8. Compositional standards of each feed additives and standards of manufacturing methods, etc.

(1) Sodium Alginate

A. Raw material for manufacturing

(a) Compositional standards

Physical and chemical properties: This product is white to yellowish-white powder with little odor.

Confirmation test:

i. 0.5 g (0.45~0.54 g) of the product is gradually added to 50 mL of water while stirring, warmed at 60~70 °C for 20 minutes while occasionally stirring to make a homogeneous solution, and allowed to cool. This is used as a sample solution. When 5 mL of this sample solution is added with 1 mL of calcium chloride test solution, gelatinous precipitation is generated within 30 seconds.

ii. When 10 mL of the sample solution of i. is added with 1 mL of dilute sulfuric acid, gelatinous precipitation is generated within 30 seconds.

iii. When 1 mL of the sample solution of i. is added with 2 mL of phloroglucinol hydrochloric acid test solution and boiled for 30 seconds, the resulting solution is reddish violet.

iv. The residue obtained by the ashing of this product gives the qualitative reaction of sodium salt.

Purity test:

i. pH: The pH of the solution (1 → 100) of this product shall be 6.0~8.0.

ii. Sulfate: 0.1 g (0.05~1.4 g) of this product is added with 20 mL of water to make it a paste, shaken well with 1 mL of hydrochloric acid, warmed in a water bath for several minutes, allowed to cool and filtered. The container is washed with 10 mL of water and the washings are also filtered with the same filter. Next, the same procedure is repeated twice with 10 mL of water each. All the filtrates are collected and added with water to make 50 mL to prepare a sample solution. A control solution is prepared using 0.4 mL of 0.005 mol/L sulfuric acid by the sulfate test method. When 10 mL of the sample and control solution are each tested for sulfate, the turbidity of the sample solution shall not be higher than that of the control solution (0.96 % or less).

iii. Heavy metal: 2.0 g (1.95~2.04 g) of this product is gently heated, carbonized, ashed at 450~500 °C, added with 2 mL of hydrochloric acid, and evaporated to dryness on a water bath. The residue is dissolved in 4 mL of diluted acetic acid and 20 mL of water and filtered. The residue on the filter paper is washed three times with 5 mL of water

each. All the filtrates are collected and added with water to make 50 mL. This is used as a sample solution. A control solution is prepared using 2.0 mL of lead standard solution. When 25 mL each of the sample and control solutions are tested for heavy metal, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

v. Starch: When 5 mL of a solution of this product in water (1 → 100) is added with 2 drops of iodine test solution and shaken, the color of the resulting solution shall not be blue to reddish violet.

vi. Gelatin: When 5 mL of a solution of this product in water (1 → 100) is added with 1 mL of ammonium molybdate solution (1 → 20) and shaken, precipitation shall not be generated within 5 minutes.

Loss on drying: 15 % or less (1 g, 105 °C, 4 hours)

Ignition residue: 33~37 % (1 g)

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium alginate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium alginate is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of sodium alginate and fillers.

Confirmation test: 1.0 g (0.95~1.04 g) of this product is gradually added to 50 mL of water while stirring and allowed to stand for an hour while occasionally stirring. Then, the supernatant obtained by 10 minutes centrifugation at 3,000 rpm is used as a sample solution, and the confirmation test of the raw materials for manufacturing of sodium alginate is hereinafter applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a capped container.

(2) Ethoxyquin

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 97.5 % or over of ethoxyquin ($C_{14}H_{19}NO$).

Physical and chemical properties:

i. This product is a yellow-brown to brown viscous fluid with a slight, specific odor.

ii. This product is easy to extremely dissolve in acetone, isopropanol, ethanol, ether, chloroform, petroleum ether or n-hexane and hardly dissolves in water.

iii. This product is gradually colored by air or light.

Confirmation test:

i. When a solution of this product in n-hexane (1 → 10,000) is irradiated with ultraviolet light (dominant wavelength: 365 nm), it exhibits blue-white fluorescence.

ii. In the measurement of the absorption spectrum of this product in isopropanol solution (1 → 20,000), the absorption maximum is at the wavelengths of 356~362 nm.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of n-hexane. The resulting solution shall be clear and its color shall not be darker than that of a mixture of 10 mL of a color control stock solution of cobalt chloride, 5 mL of a color control stock solution of copper sulfate, and 5 mL of a color control stock solution of ferric chloride.

ii. Sulfate: 2.0 g (1.95~2.04 g) of this product is weighed, added with 30 mL of water, shaken for 2 minutes, and filtered. 15 mL of the filtrate is measured and added with 1 mL of dilute hydrochloric acid and water to make 50 mL. This is used as a sample solution. When sulfate is tested using a control solution prepared with 0.25 mL of 0.005 mol/L sulfuric acid by the sulfate test method, the turbidity of the sample solution shall not be higher than that of the control solution (0.012 % or less).

iii. Heavy metal: 2.0 g (1.95~2.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (5 mg/kg or less).

iv. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Ignition residue: 0.2 % or less (1 g)

Assay: Approximately 0.1 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 1 mL of acetic anhydride and 50 mL of glacial acetic acid for nonaqueous titration and titrated with 0.1 mol/L of perchloric acid-dioxane solution (potentiometric titration). A blank test is performed in the same way and corrections are made.

0.1 mol/L of perchloric acid-dioxane solution 1 mL =21.73 mg $C_{14}H_{19}NO$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of ethoxyquin are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of ethoxyquin is applied mutatis mutandis.

C. Preparation (Part 2: liquid)

(a) Compositional standards

This product is oil liquid or water-soluble liquid of the mixture of the raw material for manufacturing of ethoxyquin and glycerin, hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats.

Content: When this product is determined, it contains ethoxyquin ($C_{14}H_{19}NO$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, 0.1 g of the raw material for manufacturing of ethoxyquin is weighed, dissolved in a little isopropanol, added with n-hexane to make 100 mL and shaken well. The resulting solution is used as a sample solution. 10 mL of this solution is measured and added with n-hexane to make 100 mL. Using the resulting solution, the confirmation test i. for the raw material for manufacturing of ethoxyquin is hereinafter applied mutatis mutandis.

ii. 50 mL of the sample solution of i. is measured, the solvent is distilled off under reduced pressure and the residue is dissolved in 1,000 mL of isopropanol. Using this

solution, the confirmation test ii. for the raw material for manufacturing of ethoxyquin is hereinafter applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.1 g of ethoxyquin ($C_{14}H_{19}NO$) is weighed to three significant digits and the value is recorded. It is dissolved in a little isopropanol, transferred to a 1,000 mL volumetric flask, added with n-hexane, shaken up well, and added with n-hexane to the graduation line to make 1,000 mL. 1 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with n-hexane to the graduation line to make 100 mL. This is used as a sample solution. Separately, approximately 0.1 g of ethoxyquin for assay is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with n-hexane, transferred to a 1,000 mL volumetric flask, added with more n-hexane to the graduation line to make 1,000 mL, and subjected to the same procedure as that for the sample solution to prepare a standard solution. For the sample solution (T), the standard solution (S), and n-hexane (B), fluorescence intensities, F_T, F_S, and F_B are measured at the excitation wavelength 365 nm and the fluorescence wavelength 415 nm using a fluorophotometer.

$$\text{Amount of ethoxyquin } (C_{14}H_{19}NO) \text{ (mg)} = \text{Amount of ethoxyquin for assay (mg)} \times \frac{F_T - F_B}{F_S - F_B}$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

D. Preparation (Part 3: powder)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of ethoxyquin and fillers.

Content: When this product is determined,it contains ethoxyquin ($C_{14}H_{19}NO$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.1 g of the raw material for manufacturing of ethoxyquin is weighed, added with 100 mL of n-hexane, shaken well and mixed, and filtered to prepare a sample solution. 10 mL of this solution is measured and added with n-hexane to make 100 mL. Using this solution, the confirmation test i. for the raw material for manufacturing of ethoxyquin is hereinafter applied mutatis mutandis.

ii. 50 mL of the sample solution of i. is measured, the solvent is distilled off under reduced pressure and the residue is dissolved in 1,000 mL of isopropanol. Using this

solution, the confirmation test ii. for the raw material for manufacturing of ethoxyquin is hereinafter applied mutatis mutandis.

Assay: Methods No. 1 is used for the amount on the label is 40 % and above and Method No. 2 is used for the label is less than 40 %.

Method No. 1: The amount of this product containing approximately 0.1 g of ethoxyquin ($C_{14}H_{19}NO$) is weighed to three significant digits and the value is recorded. It is added with 50 mL of acetone, shaken up well, and filtered. The residue is washed three times with 30 mL each of acetone. The filtrate and washings are collected together and the solvent is distilled off under reduced pressure and nitrogen stream at 40 °C. Within 30 seconds, the residue is dissolved with 1 mL of acetic anhydride and 50 mL of glacial acetic acid for nonaqueous titration, and the assay for the raw material for manufacturing of ethoxyquin is hereinafter applied mutatis mutandis.

0.1 mol/L perchloric acid-dioxane solution 1 mL =21.73 mg $C_{14}H_{19}NO$

Method No. 2: The amount of this product containing approximately 0.1 g of ethoxyquin ($C_{14}H_{19}NO$) is weighed to three significant digits and the value is recorded. It is dissolved with n-hexane, transferred to a 1,000 mL volumetric flask, shaken up well, added with more n-hexane to the graduation line to make 1,000 mL. This solution is filtered with a dried filter paper and 20 mL of the first filtrate is removed. 1 mL of the second filtrate is measured using a volumetric pipette and the assay for ethoxyquin preparation (part 2: liquid) is hereinafter applied mutatis mutandis.

$$\text{Amount of ethoxyquin } (C_{14}H_{19}NO) \text{ (mg)} = \text{Amount of ethoxyquin for assay (mg)} \times \frac{F_T - F_B}{F_S - F_B}$$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(3) Sodium Caseinate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 3 hours drying at 100 °C, it contains 14.5~15.8 % of nitrogen (N).

Physical and chemical properties: This product is white to pale yellow particles, powder or pieces with no odor or a slight, specific odor.

Confirmation test:

i. When 0.1 g (0.05~0.14 g) of this product is dissolved with 10 mL of 1 mol/L sodium hydroxide test solution, and added with acetic acid to make it slightly acidic, white flocculation is produced.

ii. When 0.1 g (0.05~0.14 g) of this product is dissolved with 10 mL of 1 mol/L sodium hydroxide test solution, added with a drop of copper sulfate test solution and shaken up, blue precipitation is produced and the solution is purple.

iii. When 0.1 g (0.05~0.14 g) of this product is ignited, it generates smoke and emits a specific odor. After the smoke generation stop, the heating is stopped and the product is allowed to cool. The black residue is added with 5 mL of dilute nitric acid, warmed and filtered. When the filtrate is added with 1 mL of ammonium molybdate test solution and warmed, yellow precipitation is produced.

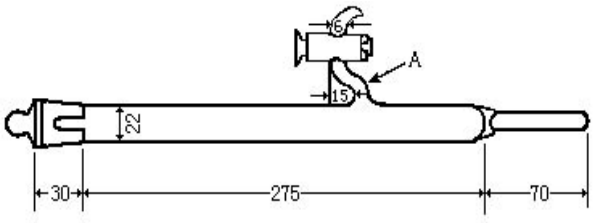
iv. The residue on ignition of this product gives the flame reaction of sodium salt.

Purity test:

i. Clarity and color of solution: This product is dried in a desiccator (reduced pressure, sulfuric acid) for 4 hours to make it fine powder. 0.1 g (0.05~0.14 g) of this powder is weighed, added with 30 mL of water, shaken up, allowed to stand for approximately 10 minutes, added with 2 mL of 0.1 mol/L sodium hydroxide test solution, and warmed at 60 °C for one hour with occasional shaking to make it dissolved. When it is allowed to cool and then added with water to make 100 mL, it is colorless and its turbidity shall be slightly cloudiness or clearer.

ii. pH: The pH of the solution (1 → 50) of this product shall be 6.0~7.5.

iii. Fat: Approximately 2.5 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 15 mL of hydrochloric acid (27 → 40), dissolved by gentle direct heating, and then heated in a water bath for 20 minutes. It is allowed to cool, added with 10 mL of ethanol, transferred to a rohrig tube, added with 25 mL of ether, and vigorously shaken up for 1 minute. Then, the mixture is added with 25 mL of petroleum ether, vigorously shaken up for 30 seconds, and allowed to stand. The upper layer solution obtained from the branch tube (A) is filtered, and the filtrate is placed in a flask, whose mass is known. Then, it is extracted twice with 15 mL of ether and 15 mL of petroleum ether in the same way. The upper layer solutions are collected in the previous flask, and ether and petroleum ether are distilled off on a water bath. When the residue is dried at 98~100 °C for 4 hours, the amount of residue shall be 1.5 % or less.



Rohrig tube

(Unit: mm)

iv. Heavy metal: 2.0 g (1.95~2.04 g) of this product is gently heated and carbonized. Then it is ashed at less than 500 °C, added with 2 mL of hydrochloric acid, and evaporated to dryness on a water bath. The residue is resolved in 4 mL of diluted acetic acid and 20 mL of water and filtered. Then, the residue on the filter paper is washed three times with 5 mL of water each. These filtrates are collected together and added with 50 mL of water. The resulting solution is used as a sample solution. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using 25 mL of the sample and control solutions each, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

v. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 15 % or less (1 g, 100 °C, 3 hours)

Ignition residue: 6 % or less (1 g)

Assay: This product is dried at 100 °C for 3 hours, approximately 0.15 g of it is weighed to the digits of 0.001 g and the value is recorded. The assay is performed by the nitrogen determination method (Kjeldahl method).

0.05 mol/L sulfuric acid 1 mL = 1.401 mg N

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium caseinate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium caseinate is applied mutatis mutandis.

(4) Sodium Carboxymethyl Cellulose

A. Raw material for manufacturing

(a) Compositional standards

Physical and chemical properties: This product is white to whitish powder, particles, or fibrous substances with no odor.

Confirmation test:

i. 0.5 g (0.45~0.54 g) of this product is gradually added to 50 mL of water while stirring and warmed at 60~70 °C for 20 minutes while occasionally stirring, then allowed to cool. This is used as a sample solution. The volume required for testing is measured and diluted fivefold with water. When a drop of this diluted solution is added with 0.5 mL of chromotropic acid test solution and heated in a water bath for 10 minutes, it turns red-purple.

ii. When 5 mL of the sample solution of i. is shaken up with 10 mL of acetone, white flocculation is produced.

iii. When 5 mL of the sample solution of i. is shaken up with 1 mL of copper sulfate test solution, pale blue flocculation is produced.

iv. The residue obtained by the ashing of this product gives the qualitative reaction of sodium salt.

Purity test:

i. pH: The pH of the solution (1 → 100) of this product shall be 6.0~8.5.

ii. Chloride: 0.1 g (0.05~0.14 g) of this product is added with 20 mL of water and 0.5 mL of hydrogen peroxide solution and heated in a water bath for 30 minutes. After cooling, it is added with water to make 100 mL and filtered through filter paper. 25 mL of this filtrate is measured and added with 6 mL of dilute nitric acid to prepare a sample solution. When chloride is tested, using a control solution prepared with 0.45 mL of 0.01 mol/L hydrochloric acid by the chloride test method, the turbidity of the sample solution shall not be higher than that of the control solution (0.64 % or less).

iii. Sulfate: 20 mL of the filtrate obtained in ii. is measured and added with 1 mL of dilute hydrochloric acid and water to make 50 mL. This solution is used as a sample solution. When sulfate is tested, using a control solution prepared with 0.4 mL of 0.05 mol/L sulfuric acid by the sulfate test method, the turbidity of the sample solution shall not be higher than that of the control solution (0.96 % or less).

iv Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

v. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed to prepare a sample solution by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Loss on drying: 12 % or less (1 g, 105 °C, 4 hours)

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium carboxymethyl cellulose are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium carboxymethyl cellulose is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder, particles, or fibrous substances of the mixture of the raw material for manufacturing of sodium carboxymethyl cellulose and fillers.

Confirmation test: According to the amount of this product on the label, 0.05 g of the raw materials for manufacturing of sodium carboxymethyl cellulose is weighed, gradually added to 50 mL of water with stirring, warmed at 60~70 °C for 20 minutes while occasionally stirring, and allowed to cool to prepare a sample solution. The confirmation test for the raw material for manufacturing of sodium carboxymethyl cellulose is hereinafter applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium carboxymethyl cellulose is applied mutatis mutandis.

(5) Formic Acid

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 98.0 % or over of formic acid (CH_2O_2).

Physical and chemical properties:

i. This product is clear liquid with a specific acid odor.

ii. This product is miscible with water, ethanol, acetone or ether.

Confirmation test:

i. The pH of the solution of this product in water (1 → 100) is 2.0~2.3.

ii. When 1 mL of this product is added with 1 mL of ethanol and 3 drops of sulfuric acid and warmed on a water bath, it emits the odor of ethyl formate.

iii. When this product is added with lead acetate solution, white crystalline precipitation is generated. When it is added with silver nitrate solution and heated, it becomes cloudy within 30 seconds.

Purity test:

i. Residue on evaporation: When 50 g (49.5~50.4 g) of this product is evaporated on a water bath and dried at 105~110 °C to constant weight, and the residue shall be 5 mg or less (0.01 % or less).

ii. Heavy metal: 2.0 g (1.95~2.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iv. Chloride: 20 g (19.5~20.4 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.3 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these samples and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (5 mg/kg or less).

Assay: Approximately 2.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with 100 mL of newly boiled and cooled water and titrated with 1 mol/L sodium hydroxide solution (indicator: 3 drops of phenolphthalein test solution). A blank test is performed in the same way and corrections are made.

1 mol/L sodium hydroxide solution 1 mL = 46.03 mg CH_2O_2

(b) Standard of storage method

It shall be stored in an acid resistance airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of formic acid are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of formic acid is applied mutatis mutandis.

(c) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

Precautions: Handle this feed additive with care because of its low pH.

C. Preparation (Part 2: liquid)

(a) Compositional standards

This product is liquid, in which the raw material for manufacturing of formic acid is mixed with water and added with food dye if necessary.

Content: When this product is determined, it contains formic acid (CH_2O_2) corresponding to 97~103 % of the amount on the label.

Confirmation test:

i. The confirmation test i. for the raw material for manufacturing of formic acid is applied mutatis mutandis.

ii. The confirmation test ii. for the raw material for manufacturing of formic acid is applied mutatis mutandis.

iii. The confirmation test iii. for the raw material for manufacturing of formic acid is applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.5 g of formic acid (CH_2O_2) is weighed to three significant digits and the value is recorded. It is dissolved with 100 mL of newly boiled and cooled water and titrated with 1 mol/L sodium hydroxide solution (potentiometric titration).

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of formic acid is applied mutatis mutandis.

(c) Standards of the label

The standards of the label of the preparation (Part 1) of formic acid are applied mutatis mutandis.

D. Preparation (Part 3: liquid)

(a) Compositional standards

This product is liquid, in which the raw material for manufacturing of formic acid is mixed with water, mixed with ammonia (limited to one meeting the standards of food additives) at a molar ratio of ammonia to formic acid, 1:4, and if necessary added with food dye.

Content: When this product is determined, it contains formic acid (CH_2O_2) corresponding to 97~103 % of the amount on the label.

Confirmation test:

i. The pH of the solution of this product in water (1 → 100) is 2.8~3.2.

ii. The confirmation test ii. for the raw material for manufacturing of formic acid is applied mutatis mutandis.

iii. The confirmation test iii. for the raw material for manufacturing of formic acid is applied mutatis mutandis.

Assay: The assay of storage method of the preparation (Part 2) of formic acid is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of formic acid is applied mutatis mutandis.

(c) Standards of the label

The standards of the label of the preparation (Part 1) of formic acid are applied mutatis mutandis.

E. Preparation (Part 4)

(a) Compositional standards

This product is powder or particles in which the raw material for manufacturing of formic acid is added with water as appropriate, and then mixed with fillers.

Content: When this product is determined, it contains formic acid (CH_2O_2) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. 5 g (4.5~5.4 g) of this product is weighed, added with 100 mL of water, stirred for 1 minute and filtered. The pH of the filtrate is 1.6~2.1.

ii. To 1 mL of the filtrate of i., the confirmation test ii. for the raw material for manufacturing of formic acid is applied mutatis mutandis.

iii. To the filtrate of i., the confirmation test iii. for the raw material for manufacturing of formic acid is applied mutatis mutandis.

Assay: Approximately 2.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 20 mL of newly boiled and cooled water to be suspended, and titrated with 1 mol/L sodium hydroxide solution (indicator: 3 drops of phenolphthalein test solution). A blank test is performed in the same way and corrections are made.

1 mol/L sodium hydroxide solution 1 mL = 46.03 mg CH_2O_2

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of formic acid is applied mutatis mutandis.

(c) Standards of the label

The standards of the label of the preparation (Part 1) of formic acid are applied mutatis mutandis.

F. Preparation (Part 5: liquid)

(a) Compositional standards

Formic acid formulation is a liquid obtained by mixing sodium hydroxide VS [limited to those conforming to the standards for food additives (excluding the part pertaining to the clarity and color of solution); however, the term “not more than 2.0%” in the “sodium carbonate” section shall be deemed to be replaced with “not more than 3.0%”] with an active ingredient for formic acid production at a molar ratio of 5.4 to 1 (formic acid to sodium hydroxide equivalent).

Content: When this product is determined, it contains formic acid (CH_2O_2) corresponding to 97~103 % of the amount on the label.

Confirmation test:

i. The pH of the solution of this product in water (1 → 100) is 2.6~3.2.

ii. The confirmation test ii. for the raw material for manufacturing of formic acid is applied mutatis mutandis.

iii. The confirmation test iii. for the raw material for manufacturing of formic acid is applied mutatis mutandis.

Assay: The assay of storage method of the preparation (Part 2) of formic acid is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of formic acid is applied mutatis mutandis.

(c) Standards of the label

The standards of the label of the preparation (Part 1) of formic acid are applied mutatis mutandis.

(6) Glycerin Fatty Acid Ester

A. Raw material for manufacturing

(a) Compositional standards

This product is esters of fatty acids and glycerin or polyglycerin and their derivatives. This product includes glycerin fatty acid ester, glycerin lactic acid fatty acid ester, glycerin citric acid fatty acid ester, glycerin succinic acid fatty acid ester, glycerin diacetyl tartaric acid fatty acid ester, polyglycerin fatty acid ester, and polyglycerin condensed ricinoleic acid ester.

Physical and chemical properties: This product is colorless to brown powder, flakes, coarse powder, particulate or waxy blocks, semiliquid, or liquid, with no odor or a specific odor.

Confirmation test:

i. Approximately 5 g of this product is added with 50 mL of dilute potassium hydroxide-ethanol test solution and heated with a reflux condenser in a water bath for an hour, then distill ethanol until almost dryness. Then, it is added with 50 mL of hydrochloric acid (1 → 10) and shaken up well. The produced fatty acids are separated by 3 extractions with 40 mL each of a mixture of petroleum ether and methyl ethyl ketone (7:1). This aqueous layer is stirred well, added with sodium hydroxide solution (1 → 9) to make it almost neutral, and concentrated in a water bath under reduced pressure. It is added with 20 mL of methanol at approximately 40 °C, shaken up well, cooled, and filtered. Methanol of the filtrate is distilled away in a water bath. A solution of this residue in methanol (1 → 10) is used as a sample solution. Separately, 9 mL of methanol is added with 1 mL of glycerin to prepare a standard solution. 5 μL each of the sample and standard solutions are spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then, they are developed approximately 15 cm from the base line with the developing solvent, a mixture of n-butanol, methanol, and chloroform (5:3:2), and then the thin layer plate is air dried and heated at 110 °C for 10 minutes to remove the solvent. When the cooled thin layer plate is sprayed with thymol-sulfuric acid test solution, heated at 110 °C for 20 minutes to give a color, white spots are observed in the same location of the sample solution for glycerin ester, and white spots or white linear spots are observed in the same location or under the sample solution for polyglycerin ester.

ii. The petroleum ether and methyl ethyl ketone layers obtained by separation in i. are collected and the solvent is distilled away, and then oily matter or white to a yellowish-white solid remains. When 0.1 g (0.05~0.14 g) of this residue is shaken with 5 mL of ether, it is dissolved.

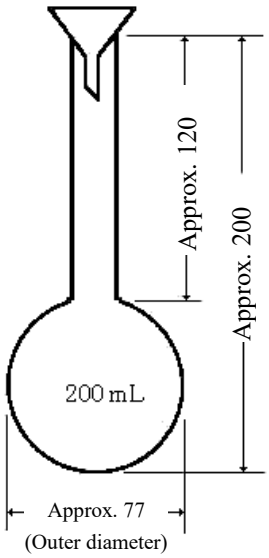
iii. Except the cases of glycerin fatty acid ester and polyglycerol ester, when 5 mL of the sample solution of i. and 50 mL of water are mixed by shaking, the resulting solution gives the reaction of lactate for glycerin lactic acid fatty acid ester, the reaction of citrate ii. for glycerin citric acid fatty acid ester, the reaction of succinate for glycerin succinic acid fatty acid ester, and the reaction of acetate and tartrate for glycerin diacetyl tartaric acid fatty acid ester.

iv. In the case of polyglycerin condensed ricinoleic acid ester, it is mixed with the petroleum ether and methyl ethyl ketone layers obtained by separation in i. and the solution is washed twice with 50 mL each of water, dehydrated with anhydrous sodium sulfate, filtered, and warmed under reduced pressure to remove the solvent. Approximately 1 g of the residue is weighed to the digits of 0.01 g, the value is recorded, and the residue is placed in a round-bottom flask as shown in the figure below. Then, it is added with 5 mL of acetic anhydride-pyridine test solution using a volumetric pipette. A small funnel is placed on the mouth of the round-bottom flask and the flask is heated in an oil bath at 95~100 °C for a drop with the height of 1 cm from the bottom immersed in the oil. It is allowed to cool and added with 1 mL of water, shaken well, further heated for 10 minutes, and allowed to cool. Then, the funnel and the neck of the flask are washed with 5 mL of ethanol and excessive volume of acetic acid is titrated with 0.5 mol/L potassium hydroxide-ethanol solution (indicator: 1 mL of phenolphthalein test solution). Separately, a blank test is performed. The hydroxyl value calculated by the following equation is 150~170. Provided, however, that the acid value is measured using approximately 0.5 g of the residue.

$$\text{Hydroxyl value} = \frac{(a-b)\times 28.05}{\text{Amount of the residue weighed (g)}} + \text{Acid value}$$

a: Consumed volume of 0.5 mol/L potassium hydroxide-ethanol solution in the blank test (mL)

b: Consumed volume of 0.5 mol/L potassium hydroxide-ethanol solution in the main test (mL)



(Unit mm)

Purity test:

i. Acid value: According to the acid value of the sample, the amount of the sample to be collected of the sample, as shown in the following table, is weighed to three significant digits and the value is recorded. It is added with 50 mL of the mixture of ethanol and ether (1:1) and as appropriate warmed to be dissolved to prepare a sample solution. It is allowed to cool and added with 0.1 mol/L potassium hydroxide-ethanol solution until the solution continuously gives a red color for 30 seconds using an indicator, phenolphthalein test solution. The acid value calculated by the following equation shall be 6.0 or less for glycerin fatty acid ester and glycerin lactic acid fatty acid ester, 12 or less for polyglycerin fatty acid ester and polyglycerin condensed ricinoleic acid ester, 100 or less for glycerin citric acid fatty acid ester, 60~120 for glycerin succinic acid fatty acid ester and glycerin diacetyl tartaric acid fatty acid ester. However, the solvent to be used is previously added with 0.1 mol/L potassium hydroxide-ethanol solution until the solution continuously gives a red color for 30 seconds using an indicator, phenolphthalein test solution.

$$\text{Acid value} = \frac{\text{Consumed volume of 0.1 mol/L potassium hydroxide-ethanol solution (mL)} \times 5.611}{\text{Amount of the residue weighed (g)}}$$

Table

Acid value	Amount of the sample to be collected
Less than 5	10 g
5 to less than 15	5 g

15 to less than 50	3 g
50 to less than 120	1 g
120 or over	0.5 g

ii. Heavy metal: 2.0 g (1.95~2.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed to prepare a sample solution by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

iv. Polyoxyethylene: 1.0 g (0.95~1.04 g) of this product is weighed, placed in a 200 mL flask, added with 25 mL of dilute potassium hydroxide-ethanol test solution. It is boiled with a ground glass joint reflux condenser in a water bath for an hour with occasional shaking. Then, it is evaporated to dryness by distilling away ethanol in a water bath or under the reduced pressure, added with 20 mL of sulfuric acid (3 → 100), shaken up well while warming, added with 15 mL of ammonium thiocyanate-cobalt nitrate test solution, shaken up well, added with 10 mL of chloroform, again shaken up and allowed to stand. The chloroform layer is not blue.

Ignition residue: 1.5 % or less (1 g)

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of glycerin fatty acid ester are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of glycerin fatty acid ester is applied mutatis mutandis.

(7) Dibutylhydroxytoluene

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 98.5 % or over of dibuthylhydroxytoluene ($C_{15}H_{24}O$)

Physical and chemical properties:

i. This product is colorless crystals or white crystalline powder or blocks, with no odor or a slight, specific odor, and with no taste.

ii. This product is easy to extremely dissolve in acetone, easy to dissolve in ethanol, and hardly dissolves in water.

Confirmation test:

i. When 5 mg (4.5~5.4 mg) of this product is added with 1~2 drops of a solution of 5-nitroso-8-oxyquinoline in sulfuric acid (1 → 100), it dissolves while becoming yellow, and then the color of the solution changes to red-brown.

ii. When 1 mL of a solution of this product in ethanol (1 → 30) is added with 3~4 drops of dilute ferric chloride test solution, the resulting solution gives no color, and it is subsequently added with small crystals of α-α′-dipyridyl, it is red. However, a dilute ferric chloride test solution which gives no color in a blank test shall be used.

