

**Ministry of Health, Labour and Welfare Notification No. 394**

The Minister of Health, Labour and Welfare has partially revised the Specifications and Standards for Food, Food Additives, Etc. (Ministry of Health and Welfare Notification No. 370, 1959), as given below, based on the provision of Paragraph 1, Article 11 of the Food Sanitation Law.

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Minister of Health, Labour and Welfare

Item 5 (9) in Section A *General Compositional Standards for Food*, Part I *Food* shall be revised as follows:

(9) Chloramphenicol Analytical Method

1. Apparatus

Liquid chromatograph-mass spectrometer (LC/MS or LC/MS/MS)

2. Reagents and test solutions

In addition to the reagents and test solutions listed below, use those listed in Section C *Reagents/Test Solutions, Etc.*, Part II *Food additives*.  
Acetonitrile: Acetonitrile produced for liquid chromatography.

Divinylbenzene-*N*-vinylpyrrolidine Copolymer Cartridge Column (60 mg): Use a polyethylene column of 12–13 mm in inner diameter packed with 60 mg of divinylbenzen-*N*-vinylpyrrolidone copolymer, or a column equivalent to the specified one in separation capability.

Divinylbenzene-*N*-vinylpyrrolidine Copolymer Cartridge Column (200 mg): Use a polyethylene column of 12–13 mm in inner diameter packed with 200 mg of divinylbenzen-*N*-vinylpyrrolidone copolymer, or a column equivalent to the specified one in separation capability.

Water Water produced for liquid chromatography.

3. Reference standard

Chloramphenicol: This product contains not less than 99% chloramphenicol.

Decomposition point: 208°C.

4. Procedure

a. Extraction

i. Honey

Weigh 5.00 g of the test sample, previously homogenized, and dissolve it in 20 ml of water.

ii. Royal jelly

Weigh 1.00 g of the test sample, previously homogenized, add 60 ml of methanol/1% metaphosphoric acid (3:2), and homogenize the mixture again. Filter the homogenized sample by suction into a rotary vacuum evaporator through a filter paper covered with a 2-mm thick layer of diatomaceous earth. Wash the residue on the filter paper with 15 ml of methanol/1% metaphosphoric acid (3:2), and filter the washings by suction. Combine the filtrates into the evaporator, and evaporate the mixture to 2 ml at 45°C or lower.

iii. Foods other than those given in sections i and ii

Weigh 5.00 g of the test sample, previously ground. For muscle, the fat layer should be removed as much as possible before grinding. To the sample measured out, add 100 ml of methanol/1% metaphosphoric acid (3:2), and homogenize the mixture again. Filter the homogenized sample by suction into a rotary vacuum evaporator through a filter paper covered with a 2-mm thick layer of diatomaceous earth. Wash the residue on the filter paper with 10 ml of methanol/1% metaphosphoric acid (3:2), and filter the washings by suction. Combine the filtrates into the evaporator, and evaporate the mixture to 30 ml at 45°C or lower.

b. Clean-up

i. Honey

Pour 5 ml of methanol and 5 ml of water into a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge column (60 mg), and discard the effluent. Pour the sample solution obtained by the extraction method described in 4-a. into the column, then pour 5 ml of 20% (vol) methanol. Discard the effluent. Pour 6 ml of 60% (vol) methanol, and collect the eluate into a rotary vacuum evaporator. Remove the methanol and water at 45°C or lower. Dissolve the residue in 1.0 ml of acetonitrile/water (3:7), and use the obtained solution as the test solution.

ii. Royal jelly

Pour 10 ml of methanol and 10 ml of water into a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge column (200 mg), and discard the effluent. Pour the sample solution obtained by the extraction method described in 4-a. into the column, then pour 4 ml of water and 4 ml of 5% (vol) methanol into the column. Discard the effluent. Pour 10 ml of 60% (vol) methanol, and collect the eluate into a rotary vacuum

evaporator. Remove the methanol and water at 45°C or lower. Dissolve the residue in 1.0 ml of acetonitrile/water (3:7), and use the obtained solution as the test solution.

iii. Foods other than those given in sections i and ii

Pour 5 ml of methanol and 5 ml of water into a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge column (60 mg), and discard the effluent. Pour the sample solution obtained by the extraction method described in 4-a. into the column, then pour 10 ml of water. Discard the effluent. Pour 10 ml of methanol, and collect the eluate into a rotary vacuum evaporator. Remove the methanol at 40°C or lower. Dissolve the residue in 1.0 ml of acetonitrile/water (3:7), and use the obtained solution as the test solution.

5. Determination

a. Qualitative tests

Perform qualitative tests under the following conditions. Test results obtained must be the same as those obtained for the reference standard.

Testing conditions

Column packing: Octadecylsilanized silica gel (2–5 µm in particle size)

Column: A stainless steel tube (2.0–6.0 mm in inner diameter, 100–250 mm in length)

Column temperature: 40°C

Mobile phase: Acetonitrile/10 mmol/l ammonium formate (3:7). The flow rate should be adjusted so that chloramphenicol flows out in approximately 5 minutes.

b. Quantitative tests

Determine the quantity from the test results obtained under the conditions specified for the qualitative tests, using either the peak height or peak area method.