C_{13}H_{14}N_4O$  [K 8488, Special class]

**1,5-Diphenylcarbonohydrazide TS** Dissolve 0.2 g of 1,5-diphenylcarbonohydrazide in 100 mL of a mixture of ethanol (95) and acetic acid (100) (9:1).

**5% Diphenyl-95% dimethylpolysiloxane for gas chromatography** Prepared for gas chromatography.

**Diphenyl ether**  $C_{12}H_{10}O$  Colorless crystals, having a geranium-like aroma. Dissolves in ethanol (95) and in diethyl ether, and practically insoluble in water.

*Specific gravity* <2.56>  $d_{25}^{25}$ : 1.072 – 1.075

*Boiling point* <2.57>: 254 – 259°C

*Melting point* <2.60>: 28°C

**Diphenyl imidazole**  $C_{15}H_{12}N_2$  White, crystals or crystalline powder, freely soluble in acetic acid (100), and sparingly soluble in methanol.

*Melting point* <2.60>: 234 – 238°C

*Loss on drying* <2.41>: not more than 0.5% (0.5 g, 105°C, 3 hours).

**Content:** not less than 99.0%. **Assay**—Dissolve about 0.3 g of diphenyl imidazole, previously dried and weighed accurately, in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS).

Each mL of 0.1 mol/L perchloric acid VS  
= 22.03 mg of  $C_{15}H_{12}N_2$

**Diphenyl phthalate**  $C_6H_4(COOCC_6H_5)_2$  White crystalline powder.

*Melting point* <2.60>: 71 – 76°C

**Purity** Related substances—Dissolve 60 mg of diphenyl phthalate in 50 mL of chloroform and use this solution as the sample solution. Perform the test with 10  $\mu\text{L}$  of the sample solution as directed in the Assay under Tolnaftate Solution: any peak other than the principal peak at the retention time of about 8 minutes and the peak of the solvent does not appear. Adjust the detection sensitivity so that the peak height of diphenyl phthalate obtained from 10  $\mu\text{L}$  of the sample solution is 50 to 100% of the full scale, and the time span of measurement is about twice as long as the retention time of diphenyl phthalate, beginning after the solvent peak.

**1,1-Diphenyl-4-piperidino-1-butene hydrochloride for thin-layer chromatography**  $C_{21}H_{25}N.HCl$  To 1 g of diphenidol hydrochloride add 30 mL of 1 mol/L hydrochloric acid TS, and heat under a reflux condenser for 1 hour. After cooling, extract twice with 30 mL-portions of chloroform, combine the chloroform extracts, wash twice with 10 mL portions of water, and evaporate chloroform under reduced pressure. Recrystallize the residue from a mixture of diethyl ether and ethanol (95) (3:1), and dry in a desiccator (in vacuum, silica gel) for 2 hours. White crystals or crystalline powder.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (250 nm): 386 – 446 (10 mg, water, 1000 mL).

*Melting point* <2.60>: 176 – 180°C

**Content:** not less than 99.0%. **Assay**—Dissolve about 0.2 g of 1,1-diphenyl-4-piperidino-1-butene hydrochloride for thin-layer chromatography, previously weighed accurately, in 20 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS  
= 16.39 mg of  $C_{21}H_{25}N.HCl$

**Diphenyl sulfone for assay**  $C_{12}H_{10}O_2S$  White, crystals or crystalline powder. It dissolves in dimethylsulfoxide.

It is used after correcting with the amount of diphenyl sulfone obtained in the Assay.

**Identification**—Proceed as directed in the Assay: it exhibits a triplet-like signal equivalent to 4 protons around  $\delta$  7.65 ppm, triplet-like signals equivalent to 2 protons around  $\delta$  7.73 ppm, and doublet-like signals equivalent to 4 protons around  $\delta$  7.99 ppm.

**Unity of peak**—Dissolve 10 mg of diphenyl sulfone for assay in 100 mL of methanol. To 10 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 10  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of diphenyl sulfone peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

**Operating conditions**

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Perilla Herb.

**Detector:** A photodiode array detector (wavelength: 234 nm, spectrum range of measurement: 220 – 400 nm).

**System suitability**

**System performance:** Proceed as directed in the system suitability in the Assay under Perilla Herb.

The unity of peak is unnecessary if the content (%) of diphenyl sulfone ( $C_{12}H_{10}O_2S$ ) is between 99.5% and 100.5%.

**Assay**—Weigh accurately 5 mg of diphenyl sulfone for as-

say and 1 mg of DSS-*d*<sub>6</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 2 mL of deuterated dimethyl sulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using DSS-*d*<sub>6</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities, *A*<sub>1</sub> (equivalent to 6 hydrogens) and *A*<sub>2</sub> (equivalent to 4 hydrogens), of the signals around δ 7.64 – 7.74 ppm and δ 7.98 – 8.01 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%)} \text{ of diphenyl sulfone (C}_{12}\text{H}_{10}\text{O}_2\text{S)} \\ = M_s \times I \times P/(M \times N) \times 0.9729$$

*M*: Amount (mg) of diphenyl sulfone for assay taken

*M<sub>s</sub>*: Amount (mg) of DSS-*d*<sub>6</sub> for nuclear magnetic resonance spectroscopy taken

*I*: Sum of the signal resonance intensities, *A*<sub>1</sub> and *A*<sub>2</sub>, based on the signal resonance intensity of DSS-*d*<sub>6</sub> for nuclear magnetic resonance spectroscopy as 9.000

*N*: Sum of numbers of the hydrogen derived from *A*<sub>1</sub> and *A*<sub>2</sub>

*P*: Purity (%) of DSS-*d*<sub>6</sub> for nuclear magnetic resonance spectroscopy

#### Operating conditions

**Apparatus:** An apparatus of nuclear magnetic resonance spectrom measurement having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

<sup>13</sup>C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

**Test for required detectability:** When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around δ 7.64 – 7.74 ppm and δ 7.98 – 8.01 ppm is not less than 100.

**System performance:** When the procedure is run with the sample solution under the above operating conditions, the signals around δ 7.64 – 7.74 ppm and δ 7.98 – 8.01 ppm are not overlapped with any signal of obvious foreign substances, and the ratios of the resonance intensities, (*A*<sub>1</sub>/6)/(*A*<sub>2</sub>/4), of each signal around δ 7.64 – 7.74 ppm and δ 7.98 – 8.01 ppm are between 0.99 and 1.01, respectively.

**System repeatability:** When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity, *A*<sub>1</sub> or *A*<sub>2</sub> to that of the reference standard for qNMR are not more than 1.0%.

#### Dipicolinic acid C<sub>7</sub>H<sub>5</sub>NO<sub>4</sub>

**White powder.**  
**Identification—**Determine the infrared absorption spectrum of dipicolinic acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 2630 cm<sup>-1</sup>,

1701 cm<sup>-1</sup>, 1576 cm<sup>-1</sup>, 1416 cm<sup>-1</sup>, 1300 cm<sup>-1</sup> and 1267 cm<sup>-1</sup>.

**Purity** Clarity and color of solution—Dissolve by warming 0.5 g of dipicolinic acid in 20 mL of ethanol (99.5), and cool: a clear, colorless liquid.

**Content:** Not less than 98.0%. **Assay—**Weigh accurately about 0.1 g of dipicolinic acid, add 25 mL of ethanol (99.5), dissolve by warming, cool, then titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 8.356 \text{ mg of C}_7\text{H}_5\text{O}_4\text{N} \end{aligned}$$

**Dipotassium hydrogen phosphate** K<sub>2</sub>HPO<sub>4</sub> [K 9017, Special class]

**Dipotassium hydrogen phosphate-citric acid buffer solution (pH 5.3)** Mix 100 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution and 38 mL of 1 mol/L citric acid TS for buffer solution, and add water to make 200 mL.

**1 mol/L Dipotassium hydrogen phosphate TS for buffer solution** Dissolve 174.18 g of dipotassium hydrogen phosphate in water to make 1000 mL.

#### Dipotassium tetraborate tetrahydrate K<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.4H<sub>2</sub>O

White, crystalline powder or powder. Slightly soluble in ethanol (99.5).

**Diprophylline** C<sub>10</sub>H<sub>14</sub>N<sub>4</sub>O<sub>4</sub> A white, powder or grain. Freely soluble in water, and slightly soluble in ethanol (95).

**Identification—**Determine the infrared absorption spectrum of the substance to be examined, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3460 cm<sup>-1</sup>, 3330 cm<sup>-1</sup>, 1651 cm<sup>-1</sup>, 1242 cm<sup>-1</sup>, 1059 cm<sup>-1</sup> and 1035 cm<sup>-1</sup>.

**α,α'-Dipyridyl** See 2,2'-bipyridyl.

#### Disodium chromotropate dihydrate

C<sub>10</sub>H<sub>6</sub>Na<sub>2</sub>O<sub>8</sub>S<sub>2</sub>.2H<sub>2</sub>O [K 8316, Special class] Preserve in light-resistant containers.

**Disodium dihydrogen ethylenediamine tetraacetate dihydrate** C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>.2H<sub>2</sub>O [K 8107, Special class]

**0.4 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS (pH 8.5)** Dissolve 148.9 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in about 800 mL of water, adjust to pH 8.5 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.

**0.04 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS** Dissolve 14.890 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL.

**0.1 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS** Dissolve 37.2 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL.

**Disodium ethylenediaminetetraacetate** See disodium dihydrogen ethylenediamine tetraacetate dihydrate.

**Disodium ethylenediaminetetraacetate copper** See copper (II) disodium ethylenediamine tetraacetate tetrahydrate.

**0.1 mol/L Disodium ethylenediaminetetraacetate TS** See 0.1 mol/L disodium dihydrogen ethylenediamine tetra-

acetate TS.

**Disodium hydrogen phosphate, anhydrous**  $\text{Na}_2\text{HPO}_4$  [K 9020, Special class]

**Disodium hydrogen phosphate-citric acid buffer solution (pH 3.0)** Dissolve 35.8 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL. To this solution add a solution of citric acid monohydrate (21 in 1000) to adjust the pH to 3.0.

**Disodium hydrogen phosphate-citric acid buffer solution (pH 4.5)** Dissolve 21.02 g of citric acid monohydrate in water to make 1000 mL, and adjust the pH to 4.5 with a solution prepared by dissolving 35.82 g of disodium hydrogen-phosphate dodecahydrate in water to make 1000 mL.

**Disodium hydrogen phosphate-citric acid buffer solution (pH 5.0)** Dissolve 7.1 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL, and adjust to pH 5.0 with a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL.

**Disodium hydrogen phosphate-citric acid buffer solution (pH 5.4)** Dissolve 1.05 g of citric acid monohydrate and 2.92 g of disodium hydrogen phosphate dodecahydrate in 200 mL of water, and adjust to pH 5.4 with phosphoric acid or sodium hydroxide TS, if necessary.

**Disodium hydrogen phosphate-citric acid buffer solution (pH 5.5)** To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS add an amount of a solution, prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL, to adjust to pH 5.5.

**Disodium hydrogen phosphate-citric acid buffer solution (pH 6.0)** Dissolve 71.6 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To this solution add a solution, prepared by dissolving 21.0 g of citric acid monohydrate in water to make 1000 mL, until the pH becomes 6.0 (ratio of volume: about 63:37).

**0.05 mol/L Disodium hydrogen phosphate-citric acid buffer solution (pH 6.0)** To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS add a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL to adjust pH 6.0.

**Disodium hydrogen phosphate-citric acid buffer solution (pH 6.8)** To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS add a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL to adjust the pH to 6.8.

**Disodium hydrogen phosphate-citric acid buffer solution (pH 7.2)** Dissolve 7.1 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL. Adjust this solution to pH 7.2 with a solution prepared by dissolving 5.3 g of citric acid monohydrate in water to make 1000 mL.

**Disodium hydrogen phosphate-citric acid buffer solution (pH 7.5)** To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS add a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL to adjust the pH to 7.5.

**Disodium hydrogen phosphate-citric acid buffer solution (pH 8.2)** Dissolve 20.7 g of anhydrous disodium hydrogen phosphate, 7.38 g of citric acid monohydrate, and 0.535 g of sodium dihydrogen phosphate dihydrate in 400 mL of water, adjust to pH 8.2 with a solution of sodium hydroxide (1 in 2), and add water to make 500 mL.

**Disodium hydrogen phosphate-citric acid buffer solution for penicillium origin  $\beta$ -galactosidase (pH 4.5)** Dissolve 71.6 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL, and adjust the pH to 4.5 with a solution prepared by dissolving 21.0 g of citric acid monohydrate in water to make 1000 mL (volume ratio: about 44:56).

**Disodium hydrogen phosphate dodecahydrate**  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  [K 9019, Special class]

**Disodium hydrogen phosphate for pH determination**  $\text{Na}_2\text{HPO}_4$  [K 9020, for pH determination]

**Disodium hydrogen phosphate TS** Dissolve 12 g of disodium hydrogen phosphate dodecahydrate in water to make 100 mL (0.3 mol/L).

**0.05 mol/L Disodium hydrogen phosphate TS** Dissolve 7.098 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

**0.5 mol/L Disodium hydrogen phosphate TS** Dissolve 70.982 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

#### Disodium 1-nitroso-2-naphthol-3,6-disulfonate

$\text{C}_{10}\text{H}_5\text{NNa}_2\text{O}_8\text{S}_2$  Yellow, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3400\text{ cm}^{-1}$ ,  $1639\text{ cm}^{-1}$ ,  $1451\text{ cm}^{-1}$ ,  $1270\text{ cm}^{-1}$ ,  $1231\text{ cm}^{-1}$ ,  $1173\text{ cm}^{-1}$ ,  $1049\text{ cm}^{-1}$ ,  $848\text{ cm}^{-1}$  and  $662\text{ cm}^{-1}$ .

**Storage**—Preserve in a light-resistant tight container.

**Dissolved acetylene**  $\text{C}_2\text{H}_2$  [K 1902]

**Distigmine bromide for assay**  $\text{C}_{22}\text{H}_{32}\text{Br}_2\text{N}_4\text{O}_4$  [Same as the monograph Distigmine Bromide. It contains not less than 99.0% of distigmine bromide ( $\text{C}_{22}\text{H}_{32}\text{Br}_2\text{N}_4\text{O}_4$ ), calculated on the anhydrous basis.]

**Distilled water for injection** [Use the water prescribed by the monographs of Water for Injection or Sterile Water for Injection in Containers. Prepared by distillation. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of relevant test.]

**2,6-Di-tert-butylcresol**  $[(\text{CH}_3)_3\text{Cl}_2\text{C}_6\text{H}_2(\text{CH}_3)\text{OH}$  A white crystalline powder. Freely soluble in ethanol (95).

**Melting point** <2.60>: 69 – 71°C

**Residue on ignition** <2.44>: not more than 0.05%.

**2,6-Di-tert-butylcresol TS** Dissolve 0.1 g of 2,6-di-tert-butylcresol in ethanol (95) to make 10 mL.

**2,6-Di-tert-butyl-p-cresol** See 2,6-di-tert-butylcresol.

**2,6-Di-tert-butyl-p-cresol TS** See 2,6-di-tert-butylcresol TS.

**1,3-Di (4-pyridyl) propane**  $\text{C}_{13}\text{H}_{14}\text{N}_2$  A light yellow powder.

**Melting point** <2.60>: 61 – 62°C

**Water** <2.48>: less than 0.1%.

**1,1'-[3,3'-Dithiobis(2-methyl-1-oxopropyl)]-L-diproline**  $\text{C}_{18}\text{H}_{28}\text{N}_2\text{O}_6\text{S}_2$  White, crystals or crystalline powder. Sparingly soluble in methanol, and practically insoluble in water.

**Identification**—Determine the infrared absorption spectrum of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-diproline according to potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at

the wave numbers of about  $2960\text{ cm}^{-1}$ ,  $1750\text{ cm}^{-1}$ ,  $1720\text{ cm}^{-1}$ ,  $1600\text{ cm}^{-1}$ ,  $1480\text{ cm}^{-1}$ ,  $1450\text{ cm}^{-1}$  and  $1185\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 0.10 g of 1,1'-[3,3'-dithiobis (2-methyl-1-oxopropyl)]-L-diproline in exactly 10 mL of methanol. Perform the test with this solution as directed in the Purity (3) under Captopril: any spot other than the principal spot at the *Rf* value of about 0.2 does not appear.

**Content:** not less than 99.0%. Assay—Weigh accurately about 0.3 g of 1,1'-[3,3'-dithiobis (2-methyl-1-oxopropyl)]-L-diproline, dissolve in 20 mL of methanol, add 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through blue-green to blue (indicator: 3 drops of bromothymol blue TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned}\text{Each mL of } 0.1 \text{ mol/L sodium hydroxide VS} \\ = 21.63 \text{ mg of } \text{C}_{18}\text{H}_{28}\text{N}_2\text{O}_6\text{S}_2\end{aligned}$$

**Dithiodiglycolic acid**  $\text{C}_4\text{H}_6\text{O}_4\text{S}_2$  Prepared for biochemistry or amino acid analysis.

**Dithiodipropionic acid**  $\text{C}_6\text{H}_{10}\text{O}_4\text{S}_2$  Prepared for biochemistry or amino acid analysis.

**Dithiothreitol**  $\text{C}_4\text{H}_{10}\text{O}_2\text{S}_2$  Crystals.

*Melting point* <2.60>: about  $42^\circ\text{C}$

**Dithizone**  $\text{C}_6\text{H}_5\text{NHNHCSN:NC}_6\text{H}_5$  [K 8490, Special class]

**Dithizone solution for extraction** Dissolve 30 mg of dithizone in 1000 mL of chloroform, and add 5 mL of ethanol (95), and store. Before use, shake a suitable volume of the solution with one-half of its volume of diluted nitric acid (1 in 100), and use the chloroform layer after discarding the water layer.

**Dithizone TS** Dissolve 25 mg of dithizone in ethanol (95) to make 100 mL. Prepare before use.

**DNA standard stock solution for interferon alfa (NAMALWA)** To  $1 \times 10^9$  Namalwa cells add 0.1 mL of proteinase K solution and 20 mL of *N*-lauroyl sarcosine sodium TS, lyse the cells by gentle stirring at  $50 \pm 1^\circ\text{C}$  for 3 hours, add 20 mL of water-saturated phenol, and stir gently at room temperature for 3 hours. Add 10 mL of a mixture of chloroform and 3-methyl-1-butanol (24:1), centrifuge, and discard the lower layer. Add 20 mL of water-saturated phenol to the upper layer, stir gently at room temperature for 2 hours, and centrifuge. Collect the lower layer, dialyze for 24 hours against dialysis buffer A, add ribonuclease A and ribonuclease T<sub>1</sub> so that each mL of the inner solution obtained contains 25  $\mu\text{g}$  of ribonuclease A and 25 units of ribonuclease T<sub>1</sub>, and stir gently at  $37 \pm 1^\circ\text{C}$  for 3 hours. Add sodium lauryl sulfate solution (1 in 10) and proteinase K solution so that each mL contains 5 mg of sodium lauryl sulfate and 50  $\mu\text{g}$  of proteinase K, and stir gently at  $50 \pm 1^\circ\text{C}$  for 2 hours. Add an equal volume of phenol-saturated TE buffer solution, stir gently at room temperature for 2 hours, and centrifuge. After removing the lower layer, repeat the same operation. Collect the upper layer, and dialyze for 10 hours against dialysis buffer B, then change the external solution to dialysis buffer C, and dialyze for 24 hours. Collect the inner solution, add 0.1 volume of acetic acid-sodium acetate buffer solution (pH 5.2) and 2.2 volume of ethanol (99.5), and stir gently. Collect the DNA precipitated by winding on a glass rod, wash with diluted ethanol (7 in 10), dry in vacuum, dissolve the residue in 4 mL of TE buffer solution, and use this solution as the standard DNA. Dilute

with water so that each mL contains exactly 40 ng of DNA following the specific absorbance of double-stranded DNA,  $E^{1\%}_{\text{cm}} (260\text{ nm})$ , is 200.

**Docetaxel hydrate**  $\text{C}_{43}\text{H}_{53}\text{NO}_{14} \cdot 3\text{H}_2\text{O}$  [Same as the namesake monograph]

**Dopamine hydrochloride for assay**  $\text{C}_8\text{H}_{11}\text{NO}_2 \cdot \text{HCl}$

[Same as the monograph Dopamine hydrochloride. When dried, it contains not less than 99.0% of dopamine hydrochloride ( $\text{C}_8\text{H}_{11}\text{NO}_2 \cdot \text{HCl}$ ).]

**Doxepin hydrochloride**  $\text{C}_{19}\text{H}_{21}\text{NO} \cdot \text{HCl}$  White, crystals or crystalline powder. Melting point:  $185 - 191^\circ\text{C}$ .

**Doxifluridine**  $\text{C}_9\text{H}_{11}\text{FN}_2\text{O}_5$  [Same as the namesake monograph]

**Doxifluridine for assay**  $\text{C}_9\text{H}_{11}\text{FN}_2\text{O}_5$  [Same as the monograph Doxifluridine. When dried, it contains not less than 99.5% of doxifluridine ( $\text{C}_9\text{H}_{11}\text{FN}_2\text{O}_5$ ).]

**Doxorubicin hydrochloride**  $\text{C}_{27}\text{H}_{29}\text{NO}_{11} \cdot \text{HCl}$  [Same as the namesake monograph]

**Dragendorff's TS** Dissolve 0.85 g of bismuth subnitrate in 10 mL of acetic acid (100) and 40 mL of water with vigorous shaking (solution A). Dissolve 8 g of potassium iodide in 20 mL of water (solution B). Immediately before use, mix equal volumes of solution A, solution B and acetic acid (100). Store solution A and solution B in light-resistant containers.

**Dragendorff's TS for spraying** Add 20 mL of diluted acetic acid (31) (1 in 5) to 4 mL of a mixture of equal volumes of solution A and solution B of Dragendorff's TS. Prepare before use.

**Dried human normal plasma powder** Freeze-dried normal plasma obtained from healthy human.

**Dried sodium carbonate**  $\text{Na}_2\text{CO}_3$  [Same as the namesake monograph]

**Droxidopa for assay**  $\text{C}_9\text{H}_{11}\text{NO}_5$  [Same as the monograph Droxidopa. When dried, it contains not less than 99.5% of droxidopa ( $\text{C}_9\text{H}_{11}\text{NO}_5$ ).]

**DSS-*d*<sub>6</sub> for nuclear magnetic resonance spectroscopy**

$\text{C}_6\text{H}_9\text{D}_6\text{NaO}_3\text{SSi}$  Sodium 3-(trimethylsilyl)-1-propanesulfonate-*d*<sub>6</sub> that the traceability to the International System of Units has been secured.

**Dydrogesterone for assay**  $\text{C}_{21}\text{H}_{28}\text{O}_2$  [Same as the monograph Dydrogesterone. When dried, it contains not less than 99.0% of dydrogesterone ( $\text{C}_{21}\text{H}_{28}\text{O}_2$ ).]

**Eagle's minimum essential medium** Dissolve 6.80 g of sodium chloride, 400 mg of potassium chloride, 115 mg of anhydrous sodium dihydrogen phosphate, 93.5 mg (as anhydrous) of magnesium sulfate, 200 mg (as anhydrous) of calcium chloride, 1.00 g of glucose, 126 mg of L-arginine hydrochloride, 73.0 mg of L-lysine hydrochloride, 31.4 mg of L-cysteine hydrochloride monohydrate, 36.0 mg of L-tyrosine, 42.0 mg of L-histidine hydrochloride monohydrate, 52.0 mg of L-isoleucine, 52.0 mg of L-leucine, 15.0 mg of methionine, 32.0 mg of phenylalanine, 48.0 mg of L-threonine, 10.0 mg of L-tryptophan, 46.0 mg of L-valine, 75.0 mg of succinic acid, 100 mg of sodium succinate hexahydrate, 1.8 mg of choline bitartrate, 1.0 mg of folic acid, 2.0 mg of myoinositol, 1.0 mg of nicotinamide, 1.0 mg of calcium D-pantothenate, 1.0 mg of pyridoxal hydrochloride, 0.1 mg of riboflavin, 1.0 mg of thiamine chloride hydrochloride, 20  $\mu\text{g}$  of biotin and 6.0 mg of phenol red in 1000 mL of water, heat

in an autoclave at 121°C for 15 minutes and cool to room temperature, then add separately sterilized 22 mL of 10% sodium hydrogen carbonate TS and 10 mL of glutamine TS.

**Eagle's minimum essential medium containing bovine serum** To Eagle's minimum essential medium add an adequate amount of bovine serum.

**Ebastine for assay**  $C_{32}H_{39}NO_2$  [Same as the monograph Ebastine. When dried, it contains not less than 99.5% of ebastine ( $C_{32}H_{39}NO_2$ ).]

**Ecabet sodium hydrate for assay**  $C_{20}H_{27}NaO_5S \cdot 5H_2O$  [Same as the monograph Ecabet Sodium Hydrate. It contains not less than 99.5% of ecabet sodium ( $C_{20}H_{27}NaO_5S$ ), calculated on the anhydrous basis.]

**E. coli protein** Process *E. coli* cells (*E. coli* N4830/pTB281) retaining a plasmid deficient in the cilmoleukin gene according to the cilmoleukin purification process in the following order; (i) extraction, (ii) butylated vinyl polymer hydrophobic column chromatography, (iii) carboxymethylated vinyl polymer ion-exchange column chromatography, and (iv) sulfopropyl-polymer ion-exchange chromatography, and during process (iv) collect the fractions corresponding to the cilmoleukin elution position. Dialyze the fractions obtained in (iv) against 0.01 mol/L acetate buffer solution (pH 5.0), and take the dialysis solution as *E. coli* protein.

*Description*—Clear and colorless solution.

*Identification*—When the absorption spectrum is determined as directed under Ultraviolet-visible Spectrophotometry <2.24>, an absorption maximum is observed in the region of 278 nm.

*Protein content*: When determining the protein content using the Assay (1) under Cilmoleukin (Genetical Recombination), the protein content per mL is 0.1 to 0.5 mg.

**E. coli protein stock solution** A solution obtained by culturing a bacteria that contains a plasmid lacking the teceleukin gene but is otherwise exactly identical to the teceleukin-producing *E. coli* strain in every function except teceleukin production, and then purified using a purification technique that is more simple than that for teceleukin. Determine the amount of protein by Bradford method using bovine serum albumin as the standard substance. Store shielded from light at -70°C.

**Edaravone for assay**  $C_{10}H_{10}N_2O$  [Same as the monograph Edaravone. When dried, it contains not less than 99.5% of edaravone ( $C_{10}H_{10}N_2O$ ).]

**Egg albumin for gel filtration molecular mass marker** Obtained from chicken egg white, for gel filtration chromatography.

**Elderberry lectin** It is a lectin derived from the Japan or Western elderberry, specifically recognizes a sugar chain with sialic acid is bound to end by α-2, 6 linkage.

**Elderberry lectin TS** Dilute biotin-labeled elderberry lectin with 0.01 mol/L tris buffer solution-sodium chloride TS (pH 7.4) so that the concentration is 10 µg/mL. Prepare before use.

**Eleutheroside B for liquid chromatography**  $C_{17}H_{24}O_9$  A white crystalline powder. Sparingly soluble in methanol, slightly soluble in water, and very slightly soluble in ethanol (99.5). Melting point: 190 – 194°C.

*Identification*—Determine the absorption spectrum of a solution of eleutheroside B for liquid chromatography in methanol (1 in 200,000) as directed under Ultraviolet-visible

Spectrophotometry <2.24>; it exhibits a maximum between 263 nm and 267 nm.

*Purity Related substances*—Dissolve 1.0 mg of eleutheroside B for liquid chromatography in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than eleutheroside B obtained from the sample solution is not larger than the peak area of eleutheroside B from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Identification under *Eleutherococcus Senticosus Rhizome*.

Time span of measurement: About 3 times as long as the retention time of eleutheroside B, beginning after the solvent peak.

#### System suitability

System performance: Proceed as directed in the system suitability in the Identification under *Eleutherococcus Senticosus Rhizome*.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of eleutheroside B obtained with 10 µL of this solution is equivalent to 3.5 to 6.5% of that with 10 µL of the standard solution.

**EMB plate medium** Melt eosin methylene blue agar medium by heating, and cool to about 50°C. Transfer about 20 mL of this medium to a Petri dish, and solidify horizontally. Place the dish with the cover slightly opened in the incubator to evaporate the inner vapor and water on the plate.

**Emedastine fumarate for assay**  $C_{17}H_{26}N_4O \cdot 2C_4H_4O_4$  [Same as the monograph Emedastine Fumarate. When dried it contains not less than 99.5% of emedastine fumarate ( $C_{17}H_{26}N_4O \cdot 2C_4H_4O_4$ ).]

**Emetine hydrochloride for assay**  $C_{29}H_{40}N_2O_4 \cdot 2HCl$  A white or light-yellow crystalline powder. Soluble in water.

*Absorbance* <2.24>  $E_{1\text{ cm}}^{1\%}$  (283 nm): 116 – 127 (10 mg, diluted methanol (1 in 2), 400 mL). [after drying in a desiccator (in vacuum, phosphorus (V) oxide, 50°C) for 5 hours].

*Melting point* <2.60>: about 250°C [with decomposition, after drying in a desiccator (in vacuum, phosphorus (V) oxide, 50°C) for 5 hours].

*Purity Related substances*—Dissolve 10 mg of emetine hydrochloride for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of peaks other than emetine obtained from the sample solution is not larger than the peak of emetine from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under *Ipecac*.

Time span of measurement: About 3 times as long as the retention time of emetine.

#### System suitability

System performance and System repeatability: Proceed as directed in the system suitability in the Assay under Ipecac.

Test for required detectability: Pipet 1 mL of the standard solution, add the mobile phase to make exactly 20 mL. Confirm that the peak area of emetine obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

#### Emetine hydrochloride for component determination

See emetine hydrochloride for assay.

**Emorfazone for assay**  $C_{11}H_{17}N_3O_3$  [Same as the monograph Emorfazone. When dried, it contains not less than 99.0% of emorfazone ( $C_{11}H_{17}N_3O_3$ ).]

**Enalapril maleate**  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$  [Same as the namesake monograph]

**Endo's medium** Melt 1000 mL of the ordinary agar medium by heating in a water bath, and adjust the pH to between 7.5 and 7.8. Add 10 g of lactose monohydrate previously dissolved in a small quantity of water, mix thoroughly, and add 1 mL of fuchsin-ethanol TS. After cooling to about 50°C, add dropwise a freshly prepared solution of sodium sulfite heptahydrate (1 in 10) until a light red color develops owing to reducing fuchsin, requiring about 10 to 15 mL of a solution of sodium sulfite heptahydrate (1 in 10). Dispense the mixture, and sterilize fractionally on each of three successive days for 15 minutes at 100°C.

**Endo's plate medium** Melt Endo's medium by heating, and cool to about 50°C. Transfer about 20 mL of this medium to a Petri dish, and solidify horizontally. Place the dishes with the cover slightly opened in the incubator to evaporate the inner vapor and water on the surface of the agar.

**Enflurane**  $C_3H_2ClF_3O$  [Same as the namesake monograph]

**Enzyme TS** The supernatant liquid is obtained as follows: To 0.3 g of an enzyme preparation potent in amylo-lytic and phosphorolytic activities, obtained from *Aspergillus oryzae*, add 10 mL of water and 0.5 mL of 0.1 mol/L hydrochloric acid TS, mix vigorously for a few minutes, and centrifuge. Prepare before use.

**Enzyme TS for glucagon** Dissolve 2 mg of  $\alpha$ -chymotrypsin in 1 mL of 0.1 mol/L ammonium hydrogen carbonate TS.

#### Eosin

See eosin Y.

#### Eosin Y

$C_{20}H_6Br_4Na_2O_5$  Red, masses or powder.

**Identification**—To 10 mL of a solution (1 in 1000) add 1 drop of hydrochloric acid: yellow-red precipitates appear.

**Eosin methylene blue agar medium** Dissolve by boiling 10 g of casein peptone, 2 g of dipotassium hydrogenphosphate and 25 to 30 g of agar in about 900 mL of water. To this mixture add 10 g of lactose monohydrate, 20 mL of a solution of eosin Y(1 in 50), 13 mL of a solution of methylene blue (1 in 200) and warm water to make 1000 mL. Mix thoroughly, dispense, sterilize by autoclaving at 121°C for not more than 20 minutes, and cool quickly by immersing in cold water, or sterilize fractionally on each of three successive days for 30 minutes at 100°C.

**Ephedrine hydrochloride**  $C_{10}H_{15}NO \cdot HCl$  [Same as the namesake monograph]

**Ephedrine hydrochloride for assay** See ephedrine hydrochloride.

#### Ephedrine hydrochloride for assay of crude drugs

$C_{10}H_{15}NO \cdot HCl$  Ephedrine hydrochloride for assay or the substance that complies with the following requirements.

White, crystals or crystalline powder. Freely soluble in water, and soluble in ethanol (95).

**Identification**—Determine the infrared absorption spectrum of ephedrine hydrochloride for assay of crude drugs, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum of Ephedrine Hydrochloride: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ :  $-33.0^{\circ}$  to  $-36.0^{\circ}$  (after drying, 0.1 g, water, 2 mL, 100 mm).

#### Melting point

<2.60>: 218–222°C

**Purity** Related substances—Dissolve 10 mg of ephedrine hydrochloride for assay of crude drugs in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than ephedrine obtained from the sample solution is not larger than the peak area of ephedrine from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Ephedra Herb.

Time span of measurement: About 3 times as long as the retention time of ephedrine, beginning after the solvent peak.

#### System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Ephedra Herb.

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of ephedrine obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

**Loss on drying** <2.41>: Not more than 0.5% (0.1 g, 105°C, 3 hours).

#### 6-Epoxycycline hydrochloride

$C_{22}H_{24}N_2O_8 \cdot HCl$  Yellow to dark yellow, crystals or crystalline powder.

**Purity** Related substances—Dissolve 20 mg of 6-epoxycycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20  $\mu$ L of the sample solution as directed in the Purity (2) under Doxycycline Hydrochloride Hydrate, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total area of the peaks other than 6-epoxycycline is not more than 10%.

**4-Epioxytetracycline**  $C_{22}H_{24}N_2O_9$  Green-brown to brown powder.

**Purity** Related substances—Dissolve 20 mg of 4-epoxytetracycline in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20  $\mu$ L of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each

peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than 4-epoxytetracycline is not more than 10%.

**Eriochrome black T**  $C_{20}H_{12}N_3NaO_7S$  [K 8736, Special class]

**Eriochrome black T-sodium chloride indicator** Mix 0.1 g of eriochrome black T and 10 g of sodium chloride, and triturate until the mixture becomes homogeneous.

**Eriochrome black T TS** Dissolve 0.3 g of eriochrome black T and 2 g of hydroxylammonium chloride in methanol to make 50 mL. Use within 1 week. Preserve in light-resistant containers.

**Erythromycin B**  $C_{37}H_{67}NO_{12}$  White to light yellow-white powder.

**Purity** Related substances—Dissolve 10 mg of erythromycin B in 1 mL of methanol, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100  $\mu$ L each of the sample solution and standard solution as directed in the Purity (3) under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin B obtained from the sample solution is not more than the peak area of erythromycin B from the standard solution.

**Erythromycin C**  $C_{36}H_{65}NO_{13}$  White to light yellow-white powder.

**Purity** Related substances—Dissolve 10 mg of erythromycin C in 1 mL of methanol, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100  $\mu$ L each of the sample solution and standard solution as directed in the Purity (3) under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin C obtained from the sample solution is not more than the peak area of erythromycin C from the standard solution.

**Essential oil** Same as the essential oil under the monograph.

**Etacrylic acid for assay**  $C_{13}H_{12}Cl_2O_4$  [Same as the monograph Etacrylic acid. When dried, it contains not less than 99.0% of etacrylic acid ( $C_{13}H_{12}Cl_2O_4$ ).]

**Ethanol** See ethanol (95).

**Ethanol (95)**  $C_2H_5OH$  [K 8102, Special class]

**Ethanol (95), methanol-free** Perform the test for methanol, by using this methanol-free ethanol (95) in place of the standard solution, as directed in Methanol Test <1.12>; it is practically colorless.

**Ethanol (99.5)**  $C_2H_5OH$  [K 8101, Special class]

**Ethanol (99.5) for liquid chromatography**  $C_2H_5OH$  A clear, colorless liquid, miscible with water.

**Purity** Ultraviolet absorbing substance—Perform the test as directed under Ultraviolet-visible Spectrophotometry

<2.24> using water as the blank: the absorbances at 210 nm, at 220 nm, at 230 nm, at 240 nm, at 254 nm and at 260 nm are not more than 0.70, 0.40, 0.20, 0.10, 0.02 and 0.01, respectively.

**Ethanol, aldehyde-free** Transfer 1000 mL of ethanol (95) to a glass-stoppered bottle, add the solution prepared by dissolving 2.5 g of lead (II) acetate trihydrate in 5 mL of water, and mix thoroughly. In a separate container, dissolve 5 g of potassium hydroxide in 25 mL of warm ethanol (95), cool, and add this solution gently, without stirring, to the first solution. After 1 hour, shake this mixture vigorously, allow to stand overnight, decant the supernatant liquid, and distil the ethanol.

**Ethanol, dehydrated** See ethanol (99.5).

**Ethanol, dilute** To 1 volume of ethanol (95) add 1 volume of water.

**Ethanol, diluted** Prepare by diluting ethanol (99.5).

**Ethanol for alcohol number determination** See Alcohol Number Determination <1.01>.

**Ethanol for disinfection** [Same as the namesake monograph]

**Ethanol for gas chromatography** Use ethanol prepared by distilling ethanol (99.5) with iron (II) sulfate heptahydrate. Preserve in containers, in which the air has been displaced with nitrogen, in a dark, cold place.

**Ethanol-free chloroform** See chloroform, ethanol-free.

**Ethanol-isotonic sodium chloride solution** To 1 volume of ethanol (95) add 19 volumes of isotonic sodium chloride solution.

**Ethanol, methanol-free** See ethanol (95), methanol-free.

**Ethanol, neutralized** To a suitable quantity of ethanol (95) add 2 to 3 drops of phenolphthalein TS, then add 0.01 mol/L or 0.1 mol/L sodium hydroxide VS until a light red color develops. Prepare before use.

**Ethenzamide**  $C_9H_{11}NO_2$  [Same as the namesake monograph].

**Ether** See diethyl ether.

**Ether, anesthetic**  $C_2H_5OC_2H_5$  [Same as the namesake monograph]

**Ether, dehydrated** See diethyl ether, dehydrated.

**Ether for purity of crude drug** See diethyl ether for purity of crude drug.

**Ethinylestradiol**  $C_{20}H_{24}O_2$  [Same as the namesake monograph]

**4'-Ethoxyacetophenone**  $C_2H_5OC_6H_4COCH_3$  White crystals.

Melting point <2.60>: 37 – 39°C

**3-Ethoxy-4-hydroxybenzaldehyde**  $C_9H_{10}O_3$  White to pale yellow-white crystalline. Freely soluble in ethanol (95), and slightly soluble in water.

Melting point <2.60>: 76 – 78°C

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.3 g of 3-ethoxy-4-hydroxybenzaldehyde, previously dried in a desiccator (phosphorous (V) oxide) for 4 hours, dissolve in 50 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (indicator: thymol blue TS).

Each mL of 0.1 mol/L sodium methoxide VS  
= 16.62 mg of  $C_9H_{10}O_3$

**p-Ethoxyphenol** See 4-ethoxyphenol.

**4-Ethoxyphenol**  $C_8H_{10}O_2$  White to light yellow-brown, crystals or crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.

*Melting point* <2.60>: 62 – 68°C

*Purity*—Dissolve 0.5 g of 4-Ethoxyphenol in 5 mL of ethanol (95), and use this solution as the sample solution. Perform the test with 1  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of substance other than 4-ethoxyphenol by the area percentage method: it is not more than 2.0%.

#### Operating conditions

Detector: Thermal conductivity detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with 180 to 250  $\mu$ m siliceous earth for gas chromatography coated with methylsilicone polymer for gas chromatography.

Column temperature: A constant temperature of about 150°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of 4-ethoxyphenol is about 5 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 4-ethoxyphenol obtained with 1  $\mu$ L of the sample solution is not less than 50% of the full scale.

Time span of measurement: 3 times as long as the retention time of 4-ethoxyphenol, beginning after the solvent peak.

**Ethyl acetate**  $CH_3COOC_2H_5$  [K 8361, Special class]

**Ethylamine hydrochloride**  $C_2H_5NH_2 \cdot HCl$  White to light yellow-brown, crystals or crystalline powder, having a deliquescent.

**Ethyl aminobenzoate**  $C_9H_{11}NO_2$  [Same as the namesake monograph]

**Ethylbenzene**  $C_6H_5C_2H_5$  A colorless liquid. Freely soluble in ethanol (99.5) and in acetone, and practically insoluble in water.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.862 – 0.872

*Boiling point* <2.57>: about 135°C

**Ethyl benzoate**  $C_6H_5COOC_2H_5$  Clear, colorless liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.502 – 1.507

*Specific gravity* <2.56>  $d_2^{20}$ : 1.045 – 1.053

**Ethyl n-caprylate**  $C_{10}H_{20}O_2$  Clear and colorless to almost colorless liquid.

*Specific gravity* <2.56>  $d_2^{20}$ : 0.864 – 0.871

*Purity* Related substances—Dissolve 0.10 g of ethyl n-caprylate in 10 mL of dichloromethane and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 5  $\mu$ L each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area from these solutions by the automatic integration method: the total peak areas other than ethyl n-caprylate obtained from the sample solution is not larger than the peak area of ethyl n-caprylate from the standard solution (1).

#### Operating conditions

Proceed the operating conditions in the Assay under Men-

tha Oil except time span of measurement.

Time span of measurement: 3 times as long as the retention time of ethyl n-caprylate, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution (1), add dichloromethane to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of ethyl n-caprylate obtained with 5  $\mu$ L of the standard solution (2) can be measured by the automatic integration method and the peak height of ethyl n-caprylate with 5  $\mu$ L of the standard solution (1) is about 20% of the full scale.

**Ethyl carbamate**  $H_2NCOOC_2H_5$  White, crystals or powder.

*Melting point* <2.60>: 48 – 50°C

*Purity* Clarity of solution—Dissolve 5 g of ethyl carbamate in 20 mL of water: the solution is clear.

**Ethyl cyanoacetate**  $NCCH_2COOC_2H_5$  Colorless or light yellow, clear liquid, having an aromatic odor. Specific gravity  $d_2^{20}$ : about 1.08.

*Identification*—To 0.5 mL of a solution of ethyl cyanoacetate in ethanol (99.5) (1 in 10,000) add a mixture of 1 mL of a solution of quinhydrone in diluted ethanol (99.5) (1 in 2) (1 in 20,000) and 1 drop of ammonia solution (28): a light blue color develops.

**Ethylenediamine**  $C_2H_8N_2$  [Same as the namesake monograph]

**Ethylenediamine TS** Dissolve 70 g of ethylenediamine in 30 g of water.

**Ethylene glycol**  $HOCH_2CH_2OH$  [K 8105, Special class]

**Ethylene glycol for water determination** Distil ethylene glycol, and collect the fraction distilling between 195°C and 198°C. The water content is not more than 1.0 mg per mL.

**Ethylene oxide** A colorless flammable gas. Use ethylene oxide from a metal cylinder.

*Boiling point* <2.57>: 9 – 12°C

**Ethyl formate**  $HCOOC_2H_5$  A clear and colorless liquid. Miscible with ethanol (95) and with acetone, and soluble in water.

*Identification*—Determine the infrared absorption spectrum of ethyl formate as directed in the liquid film method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $2980\text{ cm}^{-1}$ ,  $2930\text{ cm}^{-1}$ ,  $1718\text{ cm}^{-1}$ ,  $1470\text{ cm}^{-1}$ ,  $1449\text{ cm}^{-1}$ ,  $1387\text{ cm}^{-1}$ ,  $1302\text{ cm}^{-1}$ ,  $1181\text{ cm}^{-1}$ ,  $1004\text{ cm}^{-1}$ ,  $840\text{ cm}^{-1}$  and  $747\text{ cm}^{-1}$ .

*Purity*—(1) Perform the test with 1  $\mu$ L of ethyl formate as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of ethyl formate by the area percentage method: not less than 97.0%.

#### Operating conditions

Detector: A thermal conductivity detector.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated the inside surface with a layer about 0.25  $\mu$ m thick of polyethylene glycol 20M for gas chromatography.

Column temperature: Maintain at 50°C for 1 minute after injecting sample, raise the temperature to 150°C at the rate of 10°C per minute, and maintain at 150°C for 1 minute.

Carrier gas: Helium.

Flow rate: 41 cm per second.

Split ratio: 1:110.

Time span of measurement: About 5 times as long as the retention time of ethyl formate.

(2) Acid (as formic acid) Dissolve 0.5 g of potassium iodate and 5 g of potassium iodide in 50 mL of water, and add 2 g of ethyl formate. After allowing to stand for 10 minutes, add 2 drops of starch TS and 1.30 mL of 0.1 mol/L sodium thiosulfate VS: the solution is colorless (not more than 0.3%).

*Water* <2.48>: not more than 0.5% (1 g, coulometric titration).

**2-Ethylhexyl parahydroxybenzoate**  $C_{15}H_{22}O_3$  Pale yellow, clear viscous liquid. Miscible with methanol (99.5). Practically insoluble in water.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 1 g of 2-ethylhexyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 250.3 \text{ mg of } C_{15}H_{22}O_3 \end{aligned}$$

**Ethyl iodide** See iodoethane.

**N-Ethylmaleimide**  $C_6H_7NO_2$  White crystals, having a pungent, characteristic odor. Freely soluble in ethanol (95), and slightly soluble in water.

*Melting point* <2.60>: 43 – 46°C

**Purity** Clarity and color of solution—Dissolve 1 g of *N*-ethylmaleimide in 20 mL of ethanol (95): the solution is clear and colorless.

**Content:** not less than 99.0%. **Assay**—Dissolve about 0.1 g of *N*-ethylmaleimide, accurately weighed, in 20 mL of ethanol (95), add exactly 20 mL of 0.1 mol/L sodium hydroxide VS, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 12.51 \text{ mg of } C_6H_7NO_2 \end{aligned}$$

**N-Ethylmorpholine**  $C_6H_{13}NO$  A colorless to yellow-brown liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.439 – 1.443

*Specific gravity* <2.56>  $d_4^{20}$ : 0.908 – 0.916

**Ethyl parahydroxybenzoate**  $HOC_6H_4COOC_2H_5$  [Same as the namesake monograph]

**2-Ethyl-2-phenylmalondiamide**  $C_{11}H_{14}O_2N_2$  White, odorless crystals. Soluble in ethanol (95), and very slightly soluble in water. Melting point: about 120°C (with decomposition).

**Purity** Related substances—To 5.0 mg of 2-ethyl-2-phenylmalondiamide add 4 mL of pyridine and 1 mL of bis(trimethylsilyl)acetamide, shake thoroughly, and heat at 100°C for 5 minutes. After cooling, add pyridine to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 2  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the operating conditions in the Purity (3) under Primidone: any peak other than the peaks of 2-ethyl-2-phenylmalondiamide and the solvent does not appear. Adjust the detection sensitivity so that the peak height of 2-ethyl-2-phenylmalondiamide obtained from 2  $\mu$ L of the sample solution is about 80% of the full scale, and the time span of measurement is about twice

as long as the retention time of 2-ethyl-2-phenylmalondiamide, beginning after the solvent peak.

**Ethyl propionate**  $CH_3CH_2COOC_2H_5$  Colorless, clear liquid.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.890 – 0.892

**Etidronate disodium for assay**  $C_2H_6Na_2O_7P_2$  [Same as the monograph Etidronate Disodium. When dried, it contains not less than 99.0% of etidronate disodium ( $C_2H_6Na_2O_7P_2$ ).]

**Etilefrine hydrochloride**  $C_{10}H_{15}NO_2.HCl$  [Same as the namesake monograph]

**Etilefrine hydrochloride for assay**  $C_{10}H_{15}NO_2.HCl$  [Same as the monograph Etilefrine Hydrochloride. When dried, it contains not less than 99.0% of etilefrine hydrochloride ( $C_{10}H_{15}NO_2.HCl$ ).]

**Etizolam for assay**  $C_{17}H_{15}ClN_4S$  [Same as the monograph Etizolam. When dried, it contains not less than 99.0% of etizolam ( $C_{17}H_{15}ClN_4S$ ).]

**Eugenol for thin-layer chromatography**  $C_{10}H_{12}O_2$  Colorless to yellow, clear liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and slightly soluble in water.

**Identification**—Determine the absorption spectrum of a solution of eugenol for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 227 nm and 231 nm, and between 280 nm and 284 nm.

**Purity** Related substances—Dissolve 5 mg of eugenol for thin-layer chromatography in 1 mL of methanol. Perform the test with 1  $\mu$ L of this solution as directed in the Identification under Clove: any spot other than the principal spot with an *Rf* value of about 0.4 does not appear.

**Euodia fruit** [Same as the namesake monograph]

**Evodiamine for assay**  $C_{19}H_{17}N_3O$  White to light yellow, crystals or crystalline powder. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Correct the content based on the amount (%) obtained in the Assay.

**Identification**—Measure  $^1H$ -NMR as directed in the Assay: When the signal of the reference standard for qNMR as  $\delta$  0 ppm, it exhibits a double doublet-like signal equivalent to one proton around  $\delta$  2.82 ppm, signals equivalent to four protons which includes a singlet signal around  $\delta$  2.91 ppm and a multiplet signal around  $\delta$  2.90 ppm –  $\delta$  2.98 ppm, a double triplet-like signal equivalent to one proton around  $\delta$  3.23 ppm, a double doublet-like signal equivalent to one proton around  $\delta$  4.66 ppm, a singlet signal equivalent to one proton around  $\delta$  6.16 ppm, a triplet-like signal equivalent to one proton around  $\delta$  7.00 ppm, a triplet-like signal equivalent to one proton around  $\delta$  7.05 ppm, a doublet-like signal equivalent to one proton around  $\delta$  7.08 ppm, a triplet-like signal equivalent to one proton around  $\delta$  7.14 ppm, a doublet-like signal equivalent to one proton around  $\delta$  7.39 ppm, a doublet-like signal equivalent to one proton around  $\delta$  7.51 ppm, a multiplet signal equivalent to one proton around  $\delta$  7.52 ppm and a double doublet-like signal equivalent to one proton around  $\delta$  7.83 ppm.

**Unity of peak**—Dissolve 1 mg of evodiamine for assay in 20 mL of methanol, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of evodiamine peak

and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Goshuyuto Extract.

Detector: A photodiode array detector (wavelength: 282 nm; spectrum range of measurement: 220 – 400 nm).

#### System suitability

System performance: When the procedure is run with 10  $\mu\text{L}$  of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of evodiamine are not less than 5000 and not more than 1.5, respectively.

**Assay**—Weigh accurately 5 mg of evodiamine for assay and 1 mg of DSS- $d_6$  for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve them in 1 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure  $^1\text{H-NMR}$  as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using DSS- $d_6$  for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity  $A$  (equivalent to 1 hydrogen) of the signal around  $\delta$  6.16 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\begin{aligned} \text{Amount (\%)} \text{ of evodiamine } (\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}) \\ = M_s \times I \times P/(M \times N) \times 1.3521 \end{aligned}$$

*M*: Amount (mg) of evodiamine for assay taken

*M<sub>s</sub>*: Amount (mg) of DSS- $d_6$  for nuclear magnetic resonance spectroscopy taken

*I*: Signal resonance intensity  $A$  based on the signal resonance intensity of DSS- $d_6$  for nuclear magnetic resonance spectroscopy as 9.000

*N*: Number of the hydrogen derived from  $A$

*P*: Purity (%) of DSS- $d_6$  for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having  $^1\text{H}$  resonance frequency of not less than 400 MHz.

Target nucleus:  $^1\text{H}$ .

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

$^{13}\text{C}$  decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around  $\delta$  6.16 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal around  $\delta$  6.16 ppm is not overlapped with any signal of obvious foreign substance.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity  $A$  to that of the reference standard for qNMR is not more than 1.0%.

**Factor IIa** A lyophilized factor IIa purified from human plasma. A white to pale yellow powder. It contains not less than 2000 IU per mg of protein.

**Factor Xa** It is prepared from lyophilization of Factor Xa which has been prepared from bovine plasma. White or pale yellow, masses or powder.

**Purity** Clarity and color of solution—Dissolve 71  $\text{nkat}_{\text{s}-2222}$  of it in 10 mL water; the solution is clear and colorless or pale yellow.

**Content:** not less than 75% and not more than 125% of the label.

**Factor Xa TS** Dissolve 71  $\text{nkat}_{\text{s}-2222}$  of factor Xa in 10 mL of water.

**Famotidine for assay**  $\text{C}_{8}\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3$  [Same as the monograph Famotidine. When dried, it contains not less than 99.0% of famotidine ( $\text{C}_{8}\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3$ ), and when proceed as directed in the Purity (3), the total related substance is not more than 0.4%.]

**Fatty acid methyl esters mixture TS** Dissolve 0.50 g of a mixture of methyl myristate for gas chromatography, methyl palmitate for gas chromatography, methyl palmitoleate for gas chromatography, methyl stearate for gas chromatography, methyl oleate for gas chromatography, methyl linoleate for gas chromatography and methyl linolenate for gas chromatography, corresponding to the composition of Polysorbate 80, in heptane to make 50.0 mL.

**Fatty oil** Same as the fatty oil under the monograph.

**FBS-IMDM** Dissolve an amount of the powder for 1 L of Iscove's modified Dulbecco's powder medium, 0.1 g of kanamycin sulfate (not less than 600  $\mu\text{g}$  potency/mg), 3.0 g of sodium hydrogen carbonate and 36  $\mu\text{L}$  of 2-mercapto ethanol solution (1 in 10) in water to make 1000 mL, and sterilize by filtration. To this solution add fetal calf serum, previously heated at 56°C for 30 minutes, so that the concentration of the serum is 10 vol%.

**Fehling's TS** The copper solution—Dissolve 34.66 g of copper (II) sulfate pentahydrate in water to make 500 mL. Keep this solution in a glass-stoppered bottles in well-filled.

The alkaline tartrate solution—Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodium hydroxide in water to make 500 mL. Preserve this solution in a polyethylene container.

Before use, mix equal volumes of both solutions.

**Fehling's TS for amyloytic activity test** The copper solution—Dissolve 34.660 g of copper (II) sulfate pentahydrate, accurately weighed, in water to make exactly 500 mL. Preserve this solution in well-filled, glass-stoppered bottles.

The alkaline tartrate solution—Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodium hydroxide in water to make exactly 500 mL. Preserve this solution in polyethylene containers.

Before use, mix exactly equal volumes of both solutions.

**Felbinac for assay**  $\text{C}_{14}\text{H}_{12}\text{O}_2$  [Same as the monograph Felbinac. When dried, it contains not less than 99.0% of felbinac ( $\text{C}_{14}\text{H}_{12}\text{O}_2$ ).]

**Felodipine for assay**  $\text{C}_{18}\text{H}_{19}\text{Cl}_2\text{NO}_4$  [Same as the mono-

graph Felodipine. It contains not less than 99.5% of ferodipine ( $C_{18}H_{19}Cl_2NO_4$ ), calculated on the dried basis.]

**Ferric ammonium citrate** See ammonium iron (III) citrate.

**Ferric ammonium sulfate** See ammonium iron (III) sulfate dodecahydrate.

**Ferric ammonium sulfate TS** See ammonium iron (III) sulfate TS.

**Ferric ammonium sulfate TS, dilute** See ammonium iron (III) sulfate TS, dilute.

**Ferric chloride** See iron (III) chloride hexahydrate.

**Ferric chloride-acetic acid TS** See iron (III) chloride-acetic acid TS.

**Ferric chloride-iodine TS** See iron (III) chloride-iodine TS.

**Ferric chloride-methanol TS** See iron (III) chloride-methanol TS.

**Ferric chloride-pyridine TS, anhydrous** See iron (III) chloride-pyridine TS, anhydrous.

**Ferric chloride TS** See iron (III) chloride TS.

**Ferric chloride TS, acidic** See iron (III) chloride TS, acidic.

**Ferric chloride TS, dilute** See iron (III) chloride TS, dilute.

**Ferric nitrate** See iron (III) nitrate enneahydrate.

**Ferric nitrate TS** See iron (III) nitrate TS.

**Ferric perchlorate** See iron (III) perchlorate hexahydrate.

**Ferric perchlorate-dehydrated ethanol TS** See iron (III) perchlorate-ethanol TS.

**Ferric salicylate TS** See Iron salicylate TS

**Ferric sulfate** See iron (III) sulfate *n*-hydrate.

**Ferric sulfate TS** See iron (III) sulfate TS.

**Ferrous ammonium sulfate** See ammonium iron (II) sulfate hexahydrate.

**Ferrous sulfate** See iron (II) sulfate heptahydrate.

**Ferrous sulfate TS** See iron (II) sulfate TS.

**Ferrous sulfide** See iron (II) sulfide.

**Ferrous tartrate TS** See iron (II) tartrate TS.

**Ferrous thiocyanate TS** See iron (II) thiocyanate TS.

**Ferrous trisodium pentacyanoamine TS** See iron (II) trisodium pentacyanoamine TS.

**(E)-Ferulic acid**  $C_{10}H_{10}O_4$  White to light yellow, crystals or crystalline powder. Freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 173 – 176°C.

**Identification**—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 215 nm and 219 nm, between 231 nm and 235 nm, and between 318 nm and 322 nm.

**Purity** Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dis-

solve 1 mg of (E)-ferulic acid in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 2  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365 nm): no spot appears other than the principle spot at the *Rf* value of about 0.6.

**(E)-Ferulic acid for assay**  $C_{10}H_{10}O_4$  Use (E)-Ferulic acid.

It meets the requirements of the following 1) (E)-ferulic acid for assay 1 or 2) (E)-ferulic acid for assay 2 (Purity value by qNMR). The former is used after drying in a desiccator (silica gel) for 24 hours, and latter is used with correction for its amount based on the result obtained in the Assay.

1) (E)-Ferulic acid for assay 1

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (320 nm): 878 – 969 (5 mg, methanol, 1000 mL).

**Purity** Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 5 mg of (E)-ferulic acid for assay 1 in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than (E)-ferulic acid obtained from the sample solution is not larger than the peak area of (E)-ferulic acid from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Tokishakuyakusan Extract.

Time span of measurement: About 6 times as long as the retention time of (E)-ferulic acid, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of (E)-ferulic acid obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

**System performance:** When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (E)-ferulic acid are not less than 5000 and not more than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (E)-ferulic acid is not more than 1.5%.

2) (E)-Ferulic acid for assay 2 (Purity value by quantitative NMR)

**Unity of peak**—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 5 mg of (E)-ferulic acid for assay 2 in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spec-

tra of at least 3 points including the top of (*E*)-ferulic acid peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Tokishakuyakusan Extract.

Detector: A photodiode array detector (wavelength: 320 nm, spectrum range of measurement: 220–400 nm).

#### System suitability

System performance: When the procedure is run with 10  $\mu$ L of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (*E*)-ferulic acid are not less than 5000 and not more than 1.5, respectively.

**Assay**—Weigh accurately 5 mg of (*E*)-ferulic acid for assay 2 and 1 mg of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity *A* (equivalent to 1 hydrogen) of the signal around  $\delta$  6.06 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\begin{aligned} \text{Amount (\%)} \text{ of } (\text{E})\text{-ferulic acid } (\text{C}_{10}\text{H}_{10}\text{O}_4) \\ = M_S \times I \times P/(M \times N) \times 0.8573 \end{aligned}$$

*M*: Amount (mg) of (*E*)-ferulic acid for assay 2 taken

*M<sub>S</sub>*: Amount (mg) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

*I*: Signal resonance intensity *A* based on the signal resonance intensity of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

*N*: Number of hydrogen derived from *A*

*P*: Purity (%) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

<sup>13</sup>C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around  $\delta$  6.06 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal around  $\delta$  6.06 ppm is not overlapped with any signal

of obvious foreign substances.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity *A* to that of the reference standard for qNMR is not more than 1.0%.

**Fetal calf serum** Serum obtained from fetal calves. Interleukin-2 dependent cell growth suppression substance is removed by heat at 56°C for 30 min before use.

**Fibrinogen** Fibrinogen is prepared from human or bovine blood by fractional precipitation with ethanol or ammonium sulfate. It may contain citrate, oxalate and sodium chloride. A white amorphous solid. Add 1 mL of isotonic sodium chloride solution to 10 mg of fibrinogen. It, when warmed to 37°C, dissolves with a slight turbidity, and clots on the subsequent addition of 1 unit of thrombin.

**1st Fluid for disintegration test** See 1st fluid for dissolution test.

**1st Fluid for dissolution test** Dissolve 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid and water to make 1000 mL. It is clear and colorless, and has a pH of about 1.2.

**Fixed oil** Same as the vegetable oils under the monograph.

**FL cell** Established cell strain derived from normal human amnion. Subculture the cells in Eagle's minimum essential medium containing bovine serum.

**Flecainide acetate** C<sub>17</sub>H<sub>20</sub>F<sub>6</sub>N<sub>2</sub>O<sub>3</sub>,C<sub>2</sub>H<sub>4</sub>O<sub>2</sub> [Same as the namesake monograph]

**Flecainide acetate for assay** C<sub>17</sub>H<sub>20</sub>F<sub>6</sub>N<sub>2</sub>O<sub>3</sub>,C<sub>2</sub>H<sub>4</sub>O<sub>2</sub> [Same as the monograph Flecainide Acetate. When dried, it contains not less than 99.0% of flecainide acetate (C<sub>17</sub>H<sub>20</sub>F<sub>6</sub>N<sub>2</sub>O<sub>3</sub>,C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>). Additionally, when perform the test as directed in the Purity (3), the sample solution does not show the spot corresponding to the spot obtained from the standard solution, and when perform the test as directed in the Purity (4), the total area of the peaks other than flecainide obtained from the sample solution is not larger than the peak area of flecainide from the standard solution.]

**Flopropione** C<sub>9</sub>H<sub>10</sub>O<sub>4</sub> [Same as the namesake monograph]

**Flopropione for assay** C<sub>9</sub>H<sub>10</sub>O<sub>4</sub> [Same as the monograph Flopropione. It contains not less than 99.0% of flopropione (C<sub>9</sub>H<sub>10</sub>O<sub>4</sub>), calculated on the anhydrous basis.]

**Fluconazole for assay** C<sub>13</sub>H<sub>12</sub>F<sub>2</sub>N<sub>6</sub>O [Same as the monograph Fluconazole]

**Fludiazepam for assay** C<sub>16</sub>H<sub>12</sub>ClFN<sub>2</sub>O [Same as the monograph Fludiazepam. It meets the following additional requirements.]

**Purity** Related substances—Dissolve 25 mg of fludiazepam for assay in 50 mL of a mixture of acetonitrile and water (3:2), and use this solution as the sample solution. Pipet 5 mL of the sample solution, add a mixture of acetonitrile and water (3:2) to make exactly 50 mL. Pipet 2.5 mL of this solution, add a mixture of acetonitrile and water (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than fludiazepam ob-

tained from the sample solution is not larger than 2/5 times the peak area of fludiazepam from the standard solution, and the total area of the peaks other than fludiazepam from the sample solution is not larger than the peak area of fludiazepam from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Fludiazepam Tablets.

Time span of measurement: About 4 times as long as the retention time of fludiazepam, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of acetonitrile and water (3:2) to make exactly 10 mL. Confirm that the peak area of fludiazepam obtained with 20  $\mu$ L of this solution is equivalent to 7 to 13% of that with 20  $\mu$ L of the standard solution.

System performance: When the procedure is run with 20  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fludiazepam are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fludiazepam is not more than 2.0%.

**Fluid thioglycolate medium** See the Sterility Test <4.06>.

**Fluocinolone acetonide**  $C_{24}H_{30}F_2O_6$  [Same as the namesake monograph]

**9-Fluorenylmethyl chloroformate**  $C_{15}H_{11}ClO_2$  White, crystals or crystalline powder.

Melting point <2.60>: 60 – 63°C

**9-Fluorenylmethyl chloroformate**  $C_{15}H_{11}ClO_2$

Prepared for amino acid analysis or biochemistry.

**Fluorescamine**  $C_{17}H_{10}O_4$  A white powder.

**Fluorescein**  $C_{20}H_{12}O_5$  An yellowish red powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1597  $\text{cm}^{-1}$ , 1466  $\text{cm}^{-1}$ , 1389  $\text{cm}^{-1}$ , 1317  $\text{cm}^{-1}$ , 1264  $\text{cm}^{-1}$ , 1247  $\text{cm}^{-1}$ , 1213  $\text{cm}^{-1}$ , 1114  $\text{cm}^{-1}$  and 849  $\text{cm}^{-1}$ .

**Fluorescein sodium**  $C_{20}H_{10}Na_2O_5$  [Same as the namesake monograph].

**Fluorescein sodium TS** Dissolve 0.2 g of fluorescein sodium in water to make 100 mL.

**Fluorescence TS** Mix 400  $\mu$ L of sodium dithionite solution containing 6.27 g in 200 mL of water, 210  $\mu$ L of 2-mercaptoethanol, 321  $\mu$ L of acetic acid (100), 400  $\mu$ L of 1,2-diamino-4,5-methylenedioxobenzene solution containing 31.1 mg in 1.0 mL of water and 2669  $\mu$ L of water. Prepare before use.

**4-Fluorobenzoic acid**  $C_7H_5FO_2$  White, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1684  $\text{cm}^{-1}$ , 1606  $\text{cm}^{-1}$  and 1231  $\text{cm}^{-1}$ .

Melting point <2.60>: 182 – 188°C

**1-Fluoro-2,4-dinitrobenzene**  $C_6H_3(NO_2)_2F$  Light yellow, liquid or crystalline masses. Melting point: about 25°C.

**Identification**—Determine the infrared absorption spectrum as directed in the liquid film method as directed under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3110  $\text{cm}^{-1}$ , 1617  $\text{cm}^{-1}$ , 1538  $\text{cm}^{-1}$ , 1345  $\text{cm}^{-1}$ , 1262  $\text{cm}^{-1}$  and 743  $\text{cm}^{-1}$ .

**Storage**—Preserve in a light-resistant tight container.

**Fluorogenic substrate TS** A solution containing oxidation-reduction indicator.

**7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole**  $C_6H_2FN_3O_3$  Prepared for amino acid analysis or biochemistry.

**Fluoroquinolonic acid for thin-layer chromatography**

$C_{13}H_9ClFNO_3$  A white to light brown powder.

**Purity** Perform the test with 8  $\mu$ L of a solution of fluoroquinolonic acid for thin-layer chromatography in acetonitrile (1 in 1250) as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of the peak of fluoroquinolonic acid is not less than 98.0%.

#### Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4 mm in inside diameter and 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Diluted phosphoric acid (1 in 500).

Mobile phase B: Methanol.

Flow of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 5.5	60 → 55	40 → 45
5.5 – 14	55 → 25	45 → 75
14 – 15	25 → 15	75 → 85

Flow rate: 1.5 mL per minute (the retention time of fluoroquinolonic acid is about 8 minutes).

Time span of measurement: For 15 minutes after injection, beginning after the solvent peak.

#### System suitability

System performance: When perform the test with 8  $\mu$ L of a solution of fluoroquinolonic acid for thin-layer chromatography in acetonitrile (1 in 1250) according to the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fluoroquinolonic acid are not less than 10,000 and not more than 1.5, respectively.

**Flurazepam for assay**  $C_{21}H_{23}ClFN_3O$  [Same as the monograph Flurazepam. When dried, it contains not less than 99.3% of flurazepam ( $C_{21}H_{23}ClFN_3O$ ).]

**Flutoprazepam for assay**  $C_{19}H_{16}ClFN_2O$  [Same as the monograph Flutoprazepam. When dried, it contains not less than 99.5% of flutoprazepam ( $C_{19}H_{16}ClFN_2O$ ).]

**Folic acid**  $C_{19}H_{19}N_7O_6$  [Same as the namesake monograph]

**Folin's TS** Place 20 g of sodium tungstate (VI) dihydrate, 5 g of disodium molybdate (VI) dihydrate and about

140 mL of water in a 300-mL volumetric flask, add 10 mL of diluted phosphoric acid (17 in 20) and 20 mL of hydrochloric acid, and boil gently using a reflux condenser with ground-glass joints for 10 hours. To the mixture add 30 g of lithium sulfate monohydrate and 10 mL of water, and then add a very small quantity of bromine to change the deep green color of the solution to yellow. Remove the excess bromine by boiling for 15 minutes without a condenser, and cool. Add water to make 200 mL, and filter through a glass filter. Store it free from dust. Use this solution as the stock solution, and dilute with water to the directed concentration before use.

**Folin's TS, dilute** Titrate  $<2.50>$  Folin's TS with 0.1 mol/L sodium hydroxide VS (indicator: phenolphthalein TS), and determine the acid concentration. Prepare by adding water to Folin's TS so the acid concentration is 1 mol/L.

**Formaldehyde solution** HCHO [K 8872, Special class]

**Formaldehyde solution-sulfuric acid TS** Add 1 drop of formaldehyde solution to 1 mL of sulfuric acid. Prepare before use.

**Formaldehyde solution TS** To 0.5 mL of formaldehyde solution add water to make 100 mL.

**Formaldehyde TS, dilute** Dilute formaldehyde solution to 10 times its volume with water.

**Formalin** See formaldehyde solution.

**Formalin TS** See formaldehyde solution TS.

**Formalin-sulfuric acid TS** See formaldehyde solution-sulfuric acid TS.

**Formamide** HCONH<sub>2</sub> [K 8873, Special class]

**Formamide for water determination** HCONH<sub>2</sub> [K 8873, Special class; water content per g of formamide for water determination should be not more than 1 mg.]

**Formazin opalescence standard solution** To 15 mL of formazin stock suspension add water to make 1000 mL. Use within 24 hours after preparation. Shake thoroughly before use.

**Formic acid** HCOOH [K 8264, Special class, specific gravity: not less than 1.21].

**2-Formylbenzoic acid** CHOC<sub>6</sub>H<sub>4</sub>COOH White crystals. Melting point: 97 – 99°C.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 0.3 g of 2-formylbenzoic acid, previously dried (in vacuum, phosphorus (V) oxide, 3 hours), dissolve in 50 mL of freshly boiled and cooled water, and titrate  $<2.50>$  with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenol red TS).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 15.01 mg of C<sub>8</sub>H<sub>6</sub>O<sub>3</sub>

**Forsythia fruit** [Same as the namesake monograph.]

**Freund's complete adjuvant** A suspension of 5 mg of mycobacteria of *Corynebacterium butyricum*, killed by heating, in 10 mL of a mixture of mineral oil and arlacet A (17:3).

**Fructose** C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> [Same as the namesake monograph]

**Fructose for thin-layer chromatography** C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> Colorless to white, crystals or crystalline powder. Very soluble in water, and sparingly soluble in ethanol (99.5). It is deliquescent with the atmospheric moisture.

**Optical rotation**  $<2.49>$  [α]<sub>D</sub><sup>20</sup>: -88 – -94° (1 g, diluted ammonia solution (28) (1 in 1000), 100 mL, 100 mm. Previously, dried over silica gel as the desiccant for 3 hours).

**Purity** Related substances—Dissolve 2 mg of Fructose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer chromatography  $<2.03>$ . Spot 2 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS to the plate, and heat the plate at 105°C for 10 minutes: any spot other than the principle spot with an R<sub>f</sub> value of about 0.6 does not appear.

**Fuchsin** A lustrous, green, crystalline powder or mass, slightly soluble in water and in ethanol (95).

**Loss on drying**  $<2.41>$ : 17.5 – 20.0% (1 g, 105°C, 4 hours).

**Residue on ignition**  $<2.44>$ : not more than 0.1% (1 g).

**Fuchsin-ethanol TS** Dissolve 11 g of fuchsin in 100 mL of ethanol (95).

**Fuchsin-sulfurous acid TS** Dissolve 0.2 g of fuchsin in 120 mL of hot water, and allow the solution to cool. Add a solution prepared by dissolving 2 g of anhydrous sodium sulfite in 20 mL of water, then add 2 mL of hydrochloric acid and water to make 200 mL, and allow to stand for at least 1 hour. Prepare before use.

**Fudosteine for assay** C<sub>6</sub>H<sub>13</sub>NO<sub>3</sub>S [Same as the monograph Fudosteine]

**Fumaric acid for thin-layer chromatography** C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>

White, crystalline powder, odorless, and has a characteristic acid taste.

**Purity**—Perform the test as directed in the Identification (5) under Clemastine Fumarate: any spot other than the principal spot at the R<sub>f</sub> value of about 0.8 does not appear.

**Fuming nitric acid** See nitric acid, fuming.

**Fuming sulfuric acid** See sulfuric acid, fuming.

**Furfural** C<sub>5</sub>H<sub>4</sub>O<sub>2</sub> A clear, colorless liquid.

**Specific gravity**  $<2.56>$  d<sub>20</sub><sup>20</sup>: 1.160 – 1.165

**Distilling range**  $<2.57>$ : 160 – 163°C, not less than 95 vol%.

**D-Galactosamine hydrochloride** C<sub>6</sub>H<sub>13</sub>NO<sub>5</sub>.HCl White powder. Melting point: about 180°C (with decomposition).

**Optical rotation**  $<2.49>$  [α]<sub>D</sub><sup>20</sup>: +90 – +97° (1 g, water, 100 mL, 100 mm).

**Galactose** See D-galactose.

**D-Galactose** C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> White, crystals, granules or powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry  $<2.25>$ : it exhibits absorption at the wave numbers of about 3390 cm<sup>-1</sup>, 3210 cm<sup>-1</sup>, 3140 cm<sup>-1</sup>, 1151 cm<sup>-1</sup>, 1068 cm<sup>-1</sup>, 956 cm<sup>-1</sup>, 836 cm<sup>-1</sup>, 765 cm<sup>-1</sup> and 660 cm<sup>-1</sup>.

**Optical rotation**  $<2.49>$  [α]<sub>D</sub><sup>20</sup>: +79 – +82° (2.5 g after drying for 18 hours in a desiccator (silica gel), diluted ammonia solution (28) (1 in 300), 25 mL, 100 mm).

**Gallic acid** See gallic acid monohydrate.

**Gallic acid monohydrate** C<sub>6</sub>H<sub>2</sub>(OH)<sub>3</sub>COOH.H<sub>2</sub>O White to pale yellow-white, crystals or powder.

**Melting point** <2.60>: about 260°C (with decomposition).

**Gelatin** [Same as the monograph Gelatin. It is the gelling grade.]

**Gelatin, acid-treated** [Same as the monograph Gelatin. It is the gelling grade and its isoelectric point is at pH between 7.0 and 9.0]

**Gelatin peptone** See peptone, gelatin.

**Gelatin-phosphate buffer solution** Dissolve 13.6 g of potassium dihydrogen phosphate, 15.6 g of sodium dihydrogen phosphate dihydrate and 1.0 g of sodium azide in water to make 1000 mL, adjust the pH to 3.0 with diluted phosphoric acid (1 in 75) (solution A). Dissolve 5.0 g of acid-treated gelatin in 400 mL of the solution A by warming, after cooling, adjust the pH to 3.0 with diluted phosphoric acid (1 in 75), and add the solution A to make 1000 mL.

**Gelatin-phosphate buffer solution (pH 7.0)** Dissolve 1.15 g of sodium dihydrogen phosphate dihydrate, 5.96 g of disodium hydrogen phosphate dodecahydrate and 5.4 g of sodium chloride in 500 mL of water. Dissolve 1.2 g of gelatin to this solution by heating, and after cooling add water to make 600 mL.

**Gelatin-phosphate buffer solution (pH 7.4)** To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution add 39.50 mL of 0.2 mol/L sodium hydroxide TS and 50 mL of water. Dissolve 0.2 g of gelatin to this solution by heating, then after cooling adjust to pH 7.4 with 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

**Gelatin-tris buffer solution** Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 2.22 g of sodium chloride in 700 mL of water. Separately, dissolve 10 g of acid-treated gelatin in 200 mL of water by warming. After cooling, mix these solutions, and adjust the pH to 8.8 with dilute hydrochloric acid, and add water to make 1000 mL.

**Gelatin-tris buffer solution (pH 8.0)** Dissolve 40 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 5.4 g of sodium chloride in 500 mL of water. To this solution add 1.2 g of gelatin to dissolve by heating, adjust to pH 8.0 with dilute hydrochloric acid after cooling, and add water to make 600 mL.

**Gelatin TS** Dissolve 1 g of gelatin in 50 mL of water by gentle heating, and filter if necessary. Prepare before use.

**Geniposide for assay**  $C_{17}H_{24}O_{10}$  Use geniposide for thin-layer chromatography meeting the following additional specifications. Correct the content based on the amount (%) obtained in the Assay.

**Unity of peak**—Dissolve 5 mg of geniposide for assay in 50 mL of diluted methanol (1 in 2). To 1 mL of this solution add diluted methanol (1 in 2) to make 100 mL, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of geniposide peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

#### Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Gardenia Fruit.

Detector: A photodiode array detector (wavelength: 240 nm, spectrum range of measurement: 220 – 400 nm).

#### System suitability

System performance: Proceed as directed in the system suitability in the Assay under Gardenia Fruit.

**Assay**—Weigh accurately 10 mg of geniposide for assay and 1 mg of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities, *A*<sub>1</sub> (equivalent to 1 hydrogen) and *A*<sub>2</sub> (equivalent to 1 hydrogen), of the signals around  $\delta$  3.93 ppm and  $\delta$  4.06 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\begin{aligned} \text{Amount (\%)} \text{ of geniposide } (C_{17}H_{24}O_{10}) \\ = M_s \times I \times P / (M \times N) \times 1.7147 \end{aligned}$$

*M*: Amount (mg) of geniposide for assay taken

*M<sub>s</sub>*: Amount (mg) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

*I*: Sum of the signal resonance intensities, *A*<sub>1</sub> and *A*<sub>2</sub>, based on the signal resonance intensity of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

*N*: Sum of number of the hydrogen derived from *A*<sub>1</sub> and *A*<sub>2</sub>

*P*: Purity (%) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between -5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

<sup>13</sup>C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the two signals around  $\delta$  3.93 ppm and  $\delta$  4.06 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals of around  $\delta$  3.93 ppm and  $\delta$  4.06 ppm are not overlapped with any signal of obvious foreign substance, and the ratios of the resonance intensities, *A*<sub>1</sub>/*A*<sub>2</sub>, of each signal around  $\delta$  3.93 ppm and  $\delta$  4.06 ppm are between 0.99 and 1.01, respectively.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity, *A*<sub>1</sub> or *A*<sub>2</sub>, to that of the reference standard for qNMR is not more than 1.0%.

**Geniposide for component determination** See geniposide for assay.

**Geniposide for thin-layer chromatography**  $C_{17}H_{24}O_{10}$  White, crystals or crystalline powder. Freely soluble in water and in methanol, and soluble in ethanol (99.5). Melting point: about 160°C.

**Purity** Related substances—Dissolve 1.0 mg of geniposide for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 20  $\mu$ L of this solution as directed in the Identification (2) under Gardenia Fruit: no spot other than the principal spot at an  $R_f$  value of about 0.3 is observed.

**Gentamicin B**  $C_{19}H_{38}N_4O_{10}$  White to pale yellow-white powder. Very soluble in water, and practically insoluble in ethanol (95).

**Content:** not less than 80.0%. **Assay**—Dissolve a suitable amount of gentamicin B in 0.05 mol/L sulfuric acid TS to make the solution containing 0.1 mg of gentamicin B per mL, and use this solution as the sample solution. Perform the test with 5  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of gentamicin B by the area percentage method.

#### Operating conditions

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reagent, reaction temperature, flow rate of the mobile phase, and flow rate of the reagent: Proceed as directed in the operating conditions in the Assay under Isepamicin Sulfate.

Time span of measurement: About 3 times as long as the retention time of gentamicin B.

#### System suitability

Proceed as directed in the system suitability in the Assay under Isepamicin Sulfate.

**Gentiopicroside for thin-layer chromatography**  $C_{16}H_{20}O_9$  A white powder. Freely soluble in water and in methanol, and practically insoluble in diethyl ether. Melting point: about 110°C (with decomposition).

**Purity** Related substances—Dissolve 10 mg of gentiopicroside for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification (2) under Gentian: the spots other than the principal spot at the  $R_f$  value of about 0.4 from the sample solution are not more intense than the spot from the standard solution.

**Gentisic acid**  $C_7H_6O_4$  Light yellow crystals.

**Melting point** <2.60>: About 200°C

**Giemsa's TS** Dissolve 3 g of azure II-eosin Y and 0.8 g of azure II in 250 g of glycerin by warming to 60°C. After cooling, add 250 g of methanol, and mix well. Allow to stand for 24 hours, and filter. Store in tightly stoppered bottles.

Azure II-eosin Y is prepared by coupling eosin Y to azure II. Azure II is the mixture of equal quantities of methylene azure (azure I), prepared by oxidizing methylene blue, and methylene blue.