Purity test:

i. Melting point: The melting point of this product shall be 69.5~71.5 °C.

ii. Freezing point: The solidifying point of this product shall be 69.0 °C or over.

iii. Clarity and color of solution: When 1.0 g (0.95~1.04 g) of this product is added with 10 mL of ethanol to be dissolved, the resulting solution shall be colorless and clear.

iv. Sulfate: 0.5 g (0.45~0.54 g) of this product is weighed, added with 30 mL of water, heated in a water bath for 5 minutes with occasional shaking, allowed to cool, filtered, and added with 1 mL of dilute hydrochloric acid and water to make 50 mL. This is used as a sample solution. When sulfate is tested, using a control solution prepared with 0.2 mL of 0.05 mol/L sulfuric acid by the sulfate test method, the turbidity of the sample solution shall not be higher than that of the control solution (0.019 % or less).

v. Heavy metal: When 0.5 g (0.45~0.54 g) of this product is weighed, dissolved with 35 mL of acetone, added with 2 mL of diluted acetic acid and water to make 50 mL, and added with 2 drops of sodium sulfide test solution, the color of the resulting solution shall not be darker than that of a solution in which 35 mL of acetone is added with 2 mL of lead standard solution, 2 mL of diluted acetic acid and water to make 50 mL and added with 2 drops of sodium sulfide test solution (40 mg/kg or less).

vi. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 3 of the arsenic test method. For the sample solution, when the arsenic test is performed by the method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

vii. *p*-cresol: 1.0 g (0.95~1.04 g) of this product is weighed, added with 10 mL of water and 1 mL of strong ammonia solution, heated in a water bath for 3 minutes with occasional shaking, is allowed to cool and is filtered. The residue on the filter paper is washed with a little water. The washings and the filtrate are mixed and added with water to make 100 mL. This solution is used as a sample solution. When 3 mL of the sample solution is measured, transferred to a Nessler tube, added with 1 mL of a solution of phosphomolybdic acid in ethanol (1 → 20) and 0.2 mL of ammonia test solution, shaken up, added with water to make 50 mL, and allowed to stand for 10 minutes, the color of the resulting solution shall not be darker than that of a solution prepared with 3 mL of *p*-cresol solution (1 → 100,000) by the same procedure for the preparation of the sample solution.

Water content: 0.20 % or less (direct titration)

Ignition Residue: 0.05 % or less (2 g)

Assay: The amount required for testing of this product is weighed to three significant digits, and tested by the freezing point measurement method.

Amount of dibutylhydroxytoluene **(**$C_{15}H_{24}O$) (%) = 1.6976 T - 18.5822

T: Freezing point

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of dibutylhydroxytoluene are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of dibutylhydroxytoluene is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of dibutylhydroxytoluene and fillers.

Content: When this product is determined, it contains dibuthylhydroxytoluene ($C_{15}H_{24}O$) corresponding to 90~110 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of dibutylhydroxytoluene is applied mutatis mutandis.

Ignition Residue: 2.4 % or less (1 g)

Assay: The assay of the raw material for manufacturing of dibutylhydroxytoluene is applied mutatis mutandis.

Amount of dibutylhydroxytoluene ($C_{15}H_{24}O$) (%) = 1.6976 T - 18.5822 - R

T: Freezing point

R: Ignition Residue (%)

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of dibutylhydroxytoluene is applied mutatis mutandis.

(8) Sucrose Fatty Acid Ester

A. Raw material for manufacturing

(a) Compositional standards

This product is an ester of fatty acids derived from animal fat and plant oil and sucrose.

Physical and chemical properties: This product is white to yellow-brown powder or blocks or colorless to slightly yellow viscous resinoids with no odor or a slight, specific odor.

Confirmation test:

i. 1 g (0.5~1.4 g) of this product is added with 25 mL of potassium hydroxide-ethanol test solution, and heated with a reflux condenser in a water bath for an hour. This solution is added with 50 mL of water and distilled until approximately 30 mL of the residue liquid. After cooling, the residue liquid is added with 10 mL of dilute hydrochloric acid, shaken up well, saturated with sodium chloride, extracted twice with 30 mL each of ether. The ether layer is collected, washed with 20 mL of saturated sodium chloride solution, and dehydrated with 2 g (1.5~2.4 g) of anhydrous sodium sulfate, and ether is distilled away and then sufficiently removed by blowing air. When the residue is cooled to 10 °C, oil drops or a colorless to pale yellow-brown solid is deposited.

ii. 2 mL of the aqueous layer, which is separated from the ether layer in the test i., is measured, transferred to a test tube, warmed in a water bath to remove ether, then allowed to cool. When 1 mL of anthrone test solution is added slowly along the wall of the tube to the solution to form a layer, the boundary surface is blue to green.

Purity test:

i. Acid value: Approximately 3 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved in 60 mL of a mixture of isopropanol and water (2:1) and titrated with 0.1 mol/L of potassium hydroxide solution (indicator: 1 mL of phenolphthalein test solution). Separately, a blank test is performed in the same way. The acid value calculated by the following equation shall be 6 or less.

Acid value =

$$\frac{\text{Consumed volume of 0.1 mol/L of potassium hydroxide solution (mL)} \times 5.611}{\text{Amount of sample weighed (g)}}$$

ii. Dimethylformamide: Approximately 10 g of this product is weighed to the digits of 0.1 g and the value is recorded. It is placed in a 200 mL flask, added with 100 mL of a test solution of 5 % sodium hydroxide in methanol. On the flask a reflux condenser is placed, and then a condenser is connected to the reflux condenser. The head of the condenser is previously immersed in 10 mL of a solution of hydrochloric acid in methanol (1 → 100) in a receiver. The flask is heated in a water bath for 30 minutes. Then, the water in the reflux condenser is removed and solution is distilled to collect 50 mL of distillate. The distillate is concentrated to almost dryness in a water bath and the residue is dissolved with 10 mL of water and transferred to a separatory funnel. The receiver is washed three times with 10 mL each of water. The washings are mixed in the solution in the separatory funnel. This solution is added with 10 mL of a mixture of carbon disulfide and chloroform (1:20) and 5 mL of aqueous ammonia solution (1 → 3) and vigorously shaken up for 20 minutes. It is added with 1 mL of copper sulfate-ammonia test solution, vigorously shaken up for 1 minute, added with 5 mL of acetic acid solution (1 → 3) and then vigorously shaken up for 1 minute. The lower layer is collected and dehydrated with anhydrous sodium sulfate. This solution is used as a sample solution. The color of this solution shall not be darker than that of a solution prepared in the way in which 10 mL of dimethylamine hydrochloride standard solution is measured and placed in a separatory funnel, added with 30 mL of water and added with 10 mL of a mixture of carbon disulfide and chloroform (1:20) and hereinafter the above mentioned procedure is performed (1 mg/kg or less).

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less)

iv. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed to prepare a sample solution by Method No. 3 of the arsenic test method. For the sample solution, when the arsenic test is performed by the method using device A, the color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

v. Free sucrose: Approximately 2 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 40 mL of n-butanol and warmed on a water bath to be dissolved and extracted twice with 20 mL each of sodium chloride solution (1 →

20). The extracts are mixed together, added with 2 mL of dilute hydrochloric acid, and heated in a water bath for 30 minutes, then allowed to cool, added with 2~3 drops of phenolphthalein test solution, neutralized with 1 mol/L sodium hydroxide test solution, and added with water to make 100 mL. This solution is used as a sample solution. 20 mL of this sample solution is measured, added with 20 mL of Bertrand's test solution A and 20 mL of Bertrand's test solution B, gently boiled for 3 minutes, and allowed to stand to precipitate cuprous oxide. At this time, the supernatant shall be indigo blue. Then, the supernatant is filtered with a glass filter. The precipitation in the flask is dissolved with 20 mL of Bertrand's test solution C, is filtered with the same glass filter as mentioned before, and washed with water. The mixture of washings and the filtrate is titrated with Bertrand's test solution D. When by the following equation the content of free sugar is determined using the amount of copper calculated from the obtained titer and the amount of invert sugar specified in the Table of Bertrand's determination of sugar, it shall be 10 % or less.

$$\text{Content of free sugar} = \frac{\text{Amount of invert sugar (mg)} \times 0.95 \times 5}{\text{Amount of the sample weighed (mg)}} \times 100\ (\%)$$

Water content: Approximately 500 mg of this product is weighed to the digits of 1 mg and the value is recorded. The amount of water obtained in the test by the back titration method of the water determination method (Karl Fischer method) shall be 4 % or less. However, the test solution is measured, transferred to a dry titration flask, added with 10 mL of methanol for Karl Fischer, added with a certain volume of Karl Fischer test solution to make it have an excess of approximately 10 mL. The flask is sealed with a stopper. The solution is stirred for 20 minutes, and then titrated with water-methanol standard solution with vigorously stirring. Separately, a blank test is performed in the same way and corrections are made.

Ignition Residue: 1.5 % or less (1 g)

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sucrose fatty acid ester are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sucrose fatty acid ester is applied mutatis mutandis.

(9) Sorbitan fFty Acid Ester

A. Raw material for manufacturing

(a) Compositional standards

This product is an ester of fatty acids derived from animal fat and plant oil and sorbitan.

Physical and chemical properties: This product is white to yellow-brown liquid, powder, flakes, particles, or waxy blocks.

Confirmation test:

i. When 0.5 g (0.45~0.54 g) of this product is added with 5 mL of absolute ethanol, heated to be dissolved, added with 5 mL of dilute sulfuric acid, heated in a water bath for 30 minutes, and cooled, oil drops or a white to yellow-white solid is deposited. When the oil drops or solid is separated, added with 5 mL of ether and shaken up, it dissolves.

ii. When 2 mL of the remaining solution after the separation of fat and oil or solid in i.is measured, added with 2 mL of newly prepared catechol solution (1 → 10), shaken up, and added with 5 mL of sulfuric acid and shaken up, the resulting solution is red to red-brown.

Purity test:

i. Acid value: Approximately 5 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with 100 mL of a mixture of ethanol and ether (1:1) by warming, allowed to cool, added with 1 mL of phenolphthalein test solution, and titrated with 0.1 mol/L potassium hydroxide-ethanol solution. Separately, a blank test is performed in the same way. The acid value calculated by the following equation shall be 15 or less.

$$\text{Acid value} = \frac{\text{consumed volume of 0.1 mol/L of potassium hydroxide-ethanol solution (mL)} \times 5.611}{\text{Amount of the sample weighed (g)}}$$

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less)

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed to prepare a sample solution by Method No. 3 of the arsenic test method. For the sample solution, when the arsenic

test is performed by the method using device A, the color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Ignition Residue: 1.5 % or less (1 g)

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sorbitan fatty acid ester are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sorbitan fatty acid ester is applied mutatis mutandis.

(10) Butylated hydroxyanisole

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 99.0 % or over of butylated hydroxyanisole ($C_{11}H_{16}O_2$)

Physical and chemical properties:

i. This product is white or slightly yellow-brown granules, blocks, or powder, with a slight, specific odor and with an acrid taste.

ii. This product is easy to extremely dissolve in ethanol or acetone and hard to extremely dissolve in water.

Confirmation test:

i. When 2~3 mL of a solution of this product in ethanol (1 → 100) is shaken up with 2~3 drops of sodium borate solution (1 → 50) and small crystals of 2,6-dibromquinonechloroimide, the color of the resulting solution is livid.

ii. When 1 mL of a solution of this product in ethanol (1 → 30) is added with 3~4 drops of dilute ferric chloride test solution, the resulting solution gives no color, and it is subsequently added with small crystals of α-α'-dipyridyl, it is red. However, a dilute ferric chloride test solution which gives no color in a blank test shall be used.

Purity test:

i. Melting point: The melting point of this product shall be 62~65 °C.

ii. Clarity and color of solution: When 0.5 g (0.45~0.54 g) of this product is dissolved with 10 mL of ethanol, the solution shall be colorless and clear.

iii. Sulfate: 0.5 g (0.45~0.54 g) of this product is weighed, dissolved with 35 mL of acetone, added with 1 mL of dilute hydrochloric acid and water to make 50 mL. This is used as a sample solution. 0.2 mL of 0.005 mol/L sulfuric acid is added with 35 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL by the sulfate test method. This is used as a control solution. When sulfate is tested, using the control solution, the turbidity of the sample solution shall not be higher than that of the control solution (0.019 % or less).

iv. Heavy metal: When 0.5 g (0.45~0.54 g) of this product is weighed, dissolved with 35 mL of acetone, added with 2 mL of diluted acetic acid and water to make 50 mL. This is used as a sample solution. The color of the sample solution shall not be darker than that of a solution in which 35 mL of acetone is added with 2 mL of lead standard solution, 2 mL of diluted acetic acid and water to make 50 mL (40 mg/kg or less).

v. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 3 of the arsenic test method. For the sample solution, when the arsenic test is performed by the method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

vi. Para hydroxyanisole: 1.0 g (0.95~1.04 g) of this product is weighed and dissolved with 20 mL of a mixture of ether and petroleum benzin (1:1). This solution is added with 10 mL of water and 1 mL of 1 mol/L sodium hydroxide test solution, shaken up, and allowed to stand. Of the top and bottom layers, the bottom layer is collected. It is added with 20 mL of a mixture of ether and petroleum benzin (1:1), shaken up, and allowed to stand, and the bottom layer is collected and added with water to make 500 mL. 1 mL of this solution is transferred to a Nessler tube and added with 2 mL of 1 mol/L sodium hydroxide test solution, 5 mL of boric acid solution (3 → 100) and water to make 30 mL. When this is added with 5 mL of 4-aminoantipyrine solution (1 → 1,000), shaken up, then added with 1 mL of potassium ferricyanide solution (1 → 100), shaken up, added with water to make 50 mL and allowed to stand for 15 minutes, the color of the resulting solution shall not be darker than that of a solution prepared by which 0.6 mL of the color control stock solution of cobalt chloride is added with water to make 50 mL (approximately 0.5 % or less).

Water content: 0.30 % or less (direct titration)

Ignition Residue: 0.05 % or less (2 g)

Assay: Approximately 0.3 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 50 mL of acetone, added with 50 mL of water and 20 mL of dilute sulfuric acid, and titrated with 0.1 mol/L ceric ammonium sulfate solution

(indicator: 0.2 mL of *o*-phenanthroline test solution). A blank test is performed in the same way and corrections are made.

0.1 mol/L ceric ammonium sulfate solution 1 mL = 9.012 mg $C_{11}H_{16}O_2$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of butylated hydroxyanisole are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of butylated hydroxyanisole is applied mutatis mutandis.

(11) Propionic acid

A. Raw material for manufacturing

(a) Compositional standards

Contents: When this product is determined, it contains 99.0 % or more of propinic acid ($C_3H_6O_2$).

Physical and chemical properties:

i. This product is a clear liquid with specific odor.

ii. This product is easy to extremely dissolve in water and easy to dissolve in alcohol, ether, chloroform and acid.

iii. The pH of solution (1 → 100) of this product is 2.0~2.5.

Confirmation test:

i. The solution (1 → 10) of this product is acidic.

ii. When 1 mL of this product is added with 1 mL of ethanol and 3 drops of sulfuric acid and heated in a water bath, it emits the odor of ethyl propionate.

Purity test:

i. Residue on evaporation: When 50 mL of this product is evaporated on a water bath, and dried to a constant weight at 105~110 °C, the residue shall be 5 mg or less (0.01 % or less).

ii. Arsenic: 1 mL of this product is weighed and a sample solution is prepared by Method No.2 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 μg/mL or less).

iii. Heavy metal: 2 mL of this product is weighed and a sample solution is prepared by Method No.1 of the heavy metal test method. A control solution is prepared using 2 mL of lead standard solution. When the sample and control solution are tested for heavy metal, the color of the sample solution shall not be darker than the standard color (10 μg/mL or less).

Assay: Approximately 3 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with 100 mL of newly boiled and cooled water and titrated with 1 mol/L sodium hydroxide solution (indicator: 3 drops of phenolphthalein test solution). A blank test is performed in the same way and corrections are made.

1 mol/L sodium hydroxide solution 1 mL = 74.08 mg $C_3H_6O_2$

(b) Standard of storage method

It shall be stored in a airtight container.

B. Preparation (Part1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of propionic acid are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of propionic acid is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is piece or powder of the mixture of the raw material for manufacturing of propionic acid and fillers.

Contents: When this product is determined, it conteins propinic acid equivalent to 95~105 % of declared content.

Confirmation test: According to the amount of this product on the label, the amount containing approximately 2 g of the raw material for manufacturing of propionic acid is weighed, added with 20 mL of ethanol, boiled, and filtered. When 1 mL of the filtrate is measured, added with 3 drops of sulfuric acid and heated in a water bath, it emits the odor of ethyl propionate.

Assay: The amount of this product containing approximately 2.0 g of propionic acid ($C_3H_6O_2$) is weighed to three significant digits and the value is recorded. It is placed in a 1 L distillation flask and added with 200 mL of water, 80 g (79.5~80.4 g) of sodium chloride, 10 mL of 10% phosphoric acid solution, and a drop of silicone emulsion. The 1 L distillation flask is connected to a distillation device. A 500 mL volumetric flask is used as a receiver, added with 20 mL of water, and the bottom of the condenser is immersed in

this solution. When the distillate from water vapor approaches 500 mL, the distillation is stopped and water is added to the graduation line to make 500 mL. Ater shakeing it well, 50 mL of it is measured using a volumetric pipette and titrated with 0.1 mol/L sodium hydroxide solution (indicator: 3 drops of phenolphthalein test solution). A blank test is performed in the same way and corrections are made.

0.1 mol/L sodium hydroxide solution 1 mL = 7.408 mg $C_3H_6O_2$

(b) Standard of storage method

It shall be stored in a capped container.

(12) Calcium propionate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried at 120 °C for 2 hours and then determined, it contains 98.0 % or more of calcium propionate ($C_6H_{10}CaO_4$).

Physical and chemical properties:

i. This product is white crystals, granules or powder, with no odor, or a slight specific odor.

ii. This product is easy to dissolve in water.

Confirmation test:

i. When 0.5 g (0.45~0.54 g) of this product is dissolved with 5 mL of water, and added with 5 mL of dilute sulfuric acid and heated, it emits a specific odor.

ii. A solution of this product in water (1 → 10) gives the qualitative reaction of calcium salt i., ii., and iii..

Purity test:

i. Water-insoluble matter: 10.0 g (9.95~10.04 g) of this product is weighed, added with 100 mL of water, allowed to stand for an hour with occasional shaking, and filtered with a glass filter (G4). When the residue is washed with 30 mL of water and dried at 180 °C for 4 hours, its amount shall be 0.015 g or less.

ii. Acid and alkali: 2.0 g (1.95~2.04 g) of this product is weighed, dissolved with 20 mL of newly boiled and cooled water, added with 2 drops of phenolphthalein test solution to prepare a sample solution.When a sample solution is colorless, added with 0.3 mL of 0.1 mol/L sodium hydroxide solution, it changes red. When a sample solution is red color, added with 0.3 mL of 0.1 mol/L hydrochloric acid, the color disappears.

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No.1 of the heavy metal test method. A control solution is

prepared using 2 mL of lead standard solution. When the sample and control solution are tested for heavy metal, the color of the sample solution shall not be darker than the standard color (20 mg/kg or less).

iv. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No.1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 9.5 % or less (1 g, 120 °C, 2 hours)

Assay: This product is dried at 120 °C for 2 hours, approximately 1 g of it is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask, and added with water to graduation line to make 100 mL. 25 mL of this solution is measured using a volumetric pipette, added with 75 mL of water and 15 mL of potassium hydroxide solution (1 → 10), allowed to stand for approximately 1 minute, added with 0.1 g (0.05~0.14 g) of NN indicator, and titrated with 0.05 mol/L ethylenediaminetetraacetic acid disodium solution within 30 seconds. In this case, the end point of the titration is the point at which the color of the solution changes from reddish violet to blue. A blank test is performed in the same way and corrections are made.

0.05 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL = 9.311 mg$C_6H_{10}CaO_4$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 1 liquid)

(a) Compositional standards

This product is water-soluble liquid, a mixture of the raw material for manufacturing of calcium propionate and water.

Contents: When this product is determined, it contains calcium propionate ($C_6H_{10}CaO_4$) corresponding to 95~105 % of the amount of the label.

Confirmation test:

i. When, according to the amount of this product on the label, the amount containing approximately 0.5 g of the raw material for manufacturing of calcium propionate is weighed and heated with 5 mL of dilute sulfuric acid, it emits a specific odor.

ii. This product gives the qualitative reaction of calcium salt i., ii., and iii..

Assay: According to the amount of this product on the label, the amount containing approximately 1 g of the raw material for manufacturing of calcium propionate is weighed to three significant digits and the value is recorded. It is placed in a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. 25 mL of this solution is measured using a volumetric pipette, added with 75 mL of water and 15

mL of potassium hydroxide solution (1 → 10), allowed to stand for approximately 1 minute, added with 0.1 g (0.05~0.14 g) of NN indicator, and titrated with 0.05 mol/L ethylenediaminetetraacetic acid disodium solution within 30 seconds. In this case, the end point of the titration is the point at which the color of the solution changes from reddish violet to blue. A blank test is performed in the same way and corrections are made.

0.05 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL = 9.311 mg $C_6H_{10}CaO_4$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium propionate is applied mutatis mutandis.

C. Preparation (Part 2 powder)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of calcium propionate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium propionate is applied mutatis mutandis.

(13) Sodium propionate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried at 105 °C for 1 hours and then determined, it contains 99.0 % or more of sodium propionate ($C_3H_5NaO_2$).

Physical and chemical properties:

i. This product is white crystals, granules or crystalline powder, with no odor, or a slight specific odor.

ii. This product is easy to dissolve in water and easy to slightly dissolve in ethanol.

iii. This profuct is hygroscopic.

Confirmation test:

i. The confirmation test i. of calcium propionate is applied mutatis mutndis.

ii. The solution (1 → 10) of this product gives the qualitative reaction of sodium salt i.

Purity test:

i. Clarity and color of solution: When 1.0 g (0.95~1.04 g) of this product is weighed, dissolved with 20 mL of water, the resulting solution shall be colorless and the turbidity shall be delicately slight cloudiness or clearer.

ii. Acid and alkali: 2.0 g (1.95~2.04 g) of this product is weighed, dissolved with 20 mL of newly boiled and cooled water, added with 2 drops of phenolphthalein test solution to prepare a sample solution. When a sample solution is colorless, added with 0.3 mL of 0.1 mol/L sodium hydroxide solution, it changes red. When a sample solution is red color, added with 0.3 mL of 0.1 mol/L hydrochloric acid, the color disappears.

iii. Heavy metal: 1.0 g (0.95~1.04) of this product is weighed and a sample solution is prepared by Method No.1 of the heavy metal test method. A control solution is prepared using 2.0 mL of lead standard solution. When the sample and control solution are tested for heavy metal, the color of the sample solution shall not be darker than the standard color (20 mg/kg or less).

iv. Arsenic: 1.0 g (0.95~1.04) of this product is weighed and a sample solution is prepared by Method No.1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 5.0 % or less (1 g, 105 °C, 1 hours)

Assay: This product is dried at 105 °C for 1 hours, approximately 0.2 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is added with 40 mL of glacial acetic acid for nonaqueous titration, warmed to be dissolved, allowed to cool, and titrated with 0.1 mol/L perchloric acid (indicator: 3 drops of methylrosanilinium chloride test solution). A blank test is performed in the same way and corrections are made.

0.1 mol/L perchloric acid 1 mL = 9.606 mg $C_3H_5NaO_2$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium propionate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium propionate is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

The product is powder of the mixture of the raw material for manufacturing of sodium propionate and fillers.

Contents: When this product is determined, it contains sodium propionate ($C_3H_5NaO_2$) corresponding to 90~110 % of of the amount on the label.

Confirmation test:

i. 0.5 g (0.45~0.54 g) of this product is added with 5mL of water, stirred well and filtered.When the filtrate is added with 5 mL of dilute sulfuric acid and heated, it emits a specific odor.

ii. When 1 g (0.5~1.4 g) of this product is added with 10 mL of water, stirres, and filtered, the filtrate gives the qualitative reaction of sodium salt i..

Assay: This product is dried at 105 °C for an hour, approximately 5 g of it is weighed to the digits of 0.01 g, and the value is recorded. It is added with approximately 60 mL of water, stirred well, and filtered. The filtrate is collected directly in a 100 mL volumetric flask. The beaker used to dissolve the sample is washed with approximately 30 mL of water and the solids on the filter are washed with the washings. The filtrate and the washings are mixed and added with water to the 100 mL volumetric flask to the graduation line. This is used as a sample solution.

20 mL of the sample solution is measured using a volumetric pipette, and placed in a porcelain or platinum pot with a diameter of 50~55 mm, gently heated to evaporate water, and then gradually increasing temperature, heated for approximately 2 hours to be completely carbonized. The heating temperature shall be the temperature at which the heated pot is dark red (300~400 °C). In this case, the flame of the burner shall not contact the carbide. After cooling, the carbide in the pot is crushed well with a glass bar and put in a beaker along with the pot. It is added with approximately 50 mL of water, added with 50 mL of 0.25 mol/L sulfuric acid, covered with a watch glass, heated on a water bath for an hour, and filtered. If the filtrate is colored, the sample is newly weighed and sufficiently carbonized. The residues in the beaker and the pot and on the filter are washed well with warm water. When the washings do not change blue litmus paper to red, the washings are mixed with the filtrate and excessive acid is titrated with 0.5 mol/L sodium hydroxide solution (indicator: 3 drops of methyl red test solution). A blank test is performed in the same way.

0.25 mol/L sulfuric acid 1 mL = 48.03 mg $C_3H_5NaO_2$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium propionate is applied mutatis mutandis.

(14) Propylene glycol

A. Raw material for manufacturing

(a) Compositional standards

Contents: When this product is determined, it contains 97.5 % or more of propinic acid ($C_3H_8O_2$).

Physical and chemical properties:

i. This product is colorless, transparent, syrupy liquid, with no odor or a slight, specific odor, and with a slight bitter taste.

ii. This product mix with water, aceton, ethanol or chloroform.

iii. This product is easy to dissolve in ether.

iv. This product is hygroscopic.

Confirmation test:

i. 0.5 g (0.45~0.54 g) of this product is added with 5 mL of pyridine and 3.6 g (3.55~3.64 g) of triphenyl chloromethane, heated on a water bath for an hour with a reflux condenser, allowed to cool, dissolved with 100 mL of warm acetone, added with 0.1 g (0.05~0.14 g) of activated charcoal, shaken up well and filtered. The filtrate is evaporated to dryness on a water bath to 50 mL and allowed to stand in a refrigerator overnight. The produced crystals are collected by filtration and recrystallized three times with very little acetone until the odor of pyridine disappears. When these are dried at 105 °C for 30 minutes, the melting point is 173~179 °C.

ii. When 1 mL of this product is added with 0.5 g (0.45~0.54 g) of potassium hydrogen sulfate and gently heated, it emits a fruit-like odor.

Purity test:

i. Specific gravity: The specific gravity of this product d_{20}^{20} shall be 1.036~1.040.

ii. Acid: When 10 mL of this product is added with 50 mL of newly boiled and cooled water to mix well, added with 5 drops of phenolphthalein test solution and 0.30 mL of 0.1 mol/L sodium hydroxide solution, the resulting solution shall be red.

iii. Chloride: 2.0 g (1.95~2.04 g) of this product is weighed and a sample solution is prepared by chloride test method. A control solution is used 0.4mL of 0.01 mol/L hydrochloric acid. When the sample and control solution are tested for chloride, the turbidity of the sample solution shall not be higher than that of control solution (0.007 % or less).

iv. Heavy metal: 5.0 g (4.95~5.04 g) of this product is weighed and a sample solution is prepared by Method No.1 of the heavy metal test method. A control solution is prepared using 2.5 mL of lead standard solution. When the sample and control solution are tested for heavy metal, the color of the sample solution shall not be darker than the standard color (5 mg/kg or less).

v. Arsenic: 0.7 g (0.65~0.74 g) of this product is weighed and a sample solution is prepared by Method No.1 of the arsenic test method. The arsenic test is performed by

the method using device A. The color of absorbing solution shall not be darker than the standard color (3 mg/kg or less).

vi. Glycerin and ethylene glycol: Approximately 1 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with water to make 1,000 mL, of which 13 mL is measured, added with 0.2 g (0.15~0.24 g) of potassium periodate and 1 mL of sulfuric acid, and further added with 50 mL of water and distilled at a rate of 3-5 mL/min. It is continued distillation until the residual solution is about 1 mL.The distillate is collected in a receiver cooled in ice water. The distillate is added with water to make 500 mL to prepare a sample solution. When 1 mL of the sample solution is measured, added with 0.1 g (0.05~0.14 g) of chromotropic acid and 5 mL of sulfuric acid, heated in a water bath for 30 minutes, cooled, and added with water to make 250 mL, the color of the solution shall not be darker than a solution obtained by the same procedure for preparation of a sample solution using 1 mL of formaldehyde standard solution.

Water content: 0.20 % or less (direct titration)

Ignition residue: Approximately 20 g of this product is weighed to the digits of 0.1 g and the value is recorded. It is gently heated to boil and then the heating is stopped. It is ignited within 30 seconds to burn and allowed to cool. When the residue is moisturized with 0.2 mL of sulfuric acid and tested by the ignition residue test method, the amount shall be 0.05 % or less.

Distillation test: 184~189 °C, 95 v/v% or more

Assay: Approximately 1 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with water, transferred to a 250 mL volumetric flask, and added with water to the graduation line to make 250 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a stoppered flask, added with 10 mL of sodium periodate test solution using a volumetric pipette, further added with 4 mL of sulfuric acid (1 → 2), shaken up, and allowed to stand for 40 minutes. It is added with 5 g (4.5～5.4 g) of potassium iodide, sealed with a stopper within 30 seconds, shaken up well, allowed to stand in a dark place for 5 minutes, added with 50 mL of 0.1 mol/L sodium thiosulfate solution using a volumetric pipette, and titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 1 mL of starch test solution). Separately, a blank test is performed in the same way.

0.1 mol/L sodium thiosulfate solution 1 mL = 3.805 mg $C_3H_8O_2$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of proplene glycol are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of proplene glycol is applied mutatis mutandis.

(15) Sodium polyacrylate

A. Raw material for manufacturing

(a) Compositional standards

Physical and chemical properties: This product is white powder with no odor.

