Provisional Translation
from the Japanese Original

Ministry of Health, Labour and Welfare Notification No. 645

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.

November 30, 2006
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Minister of Health, Labour and Welfare

Item 5 (14) in Section A ***General Compositional Standards for Food***, Part I ***Food*** shall be revised as follows:

(14) Nitrofurans analytical method

Analyze 3-amino-2-oxazolidinone, 1-aminohydantoin, 3-amino-5-morpholinomethyl-2-oxazolidinone and semicarbazide.

1. Apparatus

A liquid chromatograph/tandem mass spectrometer (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 Tolutions, Etc.***, Part II ***Food Additives***. Reagents designated as “special grade” in this section must meet the requirements for “special grade” specified in the Japan Industrial Standards for the reagents.

Acetonitrile: Acetonitrile produced for liquid chromatography.

Porous diatomaceous earth column (to hold 20 ml of solution): A polyethylene column of 20-30 mm in inner diameter packed with granular porous diatomaceous earth produced for column chromatography that can hold 20 ml of solution, or a column equivalent to the specified one in separation capability.

o-Nitrobenzaldehyde: *o*-Nitrobenzaldehyde (special grade).

Water: Water produced for liquid chromatography.

3. Reference standard

3-amino-2-oxazolidinone: This product contains not less than 99% of 3-amino-2-oxazolidinone, and its decomposition point is 65-67°C.

1-aminohydantoin hydrochloride: This product contains not less than 90% of 1-aminohydantoin hydrochloride, and its decomposition point is 201-205°C.

3-amino-5-morpholinomethyl-2-oxazolidinone: This product contains not less than 99% of 3-amino-5-morpholinomethyl-2-oxazolidinone, and its decomposition point is 115-120°C.

Semicarbazide hydrochloride: This product contains not less than 99% of semicarbazide hydrochloride, and its decomposition point is 175-177°C.

4. Procedure

a. Extraction

Weigh 5.00 g of the test sample, previously ground, and homogenize it with 70 ml of 0.1 mol/l hydrochloric acid. Centrifuge the mixture at 2,500 rpm for five minutes and collect the supernatant, and then add 0.1 mol/l hydrochloric acid to make 100 ml of solution.

b. Derivatization

Transfer 10 ml of the solution obtained by the extraction described in 4-a and add 0.4 ml of 0.05 mol/l *o*-nitrobenzaldehyde-dimethyl sulfoxide solution, and then leave it to stand for 16 hours at 37°C. Add 5 ml of 0.1 mol/l dipotassium hydrogen orthophosphate solution to the solution and add approximately 0.8 ml of a 1 mol/l sodium hydroxide solution to adjust the pH to 7-8. When some residue remains in the solution, centrifuge at 2,500 rpm for five minutes and collect the supernatant.

c. Clean-up

Pour the solution obtained by the derivatization described in 4-b into a porous diatomaceous earth column (to hold 20 ml of solution). Leave the column to stand for five minutes and add 100 ml of ethyl acetate. Collect the eluate into a rotary vacuum evaporator and remove the ethyl acetate at 40°C or lower. Dissolve the residue in 1.0 ml of acetonitrile/water (1:1), which is used as the sample 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

standards under the procedure described in 4-b and 4-c.

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (2-5μm in particle size)

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

Column temperature: 40°C

Mobile phase: Use a mixture of acetonitrile and 0.1% acetic acid solution. Create a concentration gradient of 1:4 to 4:1 acetonitrile /0.1% acetic acid solution in 20 minutes. Adjust the flow rate so that the derivative of 3-amino-2-oxazolidinone flows out in approximately 12 minutes.

b. Quantitative tests

Determine the quantity from the test results obtained under the same conditions described in 5-a using either the peak height or peak area method.

Item 5 (16) in Section A ***General Compositional Standards for Food***, Part I ***Food*** shall be revised as follows:

(16) Malachite green analytical method

Analyze malachite green and leucomalachite green.

1. Apparatus

A 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 Tolutions, Etc.***, Part II ***Food Additives***. Reagents designated as “special grade” in this section must meet the requirements for “special grade” specified in the Japan Industrial Standards for the reagents.

Acetonitrile: Acetonitrile produced for liquid chromatography.

Ammonium formate: Ammonium formate (special grade).

Strongly acidic cation exchanger cartridge column (500 mg): A polyethylene column of 8-9 mm in inner diameter packed with 500 mg of benzenesulfonyl propylsilanized silica gel, or a column

equivalent to the specified one in separation capability.

Citric acid–phosphate buffer (pH 3.0)

Solution 1: Dissolve 63.0g of citric acid in water to make 1,000 mL of solution.

Solution 2: Dissolve 215 g of disodium phosphate in water to make 1,000 mL of solution.

Mix Solution 1 and Solution 2 and adjust the pH to 3.0.