**Giemsa's TS, dilute** Dilute Giemsa's TS to about 50 times its volume with a solution prepared by dissolving 4.54 g of potassium dihydrogen phosphate and 4.75 g of anhydrous disodium hydrogen phosphate in water to make

1000 mL, and filter with a filter paper. Prepare before use.

**[6]-Gingerol for assay**  $C_{17}H_{26}O_4$  [6]-Gingerol for thin-layer chromatography. It meets the requirements of the following 1) [6]-Gingerol for assay 1 or 2) [6]-Gingerol for assay 2 (Purity value by quantitative NMR). The latter is used with correction for its amount based on the result obtained in the Assay 2.

#### 1) [6]-Gingerol for assay 1

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (281 nm): 101 – 112 [7 mg, ethanol (99.5), 200 mL].

**Purity** Related substances—Dissolve 5 mg of [6]-gingerol for assay 1 in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than [6]-gingerol obtained from the sample solution is not larger than the peak area of [6]-gingerol obtained from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Hangekobokuto Extract.

Time span of measurement: About 6 times as long as the retention time of [6]-gingerol.

#### System suitability

System performance: Proceed as directed in the system suitability in the Assay (3) under Hangekobokuto Extract.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of [6]-gingerol obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

#### 2) [6]-Gingerol for assay 2 (Purity value by quantitative NMR)

**Unity of peak**—Dissolve 5 mg of [6]-gingerol for assay 2 in 5 mL of methanol, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of [6]-gingerol peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

#### Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Hangekobokuto Extract.

Detector: A photodiode array detector (wavelength: 282 nm, measuring range of spectrum: 220 – 400 nm).

#### System suitability

System performance: Proceed as directed in the system suitability in the Assay (3) under Hangekobokuto Extract.

**Assay**—Weigh accurately 5 mg of [6]-gingerol for assay 2 and 1 mg of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve both in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in

outer diameter, and measure  $^1\text{H-NMR}$  as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities,  $A_1$  (equivalent to 3 hydrogens) and  $A_2$  (equivalent to 1 hydrogen), of the signals around  $\delta$  3.56 ppm and  $\delta$  6.52 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\text{Amount (\%)} \text{ of [6]-gingerol} (\text{C}_{17}\text{H}_{26}\text{O}_4) \\ = M_s \times I \times P/(M \times N) \times 1.2997$$

$M$ : Amount (mg) of [6]-gingerol for assay 2 taken

$M_s$ : Amount (mg) of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy taken

$I$ : Sum of the signal resonance intensities,  $A_1$  and  $A_2$ , based on the signal resonance intensity of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy as 18.000

$N$ : Sum of the numbers of the hydrogen derived from  $A_1$  and  $A_2$

$P$ : Purity (%) of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having  $^1\text{H}$  resonance frequency of not less than 400 MHz.

Target nucleus:  $^1\text{H}$ .

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between -5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

$^{13}\text{C}$  decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around  $\delta$  3.56 ppm and  $\delta$  6.52 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around  $\delta$  3.56 ppm and  $\delta$  6.52 ppm are not overlapped with any signal of obvious foreign substance, and the ratio of the resonance intensities,  $(A_1/3)/A_2$ , of each signal around  $\delta$  3.56 ppm and  $\delta$  6.52 ppm is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity,  $A_1$  or  $A_2$ , to that of the reference standard for qNMR is not more than 1.0%.

**[6]-Gingerol for component determination** See [6]-gingerol for assay.

**[6]-Gingerol for thin-layer chromatography**  $\text{C}_{17}\text{H}_{26}\text{O}_4$   
A yellow-white to yellow, liquid or solid. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification**—Determine the absorption spectrum of a solution of [6]-gingerol for thin-layer chromatography in ethanol (99.5) (7 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum be-

tween 279 nm and 283 nm.

**Purity** Related substances—Dissolve 1.0 mg of [6]-gingerol for thin-layer chromatography in exactly 2 mL of methanol. Perform the test with 10  $\mu\text{L}$  of this solution as directed in the Identification under Ginger: any spot other than the principal spot at the  $R_f$  value of about 0.3 does not appear.

#### Ginsenoside Rb<sub>1</sub> for thin-layer chromatography

$\text{C}_{54}\text{H}_{92}\text{O}_{23}$  A white powder. Freely soluble in water and in methanol, and sparingly soluble in ethanol (99.5). It is hygroscopic.

**Identification** Determine the infrared absorption spectrum of ginsenoside Rb<sub>1</sub> for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3390  $\text{cm}^{-1}$ , 1650  $\text{cm}^{-1}$ , 1077  $\text{cm}^{-1}$  and 1038  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 2 mg of ginsenoside Rb<sub>1</sub> for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 2  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification (2) under Ginseng by developing the plate without entirely drying after applying the solutions: any spot other than the principal spot with an  $R_f$  value of about 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

**Ginsenoside Rc**  $\text{C}_{53}\text{H}_{90}\text{O}_{22}$  A white crystalline powder. It is odorless.

**Purity**—Dissolve 1 mg of ginsenoside Rc in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10  $\mu\text{L}$  of this solution as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay (2) under Ginseng until ginsenoside Rc is eluted: the total area of the peaks other than ginsenoside Rc and solvent peak is not larger than 1/10 times the total peak area excluding the peak area of the solvent.

**Ginsenoside Re**  $\text{C}_{48}\text{H}_{82}\text{O}_{18}$  A white crystalline powder. It is odorless.

**Purity**—Dissolve 1.0 mg of ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10  $\mu\text{L}$  of this solution as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay (1) under Ginseng until ginsenoside Re is eluted: the total area of the peaks other than ginsenoside Re and solvent peak is not larger than 1/10 times the total peak area excluding the peak area of the solvent.

#### Ginsenoside Rg<sub>1</sub> for thin-layer chromatography

$\text{C}_{42}\text{H}_{72}\text{O}_{14}$  A white, powder or crystalline powder. Very soluble in methanol and in ethanol (99.5), and soluble in water. It is hygroscopic.

**Identification** Determine the infrared absorption spectrum of ginsenoside Rg<sub>1</sub> for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3390  $\text{cm}^{-1}$ , 1642  $\text{cm}^{-1}$ , 1075  $\text{cm}^{-1}$  and 1032  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 2 mg of ginsenoside Rg<sub>1</sub> for thin-layer chromatography in 1 mL of methanol, and use this as the sample solution. Pipet 0.5 mL of the sample solution, add methanol to make exactly 25 mL, and use as the standard solution. Perform the test with 2  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification (2) under Ginseng by developing the plate

without entirely drying after applying the solutions: any spot other than the principal spot with an *Rf* value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

**Glacial acetic acid** See acetic acid (100).

**Glacial acetic acid for nonaqueous titration** See acetic acid for nonaqueous titration.

**Glacial acetic acid-sulfuric acid TS** See acetic acid sulfuric acid TS.

**$\gamma$ -Globulin** A plasma protein obtained from human serum as Cohn's II and III fractions. White crystalline powder. It contains not less than 98% of  $\gamma$ -globulin in the total protein.

**D-Glucosamine hydrochloride**  $C_6H_{13}NO_5 \cdot HCl$  White, crystals or crystalline powder.

**Content:** not less than 98%. Assay—Dissolve about 0.4 g of D-glucosamine hydrochloride, accurately weighed, in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L silver nitrate VS} \\ = 21.56 \text{ mg of } C_6H_{13}NO_5 \cdot HCl \end{aligned}$$

**Glucose**  $C_6H_{12}O_6$  [Same as the namesake monograph]

**Glucose detection TS** Dissolve 1600 units of glucose oxidase, 16 mg of 4-aminoantipyrine, 145 units of peroxidase and 0.27 g of *p*-hydroxybenzoic acid in tris buffer solution (pH 7.0) to make 200 mL.

**Glucose detection TS for penicillium origin  $\beta$ -galactosidase** Dissolve glucose oxidase (not less than 500 units), peroxidase (not less than 50 units), 10 mg of 4-aminoantipyrine and 0.1 g of phenol in phosphate buffer (pH 7.2) to make 100 mL.

**Glucose oxidase** Obtained from *Aspergillus niger*. White powder. It is freely soluble in water. It contains about 200 Units per mg. One unit indicates an amount of the enzyme which produces 1  $\mu$ mol of D-glucono- $\delta$ -lactone in 1 minute at 25°C and pH 7.0 from glucose used as the substrate.

**Glucose TS** Dissolve 30 g of glucose in water to make 100 mL. Prepare as directed under Injections.

**4'-O-Glucosyl-5-O-methylvisamminol for thin-layer chromatography**  $C_{22}H_{28}O_{10}$  White, crystals or crystalline powder. Freely soluble in methanol and in ethanol (99.5), and sparingly soluble in water.

**Identification**—Determine the absorption spectrum of a solution of 4'-O-glucosyl-5-O-methylvisamminol for thin-layer chromatography in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 286 nm and 290 nm.

**Purity** Related substances—Dissolve 1 mg of 4'-O-glucosyl-5-O-methylvisamminol for thin-layer chromatography in 1 mL of methanol. Perform the test with 5  $\mu$ L of this solution directed in the Identification under Saposhnikovia Root and Rhizome: no spots other than the principal spot at around *Rf* value of 0.3 appears.

**L-Glutamic acid**  $HOOC(CH_2)_2CH(NH_2)COOH$  [K 9047, Special class]

**L-Glutamine**  $H_2NCO(CH_2)_2CH(NH_2)COOH$  [K 9103, L(+)-glutamine, Special class]

**Glutamine TS** Dissolve 2.92 g of L-glutamine in water to make 100 mL, and sterilize by filtration through a membrane filter with a pore size not exceeding 0.22  $\mu$ m.

**7-(Glutarylglycyl-L-arginylamino)-4-methylcoumarin**

$C_{23}H_{30}N_6O_7$  White powder. It is freely soluble in acetic acid (100), sparingly soluble in dimethylsulfoxide, and practically insoluble in water.

**Absorbance** <2.24>  $E^{1\%}_{cm}$  (325 nm): 310–350 [2 mg, diluted acetic acid (100) (1 in 500), 200 mL].

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : –50°––60° [0.1 g, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm].

**Purity** Related substances—Prepare the sample solution by dissolving 5 mg of 7-(glutarylglycyl-L-arginylamino)-4-methylcoumarin in 0.5 mL of acetic acid (100), and perform the test as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (15:12:10:3) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, allow the plate to stand for 30 minutes in a box filled with iodine vapors: any observable spot other than the principal spot at the *Rf* value of about 0.6 does not appear.

**7-(Glutarylglycyl-L-arginylamino)-4-methylcoumarin TS**

Dissolve 5 mg of 7-(glutarylglycyl-L-arginylamino)-4-methylcoumarin in 0.5 to 1 mL of acetic acid (100), lyophilize, dissolve this in 1 mL of dimethylsulfoxide, and use this solution as solution A. Dissolve 30.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.6 g of sodium chloride in 400 mL of water, adjust the pH to 8.5 with dilute hydrochloric acid, add water to make 500 mL, and use this solution as solution B. Mix 1 mL of the solution A and 500 mL of the solution B before use.

**Glutathione**  $C_{10}H_{17}N_3O_6S$  [Same as the namesake monograph]

**Glycerin**  $C_3H_8O_3$  [K 8295, Glycerol, Special class, or same as the monograph Concentrated Glycerin]

**85% Glycerin**  $C_3H_8O_3$  [Same as the monograph Glycerin]

**Glycerin for gas chromatography**  $C_3H_8O_3$  [K 8295, Special class or for gas chromatography] When perform the test as directed in the Purity (11) under Concentrated Glycerin, it does not show any peak at the retention times corresponding to ethylene glycol and diethylene glycol.

**Glycine**  $H_2NCH_2COOH$  [K 8291, Special class]

**Glycolic acid**  $C_2H_4O_3$  **Purity:** not less than 98.0%.

**N-Glycolylneuraminic acid**  $C_{11}H_{19}NO_{10}$  White needle crystalline powder.

**0.1 mmol/L N-Glycolylneuraminic acid TS** Weigh accurately about 16.5 mg of *N*-glycolylneuraminic acid, and dissolve in water to make exactly 50 mL. To exactly *V* mL of this solution add water to make exactly 100 mL.

$$V(\text{mL}) = 325.3 \times 0.5/\text{amount (mg)} \text{ of } N\text{-glycolylneuraminic acid taken}$$

**Glycyrrhizic acid monoammonium salt for resolution check** See monoammonium glycyrrhizinate for solution check

**Glycyrrhizic acid for thin-layer chromatography**

$C_{42}H_{62}O_{16}$  White, crystals or crystalline powder. Freely soluble in ethanol (99.5), and practically insoluble in water.

**Identification** Determine the infrared absorption spectrum of glycyrrhetic acid for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3420\text{ cm}^{-1}$ ,  $1722\text{ cm}^{-1}$ ,  $1654\text{ cm}^{-1}$  and  $1389\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 4 mg of glycyrrhetic acid for thin-layer chromatography in 2 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification under Glycyrrhiza: the spots other than the principal spot with an *R<sub>f</sub>* value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

**40% glyoxal TS Content:** 38 – 42%. Assay—Put 1.000 g of 40% glyoxal TS in a glass-stoppered flask, add 20 mL of a solution of hydroxylammonium chloride (7 in 100) and 50 mL of water. Stopper tightly, allow to stand for 30 minutes, titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 1.0 mL of methyl red-methylene blue TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned}\text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 29.02 \text{ mg of C}_2\text{H}_2\text{O}_2\end{aligned}$$

**Goat anti-ECP antibody** Combine 1 volume of ECP standard substance (equivalent to about 1 mg of protein) and 1 volume of Freund's complete adjuvant, and immunize goats subcutaneously in the back region with this solution 5 times at 2 week intervals. Harvest blood on the 10<sup>th</sup> day after completing the immunization to obtain goat antiserum. Goat anti-ECP antibody is obtained by preparing an immobilized ECP column in which ECP standard substance is bound to sepharose 4B and then purifying by affinity column chromatography.

**Description:** Clear and colorless solution.

**Identification:** When sodium lauryl sulfate-supplemented polyacrylamide gel electrophoresis is conducted under non-reducing conditions, the molecular weight of the major band is within the range of  $1.30 \times 10^5$  to  $1.70 \times 10^5$ .

**Protein content:** When determining the protein content using Assay (1) under Celmoleukin (Genetical Recombination), the protein content per mL is 0.2 to 1.0 mg.

**Goat anti-ECP antibody TS** Dilute goat anti-ECP antibody with 0.1 mol/L carbonate buffer solution (pH 9.6) to prepare a solution containing 50  $\mu\text{g}$  protein per mL.

**Griess-Romijn's nitric acid reagent** Triturate thoroughly 1 g of 1-naphthylamine, 10 g of sulfanilic acid and 1.5 g of zinc dust in a mortar.

**Storage**—Preserve in tight, light-resistant containers.

**Griess-Romijn's nitrous acid reagent** Triturate thoroughly 1 g of 1-naphthylamine, 10 g of sulfanilic acid and 89 g of tartaric acid in a mortar.

**Storage**—Preserve in tight, light-resistant containers.

**Guaiacol**  $\text{CH}_3\text{OC}_6\text{H}_4\text{OH}$  Clear, colorless to yellow, liquid or colorless crystals, having a characteristic aroma. Sparingly soluble in water, and miscible with ethanol (95), with chloroform and with diethyl ether. Melting point: about 28°C.

**Purity**—Perform the test with 0.5  $\mu\text{L}$  of guaiacol as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the auto-

matic integration method, and calculate the amount of guaiacol by the area percentage method: It showed the purity of not less than 99.0%.

#### Operating conditions

**Detector:** A hydrogen flame-ionization detector

**Column:** A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography, 150- to 180- $\mu\text{m}$  in particle diameter, coated with polyethylene glycol 20 M at the ratio of 20%.

**Column temperature:** A constant temperature of about 200°C.

**Carrier gas:** Nitrogen.

**Flow rate:** Adjust so that the retention time of guaiacol is 4 to 6 minutes.

**Detection sensitivity:** Adjust the detection sensitivity so that the peak height of guaiacol obtained with 0.5  $\mu\text{L}$  of guaiacol is about 90% of the full scale.

**Time span of measurement:** About 3 times as long as the retention time of guaiacol.

**Guaiacol for assay**  $\text{C}_7\text{H}_8\text{O}_2$  Colorless to yellow clear liquid or colorless crystals with a characteristic, aromatic odor. Miscible with methanol and with ethanol (99.5), and sparingly soluble in water. Congealing point: 25 – 30°C.

**Identification**—Determine the infrared absorption spectrum of guaiacol for assay as directed in the ATR method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $1595\text{ cm}^{-1}$ ,  $1497\text{ cm}^{-1}$ ,  $1443\text{ cm}^{-1}$ ,  $1358\text{ cm}^{-1}$ ,  $1255\text{ cm}^{-1}$ ,  $1205\text{ cm}^{-1}$ ,  $1108\text{ cm}^{-1}$ ,  $1037\text{ cm}^{-1}$ ,  $1020\text{ cm}^{-1}$ ,  $916\text{ cm}^{-1}$ ,  $833\text{ cm}^{-1}$ , and  $738\text{ cm}^{-1}$ .

**Purity** Related substances—Perform the test with 0.5  $\mu\text{L}$  of guaiacol for assay as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of guaiacol is not more than 2.0%.

#### Operating conditions

**Detector:** A hydrogen flame-ionization detector.

**Column:** A fused silica column 0.25 mm in inside diameter and 60 m in length, coated inside with polymethylsiloxane for gas chromatography in 0.25 to 0.5  $\mu\text{m}$  in thickness.

**Column temperature:** Raise the temperature from 100°C to 130°C at a rate of 5°C per minute, raise to 140°C at a rate of 2°C per minute, raise to 200°C at a rate of 15°C per minute, and maintain at 200°C for 2 minutes.

**Injection port temperature:** 200°C.

**Detector temperature:** 250°C.

**Carrier gas:** Helium.

**Flow rate:** Adjust so that the retention time of guaiacol is about 8 minutes.

**Split ratio:** 1:50.

#### System suitability

**Test for required detectability:** Weigh accurately about 70 mg of guaiacol for assay, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of guaiacol obtained from 1  $\mu\text{L}$  of the solution for system suitability test is equivalent to 0.08 to 0.16% of that of guaiacol obtained when 0.5  $\mu\text{L}$  of guaiacol for assay is injected.

**System performance:** When the procedure is run with 1  $\mu\text{L}$  of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of guaiacol are not less than 20,000 and not more than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 1  $\mu\text{L}$  of the solution for system suitability test under the above operating conditions, the relative standard deviation

of the peak area of guaiacol is not more than 2.0%.

**Guaiifenesin**  $C_{10}H_{14}O_4$  [Same as the namesake monograph]

**Guanine**  $C_5H_5N_5O$  White to pale yellow-white powder. **Absorbance** <2.24> Weigh accurately about 10 mg of guanine, dissolve in 20 mL of dilute sodium hydroxide TS, and add 2 mL of 1 mol/L hydrochloric acid TS and 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL. Determine the absorbances,  $E_{1\%}^{1\%}$ , of this solution at 248 nm and 273 nm: they are between 710 and 770, and between 460 and 500, respectively.

**Loss on drying** <2.41>: Not more than 1.5% (0.5 g, 105°C, 4 hours).

**Haloperidol for assay**  $C_{21}H_{23}ClFNO_2$  [Same as the monograph Haloperidol]

**Hanus' TS** Dissolve 20 g of iodine monobromide in 1000 mL of acetic acid (100).

**Storage**—Preserve in light-resistant, glass-stoppered bottles, in a cold place.

**Heart infusion agar medium** Prepared for biochemical tests.

**Heavy hydrogenated solvent for nuclear magnetic resonance spectroscopy** Prepared for nuclear magnetic resonance spectroscopy. Heavy hydrogenated chloroform ( $CDCl_3$ ), heavy hydrogenated dimethyl sulfoxide [ $(CD_3)_2SO$ ], heavy water ( $D_2O$ ), and heavy hydrogenated pyridine ( $C_5D_5N$ ) are available.

**Heavy water for nuclear magnetic resonance spectroscopy**  $D_2O$  Prepared for nuclear magnetic resonance spectroscopy.

**Helium** He Not less than 99.995 vol%.

**Hematoxylin**  $C_{16}H_{14}O_6.xH_2O$  White or light yellow to brownish, crystals or crystalline powder. It is soluble in hot water and in ethanol (95), and sparingly soluble in cold water.

**Residue on ignition** <2.44>: not more than 0.1% (1 g).

**Hematoxylin TS** Dissolve 1 g of hematoxylin in 12 mL of ethanol (99.5). Dissolve 20 g of aluminum potassium sulfate 12-water in 200 mL of warm water, cool, and filter. After 24 hours, mix these two prepared solutions. Allow to stand for 8 hours in a wide-mouthed bottle without using a stopper, and filter.

**Heparin sodium** [Same as the namesake monograph]

**HEPES buffer solution (pH 7.5)** Dissolve 2.38 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid in 90 mL of water, adjust to pH 7.5 with diluted 6 mol/L sodium hydroxide TS (5 in 6), and add water to make 100 mL.

**Heptafluorobutylic acid**  $C_4HF_7O_2$  A clear and colorless liquid.

**Content:** Not less than 98.0%. **Assay**—Take 30 mL of water in a glass-stoppered flask, weigh accurately the mass of the flask, add about 4.3 g of heptafluorobutylic acid, and weigh accurately the mass of this flask. Then, add 40 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS  
= 214.0 mg of  $C_4HF_7O_2$

**Heptane**  $CH_3(CH_2)_5CH_3$  [K 9701, Special class]

**Heptane for liquid chromatography**  $C_7H_{16}$  Clear and

colorless solution.

**Purity** Ultraviolet-absorbing substances—Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances of heptane for liquid chromatography at 210 nm, 220 nm, 230 nm and 240 nm, using water as the control solution: the absorbance is not more than 0.35, not more than 0.15, not more than 0.05 and not more than 0.03, respectively.

**Heptyl parahydroxybenzoate**  $C_{14}H_{20}O_3$  White, crystals or crystalline powder.

**Melting point** <2.60>: 45 – 50°C

**Content:** Not less than 98.0%. **Assay**—Weigh accurately about 3.5 g of heptyl parahydroxybenzoate, dissolve in 50 mL of diluted *N,N*-dimethylformamide (4 in 5), and titrate <2.50> with 1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS  
= 236.3 mg of  $C_{14}H_{20}O_3$

**Hesperidin for assay**  $C_{28}H_{34}O_{15}$  Hesperidin for thin-layer chromatography. It meets the following requirement.

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : -100 – -120° (5 mg dried with silica gel for 24 hours, methanol, 50 mL, 100 mm).

**Purity** Related substances—Dissolve 2 mg of hesperidin for assay in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than hesperidin and the solvent obtained from the sample solution is not larger than the peak area of hesperidin from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Hochuekkito Extract.

Time span of measurement: About 6 times as long as the retention time of hesperidin.

**System suitability**

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Hochuekkito Extract.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of hesperidin obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

**Hesperidin for component determination** See hesperidin for assay.

**Hesperidin for thin-layer chromatography**  $C_{28}H_{34}O_{15}$

A white to light brown-yellow, crystalline powder or powder. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 245°C (with decomposition).

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (284 nm): 310 – 340 (8 mg dried in a desiccator (silica gel) for 24 hours, methanol, 500 mL).

**Purity** Related substances—Dissolve 1 mg of hesperidin for thin-layer chromatography in 2 mL of methanol. Perform the test with 20  $\mu$ L of this solution as directed in the Identification (6) under Hochuekkito Extract: no spot other than the principle spot of around *Rf* value of 0.3 appears.

**Hexaammonium heptamolybdate-cerium (IV) sulfate TS**

Dissolve 2.5 g of hexaammonium heptamolybdate tetrahydrate and 1.0 g of cerium (IV) sulfate tetrahydrate in diluted sulfuric acid (3 in 50) to make 100 mL. Prepare before use.

**Hexaammonium heptamolybdate-sulfuric acid TS** Dissolve 1.0 g of hexaammonium heptamolybdate tetrahydrate in diluted sulfuric acid (3 in 20) to make 40 mL. Prepare before use.

**Hexaammonium heptamolybdate tetrahydrate**

$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  [K 8905, Special class]

**Hexaammonium heptamolybdate TS** dissolve 21.2 g of hexaammonium heptamolybdate tetrahydrate in water to make 200 mL (10%). Prepare before use.

**1,1,1,3,3,3-Hexamethyldisilazane**  $(\text{CH}_3)_3\text{SiNHSi}(\text{CH}_3)_3$   
A colorless or practically colorless, liquid. Very soluble in diethyl ether, and reactable with water or with ethanol. Boiling point: about 125°C.

**Hexamethylenetetramine**  $(\text{CH}_2)_6\text{N}_4$  [K 8847, Special class]

**Hexamethylenetetramine TS** Dissolve exactly 2.5 g of hexamethylenetetramine in exactly 25 mL of water.

**Hexamine** See hexamethylenetetramine.

**Hexane**  $\text{C}_6\text{H}_{14}$  [K 8848, Special class]

**Hexane for liquid chromatography**  $\text{C}_6\text{H}_{14}$  Colorless, clear liquid. Miscible with ethanol (95), with diethyl ether, with chloroform and with benzene.

Boiling point: about 69°C.

**Purity** (1) Ultraviolet absorptive substances—Read the absorbances of hexane for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: not more than 0.3 at the wavelength of 210 nm, and not more than 0.01 between 250 nm and 400 nm.

(2) Peroxide—To a mixture of 100 mL of water and 25 mL of dilute sulfuric acid add 25 mL of a solution of potassium iodide (1 in 10) and 20 g of hexane for liquid chromatography. Stopper tightly, shake, and allow to stand in a dark place for 15 minutes. Titrate <2.50> this solution, while shaking well, with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction (not more than 0.0005%).

**n-Hexane for liquid chromatography** See hexane for liquid chromatography.

**Hexane for purity of crude drug**  $\text{C}_6\text{H}_{14}$  [K 8848, Special class] Use hexane meeting the following additional specification. Evaporate 300.0 mL of hexane for purity of crude drug in vacuum at a temperature not higher than 40°C, add the hexane to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of  $\gamma$ -BHC in hexane to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane to make exactly 100 mL. Further pipet 2 mL of this solution, add hexane to make exactly 100 mL, and use this solution as the standard solution I. Perform the test with exactly 1  $\mu\text{L}$  each of the sample solution and standard solution I as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the solvent peak obtained from the sample solution is not larger than the peak area of  $\gamma$ -BHC from the standard solution I.

**Operating conditions**

Proceed the operating conditions in 4.3. under Crude Drugs Test <5.01> except the time span of measurement.

Time span of measurement: About three times as long as the retention time of  $\gamma$ -BHC, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution I, add hexane to make exactly 20 mL, and use this solution as the standard solution II. Adjust the detection sensitivity so that the peak area of  $\gamma$ -BHC obtained with 1  $\mu\text{L}$  of the standard solution II can be measured by the automatic integration method, and the peak height of  $\gamma$ -BHC with 1  $\mu\text{L}$  of the standard solution I is about 20% of the full scale.

**Hexane for ultraviolet-visible spectrophotometry**  $\text{C}_6\text{H}_{14}$  [K 8848, Special class]. When determining the absorbance of hexane for ultraviolet-visible spectrophotometry as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank solution, its value is not more than 0.10 at 220 nm and not more than 0.02 at 260 nm, and it has no characteristic absorption between 260 nm and 350 nm.

**n-Hexane for ultraviolet-visible spectrophotometry** See hexane for ultraviolet-visible spectrophotometry.

**1-Hexanol**  $\text{C}_6\text{H}_{14}\text{O}$  A clear and colorless liquid.

Specific gravity  $d_{20}^{20}$ : 0.816 – 0.821

Boiling point 156 – 158°C.

**Hexyl parahydroxybenzoate**  $\text{C}_{13}\text{H}_{18}\text{O}_3$  White, crystals or crystalline powder.

Melting point <2.60>: 49 – 53°C

Content: not less than 98.0%. Assay—Weigh accurately about 0.3 g of hexyl parahydroxybenzoate, dissolve in 50 mL of diluted *N,N*-dimethylformamide (4 in 5), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L sodium hydroxide VS} \\ = 22.23 \text{ mg of } \text{C}_{13}\text{H}_{18}\text{O}_3 \end{aligned}$$

**High-density polyethylene film** Prepared for cytotoxicity test. It does not show cytotoxicity.

**Hirsutine** See hirsutine for thin-layer chromatography.

**Hirsutine for assay**  $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_3$  Hirsutine for thin-layer chromatography. It meets the following requirements.

Absorbance <2.24>:  $E_{1\text{cm}}^{1\%}$  (245 nm): 354 – 389 (5 mg calculated on the anhydrous basis, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

**Purity** Related substances—Dissolve 5 mg of hirsutine for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3), use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid-chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than hirsutine obtained from the sample solution is not larger than the peak area of hirsutine from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Uncaria Hook.

Time span of measurement: About 1.5 times as long as the retention time of hirsutine, beginning after the solvent peak.  
System suitability

System performance: Proceed as directed in the system suitability in the Assay under Uncaria Hook.

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of hirsutine obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hirsutine is not more than 1.5%.

**Hirsutine for thin-layer chromatography** C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>3</sub>  
A white or light orange, crystalline or powder. Very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 105°C.

**Identification**—Determine the absorption spectrum of a solution of hirsutine for thin-layer chromatography in a mixture of methanol and dilute acetic acid (7:3) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 287 nm and 291 nm.

**Purity** Related substances—Dissolve 1.0 mg of hirsutine for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): no spot other than the principal spot at around R<sub>f</sub> value of 0.55 appears.

**L-Histidine** C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub> [Same as the namesake monograph]

**L-Histidine hydrochloride** See L-histidine hydrochloride monohydrate.

**L-Histidine hydrochloride monohydrate**  
C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>.HCl.H<sub>2</sub>O [K 9050, Special class]

**Homatropine hydrobromide** C<sub>16</sub>H<sub>21</sub>NO<sub>3</sub>.HBr [Same as the namesake monograph]

**Honokiol** C<sub>18</sub>H<sub>18</sub>O<sub>2</sub> Odorless white, crystals or crystalline powder.

**Purity**—Dissolve 1 mg of honokiol in the mobile phase to make 10 mL, and use this solution as the sample solution. Perform the Liquid Chromatography <2.01> with 10  $\mu$ L of the sample solution as directed in the Assay under Magnolia Bark: when measure the peak areas for 2 times as long as the retention time of magnolol, the total area of peaks other than honokiol obtained from the sample solution is not larger than 1/10 times the total area of the peaks other than the solvent peak.

**Horseradish peroxidase** An oxidase (Molecular weight: about 40,000) derived from horseradish.

**Horse serum** Collect the blood from horse in a flask, coagulate, and allow to stand at room temperature until the serum is separated. Transfer the separated serum in glass containers, and preserve at -20°C.

**Human anti-thrombin** A serine protease inhibitor obtained from healthy human plasma. A protein that inhibits activities of activated blood coagulation factor II (thrombin)

and activated blood coagulation factor X. It contains not less than 6 IU per mg of protein.

**Human antithrombin III** Serine protease inhibition factor obtained from normal plasma of health human. It is a protein, which inhibits the activities of thrombin and activated blood coagulation factor X. It contains not less than 300 Units per mg protein. One unit indicates an amount of the antithrombin III which inhibits 1 unit of thrombin at 25°C under the existence of heparin.

**Human chorionic gonadotrophin TS** Weigh accurately a suitable amount of Human Chorionic Gonadotrophin according to the labeled amount, and dissolve in bovine serum albumin-sodium chloride-phosphate buffer solution (pH 7.2) so that each 1.0 mL contains 80 human chorionic gonadotrophin Units.

**Human insulin desamido substance-containing TS** Dissolve 1.5 mg of Insulin Human in 1 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at 25°C for 3 days, and when the procedure is run with this solution according to the conditions as directed in the Purity (1) under Insulin Human (Genetical Recombination), the solution contains about 5% of the desamido substance.

**Human insulin dimer-containing TS** Allow to stand Insulin Human (Genetical Recombination) at 25°C for 10 days or more, and dissolve 4 mg of this in 1 mL of 0.01 mol/L hydrochloric acid TS.

**Human normal plasma** Dissolve an amount of dried human normal plasma powder, equivalent to 1 mL of the normal plasma of human, in 1 mL of water. Store between 2°C and 10°C, and use within one week.

**Human serum albumin for assay** White to light yellow powder. Albumin content is at least 99%. Convert to the dehydrate using the following water determination method.

**Water** <2.48>: (0.2 g, volumetric titration, direct titration). However, in a dehydration solvent, use a mixture of pyridine for water determination and ethylene glycol for water determination (5:1).

**Hyaluronic acid** (C<sub>14</sub>H<sub>21</sub>NO<sub>11</sub>)<sub>n</sub> A white powder.

**Hyaluronidase** Obtained from *Streptomyces albogriseolus*. A lyophilized white powder.

**Content:** Not less than 100 units of hyaluronidase per ampule.

**Assay** (i) **Sample solution:** Dissolve the content of 1 ampoule by adding exactly 2 mL of cold water. Dilute this solution with cold water so that each mL contains exactly 1.3 to 3.8 units of hyaluronidase. Prepare before use, and store in a cold place.

(ii) **Substrate solution:** To exactly 50 mg of hyaluronic acid add 40 mL of 0.02 mol/L acetate buffer solution (pH 6.0), stir for 5 hours to dissolve, and add 0.02 mol/L acetate buffer solution (pH 6.0) to make exactly 50 mL.

(iii) **4-Dimethylaminobenzaldehyde solution:** To a mixture of 0.6 mL of water and 11.9 mL of hydrochloric acid add acetic acid to make exactly 100 mL, and dissolve 10.0 g of 4-dimethylaminobenzaldehyde in this solution. To exactly 1 mL of this solution add exactly 9 mL of acetic acid. Prepare before use.

(iv) **Borate solution:** Dissolve 4.95 g of boric acid in 40 mL of water, adjust to pH 9.1 with potassium hydroxide TS, and add water to make 100 mL.

(v) **Procedure:** Pipet 0.5 mL of substrate solution, warm at 60 ± 0.5°C for 10 minutes, add exactly 0.5 mL of sample solution, and shake immediately. After allowing this solu-

tion to stand at  $60 \pm 0.5^\circ\text{C}$  for exactly 30 minutes, add exactly 0.2 mL of borate solution, shake, heat the vessel covered with a marble in a water bath for exactly 3 minutes, and cool the vessel with running water. Then, add exactly 3 mL of 4-dimethylaminobenzaldehyde solution, shake, and allow to stand at  $37 \pm 0.5^\circ\text{C}$  for exactly 20 minutes. Determine the absorbance,  $A_1$ , at 585 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank. Separately, pipet 0.5 mL of substrate solution, allow to stand at  $60 \pm 0.5^\circ\text{C}$  for 40 minutes, add exactly 0.2 mL of borate solution, and shake. Add exactly 0.5 mL of sample solution, and shake immediately. Heat the vessel covered with a marble in a water bath for exactly 3 minutes, and cool the vessel with running water. Then, proceed in the same manner as above, and determine the absorbance,  $A_0$ , of this solution.

(vi) Calculation: Calculate the enzyme activity in 1 ampoule by the following equation, where 1 unit means the amount of enzyme which decreases 50% in absorbance at 660 nm of hyaluronic acid in 30 minutes at  $60^\circ\text{C}$  and pH 6.0.

$$\begin{aligned} \text{Hyaluronidase unit in 1 ampoule} \\ = (A_1 - A_0) \times D_m \times 3.2 \times 4 \end{aligned}$$

$D_m$ : Dilution factor for sample solution

3.2: Conversion factor to turbidity reduction unit

**Hydralazine hydrochloride**  $\text{C}_8\text{H}_8\text{N}_4\cdot\text{HCl}$  [Same as the namesake monograph]

**Hydralazine hydrochloride for assay**  $\text{C}_8\text{H}_8\text{N}_4\cdot\text{HCl}$  [Same as the monograph Hydralazine Hydrochloride. When dried, it contains not less than 99.0% of hydralazine hydrochloride ( $\text{C}_8\text{H}_8\text{N}_4\cdot\text{HCl}$ ).]

**Hydrazine monohydrate**  $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$  Colorless liquid, having a characteristic odor.

**Hydrazine sulfate** See hydrazinium sulfate.

**Hydrazinium sulfate**  $\text{N}_2\text{H}_6\text{SO}_4$  [K 8992, Special class]

**Hydrazinium sulfate TS** Dissolve exactly 1.0 g of hydrazinium sulfate in exactly 100 mL of water. Use after standing for 4–6 hours.

**Hydriodic acid HI** [K 8917, Special class]

**Hydrobromic acid** HBr [K 8509, Special class]

**Hydrochloric acid** HCl [K 8180, Special class]

**Hydrochloric acid-ammonium acetate buffer solution (pH 3.5)** Dissolve 25 g of ammonium acetate in 45 mL of 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

**Hydrochloric acid, dilute** Dilute 23.6 mL of hydrochloric acid with water to make 100 mL (10%).

**Hydrochloric acid-ethanol TS** Dilute 23.6 mL of hydrochloric acid with ethanol (95) to make 100 mL.

**0.01 mol/L Hydrochloric acid-methanol TS** To 20 mL of 0.5 mol/L hydrochloric acid TS add methanol to make 1000 mL.

**0.05 mol/L Hydrochloric acid-methanol TS** To 100 mL of 0.5 mol/L hydrochloric acid add methanol to make 1000 mL.

**Hydrochloric acid-2-propanol TS** Add 0.33 mL of hydrochloric acid to 100 mL of 2-propanol, mix, and store in a dark and cool place.

**Hydrochloric acid-potassium chloride buffer solution (pH**

**2.0)** To 10.0 mL of 0.2 mol/L hydrochloric acid VS add 88.0 mL of 0.2 mol/L potassium chloride TS, adjust the pH to  $2.0 \pm 0.1$  with 0.2 mol/L hydrochloric acid VS or 0.2 mol/L potassium chloride TS, then add water to make 200 mL.

**Hydrochloric acid, purified** Add 0.3 g of potassium permanganate to 1000 mL of diluted hydrochloric acid (1 in 2), distil, discard the first 250 mL of the distillate, and collect the following 500 mL of the distillate.

**0.001 mol/L Hydrochloric acid TS** Dilute 10 mL of 0.1 mol/L hydrochloric acid TS with water to make 1000 mL.

**0.01 mol/L Hydrochloric acid TS** Dilute 100 mL of 0.1 mol/L hydrochloric acid TS with water to make 1000 mL.

**0.02 mol/L Hydrochloric acid TS** Dilute 100 mL of 0.2 mol/L hydrochloric acid TS with water to make 1000 mL.

**0.05 mol/L Hydrochloric acid TS** Dilute 100 mL of 0.5 mol/L hydrochloric acid TS with water to make 1000 mL.

**0.1 mol/L Hydrochloric acid TS** Dilute 100 mL of 1 mol/L hydrochloric acid TS with water to make 1000 mL.

**0.2 mol/L Hydrochloric acid TS** Dilute 18 mL of hydrochloric acid with water to make 1000 mL.

**0.5 mol/L Hydrochloric acid TS** Dilute 45 mL of hydrochloric acid with water to make 1000 mL.

**1 mol/L Hydrochloric acid TS** Dilute 90 mL of hydrochloric acid with water to make 1000 mL.

**2 mol/L Hydrochloric acid TS** Dilute 180 mL of hydrochloric acid with water to make 1000 mL.

**3 mol/L Hydrochloric acid TS** Dilute 270 mL of hydrochloric acid with water to make 1000 mL.

**5 mol/L Hydrochloric acid TS** Dilute 450 mL of hydrochloric acid with water to make 1000 mL.

**6 mol/L Hydrochloric acid TS** Dilute 540 mL of hydrochloric acid with water to make 1000 mL.

**7.5 mol/L Hydrochloric acid TS** Dilute 675 mL of hydrochloric acid with water to make 1000 mL.

**10 mol/L Hydrochloric acid TS** Dilute 900 mL of hydrochloric acid with water to make 1000 mL.

**6 mol/L Hydrochloric acid TS for amino acid automatic analysis** Contains 19–21% hydrogen chloride (HCl: 36.46) for amino acid automatic analysis (constant boiling hydrochloric acid).

**Hydrochlorothiazide**  $\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$  [Same as the namesake monograph]

**Hydrocortisone**  $\text{C}_{21}\text{H}_{30}\text{O}_5$  [Same as the namesake monograph]

**Hydrocortisone acetate**  $\text{C}_{23}\text{H}_{32}\text{O}_6$  [Same as the namesake monograph]

**Hydrocotarnine hydrochloride for assay** See hydrocotarnine hydrochloride hydrate for assay.

**Hydrocotarnine hydrochloride hydrate for assay**  $\text{C}_{12}\text{H}_{15}\text{NO}_3\cdot\text{HCl}\cdot\text{H}_2\text{O}$  [Same as the monograph Hydrocotarnine Hydrochloride Hydrate. When dried, it contains not less than 99.0% of hydrocotarnine hydrochloride ( $\text{C}_{12}\text{H}_{15}\text{NO}_3\cdot\text{HCl}$ ).]

**Hydrofluoric acid HF** [K 8819, Special class] It contains not less than 46.0% of HF.

**Hydrogen** H<sub>2</sub> [K 0512, Standard substance, Third class] It contains not less than 99.99% of H<sub>2</sub>.

**Hydrogen chloride-ethanol TS** Pass dry hydrogen chloride, which is generated by slowly adding 100 mL of sulfuric acid dropwise to 100 mL of hydrochloric acid and dried by washing with sulfuric acid, through 75 g of ethanol (99.5) cooled in an ice bath until the increase in mass has reached 25 g. Prepare before use.

**Hydrogen hexachloroplatinate (IV) hexahydrate**  
H<sub>2</sub>PtCl<sub>6</sub>·6H<sub>2</sub>O [K 8153, Special class]

**Hydrogen hexachloroplatinate (IV)-potassium iodide TS** To 3 mL of hydrogen hexachloroplatinate (IV) TS add 97 mL of water and 100 mL of a solution of potassium iodide (3 in 50). Prepare before use.

**Hydrogen hexachloroplatinate (IV) TS** Dissolve 2.6 g of hydrogen hexachloroplatinate (IV) hexahydrate in water to make 20 mL (0.125 mol/L).

**Hydrogen peroxide (30)** H<sub>2</sub>O<sub>2</sub> [K 8230, Hydrogen peroxide, Special class, Concentration: 30.0 – 35.5%.]

**Hydrogen peroxide-sodium hydroxide TS** To a mixture of water and hydrogen peroxide (30) (9:1) add 3 drops of bromophenol blue TS, and then add 0.01 mol/L sodium hydroxide TS until a purple-blue color develops. Prepare before use.

**Hydrogen peroxide TS** Dilute 1 volume of hydrogen peroxide (30) with 9 volumes of water. Prepare before use (3%).

**Hydrogen peroxide TS, dilute** Dilute 1 mL of hydrogen peroxide (30) with 500 mL of water, and dilute 5 mL of this solution with water to make 100 mL. Prepare before use.

**Hydrogen peroxide water, strong** See hydrogen peroxide (30).

**Hydrogen sulfide** H<sub>2</sub>S Colorless, poisonous gas, heavier than air. It dissolves in water. Prepare by treating iron (II) sulfide with dilute sulfuric acid or dilute hydrochloric acid. Other sulfides yielding hydrogen sulfide with dilute acids may be used.

**Hydrogen sulfide TS** A saturated solution of hydrogen sulfide. Prepare by passing hydrogen sulfide into cold water.

**Storage**—Preserve in well-filled, light-resistant bottles, in a dark, cold place.

**Hydrogen tetrachloroaurate (III) tetrahydrate**  
HAuCl<sub>4</sub>·4H<sub>2</sub>O [K 8127, Special class]

**Hydrogen tetrachloroaurate (III) TS** Dissolve 1 g of hydrogen tetrachloroaurate (III) tetrahydrate in 35 mL of water.

**Hydroquinone** C<sub>6</sub>H<sub>4</sub>(OH)<sub>2</sub> [K 8738, Special class]

**Hydroxocobalamin acetate** C<sub>62</sub>H<sub>89</sub>CoN<sub>13</sub>O<sub>15</sub>P·C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>  
Dark red, crystals or powder.

**Loss on drying <2.41>**: not more than 12% (50 mg, in vacuum not exceeding 0.67 kPa, phosphorus (V) oxide, 100°C, 6 hours).

**Content**: not less than 98.0%. **Assay**—Proceed as directed in the Assay under Hydroxocobalamin Acetate.

**m-Hydroxyacetophenone** C<sub>8</sub>H<sub>8</sub>O<sub>2</sub> White to light yellow-white crystalline powder.

**Melting point <2.60>**: about 96°C

**Purity** Related substances—Perform the test with 10 μL of a solution of m-hydroxyacetophenone in 0.1 mol/L phos-

phate buffer solution (pH 4.5) (1 in 15,000) as directed in the Assay under Cefalexin: Any obstructive peaks for determination of cefalexin are not observed.

**p-Hydroxyacetophenone** C<sub>8</sub>H<sub>8</sub>O<sub>2</sub> White to pale yellow, crystals or crystalline powder. It is freely soluble in methanol.

**Melting point <2.60>**: 107 – 111°C

**Purity**—Weigh 1 mg of p-hydroxyacetophenone, add methanol and dissolve to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the Assay under Peony Root: the total area of the peaks other than p-hydroxyacetophenone obtained from the sample solution is not larger than the total area of the peaks other than the solvent peak.

**3-Hydroxybenzoic acid** HOC<sub>6</sub>H<sub>4</sub>COOH White, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum according to the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3300 cm<sup>-1</sup>, 1690 cm<sup>-1</sup>, 1600 cm<sup>-1</sup>, 1307 cm<sup>-1</sup>, 1232 cm<sup>-1</sup> and 760 cm<sup>-1</sup>.

**Melting point <2.60>**: 203 – 206°C

**Purity** Clarity of solution—Dissolve 1.0 g of 3-hydroxybenzoic acid in 20 mL of methanol: the solution is clear.

**Content**: not less than 99.0%. **Assay**—Weigh accurately about 0.2 g of 3-hydroxybenzoic acid, dissolve in 20 mL of diluted ethanol (95) (1 in 2), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of cresol red TS) until the color of the solution changes from yellow to dark orange-red. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 13.81 mg of C<sub>7</sub>H<sub>6</sub>O<sub>3</sub>

**p-Hydroxybenzoic acid** See parahydroxybenzoic acid.

**10-Hydroxy-2-(E)-decenoic acid for assay** C<sub>10</sub>H<sub>18</sub>O<sub>3</sub> 10-hydroxy-2-(E)-decenoic acid for thin-layer chromatography. It meets the requirement of the following 1) 10-Hydroxy-2-(E)-decenoic acid for assay 1 or 2) 10-Hydroxy-2-(E)-decenoic acid for assay 2 (Purity value by quantitative NMR). The latter is used with correction for its amount based on the result obtained in the Assay.

1) 10-Hydroxy-2-(E)-decenoic acid for assay 1

**Purity** Related substances—Dissolve 10 mg of 10-hydroxy-2-(E)-decenoic acid for assay 1 in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than 10-hydroxy-2-(E)-decenoic acid obtained from the sample solution is not larger than the peak area of 10-hydroxy-2-(E)-decenoic acid from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Royal Jelly.

**Time span of measurement**: About 4 times as long as the retention time of 10-hydroxy-2-(E)-decenoic acid, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of 10-hydroxy-2-(*E*)-decenoic acid obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

System performance: Dissolve 1 mg of propyl parahydroxybenzoate for resolution check in 10 mL of the sample solution. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, 10-hydroxy-2-(*E*)-decenoic acid and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 10-hydroxy-2-(*E*)-decenoic acid is not more than 1.5%.

2) 10-Hydroxy-2-(*E*)-decenoic acid for assay 2 (Purity value by quantitative NMR)

*Unity of peak*—Dissolve 1 mg of 10-hydroxy-2-(*E*)-decenoic acid for assay 2 in 50 mL of methanol, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of 10-hydroxy-2-(*E*)-decenoic acid peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

#### Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Royal Jelly.

Detector: A photodiode array detector (wavelength: 215 nm; spectrum range of measurement: 200 – 400 nm).

#### System suitability

System performance: Dissolve 1 mg each of 10-hydroxy-2-(*E*)-decenoic acid for assay 2 and propyl parahydroxybenzoate for resolution check in methanol to make 50 mL. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, 10-hydroxy-2-(*E*)-decenoic acid and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.5.

*Assay*—Weigh accurately 5 mg of 10-hydroxy-2-(*E*)-decenoic acid for assay 2 and 1 mg of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities, *A*<sub>1</sub> (equivalent to 1 hydrogen) and *A*<sub>2</sub> (equivalent to 1 hydrogen), of the signals around  $\delta$  5.54 ppm and  $\delta$  6.70 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\text{Amount (\%)} \text{ of 10-hydroxy-2-(E)-decenoic acid (C}_{10}\text{H}_{18}\text{O}_3) \\ = M_s \times I \times P/(M \times N) \times 0.8223$$

*M*: Amount (mg) of 10-hydroxy-2-(*E*)-decenoic acid for assay 2 taken

*M*<sub>s</sub>: Amount (mg) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

*I*: Sum of the signal resonance intensities, *A*<sub>1</sub> and *A*<sub>2</sub>, based on the signal resonance intensity of 1,4-BTMSB-

*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

*N*: Sum of the numbers of the hydrogen derived from *A*<sub>1</sub> and *A*<sub>2</sub>

*P*: Purity (%) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

<sup>13</sup>C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around  $\delta$  5.54 ppm and  $\delta$  6.70 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around  $\delta$  5.54 ppm and  $\delta$  6.70 ppm are not overlapped with any signal of obvious foreign substances, and the ratio of the resonance intensities, *A*<sub>1</sub>/*A*<sub>2</sub>, of each signal around  $\delta$  5.54 ppm and  $\delta$  6.70 ppm is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviations of the ratios of the resonance intensities, *A*<sub>1</sub> or *A*<sub>2</sub>, to that of the reference standard for qNMR are not more than 1.0%.

**10-Hydroxy-2-(*E*)-decenoic acid for component determination** See 10-hydroxy-2-(*E*)-decenoic acid for assay.

**10-Hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography** C<sub>10</sub>H<sub>18</sub>O<sub>3</sub> White crystalline powder. Very soluble in methanol, freely soluble in ethanol (99.5), soluble in diethyl ether, and slightly soluble in water.

*Identification*—Determine the absorption spectrum of a solution of 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography in ethanol (99.5) (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 206 nm and 210 nm.

*Melting point* <2.60>: 63 – 66°C

*Purity* Related substances—Dissolve 5.0 mg of 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography in 1 mL of diethyl ether. Perform the test with 20  $\mu$ L of this solution as directed in the Identification under Royal Jelly: no spot other than the principal spot at around *Rf* value of 0.5 appears.

**d-3-Hydroxy-cis-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5*H*)-one hydrochloride** C<sub>20</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>S.HCl To 9 g of diltiazem hydrochloride add 50 mL of ethanol (99.5), and dissolve by heating at 80°C. To this solution add slowly 50 mL of a solution of potassium hydroxide in ethanol (99.5) (33 in 500) dropwise, and heat for 4 hours with stirring. Cool in an ice bath, filter, and evaporate the filtrate to dryness. Dissolve the residue in ethanol (99.5), add slowly a solution of hydrochlo-

ric acid in ethanol (99.5) (59 in 250) to make acidic, and filter. Add diethyl ether slowly to the filtrate, and filter the crystals produced. To the crystals add ethanol (99.5), heat to dissolve, add 0.5 g of activated charcoal, allow to stand, and filter. After cooling the filtrate in an ice-methanol bath, filter the crystals formed, and wash with diethyl ether. Further, add ethanol (99.5) to the crystals, and heat to dissolve. After cooling, filter the crystals produced, and dry under reduced pressure. White, crystals or crystalline powder, having a slight, characteristic odor.

**Purity**—Dissolve 50 mg of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4-(5*H*)-one hydrochloride in chloroform to make exactly 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), chloroform, water and acetic acid (100) (12:10:3:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly iodine TS on the plate: any spot other than the principal spot does not appear.

**Water** <2.48>: not more than 1.0% (0.5 g).

**Content:** not less than 99.0%, calculated on the anhydrous basis. **Assay**—Weigh accurately about 0.5 g of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4-(5*H*)-one hydrochloride, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 40.89 mg of  $C_{20}H_{24}N_2O_3S \cdot HCl$

***d*-3-Hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepine-4 (5*H*)-one hydrochloride** See *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4 (5*H*)-one hydrochloride.

***N*-(2-Hydroxyethyl)isonicotinamide nitric ester**  
 $C_8H_9N_3O_4$  A white crystalline powder.

**Identification**—Determine the infrared absorption spectrum of *N*-(2-hydroxyethyl)isonicotinamide nitric ester as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3270  $\text{cm}^{-1}$ , 1653  $\text{cm}^{-1}$ , 1546  $\text{cm}^{-1}$  and 1283  $\text{cm}^{-1}$ .

***N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid**  
 $C_8H_{18}N_2O_4S$  White crystalline powder.

**Purity** Clarity and color of solution—Dissolve 11.9 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid in 50 mL of water: the solution is clear and colorless.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 1 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, dissolve in 60 mL of water, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (Potentiometric titration).

Each mL of 0.5 mol/L sodium hydroxide VS  
= 119.2 mg of  $C_8H_{18}N_2O_4S$

**1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol**  $C_3H_6N_4OS$

White, crystals or powder.

**Melting point** <2.60>: 136 – 141  $^{\circ}\text{C}$

**Purity** Related substances—Dissolve 0.10 g of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol in 1 mL of water, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add water to make exactly 25 mL, and use

this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1  $\mu$ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, water, methanol and formic acid (60:10:7:6) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid**  $C_{21}H_{14}N_2O_7S$  [K 8776, Special class]

**4-Hydroxyisophthalic acid**  $HOC_6H_3(COOH)_2$  White, crystals or powder.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.14 g of 4-hydroxyisophthalic acid, dissolve in 50 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 9.107 mg of  $C_8H_6O_5$

**Hydroxylamine hydrochloride** See hydroxylammonium chloride.

**Hydroxylamine hydrochloride-ferric chloride TS** See hydroxylammonium chloride-iron (III) chloride TS.

**Hydroxylamine hydrochloride TS** See hydroxylammonium chloride TS.

**Hydroxylamine perchlorate**  $NH_2OH \cdot HClO_4$  Hygroscopic, white crystals. Dissolves in water and in ethanol (95).

**Melting point** <2.60>: 87.5 – 90  $^{\circ}\text{C}$

**Hydroxylamine perchlorate-dehydrated ethanol TS** See hydroxylamine perchlorate-ethanol TS.

**Hydroxylamine perchlorate-ethanol TS** Dilute 2.99 mL of hydroxylamine perchlorate TS with ethanol (99.5) to make 100 mL.

**Storage**—Preserve in tight containers, in a cold place.

**Hydroxylamine perchlorate TS** An ethanol (99.5) solution which contains 13.4% of hydroxylamine perchlorate.

**Storage**—Preserve in tight containers, in a cold place.

**Hydroxylamine TS** Dissolve 10 g of hydroxylammonium chloride in 20 mL of water, and add ethanol (95) to make 200 mL. To this solution add, with stirring, 150 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and filter. Prepare before use.

**Hydroxylamine TS, alkaline** Mix equal volumes of a solution of hydroxylammonium chloride in methanol (7 in 100) and a solution of sodium hydroxide in methanol (3 in 25), and filter. Prepare before use.

**Hydroxylamine hydrochloride TS (pH 3.1)** See hydroxylammonium chloride TS (pH 3.1).

**Hydroxylammonium chloride**  $NH_2OH \cdot HCl$  [K 8201, Special class]

**Hydroxylammonium chloride-ethanol TS** Dissolve 34.8 g of hydroxylammonium chloride in water to make 100 mL, and use this solution as Solution A. Dissolve 10.3 g of sodium acetate trihydrate and 86.5 g of sodium hydroxide in water to make 1000 mL, and use this solution as Solution B.

Mix 1 volume of Solution A, 1 volume of Solution B and 4 volumes of ethanol (95).

**Hydroxylammonium chloride-iron (III) chloride TS**

Acidify 100 mL of a solution of iron (III) chloride hexahydrate in ethanol (95) (1 in 200) with hydrochloric acid, and dissolve 1 g of hydroxylammonium chloride in the solution.

**Hydroxylammonium chloride TS** Dissolve 20 g of hydroxylammonium chloride in water to make 65 mL, transfer it to a separator, add 2 to 3 drops of thymol blue TS, then add ammonia solution (28) until the solution exhibits a yellow color. Shake well after adding 10 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 25), allow to stand for 5 minutes, and extract this solution with 10 to 15 mL portions of chloroform. Repeat the extraction until 5 mL of the extract does not exhibit a yellow color, upon adding 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) and shaking it. Add 1 to 2 drops of thymol blue TS, add dropwise dilute hydrochloric acid to this aqueous solution until it exhibits a red color, then add water to make 100 mL.

**Hydroxylammonium chloride TS (pH 3.1)** Dissolve 6.9 g of hydroxylammonium chloride in 80 mL of water, adjust the pH to 3.1 by adding dilute sodium hydroxide TS, and add water to make 100 mL.

**4-Hydroxy-3-methoxybenzyl nonylic acid amide**

$C_{17}H_{27}NO_3$  A white crystalline powder, having a faint, characteristic odor.

**Purity** Related substances—Dissolve 10 mg of 4-Hydroxy-3-methoxybenzyl nonylic acid amide in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed in the Assay under Capsicum: when measure the peak areas 2 times as long as the retention time of capsaicin, the total area of the peaks other than 4-hydroxy-3-methoxybenzyl nonylic acid amide from the sample solution is not larger than the peak area of 4-hydroxy-3-methoxybenzyl nonylic acid amide from the standard solution.

**3-(3-Hydroxy-4-methoxyphenyl)-2-(*E*)-propenoic acid**

See (*E*)-isoferulic acid.

**3-(3-Hydroxy-4-methoxyphenyl)-2-(*E*)-propenoic acid-(*E*)-ferulic acid TS for thin-layer chromatography** See (*E*)-isoferulic acid-(*E*)-ferulic acid TS for thin-layer chromatography.

**2-[4-(2-Hydroxymethyl)-1-piperazinyl] propanesulfonic acid**  $C_8H_{18}N_2O_4S$  A white crystalline powder.

**Residue on ignition** <2.44>: not more than 0.1%.

**Content:** not less than 99%.

***N*-(3-Hydroxyphenyl)acetamide**  $C_8H_9NO_2$  White to pale yellow-white crystals. It is freely soluble in ethanol (95), and sparingly soluble in water.

**Melting point** <2.60>: 146 – 149°C

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of *N*-(3-hydroxyphenyl)acetamide in 50 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 0.1 g of *N*-(3-hydroxyphenyl)acetamide in 1000 mL of water. Pipet 10 mL of this solution, add 6.5 mL of acetonitrile and water to make exactly 50 mL, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed in the Assay under Aspoxicillin Hydrate: any peak

other than those of *N*-(3-hydroxyphenyl)acetamide and the solvent does not appear.

**3-(*p*-Hydroxyphenyl)propionic acid**  $C_9H_{10}O_3$  White to light yellow-brown, crystals or crystalline powder, having a faint, characteristic odor.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 0.2 g of 3-(*p*-hydroxyphenyl)propionic acid, previously dried (in vacuum, 60°C, 4 hours), dissolve in 5 mL of methanol, add 45 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of bromothymol blue TS).

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L sodium hydroxide VS} \\ = 16.62 \text{ mg of } C_9H_{10}O_3 \end{aligned}$$

**Hyodeoxycholic acid for thin-layer chromatography**

$C_{24}H_{40}O_4$  White to pale brown, crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification**—Determine the infrared absorption spectrum of hyodeoxycholic acid for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2940  $\text{cm}^{-1}$ , 2840  $\text{cm}^{-1}$ , 1740  $\text{cm}^{-1}$ , 1460  $\text{cm}^{-1}$ , 1340  $\text{cm}^{-1}$ , 1200  $\text{cm}^{-1}$ , 1160  $\text{cm}^{-1}$ , 1040  $\text{cm}^{-1}$  and 600  $\text{cm}^{-1}$ .

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : +7 – +10° (0.4 g, ethanol (99.5), 20 mL, 100 mm).

**Melting point** <2.60>: 198 – 205°C

**Purity** Related substances—Dissolve 20 mg of hyodeoxycholic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of the sample solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Splay evenly dilute sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: the spots other than the principal spot at the *Rf* value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

**Hypaconitine for purity**  $C_{33}H_{45}NO_{10}$  White, crystals or crystalline powder. Soluble in acetonitrile, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water. Melting point: about 175°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of hypaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3500  $\text{cm}^{-1}$ , 1728  $\text{cm}^{-1}$ , 1712  $\text{cm}^{-1}$ , 1278  $\text{cm}^{-1}$ , 1118  $\text{cm}^{-1}$ , 1099  $\text{cm}^{-1}$  and 714  $\text{cm}^{-1}$ .

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (230 nm): 217 – 252 [5 mg, ethanol (99.5), 200 mL].

**Purity** Related substances—(1) Dissolve 5.0 mg of hypaconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot obtained from the sam-

ple solution is not more intense than the spot from the standard solution.

(2) Dissolve 5.0 mg of hypaconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than hypaconitine obtained from the sample solution is not larger than the peak area of hypaconitine from the standard solution.

#### Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity (3) under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust so that the retention time of hypaconitine is about 23 minutes.

Time span of measurement: About 3 times as long as the retention time of hypaconitine, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of hypaconitine obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

System performance: Dissolve 1 mg each of hypaconitine for purity, aconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hypaconitine is not more than 1.5%.

**Water** <2.48>: not more than 1.0% (5 mg, coulometric titration).

#### Hyperoside for thin-layer chromatography C<sub>21</sub>H<sub>20</sub>O<sub>12</sub>

Yellow, crystals or crystalline powder. Slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 220°C (with decomposition).

**Identification**—Determine the absorption spectrum of a solution of hyperoside for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 255 nm and 259 nm.

**Purity** Related substances—Dissolve 1 mg of hyperoside for thin-layer chromatography in 20 mL of methanol. Perform the test with 10  $\mu$ L of this solution as directed in the Identification 2) under Crataegus Fruit: any spot other than the principal spot of around *Rf* value of 0.5 does not appear.

**Hypophosphorus acid** See phosphinic acid.

**Hypoxanthine** C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O White, crystals or crystalline powder. Freely soluble in ammonia TS, sparingly soluble in dilute hydrochloric acid and in hot water, very slightly soluble in water, and practically insoluble in methanol.

**Purity** Related substances—Dissolve 5.0 mg of hypoxan-

thine in 100 mL of a solution of ammonia solution (28) in methanol (1 in 10) to make exactly 100 mL. Proceed with this solution as directed in the Purity (4) under Mercaptopurine Hydrate: any spot other than the principal spot at the *Rf* value of about 0.2 does not appear.

**Content:** not less than 97.0% and not more than 103.0%.

**Assay**—Weigh accurately about 0.15 g of hypoxanthine, previously dried at 105°C for 3 hours, and dissolve in phosphate buffer solution (pH 7.0) to make exactly 1000 mL. Pipet 10 mL of this solution, and dilute with phosphate buffer solution (pH 7.0) to make exactly 250 mL. Read the absorbance *A* of this solution at the wavelength of 250 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of hypoxanthine (C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O)

$$= \frac{A}{779} \times 250,000$$

**Ibuprofen** C<sub>13</sub>H<sub>18</sub>O<sub>2</sub> [Same as the namesake monograph]

**Ibuprofen piconol** C<sub>19</sub>H<sub>23</sub>NO<sub>2</sub> [Same as the namesake monograph]

**Ibuprofen piconol for assay** C<sub>19</sub>H<sub>23</sub>NO<sub>2</sub> [Same as the monograph Ibuprofen Piconol. It contains not less than 99.0% of ibuprofen piconol (C<sub>19</sub>H<sub>23</sub>NO<sub>2</sub>), calculated on the anhydrous basis, and meets the following additional requirement.]

**Purity** Related substances—Dissolve 0.15 g of ibuprofen piconol for assay in the mobile phase to make 100 mL. To 10 mL of this solution add the mobile phase to make 30 mL, and use this as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas by the automatic integration method: the total area of the peaks other than ibuprofen piconol obtained from the sample solution is not larger than the peak area of ibuprofen piconol from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Perform as directed in the operating conditions in the Assay under Ibuprofen Piconol Ointment.

Time span of measurement: About 2 times as long as the retention time of ibuprofen piconol.

#### System suitability

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of ibuprofen piconol obtained with 5  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 5  $\mu$ L of the standard solution.

System performance: When the procedure is run with 5  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ibuprofen piconol are not less than 5000 and not more than 1.3, respectively.

System repeatability: When the test is repeated 6 times with 5  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ibuprofen piconol is not more than 2.0%.

**Icariin for thin-layer chromatography** C<sub>33</sub>H<sub>40</sub>O<sub>15</sub> Light yellow crystals. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 234°C (with decomposition).

**Purity** Related substances—Dissolve 1.0 mg of icariin

for thin-layer chromatography in 1 mL of methanol. Perform the test with 10  $\mu$ L of this solution as directed in the Identification under *Epimedium Herb*: no spot other than the principal spot at an *Rf* value of about 0.4 appears.

**Ifenprodil tartrate for assay**  $(C_{21}H_{27}NO_2)_2 \cdot C_4H_6O_6$  [Same as the monograph Ifenprodil Tartrate. It contains not less than 99.5% of ifenprodil tartrate [ $(C_{21}H_{27}NO_2)_2 \cdot C_4H_6O_6$ ], calculated on the anhydrous basis, and meets the following additional requirement.]

**Purity** Related substances—Dissolve 20 mg of ifenprodil tartrate for assay in 200 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than ifenprodil obtained from the sample solution is not larger than 1/2 times the peak area of ifenprodil from the standard solution. For the area of the peak, having the relative retention time of about 0.55 to ifenprodil, multiply the correction factor, 7.1.

#### Operating conditions

Detector, column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay under Ifenprodil Tartrate Fine Granules.

Mobile phase A: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 6.5 with potassium hydroxide TS, and add water to make 1000 mL. To 420 mL of this solution, add 320 mL of methanol for liquid chromatography and 260 mL of acetonitrile for liquid chromatography.

Mobile phase B: Methanol for liquid chromatography.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0.0 – 15.0	100	0
15.0 – 15.1	100 → 0	0 → 100
15.1 – 35.0	0	100

Time span of measurement: For 35 minutes after injection of the sample solution.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, add the mobile phase A to make exactly 10 mL. Confirm that the peak area of ifenprodil obtained with 20  $\mu$ L of this solution is equivalent to 7 to 13% of that with the standard solution.

System performance: When the procedure is run with 20  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ifenprodil are not less than 3500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ifenprodil is not more than 2.0%.

**Imidapril hydrochloride**  $C_{20}H_{27}N_3O_6 \cdot HCl$  [Same as the namesake monograph]

**Imidapril hydrochloride for assay**  $C_{20}H_{27}N_3O_6 \cdot HCl$  [Same as the monograph Imidapril Hydrochloride. When

dried, it contains not less than 99.0% of imidapril hydrochloride ( $C_{20}H_{27}N_3O_6 \cdot HCl$ ).]

**Imidazole**  $C_3H_4N_2$  White crystalline powder. Very soluble in water and in methanol.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (313 nm): not more than 0.031 (8 g, water, 100 mL).

*Melting point* <2.60>: 89 – 92°C

**Imidazole for thin-layer chromatography**  $C_3H_4N_2$

White crystalline powder. Very soluble in water and in methanol, and freely soluble in ethyl acetate and in dichloromethane.

*Melting point* <2.60>: 89 – 92°C

**Purity** Related substances—Dissolve 10 mg of imidazole for thin-layer chromatography in exactly 20 mL of dichloromethane, and proceed with this solution as directed in the Purity (6) under Clotrimazole: any spot other than the principal spot does not appear.

**Imidazole for water determination** Imidazole for thin-layer chromatography. Water content per g of imidazole for water determination should not be more than 1 mg.

**Imidazole hydrobromide**  $C_3H_4N_2 \cdot HBr$  White to pale yellow crystals. Melting point: about 221°C.

**Imidazole TS** Dissolve 8.25 g of imidazole in 65 mL of water, adjust the pH to 6.8 with 5 mol/L hydrochloric acid TS, and add water to make 100 mL.

**Iminodibenzyl**  $C_{14}H_{13}N$  White to light brown, crystals or crystalline powder, having a slight, characteristic odor.

*Melting point* <2.60>: 104 – 110°C

**Purity** (1) Clarity of solution—Dissolve 1.0 g of iminodibenzyl in 20 mL of methanol by heating on a water bath: the solution is clear.

(2) Related substances—Proceed as directed in the Purity (6) under Carbamazepine: any spot other than the principal spot at the *Rf* value of about 0.9 does not appear.

*Nitrogen* <1.08>: 6.8 – 7.3%.

**2,2'-Iminodiethanol hydrochloride**  $C_4H_{11}NO_2 \cdot HCl$

A light yellow liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.515 – 1.519

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.259 – 1.263

*Water* <2.48>: less than 0.1%.

**Imipramine hydrochloride**  $C_{19}H_{24}N_2 \cdot HCl$  [Same as the namesake monograph]

**Immature orange** [Same as the namesake monograph]

**Indigo carmine**  $C_{16}H_8N_2Na_2O_8S_2$  [K 8092, Special class]

**Indigo carmine TS** Dissolve 0.20 g of indigo carmine in water to make 100 mL. Use within 60 days.

**2,3-Indolinedione**  $C_8H_5NO_2$  [K 8089, Special class]

**Indometacin**  $C_{19}H_{16}ClNO_4$  [Same as the namesake monograph]

**Insulin human** [Same as the monograph Insulin Human (Genetical Recombination)]

**Interleukin-2 dependent mouse natural killer cell NKC3**

Fractionate using discontinuous concentration gradient method cells obtained by removing adhesive cells and phagocytic cells from C3H/He mouse spleen cells. Then, cultivate in soft agar containing interleukin-2 the cell fraction with potent NK activity and obtain the colonies. From among the cell lines obtained, one of the cell lines dependent on interleukin-2 that grows in liquid medium and serially subcul-

tured in liquid medium containing interleukin-2 is identified as NKC3.

**Iodine I** [K 8920, Special class]

**Iodine bromide (II) TS** Dissolve 20 g of iodine monobromide in acetic acid (100) to make 1000 mL. Store protected from light.

**Iodine for assay I** [Same as the monograph Iodine]

**Iodine monobromide** IBr Black-brown, crystals or masses. It dissolves in water, in ethanol (95), in acetic acid (100), in diethyl ether and in carbon disulfide.

*Melting point* <2.60>: 37 – 43°C

*Storage*—Preserve in light-resistant glass containers, in a cold place.

**Iodine-starch TS** To 100 mL of starch TS add 3 mL of dilute iodine TS.

**Iodine trichloride**  $\text{ICl}_3$  [K 8403, Special class]

**Iodine TS** Dissolve 14 g of iodine in 100 mL of a solution of potassium iodide (2 in 5), add 1 mL of dilute hydrochloric acid, and dilute with water to make 1000 mL (0.05 mol/L).

*Storage*—Preserve in light-resistant containers.

**Iodine TS, dilute** To 1 volume of iodine TS add 4 volumes of water.

**0.0002 mol/L Iodine TS** Measure exactly 1 mL of 0.5 mol/L iodine TS, add water to make exactly 250 mL, pipet 10 mL of the solution, and add water to make exactly 100 mL. Prepare before use.

**0.5 mol/L Iodine TS** To 12.7 g of iodine and 25 g of potassium iodide add 10 mL of water, triturate, and add water to make 100 mL.

**Iodoacetic acid**  $\text{ICH}_2\text{COOH}$  White or practically white crystals.

**Iodoethane**  $\text{C}_2\text{H}_5\text{I}$  A colorless or a dark-brown, clear liquid, having diethyl ether-like odor.

*Distilling range* <2.57>: 71.0 – 72.5°C, not less than 94 vol%.

**Iodoethane for assay**  $\text{C}_2\text{H}_5\text{I}$  Colorless to pale yellow liquid, turning brown on exposure to air and light. Miscible with ethanol (95). Specific gravity  $d_{20}^{20}$ : about 1.95; Boiling point: about 72°C.

*Refractive index* <2.45>  $n_{20}^{20}$ : 1.509 – 1.515.

*Content*: not less than 99.0%. Assay—Proceed as directed in the Assay under isopropyl iodide for assay.

Each mL of 0.1 mol/L silver nitrate VS  
= 15.60 mg of  $\text{C}_2\text{H}_5\text{I}$

*Storage*—Preserve in tight, light-resistant containers.

**Iodomethane**  $\text{CH}_3\text{I}$  [K 8919, Special class]

**Iodomethane for assay**  $\text{CH}_3\text{I}$  Colorless to dark brown, clear liquid. On exposure to light, it liberates iodine and becomes brown. Miscible with ethanol (95) and with diethyl ether, and sparingly soluble in water. Use the distillate obtained between 42.2°C and 42.6°C.

*Specific gravity* <2.56>  $d_{25}^{25}$ : 2.27 – 2.28.

*Purity*—Perform the test with 1  $\mu\text{L}$  of iodomethane for assay as directed under Gas Chromatography <2.02> according to the operating conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of iodomethane

by the area percentage method: it shows the purity of not less than 99.8%. Adjust the detection sensitivity so that the peak height of iodomethane from 1  $\mu\text{L}$  of iodomethane for assay is about 80% of the full scale.

*Content*: not less than 98.0%. Assay—Proceed as directed in the Assay under isopropyl iodide for assay.

Each mL of 0.1 mol/L silver nitrate VS  
= 14.19 mg of  $\text{CH}_3\text{I}$

**5-Iodouracil for liquid chromatography**  $\text{C}_4\text{H}_3\text{IN}_2\text{O}_2$

White crystalline powder. Melting point: about 275°C (with decomposition).

*Purity*—Dissolve 3 mg of 5-iodouracil for liquid chromatography in diluted methanol (1 in 25) to make 10 mL. Perform the test with 10  $\mu\text{L}$  of this solution as directed under Liquid Chromatography <2.01>, according to the operating conditions in the Purity under Idoxuridine Ophthalmic Solution. Determine each peak area by the automatic integration method over a time span of twice as long as the retention time of the principal peak, and calculate the amount of 5-iodouracil by the area percentage method: It shows the purity of not less than 98.5%.

*Content*: not less than 98.5%. Assay—Weigh accurately about 5 mg of 5-iodouracil for liquid chromatography, previously dried at 60°C for 3 hours under reduced pressure, dissolve in water to make exactly 250 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbance  $A$  at the wavelength of maximum absorption at about 282 nm.

Amount (mg) of 5-iodouracil ( $\text{C}_4\text{H}_3\text{IN}_2\text{O}_2$ )  
=  $\frac{A}{265} \times 2500$

**Iopamidol for assay**  $\text{C}_{17}\text{H}_{22}\text{I}_3\text{N}_3\text{O}_8$  [Same as the monograph Iopamidol]

**Totalamic acid for assay**  $\text{C}_{11}\text{H}_9\text{I}_3\text{N}_2\text{O}_4$  [Same as the monograph Totalamic Acid]

**Irbesartan for assay**  $\text{C}_{25}\text{H}_{28}\text{N}_6\text{O}$  [Same as the monograph Irbesartan]

**Irinotecan hydrochloride hydrate for assay**

$\text{C}_{33}\text{H}_{38}\text{N}_4\text{O}_6\text{.HCl.3H}_2\text{O}$  [Same as the monograph Irinotecan Hydrochloride Hydrate]

**Iron Fe** Iron in the forms of strips, sheets, granules or wires. Fe: not less than 97.7%. It is attracted by a magnet.

**Iron (II) sulfate heptahydrate**  $\text{FeSO}_4\text{.7H}_2\text{O}$  [K 8978, Special class]

**Iron (II) sulfate TS** Dissolve 8 g of iron (II) sulfate heptahydrate in 100 mL of freshly boiled and cooled water. Prepare before use.

**Iron (II) sulfide**  $\text{FeS}$  [K 8948, for hydrogen sulfide development]

**Iron (II) tartrate TS** Dissolve 1 g of iron (II) sulfate heptahydrate, 2 g of potassium sodium tartrate tetrahydrate and 0.1 g of sodium hydrogen sulfite in water to make 100 mL.

**Iron (II) thiocyanate TS** Add 3 mL of dilute sulfuric acid to 35 mL of water, and remove the dissolved oxygen by boiling the solution. Dissolve 1 g of iron (II) sulfate heptahydrate in this hot solution, cool, and then dissolve 0.5 g of potassium thiocyanate. When the solution is pale red in color, decolorize by adding reduced iron, separate the excess of reduced iron by decanting, and preserve the solution with protection from oxygen. Do not use a solution showing a

pale red color.

**Iron (II) trisodium pentacyanoamine TS** To 1.0 g of sodium pentacyanonitrosylferrate (III) dihydrate add 3.2 mL of ammonia TS, shake, stopper closely, and allow to stand in a refrigerator overnight. Add this solution to 10 mL of ethanol (99.5), filter a yellow colored precipitate by suction, wash with dehydrated diethyl ether, dry, and preserve in a desiccator. Before using, dissolve in water to make a solution of 1.0 mg/mL, and store in a refrigerator. Use within 7 days after preparation.

**Iron (III) chloride-acetic acid TS** Dissolve 0.1 g of iron (III) chloride hexahydrate in diluted acetic acid (31) (3 in 100) to make 100 mL.

**Iron (III) chloride-amidosulfuric acid TS** Dissolve 10 g of iron (III) chloride hexahydrate and 16 g of amidosulfuric acid (standard reagent) in water to make 1000 mL.

**Iron (III) chloride hexahydrate**  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  [K 8142, Special class]

**Iron (III) chloride-iodine TS** Dissolve 5 g of iron (III) chloride hexahydrate and 2 g of iodine in a mixture of 50 mL of acetone and 50 mL of a solution of L-tartaric acid (1 in 5).

**Iron (III) chloride-methanol TS** Dissolve 1 g of iron (III) chloride hexahydrate in methanol to make 100 mL.

**Iron (III) chloride-potassium hexacyanoferrate (III) TS** Dissolve 0.1 g of potassium hexacyanoferrate (III) in 20 mL of iron (III) chloride TS. Prepare before use.

**Iron (III) chloride-pyridine TS, anhydrous** Heat gradually 1.7 g of iron (III) chloride hexahydrate by direct application of flame, melt, and solidify. After cooling, dissolve the residue in 100 mL of chloroform, add 8 mL of pyridine, and filter.

**Iron (III) chloride TS** Dissolve 9 g of iron (III) chloride hexahydrate in water to make 100 mL (0.33 mol/L).

**Iron (III) chloride TS, acidic** To 60 mL of acetic acid (100) add 5 mL of sulfuric acid and 1 mL of iron (III) chloride hexahydrate TS.

**Iron (III) chloride TS, dilute** Dilute 2 mL of iron (III) chloride TS with water to make 100 mL. Prepare before use.

**Iron (III) nitrate enneahydrate**  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  [K 8559, Special class]

**Iron (III) nitrate TS** Dissolve 1 g of iron (III) nitrate enneahydrate in hydrochloric acid-potassium chloride buffer solution (pH 2.0) to make 300 mL.

**Iron (III) perchlorate-ethanol TS** Dissolve 0.8 g of iron (III) perchlorate hexahydrate in perchloric acid-ethanol TS to make 100 mL.

*Storage*—Preserve in tight containers, in a cold place.

**Iron (III) perchlorate hexahydrate**  $\text{Fe}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$  Hygroscopic, light purple crystals, and a solution in ethanol (99.5) (1 in 125) is clear and orange in color.

**Iron (III) sulfate n-hydrate**  $\text{Fe}_2(\text{SO}_4)_3 \cdot x\text{H}_2\text{O}$  [K 8981, Special class]

**Iron (III) sulfate TS** Dissolve 50 g of iron (III) sulfate n-hydrate in an excess of water, and add 200 mL of sulfuric acid and water to make 1000 mL.

**Iron-phenol TS** Dissolve 1.054 g of ammonium iron (II) sulfate hexahydrate in 20 mL of water, add 1 mL of sulfuric acid and 1 mL of hydrogen peroxide (30), heat until efferves-

cence ceases, and dilute with water to make 50 mL. To 3 volumes of this solution contained in a volumetric flask add sulfuric acid, with cooling, to make 100 volumes, yielding the iron-sulfuric acid solution. Purify phenol by distillation, discarding the first 10% and the last 5%, and collect the distillate, with exclusion of moisture, in a dry, tared, glass-stoppered flask of about twice the volume of the phenol. Stopper the flask, solidify the phenol in an ice bath, breaking the top crust with a glass rod to ensure complete crystallization, and after drying, weigh the flask. To the glass-stoppered flask add 1.13 times the mass of phenol of the iron-sulfuric acid solution, insert the stopper in the flask, and allow to stand, without cooling but with occasional shaking, until the phenol is liquefied, then shake the mixture vigorously. Allow to stand in a dark place for 16 to 24 hours. To the mixture add diluted sulfuric acid (10 in 21) equivalent to 23.5% of its mass, mix well, transfer to dry glass-stoppered bottles, and preserve in a dark place, with protection from atmospheric moisture. Use within 6 months.