Confirmation test:

i. 0.2 g (0.15~0.24 g) of this product is added with 100 mL of water, then stirred well to dissolve. This is used as a sample solution. When 10 mL of this sample solution is added with 1 mL of calcium chloride test solution and shaken up, white precipitation is generated within 30 seconds.

ii. The residue obtained by ashing of this product gives the qualitative reaction of sodium salt.

Purity test:

i. Free alkali: 0.2 g (0.15~0.24 g) of this product is added with 60 mL of water, stirred well to dissolve, added with 3 mL of calcium chloride test solution, heated on a water bath for approximately 20 minutes, is allowed to cool and is filtered. The residue on the filter is washed with water, and the washings are mixed with the filtrate and added with water to make 100 mL to prepare a sample solution. When 50 mL of the sample solution is measured and added with 2 drops of phenolphthalein test solution, it shall not be red.

ii. Sulfate: 20 mL of the sample solution i. is measured, added with 1 mL of dilute hydrochloric acid and water to make 50 mL. This is used as a sample solution. When sulfate is tested using a control solution prepared with 0.4 mL of 0.005 mol/L sulfuric acid using the sulfate test method, the turbidity of the sample solution shall not be higher than that of the control solution (0.49 % or less).

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No.2 of the heavy metal test method. A control solution is prepared using 2.0 mL of lead standard solution. When the sample and control solution

are tested for heavy metal, the color of the sample solution shall not be darker than the standard color (20 mg/kg or less).

iv. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No.3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

v. Residual monomer: Approximately 1 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is placed in a 300 mL iodine bottle, added with 100 mL of water, shaken up occasionally, and allowed to stand for 24 hours to dissolve. 10 mL of potassium bromate-potassium bromide test solution using a volumetric pipette, shaken up well, quickly added with approximately 10 mL of hydrochloric acid, and a stopper is closed within 30 seconds to be shaken up well. Approximately 20 mL of potassium iodide test solution is put in the upper part of the iodine bottle and allowed to stand in a dark place for 20 minutes. Then, the stopper is loosened, the potassium iodide test solution is poured, and the stopper is closed within 30 seconds to be shaken up well. Then, it is titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 2 mL of starch test solution). Separately, a blank test is performed in the same way. The amount of the residual monomer calculated using the following equation shall be 1 % or less.

$$\text{Amount of residual monomer} = \frac{0.0047 \times (a - b)}{\text{Amount of the sample weighed (g)}} \times 100\ (\%)$$

a: Consumed volume of 0.1 mol/L sodium thiosulfate solution in the blank test (mL)

b: Consumed volume of 0.1 mol/L sodium thiosulfate solution in the main test (mL)

vi. Low polymer: 2 g (1.95~2.04 g) of this product is added with 200 mL of water, shaken up occasionally, allowed to stand for 24 hours to dissolve. In this solution 50 mL of 10 mol/L hydrochloric acid is added dropwise with stirring, warmed in a water bath at 40 °C for 30 minutes with stirring and then is allowed to stand for 24 hours. The generated precipitation is filtered and the filtrate is added with a drop of phenolphthalein test solution, added with sodium hydroxide solution (2 → 5) until the filtrate is slightly red, and then added with hydrochloric acid (1 → 30) by dropping until the red color disappears. 200 mL of water is added, and 25 mL of calcium chloride test solution is dropped with stirring and heated in a water bath at approximately 40 °C for 30 minutes with stirring. The generated precipitation is filtered by suction using a glass filter (1G4) whose mass is known. The residue is washed three times with approximately 10 mL each of water, dried at 105 °C for 4

hours. The amount of the lower polymer calculated using the following equation shall be 5 % or less.

$$\text{Low polymer} = \frac{\text{Residue (g) x 1.0324}}{\text{Amount of the sample weighed (g)}} \text{ x 100 (\%)}$$

Loss on dring: 10 % or less (1.5g, 105°C, 4 hours)

Ignition residue: After drying 76% or less (1 g)

(b) Standard of storage method

It shall be stored in a capped container avoiding a moisture.

B. Preparation (Part1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium polyacrylate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium polyacrylate is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

The product is powder of the mixture of the raw material for manufacturing of sodium polyacrylate and fillers.

Confirmation test: According to the amount of this product on the label, the amount containing approximately 0.2 g of the raw material for manufacturing of sodium polyacrylate is weighed and dried at 140 °C for an hour. This preparation is placed in a separatory funnel, added with 100 mL of carbon tetrachloride, vigorously shaken up, and allowed to stand for 5 minutes to separate the bottom precipitation layer (layer containing sodium polyacrylate). The separated layer is added with 50 mL of a mixture of ethanol and water (1:1), stirred well, and allowed to stand for a few minutes, and the upper layer is removed by suction. At this time, sodium polyacrylate is separated to the middle zonal layer. Then the middle layer is filtered by suction using a glass filter (G3). The precipitation in the bottom layer is discarded. Then the residue on the funnel is transferred to a 200 mL erlenmeyer flask, dried, added with 100 mL of water, and stirred well to dissolve. The confirmation test i. and ii. of the raw material for manufacturing of sodium polyacrylate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium polyacrylate is applied mutatis mutandis.

(16) Polyoxyethylene Glycerol Fatty Acid Ester

A. Raw material for manufacturing

(a) Compositional standards

This product is a polyoxyethylene adduct of an ester of fatter acids derived from animal fat and plant oil and glycerin.

Physical and chemical properties: This product is a white to yellow-brown liquid, semiliquid, or waxy blocks, with no odor or a slight, specific odor.

Confirmation test:

i. When 0.5 g (0.45~0.54 g) of this product is added with 5 mL of absolute ethanol, heated to dissolve, added with 5 mL of dilute sulfuric acid, heated in a water bath for 30 minutes, and then cooled, oil drops or a white to yellow-white solid is deposited. When the oil drops or solid is separated, added with 5 mL of ether and shaken up, it dissolves.

ii. When 1 g (0.5~1.4 g) of this product is added with 20 mL of water, warmed, shaken up well, added with 10 mL of ammonium thiocyanate-cobalt nitrate test solution, shaken up well, added with 10 mL of chloroform, shaken up, and allowed to stand, the chloroform layer is blue.

Purity test:

i. Acid value: Approximately 5 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 100 mL of a solution of ethanol and ether (1:1), warmed to dissolve, allowed to cool, added with 1 mL of phenolphthalein test solution, and titrated with 0.1 mol/L potassium hydroxide-ethanol solution. Separately, a blank test is performed in the same way. The acid value calculated by the following equation shall be 6 or less.

$$\text{Acid value} = \frac{\text{Consumed volume of 0.1 mol/L potassium hydroxide-ethanol solution (mL)} \times 5.611}{\text{Amount of the sample weighed (g)}}$$

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

iv. 1,4-Dioxane: The peak height of this product obtained from the test by the 1,4-Dioxane test method shall not be higher than that of 0.2 mL of the measurement standard solution of 1,4-Dioxane (10 mg/kg or less).

Ignition residue: 1.5 % or less (1 g)

(b) Standard of storage method

It shall be stored in a lightproof sealed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of polyoxyethylene glycerolin fatty acid ester are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of polyoxyethylene glycerolin fatty acid ester is applied mutatis mutandis.

(17) Polyoxyethylene Sorbitan Fatty Acid Ester

A. Raw material for manufacturing

(a) Compositional standards

This is a polyoxyethylene adduct of an ester of fatter acids derived from animal fat and plant oil and sorbitan.

Physical and chemical properties: This product is a yellow to brown liquid, semiliquid or waxy blocks.

Confirmation test:

i. When 0.5 g (0.45~0.54 g) of this product is added with 5 mL of absolute ethanol, heated to dissolve, added with 5 mL of dilute sulfuric acid, heated in a water bath for 30 minutes, and then cooled, oil drops or a white to yellow-white solid is deposited. When the oil drops or solid is separated, added with 5 mL of ether and shaken up, it dissolves.

ii. When 1 g (0.95~1.04 g) of this product is added with 20 mL of water, warmed, shaken up well, allowed to cool, added with 10 mL of ammonium thiocyanate-cobalt nitrate test solution, shaken up well, added with 10 mL of chloroform, shaken up, and allowed to stand, the chloroform layer is blue.

Purity test:

i. Acid value: Approximately 5 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 100 mL of a solution of ethanol and ether (1:1), warmed to dissolve, allowed to cool, added with 1 mL of phenolphthalein test solution,

and titrated with 0.1 mol/L potassium hydroxide-ethanol solution. Separately, a blank test is performed in the same way. The acid value calculated by the following equation shall be 6 or less.

$$\text{Acid value} = \frac{\text{Consumed volume of 0.1 mol/L potassium hydroxide-ethanol solution (mL)} \times 5.611}{\text{Amount of the sample weighed (g)}}$$

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

iv. 1,4-Dioxane: Approximately 5 g of this product is weighed and the peak height of this product obtained from the test by the 1,4-Dioxane test method shall not be higher than that of 0.2 mL of the measurement standard solution of 1,4-Dioxane (10 mg/kg or less).

Ignition residue: 1.5 % or less (1 g)

(b) Standard of storage method

It shall be stored in a lightproof sealed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of polyoxyethylene sorbitan fatty acid ester are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of polyoxyethylene sorbitan fatty acid ester is applied mutatis mutandis.

(18) L-ascorbic Acid

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried in a desiccator (silica gel) for 24 hours and determined, it contains 99.9 % or more of L-ascorbic acid ($C_6H_8O_6$).

Physical and chemical properties:

i. This product is white crystals or crystalline powder with no odor and with a sour taste.

ii. This product is easy to dissolve in water, hard to slightly dissolve in ethanol, and hardly dissolves in ether, chloroform, benzene, or petroleum ether.

iii. The pH of the solution of this product in water (1 → 50) is 2.4~2.8.

iv. Melting point: About 190 °C (decomposition)

Confirmation test:

i. 5 mL each of a solution of this product in water (1 → 50) is measured. When 1 drop of potassium permanganate test solution is added to the solution and when 1~2 drops of 2,6-dichlorophenolindophenol sodium test solution is added to it, the color of the both test solutions disappears within 30 seconds.

ii. 0.1 g (0.05~0.14 g) of this product is dissolved in 100 mL of metaphosphoric acid solution (1 → 50) and 5 mL of this solution is measured. Iodine test solution is dropped in the solution until it is slightly yellow and then a drop of copper sulfate solution (1 → 1,000) and a drop of pyrrole are added to it. When the resulting solution is warmed in a water bath at 50 °C for 5 minutes, it is blue.

Purity test:

i. Specific rotation: Approximately 2.5 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved in water to make 25 mL. The optical rotation of this solution shall be $[\alpha]_D^{20} = +20.5° \sim +21.5°$.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

Loss on drying: 0.20 % or less (1 g, silica gel, 24 hours)

Ignition residue: 0.10 % or less (1 g)

Assay: This product is dried in a desiccator (silica gel) for 24 hours, approximately 0.2 g of it weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 50 mL of metaphosphoric acid solution (1 → 50) and titrated with 0.1 mol/L iodine solution (indicator: 1 mL of starch test solution).

0.05 mol/L iodine solution 1 mL = 8.806 mg $C_6H_8O_6$

(b) Standard of storage method

It shall be stored in a lightproof tightly sealed container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-ascorbic acid are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-ascorbic acid is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of L-ascorbic acid and carrier.

Content: When this product is determined, it contains L-ascorbic acid ($C_6H_8O_6$) corresponding to 90~120 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of L-ascorbic acid is weighed, added with 50 mL of water and for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats added with 15 mL of chloroform, shaken up, and allowed to stand. The aqueous layer is filtered to prepare a sample solution and 5 mL of this solution is measured. The confirmation test i. of the raw material for manufacturing of L-ascorbic acid is hereinafter applied mutatis mutandis.

ii. 1 mL of the test solution i. is added with 19 mL of metaphosphoric acid solution (1 → 50) to mix them. 5 mL of this solution is measured and the confirmation test ii. of the raw material for manufacturing of L-ascorbic acid is hereinafter applied mutatis mutandis.

Assay: Method No. 1 is used for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats and Method No. 2 is used for others.

Method No. 1: The amount of this product containing approximately 0.1 g of L-ascorbic acid ($C_6H_8O_6$) is weighed to three significant digits and the value is recorded. It is added with 50 mL of chloroform, shaken up, and filtered using a glass filter (G4). The residue on the filter is washed three times with 20 mL each of chloroform. When the chloroform odor on the residue disappears, the residue is filtered by extracting with 190 mL of metaphosphoric acid-acetic acid test solution. The filtrate is placed in a 200 mL volumetric flask, added with metaphosphoric acid-acetic acid test solution to the graduation line to make 200 mL. This is used a sample solution. 2 mL of the sample solution is measured using a volumetric pipette, added with 8 mL of metaphosphoric acid-acetic acid test solution and 2 mL of hydrogen peroxide test solution, shaken up,

and titrated with 2,6-dichlorophenolindophenol sodium solution for titration until pink color of the solution persists for 5 seconds. A blank test is performed in the same way and corrections are made.

2,6-dichlorophenolindophenol sodium test solution for titration 1 mL = A mg $C_6H_8O_6$

A: Amount of L-ascorbic acid ($C_6H_8O_6$) corresponding to 1 mL of 2,6-dichlorophenolindophenol sodium test solution for titration

The reference standard of L-ascorbic acid is dried in a desiccator (silica gel) for 24 hours. Approximately 0.05 g of it is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with metaphosphoric acid-acetic acid test solution and transferred to a 100 mL volumetric flask, and further added with metaphosphoric acid-acetic acid test solution to the graduation line to make 100 mL, and hereinafter subjected to the same procedure for the sample solution to obtain A.

Method No. 2: The amount of this product containing approximately 0.1 g of L-ascorbic acid ($C_6H_8O_6$) is weighed to three significant digits and the value is recorded. It is added with 150 mL of metaphosphoric acid-acetic acid test solution, vigorously shaken up and filtered. The residue on the filter is washed twice with 15 mL each of metaphosphoric acid-acetic acid test solution. The filtrate and the washings are mixed, the mixture is placed in a 200 mL volumetric flask and added with metaphosphoric acid-acetic acid test solution to the graduation line to make 200 mL, and hereinafter the method No. 1 is applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a lightproof sealed container.

(19) Calcium L-Ascorbate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 98.0 % or more of Calcium L-ascorbate ($C_{12}H_{14}CaO_{12}\cdot 2H_2O$).

Physical and chemical properties:

i. This product is white to yellowish-white crystal powder with no odor.

ii. This product is easy to dissolve in water, and hardly dissolves in acetone, ether or methanol.

Confirmation test:

i. When 2 ml of the solution of this product in water (1 → 100) is measured and added with 4 mL of 0.1 mol/L hydrochloric acid, 5~6 drops of sodium nitroprusside test

solution and 2 drops of 1 mol/L sodium hydroxide test solution, the resulting solution is blue within 30 seconds.

ii. 0.1 g (0.05~0.14 g) of this product is dissolved with 100 mL of metaphosphoric acid solution (1 → 50). When iodine test solution is dropped in 5 mL of this solution until the solution is slightly yellow, added with a drop of copper sulfate solution (1 → 1,000) and a drop of pyrrole, heated at 50 °C for 5 minutes, it is blue to blue-green.

iii. The solution of this product in water (1 → 10) gives the qualitative reaction of calcium salt.

Purity test:

i. Specific rotation: Approximately 2.5 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved in water to make 100 mL. The rotation of this solution shall be $[\alpha]_D^{20} = +95° \sim +97°$.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Ignition residue: 30.0~33.0 % (1 g)

Assay: Approximately 0.2 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 50 mL of metaphosphoric acid solution (1 → 50) and titrated with 0.05 mol/L iodine solution (indicator: 1 mL of starch test solution).

0.05 mol/L iodine solution 1 mL = 10.66 mg $C_{12}H_{14}CaO_{12}\cdot 2H_2O$

(b) Standard of storage method

It shall be stored in a lightproof tightly sealed container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of calcium L-ascorbate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium L-ascorbate is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of calcium L-ascorbate and carrier.

Content: When this product is determined, it contains calcium L-ascorbate ($C_{12}H_{14}CaO_{12}\cdot 2H_2O$) corresponding to 90~120 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.15 g of the raw material for manufacturing of calcium L-ascorbate is weighed and added with 15 mL of water, and for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats added with 5 mL of chloroform, is shaken up and allowed to stand. The aqueous layer is filtered to prepare a sample solution. 2 mL of this solution is measured and the confirmation test i. of the raw material for manufacturing of calcium L-ascorbate is hereinafter applied mutatis mutandis.

ii. 1 mL of the sample solution i. is added with 9 mL of metaphosphoric acid solution (1 → 50) to mix them. 5 mL of the solution is measured and the confirmation test ii. for the raw material for manufacturing of calcium L-ascorbate is hereinafter applied mutatis mutandis.

Assay: Method No. 1 is used for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats and Method No. 2 is used for others.

Method No. 1: The amount of this product containing approximately 0.1 g of calcium L-ascorbate ($C_{12}H_{14}CaO_{12}\cdot 2H_2O$) is weighed to three significant digits and the value is recorded. It is added with 50 mL of chloroform, shaken up, and filtered using a glass filter (G4). The residue on the filter is washed three times with 20 mL each of chloroform. When the chloroform odor on the residue disappears, the residue is filtered by extracting with approximately 190 mL of metaphosphoric acid-acetic acid test solution. The filtrate is placed in a 200 mL volumetric flask, added with metaphosphoric acid-acetic acid test solution to the graduation line to make 200 mL. This is used as a sample solution. 2 mL of the sample solution is measured using a volumetric pipette, added with 8 mL of metaphosphoric acid-acetic acid test solution and 2 mL of hydrogen peroxide test solution, shaken up, and titrated with 2,6-dichlorophenolindophenol sodium solution for titration until a pink color of the solution persists for 5 seconds. A blank test is performed in the same way and corrections are made.

2,6-dichlorophenolindophenol sodium test solution for titration 1 mL = (A × 1.210) mg $C_{12}H_{14}CaO_{12}\cdot 2H_2O$

A: Amount (mg) of L-ascorbic acid ($C_6H_8O_6$) corresponding to 1 mL of 2,6-dichlorophenolindophenol sodium test solution for titration

The reference standard of L-ascorbic acid is dried in a desiccator (silica gel) for 24 hours. Approximately 0.05 g of it is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with metaphosphoric acid-acetic acid test solution and transferred to a 100 mL volumetric flask, and added with metaphosphoric acid-acetic acid test solution to the graduation line to make 100 mL, and hereinafter subjected to the same procedure for the sample solution to obtain A

Method No. 2: The amount of this product containing approximately 0.1 g of calcium L-ascorbate ($C_{12}H_{14}CaO_{12}\cdot 2H_2O$) is weighed to three significant digits and the value is recorded. It is added with 150 mL of metaphosphoric acid-acetic acid test solution, vigorously shaken up and filtered. The residue on the filter is washed twice with 15 mL each of metaphosphoric acid-acetic acid test solution. The filtrate and the washings are mixed, the mixture is placed in a 200 mL volumetric flask and added with metaphosphoric acid-acetic acid test solution to the graduation line to make 200 mL, and hereinafter the method No. 1 is applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a lightproof sealed container.

(20) Sodium L-Ascorbate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried in a vacuum desiccator (silica gel) for 24 hours and determined, it contains 99.0 % or more of sodium L-ascorbate ($C_6H_7NaO_6$).

Physical and chemical properties:

i. This product is white to yellowish-white crystal powder with no odor.

ii. This product is easy to dissolve in water, and hardly dissolves in ethanol.

Confirmation test:

i. When 5 mL of a solution of this product in water (1 → 50) is added with 1-2 drops of 2,6-dichlorophenolindophenol sodium test solution, the color of the test solution disappears within 30 seconds.

ii. 0.1 g (0.05~0.14 g) of this product is dissolved in 100 mL of metaphosphoric acid solution (1 → 50). When 5 mL of this solution is dropped with iodine test solution

until the solution is slightly yellow, added with a drop of copper sulfate solution (1 → 1,000) and a drop of pyrrole and warmed at 50 °C for 5 minutes, it is blue to blue-green.

iii. The solution of this product in water (1 → 10) gives the qualitative reaction of sodium salt.

Purity test:

i. Specific rotation: Approximately 2.5 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved in water to make 25 mL. The rotation of this solution shall be $[\alpha]_D^{20} = +103.0° \sim +108.0°$.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Loss on drying: 0.50 % or less (1 g, reduced pressure, silica gel, 24 hours)

Assay: This product is dried in a vacuum desiccator (silica gel) for 24 hours, and approximately 0.2 g of it is weighed to the digits of 0.001 g, and the value is recorded. When it is dissolved with 50 mL of metaphosphoric acid solution (1 → 50) and titrated with 0.05 mol/L iodine solution (indicator: 1 mL starch test solution).

0.05 mol/L iodine solution 1 mL = 9.906 mg $C_6H_7NaO_6$

(b) Standard of storage method

It shall be stored in a lightproof tightly sealed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium L-ascorbiate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium L-ascorbiate is applied mutatis mutandis.

(21) Sodium-calcium-L-Ascorbic acid-2-Phosphate ester

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 60.0 % or more of sodium calcium L-ascorbic acid-2-phosphate ester ($(C_6H_6O_9P)_2Na_4Ca$).

Physical and chemical properties: This product is white to grayish brown powder.

Confirmation test:

i. 2 mg (1.5~2.4 mg) of this product is added with 0.1 mol/L hydrochloric acid solution to make 100 mL. In the measurement of the absorption spectrum of this solution, the absorption maximum is at the wavelength of 235~239 nm.

ii. 90 mg (89.5~90.4 mg) of this product is weighed, stirred with 20 mL of water, and centrifuged for 5 minutes at 3,000 rpm. The supernatant is used as a sample solution. Separately, 5 mg (4.5~5.4 mg) of the reference standard of L-ascorbic acid-2-phosphate ester tris cyclohexylammonium is weighed and dissolved with 1 mL of water to prepare a standard solution. 5 μL each of the sample and standard solution is spotted on a thin layer plate prepared using cellulose powder thin-layer chromatography (with a fluorescent agent). Then, they are developed to 10 cm with the developing solvent, a mixture of absolute ethanol, water and ammonia solution (20:10:3), and the thin layer plate is air dried. When it is irradiated with ultraviolet light (dominant wavelength: 254 nm), the spots obtained from the sample and standard solutions are dark blue and their Rf values are equal.

Purity test:

pH: The pH of the solution of this product in water (1 → 50) is 7.5~9.5.

Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 9.0 % or less (1 g, 105 °C, 4 hours)

Assay: 0.25 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is added with 40 mL of water, subjected to ultrasonic vibration for 1 minute, transferred to a 50 mL volumetric flask, added with water to the graduation line to make 50 mL. 5.0 mL of this solution measured using a volumetric pipette is transferred to a 50 mL volumetric flask and added with water to the graduation line to make 50 mL. This is used as a sample solution. 20 μL of this solution is tested by the liquid chromatography under the following conditions. The peak height or area of L-ascorbic acid-2-phosphate ester is measured from the obtained chromatogram. The concentration of L-ascorbic acid-2-

phosphate ester is obtained from a calibration curve separately prepared. Then, its content is calculated using the following equation.

Amount of sodium -calcium -L-ascorbic acidate-2-phosphate ester (%)

$$= C \times \frac{50}{W} \times \frac{319.10}{553.63}$$

C: Concentration of L-ascorbic acid-2-phosphate ester (mg/mL)

W: Amount of this product weighed (g)

Operating condition:

Detector: Ultraviolet spectrophotometer (measurement wavelength: 250 nm)

Column: A stainless tube (inner diameter: 4.6 mm, length: 150 mm) is filled with particle size 5 μm of octadecyl-silylated silica gel for liquid chromatography.

Column temperature: Constant temperature around 25 °C

Mobile phase: 13.6 g (13.55~13.64 g) of potassium dihydrogenphosphate and 4.0 mL of tetrabutylammonium hydroxide test solution are dissolved in 950 mL of water, the pH of the solution is adjusted to 6.0 using 2 mol/L sodium hydroxide test solution, and then water is added to make 1,000 mL. 950 mL of this solution is added with 50 mL of acetonitrile to mix them.

Flow rate: The flow rate is adjusted so that the retention time of L-ascorbic acid-2-phosphate ester is approximately 5 minutes.

Preparation of calibration curve:

Approximately 0.1 g of the reference standard of L-ascorbic acid-2-phosphate ester tris cyclohexylammonium is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with water, transferred to a 20 mL volumetric flask, added with water to the graduation line to make 20 mL. This is used as a standard stock solution. 1 mL of this solution contains 5 mg of L-ascorbic acid-2-phosphate ester tris cyclohexylammonium ($C_{24}H_{48}N_3O_9P$). In use, a certain volume of the standard stock solution is diluted with water so that 1 mL of it contains 0.2 mg, 0.4 mg, 0.6 mg or 0.8 mg. This is used as a standard solution. 20 μL each of these standard solutions is hereinafter tested by the liquid chromatography as the same is the case in the sample solution. The peak height or area of L-ascorbic acid-2-phosphate ester is measured from the obtained chromatogram and the calibration curve is prepared.

(b) Standard of storage method

It shall be stored in a lightproof tightly sealed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium-calcium-L-ascorbic acid-2-phosphate ester are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium-calcium-L-ascorbic acid-2-phosphate ester is applied mutatis mutandis.

(22) L-Ascorbic acid-2-Phosphate ester magnesium

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 93.0 % or more of L-ascorbic acid-2-phosphate ester magnesium ($(C_6H_6O_9P)_2Mg_3 \cdot 10H_2O$).

Physical and chemical properties:

i. This product is white to slightly yellow powder with a slight, specific odor.

ii. This product is easy to dissolve in water and hardly dissolves in acetone or methanol.

iii. The pH of the solution of this product in water (1 → 50) is 7.5~8.5.

Confirmation test:

i. 2 mg (1.5~2.4 mg) of this product is added with 0.1 mol/L of hydrochloric acid test solution to make 100 mL. In the measurement of the absorption spectrum of this product, the absorption maximum is at the wavelength of 235~239 nm.

ii. When 5 mL of a solution of this product in water (1 → 50) is added with a drop of ferric chloride test solution, the resulting solution is red-brown.

iii. 0.1 g (0.05~0.14 g) of this product is added with 2 mL of sulfuric acid and 15 mL of hydrogen peroxide solution, heated until it is approximately 5 mL, cooled, and then is added with water to make 50 mL. When 1 mL of this solution is added with 1 mL of 3 mol/L of sulfuric acid, 1 mL of ammonium molybdate test solution, and 1 mL of amidol-sodium bisulfite test solution, the resulting solution turns blue.

iv. 0.5 g (0.45~0.54 g) of this product is added with 2 mL of sulfuric acid and 30 mL of hydrogen peroxide solution and heated until it is approximately 5 mL. When it allowed to cool, added with 20 mL of water, and neutralized with 1 mol/L sodium hydroxide test solution, the resulting solution gives the qualitative reaction of magnesium salt.

Purity test:

i. Clarity and color of solution: When 1.0 g (0.95~1.04 g) of this product is dissolved with 10 mL of water, the solution shall not be darker than the control solution for color J, and should be almost clear.

ii. Specific rotation: Approximately 1.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved in water to make 50 mL. The rotation of this solution shall be $[\alpha]_D^{20} = +44° \sim +50°$.

iii. Chloride: 0.05 g (0.045~0.054 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.5 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these samples and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.35 % or less).

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

v. Arsenic: 0.2 g (0.15~0.24 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Water content: Approximately 1.6 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is placed to a 50 mL volumetric flask, dissolved with a mixture of methanol and sulfuric acid (70:1), added with more mixture of methanol and sulfuric acid (70:1) to the graduation line to make 50 mL. This is used as a sample solution. The mass of the solution is weighed to three significant digits and the value is recorded. 1 mL of this sample solution is measured using a volumetric pipette and water content is determined by the direct titration method, the Karl Fischer method. Separately, the mass of approximately 1 mL of a mixture of methanol and sulfuric acid (70:1) is weighed to three significant digits and the value is recorded. The water content of this mixture is determined in the same way. The water content of this product determined using the following equation shall be 22.0~28.0 %.

$$\text{Water content of this product (\%)} = \frac{50 \times S - B \times (w_2 - w_1) / w_3}{w_1 \times 10}$$

S: Water content in 1 mL of the test solution (mg)

B: Water content in approximately 1 mL of a mixture of methanol and sulfuric acid (70:1), the mass of which is weighed to three significant digits and the value is recorded (mg)

W_1: Amount of this product weighed (g)

W_2: Mass of 50 mL of the sample solution (g)

W_3: Mass of approximately 1 mL of a mixture of methanol and sulfuric acid (70:1) used for the measurement of B (g)

Assay: Approximately 0.3 g each of this product and approximately 0.50 g of maleic acid as an internal standard is weighed to the digits of 0.001 g and the value is recorded. They are dissolved with water to make 100 mL to prepare a sample solution. 20 μL of this solution is tested by the liquid chromatography under the following conditions. The peak areas S_{A1} and S_{M1} of L-ascorbic acid-2-phosphate ester magnesium and maleic acid, respectively, are measured from the obtained chromatogram. Separately, the peak areas S_{A2} and S_{M2} of the reference standard of L-ascorbic acid-2-phosphate ester magnesium and maleic acid, respectively, are measured by the same procedure.

Content of L-ascorbic acid-2-phosphate ester magnesium ($(C_6H_6O_9P)_2Mg_3 \cdot 10H_2O$) (%) = $(S_{A1}/W_{A1}) \times (W_{M1}/S_{M1}) \times (W_{A2}/W_{M2}) \times (S_{M2}/S_{A2}) \times 100$

W_{A1}: Amount of this product weighed (g)

W_{M1}: Collected amount of maleic acid related to this product (g)

W_{A2}: Collected amount of the reference standard of L-ascorbic acid-2-phosphate ester magnesium (g)

W_{M2}: Collected amount of maleic acid related to the reference standard of L-ascorbic acid-2-phosphate ester magnesium (g)

Operating condition:

Detector: Ultraviolet spectrophotometer (measurement wavelength: 257 nm)

Column: A stainless tube (inner diameter: 8 mm, length: 250 mm) is filled with the gel of polyvinyl alcohol cross-linked with epichlorohydrin, as a packing material (exclusion limit molecular weight (pullulan) is 1.8×10^3).