Dichloromethane: Dichloromethane (special grade).

Water: Water produced for liquid chromatography.

Sodium sulfate (anhydrous): Sodium sulfate (anhydrous)(special grade). When it contains substances that may interfere with the analysis, wash it with ethyl acetate before using.

3. Reference standard

Malachite Green Oxalate: This product contains not less than 99% of malachite green oxalate, and its decomposition point is 164ºC.

Leucomalachite Green: This product contains not less than 99% of leucomalachite green, and its decomposition point is 103ºC.

4. Procedure

a. Extraction

Weigh 5.00 g of the test sample, previously ground, and homogenize it with 10 mL of citric acid-phosphate buffer (pH 3.0). Add 15 mL of acetonitrile and shake the mixture vigorously for five minutes using a shaker. Centrifuge the mixture at 3,000 rpm for five minutes and collect the acetonitrile-water layer. Add 15mL of acetonitrile to the residue and repeat the above procedure, and then combine the acetonitrile layer with the acetonitrile-water layer.

Add 5 mL of *n*-hexane to the combined layers and shake the mixture vigorously for five minutes using a shaker and leave it to stand, and then collect the acetonitrile-water layer. Add 5 mL of *n*-hexane to the collected layer and repeat the above procedure to collect the acetonitrile-water layer.

Add 50 mL of 20% sodium chloride and 10 mL of dichloromethane to the collected layer and shake it vigorously for five minutes using the shaker and leave it to stand, and then collect the acetonitrile-dichloromethane layer.

Add an adequate amount of sodium sulfate (anhydrous) and leave it to stand for 15 minutes with occasional shaking, and then filter the mixture.

b. Clean-up

Pour 5 mL of acetonitrile into a strongly acidic cation exchanger cartridge column (500 mg) and discard the effluent. Pour the solution obtained by the extraction described in section 4–a into the column followed by 5 mL of acetonitrile and discard the effluent. Pour 10 mL of acetonitrile/ammonia water (9:1) and collect the eluate into a rotary vacuum evaporator, and then remove the acetonitrile and ammonia water at 40ºC or lower. Dissolve the residue in 1.0 mL of acetonitrile, which is used as the sample 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 standards.

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (2-5μm in particle size).

Column: A stainless tube (2.0-6.0 mm in inner diameter, and 100-250 mm in length).

Column temperature: 40°C

Mobile phase: Use a mixture of acetonitrile and 0.01 mol/L ammonium formate. Create a concentration gradient of 1:9 to 1:0 acetonitrile/0.01 mol/L ammonium formate in 20 minutes, and maintain a ratio of 1:0 for 10 minutes. Adjust the flow rate so that malachite green flows out in approximately 10 minutes.

b. Quantitative tests

Determine the quantity from the test results obtained under the same conditions described in 5-a using either the peak height or peak area method.

Food has been revised as follows:

The “enrofloxacin” section has been added after the “endrin” section.

Enrofloxacin	Cattle, muscle	0.05 ppm
	Pig, muscle	0.05 ppm
	Other terrestrial mammals, muscle	0.05 ppm
	Cattle, fat	0.05 ppm
	Pig, fat	0.05 ppm
	Other terrestrial mammals, fat	0.05 ppm
	Cattle, liver	0.1 ppm
	Pig, liver	0.1 ppm
	Other terrestrial mammals, liver	0.1 ppm
	Cattle, kidney	0.1 ppm
	Pig, kidney	0.1 ppm
	Other terrestrial mammals, kidney	0.1 ppm
	Cattle, edible offal	0.05 ppm
	Pig, edible offal	0.05 ppm
	Other terrestrial mammals, edible offal	0.05 ppm
	Milk	0.05 ppm
	Chicken, muscle	0.05 ppm
	Other poultry, muscle	0.05 ppm
	Chicken, fat	0.05 ppm
	Other poultry, fat	0.05 ppm
	Chicken, liver	0.1 ppm
	Other poultry, liver	0.1 ppm
	Chicken, kidney	0.1 ppm
	Other poultry, kidney	0.1 ppm
	Chicken, edible offal	0.1 ppm
	Other poultry, edible offal	0.1 ppm

The “tulathromycin” section has been added after the “tilmicosin” section.

Tulathromycin	Cattle, muscle	0.3 ppm
	Pig, muscle	2 ppm
	Cattle, fat	0.2 ppm
	Pig, fat	0.3 ppm
	Cattle, liver	5 ppm
	Pig, liver	4 ppm
	Cattle, kidney	3 ppm
	Pig, kidney	9 ppm
	Cattle, edible offal	3 ppm
	Pig, edible offal	5 ppm

In item 7(1) in Section A ***General Compositional Standards for Food***, Part I ***Food,*** the “enrofloxacin” and “tulathromycin” section has been deleted.