**Iron-phenol TS, dilute** To 10 mL of iron-phenol TS add 4.5 mL of water. Prepare before use.

**Iron powder** Fe A lusterless, gray to grayish black powder, being attracted by a magnet.

*Identification*—To 1 mL of a solution of iron powder in hydrochloric acid (1 in 50) add water to make 15 mL, and add 0.1 mL of potassium hexacyanoferrate (III) TS: a blue color appears.

**Iron salicylate TS** Dissolve 0.1 g of ammonium iron (III) sulfate dodecahydrate in 50 mL of diluted sulfuric acid (1 in 250), and add water to make 100 mL. Measure 20 mL of this solution, and add 10 mL of a solution of sodium salicylate (23 in 2000), 4 mL of dilute acetic acid, 16 mL of sodium acetate TS and water to make 100 mL. Prepare before use.

**Irsogladine maleate**  $\text{C}_9\text{H}_7\text{Cl}_2\text{N}_5\text{C}_4\text{H}_4\text{O}_4$  [Same as the namesake monograph]

**Irsogladine maleate for assay**  $\text{C}_9\text{H}_7\text{Cl}_2\text{N}_5\text{C}_4\text{H}_4\text{O}_4$  [Same as the monograph Irsogladine Maleate. When dried, it contains not less than 99.5% of irsogladine maleate ( $\text{C}_9\text{H}_7\text{Cl}_2\text{N}_5\text{C}_4\text{H}_4\text{O}_4$ ).]

**Isatin** See 2,3-indolinedione.

**Iscove's modified Dulbecco's fluid medium for filgrastim** A fluid medium for cell culture, containing 0.165 g of anhydrous calcium chloride, 97.67 mg of anhydrous magnesium sulfate, 0.330 g of potassium chloride, 76  $\mu\text{g}$  of potassium nitrate, 4.5 g of sodium chloride, 0.125 g of sodium dihydrogen phosphate monohydrate, 17.3  $\mu\text{g}$  of sodium selenite pentahydrate, 30 mg of glycine, 25 mg of L-alanine, 84 mg of L-arginine hydrochloride, 25 mg of L-asparagine, 30 mg of L-aspartic acid, 91.4 mg of L-cystine dihydrochloride, 75 mg of L-glutamic acid, 0.584 g of L-glutamine, 42 mg of L-histidine hydrochloride monohydrate, 0.105 g of L-isoleucine, 0.105 g of L-leucine, 0.146 g of L-lysine hydrochloride, 30 mg of L-methionine, 66 mg of L-phenylalanine, 40 mg of L-proline, 42 mg of L-serine, 95 mg of L-threonine, 16 mg of L-tryptophan, 0.104 g of disodium L-tyrosine, 94 mg of L-valine, 13  $\mu\text{g}$  of biotin, 4 mg of choline chloride, 4 mg of calcium D-pantothenate, 4 mg of folic acid, 4 mg of nicotinic acid amide, 4 mg of pyridoxal hydrochloride, 0.4 mg of riboflavin, 4 mg of thiamine hydrochloride, 13  $\mu\text{g}$  of cyanocobalamin, 7.2 mg of myoinositol, 4.5 g of glucose, 5.958 g of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 15 mg of phenol red, 0.110 g of sodium pyruvate and 3.024 g of sodium hydrogen carbonate in 1 L.

**Iscove's modified Dulbecco's powder medium** A powder to make fluid medium for cell culture, containing 0.165 g of anhydrous calcium chloride, 97.67 mg of anhydrous magnesium sulfate, 0.330 g of potassium chloride, 76 µg of potassium nitrate, 4.5 g of sodium chloride, 0.125 g of sodium dihydrogen phosphate monohydrate, 17.3 µg of sodium selenite pentahydrate, 30 mg of glycine, 25 mg of L-alanine, 84 mg of L-arginine hydrochloride, 25 mg of L-asparagine, 30 mg of L-aspartic acid, 91.4 mg of L-cystine dihydrochloride, 75 mg of L-glutamic acid, 0.584 g of L-glutamine, 42 mg of L-histidine hydrochloride monohydrate, 0.105 g of L-isoleucine, 0.105 g of L-leucine, 0.146 g of L-lysine hydrochloride, 30 mg of L-methionine, 66 mg of phenylalanine, 40 mg of L-proline, 42 mg of L-serine, 95 mg of L-threonine, 16 mg of L-tryptophan, 0.104 g of disodium L-tyrosine, 94 mg of L-valine, 13 µg of biotin, 4 mg of choline chloride, 4 mg of calcium D-pantothenate, 4 mg of folic acid, 4 mg of nicotinic acid amide, 4 mg of pyridoxal hydrochloride, 0.4 mg of riboflavin, 4 mg of thiamine hydrochloride, 13 µg of cyanocobalamin, 7.2 mg of myoinositol, 4.5 g of glucose, 5.958 g of N-2-hydroxyethylpiperazine-N-2-ethanesulfonate, 15 mg of phenol red and 0.110 g of sodium pyruvate in each L.

**Isoamyl acetate** See 3-methylbutyl acetate.

**Isoamyl alcohol** See 3-methyl-1-butanol.

**Isoamyl benzoate**  $C_{12}H_{16}O_2$

*Specific gravity* <2.56>  $d_4^{15}$ : 0.993

*Boiling point* <2.57>: 260 – 262°C

**Isoamyl parahydroxybenzoate**  $C_{12}H_{16}O_3$  White crystalline powder, having a faint characteristic odor.

It is very soluble in acetonitrile, in ethanol (95), in acetone and in diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: 62 – 64°C

**Isobutanol** See 2-methyl-1-propanol.

**Isobutyl parahydroxybenzoate**  $C_{11}H_{14}O_3$  Colorless crystals or white crystalline powder. Freely soluble in ethanol (95), and practically insoluble in water.

*Melting point* <2.60>: 74 – 78°C

*Residue on ignition* <2.44>: not more than 0.1%.

**Content:** not less than 99.0%. Assay—Weigh accurately about 1 g of isobutyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 194.2 \text{ mg of } C_{11}H_{14}O_3 \end{aligned}$$

**Isobutyl salicylate**  $C_{11}H_{14}O_3$  Colorless, clear liquid, having a characteristic odor.

*Refractive index* <2.45>  $n_D^{20}$ : 1.506 – 1.511

*Specific gravity* <2.56>  $d_4^{20}$ : 1.068 – 1.073

*Boiling point* <2.57>: 260 – 262°C

**Purity**—Perform the test with 1 µL of isobutyl salicylate as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of isobutyl salicylate by the area percentage method: It shows the purity of not less than 97.0%.

**Operating conditions**

Detector: A thermal conductivity detector.

Column: A column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography, 180 to 250 µm in particle diameter, coated with polyethylene glycol 20 M for gas chromatography at the ratio of 10%.

Column temperature: A constant temperature of about 220°C.

Carrier gas: Helium.

Flow rate: About 20 mL per minute.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of isobutyl salicylate obtained from 1 µL of the isobutyl salicylate is about 60 to 80% of the full scale.

Time span of measurement: About 3 times as long as the retention time of isobutyl salicylate.

**(E)-Isoferulic acid**  $C_{10}H_{10}O_4$  White to light yellow, crystals or crystalline powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 230°C (with decomposition).

**Identification**—Determine the absorption spectrum of a solution of (E)-isoferulic acid in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 215 nm and 219 nm, between 238 nm and 242 nm, between 290 nm and 294 nm, and between 319 nm and 323 nm.

**Purity** Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 1 mg of (E)-isoferulic acid in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 2 µL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): no spot other than the principal spot at an *Rf* value of about 0.6 appears.

**(E)-Isoferulic acid-(E)-ferulic acid TS for thin-layer chromatography** Dissolve 1 mg each of (E)-isoferulic acid and (E)-ferulic acid in 2 mL of methanol.

**Isoelectric point markers for teceleukin** Dissolve 0.02 to 0.05 mg of cytochrome C, trypsinogen, lentil-lectin basic band, lentil-lectin middle band, lentil-lectin acidic band, horse myoglobin basic band, horse myoglobin acidic band, human carbonic anhydrase B, bovine carbonic anhydrase B, and β-lactoglobulin A, in 0.1 mL of saccharose solution (3 in 10).

**L-Isoleucine**  $C_6H_{13}NO_2$  [Same as the namesake monograph]

**L-Isoleucine for assay**  $C_6H_{13}NO_2$  [Same as the monograph L-Isoleucine. When dried, it contains not less than 99.0% of L-isoleucine ( $C_6H_{13}NO_2$ ).]

**Isomalt**  $C_{12}H_{24}O_{11}$  White, powder or grain. Very soluble in water, and practically insoluble in ethanol (99.5).

**Isoniazid**  $C_6H_7N_3O$  [Same as the namesake monograph]

**Isoniazid for assay**  $C_6H_7N_3O$  [Same as the monograph Isoniazid. When dried, it contains not less than 99.0% of isoniazid ( $C_6H_7N_3O$ ).]

**Isoniazid TS** Dissolve 0.1 g of isoniazid for assay in a mixture of 50 mL of methanol and 0.12 mL of hydrochloric acid, and add methanol to make 200 mL.

**Ionicotinic acid** White, crystals or powder. Melting

point: about 315°C (decomposition).

**Isonicotinic acid amide**  $C_6H_6N_2O$  White, crystals or crystalline powder.

*Melting point* <2.60>: 155 – 158°C

*Purity* Clarity of solution—Dissolve 1.0 g of the substance to be tested in 20 mL of methanol: the solution is clear.

*Content:* not less than 99.0%. Assay—Weigh accurately about 0.3 g of isonicotinic acid amide, previously dried, and dissolve in 20 mL of acetic acid (100) by heating. After cooling, add 100 mL of benzene, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L perchloric acid VS} \\ = 11.21 \text{ mg of } C_6H_6N_2O \end{aligned}$$

**Isooctane** See octane, iso.

**Isopromethazine hydrochloride for thin-layer chromatography**  $C_{17}H_{20}N_2S.HCl$  White crystalline powder. Odorless. Freely soluble in water, in ethanol (95) and in chloroform, and practically insoluble in diethyl ether.

*Melting point* <2.60>: 186 – 195°C

*Purity* Related substances—Dissolve 5.0 mg of isopromethazine hydrochloride for thin-layer chromatography in exactly 25 mL of ethanol (95), and perform the test with this solution as directed in the Purity (3) under Promethazine Hydrochloride: any spot other than the principal spot at the *Rf* value of about 0.65 does not appear.

**Isopropanol** See 2-propanol.

**Isopropanol for liquid chromatography** See 2-propanol for liquid chromatography.

**Isopropylamine** See propylamine, iso.

**Isopropylamine-ethanol TS** To 20 mL of isopropylamine add ethanol (99.5) to make 100 mL. Prepare before use.

**Isopropyl p-aminobenzoate** See isopropyl 4-aminobenzoate.

**Isopropyl 4-aminobenzoate**  $H_2NC_6H_4COOCH(CH_3)_2$  Pale brown crystals.

*Melting point* <2.60>: 83 – 86°C

**Isopropyl benzoate**  $C_6H_5COOCH(CH_3)_2$  A clear, colorless liquid, having a characteristic odor.

*Refractive index* <2.45>  $n_D^{20}$ : 1.490 – 1.498

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.008 – 1.016

**Isopropylether** See propylether, iso.

**Isopropyl p-hydroxybenzoate** See isopropyl parahydroxybenzoate.

**Isopropyl iodide for assay**  $C_3H_7I$  Colorless, clear liquid. On exposure to light it liberates iodine and becomes brown. Miscible with ethanol (95), with diethyl ether and with petroleum benzin, and not miscible with water. Use the distillate obtained between 89.0°C and 89.5°C.

*Specific gravity* <2.56>  $d_4^{20}$ : 1.700 – 1.710

*Purity*—Perform the test with 1 μL of isopropyl iodide for assay as directed under Gas Chromatography <2.02> according to the operating conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of isopropyl iodide by the area percentage method: It shows the purity of not less than 99.8%. Adjust the detection sensitivity so that

the peak height of isopropyl iodide from 1 μL of isopropyl iodide for assay is about 80% of the full scale.

*Content:* not less than 98.0%. Assay—Transfer 10 mL of ethanol (95) into a brown volumetric flask, weigh accurately, add 1 mL of isopropyl iodide for assay, and weigh accurately again. Add ethanol (95) to make exactly 100 mL, pipet 20 mL of this solution into the second brown volumetric flask, add exactly 50 mL of 0.1 mol/L silver nitrate VS and then 2 mL of nitric acid, stopper, shake occasionally for 2 hours in a dark place, and allow to stand overnight in a dark place. Shake occasionally for 2 hours, add water to make exactly 100 mL, and filter through dry filter paper. Discard the first 20 mL of the filtrate, pipet the next 50 mL, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L silver nitrate VS} \\ = 17.00 \text{ mg of } C_3H_7I \end{aligned}$$

**Isopropyl myristate**  $C_{17}H_{34}O_2$  Colorless, clear, oily liquid, and odorless. Congeals at about 5°C. Soluble in 90% alcohol, miscible with many organic solvents and with solid oils, and insoluble in water, in glycerin and in propylene glycol.

*Refractive index* <2.45>  $n_D^{20}$ : 1.432 – 1.436

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.846 – 0.854

*Acid value* <1.13>: not more than 1.

*Saponification value* <1.13>: 202 – 212

*Iodine value* <1.13>: not more than 1.

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

**Isopropyl myristate for sterility test**  $C_{17}H_{34}O_2$  Transfer 100 mL of isopropyl myristate into a centrifuge tube, add 100 mL of twice-distilled water, and shake vigorously for 10 minutes. Then centrifuge at a rate of 1800 revolutions per minute for 20 minutes, separate the supernatant liquid (isopropyl myristate layer), and determine the pH of the residual water layer: not less than 5.5.

Treat isopropyl myristate which meets the requirements of pH determination as follows: 500 mL of isopropyl myristate, which has met the requirements of pH determination, is percolated through a 15-cm high layer of activated alumina filled in a glass column 20 mm in diameter and 20 cm in length with a slightly positive pressure in order to facilitate adequate flow, and then sterilized by filtration.

**Isopropyl parahydroxybenzoate**  $C_{10}H_{12}O_3$  Colorless fine crystals, or white crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.

*Melting point* <2.60>: 84 – 86°C

*Residue on ignition* <2.44>: not more than 0.1%.

*Content:* not less than 99.0%. Assay—Weigh accurately about 1 g of isopropyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of } 1 \text{ mol/L sodium hydroxide VS} \\ = 180.2 \text{ mg of } C_{10}H_{12}O_3 \end{aligned}$$

**4-Isopropylphenol**  $C_9H_{12}O$  White to reddish yellow, crystals or crystalline powder.

*Melting point* <2.60>: 59 – 63°C

**Isosorbide dinitrate for assay**  $C_6H_8N_2O_8$  [Same as the monograph Isosorbide Dinitrate. It contains not less than

99.0% of isosorbide dinitrate ( $C_6H_8N_2O_8$ ), calculated on the anhydrous basis, meeting the following additional specifications.]

**Purity** Related substances—Dissolve 50 mg of isosorbide dinitrate for assay in 50 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of isosorbide dinitrate obtained from the sample solution is not larger than the peak area of isosorbide dinitrate from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Isosorbide Dinitrate Tablets.

Time span of measurement: About 2 times as long as the retention time of isosorbide dinitrate, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 50 mL. Confirm that the peak area of isosorbide dinitrate obtained with 10  $\mu$ L of this solution is equivalent to 7 to 13% of that with 10  $\mu$ L of the standard solution.

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide dinitrate are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosorbide dinitrate is not more than 2.0%.

#### Isosorbide mononitrate for assay $C_6H_9NO_6$

Odorless white crystals.

**Method of purification:** To Isosorbide Mononitrate 70%/Lactose 30% add not less than 3-fold volume of ethyl acetate, shake vigorously, filter through a membrane filter with a pore size not exceeding 0.5  $\mu$ m, and evaporate the filtrate to dryness on a water bath under reduced pressure. Recrystallize the residue from a mixture of hexane and ethyl acetate (3:2), and dry under reduced pressure on silica gel for 4 hours.

**Identification**—Determine the infrared absorption spectrum of isosorbide mononitrate for assay, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of between 3210  $cm^{-1}$  and 3230  $cm^{-1}$ , and about 1651  $cm^{-1}$ , 1635  $cm^{-1}$ , 1282  $cm^{-1}$ , 1093  $cm^{-1}$  and 852  $cm^{-1}$ .

**Optical rotation** <2.49> [ $\alpha_D^{20}$ : +171 – +176° (after drying, 1 g, ethanol (95), 100 mL, 100 mm).

**Melting point** <2.60>: 89 – 92°C

**Purity** Related substances—Dissolve 50 mg of isosorbide mononitrate for assay in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to

the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than isosorbide mononitrate obtained from the sample solution is not larger than the peak area of isosorbide mononitrate from the standard solution, and the total area of the peaks other than isosorbide mononitrate from the sample solution is not larger than 2 times the peak area of isosorbide mononitrate from the standard solution. For the area of the peak, having a relative retention time of about 4.5 to isosorbide mononitrate, multiply its correction factor, 0.62.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Isosorbide Mononitrate 70%/Lactose 30%.

Time span of measurement: About 5 times as long as the retention time of isosorbide mononitrate, beginning after the solvent peak.

#### System suitability

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide mononitrate are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosorbide mononitrate is not more than 2.0%.

**Loss on drying** <2.41>: not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 0.2 g of previously dried isosorbide mononitrate for assay, put in a Kjeldahl flask, dissolve in 10 mL of water, add 3 g of Devarda's alloy and 40 mL of water, and set the flask on the apparatus as shown in the figure under Nitrogen Determination <1.08>. Put exactly 25 mL of 0.05 mol/L sulfuric acid VS and 5 drops of bromocresol green-methyl red TS in an absorption flask, and set to the apparatus to immerse the lower end of the condenser. Add 15 mL of sodium hydroxide solution (1 in 2) through the funnel, rinse cautiously the funnel with 20 mL of water, immediately close the clamp attached to the rubber tubing, then begin the distillation with steam, and continue until the distillate measures about 100 mL. Remove the absorption flask from the lower end of the condenser, rinse the end part of the condenser with a small quantity of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red to light blue-green through a light red-purple. Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of } 0.05 \text{ mol/L sulfuric acid VS} \\ = 19.11 \text{ mg of } C_6H_9NO_6 \end{aligned}$$

**Isotonic sodium chloride solution** [Same as the name-sake monograph]

**Isoxsuprine hydrochloride for assay  $C_{18}H_{23}NO_3.HCl$**  [Same as the monograph Isoxsuprine Hydrochloride]

**Japanese acid clay** Natural hydrous aluminum silicate, grayish white powder, having a particle size of about 75  $\mu$ m.

**Loss on drying** <2.41>: not more than 10% (1 g, 105°C, 4 hours).

**Water adsorbing capacity:** not less than 2.5%. Weigh accurately about 10 g of Japanese acid clay in weighing bottle, allow to stand for 24 hours with cover in a chamber, in which humidity is maintained to 80% by means of sulfuric acid (specific gravity 1.19), reweigh, and determine the increase of mass of the sample.

**Japanese zanthoxylum peel** [Same as the namesake monograph]

**Jesaconitine for purity**  $C_{35}H_{49}NO_{12}$  A white powder. Freely soluble in acetonitrile, in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

**Identification**—Determine the infrared absorption spectrum of jesaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3500\text{ cm}^{-1}$ ,  $1715\text{ cm}^{-1}$ ,  $1607\text{ cm}^{-1}$ ,  $1281\text{ cm}^{-1}$ ,  $1259\text{ cm}^{-1}$ ,  $1099\text{ cm}^{-1}$  and  $772\text{ cm}^{-1}$ .

**Absorbance** <2.24>  $E_{1\text{ cm}}^{1\%}$  (258 nm): 270 – 291 [5 mg, ethanol (99.5), 200 mL].

**Purity** Related substances—(1) Dissolve 5.0 mg of jesaconitine for purity in 2 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

(2) Dissolve 5.0 mg of jesaconitine for purity in 5 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than jesaconitine obtained from the sample solution is not larger than the peak area of jesaconitine from the standard solution.

#### Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust so that the retention time of jesaconitine is about 36 minutes.

Time span of measurement: About 3 times as long as the retention time of jesaconitine, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of jesaconitine obtained with 10  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu\text{L}$  of the standard solution.

System performance: Dissolve 1 mg of jesaconitine for purity and 5 mg each of aconitine for purity, hypaconitine for purity and mesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10  $\mu\text{L}$  of this solution under the above operating conditions, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of jesaconitine is not more than 1.5%.

**Water** <2.48>: not more than 1.0% (5 mg, coulometric

titration).

**Josamycin**  $C_{42}H_{69}NO_{15}$  [Same as the namesake monograph]

**Josamycin propionate**  $C_{45}H_{73}NO_{16}$  [Same as the namesake monograph]

**Kainic acid** See kainic acid hydrate.

**Kainic acid for assay** See kainic acid hydrate.

**Kainic acid hydrate**  $C_{10}H_{15}NO_4 \cdot H_2O$  [Same as the namesake monograph]

**Kainic acid hydrate for assay** See kainic acid hydrate.

**Kanamycin sulfate**  $C_{18}H_{36}N_4O_{11} \cdot xH_2SO_4$  [Same as the namesake monograph]

**Karl Fischer TS for water determination** See Water Determination <2.48>.

**Kerosene** It is mainly a mixture of hydrocarbons in the methane series, and a colorless, clear liquid, having not a disagreeable, characteristic odor.

**Specific gravity** <2.56>: about 0.80

**Distilling range** <2.57>: 180 – 300°C

**Ketoconazole**  $C_{26}H_{28}Cl_2N_4O_4$  [Same as the namesake monograph]

**Ketoconazole for assay**  $C_{26}H_{28}Cl_2N_4O_4$  [Same as the monograph Ketoconazole. When dried, it contains not less than 99.5% of ketoconazole ( $C_{26}H_{28}Cl_2N_4O_4$ ).]

**Kininogen** Produced by purifying from bovine plasma. Dissolve an appropriate amount of kininogen in 0.02 mol/L phosphate buffer solution (pH 8.0) so that 10 mL of the solution contains 1 mg of kininogen, and use this solution as the sample solution. Perform the following tests with the sample solution: it meets the requirement of each test.

(i) Immediately after the sample solution is prepared, add 0.1 mL of a solution of trichloroacetic acid (1 in 5) to 0.5 mL of the sample solution, shake, and centrifuge. To 0.5 mL of the supernatant liquid add 0.5 mL of gelatin-tris buffer solution (pH 8.0), and shake. To 0.1 mL of this solution add 1.9 mL of trichloroacetic acid-gelatin-tris buffer solution. Proceed with 0.1 mL of this solution as directed in the Purity (2) under Kallidinogenase, and determine the amount of kinin: kinin is not detected.

(ii) Warm 0.5 mL of the sample solution at  $30 \pm 0.5^\circ\text{C}$  for 20 minutes, and proceed as directed in (i): kinin is not detected.

(iii) Perform the test with 0.5 mL of the sample solution as directed in the Purity (2) under Kallidinogenase: the decomposition of bradykinin is not observed.

(iv) To 0.5 mL of the sample solution add 0.5 mL of 0.02 mol/L phosphate buffer solution (pH 8.0) containing 500  $\mu\text{g}$  of crystalline trypsin, previously warmed at  $30 \pm 0.5^\circ\text{C}$  for 5 minutes, warm this solution at  $30 \pm 0.5^\circ\text{C}$  for 5 minutes, add 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Then boil for 3 minutes, cool in ice immediately, and centrifuge. To 0.5 mL of the supernatant liquid add 0.5 mL of gelatin-tris buffer solution (pH 8.0), and shake. To 0.1 mL of this solution add 0.9 mL of trichloroacetic acid-gelatin-tris buffer solution. To 0.1 mL of this solution add trichloroacetic acid-gelatin-tris buffer solution to make 20 mL, then proceed as directed in (i), and determine the amount,  $B_K$ , of kinin per well. Calculate the kinin-releasing activity per mg by the following equation: not less than 10  $\mu\text{g}$  bradykinin equivalent per mg.

Kinin-releasing activity per mg ( $\mu\text{g}$  bradykinin equivalent/mg) =  $B_K \times 0.96$

**Kininogen TS** Dissolve a sufficient quantity of kininogen in 0.02 mol/L phosphate buffer solution (pH 8.0) to prepare a solution having an ability in each mL to release kinin corresponding to not less than 1  $\mu\text{g}$  of bradykinin.

**Labetalol hydrochloride**  $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_3 \cdot \text{HCl}$  [Same as the namesake monograph]

**Labetalol hydrochloride for assay**  $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_3 \cdot \text{HCl}$  [Same as the monograph Labetalol Hydrochloride. However, when dried, it contains not less than 99.0% of labetalol hydrochloride ( $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_3 \cdot \text{HCl}$ ).]

**Lactic acid**  $\text{CH}_3\text{CH}(\text{OH})\text{COOH}$  [K 8726, Special class]

**Lactic acid TS** Dissolve 12.0 g of lactic acid in water to make 100 mL.

**$\alpha$ -Lactoalbumin** White powder. Derived from milk. Molecular weight of about 14,200.

**Lactobionic acid**  $\text{C}_{12}\text{H}_{22}\text{O}_{12}$  Colorless crystals or white crystalline powder.

*Melting point* <2.60>: 113 – 118°C

**Purity**—Dissolve 0.10 g of lactobionic acid in 10 mL of a mixture of methanol and water (3:2), and perform the test with 10  $\mu\text{L}$  of this solution as directed in the Identification (2) under Erythromycin Lactobionate: the spot other than the principal spot is not found.

**$\beta$ -Lactoglobulin** Prepare from milk. White to light yellow powder.

**Nitrogen content** <1.08>: not less than 14% (calculated on the dried basis).

**Lactose** See lactose monohydrate.

**$\alpha$ -Lactose and  $\beta$ -lactose mixture (1:1)** Use a mixture of lactose monohydrate and anhydrous lactose (3:5).

**Lactose broth** After adding lactose monohydrate to ordinary broth in the ratio of 0.5%, add about 12 mL of bromothymol blue-sodium hydroxide TS to 1000 mL of the medium. Then dispense portions of about 10 mL into tubes for fermentation, and sterilize fractionally on each of three successive days for 15 to 30 minutes at 100°C by using an autoclave, or sterilize by autoclaving for not more than 20 minutes at 121°C, and cool quickly by immersing in cold water.

**Lactose broth, three times concentrated** Add lactose monohydrate to ordinary broth prepared by using 330 mL in place of 1000 mL of water in the ratio of 1.5%, and prepare according to the method of preparation under lactose broth, with 25 mL portions in tubes for fermentation.

**Lactose broth, twice concentrated** Add lactose monohydrate to ordinary broth prepared by using 500 mL in place of 1000 mL of water in the ratio of 1.0% and prepare according to the method of preparation under lactose broth.

**Lactose monohydrate**  $\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{H}_2\text{O}$  [Same as the monograph Lactose].

**Lactose substrate TS** Dissolve 6.0 g of lactose monohydrate in disodium hydrogen phosphate-citric acid buffer solution (pH 4.5) to make 100 mL.

**Lactose substrate TS for  $\beta$ -galactosidase (penicillium)** Dissolve 6.0 g of lactose monohydrate in diluted disodium hydrogen phosphate-citric acid buffer solution (pH 4.5) (1 in 10) to make 100 mL.

**Lafutidine for assay**  $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}_4\text{S}$  [Same as the monograph Lafutidine. When dried, it contains not less than 99.5% of lafutidine ( $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}_4\text{S}$ ).]

**Lanoconazole**  $\text{C}_{14}\text{H}_{10}\text{ClN}_3\text{S}_2$  [Same as the namesake monograph]

**Lanthanum-alizarin complexone TS** To 1 mL of ammonia water (28) add 10 mL of water. To 4 mL of this solution add 4 mL of a solution of ammonium acetate (1 in 5) and 192 mg of alizarin complexone, and label this solution as alizarin complexone stock solution. Dissolve 41 g of sodium acetate trihydrate in 400 mL of water, and add 24 mL of acetic acid (100). To this solution add the total volume of the alizarin complexone stock solution, add 400 mL of acetone, and label this solution as alizarin complexone solution. To 10 mL of diluted hydrochloric acid (1 in 6) add 163 mg of lanthanum (III) oxide, heat to dissolve, and label this solution as lanthanum (III) oxide solution. To the alizarin complexone solution add the lanthanum (III) oxide solution, and mix. After cooling, adjust to pH 4.7 with acetic acid (100) or ammonia water (28), and add water to make 1000 mL. Prepare before use.

**Lanthanum chloride TS** To 58.65 g of lanthanum (III) oxide add 100 mL of hydrochloric acid, and boil. After cooling, add water to make 1000 mL.

**Lanthanum (III) oxide**  $\text{La}_2\text{O}_3$  White crystals.

**Loss on ignition** <2.43>: not more than 0.5% (1 g, 1000°C, 1 hour).

**Lauromacrogol** [Same as the namesake monograph]

**Lead acetate** See lead (II) acetate trihydrate.

**Lead acetate TS** See lead (II) acetate TS.

**Lead dioxide** See lead (IV) oxide.

**Lead monoxide** See lead (II) oxide.

**Lead nitrate** See lead (II) nitrate.

**Lead subacetate TS** Place the yellowish mixture, obtained by triturating 3 g of lead (II) acetate trihydrate and 1 g of lead (II) oxide with 0.5 mL of water, in a beaker, and heat on a water bath, covering with a watch glass, until it shows a homogeneous, white to reddish white color. Then add 9.5 mL of hot water in small portions, cover it again with a watch glass, and set it aside. Decant the supernatant liquid, and adjust the specific gravity to 1.23 to 1.24 (15°C) by adding water.

**Storage**—Preserve in tightly stoppered bottles.

**Lead subacetate TS, dilute** To 2 mL of lead subacetate TS add freshly boiled and cooled water to make 100 mL. Prepare before use.

**Lead (II) acetate trihydrate**  $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$  [K 8374, Special class]

**Lead (II) acetate TS** To 9.5 g of lead (II) acetate trihydrate add freshly boiled and cooled water to make 100 mL.

**Storage**—Preserve in tightly stoppered bottles (0.25 mol/L).

**Lead (II) nitrate**  $\text{Pb}(\text{NO}_3)_2$  [K 8563, Special class]

**Lead (II) oxide**  $\text{PbO}$  [K 8090, Special class]

**Lead (IV) oxide**  $\text{PbO}_2$  A dark brown to black-brown, powder or granules.

**Identification**—A supernatant liquid of a solution of lead (IV) oxide in dilute acetic acid (1 in 100) responds to Qualita-

tive Tests <1.09> (3) for lead salt.

**Lecithin** A pale yellow to yellow-brown, powder or grains, having a characteristic odor.

It is emulsified with water. Hygroscopic.

**L-Leucine**  $C_6H_{13}NO_2$  [Same as the namesake monograph]

**L-Leucine for assay**  $C_6H_{13}NO_2$  [Same as the monograph L-Leucine. When dried, it contains not less than 99.0% of L-leucine ( $C_6H_{13}NO_2$ ).]

**Levallorphan tartrate for assay**  $C_{19}H_{25}NO.C_4H_6O_6$  [Same as the monograph Levallorphan Tartrate. When dried, it contains not less than 99.0% of levallorphan tartrate ( $C_{19}H_{25}NO.C_4H_6O_6$ ).]

**Levofloxacin hydrate for assay**  $C_{18}H_{20}FN_3O_4.\frac{1}{2}H_2O$  [Same as the monograph Levofloxacin Hydrate]

**Levothyroxine sodium** See levothyroxine sodium hydrate.

**Levothyroxine sodium for thin-layer chromatography** See levothyroxine sodium hydrate for thin-layer chromatography.

**Levothyroxine sodium hydrate**  $C_{15}H_{10}I_4NNaO_4.xH_2O$  [Same as the namesake monograph]

**Levothyroxine sodium hydrate for thin-layer chromatography**  $C_{15}H_{10}I_4NNaO_4.xH_2O$  [Same as the monograph Levothyroxine Sodium Hydrate. Proceed the test as directed in the Purity (3) under Levothyroxine Sodium Hydrate: any spot other than the principal spot at the  $R_f$  value of about 0.26 does not appear.]

**Lidocaine for assay**  $C_{14}H_{22}N_2O$  [same as the monograph Lidocaine]

**(Z)-Ligustilide for thin-layer chromatography**  $C_{12}H_{14}O_2$  A clear, yellow-green liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and practically insoluble in water.

**Identification**—Determine the absorption spectrum of a solution in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 320 nm and 324 nm.

**Purity** Related substances—Dissolve 1 mg of (Z)-ligustilide for thin-layer chromatography in 10 mL of methanol. Proceed the test with 1  $\mu$ L of this solution as directed in the Identification (5) under Hochuekkito Extract: no spot other than the principle spot of around  $R_f$  value of 0.6 appears.

**(Z)-Ligustilide TS for thin-layer chromatography** Dissolve 1 mg of (Z)-ligustilide for thin-layer chromatography in 10 mL of methanol.

**Limonene**  $C_{10}H_{16}$  Clear and colorless liquid, having a characteristic aroma and a bitter taste.

**Refractive index** <2.45>  $n_D^{20}$ : 1.472 – 1.474

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.841 – 0.846

**Purity** Related substances—Dissolve 0.1 g of limonene in 25 mL of hexane and use this solution as the sample solution. Perform the test with 2  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method and calculate the amount of them by the area percentage method: the total amount of the peaks other than limonene is not more than 3.0%.

#### Operating conditions

Proceed the operating conditions in the Assay under Eucalyptus Oil except test for time span of measurement.

Time span of measurement: About 3 times as long as the retention time of limonene, beginning after the solvent peak. System suitability

Test for required detectability: To 1 mL of the sample solution add hexane to make 100 mL. Adjust so that the peak height of limonene obtained with 2  $\mu$ L of this solution is 40 to 60% of the full scale.

#### Limonin for thin-layer chromatography

$C_{26}H_{30}O_8$  White, crystals or crystalline powder. Slightly soluble in methanol and in ethyl acetate, and practically insoluble in water and in ethanol (99.5). Melting point: about 290°C.

**Identification** Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared-visible Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1759  $\text{cm}^{-1}$ , 1709  $\text{cm}^{-1}$ , 1166  $\text{cm}^{-1}$ , 798  $\text{cm}^{-1}$  and 601  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 1 mg of limonin for thin-layer chromatography in 1 mL of ethyl acetate, and perform the test with 1  $\mu$ L of this solution as directed in the Identification (2) under Oregedokuto Extract: no spot other than the principal spot at an  $R_f$  value of about 0.4 appears.

**Liothryronine sodium**  $C_{15}H_{11}I_3NNaO_4$  [Same as the namesake monograph]

#### Liothryronine sodium for thin-layer chromatography

$C_{15}H_{11}I_3NNaO_4$  [Same as the monograph Liothryronine Sodium. Proceed as directed for the Identification (1) under Liothryronine Sodium Tablets: any spot other than the principal spot at the  $R_f$  value of about 0.3 to 0.4 does not appear.]

**Liquid paraffin** See paraffin, liquid.

**Liquiritin for thin-layer chromatography**  $C_{21}H_{22}O_9$  White, crystals or crystalline powder. Sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 210°C (with decomposition).

**Identification**—Determine the absorption spectrum of a solution of liquiritin for thin-layer chromatography in diluted methanol (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 215 nm and 219 nm, and between 275 nm and 279 nm.

**Purity** Related substances—Dissolve 1.0 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and perform the test with 1  $\mu$ L of this solution as directed in the Identification (5) under Kakkonto Extract: no spot other than the principal spot with an  $R_f$  value of about 0.4 appears.

**Lisinopril** See lisinopril hydrate.

**Lisinopril for assay** See lisinopril hydrate for assay.

**Lisinopril hydrate**  $C_{21}H_{31}N_3O_5.2H_2O$  [Same as the namesake monograph]

**Lisinopril hydrate for assay**  $C_{21}H_{31}N_3O_5.2H_2O$  [Same as the monograph Lisinopril Hydrate. It contains not less than 99.5% of lisinopril ( $C_{21}H_{31}N_3O_5$ : 405.49), calculated on the anhydrous basis.]

**Lithium acetate dihydrate**  $CH_3COOLi.2H_2O$  Colorless crystals.

**Dilute acetic acid insoluble substances**—To 40.0 g of lithi-

um acetate dihydrate add 45 mL of water, heat in a water bath to dissolve, cool, then dissolve in dilute acetic acid, and filter by suction. Wash the filter with water, dry the filter at  $105 \pm 2^\circ\text{C}$  for 1 hour, and weigh the mass of the residue after cooling: not more than 0.0025%.

**Content:** not less than 97.0%. Assay—Weigh accurately 0.3 g of lithium acetate dihydrate, add exactly 50 mL of acetic acid (100) and exactly 5 mL of acetic anhydride, dissolve by heating in a water bath, and titrate <2.50> with 0.1 mol/L perchloric acid VS after cooling (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 10.20 \text{ mg of } \text{CH}_3\text{COOLi}\cdot 2\text{H}_2\text{O} \end{aligned}$$

**Lithium bromide** LiBr White, crystals or crystalline powder. It is hygroscopic.

- Purity** (1) Chloride <1.03>: not more than 0.1%.  
(2) Sulfate <1.14>: not more than 0.01%.

**Lithium chloride** LiCl White, crystals or masses.

**Identification**—Perform the test as directed under Flame Coloration Test <1.04> (1): a persistent red color appears.

**Lithium hydroxide monohydrate** LiOH·H<sub>2</sub>O White, crystals or crystalline powder, having a hygroscopicity.

**Lithium perchlorate** LiClO<sub>4</sub> White, crystals or crystalline powder.

**Content:** not less than 98%. Assay—Accurately weigh about 0.2 g of lithium perchlorate, dissolve in 30 mL of water. Transfer the solution to a chromatographic column, prepared by pouring about 25 mL of strongly acidic ion-exchange resin (H type) for column chromatography into a chromatographic tube about 11 mm in inside diameter and about 300 mm in height (after adding 200 mL of 1 mol/L hydrochloride TS and flowing at a flow rate of 3–4 mL per minute, wash the chromatographic column with water until the color of the rinse water changes to yellowish red when adding methyl orange TS to the eluate), and flow at a flow rate of 3–4 mL per minute. Then, wash the column with about 30 mL of water at a flow rate of 3–4 mL per minute 5 times. Combine the rinse water and the eluate, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue TS). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 10.64 \text{ mg of LiClO}_4 \end{aligned}$$

**Lithium sulfate** See lithium sulfate monohydrate.

**Lithium sulfate monohydrate** Li<sub>2</sub>SO<sub>4</sub>·H<sub>2</sub>O [K 8994, Special class]

#### Lithocholic acid for thin-layer chromatography

C<sub>24</sub>H<sub>40</sub>O<sub>3</sub> White, crystals or crystalline powder. Soluble in ethanol (95), in acetic acid (100) and in acetone, slightly soluble in chloroform, and practically insoluble in water. Melting point: about 186°C.

**Purity** Related substances—Dissolve 25 mg of lithocholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 25 mL. Dilute 1.0 mL of this solution with a mixture of chloroform and ethanol (95) (9:1) to make exactly 100 mL. Perform the test with 10 μL of this solution as directed in the Purity (4) under Ursodeoxycholic Acid: any spot other than the principal spot with the R<sub>f</sub> value of about 0.7 does not appear.

**Content:** 98.0%. Assay—Weigh accurately about 0.5 g

of lithocholic acid for thin-layer chromatography, previously dried at 80°C for 4 hours under reduced pressure (phosphorus (V) oxide), dissolve in 40 mL of neutralized ethanol and 20 mL of water. Add 2 drops of phenolphthalein TS, titrate <2.50> with 0.1 mol/L sodium hydroxide VS, add 100 mL of freshly boiled and cooled water near the end point, and continue the titration.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 37.66 \text{ mg of C}_{24}\text{H}_{40}\text{O}_3 \end{aligned}$$

#### Locke-Ringer's TS

Sodium chloride	9.0 g
Potassium chloride	0.42 g
Calcium chloride dihydrate	0.24 g
Magnesium chloride hexahydrate	0.2 g
Sodium hydrogen carbonate	0.5 g
Dextrose	0.5 g
Water, freshly distilled with a hard-glass apparatus	a sufficient quantity
To make	1000 mL

Prepare before use. The constituents except dextrose and sodium hydrogen carbonate can be made up in concentrated stock solutions, stored in a dark place, and diluted before use.

**Loganin for assay** C<sub>17</sub>H<sub>26</sub>O<sub>10</sub> Loganin for thin-layer chromatography. It meets the requirement of the following 1) Loganin for assay 1 or 2) Loganin for assay 2 (Purity value by qNMR). The former is used after drying in a desiccator (silica gel) for 24 hours, and the latter is used with correction for its amount based on the result obtained in the Assay.

#### 1) Loganin for assay 1

**Absorbance** <2.24> E<sub>1cm</sub><sup>1%</sup> (235 nm): 275–303 [5 mg after drying in a desiccator (silica gel) for 24 hours, methanol, 500 mL]

**Purity** Related substances—Dissolve 2 mg of loganin for assay 1 in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than loganin obtained from the sample solution is not larger than the peak area of loganin from the standard solution.

#### Operating conditions

**Detector, column, column temperature, mobile phase and flow rate:** Proceed as directed in the operating conditions in the Assay (1) under Goshajinkigan Extract.

**Time span of measurement:** About 3 times as long as the retention time of loganin.

#### System suitability

**System performance and system repeatability:** Proceed as directed in the system suitability in the Assay (1) under Goshajinkigan Extract.

**Test for required detectability:** Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of loganin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

#### 2) Loganin for assay 2 (Purity value by qNMR)

**Unity of peak**—Dissolve 2 mg of loganin for assay 2 in 5 mL of the mobile phase, and use this solution as the sample

solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of loganin peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

#### Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Goshajinkigan Extract.

Detector: A photodiode array detector (wavelength: 238 nm, spectrum range of measurement: 220 – 400 nm).

#### System suitability

System performance: Proceed as directed in the system suitability in the Assay (1) under Goshajinkigan Extract.

**Assay**—Weigh accurately 5 mg of loganin for assay 2 and 1 mg of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity *A* (equivalent to 1 hydrogen) of the signal around  $\delta$  7.14 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\text{Amount (\%)} \text{ of loganin (C}_{17}\text{H}_{26}\text{O}_{10}) \\ = M_s \times I \times P/(M \times N) \times 1.7235$$

*M*: Amount (mg) of loganin for assay 2 taken

*M<sub>s</sub>*: Amount (mg) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

*I*: Signal resonance intensity *A* based on the signal resonance intensity of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

*N*: Number of the hydrogen derived from *A*

*P*: Purity (%) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

<sup>13</sup>C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around  $\delta$  5.02 ppm and  $\delta$  7.14 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals around  $\delta$  5.02 ppm and  $\delta$  7.14 ppm are not

overlapped with any signal of obvious foreign substance. Furthermore, when determined the resonance intensities *A*<sub>1</sub> and *A*, both equivalent to 1 hydrogen, of each signal around  $\delta$  5.02 ppm and  $\delta$  7.14 ppm, the ratio of them, *A*<sub>1</sub>/*A*, is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity *A* to that of the reference standard for qNMR is not more than 1.0%.

**Loganin for component determination** See loganin for assay.

#### Loganin for thin-layer chromatography C<sub>17</sub>H<sub>26</sub>O<sub>10</sub>

White, crystals or crystalline powder. Soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (99.5). Melting point: 221 – 227°C.

**Purity** Related substances—Dissolve 1.0 mg of loganin for thin-layer chromatography in 2 mL of methanol. Perform the test with 10  $\mu$ L of this solution as directed in the Identification under Cornus Fruit: any spot other than the principal spot at the *Rf* value of about 0.4 does not appear.

**Losartan potassium** C<sub>22</sub>H<sub>22</sub>ClKN<sub>6</sub>O [Same as the namesake monograph]

**Lovastatin** C<sub>24</sub>H<sub>36</sub>O<sub>5</sub> White, crystals or crystalline powder. Soluble in acetonitrile and in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

*Optical rotation* <2.49> [ $\alpha$ ]<sub>D</sub><sup>20</sup>: +325 – +340° (50 mg calculated on the dried basis, acetonitrile, 10 mL, 100 mm).

*Loss on drying* <2.41>: Not more than 1.0% (1 g, under reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

#### Low-molecular mass heparin for calculation of molecular mass

It is a low-molecular mass heparin with a disaccharide unit prepared, and display the molecular mass distribution between 600 and more than 10,000. When the average of molecular mass of Low-molecular mass heparin international standard is determined as a reference with this, the difference compared as a reference with the Low-molecular mass heparin international standard is not less than 5%.

#### Luteolin for thin-layer chromatography C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>

Light yellow to yellow-brown crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 310°C (with decomposition).

**Purity** Related substances—Dissolve 1.0 mg of luteolin for thin-layer chromatography in 1 mL of methanol. Proceed the test with 10  $\mu$ L of this solution as directed in the Identification under Chrysanthemum Flower: any spot other than the principal spot at an *Rf* value of about 0.7 does not appear.

**Lysate reagent** A lyophilized product obtained from amebocyte lysate of horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). Amebocyte lysate preparations which do not react to  $\beta$ -glucans are available: they are prepared by removing the G factor reacting to  $\beta$ -glucans from amebocyte lysate or by inhibiting the G factor reacting system of amebocyte lysate.

**Lysate TS** Dissolve a lysate reagent in water for bacterial endotoxins test, or in a suitable buffer, by gentle stirring.

**Lysil endopeptidase** White, powder or masses. An exotoxin produced by *Achromobacter*. Molecular weight:

27,500.

**L-Lysine hydrochloride** C<sub>6</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>·HCl [Same as the namesake monograph]

**Lysyl endopeptidase** A protease obtained from *Lysobacter enzymogenes*. It contains about 150 units per mg, where 1 unit is an enzyme amount which hydrolyzes 1 μmol of tosyl-glycyl-prolyl-lysine-4-nitroanilide acetate per minute at pH 7.7 and 25°C.

**Macrogol 600** HOCH<sub>2</sub>(CH<sub>2</sub>OCH<sub>2</sub>)<sub>n</sub>CH<sub>2</sub>OH, n = 11 – 13 Clear, colorless, viscous liquid or a white, petrolatum-like solid, having a faint, characteristic odor. Very soluble in water, in ethanol (95), in acetone and in macrogol 400, soluble in diethyl ether, and practically insoluble in petroleum benzene. Congealing point: 18 – 23°C.

*Average molecular mass:* When perform the test as directed in the Average molecular mass test under Macrogol 400, it is between 570 and 630.

**Magnesia TS** Dissolve 5.5 g of magnesium chloride hexahydrate and 7 g of ammonium chloride in 65 mL of water, add 35 mL of ammonia TS, allow the mixture to stand for a few days in tightly stoppered bottles, and filter. If the solution is not clear, filter before use.

**Magnesium** Mg [K 8875, Special class]

**Magnesium chloride** See magnesium chloride hexahydrate.

**Magnesium chloride hexahydrate** MgCl<sub>2</sub>·6H<sub>2</sub>O [K 8159, Special class]

**Magnesium nitrate** See magnesium nitrate hexahydrate.

**Magnesium nitrate hexahydrate** Mg(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O [K 8567, Special class]

**Magnesium oxide** MgO [K 8432, Special class]

**Magnesium powder** Mg [K 8876, Special class]

**Magnesium sulfate** See magnesium sulfate heptahydrate.

**Magnesium sulfate heptahydrate** MgSO<sub>4</sub>·7H<sub>2</sub>O [K 8995, Special class]

**Magnesium sulfate TS** Dissolve 12 g of magnesium sulfate heptahydrate in water to make 100 mL (0.5 mol/L).

**Magnoflorine iodide for assay** C<sub>20</sub>H<sub>24</sub>INO<sub>4</sub> White to light yellowish white, crystals or crystalline powder. Slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5). Melting point: about 250°C (with decomposition).

It is used after correcting with the amount of magnoflorine iodide obtained in the Assay.

**Identification** (1) Determine the absorption spectrum of a solution of magnoflorine iodide for assay in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 221 nm and 225 nm.

(2) Determine the infrared absorption spectrum of magnoflorine iodide for assay as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3170 cm<sup>-1</sup>, 3000 cm<sup>-1</sup>, 2840 cm<sup>-1</sup>, 1459 cm<sup>-1</sup>, 1231 cm<sup>-1</sup>, 1122 cm<sup>-1</sup> and 833 cm<sup>-1</sup>.

**Absorbance** <2.24> E<sub>1cm</sub><sup>1%</sup> (223 nm): 1066 – 1132 (5 mg, methanol, 1000 mL).

**Purity** Related substances—Dissolve 5 mg of magnoflo-

rine iodide for assay in 2 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and formic acid (5:3:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: the spot other than the principal spot at the R<sub>f</sub> value of about 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

**Unity of peak:** Dissolve 5 mg of magnoflorine iodide for assay in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of magnoflorine peak and around the two middle peak heights of before and after the top: no difference is observed in the shape between their spectra.

#### Operating conditions

**Column, column temperature, and mobile phase:** Proceed as directed in the operating conditions in the Assay (4) under Kakkontokasenkyushin'i Extract.

**Detector:** A photodiode array detector (wavelength: 303 nm; spectrum range of measurement: 220 – 400 nm).

**Flow rate:** Adjust so that the retention time of magnoflorine is about 20 minutes.

#### System suitability

**System performance:** To 1 mL of the sample solution add a mixture of water and methanol (1:1) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of magnoflorine are not less than 5000 and not more than 1.5, respectively.

**System repeatability:** To 1 mL of the sample solution add a mixture of water and methanol (1:1) to make 100 mL. When the test is repeated 6 times with 10 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of magnoflorine is not more than 1.5%.

**Assay**—Weigh accurately 5 mg of magnoflorine iodide for assay and 1 mg of DSS-d<sub>6</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure <sup>1</sup>H-NMR spectrum as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using DSS-d<sub>6</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the signal integrated intensity A (equivalent to 3 hydrogen) around δ 6.94 – δ 7.05 ppm [the integrated intensities A<sub>1</sub> (equivalent to 2 hydrogen) and A<sub>2</sub> (equivalent to 1 hydrogen) of the signals around δ 6.96 ppm and δ 7.04 ppm], assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%)} \text{ of magnoflorine iodide (C}_{20}\text{H}_{24}\text{INO}_4\text{)} \\ = M_s \times I \times P/(M \times N) \times 2.0918$$

**M:** Amount (mg) of magnoflorine iodide for assay taken

**M<sub>s</sub>:** Amount (mg) of DSS-d<sub>6</sub> for nuclear magnetic resonance spectroscopy taken

*I*: The signal integrated intensity  $A$  based on the signal integrated intensity of DSS- $d_6$  for nuclear magnetic resonance spectroscopy as 9.000

*N*: Number of hydrogen derived from  $A$

*P*: Purity (%) of DSS- $d_6$  for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectroscopy apparatus having  $^1\text{H}$  resonance frequency of not less than 400 MHz.

Target nucleus:  $^1\text{H}$ .

Digital resolution: 0.25 Hz or lower.

Measuring spectrum width: 20 ppm or upper, including between  $-5$  ppm and  $15$  ppm.

Spinning: off.

Pulse angle:  $90^\circ$ .

$^{13}\text{C}$  decoupling: on.

Delay time: Repeating pulse waiting time 60 seconds or longer.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature of  $20 - 30^\circ\text{C}$ .

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around  $\delta 6.94 - \delta 7.05$  ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals of around  $\delta 6.96 - \delta 7.04$  ppm are not overlapped with any signal of obvious foreign substance, and the ratio of the integrated intensity of each signal ( $A_1/2)/A_2$ ) is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the integrated intensity  $A$  to that of the reference standard for qNMR is not more than 1.0%.

**Magnolia flower** [Same as the namesake monograph]

**Magnolol for assay**  $\text{C}_{18}\text{H}_{18}\text{O}_2$  Use magnolol for thin-layer chromatography meeting the following additional specifications. Correct the content based on the amount (%) obtained in the Assay.

**Unity of peak**—Dissolve 5 mg of magnolol for assay in 10 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Perform the test with  $10\ \mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of magnolol peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

#### Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Magnolia Bark.

Detector: A photodiode array detector (wavelength: 289 nm, spectrum range of measurement: 220 – 400 nm).

#### System suitability

System performance: Proceed as directed in the system suitability in the Assay under Magnolia Bark.

**Assay**—Weigh accurately 5 mg of magnolol for assay and 1 mg of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spec-

troscopy using an ultramicrobalance, dissolve in 1 mL of deuterated chloroform for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure  $^1\text{H-NMR}$  as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities,  $A_1$  (equivalent to 2 hydrogen) and  $A_2$  (equivalent to 2 hydrogen), of the signals around  $\delta 6.70$  ppm and  $\delta 6.81$  ppm assuming the signal of the reference standard for qNMR as  $\delta 0$  ppm.

$$\begin{aligned} \text{Amount (\%)} \text{ of magnolol} (\text{C}_{18}\text{H}_{18}\text{O}_2) \\ = M_s \times I \times P / (M \times N) \times 1.1758 \end{aligned}$$

*M*: Amount (mg) of magnolol for assay taken

*M<sub>s</sub>*: Amount (mg) of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy taken

*I*: Sum of the signal resonance intensities,  $A_1$  and  $A_2$ , based on the signal resonance intensity of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy as 18.000

*N*: Sum of numbers of the hydrogen derived from  $A_1$  and  $A_2$

*P*: Purity (%) of 1,4-BTMSB- $d_4$  for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having  $^1\text{H}$  resonance frequency of not less than 400 MHz.

Target nucleus:  $^1\text{H}$ .

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between  $-5$  ppm and  $15$  ppm.

Spinning: off.

Pulse angle:  $90^\circ$ .

$^{13}\text{C}$  decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between  $20^\circ\text{C}$  and  $30^\circ\text{C}$ .

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the two signals of around  $\delta 6.70$  ppm and  $\delta 6.81$  ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals of around  $\delta 6.70$  ppm and  $\delta 6.81$  ppm are not overlapped with any signal of obvious foreign substance, and the ratios of the resonance intensities,  $A_1/A_2$ , of each signal around  $\delta 6.70$  ppm and  $\delta 6.81$  ppm are between 0.99 and 1.01, respectively.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity,  $A_1$  or  $A_2$ , to that of the reference standard for qNMR is not more than 1.0%.

**Magnolol for component determination** See magnolol for assay.

**Magnolol for thin-layer chromatography**  $\text{C}_{18}\text{H}_{18}\text{O}_2$  Odorless, white, crystals or crystalline powder. Freely solu-

ble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 102°C.

**Identification**—Determine the absorption spectrum of a solution of magnolol for thin-layer chromatography in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 287 nm and 291 nm.

**Purity** Related substances—Dissolve 1.0 mg of magnolol for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, acetone and acetic acid (100) (20:15:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot of around  $R_f$  value of 0.5 does not appear.

**Malachite green** See malachite green oxalate.

**Malachite green oxalate**  $C_{52}H_{54}N_4O_{12}$  [K 8878, Malachite green (oxalate), Special class]

**Maleic acid**  $C_4H_4O_4$  A white crystalline powder.

**Identification**—Determine the infrared absorption spectrum of maleic acid as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1706  $cm^{-1}$ , 1637  $cm^{-1}$ , 1587  $cm^{-1}$ , 1567  $cm^{-1}$ , 1436  $cm^{-1}$ , 1263  $cm^{-1}$ , 876  $cm^{-1}$  and 786  $cm^{-1}$ .

**4-(*N*-Maleimidomethyl)cyclohexane-1-carboxylic acid *N*-succinimidyl ester**  $C_{16}H_{18}N_2O_2$  Colorless crystals, which is decomposed by acid or alkali.

**Maltitol**  $C_{12}H_{24}O_{11}$  A white crystalline powder. Very soluble in water, and practically insoluble in ethanol (99.5).

**Maltose** See maltose monohydrate.

**Maltose monohydrate**  $C_{12}H_{22}O_{11} \cdot H_2O$  [Same as the namesake monograph].

**Maltotriose**  $C_{18}H_{32}O_{16}$  A white powder.

**Identification**—Determine the infrared absorption spectrum of maltotriose as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3420  $cm^{-1}$ , 1420  $cm^{-1}$ , 1153  $cm^{-1}$  and 1024  $cm^{-1}$ .

**Manganese dioxide**  $MnO_2$  Black to black-brown, masses or powder.

**Identification**—To 0.5 g of manganese dioxide add 20 mL of water and 3 mL of hydrochloric acid, and 3 mL of hydrogen peroxide (30). Alkalinize the solution with ammonia solution (28) while cooling, and add 25 mL of hydrogen sulfide TS; pale red precipitates appear.

**Manganese (II) nitrate hexahydrate**  $Mn(NO_3)_2 \cdot 6H_2O$  [K 8568, Special class]

**Mangiferin for assay**  $C_{19}H_{18}O_{11}$  Yellow crystals or crystalline powder. It is practically insoluble in water or ethanol (99.5). It is used after correcting with the amount (%) of mangiferin obtained in the Assay.

**Identification**—Measure  $^1H$ -NMR as directed in the Assay: When the signal of the reference standard for qNMR as  $\delta$  0 ppm, it exhibits a multiple signal equivalent to one proton around  $\delta$  3.15 ppm, a multiple signal equivalent to one proton around  $\delta$  3.19 ppm, a multiple signal equivalent to one proton around  $\delta$  3.22 ppm, a multiple signal equiv-

alent to one proton around  $\delta$  3.43 ppm, a doublet-like signal equivalent to one proton around  $\delta$  3.71 ppm, a triplet-like signal equivalent to one proton around  $\delta$  4.07 ppm, a doublet-like signal equivalent to one proton around  $\delta$  4.61 ppm, a single signal equivalent to one proton around  $\delta$  6.40 ppm, a single signal equivalent to one proton around  $\delta$  6.89 ppm, and a single signal equivalent to one proton around  $\delta$  7.40 ppm.

**Unity of peak**—Dissolve 1 mg of mangiferin for assay in 20 mL of diluted methanol (1 in 2) and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of mangiferin peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

#### Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Byakkokaninjinto Extract.

Detector: A photodiode array detector (wavelength: 367 nm, spectrum range of measurement: 220 – 400 nm).

#### System suitability

System performance: When the procedure is run with 10  $\mu$ L of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mangiferin are not less than 5000 and not more than 1.5, respectively.

**Assay**—Weigh accurately 5 mg of mangiferin for assay and 1 mg of DSS- $d_6$  for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure  $^1H$ -NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using DSS- $d_6$  for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities,  $A_1$  (equivalent to 1 hydeogen),  $A_2$  (equivalent to 1 hydeogen), and  $A_3$  (equivalent to 1 hydeogen), of the signals around  $\delta$  6.40 ppm,  $\delta$  6.89 ppm, and  $\delta$  7.40 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\begin{aligned} \text{Amount (\%)} \text{ of mangiferin } (C_{19}H_{18}O_{11}) \\ = M_S \times I \times P / (M \times N) \times 1.8824 \end{aligned}$$

$M$ : Amount (mg) of mangiferin for assay taken

$M_S$ : Amount (mg) of DSS- $d_6$  for nuclear magnetic resonance spectroscopy taken

$I$ : Sum of the signal resonance intensities,  $A_1$ ,  $A_2$  and  $A_3$ , based on the signal resonance intensity of DSS- $d_6$  for nuclear magnetic resonance spectroscopy as 9.000

$N$ : Sum of numbers of the hydeogen derived from  $A_1$ ,  $A_2$  and  $A_3$

$P$ : Purity (%) of DSS- $d_6$  for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having  $^1H$  resonance frequency of not less than 400 MHz.

Target nucleus:  $^1H$ .

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

$^{13}\text{C}$  decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around  $\delta$  6.40 ppm,  $\delta$  6.89 ppm, and  $\delta$  7.40 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the three signals around  $\delta$  6.40 ppm,  $\delta$  6.89 ppm, and  $\delta$  7.40 ppm are not overlapped with any signal of obvious foreign substance, and the ratios of the resonance intensities,  $A_1/A_2$ ,  $A_1/A_3$ , and  $A_2/A_3$ , of each signal around  $\delta$  6.40 ppm,  $\delta$  6.89 ppm, and  $\delta$  7.40 ppm are between 0.99 and 1.01, respectively.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity,  $A_1$  or  $A_2$  or  $A_3$ , to that of the reference standard for qNMR is not more than 1.0%.

**Manninotriose for thin-layer chromatography**  $\text{C}_{18}\text{H}_{32}\text{O}_{16}$  A white powder. Very soluble in water, and practically insoluble in ethanol (99.5). It is hygroscopic. It is deliquescent with the atmospheric moisture.

*Optical rotation* <2.49>  $[\alpha]_{\text{D}}^{20}$ : +159 – +170° (50 mg calculated on the anhydrous basis, diluted ammonia solution (28) (1 in 1000), 5 mL, 100 mm).

**Purity** Related substances—Dissolve 3 mg of manninotriose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer chromatography <2.03>. Spot 2  $\mu\text{L}$  of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS to the plate, and heat the plate at 105°C for 10 minutes: a spot other than the principle spot with an *R<sub>f</sub>* value of about 0.4 is not observed.

**D-Mannitol**  $\text{C}_6\text{H}_{14}\text{O}_6$  [Same as the monograph D-Mannitol]

**D-Mannosamine hydrochloride**  $\text{C}_6\text{H}_{13}\text{NO}_5\cdot\text{HCl}$  White powder. Melting point: about 168°C (with decomposition).

*Optical rotation* <2.49>  $[\alpha]_{\text{D}}^{20}$ : -4.2 – -3.2° (0.4 g, water, 20 mL, 100 mm).

**D-Mannose**  $\text{C}_6\text{H}_{12}\text{O}_6$  White, crystal or crystalline powder. It is very soluble in water. Melting point: about 132°C (with decomposition).

*Optical rotation* <2.49>  $[\alpha]_{\text{D}}^{20}$ : +13.7 – +14.7° (4 g, diluted ammonia TS (1 in 200), 20 mL, 100 mm).

**Marker protein for celmoleukin molecular mass determination** Add 10  $\mu\text{L}$  of cytochrome C prepared to a concentration of 2 mg per mL to 10  $\mu\text{L}$  of a commercially available marker protein with a known molecular weight (6 ingredients: phosphorylase b, bovine serum albumin, ovalbumin, carbonic dehydratase, soy trypsin inhibitor, and lysozyme) and then dilute 10-fold with buffer solution for celmoleukin.

**Meat extract** Concentrated extract of fresh meat of bovine, equine or other animals. A yellow-brown to dark brown, paste-like mass, having a meat-like odor.

**Mebendazole**  $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_3$  White powder. Practically insoluble in water and in ethanol (95).

**Medium for float culture** Dissolve 6.000 g of sodium chloride, 0.400 g of potassium chloride, 0.677 g of anhydrous sodium dihydrogen phosphate, 0.100 g of calcium nitrite tetrahydrate, 0.100 g of magnesium sulfate heptahydrate, 2.000 g of glucose, 0.164 g of sodium succinate hexahydrate, 46 mg of succinic acid, 0.240 g of L-arginine hydrochloride, 56.8 mg of L-asparagine monohydrate, 20 mg of L-aspartic acid, 72.9 mg of L-cysteine hydrochloride monohydrate, 20 mg of L-glutamic acid, 1 mg of glutathione, 10 mg of glycine, 20.3 mg of L-histidine hydrochloride monohydrate, 20 mg of L-hydroxyproline, 50 mg of L-isoleucine, 40 mg of L-lysine hydrochloride, 15 mg of methionine, 20 mg of L-threonine, 5 mg of L-tryptophan, 20 mg of L-valine, 50 mg of L-leucine, 15 mg of L-phenylalanine, 20 mg of L-proline, 30 mg of L-serine, 20 mg of L-tyrosine, 0.2 mg of D-biotin (crystals), 0.25 mg of calcium pantothenate, 3 mg of choline chloride, 35 mg of *i*-inositol, 1 mg of 4-aminobenzoic acid, 5  $\mu\text{g}$  of cyanocobalamin, 1 mg of folic acid, 1 mg of nicotinamide, 0.2 mg of riboflavin, 1 mg of thiamine hydrochloride, 1 mg of pyridoxine hydrochloride, and 5 mg of phenol red in a suitable amount of water, add 1 mL of kanamycin sulfate solution (3 in 50), add water to make 1000 mL, and then sterilize by autoclaving at 121°C for 15 minutes. After cooling, add 10 mL of L-glutamine solution (3 in 100) and 20 mL of 7% sodium bicarbonate injection, and then mix. Store at 4°C.

**Mefloquin hydrochloride**  $\text{C}_{17}\text{H}_{16}\text{F}_6\text{N}_2\text{O}\cdot\text{HCl}$  [Same as the namesake monograph]

**Mefruside for assay**  $\text{C}_{13}\text{H}_{19}\text{ClN}_2\text{O}_5\text{S}_2$  [Same as the monograph Mefruside. When dried, it contains not less than 99.0% of mefruside ( $\text{C}_{13}\text{H}_{19}\text{ClN}_2\text{O}_5\text{S}_2$ ).]

**Meglumine**  $\text{C}_7\text{H}_{17}\text{NO}_5$  [same as the namesake monograph]

**Mentha herb** [Same as the namesake monograph]

**Mentha oil** [Same as the namesake monograph]

**Menthol**  $\text{C}_{10}\text{H}_{20}\text{O}$  [Same as the monograph *dl*-Menthol or *l*-Menthol]

***l*-Menthol for assay**  $\text{C}_{10}\text{H}_{20}\text{O}$  [Same as the monograph *l*-Menthol. It contains not less than 99.0% of *l*-menthol ( $\text{C}_{10}\text{H}_{20}\text{O}$ ) and meets the following additional specifications.]

*Optical rotation* <2.49>  $[\alpha]_{\text{D}}^{20}$ : -48.0 – -51.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

**Purity** Related substances—Dissolve 0.10 g of *l*-menthol for assay in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 5  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than *l*-menthol obtained from the sample solution is not larger than the peak area of *l*-menthol from the standard solution (1).

**Operating conditions**

Proceed the operating conditions in the Assay under Mentha Oil except time span of measurement.

Time span of measurement: About twice as long as the retention time of *l*-menthol, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution (1), add dichloromethane to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of *l*-menthol obtained with 5  $\mu$ L of the standard solution (2) can be measured and the peak height of *l*-menthol with 5  $\mu$ L of the standard solution (1) is about 20% of the full scale.

#### Mepivacaine hydrochloride for assay C<sub>15</sub>H<sub>22</sub>N<sub>2</sub>O.HCl

[Same as the monograph Mepivacaine Hydrochloride. When dried, it contains not less than 99.0% of mepivacaine hydrochloride (C<sub>15</sub>H<sub>22</sub>N<sub>2</sub>O.HCl).]

**Mequitazine for assay** C<sub>20</sub>H<sub>22</sub>N<sub>2</sub>S [Same as the monograph Mequitazine. When dried, it contains not less than 99.5% of mequitazine (C<sub>20</sub>H<sub>22</sub>N<sub>2</sub>S).]

**Mercapto acetic acid** HSCH<sub>2</sub>COOH [K 8630, Special class] Place in an ampule, and preserve in a dark, cold place. Do not use after storing for a long period.

**Mercaptoethanesulfonic acid** C<sub>2</sub>H<sub>6</sub>O<sub>3</sub>S<sub>2</sub> Prepared for amino acid analysis or biochemistry.

**2-Mercaptoethanol** HSCH<sub>2</sub>CH<sub>2</sub>OH Clear and colorless liquid.

Specific gravity <2.56> d<sub>4</sub><sup>20</sup>: 1.112 – 1.117

Content: not less than 97.0%. Assay—Perform the test with 0.6  $\mu$ L of the substance to be examined as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas of each component by the automatic integration method.

Content (%) = (the peak area of 2-mercaptopropanoic acid/the total of the peak areas of each component)  $\times$  100

#### Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (177–250  $\mu$ m in particle diameter) coated in 20% with 50% phenyl-methyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Carrier gas: Helium.

Flow rate: about 50 mL per minute (the retention time of 2-mercaptopropanoic acid is 3 to 4 minutes.)

Time span of measurement: About 7 times as long as the retention time of 2-mercaptopropanoic acid.

**2-Mercaptoethanol for epoetin beta** HSCH<sub>2</sub>CH<sub>2</sub>OH Prepared for study of sulfoprotein.

**Mercaptopurine** See mercaptopurine hydrate.

**Mercaptopurine hydrate** C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>S.H<sub>2</sub>O [Same as the namesake monograph]

**Mercuric acetate** See mercury (II) acetate.

**Mercuric acetate TS for nonaqueous titration** See mercury (II) acetate TS for nonaqueous titration.

**Mercuric chloride** See mercury (II) chloride.

**Mercury** Hg [K 8572, Special class]

**Mercury (II) acetate** Hg(CH<sub>3</sub>COO)<sub>2</sub> White, crystals or crystalline powder.

**Identification**—(1) Dissolve 1 g of mercury (II) chloride

in 1 mL of diluted nitric acid (1 in 7), add 20 mL of water, and use this solution as the sample solution. To 10 mL of the sample solution add 0.8 mL of iron (III) chloride TS: a red-brown color is developed.

(2) To 10 mL of the sample solution obtained in (1) add 2 mL of potassium iodate TS: a red precipitate is produced.

**Storage**—Preserve in a light-resistant tight container.

**Mercury (II) acetate TS for nonaqueous titration** Dissolve 5 g of mercury (II) acetate in acetic acid (100) for nonaqueous titration to make 100 mL.

**Mercury (II) chloride** HgCl<sub>2</sub> [K 8139, Special class]

**Mesaconitine for purity** C<sub>33</sub>H<sub>45</sub>NO<sub>11</sub> White, crystals or crystalline powder. Slightly soluble in acetonitrile and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water. Melting point: about 190°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of mesaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3510 cm<sup>-1</sup>, 1713 cm<sup>-1</sup>, 1277 cm<sup>-1</sup>, 1116 cm<sup>-1</sup>, 1098 cm<sup>-1</sup> and 717 cm<sup>-1</sup>.

**Absorbance** <2.24> E<sub>1</sub><sup>cm</sup> (230 nm): 211 – 247 (5 mg, ethanol (99.5), 200 mL).

**Purity** Related substances—(1) Dissolve 5.0 mg of mesaconitine for purity in 2 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification under Processed Aconite Root: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

(2) Dissolve 5.0 mg of mesaconitine for purity in 5 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than mesaconitine obtained from the sample solution is not larger than the peak area of mesaconitine from the standard solution.

#### Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity (3) under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust so that the retention time of mesaconitine is about 19 minutes.

Time span of measurement: About 3 times as long as the retention time of mesaconitine, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of mesaconitine with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

**System performance**: Dissolve 1 mg each of mesaconitine

for purity, aconitine for purity and hypaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mesaconitine is not more than 1.5%.

**Water** <2.48>: not more than 1.0% (5 mg, coulometric titration).

**Mesalazine for assay**  $C_7H_7NO_3$  [Same as the monograph Mesalazine. When dried, it contains not less than 99.0% of mesalazine ( $C_7H_7NO_3$ ).]

**Mesityl oxide**  $CH_3COCH = C(CH_3)_2$  A colorless or pale yellow, clear liquid, having a characteristic odor.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.850 – 0.860

**Metacresol purple**  $C_{21}H_{18}O_5S$  [K 8889, Special class]

**Metacresol purple TS** Dissolve 0.10 g of metacresol purple in 13 mL of 0.01 mol/L sodium hydroxide TS, and add water to make 100 mL. Filter if necessary.

**Metacycline hydrochloride**  $C_{22}H_{22}N_2O_8.HCl$  Yellow to dark yellow, crystals or crystalline powder.

**Purity** Related substances—Dissolve 20 mg of metacycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20  $\mu$ L of the sample solution as directed in the Purity (2) under Doxycycline Hydrochloride Hydrate, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total area of peaks other than metacycline is not more than 10%.

**Metallic sodium** See sodium.

**Metanil yellow**  $C_{18}H_{14}N_3NaO_3S$  Yellow-brown powder. Sparingly soluble in water, and very slightly soluble in ethanol (95) and in *N,N*-dimethylformamide.

**Metanil yellow TS** Dissolve 0.1 g of metanil yellow in 200 mL of *N,N*-dimethylformamide.

**Metaphosphoric acid**  $HPO_3$  A colorless, deliquescent, stick or masses.

**Identification**—(1) Dissolve 1 g of metaphosphoric acid in 50 mL of water, and use this solution as the sample solution. To 10 mL of the sample solution add 0.2 mL of ammonia TS and 1 mL of silver nitrate TS: a yellowish white precipitate is produced.

(2) To 10 mL of the sample solution obtained in (1) add 10 mL of albumin TS: a white precipitate is produced.

**Metaphosphoric acid-acetic acid TS** Dissolve 15 g of metaphosphoric acid and 40 mL of acetic acid (100) in water to make 500 mL. Preserve in a cold place, and use within 2 days.

**Metenolone enanthate**  $C_{27}H_{42}O_3$  [Same as the namesake monograph]

**Metenolone enanthate for assay**  $C_{27}H_{42}O_3$  To 1 g of metenolone enanthate add 30 mL of water, and add slowly 70 mL of methanol with warming to dissolve. Filter while hot, and allow the filtrate to stand on a water bath for 30 minutes. Allow to stand overnight in a cold place, collect the crystals thus formed, and wash with a small amount of

diluted methanol (1 in 3). Recrystallize in the same manner, and dry the crystals in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours. It is white, odorless crystals.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (242 nm): 321 – 328 (1 mg, methanol, 100 mL).

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : +40 – +42° (0.2 g, chloroform, 10 mL, 100 mm).

**Melting point** <2.60>: 69 – 72°C

**Purity** Related substances—Dissolve 50 mg of metenolone enanthate for assay in chloroform to make exactly 10 mL, and perform the test with 10  $\mu$ L of this solution as directed in the Purity (3) under Metenolone Enanthate: any spot other than the principal spot does not appear.

**Metformin hydrochloride for assay**  $C_4H_{11}N_5.HCl$

[Same as the monograph Metformin Hydrochloride. When dried, it contains not less than 99.0% of metformin hydrochloride ( $C_4H_{11}N_5.HCl$ ).]

**Methanesulfonic acid**  $CH_3SO_3H$  Clear, colorless liquid or colorless or white, crystalline mass, having a characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether.

**Congealing point** <2.42>: 15 – 20°C

**Specific gravity** <2.56>  $d_{20}^{20}$ : 1.483 – 1.488

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 2 g of methanesulfonic acid, dissolve in 40 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of bromothymol blue TS).

Each mL of 1 mol/L sodium hydroxide VS  
= 96.11 mg of  $CH_3SO_3H$

**Methanesulfonic acid TS** To 35 mL of methanesulfonic acid add 20 mL of acetic acid (100) and water to make 500 mL.

**0.1 mol/L Methanesulfonic acid TS** To 4.8 g of methanesulfonic acid add water to make 500 mL.

**Methanol**  $CH_3OH$  [K 8891, Special class]

**Methanol, anhydrous**  $CH_4O$  To 1000 mL of methanol add 5 g of magnesium powder. After the evolving of a gas is stopped, distill the solution, and preserve the distillate protecting from moisture. Water content per mL is not more than 0.3 mg.

**Methanol for liquid chromatography**  $CH_3OH$  A clear, colorless liquid. Miscible with water.

**Purity** Ultraviolet-absorbing substances—Perform the test as directed in Ultraviolet-visible Spectrophotometry <2.24> using water as the blank: the absorbances at 210 nm, at 220 nm, at 230 nm, at 240 nm and at 254 nm are not more than 0.70, 0.30, 0.15, 0.07 and 0.02, respectively.

**Methanol for water determination** To 1000 mL of methanol add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of methanol. Preserve the methanol, protecting it from moisture. The water content of this methanol should not be more than 0.1 mg per mL.

**Methanol-free ethanol** See ethanol (95), methanol-free.

**Methanol-free ethanol (95)** See ethanol (95), methanol-free.

**Methanol, purified** Distil methanol before use.

**Methionin** See L-methionine.

**L-Methionine**  $C_5H_{11}NO_2S$  [Same as the namesake

monograph]

**Methotrexate**  $C_{20}H_{22}N_8O_5$  [Same as the namesake monograph]

**4'-Methoxyacetophenone**  $C_9H_{10}O_2$  White to light brown, crystals or crystalline powder.

*Melting point* <2.60>: 34 – 39°C

**4-Methoxybenzaldehyde**  $C_8H_8O_2$  Clear, colorless to light yellow liquid. Miscible with ethanol (95) and with diethyl ether, and practically insoluble in water.

*Specific gravity* <2.56>  $d_4^{20}$ : 1.123 – 1.129

**Content:** not less than 97.0%. **Assay**—Weigh accurately about 0.8 g of 4-methoxybenzaldehyde, add exactly 75 mL of hydroxylamine TS, shake well, allow to stand for 30 minutes, and titrate <2.50> with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS) until the color of the solution changes from blue through green to yellow-green. Perform a blank determination in the same manner.

Each mL of 0.5 mol/L hydrochloric acid VS  
= 68.08 mg of  $C_8H_8O_2$

**4-Methoxybenzaldehyde-acetic acid TS** To 0.5 mL of 4-methoxybenzaldehyde add acetic acid (100) to make 100 mL.

**4-Methoxybenzaldehyde-sulfuric acid TS** To 9 mL of ethanol (95) add 0.5 mL of 4-methoxybenzaldehyde and 0.5 mL of sulfuric acid, and mix thoroughly.

**4-Methoxybenzaldehyde-sulfuric acid-acetic acid-ethanol**

**TS for spraying** To 9 mL of ethanol (95) add 0.5 mL of 4-methoxybenzaldehyde, mix gently, add gently 0.5 mL of sulfuric acid and 0.1 mL of acetic acid (100) in this order, and mix well.

**4-Methoxybenzaldehyde-sulfuric acid-acetic acid TS** To 50 mL of acetic acid (100) add 1 mL of sulfuric acid and 0.5 mL of 4-methoxybenzaldehyde, and stir well. Prepare before use.

**(E)-2-Methoxycinnamaldehyde for thin-layer chromatography**  $C_{10}H_{10}O_2$  White to yellow, crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. *Melting point*: 44 – 50°C.

**Identification**—(1) Determine the absorption spectrum of a solution of (E)-2-methoxycinnamaldehyde for thin-layer chromatography in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 282 nm and 286 nm, and between 331 nm and 335 nm.

(2) Determine the infrared absorption spectrum of (E)-2-methoxycinnamaldehyde for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1675  $\text{cm}^{-1}$ , 1620  $\text{cm}^{-1}$ , 1490  $\text{cm}^{-1}$ , 1470  $\text{cm}^{-1}$ , 1295  $\text{cm}^{-1}$ , 1165  $\text{cm}^{-1}$ , 1130  $\text{cm}^{-1}$ , 1025  $\text{cm}^{-1}$  and 600  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 10 mg of (E)-2-methoxycinnamaldehyde for thin-layer chromatography in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this as the standard solution. Perform the test with 5  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification (5) (ii) under Goshajinkigan Extract: the spots other than the principal spot appeared at *R<sub>f</sub>* value of about 0.4 obtained from the sample solution are not more intense than the spot from the standard solution.

**2-Methoxyethanol**  $CH_3OCH_2CH_2OH$  [K 8895, Special class]

**2-Methoxy-4-methylphenol**  $C_8H_{10}O_2$  Colorless to pale yellow liquid. Miscible with methanol and with ethanol (99.5), and slightly soluble in water. Congealing point: 3 – 8°C.

**Identification**—Determine the infrared absorption spectrum of 2-methoxy-4-methylphenol as directed in the ATR method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1511  $\text{cm}^{-1}$ , 1423  $\text{cm}^{-1}$ , 1361  $\text{cm}^{-1}$ , 1268  $\text{cm}^{-1}$ , 1231  $\text{cm}^{-1}$ , 1202  $\text{cm}^{-1}$ , 1148  $\text{cm}^{-1}$ , 1120  $\text{cm}^{-1}$ , 1031  $\text{cm}^{-1}$ , 919  $\text{cm}^{-1}$ , 807  $\text{cm}^{-1}$  and 788  $\text{cm}^{-1}$ .

**Purity** Related substances—Perform the test with 0.2  $\mu\text{L}$  of 2-methoxy-4-methylphenol as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than 2-methoxy-4-methylphenol is not more than 3.0%.

**Operating conditions**

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 60 m in length, coated inside with polymethylsiloxane for gas chromatography in 0.25 to 0.5  $\mu\text{m}$  in thickness.

Column temperature: Inject at a constant temperature of about 100°C, raise the temperature to 130°C at a rate of 5°C per minute, raise to 140°C at a rate of 2°C per minute, raise to 200°C at a rate of 15°C per minute, and maintain at 200°C for 2 minutes.