Column temperature: 40 °C

Mobile phase: A solution for which 7.10 g (7.095~7.104 g) of anhydrous sodium sulfate and 5.76 g (5.755~5.764 g) are dissolved in water to make 1,000 mL.

Flow rate: 0.7 mL per minute

(b) Standard of storage method

It shall be stored in a lightproof tightly sealed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-ascorbic acid-2-phosphate ester magnesium are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-ascorbic acid-2-phosphate ester magnesium is applied mutatis mutandis.

(23) Astaxanthin

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 97.0~102.0 % of astaxanthin ($C_{40}H_{52}O_4$).

Physical and chemical properties: This product is reddish violet to dark purple, crystalline powder with a slight, specific odor.

Confirmation test:

i. When 0.5 mL of a solution of this product in chloroform (1 → 10,000) is added with 1 mL of antimony trichloride test solution, the resulting solution is bluish purple within 30 seconds.

ii. In the measurement of the absorption spectrum of the sample solution obtained by the assay using chloroform as a control, the absorption maximum is at the wavelengths of 487~491 nm.

Purity test:

i. Clarity and color of solution: When 0.01 g (0.005~0.014 g) of this product is dissolved with 10 mL of chloroform, the resulting solution shall be red and clear.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 0.5 % or less (0.5 g, 105 °C, 4 hours)

Ignition residue: 0.10 % or less (0.5 g)

Assay: Approximately 0.035 g of this product is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with chloroform, transferred to a 200 mL volumetric flask, and added with more chloroform to the graduation line to make 200 mL. 5 mL of this solution is measured using a volumetric pipette and put in a 50 mL volumetric flask and added with chloroform to the graduation line to make 50 mL. Then, 5 mL of this solution is measured using a volumetric pipette and put in a 50 mL volumetric flask and added with chloroform to the graduation line to make 50 mL. This is used as a sample

solution. The absorbance A of this solution is measured at a wavelength of nearly 489 nm using chloroform as a control.

$$\text{Amount of astaxanthin } (C_{40}H_{52}O_4)\ (\text{mg}) = \frac{A}{1{,}975} \times 200{,}000$$

(b) Standard of storage method

It shall be put in a lightproof hermetically sealed container with inert gas replaced with air and stored.

B. Preparation (Part 1)

(a) Compositional standards

This product is particles of the mixture of the raw material for manufacturing of Astaxanthin and carrier.

Content: When this product is determined, it contains Astaxanthin ($C_{40}H_{52}O_4$) corresponding to 90~120 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 1 mg of the raw material for manufacturing of astaxanthin is weighed, added with 1 mL of warmed water at 60 °C, and sonicated in a water bath at 60 °C for approximately 5 minutes. It is allowed to cool, added with 10 mL of chloroform and shaken up well. 1 mL of the chloroform layer is collected and added with 1 mL of antimony trichloride test solution. The resulting solution is bluish-purple within 30 seconds.

ii. In the measurement of the absorption spectrum of the sample solution obtained by the assay using chloroform as a control, the absorption maximum is at the wavelengths of 485~489 nm.

iii. 20 mL of the supernatant obtained by the assay is collected and the solvent is distilled away under reduced pressure. The residue is added with 0.5 mL of chloroform and 10 μL of this solution is spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then it is developed approximately 15 cm with the developing solvent, a mixture of cyclohexane and dioxane (3:2) in a dark place. When the thin layer is air dried in a dark place, red spots are observed at the Rf value of approximately 0.4.

Assay: The amount of this product containing approximately 5 mg of astaxanthin ($C_{40}H_{52}O_4$) is weighed to three significant digits and the value is recorded. It is added with approximately 5 mL of warmed water at 60 °C, and sonicated in a water bath at 60 °C for approximately 5 minutes until the particles disappear. It is allowed to cool, transferred to a 200 mL volumetric flask, added with 100 mL of absolute ethanol, shaken up well, then added with chloroform to the graduation line to make 200 mL.

Approximately 40 mL of this solution is measured and centrifuged for 5 minutes at 3,000 rpm. 5 mL of the supernatant is measured using a volumetric pipette and the solvent is distilled away in a water bath at 50 °C under nitrogen stream. The residue is dissolved with chloroform by 5-minute sonication, transferred to a 50 mL volumetric flask and added with chloroform to the graduation line to make 50 mL. This is used as a sample solution. The absorbance A of this solution is measured at a wavelength of nearly 487 nm using chloroform as a control.

$$\text{Amount of astaxanthin } (C_{40}H_{52}O_4)\ (\text{mg}) = \frac{A}{1{,}830} \times 200{,}000$$

(b) Standard of storage method

It shall be stored in a lightproof tightly sealed container.

C. Preparation (Part 2: liquid)

(a) Compositional standards

This product is suspension of the mixture of the preparation (Part 1), in which the raw material for manufacturing of astaxanthin is mixed with edible starch, mixed with vegetable oil, light anhydrous silicic acid, glycerine fatty acid ester or polyoxyethylene sorbitan fatty acid ester.

Content: When this product is determined, it contains astaxanthin ($C_{40}H_{52}O_4$) corresponding to 90~120 % of the amount on the label.

Confirmation test: The confirmation test of the preparation (Part 1) of astaxanthin is applied mutatis mutandis.

Assay: The assay of the preparation (Part 1) of astaxanthin is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the preparation (Part 1) of astaxanthin is applied mutatis mutandis.

(c) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

Valid period: 3 months from the day of the manufacture

“有効期間　製造の日から３か月”

(24) Acetomenaphthone

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following 3-hour drying at 80 °C, it contains 98.0 % or more of astaxanthin ($C_{15}H_{14}O_4$).

Physical and chemical properties: This product is white crystalline powder with no odor or a slight acetic acid odor.

Confirmation test:

i. 0.03 g (0.025~0.034 g) of this product is dissolved with 2 mL of glacial acetic acid, added with 2 mL of dilute hydrochloric acid, and heated in a water bath for 5 minutes. It is allowed to cool, added with a drop of strong hydrogen peroxide solution, warmed for a while, shaken up, added with 2 mL of water and 3 mL of chloroform, and shaken up, and then the chloroform layer is yellow.

ii. 1 mL of the chloroform layer of i. is measured and warmed to evaporate chloroform. When the residue is dissolved with 1 mL of ethanol, added with 1 mL of ammonia test solution, shaken up, and added with 3 drops of ethyl cyanoacetate, the resulting solution is purple-blue. When 1 mL of sodium hydroxide solution (1 → 3) is added to this solution, the solution turns to green, and then to brownish.

iii. 0.2 g (0.15~0.24 g) of this product is added with 3 mL of 1 mol/L sodium hydroxide test solution, heated for several minutes, allowed to cool, and neutralized with dilute sulfuric acid. The generated precipitation is filtered. The filtrate gives the qualitative reaction of acetate.

Purity test:

i. Melting point: The melting point of this product shall be 112~115 °C.

ii. Zinc: 1.0 g (0.95~1.04 g) of this product is weighed, added with 10 mL of dilute hydrochloric acid, heated until acetomenaphthone becomes oily, cooled within 30 seconds, and filtered. The filter paper is washed with 30 mL of boiling water. When the mixture of the filtrate and the washings are added with water to make 50 mL, added with 1.0 mL of potassium ferrocyanide test solution, shaken up, and allowed to stand for 5 minutes, the turbidity of this solution shall be lower than or equal to that shown when 5.0 mL of zinc reference standard is added with 10 mL of dilute hydrochloric acid and subjected hereinafter to the same procedure for the sample solution (50 mg/kg or less).

Loss on drying: 0.5 % or less (1 g, 80 °C, 3 hours)

Ignition residue: 0.10 % or less (1 g)

Assay: This product is dried at 80 °C for 3 hours, approximately 0.25 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is placed in a 150 mL saponification flask, dissolved with 20 mL of glacial acetic acid, added with 15 mL of dilute hydrochloric acid, boiled for 15 minutes with a reflux condenser, and water-cooled.

Within 30 seconds after the water-cooling, it is titrated with 0.1 mol/L ceric ammonium sulfate solution (indicator: 3 drops of *o*-phenanthroline test solution). A blank test is performed in the same way and corrections are made.

0.1 mol/L ceric ammonium sulfate solution 1 mL = 12.91 mg $C_{15}H_{14}O_4$

(b) Standard of storage method

It shall be stored in a lightproof tightly sealed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of acetomenaphthone are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of acetomenaphthone is applied mutatis mutandis.

(25) β-apo-8'-carotene acid ethyl ester

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 96.0~102.5 % of β-apo-8'-carotenne acid ethyl ester ($C_{32}H_{44}O_2$).

Physical and chemical properties:

i. This product is red-brown to reddish violet crystalline powder.

ii. This product is easy to dissolve in chloroform, is hard to dissolve in ether, hard to extremely dissolve in cyclohexane, and hardly dissolves in absolute ethanol and water.

iii. This product gradually changes by oxygen and light.

iv. Melting point: About 132 °C (decomposition)

Confirmation test:

i. 1 mg (0.5~1.4 mg) of this product is dissolved with 10 mL of chloroform. When 0.5 mL of this solution is measured and added with 1 mL of antimony trichloride test solution, the resulting solution is green.

ii. In the measurement of the absorption spectrum of the sample solution obtained by the assay, the absorption maximum is at the wavelengths of 446~450 nm and 471~475 nm and the absorption minimum is at 461~467 nm. When the absorbances at the former and latter absorption maximum are A1 and A2, respectively, A1/A2 is 0.82~0.86.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 0.5 % or less (1 g, reduced pressure, silica gel, 3 hours)

Ignition residue: 0.10 % or less (1 g)

Assay: Approximately 10 mg of this product is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with cyclohexane, transferred to a 200 mL volumetric flask and added with more cyclohexane to the graduation line to make 200 mL. 4 mL of this solution is measured using a volumetric flask, and transferred to a 100 mL volumetric flask, and added with cyclohexane to the graduation line to make 100 mL. This is used as a sample solution. The absorbance A of this solution is measured at a wavelength of nearly 448 nm using cyclohexane as a control.

$$\text{Amount of β-apo-8'-carotene acid ethyl ester } (C_{32}H_{44}O_2)\text{ (mg)} = \frac{A}{2{,}550} \times 50{,}000$$

(b) Standard of storage method

Store in airtight container shielded from light, replace air with nitrogen gas and save.

B. Preparation

(a) Component standard

This product is particles of the mixture of the raw material for manufacturing of β-apo-8'-carotenic acid ethyl ester and fillers.

Content: When this product is determined, it contains β-apo-8'-carotenic acid ethyl ester ($C_{32}H_{44}O_2$) corresponding to 90~120 % of the amount on the label.

Confirmation test: 5 mL of the supernatant obtained by the assay is measured and the solvent is distilled away in a water bath at 50 °C under nitrogen stream. The residue is added with 0.5 mL of chloroform and 10 μL of this solution is spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then it is developed approximately 15 cm with the developing solvent, a mixture of dichloromethane and ether (9:1) in a dark place. When the thin layer is air dried in a dark place, red spots are observed at the Rf value of approximately 0.7.

Assay: The amount of this product containing approximately 10 mg of β-apo-8'-carotene acid ethyl ester ($C_{32}H_{44}O_2$) is weighed to three significant digits and the value is recorded. It is added with 10 mL of warmed water at 60 °C, warmed in a water bath at 60 °C, stirred under nitrogen stream to make it a complete suspension. It is allowed to cool and transferred to a 250 mL volumetric flask, added with 50 mL of absolute ethanol and 100 mL of ether, vigorously shaken up for 3 minutes and added with ether to the graduation line to make 250 mL. Then, it is allowed to stand in a dark place for 15 minutes to precipitate insoluble matter and the 5 mL of the supernatant is measured using a volumetric pipette, and placed in a 100 mL a round-bottom flask, and the solvent is distilled away in a water bath at 50 °C under nitrogen stream. After cooling, the residue is dissolved with cyclohexane and added with more cyclohexane to the graduation line to make 100 mL. This is used as a sample solution. The absorbance A of this solution is measured at a wavelength of nearly 449 nm using cyclohexane as a control.

$$\text{Amount of β-apo-8'-carotene acid ethyl ester } (C_{32}H_{44}O_2)\text{ (mg)} = \frac{A}{2{,}430} \times 50{,}000$$

(b) Standard of storage method

Store in an airtight container shielded from light.

(26) Aminoacetic acid

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 3 hours, it contains 98.5 % or more of aminoacetic acid ($C_2H_5NO_2$).

Physical and chemical properties:

i. This product is white crystals or crystalline powder with a sweet taste.

ii. This product is soluble in water and hardly soluble in ethanol or ether.

iii. The pH of the aqueous solution (1 → 10) of this product is 5.5~7.0.

Confirmation test:

i. When 5 mL of the solution of this product in water (1 → 10) is added with 5 drops of dilute hydrochloric acid and 1 mL of sodium nitrite test solution, colorless gas is generated.

ii. 5 drops of the solution, which completes the reaction of i., is placed in a small test tube, boiled for a while, evaporated to dryness in a desiccator at 120 °C, and allowed to cool. When the residue is added with 5~6 drops of chromotropic acid test solution and heated in a water bath for 10 minutes, the solution is dark purple.

iii. When 5 mL of a solution of this product in water (1 → 1,000) is added with 1 mL of ninhydrin test solution and heated for 3 minutes, the solution is purple.

Purity test:

i. Clarity and color of solution: When 1.0 g (0.95~1.04 g) is dissolved with 10 mL of water, the solution shall be colorless and clear.

ii. Chloride: 0.5 g (0.45~0.54 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.30 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these samples and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.021 %).

iii. Ammonium salt: 0.1 g (0.05~0.14 g) of this product is placed in a distilling flask, dissolved with 70 mL of water, added with 1 g (0.5~1.4 g) of magnesium oxide, and distilled until the volume of distillate liquid reaches 40 mL. A receiver is a Nessler tube containing 2 mL of 0.1 mol/L hydrochloric acid, in which the bottom of the condenser is immersed. When the distillate liquid is added with 5 mL of 1 mol/L sodium hydroxide test solution and water to make 50 mL and added with 1 mL of Nessler reagent solution, the color shall not be darker than that obtained when 2 mL of ammonium reference standard is placed in a Nessler tube, added with water to make 40 mL, and hereinafter subjected to the same procedure as one for the sample (0.02 % or less).

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

v. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 0.3 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 0.1 % or less (1 g)

Assay: This product is dried at 105 °C for 3 hours, approximately 0.5 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolves in 3 mL of formic acid, added with 50 mL of glacial acetic acid for nonaqueous titration, and titrated with 0.1 mol/L perchloric acid (indicator: 0.5 mL of α-naphtholbenzein test solution). In this case,

the end point of the titration is the point at which the color of the solution changes from brown to green. A blank test is performed in the same way and corrections are made.

01.mol/L perchloric acid 1 mL = 7.507 mg $C_2H_5NO_2$

(b)Standard of strong method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The Compositional standards of raw material for manufacturing of aminoacetic acid are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of aminoacetic acid is applied mutatis mutandis.

(27) DL-alanine

A. Raw material for manufacturing

(a) Compositional standard

Content: When this product is determined after dried at 105 °C for 3 hours, it contains 98.5 % or more of DL-alanine ($C_3H_7NO_2$).

Physical and chemical properties:

i. This product is colorless to white crystalline powder with a sweet taste.

ii. This product is easy to dissolve in water, it is difficult to dissolve in organic solvents.

iii. The pH of the aqueous solution (1 → 10) of this product is 5.5~7.0.

Confirmation test:

i. When 0.2 g (0.15~0.24 g) of this product is dissolved in 10 mL of dilute sulfuric acid, added with 0.1 g (0.05~0.14 g), and boiled, it emits the odor of acetaldehyde.

ii. When 5 ml of a solution of this product in water (1 → 1,000) is added with 1 mL of ninhydrin test solution and heated for 3 minutes, it is purple.

Purity test:

i. Clarity and color of solution: When 1.0 g (0.95~1.04 g) of this product is dissolved in 10 mL of water, the solution shall be colorless and clear.

ii. Chloride: When chloride is tested using a sample solution prepared with 0.5 g (0.45~0.54 g) of this product by the chloride test method and a control solution prepared with 0.3 mL of 0.01 mol/L hydrochloric acid, the turbidity of the sample solution shall not be higher than that of the control solution (0.021 % or less).

iii. Ammonium salt: The purity test iii. of the raw material for manufacturing of aminoacetic acid is applied mutatis mutandis, provided that “2 mL of ammonium reference standard” shall be replaced by “3 mL of ammonium reference standard” (0.03 % or less).

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less). v. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 0.3 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 0.20 % or less (1 g)

Assay: This product is dried at 105 °C for 3 hours, approximately 0.2 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolved in 3 mL of formic acid, added with 50 mL of glacial acetic acid for nonaqueous titration, and titrated with 0.1 mol/L perchloric acid (indicator: 10 drops of α-naphtholbenzein test solution). In this case, the end point of the titration is the point at which the color of the solution changes from brown to green. A blank test is performed in the same way and corrections are made.

01.mol/L perchloric acid 1 mL = 8.909 mg $C_3H_7NO_2$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of DL-alanine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of DL-alanine is applied mutatis mutandis.

(28) L-arginine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 3 hours, it contains 98.0 % or more of L-arginine ($C_6H_{14}N_4O_2$).

Physical and chemical properties:

i. This product is pale yellow to pale yellow-brown crystals or crystalline powder with a slight, specific odor.

ii. The pH of a solution or a suspension of this product in water (1 → 10) is 10.5~12.0.

Confirmation test:

i. When 5 mL of a solution of this product in water (1 → 1,000) is added with 1 mL of ninhydrin test solution and heated for 3 minutes, the solution is reddish violet to purple.

ii. When 2 mL of a solution of this product in water (1 → 20,000) is added with 1 mL of 8-oxyquinoline test solution and 1 mL of N-bromosuccinimide solution (1 → 1,000), the solution is red-yellow.

Purity test:

i. Specific rotation: This product is dried at 105 °C for 3 hours, approximately 4 g of it is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved in 6 mol/L hydrochloric acid to make 50 mL and filtered using a membrane filter (0.45 μm). The rotation of this solution shall be

$[\alpha]_D^{20}$ = + 25.5 to + 29.5°.

ii. Ammonium salt: The purity test iii. of the raw material for manufacturing of aminoacetic acid is applied mutatis mutandis, provided that “0.1 g” and “2 mL of ammonium reference standard” shall be replaced by “0.02 g” and “3 mL of ammonium reference standard”, respectively (0.15 % or less).

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed in a decomposition flask, added with 10 mL of nitric acid and 5 mL of sulfuric acid, and gently heated. If the solution is still brown, it is allowed to cool, then is heated with 1~2 mL of additional nitric acid. This procedure is repeated until the solution becomes colorless to slightly yellow. It is allowed to cool, added with 0.5 mL of perchloric acid, and heated until white smoke emerges. It is allowed to cool, added with 15 mL of saturated ammonium oxalate solution and again heated until white smoke emerges. It is allowed to cool and added with water to make approximately 10 mL. This is used as a sample solution.

When the sample solution is tested on arsenic by the method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 1.0 % or less (1 g, 105 °C., 3 hours)

Ignition residue: 1.0 % or less (1 g)

Assay: This product is dried at 105 °C for 3 hours, approximately 0.2 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 0.5 mL of water and 50 mL of glacial acetic acid, and titrated with 0.1 mol/L perchloric acid (indicator: 1 mL of α-naphtholbenzein test solution). Provided, however, that the end point of the titration is the point at which the color of the solution changes from orange-yellow through yellowish green to green. A blank test is performed in the same way and corrections are made.

0.1 mol/L perchloric acid 1 mL = 8.710 mg $C_6H_{14}N_4O_2$

(b) Standard of storage method

Store in a light-tight closed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-arginine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-arginine is applied mutatis mutandis.

(29) L- Isoleucine

A. Raw material for manufacturing

(a) Compositional standards

Content: L-isoleucine, after drying at 105 °C for four hours, contains not less than 90.0% of L-isoleucine (C6H13NO2).

Physical and chemical properties:

i. L-isoleucine occurs as white crystals or crystalline powder.

ii. The pH of an aqueous solution of L-isoleucine or a suspension of L-isoleucine in water (0.2 → 20) is between 4.5 ~ 7.0.

Identification: To 5 mL of an aqueous solution of L-isoleucine (1 → 1000), add 1 mL of ninhydrin solution (1 → 1000), and heat for three minutes: a purple color develops.

Purity

i. Specific rotation: Weigh about 2 g of L-isoleucine, previously dried at 105 °C for four hours, to the order of 0.01 g, record the value, dissolve in 6 mol/L hydrochloric acid TS to make 50 mL, filter if necessary, and determine the optical rotation of this solution: It shall be between +38.0 ~ +41.5°.

ii. Ammonium salt: Proceed as directed in the Purity (3) for the active ingredients for aminoacetic acid production. In this case, the term "0.1 g" shall be deemed to be replaced with "0.01 g," and the term "2 mL of Standard Ammonium Solution" shall be deemed to be replaced with "3 mL of Standard Ammonium Solution" (not more than 0.30%).

iii. Lead: Perform the test for lead with 0.5 g (0.45 ~ 0.54 g) of L-isoleucine as directed under Lead Test (Method 1 of Atomic Absorption Spectrophotometry): the amount of lead shall be not more than 2 μg/g. Pipet 0.5 mL of standard lead solution using a volumetric pipette, transfer it into a 50-mL volumetric flask, add nitric acid (1 → 150) to the marked line to make 50 mL, and use this solution as the standard solution.

iv. Arsenic: Weigh 1.0 g (0.95 ~1.04 g) of L-isoleucine, transfer to a decomposition flask, add 10 mL of nitric acid and 5 mL of sulfuric acid, and heat gently. If a brown color still develops in the solution, allow to cool, add 1 to 2 mL of nitric acid, heat, and repeat this procedure until the solution becomes colorless to pale yellow. After allowing to cool, add 0.5 mL of perchloric acid, and heat until white fumes are evolved. After allowing to cool, add 15 mL of saturated ammonium oxalate solution, and heat again until white fumes are evolved. After allowing to cool, add water to make about 10 mL, and use this solution as the sample solution to perform the test for arsenic by using Apparatus A . At this time, the color of the absorbing liquid shall have no more color than the standard color (not more than 2 μg/g).

Loss on drying: Not more than 2.0% (5 g, 105°C, 4 hours)

Residue on ignition: Not more than 1.0% (1 g)

Assay: Weigh about 0.25 g of L-isoleucine, previously dried at 105 °C for four hours, to four significant figures, record the value, dissolve it in water, transfer to a 250-mL volumetric flask, add water to the marked line to make 250 mL, and use this solution as the sample solution. Perform the test with 5 μL of this sample solution as directed under Liquid Chromatography according to the following conditions. Based on the chromatogram obtained, determine the peak area of L-isoleucine, calculate the concentration of L-isoleucine by a calibration curve obtained separately, and calculate the content.

Operating conditions

Apparatus: The apparatus consists of two pumps for flowing the mobile phase and the chromogenic liquid, a sample introduction part, a column, a reaction vessel, a detector, and a recording apparatus, and use the column and the reaction vessel that can be kept at a constant temperature.

Detector: A fluorometer (excitation wavelength: 338 nm, fluorescence wavelength: 425 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 150 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter)

Column temperature: 60 °C

Reaction vessel (reaction coil): A column about 0.25 mm in inside diameter and about 3.0 m in length

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.62 g of sodium 1-octanesulfonate in water to make 1000 mL. To this solution, add 266 mL of methanol, and adjust the pH to 2.5 with phosphoric acid.

Reaction solution: Dissolve 13.25 g of potassium hydroxide, 15.00 g of boric acid, 0.35 g of o-phthalaldehyde, 1 mL of 2-mercaptoethanol, 5 mL of methanol, and 1.25 mL of 3.5% polyoxyethylene (23) lauryl ether in water to make 1000 mL.

Mobile phase flow rate: 1.0 mL per minute

Reaction solution flow rate: 0.5 mL per minute

Reaction temperature: 60 °C

Creating calibration curve

Weigh about 0.25 g of L-isoleucine for assay to the order of 0.001 g, record the value, add water to dissolve it, transfer to a 50 mL volumetric flask, add water to the marked line to make 50 mL, and use this solution as the standard stock solution [each mL of this solution contains 5 mg of L-isoleucine ($C_6H_{13}NO_2$)]. When using it, dilute a fixed volume of the standard stock solution with water exactly so that each mL contains 0.5 mg, 1.0 mg, and 1.5 mg. Filter each solution through a 0.45 μm membrane filter as necessary, and use these solutions as the standard solutions. Perform the test with 5 μL each of the standard solutions as directed under Liquid Chromatography in the same manner as for the sample solution. Based on the chromatogram obtained, determine the peak area of L-isoleucine, and prepare a calibration curve.

(b)Standards for manufacturing method

Cultivate Corynebacterium glutamicum strain KCCM 80189, and after the completion of culturing, filter the culture to remove bacterial bodies and isolate the L-isoleucine crude crystalline fraction. Purify a crude crystal and dry the resulting solid to produce the product.

(c) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-isoleucine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-isoleucine is applied mutatis mutandis.

(30) Inositol

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following 4hours drying at 105 °C, it contains 97.0 % or more of inositol ($C_6H_{12}O_6$).

Physical and chemical properties:

i. This product is white crystals or crystalline powder with no odor and with a sweet taste.

ii. This product is easy to dissolve in water and hardly dissolves in ether, ethanol, and chloroform.

iii. A solution of this product in water (1 → 10) is neutral.

iv. This product is not optical rotatory.

Confirmation test:

i. 1 mL of a solution of this product in water (1 → 50) is added with 6 mL of nitric acid and evaporated to dryness on a water bath. When the residue is added with 0.5 mL of strontium nitrate solution (1 → 10) and again evaporated to dryness in a water bath, it changes through reddish violet to orange.

ii. When 4 mL of a solution of this product in water (1 → 100) is added with 1 mL of lead subacetate test solution, shaken up, and heated in a water bath for 5 minutes, the solution becomes a translucent gel.

iii. The melting point of hexa-acetyl-inositol obtained by the assay is 212~216 °C.

Purity test:

i. Clarity and color of solution: When 1.0 g (0.95~1.04 g) is dissolved with 10 mL of water, the resulting solution shall be colorless and clear.

ii. Melting point: The melting point of this product shall be 223~227 °C.

iii. Chloride: 2.0 g (1.95~2.04 g) of this product is weighed to prepare a sample solution by the chloride test method. When sulfate is tested using a control solution prepared with 0.30 mL of 0.01 mol/L hydrochloric acid using the chloride test method, the turbidity of the sample solution shall not be higher than that of the control solution (0.005 % or less).

iv. Sulfate: 4.0 g (3.95~4.04 g) of this product is weighed to prepare a sample solution by the sulfate test method. When sulfate is tested using a control solution prepared with 0.50 mL of 0.005 mol/L sulfuric acid using the sulfate test method, the turbidity of the sample solution shall not be higher than that of the control solution (0.006 % or less).

v. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.5 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (25 mg/kg or less).

vi. Iron: When 2.0 g (1.95~2.04 g) of this product is weighed, dissolved with 40 mL of water, and added with 2 mL of hydrochloric acid, 0.04 g (0.035~0.044 g) of ammonium persulfate and 2 mL of ammonium thiocyanate test solution, the color of the resulting solution shall not be darker than that obtained when 1.0 mL of iron reference standard is measured, added with 40 mL of water, and hereinafter subjected to the same procedure as the one for the sample (5 mg/kg or less).

vii. Calcium: When 1.0 g (0.95~1.04 g) of this product is dissolved with 10 mL of water, added with 1 mL of ammonium oxalate test solution, and allowed to stand for 1 minute, the resulting solution shall be clear.

viii. Fehling test solution reducing substance: When 0.5 g (0.45~0.54 g) of this product is dissolved with 10 mL of water, added with 5 mL of Fehling test solution, boiled for 3 minutes, and allowed to stand for 30 minutes, orange-yellow to red precipitation shall not be generated.

Loss on drying: 0.5 % or less (1 g, 105 °C, 4 hours)

Ignition residue: 0.10 % or less (1 g)

Assay: This product is dried at 105 °C for 4 hours, and approximately 0.2 g of it is weighed to the digits of 0.001 g, the value is recorded. It is placed in a 250 mL beaker and added with 5 mL of a mixture of 1 mL of dilute sulfuric acid and 50 mL of acetic anhydride. The beaker is covered with a watch glass, heated on a water bath for 20 minutes, and cooled with ice. Then it is added with 100 mL of water, boiled for 20 minutes and allowed to cool. The contents of the beaker are placed in a 250 mL separatory funnel, and then washed with a little water. Then the beaker is washed

sequentially with 30, 25, 20, 15, 10, and 10 mL of chloroform and extracted sequentially. All chloroform extracts are collected together and washed with 10 mL of water and the chloroform layer is filtered with absorbent cotton. The aqueous layer and the absorbent cotton are washed with 10 mL of chloroform. The mixture of the filtrate and the washings are evaporated to dryness on a water bath, dried at 105 °C for 2 hours, allowed to cool, and the mass is weighed. The mass is the amount of hexa-acetyl-inositol ($C_{18}H_{24}O_{12}$).

Amount of inositol ($C_6H_{12}O_6$) (mg) =

Amount of hexa-acetyl-inositol ($C_{18}H_{24}O_{12}$) (mg) × 0.4167

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of inositol are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of inositol is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of inositol and fillers.

Content: When this product is determined, it contains inositol ($C_6H_{12}O_6$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of inositol is weighed, added with 50 mL of water, shaken up well, and filtered. To the filtrate, the confirmation test i. for the raw material for manufacturing of inositol is hereinafter applied mutatis mutandis.

ii. According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of inositol is weighed, added with 100 mL of water, shaken up well, and filtered. To the filtrate, the confirmation test ii. for the raw material for manufacturing of inositol is hereinafter applied mutatis mutandis.

iii. The melting point of hexa-acetyl-inositol obtained by the assay is 212~216 °C.