Injection port temperature: 200°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of 2-methoxy-4-methylphenol is about 10 minutes.

Split ratio: 1:50.

**System suitability**

**System performance:** Dissolve 60 mg of 2-methoxy-4-methylphenol in methanol to make 100 mL, and use this solution as the solution for system suitability test. Proceed with 1  $\mu\text{L}$  of the solution for system suitability test under the above operating conditions, the symmetry factor of the peak of 2-methoxy-4-methylphenol is not more than 1.5.

**System repeatability:** When the test is repeated 6 times with 1  $\mu\text{L}$  of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of 2-methoxy-4-methylphenol is not more than 2.0%.

**1-Methoxy-2-propanol**  $C_4H_{10}O_2$  A colorless, clear liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.402 – 1.405

*Specific gravity* <2.56>  $d_4^{20}$ : 0.920 – 0.925

**Purity** Clarity of solution—To 5 mL of 1-methoxy-2-propanol add 20 mL of water, and mix: the solution is clear.

**Water** <2.48>: not more than 0.5% (5 g).

**Content:** not less than 98.0%. **Assay**—Proceed as directed under Gas Chromatography <2.02> using the area percentage method according to the following conditions:

**Operating conditions**

Detector: Thermal conductivity detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180  $\mu\text{m}$ ) coated with polyethylene glycol 20 M for gas chromatography in 20%.

Column temperature: A constant temperature of about 90°C.

Carrier gas: Helium.

Flow rate: A constant flow rate of 20 mL per minute.

**Methyl acetate**  $\text{CH}_3\text{COOCH}_3$  [K 8382, Special class]

**Methyl 4-aminobenzoate**  $\text{H}_2\text{NC}_6\text{H}_4\text{COOCH}_3$ , Pale yellow, crystals or crystalline powder.

*Melting point* <2.60>: 111 – 114°C

**p-Methyl aminophenol sulfate** See 4-methyl aminophenol sulfate.

**4-Methylaminophenol sulfate**  $(\text{HOCC}_6\text{H}_4\text{NHCH}_3)_2\text{H}_2\text{SO}_4$

White to pale yellow or very pale gray, crystals or crystalline powder. Melting point: about 260°C (with decomposition).

**p-Methyl aminophenol sulfate TS** See 4-methyl aminophenol sulfate TS.

**4-Methyl aminophenol sulfate TS** Dissolve 0.35 g of 4-methyl aminophenol sulfate and 20 g of sodium hydrogen sulfite in water to make 100 mL. Prepare before use.

**2-Methylaminopyridine**  $\text{C}_6\text{H}_8\text{N}_2$  A light yellow liquid.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.050 – 1.065

*Boiling point* <2.57>: 200 – 202°C

*Water* <2.48>: less than 0.1%.

**2-Methylamino pyridine for water determination** Distill and preserve 2-methylaminopyridine, protecting it from moisture. The water content of this 2-methylaminopyridine should not be more than 1 mg per mL.

**Methyl arachidate for gas chromatography**  $\text{C}_{21}\text{H}_{42}\text{O}_2$  White to light yellow, crystals or crystalline masses.

*Melting point* <2.60>: 45 – 50°C

**Methyl behenate**  $\text{C}_{23}\text{H}_{46}\text{O}_2$  White, odorless and tasteless, scaly crystals or powder. Dissolves in acetone, in diethyl ether and in chloroform.

*Melting point* <2.60>: 54°C

*Saponification value* <1.13>: 155.5 – 158.5

**Methyl benzoate**  $\text{C}_6\text{H}_5\text{COOCH}_3$  Clear, colorless liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.515 – 1.520

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.087 – 1.095

**Purity**—Dissolve 0.1 mL of methyl benzoate in the mobile phase in Assay under Thiamine Chloride Hydrochloride to make 50 mL. Perform the test as directed under Liquid Chromatography <2.01> with 10  $\mu\text{L}$  of this solution according to the operating conditions in the Assay under Thiamine Chloride Hydrochloride. Determine each peak area by the automatic integration method in a range about twice the retention time of methyl benzoate, and calculate the amount of methyl benzoate by the area percentage method: it shows the purity of not less than 99.0%.

**Methyl benzoate for estriol test**  $\text{C}_6\text{H}_5\text{COOCH}_3$  Clear, colorless liquid, having a characteristic odor.

*Refractive index* <2.45>  $n_D^{20}$ : 1.515 – 1.520

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.087 – 1.095

*Acid value* <1.13>: not more than 0.5.

**4-Methylbenzophenone**  $\text{C}_{14}\text{H}_{12}\text{O}$  White crystals.

**3-Methyl-2-benzothiazolonehydrazone hydrochloride monohydrate**  $\text{C}_8\text{H}_{10}\text{ClN}_3\text{S}\cdot\text{H}_2\text{O}$  A white to light yellow-white crystalline powder. Melting point: about 270°C (with decomposition).

**D-(+)- $\alpha$ -Methylbenzylamine**  $\text{C}_6\text{H}_5\text{CH}(\text{CH}_3)\text{NH}_2$  Colorless or pale yellow, clear liquid, having an amine like odor. Very soluble in ethanol (95) and in acetone, and slightly soluble in water.

*Refractive index* <2.45>  $n_D^{20}$ : 1.524 – 1.529

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +37 – +41° (50 mm).

**Purity**—Perform the test with 0.6  $\mu\text{L}$  of D-(+)- $\alpha$ -methylbenzylamine as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of D-(+)- $\alpha$ -methylbenzylamine by the area percentage method: not less than 98.0%.

**Operating conditions**

**Detector:** A hydrogen flame-ionization detector.

**Column:** A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (180 to 250  $\mu\text{m}$  in particle diameter) coated with polyethylene glycol 20 M for gas chromatography and potassium hydroxide at the ratio of 10% and 5%, respectively.

**Column temperature:** A constant temperature of about 140°C.

**Carrier gas:** Helium.

**Flow rate:** Adjust so that the retention time of D-(+)- $\alpha$ -methylbenzylamine is about 5 minutes.

**Selection of column:** To 5 mL of D-(+)- $\alpha$ -methylbenzylamine add 1 mL of pyridine. Proceed with 0.6  $\mu\text{L}$  of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pyridine and D-(+)- $\alpha$ -methylbenzylamine in this order with the resolution between these peaks being not less than 3.

**Detection sensitivity:** Adjust the detection sensitivity so that the peak height of D-(+)- $\alpha$ -methylbenzylamine obtained with 0.6  $\mu\text{L}$  of the D-(+)- $\alpha$ -methylbenzylamine is not less than about 90% of the full scale.

**Time span of measurement:** About 3 times as long as the retention time of D-(+)- $\alpha$ -methylbenzylamine.

**3-Methyl-1-butanol**  $\text{C}_5\text{H}_{12}\text{O}$  [K 8051, Special class]

**3-Methylbutyl acetate**  $\text{CH}_3\text{COOCH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$  A clear and colorless liquid. Boiling point: about 140°C.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.868 – 0.879

**Storage:** Preserve in a light-resistant tight container.

**Methyl cellosolve** See 2-methoxyethanol.

**Methylcyclohexane**  $\text{C}_7\text{H}_{14}$  A clear and colorless liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.420 – 1.425

*Density* <2.56> (20°C): 0.766 – 0.772 g/mL

**Methyl docosanate**  $\text{C}_{23}\text{H}_{46}\text{O}_2$  White, tabular crystals or crystalline powder.

*Melting point* <2.60>: 51.0 – 56.0°C

**Methyldopa** See methyldopa hydrate.

**Methyldopa for assay** See methyldopa hydrate for assay.

**Methyldopa hydrate**  $\text{C}_{10}\text{H}_{13}\text{NO}_4 \cdot 1\frac{1}{2}\text{H}_2\text{O}$  [Same as the namesake monograph]

**Methyldopa hydrate for assay**  $\text{C}_{10}\text{H}_{13}\text{NO}_4 \cdot 1\frac{1}{2}\text{H}_2\text{O}$

[Same as the monograph Methyldopa Hydrate. It contains not less than 99.0% of methyldopa ( $\text{C}_{10}\text{H}_{13}\text{NO}_4$ ), calculated on the anhydrous basis.]

**N,N'-Methylenabisacrylamide**  $\text{CH}_2(\text{NHCOCHCH}_2)_2$

White crystalline powder.

**Content:** not less than 97.0%.

**Methyl eicosenoate for gas chromatography**  $\text{C}_{21}\text{H}_{40}\text{O}_2$  A clear and colorless, liquid.

**Methylene blue** See methylene blue trihydrate.

**Methylene blue-sulfuric acid-sodium dihydrogenphosphate TS** To 30 mL of a solution of methylene blue (1 in

1000) add 500 mL of water, 6.8 mL of sulfuric acid and 50 g of sodium dihydrogenphosphate dihydrate, dissolve, and add water to make 1000 mL.

**Methylene blue trihydrate**  $C_{16}H_{18}ClN_3S \cdot 3H_2O$  [K 8897, Special class]

**Methylene blue TS** Dissolve 0.1 g of methylene blue trihydrate in water to make 100 mL. Filter if necessary.

**dl-Methylephedrine hydrochloride**  $C_{11}H_{17}NO \cdot HCl$  [Same as the namesake monograph]

**dl-Methylephedrine hydrochloride for assay** [Same as the monograph *dl*-Methylephedrine Hydrochloride]

**Methylergometrine maleate for assay**  $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$  [Same as the monograph Methylergometrine Maleate. When dried, it contains not less than 99.0% of methylergometrine maleate ( $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ ).]

**Methyl ethyl ketone** See 2-butanone.

**Methyl iodide** See iodomethane.

**Methyl iodide for assay** See iodomethane for assay.

**Methyl isobutyl ketone** See 4-methyl-2-pentanone.

**Methyl laurate for gas chromatography**  $C_{13}H_{26}O_2$  A colorless to yellow, liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.431 – 1.433

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.870 – 0.872

**Methyl lignocerate for gas chromatography**  $C_{25}H_{50}O_2$  A white crystalline powder.

*Melting point* <2.60>: 58 – 61 °C

**Methyl linoleate for gas chromatography**  $C_{19}H_{34}O_2$  A colorless to light yellow, liquid.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.880 – 0.889

**Methyl linolenate for gas chromatography**  $C_{19}H_{32}O_2$  A colorless to light yellow, liquid.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.890 – 0.901

**3-O-Methylmethyldopa for thin-layer chromatography**  $C_{11}H_{15}NO_4$

**Purity** Related substances—Dissolve 5 mg of 3-O-methylmethyldopa for thin-layer chromatography in methanol to make exactly 100 mL. Perform the test with 20  $\mu$ L of this solution as directed in the Purity (5) under Metyldopa Hydrate: any spot other than the principal spot at the *Rf* value of about 0.7 does not appear.

**Methyl myristate for gas chromatography**  $C_{15}H_{30}O_2$  A colorless to light yellow, liquid.

*Specific gravity* <2.56>  $d_{20}^{20}$ : about 0.866 – 0.874

**2-Methyl-5-nitroimidazole for thin-layer chromatography**  $C_4H_5N_3O_2$  White crystalline powder. Slightly soluble in water and in acetone. Melting point: about 253 °C (with decomposition).

**Purity** Related substances—Dissolve 40 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in 8 mL of acetone, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test as directed in the Purity (2) under Metronidazole: the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

**Methyl oleate for gas chromatography**  $C_{19}H_{36}O_2$  A clear, colorless to light yellow, liquid.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.866 – 0.882

**Methyl orange**  $C_{14}H_{14}N_3NaO_3S$  [K 8893, Special class]

**Methyl orange-boric acid TS** Add 0.5 g of methyl orange and 5.2 g of boric acid in 500 mL of water, and dissolve by warming on a water bath. After cooling, wash this solution with three 50-mL portions of chloroform.

**Methyl orange TS** Dissolve 0.1 g of methyl orange in 100 mL of water, and filter if necessary.

**Methyl orange-xilenecyanol FF TS** Dissolve 1 g of methyl orange and 1.4 g of xylene cyanol FF in 500 mL of dilute ethanol.

**Methyl palmitate for gas chromatography**  $C_{17}H_{34}O_2$  White, crystals or waxy masses.

*Congealing point* <2.42>: 25 – 31 °C

**Methyl palmitoleate for gas chromatography**  $C_{17}H_{32}O_2$  *Specific gravity* <2.56>  $d_{20}^{20}$ : 0.876 – 0.881

**Methyl parahydroxybenzoate**  $HOC_6H_4COOCH_3$  [Same as the namesake monograph]

**Methyl parahydroxybenzoate for resolution check**

$C_8H_8O_3$  Colorless crystals or a white crystalline powder. Freely soluble in methanol, in ethanol (95) and in acetone, and very slightly soluble in water. Melting point: 125 – 128 °C.

**Identification**—Determine the infrared absorption spectrum of methyl parahydroxybenzoate for resolution check as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum of Methyl Parahydroxybenzoate or the spectrum of Methyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** Related substances—Dissolve 50 mg of methyl parahydroxybenzoate for resolution check in 2.5 mL of methanol, and add the mobile phase to make 50 mL. To 10 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: total area of the peaks other than methyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of methyl parahydroxybenzoate from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Methyl Parahydroxybenzoate.

Time span of measurement: About 5 times as long as the retention time of methyl parahydroxybenzoate.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of methyl parahydroxybenzoate obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

**System performance**: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of methyl parahydroxybenzoate are not less than 2500 and not more than 2.0, respectively.

**System repeatability**: When the test is repeated 6 times

with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methyl parahydroxybenzoate is not more than 5.0%.

**4-Methylpentan-2-ol** C<sub>6</sub>H<sub>14</sub>O A clear and colorless, volatile liquid.

Refractive index <2.45>  $n_D^{20}$ : about 1.411

Specific gravity <2.56>  $d_{20}^{20}$ : about 0.802

Boiling point <2.57>: about 132°C

**4-Methyl-2-pentanone** CH<sub>3</sub>COCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub> [K 8903, Special class]

**3-Methyl-1-phenyl-5-pyrazolone** C<sub>10</sub>H<sub>10</sub>N<sub>2</sub>O [K 9548, Special class]

**Methyl prednisolone** C<sub>22</sub>H<sub>30</sub>O<sub>3</sub> [Same as the namesake monograph]

**2-Methyl-1-propanol** (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>OH [K 8811, Special class]

**N-Methylpyrrolidine** C<sub>5</sub>H<sub>11</sub>N Colorless, clear liquid, having a characteristic order.

**Identification**—Determine the spectrum of N-methylpyrrolidine in a solution of deuterated chloroform for nuclear magnetic resonance spectroscopy (2 in 25) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (<sup>1</sup>H): it exhibits a big signal, at around  $\delta$  2.3 ppm.

**Content:** not less than 95%. **Assay**—Put 30 mL of water in a beaker, weigh accurately the beaker, add dropwise about 0.15 g of N-methylpyrrolidine, weigh accurately the beaker again, and titrate <2.50> with 0.05 mol/L sulfuric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.05 mol/L sulfuric acid VS} \\ = 8.515 \text{ mg of C}_5\text{H}_{11}\text{N} \end{aligned}$$

**Methyl red** C<sub>15</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub> [K 8896, Special class]

**Methyl red-methylene blue TS** Dissolve 0.1 g of methyl red and 0.1 g of methylene blue in ethanol (95) to make 100 mL, and filter if necessary.

**Storage**—Preserve in light-resistant containers.

**Methyl red-sodium hydroxide TS** Dissolve 50 mg of methyl red in a mixture of 1.86 mL of 0.1 mol/L sodium hydroxide VS and 50 mL of ethanol (95), and add water to make 100 mL.

**Methyl red TS** Dissolve 0.1 g of methyl red in 100 mL of ethanol (95), and filter if necessary.

**Methyl red TS, dilute** Dissolve 25 mg of methyl red in 100 mL of ethanol (99.5), and filter if necessary. Prepare before use.

**Methyl red TS for acidity or alkalinity test** To 0.1 g of methyl red add 7.4 mL of 0.05 mol/L sodium hydroxide VS or 3.7 mL of 0.1 mol/L sodium hydroxide VS, triturate to dissolve in a mortar, and add freshly boiled and cooled water to make 200 mL.

**Storage**—Preserve in light-resistant, glass-stoppered bottles.

**Methylrosaniline chloride** See crystal violet.

**Methylrosaniline chloride TS** See crystal violet TS.

**Methyl salicylate** C<sub>8</sub>H<sub>8</sub>O<sub>3</sub> [Same as the namesake monograph]

**Methylsilicone polymer for gas chromatography** Pre-

pared for gas chromatography.

**Methyl stearate for gas chromatography** C<sub>19</sub>H<sub>38</sub>O<sub>2</sub> White, crystals or crystalline masses.

Melting point <2.60>: 36 – 42°C

**Methyltestosterone** C<sub>20</sub>H<sub>30</sub>O<sub>2</sub> [Same as the namesake monograph]

**1-Methyl-1*H*-tetrazole-5-thiol** C<sub>2</sub>H<sub>4</sub>N<sub>4</sub>S White, crystals or crystalline powder.

**Identification** (1) Determine the ultraviolet-visible absorption spectrum of a solution of 1-methyl-1*H*-tetrazole-5-thiol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 222 nm and 226 nm.

(2) Determine the infrared absorption spectrum of 1-methyl-1*H*-tetrazole-5-thiol according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3060  $\text{cm}^{-1}$ , 2920  $\text{cm}^{-1}$ , 2780  $\text{cm}^{-1}$ , 1500  $\text{cm}^{-1}$ , 1430  $\text{cm}^{-1}$  and 1410  $\text{cm}^{-1}$ .

Melting point <2.60>: 125 – 129°C

**Purity** Related substances—Dissolve 0.10 g of 1-methyl-1*H*-tetrazole-5-thiol in exactly 100 mL of water. Perform the test with 1  $\mu$ L of this solution as directed in the Purity (4) under Cefmetazole Sodium: any spot other than the principal spot at the R<sub>f</sub> value of about 0.77 does not appear.

**1-Methyl-1*H*-tetrazole-5-thiol for liquid chromatography** C<sub>2</sub>H<sub>4</sub>N<sub>4</sub>S White, crystals or crystalline powder. Very soluble in methanol, and freely soluble in water.

Melting point <2.60>: 123 – 127°C

**Loss on drying** <2.41>: not more than 1.0% (1 g, in vacuum, phosphorous (V) oxide, 2 hours).

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 0.2 g of 1-methyl-1*H*-tetrazole-5-thiol, previously dried, dissolve in 80 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (indicator: 3 drops of thymol blue-*N,N*-dimethylformamide TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium methoxide VS} \\ = 11.61 \text{ mg of C}_2\text{H}_4\text{N}_4\text{S} \end{aligned}$$

**Methylthymol blue** C<sub>37</sub>H<sub>43</sub>N<sub>2</sub>NaO<sub>13</sub>S [K 9552, Special class]

**Methylthymol blue-potassium nitrate indicator** Mix 0.1 g of methylthymol blue with 9.9 g of potassium nitrate, and triturate until the mixture becomes homogeneous.

**Sensitivity**—When 20 mg of methylthymol blue-potassium nitrate indicator is dissolved in 100 mL of 0.02 mol/L sodium hydroxide VS, the solution is slightly blue in color. On adding 0.05 mL of 0.01 mol/L barium chloride VS to this solution, the solution shows a blue color, then on the subsequent addition of 0.1 mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, it becomes colorless.

**Methylthymol blue-sodium chloride indicator** Mix 0.25 g of methylthymol blue and 10 g of sodium chloride, and grind to homogenize.

**Methyl yellow** C<sub>14</sub>H<sub>15</sub>N<sub>3</sub> [K 8494, Special class]

**Methyl yellow TS** Dissolve 0.1 g of methyl yellow in 200 mL of ethanol (95).

**Metoclopramide for assay** C<sub>14</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>2</sub> [Same as the monograph]

**Metoprolol tartrate for assay** (C<sub>15</sub>H<sub>25</sub>NO<sub>3</sub>)<sub>2</sub>.C<sub>4</sub>H<sub>6</sub>O<sub>6</sub>

[Same as the monograph Metoprolol Tartrate. When dried, it contains not less than 99.5% of metoprolol tartrate ( $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ ).]

**Metronidazole**  $C_6H_9N_3O_3$  [Same as the namesake monograph]

**Metronidazole for assay**  $C_6H_9N_3O_3$  [Same as the monograph Metronidazole. It meets the following additional requirement.]

**Purity** Related substances—Dissolve about 25 mg of metronidazole for assay in 100 mL of a mixture of water and methanol (4:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mixture of water and methanol (4:1) to make exactly 50 mL. Pipet 2.5 mL of this solution, add the mixture of water and methanol (4:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than metronidazole obtained from the sample solution is not more than the peak area of metronidazole from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Metronidazole Tablets.

Time span of measurement: About 4 times as long as the retention time of metronidazole.

#### System suitability

Test for required detectability: Measure exactly 2 mL of the standard solution, add a mixture of water and methanol (4:1) to make exactly 20 mL. Confirm that the peak area of metronidazole obtained with 10  $\mu$ L of this solution is equivalent to 7 to 13% of that with the standard solution.

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metronidazole are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metronidazole is not more than 2.0%.

**Miconazole nitrate**  $C_{18}H_{14}Cl_4N_2O \cdot HNO_3$  [Same as the namesake monograph]

**Microplate** A plate having multiple wells. It ordinarily has 96 or more wells and is about 128 mm  $\times$  about 85 mm  $\times$  about 14 mm in size. Select the material, surface treatment, and other conditions of the microplate suitable to tests for use.

**Miglitol**  $C_8H_{17}NO_5$  [Same as the namesake monograph]

**Milk casein** See casein, milk.

**Milk of lime** Place 10 g of calcium oxide in a mortar, and add gradually 40 mL of water under grinding.

**Minocycline hydrochloride**  $C_{23}H_{27}N_3O_7 \cdot HCl$  [Same as the namesake monograph]

**Mitiglinide calcium hydrate**  $C_{38}H_{48}CaN_2O_6 \cdot 2H_2O$  [Same as the namesake monograph]

**Mixture of petroleum hexamethyl tetracosane branching hydrocarbons (L) for gas chromatography** Prepared for

gas chromatography.

**Molecular mass marker for epoetin alfa** A solution containing about 0.4 mg each of egg albumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme in 200  $\mu$ L.

**Molecular mass marker for interferon alfa** Molecular mass known marker proteins, which are adjusted for molecular mass determination [4 components: egg albumin, carbonic anhydrase, soybean trypsin inhibitor, and  $\alpha$ -lactalbumin].

**Identification** Use a solution of the molecular mass marker for interferon alfa as the sample solution. Separately, add water to an amount of egg albumin so that each mL contains 100  $\mu$ g as protein content of egg albumin, and use this solution as the standard solution. Perform SDS-polyacrylamide gel electrophoresis with the sample solution and standard solution under the test conditions for molecular mass of Interferon Alfa (NAMALWA). The sample solution indicates 4 main electrophoretic bands. In addition, the mobility of egg albumin obtained from the sample solution corresponds with that of the band from the standard solution.

**Molecular mass marker for nartograstim test** A solution containing the following proteins. Egg albumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme.

**Molecular mass standard stock solution** Dissolve 1.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 3.2 g of sodium lauryl sulfate in a suitable amount of water, adjust to pH 6.8 with 6 mol/L hydrochloric acid TS, 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS, add 32 mg of bromophenol blue and 16 mL of glycerin, and add water to make 40 mL. To 500  $\mu$ L of this solution add 100  $\mu$ L of molecular mass marker for epoetin alfa and 1400  $\mu$ L of water, and heat at 100°C for 5 minutes. It meets the following requirement.

**Identification**—Dissolve 0.1 mg each of egg albumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme in 250  $\mu$ L of buffer solution for epoetin alfa sample, add water to them to make 1 mL and heat at 100°C for 5 minutes, and use these solutions as each standard solution. When perform the test with the solution to be examined and each standard solution by the SDS-polyacrylamide gel electrophoresis as directed in the Molecular mass under Epoetin Alfa (Genetical recombination), the each band in the chromatogram obtained from the solution to be examined shows the same mobility as the band corresponding to egg albumin, carbonic anhydrase, soybean trypsin inhibitor or lysozyme obtained from each standard solution.

**Molecular mass markers for teceleukin** Dissolve 0.4 mg each of lysozyme, soybean trypsin inhibitor, carbonic anhydrase, egg albumin, bovine serum albumin, and phosphorylase b in 200  $\mu$ L of diluted glycerin (1 in 2).

**Molybdenum (VI) oxide**  $MoO_3$  A white to yellowish green powder.

**Identification**—Dissolve 0.5 g of molybdenum (VI) oxide in 5 mL of ammonia solution (28), acidify 1 mL of this solution with a suitable amount of nitric acid, add 5 mL of sodium phosphate TS, and warm: yellow precipitates appear.

**Molybdenum (VI) oxide-citric acid TS** To 54 g of molybdenum (VI) oxide and 11 g of sodium hydroxide add 200 mL of water, and dissolve by heating while stirring. Separately, dissolve 60 g of citric acid monohydrate in 250 mL of water, and add 140 mL of hydrochloric acid. Mix these solutions, filter if necessary, add water to make 1000 mL, and add a so-

lution of potassium bromate (1 in 100) until a yellow-green color appears.

**Storage**—Preserve in tightly stoppered containers, protected from light.

**Molybdenum-sulfuric acid TS** Dissolve 2.5 g of hexaammonium heptamolybdate tetrahydrate in 20 mL of water by heating. To this solution add a solution, prepared by careful adding 28 mL of sulfuric acid to 50 mL of water, mixing and cooling, and add water to make 100 mL. Reserve in a polyethylene container.

**Molybdenum trioxide** See molybdenum (VI) oxide.

**Molybdenum trioxide-citric acid TS** See molybdenum (VI) oxide-citric acid TS.

**Monoammonium glycyrrhizinate for resolution check**

$C_{42}H_{61}O_{16}NH_4$  Mainly composed with monoammonium glycyrrhizinate and its isomers. It is white, crystals or crystalline powder.

**Identification**—Dissolve 1 mg of monoammonium glycyrrhizinate for resolution check in 2 mL of diluted ethanol (2 in 5). Perform the test with 2  $\mu$ L of this solution as directed under Liquid Chromatography <2.01> according to the following conditions: a peak having a relative retention time of about 0.9 to glycyrrhizic acid is observed, and when performed the test with these two peaks by liquid chromatography-mass spectrometry (ESI method, positive mode) their mass charge ratios ( $m/z$  values) are observed at 823 or 840 or at the both of them, respectively.

**Operating conditions**

**Detector**: An ultraviolet absorption photometer (wavelength: 254 nm) and mass spectrometer.

**Column**: A stainless steel column 2 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3  $\mu$ m in particle diameter).

**Column temperature**: A constant temperature of about 40°C.

**Mobile phase**: Dissolve 0.63 g of ammonium formate in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.

**Flow rate**: About 0.5 mL per minute.

**Monobasic ammonium phosphate** See ammonium dihydrogenphosphate.

**0.02 mol/L Monobasic ammonium phosphate TS** See 0.02 mol/L ammonium dihydrogenphosphate TS.

**Monobasic potassium phosphate** See potassium dihydrogenphosphate.

**Monobasic potassium phosphate for pH determination**  
See potassium dihydrogenphosphate for pH determination.

**0.05 mol/L Monobasic potassium phosphate (pH 3.0)**  
See 0.05 mol/L potassium dihydrogenphosphate (pH 3.0).

**0.05 mol/L Monobasic potassium phosphate TS (pH 4.7)**  
See 0.05 mol/L potassium dihydrogenphosphate TS (pH 4.7).

**0.02 mol/L Monobasic potassium phosphate TS** See 0.02 mol/L potassium dihydrogenphosphate TS.

**0.05 mol/L Monobasic potassium phosphate TS** See 0.05 mol/L potassium dihydrogenphosphate TS.

**0.2 mol/L Monobasic potassium phosphate TS** See 0.2 mol/L potassium dihydrogenphosphate TS.

**0.2 mol/L Monobasic potassium phosphate TS for buffer solution** See 0.2 mol/L potassium dihydrogenphosphate

TS for buffer solution.

**Monobasic sodium phosphate** See sodium dihydrogenphosphate dihydrate.

**0.05 mol/L Monobasic sodium phosphate TS (pH 2.6)**

See 0.05 mol/L sodium dihydrogenphosphate TS (pH 2.6).

**0.05 mol/L Monobasic sodium phosphate TS (pH 3.0)**

See 0.05 mol/L sodium dihydrogenphosphate TS (pH 3.0).

**0.1 mol/L Monobasic sodium phosphate TS (pH 3.0)**

See 0.1 mol/L sodium dihydrogenphosphate TS (pH 3.0).

**0.05 mol/L Monobasic sodium phosphate TS** See 0.05 mol/L sodium dihydrogenphosphate TS.

**0.1 mol/L Monobasic sodium phosphate TS** See 0.1 mol/L sodium dihydrogenphosphate TS.

**2 mol/L Monobasic sodium phosphate TS** See 2 mol/L sodium dihydrogenphosphate TS.

**Monoethanolamine** See 2-Aminoethanol.

**Morphine hydrochloride** See morphine hydrochloride hydrate.

**Morphine hydrochloride for assay** See morphine hydrochloride hydrate for assay.

**Morphine hydrochloride hydrate**  $C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$   
[Same as the namesake monograph]

**Morphine hydrochloride hydrate for assay**

$C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$  [Same as the monograph Morphine Hydrochloride Hydrate. It contains not less than 99.0% of morphine hydrochloride ( $C_{17}H_{19}NO_3 \cdot HCl$ ), calculated on the anhydrous basis.]

**3-(*N*-Morpholino)propanesulfonic acid**  $C_7H_{15}NO_4S$

White crystalline powder, freely soluble in water, and practically insoluble in ethanol (99.5%).

**Melting point** <2.60>: 275 – 280°C

**0.02 mol/L 3-(*N*-Morpholino)propanesulfonic acid buffer solution (pH 7.0)** Dissolve 4.2 g of 3-(*N*-morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with dilute sodium hydroxide TS, and add water to make 1000 mL.

**0.1 mol/L 3-(*N*-Morpholino)propanesulfonic acid buffer solution (pH 7.0)** Dissolve 20.92 g of 3-(*N*-morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with sodium hydroxide TS, and add water to make 1000 mL.

**0.02 mol/L 3-(*N*-Morpholino)propanesulfonic acid buffer solution (pH 8.0)** Dissolve 4.2 g of 3-(*N*-morpholino)propanesulfonic acid in 700 mL of water, adjust the pH to 8.0 with dilute sodium hydroxide TS, and add water to make 1000 mL.

**Mosapride citrate for assay** See mosapride citrate hydrate for assay.

**Mosapride citrate hydrate for assay**

$C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7 \cdot 2H_2O$  [Same as the monograph Mosapride Citrate Hydrate. It contains not less than 99.0% of mosapride citrate ( $C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$ ) calculated on the anhydrous basis.]

**Mouse anti-epoetin alfa monoclonal antibody** A solution of the monoclonal antibody in phosphate-buffered sodium chloride TS, which is obtained from mouse immunized with a synthetic peptide having the amino acid sequence corre-

sponding to N-terminal 20 residues of epoetin alfa (genetical recombination). When perform the Western blotting against Epoetin Alfa RS, it is reactable.

**MTT TS** Dissolve 8 g of sodium chloride, 0.2 g of potassium chloride, 1.15 g of anhydrous disodium hydrogen phosphate and 0.2 g of potassium dihydrogen phosphate in water to make 1000 mL, and sterilize in an autoclave for 15 minutes at 121°C to make the PBS(–) solution. Dissolve 0.3 g of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide in this PBS(–) solution to make 100 mL. Sterilize by membrane filtration (pore size, 0.45 µm), and store in a cool place shielded from light.

**Murexide**  $C_8H_8N_6O_6$  Red-purple powder. Practically insoluble in water, in ethanol (95) and in diethyl ether.

**Purity** Clarity of solution—Dissolve 10 mg of murexide in 100 mL of water; the solution is clear.

**Residue on ignition** <2.44>: not more than 0.1% (1 g).

**Sensitivity**—Dissolve 10 mg of murexide in 2 mL of ammonia-ammonium chloride buffer solution (pH 10.0), and add water to make 100 mL, and use this solution as the sample solution. Separately, add 2 mL of ammonia-ammonium chloride buffer solution (pH 10.0) to 5 mL of diluted Standard Calcium Solution (1 in 10), add water to make 25 mL, and render the solution to pH 11.3 with sodium hydroxide TS. Add 2 mL of the sample solution and water to this solution to make 50 mL: a red-purple color develops.

**Murexide-sodium chloride indicator** Prepared by mixing 0.1 g of murexide and 10 g of sodium chloride and grinding to get homogeneous.

**Storage**—Preserve in light-resistant containers.

**Myoglobin** A hemoprotein obtained from horse heart muscle. White crystalline powder. It contains not less than 95% of myoglobin in the total protein.

**Myoinositol**  $C_6H_6(OH)_6$  White, crystals or crystalline powder.

**Myristicin for thin-layer chromatography**  $C_{11}H_{12}O_3$  Colorless, clear liquid, having a characteristic odor. Miscible with ethanol (95), and practically insoluble in water.

**Identification**—Determine the infrared absorption spectrum of myristicin for thin-layer chromatography as directed in the liquid film method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3080 cm<sup>-1</sup>, 2890 cm<sup>-1</sup>, 1633 cm<sup>-1</sup>, 1508 cm<sup>-1</sup>, 1357 cm<sup>-1</sup>, 1318 cm<sup>-1</sup>, 1239 cm<sup>-1</sup>, 1194 cm<sup>-1</sup>, 1044 cm<sup>-1</sup>, 994 cm<sup>-1</sup>, 918 cm<sup>-1</sup>, 828 cm<sup>-1</sup> and 806 cm<sup>-1</sup>.

**Purity** Related substances—Dissolve 20 mg of myristicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 5 µL each of the sample solution and standard solution as directed in the Identification under Nutmeg: the spots other than the principle spot at the *Rf* value of about 0.4 obtained from the sample solution are not more intense than the spot from the standard solution.

**NADH peroxidase** One unit indicates an amount of the enzyme which consumes 1 µmol of β-NADH in 1 minute at 25°C and pH 8.0 using β-nicotinamide adenine dinucleotide (β-NADH) and hydrogen peroxide as the substrate.

**NADH peroxidase TS** Suspend NADH peroxidase in ammonium sulfate TS so that each mL contains 10 units of the activity.

**Storage**—Between 0 and 8°C.

**Naftopidil for assay**  $C_{24}H_{28}N_2O_3$  [Same as the monograph Naftopidil. When dried, it contains not less than 99.5% of naftopidil ( $C_{24}H_{28}N_2O_3$ ).]

**Nalidixic acid**  $C_{12}H_{12}N_2O_3$  [Same as the namesake monograph]

**Namalwa cell** Human cell line derived from B lymphoblasts, taken from patients with Burkitt's lymphoma.

**Naphazoline hydrochloride**  $C_{14}H_{14}N_2.HCl$  [Same as the namesake monograph]

**Naphazoline nitrate**  $C_{14}H_{14}N_2.HNO_3$  [Same as the namesake monograph]

**Naphazoline nitrate for assay**  $C_{14}H_{14}N_2.HNO_3$  [Same as the monograph Naphazoline Nitrate. When dried, it contains not less than 99.0% of naphazoline nitrate ( $C_{14}H_{14}N_2.NHO_3$ ).]

**Naphthalene**  $C_{10}H_8$  Colorless flake-like or lustrous stick-like crystals, having a characteristic odor.

**Melting point** <2.60>: 78 – 82°C

**1,3-Naphthalenediol**  $C_{10}H_8O_2$  Red-brown crystals or gray-brown powder. Freely soluble in water, in methanol and in ethanol (99.5). Melting point: about 124°C.

**1,3-Naphthalenediol TS** Dissolve 50 mg of 1,3-naphthalenediol in 25 mL of ethanol (99.5), and add 2.5 mL of phosphoric acid.

**2-Naphthalenesulfonic acid** See 2-naphthalenesulfonic acid monohydrate.

**2-Naphthalenesulfonic acid monohydrate**  $C_{10}H_8O_3S.H_2O$  White to pale yellow-white powder. Very soluble in water, in methanol and in ethanol (95), and sparingly soluble in diethyl ether and in chloroform.

**Water** <2.48>: 7.0 – 11.5% (0.5 g, volumetric titration, direct titration).

**Content:** not less than 95.0%, calculated on the anhydrous basis. **Assay**—Weigh accurately about 0.5 g of 2-naphthalenesulfonic acid monohydrate, dissolve in 30 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L sodium hydroxide VS} \\ = 20.82 \text{ mg of } C_{10}H_8O_3S \end{aligned}$$

**1-Naphthol**  $C_{10}H_7OH$  [K 8698, Special class] Preserve in light-resistant containers.

**2-Naphthol**  $C_{10}H_7OH$  [K 8699, Special class] Preserve in light-resistant containers.

**α-Naphthol** See 1-naphthol.

**β-Naphthol** See 2-naphthol.

**p-Naphtholbenzein**  $C_{27}H_{18}O_2$  [K 8693, Special class]

**α-Naphtholbenzein** See *p*-naphtholbenzein.

**p-Naphtholbenzein TS** Dissolve 0.2 g of *p*-naphtholbenzein in acetic acid (100) to make 100 mL.

**Purity** Clarity and color of solution—Dissolve 0.10 g of *p*-naphtholbenzein in 100 mL of ethanol (95); the solution is red in color and clear.

**Sensitivity**—Add 100 mL of freshly boiled and cooled water to 0.2 mL of a solution of *p*-naphtholbenzein in ethanol (95) (1 in 1000), and add 0.1 mL of 0.1 mol/L sodium hydroxide VS: a green color develops. Add subse-

quently 0.2 mL of 0.1 mol/L hydrochloric acid VS: the color of the solution changes to yellow-red.

**α-Naphtholbenzein TS** See *p*-naphtholbenzein TS.

**1-Naphthol-sulfuric acid TS** Dissolve 1.5 g of 1-naphthol in 50 mL of ethanol (95), add 3 mL of water and 7 mL of sulfuric acid, and mix well. Prepare before use.

**1-Naphthol TS** Dissolve 6 g of sodium hydroxide and 16 g of anhydrous sodium carbonate in water to make 100 mL. In this solution dissolve 1 g of 1-naphthol. Prepare before use.

**2-Naphthol TS** Dissolve 1 g of 2-naphthol in sodium carbonate TS to make 100 mL. Prepare before use.

**α-Naphthol TS** See 1-naphthol TS.

**β-Naphthol TS** See 2-naphthol TS.

**Naphthoresorcin-phosphoric acid TS** Dissolve 0.2 g of 1,3-dihydroxynaphthalene in ethanol (99.5) to make 100 mL. To this solution add 10 mL of phosphoric acid.

**1-Naphthylamine**  $C_{10}H_7NH_2$  [K 8692, Special class] Preserve in light-resistant containers.

**α-Naphthylamine** See 1-naphthylamine.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate** See *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS** See *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate-acetone TS.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate TS** See *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS.

***N*-1-Naphthylethylenediamine dihydrochloride**  $C_{10}H_7NH_2CH_2CH_2NH_2 \cdot 2HCl$  [K 8197, Special class]

**Naphthylethylenediamine TS** Dissolve 0.1 g of *N*-1-naphthylethylenediamine dihydrochloride in water to make 100 mL. Prepare before use.

**Naringin for thin-layer chromatography**  $C_{27}H_{32}O_{14}$  White to light yellow crystalline powder. Freely soluble in ethanol (95) and in acetone, and slightly soluble in water. Melting point: about 170°C (with decomposition).

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : -87 - -93° (0.1 g, ethanol (95), 10 mL, 100 mm).

**Purity** Related substances—Proceed with 10  $\mu$ L of a solution, prepared by dissolving 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), as directed in the Identification under Bitter Orange Peel: any spot other than the principal spot with an *Rf* value of about 0.4 does not appear.

**Neocarzinostatin**  $C_{511}H_{768}N_{132}O_{179}S_4$  A white or pale yellow-white powder.

**Identification**—Determine the absorption spectrum of a solution of the substance to be examined (1 in 3000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 270 nm and 275 nm, and shoulders between 288 nm and 292 nm and between 330 nm and 360 nm.

**Purity**—Dissolve 10 mg of the substance to be examined in the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 0.25 mL of the sample solution as directed under Liquid Chromatography <2.01>, determine each peak area by the automatic integration method, and calculate the amount of neocarzinostatin by the area percentage method: not less than 90.0%.

#### Operating conditions

**Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).

**Column:** Pre-column is a stainless steel column 7.5 mm in inside diameter and 75 mm in length, packed with silica gel for liquid chromatography (10  $\mu$ m in particle size). Separation column is a stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with silica gel for liquid chromatography (10  $\mu$ m in particle size), which is coupled to the pre-column.

**Column temperature:** A constant temperature of about 25°C.

**Mobile phase:** Dissolve 3.78 g of potassium dihydrogen phosphate and 5.52 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

**Flow rate:** Adjust so that the retention time of neocarzinostatin is about 21 minutes.

**Time span of measurement:** About 2 times as long as the retention time of neocarzinostatin.

#### System suitability

**System performance:** When the procedure is run with 0.25 mL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of neocarzinostatin are not less than 2000 and not more than 2.5, respectively.

**System repeatability:** When the test is repeated 6 times with 0.25 mL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of neocarzinostatin is not more than 2.0%.

**Water** <2.48> Not more than 10.0% (10 mg, coulometric titration).

**Neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)** Condensate of neocarzinostatin and styrene-maleic acid alternating copolymer partial butyl ester in a rate of 2:3 by amide bond. Average molecular mass: about 28,400. A pale yellow powder.

**Identification**—Dissolve 4 mg of the substance to be examined in 0.05 mol/L phosphate buffer solution (pH 7.0) to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 266 nm and 270 nm, and shoulders between 257 nm and 262 nm, between 286 nm and 291 nm and between 318 nm and 348 nm.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (268 nm): 13.0 - 17.5 [4 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution (pH 7.0), 10 mL].

#### Purity (i) Test solutions

**Solution A:** Dissolve 36.6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.23 mL of *N,N,N',N'*-tetramethylethylenediamine and water to make 100 mL.

**Solution B:** Dissolve 33.3 g of acrylamide and 0.89 g of *N,N'*-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

**Solution C:** Dissolve 5.98 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.46 mL of *N,N,N',N'*-tetramethylethylenediamine and water to make 100 mL.

**Solution D:** Dissolve 10.0 g of acrylamide and 2.5 g of *N,N'*-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

**Solution E:** Dissolve 4 mg of riboflavin in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

**Solution F:** Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycine in water to make 500 mL.

Buffer solution for sample: To 50 mL of Solution C add 20 mL of water and 10 mL of glycerin solution (3 in 5).

(ii) Gels

Resolving gel: Mix 2.5 mL of Solution A and 7.5 mL of Solution B. Mix the mixture with 10 mL of freshly prepared ammonium peroxodisulfate solution (7 in 5000) after degassing under reduced pressure. Pour this mixture into a glass tube, 5 mm in inside diameter and 10 cm in length, to make 7 cm height, put water gently on the upper surface of the mixture, and allow to polymerize for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

Stacking gel: Mix 1 mL of Solution C, 2 mL of Solution D, 1 mL of Solution E and 4 mL of water, pour 0.2 mL of the mixture on the resolving gel, put water gently on the upper surface of the mixture, and allow to polymerize under a fluorescent light for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

iii) Sample solution Dissolve 3.0 mg of the substance to be examined in the buffer solution for sample to make 10 mL.

(iv) Procedure Mount the gel in electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the upper reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100  $\mu$ L of the sample solution onto the surface of the gel, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached 5 cm from the upper end of the gel.

(v) Staining and decolorization Dissolve 0.1 g of Coomassie brilliant blue G-250 in 100 mL of trichloroacetic acid solution (1 in 2), and mix 1 volume of this solution and 2 volumes of water before using. Immerse the gels for 15 hours in this mixture, and transfer into about 20 mL of acetic acid (100) solution (7 in 100) to remove the excess of dye. Replace the acetic acid (100) solution (7 in 100) until the background of the gel becomes colorless.

(vi) Determination Determine the peak area,  $A_T$ , of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) and the total area,  $A$ , of the peaks other than neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), based on the absorbance at 600 nm of the gel determined by using a densitometer. Calculate the amount of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) by the following formula: not less than 90.0%.

Amount (%) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)  
 $= A_T / (A_T + A) \times 100$

Water <2.48> Not more than 12.0% (10 mg, coulometric titration).

**Neutral alumina containing 4% of water** Take 50 g of neutral alumina for column chromatography, previously dried at 105°C for 2 hours, in a tight container, add 2.0 mL of water, shake well to make homogeneous, and allow to stand for more than 2 hours.

**Neutral detergent** Synthetic detergent containing anionic or non-ionic surfactant, and pH of its 0.25% solution is between 6.0 and 8.0. Dilute to a suitable concentration before use.

**Neutralized ethanol** See ethanol, neutralized.

**Neutral red**  $C_{15}H_{17}N_4Cl$  Slightly metallic, dark green powder or masses.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3310  $\text{cm}^{-1}$ , 3160  $\text{cm}^{-1}$ , 1621  $\text{cm}^{-1}$ , 1503  $\text{cm}^{-1}$ , 1323  $\text{cm}^{-1}$ , 1199  $\text{cm}^{-1}$  and 732  $\text{cm}^{-1}$ .

**Neutral red TS** Dissolve 0.1 g of neutral red in acetic acid (100) to make 100 mL.

**Neutral red-Eagle's minimum essential medium containing bovine serum** To Eagle's minimum essential medium containing bovine serum, which contains *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid but not sodium hydrogen carbonate, add a solution of neutral red (1 in 100), and adjust to pH 6.7 – 6.8 with sodium hydroxide TS.

**NFS-60 cell** Prepared from leukemia mouse, infected with retrovirus (Cas-Br-M). After conditioning with a suitable medium, preserve the strain established by J. N. Ihle, *et al.* (*Proc. Natl. Acad. Sci. USA*, 1985, 82, 6687) at not exceeding –150°C in conveniently sized packets.

**Nicardipine hydrochloride for assay**  $C_{26}H_{29}N_3O_6.HCl$  [Same as the monograph Nicardipine Hydrochloride. When dried, it contains not less than 99.0% of nicardipine hydrochloride ( $C_{26}H_{29}N_3O_6.HCl$ ).]

**Nicergoline for assay**  $C_{24}H_{26}BrN_3O_3$  [Same as the monograph Nicergoline, or Nicergoline purified according to the method below. When dried, it contains not less than 99.0% of nicergoline ( $C_{24}H_{26}BrN_3O_3$ ), and when perform the test of the Purity (2) under Nicergoline, the total area of the peaks other than nicergoline obtained from the sample solution is not more than 2.5 times the peak area of nicergoline from the standard solution.]

Method of purification: Dissolve 1 g of Nicergoline in 20 mL of acetonitrile, allow to stand in a dark place for about 36 hours, filter, and dry the crystals so obtained at 60°C for 2 hours in vacuum.]

**Nickel (II) sulfate hexahydrate**  $NiSO_4.6H_2O$  [K 8989, Special class]

**Nicomol for assay**  $C_{34}H_{32}N_4O_9$  [Same as the monograph Nicomol. When dried, it contains not less than 99.0% of nicomol ( $C_{34}H_{32}N_4O_9$ ).]

**Nicotinamide**  $C_6H_6N_2O$  [Same as the namesake monograph]

**$\beta$ -Nicotinamide-adenine dinucleotide ( $\beta$ -NAD)**  $C_{21}H_{27}N_7O_{14}P_2$  [K 9802  $\beta$ -NAD<sup>+</sup>, and meets the following requirement.]

**Content:** not less than 94.5%. Assay—Weigh accurately about 25 mg of  $\beta$ -nicotinamide-adenine dinucleotide, and dissolve in water to make exactly 25 mL. Pipet 0.2 mL of this solution, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL, and use this solution as the sample solution. Determine the absorbances,  $A_T$  and  $A_B$ , of the sample solution and 0.1 mol/L phosphate buffer solution (pH 7.0) at 260 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Amount (mg) of  $\beta$ -nicotinamide-adenine dinucleotide ( $C_{21}H_{27}N_7O_{14}P_2$ )

$$= \frac{0.6634 \times 10}{17.6 \times 0.20} \times (A_T - A_B) \times 25$$

**$\beta$ -Nicotinamide adenine dinucleotide TS** Dissolve 40 mg of  $\beta$ -nicotinamide adenine dinucleotide ( $\beta$ -NAD) in 10 mL of water. Prepare before use.

**$\beta$ -Nicotinamide adenine dinucleotide reduced form ( $\beta$ -NADH)**  $C_{21}H_{27}N_7O_{14}P_2.Na_2$  A white to light yellow-white powder.

**Absorbance ratio:** Determine the absorbances at 260 nm and at 340 nm,  $A_{260}$  and  $A_{340}$ , of a solution of  $\beta$ -nicotinamide adenine dinucleotide reduced form ( $\beta$ -NADH) in diluted phosphate buffer solution (pH 7.4) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; the result of  $A_{260}/A_{340}$  is between 2.2 and 2.4.

**Water <2.48>:** not more than 8.0% (0.3 g, volumetric titration, direct titration).

**$\beta$ -Nicotinamide adenine dinucleotide reduced form TS** Dissolve 0.4 mg of  $\beta$ -nicotinamide adenine dinucleotide reduced form ( $\beta$ -NADH) in 1 mL of 0.6 mol/L 2,2',2"-nitriolotriethanol hydrochloride buffer solution (pH 8.0). Prepare before use.

**Nicotinic acid**  $C_6H_5NO_2$  White, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum of nicotinic acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $2440\text{ cm}^{-1}$ ,  $1707\text{ cm}^{-1}$ ,  $1418\text{ cm}^{-1}$ ,  $811\text{ cm}^{-1}$ ,  $747\text{ cm}^{-1}$  and  $641\text{ cm}^{-1}$ .

**Nifedipine**  $C_{17}H_{18}N_2O_6$  [Same as the namesake monograph]

**Nifedipine for assay**  $C_{17}H_{18}N_2O_6$  [Same as the monograph Nifedipine. When dried, it contains not less than 99.0% of nifedipine ( $C_{17}H_{18}N_2O_6$ ) and meets the following requirement.]

**Purity Related substances**—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 25 mg of nifedipine for assay in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area by the automatic integration method: the total area of the peaks other than nifedipine obtained from the sample solution is not larger than the peak area of nifedipine from the standard solution.

#### Operating conditions

**Detector:** An ultraviolet absorption photometer (wavelength: 230 nm).

**Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** Adjust to pH 6.1 of a mixture of methanol and diluted 0.05 mol/L disodium hydrogen phosphate TS (1 in 5) (11:9) with phosphoric acid.

**Flow rate:** Adjust so that the retention time of nifedipine is about 6 minutes.

**Time span of measurement:** About 2 times as long as the retention time of nifedipine, beginning after the solvent peak.

#### System suitability

**Test for required detectability:** To exactly 5 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of nifedipine obtained with 10  $\mu$ L of this solution is equivalent to 18 to 32% of that with 10  $\mu$ L of the standard solution.

**System performance:** When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nifedipine are not less than 4000 and not more than 1.2, respectively.

**System repeatability:** When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nifedipine is not more than 2.0%.

**Nile blue**  $C_{20}H_{20}ClN_3O$  Blue-green powder.

**Ninhydrin**  $C_9H_6O_4$  [K 8870, Special class]

**Ninhydrin TS** Dissolve 0.2 g of ninhydrin in water to make 10 mL. Prepare before use.

**Ninhydrin-acetic acid TS** Dissolve 1.0 g of ninhydrin in 50 mL of ethanol (95), and add 10 mL of acetic acid (100).

**Ninhydrin-L-ascorbic acid TS** Dissolve 0.25 g of ninhydrin and 10 mg of L-ascorbic acid in water to make 50 mL. Prepare before use.

**Ninhydrin-butanol TS** Dissolve 0.3 g of ninhydrin in 100 mL of 1-butanol, and add 3 mL of acetic acid (100).

**Ninhydrin-citric acid-acetic acid TS** Dissolve 70 g of citric acid monohydrate in 500 mL of water, add 58 mL of acetic acid (100), 70 mL of a solution of sodium hydroxide (21 in 50) and water to make 1000 mL. In 100 mL of this solution dissolve 0.2 g of ninhydrin.

**Ninhydrin-ethanol TS for spraying** Dissolve 1 g of ninhydrin in 50 mL of ethanol (95).

**Ninhydrin-stannous chloride TS** See ninhydrin-tin (II) chloride TS.

**Ninhydrin-sulfuric acid TS** Dissolve 0.1 g of ninhydrin in 100 mL of sulfuric acid. Prepare before use.

**Ninhydrin-tin (II) chloride TS** Dissolve 21.0 g of citric acid monohydrate in water to make 200 mL, adjust the pH to  $5.6 \pm 0.2$  with sodium hydroxide TS, add water to make 500 mL, and dissolve 1.3 g of tin (II) chloride dihydrate. To 50 mL of the solution, add 50 mL of a 2-methoxyethanol solution of ninhydrin (1 in 25). Prepare before use.

**0.2% Ninhydrin-water saturated 1-butanol TS** Dissolve 2 g of ninhydrin in 1-butanol saturated with water to make 1000 mL.

**Nitrendipine for assay**  $C_{18}H_{20}N_2O_6$  [Same as the monograph Nitrendipine. It, when dried, contains not less than 99.0% of nitrendipine ( $C_{18}H_{20}N_2O_6$ ), and meets the following requirement. When perform the test as directed in the Purity (2) under Nitrendipine, the area of the peak of dimethyl ester, having the relative retention time of about 0.8 to nitrendipine obtained from the sample solution is not larger than 1/2 times the peak area of nitrendipine from the standard solution, the area of the peak other than nitrendipine and the dimethyl ester is not larger than 1/5 times the peak area of nitrendipine from the standard solution, and the total area of the peak other than nitrendipine is not larger than 1/2 times the peak area of nitrendipine from the standard solution.]

**Nitric acid**  $\text{HNO}_3$  [K 8541, Special class, Concentration: 69 – 70%, Density: about 1.42 g/mL]

**Nitric acid, dilute** Dilute 10.5 mL of nitric acid with water to make 100 mL.

**Nitric acid, fuming** [K 8739, Special class, Concentration: not less than 97%, Density: about 1.52 g/mL]

**Nitric acid TS, 2 mol/L** Dilute 12.9 mL of nitric acid with water to make 100 mL.

**Nitrilotriacetic acid**  $\text{C}_6\text{H}_9\text{NO}_6$  A white crystalline powder. Melting point: about 240°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of nitrilotriacetic acid as directed in the paste method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1718  $\text{cm}^{-1}$ , 1243  $\text{cm}^{-1}$ , 1205  $\text{cm}^{-1}$ , 968  $\text{cm}^{-1}$ , 903  $\text{cm}^{-1}$ , 746  $\text{cm}^{-1}$  and 484  $\text{cm}^{-1}$ .

**Loss on drying** <2.41>: not more than 0.5% (1 g, 105°C, 3 hours).

**Content:** not less than 97.0%. **Assay**—Weigh accurately about 0.2 g of nitrilotriacetic acid, dissolve in 50 mL of water by heating, and titrate <2.50> after cooling with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 9.557 mg of  $\text{C}_6\text{H}_9\text{NO}_6$

**2,2',2"-Nitrilotriethanol**  $(\text{CH}_2\text{CH}_2\text{OH})_3\text{N}$  [K 8663, Special class]

#### 2,2',2"-Nitrilotriethanol buffer solution (pH 7.8)

Dissolve 149.2 g of 2,2',2"-nitrilotriethanol in about 4500 mL of water, adjust to pH 7.8 with diluted 6 mol/L hydrochloric acid TS (2 in 3), and add water to make 5000 mL.

#### 2,2',2"-Nitrilotriethanol hydrochloride

$(\text{CH}_2\text{CH}_2\text{OH})_3\text{N}\cdot\text{HCl}$  White, crystals or powder.

**Purity** Clarity of solution—A solution (1 in 20) is clear.

**Content:** not less than 98%. **Assay**—Dissolve 0.3 g of 2,2',2"-nitrilotriethanol hydrochloride, accurately weighed, in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS  
= 18.57 mg of  $(\text{CH}_2\text{CH}_2\text{OH})_3\text{N}\cdot\text{HCl}$

**0.6 mol/L 2,2',2"-Nitrilotriethanol hydrochloride buffer solution (pH 8.0)** Dissolve 5.57 g of 2,2',2"-nitrilotriethanol hydrochloride in 40 mL of water, adjust to pH 8.0 with 5 mol/L sodium hydroxide TS, and add water to make 50 mL.

**3-Nitroaniline**  $\text{C}_6\text{H}_5\text{N}_2\text{O}_2$  Yellow, crystals or crystalline powder.

**Melting point** <2.60>: 112 – 116°C

**4-Nitroaniline**  $\text{C}_6\text{H}_4\text{NO}_2\text{NH}_2$  Yellow to yellowish-red, crystals or crystalline powder.

**Melting point** <2.60>: 147 – 150°C.

**Storage**—Preserve in a light-resistant tight container.

**p-Nitroaniline** See 4-nitroaniline.

**p-Nitroaniline-sodium nitrite TS** See 4-nitroaniline-sodium nitrite TS.

**4-Nitroaniline-sodium nitrite TS** To 90 mL of a solution of 0.3 g of 4-nitroaniline in 100 mL of 10 mol/L hydrochloric acid TS add 10 mL of a solution of sodium nitrite (1 in

20), and mix well. Prepare before use.

**o-Nitrobenzaldehyde** See 2-nitrobenzaldehyde.

**2-Nitrobenzaldehyde**  $\text{O}_2\text{NC}_6\text{H}_4\text{CHO}$  Pale yellow, crystals or crystalline powder.

**Melting point** <2.60>: 42 – 44°C

**Nitrobenzene**  $\text{C}_6\text{H}_5\text{NO}_2$  [K 8723, Special class]

**p-Nitrobenzenediazonium chloride TS** See 4-nitrobenzenediazonium chloride TS.

**4-Nitrobenzenediazonium chloride TS** Dissolve 1.1 g of 4-nitroaniline in 1.5 mL of hydrochloric acid, add 1.5 mL of water, and then add a solution prepared by dissolving 0.5 g of sodium nitrite in 5 mL of water, while cooling in an ice bath. Prepare before use.

**p-Nitrobenzenediazonium chloride TS for spraying** See 4-nitrobenzenediazonium chloride TS for spraying.

**4-Nitrobenzenediazonium chloride TS for spraying** Dissolve 0.4 g of 4-nitroaniline in 60 mL of 1 mol/L hydrochloric acid TS, and add, while cooling in an ice bath, sodium nitrite TS until the mixture turns potassium iodide-starch paper to blue in color. Prepare before use.

**p-Nitrobenzenediazonium fluoroborate** See 4-nitrobenzenediazonium fluoroborate.

#### 4-Nitrobenzenediazonium fluoroborate

$\text{O}_2\text{NC}_6\text{H}_4\text{N}_2\text{BF}_4$  Light yellow-white, almost odorless powder. Freely soluble in dilute hydrochloric acid, slightly soluble in water, and very slightly solute in ethanol (95) and in chloroform. Melting point: about 148°C (with decomposition).

**Identification**—Add 1 mL each of a solution of phenol (1 in 1000) and sodium hydroxide TS to 10 mL of a solution of 4-nitrobenzenediazonium fluoroborate (1 in 1000): a red color develops.

**Loss on drying** <2.41>: not more than 1.0% (1 g, silica gel, 2 hours).

**p-Nitrobenzoyl chloride** See 4-nitrobenzoyl chloride.

**4-Nitrobenzoyl chloride**  $\text{O}_2\text{NC}_6\text{H}_4\text{COCl}$  Light yellow crystals.

**Melting point** <2.60>: 70 – 74°C

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.5 g of 4-nitrobenzoyl chloride, add an excess of silver nitrate-ethanol TS, and boil under a reflux condenser for 1 hour. After cooling, filter the precipitate, wash with water, dry at 105°C to constant mass, and weigh. The mass of 4-nitrobenzoyl chloride, multiplied by 1.107, represents the mass of 4-nitrobenzoyl chloride ( $\text{C}_7\text{H}_4\text{ClNO}_3$ ).

**p-Nitrobenzyl chloride** See 4-nitrobenzyl chloride.

**4-Nitrobenzyl chloride**  $\text{O}_2\text{NC}_6\text{H}_4\text{CH}_2\text{Cl}$  Light yellow, crystals or crystalline powder. Soluble in ethanol (95).

**Melting point** <2.60>: 71 – 73°C

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.5 g of 4-nitrobenzyl chloride, add 15 mL of a solution prepared by dissolving 4 g of silver nitrate in 10 mL of water and adding ethanol (95) to make 100 mL, and heat on a water bath under a reflux condenser for 1 hour. After cooling, filter the precipitate with a glass filter, wash with water, dry at 105°C to constant mass, and weigh. The mass of the precipitate represents the amount of silver chloride ( $\text{AgCl}$ : 143.32).

Amount (mg) of 4-nitrobenzyl chloride ( $\text{C}_7\text{H}_4\text{ClNO}_2$ )  
= amount (mg) of silver chloride ( $\text{AgCl}$ ) × 1.1972

**4-(4-Nitrobenzyl)pyridine**  $C_{12}H_{10}N_2O_2$  Pale yellow, crystalline powder. Freely soluble in acetone, and soluble in ethanol (95).

*Melting point* <2.60>: 69 – 71°C

**Nitroethane**  $C_2H_5NO_2$

*Density* <2.56>: 1.048 – 1.053 g/cm<sup>3</sup> (20°C)

*Water* <2.48>: not more than 0.1%.

**Nitrogen**  $N_2$  [Same as the namesake monograph]

**Nitrogen monoxide** NO A colorless gas. Prepare by adding sodium nitrite TS to a solution of iron (II) sulfate heptahydrate in dilute sulfuric acid. Nitrogen monoxide from a metal cylinder may be used.

**Nitromethane**  $CH_3NO_2$  [K 9523, Special class]

**2-Nitrophenol**  $C_6H_5NO_3$  A yellow crystalline powder.

*Melting points* <2.60>: 44.5 – 49.0°C

**3-Nitrophenol**  $C_6H_5NO_3$  A light yellow crystalline powder.

*Melting point* <2.60>: 96 – 99°C

**4-Nitrophenol**  $C_6H_5NO_3$  [K 8721, *p*-nitrophenol, Special class]

***o*-Nitrophenyl-β-D-galactopyranoside** See 2-nitrophenyl-β-D-galactopyranoside.

**2-Nitrophenyl-β-D-galactopyranoside**  $C_{12}H_{15}NO_8$

White crystalline powder. Odorless. It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

*Melting point* <2.60>: 193 – 194°C

**Purity** Clarity and color of solution—A solution of 2-nitrophenyl-β-D-galactopyranoside (1 in 100) is clear and colorless.

**Loss on drying** <2.41>: not more than 0.1% (0.5 g, 105°C, 2 hours).

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 50 mg of 2-nitrophenyl-β-D-galactopyranoside, previously dried, dissolve in water to make exactly 100 mL. Pipet 20 mL of this solution, and add water to make exactly 50 mL. Determine the absorbance, *A*, of this solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of 2-nitrophenyl-}\beta\text{-D-galactopyranoside} \\ = \frac{A}{133} \times 25,000 \end{aligned}$$

**1-Nitroso-2-naphthol**  $C_{10}H_7NO_2$  A yellow-brown to red-brown crystalline powder.

*Melting point* <2.60>: 106 – 110°C

**Storage**—Preserve in a light-resistant tight container.

**1-Nitroso-2-naphthol TS** Dissolve 60 mg of 1-nitroso-2-naphthol in 80 mL of acetic acid (100), and add water to make 100 mL.

**α-Nitroso-β-naphthol** See 1-nitroso-2-naphthol.

**α-Nitroso-β-naphthol TS** See 1-nitroso-2-naphthol TS.

**Nitrous oxide**  $N_2O$  Colorless and odorless gas. Use nitrous oxide from a metal cylinder.

**NK-7 cells** Cells derived from mouse NK cells.

**NN Indicator** Mix 0.5 g of 2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid with 50 g of anhydrous sodium sulfate, and triturate until the mixture becomes homogeneous.

**Nodakenin for thin-layer chromatography**  $C_{20}H_{24}O_9$

White powder. Slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5). Melting point: about 220°C (with decomposition).

**Identification**—Determine the absorption spectrum of a solution of nodakenin for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 333 nm and 337 nm.

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : +50 – +68° (5 mg, methanol, 10 mL, 100 mm).

**Purity** Related substances—Dissolve 1 mg of nodakenin for thin-layer chromatography in 3 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed with 5 µL each of these solutions as directed in the Identification (2) under Peucedanum Root: the spot other than the principal spot of around *Rf* value of 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

**Nonessential amino acid TS** Dissolve 89 mg of L-alanine, 150 mg of L-asparagine monohydrate, 133 mg of L-aspartic acid, 147 mg of L-glutamic acid, 75 mg of glycine, 115 mg of L-proline and 105 mg of L-serine in 100 mL of water, and sterilize by filtration through a membrane filter with a pore size not exceeding 0.22 µm.

**Nonylphenoxypoly(ethyleneoxy)ethanol for gas chromatography** Prepared for gas chromatography.

**L-Norleucine**  $C_6H_{13}NO_2$  White, crystals or powder. Dissolves in water.

**Normal agar media for tecfleukin** Dissolve 5.0 g of meat extract, 10.0 g of peptone, 5.0 g of sodium chloride, and 15.0 to 20.0 g of agar in water to make 1000 mL, and sterilize. Adjust the pH to 6.9 to 7.1.

**Nortriptyline hydrochloride**  $C_{19}H_{21}N.HCl$  [Same as the namesake monograph]

**Nortriptyline hydrochloride for assay**  $C_{19}H_{21}N.HCl$  [Same as the monograph Nortriptyline Hydrochloride. When dried, it contains not less than 99.0% of nortriptyline hydrochloride ( $C_{19}H_{21}N.HCl$ ).]

**Nuclease-free water** See water, nuclease-free.

**n-Octadecane**  $C_{18}H_{38}$  Colorless or white solid at ordinary temperature.

**Purity** Clarity of solution—A solution of *n*-octadecane in chloroform (1 in 25) is clear.

**Octadecylsilanized silica gel for pretreatment** Prepared for pretreatment.

**n-Octane**  $C_8H_{18}$

*Specific gravity* <2.56>  $d_4^{20}$ : 0.700 – 0.705

**Purity**—Perform the test with 2 µL of *n*-octane as directed under Gas Chromatography <2.02> according to the operating conditions in the Assay under Hypromellose. Determine each peak area by the automatic integration method, and calculate the amount of *n*-octane by the area percentage method: not less than 99.0%.

**Octane, iso** A colorless liquid. Practically insoluble in water. Miscible with diethyl ether and with chloroform.

**Purity**—Determine the absorbances of isoctane at 230 nm, 250 nm and 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank solution:

these values are not more than 0.050, 0.010 and 0.005, respectively.

**1-Octanol**  $\text{CH}_3(\text{CH}_2)_6\text{CH}_2\text{OH}$  [K 8213, Special class]

**Octyl alcohol** See 1-octanol.

**n-Octylbenzene**  $\text{C}_{14}\text{H}_{22}$  Clear and colorless liquid, having a characteristic odor.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.854 – 0.863

*Distillation test* <2.57>: 263 – 265°C, not less than 95 vol%.

**Oflloxacin**  $\text{C}_{18}\text{H}_{20}\text{FN}_3\text{O}_4$  [Same as the namesake monograph]

**Oflloxacin demethyl substance** ( $\pm$ )-9-Fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-10-(1-piperazinyl)-pyrido[1,2,3-*d*]-[1,4]benzoxazine-6-carboxylic acid  $\text{C}_{17}\text{H}_{18}\text{FN}_3\text{O}_4$  White to light green-yellow-white, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum of ofloxacin demethyl substance as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3050  $\text{cm}^{-1}$ , 2840  $\text{cm}^{-1}$ , 1619  $\text{cm}^{-1}$ , 1581  $\text{cm}^{-1}$ , 1466  $\text{cm}^{-1}$ , 1267  $\text{cm}^{-1}$ , 1090  $\text{cm}^{-1}$ , 1051  $\text{cm}^{-1}$  and 816  $\text{cm}^{-1}$ .

**Oleic acid**  $\text{C}_{18}\text{H}_{34}\text{O}_2$  Occurs as a colorless or pale yellow transparent liquid and has a slightly distinct odor. It is miscible with ethanol (95) and with diethyl ether, and practically insoluble in water.

*Specific gravity* <2.56>  $d_2^{20}$ : about 0.9.

**Content:** not less than 99.0%. **Assay**—To 40  $\mu\text{L}$  of oleic acid add 1 mL of a solution of boron trifluoride in methanol (3 in 20), mix, and heat on a water bath for 3 minutes. After cooling, add 10 mL of petroleum ether and 10 mL of water, shake, collect the ether layer after allowing to stand, and use this solution as the sample solution. Perform the test with 0.2  $\mu\text{L}$  of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of methyl oleate by the area percentage method.

#### Operating conditions

**Detector:** A hydrogen flame-ionization detector

**Column:** A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (149 – 177  $\mu\text{m}$ ) coated with methyl polyacrylate in a rate of 5 – 10%.

**Column temperature:** A constant temperature of about 220°C.

**Carrier gas:** Helium.

**Flow rate:** Adjust so that the retention time of methyl oleate is about 10 minutes.

**Time span of measurement:** About 2 times as long as the retention time of methyl oleate, beginning after the solvent peak.

**Olive oil** [Same as the namesake monograph]

**Olopatadine hydrochloride for assay**  $\text{C}_{21}\text{H}_{23}\text{NO}_3\cdot\text{HCl}$  [Same as the monograph Olopatadine Hydrochloride. When dried, it contains not less than 99.5% of olopatadine hydrochloride ( $\text{C}_{21}\text{H}_{23}\text{NO}_3\cdot\text{HCl}$ ).]

**Omeprazole for assay**  $\text{C}_{17}\text{H}_{19}\text{N}_3\text{O}_3\text{S}$  [Same as the monograph Omeprazole]

**Ophiopogon root** [Same as the namesake monograph]

**Orcine**  $\text{C}_7\text{H}_3\text{O}_2$  White to light red-brown, crystals or crystalline powder, having an unpleasant sweet taste. It turns

to red in color when oxidized in air. Soluble in water, in ethanol (95), and in diethyl ether.

*Meting point* <2.60>: 107 – 111°C

**Orcine-ferric chloride TS** See orcine-iron (III) chloride TS.

**Orcine-iron (III) chloride TS** Dissolve 10 mg of orcine in 1 mL of a solution of iron (III) chloride hexahydrate in hydrochloric acid (1 in 1000). Prepare before use.

**Ordinary agar medium** Dissolve 25 to 30 g of agar in 1000 mL of ordinary broth with the aid of heat, add water to make up for the loss, adjust the pH to between 6.4 and 7.0, and filter. Dispense the filtrate, and sterilize by autoclaving. When powdered agar is used, 15 to 20 g of it is dissolved.

**Ordinary broth** Dissolve 5 g of beef extract and 10 g of peptone in 1000 mL of water by gentle heating. Adjust the pH of the mixture between 6.4 and 7.0 after sterilization, cool, add water to make up for the loss, and filter. Sterilize the filtrate by autoclaving for 30 minutes at 121°C.

**Osthole for thin-layer chromatography**  $\text{C}_{15}\text{H}_{16}\text{O}_3$  A white crystalline powder, having no odor. Freely soluble in methanol and in ethyl acetate, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 83 – 84°C.

**Purity** Related substances—Dissolve 1.0 mg of osthole for thin-layer chromatography in 1 mL of methanol. Perform the test with 10  $\mu\text{L}$  of this solution as directed in the Identification under Cnidium Monnier Fruit: on spot appears other than the principal spot at around *Rf* value of 0.3.

**Oxalate pH standard solution** See pH Determination <2.54>.

**Oxalic acid** See oxalic acid dihydrate.

**Oxalic acid dihydrate**  $\text{H}_2\text{C}_2\text{O}_4\cdot2\text{H}_2\text{O}$  [K 8519, Special class]

**Oxalic acid TS** Dissolve 6.3 g of oxalic acid dihydrate in water to make 100 mL (0.5 mol/L).

**Oxycodone hydrochloride for assay** See oxycodone hydrochloride hydrate for assay.

**Oxycodone hydrochloride hydrate for assay**

$\text{C}_{18}\text{H}_{21}\text{NO}_4\cdot\text{HCl}\cdot3\text{H}_2\text{O}$  [Same as the monograph Oxycodone Hydrochloride Hydrate. It contains not less than 99.0% of oxycodone hydrochloride ( $\text{C}_{18}\text{H}_{21}\text{NO}_4\cdot\text{HCl}$ ), calculated on the anhydrous basis.]

**Oxygen**  $\text{O}_2$  [K 1101]

**Oxygen reference gas for assay** Containing not less than 99.99 vol% of oxygen in a sealed pressure-resistant container.

**Oxygen span gas for assay** Containing not less than 99.7 vol% of oxygen in a sealed pressure-resistant container.

**Oxygen zero gas for assay** Containing not less than 99.99 vol% of nitrogen or argon in a sealed pressure-resistant container. Or, containing oxygen of 98 to 99 vol% of a minimum scale within the measuring range, and nitrogen or argon is employed as a diluent gas.

**2-Oxy-1-(2'-oxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid** See 2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid.

**8-Oxyquinoline** See 8-quinolinol.

**Oxytocin**  $\text{C}_{43}\text{H}_{66}\text{N}_{12}\text{O}_{12}\text{S}_2$  [Same as the namesake

monograph]

**Paeoniflorin for thin-layer chromatography**  $C_{23}H_{28}O_{11}$   
A white powder. Freely soluble in water, in methanol and in ethanol (99.5).

**Identification** Determine the infrared absorption spectrum of paeoniflorin to be examined as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3410\text{ cm}^{-1}$ ,  $1711\text{ cm}^{-1}$ ,  $1279\text{ cm}^{-1}$ ,  $823\text{ cm}^{-1}$  and  $714\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 1 mg of paeoniflorin to be examined in exactly 1 mL of methanol. Perform the test with  $20\text{ }\mu\text{L}$  of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot with an *R<sub>f</sub>* value of about 0.3 does not appear.

**Paeonol for assay**  $C_9H_{10}O_3$  Use paeonol for thin-layer chromatography meeting the following additional specifications. Correct the content based on the amount (%) obtained in the Assay.

**Unity of peak**—Dissolve 5 mg of paeonol for assay in 50 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with  $10\text{ }\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of paeonol peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

#### Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Moutan Bark.

Detector: A photodiode array detector (wavelength: 274 nm, spectrum range of measurement: 220 – 400 nm).

#### System suitability

System performance: Proceed as directed in the system suitability in the Assay under Moutan Bark.

**Assay**—Weigh accurately 5 mg of paeonol for assay and 1 mg of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities, *A*<sub>1</sub> (equivalent to 2 hydrogen) and *A*<sub>2</sub> (equivalent to 1 hydrogen), of the signals around  $\delta$  6.17 – 6.25 ppm and  $\delta$  7.54 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\begin{aligned}\text{Amount (\%)} \text{ of paeonol } (C_9H_{10}O_3) \\ = M_S \times I \times P / (M \times N) \times 0.7336\end{aligned}$$

*M*: Amount (mg) of paeonol for assay taken

*M<sub>S</sub>*: Amount (mg) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

*I*: Sum of the signal resonance intensities, *A*<sub>1</sub> and *A*<sub>2</sub>, based on the signal resonance intensity of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

*N*: Sum of numbers of the hydrogen derived from *A*<sub>1</sub> and *A*<sub>2</sub>

*P*: Purity (%) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic

resonance spectroscopy

#### Operating conditions

Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

<sup>13</sup>C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, SN ratio of the two signals of around  $\delta$  6.17 –  $\delta$  6.25 ppm and  $\delta$  7.54 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals of around  $\delta$  6.17 –  $\delta$  6.25 ppm and  $\delta$  7.54 ppm are not overlapped with any signal of obvious foreign substance, and the ratios of the resonance intensities, (*A*<sub>1</sub>/2)/*A*<sub>2</sub>, of each signal around  $\delta$  6.17 –  $\delta$  6.25 ppm and  $\delta$  7.54 ppm are between 0.99 and 1.01, respectively.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity, *A*<sub>1</sub> or *A*<sub>2</sub>, to that of the reference standard for qNMR is not more than 1.0%.

**Paeonol for component determination** See paeonol for assay.

#### Paeonol for thin-layer chromatography

$C_9H_{10}O_3$   
White, crystals or crystalline powder, having a specific odor. Freely soluble in methanol and in diethyl ether, and slightly soluble in water. Melting point: about 50°C.

**Purity** Related substances—Dissolve 1.0 mg of paeonol for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with  $10\text{ }\mu\text{L}$  of this solution as directed in the Identification under Moutan Bark: any spot other than the principal spot at the *R<sub>f</sub>* value of near 0.5 does not appear.

**Palladium chloride** See palladium (II) chloride.

**Palladium chloride TS** See palladium (II) chloride TS.

**Palladium (II) chloride**  $PdCl_2$  [K 8154, Special class]

**Palladium (II) chloride TS** Dissolve 0.2 g of palladium (II) chloride in 500 mL of 0.25 mol/L sulfuric acid TS, by heating if necessary, cool, and add 0.25 mol/L sulfuric acid TS to make 1000 mL.

**Palmatin chloride**  $C_{21}H_{22}ClNO_4$  A yellow-brown crystalline powder.

**Purity** Related substances—Dissolve 1 mg of palmatin chloride in 10 mL of methanol, and use this solution as the sample solution. Proceed with  $20\text{ }\mu\text{L}$  of the sample solution as directed in the Assay under Phellodendron Bark: when measure the peak areas for 2 times the retention time of berberine, the total area of the peaks other than palmatin is not larger than 1/10 times the total area except the area of sol-

vent peak.

**Palmitic acid for gas chromatography**  $C_{16}H_{32}O_2$  [K 8756, Special class]

**Pancreatic digest of casein** See Peptone, casein.

**Pancreatic digest of gelatin** See Peptone, gelatin.

**Papaic digest of soya bean** See Peptone, soybean.

**Papaverine hydrochloride**  $C_{20}H_{21}NO_4 \cdot HCl$  [Same as the namesake monograph]

**Papaverine hydrochloride for assay**  $C_{20}H_{21}NO_4 \cdot HCl$  [Same as the monograph Papaverine Hydrochloride. When dried, it contains not less than 99.0% of papaverine hydrochloride ( $C_{20}H_{21}NO_4 \cdot HCl$ ).]

**Paraffin** [Same as the namesake monograph]

**Paraffin, light liquid** [Same as the namesake monograph]

**Parahydroxybenzoic acid**  $C_7H_6O_3$  White crystals.

*Melting point* <2.60>: 212 – 216°C

**Content:** not less than 98.0 %. Assay—Weigh accurately about 0.7 g of parahydroxybenzoic acid, dissolve in 50 mL of acetone, add 100 mL of water, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Each mL of 0.5 mol/L sodium hydroxide VS} \\ = 69.06 \text{ mg of } C_7H_6O_3$$

**Particle counter apparatus** An apparatus that is able to count the fine particles derived from reticulocyte similar cells.

**Patent blue**  $C_{27}H_{31}N_2NaO_6S_2$  Red-purple-brown to dark red-brown, crystalline powder to powder, or masses.

**Identification**—(1) To 5 mg of patent blue add 20 mL of ethanol (99.5); a dark blue color develops.

(2) Determine the infrared absorption spectrum of patent blue as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $1580\text{ cm}^{-1}$ ,  $1420\text{ cm}^{-1}$ ,  $1340\text{ cm}^{-1}$ ,  $1180\text{ cm}^{-1}$ ,  $1150\text{ cm}^{-1}$ ,  $1070\text{ cm}^{-1}$ ,  $1030\text{ cm}^{-1}$ ,  $910\text{ cm}^{-1}$ ,  $790\text{ cm}^{-1}$ ,  $700\text{ cm}^{-1}$  and  $620\text{ cm}^{-1}$ .

**2-fold PCR reaction solution containing SYBR Green** 2-Fold reaction solution for real-time PCR, containing SYBR Green.

**Peanut oil** [Same as the namesake monograph]

**Pemirolast potassium**  $C_{10}H_7KN_6O$  [Same as the namesake monograph]

**Pentane**  $CH_3(CH_2)_3CH_3$  Clear and colorless liquid.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.620 – 0.650

*Distilling range* <2.57>: 35.5 – 37°C, not less than 98 vol%.

**Peptic digest of animal tissue** See Peptone, animal tissue.

**Peptone** Prepared for microbial test.

**Peptone, animal tissue** Prepared for microbial test.

**Peptone, casein** Grayish yellow powder, having a characteristic but not putrescent odor. It dissolves in water, but not in ethanol (95) and in diethyl ether.

*Loss on drying* <2.41>: not more than 7% (0.5 g, 105°C, constant mass).

*Residue on ignition* <2.44>: not more than 15% (0.5 g).

**Degree of digestion**—Dissolve 1 g of casein peptone in 10 mL of water, and perform the following test using this solution as the sample solution:

(1) Overlay 1 mL of the sample solution with 0.5 mL of a mixture of 1 mL of acetic acid (100) and 10 mL of dilute ethanol: no ring or precipitate forms at the junction of the two liquids, and on shaking, no turbidity results.

(2) Mix 1 mL of the sample solution with 4 mL of a saturated solution of zinc sulfate heptahydrate: a small quantity of precipitate is produced (proteoses).

(3) Filter the mixture of (2), and to 1 mL of the filtrate add 3 mL of water and 4 drops of bromine TS: a red-purple color is produced.

*Nitrogen content* <1.08>: not less than 10% (105°C, constant mass, after drying).

**Peptone, gelatin** Prepared for microbial test.

**Peptone, soybean** Prepared for microbial test.

**Perchloric acid**  $HClO_4$  [K 8223, Special class, Density: about 1.67 g/mL. Concentration: 70.0 – 72.0%]

**Perchloric acid-dehydrated ethanol TS** See perchloric acid-ethanol TS.

**Perchloric acid-ethanol TS** Add cautiously 25.5 mL of perchloric acid to 50 mL of ethanol (99.5), cool, and add ethanol (99.5) to make 100 mL (3 mol/L).

**Formic acid** Mix 9 volumes of formic acid and 1 volume of hydrogen peroxide (30), and leave at room temperature for 2 hours.

**Storage**—Store in a cool place.

**Perillaldehyde for assay**  $C_{10}H_{14}O$  Perillaldehyde for thin-layer chromatography meeting the following specifications.

*Absorbance* <2.24>  $E_{cm}^{\%}$  (230 nm): 850 – 950 (10 mg, methanol, 2000 mL).

**Purity** Related substances—Dissolve 10 mg of perillaldehyde for assay in 250 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the total area of the peaks other than perillaldehyde obtained from the sample solution is not larger than perillaldehyde from the standard solution.

**Operating conditions**

**Detector:** An ultraviolet absorption photometer (wavelength: 230 nm).

**Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of water and acetonitrile (13:7).

**Flow rate:** 1.0 mL per minute.

**Time span of measurement:** About 3 times as long as the retention time of perillaldehyde, beginning after the solvent peak.

**System suitability**

**Test for required detectability:** Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm

that the peak area of perillaldehyde obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

**System performance:** Dissolve 1 mg of (*E*)-asarone in 50 mL of the standard solution. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, perillaldehyde and (*E*)-asarone are eluted in this order with the resolution between these peaks being not less than 1.5.

**System repeatability:** When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of perillaldehyde is not more than 1.5%.

**Perillaldehyde for component determination** See perillaldehyde for assay.

**Perillaldehyde for thin-layer chromatography**  $C_{10}H_{14}O$   
Colorless to light brown transparent liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and very slightly soluble in water.

**Identification**—Determine the infrared absorption spectrum of perillaldehyde for thin-layer chromatography as directed in the liquid film method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3080  $cm^{-1}$ , 2930  $cm^{-1}$ , 1685  $cm^{-1}$ , 1644  $cm^{-1}$ , 1435  $cm^{-1}$  and 890  $cm^{-1}$ .

**Purity Related substances**—Dissolve 1.0 mg of perillaldehyde for thin-layer chromatography in 10 mL of methanol, and perform the test with 10  $\mu$ L of this solution as directed in the Identification under Perilla Herb: no spot other than the principal spot at around *R<sub>f</sub>* value of 0.5 appears.

**Peroxidase** Obtained from horse-radish. A red-brown powder. It is freely soluble in water. It contains about 250 units per mg. One unit indicates an amount of the enzyme which produces 1 mg of purpurogallin in 20 seconds at 20°C and pH 6.0, from pyrogallol and hydrogen peroxide (30) used as the substrate.

**Peroxidase labeled anti-rabbit antibody** It is prepared as follows: Immunize small animals with rabbit immunoglobulin G to obtain the antiserum. From the obtained antiserum the specific antibody is separated by the affinity chromatography using a column coupled with rabbit immunoglobulin G, and the specific antibody is labeled with peroxidase by the periodic acid method.

**Peroxidase labeled anti-rabbit antibody TS** Dissolve 0.10 g of bovine serum albumin in phosphate-buffered sodium chloride TS to make 100 mL. To 15 mL of this solution add 5  $\mu$ L of peroxidase labeled anti-rabbit antibody. Prepare before use.

**Peroxidase-labeled avidin** A solution of avidin conjugated with horseradish peroxidase in an appropriate buffer solution.

**Peroxidase-labeled avidin TS** Dilute peroxidase-labeled avidin with 0.01 mol/L tris buffer solution-sodium chloride TS (pH 7.4) so that the concentration of peroxidase-labeled avidin is 0.3  $\mu$ g/mL. Prepare before use.

**Peroxidase-labeled bradykinin** A solution of horseradish origin peroxidase-binding bradykinin in gelatin-phosphate buffer solution (pH 7.0). A colorless to light brown clear solution.

**Peroxidase-labeled bradykinin TS** To 0.08 mL of peroxidase-labeled bradykinin, 8 mg of sodium tetraborate decahydrate, 8 mg of bovine serum albumin and 0.8 mL of gelatin-phosphate buffer solution (pH 7.0) add water to

make 8 mL, and lyophilize. Dissolve this in 8 mL of water. Prepare before use.

**Perphenazine maleate for assay**  $C_{21}H_{26}ClN_3OS.2C_4H_4O_4$   
[Same as the monograph Perphenazine Maleate. When dried, it contains not less than 99.0% of perphenazine maleate ( $C_{21}H_{26}ClN_3OS.2C_4H_4O_4$ ).]

**Pethidine hydrochloride for assay**  $C_{15}H_{21}NO_2.HCl$   
[Same as the monograph Pethidine Hydrochloride. When dried, it contains not less than 99.0% of pethidine hydrochloride  $C_{15}H_{21}NO_2.HCl$ .]

**Petrolatum** [Same as the monograph Yellow Petrolatum or White Petrolatum]

**Petroleum benzine** [K 8594, Special class]

**Petroleum ether** [K 8593, Special class]

**Peucedanum·ledebourielloides for purity** Powder of the root and rhizome of *Peucedanum ledebourielloides* K. T. Fu (*Umbelliferae*).

**Identification**—Place 1.0 g of peucedanum·ledebourielloides for purity in a glass-stoppered centrifuge tube, add 5 mL of hexane, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (20:10:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): blue fluorescent spots at *R<sub>f</sub>* values of about 0.35 (agasyllin) and about 0.4 [xanthalin ( $C_{24}H_{26}O_7$ )] are observed.

**Phenacetin**  $C_{10}H_{13}NO_2$  White, crystals or crystalline powder. Soluble in ethanol (95), and very slightly soluble in water.

**Melting point** <2.60>: 134 – 137°C

**Loss on drying** <2.41>: not more than 0.5% (1 g, 105°C, 2 hours).

***o*-Phenanthroline** See 1,10-phenanthroline monohydrate.

***o*-Phenanthroline hydrochloride** See 1,10-phenanthroline monohydrate.

**1,10-Phenanthroline monohydrate**  $C_{12}H_8N_2\cdot H_2O$   
[K 8789, Special class]

***o*-Phenanthroline TS** See 1,10-phenanthroline TS.

**1,10-Phenanthroline TS** Dissolve 0.15 g of 1,10-phenanthroline monohydrate in 10 mL of a freshly prepared iron (II) sulfate heptahydrate solution (37 in 2500) and 1 mL of dilute sulfuric acid. Preserve in tightly stoppered containers.

**1,10-Phenanthrolinium chloride monohydrate**  
 $C_{12}H_8N_2\cdot HCl\cdot H_2O$  [K 8202, Special class]

**Phenethylamine hydrochloride**  $C_6H_5CH_2CH_2NH_2\cdot HCl$   
White, crystals or crystalline powder.

**Melting point:** <2.60> 220 – 225°C

**Phenobarbital for assay**  $C_{12}H_{12}N_2O_3$  [Same as the monograph Phenobarbital]

**Phenol**  $C_6H_5OH$  [K 8798, Special class]

**Phenol for assay**  $C_6H_5OH$  [K 8798, Phenol, Special class]

**Phenol-hydrochloric acid TS** Dissolve 0.2 g of phenol in 10 mL of 6 mol/L hydrochloric acid TS.

**Phenolphthalein**  $C_{20}H_{14}O_4$  [K 8799, Special class]

**Phenolphthalein-thymol blue TS** Solution A: Dissolve 0.1 g of phenolphthalein in 100 mL of diluted ethanol (4 in 5). Solution B: Dissolve 0.1 g of thymol blue in 50 mL of a mixture of ethanol (95) and dilute sodium hydroxide TS (250:11), add water to make 100 mL. Mix 2 volumes of solution A and 3 volumes of solution B before use.

**Phenolphthalein TS** Dissolve 1 g of phenolphthalein in 100 mL of ethanol (95).

**Phenolphthalein TS, alkaline** See Alcohol Number Determination <1.01>.

**Phenolphthalein TS, dilute** Dissolve 0.1 g of phenolphthalein in 80 mL of ethanol (95), and add water to make 100 mL.

**Phenol red**  $C_{19}H_{14}O_5S$  [K 8800, Special class]

**Phenol red TS** Dissolve 0.1 g of phenol red in 100 mL of ethanol (95), and filter if necessary.

**Phenol red TS, dilute** To 235 mL of a solution of ammonium nitrate (1 in 9400) add 105 mL of 2 mol/L sodium hydroxide TS and 135 mL of a solution prepared by dissolving 24 g of acetic acid (100) in water to make 200 mL. To this solution add 25 mL of a solution prepared by dissolving 33 mg of phenol red in 1.5 mL of 2 mol/L sodium hydroxide TS and adding water to make 100 mL. If necessary, adjust the pH to 4.7.

**Phenol-sodium nitroprusside TS** See phenol-sodium pentacyanonitrosylferrate (III) TS.

**Phenol-sodium pentacyanonitrosylferrate (III) TS** Dissolve 5 g of phenol and 25 mg of sodium pentacyanonitrosylferrate (III) dihydrate in sufficient water to make 500 mL. Preserve in a dark, cold place.

**Phenolsulfonphthalein for assay**  $C_{19}H_{14}O_5S$  [Same as the monograph Phenolsulfonphthalein. When dried, it contains not less than 99.0% of phenolsulfonphthalein ( $C_{19}H_{14}O_5S$ ).]

**Phenylalanine** See L-phenylalanine.

**L-Phenylalanine**  $C_9H_{11}NO_2$  [Same as the namesake monograph]

**H-D-phenylalanyl-L-pipecolyl-L-arginyl-p-nitroanilide dihydrochloride** A white powder. Slightly soluble in water.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (316 nm): 192 – 214 (10 mg, water, 300 mL).

**Phenyl benzoate**  $C_6H_5COOC_6H_5$  White, crystals or crystalline powder, having a slight, characteristic odor.

*Melting point* <2.60>: 68 – 70°C

*Purity* Clarity of solution—Dissolve 1.0 g of phenyl benzoate in 20 mL of methanol: the solution is clear.

**25% Phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography** Prepared for gas chromatography.

***o*-Phenylenediamine**  $H_2NC_6H_4NH_2$  White to dark brown, crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and soluble in water.

*Content:* not less than 95.0%. Assay—Accurately weigh about 0.15 g of *o*-phenylenediamine, add 50 mL of acetic acid for nonaqueous titration to dissolve, and then titrate <2.50> with 0.1 mol/L of perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 10.81 mg of  $H_2NC_6H_4NH_2$

#### ***o*-Phenylenediamine dihydrochloride**

$H_2NC_6H_4NH_2 \cdot 2HCl$  White to pale yellow or pale red, crystals or crystalline powder.

*Purity* Clarity—a solution (1 in 20) is clear.

*Content:* not less than 98.0%. Assay—Weigh accurately about 0.15 g of *o*-phenylenediamine dihydrochloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 9.053 mg of  $H_2NC_6H_4NH_2 \cdot 2HCl$

**1,3-Phenylenediamine hydrochloride**  $C_6H_8N_2 \cdot 2HCl$  A white or faintly reddish crystalline powder. It is colored to red or brown by light.

*Identification*—To 3 mL of a solution of 1,3-phenylenediamine hydrochloride (1 in 6000) add 0.5 mL of a solution of sodium nitrite (3 in 20,000), then add 2 to 3 drops of hydrochloric acid: a yellow color is produced.

**(S)-1-Phenylethyl isocyanate**  $C_6H_5CH(CH_3)NCO$

Colorless to light yellow, clear liquid, having a characteristic odor.

*Optical rotation* <2.49>  $\alpha_D^{20}$ : –8.5 – –11.5° (100 mm).

*Specific gravity* <2.56>  $d_4^{20}$ : 1.040 – 1.050

**Phenylfluorone**  $C_{19}H_{12}O_5$  [K 9547, Special class]

**Phenylfluorone-ethanol TS** Dissolve 50 mg of phenylfluorone in ethanol (95) and in 10 mL of diluted hydrochloric acid (1 in 3), and add ethanol (95) to make exactly 500 mL.

**D-Phenylglycine**  $C_8H_9NO_2$  White, crystals or crystalline powder. Slightly soluble in water.

*Loss on drying* <2.41>: not more than 0.5% (1 g, 105°C, 3 hours).

*Content:* not less than 98.5%. Assay—Weigh accurately about 0.3 g of D-phenylglycine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 15.12 mg of  $C_8H_9NO_2$

**Phenylhydrazine**  $C_6H_5NHNH_2$  Colorless or light yellow, clear liquid, having a faint aromatic odor.

*Content:* not less than 99.0%. Assay—Weigh accurately about 1 g of phenylhydrazine, add 30 mL of diluted hydrochloric acid (1 in 100) and water to make exactly 100 mL. Put exactly 20 mL of this solution in a glass-stoppered conical flask, and add 40 mL of diluted hydrochloric acid (3 in 4). After cooling, add 5 mL of chloroform, and titrate <2.50> with 0.05 mol/L potassium iodate VS while shaking vigorously until the red color of the chloroform layer disappears. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L potassium iodate VS  
= 5.407 mg of  $C_6H_5NHNH_2$

**Phenylhydrazine hydrochloride** See phenylhydrazinium chloride.

**Phenylhydrazine hydrochloride TS** See phenylhydrazinium chloride TS.

**Phenylhydrazinium chloride**  $C_6H_5NHNH_2 \cdot HCl$

[K 8203, Special class]

**Phenylhydrazinium chloride TS** Dissolve 65 mg of phenylhydrazinium chloride recrystallized from dilute ethanol, in 100 mL of a solution previously prepared by adding cautiously 170 mL of sulfuric acid to 80 mL of water.

**Phenyl isothiocyanate** C<sub>7</sub>H<sub>5</sub>NS Prepared for amino acid analysis or biochemistry.

**1-phenyl-3-methyl-5-pyrazolone** See 3-methyl-1-phenyl-5-pyrazolone.

**50% Phenyl-50% methylpolysiloxane for gas chromatography** Prepared for gas chromatography.

**5% Phenyl-methyl silicone polymer for gas chromatography** Prepared for gas chromatography.

**35% Phenyl-methyl silicone polymer for gas chromatography** Prepared for gas chromatography.

**50% Phenyl-methyl silicone polymer for gas chromatography** Prepared for gas chromatography.

**65% Phenyl-methyl silicone polymer for gas chromatography** Prepared for gas chromatography.

**Phenylpiperazine hydrochloride** See 1-phenylpiperazine monohydrochloride.

**1-Phenylpiperazine monohydrochloride** C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>.HCl A white powder. Melting point: about 247°C (with decomposition).

**Phenytoin for assay** C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub> [Same as the monograph Phenytoin. It meets the following requirements.]

**Purity** Related substances—Dissolve 25 mg of phenytoin for assay in 50 mL of the mobile phase, and use this solution as the sample solution. To exactly 1 mL of the sample solution add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than phenytoin obtained from the sample solution is not larger than the peak area of phenytoin from the standard solution.

#### Operating conditions

Column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay under Phenytoin Tablets.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution (pH 3.5) and acetonitrile for liquid chromatography (11:9).

Time span of measurement: About 5 times as long as the retention time of phenytoin, beginning after the solvent peak.

#### System suitability

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of phenytoin obtained with 10 µL of this solution is equivalent to 8 to 12% of that with 10 µL of the standard solution.

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of phenytoin are not less than 6000 and not more than 2.0, respectively.

**System repeatability:** When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phenytoin is not more than 2.0%.

**Phloroglucin** See phloroglucinol dihydrate.

**Phloroglucin dihydrate** See phloroglucinol dihydrate.

**Phloroglucinol dihydrate** C<sub>6</sub>H<sub>3</sub>(OH)<sub>3</sub>.2H<sub>2</sub>O White to pale yellow, crystals or crystalline powder.

*Melting point* <2.60>: 215 – 219°C (after drying).

*Loss on drying* <2.41>: 18.0 – 24.0% (1 g, 105°C, 1 hour).

**Phosphatase, alkaline** Obtained from bovine small intestine. A white to grayish white or yellow-brown, lyophilized powder.

It contains not less than 1 unit per mg, not containing any saline. One unit indicates an amount of the enzyme which produces 1 µmol of 4-nitrophenol at 37°C and pH 9.8 in 1 minute from 4-nitrophenyl phosphate used as the substrate.

**Phosphatase TS, alkaline** Dissolve 0.1 g of alkaline phosphatase in 10 mL of boric acid-magnesium chloride buffer solution (pH 9.0). Prepare before use.

**Phosphate-buffered sodium chloride TS** Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 2.9 g of disodium hydrogen phosphate dodecahydrate, and 0.2 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.01 mol/L Phosphate buffer-sodium chloride TS (pH 7.4)** Dissolve 2.93 g of disodium hydrogen phosphate dodecahydrate, 0.25 g of potassium dihydrogen phosphate, and 9 g of sodium chloride in water to make 1000 mL.

#### Phosphate buffer solution for assay of bupleurum root

To 100 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 59 mL of 0.2 mol/L sodium hydroxide TS.

**Phosphate buffer solution for component determination of bupleurum root** See phosphate buffer solution for assay of bupleurum root.

**Phosphate buffer solution for cytotoxicity test** Dissolve 0.20 g of potassium chloride, 0.20 g of potassium dihydrogen phosphate, 8.00 g of sodium chloride and 1.15 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL, and sterilize in an autoclave at 121°C for 15 minutes.

**Phosphate buffer solution for epoetin alfa** Dissolve 0.247 g of sodium dihydrogen phosphate dihydrate, 0.151 g of disodium hydrogen phosphate dodecahydrate and 8.77 g of sodium chloride in water to make 1000 mL.

**Phosphate buffer solution for microplate washing** Dissolve 0.62 g of sodium dihydrogen phosphate dihydrate, 9.48 g of disodium hydrogen phosphate dodecahydrate, 52.6 g of sodium chloride, 3.0 g of polysorbate 80 and 1.8 g of polyoxyethylene (40) octylphenyl ether in water to make 600 mL. Dilute this solution 10 times with water before use.

**Phosphate buffer solution for pancreatin** Dissolve 3.3 g of anhydrous disodium hydrogen phosphate, 1.4 g of potassium dihydrogen phosphate and 0.33 g of sodium chloride in water to make 100 mL.

#### Phosphate buffer solution for processedaconite root

Dissolve 19.3 g of disodium hydrogen phosphate dodecahydrate in 3660 mL of water, and add 12.7 g of phosphoric acid.

**Phosphate buffer solution (pH 3.0)** Dissolve 136 g of po-

tassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.0 with phosphoric acid.

**0.02 mol/L Phosphate buffer solution (pH 3.0)** Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10).

**Phosphate buffer solution (pH 3.1)** Dissolve 136.1 g of potassium dihydrogen phosphate in 500 mL of water, and add 6.3 mL of phosphoric acid and water to make 1000 mL.

**0.02 mol/L Phosphate buffer solution (pH 3.5)** Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10).

**0.05 mol/L Phosphate buffer solution (pH 3.5)** To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add a suitable amount of a solution of diluted phosphoric acid (49 in 10,000) to make a solution having (pH 3.5).

**Phosphate buffer solution (pH 4.0)** Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 4.0 with diluted phosphoric acid (1 in 10).

**0.1 mol/L Phosphate buffer solution (pH 4.5)** Dissolve 13.61 g of potassium dihydrogen phosphate in 750 mL of water, adjust to pH 4.5 with potassium hydroxide TS, and add water to make 1000 mL.

**0.1 mol/L Phosphate buffer solution (pH 5.3)** Dissolve 0.44 g of disodium hydrogen phosphate dodecahydrate and 13.32 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 5.3 with sodium hydroxide TS or phosphoric acid, and add water to make 1000 mL.

**0.1 mol/L Phosphate buffer solution (pH 7)** Dissolve 13.6 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to  $7 \pm 0.4$  with sodium hydroxide TS, and add water to make 1000 mL.

**1/15 mol/L Phosphate buffer solution (pH 5.6)** Dissolve 9.07 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 5.6 with potassium hydroxide TS, and add water to make 1000 mL.

**Phosphate buffer solution (pH 5.9)** Dissolve 6.8 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 5.9 with diluted potassium hydroxide TS (1 in 10), and add water to make 1000 mL.

**Phosphate buffer solution (pH 6.0)** Dissolve 8.63 g of potassium dihydrogen phosphate and 1.37 g of anhydrous disodium hydrogen phosphate in 750 mL of water, adjust the pH to 6.0 with sodium hydroxide TS or diluted phosphoric acid (1 in 15), and add water to make 1000 mL.

**0.05 mol/L Phosphate buffer solution (pH 6.0)** To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution add 5.70 mL of 0.2 mol/L sodium hydroxide TS and water to make 200 mL.

**Phosphate buffer solution (pH 6.2)** Dissolve 9.08 g of potassium dihydrogen phosphate in 1000 mL of water (solution A). Dissolve 9.46 g of anhydrous disodium hydrogen phosphate in 1000 mL of water (solution B). Mix 800 mL of the solution A and 200 mL of the solution B, and adjust the pH to 6.2 with the solution A or the solution B if necessary.

**Phosphate buffer solution (pH 6.5)** Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 15.20 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

#### Phosphate buffer solution for antibiotics (pH 6.5)

Dissolve 10.5 g of disodium hydrogen phosphate dodecahydrate and 5.8 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.5 with sodium hydroxide TS, and add water to make 1000 mL.

**Phosphate buffer solution (pH 6.8)** Dissolve 3.40 g of potassium dihydrogen phosphate and 3.55 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

**0.01 mol/L Phosphate buffer solution (pH 6.8)** Dissolve 1.36 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 6.8 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

**0.1 mol/L Phosphate buffer solution (pH 6.8)** Dissolve 6.4 g of potassium dihydrogen phosphate and 18.9 g of disodium hydrogen phosphate dodecahydrate in 750 mL of water, adjust the pH to 6.8 with sodium hydroxide TS and add water to make 1000 mL.

**Phosphate buffer solution (pH 7.0)** Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 29.54 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

**0.05 mol/L Phosphate buffer solution (pH 7.0)** Dissolve 4.83 g of dipotassium hydrogen phosphate and 3.02 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to (pH 7.0) with phosphoric acid or potassium hydroxide TS.

**0.1 mol/L Phosphate buffer solution (pH 7)** Dissolve 13.6 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to  $7 \pm 0.4$  with sodium hydroxide TS, and add water to make 1000 mL.

**0.1 mol/L Phosphate buffer solution (pH 7.0)** Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL (solution A). Dissolve 6.8 g of potassium dihydrogen phosphate in water to make 500 mL (solution B). To a volume of solution A add solution B until the mixture is adjusted to pH 7.0 (about 2:1 by volume of solutions A and B).

**Phosphate buffer solution (pH 7.2)** Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 34.7 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

**Phosphate buffer solution (pH 7.4)** Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 39.50 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

**0.02 mol/L Phosphate buffer solution (pH 7.5)** Dissolve 2.72 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 7.5 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

**0.03 mol/L Phosphate buffer solution (pH 7.5)** Dissolve 4.083 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 7.5 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

**Phosphate buffer solution (pH 8.0)** Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 46.1 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

**0.02 mol/L Phosphate buffer solution (pH 8.0)** To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 300 mL of water, adjust the pH to 8.0 with sodium hydrox-

ide TS, and add water to make 500 mL.

**0.1 mol/L Phosphate buffer solution (pH 8.0)** Dissolve 13.2 g of anhydrous disodium hydrogen phosphate and 0.91 g of potassium dihydrogen phosphate in about 750 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL.

**0.1 mol/L Phosphate buffer solution for antibiotics (pH 8.0)** Dissolve 16.73 g of dipotassium hydrogen phosphate and 0.523 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 8.0 with phosphoric acid, and add water to make 1000 mL.

**0.2 mol/L Phosphate buffer solution (pH 10.5)** Dissolve 34.8 g of dipotassium hydrogen phosphate in 750 mL of water, adjust to pH 10.5 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.

**Phosphate buffer solution (pH 12)** To 5.44 g of anhydrous disodium hydrogen phosphate add 36.5 mL of sodium hydroxide TS and about 40 mL of water, dissolve by shaking well, and add water to make 100 mL.

**0.01 mol/L Phosphate buffer solution** Dissolve 1.15 g of anhydrous disodium hydrogen phosphate, 0.2 g of potassium dihydrogen phosphate, 8.0 g of sodium chloride and 0.2 g of potassium chloride in water to make 1000 mL.

**Phosphinic acid**  $\text{H}_3\text{PO}_2$  Colorless or pale yellow viscous liquid.

**Identification**—(1) To 0.5 mL of phosphinic acid add 0.5 mL of hydrogen peroxide (30) and 0.5 mL of diluted sulfuric acid (1 in 6), and evaporate to nearly dryness on a water bath. After cooling, add 10 mL of water and 5 mL of ammonia TS, and add 5 mL of magnesia TS; a white precipitate is produced.

(2) To 1 mL of phosphinic acid add the mixture of iodine TS (1 mL) and water (20 mL); the iodine color disappears.

**Content:** 30.0 – 32.0%. **Assay**—Weigh accurately about 1.5 g of phosphinic acid, and dissolve in water to make exactly 250 mL. Pipet 25 mL of this solution into an iodine bottle, add exactly 50 mL of 0.05 mol/L bromine VS, 100 mL of water and 10 mL of diluted sulfuric acid (1 in 6), immediately stoppered, gently shake, and allow to stand for 3 hours. Then add 20 mL of potassium iodide TS, stopper immediately, shake vigorously, and titrate <2.50> liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

$$\begin{aligned} \text{Each mL of 0.05 mol/L bromine VS} \\ = 1.650 \text{ mg of } \text{H}_3\text{PO}_2 \end{aligned}$$

**Phosphate TS** Dissolve 2.0 g of dipotassium hydrogen phosphate and 8.0 g of potassium dihydrogen phosphate in water to make 1000 mL.

**Phosphomolybdic acid** See phosphomolybdic acid *n*-hydrate.

**Phosphomolybdic acid *n*-hydrate**  $\text{P}_2\text{O}_5 \cdot 24\text{MoO}_3 \cdot x\text{H}_2\text{O}$  Yellow, crystals or crystalline powder.

**Identification** (1) To 10 mL of a solution (1 in 10) add 0.5 mL of ammonia TS; yellow precipitates appear, which disappear by the addition of 2 mL of ammonia TS, and yellow precipitates appear by further addition of 5 mL of diluted nitric acid (1 in 2).

(2) To 5 mL of a solution (1 in 10) add 1 mL of ammonia TS and 1 mL of magnesia TS; white precipitates appear.

**Phosphoric acid**  $\text{H}_3\text{PO}_4$  [K 9005, Special class]

**Phosphoric acid-acetic acid-boric acid buffer solution (pH 2.0)** Dissolve 6.77 mL of phosphoric acid, 5.72 mL of acetic acid (100) and 6.18 g of boric acid in water to make 1000 mL. Adjust the pH of this solution to 2.0 with 0.5 mol/L sodium hydroxide VS.

**Phosphoric acid-sodium sulfate buffer solution (pH 2.3)** Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water, and add 2.7 mL of phosphoric acid. If necessary, adjust to pH 2.3 with 2-aminoethanol.

**Phosphorus pentoxide** See phosphorus (V) oxide.

**Phosphorus, red P** An odorless dark red powder. Practically insoluble in carbon disulfide and in water.

**Purity** Free phosphoric acid: Not more than 0.5%.

To 5 g add 10 mL of a solution of sodium chloride (1 in 5), mix, then add 50 mL of the solution of sodium chloride (1 in 5), allow to stand for 1 hour, and filter. Wash the residue with three 10-mL portions of the solution of sodium chloride (1 in 5), combine the filtrate and the washings, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of thymol blue TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 4.90 \text{ mg of } \text{H}_3\text{PO}_4 \end{aligned}$$

**Phosphorus (V) oxide**  $\text{P}_2\text{O}_5$  [K 8342, Special class]

**Phosphotungstic acid** See phosphotungstic acid *n*-hydrate.

**Phosphotungstic acid *n*-hydrate**  $\text{P}_2\text{O}_5 \cdot 24\text{WO}_3 \cdot x\text{H}_2\text{O}$  White to yellowish green, crystals or crystalline powder.

**Identification**—To 5 mL of a solution (1 in 10) add 1 mL of acidic tin (II) chloride TS, and heat: blue precipitates appear.

**Phosphotungstic acid TS** Dissolve 1 g of phosphotungstic acid *n*-hydrate in water to make 100 mL.

***o*-Phthalaldehyde**  $\text{C}_6\text{H}_4(\text{CHO})_2$  Light yellow to yellow crystals.

**Content:** not less than 99%. **Assay**—Dissolve 1 g of *o*-phthalaldehyde in 10 mL of ethanol (95). Perform the test with 2  $\mu\text{L}$  of this solution as directed in Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method.

$$\text{Content (\%)} = \frac{\text{peak area of } o\text{-phthalaldehyde}}{\text{total area of all peaks}} \times 100$$

**Operating conditions**

**Detector:** A thermal conductivity detector.

**Column:** A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography treated with acid and silane (177 – 250  $\mu\text{m}$ ), coated with methyl silicon polymer for gas chromatography in 10%.

**Column temperature:** A constant temperature of about 180°C.

**Carrier gas:** Helium.

**Flow rate:** Adjust so that the retention time of *o*-phthalaldehyde is 3 – 4 minutes.

**Time span of measurement:** About 7 times as long as the retention time of *o*-phthalaldehyde, beginning after the solvent peak.

**Phthalate buffer solution (pH 5.8)** Dissolve 100.0 g of potassium hydrogen phthalate in about 800 mL of water, adjust to pH 5.8 with a solution of sodium hydroxide (1 in 2), and add water to make 1000 mL.

**Phthalein purple**  $C_{32}H_{32}N_2O_{12}.xH_2O$  Yellow-white to brown powder. Soluble in ethanol (95), and practically insoluble in water.

**Sensitivity test**—Dissolve 10 mg of phthalein purple in 1 mL of ammonia solution (28), and add water to make 100 mL. To 5 mL of this solution add 95 mL of water, 4 mL of ammonia solution (28), 50 mL of ethanol (95) and 0.1 mL of diluted barium chloride TS (1 in 5): the solution shows a blue-purple color which disappears on the addition of 0.15 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

**Phthalic acid**  $C_8H_6O_4$  Colorless or white crystalline powder. Soluble in methanol and in ethanol (95), sparingly soluble in water, and practically insoluble in chloroform. Melting point: about 200°C (with decomposition).

**Content:** not less than 98%. Assay—Weigh accurately about 2.8 g of phthalic acid, add exactly 50 mL of 1 mol/L sodium hydroxide VS and 25 mL of water, and dissolve by heating on a hot plate. After cooling, add 5 drops of phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS. Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 83.07 \text{ mg of } C_8H_6O_4 \end{aligned}$$

**Phthalic anhydride**  $C_8H_4O_3$  White, crystals or crystalline powder.

*Melting point* <2.60>: 131 – 134°C

**Phthalimide**  $C_8H_5NO_2$  White to pale brown, crystals or powder.

*Melting point* <2.60>: 232 – 237°C

**Purity** Clarity—1.0 g of phthalimide dissolves in 20 mL of sodium hydroxide TS as a slight turbid solution.

**Content:** not less than 98.0%. Assay—Weigh accurately about 0.3 g of the substance to be tested, dissolve in 40 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium methoxide VS} \\ = 14.71 \text{ mg of } C_8H_5NO_2 \end{aligned}$$

**Phytanadione**  $C_{31}H_{46}O_2$  [Same as the namesake monograph]

**Picric acid** See 2,4,6-trinitrophenol.

**Picric acid-ethanol TS** See 2,4,6-trinitrophenol-ethanol TS.

**Picric acid TS** See 2,4,6-trinitrophenol TS.

**Picric acid TS, alkaline** See 2,4,6-trinitrophenol TS, alkaline.

**Pig bile powder for thin-layer chromatography** A yellow-gray to yellow-brown powder, having a characteristic odor and a bitter taste. It is practically insoluble in water, in methanol and in ethanol (99.5).

**Identification**—To 0.1 g of pig bile powder for thin-layer chromatography in a screw-capped test tube, add 1 mL of sodium hydroxide solution (3 in 25), and shake. Heat the tube in an oil bath at 120°C for 4 hours, allow to cool to a lukewarm temperature, add 2 mL of 3 mol/L hydrochloric acid TS and 2 mL of ethyl acetate, shake at 50°C for 30 minutes, and separate ethyl acetate layer as the sample solution. Separately, dissolve 10 mg of hyodeoxycholic acid for thin-layer chromatography in 5 mL of methanol, and use

this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: one of the several spots obtained from the sample solution shows the same color tone and the same *Rf* value as the spot from the standard solution.

**Pilocarpine hydrochloride for assay**  $C_{11}H_{16}N_2O_2.HCl$  [Same as the monograph Pilocarpine Hydrochloride. It meets the following additional requirements.]

**Purity** Related substances—Dissolve 40 mg of pilocarpine hydrochloride for assay in 100 mL of phosphate buffer solution (pH 4.0) and use this solution as the sample solution. Pipet 1 mL of the sample solution, add phosphate buffer solution (pH 4.0) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.78 and about 0.92 to pilocarpine obtained from the sample solution, is not larger than 1/2 times the peak area of pilocarpine from the standard solution, the area of the peak, other than pilocarpine and the peaks mentioned above from the sample solution, is not larger than 1/5 times the peak area of pilocarpine from the standard solution, and the total area of the peaks other than pilocarpine from the sample solution is not larger than the peak area of pilocarpine from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Pilocarpine Hydrochloride Tablets.

Time span of measurement: About 1.3 times as long as the retention time of pilocarpine, beginning after the solvent peak.

#### System suitability

Proceed as directed in the system suitability in the Purity under Pilocarpine Hydrochloride Tablets.

#### Pilsicainide hydrochloride hydrate for assay

$C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$  [Same as the monograph Pilsicainide Hydrochloride Hydrate. It contains not less than 99.3% of pilsicainide hydrochloride hydrate ( $C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$ ).]

**Piperacillin hydrate**  $C_{23}H_{27}N_5O_7S.H_2O$  [Same as the namesake monograph]

**Piperidine hydrochloride**  $C_5H_{11}N.HCl$  A white crystalline powder. Dissolves in water and in methanol. The pH of a solution of 1.0 g of piperidine hydrochloride in 20 mL of water is between 3.0 and 5.0.

*Melting point* <2.60>: 247 – 252°C

**Purity** Clarity and color of solution—Dissolve 1.0 g of piperidine hydrochloride in 20 mL of water: the solution is clear and colorless.

**Residue on ignition** <2.44>: not more than 0.1% (1 g).

**Content:** not less than 99.0%. Assay—Dissolve about 0.25 g of piperidine hydrochloride, accurately weighed, in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same

manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 12.16 mg of C<sub>5</sub>H<sub>11</sub>N.HCl

**Plantago seed for thin-layer chromatography** [Same as the monograph Plantago Seed meeting the following additional specifications.]

**Identification** (1) To 1 g of pulverized plantago seed for thin-layer chromatography add 3 mL of methanol, and warm on a water bath for 3 minutes. After cooling, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 µL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 10 minutes: spots equivalent to those described below appear.

Rf value	Color and shape of the spot
Around 0	A strong spot, very dark blue
Around 0.08	A very dark blue spot
Around 0.1 – 0.2	A leading spot, very dark blue
Around 0.25	A strong spot, deep blue (corresponding to plantagoguanidinic acid)
Around 0.35	A strong spot, dark grayish blue (corresponding to geniposidic acid)
Around 0.45	A weak spot, grayish yellowish green
Around 0.50	A strong spot, deep yellow-green (corresponding to verbascoside)
Around 0.6	A weak spot, light blue
Around 0.85	A deep blue spot
Around 0.9 – 0.95	A tailing spot, grayish blue

(2) Proceed with the sample solution and the standard solution obtained under (1) as directed in the method under (1), except using a mixture of ethyl acetate, water and formic acid (6:1:1) as developing solvent: spots equivalent to those described below appear.

Rf value	Color and shape of the spot
Around 0	A yellow-greenish dark gray spot
Around 0.05	A weak spot, dark grayish yellow-green
Around 0.2	A weak spot, dark green
Around 0.25	A strong spot, dark reddish purple (corresponding to geniposidic acid)
Around 0.35	A weak spot, bright blue
Around 0.4 – 0.45	A weak tailing spot, dull greenish blue
Around 0.45	A strong spot, deep yellow-green (corresponding to verbascoside)
Around 0.5	A strong spot, deep blue (corresponding to plantagoguanidinic acid)
Around 0.95	A strong spot, dark grayish blue-green
Around 0.97	A dark grayish blue-green spot

**Platycodon D for thin-layer chromatography** C<sub>57</sub>H<sub>92</sub>O<sub>28</sub>  
A white powder. Freely soluble in methanol.

**Identification** Determine the infrared absorption spectrum of platycodon D for thin-layer chromatography as di-

rected in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1734 cm<sup>-1</sup>, 1637 cm<sup>-1</sup>, 1385 cm<sup>-1</sup>, 825 cm<sup>-1</sup> and 783 cm<sup>-1</sup>.

**Purity** Related substances—Dissolve 2 mg of platycodon D for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (5:3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray dilute sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: the spots other than the principal spot with an R<sub>f</sub> value of about 0.5 obtained from the sample solution are not more intense than the spot from the standard solution.

**Platycodon Root** [Same as the namesake monograph.]

**Polyacrylamide gel for epoetin alfa** A polyacrylamide gel composed with the resolving gel 12.5% in acrylamide concentration.

**Polyacrylamide gel for filgrastim** A polyacrylamide gel composed with the resolving gel 15% in acrylamide concentration.

**Polyacrylamide gel for nartograstim** A polyacrylamide gel composed with the resolving gel 14% in acrylamide concentration.

**Polyalkylene glycol for gas chromatography** Prepared for gas chromatography.

**Polyalkylene glycol monoether for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 15000-diepoxyde for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol ester for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 20 M for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 400 for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 600 for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 1500 for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 6000 for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 2-nitroterephthalate for gas chromatography** Prepared for gas chromatography.

**Polygala root** [Same as the namesake monograph]

**Polymethyl acrylate for gas chromatography** Prepared for gas chromatography.

**Polymethylsiloxane for gas chromatography** Prepared for gas chromatography.

**Polyoxyethylene hydrogenated castor oil 60** A nonionic surfactant prepared by addition polymerization of ethylene oxide with hydrogenated castor oil. Average molar number

of ethylene oxide added is about 60. A white or pale yellow petrolatum-like or waxy substance, having a faint, characteristic odor and a slight bitter taste. Very soluble in ethyl acetate and in chloroform, freely soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

**Identification** (1) To 0.5 g of polyoxyethylene hydrogenated castor oil 60 add 10 mL of water and 5 mL of ammonium thiocyanate-cobalt (II) nitrate TS, and shake thoroughly. Add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

(2) To 0.2 g of polyoxyethylene hydrogenated castor oil 60 add 0.5 g of potassium bisulfate, and heat: an acrolein-like, irritating odor is perceptible.

(3) To 0.5 g of polyoxyethylene hydrogenated castor oil 60 add 10 mL of water, shake, and add 5 drops of bromine TS: the color of the test solution does not disappear.

**Congealing point** <2.42>: 30 – 34°C

**pH** <2.54>—To 1.0 g of polyoxyethylene hydrogenated castor oil 60 add 20 mL of water, and dissolve by heating: the pH of the solution is between 3.6 and 6.0.

**Acid value** <1.13>: not more than 1.0.

**Saponification value** <1.13>: 41 – 51

**Hydroxyl value** <1.13>: 39 – 49

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of polyoxyethylene hydrogenated castor oil 60 in 20 mL of ethanol (95): the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of polyoxyethylene hydrogenated castor oil 60 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of polyoxyethylene hydrogenated castor oil 60 according to Method 3, and perform the test (not more than 2 ppm).

**Water** <2.48>: not more than 2.0% (1 g).

**Residue on ignition** <2.44>: not more than 0.1% (1 g).

**Storage**—Preserve in tight containers.

#### Polyoxyethylene (23) lauryl ether

$C_{12}H_{25}(OCH_2CH_2)_nOH$  White masses. Melting point: about 40°C.

**Polyoxyethylene (40) octylphenyl ether** Obtained by the addition polymerization with ethylene oxide to octylphenol. A colorless or white to pale yellow, liquid, vaseline-like or waxy, having slightly a characteristic odor.

**pH** <2.54>: 7.0 – 9.5 (5 w/v%, 25°C).

**Specific gravity** <2.56>  $d_4^{25}$ : 1.10 – 1.11

**Purity** Clarity of solution—Dissolve 1.0 g of polyoxyethylene (40) octylphenyl ether in 20 mL of water: the solution is clear.

**Polysorbate 20** Chiefly consists of addition polymer of sorbitan monolaurate and ethylene oxide. Pale yellow to yellow liquid, having a faint, characteristic odor.

**Identification** (1) To 0.5 g of polysorbate 20 add 10 mL of water and 10 mL of sodium hydroxide TS, boil for 5 minutes, and acidify with dilute hydrochloric acid: an oily fraction is separated.

(2) To 0.5 g of polysorbate 20 add 10 mL of water, shake, and add 5 drops of bromine TS: the red color of the test solution does not disappear.

(3) Place 0.1 g of polysorbate 20 in a flask, dissolve in 2 mL of a solution of sodium hydroxide in methanol (1 in 50), and heat under a reflux condenser for 30 minutes. Add 2 mL of boron trifluoride-methanol TS through the condenser, and heat for 30 minutes. Then, add 4 mL of heptane through the condenser, and heat for 5 minutes. After cooling, add 10

mL of saturated sodium chloride solution, shake for about 15 seconds, then add sufficient saturated sodium chloride solution such that the upper layer of the content reaches the neck of the flask. Take 2 mL of the upper layer, wash 3 times with each 2-mL portion of water, dry with anhydrous sodium sulfate, and use this solution as the sample solution. Separately, dissolve 50 mg of methyl laurate for gas chromatography, 50 mg of methyl palmitate for gas chromatography, 80 mg of methyl stearate for gas chromatography and 100 mg of methyl oleate for gas chromatography in heptane to make 50 mL, and use this solution as the standard solution. Perform the test with 1  $\mu$ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions: the retention time of the principal peak obtained from the sample solution is the same with that of the peak of methyl laurate from the standard solution.

#### Operating conditions

**Detector**: A hydrogen flame-ionization detector.

**Column**: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated the inside surface with polyethylene glycol 20 M for gas chromatography 0.5  $\mu$ m in thickness.

**Column temperature**: Inject at a constant temperature of 80°C, raise the temperature at the rate of 10°C per minute to 220°C, and maintain the temperature at 220°C for 40 minutes.

**Injection port temperature**: A constant temperature of about 250°C.

**Detector temperature**: A constant temperature of about 250°C.

**Carrier gas**: Helium.

**Flow rate**: Adjust so that the retention time of the peak of methyl laurate is about 10 minutes.

**Split ratio**: 1:50.

#### System suitability

**System performance**: When the procedure is run with 1  $\mu$ L of the standard solution under the above operating conditions, methyl laurate, methyl palmitate, methyl stearate and methyl oleate are eluted in this order, and the resolution between the peaks of methyl stearate and methyl oleate is not less than 2.0.

**Acid value** <1.13>: not more than 4.0.

**Saponification value** <1.13>: 43 – 55

**Loss on drying** <2.41>: not more than 3.0% (5 g, 105°C, 1 hour).

**Residue on ignition**—Weigh accurately about 3 g of polysorbate 20, heat gently at first, and ignite gradually (800 – 1200°C) until the residue is completely incinerated. If any carbonized substance remains, extract with hot water, filter through a filter paper for quantitative analysis (5C), and ignite the residue with the filter paper. Add the filtrate to it, evaporate to dryness, and ignite carefully until the carbonized substance does not remain. If any carbonized substance still remains, add 15 mL of ethanol (95), crush the carbonized substance with a glass rod, burn the ethanol, and ignite carefully. Cool in a desiccator (silica gel), and weigh the residue accurately: not more than 1.0%.

**Polysorbate 20 for epoetin beta** A clear to slightly turbid, yellow-brown liquid.

**Viscosity** <2.53>: 300 – 500 mPa·s

**Acid value** <1.13>: not more than 3.

**Saponification value** <1.13>: 40 – 50

**Hydroxyl value** <1.13>: 95 – 110

**Water** <2.48>: not more than 5.0%.

**Polysorbate 80** [Same as the namesake monograph].

**Polyvinyl alcohol**  $(-\text{CH}_2\text{CHOH}-)_n$  [K 9550, Special class]

**Polyvinyl alcohol I** Colorless to white or pale yellow, granules or powder. It is odorless, or has a faint odor of acetic acid. It is tasteless. Practically insoluble in ethanol (95) and in diethyl ether. To polyvinyl alcohol I add water, and heat: a clear, viscous solution is obtained. Polyvinyl alcohol I is hygroscopic.

**Viscosity** <2.53> 25.0 – 31.0 mm<sup>2</sup>/s. Weigh 4.000 g of polyvinyl alcohol I, previously dried, add 95 mL of water, allow to stand for 30 minutes, and heat to dissolve on a water bath under a reflux condenser for 2 hours while stirring. After cooling, add water to make 100.0 g, and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1°C as directed in Method 1.

**pH** <2.54>—The pH of a solution of 1.0 g of polyvinyl alcohol I in 25 mL of water is between 5.0 and 8.0.

**Purity** Clarity and color of solution—To 20 mL of water add 1.0 g of polyvinyl alcohol I, disperse by thorough stirring, warm between 60°C and 80°C for 2 hours, and cool: the solution is clear and colorless.

**Saponification value** 98.0 – 99.0 mol%. Weigh accurately about 3.0 g of polyvinyl alcohol I, previously dried, transfer to a glass-stoppered conical flask, add 100 mL of water, and dissolve by heating on a water bath. After cooling, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then add exactly 30 mL of 0.05 mol/L sulfuric acid VS, shake thoroughly, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction. However, when the volume of 0.1 mol/L sodium hydroxide VS consumed in the test is 25 mL or more, use about 2.0 g of the sample.

$$\text{Saponification value (mol\%)} = 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{0.6005 \times (a - b)}{\text{amount (g) of polyvinyl alcohol I taken}}$$

- a: Volume (mL) of 0.1 mol/L sodium hydroxide VS consumed in the test  
b: Volume (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank test

**Polyvinyl alcohol II** Colorless to white or pale yellow, granules or powder. It is odorless, or has a faint odor of acetic acid. It is tasteless. Practically insoluble in ethanol (95) and in diethyl ether. To polyvinyl alcohol II add water, and heat: a clear, viscous solution is obtained. Polyvinyl alcohol II is hygroscopic.

**Viscosity** <2.53> 4.6 – 5.4 mm<sup>2</sup>/s. Weigh 4.000 g of polyvinyl alcohol II, previously dried, add 95 mL of water, allow to stand for 30 minutes, and dissolve by stirring on a water bath between 60°C and 80°C for 2 hours. After cooling, add water to make 100.0 g, and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1°C as directed in Method 1.

**pH** <2.54>—The pH of a solution of 1.0 g of polyvinyl alcohol II in 25 mL of water is between 5.0 and 8.0.

**Purity** Clarity and color of solution—To 20 mL of water add 1.0 g of polyvinyl alcohol II, disperse by thorough stirring, heat on a water bath for 2 hours, and cool: the solution is clear and colorless.

**Saponification value** 86.5 – 89.5 mol%. Weigh accurately about 2 g of polyvinyl alcohol II, previously dried, transfer to a glass-stoppered conical flask, add 100 mL of

water, and warm while stirring for 2 hours. After cooling, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then add exactly 30 mL of 0.25 mol/L sulfuric acid VS, shake thoroughly, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Saponification value (mol\%)} = 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{3.0025 \times (a - b)}{\text{amount (g) of polyvinyl alcohol II taken}}$$

a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test

b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank test

**Polyvinyl alcohol TS** Weigh exactly 0.50 g of polyvinyl alcohol, and add water to make exactly 100 mL.

**Polyvinylidene fluoride membrane** For Western blotting.

**Potassium acetate** CH<sub>3</sub>COOK [K 8363, Special class]

**Potassium acetate TS** Dissolve 10 g of potassium acetate in water to make 100 mL (1 mol/L).

**Potassium aluminum sulfate** See aluminum potassium sulfate dodecahydrate.

**Potassium bicarbonate** See potassium hydrogen carbonate.

**Potassium biphenylate** See potassium hydrogen phthalate.

**Potassium biphenylate buffer solution (pH 3.5)** See potassium hydrogen phthalate buffer solution (pH 3.5).

**Potassium biphenylate buffer solution (pH 4.6)** See potassium hydrogen phthalate buffer solution (pH 4.6).

**Potassium biphenylate buffer solution (pH 5.6)** See potassium hydrogen phthalate buffer solution (pH 5.6).

**Potassium biphenylate for pH determination** See potassium hydrogen phthalate for pH determination.

**Potassium biphenylate (standard reagent)** See potassium hydrogen phthalate (standard reagent).

**0.2 mol/L Potassium biphenylate TS for buffer solution** See 0.2 mol/L potassium hydrogen phthalate TS for buffer solution.

**Potassium bisulfate** See potassium hydrogen sulfate.

**Potassium bromate** KBrO<sub>3</sub> [K 8530, Special class]

**Potassium bromide** KBr [K 8506, Special class]

**Potassium bromide for infrared spectrophotometry**

Crush homocrystals of potassium bromide or potassium bromide, collect a powder passed through a No. 200 (75 µm) sieve, and dry at 120°C for 10 hours or at 500°C for 5 hours. Prepare tablets with this powder, and determine the infrared absorption spectrum <2.25>: any abnormal absorption does not appear.

**Potassium carbonate** K<sub>2</sub>CO<sub>3</sub> [K 8615, Special class]

**Potassium carbonate, anhydrous** See potassium carbonate.

**Potassium carbonate-sodium carbonate TS** Dissolve

1.7 g of potassium carbonate and 1.3 g of anhydrous sodium carbonate in water to make 100 mL.

**Potassium chloride**  $\text{KClO}_3$  [K 8207, Special class]

**Potassium chloride**  $\text{KCl}$  [K 8121, Special class]

**Potassium chloride for assay**  $\text{KCl}$  [Same as the monograph, Potassium Chloride]

**Potassium chloride for conductivity measurement** [K 8121, Potassium chloride for conductivity measurement]

**Potassium chloride for infrared spectrophotometry**

Crush homocrystals of potassium chloride or potassium chloride, collect the powder passed through a No. 200 (75  $\mu\text{m}$ ) sieve, and dry at 120°C for 10 hours or at 500°C for 5 hours. Prepare tablets with this powder, and determine the infrared absorption spectrum <2.25>; any abnormal absorption does not appear.

**Potassium chloride-hydrochloric acid buffer solution** To 250 mL of a solution of potassium chloride (3 in 20) add 53 mL of 2 mol/L hydrochloric acid TS and water to make 1000 mL.

**Potassium chloride TS, acidic** Dissolve 250 g of potassium chloride in water to make 1000 mL, and add 8.5 mL of hydrochloric acid.

**0.2 mol/L Potassium chloride TS** Dissolve 14.9 g of potassium chloride in water to make 1000 mL. Prepare before use.

**Potassium chromate**  $\text{K}_2\text{CrO}_4$  [K 8312, Special class]

**Potassium chromate TS** Dissolve 10 g of potassium chromate in water to make 100 mL.

**Potassium cyanide**  $\text{KCN}$  [K 8443, Special class]

**Potassium cyanide TS** Dissolve 1 g of potassium cyanide in water to make 10 mL. Prepare before use.

**Potassium dichromate**  $\text{K}_2\text{Cr}_2\text{O}_7$  [K 8517, Special class]

**Potassium dichromate (standard reagent)**  $\text{K}_2\text{Cr}_2\text{O}_7$  In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

**Potassium dichromate-sulfuric acid TS** Dissolve 0.5 g of potassium dichromate in diluted sulfuric acid (1 in 5) to make 100 mL.

**Potassium dichromate TS** Dissolve 7.5 g of potassium dichromate in water to make 100 mL.

**Potassium dihydrogen phosphate**  $\text{KH}_2\text{PO}_4$  [K 9007, Special class]

**Potassium dihydrogen phosphate for pH determination**  $\text{KH}_2\text{PO}_4$  [K 9007, for pH determination]

**0.1 mol/L Potassium dihydrogen phosphate TS (pH 2.0)**

Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL. Adjust the pH to 2.0 with phosphoric acid.

**0.05 mol/L Potassium dihydrogen phosphate (pH 3.0)**

Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid.

**0.25 mol/L Potassium dihydrogen phosphate TS (pH 3.5)**

Dissolve 34 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 3.5 with phosphoric acid, and add water to make 1000 mL.

**0.01 mol/L Potassium dihydrogen phosphate TS (pH 4.0)**

Dissolve 1.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

**0.05 mol/L Potassium dihydrogen phosphate TS (pH 4.7)**

Dissolve 6.80 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to exactly 4.7 with dilute sodium hydrochloride TS, and add water to make 1000 mL.

**0.02 mol/L Potassium dihydrogen phosphate TS** Dissolve 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.05 mol/L Potassium dihydrogen phosphate TS** Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.33 mol/L Potassium dihydrogen phosphate TS** Dissolve 4.491 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.1 mol/L Potassium dihydrogen phosphate TS** Dissolve 13.61 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.2 mol/L Potassium dihydrogen phosphate TS** Dissolve 27.22 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.2 mol/L Potassium dihydrogen phosphate TS for buffer solution** Dissolve 27.218 g of potassium dihydrogen phosphate for pH determination in water to make 1000 mL.

**Potassium disulfate**  $\text{K}_2\text{S}_2\text{O}_7$  [K 8783, Special class]

**Potassium ferricyanide** See potassium hexacyanoferrate (III).

**Potassium ferricyanide TS** See potassium hexacyanoferrate (III) TS.

**Potassium ferricyanide TS, alkaline** See potassium hexacyanoferrate (III) TS, alkaline.

**Potassium ferrocyanide** See potassium hexacyanoferrate (II) trihydrate.

**Potassium ferrocyanide TS** See potassium hexacyanoferrate (II) TS.

**Potassium guaiacolsulfonate**  $\text{C}_7\text{H}_7\text{KO}_5\text{S}$  [Same as the namesake monograph]

**Potassium hexacyanoferrate (II) trihydrate**

$\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  [K 8802, Special class]

**Potassium hexacyanoferrate (II) TS** Dissolve 1 g of potassium hexacyanoferrate (II) trihydrate in water to make 10 mL (0.25 mol/L). Prepare before use.

**Potassium hexacyanoferrate (III)**  $\text{K}_3\text{Fe}(\text{CN})_6$  [K 8801, Special class]

**Potassium hexacyanoferrate (III) TS** Dissolve 1 g of potassium hexacyanoferrate (III) in water to make 10 mL (0.3 mol/L). Prepare before use.

**Potassium hexacyanoferrate (III) TS, alkaline** Dissolve 1.65 g of potassium hexacyanoferrate (III) and 10.6 g of anhydrous sodium carbonate in water to make 1000 mL. Preserve in light-resistant containers.

**Potassium hexahydroxoantimonate (V)**  $\text{K}[\text{Sb}(\text{OH})_6]$

White, granules or crystalline powder.

**Identification**—To 1 g add 100 mL of water, and dissolve by warming. To 20 mL of this solution add 0.2 mL of sodium chloride TS; white precipitates appear. Rubbing the in-

side wall of the vessel with a glass rod accelerates the forming of the precipitates.

**Potassium hexahydroxoantimonate (V) TS** To 2 g of potassium hexahydroxoantimonate (V) add 100 mL of water. Boil the solution for about 5 minutes, cool quickly, add 10 mL of a solution of potassium hydroxide (3 in 20), allow to stand for 1 day, and filter.

**Potassium hydrogen carbonate**  $\text{KHCO}_3$  [K 8621, Special class]

**Potassium hydrogen phthalate**  $\text{C}_6\text{H}_4(\text{COOK})(\text{COOH})$  [K 8809, Special class]

**Potassium hydrogen phthalate (standard reagent)**  $\text{C}_6\text{H}_4(\text{COOK})(\text{COOH})$  In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

**Potassium hydrogen phthalate buffer solution (pH 3.5)** Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 7.97 mL of 0.2 mol/L hydrochloric acid VS with water to make 200 mL.

**Potassium hydrogen phthalate buffer solution (pH 4.6)** Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 12.0 mL of 0.2 mol/L sodium hydroxide VS with water to make 200 mL.

**0.3 mol/L Potassium hydrogen phthalate buffer solution (pH 4.6)** Dissolve 61.26 g of potassium hydrogen phthalate in about 800 mL of water, adjust the pH to 4.6 with sodium hydroxide TS, and add water to make 1000 mL.

**Potassium hydrogen phthalate buffer solution (pH 5.6)** Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 39.7 mL of 0.2 mol/L sodium hydroxide VS with water to make 200 mL.

**Potassium hydrogen phthalate for pH determination**  $\text{C}_6\text{H}_4(\text{COOK})(\text{COOH})$  [K 8809, For pH determination]

**0.2 mol/L Potassium hydrogen phthalate TS for buffer solution** Dissolve 40.843 g of potassium hydrogen phthalate for pH determination in water to make 1000 mL.

**Potassium hydrogen sulfate**  $\text{KHSO}_4$  [K 8972, Special class]

**Potassium hydroxide**  $\text{KOH}$  [K 8574, Special class]

**Potassium hydroxide-ethanol TS** Dissolve 10 g of potassium hydroxide in ethanol (95) to make 100 mL. Prepare before use.

**0.1 mol/L Potassium hydroxide-ethanol TS** To 1 mL of dilute potassium hydroxide-ethanol TS add ethanol (95) to make 5 mL. Prepare before use.

**Potassium hydroxide-ethanol TS, dilute** Dissolve 35 g of potassium hydroxide in 20 mL of water, and add ethanol (95) to make 1000 mL (0.5 mol/L). Preserve in tightly stoppered bottles.

**Potassium hydroxide TS** Dissolve 6.5 g of potassium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.

**0.02 mol/L Potassium hydroxide TS** Dilute 2 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

**0.05 mol/L Potassium hydroxide TS** Dilute 5 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

**8 mol/L Potassium hydroxide TS** Dissolve 52 g of potassium hydroxide in water to make 100 mL. Preserve in polyethylene bottles.

**Potassium iodate**  $\text{KIO}_3$  [K 8922, Special class]

**Potassium iodate (standard reagent)**  $\text{KIO}_3$  In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

**Potassium iodide**  $\text{KI}$  [K 8913, Special class]

**Potassium iodide for assay** [Same as the monograph Potassium Iodide]

**Potassium iodide-starch TS** Dissolve 0.5 g of potassium iodide in 100 mL of freshly prepared starch TS. Prepare before use.

**Potassium iodide TS** Dissolve 16.5 g of potassium iodide in water to make 100 mL. Preserve in light-resistant containers. Prepare before use (1 mol/L).

**Potassium iodide TS, concentrated** Dissolve 30 g of potassium iodide in 70 mL of water. Prepare before use.

**Storage**—Preserve in light-resistant containers.

**Potassium iodide TS, saturated** Saturate 20 g of potassium iodide in 10 mL of fleshly boiled and cooled water. Prepare before use.

**Potassium iodide-zinc sulfate TS** Dissolve 5 g of potassium iodide, 10 g of zinc sulfate heptahydrate, and 50 g of sodium chloride in water to make 200 mL.

**Potassium methanesulfonate**  $\text{CH}_3\text{SO}_3\text{K}$  White, crystals or crystalline powder.

**Purity** Clarity and color of solution—Dissolve 1.0 g of potassium methanesulfonate in 20 mL of water: the solution is transparent and colorless.

**Content:** not less than 98.0%. **Assay**—Dissolve about 0.1 g of potassium methanesulfonate, accurately weighed, in 10 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L perchloric acid VS} \\ = 13.42 \text{ mg of } \text{CH}_3\text{SO}_3\text{K} \end{aligned}$$

**Potassium naphthoquinone sulfonate** See potassium 1,2-naphthoquinone-4-sulfonate.

**Potassium 1,2-naphthoquinone-4-sulfonate**  $\text{C}_{10}\text{H}_5\text{O}_2\text{SO}_3\text{K}$  [K 8696, Special class]

**Potassium 1,2-naphthoquinone-4-sulfonate TS** Dissolve 0.5 g of potassium 1,2-naphthoquinone-4-sulfonate in water to make 100 mL. Prepare before use.

**Potassium nitrate**  $\text{KNO}_3$  [K 8548, Special class]

**Potassium nitrite**  $\text{KNO}_2$  A white to pale yellow crystalline powder. It is deliquescent.

**Identification**—(1) Dissolve 1 g of potassium nitrite in 20 mL of water, and use this solution as the sample solution. To 5 mL of the sample solution add 1 mL of sulfuric acid: a yellow-brown gas is evolved.

(2) The sample solution obtained in (1) responds to Qualitative Tests <1.09> (1) for potassium salt.

**Storage**—Preserve in a light-resistant tight container.

**Potassium periodate**  $\text{KIO}_4$  [K 8249, Special class]

**Potassium periodate TS** To 2.8 g of potassium periodate

add 200 mL of water, dissolve by adding dropwise 20 mL of sulfuric acid under shaking, cool, and add water to make 1000 mL.

**1.6% Potassium periodate-0.2% potassium permanganate TS, alkaline** Dissolve 1 g of potassium permanganate, 8 g of potassium periodate and 10 g of potassium carbonate in 500 mL of water. After allowing to stand for 16 hours, filter through a filter paper.

**Potassium permanganate**  $\text{KMnO}_4$  [K 8247, Special class]

**Potassium permanganate TS** Dissolve 3.3 g of potassium permanganate in water to make 1000 mL (0.02 mol/L).

**Potassium permanganate TS, acidic** To 100 mL of potassium permanganate TS add 0.3 mL of sulfuric acid.

**Potassium peroxodisulfate**  $\text{K}_2\text{S}_2\text{O}_8$  [K 8253, Special class]

**Potassium persulfate** See potassium peroxodisulfate.

**Potassium pyroantimonate** See potassium hexahydroxoantimonate (V).

**Potassium pyroantimonate TS** See potassium hexahydroxoantimonate (V) TS.

**Potassium pyrophosphate**  $\text{K}_4\text{O}_7\text{P}_2$  White crystalline powder, very soluble in water. Melting point: 1109°C.

**Potassium pyrosulfate** See potassium disulfate.

**Potassium sodium tartarate** See potassium sodium tartrate tetrahydrate.

**Potassium sodium tartarate tetrahydrate**

$\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$  [K 8536, (+)-Potassium sodium tartrate tetrahydrate, Special class]

**Potassium sulfate**  $\text{K}_2\text{SO}_4$  [K 8962, Special class]

**Potassium sulfate TS** Dissolve 1 g of potassium sulfate in water to make 100 mL.

**Potassium tartrate**  $2\text{C}_4\text{H}_4\text{K}_2\text{O}_6 \cdot \text{H}_2\text{O}$  [K 8535, (+)-Potassium Tartrate-Water (2/1), Special class]

**Potassium tellurite**  $\text{K}_2\text{TeO}_3$  White, powder or small masses obtained by melting an equimolar mixture of tellurium dioxide and potassium carbonate in a stream of carbon dioxide. Soluble in water.

**Content:** not less than 90.0%. **Assay**—Dissolve about 1.0 g of potassium tellurite, accurately weighed, in 100 mL of water, add 5 mL of diluted acetic acid (31) (1 in 3), and boil. After cooling, filter by suction through a crucible glass filter (1G4) [previously dried at  $105 \pm 2^\circ\text{C}$  for 1 hour to constant mass (*b* (g))]. Wash the filtrate with water, dry the glass filter at  $110^\circ\text{C}$  for 3 hours, and measure the mass *a* (g).

Content (%) of potassium tellurite ( $\text{K}_2\text{TeO}_3$ )

$$= \frac{(a - b) \times 1.5902}{S} \times 100$$

*S*: Amount (g) of potassium tellurite taken

**Potassium tetraxalate for pH determination** See potassium trihydrogen dioxalate dihydrate for pH determination.

**Potassium tetraphenylborate TS** Add 1 mL of acetic acid (31) to 50 mL of a solution of potassium biphthalate (1 in 500), then to this solution add 20 mL of a solution of sodium tetraphenylborate (7 in 1000), shake well, and allow to stand for 1 hour. Collect the produced precipitate on filter paper,

and wash it with water. To 1/3 quantity of the precipitate add 100 mL of water, warm, with shaking, at about  $50^\circ\text{C}$  for 5 minutes, cool quickly, allow to stand for 2 hours with occasional shaking, and filter, discarding the first 30 mL of the filtrate.

**Potassium thiocyanate**  $\text{KSCN}$  [K 9001, Special class]

**Potassium thiocyanate TS** Dissolve 1 g of potassium thiocyanate in water to make 10 mL.

**Potassium trihydrogen dioxalate dihydrate for pH determination**  $\text{KH}_3(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$  [K 8474, for pH determination]

**Potato extract** Prepared for microbial test.

**Potato starch** [Same as the namesake monograph]

**Potato starch TS** Prepare as directed under starch TS with 1 g of potato starch.

**Potato starch TS for amylolytic activity test** Dry about 1 g of potato starch, accurately weighed, at  $105^\circ\text{C}$  for 2 hours, and measure the loss. Weigh accurately an amount of potato starch, equivalent to 1.000 g on the dried basis, place into a conical flask, add 20 mL of water, and make it pasty by gradually adding 5 mL of a solution of sodium hydroxide (2 in 25) while shaking well. Heat in a water bath for 3 minutes while shaking, add 25 mL of water, and cool. Neutralize exactly with 2 mol/L hydrochloric acid TS, add 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution (pH 5.0) and add water to make exactly 100 mL. Prepare before use.

**Potency measuring medium for nartograstim test** Dissolve 10.4 g of RPMI-1640 medium in a suitable amount of water, add 16 mL of sodium hydrogen carbonate solution (3 in 40), then add water to make 1000 mL, adjust to pH 7.0 by passing carbon dioxide through the solution, and sterilize by filtration. To 90 mL of this solution add 10 mL of fetal bovine serum, previously heat at  $56^\circ\text{C}$  for 30 minutes, 1 mL of a solution dissolved  $1.0 \times 10^5$  units of potassium benzylpenicillin and 0.1 g (potency) of streptomycin sulfate in 10 mL of isotonic sodium chloride solution, and add 5  $\mu\text{L}$  of 2-mercaptoethanol solution (9 in 125). Sterilize this solution by filtration.

**Potency measuring medium for teceleukin** Add 100 mL of fetal calf serum to 1000 mL of medium for float culture. Store at  $4^\circ\text{C}$ .

**Powdered tragacanth** [Same as the namesake monograph]

**( $\pm$ )-Praeruptorin A for thin-layer chromatography**

$\text{C}_{21}\text{H}_{22}\text{O}_7$  White, crystals or crystalline powder. Soluble in methanol, sparingly slightly soluble in ethanol (99.5), and practically insoluble in water.

**Identification**—Determine the absorption spectrum of a solution of ( $\pm$ )-praeruptorin A for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 320 nm and 324 nm.

**Melting point** <2.60>: 152–156°C

**Purity** Related substances—Dissolve 2 mg of ( $\pm$ )-praeruptorin A for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed with 5  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification (1) under Peucedanum

**Root:** the spot other than the principal spot of around *Rf* value of 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

**Pravastatin sodium**  $C_{23}H_{35}NaO_7$  [Same as the namesake monograph]

**Prazepam for assay**  $C_{19}H_{17}ClN_2O$  [Same as the monograph Prazepam. When dried, it contains not less than 99.0% of prazepam ( $C_{19}H_{17}ClN_2O$ ).]

**Prednisolone**  $C_{21}H_{28}O_5$  [Same as the namesake monograph]

**Prednisolone acetate**  $C_{23}H_{30}O_6$  [Same as the namesake monograph]

**Prednisone**  $C_{21}H_{26}O_5$  White crystalline powder. Slightly soluble in methanol, in ethanol (95) and in chloroform, and very slightly soluble in water.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +167 – +175° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

*Loss on drying* <2.41>: not more than 1.0% (1 g, 105°C, 3 hours).

**Content:** 96.0 – 104.0%. Assay—Weigh accurately about 20 mg of prednisone, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, dilute with methanol to make exactly 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and read the absorbance *A* at the wavelength of maximum absorption at about 238 nm.

$$\text{Amount (mg) of prednisone } (C_{21}H_{26}O_5) = \frac{A}{440} \times 20,000$$

**Primary antibody TS** To a mixture of 1.5 mL of blocking TS for epoetin alfa and 13.5 mL of sodium azide-phosphate-buffered sodium chloride TS add a volume of mouse anti-epoetin alfa monoclonal antibody corresponding to 100 µg of protein, 50 µL of a solution of aprotinin containing  $1 \times 10^5$  units in 5 mL of water and 100 µL of phenylmethylsulfonyl fluoride solution containing 1.74 mg in 100 mL of methanol.

**Primer F** A primer corresponding to the Alu sequence. Synthesize an oligonucleotide which nucleotide sequence is represented by “5'-CATCCTGGCYAACAYGGTGAAAC-3'”, and use.

**Primer F TS** To primer F add TE buffer solution so that primer F is 100 µmol/L. Then add tris-glycine buffer solution (pH 6.8) so that primer F is 25 µmol/L.

**Primer R** A primer corresponding to the Alu sequence. Synthesize an oligonucleotide which nucleotide sequence is represented by “5'-ATTCTCCTGCCTCAGCCTCC-3'”, and use.

**Primer R TS** To primer R add TE buffer solution so that primer R is 100 µmol/L. Then, add tris-glycine buffer solution (pH 6.8) so that primer R is 25 µmol/L.

**Probencid**  $C_{13}H_{19}NO_4S$  [Same as the namesake monograph]

**Procainamide hydrochloride**  $C_{13}H_{21}N_3O.HCl$  [Same as the namesake monograph]

**Procainamide hydrochloride for assay**  $C_{13}H_{21}N_3O.HCl$  [Same as the monograph Procainamide Hydrochloride. When dried, it contains not less than 99.0% of procainamide hydrochloride ( $C_{13}H_{21}N_3O.HCl$ ).]

**Procaine hydrochloride**  $C_{13}H_{20}N_2O_2.HCl$  [Same as the namesake monograph]

**Procaine hydrochloride for assay** See procaine hydrochloride.

**Procaterol hydrochloride** See procaterol hydrochloride hydrate.

**Procaterol hydrochloride hydrate**  $C_{16}H_{22}N_2O_3.HCl \cdot \frac{1}{2}H_2O$  [Same as the namesake monograph]

**Progesterone**  $C_{21}H_{30}O_2$  [Same as the namesake monograph]

**L-Proline**  $C_5H_9NO_2$  [K 9107, L(-)-proline, Special class]

**Propafenone hydrochloride for assay**  $C_{21}H_{27}NO_3.HCl$  [Same as the monograph Propafenone Hydrochloride. When dried, it contains not less than 99.0% of propafenone hydrochloride ( $C_{21}H_{27}NO_3.HCl$ ). When perform the test as directed in the Purity (2), the total area of the peaks other than propafenone is not larger than 3 times the peak area of propafenone from the standard solution.]

**n-Propanol** See 1-propanol.

**1-Propanol**  $CH_3CH_2CH_2OH$  [K 8838, Special class]

**2-Propanol**  $(CH_3)_2CHOH$  [K 8839, Special class]

**2-Propanol for vitamin A assay**  $(CH_3)_2CHOH$  [K 8839, Special class] When the absorbances at 300 nm and between 320 nm and 350 nm are determined as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control, they are not more than 0.05 and not more than 0.01, respectively. If necessary, purify by distillation.

**2-Propanol for liquid chromatography**  $(CH_3)_2CHOH$

Clear, colorless and volatile liquid, having a characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether. Boiling point: about 82°C.

*Refractive index* <2.45>  $n_D^{20}$ : 1.376 – 1.378

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.785 – 0.788

**Purity** (1) Ultraviolet absorbing substances—Perform the test with 2-propanol for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbance at 230 nm is not more than 0.2; at 250 nm, not more than 0.03; and between 280 nm and 400 nm, not more than 0.01.

(2) Peroxide—Mix 100 mL of water and 25 mL of dilute sulfuric acid, and add 25 mL of a solution of potassium iodide (1 in 10). Add this solution to 20 g of 2-propanol for liquid chromatography. Stopper tightly, shake, allow to stand for 15 minutes in a dark place, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction (not more than 0.0005%).

**Propanol, iso** See 2-propanol.

**Propranolol hydrochloride for assay**  $C_{16}H_{21}NO_2.HCl$

[Same as the monograph Propranolol Hydrochloride. When dried, it contains not less than 99.5% of propranolol hydrochloride ( $C_{16}H_{21}NO_2.HCl$ ).]

**Propantheline bromide**  $C_{23}H_{30}BrNO_3$  [Same as the namesake monograph]

**Propionic acid**  $CH_3CH_2COOH$  Colorless liquid.

**Purity**—Clarity and color of solution—Dissolve 1.0 g of

propionic acid in 20 mL of ethanol (95): the solution is clear and colorless.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.998 – 1.004

**Distilling range** <2.57>: 139 – 143°C, not less than 95 vol%.

**Propylamine, iso**  $(\text{CH}_3)_2\text{CHNH}_2$  Colorless liquid, having a characteristic, amine-like odor. Miscible with water, with ethanol (95) and with diethyl ether.

**Refractive index** <2.45>  $n_D^{20}$ : 1.374 – 1.376

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.685 – 0.690

**Distilling range** <2.57>: 31 – 33°C, not less than 95 vol%.

**Propyl benzoate**  $\text{C}_6\text{H}_5\text{COOC}_3\text{H}_7$  Clear, colorless liquid, having a characteristic odor.

**Refractive index** <2.45>  $n_D^{20}$ : 1.498 – 1.503

**Specific gravity** <2.56>  $d_{20}^{20}$ : 1.022 – 1.027

**Propylene carbonate**  $\text{C}_4\text{H}_6\text{O}_3$  Colorless liquid.

**Boiling point** <2.57>: 240 – 242°C

**Water** <2.48>: less than 0.1%.

**Propylene carbonate for water determination** To 1000 mL of propylene carbonate add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear propylene carbonate layer. Preserve this protecting from moisture. The water content should not be more than 0.3 mg per mL.

**Propylene glycol**  $\text{CH}_3\text{CH}(\text{OH})\text{CH}_2\text{OH}$  [K 8837, Special class]

**Propylene glycol cefatrizine**  $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2\text{.C}_3\text{H}_8\text{O}_2$

[Same as the namesake monograph]

**Propylene glycol for gas chromatography**  $\text{C}_3\text{H}_8\text{O}_2$  [K 8837, Special class] When perform the test as directed in the Purity (7) under Propylene Glycol, it does not show any peak at the retention times corresponding to ethylene glycol and diethylene glycol.

**Propylether, iso**  $(\text{CH}_3)_2\text{CHOCH}(\text{CH}_3)_2$  Clear, colorless liquid, having a characteristic odor. Not miscible with water.

**Refractive index** <2.45>  $n_D^{20}$ : 1.368 – 1.369

**Specific gravity** <2.56>  $d_4^{20}$ : 0.723 – 0.725

#### Propyl parahydroxybenzoate

$\text{HOOC}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{CH}_3$  [Same as the namesake monograph]

#### Propyl parahydroxybenzoate for resolution check

$\text{C}_{10}\text{H}_{12}\text{O}_3$  Colorless crystals or a white crystalline powder. Freely soluble in methanol, in ethanol (95) and in acetone, and very slightly soluble in water. Melting point: 96 – 99°C.

**Identification**—Determine the infrared absorption spectrum of propyl parahydroxybenzoate for resolution check as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum of Propyl Parahydroxybenzoate or the spectrum of Propyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** Related substances—Dissolve 50 mg of propyl parahydroxybenzoate for resolution check in 2.5 mL of methanol, and add the mobile phase to make 50 mL. To 10 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine areas of all peaks of both solutions by the automatic integration method: the total area of the peaks other than prostaglandin A<sub>1</sub> obtained from the sample solution is not larger than the peak area of prostaglandin A<sub>1</sub> from the standard solution.

<2.01> according to the following conditions. Determine each peak area by the automatic integration method: total area of the peaks other than propyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of propyl parahydroxybenzoate from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Propyl Parahydroxybenzoate.

Time span of measurement: About 2.5 times as long as the retention time of propyl parahydroxybenzoate.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of propyl parahydroxybenzoate obtained with 10  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu\text{L}$  of the standard solution.

System performance: When the procedure is run with 10  $\mu\text{L}$  of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propyl parahydroxybenzoate are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propyl parahydroxybenzoate is not more than 5.0%.

**Propylthiouracil for assay**  $\text{C}_7\text{H}_{10}\text{N}_2\text{OS}$  [Same as the monograph Propylthiouracil. When dried, it contains not less than 99.0% of propylthiouracil ( $\text{C}_7\text{H}_{10}\text{N}_2\text{OS}$ ).]

**Prostaglandin A<sub>1</sub>**  $\text{C}_{20}\text{H}_{32}\text{O}_4$  White, crystals or crystalline powder. Very soluble in ethanol (95) and in ethyl acetate, and very slightly soluble in water.

**Purity** Related substances—Dissolve 5 mg of prostaglandin A<sub>1</sub> in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine areas of all peaks of both solutions by the automatic integration method: the total area of the peaks other than prostaglandin A<sub>1</sub> obtained from the sample solution is not larger than the peak area of prostaglandin A<sub>1</sub> from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed the operating conditions in the Assay under Alprostadil Alfadex.

**Detection sensitivity:** Adjust the detection sensitivity so that the peak height of prostaglandin A<sub>1</sub> obtained with 10  $\mu\text{L}$  of the standard solution is 5 to 10% of the full scale.

Time span of measurement: About twice as long as the retention time of prostaglandin A<sub>1</sub>, beginning after the solvent peak.

**Protein digestive enzyme TS** A solution of lysyl endopeptidase in 0.05 mol/L tris buffer solution (pH 8.6) (1 in 50,000).

**Pseudoephedrine hydrochloride**  $\text{C}_{10}\text{H}_{15}\text{NO.HCl}$  White, crystals or crystalline powder. Freely soluble in water, in methanol and in acetic acid (100), soluble in ethanol (99.5), and practically insoluble in acetic anhydride. Melting point: 182 – 186°C.

**Purity** Related substances—Dissolve 1 mg of pseudoephedrine hydrochloride in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the

test with 10  $\mu$ L of the sample solution for twice as long as the retention time of ephedrine as directed in the Assay (1) under Kakkonto Extract: the total area of the peaks other than pseudoephedrine and the solvent is not larger than 1/10 times the total area of the peaks other than the solvent.

**Puerarin for thin-layer chromatography**  $C_{21}H_{20}O_9$

White, crystals or crystalline powder. Freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water.

**Identification** Determine the infrared absorption spectrum of puerarin to be examined as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3370  $cm^{-1}$ , 1632  $cm^{-1}$ , 1447  $cm^{-1}$ , 1060  $cm^{-1}$  and 836  $cm^{-1}$ .

**Purity** Related substances—Dissolve 1 mg of puerarin to be examined in methanol to make exactly 1 mL. Perform the test with 2  $\mu$ L of this solution as directed in the Identification under Pueraria Root: any spot other than the principal spot with an *R<sub>f</sub>* value of about 0.4 does not appear.

**Pullulanase** An enzyme obtained from *Klebsiella pneumoniae*. White crystals. It contains not less than 30 units per mg. One unit is an enzymatic activity to produce 1  $\mu$ mol of maltotriose from pullulan per minute at pH 5.0 and 30°C.

**Pullulanase TS** A solution of pullulanase containing 10 units per mL.

**Purified hydrochloric acid** See hydrochloric acid, purified.

**Purified sodium hyaluronate** See sodium hyaluronate, purified.

**Purified methanol** See methanol, purified.

**Purified sulfuric acid** See sulfuric acid, purified.

**Purified water** [Use the water prescribed by the monographs of Purified Water or Purified Water in Containers. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of the relevant test.]