Assay: According to the amount of this product on the label, the amount containing approximately 1.0 g of inositol ($C_6H_{12}O_6$) is weighed to three significant digits and the

value is recorded. It is added with 60 mL of water, shaken up well, and filtered. The residue on the filter is washed twice with 15 mL of water. The filtrate and the washings are collected together, transferred to a 100 mL volumetric flask, added with water to the graduation line to make 100 mL. 20 mL of this solution is measured using a volumetric pipette, placed in a 250 mL beaker, evaporated to dryness on a water bath, and dried at 105 °C for an hour. It is allowed to cool, and the assay for the raw material for manufacturing of inositol is hereinafter applied mutatis mutandis.

Amount of inositol ($C_6H_{12}O_6$) (mg)

= Amount of hexa-acetyl-inositol ($C_{18}H_{24}O_{12}$) (mg) × 0.4167

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of inositol is applied mutatis mutandis.

(31) Ergocalciferol

A. Raw material for manufacturing

(a) Compositional standards

Physical and chemical properties:

i. This product is white crystals with no odor.

ii. This product is easy to dissolve in ethanol, acetone, ether or chloroform, easy to slightly dissolve in fat and oil, and hardly dissolves in water.

iii. This product changes by air or light.

Confirmation test:

i. When 0.5 mg (0.45~0.54 mg) is dissolved with 5 mL of chloroform, added with 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shaken up, the resulting solution is red and within 30 seconds changes from purple through blue to green.

ii. 0.05 g (0.045~0.054 g) of this product is dissolved with 1 mL of anhydrous pyridine, added with the solution of 0.05 g (0.045~0.054 g) of 3,5-dinitrobenzoyl chloride in 1 mL of anhydrous pyridine, and heated on a water bath for 10 minutes with a reflux glass tube. It is allowed to cool and added with 5 mL of water, and the generated precipitation is filtered, washed with water, recrystallized twice with acetone as a solvent, and dried in a desiccator (reduced pressure, silica gel) for 2 hours, and then the melting point is 147~149 °C.

Purity test:

i. Absorbance: 0.01 g of this product is weighed to the digits of 0.0001 g and the value is recorded. It is dissolved in ethanol to make 1,000 mL. The absorbance of this solution measured at wavelength of 265 nm, shall be $E_{1\,cm}^{1\,\%}$ = 445~485.

ii. Specific rotation: 0.3 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved in ethanol within 30 minutes to make 20 mL. The rotation of this solution measured within 30 minutes shall be $[\alpha]_D^{20} = +102\sim+107°$.

iii. Melting point: This product is placed in a capillary and dried in a desiccator (reduced pressure, 2.67 kPa or less) for 3 hours. When the capillary is sealed by melting within 30 seconds and heated in a bath heated at a temperature, which is approximately 10 °C less than the predicted melting point, at the rate of 3 °C increase per 1 minute, the melting point shall be 115~118 °C.

iv. Ergosterol: When 0.010 g (0.0095~0.0104 g) of this product is dissolved with 2.0 mL of absolute ethanol (9 → 10), added with a solution of 0.020 g (0.0195~0.0204 g) of digitonin in 2.0 mL of absolute ethanol (9 → 10), and allowed to stand for 18 hours, precipitation shall not be generated.

(b) Standard of storage method

It shall be put in a lightproof sealed container with nitrogen replaced with air and stored in a cold place.

B. Preparation (Part 1: liquid)

(a) Compositional standards

This product is oil liquid or water-soluble liquid of the mixture of the raw material for manufacturing of ergocalciferol and hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil, or animal fats.

Content: When this product is determined, it contains ergocalciferol ($C_{28}H_{44}O$) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing vitamin D powder are applied mutatis mutandis.

Assay: The test is performed using the vitamin D assay.

(b) Standard of storage method

It shall be put in a lightproof air tight container making it almost full or with nitrogen replaced with air.

C. Preparation (Part 2: powder)

(a) Compositional standards

It is powder or particles, in which the raw material for manufacturing of ergocalciferol and fillers are mixed.

Content: When this product is determined, it contains ergocalciferol ($C_{28}H_{44}O$) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing vitamin D powder are applied mutatis mutandis.

Assay: The test is performed using the vitamin D assay.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(32) Potassium Chloride

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following 2 hours drying at 130 °C, it contains 99.0 % or more of potassium chloride (KCl).

Physical and chemical properties:

i. This product is colorless or white crystals or crystalline powder with no odor and with a salty taste.

ii. This product is easy to dissolve in water, and hardly dissolves in ethanol.

iii. Solution of this product in water (1 → 50) is neutral.

Confirmation test: A solution of this product in water (1 → 50) gives the qualitative reactions of potassium salt and chloride.

Purity test:

i. Clarity and color of solution: When 1.0 g (0.95~1.04 g) of this product is dissolved with 5 mL of water, the solution shall be colorless and clear.

ii. Acid and alkali: When 5.0 g (4.95~5.04 g) of this product is weighed, dissolved with 50 mL of newly boiled and cooled water, added with 3 drops of phenolphthalein test solution, it shall not be red. When this solution is added with 0.50 mL of 0.01 mol/L sodium hydroxide solution, the resulting solution shall be red.

iii. Bromide: 1.0 g (0.95~1.04 g) of this product is weighed and dissolved with water to make 100 mL. When 5 mL of this solution is measured, added with 3 drops of dilute hydrochloric acid and 1 mL of chloroform and 3 drops of chloramine test solution are dropped in it with shaking, the chloroform layer shall not be yellow to yellow-red.

iv. Iodide: When 0.5 g (0.45~0.54 g) of this product is weighed, dissolved with 10 mL of water, added with 3 drops of ferric chloride test solution and 1 mL of chloroform, shaken up, allowed to stand for 30 minutes, and again shaken up, the chloroform layer shall not be reddish violet to purple.

v. Heavy metal: 4.0 g (3.95~4.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (5 mg/kg or less).

vi. Calcium and magnesium: When 0.20 g (0.195~0.204 g) of this product is weighed, dissolved with 20 mL of water, added with 2 mL of ammonia test solution, 2 mL of ammonium oxalate test solution, and 2 mL of disodium hydrogen phosphate test solution, and allowed to stand for 5 minutes, the solution shall not have opacity.

vii. Sodium: When 1.0 g (0.95~1.04 g) of this product is weighed, dissolved with 20 mL of water, and subjected to the flame coloration test, it shall not be continuously yellow.

viii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 0.5 % or less (1 g, 130 °C, 2 hours)

Assay: This product is dried at 130 °C for 2 hours, approximately 0.2 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 50 mL of water and titrated with 0.1 mol/L silver nitrate solution (indicator: 1 mL of potassium chromate test solution) with vigorous shaking. A blank test is performed in the same way and corrections are made.

0.1 mol/L silver nitrate solution 1 mL = 7.455 mg KCl

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of potassium chloride are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of potassium chloride is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of potassium chloride and fillers.

Content: When this product is determined, it contains potassium chloride (KCl) corresponding to 90~110 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of potassium chloride is weighed, added with 50 mL of water, shaken up well, and filtered. It is neutralized with dilute nitric acid as appropriate, and to this solution the confirmation test for the raw material for manufacturing of potassium chloride is applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.2 g of potassium chloride (KCl) is weighed to three significant digits, and the value is recorded. It is added with 20 mL of water, shaken up well, and filtered. The residue on the filter is washed six times with 5 mL of water, the filtrate and the washings are collected together and neutralized with dilute nitric acid as appropriate. The assay for the raw material for manufacturing of potassium chloride is hereinafter applied mutatis mutandis.

0.1 mol/L silver nitrate solution 1 mL = 7.455 mg KCl

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of potassium chloride is applied mutatis mutandis.

(33) Choline Chloride

A. Raw material for manufacturing

(a) Compositional standards

Content: By the determination, this product contains 96.0 % or more of choline chloride ($C_5H_{14}ClNO$) on the dried basis.

Physical and chemical properties:

i. This is colorless, almost clear, and viscous fluid with a slight, specific odor.

ii. This product is miscible with ethanol and hardly dissolves in ether, chloroform, or benzene.

iii. The pH of this product is 6.5~8.0.

iv. This product is hygroscopic and absorbs carbon dioxide to emit an amine odor.

Confirmation test:

i. When 5 mL of a solution of this product in water (1 → 100) is added with 3 mL of reinecke salt test solution, red precipitation is generated.

ii. When 5 mL of a solution of this product in water (1 → 10) is added with 2 g (1.5~2.4 g) of potassium hydroxide and warmed, it emits an amine odor and its gas changes wet red litmus paper to blue.

iii. A solution of this product in water (1 → 10) gives the qualitative reaction of chloride.

Purity test:

i. Ethylene glycol: 10.0 g (9.95~10.04 g) of this product is weighed into a stoppered flask, added with 25 mL of periodic acid test solution, allowed to stand in a dark place for 30 minutes, added with 20 mL of potassium iodide test solution, shaken up, and titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 1 mL of starch test solution). A blank test is performed in the same way and corrections are made. The amount of ethylene glycol shall be 1.0 % or less.

0.1 mol/L sodium thiosulfate solution 1 mL = 6.207 mg $C_2H_6O_2$

ii. Trimethylamine: 1.0 g (0.95~1.04 g) of this product is weighed, dissolved with water, transferred to a 100 mL volumetric flask, added with more water to the graduation line to make 100 mL. This is used as a sample solution. 5 mL of the sample solution is measured, transferred to a separatory funnel, added with 1 mL formalin-magnesium carbonate test solution, 10 mL of toluene and 3 mL of saturated potassium carbonate, vigorously shaken up for 1 minute, and allowed to stand for 5 minutes. The toluene layer is transferred to a stoppered test tube containing 0.5 g (0.45~0.54 g) of anhydrous sodium sulfate and toluene is dehydrated. When 5 mL of this toluene solution is added with 5 mL of picric acid-toluene test solution, the color of the resulting solution shall not be darker than that of a solution prepared with 5 mL of trimethylamine reference standard solution by the same procedure for the preparation of the sample solution.

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

Loss on drying: 30 % or less (0.5 g, reduced pressure, phosphorus pentoxide, 5 hours)

Ignition residue: 0.20 % or less (1 g)

Assay: Approximately 0.3 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is completely dissolved with 50 mL of mercuric acetate test solution fornonaqueous titration, added with 10 mL of mercuric acetate test solution for nonaqueoustitration and titrated with 0.1 mol/L perchloric acid (indicator: 2 drops of methylrosanilinium chloride test solution). In this case, the end point of the titration is the point at which the color of the solution changes from purple through blue to green. A blank test is performed in the same way and corrections are made.

0.1 mol/L perchloric acid 1 mL = 13.96 mg $C_5H_{14}ClNO$

(b) Standard of storage method

It shall be stored in an airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of choline chloride are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of choline chloride is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particle of the mixture of the raw material for manufacturing of choline chloride and fillers.

Content: When this product is determined, it contains choline chloride ($C_5H_{14}ClNO$) corresponding to 90~110 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount of this product containing 1 g of the raw material for manufacturing of choline chloride is weighed, added with water to make 100 mL, shaken up well, and filtered. To the filtrate, the confirmation test i. for the raw material for manufacturing choline chloride is hereinafter applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.3 g of choline chloride ($C_5H_{14}ClNO$) is measured to three significant digits and the value is recorded. It is added with 50 mL of methanol and sufficiently eluted for 30 minutes with occasional shaking-up, filtered, and washed with 20, 15, and 15 ml of methanol. The filtrate and the washings are collected together and evaporated to dryness on a water bath. It is added with 50 mL of glacial acetic acid for nonaqueous titration and warmed on a water bath to completely dissolve. The assay for the raw material for manufacturing of choline chloride is hereinafter applied mutatis mutandis.

0.1 mol/L perchloric acid 1 mL = 13.96 mg $C_5H_{14}ClNO$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of choline chloride is applied mutatis mutandis.

(34) Dibenzoyl Thiamine Hydrochloride

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried in a desiccator (reduced pressure, silica gel) for 24 hours and determined, it contains 97.0 % or more of dibenzoyl thiamine hydrochloride ($C_{26}H_{26}N_4O_4S \cdot HCl$).

Physical and chemical properties:

i. This product is white crystalline powder with no odor.

ii. This product is easy to dissolve in chloroform, hard to dissolve in ethanol or acetone, hard to extremely dissolve in water, and hardly dissolves in ether.

Confirmation test:

i. When 0.1 g (0.05~0.14 g) of this product is dissolved with 10 mL of methanol, added with 1 mL of dilute nitric acid, and then added with 1 mL of silver nitrate test solution, white a precipitation is generated.

ii. 5 mg (4.5~5.4 mg) of this product is added with 1 mL of methanol, warmed to dissolve, added with 2 mL of water, 2 mL of cysteine hydrochloride test solution (1 → 100) and 2 mL of pH 7.0 phosphate buffer, shaken up, and allowed to stand for 30 minutes. Then it is added with 1 mL of potassium ferricyanide test solution, 5 mL of 0.5 mol/L sodium hydroxide test solution and 5 mL of isobutanol, vigorously shaken up for 2 minutes, allowed to stand to separate the solution into two layers, then is irradiated with ultraviolet rays from above. When the top of the upper layer is observed from a perpendicular direction to the direction of the irradiation, bluish-purple fluorescence is observed. This fluorescence disappears when the solution is made acidic, and reappears when it is made alkaline.

iii. 30 mg (29.5~30.4 mg) of this product is added with 7 mL of 0.1 mol/L hydrochloric acid test solution, heated in a water bath to dissolve, added with 2 mL of a mixture of hydroxylamine hydrochlorides solution (3 → 20) and sodium hydroxide solution (3 → 20) (1:1), shaken up for a minute, and added with 0.8 mL of hydrochloric acid and 0.5 mL of ferric chloride test solution. The resulting solution is purple.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is added with 10 mL of water and heated in a water bath to dissolve. The resulting solution shall be clear or almost clear.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using

these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

Loss on drying: 11.0 % or less (1 g, reduced pressure, silica gel, 5 hours)

Ignition residue: 0.20 % or less (1 g)

Assay: This product is dried in a desiccator (reduced pressure, silica gel) for 24 hours, approximately 0.4 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 40 mL of methanol and 40 mL of 0.1 mol/L hydrochloric acid testsolution, transferred to a 1,000 mL volumetric flask and added with water to the graduation line to make 1,000 mL. This is used as a sample solution. 5 mL of this solution is measured using a volumetric pipette, transferred to a 250 mL volumetric flask, and added with 0.1 mol/L hydrochloric acid test solution to the graduation line to make 250 mL. The absorbance A of this solution is measured at a wavelength of 237 nm. Separately, a blank test is performed in the same way and the absorbance A_0 is measured.

$$\text{Amount of dibenzoyl thiamine hydrochloride } (C_{26}H_{26}N_4O_4S\cdot HCl)\ (mg) = \frac{A - A_0}{526} \times 500{,}000$$

(b) Standard of storage method

It shall be stored in an airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of dibenzoyl thiamine hydrochloride are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of dibenzoyl thiamine hydrochloride is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particle of the mixture of the raw material for manufacturing of dibenzoy thiamine hydrochloride and fillers.

Content: When this product is determined, it contains dibenzoyl thiamine hydrochloride ($C_{26}H_{26}N_4O_4S\cdot HCl$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.025 g of the raw material for manufacturing of dibenzoyl thiamine hydrochloride is weighed, added with 15 mL of a mixture of 0.1 mol/L hydrochloric acid test solution and ethanol (3:7), vigorously shaken up for 1 minute, and filtered. 5 mL of the filtrate is added with 2 mL of a mixture of hydroxylamine hydrochlorides solution (3 → 20) and

sodium hydroxide solution (3 → 20) (1:1), shaken up for 1 minute, added with 0.8 mL of hydrochloric acid and 0.5 mL of mercuric chloride test solution, and filtered. The resulting solution is purple.

ii. 0.3 mL of the filtrate in i. is added with 2 mL of cysteine hydrochloride test solution (1 → 100) and 2 mL of pH 7.0 phosphate buffer, shaken up, and allowed to stand for 30 minutes. Then, it is added with 3 mL of cyanogen bromide test solution for dibenzoyl thiamine for assay, 5 mL of 1 mol/L sodium hydroxide test solution and 5 mL of isobutanol, vigorously shaken up for 2 minutes, allowed to stand to separate the solution into two layers. When it is irradiated with ultraviolet rays from above and the top of the upper layer is observed from a perpendicular direction to the direction of the irradiation, bluish~purple fluorescence is observed. This fluorescence disappears when the solution is made acidic, and reappears when it is made alkaline.

Assay: The amount of this product containing approximately 0.03 g of dibenzoyl thiamine hydrochloride ($C_{26}H_{26}N_4O_4S \cdot HCl$) is weighed to three significant digits and the value is recorded. It is added with 200 mL of a mixture of 0.1 mol/L hydrochloric acid test solution and ethanol (3:7) using a volumetric pipette, is vigorously shaken up for 5 minutes, and filtered. 2 mL of the filtrate is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, added with 5 mL of cysteine hydrochloride solution (1 → 20), added with dilute sodium hydroxide test solution to make it pH 7.0~7.5, and added with water to the graduation line to make 100 mL. This solution is shaken up well and allowed to stand for 60 minutes to prepare a sample solution. Separately, the reference standard of thiamine hydrochloride is dried at 105 °C for 2 hours, approximately 0.02 g of it is weighed to the digits of 0.0001 g and the value is recorded. It is added with 200 mL of a mixture of 0.1 mol/L hydrochloric acid test solution and ethanol (3:7) using a volumetric pipette to dissolve. 2 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, added with 5 mL of cysteine hydrochloride solution (1 → 20), added with dilute sodium hydroxide test solution to make it pH 7.0~7.5, and added with water to the graduation line to make 100 mL. This is used as a standard solution. 2 mL of the sample solution is measured using a volumetric pipette into stoppered centrifugal precipitation tubes T and T', added with 3 mL of acidic potassium chloride test solution, then added with 1 g (0.5~1.4 g) of anhydrous sodium sulfate, warmed in a water bath at 50~60 °C for 20 minutes with occasional, gentle shaking-up to make it almost dissolve. It is cooled to room temperature. The stoppered centrifugal precipitation tube T is added with 3 mL of cyanogen bromide test solution for dibenzoyl thiamine assay, shaken up, added with 2 mL of sodium hydroxide solution (3 → 10) within 30 seconds, shaken up, added with 15

mL of isobutanol using a volumetric pipette, tightly stoppered, and shaken up for 2 minutes. The stoppered centrifugal precipitation tube T' is added with 2 mL of sodium hydroxide solution (3isobutanol using a volumetric pipette, tightly stoppered, and shaken updibenzoyl thiamine assay, shaken up, added with 15 mL of isobutanol using a volumetric pipette, tightly stoppered, and vigorously shaken up for 2 minutes. Separately, 2 mL of the standard solution is measured using a volumetric pipette into stoppered centrifugal precipitation tubes S and S', and subjected to the same procedure for the sample solution. Each centrifugal precipitation tube is centrifuged and each isobutanol layer is collected. Fluorescence intensities F_T, $F_{T'}$, F_S, and $F_{S'}$ of the collected layers are measured at the maximum wavelength of excitation at around 370 nm and at the maximum wavelength of fluorescence at around 440 nm using a fluorometer.

Amount of dibenzoyl thiamine hydrochloride ($C_{26}H_{26}N_4O_4S \cdot HCl$) (mg)

$$= \text{Amount of reference standard of thiamine hydrochloride (mg)} \times \frac{F_T - F_{T'}}{F_S - F_{S'}} \times 1.5627$$

(b) Standard of storage method

It shall be stored in a capped container.

(35) Thiamine Hydrochloride A. Raw material for manufacturing

A. Raw material for manufacturing

(a) Compositional standards

Content: This product contains 98.0 ~102.0 % of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) when you determine the quantity of it after drying for 2 hours at 105 degree.

Physical and chemical properties:

i. This product is white crystals or crystalline powder with no odor or a slight, specific odor.

ii. This product is easy to dissolve in water, hard to dissolve in ethanol or glycerin, and hardly dissolves in ether or benzene.

iii. Melting point: About 245 °C (decomposition)

Confirmation test:

i. When 2 mL of a solution of this product in water (1 → 500) is added with 2~3 drops of iodine test solution, with 2~3 drops of Mayer's test solution, and with 1 mL of picric acidtest solution, red-brown precipitation or opacity, yellowish white precipitation or opacity and yellow precipitation or opacity are generated, respectively.

ii. When 1 mL of a solution of this product in water (1 → 500) is added with 1 mL of lead acetate test solution and 1 mL of sodium hydroxide solution (1 → 10) and warmed, the solution turns through yellow to brown. It is allowed to stand and then blackish brown precipitation is generated.

iii. When 5 mL of a solution of this product in water (1 → 500) is added with 2.5 mL of 1 mol/L sodium hydroxide test solution and 0.5 mL of potassium ferricyanide test solution, then added with 5 mL of isobutanol, vigorously shaken up for 2 minutes, allowed to stand, and observed under ultraviolet, the isobutanol layer emits blue-violet fluorescence. This fluorescence disappears when the solution is made acidic, and reappears when it is made alkaline.

iv. A solution of this product in water (1 → 500) gives the qualitative reaction of chloride.

Purity test:

i. pH: 1.0 g (0.95~1.04 g) of this product is dissolved in water to make 100 mL. The pH of this solution shall be 2.7~3.4.

ii. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved in water to make 10 mL. The color of this solution shall not be darker than that of a solution prepared by which 1.5 mL of 0.017 mol/L potassium dichromate solution is added with water to make 1,000 mL.

iii. Sulfate: 1.5 g (1.45~1.54 g) of this product is weighed to prepare a sample solution by the sulfate test method. A control solution is prepared using 0.35 mL of 0.005 mol/L sulfuric acid. When sulfate is tested using these samples and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.011 % or less).

iv. Nitrate: When 0.5 g (0.45~0.54 g) of this product is dissolved with 25 mL of water. 2 mL of this solution is added with 2 mL of sulfuric acid, shaken up, and allowed to cool, and on it the layer of ferrous sulfate test solution is formed, at the boundary surface a dark brown ring shall not be produced.

v. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

Loss on drying: 5.0 % or less (0.5 g, 105 °C, 2 hours)

Ignition residue: 0.20 % or less (1 g)

Assay: This product and the reference standard of thiamine hydrochloride are separately dried at 105 °C for 2 hours. Approximately 0.1 g each is weighed to the digits of 0.001 g and the value is recorded. Each of them is dissolved with 0.001 mol/L hydrochloric acid test solution, transferred to a 200 mL volumetric flask, and added with 0.001 mol/L hydrochloric acid test solution to the graduation line to make 200 mL. 2 mL each of these solutions is measured using a volumetric pipette and transferred to a 50 mL volumetric flask, added with 0.001 mol/L hydrochloric acid test to the graduation line to make 50 mL. They are used as a sample solution and a standard solution. 5 mL of the sample solution is measured using a volumetric pipette into a stoppered test tube T and T'. The tube T is added with 3.0 mL of cyanogen bromide, shaken up, added with 5.0 mL of sodium hydroxide solution (1 → 10) within 30 seconds and shaken up. The tube T' is added with 5.0 mL of sodium hydroxide solution (1 → 10), shaken up, added with 3.0 mL of cyanogen bromide and shaken up.Separately, 5 mL of the standard solution is measured using a volumetric pipette into stoppered test tubes S and S', and the same procedure for the sample solution is performed.Absorbance of each solution, A_T, $A_{T'}$, A_S, $A_{S'}$ at a wavelength 368 nm measured using water as a control solution.

Amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) (mg)

$$= \text{Amount of reference standard of thiamine hydrochloride (mg)} \times \frac{A_T - A_{T'}}{A_S - A_{S'}}$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of thiamine hydrochloride are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of thiamine hydrochloride is applied mutatis mutandis.

(36) L- Histidine hydrochloride

A. Raw material for manufacturing

(a) Compositional standards

Content: L-histidine hydrochloride, after drying at 105 °C for three hours, contains not less than 98.0% of L-histidine hydrochloride monohydrate ($C_6H_9N_3O_2.HCl.H_2O$).

Physical and chemical properties

i. L-histidine Monohydrochloride occurs as white crystals or crystalline powder.

ii. The pH of an aqueous solution of L-histidine hydrochloride (1 → 10) is between 3.5 ~ 4.5.

Identification

i. To 5 mL of an aqueous solution of L-histidine hydrochloride (1 → 1000), add 1 mL of ninhydrin solution (1 → 1000), and heat for three minutes: a purple color develops.

ii. An aqueous solution of L-histidine hydrochloride (1 → 10) responds to the Qualitative Tests for chloride.

Purity

i. Specific rotation: Weigh about 5.5 g of L-histidine hydrochloride to the order of 0.01 g, record the value calculated on the dried basis, dissolve in 6 mol/L hydrochloric acid TS to make 50 mL, filter if necessary, and determine the optical rotation of this solution: It shall be between + 8.5 ~ + 10.5°.

ii. Ammonium salt: Proceed as directed in the Purity (3) for the active ingredients for aminoacetic acid production. In this case, the term “0.1 g” shall be deemed to be replaced with “0.05 g,” and the term “1 mL” shall be deemed to be replaced with “0.5 mL” (not more than 0.04 %).

iii. Lead: Perform the test for lead with 5.0 g (4.95 ~ 5.04 g) of L-histidine hydrochloride as directed under Lead Test (Method 1 of Atomic Absorption Spectrophotometry): The amount of lead shall be not more than 2 μg/g.

Pipet 1.0 mL of standard lead solution using a volumetric pipette, transfer it into a 10 mL volumetric flask, add nitric acid (1 →150) to the marked line to make 10 mL, and use this solution as the standard solution.

iv. Arsenic: Transfer 1.0 g (0.95 ~ 1.04 g) of L-histidine hydrochloride to a decomposition flask, add 10 mL of nitric acid and 5 mL of sulfuric acid, and heat gently. If a brown color still develops in the solution, allow to cool, add 1 to 2 mL of nitric acid, heat, and repeat this procedure until the solution becomes colorless to pale yellow. After allowing to cool, add 0.5 mL of perchloric acid, and heat until white fumes are evolved. After allowing to cool, add 15 mL of saturated ammonium oxalate solution, and heat again until white fumes are evolved. After allowing to cool, add water to make about 10 mL, and use this solution as the sample solution to perform the test for arsenic by using Apparatus A .

At this time, the color of the absorbing liquid shall have no more color than the standard color (not more than 2 μg/g).

Loss on drying: Not more than 0.3% (3 g, 105 °C, 3 hours)

Residue on ignition: Not more than 0.1% (1 g)

Assay: Weigh about 0.5 g of L-histidine hydrochloride, previously dried at 105 °C for three hours, to the order of 0.1 mg, record the value, add water to dissolve it, transfer to a 1000 mL volumetric flask, add water to the marked line to make 1000 mL, and use this solution as the sample solution. Perform the test with 5 μL of this solution as directed under liquid chromatography according to the following conditions. Based on the chromatogram obtained, determine the peak area of histidine, and calculate the concentration of L-histidine hydrochloride monohydrate by a calibration curve obtained separately, and calculate the content.

Operating conditions

Detector: An ultraviolet absorption photometer (measuring wavelength: 210 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 150 mm in length packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: 35 °C

Mobile phase: Dissolve 2.2 g (2.265 ～ 2.274 g) of potassium dihydrogen phosphate and 1.08 g (1.075 ～ 1.084 g) of sodium 1-octanesulfonate in 850 mL of water, adjust the pH to 2.5 with phosphoric acid, add and mix 100 mL of acetonitrile for liquid chromatography, and add water to make 1000 mL.

Flow rate: 1.0 mL per minute

Creating calibration curve

Weigh about 0.05 g, 0.1 g, 0.5 g, and 1 g of L-histidine hydrochloride for assay to the order of 0.1 mg, record the values, add about 800 mL of water to each to dissolve it, transfer to 1000 mL volumetric flasks, add water to the marked line to make 1000 mL, and use these solutions as the standard solutions containing 0.05 mg, 0.1 mg, 0.5 mg, and 1 mg per mL. Perform the test with 5 μL each of the standard solutions as directed under Liquid Chromatography in the same manner as for the sample solution. Based on the chromatogram obtained, determine the peak area of histidine and prepare a calibration curve.

(b) Standard of storage method

Culture a histidine-producing strain of Corynebacterium glutamicum aerobically, and after the completion of culturing, filter the culture to remove bacterial bodies and isolate the L-histidine crude crystalline fraction. Purify a crude crystal and dry the resulting solid to produce the product.

(c) Standard of storage method

It shall be stored in a capped container.

B. Preparatio

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-histidine hydrochloride are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-histidine hydrochloride is applied mutatis mutandis.

(37) Pyridoxine Hydrochloride

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 4 hours drying in a desiccator (reduced pressure, silica gel), it contains 98.0% or more of pyridoxine hydorochloride ($C_8H_{11}NO_3 \cdot HCl$).

Physical and chemical properties:

i. This product is white-slightly yellow crystalline powder with no odor and with bitter and sour tastes.

ii. This product is easy to dissolve in water, hard to dissolve in ethanol, and hardly dissolves in acetone, ether or chloroform.

iii. The pH of solution (1 → 50) of this product is 2.5~3.5.

iv. This product gradually changes by light.

v. Melting point: About 206 °C (decomposition)

Confirmation test:

i. When 1 mL of a solution of this product in water (1 → 1,000) is added with a drop of ferric chloride test solution, the resulting solution is orange-brown. The color changes to yellow by adding a drop of hydrochloric acid.

ii. When 1 mL of solution of this product in water (1 → 10,000) is added with 2 mL of newly prepared a solution of 2,6-dibromequinonechloroimide in ethanol (1 → 4,000) and a drop of ammonia test solution, the resulting solution is blue. When 1 mL of a solution of this product in water (1 → 10,000) is added with 1 mL of a saturated solution of boric acid and subjected to the same procedure, the solution is not blue.

iii. 0.5 g (0.45~0.54 g) of this product is added with 1 mL of water, warmed to dissolve, allowed to cool, added with 6 mL of picric acid test solution, and allowed to stand for 2~3 hours. The deposited crystals are collected by filtration, washed with a little ice water, dried at 105 °C for 2 hours, and the melting point is 156~159 °C (decomposition).

iv. A solution of this product in water (1 → 10) gives the qualitative reaction of chloride.