**Pyrazole**  $C_3H_4N_2$  White to pale yellow, crystals or crystalline powder.

*Melting point* <2.60>: 67 – 71°C

**Pyridine**  $C_5H_5N$  [K 8777, Special class]

**Pyridine-acetic acid TS** Dilute 20 mL of pyridine with sufficient diluted acetic acid (100) (1 in 25) to make 100 mL. Prepare before use.

**Pyridine, dehydrated**  $C_5H_5N$  To 100 mL of pyridine add 10 g of sodium hydroxide, and allow to stand for 24 hours. Decant the supernatant liquid, and distill.

**Pyridine for water determination** Add potassium hydroxide or barium oxide to pyridine, stopper tightly, and allow to stand for several days. Distill and preserve the purified and dried pyridine, protecting it from moisture. The water content of this pyridine for water determination should not be more than 1 mg per mL.

**0.2 mol/L Pyridine-formic acid buffer solution (pH 3.0)**

To 15.82 g of pyridine add 900 mL of water, mix well, adjust the pH to 3.0 with diluted formic acid (1 in 2), and add water to make 1000 mL.

**Pyridine-pyrazolone TS** Dissolve, with thorough shaking, 0.1 g of 3-methyl-1-phenyl-5-pyrazolone in 100 mL of water by heating between 65°C and 70°C, and cool below

30°C. Mix this solution with a solution prepared by dissolving 20 mg of bis-(1-phenyl-3-methyl-5-pyrazolone) in 20 mL of pyridine. Prepare before use.

**Pyridoxine hydrochloride**  $C_8H_{11}NO_3 \cdot HCl$  [Same as the namesake monograph]

**1-(2-Pyridylazo)-2-naphthol**  $C_{15}H_{11}N_3O$  Orange-yellow or orange-red powder.

**Absorbance**—Dissolve 25 mg of 1-(2-pyridylazo)-2-naphthol in methanol to make exactly 100 mL. Pipet 2.0 mL of this solution, and add methanol to make exactly 50 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; absorbance at the wavelength of 470 nm is not less than 0.55.

*Melting point* <2.60>: 137 – 140°C

**Purity** Clarity and color of solution—Dissolve 25 mg of 1-(2-pyridylazo)-2-naphthol in 100 mL of methanol: the solution is clear and orange-yellow.

**Residue on ignition** <2.44>: not more than 1.0%.

**Sensitivity**—On adding 50 mL of water, 30 mL of methanol and 10 mL of acetic acid-sodium acetate buffer solution (pH 5.5) to 0.2 mL of a solution of 1-(2-pyridylazo)-2-naphthol in methanol (1 in 4000), the solution is yellow in color. Add 1 drop of a solution of copper (II) chloride dihydrate (1 in 600) to this solution: the solution is red-purple in color. Add a subsequent 1 drop of diluted 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (1 in 10): the color of the solution changes to yellow again.

**1-(4-Pyridyl)pyridinium chloride hydrochloride**

$C_{10}H_9ClN_2 \cdot HCl$  White to yellow-white crystalline powder. Very soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

*Melting point* <2.60>: 154 – 156°C

**Pyrogallol**  $C_6H_3(OH)_3$  [K 8780, Special class]

**L-Pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride**  $C_{19}H_{26}N_8O_6 \cdot HCl$  White to light yellow powder. Freely soluble in water, in methanol and in acetic acid (100).

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (316 nm): 242 – 268 (2 mg, water, 100 mL).

**Optical rotation** <2.49>  $[\alpha]_D^{25}$ : –51 – –56° [0.1 g, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm].

**Purity** Related substances—Dissolve 50 mg of L-pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu$ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (15:12:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

**L-Pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride TS** Dissolve 25 mg of L-pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride and 40 mg of D-mannitol in 2 to 3 mL of water, lyophilize, and add 16.7 mL of water to dissolve. To 1 volume of this solution add 9 volumes of water before use.

**Pyrrole**  $C_4H_5N$  Clear, colorless liquid, having a characteristic odor. Soluble in ethanol (95) and in diethyl ether,

and practically insoluble in water.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.965 – 0.975

**Pyrophosphate buffer solution (pH 9.0)** Dissolve 3.3 g of potassium pyrophosphate, 15 mg of dithiothreitol and 40 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 70 mL of water, adjust the pH with a solution of citric acid monohydrate (21 in 100) to exactly 9.0, and add water to make 100 mL.

**2-Pyrrolidone**  $C_4H_7NO$  Clear, colorless to pale yellow liquid, or white to pale yellow, masses or powder. Odorless.

**Congealing point** <2.42>: 22 – 26°C

**Purity** Dissolve about 1 g of 2-pyrrolidone in 10 mL of methanol, and use this solution as the sample solution. Perform the test with 1  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of 2-pyrrolidone by the area percentage method: not less than 98.0%.

#### Operating conditions

Detector: Hydrogen flame-ionization detector.

Column: A capillary glass column 0.53 mm in inside diameter and 30 m in length, coated with a 1.0- $\mu$ m layer of polyethylene glycol 20 M for gas chromatography on the inner surface.

Column temperature: Maintain the temperature at 80°C for 1 minute, then raise at the rate of 10°C per minute to 190°C, and maintain at this temperature for 20 minutes.

Temperature of sample vaporization chamber: A constant temperature of about 200°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of 2-pyrrolidone is about 10 minutes.

Split ratio: 1:20.

Time span of measurement: About 2 times as long as the retention time of 2-pyrrolidone.

**Water** <2.48> Not more than 0.2% (5 g, volumetric titration, direct titration).

**0.05 mol/L Pyrophosphate buffer solution (pH 9.0)** Dissolve 0.83 g of potassium pyrophosphate in 40 mL of water, adjust the pH with 1 mol/L hydrochloric acid TS to 9.0, and add water to make 50 mL. Adjust the temperature to 22 ± 2°C before use.

**Quinapril hydrochloride for assay**  $C_{25}H_{30}N_2O_5 \cdot HCl$

[Same as the monograph Quinapril Hydrochloride. When perform the test as directed in the Purity (2) of Quinapril Hydrochloride, the area of the peaks, having the relative retention time of about 0.5 and about 2.0 to quinapril obtained from the sample solution, is not larger than the peak area of quinapril from the standard solution, the area of peak other than quinapril and the peak mentioned above from the sample solution is not larger than 2/5 times the peak area of quinapril from the standard solution, and the total area of the peaks other than quinapril from the sample solution is not larger than 2 times the peak area of quinapril from the standard solution.]

**Quinhydrone**  $C_6H_4(OH)_2 \cdot C_6H_4O_2$  Green, crystals or crystalline powder.

**Melting point** <2.60>: 169 – 172°C

**Quinidine sulfate** See quinidine sulfate hydrate.

**Quinidine sulfate hydrate**  $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$   
[Same as the namesake monograph]

**Quinine sulfate** See quinine sulfate hydrate.

**Quinine sulfate hydrate**  $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$

[Same as the namesake monograph]

**Quinoline**  $C_9H_7N$  [K 8279, Special class]

**Quinoline TS** Mix 50 mL of quinoline with 360 mL of diluted hydrochloric acid (1 in 6), previously heated, cool, and filter if necessary.

**8-Quinolinol**  $C_9H_7NO$  [K 8775, Special class]

**Rabbit anti-nartogristim antibody** Dissolve the antibody obtained from rabbit antiserum, prepared by immunizing with Nartogristim (Genetical Recombination), in tris-acetic acid buffer solution (pH 8.0) so that each mL contains 1 mg of rabbit anti-nartogristim antibody. Storage at –80°C.

**Performance test:** When perform the test by Ouchterlony method, a precipitation line is appeared against Nartogristim (Genetical Recombination).

**Protein concentration:** Determine the absorbance at 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, and calculate the protein concentration using the specific absorbance  $E_{1\text{cm}}^{1\%}$  15.

**Rabbit anti-nartogristim antibody TS** To rabbit anti-nartogristim antibody add bovine serum albumin TS for nartogristim test so that each mL contains 0.2  $\mu$ g of rabbit anti-nartogristim antibody. Prepare before use.

**Raney nickel catalyst** Grayish black powder. An alloy containing 40 to 50% of nickel and 50 to 60% of aluminum.

**Ranitidinediamine**  $(C_{10}H_{18}N_2OS)_2 \cdot C_4H_4O_4$  White to pale yellow crystalline powder.

**Identification**—Determine the infrared absorption spectrum of ranitidinediamine as directed in the paste method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 2780  $\text{cm}^{-1}$ , 1637  $\text{cm}^{-1}$ , 1015  $\text{cm}^{-1}$  and 788  $\text{cm}^{-1}$ .

**Content:** not less than 95%. **Assay**—Weigh accurately about 0.1 g of ranitidinediamine, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple to green through blue (indicator: crystal violet TS). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 13.62 mg of  $(C_{10}H_{18}N_2OS)_2 \cdot C_4H_4O_4$

**Rebamipide for assay**  $C_{19}H_{15}ClN_2O_4$  [Same as the monograph Rebamipide. When dried, it contains not less than 99.5% of rebamipide ( $C_{19}H_{15}ClN_2O_4$ ).]

**Reduced iron** See iron powder.

**Reduction buffer solution for nartogristim sample** Mix 0.8 mL of sodium lauryl sulfate solution (1 in 10), 0.5 mL of 0.5 mol/L tris buffer solution (pH 6.8), 0.4 mL of glycerin, 0.3 mL of 2-mercaptoethanol and 0.1 mL of bromophenol blue solution (1 in 200). Prepare before use.

**Reduction liquid for molecular mass determination** Dissolve 10.6 g of sodium lauryl sulfate and 3.9 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 60 mL of water, adjust to pH 6.8 with hydrochloric acid, dissolve 31 g of sucrose, and add water to make 100 mL. To 97 mL of this solution add 3 mL of bromophenol blue solution (11 in 2500). To 0.4 mL of this solution add 0.1 mL of 2-mercaptoethanol. Prepare before use.

**Reference anti-interleukin-2 antibody for teceleukin**

Monoclonal antibody obtained from a fusion cell strain

from mouse spleen cells sensitized to teceleukin and mouse melanoma cells, or alternately, rabbit antiserum towards human interleukin-2, that is purified using affinity chromatography. When determining the neutralizing activity, taking 1 neutralizing unit as the titer that neutralizes one unit of activity of teceleukin, contains at least 2000 neutralizing units per mL.

**Reference anti-interleukin-2 antiserum TS** Anti-interleukin-2 antiserum is diluted with culture media for celmoleukin, so that the diluted antiserum solution neutralizes the same volume of about 800 unit per mL solution of Cel-moleukin (Genetical Recombination).

**Reference suspension 1** To 5.0 mL of formazin opalescence standard solution add 95.0 mL of water. Mix and shake before use.

**Reinecke salt** See reinecke salt monohydrate.

**Reinecke salt monohydrate**  $\text{NH}_4[\text{Cr}(\text{NH}_3)_2(\text{SCN})_4] \cdot \text{H}_2\text{O}$   
Dark red, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum of Reinecke salt monohydrate as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3310\text{ cm}^{-1}$ ,  $2130\text{ cm}^{-1}$ ,  $1633\text{ cm}^{-1}$ ,  $1400\text{ cm}^{-1}$ ,  $1261\text{ cm}^{-1}$  and  $711\text{ cm}^{-1}$ .

**Reinecke salt TS** To 20 mL of water add 0.5 g of Reinecke salt monohydrate, shake frequently for 1 hour, then filter. Use within 48 hours.

**Resazurin**  $\text{C}_{12}\text{H}_6\text{NNaO}_4$  Brownish purple powder. It dissolves in water and the solution is purple in color.

**Residue on ignition** <2.44>: not less than 28.5% (1 g).

**Resazurin solution** Prepared for the test for measurement of living cell.

**Resibufogenin for assay**  $\text{C}_{24}\text{H}_{32}\text{O}_4$  Odorless white crystalline powder.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (300 nm): 131 – 145 (10 mg, methanol, 250 mL), dried in a desiccator (silica gel) for 24 hours.

**Purity** Related substances—Weigh accurately 40 mg of resibufogenin for assay and proceed as directed in the Purity under bufalin for assay.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 10 mg of resibufogenin for assay, previously dried in a desiccator (silica gel) for 24 hours, add methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of resibufogenin by the area percentage method.

#### Operating conditions

**Detector:** An ultraviolet absorption photometer (wavelength: 300 nm).

**Column:** A stainless steel column about 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 mm in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of water and acetonitrile (1:1).

**Flow rate:** Adjust so that the retention time of resibufogenin is about 9 minutes.

**Time span of measurement:** About twice as long as the retention time of resibufogenin, beginning after the peak of

solvent.

#### System suitability

**Test for required detectability:** Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of resibufogenin obtained with 20  $\mu\text{L}$  of standard solution (2) can be measured by the automatic integration method and the peak height of resibufogenin with 20  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

**System performance:** Dissolve 10 mg each of resibufogenin for assay, bufalin for assay and cinobufagin for assay in methanol to make 200 mL. When the procedure is run with 20  $\mu\text{L}$  of this solution according to the above operating conditions, bufalin, cinobufagin and resibufogenin are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

**Resibufogenin for component determination** See resibufogenin for assay.

**Resibufogenin for thin-layer chromatography**  $\text{C}_{24}\text{H}_{32}\text{O}_4$   
White crystalline powder having no odor. It is freely soluble in acetone and in methanol.

**Purity** Related substances—Dissolve 5.0 mg of resibufogenin for thin-layer chromatography in exactly 5 mL of acetone. Perform the test with 5  $\mu\text{L}$  of this solution as directed in the Identification under Toad Cake: no spot other than the principal spot with an *Rf* value of about 0.4 appear.

**Resolving gel for celmoleukin** Prepare the resolving gel in tris buffer solution (pH 8.8) using ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine so the concentrations of acrylamide and sodium lauryl sulfate are 13.5% and 0.1%, respectively.

**Resorcin** See resorcinol.

**Resorcinol**  $\text{C}_6\text{H}_4(\text{OH})_2$  [K 9032, Special class]

**Resorcinol sulfuric acid TS** Dissolve 0.1 g of resorcinol in 10 mL of diluted sulfuric acid (1 in 10).

**Resorcinol TS** Dissolve 0.1 g of resorcinol in 10 mL of hydrochloric acid. Prepare before use.

**Resorcinol-copper (II) sulfate TS** Dissolve 0.1 g of resorcinol in 5 mL of water, add 125  $\mu\text{L}$  of 0.1 mol/L copper (II) sulfate solution, 24 mL of hydrochloric acid, and add water to make 50 mL. Prepare this TS at least 4 hours before the time of use.

**Resorcin sulfuric acid TS** See resorcinol sulfuric acid TS.

**Resorcin TS** See resorcinol TS.

**L-Rhamnose monohydrate**  $\text{C}_6\text{H}_{12}\text{O} \cdot \text{H}_2\text{O}$  White crystalline powder having sweet taste. Freely soluble in water, and sparingly soluble in ethanol (95).

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : +7.8 – +8.3° (1 g, 20 mL of water, 2 drops of ammonia TS, 100 mm).

**Melting point** <2.60>: 87 – 91°C

**Purity** Related substances—Dissolve 1.0 mg of L-rhamnose monohydrate in 1 mL of water, and add methanol to make exactly 10 mL. Proceed with 20  $\mu\text{L}$  of this solution as directed in the Identification under Acacia: any spot other than the principal spot at the *Rf* value of about 0.5 does not appear.

**Rhaponticin for purity**  $\text{C}_{21}\text{H}_{24}\text{O}_9$  A white to pale yellow-brown crystalline powder, having no odor. Slightly solu-

ble in methanol, and practically insoluble in water and in ethanol (99.5).

**Identification**—Determine the infrared absorption spectrum of rhabdomicin for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $1612\text{ cm}^{-1}$ ,  $1577\text{ cm}^{-1}$ ,  $1513\text{ cm}^{-1}$ ,  $948\text{ cm}^{-1}$ ,  $831\text{ cm}^{-1}$  and  $798\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 4 mg of rhabdomicin for purity in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with  $10\text{ }\mu\text{L}$  each of the sample solution and standard solution as directed in the Purity (3) under Rhubarb; the spot other than the principal spot that appears at an *Rf* value of about 0.3 obtained with the sample solution is not more intense than the spot with the standard solution.

**Rhein for assay**  $\text{C}_{15}\text{H}_8\text{O}_6$  Use rhein for thin-layer chromatography meeting the following additional specifications. Its content is corrected based on the amount (%) of rhein obtained in the Assay.

**Absorbance** <2.24>  $E_{1\text{ cm}}^{1\%}$  (257 nm): 678 – 720 (3 mg, methanol, 500 mL).

**Unity of peak**—Dissolve 1 mg of rhein for assay in 100 mL of acetone, and use this solution as the sample solution. Perform the test with  $10\text{ }\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of rhein peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

#### Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ( $5\text{ }\mu\text{m}$  in particle diameter).

Column temperature: A constant temperature of about  $50^\circ\text{C}$ .

Mobile phase: A mixture of water, acetonitrile, and phosphoric acid (650:350:1).

Flow rate: Adjust so that the retention time of rhein is about 14 minutes.

#### System suitability

System performance: Proceed as directed in the system suitability in the Assay (5) under Otsujito Extract.

**Assay**—Weigh accurately 5 mg of rhein for assay and 1 mg of DSS- $d_6$  for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated dimethyl sulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure  $^1\text{H-NMR}$  as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using DSS- $d_6$  for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity *A* (equivalent to 1 hydrogen) of the signal around  $\delta 8.16\text{ ppm}$  assuming the signal of the reference standard for qNMR as  $\delta 0$ .

$$\begin{aligned} \text{Amount (\%)} \text{ of rhein} (\text{C}_{15}\text{H}_8\text{O}_6) \\ = M_S \times I \times P / (M \times N) \times 1.2668 \end{aligned}$$

*M*: Amount (mg) of rhein for assay taken

*M<sub>S</sub>*: Amount (mg) of DSS- $d_6$  for nuclear magnetic reso-

nance spectroscopy taken

*I*: Signal resonance intensity based on the signal resonance intensity of DSS- $d_6$  for nuclear magnetic resonance spectroscopy as 9.000

*N*: Number of the hydrogen derived from *A*

*P*: Purity (%) of DSS- $d_6$  for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having  $^1\text{H}$  resonance frequency of not less than 400 MHz.

Target nucleus:  $^1\text{H}$ .

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between  $-5\text{ ppm}$  and  $15\text{ ppm}$ .

Spinning: off.

Pulse angle:  $90^\circ$ .

$^{13}\text{C}$  decoupling: on.

Delay time: Repeating pulse waiting time is not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between  $20^\circ\text{C}$  and  $30^\circ\text{C}$ .

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal of around  $\delta 8.16\text{ ppm}$  is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal of around  $\delta 8.16\text{ ppm}$  is not overlapped with any signal of obvious foreign substance.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the resonance intensity *A* to that of the reference standard for qNMR is not more than 1.0%.

**Rhein for thin-layer chromatography**  $\text{C}_{15}\text{H}_8\text{O}_6$  A yellow to reddish yellow powder. Very slightly soluble in acetone, and practically insoluble in water, in methanol, and in ethanol (99.5).

**Identification**—Determine the absorption spectrum of a solution in methanol (3 in 500,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 228 nm and 232 nm, between 255 nm and 259 nm, and between 429 nm and 433 nm.

**Purity** Related substances—Dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and perform the test with  $2\text{ }\mu\text{L}$  of this solution as directed in the Identification (1) under Daiokanzoto Extract: no spot other than the principal spot at an *Rf* value of about 0.3 appears.

**Rhyncophylline for assay**  $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_4$  Rhyncophylline for thin-layer chromatography. It meets the following requirements.

**Absorbance** <2.24>:  $E_{1\text{ cm}}^{1\%}$  (245 nm): 473 – 502 (5 mg of the dried substance in a desiccator (silica gel) for 24 hours, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

**Purity** Related substances—Dissolve 5 mg of rhyncophylline for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly  $20\text{ }\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid-chromatography

<2.01> according to the following conditions. Determine the peak area of each solution by the automatic integration method: the total area of the peaks other than rhyncophylline obtained from the sample solution is not larger than the peak area of rhyncophylline from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Uncaria Hook.

Time span of measurement: About 4 times as long as the retention time of rhyncophylline, beginning after the solvent peak.

#### System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Uncaria Hook.

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of rhyncophylline obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

**Rhynchophylline for component determination** See rhynchophylline for assay.

#### Rhynchophylline for thin-layer chromatography

$C_{22}H_{28}N_2O_4$  White, crystals or crystalline powder. Slightly soluble in ethanol (99.5) and in acetone, and practically insoluble in water. Melting point: 205 – 209°C.

**Identification**—Determine the absorption spectrum of a solution of rhyncophylline for thin-layer chromatography in a mixture of methanol and dilute acetic acid (7:3) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 242 nm and 246 nm.

**Purity** Related substances—Dissolve 1.0 mg of rhyncophylline for thin-layer chromatography in 1 mL of acetone, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): no spot other than the principal spot at around  $R_f$  value of 0.5 appears.

**Ribavirin**  $C_8H_{12}N_4O_5$  [Same as the namesake monograph]

**Riboflavin**  $C_{17}H_{20}N_4O_6$  [Same as the namesake monograph]

**Riboflavin sodium phosphate**  $C_{17}H_{20}N_4NaO_9P$  [Same as the namesake monograph]

**Ribonuclease A for gel filtration molecular mass marker** Obtained from bovine pancreas, for gel filtration chromatography.

#### Rilmazafone hydrochloride hydrate

$C_{21}H_{20}Cl_2N_6O_3 \cdot HCl \cdot 2H_2O$  [Same as the namesake monograph]

**Risperidone for assay**  $C_{23}H_{27}FN_4O_2$  [Same as the monograph Risperidone. It contains not less than 99.5% of risperidone ( $C_{23}H_{27}FN_4O_2$ ), calculated on the dried basis.]

**Ritodrine hydrochloride**  $C_{17}H_{21}NO_3 \cdot HCl$  [Same as the namesake monograph]

**Rose Bengal**  $C_{20}H_2Cl_4I_4Na_2O_5$  [Special class] A red-brown powder, and shows a purple red when dissolved in water.

**Rosmarinic acid for assay**  $C_{18}H_{16}O_8$  Use rosmarinic acid for thin-layer chromatography meeting the following additional specifications. Its content is corrected based on the amount (%) of rosmarinic acid obtained in the Assay.

**Unity of peak**—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 1 mg of rosmarinic acid for assay in 50 mL of ethanol, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of rosmarinic acid peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

#### Operating conditions

Detector: A photodiode array detector (wavelength: 330 nm, spectrum range of measurement: 220 – 400 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (1 in 100) and methanol (13:7).

Flow rate: Adjust so that the retention time of rosmarinic acid is about 10 minutes.

#### System suitability

System performance: When the procedure is run with 10  $\mu$ L of the sample solution, previously irradiated ultraviolet light (main wavelength: 365 nm) for 30 minutes, under the above operating conditions, an obvious peak is observed just before the peak of rosmarinic acid, and the resolution between these peaks is not less than 1.5.

**Assay**—Weigh accurately 5 mg of rosmarinic acid for assay and 1 mg of DSS- $d_6$  for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure  $^1H$ -NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using DSS- $d_6$  for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity  $A$  (equivalent to 1 hydrogen) of the signal around  $\delta$  6.27 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\begin{aligned} \text{Amount (\%)} \text{ of rosmarinic acid } &(C_{18}H_{16}O_8) \\ &= M_S \times I \times P / (M \times N) \times 1.6059 \end{aligned}$$

$M$ : Amount (mg) of rosmarinic acid for assay 2 taken

$M_S$ : Amount (mg) of DSS- $d_6$  for nuclear magnetic resonance spectroscopy taken

$I$ : Signal resonance intensity  $A$  based on the signal resonance intensity of DSS- $d_6$  for nuclear magnetic resonance spectroscopy as 9.000

$N$ : Number of the hydrogen derived from  $A$

$P$ : Purity (%) of DSS- $d_6$  for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having  $^1H$  resonance frequency of not less than 400 MHz.

Target nucleus:  $^1H$ .

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between -5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

$^{13}\text{C}$  decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal of around  $\delta$  6.27 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal of around  $\delta$  6.27 ppm is not overlapped with any obvious signal of foreign substance.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity  $A$  to that of the reference standard for qNMR is not more than 1.0%.

**Rosmarinic acid for component determination** See rosmarinic acid for assay.

#### Rosmarinic acid for thin-layer chromatography

$\text{C}_{18}\text{H}_{16}\text{O}_8$  White to pale yellow, crystals or crystalline powder. Freely soluble in ethanol (99.5), and slightly soluble in water. Melting point: about 170°C (with decomposition).

**Identification**—Determine the absorption spectrum of a solution of rosmarinic acid for thin-layer chromatography (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 215 nm and 219 nm and between 322 nm and 326 nm.

**Purity Related substances**—Conduct this procedure using light-resistance vessels. Dissolve 10 mg of rosmarinic acid for thin-layer chromatography in 2 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Proceed with 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification (2) under Hanekobokuto Extract: the spot other than the principal spot of around  $R_f$  value of 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

**Rosuvastatin calcium** ( $\text{C}_{22}\text{H}_{27}\text{FN}_3\text{O}_6\text{S}_2\text{Ca}$ ) [Same as the namesake monograph]

**Rosuvastatin calcium enantiomer** ( $\text{C}_{22}\text{H}_{27}\text{FN}_3\text{O}_6\text{S}_2\text{Ca}$ ) White powder.

**Identification**—(1) Proceed the test as directed in the system performance of the system suitability in the Purity (4) under Rosuvastatin Calcium: the relative retention time of rosuvastatin enantiomer to rosuvastatin peak is about 0.92.

(2) Determine the  $^1\text{H}$  spectrum of a solution of Rosuvastatin calcium enantiomer in deuterated dimethyl sulfoxide for nuclear magnetic resonance spectroscopy (3 in 100) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double triplet signal A at around  $\delta$  1.5 ppm, a multiplet signal B at around  $\delta$  4.2 ppm, a double doublet signal C at around  $\delta$  5.5 ppm, a double doublet sig-

nal D at around  $\delta$  6.5 ppm, a multiplet signal E at around  $\delta$  7.3 ppm, and a multiplet signal F at around  $\delta$  7.7 ppm. The ratio of integrated intensity of each signal, A:B:C:D:E:F, is about 1:1:1:1:2:2.

**Roxatidine acetate hydrochloride**  $\text{C}_{19}\text{H}_{28}\text{N}_2\text{O}_4\text{HCl}$

[Same as the namesake monograph]

**RPMI-1640 powdered medium** Powdered medium for cell culture containing 6 g of sodium chloride, 400 mg of potassium chloride, 800 mg of anhydrous sodium dihydrogen phosphate, 100 mg of anhydrous calcium nitrate, 49 mg of anhydrous magnesium sulfate, 2 g of dextrose, 200 mg of L-arginine, 1 mg of glutathione, 50 mg of L-isoleucine, 15 mg of L-phenylalanine, 5 mg of L-tryptophan, 0.2 mg of biotin, 1 mg of nicotinamide, 1 mg thiamine hydrochloride, 300 mg of L-glutamine, 56.8 mg of L-asparagine, 10 mg of glycine, 50 mg of L-leucine, 20 mg of L-proline, 20 mg of L-tyrosine, 0.25 mg of D-calcium pantothenate, 5  $\mu\text{g}$  of cyanocobalamin, 1 mg of aminobenzoic acid, 20 mg of L-aspartic acid, 15 mg of L-histidine, 40 mg of L-lysine hydrochloride, 30 mg of L-serine, 20 mg of L-valine, 1 mg of folic acid, 1 mg of pyridoxine hydrochloride, 20 mg of L-glutamic acid, 20 mg of L-hydroxyproline, 15 mg of L-methionine, 20 mg of L-threonine, 3 mg of choline chloride, 35 mg of *i*-inositol, 0.2 mg of riboflavin, 59 mg of L-cystine, and 5 mg of phenol red per 1 L.

**Rubidium chloride**  $\text{RbCl}$  White, crystals or crystalline powder.

**Content**: Not less than 99.0%. Assay—Weigh accurately about 0.2 g of rubidium chloride, dissolve in 100 mL of water, add 5 mL of diluted nitric acid (1 in 2), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L silver nitrate VS} \\ = 12.09 \text{ mg of RbCl} \end{aligned}$$

**Rutin for thin-layer chromatography**  $\text{C}_{27}\text{H}_{30}\text{O}_{16}$  Pale yellow to yellow-green, crystals or crystalline powder, having no odor. Soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of rutin for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 255 nm and 259 nm, and between 356 nm and 360 nm.

(2) Determine the infrared absorption spectrum of rutin for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1655  $\text{cm}^{-1}$ , 1600  $\text{cm}^{-1}$ , 1507  $\text{cm}^{-1}$  and 1363  $\text{cm}^{-1}$ .

**Purity Related substances**—Dissolve 10 mg of rutin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 2  $\mu\text{L}$  each of the sample solution and standard solution as directed in Identification (1) under Crataegus Fruit: the spot other than the principal spot appeared at an  $R_f$  value of about 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

**Saccharated pepsin** [Same as the namesake monograph]

**Saikosaponin a for assay**  $\text{C}_{42}\text{H}_{68}\text{O}_{13}$  Use saikosaponin a for thin-layer chromatography meeting the following additional specifications, 1) Saikosaponin a for assay 1 or 2) Saikosaponin a for assay 2 (Purity value by quantitative NMR).

The former is used after drying in a desiccator (silica gel) for 24 hour, and the latter is corrected based on the amount (%) obtained in the Assay.

1) Saikosaponin a for assay 1

*Absorbance <2.24> E<sub>cm</sub>% (206 nm): 65 – 73 (15 mg, methanol, 200 mL). Previously dried in a desiccator (in vacuum, silica gel) for 24 hours.*

*Purity Related substances—Dissolve 10 mg of saikosaponin a for assay 1 in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than saikosaponin a obtained from the sample solution is not more than the peak area of saikosaponin a from the standard solution.*

Operating conditions

Detector, and column: Proceed as directed in the operating conditions in the Assay under *Bupleurum Root*.

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: Adjust so that the retention time of saikosaponin a is about 16 minutes.

Time span of measurement: About 6 times as long as the retention time of saikosaponin a, beginning after the solvent peak.

System suitability

Test for required detectability: Measure exactly 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of saikosaponin a obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

System performance: Dissolve 6 mg each of saikosaponin a for assay and saikosaponin b<sub>2</sub> for assay in methanol to make 100 mL. When the procedure is run with 20  $\mu$ L of this solution under the above operating conditions, saikosaponin a and saikosaponin b<sub>2</sub> are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin a is not more than 1.0%.

2) Saikosaponin a for assay 2 (Purity value by quantitative NMR)

*Unity of peak—Dissolve 1 mg of saikosaponin a for assay 2 in 2 mL of methanol, and use this solution as the sample solution. Perform the test with 20  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of saikosaponin a peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.*

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under *Bupleurum Root*.

Detector: A photodiode array detector (wavelength: 206 nm, spectrum range of measurement: 200 – 400 nm).

System suitability

System performance: Dissolve 1 mL of the sample solution and 1 mg of saikosaponin d for assay 2 in 2 mL of methanol. To 1 mL of this solution add methanol to make

10 mL. When the procedure is run with 20  $\mu$ L of this solution under the above operating conditions, saikosaponin a and saikosaponin d are eluted in this order, and the numbers of theoretical plates and the symmetry factors of these peaks are not less than 4000 and not more than 1.4, respectively.

*Assay—Weigh accurately 5 mg of saikosaponin a for assay 2 and 1 mg of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity A (equivalent to 1 hydrogen atom) of the signal around  $\delta$  5.70 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.*

$$\text{Amount (\%)} \text{ of saikosaponin a (C}_{42}\text{H}_{68}\text{O}_{13}) \\ = M_s \times I \times P / (M \times N) \times 3.4480$$

M: Amount (mg) of saikosaponin a for assay 2 taken

M<sub>s</sub>: Amount (mg) of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

I: Signal resonance intensity A based on the signal resonance intensity of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

N: Number of the hydrogen derived from A

P: Purity (%) of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

<sup>13</sup>C Decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around  $\delta$  5.70 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal of around  $\delta$  5.70 ppm is not overlapped with any obvious signal of foreign substance.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity A to that of the reference standard for qNMR is not more than 1.0%.

**Saikosaponin a for component determination** See saikosaponin a for assay.

**Saikosaponin a for thin-layer chromatography**

C<sub>42</sub>H<sub>68</sub>O<sub>13</sub> A white, crystalline powder or powder. Freely

soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 225 – 232°C (with decomposition).

**Purity** Related substances—Dissolve 1.0 mg of saikosaponin a for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 10  $\mu$ L of this solution as directed in the Identification (2) under Bupleurum Root: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.4 does not appear.

**Saikosaponins a and d standard TS for assay** Prepare as described in the following 1), 2)-1 or 2)-2.

1) Weigh accurately about 10 mg each of saikosaponin a for assay (for assay 1) and saikosaponin d for assay (for assay 1), previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 200 mL, and use this solution as the saikosaponins a and d standard TS for assay.

2)-1 Weigh accurately about 10 mg each of saikosaponin a (for assay 2) and saikosaponin d (for assay 2), and dissolve in methanol to make exactly 100 mL. Pipet 500  $\mu$ L of this solution, and evaporate the solvent under low pressure (in vacuo). Before using, add exactly 1 mL of methanol, and use this solution as the saikosaponins a and d TS for assay. It contains 10 mg each of saikosaponin a for assay and saikosaponin d for assay in 200 mL of methanol. This standard TS is corrected by the content obtained in the Assay for saikosaponin a for assay (for assay 2) and saikosaponin d for assay (for assay 2).

2)-2 Weigh accurately about 10 mg each of saikosaponin a (for assay 2) and saikosaponin d (for assay 2), dissolve in methanol to make exactly 200 mL, and use this solution as the saikosaponins a and d TS for assay. This standard TS is corrected by the content obtained in the Assay for saikosaponin a for assay (for assay 2) and saikosaponin d for assay (for assay 2).

**Saikosaponin b<sub>2</sub> for assay** C<sub>42</sub>H<sub>68</sub>O<sub>13</sub> Saikosaponin b<sub>2</sub> for thin-layer chromatography. It meets the the following requirements. It is used with correction for its amount based on the result obtained in the Assay.

**Unity of peak**—Dissolve 1 mg of saikosaponin b<sub>2</sub> for assay in 50 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of saikosaponin b<sub>2</sub> peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

#### Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Saireito Extract.

Detector: A photodiode array detector (wavelength: 252 nm, spectrum range of measurement: 220 – 400 nm).

#### System suitability

System performance: Proceed as directed in the system suitability in the Assay (1) under Saireito Extract.

**Assay**—Weigh accurately 5 mg of saikosaponin b<sub>2</sub> for assay and 1 mg of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.27> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-

BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity *A* (equivalent to 1 hydrogen) of the signal around  $\delta$  6.20 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\text{Amount (\%)} \text{ of saikosaponin b}_2 (\text{C}_{42}\text{H}_{68}\text{O}_{13}) \\ = M_s \times I \times P / (M \times N) \times 3.4480$$

*M*: Amount (mg) of saikosaponin b<sub>2</sub> for assay taken

*M<sub>s</sub>*: Amount (mg) of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

*I*: Signal resonance intensity *A* based on the signal resonance intensity of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

*N*: Number of the hydrogen derived from *A*

*P*: Purity (%) of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°

<sup>13</sup>C Decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal of around  $\delta$  6.20 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal of around  $\delta$  6.20 ppm is no overlapped with any obvious signal of foreign substance.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity *A* to that of the reference standard for qNMR is not more than 1.0%.

**Saikosaponin b<sub>2</sub> for component determination** See saikosaponin b<sub>2</sub> for assay.

**Saikosaponin b<sub>2</sub> standard TS for assay** Prepare as described in the following 1) or 2).

1) Weigh accurately about 10 mg of saikosaponin b<sub>2</sub> for assay, and dissolve in methanol to make exactly 250 mL. Pipet 500  $\mu$ L of this solution, and evaporate the solvent under reduced pressure. Before using, add exactly 2 mL of a mixture of water and methanol (1:1), and use this solution as the saikosaponin b<sub>2</sub> standard TS for assay. It contains 10 mg of saikosaponin b<sub>2</sub> for assay in 1000 mL of a mixture of water and methanol (1:1). This standard TS is corrected by the content obtained in the Assay for saikosaponin b<sub>2</sub> for assay.

2) Weigh accurately about 10 mg of saikosaponin b<sub>2</sub> for assay, dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 100

mL, and use this solution as saikosaponin b<sub>2</sub> standard TS for assay. This standard TS is corrected by the content obtained in the Assay for saikosaponin b<sub>2</sub> for assay.

#### Saikosaponin b<sub>2</sub> for thin-layer chromatography

C<sub>42</sub>H<sub>68</sub>O<sub>13</sub> White, crystals or crystalline powder. Freely soluble in ethanol (99.5), soluble in methanol, and practically insoluble in water. Melting point: about 240°C.

**Identification**—Determine the absorption spectrum of a solution of saikosaponin b<sub>2</sub> for thin-layer chromatography in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 241 nm and 245 nm, between 250 nm and 254 nm, and between 259 nm and 263 nm.

**Purity Related substances**—Dissolve 2 mg of saikosaponin b<sub>2</sub> for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Proceed the test with 10 µL each of the sample solution and standard solution as directed in the Identification (1) under Saireito Extract: the spot other than the principle spot, having R<sub>f</sub> value of about 0.3, obtained from the sample solution is not more intense than the spot from the standard solution.

**Saikosaponin d for assay** C<sub>42</sub>H<sub>68</sub>O<sub>13</sub> A white, crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 240°C. It meets the requirements of the following Saikosaponin d for assay 1 and Saikosaponin d for assay 2 (Purity value by quantitative NMR). The former is used after drying in a desiccator (silica gel) for 24 hour in the Assay 1, and the latter is corrected the content based on the amount (%) obtained in the Assay 2.

#### 1) Saikosaponin d for assay 1

**Absorbance** <2.24> E<sub>1</sub><sup>cm</sup> (206 nm): 66 – 74 (15 mg, methanol, 200 mL). Previously dried in a desiccator (in vacuum, silica gel) for 24 hours.

#### Purity Related substances—

(1) Dissolve 2.0 mg of saikosaponin d for assay in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this as the standard solution. Proceed the test with 10 µL each of the sample solution and standard solution as directed in the Identification (2) under Bupleurum Root: the spot other than the principal spot around R<sub>f</sub> value of 0.4 obtained from the sample solution is not larger and not more intense than the spot from the standard solution.

(2) Dissolve 10 mg of saikosaponin d for assay in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than saikosaponin d obtained from the sample solution is not more than the peak area of saikosaponin d from the standard solution.

#### Operating conditions

**Detector, and column:** Proceed as directed in the operating conditions in the Assay under Bupleurum Root.

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of water and acetonitrile (11:9).

**Flow rate:** Adjust so that the retention time of saikosapo-

nin d is about 13 minutes.

**Time span of measurement:** About 4 times as long as the retention time of saikosaponin d, beginning after the solvent peak.

#### System suitability

**Test for required detectability:** Measure exactly 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of saikosaponin d obtained with 20 µL of this solution is equivalent to 3.5 to 6.5% of that with 20 µL of the standard solution.

**System performance:** Dissolve 6 mg each of saikosaponin d for assay and saikosaponin a for assay in methanol to make 100 mL. When the procedure is run with 20 µL of this solution under the above operating conditions, saikosaponin a and saikosaponin d are eluted in this order with the resolution between these peaks being not less than 1.5.

**System repeatability:** When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin d is not more than 1.0%.

#### 2) Saikosaponin d for assay 2 (Purity value by quantitative NMR)

**Unity of peak**—Dissolve 1 mg of saikosaponin d for assay in 2 mL of methanol, and use this solution as the sample solution. Perform the test with 20 µL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of saikosaponin d peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

#### Operating conditions

**Column, column temperature, mobile phase, and flow rate:** Proceed as directed in the operating conditions in the Assay under Bupleurum Root.

**Detector:** A photodiode array detector (wavelength: 206 nm, spectrum range of measurement: 200 – 400 nm).

#### System suitability

**System performance:** Dissolve 1 mL of the sample solution and 1 mg of saikosaponin a for assay 2 in 2 mL of methanol. To 1 mL of this solution add methanol to make 10 mL. When the procedure is run with 20 µL of this solution under the above operating conditions, saikosaponin a and saikosaponin d are eluted in this order, and the numbers of theoretical plates and the symmetry factors of these peaks are not less than 4000 and not more than 1.4, respectively.

**Assay**—Weigh accurately 5 mg of saikosaponin d for assay 2 and 1 mg of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity A (equivalent to 1 hydrogen atom) of the signal around δ 5.70 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%)} \text{ of saikosaponin d (C}_{42}\text{H}_{68}\text{O}_{13}) \\ = M_s \times I \times P/(M \times N) \times 3.4480$$

**M:** Amount (mg) of saikosaponin a for assay 2 taken

**M<sub>s</sub>:** Amount (mg) of 1,4-BTMSB-d<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

**I:** Signal resonance intensity A based on the signal

resonance intensity of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

*N*: Number of the hydrogen derived from *A*

*P*: Purity (%) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between -5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°

<sup>13</sup>C Decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal of around δ 5.70 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal of around δ 5.70 ppm is no overlapped with any obvious signal of foreign substance.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity *A* to that of the reference standard for qNMR is not more than 1.0%.

**Saikosaponin d for component determination** See saikosaponin d for assay.

**Salicylaldazine** C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub> Dissolve 0.30 g of hydrazinium sulfate in 5 mL of water. To this solution add 1 mL of acetic acid (100) and 2 mL of a freshly prepared solution of salicylaldehyde in 2-propanol (1 in 5), shake well, and allow to stand until a yellow precipitate is produced. Extract with two 15 mL portions of dichloromethane, to the combined dichloromethane extracts add 5 g of anhydrous sodium sulfate, shake, decant or filter, and evaporate the dichloromethane in the supernatant liquid or filtrate. Dissolve the residue in a warmed mixture of toluene and methanol (3:2), and cool. Filter the crystals produced, and dry in a desiccator (in vacuum, silica gel) for 24 hours. It is a yellow, crystalline powder.

*Melting point* <2.60>: 213 – 219°C

*Purity* Related substances—Dissolve 90 mg of salicylaldazine in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene to make exactly 100 mL, and perform the test with this solution as directed in the Purity (6) under Povidone: any spot other than the principal spot does not appear.

**Salicylaldehyde** HOC<sub>6</sub>H<sub>4</sub>CHO [K 8390, Special class]

**Salicylamide** C<sub>7</sub>H<sub>7</sub>NO<sub>2</sub> White, crystals or crystalline powder, and it is odorless and tasteless. Very soluble in *N,N*-dimethylformamide, freely soluble in ethanol (95), soluble in propylene glycol, sparingly soluble in diethyl ether, and slightly soluble in water and in chloroform. It dissolves in sodium hydroxide TS.

*Melting point* <2.60>: 139 – 143°C

*Purity* Ammonium <1.02>—Shake 1.0 g of salicylamide with 40 mL of water, and filter through filter paper previously washed well with water. Discard the first 10 mL of the filtrate, transfer the subsequent 20 mL to a Nessler tube, and add water to make 30 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: transfer 2.5 mL of Standard Ammonium Solution to a Nessler tube, and add water to make 30 mL.

*Loss on drying* <2.41>: not more than 0.5% (1 g, silica gel, 4 hours).

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

*Content*: not less than 98.5%. Assay—Weigh accurately about 0.2 g of salicylamide, previously dried, dissolve in 70 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination in the same manner with a solution of 70 mL of *N,N*-dimethylformamide in 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 13.71 mg of C<sub>7</sub>H<sub>7</sub>NO<sub>2</sub>

**Salicylic acid** HOC<sub>6</sub>H<sub>4</sub>COOH [K 8392, Special class]

**Salicylic acid for assay** HOC<sub>6</sub>H<sub>4</sub>COOH [K 8392, Special class]

**Salicylic acid TS** Dissolve 0.1 g of salicylic acid in 10 mL of sulfuric acid. Prepare before use.

**Salmon sperm DNA** Salmon sperm or nuclear fraction extracted from salmon sperm, which is sonicated and dried.

**Santonin** C<sub>15</sub>H<sub>18</sub>O<sub>3</sub> [Same as the namesake monograph]

**Santonin for assay** C<sub>15</sub>H<sub>18</sub>O<sub>3</sub> [Same as the monograph Santonin. It contains not less than 99.0% of santonin (C<sub>15</sub>H<sub>18</sub>O<sub>3</sub>).]

**Sarpogrelate hydrochloride** C<sub>24</sub>H<sub>31</sub>NO<sub>6</sub>.HCl [Same as the namesake monograph]

**Sarsasapogenin for thin-layer chromatography** C<sub>27</sub>H<sub>44</sub>O<sub>3</sub> It is a white or slightly greyish white, crystalline powder or powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

*Identification* Determine the infrared absorption spectrum of the substance to be examined as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 2930 cm<sup>-1</sup>, 1448 cm<sup>-1</sup>, 1173 cm<sup>-1</sup>, 985 cm<sup>-1</sup> and 850 cm<sup>-1</sup>.

*Purity* Related substances—Dissolve 1 mg of sarsasapogenin for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Perform the test with 5 μL each of the sample solution and standard solution as directed in the Identification (2) under Anemarrhena Rhizome: any spot other than the principal spot with an *Rf* value of about 0.4 obtained from the sample solution is not more intense than the spot from the standard solution.

**Saussurea root** [Same as the namesake monograph]

**Schisandrin for thin-layer chromatography** C<sub>24</sub>H<sub>32</sub>O<sub>7</sub> White, crystals or crystalline powder. Freely soluble in meth-

anol and in diethyl ether, and practically insoluble in water.

**Melting point**  $<2.60>$ : 130–135°C

**Purity** Related substances—Dissolve 1.0 mg of schisan-drin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 5  $\mu$ L of this solution as directed in the Identification under Schisandra Fruit: any spot other than the principal spot at the  $R_f$  value of about 0.4 does not appear.

**Scopolamine hydrobromide** See scopolamine hydrobromide hydrate.

**Scopolamine hydrobromide for thin-layer chromatography** See scopolamine hydrobromide hydrate for thin-layer chromatography.

#### Scopolamine hydrobromide hydrate

$C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$  [Same as the namesake monograph]

**Scopolamine hydrobromide hydrate for thin-layer chromatography**  $C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$  [Same as the monograph Scopolamine Hydrobromide Hydrate], or scopolamine hydrobromide hydrate meeting the following requirements. Colorless or white crystals, or white, grains or powder. Freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

**Identification** Determine the infrared absorption spectrum of scopolamine hydrobromide hydrate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry  $<2.25>$ : it exhibits absorption at the wave numbers of about  $1731\text{ cm}^{-1}$ ,  $1204\text{ cm}^{-1}$ ,  $1070\text{ cm}^{-1}$  and  $735\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 5 mg of scopolamine hydrobromide hydrate for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography  $<2.03>$ . Spot 20  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia solution (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: any spot other than the principal spot with an  $R_f$  value of about 0.6 obtained from the sample solution is not more intense than the spot from the standard solution.

#### Scopoletin for thin-layer chromatography

$C_{10}H_8O_4$   
White or light brown, crystalline powder or powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 206°C.

**Identification**—(1) Determine the absorption spectrum of a solution of scopoletin for thin-layer chromatography in methanol (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry  $<2.24>$ : it exhibits maxima between 226 nm and 230 nm, between 295 nm and 299 nm and between 343 nm and 347 nm.

(2) Determine the infrared absorption spectrum of scopoletin for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry  $<2.25>$ : it exhibits absorption at the wave numbers of about  $3340\text{ cm}^{-1}$ ,  $1702\text{ cm}^{-1}$ ,  $1566\text{ cm}^{-1}$ ,  $1436\text{ cm}^{-1}$  and  $923\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 1.0 mg of scopoletin for thin-layer chromatography in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of

the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 5  $\mu$ L each of the sample solution and standard solution as directed in the Identification under Artemisia Leaf: the spot other than the principal spot, having an  $R_f$  value of about 0.4, obtained from the sample solution is not more intense than the spot from the standard solution.

**Sea sand** A mixture of white, grey, brown or black grains, 0.3 to 1.0 mm in particle size.

**Secondary antibody TS** To a mixture of 1.5 mL of blocking TS for epoetin alfa and 13.5 mL of sodium azide-phosphate-buffered sodium chloride TS, add 1 drop of biotinylated equine anti-mouse IgG antibody.

**2nd Fluid for disintegration test** To 250 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 118 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. It is clear and colorless, and has a pH about 6.8.

**2nd Fluid for dissolution test** A mixture of phosphate buffer solution (pH 6.8) and water (1:1).

**Selenious acid**  $H_2SeO_3$  Colorless or white crystals. It is hygroscopic.

**Identification**—(1) Dissolve 0.2 g of selenious acid in 20 mL of water, and use this solution as the sample solution. To 10 mL of the sample solution add 2 mL of tin (II) chloride TS: a red precipitate is produced.

(2) To 10 mL of the sample solution obtained in (1) add 1 mL of diluted hydrochloric acid (2 in 3) and 1 mL of potassium iodide TS: a brown color is produced.

**Storage**—Preserve in a light-resistant tight container.

**Selenious acid-sulfuric acid TS** Dissolve 50 mg of selenious acid in 10 mL of sulfuric acid.

**Selenium** Se [K 8598, Special class]

**Selenium dioxide**  $SeO_2$  White, crystals or crystalline powder.

**Identification**—(1) To 10 mL of a solution of selenium dioxide (1 in 100) add 2 mL of tin (II) chloride TS: a red precipitate is produced.

(2) To 10 mL of a solution of selenium dioxide (1 in 100) add 1 mL of diluted hydrochloric acid (2 in 3) and 1 mL of potassium iodide TS: a brown color is produced.

**Content**: not less than 97.0%. **Assay**—Weigh accurately about 0.6 g of selenium dioxide, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution into an iodine bottle, add 80 mL of water, 3 g of potassium iodide and 5 mL of diluted hydrochloric acid (2 in 3), allow to stand at a dark place for 5 minutes, and titrate  $<2.50>$  with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L sodium thiosulfate VS} \\ = 2.774 \text{ mg of } SeO_2 \end{aligned}$$

**Semicarbazide acetate TS** Place 2.5 g of semicarbazide hydrochloride, 2.5 g of anhydrous sodium acetate and 30 mL of methanol in a flask, heat on a water bath for 2 hours, cool to 20°C, and filter. To the filtrate add methanol to make 100 mL. Preserve in a cold place. Do not use the solution showing a yellow color.

**Semicarbazide hydrochloride**  $H_2NNHCONH_2 \cdot HCl$   
White to light yellow crystals.

**Identification** (1) To 10 mL of a solution of semicarbazide hydrochloride (1 in 100) add 1 mL of silver nitrate

TS: white precipitates appear.

(2) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3420\text{ cm}^{-1}$ ,  $3260\text{ cm}^{-1}$ ,  $2670\text{ cm}^{-1}$ ,  $1684\text{ cm}^{-1}$ ,  $1582\text{ cm}^{-1}$ ,  $1474\text{ cm}^{-1}$ ,  $1386\text{ cm}^{-1}$ ,  $1210\text{ cm}^{-1}$ ,  $1181\text{ cm}^{-1}$ ,  $770\text{ cm}^{-1}$  and  $719\text{ cm}^{-1}$ .

**Sendai virus** RNA virus of *Paramyxoviridae*, which is grown in the allantoic cavity of embryonated chicken eggs. Measure hemagglutination titer (HA titer) with chicken red blood cells, and use it with 800 to 3200 HA titer/mL.

**Sennoside A for thin-layer chromatography**  $\text{C}_{42}\text{H}_{38}\text{O}_{20}$

A yellow powder. Practically insoluble in water and in ethanol (99.5).

**Identification** Determine the infrared absorption spectrum of sennoside A to be examined as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3420\text{ cm}^{-1}$ ,  $1712\text{ cm}^{-1}$ ,  $1637\text{ cm}^{-1}$ ,  $1597\text{ cm}^{-1}$  and  $1074\text{ cm}^{-1}$ .

**Purity** Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 1 mg of sennoside A to be examined in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add a mixture of tetrahydrofuran and water (7:3) to make exactly 25 mL, and use this solution as the standard solution. Then, perform the test with 5  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification (2) under Senna Leaf: any spot other than the principal spot with an *R<sub>f</sub>* value of about 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

**L-Serine**  $\text{C}_3\text{H}_7\text{NO}_3$  [K 9105, Special class]

**Sesame oil** [Same as the namesake monograph]

**Sesamin for thin-layer chromatography**  $\text{C}_{20}\text{H}_{18}\text{O}_6$

White, crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** Determine the absorption spectrum of a solution of sesame for thin-layer chromatography in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 235 nm and 239 nm and between 285 nm and 289 nm.

**Melting point** <2.60>: 122 – 124°C

**Purity** Related substances—Dissolve 2.0 mg of sesamin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 5  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification under Sesame: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**[6]-Shogaol for assay**  $\text{C}_{17}\text{H}_{24}\text{O}_3$  [6]-Shogaol for thin-layer chromatography. It meets the following 1) [6]-Shogaol for assay 1 or 2) [6]-Shogaol for assay 2 (Purity value by quantitative NMR). The latter is used with correction for its amount based on the result obtained in the Assay.

1) [6]-Shogaol for assay 1

**Absorbance** <2.24>:  $E_{1\text{cm}}^{1\%}$  (225 nm): 727 – 781 (5 mg, ethanol (99.5), 500 mL).

**Purity** Related substances—Dissolve 5 mg of [6]-shogaol for assay in 10 mL of a mixture of acetonitrile and water

(2:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (2:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than [6]-shogaol obtained from the sample solution is not larger than the peak area of [6]-shogaol from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions under Assay (2) of Mukoi-Daikenchuto Extract.

Time span of measurement: 3 times as long as the retention time of [6]-shogaol, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of acetonitrile and water (2:1) to make exactly 20 mL. Confirm that the peak area of [6]-shogaol obtained with 10  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu\text{L}$  of the standard solution.

System performance: When the procedure is run with 10  $\mu\text{L}$  of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5%, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-shogaol is not more than 1.5%.

2) [6]-Shogaol for assay 2 (Purity value by quantitative NMR)

**Unity of peak**—Dissolve 5 mg of [6]-shogaol for assay 2 in 10 mL of a mixture of acetonitrile and water (2:1), and use this solution as the sample solution. Perform the test with 10  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of [6]-shogaol peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

**Operating conditions**

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (2) under Mukoi-Daikenchuto Extract.

Detector: A photodiode array detector (wavelength: 225 nm, spectrum range of measurement: 220 – 400 nm).

**System suitability**

System performance: Proceed as directed in the system suitability in the Assay (2) under Mukoi-Daikenchuto Extract.

**Assay**—Weigh accurately 5 mg of [6]-shogaol for assay 2 and 1 mg of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity *A* (equivalent to 3 hydrogens) of the signal around  $\delta$  3.57 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\text{Amount (\%)} \text{ of [6]-shogaol } (C_{17}H_{24}O_3) \\ = M_S \times I \times P/(M \times N) \times 1.2202$$

*M*: Amount (mg) of [6]-shogaol for assay 2 taken

*M<sub>S</sub>*: Amount (mg) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

*I*: Signal resonance intensity *A* based on the signal resonance intensity of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

*N*: Number of the hydrogen derived from *A*

*P*: Purity (%) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy

#### Operating conditions

**Apparatus:** A nuclear magnetic resonance spectrometer having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between – 5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

<sup>13</sup>C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

**Test for required detectability:** When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around δ 3.57 ppm and δ 6.37 – 6.43 ppm is not less than 100.

**System performance:** When the procedure is run with the sample solution under the above operating conditions, the two signals around δ 3.57 ppm and δ 6.37 – 6.43 ppm are not overlapped with any signal of obvious foreign substance. Furthermore, when determined the resonance intensities, *A* (equivalent to 3 hydrogens) and *A*<sub>1</sub> (equivalent to 2 hydrogens) of each signal around δ 3.57 ppm and δ 6.37 – 6.43 ppm, the ratio of the resonance intensities, (*A*<sub>1</sub>/2)/(*A*/3), of each signal around δ 3.57 ppm and δ 6.37 – 6.43 ppm is between 0.99 and 1.01.

**System repeatability:** When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity *A* to that of the reference standard for qNMR is not more than 1.0%.

#### [6]-Shogaol for thin-layer chromatography C<sub>17</sub>H<sub>24</sub>O<sub>3</sub>

A pale yellow, clear liquid. Miscible with methanol and with ethanol (99.5), and practically insoluble in water.

**Purity** Related substances—Dissolve 1.0 mg of [6]-shogaol for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: any spot other than the principal spot at an *Rf* value of about 0.5 does not appear.

**Silica gel** An amorphous, partly hydrated silicic acid occurring in glassy granules of various sizes. When used as a desiccant, it is frequently coated with a substance that

changes color when the capacity to absorb water is exhausted. Such colored products may be regenerated by being heated at 110°C until the gel assumes the original color.

**Loss on ignition** <2.43>: not more than 6% (2 g, 950 ± 50°C).

**Water absorption:** not less than 31%. Weigh accurately about 10 g of silica gel, and allow to stand for 24 hours in a closed container in which the atmosphere is maintained at 80% relative humidity with sulfuric acid having a specific gravity of 1.19. Weigh again, and calculate the increase in mass.

**Siliceous earth** [K 8330, Diatomaceous earth, First class]

**Silicone oil** Colorless clear liquid, having no odor.

**Viscosity** <2.53>: 50 – 100 mm<sup>2</sup>/s.

**Silicone resin** Light gray, half-clear, viscous liquid or a pasty material. It is almost odorless.

**Viscosity and refractive index**—Place 15 g of silicone resin in a Soxhlet extractor, then extract with 150 mL of carbon tetrachloride for 3 hours. The kinematic viscosity of the residual liquid, obtained by evaporating carbon tetrachloride from the extract on a water bath, is 100 to 1100 mm<sup>2</sup>/s (25°C). Its refractive index is 1.400 to 1.410 (25°C).

**Specific gravity** <2.56> *d*: 0.98 – 1.02

**Loss on drying** <2.41>: 0.45 – 2.25 g with the extracted residue obtained in the Viscosity and refractive index (100°C, 1 hour).

**Silicotungstic acid 26-water** SiO<sub>2</sub>.12WO<sub>3</sub>.26H<sub>2</sub>O

White to slightly yellowish, crystals. Deliquescent. Very soluble in water and in ethanol (95).

**Purity** Clarity and color of solution—a solution (1 in 20) is clear and colorless.

**Loss on ignition** <2.43>: 14 – 15% (2 g, dry at 110°C for 2 hours then 700 – 750°C, constant mass).

**Silodosin** C<sub>25</sub>H<sub>32</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub> [Same as the namesake monograph]

**Silver chromate-saturated potassium chromate TS** Dissolve 5 g of potassium chromate in 50 mL of water, add silver nitrate TS until a pale red precipitate is produced, and filter. To the filtrate add water to make 100 mL.

**Silver diethyldithiocarbamate** See silver *N,N*-diethyldithiocarbamate.

**Silver nitrate** AgNO<sub>3</sub> [K 8550, Special class]

**Silver nitrate-ammonia TS** Dissolve 1 g of silver nitrate in 20 mL of water, and add ammonia TS dropwise with stirring until the precipitate is almost entirely dissolved.

**Storage**—Preserve in tight, light-resistant containers.

**Silver nitrate TS** Dissolve 17.5 g of silver nitrate in water to make 1000 mL (0.1 mol/L).

**Storage**—Preserve in light-resistant containers.

**Silver *N,N*-diethyldithiocarbamate** C<sub>5</sub>H<sub>10</sub>AgNS<sub>2</sub> [K 9512, Special class]

**Sindbis virus** RNA virus of *Togaviridae*, proliferated by chick embryo cell primary culture. Determine the number of plaques on the cell culture, and use the virus with not less than 1 × 10<sup>8</sup> PFU/mL.

**Sinomenine for assay** C<sub>19</sub>H<sub>23</sub>NO<sub>4</sub> Sinomenine for thin-layer chromatography. It meets the requirements of the following 1) sinomenine for assay 1 or 2) sinomenine for assay 2 (Purity value by quantitative NMR). The former is used after drying in a desiccator (silica gel) for 24 hours, and lat-

ter is used with correction for its amount based on the result obtained in the Assay.

### 1) Sinomenine for assay 1

**Identification** Determine the absorption spectrum of a solution of sinomenine for assay in methanol (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits the maximum between 259 nm and 263 nm.

**Purity** Related substances—Dissolve 5 mg of sinomenine for assay 1 in 10 mL of a mixture of water and acetonitrile (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than sinomenine obtained from the sample solution is not larger than the peak area of sinomenine from the standard solution.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Boiogito Extract.

Detector: An ultraviolet absorption photometer (wavelength: 261 nm).

Time span of measurement: About 3 times as long as the retention time of sinomenine, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and acetonitrile (7:3) to make exactly 20 mL. Confirm that the peak area of sinomenine obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of sinomenine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sinomenine is not more than 1.5%.

### 2) Sinomenine for assay 2 (Purity value by quantitative NMR)

**Unity of peak**—Dissolve 5 mg of sinomenine for assay 2 in 10 mL of a mixture of water and acetonitrile (7:3), and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of sinomenine peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Boiogito Extract.

Detector: A photodiode array detector (wavelength: 261 nm, spectrum range of measurement: 220 – 400 nm).

#### System suitability

System performance: When the procedure is run with 10  $\mu$ L of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of sinomenine are not less than 5000 and not more than 1.5, respectively.

**Assay**—Weigh accurately 5 mg of sinomenine for assay 2 and 1 mg of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated acetone for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure <sup>1</sup>H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensity *A* (equivalent to 1 hydrogen) of the signal around  $\delta$  5.42 ppm assuming the signal of the reference standard for qNMR as  $\delta$  0 ppm.

$$\text{Amount (\%)} \text{ of sinomenine (C}_{19}\text{H}_{23}\text{NO}_4\text{)} \\ = M_s \times I \times P / (M \times N) \times 1.4543$$

*M*: Amount (mg) of sinomenine for assay 2 taken

*M<sub>s</sub>*: Amount (mg) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy taken

*I*: Signal resonance intensity *A* based on the signal resonance intensity of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy as 18.000

*N*: Number of hydrogen derived from *A*

*P*: Purity (%) of 1,4-BTMSB-*d*<sub>4</sub> for nuclear magnetic resonance spectroscopy

#### Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having <sup>1</sup>H resonance frequency of not less than 400 MHz.

Target nucleus: <sup>1</sup>H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between –5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

<sup>13</sup>C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

#### System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around  $\delta$  5.42 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal around  $\delta$  5.42 ppm is not overlapped with any signal of obvious foreign substances.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity *A* to that of the reference standard for qNMR is not more than 1.0%.

**Sinomenine for thin-layer chromatography** C<sub>19</sub>H<sub>23</sub>NO<sub>4</sub> A white or pale brown crystalline powder. Freely soluble in methanol, soluble in ethanol (99.5), and very slightly soluble in water.

**Identification** Determine the infrared absorption spectrum of sinomenine for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 2830 cm<sup>-1</sup>, 1687 cm<sup>-1</sup>, 1630 cm<sup>-1</sup>, 1441 cm<sup>-1</sup> and 1279 cm<sup>-1</sup>.

**Purity** Related substances—Dissolve 5 mg of sinomenine for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification (1) under Boiogito Extract: the spot other than the principal spot at an *Rf* value of about 0.2 obtained from the sample solution is not more colored than the spot from the standard solution.

**Sivelestat sodium hydrate**  $C_{20}H_{21}N_2NaO_7S \cdot 4H_2O$   
[Same as the namesake monograph]

**Soda lime** [K 8603, For carbon dioxide absorption]

**Sodium** Na [K 8687, special class]

**Sodium acetate** See sodium acetate trihydrate.

**Sodium acetate-acetone TS** Dissolve 8.15 g of sodium acetate trihydrate and 42 g of sodium chloride in 100 mL of water, and add 68 mL of 0.1 mol/L hydrochloric acid VS, 150 mL of acetone and water to make 500 mL.

**Sodium acetate, anhydrous**  $CH_3COONa$  [K 8372, Special class]

**Sodium acetate trihydrate**  $CH_3COONa \cdot 3H_2O$  [K 8371, Special class]

**Sodium acetate TS** Dissolve 13.6 g of sodium acetate trihydrate in water to make 100 mL (1 mol/L).

**Sodium azide**  $NaN_3$  [K 9501, Special class]

**Sodium azide-phosphate-buffered sodium chloride TS** Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 2.9 g of disodium hydrogen phosphate dodecahydrate and 0.2 g of potassium dihydrogen phosphate in water to make 1000 mL. Dissolve 0.25 g of sodium azide in this solution.

**Sodium benzoate**  $C_7H_5NaO_2$  [Same as the namesake monograph]

**Sodium bicarbonate** See sodium hydrogen carbonate.

**Sodium bicarbonate for pH determination** See sodium hydrogen carbonate for pH determination.

**Sodium bicarbonate TS** See sodium hydrogen carbonate TS.

**7% Sodium bicarbonate injection** [Same as the monograph Sodium Bicarbonate Injection. However, labeled amount should be 7 w/v%.]

**Sodium bismuthate** See bismuth sodium trioxide.

**Sodium bisulfite** See sodium hydrogen sulfite.

**Sodium bisulfite TS** See sodium hydrogen sulfite TS.

**Sodium bitartrate** See sodium hydrogen tartrate monohydrate.

**Sodium bitartrate TS** See sodium hydrogen tartrate TS.

**Sodium borate** See sodium tetraborate decahydrate.

**Sodium borate for pH determination** See sodium tetraborate decahydrate for pH determination.

**Sodium borohydride**  $NaBH_4$  White to grayish white, crystals, powder or masses. Freely soluble in water.

**Content:** not less than 95%. Assay—Weigh accurately 0.25 g of sodium borohydride, dissolve in 20 mL of diluted

sodium hydroxide TS (3 in 10), and add water to make exactly 500 mL. Pipet 20 mL of this solution, put in a glassstoppered iodine flask, and cool in ice. Add exactly 40 mL of iodine TS, allow to stand at a dark place for 10 minutes, add exactly 10 mL of diluted sulfuric acid (1 in 6), and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (back titration) (indicator: starch solution). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 0.4729 mg of  $NaBH_4$

**Sodium bromide**  $NaBr$  [K 8514, Special class]

**Sodium carbonate** See sodium carbonate decahydrate.

**Sodium carbonate, anhydrous**  $Na_2CO_3$  [K 8625, Sodium carbonate, Special class]

**Sodium carbonate decahydrate**  $Na_2CO_3 \cdot 10H_2O$   
[K 8624, Special class]

**Sodium carbonate for pH determination**  $Na_2CO_3$   
[K 8625, for pH determination]

**Sodium carbonate (standard reagent)**  $Na_2CO_3$  In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

**Sodium carbonate TS** Dissolve 10.5 g of anhydrous sodium carbonate in water to make 100 mL (1 mol/L).

**0.55 mol/L Sodium carbonate TS** Dissolve 5.83 g of anhydrous sodium carbonate in water to make 100 mL.

**Sodium chloride**  $NaCl$  [K 8150, Special class]

**Sodium chloride (standard reagent)**  $NaCl$  In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

**Sodium chloride for assay**  $NaCl$  [Same as the monograph, Sodium Chloride]

**Sodium chloride TS** Dissolve 10 g of sodium chloride in water to make 100 mL.

**0.1 mol/L Sodium chloride TS** Dissolve 6 g of sodium chloride in water to make 1000 mL.

**0.2 mol/L Sodium chloride TS** Dissolve 11.7 g of sodium chloride in water to make 1000 mL.

**1 mol/L Sodium chloride TS** Dissolve 29.22 g of sodium chloride in water to make 500 mL.

**Sodium cholate hydrate**  $C_{24}H_{39}O_5Na \cdot H_2O$  A white powder.

**Identification**—Determine the infrared absorption spectrum of sodium cholate hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3400\text{ cm}^{-1}$ ,  $2940\text{ cm}^{-1}$ ,  $1579\text{ cm}^{-1}$ ,  $1408\text{ cm}^{-1}$  and  $1082\text{ cm}^{-1}$ .

**Water** <2.48>: 3.5 – 5.0% (40 mg, coulometric titration).

**Content:** not less than 99.0% of sodium cholate ( $C_{24}H_{39}O_5Na$ ), calculated on the anhydrous basis.

**Assay**—Weigh accurately about 0.35 g of sodium cholate hydrate, dissolve in 60 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 43.06 mg of  $C_{24}H_{39}O_5Na$

**Sodium citrate** See sodium citrate hydrate.

**Sodium citrate hydrate**  $C_6H_5Na_3O_7 \cdot 2H_2O$  [K 8288, Trisodium citrate dihydrate, or same as the namesake monograph]

**0.1 mol/L Sodium citrate TS** Dissolve 29.4 g of trisodium citrate dihydrate in water to make 1000 mL

**Sodium cobaltinitrite** See sodium hexanitrocobaltate (III).

**Sodium cobaltinitrite TS** See sodium hexanitrocobaltate (III) TS.

**Sodium 1-decanesulfonate**  $C_{10}H_{21}NaO_3S$  A white powder.

*Purity* Clarity and color of solution—Dissolve 1.0 g of sodium decanesulfonate in 20 mL of water: the solution is clear and colorless.

*Loss on drying* <2.41>: not more than 3.0% (1 g, 105°C, 3 hours).

*Content:* not less than 98.0%. *Assay*—Weigh accurately about 0.45 g of sodium 1-decanesulfonate, dissolve in 50 mL of water, and pass through a column, about 1.2 cm in inside diameter and about 25 cm in length, packed with about 20 mL of strongly acidic ion-exchange resin (0.3 to 1.0 mm, H type) for column chromatography at a flow rate of about 4 mL per minute. Wash with 150 mL of water at a flow rate of about 4 mL per minute. Combine the washing and the elute, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 24.43 mg of  $C_{10}H_{21}NaO_3S$

**0.0375 mol/L Sodium 1-decanesulfonate TS** Dissolve 3.665 g of sodium 1-decanesulfonate in 400 mL of water.

**Sodium desoxycholate**  $C_{24}H_{39}NaO_4$  White, odorless, crystalline powder.

*Identification*—Determine the infrared absorption spectrum of sodium desoxycholate, previously dried, according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $3400\text{ cm}^{-1}$ ,  $2940\text{ cm}^{-1}$ ,  $1562\text{ cm}^{-1}$  and  $1408\text{ cm}^{-1}$ .

*Purity* Related substances—Dissolve 0.10 g of sodium desoxycholate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol and acetic acid (100) (80:40:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly concentrated sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

**Sodium 2,6-dichloroindophenol-sodium acetate TS** Mix before use an equal volume of sodium 2,6-dichloroindophenol dihydrate solution (1 in 20) and acetic acid-sodium acetate TS (pH 7.0).

**Sodium diethyldithiocarbamate** See sodium *N,N*-diethyldithiocarbamate trihydrate.

**Sodium *N,N*-diethyldithiocarbamate trihydrate**  $(C_2H_5)_2NCS_2Na \cdot 3H_2O$  [K 8454, Special class]

**Sodium di-2-ethylhexyl sulfosuccinate**

$C_8H_{17}COOCH_2(C_8H_{17}COO)CHSO_3Na$  White or translucent white mucilaginous soft masses. Sparingly soluble in water.

*Purity* Clarity and color of solution—A solution prepared by dissolving 1.0 g of sodium di-2-ethylhexyl sulfosuccinate in 100 mL of water is clear and colorless.

*Loss on drying* <2.41>: not more than 5.0% (1 g, 105°C, 2 hours).

**Sodium dihydrogen phosphate** See sodium dihydrogen phosphate dihydrate.

**Sodium dihydrogen phosphate anhydrous**  $NaH_2PO_4$  A white, powder or crystalline powder. Freely soluble in water, and very slightly soluble in ethanol (99.5). It has a hygroscopic property.

A solution is acidic.

**Sodium dihydrogen phosphate dihydrate**

$NaH_2PO_4 \cdot 2H_2O$  [K 9009, Special class]

**Sodium dihydrogen phosphate-ethanol TS** To 500 mL of sodium dihydrogen phosphate solution (39 in 2500) add 200 mL of water, and add 300 mL of ethanol (99.5).

**Sodium dihydrogen phosphate monohydrate**

$NaH_2PO_4 \cdot H_2O$  White, crystals or crystalline powder. It slightly deliquesces in moist air. It is freely soluble in water, and practically insoluble in ethanol (99.5).

*pH* <2.54>—The pH of a solution of 1.0 g of sodium dihydrogen phosphate monohydrate in 20 mL of water is between 4.1 and 4.5.

**Sodium dihydrogen phosphate TS (pH 2.2)** Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate in 800 mL of water, adjust the pH to 2.2 with phosphoric acid, and add water to make 1000 mL.

**Sodium dihydrogen phosphate TS (pH 2.5)** Dissolve 2.7 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid.

**0.05 mol/L Sodium dihydrogen phosphate TS (pH 2.6)**

Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 2.6 with phosphoric acid and add water to make 1000 mL.

**0.05 mol/L Sodium dihydrogen phosphate TS (pH 3.0)**

Dissolve 3.45 g of sodium dihydrogen phosphate dihydrate in 500 mL of water (solution A). Dilute 2.45 g of phosphoric acid with water to make 500 mL (solution B). To a volume of solution A add solution B until the mixture is adjusted to (pH 3.0).

**0.1 mol/L Sodium dihydrogen phosphate TS (pH 3.0)**

Dissolve 15.60 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 1000 mL.

**0.05 mol/L Sodium dihydrogen phosphate TS (pH 5.5)**

Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 5.5 with sodium hydroxide TS, and add water to make 1000 mL.

**0.01 mol/L sodium dihydrogen phosphate TS (pH 7.5)**

Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 7.5 with sodium hydroxide

TS, and add water to make 1000 mL.

**0.05 mol/L Sodium dihydrogen phosphate TS** Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

**0.1 mol/L Sodium dihydrogen phosphate TS** Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 450 mL of water, adjust to a pH of 5.8 exactly with sodium hydroxide TS, and add water to make 500 mL.

**2 mol/L Sodium dihydrogen phosphate TS** Dissolve 312.02 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

**Sodium disulfite**  $\text{Na}_2\text{S}_2\text{O}_5$  [K 8501, First class]

**Sodium disulfite TS** Dissolve 0.10 g of sodium disulfite in 10 mL of 1 mol/L hydrochloric acid TS, and add acetone to make 100 mL.

**Sodium dithionite**  $\text{Na}_2\text{S}_2\text{O}_4$  A white to grayish white crystalline powder, having a strong irritating odor. It is decomposed with moisture or atmospheric oxygen.

*Identification*—(1) Dissolve 0.5 g of sodium dithionite in 50 mL of water, and use this solution as the sample solution. To 10 mL of the sample solution add 1 mL of copper (II) sulfate TS; a grayish brown color is produced.

(2) The sample solution obtained in (1) responds to Qualitative Tests <1.09> (1) for sodium salt.

*Storage*—Preserve in a light-resistant tight container.

**Sodium dodecylbenzene sulfonate**  $\text{C}_{18}\text{H}_{29}\text{SO}_3\text{Na}$

White, crystalline powder or mass.

*pH* <2.54>—The pH of a solution of 0.5 g of sodium dodecylbenzene sulfonate in 50 mL of freshly boiled and cooled water is between 5.0 and 7.0. Measure the pH at 25°C passing nitrogen with stirring.

*Loss on drying* <2.41>: not more than 0.5% (1 g, 105°C, 2 hours).

*Content*: not less than 99.0%. *Assay*—Weigh accurately about 40 mg of sodium dodecylbenzene sulfonate, previously dried, and perform the test as directed in (4) Sulfur in the Procedure of determination under Oxygen Flask Combustion Method <1.06>, using a mixture of 20 mL of water and 2 mL of strong hydrogen peroxide water as absorbing solution.

Each mL of 0.005 mol/L barium perchlorate VS  
= 1.742 mg of  $\text{C}_{18}\text{H}_{29}\text{SO}_3\text{Na}$

**Sodium fluoride**  $\text{NaF}$  [K 8821, Special class]

**Sodium fluoride (standard reagent)**  $\text{NaF}$  In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

**Sodium fluoride-hydrochloric acid TS** Dissolve 0.5 g of sodium fluoride in 100 mL of 0.5 mol/L hydrochloric acid TS. Prepare before use.

**Sodium fluoride TS** Dissolve 0.5 g of sodium fluoride in 100 mL of 0.1 mol/L hydrochloric acid TS. Prepare before use.

**Sodium gluconate**  $\text{C}_6\text{H}_{11}\text{NaO}_7$  A white or pale yellow-brown crystalline powder.

*Purity* Clarity and color of solution—A solution obtained by dissolving 1.0 g of sodium gluconate in 10 mL of water is clear and colorless or pale yellow.

**Sodium glycocholate for thin-layer chromatography**  
 $\text{C}_{26}\text{H}_{42}\text{NNaO}_6$  White to pale brown, crystalline powder or

powder. Freely soluble in water and in methanol, and slightly soluble in ethanol (99.5).

*Identification*—(1) Determine the infrared absorption spectrum of sodium glycocholate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 2940  $\text{cm}^{-1}$ , 1599  $\text{cm}^{-1}$ , 1398  $\text{cm}^{-1}$ , 1309  $\text{cm}^{-1}$ , 1078  $\text{cm}^{-1}$ , 1040  $\text{cm}^{-1}$ , 982  $\text{cm}^{-1}$  and 915  $\text{cm}^{-1}$ .

(2) Sodium glycocholate for thin-layer chromatography responds to Qualitative Tests <1.09> (1) for sodium salt.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +25 – +35° (60 mg, methanol, 20 mL, 100 mm).

*Purity* Related substances—Dissolve 5 mg of sodium glycocholate for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of the sample solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Proceed with 5  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification under Bear Bile: the spots other than the principal spot with an *Rf* value of about 0.2 obtained from the sample solution are not more intense than the spot from the standard solution.

**Sodium 1-heptane sulfonate**  $\text{C}_7\text{H}_{15}\text{NaO}_3\text{S}$  White, crystals or crystalline powder.

*Purity* Clarity and color of solution—Dissolve 1.0 g of sodium 1-heptane sulfonate in 10 mL of water: the solution is clear and colorless.

*Loss on drying* <2.41>: not more than 3.0% (1 g, 105°C, 3 hours).

*Content*: not less than 98.0%. *Assay*—Dissolve about 0.4 g of sodium 1-heptane sulfonate, previously dried and weighed accurately, in 50 mL of water, transfer to a chromatographic column, prepared by packing a chromatographic tube 9 mm in inside diameter and 160 mm in height with 10 mL of strongly acidic ion exchange resin for column chromatography (425 to 600  $\mu\text{m}$  in particle diameter, H type), and flow at a flow rate of about 4 mL per minute. Wash the column at the same flow rate with 150 mL of water, combine the washings with the effluent solution, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS) until the color of the solution changes from yellow to blue.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 20.23 mg of  $\text{C}_7\text{H}_{15}\text{NaO}_3\text{S}$

**Sodium 1-hexane sulfonate**  $\text{C}_6\text{H}_{13}\text{NaO}_3\text{S}$  White, crystals or crystalline powder.

*Loss on drying* <2.41>: not more than 3.0% (1 g, 105°C, 2 hours).

*Content*: not less than 98.0%. *Assay*—Weigh accurately about 0.4 g of sodium 1-hexane sulfonate, previously dried, and dissolve in 25 mL of water. Transfer 15–20 mL of this solution into a chromatographic column about 11 mm in diameter and about 500 mm in height of strongly acidic ion exchange resin for column chromatography (246  $\mu\text{m}$  to 833  $\mu\text{m}$  in particle diameter, type H), and elute at the rate of 5 – 10 mL per minute, then wash the column with five 50-mL portions of water at the rate of 5 – 10 mL per minute. Combine the washings to the eluate, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 18.82 mg of  $\text{C}_6\text{H}_{13}\text{NaO}_3\text{S}$

**Sodium hexanitrocobaltate (III)**  $\text{Na}_3\text{Co}(\text{NO}_2)_6$   
[K 8347, Special class]

**Sodium hexanitrocobaltate (III) TS** Dissolve 10 g of sodium hexanitrocobaltate (III) in water to make 50 mL, and filter if necessary. Prepare before use.

**Sodium hyaluronate, purified**  $(\text{C}_{14}\text{H}_{20}\text{NNaO}_{11})_n$  [Same as the namesake monograph]

**Sodium hyaluronate for assay**  $(\text{C}_{14}\text{H}_{20}\text{NNaO}_{11})_n$  [Same as the monograph Purified Sodium Hyaluronate. It contains not less than 99.0% of sodium hyaluronate  $[(\text{C}_{14}\text{H}_{20}\text{NNaO}_{11})_n]$ , calculated on the dried basis.]

**Sodium hydrogen carbonate**  $\text{NaHCO}_3$  [K 8622, Special class]

**Sodium hydrogen carbonate for pH determination**  $\text{NaHCO}_3$  [K 8622, for pH determination]

**Sodium hydrogen carbonate TS** Dissolve 5.0 g of sodium hydrogen carbonate in water to make 100 mL.

**10% Sodium hydrogen carbonate TS** Dissolve 10 g of sodium hydrogen carbonate in water to make 100 mL, and sterilize in a tight container in an autoclave at 121°C for 15 minutes or by filtration through a membrane filter with a pore size not exceeding 0.22  $\mu\text{m}$ .

**7% Sodium hydrogen carbonate injection** See 7% sodium bicarbonate injection.

**Sodium hydrogen sulfite** [K 8059, Special class]

**Sodium hydrogen sulfite TS** Dissolve 10 g of sodium hydrogen sulfite in water to make 30 mL. Prepare before use.

**Sodium hydrogen tartrate monohydrate**

$\text{NaHC}_4\text{H}_4\text{O}_6 \cdot \text{H}_2\text{O}$  [K 8538, (+)-Sodium hydrogen tartrate monohydrate, Special class]

**Sodium hydrogen tartrate TS** Dissolve 1 g of sodium hydrogen tartrate monohydrate in water to make 10 mL (0.5 mol/L). Prepare before use.

**Sodium hydroxide**  $\text{NaOH}$  [K 8576, Special class]

**Sodium hydroxide-dioxane TS** Dissolve 0.80 g of sodium hydroxide in a mixture of 1,4-dioxane and water (3:1) to make 100 mL.

**Sodium hydroxide-methanol TS** Dissolve by thorough shaking 4 g of sodium hydroxide in methanol to make 100 mL. To the supernatant liquid obtained by centrifugation add methanol to make 500 mL. Prepare before use.

**Sodium hydroxide TS** Dissolve 4.3 g of sodium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.

**Sodium hydroxide TS, dilute** Dissolve 4.3 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use (0.1 mol/L).

**0.01 mol/L Sodium hydroxide TS** Dilute 10 mL of sodium hydroxide TS with water to make 1000 mL. Prepare before use.

**0.05 mol/L Sodium hydroxide TS** To 10 mL of 0.5 mol/L sodium hydroxide TS add water to make 100 mL.

**0.2 mol/L Sodium hydroxide TS** Dissolve 8.0 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use.

**0.5 mol/L Sodium hydroxide TS** Dissolve 22 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

**2 mol/L Sodium hydroxide TS** Dissolve 86 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

**4 mol/L Sodium hydroxide TS** Dissolve 168 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

**5 mol/L Sodium hydroxide TS** Dissolve 210 g of sodium hydroxide in water to make 1000 mL. Preserve in a polyethylene bottle.

**6 mol/L Sodium hydroxide TS** Dissolve 252 g of sodium hydroxide in water to make 1000 mL. Preserve in a polyethylene bottle.

**8 mol/L Sodium hydroxide TS** Dissolve 336 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

**Sodium hypobromite TS** To 8 mL of bromine TS add 25 mL of water and 25 mL of sodium carbonate TS. Prepare before use.

**Sodium hypochlorite-sodium hydroxide TS** To a volume of sodium hypochlorite TS for ammonium limit test, equivalent to 1.05 g of sodium hypochlorite ( $\text{NaClO}$ : 74.44), add 15 g of sodium hydroxide and water to make 1000 mL. Prepare before use.

**Sodium hypochlorite TS** Prepare the solution by passing chlorine into sodium hydroxide TS while cooling with ice, so as to contain 5% of sodium hypochlorite ( $\text{NaClO}$ : 74.44). Prepare before use.

**10% Sodium hypochlorite TS** Prepare by introducing chlorine into an aqueous solution of sodium hydroxide while ice-cooling so that the content of sodium hypochlorite ( $\text{NaClO}$ : 74.44) is 10%. Prepare before use.

**Sodium hypochlorite TS for ammonium limit test**

Clear, colorless or light green-yellow solution prepared by passing chlorine into sodium hydroxide or sodium carbonate decahydrate solution, having the odor of chlorine.

**Content:** not less than 4.2 g/d L as sodium hypochlorite ( $\text{NaClO}$ : 74.44). **Assay**—Pipet 10 mL of sodium hypochlorite TS for ammonium limit test, and add water to make exactly 100 mL. Transfer exactly 10 mL of this solution to a glass-stoppered flask, add 90 mL of water, then add 2 g of potassium iodide and 6 mL of diluted acetic acid (31) (1 in 2), stopper tightly, shake well, and allow to stand for 5 minutes in a dark place. Titrate  $<2.50>$  the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 3.722 mg of  $\text{NaClO}$ .

**Sodium L-lactate solution for assay**  $\text{C}_3\text{H}_5\text{NaO}_3$  [Same as the monograph, Sodium L-Lactate Solution]

**Sodium lauryl sulfate** [Same as the namesake monograph]

**Sodium lauryl sulfate TS** Dissolve 100 g of sodium lauryl sulfate in 900 mL of water, add 10 mL of 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**0.2% Sodium lauryl sulfate TS** Dissolve 0.1 g of sodium

lauryl sulfate in 0.1 mol/L sodium phosphate buffer (pH 7.0) to make 50 mL.

**Sodium metabisulfite** See sodium disulfite.

**Sodium metabisulfite TS** See sodium disulfite TS.

**Sodium, metallic** See sodium

**Sodium 1-methyl-1*H*-tetrazole-5-thiolate** See sodium 1-methyl-1*H*-tetrazole-5-thiolate dihydrate.

**Sodium 1-methyl-1*H*-tetrazole-5-thiolate dihydrate**

$C_2H_3N_4NaS \cdot 2H_2O$  White, crystals or crystalline powder.

*Melting point* <2.60>: 90 – 94°C

*Purity* Related substances—Dissolve 10 mg of sodium 1-methyl-1*H*-tetrazole-5-thiolate dihydrate in 10 mL of water, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

**Sodium molybdate** See sodium molybdate (VI) dihydrate.

**Sodium molybdate (VI) dihydrate**  $Na_2MoO_4 \cdot 2H_2O$  [K 8906, disodium molybdate (VI) dihydrate, Special class]

**Sodium 2-naphthalenesulfonate**  $C_{10}H_7NaO_3S$  Pale brown, crystals or powder.

*Content:* not less than 98.0%.

**Sodium  $\beta$ -naphthoquinone sulfonate**  $C_{10}H_5NaO_3S$  Yellow to orange-yellow, crystals or crystalline powder. Soluble in water, and practically insoluble in ethanol (95).

*Loss on drying* <2.41>: Not more than 2.0% (1 g, in vacuum, 50°C).

*Residue on ignition* <2.44>: 26.5 – 28.0% (1 g, after drying).

**Sodium naphthoquinone sulfonate TS** Dissolve 0.25 g of sodium  $\beta$ -naphthoquinone sulfonate in methanol to make 100 mL.

**Sodium nitrate**  $NaNO_3$  [K 8562, Special class]

**Sodium nitrite**  $NaNO_2$  [K 8019, Special class]

**Sodium nitrite TS** Dissolve 10 g of sodium nitrite in water to make 100 mL. Prepare before use.

**Sodium nitroprusside** See sodium pentacyanonitrosylferrate (III) dihydrate.

**Sodium nitroprusside TS** See sodium pentacyanonitrosylferrate (III) TS.

**Sodium 1-nonanesulfonate**  $CH_3(CH_2)_8SO_3Na$  White crystalline powder. Freely soluble in water.

*Loss on drying* <2.41>: Not more than 1.0% (1 g, 105°C, 3 hours).

*Residue on ignition* <2.44>: 30 – 32% (0.5 g).

**Sodium 1-octane sulfonate**  $CH_3(CH_2)_7SO_3Na$  White, crystals or powder.

*Residue on ignition* <2.44>: 32.2 – 33.0% (1.0 g).

**Sodium oxalate (standard reagent)**  $C_2Na_2O_4$  In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

### Sodium pentacyanoammine ferroate (II) *n*-hydrate

$Na_3[Fe(CN)_5NH_3] \cdot xH_2O$  A light yellow to light green-yellow crystalline powder.

*Identification*—(1) Dissolve 0.2 g of sodium pentacyanoammine ferroate (II) *x*-hydrate in 5 mL of water, add 2 mL of sodium hydroxide solution (1 in 10), and heat: ammonia gas is evolved and a brown precipitate is produced.

(2) Dissolve 0.25 g of sodium pentacyanoammine ferroate (II) *n*-hydrate in 20 mL of water. To 1 mL of this solution add 0.2 mL of iron (II) sulfate TS: a green-blue color develops, which changes to a dark blue color on the addition of 2 drops of diluted sodium hypochlorite TS (2 in 5) and 0.2 mL of acetic acid (100).

### Sodium pentacyanonitrosylferrate (III) dihydrate

$Na_2[Fe(CN)_5(NO)] \cdot 2H_2O$  [K 8722, Special class]

**Sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS** Mix an equal volume of a solution of sodium pentacyanonitrosylferrate (III) dihydrate (1 in 10), a solution of potassium hexacyanoferrate (III) (1 in 10) and a solution of sodium hydroxide (1 in 10), and allow to stand for 30 minutes. Use after the color of the solution is changed from a dark red to yellow. Prepare before use.

**Sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS, dilute** To 5 mL of a solution of pentacyanonitrosylferrate (III) dihydrate (3 in 50) add 5 mL of a solution of potassium hexacyanoferrate (III) (13 in 200) and 2.5 mL of a solution of sodium hydroxide (1 in 10), add water to make 25 mL, mix, and use after changing the color of the solution from a dark red to light yellow. Prepare before use.

**Sodium pentacyanonitrosylferrate (III) TS** Dissolve 1 g of sodium pentacyanonitrosylferrate (III) dihydrate in water to make 20 mL. Prepare before use.

**Sodium 1-pentane sulfonate**  $C_5H_{11}NaO_3S$  White, crystals or crystalline powder. Freely soluble in water, and practically insoluble in acetonitrile.

*Purity* Clarity and color of solution—Dissolve 1.0 g of sodium 1-pentane sulfonate in 10 mL of water: the solution is colorless and clear.

*Water* <2.48>: not more than 3.0% (0.2 g).

*Content:* not less than 99.0%, calculated on the anhydrous basis. Assay—Dissolve about 0.3 g of sodium 1-pentane sulfonate, accurately weighed, in 50 mL of water. Transfer this solution to a chromatographic column, prepared by pouring 10 mL of strongly acidic ion-exchange resin (H type) (425 – 600  $\mu$ m in particle diameter) into a chromatographic tube, 9 mm in inside diameter and 160 mm in height, and elute at the rate of about 4 mL per minute. Wash the chromatographic column with 50 mL of water at the rate of about 4 mL per minute, and wash again with 100 mL of water in the same manner. Combine the washings with the eluate, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS) until the yellow color of the solution changes to blue.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 17.42 mg of  $C_5H_{11}NaO_3S$

**Sodium perchlorate** See sodium perchlorate monohydrate.

### Sodium perchlorate monohydrate

$NaClO_4 \cdot H_2O$  [K 8227, Special class]

**Sodium periodate**  $NaIO_4$  [K 8256, Special class]

**Sodium periodate TS** Dissolve 60.0 g of sodium perio-

date in 120 mL of 0.05 mol/L sulfuric acid TS, and add water to make 1000 mL. Keep in a light-resistant vessel.

**Sodium peroxide**  $\text{Na}_2\text{O}_2$  [K 8231, Special class]

**Sodium *p*-phenol sulfonate** See sodium *p*-phenol sulfonate dihydrate.

**Sodium *p*-phenol sulfonate dihydrate**  $\text{C}_6\text{H}_5\text{O}_4\text{NaS}\cdot2\text{H}_2\text{O}$

White to light yellow, crystals or crystalline powder, having a specific odor.

**Identification** (1) To 10 mL of a solution of sodium *p*-phenol sulfonate dihydrate (1 in 10) add 1 drop of iron (III) chloride TS: a purple color develops.

(2) Determine the absorption spectrum of a solution of sodium *p*-phenol sulfonate dihydrate (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 269 nm and 273 nm and between 276 nm and 280 nm.

**Purity** Clarity and color of solution—Dissolve 1.0 g of sodium *p*-phenol sulfonate dihydrate in 25 mL of water: the solution is clear and colorless.

**Content:** not less than 90.0%. **Assay**—Dissolve about 0.5 g of sodium *p*-phenol sulfonate dihydrate, accurately weighed, in 50 mL of water. Transfer the solution to a chromatographic column, prepared by pouring 20 mL of strongly acidic ion exchange resin (H type) for column chromatography (150 to 300  $\mu\text{m}$  in particle diameter) into a chromatographic tube about 1 cm in inside diameter and about 30 cm in height, and allow to flow. Wash the chromatographic column with water until the washing is no longer acidic, combine the washings with the above effluent solution, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of bromocresol green-methyl red TS). Separately, dissolve 0.5 g of sodium *p*-phenol sulfonate dihydrate, weighed accurately, in 50 mL of water and titrate with 0.1 mol/L sodium hydroxide VS, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 23.22 mg of  $\text{C}_6\text{H}_5\text{O}_4\text{NaS}\cdot2\text{H}_2\text{O}$

**Sodium phosphate** See trisodium phosphate dodecahydrate.

**Sodium phosphate TS** Dissolve 5.68 g of anhydrous disodium hydrogen phosphate and 6.24 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

#### 0.1 mol/L Sodium phosphate buffer solution (pH 7.0)

Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL. Add to this solution to a 500 mL solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate in water until the pH becomes 7.0.

**Sodium pyruvate**  $\text{CH}_3\text{COCOONa}$  A white to pale yellow crystalline powder. Freely soluble in water, and slightly soluble in ethanol (99.5) and in acetone.

**Identification** (1) Determine the infrared absorption spectrum of sodium pyruvate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1710  $\text{cm}^{-1}$ , 1630  $\text{cm}^{-1}$ , 1410  $\text{cm}^{-1}$ , 1360  $\text{cm}^{-1}$ , 1190  $\text{cm}^{-1}$ , 1020  $\text{cm}^{-1}$ , 980  $\text{cm}^{-1}$ , 830  $\text{cm}^{-1}$ , 750  $\text{cm}^{-1}$ , 630  $\text{cm}^{-1}$  and 430  $\text{cm}^{-1}$ .

(2) A solution of sodium pyruvate (1 in 20) responds to Qualitative Tests <1.09> (1) for sodium salt.

**Content:** Not less than 97.0%. **Assay**—Weigh accurately about 0.4 g of sodium pyruvate, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution into an iodine bottle, cool to 10°C or lower, add exactly 40 mL of

0.05 mol/L iodine VS, then add 20 mL of a solution of sodium hydroxide (17 in 100), and allow to stand at a dark place for 2 hours. Then add 15 mL of diluted sulfuric acid (1 in 6), and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS  
= 1.834 mg of  $\text{C}_3\text{H}_3\text{NaO}_3$

**100 mmol/L Sodium pyruvate TS** Dissolve 1.1 g of sodium pyruvate in water to make 100 mL, and sterilize by filtration through a membrane filter with a pore size not exceeding 0.22  $\mu\text{m}$ .

**Sodium salicylate**  $\text{HOCH}_2\text{COONa}$  [K 8397, Special class]

**Sodium salicylate-sodium hydroxide TS** Dissolve 1 g of sodium salicylate in 0.01 mol/L sodium hydroxide VS to make 100 mL.

**Sodium selenite**  $\text{Na}_2\text{SeO}_3$  A white crystalline powder.

**Identification**—(1) Dissolve 1 g of sodium selenite in 100 mL of water, and use this solution as the sample solution. To 10 mL of the sample solution add 2 mL of tin (II) chloride TS: a red precipitate is produced.

(2) The sample solution obtained in (1) responds to Qualitative Tests <1.09> (1) for sodium salt.

**Storage**—Preserve in a light-resistant tight container.

**Sodium stearyl fumarate**  $\text{C}_{22}\text{H}_{39}\text{NaO}_4$  A white crystalline powder.

**Identification** (1) Determine the infrared absorption spectrum of sodium stearyl fumarate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 2950  $\text{cm}^{-1}$ , 2920  $\text{cm}^{-1}$ , 2850  $\text{cm}^{-1}$ , 1720  $\text{cm}^{-1}$ , 1610  $\text{cm}^{-1}$ , 1313  $\text{cm}^{-1}$ , 1186  $\text{cm}^{-1}$ , 980  $\text{cm}^{-1}$ , and 665  $\text{cm}^{-1}$ .

(2) Sodium stearyl fumarate responds to Qualitative Tests <1.09> (1) for sodium salt.

**Sodium *p*-styrenesulfonate**  $\text{C}_8\text{H}_7\text{NaO}_3\text{S}$  White, crystals or crystalline powder. Freely soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Recrystallize from diluted ethanol (1 in 2), and dry in vacuum.

**Identification**—Determine the infrared absorption spectrum of sodium *p*-styrenesulfonate according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1236  $\text{cm}^{-1}$ , 1192  $\text{cm}^{-1}$ , 1136  $\text{cm}^{-1}$ , 1052  $\text{cm}^{-1}$ , 844  $\text{cm}^{-1}$  and 688  $\text{cm}^{-1}$ .

**Purity**—Perform the test with 10  $\mu\text{L}$  of a solution of sodium *p*-styrenesulfonate (1 in 1000) as directed in the Assay under Panipenem: Any obstructive peaks for determination of panipenem are not observed.

**Sodium sulfate** See sodium sulfate decahydrate.

**Sodium sulfate, anhydrous**  $\text{Na}_2\text{SO}_4$  [K 8987, Special class]

**Sodium sulfate decahydrate**  $\text{Na}_2\text{SO}_4\cdot10\text{H}_2\text{O}$  [K 8986, Special class]

**Sodium sulfide** See sodium sulfide enneahydrate.

**Sodium sulfide enneahydrate**  $\text{Na}_2\text{S}\cdot9\text{H}_2\text{O}$  [K 8949, Special class]

**Sodium sulfide TS** Dissolve 5 g of sodium sulfide enneahydrate in a mixture of 10 mL of water and 30 mL of glycerin. Or dissolve 5 g of sodium hydroxide in a mixture of 30 mL of water and 90 mL of glycerin, saturate a half volume of this solution with hydrogen sulfide, while cooling, and mix with the remaining half. Preserve in well-filled, light-resistant bottles. Use within 3 months.

**Sodium sulfite** See sodium sulfite heptahydrate.

**Sodium sulfite, anhydrous**  $\text{Na}_2\text{SO}_3$  [K 8061, Sodium sulfite, Special class]

**Sodium sulfite heptahydrate**  $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$  [K 8060, Special class]

**1 mol/L Sodium sulfite TS** Dissolve 1.26 g of anhydrous sodium sulfite in water to make 10 mL.

**Sodium sulfite-sodium dihydrogen phosphate TS** Mix 1.5 mL of a solution dissolved 1.26 g of anhydrous sodium sulfite in 100 mL of water and 98.5 mL of a solution dissolved 1.56 g of sodium dihydrogen phosphate dihydrate in 100 mL of water. Prepare before use.

**Sodium tartrate** See sodium tartrate dihydrate.

**Sodium tartrate dihydrate**  $\text{C}_4\text{H}_4\text{Na}_2\text{O}_6 \cdot 2\text{H}_2\text{O}$  [K 8540, sodium (+)-tartrate dihydrate, Special class]

**Sodium taurooursodeoxycholate for thin-layer chromatography**  $\text{C}_{26}\text{H}_{44}\text{NNaO}_6\text{S}$  White to pale brown, crystalline powder or powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5).

**Identification**—(1) Determine the infrared absorption spectrum of sodium taurooursodeoxycholate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $2930\text{ cm}^{-1}$ ,  $1645\text{ cm}^{-1}$ ,  $1556\text{ cm}^{-1}$ ,  $1453\text{ cm}^{-1}$ ,  $1215\text{ cm}^{-1}$  and  $1049\text{ cm}^{-1}$ .

(2) Sodium taurooursodeoxycholate for thin-layer chromatography responds to Qualitative Tests <1.09> (1) for sodium salt.

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : +40 – +50° (40 mg, methanol, 20 mL, 100 mm).

**Purity** Related substances—Dissolve 10 mg of sodium taurooursodeoxycholate for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of the sample solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Perform the test with 5  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification under Bear Bile: the spots other than the principal spot with an *R<sub>f</sub>* value of about 0.2 obtained from the sample solution are not more intense than the spot from the standard solution.

**Sodium tetraborate-calcium chloride buffer solution (pH 8.0)** Dissolve 0.572 g of sodium tetraborate decahydrate and 2.94 g of calcium chloride dihydrate in 800 mL of freshly boiled and cooled water, adjust the pH to 8.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Sodium tetraborate decahydrate**  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  [K 8866, Special class]

**Sodium tetraborate decahydrate for pH determination** [K 8866, for pH standard solution]

**Sodium tetraborate-sulfuric acid TS** To 9.5 g of sodium tetraborate decahydrate add 1000 mL of sulfuric acid, and

dissolve by shaking for a night.

**Purity:** To 5 mL of sodium tetraborate-sulfuric acid TS, previously cooled in ice water, add gently 1 mL of water, stir while cooling, then heat in a water bath for 10 minutes, and cool in ice water. Add exactly 0.2 mL of carbazole TS, stir thoroughly, then heat in a water bath for 15 minutes, and cool in ice water to room temperature: a green color does not appear.

**Sodium tetraphenylborate**  $(\text{C}_6\text{H}_5)_4\text{BNa}$  [K 9521, Special class]

**Sodium thioglycolate**  $\text{HSCH}_2\text{COONa}$  A white powder, having a characteristic odor.

**Identification** (1) To a solution (1 in 10) add 0.1 mL of ammonia solution (28) and 1 drop of iron (III) chloride TS: a dark red-purple color appears.

(2) Perform the test as directed under Flame Coloration Test <1.04> (1): a yellow color appears.

**Purity** Clarity and color of solution—Dissolve 1 g in 10 mL of water: the solution is clear and colorless.

**Sodium thiosulfate** See sodium thiosulfate pentahydrate.

**Sodium thiosulfate pentahydrate**  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  [K 8637, Special class]

**Sodium thiosulfate TS** Dissolve 26 g of sodium thiosulfate pentahydrate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000 mL (0.1 mol/L).

**Sodium toluenesulfonchloramide trihydrate**

$\text{C}_7\text{H}_7\text{ClINaO}_2\text{S} \cdot 3\text{H}_2\text{O}$  [K 8318, Sodium *p*-toluenesulfonchloramide trihydrate, Special class]

**Sodium toluenesulfonchloramide TS** Dissolve 1 g of sodium toluenesulfonchloramide trihydrate in water to make 100 mL. Prepare before use.

**Sodium tridecanesulfonate**  $\text{C}_{13}\text{H}_{27}\text{SO}_3\text{Na}$  White, crystals or powder.

**Purity** Absorbance—Dissolve 1.43 g of sodium tridecanesulfonate in 1000 mL of water, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbances at 230 nm and 254 nm are not more than 0.05 and 0.01, respectively.

**Sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy**  $(\text{CH}_3)_3\text{SiCH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{Na}$  Prepared for nuclear magnetic resonance spectroscopy.

**Sodium 3-trimethylsilylpropionate-d<sub>4</sub> for nuclear magnetic resonance spectroscopy**  $(\text{CH}_3)_3\text{SiCD}_2\text{CD}_2\text{COONa}$  Prepared for nuclear magnetic resonance spectroscopy.

**Sodium 2,4,6-trinitrobenzenesulfonate dihydrate**

$\text{C}_6\text{H}_2\text{N}_3\text{NaO}_6\text{S} \cdot 2\text{H}_2\text{O}$  White or pale yellowish, crystals or powder.

**Sodium tungstate** See sodium tungstate (VI) dihydrate.

**Sodium tungstate (VI) dihydrate**  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  [K 8612, Special class]

**Sodium valproate for assay**  $\text{C}_8\text{H}_{15}\text{NaO}_2$  [Same as the monograph Sodium Valproate. When dried, it contains not less than 99.0% of sodium valproate ( $\text{C}_8\text{H}_{15}\text{NaO}_2$ ).]

**Soluble starch** See starch, soluble.

**Soluble starch TS** Triturate 1 g of soluble starch in 10 mL of cooled water, pour gradually into 90 mL of boiled water while constantly stirring, boil gently for 3 minutes, and cool. Prepare before use.

**Sorbitan sesquioleate** [Same as the namesake monograph]

**D-Sorbitol** C<sub>6</sub>H<sub>14</sub>O<sub>6</sub> [Same as the namesake monograph]

**D-Sorbitol for gas chromatography** Prepared for gas chromatography.

**Soybean-casein digest medium** See Sterility Test <4.06>.

**Soybean oil** [Same as the namesake monograph]

**Soybean peptone** See peptone, soybean.

**Stachyose for thin-layer chromatography** C<sub>24</sub>H<sub>42</sub>O<sub>21</sub> A white powder. Very soluble in water, and practically insoluble in ethanol (99.5). It is deliquescent with the atmospheric moisture.

*Optical rotation* <2.49> [α]<sub>D</sub><sup>20</sup>: +144 – +154° (50 mg calculated on the anhydrous basis, diluted ammonia solution (28) (1 in 1000), 5 mL, 100 mm).

**Purity** Related substances—Dissolve 2 mg of stachyose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer chromatography <2.03>. Spot 2 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS to the plate, and heat at 105°C for 10 minutes: a spot other than the principle spot with an R<sub>f</sub> value of about 0.5 is not observed.

**Stacking gel for celmoleukin** In 0.5 mol/L Tris buffer solution (pH 6.8), prepare stacking the gel using ammonium persulfate and N,N,N',N'-tetramethylenediamine so the acrylamide concentration is 5.2% and the sodium lauryl sulfate concentration is 0.1%.

**Stannous chloride** See tin (II) chloride dihydrate.

**Stannous chloride-sulfuric acid TS** See tin (II) chloride-sulfuric acid TS.

**Stannous chloride TS** See tin (II) chloride TS.

**Stannous chloride TS, acidic** See tin (II) chloride TS, acidic.

**Starch** [K 8658, Special class]

**Starch-sodium chloride TS** Saturate starch TS with sodium chloride. Use within 5 to 6 days.

**Starch, soluble** [K 8659, Starch, soluble, First class]

**Starch TS** Triturate 1 g of starch with 10 mL of cold water, and pour the mixture slowly, with constant stirring, into 200 mL of boiling water. Boil the mixture until a thin, translucent fluid is obtained. Allow to settle, and use the supernatant liquid. Prepare before use.

**Stearic acid for gas chromatography** C<sub>18</sub>H<sub>36</sub>O<sub>2</sub> [K 8585, Special class]

**Stearyl alcohol** [Same as the namesake monograph]

**Sterile purified water** [Same as the monograph Sterile Purified Water in Containers. It is not necessary to confirm if they meet all of the requirement, provided that they are confirmed to be suitable for the purpose of the relevant test.]

**Strong ammonia water** See ammonia solution (28).

**Strong cupric acetate TS** See copper (II) acetate TS,

strong.

**Strong hydrogen peroxide water** See hydrogen peroxide (30).

**Strongly acidic ion exchange resin** Contains strong acid ion exchange residues. Particle diameter is about 100 μm.

**Strongly basic ion exchange resin** Contains strong basic ion exchange residues. Particle diameter is about 100 μm.

**Strontium TS** Dissolve 76.5 g of strontium chloride in water to make exactly 500 mL. Pipet 20 mL of this solution, and add water to make exactly 1000 mL (1000 ppm).

**Strontium chloride** See strontium chloride hexahydrate.

**Strontium chloride hexahydrate** SrCl<sub>2</sub>.6H<sub>2</sub>O [K 8132, Special class]

**Strychnine nitrate for assay** C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>.HNO<sub>3</sub> To 1 g of strychnine nitrate add 14 mL of water and about 10 mg of active carbon, heat in a water bath for 10 minutes, filter while hot, cool the filtrate quickly to form crystals, and filter the crystals. Add 8 mL of water to the crystals, dissolve by heating in a water bath, filter while hot, cool quickly, and filter the crystals formed. Repeat this procedure with 8 mL of water, and dry the crystals in a desiccator (in vacuum, silica gel) for 24 hours. Colorless or white, crystals or crystalline powder. Sparingly soluble in water and in glycerin, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

**Purity** Related substances—Dissolve 35 mg of strychnine nitrate for assay in 100 mL of the mobile phase and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 20 μL each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than strychnine obtained from the sample solution is not larger than the peak area of strychnine from the standard solution (1).

#### Operating conditions

Proceed the operating conditions in the Assay under Nux Vomica except time span of measurement.

Time span of measurement: About 3 times as long as the retention time of strychnine, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 40 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of strychnine obtained with 20 μL of the standard solution (2) can be measured by the automatic integration method and the peak height of strychnine with 20 μL of the standard solution (1) is about 20% of the full scale.

*Loss on drying* <2.41>: not more than 0.5% (0.2 g, 105°C, 3 hours).

**Content:** not less than 99.0% calculated on the dried basis. Assay—Dissolve about 0.5 g of strychnine nitrate for assay, accurately weighed, in 40 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), heat if necessary, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 39.74 mg of C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>.HNO<sub>3</sub>

**Styrene** C<sub>8</sub>H<sub>8</sub> Colorless, clear liquid.

*Specific gravity* <2.56> *d*: 0.902 – 0.910

**Purity**—Perform the test with 1  $\mu$ L of styrene as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method and calculate the amount of styrene by the area percentage method: it shows the purity of not less than 99%.

#### Operating conditions

Detector: Thermal conductivity detector.

Column: A glass column, about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth (180 to 250  $\mu$ m in particle diameter) coated with polyethylene glycol 20 M at the ratio of 10%.

Column temperature: A constant temperature of about 100°C.

Temperature of sample vaporization chamber: A constant temperature of about 150°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of styrene is about 10 minutes.

Time span of measurement: About twice as long as the retention time of styrene.

**Styrene-maleic acid alternating copolymer partial butyl ester** Polymerize styrene and maleic anhydride using cumene as solvent, and add 1-butanol or water to the maleic anhydride groups. Average molecular mass: about 1600. A white to pale yellow-white powder.

**Identification**—Dissolve 5 mg of the substance to be examined in sodium hydrogen carbonate solution (1 in 15) to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 256 nm and 260 nm, and a shoulder between 251 nm and 256 nm.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (258 nm): 6.3 – 7.3 [5 mg calculated on the anhydrous basis, sodium hydrogen carbonate solution (1 in 15), 10 mL].

#### Purity (i) Test solutions

Solution A: Dissolve 36.6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.23 mL of *N,N,N',N'*-tetramethylethylenediamine and water to make 100 mL.

Solution B: Dissolve 33.3 g of acrylamide and 0.89 g of *N,N'*-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution C: Dissolve 5.98 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.46 mL of *N,N,N',N'*-tetramethylethylenediamine and water to make 100 mL.

Solution D: Dissolve 10.0 g of acrylamide and 2.5 g of *N,N'*-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution E: Dissolve 4 mg of riboflavin in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution F: Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycine in water to make 500 mL.

Buffer solution for sample: To 50 mL of Solution C add 20 mL of water and 10 mL of glycerin solution (3 in 5).

#### (ii) Gels

Resolving gel: Mix 2.5 mL of Solution A and 7.5 mL of Solution B. Mix the mixture with 10 mL of freshly prepared ammonium peroxodisulfate solution (7 in 5000) after degassing under reduced pressure. Pour this mixture into a glass tube, 5 mm in inside diameter and 10 cm in length, to make 7 cm height, put water gently on the upper surface of the mix-

ture, and allow to polymerize for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

Stacking gel: Mix 1 mL of Solution C, 2 mL of Solution D, 1 mL of Solution E and 4 mL of water, pour 0.2 mL of the mixture on the resolving gel, put water gently on the upper surface of the mixture, and allow to polymerize under a fluorescent light for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

(iii) Sample solution Dissolve 3.0 mg of the substance to be examined in the buffer solution for sample to make 20 mL.

(iv) Procedure Mount the gel in an electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the upper reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100  $\mu$ L of the sample solution onto the surface of the gel, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached 5 cm from the upper end of the gel.

(v) Staining and decolorization Dissolve 0.1 g of Coomassie brilliant blue G-250 in 100 mL of trichloroacetic acid solution (1 in 2), and mix 1 volume of this solution and 2 volumes of water before using. Immerse the gels for 15 hours in this mixture, and transfer into about 20 mL of acetic acid (100) solution (7 in 100) to remove the excess of dye. Replace the acetic acid (100) solution until the background of the gel becomes colorless.

(vi) Determination Determine the peak area,  $A_T$ , of styrene-maleic acid alternating copolymer partial butyl ester and the total area,  $A$ , of the peaks other than styrene-maleic acid alternating copolymer partial butyl ester, based on the absorbance at 600 nm of the gel determined by using a densitometer. Calculate the amount of styrene-maleic acid alternating copolymer partial butyl ester by the following formula: not less than 98.0%.

$$\begin{aligned} \text{Amount (\%)} \text{ of styrene-maleic acid alternating} \\ \text{copolymer partial butyl ester} \\ = A_T / (A_T + A) \times 100 \end{aligned}$$

**Water** <2.48>: Not more than 10.0% (10 mg, coulometric titration).

**Subculture medium for nartograstim test** Dissolve an amount of Nartograstim (Genetical Recombination), equivalent to 0.20 mg in 20 mL of phosphate-buffered sodium chloride TS. To 0.1 mL of this solution add 100 mL of potency measuring medium for nartograstim test.

**Substrate buffer for cemoleukin** Dissolve 32.4 g of tripotassium citrate monohydrate in water to make 1000 mL, and add 1 mol/L citric acid TS for buffer solution to adjust the pH to 5.5. To 100 mL of this solution add and dissolve 0.44 g of *o*-phenylenediamine and then 60  $\mu$ L of hydrogen peroxide (30). Prepare at the time of use.

**Substrate TS for epoetin alfa** Dissolve 30 mg of 4-chloro-1-naphthol in 10 ml of methanol, and use as Solution A. Mix 30  $\mu$ L of hydrogen peroxide (30) and 50 mL of 0.02 mol/L tris buffer solution (pH 7.5) and use as Solution B. Mix Solutions A and B before use.

**Substrate TS for interferon alfa confirmation** Dissolve 9 mg of 3,3'-diaminobenzidine tetrahydrochloride in phosphate-buffered sodium chloride TS to make 30 mL. Add 5

$\mu\text{L}$  of hydrogen peroxide (30) to this solution. Prepare before use.

**Substrate TS for peroxidase determination** Dissolve 0.195 mL of hydrogen peroxidase (30), 8.38 g of disodium hydrogen phosphate dodecahydrate and 1.41 g of citric acid monohydrate in water to make 300 mL. To 15 mL of this solution add 13 mg of *o*-phenylenediamine dihydrochloride before use.

**Substrate TS for kallidinogenase assay (1)** Dissolve an appropriate amount of *H*-D-valyl-L-leucyl-L-arginine-4-nitroanilide dihydrochloride in 0.1 mol/L tris buffer solution (pH 8.0) to prepare a solution containing 1 mg of *H*-D-valyl-L-leucyl-L-arginine-4-nitroanilide dihydrochloride in 5 mL.

**Substrate TS for kallidinogenase assay (2)** Dissolve 17.7 mg of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester hydrochloride in 0.1 mol/L tris buffer solution (pH 8.0) to make 100 mL.

**Substrate TS for kallidinogenase assay (3)** Suspend 0.6 g of milk casein purified by the Hammerstein's method in 80 mL of 0.05 mol/L sodium hydrogen phosphate TS, and dissolve by warming at 65°C for 20 minutes. After cooling, adjust to pH 8.0 with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 100 mL. Prepare before use.

**Substrate TS for kallidinogenase assay (4)** Dissolve 25 mg of *H*-D-valyl-L-leucyl-L-arginine-4-nitroanilide dihydrochloride in 28.8 mL of water.

**Substrate TS for lysozyme hydrochloride** To a suitable amount of dried cells of *Micrococcus luteus* add a suitable amount of phosphate buffer solution (pH 6.2) gently shake to make a suspension, and add the substrate cells or the same buffer solution so that the absorbance of the suspension at 640 nm is about 0.65. Prepare before use.

***N*-Succinimidyl 4-(*N*-maleidomethyl)cyclohexane-1-carboxylate** See 4-(*N*-Maleidomethyl cyclohexane-1-carboxylic acid *N*-succinimidyl ester.

**Succinic acid**  $\text{C}_4\text{H}_6\text{O}_4$  Colorless or white crystalline powder. Very soluble in hot water, soluble in water and in ethanol (99.5), and sparingly soluble in diethyl ether.

*Melting point* <2.60>: About 185°C.

*Residue on ignition* <2.44>: not more than 0.02% (1 g).

**Content:** not less than 99.5%. **Assay**—Weigh accurately about 1 g of succinic acid, dissolve in 50 mL of water, add 5 drops of phenolphthalein TS, and titrate <2.50> with 1 mol/L sodium hydroxide VS. Perform a blank titration in the same manner, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS  
= 59.05 mg of  $\text{C}_4\text{H}_6\text{O}_4$

**Succinic acid, anhydrous**  $\text{C}_4\text{H}_4\text{O}_3$  White or pale yellow-white, crystals or flakes. It is odorless. Soluble in water, freely soluble in hot water, and sparingly soluble in ethanol (95).

**Purity** (1) Chloride <1.03>: not more than 0.005%.

(2) Iron <1.10>: not more than 0.001%.

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

**Content:** not less than 98.0%. **Assay**—Dissolve about 1 g of anhydrous succinic acid, accurately weighed, in 50 mL of water by warming, cool, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS  
= 50.04 mg of  $\text{C}_4\text{H}_4\text{O}_3$ .

**Sucrose**  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$  [K 8383, Special class]

**Sucrose for optical rotation**  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$  [K 8383, Sucrose, Special class]

**Sudan III**  $\text{C}_{22}\text{H}_{16}\text{N}_4\text{O}$  Red-brown powder. It dissolves in acetic acid (100) and in chloroform, and insoluble in water, in ethanol (95), in acetone and in diethyl ether.

*Melting point* <2.60>: 170 – 190°C

**Sudan III TS** Dissolve 10 mg of sudan III in 5 mL of ethanol (95), filter, and add 5 mL of glycerin to the filtrate. Prepare before use.

**Sulbactam sodium for sulbactam penicillamine**

$\text{C}_8\text{H}_{10}\text{NNaO}_5\text{S}$  White to yellowish white crystalline powder. Freely soluble in water, and slightly soluble in ethanol (95).

**Identification**—Determine the infrared absorption spectrum of sulbactam sodium for sulbactam penicillamine according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits the absorption at the wave numbers of about 1780  $\text{cm}^{-1}$ , 1600  $\text{cm}^{-1}$ , 1410  $\text{cm}^{-1}$ , 1400  $\text{cm}^{-1}$ , 1320  $\text{cm}^{-1}$ , 1300  $\text{cm}^{-1}$ , 1200  $\text{cm}^{-1}$  and 1130  $\text{cm}^{-1}$ .

*Water* <2.48>: not more than 1.0% (0.5 g).

**Content:** not less than 875  $\mu\text{g}$  (potency) per mg, calculated on the anhydrous basis. **Assay**—Weigh accurately an amount of sulbactam sodium for sulbactam penicillamine and Sulbactam RS, equivalent to about 0.10 g (potency), dissolve each in a suitable volume of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10  $\mu\text{L}$  each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios,  $Q_T$  and  $Q_S$ , of the peak area of sulbactam to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g } (\text{potency})] \text{ of sulbactam } (\text{C}_8\text{H}_{11}\text{NO}_5\text{S}) \\ = M_S \times Q_T/Q_S \times 1000 \end{aligned}$$

$M_S$ : amount [mg (potency)] of Sulbactam RS taken

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000).

**Operating conditions**

**Detector:** An ultraviolet absorption photometer (wavelength: 220 nm).

**Column:** A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10  $\mu\text{m}$  in particle diameter).

**Column temperature:** A constant temperature of about 35°C.

**Mobile phase:** To 750 mL of 0.005 mol/L tetrabutylammonium hydroxide TS add 250 mL of acetonitrile for liquid chromatography.

**Flow rate:** Adjust so that the retention time of sulbactam is about 6 minutes.

**System suitability**

**System performance:** When the procedure is run with 10  $\mu\text{L}$  of the standard solution according to the above operating conditions, sulbactam and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

**System repeatability:** When the test is repeated 6 times with 10  $\mu\text{L}$  of the standard solution according to the above operating conditions, the relative standard deviation of the

peak areas of sulbactam is not more than 2.0%.

**Sulfamic acid (standard reagent)** See amido sulfuric acid (standard reagent).

**Sulfanilamide**  $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2$  [K 9066, Special class]

#### Sulfanilamide for titration of diazotization

$\text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2$  [K 9066, For titration of diazotization]

**Sulfanilic acid**  $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_3\text{H}$  [K 8586, Special class]

**Sulfathiazole**  $\text{C}_9\text{H}_9\text{N}_3\text{O}_3\text{S}_2$  White crystalline powder.  
Melting point <2.60>: 200 – 204°C

**Sulfite oxidase** One unit indicates an amount of the enzyme which consumes 1  $\mu\text{mol}$  of oxygen in 1 minute at 25°C and pH 8.0 using sulfur dioxide and oxygen as the substrate.

**Sulfite oxidase TS** Suspend sulfite oxidase in ammonium sulfate TS so that each mL contains 2.5 units of the activity.

*Storage*—Between 0 and 8°C.

**Sulfosalicylic acid** See 5-sulfosalicylic acid dihydrate.

**5-Sulfosalicylic acid dihydrate**  $\text{C}_7\text{H}_6\text{O}_6\text{S} \cdot 2\text{H}_2\text{O}$   
[K 8589, Special class]

**Sulfosalicylic acid TS** Dissolve 5 g of 5-sulfosalicylic acid dihydrate in water to make 100 mL.

**Sulfur** S [K 8088, Special class]

**Sulfur dioxide**  $\text{SO}_2$  Prepare by adding sulfuric acid dropwise to a concentrated solution of sodium bisulfite. Colorless gas, having a characteristic odor.

**Sulfuric acid**  $\text{H}_2\text{SO}_4$  [K 8951, Special class]

**Sulfuric acid, dilute** Cautiously add 5.7 mL of sulfuric acid to 10 mL of water, cool, and dilute with water to make 100 mL (10%).

**Sulfuric acid-ethanol TS** With stirring, add slowly 3 mL of sulfuric acid to 1000 mL of ethanol (99.5), and cool.

**Sulfuric acid for readily carbonizable substances** To sulfuric acid, the content of which has previously been determined by the following method, add water cautiously, and adjust the final concentration to 94.5% to 95.5% of sulfuric acid ( $\text{H}_2\text{SO}_4$ ). When the concentration is changed owing to absorption of water during storage, prepare freshly.

**Assay**—Weigh accurately about 2 g of sulfuric acid in a glass-stoppered flask rapidly, add 30 mL of water, cool, and titrate <2.50> the solution with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of bromothymol blue TS).

Each mL of 1 mol/L sodium hydroxide VS  
= 49.04 mg of  $\text{H}_2\text{SO}_4$

**Sulfuric acid, fuming**  $\text{H}_2\text{SO}_4 \cdot n\text{SO}_3$  [K 8741, Special class]

**Sulfuric acid-hexane-methanol TS** To 230 mL of a mixture of methanol and hexane (3:1) add cautiously 2 mL of sulfuric acid.

**Sulfuric acid-methanol TS** Prepare carefully by adding 60 mL of sulfuric acid to 40 mL of methanol.

**0.05 mol/L Sulfuric acid-methanol TS** Add gradually 3 mL of sulfuric acid to 1000 mL of methanol, while stirring, and allow to cool.

**Sulfuric acid-monobasic sodium phosphate TS** See sulfuric acid-sodium dihydrogenphosphate TS.

**Sulfuric acid, purified** Place sulfuric acid in a beaker, heat until white fumes are evolved, then heat for 3 minutes cautiously and gently. Use after cooling.

**Sulfuric acid-sodium dihydrogenphosphate TS** Add 6.8 mL of sulfuric acid to 500 mL of water, then dissolve 50 g of sodium dihydrogenphosphate dihydrate in this solution, and add water to make 1000 mL.

**Sulfuric acid-sodium hydroxide TS** With stirring add slowly 120 mL of sulfuric acid to 1000 mL of water, and cool (solution A). Dissolve 88.0 g of sodium hydroxide in 1000 mL of freshly boiled and cooled water (solution B). Mix equal volumes of solution A and solution B.

**Sulfuric acid TS** Cautiously add 1 volume of sulfuric acid to 2 volumes of water, and while warming on a water bath add dropwise potassium permanganate TS until a pale red color of the solution remains.

**0.05 mol/L Sulfuric acid TS** Dilute 100 mL of 0.5 mol/L sulfuric acid TS with water to make 1000 mL.

**0.25 mol/L Sulfuric acid TS** With stirring, add slowly 15 mL of sulfuric acid to 1000 mL of water, then cool.

**0.5 mol/L Sulfuric acid TS** With stirring, add slowly 30 mL of sulfuric acid to 1000 mL of water, then cool.

**1 mol/L Sulfuric acid TS** Add 60 mL of sulfuric acid in 1000 mL of water slowly with stirring, then allow to cool.

**2 mol/L Sulfuric acid TS** To 1000 mL of water add gradually 120 mL of sulfuric acid with stirring, and cool.

**5 mol/L Sulfuric acid TS** Add 300 mL of sulfuric acid in 1000 mL of water slowly with stirring, then allow to cool.

**Sulfurous acid solution** A clear and colorless liquid containing more than 5% of  $\text{SO}_2$ , having a pungent odor. Specific gravity: about 1.03 g/mL.

**Identification**—To 1 mL of iodine TS add 20 mL of water, and add 1 mL of sulfurous acid solution: the color of the solution disappears, and this solution forms a white precipitate upon addition of 1 mL of barium chloride TS.

*Storage*—Preserve at a cold place.

**Sulpiride for assay**  $\text{C}_{15}\text{H}_{23}\text{N}_3\text{O}_4\text{S}$  [Same as the monograph Sulpiride. When dried, it contains not less than 99.0% of sulpiride ( $\text{C}_{15}\text{H}_{23}\text{N}_3\text{O}_4\text{S}$ ).]

**Sulpyrine** See sulpyrine hydrate.

**Sulpyrine for assay** See sulpyrine hydrate for assay.

**Sulpyrine hydrate**  $\text{C}_{13}\text{H}_{16}\text{N}_3\text{NaO}_4\text{S} \cdot \text{H}_2\text{O}$  [Same as the namesake monograph]

**Sulpyrine hydrate for assay**  $\text{C}_{13}\text{H}_{16}\text{N}_3\text{NaO}_4\text{S} \cdot \text{H}_2\text{O}$  [Same as the monograph Sulpyrine Hydrate. Calculated on the dried basis, it contains not less than 99.0% of sulpyrine ( $\text{C}_{13}\text{H}_{16}\text{N}_3\text{NaO}_4\text{S}$ ).]

**Suxamethonium chloride for thin-layer chromatography** See suxamethonium chloride hydrate for thin-layer chromatography.

**Suxamethonium chloride hydrate for thin-layer chromatography**  $\text{C}_{14}\text{H}_{30}\text{Cl}_2\text{N}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$  [Same as the monograph Suxamethonium Chloride Hydrate]

**Sweet hydrangea leaf dihydroisocoumarin for thin-layer chromatography** White to light yellow-brown crystalline powder of mainly two components, generally obtained from activated charcoal treated fraction of acetone or methanol extracts of crumpling upped leaves or tip branches of

**Hydrangea macrophylla** Seringe var. *thunbergii* Makino (Saxifragaceae).

**Identification** Dissolve 2 mg of the substance to be examined in 1 mL of methanol, and perform the test with 5  $\mu$ L of this solution as directed in the Identification under Sweet Hydrangea Leaf: two consecutive spots are observed at *Rf* values of about 0.3.

**Swertia herb** [Same as the namesake monograph]

**Swertiamarin for thin-layer chromatography**  $C_{16}H_{22}O_{10}$   
A white to light yellow powder. Freely soluble in water and in ethanol (95).

**Identification** Determine the infrared absorption spectrum of swertiamarin for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3380  $\text{cm}^{-1}$ , 1693  $\text{cm}^{-1}$ , 1618  $\text{cm}^{-1}$  and 1068  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 2 mg of swertiamarin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu$ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot with an *Rf* value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

**Synthetic zeolite for drying** A mixture of 6( $\text{Na}_2\text{O}$ ).6( $\text{Al}_2\text{O}_3$ ).12( $\text{SiO}_2$ ) and 6( $\text{K}_2\text{O}$ ).6( $\text{Al}_2\text{O}_3$ ).12( $\text{SiO}_2$ ) prepared for drying. Usually, use the spherically molded form, 2 mm in diameter, prepared by adding a binder. White to grayish white, or color transition by adsorbing water. Average fine pore diameter is about 0.3 nm, and the surface area is 500 to 700  $\text{m}^2$  per g.

**Loss on ignition** <2.43>: not more than 2.0% [2 g, 550–600°C, 4 hours, allow to stand in a desiccator (phosphorus (V) oxide).]

**System suitability test solution for filgrastim** Filgrastim (Genetical Recombination) containing about 2% charge isomer.

**Talc** [Same as the namesake monograph]

**Taltirelin hydrate for assay**  $C_{17}H_{23}N_7O_5 \cdot 4H_2O$  [Same as the monograph Taltirelin Hydrate. It contains not less than 99.0% of taltirelin ( $C_{17}H_{23}N_7O_5$ ), calculated on the anhydrous basis.]

**Tamsulosin hydrochloride**  $C_{20}H_{28}N_2O_5S \cdot HCl$  [Same as the namesake monograph]

**Tamsulosin hydrochloride for assay**  $C_{20}H_{28}N_2O_5S \cdot HCl$  [Same as the monograph Tamsulosin Hydrochloride. When dried, it contains not less than 99.0% of tamsulosin hydrochloride ( $C_{20}H_{28}N_2O_5S \cdot HCl$ ).]

**Tannic acid** [Same as the namesake monograph]

**Tannic acid TS** Dissolve 1 g of tannic acid in 1 mL of ethanol (95), and add water to make 10 mL. Prepare before use.

**Tartaric acid** See L-tartaric acid.

**L-Tartaric acid**  $C_4H_6O_6$  [K 8532, L(+)-Tartaric acid, Special class].

**Tartrate buffer solution (pH 3.0)** Dissolve 1.5 g of L-tartaric acid and 2.3 g of sodium tartarate dihydrate in water to make 1000 mL.

**Taurine**  $H_2NCH_2CH_2SO_3H$  White, crystals or crystalline powder.

**Contents:** not less than 95.0%. Assay—Weigh accurately about 0.2 g, dissolve in 50 mL of water, add 5 mL of formaldehyde solution, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 12.52 mg of  $C_2H_7NO_3S$

**Telmisartan for assay**  $C_{33}H_{30}N_4O_2$  [Same as the monograph Telmisartan]

**Temocapril hydrochloride for assay**  $C_{23}H_{28}N_2O_5S_2 \cdot HCl$  [Same as the monograph Temocapril Hydrochloride. It contains not less than 99.5% of temocapril hydrochloride ( $C_{23}H_{28}N_2O_5S_2 \cdot HCl$ : 513.07), calculated on the anhydrous basis.]

**Terbinafine hydrochloride for assay**  $C_{21}H_{25}N \cdot HCl$   
[Same as the monograph Terbinafine Hydrochloride]

**Terephthalic acid**  $C_6H_4(COOH)_2$  White, crystals or crystalline powder. Slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

**Residue on ignition** <2.44>: not more than 0.3% (1 g).

**Content:** not less than 95.0%. Assay—Weigh accurately about 2 g of terephthalic acid, dissolve in exactly 50 mL of 1 mol/L sodium hydroxide VS, and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner.

Each mL of 1 mol/L sodium hydroxide VS  
= 83.07 mg of  $C_8H_6O_4$

**Terphenyl**  $C_{18}H_{14}$  White crystalline powder.

**Identification**—Determine the absorption spectrum of a solution of terphenyl in methanol (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 276 nm and 280 nm.

**Melting point** <2.60>: 208–213°C

**p-Terphenyl** See terphenyl.

**Test bacteria inoculation medium for teceleukin** Dissolve 6.0 g of peptone, 3.0 g of yeast extract, 1.5 g of meat extract, 1.0 g of glucose, and 13.0 to 20.0 g of agar in water to make 1000 mL and sterilize. The pH is 6.5 to 6.6.

**Test bacteria inoculation medium slant for teceleukin**

Sterilized slant culture obtained by adding approximately 9 mL of test bacteria inoculation medium for teceleukin to a test tube with an inside diameter of 16 mm.

**Testosterone**  $C_{19}H_{28}O_2$  White, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum of testosterone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits the absorption at the wave numbers of about 3530  $\text{cm}^{-1}$ , 3380  $\text{cm}^{-1}$ , 1612  $\text{cm}^{-1}$ , 1233  $\text{cm}^{-1}$ , 1067  $\text{cm}^{-1}$  and 1056  $\text{cm}^{-1}$ .

**Testosterone propionate**  $C_{22}H_{32}O_3$  [Same as the name-

sake monograph]

**Tetrabromophenolphthalein ethyl ester potassium salt** C<sub>22</sub>H<sub>13</sub>Br<sub>4</sub>KO<sub>4</sub> [K 9042, Special class]

**Tetrabromophenolphthalein ethyl ester TS** Dissolve 0.1 g of tetrabromophenolphthalein ethyl ester potassium salt in acetic acid (100) to make 100 mL. Prepare before use.

**Tetra-n-butylammonium bromide** [CH<sub>3</sub>(CH<sub>2</sub>)<sub>4</sub>NBr] White, crystals or crystalline powder, having a slight, characteristic odor.

*Melting point* <2.60>: 101 – 105°C

*Purity* Clarity and color of solution—Dissolve 1.0 g of tetra-n-butylammonium bromide in 20 mL of water: the solution is clear and colorless.

*Content:* not less than 98.0%. Assay—Dissolve about 0.5 g of tetra-n-butylammonium bromide, accurately weighed, in 50 mL of water, add 5 mL of dilute nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS while strongly shaking (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 32.24 mg of C<sub>16</sub>H<sub>36</sub>NBr

**Tetra-n-butylammonium chloride** C<sub>16</sub>H<sub>36</sub>ClN White crystals, and it is deliquescent.

*Water* <2.48>: not more than 6.0% (0.1 g).

*Content:* not less than 95.0%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.25 g of tetra-n-butylammonium chloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS  
= 27.79 mg of C<sub>16</sub>H<sub>36</sub>ClN

#### Tetrabutylammonium dihydrogen phosphate

(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NH<sub>2</sub>PO<sub>4</sub> White powder. It is soluble in water. For Eribulin Mesilate, when perform the test as directed in the system suitability in the Purity (2) under Eribulin Mesilate, the height of a peak appeared in the gradient mode is not more than 6 times the peak height of eribulin obtained from the standard solution.

*Content:* not less than 97.0%. Assay—Weigh accurately 1.5 g of tetrabutylammonium dihydrogen phosphate, dissolve in 80 mL of water, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS  
= 169.7 mg of (C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NH<sub>2</sub>PO<sub>4</sub>

#### Tetrabutylammonium hydrogensulfate

C<sub>16</sub>H<sub>37</sub>NO<sub>4</sub>S

White crystalline powder.

*Content:* not less than 98.0%. Assay—Weigh accurately about 0.7 g of tetrabutylammonium hydrogensulfate, dissolve in 100 mL of freshly boiled and cooled water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromocresol green-methyl red TS).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 33.95 mg of C<sub>16</sub>H<sub>37</sub>NO<sub>4</sub>S

**40% Tetrabutylammonium hydroxide TS** A solution containing 40 g/dL of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH: 259.47].

*Content:* 36 – 44 g/dL. Assay—Pipet 10 mL of 40% tetrabutylammonium hydroxide TS, and titrate <2.50> with 1

mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 1 mol/L hydrochloric acid VS  
= 259.5 mg of C<sub>16</sub>H<sub>37</sub>NO

**0.005 mol/L Tetrabutylammonium hydroxide TS** To 10 mL of tetrabutylammonium hydroxide TS add 700 mL of water, adjust to pH 4.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL.

**Tetrabutylammonium hydroxide-methanol TS** Methanol solution containing 25 g/dL of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH: 259.47]. Colorless to pale yellow solution, having an ammonium-like odor.

*Content:* 22.5 – 27.5 g/dL. Assay—Pipet 15 mL of tetrabutylammonium hydroxide-methanol TS and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 1 mol/L hydrochloric acid VS  
= 259.5 mg of C<sub>16</sub>H<sub>37</sub>NO

**10% Tetrabutylammonium hydroxide-methanol TS** A methanol solution containing 10 g/dL of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH: 259.47].

*Content:* 9.0 – 11.0 g/dL. Assay—Pipet 2 mL of 10% tetrabutylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS  
= 25.95 mg of C<sub>16</sub>H<sub>37</sub>NO

**Tetrabutylammonium hydroxide TS** A solution containing 13 g/dL of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH: 259.47].

*Content:* 11.7 – 14.3 g/dL. Assay—Pipet a quantity, equivalent to about 0.3 g of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH], transfer to a glass-stoppered flask containing 15 mL of water, accurately weighed, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS  
= 25.95 mg of C<sub>16</sub>H<sub>37</sub>NO

**Tetrabutylammonium phosphate** See tetrabutylammonium dihydrogen phosphate.

**Tetracycline** C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub> Yellow to dark yellow, crystals or crystalline powder. Sparingly soluble in ethanol, and very slightly soluble in water.

*Content:* it contains not less than 870 µg (potency) per mg. Assay—Proceed as directed in the Assay under Tetracycline Hydrochloride. However, use the following formula.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of tetracycline (C}_{22}\text{H}_{24}\text{N}_2\text{O}_8) \\ = M_S \times (A_T/A_S) \times 1000$$

M<sub>S</sub>: Amount [mg (potency)] of Tetracycline Hydrochloride RS taken

**Tetracycline Hydrochloride** C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>.HCl Yellow, crystals or crystalline powder.

*Purity* Related substances—Dissolve 20 mg of tetracycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make 25 mL, and use this solution as the sample solution. Proceed the test with 20 µL of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area per-

centage method: the total amount of the peaks other than tetracycline is not more than 10%.

**Tetradecyl trimethylammonium bromide**

$\text{CH}_3(\text{CH}_2)_{13}\text{N}(\text{CH}_3)_3\text{Br}$  A white powder.

**Purity** Clarity and color of solution—Dissolve 1.0 g of tetradecyl trimethylammonium bromide in 20 mL of water; the solution is clear and colorless.

**Content:** not less than 98.0%. Assay—Weigh accurately about 0.5 g of tetradecyl trimethylammonium bromide, dissolve in 100 mL of water, add 5 mL of a mixture of water and nitric acid (2:1), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank test in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L silver nitrate VS} \\ = 33.64 \text{ mg of } \text{C}_{17}\text{H}_{38}\text{NBr} \end{aligned}$$

**Tetraethylammonium hydroxide TS** A solution containing 10% of tetraethylammonium hydroxide  $[(\text{C}_2\text{H}_5)_4\text{NOH}]$ : 147.26]. A clear, colorless liquid, having a strong ammonia odor. It is a strong basic and easily absorbs carbon dioxide from the air.

**Content:** 10.0 – 11.0% Assay—Weigh accurately about 3 g of tetraethylammonium hydroxide in a glass-stoppered flask containing 15 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L hydrochloric acid VS} \\ = 14.73 \text{ mg of } \text{C}_8\text{H}_{21}\text{NO} \end{aligned}$$

**Tetra-n-heptylammonium bromide**  $[\text{CH}_3(\text{CH}_2)_6\text{NBr}]$

White, crystals or crystalline powder, having a slight, characteristic odor.

**Melting point** <2.60>: 89 – 93°C

**Content:** not less than 98.0%. Assay—Dissolve about 0.5 g of tetra-n-heptylammonium bromide, accurately weighed, in 50 mL of diluted acetonitrile (3 in 5), and 5 mL of dilute nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS while strongly shaking (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L silver nitrate VS} \\ = 49.07 \text{ mg } \text{C}_{28}\text{H}_{60}\text{NBr} \end{aligned}$$

**Tetrahydrofuran**  $\text{CH}_2(\text{CH}_2)_2\text{CH}_2\text{O}$  [K 9705, Special class]

**Tetrahydrofuran for gas chromatography** Use tetrahydrofuran prepared by distilling with iron (II) sulfate heptahydrate.

**Storage**—Preserve in containers, in which the air has been displaced by nitrogen, in a dark, cold place.

**Tetrahydrofuran for liquid chromatography**  $\text{C}_4\text{H}_8\text{O}$

Clear and colorless liquid.

**Refractive index** <2.45>  $n_D^{20}$ : 1.406 – 1.409

**Density** <2.56> 0.884 – 0.889 g/mL (20°C)

**Purity** Ultraviolet absorbing substances—Determine the absorption spectrum of tetrahydrofuran for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbances at 240 nm, 254 nm, 280 nm, 290 nm, and between 300 nm and 400 nm are not more than 0.35, 0.20, 0.05, 0.02 and 0.01, respectively.

**Peroxide**—Perform the test according to the method described in JIS K 9705: not more than 0.01%.

**Tetrahydroxyquinone**  $\text{C}_6\text{H}_4\text{O}_6$  Dark blue crystals. Its

color changes to yellow on exposure to light. Soluble in ethanol (95) and sparingly soluble in water.

**Tetrahydroxyquinone indicator** Mix 1 g of tetrahydroxyquinone with 100 g of sucrose homogeneously.

**Tetrakishydroxypropylethylenediamine for gas chromatography** Prepared for gas chromatography.

**Tetramethylammonium hydroxide**  $(\text{CH}_3)_4\text{NOH}$  Ordinarily, available as an approximately 10% aqueous solution, which is clear and colorless, and has a strong ammonia-like odor. Tetramethylammonium hydroxide is a stronger base than ammonia, and rapidly absorbs carbon dioxide from the air. Use a 10% aqueous solution.

**Purity** Ammonia and other amines—Weigh accurately a quantity of the solution, corresponding to about 0.3 g of tetramethylammonium hydroxide  $[(\text{CH}_3)_4\text{NOH}]$ , in a weighing bottle already containing 5 mL of water. Add a slight excess of 1 mol/L hydrochloric acid TS (about 4 mL), and evaporate on a water bath to dryness. The mass of the residue (tetramethylammonium chloride), dried at 105°C for 2 hours and multiplied by 0.8317, represents the quantity of tetramethylammonium hydroxide  $[(\text{CH}_3)_4\text{NOH}]$ , and corresponds to  $\pm 0.2\%$  of that found in the Assay.

**Residue on evaporation:** not more than 0.02% (5 mL, 105°C, 1 hour).

**Content:** not less than 98% of the labeled amount. Assay—Accurately weigh a glass-stoppered flask containing about 15 mL of water. Add a quantity of the solution, equivalent to about 0.2 g of tetramethylammonium hydroxide  $[(\text{CH}_3)_4\text{NOH}]$ , weigh again, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: methyl red TS).

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L hydrochloric acid VS} \\ = 9.115 \text{ mg of } \text{C}_4\text{H}_{13}\text{NO} \end{aligned}$$

**Tetramethylammonium hydroxide-methanol TS** A methanol solution containing of 10 g/dL of tetramethylammonium hydroxide  $[(\text{CH}_3)_4\text{NOH}]$ : 91.15]

**Content:** 9.0 – 11.0 g/dL. Assay—Pipet 2 mL of tetramethylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: bromocresol green-methyl red TS).

$$\begin{aligned} \text{Each mL of } 0.1 \text{ mol/L hydrochloric acid VS} \\ = 9.115 \text{ mg of } \text{C}_4\text{H}_{13}\text{NO} \end{aligned}$$

**Tetramethylammonium hydroxide TS** Pipet 15 mL of tetramethylammonium hydroxide, and add ethanol (99.5) to make exactly 100 mL.

**Tetramethylammonium hydroxide TS (pH 5.5)** To 10 mL of tetramethylammonium hydroxide add 990 mL of water, and adjust the pH to 5.5 with diluted phosphoric acid (1 in 10).

**3,3',5,5'-Tetramethylbenzidine dihydrochloride dihydrate**  $\text{C}_{16}\text{H}_{22}\text{Cl}_2\text{N}_2\cdot2\text{H}_2\text{O}$  White to pale red-white crystalline powder.

***N,N,N',N'*-Tetramethylethylenediamine**

$(\text{CH}_3)_2\text{NCH}_2\text{CH}_2\text{N}(\text{CH}_3)_2$  Pale yellow clear liquid.

**Specific gravity** <2.56>  $d_4^{20}$ : 0.774 – 0.799

**Content:** not less than 99.0%.

**Tetramethylsilane for nuclear magnetic resonance spectroscopy**  $(\text{CH}_3)_4\text{Si}$  Prepared for nuclear magnetic resonance spectroscopy.

**Tetra-n-pentylammonium bromide**  $[\text{CH}_3(\text{CH}_2)_4\text{NBr}]$  White, crystals or crystalline powder. It is hygroscopic.

*Melting point* <2.60>: 100 – 101°C

**Tetraphenylboron sodium** See sodium tetraphenylborate.

**Tetra-n-propylammonium bromide**  $[CH_3CH_2CH_2]_4NBr$   
White, crystals or crystalline powder.

**Purity** Clarity and color of solution—Dissolve 1.0 g of tetra-n-propylammonium bromide in 20 mL of water: the solution is clear and colorless.

**Content:** not less than 98.0%. Assay—Weigh accurately about 0.4 g of tetra-n-propylammonium bromide, dissolve in 50 mL of water, add 5 mL of dilute nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS while shaking strongly (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L silver nitrate VS} \\ = 26.63 \text{ mg of } C_{12}H_{28}NBr \end{aligned}$$

**Theophylline**  $C_7H_8N_4O_2$  White powder. Slightly soluble in water.

*Melting point* <2.60>: 269 – 274°C

**Purity** Caffeine, theobromine or paraxanthine—To 0.20 g of theophylline add 5 mL of potassium hydroxide TS or 5 mL of ammonia TS: each solution is clear.

**Loss on drying** <2.41>: not more than 0.5% (1 g, 105°C, 4 hours).

**Content:** not less than 99.0%. Assay—Weigh accurately about 0.25 g of theophylline, previously dried, dissolve it in 40 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (indicator: 3 drops of thymol blue-*N,N*-dimethylformamide TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium methoxide VS} \\ = 18.02 \text{ mg of } C_7H_8N_4O_2 \end{aligned}$$

**Theophylline for assay**  $C_7H_8N_4O_2$  [Same as the monograph Theophylline meeting the following additional specifications.]

**Purity** Related substances—Dissolve 50 mg of theophylline for assay in water to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the total area of peaks other than theophylline obtained from the sample solution is not larger than the peak area of theophylline from the standard solution.

#### Operating conditions

**Detector:** An ultraviolet absorption photometer (wavelength: 270 nm).

**Column:** A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of diluted acetic acid (100) (1 in 100) and methanol (4:1).

**Flow rate:** Adjust so that the retention time of theophylline is about 10 minutes.

**Time span of measurement:** About 3 times as long as the retention time of theophylline.

#### System suitability

**Test for required detectability:** Pipet 5 mL of the standard solution, and add water to make exactly 25 mL. Confirm that the peak area of theophylline obtained with 20 μL of this solution is equivalent to 15 to 25% of that of theophylline with 20 μL of the standard solution.

**System performance:** When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of theophylline are not less than 3000 and not more than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of theophylline is not more than 3.0%.

**Thermolysin** It has the activity of 50 – 100 units per mg protein. Origin: *Bacillus thermoproteolyticus rokko*.

**Thiamine nitrate**  $C_{12}H_{17}N_5O_4S$  [Same as the namesake monograph]

**Thianthol** [Same as the monograph Thianthol. Proceed as directed in the Identification (3) under Sulfur, Salicylic Acid and Thianthol Ointment: any spot other than the principal spot does not appear.]

**3-Thienylethylpenicillin sodium**  $C_{14}H_{15}N_2NaO_4S_2$

White to pale yellow-white powder. Very soluble in water, freely soluble in methanol, and sparingly soluble in ethanol (95).

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +265 – +290° (0.5 g calculated on the anhydrous bases, water, 50 mL, 100 mm).

**Water** <2.48>: Not more than 10.0% (0.2 g, volumetric titration, direct titration).

**Content:** not less than 90% calculated on the anhydrous basis. Assay—Weigh accurately about 0.1 g of 3-thienylethylpenicillin sodium, dissolve in 35 mL of water, add 0.75 mL of 0.1 mol/L hydrochloric acid TS, and adjust to pH 8.5 with 0.1 mol/L sodium hydroxide TS. To this solution add 2 mL of a penicillinase solution prepared by dissolving penicillinase, equivalent to 513,000 Levy units, in 25 mL of water and neutralizing with dilute sodium hydroxide TS until a pale red color appears with 1 drop of a solution of phenolphthalein in ethanol (95) (1 in 1000) as indicator, and allow to stand at 25°C for 5 minutes. Titrate <2.50> this solution with 0.1 mol/L sodium hydroxide VS until the solution reaches pH 8.5 (potentiometric titration). Use the water freshly boiled and cooled.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 36.24 \text{ mg of } C_{14}H_{15}N_2NaO_4S_2 \end{aligned}$$

**Thioacetamide**  $C_2H_5NS$  A white crystalline powder or colorless crystals, having a characteristic odor. Freely soluble in water and in ethanol (99.5). Melting point: 112 – 116°C.

**Thioacetamide-alkaline glycerin TS** To 0.2 mL of a solution of thioacetamide (1 in 25) add 1 mL of alkaline glycerin TS, and heat for 20 seconds in a water bath. Prepare before use.

**Thioacetamide TS** To 0.2 mL of a solution of thioacetamide (1 in 25) add 1 mL of a mixture of 15 mL of sodium hydroxide TS, 5 mL of water and 20 mL of 85% glycerin, and heat in a water bath for 20 seconds. Prepare before use.

**Thiodiglycol**  $S(CH_2CH_2OH)_2$  [ $\beta$ -Thiodiglycol for amino acid autoanalysis] Colorless or pale yellow, clear liquid.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 1.180 – 1.190

**Water** <2.48>: not more than 0.7%.

**Thioglycolate medium I for sterility test** See fluid thioglycolate medium.

**Thioglycolate medium II for sterility test** See alternative thioglycolate medium.

**Thioglycolic acid** See mercapto acetic acid.

**Thionyl chloride**  $\text{SOCl}_2$  A colorless or light yellow, clear liquid, having a pungent odor.

**Specific gravity** <2.56>  $d_{20}^{20}$ : about 1.65 (Method 3).

**Content:** not less than 95.0%. Assay—Weigh accurately 0.1 g of thionyl chloride in a weighing bottle, put the bottle in a glass-stoppered conical flask containing 50 mL of water cooled to about 5°C, stopper immediately, dissolve the sample thoroughly, and transfer the solution to a 200-mL beaker. Wash the conical flask and the weighing bottle in it with 30 mL of water, and combine the washings and the solution in the beaker. Add 1 drop of an aqueous solution of polyvinyl alcohol (1 in 10), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned}\text{Each mL of 0.1 mol/L silver nitrate VS} \\ = 5.949 \text{ mg of } \text{SOCl}_2\end{aligned}$$

**Thiopental for assay**  $\text{C}_{11}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$  Dissolve 10 g of thiopental sodium in 300 mL of water. To this solution add slowly 50 mL of dilute hydrochloric acid with stirring. Take the produced crystals by filtration, wash with water until the filtrate indicates no reaction to chloride, and air-dry. Add diluted ethanol (3 in 5), dissolve by heating in a water bath, allow to stand, and take the produced crystals by filtration. Air-dry the crystals, and dry again at 105°C for 4 hours. White, odorless crystals.

**Melting point** <2.60>: 159 – 162°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of thiopental for assay in 10 mL of ethanol (99.5); the solution is clear and light yellow.

(2) Related substances—Dissolve 50 mg of thiopental for assay in 15 mL of acetonitrile, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase in the Purity (4) under Thiopental Sodium to make exactly 200 mL, and use this solution as the standard solution. Proceed as directed in Purity (4) under Thiopental Sodium.

**Loss on drying** <2.41>: not more than 0.20% (1 g, 105°C, 3 hours).

**Content:** not less than 99.0%. Assay—Weigh accurately about 0.35 g of thiopental for assay, previously dried, dissolve in 5 mL of ethanol (99.5) and 50 mL of chloroform, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned}\text{Each mL of 0.1 mol/L potassium hydroxide-ethanol VS} \\ = 24.23 \text{ mg of } \text{C}_{11}\text{H}_{18}\text{N}_2\text{O}_2\text{S}\end{aligned}$$

**Thiopental sodium**  $\text{C}_{11}\text{H}_{17}\text{N}_2\text{NaO}_2\text{S}$  [Same as the namesake monograph]

**Thiosemicarbazide**  $\text{H}_2\text{NCSNHNH}_2$  White, crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorp-

tion at the wave numbers of about 3370  $\text{cm}^{-1}$ , 3180  $\text{cm}^{-1}$ , 1648  $\text{cm}^{-1}$ , 1622  $\text{cm}^{-1}$ , 1535  $\text{cm}^{-1}$ , 1288  $\text{cm}^{-1}$ , 1167  $\text{cm}^{-1}$ , 1003  $\text{cm}^{-1}$  and 803  $\text{cm}^{-1}$ .

**Thiourea**  $\text{H}_2\text{NCSNH}_2$  [K 8635, Special class]

**Thiourea TS** Dissolve 10 g of thiourea in water to make 100 mL.

**L-Threonine**  $\text{C}_4\text{H}_9\text{NO}_3$  [Same as the namesake monograph]

**Threoprocaterol hydrochloride**  $\text{C}_{16}\text{H}_{22}\text{N}_2\text{O}_3\text{.HCl}$  To procaterol hydrochloride add 10 volumes of 3 mol/L hydrochloric acid TS, heat, and reflux for 3 hours. After cooling, neutralize (pH 8.5) with sodium hydroxide TS, and collect the crystals produced. Suspend the crystals in water, dissolve by acidifying the solution at pH 1 to 2 with addition of hydrochloric acid, neutralize (pH 8.5) by adding sodium hydroxide TS, and separate the crystals produced. Suspend the crystals in 2-propanol, and acidify the solution at pH 1 to 2 by adding hydrochloric acid. The crystals are dissolved and reproduced. Collect the crystals, dry at about 60°C while passing air. White to pale yellow-white, odorless, crystals or crystalline powder. Melting point: about 207°C (with decomposition).

**Purity**—Dissolve 0.10 g of threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 2  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the operating conditions in the Purity (3) under Procaterol Hydrochloride Hydrate. Measure each peak area by the automatic integration method, and calculate the amount of threoprocaterol by the area percentage method: it shows the purity of not less than 95.0%. Adjust the detection sensitivity so that the peak height of threoprocaterol obtained with 2  $\mu\text{L}$  of the solution prepared by diluting 5.0 mL of the sample solution with diluted methanol (1 in 2) to make 100 mL, is 5 to 10% of the full scale, and the time span of measurement is about twice as long as the retention time of threoprocaterol, beginning after the solvent peak.

**Thrombin** [Same as the namesake monograph]

**Thymine for liquid chromatography**  $\text{C}_5\text{H}_6\text{N}_2\text{O}_2$  Occurs as a white powder.

**Purity**—Dissolve 10 mg of the substance to be examined in 100 mL of methanol, add the mobile phase to make 250 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu\text{L}$  each of these solutions as directed in the Purity (3) under Zidovudine. Determine each peak area by the automatic integration method: the total area of peaks other than thymine obtained from the sample solution is not larger than that from the standard solution. However, the time span of measurement is about 10 times the retention time of thymine, beginning after the solvent peak.

**Thymol**  $\text{CH}_3\text{C}_6\text{H}_3(\text{OH})\text{CH}(\text{CH}_3)_2$  [Same as the namesake monograph]

**Thymol blue**  $\text{C}_{27}\text{H}_{30}\text{O}_5\text{S}$  [K 8643, Special class]

**Thymol blue-N,N-dimethylformamide TS** Dissolve 0.1 g of thymol blue in 100 mL of *N,N*-dimethylformamide.

**Thymol blue-1,4-dioxane TS** Dissolve 50 mg of thymol blue in 100 mL of 1,4-dioxane, and filter if necessary. Prepare before use.

**Thymol blue TS** Dissolve 0.1 g of thymol blue in 100 mL of ethanol (95), and filter if necessary.

**Thymol blue TS, dilute** Dissolve 50 mg of thymol blue in 100 mL of ethanol (99.5), and filter if necessary. Prepare before use.

**Thymol for assay**  $C_{10}H_{14}O$  [Same as the monograph Thymol. It contains not less than 99.0% of thymol ( $C_{10}H_{14}O$ ).]

**Thymol for spraying test solution**  $C_{10}H_{14}O$  White, crystals or crystalline powder, having an aromatic odor. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $2960\text{ cm}^{-1}$ ,  $1420\text{ cm}^{-1}$ ,  $1290\text{ cm}^{-1}$ ,  $1090\text{ cm}^{-1}$  and  $810\text{ cm}^{-1}$ .

**Melting point** <2.60>:  $49 - 52^\circ\text{C}$

**Purity** Other phenols—Shake vigorously 1.0 g of the substance to be examined with 20 mL of warm water for 1 minute, and filter. To 5 mL of the filtrate add 1 drop of a solution of iron (III) chloride hexahydrate (27 in 100); the solution reveals a green but not a blue to purple color.

**Thymolphthalein**  $C_{28}H_{30}O_4$  [K 8642, Special class]

**Thymolphthalein TS** Dissolve 0.1 g of thymolphthalein in 100 mL of ethanol (95), and filter if necessary.

**Thymol-sulfuric acid-methanol TS for spraying** Dissolve 1.5 g of thymol for spraying test solution in 100 mL of methanol, and add 5.7 mL of sulfuric acid.

**Tiaramide hydrochloride for assay**  $C_{15}H_{18}ClN_3O_3S.HCl$  [Same as the monograph Tiaramide Hydrochloride. When dried, it contains not less than 99.0% of tiaramide hydrochloride ( $C_{15}H_{18}ClN_3O_3S.HCl$ ).]

**Tiapride hydrochloride for assay**  $C_{15}H_{24}N_2O_4S.HCl$  [Same as the monograph Tiapride Hydrochloride]

**Ticlopidine hydrochloride for assay**  $C_{14}H_{14}ClNS.HCl$  [Same as the monograph Ticlopidine Hydrochloride. It meets the following additional requirements.]

**Purity** Related substances—Dissolve 0.2 g of ticlopidine hydrochloride for assay in 100 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 1 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly  $10\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ticlopidine obtained from the sample solution is not larger than the peak area of ticlopidine from the standard solution, and the total area of the peaks other than ticlopidine from the sample solution is not larger than 2 times the peak area of ticlopidine from the standard solution.

#### Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu\text{m}$  in particle diameter).

Column temperature: A constant temperature of about

$40^\circ\text{C}$ .

Mobile phase: A mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and methanol (1:1).

Flow rate: Adjust so that the retention time of ticlopidine is about 8 minutes.

Time span of measurement: About 7 times as long as the retention time of ticlopidine, beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of ticlopidine obtained with  $10\mu\text{L}$  of this solution is equivalent to 14 to 26% of that with  $10\mu\text{L}$  of the standard solution.

System performance: When the procedure is run with  $10\mu\text{L}$  of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ticlopidine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with  $10\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ticlopidine is not more than 2.0%.

**Tin Sn** [K 8580, Special class]

**Tin (II) chloride dihydrate**  $SnCl_2 \cdot 2H_2O$  [K 8136, Special class]

**Tin (II) chloride-hydrochloric acid TS** To 20 g of tin add 85 mL of hydrochloric acid, heat until hydrogen gas no longer are evolved, and allow to cool. Mix 1 volume of this solution and 10 volume of dilute hydrochloric acid. Prepare before use.

**Tin (II) chloride-sulfuric acid TS** Dissolve 10 g of tin (II) chloride dihydrate in diluted sulfuric acid (3 in 200) to make 100 mL.

**Tin (II) chloride TS** Dissolve 1.5 g of Tin (II) chloride dihydrate in 10 mL of water containing a small amount of hydrochloric acid. Preserve in glass-stoppered bottles in which a fragment of tin has been placed. Use within 1 month.

**Tin (II) chloride TS, acidic** Dissolve 8 g of Tin (II) chloride dihydrate in 500 mL of hydrochloric acid. Preserve in glass-stoppered bottles. Use within 3 months.

**Tipepidine hibenzate for assay**  $C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4$

[Same as the monograph Tipepidine Hibenzate. When dried, it contains not less than 99.0% of tipepidine hibenzate ( $C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4$ ).]

**Titanium dioxide** See titanium (IV) oxide.

**Titanium dioxide TS** See titanium (IV) oxide TS.

**Titanium (III) chloride (20)**  $TiCl_3$  [K 8401, Titanium (III) chloride solution, Special class] Store in light-resistant, glass-stoppered containers.

**Titanium (III) chloride-sulfuric acid TS** Mix carefully 20 mL of titanium (III) chloride TS and 13 mL of sulfuric acid, add carefully hydrogen peroxide (30) in small portions until a yellow color develops, and heat until white fumes evolve. After cooling, add water, heat again in the same manner, repeat this procedure until the solution is colorless, and add water to make 100 mL.