Purity test: Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 3.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (30 mg/kg or less).

Loss on drying: 0.30 % or less (1 g, reduced pressure, silica gel, 4 hours)

Ignition residue: 0.10 % or less (1 g)

Assay: This product is dried in a desiccator (reduced pressure, silica gel) for 4 hours. Approximately 0.4 g of it is weighed to the digits of 0.001 g and the value is recorded. It is added with 10 mL of mercuric acetate test solution for nonaqueous titration, shaken up to dissolve, added with 60 mL of glacial acetic acid for nonaqueous titration and titrated with 0.1 mol/L perchloric acid (indicator: 2 drops of methylrosanilinium chloride test solution). In this case, the end point of the titration is the point at which the color of the solution changes from purple to blue. A blank test is performed in the same way and corrections are made.

0.1 mol/L perchloric acid 1 mL = 20.56 mg $C_8H_{11}NO_3$・HCl

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Pyridoxine hydrochloride are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Pyridoxine hydrochloride is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of pyridoxine hydorochloride and fillers.

Content: When this product is determined, it contains pyridoxine hydorochloride ($C_8H_{11}NO_3$· HCl) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.01 g of the raw material for manufacturing of pyridoxine hydrochloride is weighed, added with 10 mL of water, and for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fat added with 3 mL of

chloroform, shaken up, and allowed to stand, and the aqueous layer is filtered to prepare a sample solution. 1 mL of this solution is measured, and hereinafter the confirmation tests i. for the raw material for manufacturing of pyridoxine hydrochloride is applied mutatis mutandis.

ii. 1 mL of the sample solution i. is measured and added with water to make 10 mL. 1 mL of this solution is measured, and hereinafter the confirmation tests ii. for the raw material for manufacturing of pyridoxine hydrochloride is applied mutatis mutandis.

iii. When 1 mL of the sample solution i. is measured and added with 0.5 mL of phosphotungstic acid test solution, the resulting solution becomes cloudy.

Assay: Method No. 1 is used for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats and Method No. 2 is used for others.

Method No. 1: The amount of this product containing approximately 0.02 g of pyridoxine hydrochloride ($C_8H_{11}NO_3$・HCl) is weighed to three significant digits and the value is recorded. It is added with 50 mL of chloroform, shaken up, and filtered using a glass filter (G4). The residue on the filter is washed three times with 20 mL of chloroform. When the chloroform odor on the residue disappears, the residue is filtered by extracting with approximately 90 mL of warm water, allowed to cool, transferred to a 100 mL volumetric flask. The filtrate is added with water to the graduation line to make 100 mL. 25 mL of this solution is measured using a volumetric pipette into a 200 mL volumetric flask and added with water to the graduation line to make 200 mL. This is used as a sample solution. Separately, the reference standard of pyridoxine hydrochloride is dried in a desiccator (reduced pressure, silica gel) for 4 hours. Approximately 0.1 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask and added with more water to make 100 mL. 5 mL of this solution is measured using a volumetric pipette, transferred to a 200 mL volumetric flask, added with water to the graduation line to make 200 mL. This is used as a standard solution. 1 mL of the sample solution is measured using a volumetric pipette, placed in a 25 mL volumetric flask, added with 2.0 mL of barbital buffer, 9.0 mL of isopropanol and 2.0 mL of newly prepared solution of 2,6-dibromequinonechloroimide in ethanol (1 → 4,000), shaken up well, added with isopropanol to the graduation line to make 25 mL, and allowed to stand for 90 minutes. For the resulting solution, the absorbance A_T at the maximum wavelength at around a wavelength of 650 nm is measured. The solution prepared using 1 mL of water instead of the sample solution by the same procedure for the sample solution is used as a control solution. Separately, 1 mL of the standard solution is measured using

a volumetric pipette. Its absorbance A_S is measured by the same procedure for the sample solution.

Amount of pyridoxine hydrochloride ($C_8H_{11}NO_3$・HCl) (mg)

$$= \text{Amount of reference standard of pyridoxine hydrochloride (mg)} \times \frac{A_T}{A_S} \times \frac{1}{5}$$

Method No. 2: The amount of this product containing approximately 0.02 g of pyridoxine hydrochloride ($C_8H_{11}NO_3$・HCl) is weighed to three significant digits and the value is recorded. It is added with 60 mL of water, vigorously shaken up, and filtered and the residue is washed twice with 10 mL of water. The filtrate and the washings are collected together, transferred to a 100 mL volumetric flask, added with water to the graduation line to make 100 mL. 25 mL of this solution is measured using a volumetric pipette, transferred to a 200 mL volumetric flask, added with water to the graduation line to make 200 mL. This solution is used as a sample solution and hereinafter the Method No. 1 is applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a lightproof capped container..

(38) L-Lysine Monohydrochloride

L-lysine monohydrochloride (Part 1)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 3 hours, it contains 98.5 % or more of L-lysine monohydrochloride ($C_6H_{14}N_2O_2$·HCl).

Physical and chemical properties:

i. This product is white to light brown powder with no odor or a slight, specific odor.

ii. This product is easy to dissolve in water and extremely hard to dissolve in ethanol.

iii. The pH of a solution (1 → 10) of this product is 5.0~6.0.

Confirmation test:

i. When 5 mL of a solution of this product in water (1 → 1,000) is added with 1 mL of ninhydrin test solution, heated for 3 minutes, added with 20 mL of water, and allowed to stand for 15 minutes, the resulting solution is reddish violet.

ii. A solution of this product in water (1 → 10) gives the qualitative reaction of chloride.

iii. 0.1 g (0.05~0.14 g) each of this product and Japanese pharmacopeia lysine hydrochloride is weighed and dissolved with 10 mL of water. They are used as sample and standard solutions. These solutions are tested by the thin layer chromatography. 5

μL each of the sample and standard solutions is spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then it is developed approximately 10 cm with the developing solvent, a mixture of n-propanol and strong ammonia solution (67:33) and the thin layer is air dried. When it is sprayed with a solution of ninhydrin in acetone (1 → 50) and dried at 80 °C for 5 minutes, the spots obtained from the sample and standard solutions are purple and their Rf values are equal.

Purity test:

i. Specific rotation: This product is dried at 105 °C for 3 hours. Approximately 4 g of it is weighed to the digits of 0.01 g and the value is recorded. It is dissolved in hydrochloric acid (13 → 25) to make 50 mL and as appropriate filtered using a membrane filter (0.45 μm). The rotation of this solution measured at a layer length 100 mm shall be $[\alpha]_D^{20}$ = +18.0 ~ +21.5°.

ii. Ammonium salt: The purity test iii. for the raw material for manufacturing of aminoacetic acid is applied mutatis mutandis. In this case, “0.1 g” and “1 mL” shall be replaced by “0.05 g” and “0.5 mL”, respectively (0.04 % or less).

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 3.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (30 mg/kg or less).

iv. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 1.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 0.3 % or less (1 g)

Assay: This product is dried at 105 °C for 3 hours. Approximately 0.2 g of it is weighed to the digits of 0.001 g and the value is recorded. It is added with 10 mL of mercuric acetate test solution for nonaqueous titration, warmed to dissolve, allowed to cool, added with 50 mL of glacial acetic acid for nonaqueous titration, and titrated with 0.1 mol/L perchloric acid (indicator: 10 drops of *α*-naphtholbenzein test solution). In this case, the end point of the titration is the point at which the color of the solution changes from orange to yellow-green. A blank test is performed in the same way and corrections are made.

0.1 mol/L perchloric acid 1 mL = 9.133 mg $C_6H_{14}N_2O_2 \cdot HCl$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-lysine monohydrochloride (part 1) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-lysine monohydrochloride (part 1) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of L-lysine monohydrochloride (part 1) and excipient.

Content: When this product is determined, it contains L-lysine monohydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.1 g of raw material for manufacturing of L-lysine monohydrochloride (Part 1) is weighed, added with 100 mL of water, shaken up and filtered. 5 mL of the filtrate is added with 1 mL of ninhydrin test solution, hereinafter the confirmation test i. of the raw material for manufacturing of L-lysine monohydrochloride (Part 1) is applied mutatis mutandis.

ii. According to the amount of this product on the label, the amount containing 0.1 g of raw material for manufacturing of L-lysine monohydrochloride (Part 1) is weighed, added with 10 mL of water, shaken up and filtered. The filtrate is used as a sample solution. Separately, 0.1 g (0.05~0.14 g) of lysine hydrochloride is weighed and added with 10 mL of water to prepare a standard solution. The confirmation test iii. of the raw material for manufacturing of L-lysine monohydrochloride (Part 1) is hereinafter applied mutatis mutandis.

Assay: The amount of this product containing approximately 1.0 g of L-lysine monohydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$) is weighed to three significant digits and the value is recorded. It is dissolved with water, placed in a 500 mL volumetric flask, added with water to the graduation line, shaken up well to make 500 mL, and filtered using a dried filter paper. 20 mL of the first filtrate is removed, and 20 mL of the next filtrate is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, added with water to the graduation line to make 100 mL. This is used as a sample solution. Separately, lysine hydrochloride is dried at 105 °C for 3 hours. Approximately 0.05 g and 0.03 g of it are weighed to the digits of 0.1 mg and the values are recorded. Each of them is dissolved with water, transferred to a 100 mL volumetric flask, added with water to the

graduation line to make 100 mL. The two solutions are used as standard solutions S and S′. 2 mL each of the sample solution and the standard solutions S and S′ is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, added with 4 mL of ninhydrin test solution for assay using a volumetric pipette, heated in a water bath for 20 minutes, allowed to cool, and then added with water to the graduation line to make 50 mL. The absorbances A_T, A_S and A_S at the maximum wavelength at around wavelength 475 nm are measured for the sample solution and the standard solutions S and S′, respectively.

Amount of L-lysine monohydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$) (mg)

$$= b + \frac{A_T - A_{S'}}{A_S - A_{S'}} \times (a - b)$$

a: Standard S: Amount of L-lysine monohydrochloride in 1 mL (mg)

b: Standard S′: Amount of L-lysine monohydrochloride in 1 mL (mg)

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-lysine monohydrochloride (part 1) is applied mutatis mutandis.

L-Lysine Monohydrochloride (Part 2)

A. Raw material for manufacturing

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-lysine monohydrochloride (part 1) are applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the L-lysine producing recombinant, whose host is the strain belonging to *Escherichia coli*, is cultured. After the cultivation, the bacterial cells are heat-treated to separate L-lysine, and dried.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-lysine monohydrochloride (part 1) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-lysine monohydrochloride (part 2) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-lysine monohydrochloride (part 2) is applied mutatis mutandis.

(39) L-Carnitine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 98.0~102.0 % of L-carnitine ($C_7H_{15}NO_3$) at dehydration product conversion.

Physical and chemical properties:

i. This product is white powder.

ii. The pH of a solution of this product in water (1 → 20) is 6.5 to 8.5.

Confirmation test: When the infrared absorption spectrum of this product is measured using the potassium bromide disk method of the infrared absorption spectroscopy, main absorptions are observed at wavenumbers around 3,460 cm^{-1}, 1,600 cm^{-1}, 1,479 cm^{-1}, 1,393 cm^{-1}, and 945 cm^{-1}.

Purity test:

i. Specific rotation: 10 g of this product is weighed to the digit of 0.01 g and the value is recorded. It is dissolved with water to make 100 mL. The optical rotation of this solution shall be $[\alpha]_D^{20}$ = -30.0 ~ -32.0°.

ii. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 10 mL of water. The resulting solution shall be colorless and clear.

iii. Chloride: 5.0 g (4.95~5.04 g) of this product is weighed to prepare a sample solution using the chloride test method. A control solution is prepared using 2.80 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these sample and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.02 % or less).

iv. Sulfate: 5.0 g (4.95~5.04 g) of this product is weighed to prepare a sample solution using the sulfate test method. A control solution is prepared using 3.10 mL of 0.005 mol/L sulfuric acid. When sulfate is tested using these sample and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.03 % or less).

v. Lead: 5.0 g (4.95~5.04 g) of this product is weighed. When lead is tested using the lead test method (Method No. 1 of the atomic absorption spectrophotometry), the amount of lead shall be 1 μg/g or less. In so doing, 0.5 mL of lead standard solution is measured using a volumetric pipette and put in a 10 mL volumetric flask, added with nitric acid (1 → 150) up to the capacity mark to make 10 mL. This is used as a standard solution.

vi. Potassium: 5.0 g (4.95~5.04 g) of this product is weighed in a platinum crucible, gently heated to carbonize, and heated at 500 °C or less to incinerate. The residue is

transferred into a 100 mL tall form beaker with a small amount of water, 10 mL of hydrochloric acid is gradually added, boiled for several minutes and allowed to cool. Transfer this solution into a 250 mL volumetric flask with water, added with water up to the capacity mark, and filtered with filter paper. 50 mL of the filtrate is put in a 100 mL volumetric flask using a volumetric pipette. 10 mL of interference suppression agent solution is added, and water is added up to the capacity mark to make 100 mL. Using this as a sample solution, a test is performed by atomic absorption spectrometry (flame type).

Simultaneously, 10 mL of hydrochloric acid is put in a 250 mL volumetric flask, and added with water up to the capacity mark to make 250 mL. 50 mL of the filtrate is measured using a volumetric pipette and put in a 100 mL volumetric flask. 10 mL of interference suppression agent solution is added, and added with water up to the capacity mark to make 100 mL. A blank test is performed using this solution and corrections are made.

Separately, 2 mL of potassium standard solution is measured using a volumetric pipette and put in a 100 mL volumetric flask. 10 mL of interference suppression agent solution is added, and added with water up to the capacity mark to make 100 mL. This is used as a standard solution.

When the sample and standard solutions are tested by atomic absorption spectrophotometry at the wavelength of 766.5 nm, using a potassium hollow cathode lamp as the light source, acetylene as a flammable gas, and air as a combustion-supporting gas, the absorbance of the sample solution shall be equal to or lower than that of the standard solution (0.2 % or less).

Preparation of interference suppression agent solution: 12.5 g (12.45~12.54 g) of calcium carbonate [special grade] ($CaCO_3$) is weighed and put in a beaker, added with a small amount of water, and added with 105 mL of hydrochloric acid gradually. This solution is boiled, allowed to cool, and added with water to make 1,000 mL.

vii. Sodium: The sample solution obtained in vi. is tested by atomic absorption spectrometry (flame type). A blank test is performed in the same way as vi. and corrections are made. Separately, 1 mL of sodium standard solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark. This is used as a standard solution. When the sample and standard solutions are tested by atomic absorption spectrophotometry at the wavelength of 589.0 nm, using a sodium hollow cathode lamp as the light source, acetylene as a flammable gas, and air as a combustion-supporting gas, the absorbance of the sample solution shall be equal to or lower than that of the standard solution (0.1 % or less).

Water content: 4.0 % or less (direct titration)

Ignition residue: 0.1 % or less (1 g)

Assay: Approximately 3.0 g of this product is weighed to the digit of 0.001 g and the value is recorded. It is dissolved with 40 mL of acetone and 20 mL of water and titrated with 1 mol/L hydrochloric acid (potentiometric titration). A blank test is performed in the same way and corrections are made.

1 mol/L hydrochloric acid 1 mL = 161.20 mg $C_7H_{15}NO_3$

(b) Standard of storage method

It shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

This product is a powder mixture of the raw material for manufacturing of L-Carnitine and hydrated amorphous silicon oxide.

Content: When this product is determined, it contains L-carnitine ($C_7H_{15}NO_3$) corresponding to 90~110 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of L-Carnitine is applied mutatis mutandis.

Assay: The assay of the raw material for manufacturing of L-Carnitine is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-Carnitine is applied mutatis mutandis.

(40) *β*-Carotene

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried in a desiccator (reduced pressure, silica gel) for 4 hours, it contains 98.0 % or over of *β*-carotene ($C_{40}H_{56}$).

Physical and chemical properties: This product is reddish violet to dark red crystals or crystalline powder with a slight, specific odor and taste.

Confirmation test:

i. 10 mL of a solution of this product in chloroform (1 → 1,000) is orange. When this solution is added with 1 mL of antimony trichloride test solution, it is blue-green.

ii. The sample solution obtained by the assay is measured for its absorption spectrum using cyclohexane as a control. The absorption maximum is at wavelengths of 454~457 nm and 481~484 nm.

Purity test:

i. Melting point: 176~183 °C (degradation)

ii. Clarity and color of solution: 0.1 g (0.05~0.14 g) of this product is dissolved with 10 mL of chloroform. The resulting solution shall be dark red and clear.

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

v. Absorption ratio: For the sample stock solution and the sample solution obtained by the assay, the absorptions A_1 and A_2 at the wavelengths of 340 and 362 nm of the sample stock solution and absorptions A_3, A_4 and A_5 at the wavelengths of 434, 455 and 483 of the sample solution are measured using cyclohexane as a control. A_2/A_1, $(A_4 \times 10)/A_1$, A_4/A_3, and A_4/A_5 shall be 1.00 or more, 15.0 or more, 1.30~1.60, and 1.05~1.25, respectively.

Loss on drying: 1.0 % or less (1 g, silica gel, reduced pressure, 4 hours)

Ignition residue: 0.10 % or less (1 g)

Assay: This product is dried in a desiccator (reduced pressure, silica gel) for 4 hours, approximately 0.04 g of it is weighed to the digits of 0.1 mg, and the value is recorded. It is dissolved with 10 mL of chloroform, transferred to a 100 mL volumetric flask, and added with cyclohexane to the graduation line to make 100 mL. 5 mL of this solution is weighed using a volumetric pipette into a 100 mL volumetric flask, and added with cyclohexane to the graduation line to make 100 mL. This is used as a sample stock solution. 10 mL of the sample stock solution is measured using a volumetric pipette into a 100 mL volumetric flask, and added with cyclohexane to the graduation line to make 100 mL. This is used as a sample solution. The absorbance A of this solution is measured at the maximum wavelength at around wavelength 455 nm using cyclohexane as a control.

$$\text{Amount of } \beta\text{-carotene } (C_{40}H_{56}) \text{ (mg)} = \frac{A}{2{,}450} \times 200{,}000$$

(b) Standard of storage method

It shall be stored in a lightproof capped container in which air is replaced with inert gas.

B. Preparation

(a) Compositional standards

This product is particles of the mixture of the raw material for manufacturing of *β*-carotene and excipient.

Content: When this product is determined, it contains *β*-carotene ($C_{40}H_{56}$) corresponding to 90~130 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.01 g of the raw material for manufacturing of *β*-carotene is weighed, added with 100 mg (99.5~100.4 mg) of trypsin, added with 3 mL of water, occasionally shaken up in a water bath at 40 °C for 5 minutes, sonicated for 10 minutes to be suspended if the particles remain. It is allowed to cool, added with 20 mL of chloroform, shaken up well, and centrifuged for 5 minutes at 3,000 rpm, and then the chloroform layer is orange. When 2 mL of the chloroform layer is measured, added with 0.5 mL of antimony trichloride test solution, and allowed to stand, the solution is blue-green.

ii. The sample solution obtained by the assay is measured for its absorption spectrum using cyclohexane as a control. The absorption maximum is at wavelengths of 454~457 nm and 481~484 nm.

Assay: The amount of this product containing approximately 5 mg of *β*-carotene ($C_{40}H_{56}$) is weighed to three significant digits and the value is recorded. It is transferred to a 100 mL volumetric flask, added with 100 mg (99.5~100.4 mg) of trypsin, added with 3 mL of water, occasionally shaken up in a water bath at 40 °C for approximately 5 minutes. If the particles remain, it is sonicated for 10 minutes to be suspended. It is allowed to cool, added with 30 mL of absolute ethanol, shaken up well, and added with ether to the graduation line to make 100 mL. This solution is shaken up well, and some of it is centrifuged for 5 minutes at 3,000 rpm. 2 mL of the supernatant is measured using a volumetric pipette and the solvent is distilled away in a water bath at 50 °C under nitrogen stream. The residue is dissolved with approximately 0.5 mL each of absolute ethanol and chloroform, transferred to a 50 mL volumetric flask, added with cyclohexane to the graduation line to make 50 mL. This is used as a sample solution. The absorbance A of the sample solution of the maximum wavelength at wavelength around 455 nm is measured using cyclohexane as a control.

$$\text{Amount of } \beta\text{-carotene } (C_{40}H_{56}) \text{ (mg)} = \frac{A}{2{,}230} \times 25{,}000$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

(41) Canthaxanthin

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 96.0~102.0 % of canthaxanthin ($C_{40}H_{52}O_2$).

Physical and chemical properties:

i. This product is reddish violet to dark reddish violet crystalline powder.

ii. This product is easy to dissolve in chloroform and hardly dissolves in absolute ethanol, ether, cyclohexane and water.

iii. This product gradually changes by oxygen and light.

iv. Melting point: About 209 °C (degradation)

Confirmation test:

i. When 0.5 mL of a solution of this product in chloroform (1 → 10,000) is added with 1 mL of antimony trichloride test solution, the resulting solution is dark blue.

ii. The sample solution obtained by the assay is measured for its absorption spectrum using cyclohexane as a control. The absorption maximum is at wavelengths of 470~476 nm.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 0.5 % or less (1 g, reduced pressure, silica gel, 3 hours)

Ignition residue: 0.1 % or less (1 g)

Assay: Approximately 10 mg of this product is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with chloroform, transferred to a 200 mL volumetric flask, and added with chloroform to the graduation line to make 200 mL. 4 mL of this

solution is measured using a volumetric pipette to a 100 mL volumetric flask, and added with cyclohexane to the graduation line to make 100 mL. This is used as a sample solution. The absorbance A of this solution at the maximum wavelength at around wavelength 473 nm is measured using cyclohexane as a control.

$$\text{Amount of canthaxanthin } (C_{40}H_{52}O_2)\ (\text{mg}) = \frac{A}{2{,}200} \times 50{,}000$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container in which air is replaced with nitrogen gas.

B. Preparation

(a) Compositional standards

This product is particles of the mixture of the raw material for manufacturing of canthaxanthin and excipient.

Content: When this product is determined, it contains canthaxanthin ($C_{40}H_{52}O_2$) corresponding to 90~120 % of the amount on the label.

Confirmation test: 5 mL of the filtrate obtained by the assay is measured and the solvent is distilled away in a water bath at 50 °C under nitrogen stream. The residue is dissolved with 5 mL of chloroform to prepare a sample solution. 10 μL of this solution is spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then it is developed approximately 15 cm with the developing solvent, a mixture of dichloromethane and ether (9:1) in a dark place. When the thin layer plate is air dried in a dark place, the canthaxanthin spot obtained from the sample solution is red and its Rf value is approximately 0.4.

Assay: The amount of this product containing approximately 10 mg of canthaxanthin ($C_{40}H_{52}O_2$) is weighed to three significant digits and the value is recorded. It is added with 10 mL of water warmed at 60 °C, warmed in a water bath at 60 °C, and stirred under nitrogen stream to make it a complete suspension. It is allowed to cool, transferred to a 250 mL volumetric flask and added with 100 mL of absolute ethanol. Then it is added with chloroform to the graduation line to make 250 mL and filtered. 20 mL of the first filtrate is removed, 5 mL of the next filtrate is measured using a volumetric pipette to a 100 mL round-bottom flask, and the solvent is distilled away in a water bath at 50 °C under nitrogen stream. The residue is moisturized with several drops of absolute ethanol, added with 50 mL of cyclohexane, and allowed to stand at 50 °C for approximately 2 minutes to dissolve completely. This solution is allowed to cool, transferred to a 100 mL volumetric flask, added with cyclohexane to the graduation line to make 100 mL. This is used as a sample solution. The absorbance A of this solution at the maximum wavelength at around wavelength 470 nm is measured using cyclohexane as a control.

$$\text{Amount of canthaxanthin } (C_{40}H_{52}O_2)\ (\text{mg}) = \frac{A}{1{,}970} \times 50{,}000$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

(42) Guanidinoacetic Acid

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined,it contains guanidinoacetic acid ($C_3H_7N_3O_2$) at no less than 96.5 %.

Physical and chemical properties:

i. This product is white to light brown powder.

ii. The pH of an aqueous suspension of this product (1 → 10) is 8.0~9.0.

iii. This product is slightly insoluble in water, and extremely insoluble in ethanol.

Confirmation test:

i. When 1 mL of ninhydrin test solution is added to 5 mL of an aqueous solution of this product (1 → 1,000) and heated for 3 minutes, the solution does not develop a color.

ii. When 1 mL of 8-oxyquinoline test solution and 1 mL of N-bromosuccinimide solution (1 → 1,000) are added to 2 mL of an aqueous solution of this product (1 → 20,000), the solution develops a red-yellow color.

Purity test:

i. Lead: Weigh 2.0 g (1.95~2.04 g) of this product and when a test for lead is performed by the lead limit test (atomic absorption spectrometry No.1), the amount shall be no more than 5 μg/g.

ii. Arsenic: Weigh 1.0 g (0.95~1.04 g) of this product, prepare a sample solution by according to the arsenic limit test method3, and when a test for arsenic is performed by the method using apparatus A, the color of absorption solution shall not be deeper than the standard color (no more than 2 μg/g).

iii. Dicyandiamide: Weigh 0.500 g (0.4995~0.5004 g) of this product, add water to dissolve with sonicating if necessary, place in a 1,000 mL volumetric flask, further add water to the capacity mark (i.e. volume up to 1,000 mL), filter with a membrane filter

(0.45 μm), and serve the filtrate as the sample solution. Separately, weigh 10.0 mg (9.95~10.04 mg) of dicyandiamide reference standard, add water to dissolve, place in a 1,000 mL measuring flask, and further add water, to the capacity mark (i.e. volume up to 1,000 mL). Measure 25 mL of this solution using a volumetric pipette, place in a 100 mL measuring flask, and add water to the capacity mark (i.e. volume up to 100 mL) to prepare the standard solution. Measure 20 μL each of the sample solution and standard solution and when a test is performed by liquid chromatography under the conditions below, the peak area of dicyandiamide in the sample solution shall be no more than the peak area of dicyandiamide in the standard solution multiplied by the purity of dicyandiamide reference standard (no more than 0.5 %).

Operating condition

Detector: Ultraviolet absorptiometer (measuring wavelength: 220 nm)

Column: Fill a stainless steel tube of 4.6 mm in inner diameter and 250 mm in length with octadecylsilyl silica gel for liquid chromatography of 5 μm in particle diameter.

Mobile phase: Water

Flow rate: Approximately 1.5 mL per minute.

iv. Cyanamide: Weigh 0.400 g (0.3995~0.4004 g) of this product, add water to dissolve with sonicating if necessary, place in a 100 mL volumetric flask, further add water to the marked line (i.e. volume up to 100 mL), filter with a membrane filter (0.45 μm), and serve the filtrate as the sample solution. Separately, weigh 24.0 mg (23.95~24.04 mg) of cyanamide reference standard, add water to dissolve, place in a 1,000 mL measuring flask, and further add water to the capacity mark (i.e. volume up to 1,000 mL). Measure 10 mL of this solution using a volumetric pipette, place in a 200 mL measuring flask, and add water to the marked line (i.e. volume up to 200 mL) to prepare the standard solution. Measure 50 μL each of the sample solution and standard solution and when a test is performed by liquid chromatography under the conditions below, the peak area of cyanamide in the sample solution shall be no more than the peak area of cyanamide in the standard solution multiplied by the purity of cyanamide reference standard (no more than 0.03%).

Operating condition

Detector: Ultraviolet spectrophotometer (measuring wavelength: 210 nm)

Column: Connect the columns i to iii below in this order, and use i at the sample injection device side and iii at the detector side.

i: Fill a resin tube of 4.6 mm in inner diameter and 100 mm in length with porous graphite carbon for liquid chromatography of 7 μm in particle diameter.

ii: Fill a resin tube of 4 mm in inner diameter and 50 mm in length with anion exchange resin for liquid chromatography of 9 μm in particle diameter.

iii: Fill a resin tube of 4 mm in inner diameter and 250 mm in length with anion exchange resin for liquid chromatography of 9 μm in particle diameter.

Column temperature: A constant temperature around 30 °C

Mobile phase: Add 4 mL of 1-mol/L sodium hydroxide test solution to 1,000 mL of water.

Flow rate: 1.0 mL per minute

v. Melamine Weigh 0.100 g (0.0995~0.1004 g) of this product, place in a 50 mL container for homogenizer, add 50 μL of internal standard solution using a micropipet, further add 25 mL of water-acetonitrile mixture solution (1:1) and 10 mL of n-hexane, agitate using a homogenizer for 30 seconds, centrifuge at 900×g for 5 minutes, and serve the lower layer as the extract.

Measure 1 mL of the extract using a micropipet, place in an ethylenediamine-n-propylsilyl silica gel mini cartridge column previously washed serially with 5 mL of methanol and 5 mL of water-acetonitrile mixture solution (1:1), then add 3 mL of water-acetonitrile mixture solution (1:1) and collect the eluate.

Add 130 μL of 1 mol/L hydrochloric acid test solution to this eluate, place it in a strongly acidic cation exchanger mini cartridge column previously washed serially with 5 mL of ammonia water-methanol mixture solution (1:19), 5 mL of methanol and 5 mL of water, add serially 2 mL of 0.1 mol/L hydrochloric acid test solution and 1 mL

of methanol, and discard the effluent. Elute using 5 mL of ammonia water-methanol mixture solution (1:19), distill the solvent off in a nitrogen stream at 45°C, add 2 mL of formic acid (1 → 1,000)-acetonitrile mixture solution (1:1) to the residue, and sonicate for 5 seconds to prepare the sample solution.

Separately, weigh 0.01 g (0.0095~0.0104 g) of melamine reference standard, add water-acetonitrile mixture solution (1:1) to dissolve, place in a 100 mL volumetric flask, and further add water-acetonitrile mixture solution (1:1) to the marked line (i.e. volume up to 100 mL) to prepare the standard stock solution.

Accurately dilute certain quantities of the standard stock solution with formic acid (1 → 1,000)-acetonitrile mixture solution (1:1) to prepare some solutions containing amounts equivalent to 0.0025, 0.005, 0.01, 0.025 and 0.05 μg in 1 mL. Add an internal standard solution at a rate of 1 μL per 1 mL of each solution to prepare the standard solutions.

For 1 μL of the sample solution and standard solutions, when test for melamine is performed by liquid chromatography / mass spectrometry under the conditions below, the amount shall be no more than 20 μg/g.

Operating condition

Detector: Mass spectrometer (two mass spectrometry parts shall be serially connected, and a collision cell shall be placed in between)

Column: Fill a stainless steel tube of 2 mm in inner diameter and 150 mm in length with a column packing material in which carbamoyl group is introduced to the surface of silica gel substrate of 3 μm in particle diameter.

Mobile phase: Add 1,000 mL of water to 0.77 g (0.765~0.774 g) of ammonium acetate to dissolve, and serve it as Solution A. Solution B is acetonitrile, and feed under the conditions below.

0~5 min	Solution B at 95%
5~10 min	Solution B at 95% → Solution B at 90%
10~20 min	Solution B at 90%

Flow rate: 0.2 mL per minute

Ionization method: Electrospray ionization method (cation detection mode)

Measurement mode: Selected reaction monitoring

Set mass number: m/z 127 → 85 (melamine quantification ion)
m/z 127 → 68 (melamine confirmation ion)
m/z 130 → 87 (internal standard melamine-$^{15}N_3$ quantification ion)

Preparation of internal standard solution Weigh 0.005 g (0.0045~0.0054 mg) of melamine-$^{15}N_3$ reference standard, add water-acetonitrile mixture solution (1:1) to dissolve, place in a 50-mL volumetric flask, and further add water-acetonitrile mixture solution (1:1) to the marked line (i.e. volume up to 50 mL) to prepare the internal standard stock solution. Accurately dilute a certain quantity of the internal standard stock solution with water-acetonitrile mixture solution (1:1) to contain an amount equivalent to 10 μg in 1 mL.

Ethylenediamine-n-propylsilyl silica gel mini cartridge column A propropylene column tube filled with 500 mg of ethylenediamine-n-propylsilyl silica gel or a column with equivalent separation characteristics.

Strongly acidic cation exchanger mini cartridge column A propropylene column tube filled with 500 mg of strongly acidic cation exchanger or a column with equivalent separation characteristics.

Water content No more than 1.0 % (direct titration)

Quantification method Weigh approximately 10 mg of this product to the 0.01 mg digit, record the value, add approximately 200 mL of water to dissolve with sonicating if necessary, place in a 250-mL volumetric flask, further add water to the marked line (i.e. volume up to 250 mL), filter with a membrane filter (0.45 μm) if necessary, and serve the filtrate as the sample solution. For 10 μL of this solution, perform testing by liquid chromatography under the conditions below. Calculate the peak area of guanidinoacetic acid from the resulting chromatogram to calculate the concentration of guanidinoacetic acid using a separately prepared calibration curve and calculate the content.

Operating condition

Detector: Ultraviolet spectrophotometer (measuring wavelength: 200 nm)

Column: Fill a stainless steel tube of 4.6 mm in inner diameter and 250 mm in length with a column packing material for hydrophilic interaction chromatography of 5 μm in particle diameter.

Column temperature: A constant temperature around 30°C

Mobile phase: Water-acetonitrile mixture solution (3:7)

Flow rate: Approximately 1.0 mL per minute

Preparation of calibration curve

Weigh approximately 25 mg of guanidinoacetic acid reference standard to the 0.01 mg digit, record the value, add approximately 200 mL of water to dissolve with sonicating if necessary, place in a 250 mL volumetric flask, and further add water to the marked line (i.e. volume up to 250 mL) to prepare the standard stock solution. Add water to certain quantities of the standard stock solution , accurately dilute the solutions to contain amounts equivalent to 1, 3, 10 and 50 μg in 1 mL, filter the solutions and standard stock solution with a membrane filter (0.45 μm) if necessary, and serve the filtrate as standard solutions. For 10 μL each of the standard solutions, perform testing by liquid chromatography in the same manner as the case for sample solution. Calculate the peak area of guanidinoacetic acid from the resulting chromatogram to prepare a calibration curve.

(b) Standard of storage method

Shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

This product is powder or granule in which starch is mixed into source material for manufacturing guanidinoacetic acid.

Content When quantified, this product contains guanidinoacetic acid ($C_3H_7N_3O_2$) at no less than 95.5 %.

Confirmation Apply mutatis mutandis the confirmation for source material for manufacturing guanidinoacetic acid.

Quantification method Apply mutatis mutandis the quantification method for source material for manufacturing guanidinoacetic acid.

(b) Standard of storage method

Apply mutatis mutandis the standards for methods of storage for source material for manufacturing guanidinoacetic acid.

(43) Ferric Citrate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 16.5~18.5 % of iron (Fe).

Physical and chemical properties:

i. This product is red-brown, transparent small lamina or brown powder.

ii. This product is slightly hard to dissolve in cold water, easy to slightly dissolve in hot water, and hardly dissolves in ethanol.

iii. The pH of a solution (1 → 20) of this product in water is 1.0~2.0.

iv. A solution of this product in water is gradually reduced by light to become ferrous citrate.

Confirmation test:

i. 10 mL of a solution of this product in water (1 → 20) gives the qualitative reaction i. of ferric salt.

ii. 10 mL of a solution of this product in water (1 → 20) is added with 15 mL of 1 mol/L sodium hydroxide test solution and heated on a water bath for 10 minutes, with stirring well. It is allowed to cool and is filtered. 10 mL of the filtrate is neutralized with acetic acid. The resulting solution gives the qualitative reaction iii. of citrate.

Purity test:

i. Clarity and color of solution: 1 g (0.5~1.4 g) of this product is added with 20 mL of water and heated in a water bath to dissolve. The resulting solution shall be almost clear or clear.

ii. Lead: When 0.5 g (0.45~0.54 g) of this product is weighed and subjected to the lead test method (Method No. 1 of atomic absorption spectrophotometry), the content of lead shall be 20 mg/kg or less.

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 2 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Assay: Approximately 1.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is placed in a stoppered flask, added with 5 mL of hydrochloric acid and 30 mL of water, and dissolved by heating. It is allowed to cool, added with 4.0 g (3.95~4.04

g) of potassium iodide, tightly stoppered, allowed to stand for 15 minutes in a dark place, added with 100 mL of water, and titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 1 mL of starch test solution). A blank test is performed in the same way and corrections are made.

0.1 mol/L sodium thiosulfate solution 1 mL = 5.585 mg Fe

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of ferric citrate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of ferric citrate is applied mutatis mutandis.

(44) Calcium gluconate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 80 °C for 2 hours, it contains 98.0~104.0 % of calcium gluconate ($C_6H_{12}CaO_{14}\cdot H_2O$).

Physical and chemical properties: This product is white crystalline or particulate powder.

Confirmation test:

i. A solution of this product in water (1 → 40) gives the qualitative reaction of calcium salt.

ii. When 1 mL of a solution of this product in water (1 → 40) is added with a drop of ferric chloride test solution, the resulting solution is deep yellow.

iii. 5 mL of a solution of this product in water (1 → 10) is added with 0.7 mL of acetic acid and 1 mL of newly distilled phenylhydrazine, heated on a water bath for 30 minutes and allowed to cool. The inner wall is rubbed with a glass bar, and then crystals are deposited. The crystals are collected by filtration, dissolved with 10 mL of boiling water, added with a little activated charcoal, and filtered. When it is allowed to cool, the inner wall is rubbed with a glass bar, the deposited crystals are dried, and the melting point is 192~202 °C (degradation).

Purity test:

i. Clarity and color of solution: When 1.0 g (0.95~1.04 g) of this product is added with 20 mL of water and dissolved by warming, the resulting solution shall be colorless and almost clear.

ii. pH: The pH of a solution of this product in water (1 → 20) shall be 6.0~8.0.

iii. Chloride: 0.30 g (0.295~0.304 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.60 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these samples and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.071 % or less).

iv. Sulfate: 0.50 g (0.495~0.504 g) of this product is weighed to prepare a sample solution by the sulfate test method. A control solution is prepared using 0.50 mL of 0.005 mol/L sulfate. When sulfate is tested using these samples and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.048 % or less).

v. Heavy metal: 2.0 g (1.95~2.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

vi. Arsenic: 0.50 g (0.495~0.504 g) of this product is weighed, added with 5 mL of water, and dissolved by warming. The resulting solution is added with 5 mL of sulfuric acid (3 → 50) and 1 mL of bromine test solution and heat-concentrated on a water bath to make 5 mL. This is used as a sample solution. For the sample solution the arsenic test is performed using the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

vii. Sucrose or reducing sugar: 0.50 g (0.495~0.504 g) of this product is weighed, added with 10 mL of water and 2 mL of hydrochloric acid (1 → 4) and boiled for 2 minutes. It is allowed to cool, added with 5 mL of anhydrous sodium carbonate solution (1 → 8), allowed to stand for 5 minutes, and added with water to make 20 mL. When 5 mL of this solution is measured, added with 2 mL of Fehling′s test solution, boiled for a minute, orange-yellow to red precipitation shall not be generated within 30 seconds.

Loss on drying: 0.50 % or less (1 g, 80 °C, 2 hours)

Assay: This product is dried at 80 °C for 2 hours. Approximately 0.4 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 100 mL of water, added with 10 mL of potassium hydroxide solution (1 → 10), allowed to stand for

approximately a minute, added with 0.1 g of NN indicator, and titrated with 0.05 mol/L ethylene-diamine-tetraacetic acid disodium solution within 30 seconds. In this case, the end point of the titration is the point at which the red color of the solution completely disappears and changes to blue.

0.05 mol/L ethylene-diamine-tetraacetic acid disodium solution 1 mL

= 22.42 mg $C_6H_{12}CaO_{14}\cdot H_2O$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of calcium gluconate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium gluconate is applied mutatis mutandis.

(45) Monosodium L-glutamate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 100 °C for 5 hours, it contains 99.0 % or over of monosodium L-glutamate ($C_5H_8NNaO_4\cdot H_2O$).

Physical and chemical properties:

i. This product is colorless to white pillar crystals or white crystalline powder with a specific taste.

ii. The pH of a solution of this product in water (1 → 10) is 6.7~7.2.

Confirmation test:

i. 5 mL of a solution of this product in water (1 → 1,000) is added with 1 mL of ninhydrin test solution and heated for 3 minutes. The resulting solution is purple.

ii. A solution of this product in water (1 → 10) gives the qualitative reaction of sodium salt.

Purity test:

i. Specific rotation: Approximately 5 g of this product is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved in 2.5 mol/L hydrochloric acid test solution to make 50 mL. The rotation of this solution measured at a path length 100 mm shall be $[\alpha]_D^{20} = +24.8 \sim +25.3°$

ii. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved in 10 mL of water. The resulting solution shall be colorless and clear.

iii. Chloride: 0.10 g (0.095~0.104 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.2 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these samples and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.071 % or less).

iv. Ammonium salt: The purity test iii. for the raw material for manufacturing of aminoacetic acid is applied mutatis mutandis (0.02 % or less).

v. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 1.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

vi. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 0.5 % or less (1 g, 100 °C, 5 hours)

Assay: This product is dried at 100 °C for 5 hours, approximately 0.15 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved in 3 mL of formic acid, added with 50 mL of glacial acetic acid for nonaqueous titration, and titrated with 0.1 mol/L perchloric acid (indicator: 0.5 mL of α-naphtholbenzein test solution). In this case, the end point of the titration is the point at which the color of the solution changes from brown to green. A blank test is performed in the same way and corrections are made.

0.1 mol/L perchloric acid 1 mL = 9.357 mg $C_5H_8NNaO_4 \cdot H_2O$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of monosodium L-glutamate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of monosodium L-glutamate is applied mutatis mutandis.

(46) Iron and Sodium Succinate Citrate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 10.0~11.0 % of iron (Fe).

Physical and chemical properties:

i. This product is blue-white to green-whitish powder with no odor.

ii. This product is easy to slightly dissolve in water and hardly dissolves in ethanol.

iii. This product is stable against heat but gradually oxidized by light and becomes brown.

Confirmation test:

i. A solution of this product in water (1 → 100) gives the qualitative reaction of ferrous salt.

ii. When 5 mL of a solution of this product in water (1 → 100) is added with 2 mL of strong ammonia solution, the resulting solution is red-brown and precipitation is not generated.

iii. The residue obtained by the ashing of 3.0 g (2.95~3.04 g) of this product gives the qualitative reaction of sodium salt.

iv. 0.5 g (0.45~0.54 g) of this product is added with 5 mL of water and 10 mL of potassium hydroxide test solution and heated on a water bath for 10 minutes with sufficient stirring. It is allowed to cool and is filtered. 10 mL of the filtrate is measured and neutralized with acetic acid. The resulting solution gives the qualitative reaction iii. of citrate.

v. 0.2 mL of the solution neutralized with acetic acid in iv. is measured into a porcelain pot, added with a drop of dilute hydrochloric acid to make it acidic, and evaporated to dryness. The residue is added with 5 drops of thionyl chloride and heated to almost dryness. Then, it is added with 5 drops of saturated ethanol solution of hydroxylamine hydrochloride and is further added with dilute potassium hydroxide-ethanol test solution to make it alkaline. It is boiled for approximately 30 seconds, added with a drop of dilute hydrochloric acid to make it acidic, and added with a drop of ferric chloride test solution. The resulting solution is dark purple.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is added with 10 mL of water and dissolved by warming. The resulting solution shall be dark green-brown and clear.

ii. Lead: 2.0 g (1.95~2.04 g) of this product is weighed, added with 4 mL of nitric acid and 30 mL of water, boiled for 10 minutes, allowed to cool, transferred to a 50 mL

volumetric flask, and added with water to the graduation line to make 50 mL. 25 mL of this solution is measured using a volumetric pipette, and this is used as a sample solution. When lead is tested using the lead test method (dithizone method) for the sample solution, the amount shall be 10 mg/kg or less.

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 2 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iv. Ferric salt: 2.0 g (1.95~2.04 g) of this product is placed in a stoppered flask, dissolved with 5 mL of hydrochloric acid and 30 mL of water, added with 4.0 g (3.95~4.04 g) of potassium iodide. The flask is closed with a stopper and placed for 15 minutes in a dark place. When the solution is added with 2 mL of starch test solution and shaken up well, it gives color, but the color shall disappear when it is added with 1 mL of 0.1 mol/L sodium thiosulfate.

Assay: Approximately 1.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is placed in an iodine bottle, added with 25 mL of dilute sulfuric acid and 2 mL of nitric acid, boiled for 10 minutes, allowed to cool, added with 20 mL of water and 4.0 g (3.95~4.04 g) of potassium iodide. The iodine bottle is closed with a stopper and placed in a dark place for 15 minutes. Then the solution is added with 100 mL of water and titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 3 mL of starch test solution). A blank test is performed in the same way and corrections are made.

0.1 mol/L sodium thiosulfate solution 1 mL = 5.585 mg Fe

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of iron and sodium succinate citrate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of iron and sodium succinate citrate is applied mutatis mutandis.

(45) Cholecalciferol

A. Raw material for manufacturing

(a) Compositional standards

Physical and chemical properties:

i. This product is white crystals with no odor.

ii. This product is easy to dissolve in ethanol or chloroform and easy to slightly dissolve in fatty oil and hardly dissolves in water.

iii. This product changes by air or light.

Confirmation test: The confirmation test for the raw material for manufacturing of ergocalciferol is applied mutatis mutandis. In this case, “147~149 °C” shall be replaced by “133~135 °C”.

Purity test:

i. Absorbance: The purity test i. of the raw material for manufacturing of ergocalciferol is applied mutatis mutandis. In this case, “445~485” shall be replaced by “450~490”.

ii. Specific rotation: The purity test ii. of the raw material for manufacturing of ergocalciferol is applied mutatis mutandis. In this case, “0.3 g” and “+102 ~ +107°” shall be replaced by “0.1 g” and “+103 ~ +112°”.

iii. Melting point: The purity test iii. of the raw material for manufacturing of ergocalciferol is applied mutatis mutandis. In this case, “115~118 °C” shall be replaced by “84~88 °C”.

iv. 7-dehydrocholesterol: When 0.01 g (0.0095~0.0104 g) of this product is dissolved with 2.0 mL of absolute ethanol (9 → 10), added with a solution of 0.02 g (0.0195~0.0204 g) of digitonin in 2.0 mL of absolute ethanol (9 → 10), and is allowed to stand for 18 hours, precipitation shall not be generated.

(b) Standard of storage method

It shall be stored in a lightproof capped container in which air is replaced with nitrogen gas. This product shall be stored at a cool place.

B. Preparation (Part 1 liquid)

(a) Compositional standards

This product is oil liquid or water-soluble liquid of the mixture of the raw material for manufacturing of cholecalciferol and hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats.

Content: When this product is determined, it contains cholecalciferol ($C_{27}H_{44}O$) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin D powder are applied mutatis mutandis.

Assay: The test is performed by vitamin D determination method.

(b) Standard of storage method

This product shall be stored in a lightproof sealed container in which air is replaced with nitrogen and shall be stored at a cool place.

C. Preparation (Part 2 powder)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of cholecalciferol and excipient.

Content: When this product is determined, it contains cholecalciferol ($C_{27}H_{44}O$) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin D powder are applied mutatis mutandis.

Assay: The test is performed by vitamin D determination method.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(46) *dl*-α-Tocopherol Acetate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 96.0 % or over of *dl*-α-tocopherol acetate ($C_{31}H_{52}O_3$).

Physical and chemical properties:

i. This is colorless to yellow, clear, viscous fluid with no odor.

ii. This product is miscible with acetone, ether, chloroform, or vegetable oil.

iii. This product is easy to dissolve in ethanol and hardly dissolves in water.

iv. This product is not rotatory.

v. This product changes by air and light.

Confirmation test: When 10 mL of the sample solution obtained by the assay is added with 2 mL of nitric acid and heated at 75 °C for 15 minutes, the resulting solution is red to orange.

Purity test:

i. Clarity and color of solution: 0.1 g (0.05~0.14 g) of this product is dissolved with absolute ethanol to make 10 mL. The resulting solution is clear and its color shall not be darker than that of a solution prepared by which 0.5 mL of the color control stock solution of ferric chloride is added with 0.5 mol/L hydrochloric acid test solution to make 100 mL.

ii. Absorbance: 0.01 g of this product is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved in absolute ethanol to make 100 mL. The absorbance of this solution measured at a wavelength of 284 nm shall be $E_{1\ cm}^{1\%}$ = 41.0~45.0.

iii. Refractive index: The refractive index, n_D^{20}, of this product shall be 1.494~1.499.

iv. Specific gravity: The specific gravity, d_{20}^{20}, of this product shall be 0.952~0.966.

v. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

vi. Free-*α*-tocopherol: Approximately 1 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with 100 mL of a solution of sulfuric acid in ethanol (3 → 200), added with 20 mL of water and titrated with 0.01 mol/L ceric ammonium sulfate with sufficient stirring (indicator: 2 drops of diphenylamine test solution). A blank test is performed in the same way and corrections are made. The amount of free-*α*-tocopherol shall be 0.5 % or less. In this case, it must be noted that the assay is applied mutatis mutandis for the procedure.

0.01 mol/L ceric ammonium sulfate solution 1 mL = 2.154 mg $C_9H_{50}O_2$

Assay: Approximately 0.25 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is placed in a 100 mL brown round-bottom flask, dissolved with 25 mL of absolute ethanol, added with 20 mL of a solution of sulfuric acid in ethanol (3 → 20), boiled for 3 hours with a reflux condenser. It is allowed to cool, transferred to a 200 mL brown volumetric flask, added with absolute ethanol to the graduation line to make 200 mL. This is used as a sample solution. 50 mL of the sample solution is measured using a volumetric pipette, added with 50 mL of a solution of sulfuric acid in ethanol (3 → 20) and 20 mL of water, and titrated with 0.01 mol/L ceric ammonium sulfate with sufficient stirring (indicator: 2 drops of diphenylamine test solution). In this case, the procedure shall be performed away from direct sunlight in a place as dark as possible. The dropping rate shall be 25 drops per 10 seconds and the end point of the titration is the point at which the blue-violet color of the solution is maintained for 10 seconds. A blank test is performed in the same way and corrections are made.

0.01 mol/L ceric ammonium sulfate solution 1 mL = 2.364 mg $C_{31}H_{52}O_3$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of *dl*-α-tocopherol acetate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of *dl*-α-tocopherol acetate is applied mutatis mutandis.

C. Preparation (Part 2 liquid)

(a) Compositional standards

This product is oil liquid or water-soluble liquid of the mixture of the raw material for manufacturing of *dl*-α-tocopherol acetate and hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats.

Content: When this product is determined, it contains *dl*-α-tocopherol acetate ($C_{31}H_{52}O_3$) corresponding to 90~120 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin E powder is applied mutatis mutandis.

Assay: The determination method of the raw material for manufacturing of vitamin E powder is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of *dl*-α-tocopherol acetate is applied mutatis mutandis.

D. Preparation (Part 3: powder)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of *dl*-α-tocopherol acetate and excipient.

Content: When this product is determined, it contains *dl*-α-tocopherol acetate ($C_{31}H_{52}O_3$) corresponding to 90~120 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin E powder is applied mutatis mutandis.

Assay: The determination method of the raw material for manufacturing of vitamin E powder is applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(49) Magnesium oxide

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is ignited at 1,000 °C for 30 minutes and then determined, it contains 96.0 % or more of magnesium oxide (MgO).

Physical and chemical properties:

i. This product is white or whitish powder with no odor.

ii. This product is slightly dissolved in water resulting in weak alkaline.

iii. This product is easy to dissolve in dilute hydrochloric acid.

Confirmation test: A solution of 1 g (0.5~1.4 g) of this product in 25 mL of hydrochloric acid (1 → 4) gives the qualitative reaction of magnesium salt.

Purity test:

i. Water-soluble matter: 2.0 g (1.95~2.04 g) of this product is weighed, added with 100 mL of water, heated in a water bath for 5 minutes, and filtered within 30 seconds. After cooling, 25 mL of the filtrate is measured, evaporated to dryness in a water bath, and dried at 105 °C for an hour, and then the amount of the residue is 10 mg or less (2 % or less).

ii. Hydrochloric acid-insoluble matter: 2.0 g (1.95~2.04 g) of this product is weighed, added with 75 mL of water, added by dropping hydrochloric acid until it no longer dissolves while shaking it up, and is boiled for 5 minutes. It is allowed to cool and is filtered. When the residue on the filter paper is washed with water until the washings does not give the reaction of chloride and ignited together with the filter paper at 1,000 °C for 30 minutes, the amount of the residue is 20 mg or less (1 % or less).

iii. Free alkali: When 50 mL of the filtrate in i. is measured, added with 2 drops of methyl red test solution, and is further added with 2.0 mL of 0.05 mol/L sulfuric acid, the red color of the solution does not disappear.

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, dissolved with 25 mL of hydrochloric acid (1 → 4), and concentrated in a water bath. At almost the end of evaporation, the residue is stirred well to make it fine powder. It is dissolved with 20 mL of water, evaporated to dryness in the same way, and dissolved with 20 mL of water. As appropriate it is filtered, and added with 2 mL of acetic acid (1 → 20) and water to make 50 mL. This is used as a sample solution. A control solution is prepared with 2.0 mL of lead standard solution by Method No. 2 of the heavy metals test method and the test for heavy metal is performed. The color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

v. Calcium oxide: 50 mL of solution A of the assay is measured, added with water to make 300 mL, added with 10 mL of potassium hydroxide solution (1 → 10), allowed to stand for a minute, and titrated with 0.01 mol/L ethylenediaminetetraacetic acid disodium solution (indicator: approximately 0.1 g of NN indicator) using a

microburette, and its consumed volume shall be b mL. The end shall be the time when the color of the sample solution, reddish violet, is completely consumed and changed to blue. The content calculated using the following equation is 1.5 % or less.

$$\text{Amount of calcium oxide (CaO) (\%)} = \frac{\text{b (mL)} \times 0.5608}{\text{Amount of the sample weighed (g)}}$$

vi. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and dissolved with 10 mL of hydrochloric acid (1 → 4) to prepare a sample solution. For the sample solution, when the arsenic test is performed by the method using device A, the color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Loss on ignition: 10.0 % or less (1 g, 1,000 °C, 30 minutes)

Assay: This product is dried at 1,000 °C for 30 minutes, approximately 0.5 g it is weighed to the digits of 0.001 g, and the value is recorded. It is moisturized with 5 mL of water, added with 10 mL of hydrochloric acid and 10 mL of perchloric acid, covered with a watch glass, gradually heated. After concentrated white smoke starts to be generated, it is heated for another 10 minutes. It is allowed to cool, added with approximately 50 mL of warm water and 5 mL of hydrochloric acid (1 → 2), heated a little, and filtered within 30 seconds. The filtrate is transferred to a 500 mL volumetric flask and added with water to make 500 mL. This is used as solution A. 10 ml of solution A is measured using a volumetric pipette, added with water to make 100 mL, added with 5 mL of pH 10.7 ammonia-ammonium chloride buffer and 2 drops of eriochrome black T test solution, and titrated with 0.01 mol/L ethylenediaminetetraacetic acid disodium solution within 30 seconds, and the consumed volume, a mL, is determined. The end shall be the time when the color of the sample solution changes from red to blue. The content is calculated using the consumed volume, obtained in the purity test v., b mL, by the following equation.

$$\text{Amount of magnesium oxide (MgO) (\%)} = \frac{(\text{a} - 0.2\text{b}) \times 2.0152}{\text{Amount of the sample weighed (g)}}$$

(b) Standard of storage method

It shall be stored in an airtight container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of magnesium oxide are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of magnesium oxide is applied mutatis mutandis.

(50) Cyanocobalamin

A. Raw material for manufacturing

(a) Compositional standards

Content: By the determination, this product contains 95.0 % or more of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) on the dried basis.

Physical and chemical properties:

i. This product is dark red crystals or powder.

ii. This product is hard to lightly dissolve in water, hard to dissolve in ethanol, and hardly dissolves in acetone, ether, and chloroform.

iii. This product is hygroscopic.

Confirmation test:

i. In the measurement of the absorption spectrum of sample solution of this product prepared for the assay, the absorption maximum was shown at the wavelengths of 277~279 nm, 360~362 nm, and 548~552 nm. When the absorbance at absorption maximum each is defined as A_1, A_2, and A_3, A_1/A_2 and A_3/A_2 are 0.53~0.59 and 0.29~0.32, respectively.

ii. 1 mg (0.5~1.4 mg) of this product is mixed with 0.05 g (0.045~0.054 g) of potassium acid sulfate, then ignited and melted. After cooling, the melt is crushed with a glass bar, added with 3 mL of water, dissolved by boiling, added with a drop of phenolphthalein test solution, and added with 1 mol/L sodium hydroxide test solution by dropping until the solution becomes pale red. Then, it is added with 0.5 g (0.45~0.54 g) of sodium acetate, 0.5 mL of diluted acetic acid, and 0.5 mL of 1-nitroso-2-naphthol-3, 6-disulfonic acid disodium solution (1 → 500). The resulting solution becomes red to orange-red within 30 seconds. The red color of the solution does not disappear even when it is added with 0.5 mL more of hydrochloric acid and boiled for a minute.

iii. 5 mg (4.5~5.4 mg) of this product is placed in a 50 mL distilling flask, dissolved with 5 mL of water, and added with 2.5 mL of hypophosphorous acid. The flask is put with a short condenser and the tip of the condenser immersed in 1 mL of a sodium hydroxide solution (1 → 50) in a test tube. Then it is gently boiled for 10 minutes and distilled until the volume of distillate liquid reaches 1 mL. The solution in the test tube is added with 4 drops of saturated solution of ammonium ferrous sulfate, gently shaken up, added with 30 mg (25~34 mg) of sodium fluoride, heated until boiling, within 30 seconds added with sulfuric acid (1 → 7) by dropping until the solution becomes clear, and added with 3~5 drops more of sulfuric acid (1 → 7). The resulting solution is blue to blue-green.

Purity test: Pseudocyanocobalamin: 1.0 mg (0.95~1.04 mg) of this product is dissolved with 20 mL of water, transferred to a separatory funnel, added with 5 mL of a mixture of *m*-cresol and carbon tetrachloride (1:1), vigorously shaken up for a minute, and allowed to stand. The bottom layer is transferred to another separatory funnel, added with 5 ml of sulfuric acid (1 → 7) and then is vigorously shaken up. As appropriate, it is centrifuged, and then the color of the supernatant shall be colorless, or shall not be darker than that of a solution prepared by which 0.6 mL of 0.02 mol/L potassium permanganate solution is added with water to make 1 L.

Loss on drying: 12 % or less (0.05 g, reduced pressure, 0.67 kPa or less, phosphorus pentaoxide, 100 °C, 4 hours)

Assay: Approximately 0.02 g each of this product and the reference standard of cyanocobalamin (the loss on drying is previously measured in the same way for this product) is weighed to the digits of 0.1 mg, and the values are recorded. Each of them is dissolved with water, transferred to a 1 L volumetric flask, added with water to the graduation line to make 1 L. They are used as sample and standard solutions. The absorbance, A_T and A_S at a wavelength of 361 nm of sample and standard solutions, respectively, are measured.

Amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) (mg) =

Amount of the reference standard of cyanocobalamin on the dried basis (mg) × $\frac{A_T}{A_S}$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of cyanocobalamin and excipient.

Content: When this product is determined, it contains cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) corresponding to 90~120 % of the amount on the label.

Confirmation test: This product is made into powder. The residue obtained by pretreatment by Method No. 1 of the assay for cyanocobalamin is placed in a centrifugal precipitation tube for that which is granulated and coated with hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats. For others, the amount containing 2 mg of the raw material for manufacturing of cyanocobalamin is placed in a centrifugal precipitation tube. It is added with 5 mL of water, vigorously shaken up for 10 minutes, and centrifuged for 5 minutes. The supernatant is used as a sample solution. Separately, 2 mg (1.5~2.4 mg) of the reference standard of cyanocobalamin is weighed, dissolved with

5 mL of water to prepare a standard solution. 5 μL each of the sample and standard solutions is spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then it is developed approximately 12 cm with the developing solvent, a mixture of methanol and water (19:1). When the thin layer plate is air dried, the spots obtained from the sample and standard solutions are red and their Rf values are equal.