**Titanium (III) chloride TS** To titanium (III) chloride (20) add dilute hydrochloric acid to obtain a solution containing 15 g/dL of titanium (III) chloride ( $TiCl_3$ ). Prepare before use.

**Content:** 14.0 – 16.0 g/dL. **Assay**—To exactly 2 mL of titanium (III) chloride TS add 200 mL of water and 5 mL of a hydrochloric acid solution (2 in 3), and titrate <2.50> with 0.1 mol/L ammonium iron (III) sulfate VS under carbon dioxide until a slight red color develops in the solution (indicator: 5 mL of ammonium thiocyanate TS).

Each mL of 0.1 mol/L ferric ammonium sulfate VS  
= 15.42 mg of  $\text{TiCl}_3$

**Titanium (IV) oxide**  $\text{TiO}_2$  [K 8703, Special class]

**Titanium (IV) oxide TS** To 100 mL of sulfuric acid add 0.1 g of titanium (IV) oxide, and dissolve by gradually heating on a flame with occasional gentle shaking.

**Titanium trichloride** See titanium (III) chloride (20).

**Titanium trichloride-sulfuric acid TS** See titanium (III) chloride-sulfuric acid TS.

**Titanium trichloride TS** See titanium (III) chloride TS.

**Titanium yellow**  $\text{C}_{28}\text{H}_{19}\text{N}_5\text{Na}_2\text{O}_6\text{S}_4$  A dark yellow to dark yellow-brown, powder or masses.

**Identification**—Determine the infrared absorption spectrum of titanium yellow, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1603  $\text{cm}^{-1}$ , 1467  $\text{cm}^{-1}$ , 1394  $\text{cm}^{-1}$ , 1306  $\text{cm}^{-1}$ , 1040  $\text{cm}^{-1}$ , 988  $\text{cm}^{-1}$ , 820  $\text{cm}^{-1}$  and 644  $\text{cm}^{-1}$ .

**Storage**—Preserve in a light-resistant tight container.

**Tocopherol**  $\text{C}_{29}\text{H}_{50}\text{O}_2$  [Same as the namesake monograph]

**Tocopherol acetate**  $\text{C}_{31}\text{H}_{52}\text{O}_3$  [Same as the namesake monograph]

**Tocopherol calcium succinate**  $\text{C}_{66}\text{H}_{106}\text{CaO}_{10}$  [Same as the namesake monograph]

**Tocopherol succinate**  $\text{C}_{33}\text{H}_{54}\text{O}_5$  Wet 0.5 g of tocopherol calcium succinate with 5 mL of acetic acid (100), add 10 mL of toluene, and warm at 70°C for 30 minutes with occasional shaking. After cooling, add 30 mL of water, shake thoroughly, and allow to stand. Remove the water layer, wash the toluene layer with several 30-mL portions of water until the washings become neutral, and allow to stand. Shake the toluene extract with 3 g of anhydrous sodium sulfate, decant the toluene layer, distil the toluene under reduced pressure, and obtain a light yellow, viscous liquid. When preserved at room temperature for a long time, it becomes a pale yellowish solid.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (286 nm): 38.0 – 42.0 (10 mg, chloroform, 100 mL).

**Tolbutamide**  $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$  [Same as the namesake monograph]

**Toluene**  $\text{C}_6\text{H}_5\text{CH}_3$  [K 8680, Special class]

***o*-Toluene sulfonamide**  $\text{C}_7\text{H}_9\text{NO}_2\text{S}$  Colorless crystals or white crystalline powder. Soluble in ethanol (95), and sparingly soluble in water.

**Melting point** <2.60>: 157 – 160°C

**Purity** *p*-Toluene sulfonamide—Use a solution of *o*-toluene sulfonamide in ethyl acetate (1 in 5000) as the sample solution. Perform the test with 10  $\mu\text{L}$  of the sample solution as directed under Gas Chromatography <2.02> according to the operating conditions in the Purity (5) under Saccharin Sodium Hydrate: any peak other than the peak of *o*-toluene sulfonamide does not appear. Adjust the flow rate so that

the retention time of *o*-toluene sulfonamide is about 10 minutes, and adjust the detection sensitivity so that the peak height of *o*-toluene sulfonamide obtained from 10  $\mu\text{L}$  of the sample solution is about 50% of the full scale. Time span of measurement is about twice as long as the retention time of *o*-toluene sulfonamide, beginning after the solvent peak.

**Water** <2.48>: not more than 0.5% (4 g, use 25 mL of methanol for water determination and 5 mL of pyridine for water determination).

**Content:** not less than 98.5%, calculated on the anhydrous basis. **Assay**—Weigh accurately about 25 mg of *o*-toluene sulfonamide, and perform the test as directed under Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS  
= 1.712 mg of  $\text{C}_7\text{H}_9\text{NO}_2\text{S}$

***p*-Toluenesulfonamide**  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{NH}_2$  White, crystals or crystalline powder. Melting point: about 137°C.

**Purity** Related substances—Dissolve 30 mg of *p*-toluenesulfonamide in acetone to make exactly 200 mL. Spot 10  $\mu\text{L}$  of this solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, cyclohexane and diluted ammonia solution (28) (10 in 11) (200:100:60:23) to a distance of about 12 cm, and air-dry the plate. Heat the plate at 110°C for 10 minutes, and immediately expose to chlorine for 2 minutes. Expose the plate to cold wind until a very pale blue color develops when 1 drop of potassium iodide-starch TS is placed on a site below the starting line on the plate. Spray evenly potassium iodide-starch TS on the plate: no spot other than the principal spot at an *Rf* value of about 0.6 appears.

***p*-Toluene sulfonic acid** See *p*-toluenesulfonic acid monohydrate.

***p*-Toluenesulfonic acid monohydrate**

$\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3\text{H.H}_2\text{O}$  [K 8681, Special class]

***o*-Toluec acid**  $\text{C}_8\text{H}_8\text{O}_2$  White, crystals or crystalline powder.

**Melting point** <2.60>: 102 – 105°C

**Content:** not less than 98.0%.

**Toluidine blue** See toluidine blue O

**Toluidine blue O**  $\text{C}_{15}\text{H}_{16}\text{ClN}_3\text{S}$  Dark green powder, soluble in water, and slightly soluble in ethanol (95).

**Identification**—

- (1) A solution (1 in 100) shows a blue to purple color.
- (2) A solution in ethanol (95) (1 in 200) shows a blue color.
- (3) A solution shows a maximum absorption at around 630 nm.

**Tranilast for assay**  $\text{C}_{18}\text{H}_{17}\text{NO}_5$  [Same as the monograph Tranilast. When dried, it contains not less than 99.5% of tranilast ( $\text{C}_{18}\text{H}_{17}\text{NO}_5$ ).]

**Triamcinolone acetonide**  $\text{C}_{24}\text{H}_{31}\text{FO}_6$  [Same as the namesake monograph]

**Trichloroacetic acid**  $\text{CCl}_3\text{COOH}$  [K 8667, Special class]

**Trichloroacetic acid-gelatin-tris buffer solution** To 1 volume of a solution of trichloroacetic acid (1 in 5) add 6 volume of gelatin-tris buffer solution (pH 8.0) and 5 volume of water.

**Trichloroacetic acid TS** Dissolve 1.80 g of trichloroacetic acid, 2.99 g of sodium acetate trihydrate and 1.98 g of acetic acid (31) in water to make 100 mL.

**Trichloroethylene**  $C_2HCl_3$  [K 8666, Special class]

**Trichlorofluoromethane**  $CCl_3F$  A colorless liquid or gas.

*Specific gravity* <2.56>  $d_4^{17.2}$ : 1.494

*Boiling point* <2.57>: 23.7°C

**1,1,2-Trichloro-1,2,2-trifluoroethane**  $CFCl_2CF_2Cl$

Colorless volatile liquid. Miscible with acetone and with diethyl ether, and not with water.

**Purity** Related substances—Perform the test with 0.1  $\mu$ L of 1,1,2-trichloro-1,2,2-trifluoroethane as directed under Gas Chromatography <2.02> according to the operating conditions in the Purity (5) under Halothane: any peak other than the peak of 1,1,2-trichloro-1,2,2-trifluoroethane does not appear.

**Tricine**  $C_6H_{13}NO_5$  White crystalline powder. Melting point: 182 to 184°C (with decomposition).

**Trentine hydrochloride for assay**  $C_6H_{18}N_4 \cdot 2HCl$

[Same as the monograph Trentine Hydrochloride or purified Trentine Hydrochloride according to the method of purification shown below. It contains not less than 98.0% of trentine hydrochloride ( $C_6H_{18}N_4 \cdot 2HCl$ ), calculated on the dried basis, and meets the following requirements.]

**Purity** Related substances—Dissolve 0.10 g of trentine hydrochloride for assay in 10 mL of methanol, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 3 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 3  $\mu$ L each of the sample solution and standard solution on two plates of silica gel for thin-layer chromatography. Develop one of the plates with a mixture of 2-propanol and ammonia solution (28) (3:2) to a distance of about 6 cm, and air-dry the plate. Spray evenly ninhydrin-butanol TS on the plate, and heat the plate at 130°C for 5 minutes: the spot other than the principal spot and the spot nearby the original point obtained from the sample solution is not more intense than the spot from the standard solution. Develop the other plate with a mixture of ammonia solution (28), diethyl ether, acetonitrile and ethanol (99.5) (10:4:3:3), and perform the test in the same manner as above: the spot nearby the original point from the sample solution is not more intense than the spot from the standard solution.

**Method of purification:** Dissolve Trentine Hydrochloride in water while warming, and recrystallize by addition of ethanol (99.5). Or dissolve Trentine Hydrochloride in water while warming, allow to stand after addition of activated charcoal in a cool and dark place for one night, and filter. To the filtrate add ethanol (99.5), allow to stand in a cool and dark place, and recrystallize. Dry the crystals under reduced pressure not exceeding 0.67 kPa at 40°C until ethanol odor disappears.

**Triethanolamine** See 2,2',2"-nitrilotriethanol.

**Triethylamine**  $(C_2H_5)_3N$  Clear colorless liquid, having a strong amines odor. Miscible with methanol, with ethanol (95) and with diethyl ether.

*Specific gravity* <2.56>  $d_4^{25}$ : 0.722 – 0.730

*Boiling point* <2.57>: 89 – 90°C

**Triethylamine buffer solution (pH 3.2)** To 4 mL of triethylamine add 2000 mL of water, and adjust the pH to 3.2 with phosphoric acid.

**Triethylamine for epoetin beta**  $(C_2H_5)_3N$  A clear and

colorless liquid.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.724 – 0.730

*Water* <2.48>: not more than 0.2%.

**1% Triethylamine-phosphate buffer solution (pH 3.0)**

Dissolve 10 g of triethylamine in 950 mL of water, adjust the pH to 3.0 with phosphoric acid, and make exactly 1000 mL.

**Triethylamine-phosphate buffer solution (pH 5.0)** To 1.0 mL of triethylamine add 900 mL of water, adjust the pH to 5.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL.

**Trifluoroacetic acid**  $CF_3COOH$  Colorless, clear liquid, having a pungent odor. Miscible well with water.

*Specific gravity* <2.56>  $d_2^{20}$ : 1.535

*Boiling point* <2.57>: 72 – 73°C

**Trifluoroacetic acid for epoetin beta**  $CF_3COOH$

A clear and colorless liquid.

**Purity:** When determine the absorbance of 50 vol% solution of trifluoroacetic acid for epoetin beta as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.10 at 270 nm, not more than 0.02 at 280 nm, and not more than 0.01 between 300 nm and 400 nm.

**Trifluoroacetic acid for nuclear magnetic resonance spectroscopy**  $CF_3COOH$  Prepared for nuclear magnetic resonance spectroscopy.

**Trifluoroacetic acid TS** To 1 mL of trifluoroacetic acid add water to make 1000 mL.

**Trifluoroacetic anhydride for gas chromatography**

$(CF_3CO)_2O$  Colorless, clear liquid, having a pungent odor.

*Boiling point* <2.57>: 40 – 45°C

**Trimetazidine hydrochloride for assay**  $C_{14}H_{22}N_2O_3 \cdot 2HCl$

[Same as the monograph Trimetazidine Hydrochloride. It contains not less than 99.0% of trimetazidine hydrochloride ( $C_{14}H_{22}N_2O_3 \cdot 2HCl$ ), calculated on the anhydrous basis.]

**Trimethylsilyl imidazole**  $C_6H_{12}N_2Si$  Clear, colorless to pale yellow liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.4744 – 1.4764

**2,4,6-trinitrobenzenesulfonic acid** See 2,4,6-trinitrobenzenesulfonic acid dihydrate.

**2,4,6-Trinitrobenzenesulfonic acid dihydrate**

$C_6H_2(NO_2)_3SO_3H \cdot 2H_2O$  Pale yellow to light yellow powder.

*Water* <2.48>: 11 – 15% (0.1 g, volumetric titration, direct titration).

**Content:** not less than 98%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.3 g of 2,4,6-trinitrobenzenesulfonic acid, dissolve in 50 mL of a mixture of water and ethanol (99.5) (1:1), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 29.32 mg of  $C_6H_2(NO_2)_3SO_3H$

**2,4,6-Trinitrophenol**  $HOC_6H_2(NO_2)_3$  Light yellow to yellow, moist crystals. It is added 15 to 25% of water for the sake of safety, because it might explode by heating, mechanical shocking and friction when it is dried.

**Identification**—To 0.1 g add 10 mL of water, dissolve by warming, and add 12 mL of a mixture of 1% copper (II) sulfate solution and ammonia TS (5:1): green precipitates appear.

**Content:** not less than 99.5%. Assay—Weigh accurately about 0.25 g, previously dried in a desiccator (silica gel) for 24 hours, dissolve in 50 mL of water by warming, and titrate <2.50> with 0.1 mL sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 22.91 mg of  $\text{HOC}_6\text{H}_2(\text{NO}_2)_3$

**2,4,6-Trinitrophenol-ethanol TS** Dissolve 1.8 g of 2,4,6-trinitrophenol in 50 mL of diluted ethanol (99.5) (9 in 10) and 30 mL of water, and add water to make 100 mL.

**2,4,6-Trinitrophenol TS** Dissolve 1 g of 2,4,6-trinitrophenol in 100 mL of hot water, cool, and filter if necessary.

**2,4,6-Trinitrophenol TS, alkaline** Mix 20 mL of 2,4,6-trinitrophenol TS with 10 mL of a solution of sodium hydroxide (1 in 20), and add water to make 100 mL. Use within 2 days.

**Triphenylantimony**  $\text{Sb}(\text{C}_6\text{H}_5)_3$  White to pale yellow-brown, crystals or crystalline powder or masses.

**Content:** not less than 95.0%. Assay—Weigh accurately about 0.3 g of triphenylantimony, dissolve in 100 mL of ethanol (95), add 1 g of sodium hydrogen carbonate, and titrate <2.50> with 0.05 mol/L iodine VS. Perform a blank determination in the same manner.

Each mL of 0.05 mol/L iodine VS  
= 17.65 mg of  $\text{Sb}(\text{C}_6\text{H}_5)_3$

**Triphenylchloromethane**  $(\text{C}_6\text{H}_5)_3\text{CCl}$  White to grayish or yellowish white, crystals or crystalline powder.

*Melting point* <2.60>: 107 – 115°C

**Triphenylmethane**  $\text{C}_{19}\text{H}_{16}$  A white to pale yellow, crystalline powder.

*Melting point* <2.60>: 93 – 95°C

**Triphenylmethanol for thin-layer chromatography**  
 $\text{C}_{19}\text{H}_{15}\text{OH}$  Occurs as a white powder.

**Purity**—Dissolve 0.1 g of triphenylmethanol for thin-layer chromatography in 100 mL of methanol and perform the test as directed in the Purity (2) under Zidovudine: spots other than the principal spot with an *Rf* value of about 0.73 are not observed.

**Triphenyltetrazolium chloride** See 2,3,5-triphenyl-2*H*-tetrazolium chloride.

**Triphenyltetrazolium chloride TS** See 2,3,5-triphenyl-2*H*-tetrazolium chloride TS.

**2,3,5-Triphenyl-2*H*-tetrazolium chloride**  $\text{C}_{19}\text{H}_{15}\text{ClN}_4$   
[K 8214, Special class]

**2,3,5-Triphenyl-2*H*-tetrazolium chloride TS** Dissolve 0.25 g of 2,3,5-triphenyl-2*H*-tetrazolium chloride in ethanol (99.5) to make 100 mL. Prepare before use.

**2,3,5-Triphenyl-2*H*-tetrazolium chloride-methanol TS for spraying** Solution A: A solution of 2,3,5-triphenyl-2*H*-tetrazolium chloride in methanol (1 in 25). Solution B: A solution of sodium hydroxide in methanol (1 in 125). Mix an equal volume of the solution A and solution B just before use.

**Tripotassium citrate monohydrate**  $\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$  White, crystals or crystalline powder. Very soluble in water, and practically insoluble in ethanol (95).

**Content:** 99.0% or more Assay—Accurately weigh about 0.2 g of tripotassium citrate monohydrate, add 50 mL of

acetic acid for nonaqueous titration, dissolve by warming on a water bath, cool, and then titrate <2.50> with 0.1 mol/L of perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 32.44 mg of  $\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$

**Tris-acetic acid buffer solution (pH 6.5)** Dissolve 13.57 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 6.73 g of acetic acid (100) in water to make 1000 mL.

**Tris-acetic acid buffer solution (pH 8.0)** Dissolve 1.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 800 mL of water, adjust to pH 8.0 with acetic acid (100), and add water to make 1000 mL.

**Tris buffer solution for bacterial endotoxins test** Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 800 mL of water for bacterial endotoxins test, add 100 mL of 0.1 mol/L hydrochloric acid TS and water for bacterial endotoxins test to make 1000 mL, and sterilize by heating in an autoclave at 121°C for 90 minutes.

**Tris buffer solution (pH 6.8)** Dissolve 30.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 2.0 g of sodium lauryl sulfate in 800 mL of water, adjust to pH 6.8 with 5 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**0.5 mol/L Tris buffer solution (pH 6.8)** Dissolve 6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 50 mL of water, add 2 mol/L hydrochloric acid TS to adjust the pH to 6.8, and then add water to make 100 mL. Filter if necessary.

**0.05 mol/L Tris buffer solution (pH 7.0)** Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol in about 750 mL of water, adjust to pH 7.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Tris buffer solution (pH 7.0)** Dissolve 24.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 1000 mL of water, and adjust the pH to 7.0 with 0.1 mol/L hydrochloric acid TS.

**0.1 mol/L Tris buffer solution (pH 7.3)** Dissolve 2.42 g of 2-amino-2-hydroxymethyl-1,3-propanediol in a suitable amount of water, adjust to pH 7.3 with hydrochloric acid or 6 mol/L hydrochloric acid TS, and add water to make 200 mL.

**0.02 mol/L Tris buffer solution (pH 7.4)** Dissolve 2.4 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 800 mL of water, adjust to pH 7.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**0.02 mol/L Tris buffer solution (pH 7.5)** Dissolve 2.4 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 29.2 g of sodium chloride in a suitable amount of water, adjust to pH 7.5 with hydrochloric acid, and add water to make 1000 mL.

**1 mol/L Tris buffer solution (pH 7.5)** Dissolve 12.11 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 90 mL of water, adjust to pH 7.5 with hydrochloric acid, and add water to make 100 mL.

**0.1 mol/L Tris buffer solution (pH 8.0)** Dissolve 2.42 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 100 mL of water, adjust the pH to 8.0 with 0.2 mol/L hydrochloric acid TS, and add water to make 200 mL.

**1 mol/L Tris buffer solution (pH 8.0)** Dissolve 121 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 800 mL of water, adjust to pH 8.0 with hydrochloric acid, add water to

make 1000 mL, and sterilize in an autoclave.

**0.2 mol/L Tris buffer solution (pH 8.1)** Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 1000 mL, and adjust to pH 8.1 with hydrochloric acid.

**0.5 mol/L Tris buffer solution (pH 8.1)** Dissolve 12.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 100 mL of water, adjust to pH 8.1 with 1 mol/L hydrochloric acid TS, and add water to make 200 mL.

**Tris buffer solution (pH 8.2)** Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 0.5 g of polysorbate 20 in 800 mL of water, adjust to pH 8.2 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Tris buffer solution (pH 8.3)** Dissolve 3.03 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 1.0 g of sodium lauryl sulfate, and 14.4 g of glycine in 900 mL of water, adjust to pH 8.3 with 5 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Tris buffer solution (pH 8.4)** Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 10.2 g of sodium chloride in 800 mL of water, adjust to pH 8.4 with 1 mol/L hydrochloride TS, and add water to make 1000 mL.

**0.05 mol/L Tris buffer solution (pH 8.6)** Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 950 mL of water, add 2 mol/L hydrochloric acid TS to adjust the pH to 8.6, and then add water to make 1000 mL.

**Tris buffer solution (pH 8.8)** Dissolve 22.7 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 1.5 g of sodium lauryl sulfate in 140 mL of water, adjust to pH 8.8 with 5 mol/L hydrochloric acid TS, and add water to make 200 mL.

**1.5 mol/L Tris buffer solution (pH 8.8)** Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 75 mL of water, add 5 mol/L hydrochloric acid TS to adjust the pH to 8.8, and then add water to make 100 mL. Filter if necessary.

**Tris buffer solution (pH 9.5)** Dissolve 36.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 1000 mL of water, and adjust the pH to 9.5 by adding 1 mol/L hydrochloric acid TS.

**0.01 mol/L tris buffer solution - sodium chloride TS (pH 7.4)** Dissolve 1.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 29.2 g of sodium chloride and 0.5 g of polysorbate 20 in 800 mL of water, adjust to pH 7.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Tris(4-t-butylphenyl)phosphate [(CH<sub>3</sub>)<sub>3</sub>CC<sub>6</sub>H<sub>4</sub>O]<sub>3</sub>PO**  
White, crystals or crystalline powder.

*Melting point <2.60>: 100 – 104°C*

**Tris-calcium chloride buffer solution (pH 6.5)** Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 15 mg of calcium chloride dihydrate in 800 mL of water, adjust to pH 6.5 with dilute hydrochloric acid, and add water to make 1000 mL.

**Tris-glycine buffer solution (pH 6.8)** Dissolve 1.22 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 0.76 g of glycine, 8.8 g of sodium chloride and 0.1 g of polysorbate 80 in 800 mL of water, adjust to pH 6.8 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**0.2 mol/L Tris-hydrochloride buffer solution (pH 7.4)** Dissolve 6.61 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 0.97 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 250 mL.

#### 0.05 mol/L Tris-hydrochloride buffer solution (pH 7.5)

Dissolve 6.35 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 1.18 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 1000 mL.

**Tris-sodium chloride buffer solution (pH 8.0)** Dissolve 2.42 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 1.64 g of sodium chloride in 900 mL of water, adjust to pH 8.0 with dilute hydrochloric acid, and add water to make 1000 mL.

**Trishydroxymethylaminomethane** See 2-amino-2-hydroxymethyl-1,3-propanediol.

**Trisodium citrate dihydrate** See sodium citrate hydrate.

**0.1 mol/L Trisodium citrate TS** Dissolve 29.4 g of trisodium citrate dihydrate in water to make 1000 mL.

**Trisodium ferrous pentacyanoamine TS** See iron (II) trisodium pentacyanoamine TS.

**Trisodium phosphate dodecahydrate** Na<sub>3</sub>PO<sub>4</sub>.12H<sub>2</sub>O  
[K 9012, Special class]

**Trypsin** Obtained from bovine or hog pancreas, and prepared for biochemistry or to meet the following requirements. White to light yellow, crystals or powder.

*Loss on drying:* Not more than 5.0% (60°C, in vacuum, 4 hours).

**Content:** Not less than 220 trypsin units per mg. Assay (i) Sample solution—Weigh accurately about 20 mg of the substance to be assayed, and dissolve in 0.001 mol/L hydrochloric acid TS so that each mL contains about 3000 trypsin units. To a suitable amount of this solution add 0.001 mol/L hydrochloric acid TS so that each mL contains about 40 trypsin units, and use this solution as the sample solution.

(ii) Diluting solution—Dissolve 4.54 g of potassium dihydrogen phosphate in water to make exactly 500 mL (Solution I). Dissolve 4.73 g of anhydrous disodium hydrogen phosphate in water to make exactly 500 mL (Solution II). To 80 mL of Solution II add a suitable amount of Solution I to adjust to pH 7.6.

(iii) Substrate solution—Dissolve 85.7 mg of *N*-α-benzoyl-L-ethylarginine hydrochloride in water to make exactly 100 mL, and use this solution as the substrate stock solution. Pipet 10 mL of the substrate stock solution add the diluting solution to make exactly 100 mL, and use this solution as the substrate solution. The substrate solution gives an absorbance of between 0.575 and 0.585 at 253 nm when determined as directed under Ultraviolet-visible spectrophotometry <2.24> using water as the blank. If necessary adjust the absorbance by addition of the diluting solution or substrate stock solution.

(iv) Procedure—Transfer exactly 3 mL of the substrate solution, previously warmed to 25 ± 0.1°C, into a 1-cm quartz cell, add exactly 0.2 mL of the sample solution, immediately start the timer, and determine the change of the absorbance at 253 nm at 25 ± 0.1°C for 5 minutes, using the control prepared by adding exactly 0.2 mL of 0.001 mol/L hydrochloric acid TS to exact 3 mL of the substrate solution. Obtain the variation per minute of the absorbance, A, from the part where the changing rate of the absorbance is constant for at least 3 minutes.

(v) Calculation—Calculate trypsin unit per mg using the following equation. Where, one trypsin unit is the quantity of enzyme that gives the variation of the absorbance 0.003 per minute.

$$\text{Trypsin unit per mg} = A/0.003 \times 1/M$$

**M:** Amount (mg) of the substance to be assayed in 0.2 mL of the sample solution

**Storage**—At a cold place.

**Trypsin for epoetin alfa liquid chromatography** Bovine pancreas origin. It has not less than 180 units per mg, as 1 unit is equivalent to the amount of enzyme necessary to hydrolysis 1  $\mu$ mol of *p*-toluenesulfonyl-L-arginine methyl ester per minute at 25°C, pH 8.2.

**Trypsin for liquid chromatography** An enzyme obtained from the bovine pancreas. This one part digests 250 parts of casein in the following reaction system.

**Casein solution**—To 0.1 g of milk casein add 30 mL of water, disperse the casein well, add 1.0 mL of diluted sodium hydroxide TS (1 in 10) to dissolve, and add water to make 50 mL. Prepare before use.

**Sample solution**—Dissolve 10 mg of trypsin for liquid chromatography in 500 mL of water.

**Procedure**—To 5 mL of the casein solution add 2 mL of the sample solution and 3 mL of water, mix, then allow to stand at 40°C for 1 hour, and add 3 drops of a mixture of ethanol (95), water and acetic acid (100) (10:9:1): no precipitate appears.

**Trypsin inhibitor** Produced by purifying soybean. Each mg of trypsin inhibitor inhibits 10,000 to 30,000 BAEE Units of trypsin. One BAEE Unit means a trypsin activity to indicate an absorbance difference of 0.001 at 253 nm per minute when 3.2 mL of the solution is reacted at 25°C and pH 7.6, using *N*- $\alpha$ -benzoyl-L-arginine ethyl ester as substrate.

**Trypsin inhibitor TS** Dissolve 5 mg of trypsin inhibitor in 0.05 mol/L phosphate buffer solution (pH 7.0) to make 10 mL.

**Trypsin TS** Dissolve 0.5 g of trypsin and 0.2 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in phosphate buffer solution for cytotoxicity test to make 1000 mL, and sterilize by filtration through a membrane filter with a pore size not exceeding 0.22  $\mu$ m.

**Trypsin TS for epoetin alfa** Dissolve 0.5 mg of trypsin for epoetin alfa liquid chromatography in 2.5 mL of water.

**Trypsin TS for test of elcatonin** Dissolve 5 mg of trypsin for liquid chromatography in 20 mL of a solution of ammonium hydrogen carbonate (1 in 100). Prepare before use.

**Trypsin TS for test of ulinastatin** Dissolve crystalline trypsin for ulinastatin assay in ice-cooled 1 mmol/L hydrochloric acid TS containing 1 mmol/L calcium chloride dihydrate so that each mL of the solution contains 180  $\mu$ g of trypsin. Prepare before use, and preserve in an ice-cooled water bath.

**L-Tryptophan**  $C_{11}H_{12}N_2O_2$  [Same as the namesake monograph]

**Tulobuterol for assay**  $C_{12}H_{18}ClNO$  [Same as the monograph Tulobuterol. It contains not less than 99.0% of tulobuterol ( $C_{12}H_{18}ClNO$ ), calculated on the anhydrous basis.]

**Turpentine oil** [Same as the namesake monograph]

**L-Tyrosine**  $C_9H_{11}NO_3$  White, crystals or crystalline powder. Odorless and tasteless. Freely soluble in formic acid, very slightly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether. It dissolves in dilute hydrochloric acid and in dilute nitric acid.

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : -10.5 – -12.5° (after dry-

ing, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

**Loss on drying** <2.41>: not more than 0.30% (1 g, 105°C, 3 hours).

**Content**: not less than 99.0%. **Assay**—Weigh accurately about 0.3 g of L-tyrosine, previously dried, dissolve in 6 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 18.12 mg of  $C_9H_{11}NO_3$

**Ubenimex for assay**  $C_{16}H_{24}N_2O_4$  [Same as the monograph Ubenimex. When dried, it contains not less than 99.0% of ubenimex ( $C_{16}H_{24}N_2O_4$ ).]

**Ubiquinone-9** Yellow to orange crystalline powder. Odorless and no taste.

**Absorbance** <2.24>  $E_{1\text{ cm}}^{1\%}$  (275 nm): 163 – 190 (ethanol (99.5))

**Melting point** <2.60>: about 44°C

**Umbelliferone for thin-layer chromatography**  $C_9H_6O_3$

White or light brown powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 232°C.

**Identification**—(1) Determine the absorption spectrum of a solution of umbelliferone for thin-layer chromatography in methanol (1 in 300,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 214 nm and 218 nm, and between 322 nm and 326 nm.

(2) Determine the infrared absorption spectrum of umbelliferone for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 3160  $\text{cm}^{-1}$ , 1681  $\text{cm}^{-1}$ , 1604  $\text{cm}^{-1}$ , 1323  $\text{cm}^{-1}$ , 990  $\text{cm}^{-1}$  and 903  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 1.0 mg of umbelliferone for thin-layer chromatography in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 5  $\mu$ L each of the sample solution and standard solution as directed in the Identification under Artemisia Leaf: the spot other than the principal spot having an *Rf* value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

**Uracil**  $C_4H_4N_2O_2$  Needle crystals. Freely soluble in hot water, and slightly soluble in cold water.

**Melting point** <2.60>: 335°C

**Urea**  $H_2NCONH_2$  [K 8731, Special class]

**Urea-EDTA TS** Dissolve 48.0 g of urea and 0.2 g of disodium ethylenediamine tetraacetate dihydrate in 0.5 mol/L tris buffer solution (pH 8.1) to make 100 mL.

**Urethane** See ethyl carbamate.

**Ursodeoxycholic acid**  $C_{24}H_{40}O_4$  [Same as the namesake monograph]

**Ursodeoxycholic acid for assay**  $C_{24}H_{40}O_4$  [Same as the monograph Ursodeoxycholic Acid. However, when dried, it contains not less than 99.0% of ursodeoxycholic acid ( $C_{24}H_{40}O_4$ ) meeting the following additional specifications.]

**Purity** Related substances—Dissolve 0.15 g of ursodeoxycholic acid for assay in 5 mL of methanol for liquid chromatography, and use this solution as the sample solution. Pipet 2 mL of the sample solution and add methanol for liq-

uid chromatography to make exactly 50 mL. Pipet 2.5 mL of this solution, add methanol for liquid chromatography to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 2.5 to ursodeoxycholic acid, obtained from the sample solution is not larger than the peak area of ursodeoxycholic acid from the standard solution, and the area of the peak, having the relative retention time of about 5.5, from the sample solution is not larger than 1/5 times the peak area of ursodeoxycholic acid from the standard solution. Furthermore, the total area of the peaks other than ursodeoxycholic acid and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of ursodeoxycholic acid from the standard solution.

#### Operating conditions

**Detector:** An ultraviolet absorption photometer (wavelength: 210 nm).

**Column:** A stainless steel column 3 mm in inside diameter and 7.5 cm in length, packed with octylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of methanol for liquid chromatography, diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (96:69:35).

**Flow rate:** Adjust so that the retention time of ursodeoxycholic acid is about 2.3 minutes.

**Time span of measurement:** About 7 times as long as the retention time of ursodeoxycholic acid, beginning after the solvent peak.

#### System suitability

**Test for required detectability:** Pipet 2 mL of the standard solution, and add methanol for liquid chromatography to make exactly 20 mL. Confirm that the peak area of ursodeoxycholic acid obtained with 5  $\mu$ L of this solution is equivalent to 8 to 12% of that with 5  $\mu$ L of the standard solution.

**System performance:** To 30 mg of chenodeoxycholic acid for thin-layer chromatography and 30 mg of lithocholic acid for thin-layer chromatography, add 1 mL of the sample solution, dissolve in methanol for liquid chromatography to make 50 mL. When the procedure is run with 5  $\mu$ L of this solution under the above operating conditions, ursodeoxycholic acid, chenodeoxycholic acid, and lithocholic acid are eluted in this order with the resolution between these peaks being not less than 7, respectively.

**System repeatability:** When the test is repeated 6 times with 5  $\mu$ L of the standard solution under the above conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0%.

**n-Valeric acid**  $\text{CH}_3(\text{CH}_2)_3\text{COOH}$  Clear, colorless to pale yellow liquid, having a characteristic odor. Miscible with ethanol (95) and with diethyl ether, and soluble in water.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.936 – 0.942

*Distilling range* <2.57>: 186 – 188°C, not less than 98 vol%.

**L-Valine**  $\text{C}_5\text{H}_{11}\text{NO}_2$  [Same as the namesake monograph]

**L-Valine for assay**  $\text{C}_5\text{H}_{11}\text{NO}_2$  [Same as the monograph]

L-Valine. When dried, it contains not less than 99.0% of L-valine ( $\text{C}_5\text{H}_{11}\text{NO}_2$ ).]

**Valsartan**  $\text{C}_{24}\text{H}_{29}\text{N}_5\text{O}_3$  [Same as the namesake monograph]

**H-D-Valyl-L-leucyl-L-arginine-4-nitroanilide dihydrochloride**  $\text{C}_{23}\text{H}_{38}\text{N}_8\text{O}_5 \cdot 2\text{HCl}$  White to pale yellow, powder or masses. Sparingly soluble in water.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (316 nm): 214 – 236 (10 mg, water, 500 mL).

**Vanadium pentoxide** See vanadium (V) oxide.

**Vanadium pentoxide TS** See vanadium (V) oxide TS.

**Vanadium pentoxide TS, dilute** See vanadium (V) oxide TS, dilute.

**Vanadium (V) oxide**  $\text{V}_2\text{O}_5$  Orangish yellow to yellow-brown powder.

*Identification*—Dissolve 0.3 g in 10 mL of ammonia TS and 15 mL of water. To 2 mL of this solution add 20 mL of water, mix, and add gently 1 mL of copper (II) sulfate TS: yellow precipitates appear.

**Vanadium (V) oxide TS** Add vanadium (V) oxide to phosphoric acid, saturate with vanadium (V) oxide by shaking vigorously for 2 hours, and filter through a glass filter.

**Vanadium (V) oxide TS, dilute** Dilute 10 mL of vanadium (V) oxide TS with water to make 100 mL. Prepare before use.

**Vanillin**  $\text{C}_6\text{H}_3\text{CHO}(\text{OCH}_3)(\text{OH})$  A white to light yellow crystalline powder, having a characteristic odor.

*Melting point* <2.60>: 80.5 – 83.5°C

*Storage*—Preserve in a light-resistant tight container.

**Vanillin-hydrochloric acid TS** Dissolve 5 mg of vanillin in 0.5 mL of ethanol (95), and to this solution add 0.5 mL of water and 3 mL of hydrochloric acid. Prepare before use.

**Vanillin-sulfuric acid-ethanol TS** Dissolve 3 g of vanillin in ethanol (99.5) to make 100 mL, and add 0.5 mL of sulfuric acid.

**Vanillin-sulfuric acid-ethanol TS for spraying** Dissolve 3 g of vanillin in 30 mL of ethanol (99.5), and add 100 mL of dilute sulfuric acid.

**Vanillin-sulfuric acid TS** Add cautiously 75 mL of sulfuric acid to 25 mL of ice-cold ethanol (95). After cooling, add 1 g of vanillin to dissolve. Prepare before use.

**Vasopressin**  $\text{C}_{46}\text{H}_{65}\text{N}_{15}\text{O}_{12}\text{S}_2$  A white powder.

*Constituent amino acids*—Perform the test as directed in the Constituent amino acids under Oxytocin, and calculate the respective molar ratios with respect to glycine: 0.9 – 1.1 for aspartic acid, 0.9 – 1.1 for glutamic acid, 0.9 – 1.1 for proline, 0.8 – 1.1 for tyrosine, 0.9 – 1.1 for phenylalanine, 0.9 – 1.1 for arginine and 0.8 – 1.1 for cystine, and not more than 0.03 for other amino acids.

**Verapamil hydrochloride for assay**  $\text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_4 \cdot \text{HCl}$  [Same as the monograph Verapamil Hydrochloride. When dried, it contains not less than 99.0% of verapamil hydrochloride ( $\text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_4 \cdot \text{HCl}$ )].

**Verbascoside for thin-layer chromatography**  $\text{C}_{29}\text{H}_{36}\text{O}_{15}$  A white to very pale yellow, odorless, crystalline powder or powder. Soluble in methanol, sparingly soluble in ethanol (99.5), and slightly soluble in water.

*Identification* Determine the infrared absorption spectrum of verbascoside for thin-layer chromatography as di-

rected in the potassium bromide disk method under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about  $1604\text{ cm}^{-1}$ ,  $1446\text{ cm}^{-1}$ ,  $1272\text{ cm}^{-1}$  and  $815\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 10 mg of verbasco-side for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with  $20\text{ }\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification under Cistanche Herb: the spot other than the principal spot at an *Rf* value of about 0.35 obtained from the sample solution is not more intense than the spot from the standard solution.

**Vinblastine sulfate**  $\text{C}_{46}\text{H}_{58}\text{N}_4\text{O}_9\text{H}_2\text{SO}_4$  [Same as the namesake monograph]

**Vincristine sulfate**  $\text{C}_{46}\text{H}_{56}\text{N}_4\text{O}_{10}\text{H}_2\text{SO}_4$  [Same as the namesake monograph]

**Vinyl acetate**  $\text{C}_4\text{H}_6\text{O}_2$  Clear, colorless liquid.

*Specific gravity* <2.56>: 0.932 – 0.936

*Water* <2.48>: not more than 0.2%

**Vinyl chloride**  $\text{C}_2\text{H}_3\text{Cl}$  Colorless gas.

*Boiling point* <2.57>:  $-14^\circ\text{C}$

*Melting point* <2.60>:  $-160^\circ\text{C}$

**2-Vinylpyridine**  $\text{C}_7\text{H}_7\text{N}$  A clear, colorless or dark brown liquid.

*Refractive index* <2.45>  $n_{\text{D}}^{20}$ : 1.546 – 1.552

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.975 – 0.982

**4-Vinylpyridine**  $\text{C}_7\text{H}_7\text{N}$  A pale yellow to black-brown liquid.

*Refractive index* <2.45>  $n_{\text{D}}^{20}$ : 1.5500 – 1.5530

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.9850 – 0.9880

**1-Vinyl-2-pyrrolidone**  $\text{C}_6\text{H}_9\text{NO}$  Clear liquid.

**Purity**—Perform the test with  $0.5\text{ }\mu\text{L}$  of 1-vinyl-2-pyrrolidone as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area of the solutions by the automatic integration method, and calculate the amount of 1-vinyl-2-pyrrolidone by the area percentage method: it is not less than 99.0%.

**Operating conditions**

Detector: A hydrogen flame-ionization detector.

Column: A hollow, capillary glass column about 0.53 mm in inside diameter and about 30 m in length, having an about  $1.0\text{ }\mu\text{m}$  layer of polyethylene glycol 20 M for gas chromatography on the inner side.

Column temperature: Maintain the temperature at  $80^\circ\text{C}$  for 1 minute, then raise at the rate of  $10^\circ\text{C}$  per minute to  $190^\circ\text{C}$ , and hold constant to the temperature for 20 minutes.

Temperature of sample vaporization chamber: A constant temperature of about  $190^\circ\text{C}$ .

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of 1-vinyl-2-pyrrolidone is about 15 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 1-vinyl-2-pyrrolidone with  $0.5\text{ }\mu\text{L}$  of 1-vinyl-2-pyrrolidone is about 70% of the full scale.

Time span of measurement: About twice as long as the retention time of 1-vinyl-2-pyrrolidone.

**Water** <2.48>—Take 50 mL of methanol for water determination and 10 mL of butyrolactone in a dry titration flask, and titrate with Karl Fischer TS for water determination until end point. Weigh accurately about 2.5 g of 1-vinyl-2-pyrrolidone, transfer immediately to a titration flask, and

perform the test: water is not more than 0.1%.

**V8 protease** A protease obtained from *Staphylococcus aureus* strain. When an amount of the enzyme hydrolyzes 1  $\mu\text{mol}$  of *N-t*-butoxycarbonyl-L-glutamic acid- $\alpha$ -phenyl ester in 1 minute at pH 7.8 and  $37^\circ\text{C}$  is defined as 1 unit, it contains 500 – 1000 units per mg.

**V8 protease for insulin glargine** A protease obtained from *Staphylococcus aureus* strain. When an amount of the enzyme which hydrolyzes 1  $\mu\text{mol}$  of carbobenzoxy-phenylalanyl-leucyl-glutamyl-4-nitroanilide in 1 minute at pH 7.8 and  $25^\circ\text{C}$  is defined as 1 unit, it contains not less than 20 units per mg.

**V8 protease TS** Dissolve V8 protease in water to make a solution of 1 mg per mL. Keep at a cold place and use within 6 days after preparation.

**Voglibose for assay**  $\text{C}_{10}\text{H}_{21}\text{NO}_7$  [Same as the monograph Voglibose]

**Voriconazole**  $\text{C}_{16}\text{H}_{14}\text{F}_3\text{N}_5\text{O}$  [Same as the namesake monograph]

**Warfarin potassium for assay**  $\text{C}_{19}\text{H}_{15}\text{KO}_4$  [Same as the monograph Warfarin Potassium. When dried, it contains not less than 99.0% of warfarin potassium ( $\text{C}_{19}\text{H}_{15}\text{KO}_4$ ).]

**Washing fluid for nartograstim test** Dissolve 1 mL of polysorbate 20 in phosphate-buffered sodium chloride TS to make 1000 mL.

**25% Water containing benzoyl peroxide** See Benzoyl peroxide, 25% water containing.

**Water for ammonium limit test** To 1500 mL of water add cautiously 4.5 mL of sulfuric acid, distil using a hard glass distiller, discard the sufficient volume of first distillate, and use the remaining distillate (ammonium-free water) as the water for ammonium limit test.

**Purity**—Mix 40 mL of water for ammonium limit test with 6.0 mL of phenol-sodium pentacyanonitrosylferrate (III) TS. Add 4.0 mL of sodium hypochlorite-sodium hydroxide TS, mix, and allow to stand for 60 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbance at  $640\text{ nm}$  is not more than 0.010.

**Water for bacterial endotoxins test** Use the water prescribed by the monographs of Water for Injection or Sterile Water for Injection in Containers, or the water produced by other procedures that shows no reaction with the lysate reagent employed, at the detection limit of the reagent, and is suitable for bacterial endotoxins test.

**Water for ICP analysis** See Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry <2.63>.

**Water for injection** [Use the water prescribed by the monographs of Water for Injection or Sterile Water for Injection in Containers. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of relevant test.]

**Water, nuclease-free** Water in which nuclease is not included.

**Water, sterile purified** [Use the water prescribed by the monograph of Sterile Purified Water in Containers. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to

be used is suitable for the purpose of relevant test.]

**Wijs' TS** Transfer 7.9 g of iodine trichloride and 8.9 g of iodine to separate flasks, dissolve each with acetic acid (100), mix both solutions, and add acetic acid (100) to make 1000 mL. Preserve in light-resistant, glass containers.

**Wogonin for thin-layer chromatography** C<sub>16</sub>H<sub>12</sub>O<sub>5</sub> Yellow, crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 204 – 208°C.

**Identification**—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 207 nm and 211 nm, and between 273 nm and 277 nm.

**Purity** Related substances—Dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and perform the test with 10 µL of this solution as directed in the Identification (3) under Saireito Extract: no spot other than the principal spot at an R<sub>f</sub> value of about 0.4 appears.

**Xanthene** C<sub>13</sub>H<sub>10</sub>O White to light yellow, crystals or crystalline powder, having a slight, characteristic odor.

*Melting point* <2.60>: 98 – 102°C

*Water* <2.48>: not more than 0.5% (0.15 g).

**Xanthene-9-carboxylic acid** C<sub>14</sub>H<sub>10</sub>O<sub>3</sub> Dissolve 0.25 g of propantheline bromide in 5 mL of water and 10 mL of sodium hydroxide TS, heat the mixture to boiling, then continue to heat for 2 minutes. Cool to 60°C, add 5 mL of dilute sulfuric acid, cool, filter the precipitate, and wash thoroughly with water. Recrystallize the residue from dilute ethanol, and dry for 3 hours in a desiccator (in vacuum, silica gel).

*Melting point* <2.60>: 217 – 222°C

**Xanthone** C<sub>13</sub>H<sub>8</sub>O<sub>2</sub> Light yellow powder. Freely soluble in chloroform, and slightly soluble in hot water and in diethyl ether.

*Melting point* <2.60>: 174 – 176°C

**Purity** Related substances—Dissolve 50 mg of xanthone in chloroform to make exactly 10 mL. Perform the test with 5 µL of this solution as directed in the Purity under Propantheline Bromide: any spot other than the principal spot at the R<sub>f</sub> value of about 0.7 does not appear.

**Xanthydrol** C<sub>13</sub>H<sub>10</sub>O<sub>2</sub> White to pale yellow powder. Dissolves in ethanol (95), in acetic acid (100), in chloroform, and in diethyl ether, and is practically insoluble in water.

*Melting point* <2.60>: 121 – 124°C

*Residue on ignition* <2.44>: not more than 2.0% (0.5 g).

**Xylene** C<sub>6</sub>H<sub>4</sub>(CH<sub>3</sub>)<sub>2</sub> [K 8271, First class]

**o-Xylene** C<sub>6</sub>H<sub>4</sub>(CH<sub>3</sub>)<sub>2</sub> Colorless, clear liquid.

*Refractive index* <2.45> n<sub>D</sub><sup>20</sup>: 1.501 – 1.506

*Specific gravity* <2.56> d<sub>4</sub><sup>20</sup>: 0.875 – 0.885

*Distilling range* <2.57>: 143 – 146°C, not less than 95 vol%.

**Xylene cyanol FF** C<sub>25</sub>H<sub>27</sub>N<sub>2</sub>NaO<sub>6</sub>S<sub>2</sub> [K 8272, Special class]

**Xylenol orange** C<sub>31</sub>H<sub>30</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>13</sub>S [K 9563, Special class]

**Xylenol orange TS** Dissolve 0.1 g of xylenol orange in water to make 100 mL.

**Xylitol** C<sub>5</sub>H<sub>12</sub>O<sub>5</sub> [Same as the namesake monograph]

**Xylose** See D-xylose.

**D-Xylose** C<sub>5</sub>H<sub>10</sub>O<sub>5</sub> [Same as the monograph D-Xylose]

of the Japanese Standards of Food Additives]

**Yeast extract** A peptone-like substance which represents all the soluble product of yeast cells (*Saccharomyces*) prepared under optimum conditions, clarified, and dried by evaporating to a powder. Yeast extract (1 g) represents not less than 7.5 g of yeast. A reddish yellow to brown powder, having a characteristic but not putrescent odor. Soluble in water, forming a yellow to brown solution, having a slight acidic reaction. It contains no added carbohydrate.

**Purity** (1) Chloride <1.03> (calculated as NaCl): not more than 5%.

(2) Coagulable protein—On heating a solution of yeast extract (1 in 20) to boiling, no precipitate is produced.

*Loss on drying* <2.41>: not more than 5% (105°C, constant mass).

*Residue on ignition* <2.44>: not more than 15% (0.5 g).

*Nitrogen content* <1.08>: 7.2 – 9.5% (after drying).

**Yellow beeswax** [Same as the namesake monograph]

**Zaltoprofen** C<sub>17</sub>H<sub>14</sub>O<sub>3</sub>S [Same as the namesake monograph]

**Zaltoprofen for assay** C<sub>17</sub>H<sub>14</sub>O<sub>3</sub>S [Same as the monograph Zaltoprofen. When dried, it contains not less than 99.5% of zaltoprofen (C<sub>17</sub>H<sub>14</sub>O<sub>3</sub>S)].

**Zero oxygen gas for assay** Nitrogen or argon, not less than 99.99 vol%, packed in a pressure-resistant sealed container. Or nitrogen or argon, 98 to 99 vol%, packed in a pressure-resistant sealed container containing oxygen within a minimum scale in the range of measurement.

**Zinc** Zn [K 8012, Special class]

**Zinc (standard reagent)** Zn In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

**Zinc acetate** See zinc acetate dihydrate.

**0.25 mol/L Zinc acetate buffer solution (pH 6.4)** Dissolve 54.9 g of zinc acetate dihydrate in 150 mL of acetic acid (100) and 600 mL of water, add 150 mL of ammonia water (28), gently mix, and allow to cool to a room temperature. Adjust to pH 6.4 with ammonia water (28), and add water to make 1000 mL.

**Zinc acetate dihydrate** Zn(CH<sub>3</sub>COO)<sub>2</sub>·2H<sub>2</sub>O [K 8356, Special class]

**Zinc, arsenic-free** See zinc for arsenic analysis.

**Zinc chloride** ZnCl<sub>2</sub> [K 8111, Special class]

**Zinc chloride TS** Dissolve 10 g of zinc chloride and 10 g of potassium hydrogen phthalate in 900 mL of water, adjust the pH to 4.0 with sodium hydroxide TS, and add water to make 1000 mL.

**0.04 mol/L Zinc chloride TS** Dissolve 5.452 g of zinc chloride in water to make 1000 mL.

**Zinc dibutyldithiocarbamate** [(C<sub>4</sub>H<sub>9</sub>)<sub>2</sub>NCSS]<sub>2</sub>Zn A white powder. Melting point: 106 – 110°C.

**Content:** Not less than 95.0%. **Assay**—Weigh accurately 1.0 g of zinc dibutyldithiocarbamate, add 10 mL of water and 5 mL of hydrochloric acid, and evaporate to dryness on a hot plate. To the residue add 15 mL of diluted hydrochloric acid (1 in 3), dissolve by warming, then add 50 mL of water and 40 mL of ammonia-ammonium chloride buffer solution (pH 10.7) and titrate <2.50> with 0.1 mol/L disodi-

um dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to blue (indicator: 0.1 mL of eriochrome black T TS).

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 47.41 mg of  $[(C_4H_9)_2NCSS]_2Zn$

**Zinc diethyldithiocarbamate**  $[(C_2H_5)_2NCSS]_2Zn$

A white to pale yellow powder. Melting point: 177 – 182°C.

**Content:** 94.0 – 108.0%. Assay—Weigh accurately about 0.8 g of zinc diethyldithiocarbamate, add 50 mL of water and 15 mL of diluted hydrochloric acid (1 in 3), and boil to dissolve. After cooling, add 40 mL of ammonia-ammonium chloride buffer solution (pH 10.7) and titrate <2.50> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to blue (indicator: 0.1 mL of eriochrome black T TS).

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 36.19 mg of  $[(C_2H_5)_2NCSS]_2Zn$

**Zinc disodium ethylenediamine tetraacetate** See zinc disodium ethylenediamine tetraacetate tetrahydrate.

**Zinc disodium ethylenediamine tetraacetate tetrahydrate**  $C_{10}H_{12}N_2Na_2O_8Zn \cdot 4H_2O$  White powder. The pH of a solution of zinc disodium ethylenediamine tetraacetate tetrahydrate (1 in 100) is between 6.0 and 9.0.

**Purity** Clarity and color of solution—Dissolve 0.10 g of zinc disodium ethylenediamine tetraacetate tetrahydrate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

**Content:** not less than 98.0%. Assay—Dissolve about 0.5 g of zinc disodium ethylenediamine tetraacetate tetrahydrate, accurately weighed, in water to make exactly 100 mL. Pipet 10 mL of this solution, adjust the pH to about 2 with 80 mL of water and dilute nitric acid, and titrate <2.50> with 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylenol orange TS).

Each mL of 0.01 mol/L bismuth nitrate VS  
= 4.716 mg of  $C_{10}H_{12}N_2Na_2O_8Zn \cdot 4H_2O$

**Zinc dust** See zinc powder.

**Zinc for arsenic analysis** Zn [K 8012] Use granules of about 800  $\mu m$ .

**Zinc iodide-starch TS** To 100 mL of boiling water add a solution of 0.75 g of potassium iodide in 5 mL of water, a solution of 2 g of zinc chloride in 10 mL of water and a smooth suspension of 5 g of starch in 30 mL of water, with stirring. Continue to boil for 2 minutes, then cool.

**Sensitivity**—Dip a glass rod into a mixture of 1 mL of 0.1 mol/L sodium nitrite VS, 500 mL of water and 10 mL of hydrochloric acid, and touch on zinc iodide-starch TS: an apparently blue color appears.

**Storage**—Preserve in tightly stoppered bottles, in a cold place.

**Zincon**  $C_{20}H_{15}N_4NaO_6S$  A dark red to purple powder.

**Identification**—Determine the infrared absorption spectrum of zincon, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>; it exhibits absorption at the wave numbers of about 1604  $cm^{-1}$ , 1494  $cm^{-1}$ , 1294  $cm^{-1}$ , 1194  $cm^{-1}$ , 1110  $cm^{-1}$ , 1046  $cm^{-1}$  and 764  $cm^{-1}$ .

**Storage**—Preserve in a light-resistant tight container.

**Zincon TS** Dissolve 0.1 g of zincon in 2 mL of 1 mol/L sodium hydroxide VS, and add water to make 100 mL.

**Zinc powder** Zn [K 8013, for nitrogen oxides analysis or arsenic analysis]

**Zinc sulfate** See zinc sulfate heptahydrate.

**Zinc sulfate for volumetric analysis** See zinc sulfate heptahydrate.

**Zinc sulfate heptahydrate**  $ZnSO_4 \cdot 7H_2O$  [K 8953, Special class]

**Zinc sulfate TS** Dissolve 10 g of zinc sulfate heptahydrate in water to make 100 mL.

**Zirconyl-alizarin red S TS** Dissolve 0.2 g of zirconyl nitrate dihydrate in 5 mL of dilute hydrochloric acid, add 10 mL of alizarin red S TS, and then add water to make 30 mL.

**Zirconyl-alizarin S TS** See zirconyl-alizarin red S TS.

**Zirconyl nitrate** See zirconyl nitrate dihydrate.

**Zirconyl nitrate dihydrate**  $ZrO(NO_3)_2 \cdot 2H_2O$  A white crystalline powder. Freely soluble in water.

**Identification**—(1) To 5 mL of a solution (1 in 20) add 5 mL of sodium hydroxide TS: a white, milky precipitate is formed.

(2) To 10 mL of a solution (1 in 20) add 10 mL of sulfuric acid, cool, and superimpose 2 mL of iron (II) sulfate TS: a brown ring is produced at the zone of contact.

**Zolpidem tartrate for assay**  $(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6$

[Same as the monograph Zolpidem Tartrate. It contains not less than 99.5% of zolpidem tartrate  $[(C_{19}H_{21}N_3O)_2 \cdot C_4H_6O_6]$ , calculated on the anhydrous basis.]

**Zopiclone for assay**  $C_{17}H_{17}ClN_6O_3$  [Same as the monograph Zopiclone. It contains not less than 99.5% of zopiclone  $(C_{17}H_{17}ClN_6O_3)$ , calculated on the dried basis.]

## 9.42 Solid Supports/Column Packings for Chromatography

**$\alpha_1$ -Acid glycoprotein binding silica gel for liquid chromatography** Silica gel bond  $\alpha_1$ -acid glycoprotein, prepared for liquid chromatography.

**Aminopropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Amylose tris-(3,5-dimethylphenylcarbamate)-coated silica gel for liquid chromatography** Prepared for liquid chromatography.

**Butylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Carbamoyl group bound silica gel for liquid chromatography** Prepared for liquid chromatography.

**Cellulose derivative-coated silica gel for liquid chromatography** Prepared for liquid chromatography.

**Cellulose for thin-layer chromatography** Prepared for thin-layer chromatography.

**Cellulose with fluorescent indicator for thin-layer chromatography** Use cellulose for thin-layer chromatography containing a suitable fluorescent substance.

**Cellulose tris (4-methyl benzoate)-coated silica gel for liq-**

**Liquid chromatography** Prepared for liquid chromatography.

**18-Crown ether-immobilized silica gel for liquid chromatography** Prepared for liquid chromatography.

**14% Cyanopropylphenyl-86% dimethyl silicone polymer for gas chromatography** Prepared for gas chromatography.

**Cyanopropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**$\beta$ -Cyclodextrin binding silica gel for liquid chromatography** A silica gel bound with  $\beta$ -cyclodextrin, prepared for liquid chromatography.

**Dextran-highly cross-linked agarose gel filtration carrier for liquid chromatography** Prepared for liquid chromatography.

**DEAE-cross-linking dextran anion exchanger (Cl type), slightly alkaline** Slightly alkaline anion exchanger prepared by introducing diethylaminoethyl group into cross-linking dextran of gel filtration carrier.

**Diethylaminoethyl cellulose for column chromatography** Prepared for column chromatography.

**Diethylaminoethyl group bound to synthetic polymer for liquid chromatography** Produced by binding diethylaminoethyl group to a hydrophilic synthetic polymer, for liquid chromatography. Exchange volume is about 0.1 mg equivalents/cm<sup>3</sup>.

**Dimethylaminopropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Dimethylsilanized silica gel with fluorescent indicator for thin-layer chromatography** Dimethylsilanized silica gel for thin-layer chromatography to which a fluorescent indicator is added.

**Diol silica gel for liquid chromatography** Prepared for liquid chromatography.

**Divinylbenzene-methacrylate co-polymer for liquid chromatography** Prepared for liquid chromatography.

**Divinylbenzene-N-vinyl pyrrolidone copolymer for column chromatography** Prepared for column chromatography.

**Ethylsilanized silica gel for column chromatography** Prepared for column chromatography.

**Fluorosilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linkage: 8 %)** Prepared for liquid chromatography.

**Gel type strong acid ion-exchange resin for liquid chromatography (degree of cross-linkage: 6 %)** Prepared for liquid chromatography.

**Gel type strong basic ion-exchange resin for liquid chromatography** Prepared for liquid chromatography.

**Glycol etherified silica gel for liquid chromatography** Glycol group is bound to silica gel for liquid chromatography.

**Graphite carbon for gas chromatography** Prepared for gas chromatography.

**Graphite carbon for liquid chromatography** Prepared

for liquid chromatography.

**Hexasilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Human albumin chemically bonded silica gel for liquid chromatography** Prepared for liquid chromatography.

**Hydrophilic silica gel for liquid chromatography** Diolized porous silica gel prepared for liquid chromatography (5–10  $\mu\text{m}$  in particle diameter).

**2-Hydroxypropyl- $\beta$ -cyclodextrin onto silica gel for liquid chromatography** Prepared for liquid chromatography.

**Hydroxypropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Neutral alumina for chromatography** Prepared for chromatography (75 – 180  $\mu\text{m}$  in particle diameter).

**Neutral alumina for column chromatography** Prepared for column chromatography.

**Octadecylsilanized monolithic silica for liquid chromatography** Prepared for liquid chromatography.

**Octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography** Prepared for liquid chromatography.

**Octadecylsilanized porous glass for liquid chromatography** Prepared for liquid chromatography.

**Octadecylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Octadecylsilanized silica gel for thin-layer chromatography** Octadecylsilanized silica gel prepared for thin-layer chromatography.

**Octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography** Octadecylsilanized silica gel for thin-layer chromatography containing fluorescent indicator.

**Octadecylsilanized silicone polymer coated silica gel for liquid chromatography** Prepared for liquid chromatography.

**Octadecyl-strong anion exchange-silanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Octylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Ovomucoid-chemically bonded amino silica gel for liquid chromatography** Prepared for liquid chromatography.

**Palmitamide propylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Pentaethylenehexaaminated polyvinyl alcohol polymer beads for liquid chromatography** Prepared for liquid chromatography.

**Perfluorohexylpropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Phenylated silica gel for liquid chromatography** Prepared for liquid chromatography.

**Phenylhexylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Phenylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Polyamide for column chromatography** Prepared for column chromatography.

**Polyamide for thin-layer chromatography** Prepared for thin-layer chromatography.

**Polyamide with fluorescent indicator for thin-layer chromatography** Add a fluorescent indicator to polyamide for thin-layer chromatography.

**Poly tetrafluoroethylene for gas chromatography** Prepared for gas chromatography.

**Porous acrylonitrile-divinylbenzene copolymer for gas chromatography (pore diameter: 0.06 – 0.08 µm, 100 – 200 m<sup>2</sup>/g)** Prepared for gas chromatography.

**Porous ethyl vinylbenzene-divinylbenzene copolymer for gas chromatography** Prepared for gas chromatography.

**Porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0075 µm, 500 – 600 m<sup>2</sup>/g)** A porous ethylvinylbenzene-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.0075 µm, and surface area is 500 to 600 m<sup>2</sup>/g.

**Porous polymer beads for gas chromatography** Prepared for gas chromatography.

**Porous polymethacrylate for liquid chromatography** Prepared for liquid chromatography.

**Porous silica gel for gas chromatography** Prepared for gas chromatography.

**Porous silica gel for liquid chromatography** Prepared for liquid chromatography.

**Porous styrene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0085 µm, 300 – 400 m<sup>2</sup>/g)** Prepared for gas chromatography. The average pore diameter is 0.0085 µm, and surface area is 300 to 400 m<sup>2</sup>/g.

**Porous styrene-divinylbenzene copolymer for gas chromatography (0.3 – 0.4 µm in mean pore size, not exceeding 50 m<sup>2</sup>/g)** Prepared for gas chromatography.

**Porous styrene-divinylbenzene copolymer for liquid chromatography** Prepared for liquid chromatography.

**Quaternary alkylaminated styrene-divinylbenzene copolymer for liquid chromatography** Prepared for liquid chromatography.

**Quaternary ammonium group bound to hydrophilic vinyl polymer gel for liquid chromatography** Prepared for liquid chromatography.

**Silica gel coated with cellulose tris(4-methylbenzoate) for liquid chromatography** Prepared for liquid chromatography.

**Silica gel for gas chromatography** Prepared for gas chromatography.

**Silica gel for liquid chromatography** Prepared for liquid chromatography.

**Silica gel with attached carbamoyl groups for liquid chromatography** Prepared for liquid chromatography.

**Silica gel for thin-layer chromatography** Prepared for thin-layer chromatography.

**Silica gel for thin-layer chromatography (particle size 5 – 7 µm, with fluorescent indication)** Prepared for high-performance thin-layer chromatography.

**Silica gel with complex fluorescent indicator for thin-layer chromatography** A silica gel for thin-layer chromatography containing suitable complex fluorescent indicators.

**Silica gel with fluorescent indicator for thin-layer chromatography** A silica gel for thin-layer chromatography containing a suitable fluorescent indicator.

**Siliceous earth for chromatography** A siliceous earth prepared for chromatography.

**Siliceous earth for gas chromatography** A siliceous earth prepared for gas chromatography.

**Slightly acidic ion-exchange silica gel for liquid chromatography** Prepared for liquid chromatography.

**Spherical porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography** Prepared for gas chromatography.

**Strongly acidic ion-exchange non-porous resin for liquid chromatography** Prepared for liquid chromatography.

**Strongly acidic ion-exchange resin for column chromatography** Prepared for column chromatography.

**Strongly acidic ion-exchange resin for liquid chromatography** Prepared for liquid chromatography.

**Strongly acidic ion-exchange silica gel for liquid chromatography** Prepared for liquid chromatography.

**Strongly basic ion-exchange resin for column chromatography** Prepared for column chromatography.

**Strongly basic ion-exchange resin for liquid chromatography** Prepared for liquid chromatography.

**Styrene-divinylbenzene copolymer for liquid chromatography** Prepared for liquid chromatography.

**Sulfonamide group bound to hexadecylsilanized silica gel for liquid chromatography** Prepared for column chromatography.

**Synthetic magnesium silicate for column chromatography** Prepared for column chromatography (150 – 250 µm in particle diameter).

**Terephthalic acid for gas chromatography** C<sub>6</sub>H<sub>4</sub>(COOH)<sub>2</sub> Prepared for gas chromatography.

**Tetrafluoroethylene polymer for gas chromatography** Prepared for gas chromatography.

**Triacetyltsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Trimethylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Weakly acidic CM-bridged cellulose cation exchanger (H type)** Weakly acidic cation exchanger, intensified by crosslinking porous spherical cellulose, into which carboxymethyl groups have been introduced.

**Weakly acidic ion exchange resin for liquid chromatography** Prepared for liquid chromatography.

**Weakly acidic ion exchange silica gel for liquid chromatography** Prepared for liquid chromatography.

**Zeolite for gas chromatography (0.5 nm in pore diameter)** Prepared for gas chromatography.

## 9.43 Filter Papers, Filters for filtration, Test Papers, Crucibles, etc.

**Filter paper** [P 3801, Filter paper (for chemical analysis), Filter paper for qualitative analysis]

No.1: For bulky gelatinous precipitate

No.2: For moderate-sized precipitate

No.3: For fine precipitate

No.4: Hardened filter paper for fine precipitate

**Filter paper for quantitative analysis** [P 3801, Filter paper (for chemical analysis), Filter paper for quantitative analysis]

No. 5A: For bulky gelatinous precipitate

No. 5B: For moderate-sized precipitate

No. 5C: For fine precipitate

No. 6: Thin filter paper for fine precipitate

**Porcelain crucible** [R 1301, Porcelain crucible for chemical analysis]

**Sintered glass filter** [R 3503, Glass appliance for chemical analysis, Buchner funnel glass filter]

G3: 20–30  $\mu\text{m}$  in pore size

G4: 5–10  $\mu\text{m}$  in pore size

**Sintered glass filter for cupric oxide filtration** A glass filter with a pore size of 10 – 16  $\mu\text{m}$ .

**Blue litmus paper** See litmus paper, blue.

**Congo red paper** Immerse filter paper in congo red TS, and air-dry.

**Glass fiber** See glass wool.

**Glass wool** [K 8251, Special class]

**Lead acetate paper** See lead (II) acetate paper.

**Lead (II) acetate paper** Usually, immerse strips of filter paper, 6 cm  $\times$  8 cm in size, in lead (II) acetate TS, drain off the excess liquid, and dry the paper at 100°C, avoiding contact with metals.

**Litmus paper, blue** [K 9071, Litmus paper, Blue litmus paper]

**Litmus paper, red** [K 9071, Litmus paper, Red litmus paper]

**Peroxide test strip** A strip that is prepared to be able to assay the concentration of hydrogen peroxide in the range of 0 to 25 ppm. The test strips have the suitable color scale covering the range from 0 to 25 ppm hydrogen peroxide.

**Phosgene test paper** Dissolve 5 g of 4-dimethylaminobenzaldehyde and 5 g of diphenylamine in 100 mL of ethanol (99.5). Immerse a filter paper 5 cm in width in this solution, and allow to dry spontaneously while the paper is suspended in a dark place under clear air. Then cut off the 5-cm portions from the upper side and lower side of the paper, and cut the remaining paper to a length of 7.5 cm.

*Storage*—Preserve in tight, light-resistant containers. Do not use the paper, which has changed to a yellow color.

**Potassium iodide-starch paper** Impregnate filter paper with a mixture of equivalent volumes of a solution of potassium iodate (1 in 20) and freshly prepared starch TS, and dry in a clean room.

*Storage*—Preserve in a glass-stoppered bottle, protected

from light and moisture.

**Potassium iodide-starch paper** Impregnate filter paper with freshly prepared potassium iodide-starch TS, and dry in a clean room.

*Storage*—Store in a glass-stoppered bottle, protected from light and moisture.

**Red litmus paper** See litmus paper, red.

**Turmeric paper** Macerate 20 g of powdered dried rhizome of *Curcuma longa* Linné with four 100 mL-portions of cold water, decant the supernatant liquid each time, and discard it. Dry the residue at a temperature not over 100°C. Macerate the dried residue with 100 mL of ethanol (95) for several days, and filter. Immerse filter paper in this ethanol decoction, and allow the ethanol (95) to evaporate spontaneously in clean air.

*Sensitivity*—Dip a strip of turmeric paper, about 1.5 cm length, in a solution of 1 mg of boric acid in a mixture of 1 mL of hydrochloric acid and 4 mL of water, after 1 minute remove the paper from the liquid, and allow it to dry spontaneously: the yellow color changes to brown. When the strip is moistened with ammonia TS, the color of the strip changes to greenish black.

**Zinc iodide-starch paper** Impregnate the filter paper for quantitative analysis with freshly prepared zinc iodide-starch TS, and dry in the clean room.

*Storage*—Preserve in a glass-stoppered bottle, protected from light and moisture.

## 9.44 Standard Particles, etc.

**$\alpha$ -Alumina for specific surface area determination**

$\alpha\text{-Al}_2\text{O}_3$  Prepared for specific surface area determination.

**Calibration ball for particle density measurement**

Calibration ball with a known volume prepared for measurement of particle density. The volume of the calibration ball must be accurately determined to the nearest 0.001  $\text{cm}^3$ .

**Indium for thermal analysis** Prepared for thermal analysis.

*Content*: not less than 99.99%.

**Standard particles for calibrating light-shielded automatic fine particle counter** Use plastic spherical particles of known size and number.

**Tin for thermal analysis Sn** [K 8580 (Tin). Content: not less than 99.99%]

## Measuring Instruments and Appliances, Thermometers, etc.

### 9.61 Optical Filters for Wavelength and Transmission Rate Calibration

Use optical filters for wavelength calibration and those for transmission rate calibration shown in Table 9.61-1 and Table 9.61-2, respectively. The optical filters for transmission rate calibration are also used for the calibration of absorbances.

**Table 9.61-1** Optical Filters for Wavelength Calibration

Type of filter	Range of wavelength calibration (nm)	Product name
Neodymium optical filter for wavelength calibration	400 – 750	JCRM 001
Holmium optical filter for wavelength calibration	250 – 600	JCRM 002

**Table 9.61-2** Optical Filters for Transmission Rate Calibration

Type of filter	Transmission rate for calibration (%)	Product name
Optical filter for calibration within the visible wavelength range	1	JCRM 101
	10	JCRM 110
	20	JCRM 120
	30	JCRM 130
	40	JCRM 140
	50	JCRM 150
Optical filter for calibration within the ultraviolet wavelength range	10	JCRM 210 A
	50	JCRM 250 A
Optical filter for calibration within the near-ultraviolet wavelength range	10	JCRM 310
	30	JCRM 330
	50	JCRM 350

### 9.62 Measuring Instruments, Appliances

Measuring Instruments are the instruments or machines used for measuring mass or volume in the JP tests, and Appliances are the instruments specified in order to make test conditions as consistent as possible in those tests.

**Balances and weights** (1) Chemical balances—Use balances readable to the extent of 0.1 mg.

(2) Semimicrobalances—Use balances readable to the extent of 10 µg.

(3) Microbalances—Use balances readable to the extent of 1 µg.

(4) Ultramicrobalances—Use balances readable to the extent of 0.1 µg.

(5) Weights—Use calibrated weights.

**Carbon dioxide measuring detector tube** [Gas detector tube measurement system K 0804] Packed with measurement packing for carbon dioxide.

**Carbon monoxide measuring detector tube** [Gas detector tube measurement system K 0804] Packed with measurement packing for carbon monoxide.

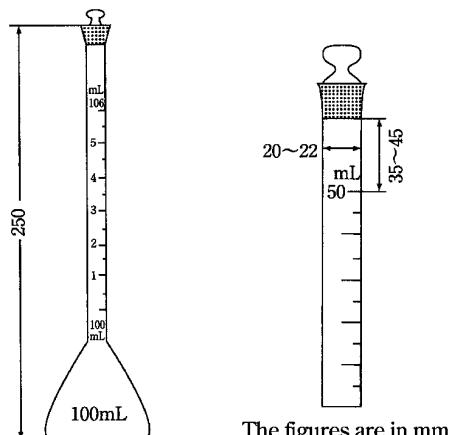
**Cassia flask** Use glass-stoppered flasks, shown in Fig. 9.62-1, made of hard glass and having graduation lines of volume on the neck.

**Gas mixer** Use the apparatus, shown in Fig. 9.62-3, made of hard glass.

**Nessler tube** Use colorless, glass-stoppered cylinders 1.0 to 1.5 mm in thickness, shown in Fig. 9.62-2, made of hard glass. The difference of the height of the graduation line of 50 mL from the bottom among cylinders does not exceed 2 mm.

**Sieves** Sieves conform to the specifications in Table 9.62-1. Use the sieve number of nominal size as the designation.

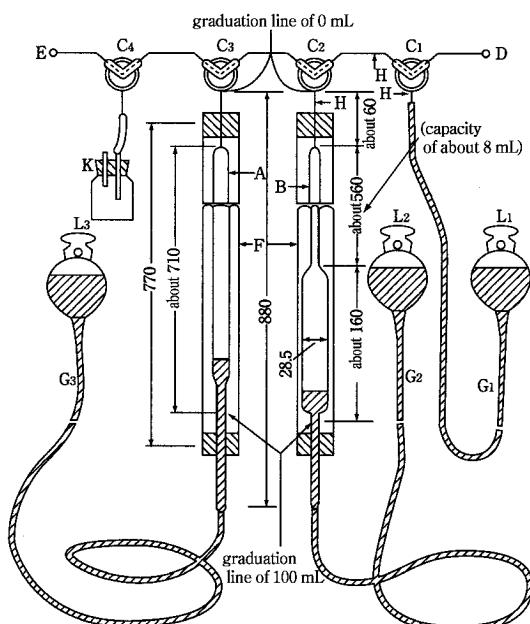
**Volumetric measures** Use volumetric flasks, transfer pipets, piston pipets, burets and measuring cylinders conforming to the Japanese Industrial Standards. For volumetric glassware with Class A error limits specified in the



The figures are in mm.

**Fig. 9.62-1**

**Fig. 9.62-2**



The figures are in mm.

- A: Gas buret (capacity of 100 mL, about 13.7 mm in inside diameter, graduated in 0.2 mL divisions, and graduated in 0.1 mL divisions at the lower, narrow part).
- B: Gas buret (capacity of 100 mL, about 4.2 mm in inside diameter at the upper stem with graduation in 0.02-mL division, about 28.5 mm in inside diameter at the lower stem with graduation in 1-mL divisions).
- C: (C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub> and C<sub>4</sub>): Three-way stopcock.
- D: Inlet of sample (bent forward at 20 mm in length).
- E: Outlet of mixed gas (bent forward at 20 mm in length).
- F: Jacket (about 770 mm in length, about 40 mm in outside diameter, almost completely filled with water at room temperature).
- G: Rubber pressure tubing, about 4 mm in inside diameter (G<sub>1</sub>: about 80 cm in length; G<sub>2</sub> and G<sub>3</sub>: about 120 cm in length).
- H: Heavy-wall capillary tube (about 1 mm in inside diameter).
- K: Receiver.
- L: Leveling bulb (L<sub>1</sub>: filled with about 50 mL of mercury; L<sub>2</sub> and L<sub>3</sub>: filled with about 150 mL of mercury).

Fig. 9.62-3

Japanese Industrial Standards, use instruments conforming to the standards. Volumetric glassware conforming to Class A error limits as per appropriate international standards published by international organizations may also be used.

## 9.63 Thermometers

**Thermometers** Ordinarily, use calibrated thermometers with an immersion line (rod) or calibrated total immersion mercury-filled thermometers according to the Japanese Industrial Standards. Use the thermometers with the immersion line (rod), shown in Table 9.63-1, for the tests in Congealing Point, Melting Point (Method 1), Boiling Point and Distilling Range.

**Table 9.62-1** Specification of Sieves

Sieve number	Nominal size ( $\mu\text{m}$ )	Nominal opening (mm)	Permissible variation of opening (mm)		Diameter of wire (mm)		
			Average	Maximum	Recommended	Maximum	Minimum
3.5	5600	5.60	$\pm 0.18$	0.47	1.60	1.90	1.30
4	4750	4.75	$\pm 0.15$	0.41	1.60	1.90	1.30
4.7	4000	4.00	$\pm 0.13$	0.37	1.40	1.70	1.20
5.5	3350	3.35	$\pm 0.11$	0.32	1.25	1.50	1.06
6.5	2800	2.80	$\pm 0.09$	0.29	1.12	1.30	0.95
7.5	2360	2.36	$\pm 0.08$	0.25	1.00	1.15	0.85
8.6	2000	2.00	$\pm 0.07$	0.23	0.90	1.04	0.77
10	1700	1.70	$\pm 0.06$	0.20	0.80	0.92	0.68
12	1400	1.40	$\pm 0.05$	0.18	0.71	0.82	0.60
14	1180	1.18	$\pm 0.04$	0.16	0.63	0.72	0.54
16	1000	1.00	$\pm 0.03$	0.14	0.56	0.64	0.48
18	850	0.850	$\pm 0.029$	0.127	0.500	0.580	0.430
22	710	0.710	$\pm 0.025$	0.112	0.450	0.520	0.380
26	600	0.600	$\pm 0.021$	0.101	0.400	0.460	0.340
30	500	0.500	$\pm 0.018$	0.089	0.315	0.360	0.270
36	425	0.425	$\pm 0.016$	0.081	0.280	0.320	0.240
42	355	0.355	$\pm 0.013$	0.072	0.224	0.260	0.190
50	300	0.300	$\pm 0.012$	0.065	0.200	0.230	0.170
60	250	0.250	$\pm 0.0099$	0.058	0.160	0.190	0.130
70	212	0.212	$\pm 0.0087$	0.052	0.140	0.170	0.120
83	180	0.180	$\pm 0.0076$	0.047	0.125	0.150	0.106
100	150	0.150	$\pm 0.0066$	0.043	0.100	0.115	0.085
119	125	0.125	$\pm 0.0058$	0.038	0.090	0.104	0.077
140	106	0.106	$\pm 0.0052$	0.035	0.071	0.082	0.060
166	90	0.090	$\pm 0.0046$	0.032	0.063	0.072	0.054
200	75	0.075	$\pm 0.0041$	0.029	0.050	0.058	0.043
235	63	0.063	$\pm 0.0037$	0.026	0.045	0.052	0.038
282	53	0.053	$\pm 0.0034$	0.024	0.036	0.041	0.031
330	45	0.045	$\pm 0.0031$	0.022	0.032	0.037	0.027
391	38	0.038	$\pm 0.0029$	0.020	0.030	0.035	0.024

**Table 9.63-1** Thermometers with Immersion Line

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Liquid	Mercury	Mercury	Mercury	Mercury	Mercury	Mercury
Gas filled above liquid	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon
Temperature range	-17 - 50°C	40 - 100°C	90 - 150°C	140 - 200°C	190 - 250°C	240 - 320°C
Minimum graduation	0.2°C	0.2°C	0.2°C	0.2°C	0.2°C	0.2°C
Longer graduation lines at Graduation numbered at	each 1°C	each 1°C	each 1°C	each 1°C	each 1°C	each 1°C
Total length (mm)	280 - 300	280 - 300	280 - 300	280 - 300	280 - 300	280 - 300
Stem diameter (mm)	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3
Bulb length (mm)	12 - 18	12 - 18	12 - 18	12 - 18	12 - 18	12 - 18
Distance from bottom of bulb to graduation at the lowest temperature (mm)	75 - 90	75 - 90	75 - 90	75 - 90	75 - 90	75 - 90
Distance from top of thermometer to graduation at the highest temperature (mm)	35 - 65	35 - 65	35 - 65	35 - 65	35 - 65	35 - 65
Distance from bottom of bulb to immersion line(mm)	58 - 62	58 - 62	58 - 62	58 - 62	58 - 62	58 - 62
Shape of top of thermometer	loop	loop	loop	loop	loop	loop
Test temperature	-15°C, 15°C, 45°C	45°C, 70°C, 95°C	95°C, 120°C, 145°C	145°C, 170°C, 195°C	195°C, 220°C, 245°C	245°C, 280°C, 315°C
Maximum scale error at any point	0.2°C	0.2°C	0.2°C	0.2°C	195°C : 0.2°C 220°C : 0.3°C 245°C : 0.3°C	245°C : 0.3°C 280°C : 0.4°C 315°C : 0.5°C