Assay: This product is made into powder and used as a sample. Method No. 2 is used for the content of cyanocobalamin on the label is 0.1 % or less, and Method No. 1 for others. Provided, however that in the measurement of the absorption spectrum at wavelengths of 300~600 nm of the sample solution obtained by Method No. 1 using water as a control, when the absorption maximum is not at wavelengths of 360~362 nm or 548~552 nm, or when the ratio of absorbances A_1 and A_2, A_1/A_2, is not within 0.29~0.32, Method No. 2 is used.

Method No. 1: For that which is granulated and coated with hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats, the amount of this product containing approximately 2 mg of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) is weighed to three significant digits and the value is recorded. It is added with 50 mL of chloroform, shaken up, and filtered using a glass filter (G4). The residue on the filter is washed and filtered three times with 20 mL of chloroform, and air dried until the chloroform odor on the residue disappears. The residue is extracted and filtered while adding 80 mL of water. The filtrate is placed in a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. This is used as a sample solution. For others, the amount of this product containing approximately 2 mg of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) is weighed to three significant digits and the value is recorded. It is placed in a 100 mL volumetric flask, added with 80 mL of water, shaken up well, added with more water to the graduation line to make 100 mL. It is centrifuged for approximately 10 minutes at 3,000 rpm as appropriate, and filtered. The first filtrate 20 mL is removed and the next filtrate is used as a sample solution.

Separately, approximately 0.02 g of the reference standard of cyanocobalamin (the loss on drying is previously measured in the same way for the raw material for manufacturing of cyanocobalamin) is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with water, transferred to a 1 L volumetric flask, and added with more water to the graduation line to make 1 L. This is used as a standard solution.

For each of them the assay of the raw material for manufacturing of cyanocobalamin is hereinafter applied mutatis mutandis.

Amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) (mg)

= Amount of the reference standard of cyanocobalamin on the dried basis (mg)

$$\times \frac{A_T}{A_S} \times \frac{1}{10}$$

Method No. 2: The amount of this product containing approximately 2.5 mg of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) is weighed to three significant digits and the value is recorded. It is added with 10 mL of water using a volumetric pipette, vigorously shaken up for 10 minutes, and centrifuged for 10 minutes at 2,700 rpm. 3 mL of the supernatant is measured using a volumetric pipette, added with 250 mg (249.5~250.4 mg) of DEAE-Sephadex A-25, allowed to stand for 30 minutes with occasional shaking-up, and filtered using a membrane filter (0.8 μm). 400 μL of the filtrate is measured using a micropipette, and spotted in a band shape at the height of approximately 2 cm from the lower end of a layer plate prepared using silica gel for thin-layer chromatography. Simultaneously, 5 μL of the standard solution of cyanocobalamin (1 → 2,500) is spotted on the left side of the thin layer not to be overlapped with the spot of the sample. Then, it is developed approximately 12 cm with the developing solvent, a mixture of methanol and water (19:1) and the thin layer plate is air dried. It is confirmed that the locations of the red spots of cyanocobalamin obtained from the standard solution and the sample are consistent with each other, and then the area at 1 cm up and down from the center of the band like spot obtained from the sample is scraped and placed in a glass column (inner diameter: 1 cm, length: 20 cm) previously added with 5 mL of a mixture of methanol and water (19:1). The red color of cyanocobalamin is completely eluted using 50 mL of a mixture of methanol and water (19:1). The eluate is collected, distilled using an aspirator in a water bath at 60 °C under reduced pressure with shaking. The residue is dissolved with water, transferred to a 5 mL volumetric flask, added with water to the graduation line to make 5 mL and filtered using a membrane filter (0.8 μm). The filtrate is used as a sample solution. 1 g (0.5~1.4 g) of silica gel for thin-layer chromatography is weighed, placed in a glass column previously added with 5 mL of a mixture of methanol and water (19:1) and washed with 50 mL of a mixture of methanol and water (19:1). The washings are collected, diluted using an aspirator in a water bath at 60 °C under reduced pressure with shaking, added with water to make 5 mL, and filtered using a membrane filter (0.8 μm). The filtrate is used as a blank test solution. Separately, approximately 0.02 g of the reference standard of cyanocobalamin (the loss on drying is previously measured in the same way for the raw material for manufacturing of cyanocobalamin) is weighed to the digits of 0.1 mg and the value is recorded. It is

dissolved with water, transferred to a 1 L volumetric flask, and added with more water to the graduation line to make 1 L. This is used as a standard solution. The absorbances, A_T, A_B, and A_S, at the maximum wavelength at around wavelength 361 nm for the sample solution, the blank test solution, and the standard solution are measured, using water as a control.

Amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) (mg)

= Amount of the reference standard of cyanocobalamin on the dried basis (mg)

$$\times \frac{A_T - A_B}{A_S} \times \frac{1}{8}$$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(51) Thiamine Mononitrate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 2 hours, it contains 98.0~102.0 % of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$)

Physical and chemical properties:

i. This product is white to slightly yellow-white crystals or crystalline powder with no odor or a slight, specific odor.

ii. This product is slightly hard to dissolve in water, extremely hard to dissolve in ethanol, and hardly dissolves in chloroform.

iii. Melting point: About 193 °C (degradation)

Confirmation test:

i. The confirmation tests i., ii. and iii. for the raw material for manufacturing of thiamine hydrochloride are applied mutatis mutandis.

ii. A solution of this product in water (1 → 50) gives the qualitative reactions i. and ii. of nitrate.

Purity test:

i. pH: 1.0 g (0.95~1.04 g) of this product is dissolved with water to make 100 mL. The pH of this solution shall be 6.5~8.0.

ii. Chloride: 0.20 g (0.195~0.204 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.3 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these sample and control

solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.053 % or less).

iii. Sulfate: 1.5 g (1.45~1.54 g) of this product is dissolved with 30 mL of water and 2 mL of dilute hydrochloric acid and added with water to make 50 mL. This is used as a sample solution. 0.35 mL of 0.005 mol/L sulfuric acid is added with 2 mL of dilute hydrochloric acid and water to make 50 mL. This is used as a control solution. When sulfate is tested using these solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.011 % or less).

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less). Provided, however, that the sample shall be warmed to dissolve.

Loss on drying: 1.0 % or less (0.5 g, 105 °C, 2 hours)

Ignition residue: 0.20 % or less (1 g)

Assay: This product and the reference standard of thiamine hydrochloride is dried at 105 °C for 2 hours each. The assay for the raw material for manufacturing of thiamine hydrochloride is hereinafter applied mutatis mutandis.

Amount of thiamine nitrate ($C_{12}H_{17}N_5O_4S$) (mg)

=Amount of the reference standard of thiamine hydrochloride (mg)

$$\times \frac{A_T - A_{T'}}{A_S - A_{S'}} \times 0.9706$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of thiamine mononitrate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of thiamine mononitrate is applied mutatis mutandis.

C. Preparation (part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of thiamine mononitrate and excipient.

Content: When this product is determined, it contains thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.02 g of the raw material for manufacturing of thiamine mononitrate is added with 50 mL of water and 10 mL of diluted acetic acid, and for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fat, added with 20 mL of chloroform, shaken up, and allowed to stand, and the aqueous layer is filtered. When 10 mL of the first filtrate is removed, and 5 mL of the next filtrate is measured and added with 2~3 drops of Mayer′s test solution, yellowish white precipitation or opacity is generated.

ii. 1 mL of the filtrate of i. is measured and added with water to make 20 mL. 5 mL of this solution is measured and the confirmation test iii. for the raw material for manufacturing of thiamine mononitrate is hereinafter applied mutatis mutandis.

Assay: Method No. 1 is used for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats and Method No. 2 is used for others.

Method No. 1: The amount of this product containing approximately 0.02 g of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) is weighed to three significant digits and the value is recorded. It is added with 50 mL of chloroform, shaken up, and filtered using a glass filter (G4). The residue on the filter is washed three times with 20 mL each of chloroform. When the chloroform odor on the residue disappears, the residue is filtered while extracting with approximately 190 mL of warmed 0.1 mol/L hydrochloric acid test solution, and allowed to cool. The filtrate is transferred to a 200 mL volumetric flask and added with 0.1 mol/L hydrochloric acid test solution to the graduation line to make 200 mL. 2 mL of this solution is measured using a volumetric pipette to a 100 mL volumetric flask and added with 0.001 mol/L hydrochloric acid test solution to the graduation line to make 100 mL. This is used as a sample solution. Separately, the reference standard of thiamine hydrochloride is dried at 105 °C for 2 hours, approximately 0.1 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 0.1 mol/L hydrochloric acid test solution, transferred to a 1 L volumetric flask, and is added with 0.1 mol/L hydrochloric acid test solution to the graduation line to make 1 L. 2 mL of this solution is measured using a volumetric pipette to a 100 mL volumetric flask and is added with 0.001 mol/L hydrochloric acid test solution to the graduation line to make 100 mL. This is used as a standard solution. 2 mL each of the sample solutions is measured using a volumetric pipette into

stoppered centrifugal precipitation tubes T and T′ and added with 3 mL each of acidic potassium chloride test solution. The stoppered centrifugal precipitation tube T is added with 3 mL of cyanogen bromide test solution, shaken up, added with 2 mL of sodium hydroxide solution (3 → 10) within 30 seconds, shaken up, added with 15 mL of isobutanol using a volumetric pipette, tightly stoppered, and vigorously shaken up for 2 minutes. The stoppered centrifugal precipitation tube T′ is added with 2 mL of sodium hydroxide solution (3 → 10), shaken up, added with 3 mL of cyanogen bromide test solution, shaken up, added with 15 mL of isobutanol using a volumetric pipette, tightly stoppered, and vigorously shaken up for 2 minutes. Separately, 2 mL of the standard solution is measured using a volumetric pipette into stoppered centrifugal precipitation tubes S and S′ and is subjected to the same procedure of the sample solution. Each centrifugal precipitation tube is centrifuged for 2 minutes at a slow speed. Then each isobutanol layer is collected into another tubes, as appropriate gradually added with 1~2 g of anhydrous sodium sulfate, gently shaken up, and allowed to stand. The clear isobutanol solution is collected. As for each isobutanol solution, the fluorescence intensities F_T, $F_{T'}$, F_S, and $F_{S'}$ are measured at an excitation wavelength of approximately 370 nm and a fluorescence wavelength of approximately 440 nm.

Amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) (mg)

= Amount of the reference standard of thiamine hydrochloride (mg)

$$\times \frac{F_T - F_{T'}}{F_S - F_{S'}} \times 0.1941$$

Method No. 2: The amount of this product containing approximately 0.02 g of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) is weighed to three significant digits and the value is recorded. It is added with 150 mL of warmed 0.1 mol/L hydrochloric acid test solution, shaken up, and filtered. The residue on the filter is washed twice with 20 mL each of 0.1 mol/L hydrochloric acid test solution. The filtrate and the washings are collected together into a 200 mL volumetric flask and added with 0.1 mol/L hydrochloric acid test solution to the graduation line to make 200 mL. 2 mL of this solution is measured using a volumetric pipette into a 100 mL volumetric flask, added with 0.001 mol/L hydrochloric acid test solution to the graduation line to make 100 mL. This is used as a sample solution. Hereinafter the Method No. 1 is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of thiamine mononitrate is applied mutatis mutandis.

(52) Aluminum Hydroxide

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 33.0~36.0 % of Aluminum (Al).

Physical and chemical properties:

i. This product is white powder with no odor.

ii. This product hardly dissolves in water or ethanol.

iii. This product almost dissolves in dilute hydrochloric acid or 1 mol/L of sodium hydroxide test solution.

Confirmation test: 0.2 g (0.15~0.24 g) of this product is added with 20 mL of dilute hydrochloric acid, warmed, and filtered. The resulting filtrate gives the qualitative reaction of aluminum salt.

Purity test:

i. Heavy metal: 2.0 g (1.95~2.04 g) of this product is weighed, added with 8 mL of hydrochloric acid and 5 mL of water, shaken up well, gently heated until boiling, and evaporated to dryness on a water bath. The residue is added with 30 mL of water, shaken up well by heating, allowed to cool, and filtered. The filtrate is dropped with 2 mL of diluted acetic acid and 5 mL of ammonia test solution, warmed while stirring until the solution becomes transparent, allowed to cool, added with water to make 50 mL. This is used as a sample solution. 2.0 mL of lead standard solution is added with 8 mL of hydrochloric acid and 5 mL of water, evaporated to dryness on a water bath, and subjected to the same procedure as the sample. This is used as a control solution. When the test for heavy metal is performed using these sample and control solutions, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

ii. Arsenic: 0.8 g (0.75~0.84 g) of this product is weighed and added with 10 mL of dilute sulfuric acid, gently heated while shaking until boiling, allowed to cool and filtered. 2.5 mL of filtrate is added with water to make 5 mL, which is used as a sample solution. When the sample solution is tested for arsenic performed using device A, the color of the absorbing solution shall not be darker than the standard color (10 mg/kg or less).

Assay: Approximately 2.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 15 mL of hydrochloric acid, heated on a water bath for 30 minutes while shaking up, transferred to a 500 mL volumetric flask and allowed to cool, and added with water to make 500 mL. 20 mL of this solution is measured using a volumetric pipette, added with 30 mL of 0.05 mol/L ethylenediaminetetraacetic acid

disodium solution using a volumetric pipette, added with 20 mL of pH 4.8 acetic acid-ammonium acetate buffer, boiled for 5 minutes, allowed to cool and added with 55 mL of ethanol, and titrated with 0.05 mol/L zinc acetate solution (indicator: 2 mL of dithizone test solution). In this case, the end of titration is the time when the color of the solution changes from pale dark green to pale red. A blank test is performed in the same way.

0.05 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL = 1.349 mg Al

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of aluminum hydroxide are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of aluminum hydroxide is applied mutatis mutandis.

(53) Taurine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 2 hours, it contains 99.0~101.0 % of taurine ($C_2H_7NO_3S$).

Physical and chemical properties:

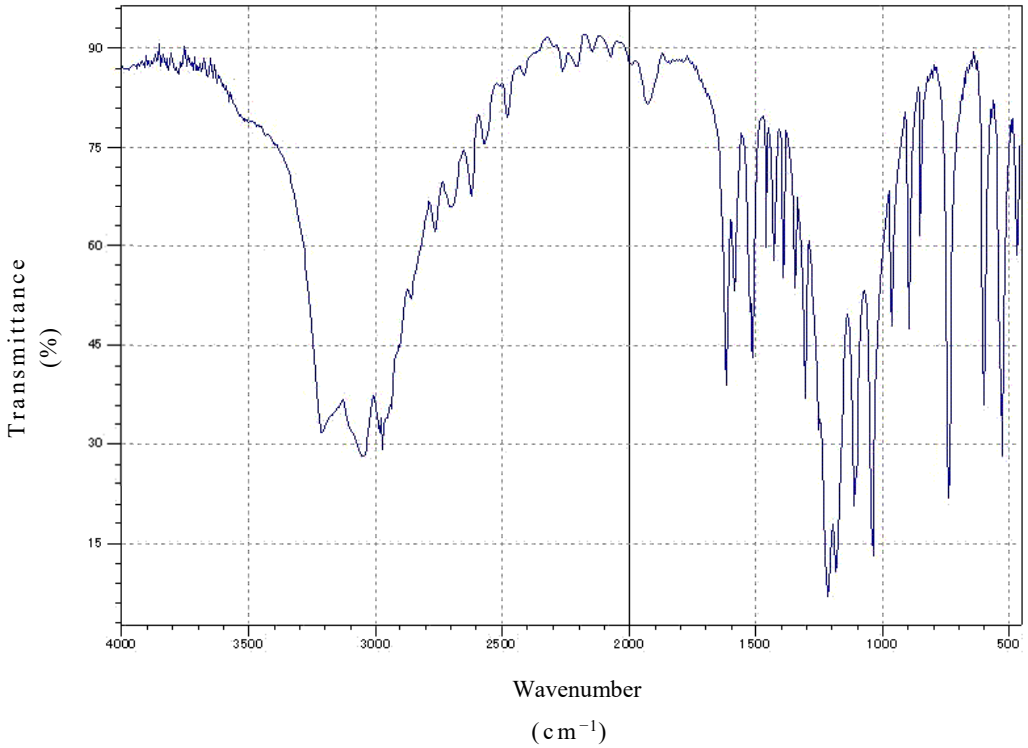
i. This product is colorless or white crystals or white crystalline powder.

ii. This product is slightly easy to dissolve in water and hardly dissolves in absolute ethanol.

iii. 1.0 g (0.95~1.04 g) of this product is dissolved in 20 mL of newly boiled and cooled water. The pH of the resulting solution is 4.1~5.6.

Confirmation test: This product is tested by the potassium bromide disk method of the infrared absorption spectroscopy and the spectrum of this product is compared with reference spectrum of this product. Both spectra show absorption with the similar intensity at the same wavenumber.

Reference spectrum of this product



Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of water. The resulting solution shall be colorless and clear.

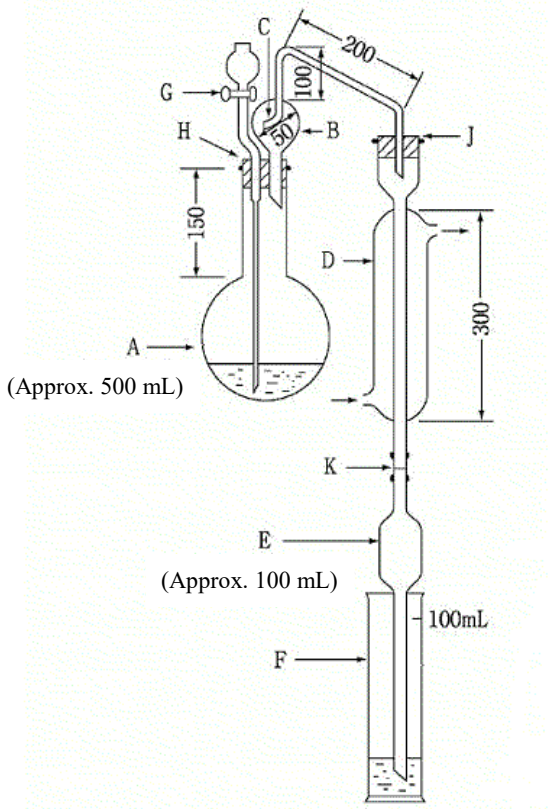
ii. Chloride: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.3 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these sample and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.011 % or less).

iii. Sulfate: 2.0 g (1.95~2.04 g) of this product is weighed to prepare a sample solution by the sulfate test method. A control solution is prepared using 0.4 mL of 0.005 mol/L sulfuric acid. When sulfate is tested using these sample and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.010 % or less).

iv. Ammonium salt

A device described in Figure is used.

A: Distilling flask
B: Spray strip
C: Small hole
D: Condenser
E: Back-flow stopper
F: Receiver (measuring cylinder)
G: Cock
H and J: Rubber stopper
K: Rubber tube



Figures are described in mm.

Operation procedures

Preparation of sample and standard solutions: 0.25 g (0.245~0.254 g) of this product is placed in distilling flask A, dissolved with 140 mL of water, added with 2 g (1.5~2.4 g) of magnesium oxide, and distilled to collect 60 mL of distillate. 20 mL of boric acid solution (1 → 200) as an absorbing solution is put in a receiver F (measuring cylinder). The bottom edge of the condenser is immersed in the absorbing solution and the heating temperature is adjusted to provide a distillation rate of 5~7 mL/min. The bottom edge of the condenser is lifted from the surface of the solution and washed with a little amount of water and water is added into the receiver to make 100 mL. This is used as a sample solution. For preparation of a standard solution, 5.0 mL of ammonium standard solution is put in distilling flask A, and is hereinafter subjected to the same procedure of preparation as the sample solution.

Tests of sample and standard solutions: 30 mL each of the sample and standard solutions is transferred to each Nessler tube, added with 6.0 mL of phenol-sodium nitroprusside test solution to mix them. Then, it is added with 4 mL of sodium hypochlorite-sodium hydroxide test solution and water to make 50 mL, mixed, and allowed to stand for 60

minutes. Each Nessler tubes are observed from above or the side using a white background to compare the colors of these solutions. The color of the sample solution shall not be darker than that of the standard solution (0.02 % or less).

Preparation of phenol-sodium nitroprusside test solution: 5 g (4.5~5.4 g) of phenol and 25 mg (24.5~25.4 mg) of sodium nitroprusside are dissolved in water to make 500 mL. It is stored in a cool, dark place.

Preparation of sodium hypochlorite-sodium hydroxide test solution: The volume of sodium hypochlorite test solution for the ammonia test containing 1.05 g of sodium hypochlorite is dissolved with 15 g (14.5~15.4 g) of sodium hydroxide and water to make 1 L. It is prepared at time of use.

v. Heavy metal: 2.0 g (1.95~2.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

vi. Analog: 1.0 g (0.95~1.04 g) of this product is dissolved in 50 mL of water to prepare a sample solution. 1 mL of this sample solution is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, and added with water to the graduation line to make 50 mL. 1 mL of this solution is measured using a volumetric pipette and added with water to make 10 mL. This is used as a standard solution. 5 μL each of the sample and standard solutions is spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then it is developed approximately 10 cm with the developing solvent, a mixed solution of water, absolute ethanol, n-butanol, and glacial acetic acid (150:150:100:1), and the thin layer is air dried. When it is evenly sprayed with ninhydrin-butanol test solution and heated at 105 °C for 5 minutes, the number of the spots other than the main spot obtained from the sample solution is one or less, and the color of such spots is not darker than that obtained from the standard solution.

Loss on drying: 0.20 % or less (1 g, 105 °C, 2 hours)

Ignition residue: 0.1 % or less (1 g)

Assay: This product is dried at 105 °C for 2 hours. Approximately 0.2 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 50 mL of water, added with 5 mL of formalin, and titrated with 0.1 mol/L sodium hydroxide solution (potentiometric titration). A blank test is performed in the same way and corrections are made.

0.1 mol/L sodium hydroxide solution 1 mL = 12.52 mg $C_2H_7NO_3S$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of taurine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of taurine is applied mutatis mutandis.

(54) Zinc Carbonate

A. Raw material for manufacturing

(a) Compositional standards

Content: This product is basic zinc carbonate. When this product is determined, it contains 57.0~60.0 % of zinc (Zn).

Physical and chemical properties:

i. This product is white powder with no odor.

ii. This product dissolves in dilute hydrochloric acid or ammonia test solution and hardly dissolves in water or ethanol.

Confirmation test:

i. A solution of this product in dilute hydrochloric acid (1 → 10) gives the qualitative reaction of salt.

ii. This product gives the qualitative reaction i. of carbonate.

Purity test:

i. Clarity and color of solution: When 2.0 g (1.95~2.04 g) of this product is dissolved with 10 mL of water and 30 mL of dilute sulfuric acid, the resulting solution shall be colorless and clear.

ii. Heavy metal: 2.0 g (1.95~2.04 g) of this product is added with 10 mL of water and 10 mL of hydrochloric acid (47 → 100), dissolved while warming, allowed to cool and is added with 3 mL of ammonia solution (4 → 5). Then, the precipitation is dissolved with hydrochloric acid (47 → 100) and added with water to make 40 mL. This is used as a sample solution. 15 mL of this sample solution is measured, added with 30 mL of potassium cyanide test solution and water to make 50 mL, added with 0.5 mL of sodium sulfide and allowed to stand for 5 minutes. The color of the resulting solution shall not be darker than that of a solution prepared with the way in which 5 mL of the

sample solution is added with 1.5 mL of lead standard solution, 30 mL of potassium cyanide test solution and water to make 50 mL, added with 0.5 mL of sodium sulfide test solution, and allowed to stand for 5 minutes (30 mg/kg or less).

iii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed and dissolved with 5 mL of dilute hydrochloric acid to prepare a sample solution. For the sample solution, when arsenic test is performed by the arsenic test method using device B, the color of mercuric bromide paper shall not be darker than the standard color (5 mg/kg or less).

Loss on drying: 3.0 % or less (1 g, 105 °C, 2 hours)

Assay: Approximately 1.5 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with 10 mL of water and 10 mL of hydrochloric acid (1 → 2), transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette, added with 80 mL of water, and added with sodium hydroxide solution (1 → 50) until slight precipitation is generated. Then it is added with 5 mL of pH 10.7 ammonia-ammonium chloride buffer and titrated with 0.05 mol/L ethylene-diamine-tetraacetic acid disodium solution (indicator: 0.04 g (0.035~0.044 g) of eriochrome black T-sodium chloride indicator).

0.05 mol/L ethylene-diamine-tetraacetic acid disodium solution 1 mL = 3.269 mg Zn

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of zinc carbonate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of zinc carbonate is applied mutatis mutandis.

(55) Cobaltous Carbonate

A. Raw material for manufacturing

(a) Compositional standards

Content: This product is basic cobaltous carbonate. When it is determined, it contains 47.0~52.0 % of cobalt (Co).

Physical and chemical properties:

i. This product is pale red or dark purple powder with no odor.

ii. This product dissolves in dilute hydrochloric acid and hardly dissolves in water or ethanol.

Confirmation test:

i. When 1.0 g (0.95~1.04 g) of this product is dissolved with 10 mL of dilute hydrochloric acid and added with 1 mL of α-nitroso-β-naphthol test solution, red-brown precipitation is generated.

ii. This product gives the qualitative reaction i. of carbonate.

Purity test:

i, Clarity and color of solution: When 2.0 g (1.95~2.04 g) of this product is dissolved with 10 mL of water and 30 mL of dilute hydrochloric acid, the resulting solution shall be reddish violet and clear.

ii. Lead: 1.0 g (0.95~1.04 g) of this product is weighed, dissolved with nitric acid (1 → 150), transferred to a 20 mL volumetric flask, and added with nitric acid (1 → 150) to the graduation line to make 20 mL. This is used as a sample solution. When lead is tested by the lead test method (Method 1 of Atomic Absorption Spectrophotometry) for the sample solution, the amount shall be 30 mg/kg or less.

iii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed and dissolved with 5 mL of dilute hydrochloric acid to prepare a sample solution. For the sample solution, when arsenic test is performed by the arsenic test method using device B, the color of mercuric bromide paper shall not be darker than the standard color (5 mg/kg or less).

Loss on drying: 3.0 % or less (1 g, 70 °C, 2 hours)

Assay: Approximately 1.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with 10 mL of water and 5 mL of hydrochloric acid (1 → 2), transferred to a 250 mL volumetric flask, added with water to the graduation line to make 250 mL. 10 mL of this solution is measured using a volumetric pipette, added with 100 mL of water, and titrated with 0.01 mol/L ethylene-diamine-tetraacetic acid disodium solution, while adjusting to pH 8.0 with ammonia test solution (indicator: 0.20 g (0.195~0.204 g) of murexide indicator).

0.01 mol/L ethylene-diamine-tetraacetic acid disodium solution 1 mL = 0.5893 mg Co

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of cobaltous carbonate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of cobaltous carbonate is applied mutatis mutandis.

(56) Sodium Bicarbonate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried in a desiccator (silica gel) for 4 hours and determined, it contains 99.0 % or more of sodium bicarbonate ($NaHCO_3$).

Physical and chemical properties:

i. This product is white crystals or crystalline powder with no odor.

ii. This product is easy to slightly dissolve in water and hardly dissolves in ethanol.

iii. This product is gradually degraded in wet air.

Confirmation test: A solution of this product in water (1 → 30) gives the qualitative reactions of sodium salt and bicarbonate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of water. The resulting solution shall be colorless and clear.

ii. Chloride: 0.40 g (0.395~0.404 g) of this product is weighed, added with 4 mL of dilute nitric acid, heated until boiling, allowed to cool, and is added with 6 mL of dilute nitric acid and water to make 50 mL. This is used as a sample solution. When chloride is tested by the chloride test method using a control solution prepared with 0.4 mL of 0.01 mol/L hydrochloric acid, the opacity of the sample solution shall not be higher than that of the control solution (0.040 % or less).

iii. Carbonate: 1.0 g (0.95~1.04 g) of this product is weighed, added with 20 mL of newly boiled and cooled water, extremely gently shaken at 15 °C or under to dissolve, and added with 2.0 mL of 0.1 mol/L hydrochloric acid and 2 drops of phenolphthalein test solution. The resulting solution shall not be red within 30 seconds.

iv. Ammonium salt: When 1.0 g (0.95~1.04 g) of this product is weighed and heated, the generated gas shall not change moist red litmus paper to blue.

v. Heavy metal: 4.0 g (3.95~4.04 g) of this product is weighed, added with 5 mL of water and 4.5 mL of hydrochloric acid, and evaporated to dryness on a water bath. The residue is dissolved with 2 mL of diluted acetic acid, 35 mL of water and a drop of ammonia test solution, and added with more water to make 50 mL. This is used as a sample solution. 4.5 mL of hydrochloric acid is evaporated to dryness and added with 2 mL of diluted acetic acid, 4.0 mL of lead standard solution and water to make 50

mL. This is used as a control solution. When these sample and control solutions are tested for heavy metal, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

vi. Arsenic: 0.70 g (0.695~0.704 g) of this product is weighed, added with 3 mL of water and 2 mL of hydrochloric acid to prepare a sample solution. When the sample solution is tested for arsenic by the method using device A, the color of the absorbing solution shall not be darker than the standard color (2.8 mg/kg or less).

Assay: This product is dried in a desiccator (silica gel) for 4 hours, approximately 2.0 g of it is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved with 25 mL of water and titrated with 0.5 mol/L sulfuric acid. When the color of the solution changes blue to yellow-green, it is carefully boiled, allowed to cool, and titrated until the color changes to greenish yellow (indicator: 2 drops of bromcresol green test solution). A blank test is performed in the same way and corrections are made.

0.5 mol/L sulfuric acid 1 mL = 84.01 mg $NaHCO_3$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium bicarbonate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium bicarbonate is applied mutatis mutandis.

(57) Magnesium Carbonate

A. Raw material for manufacturing

(a) Compositional standards

Content: This product is hydrous basic magnesium carbonate or hydrous magnesium orthocarbonate. When it is determined, it contains 40.0~43.5 % of magnesium oxide (MgO).

Physical and chemical properties:

i. This product is white powder with no odor.

ii. This product hardly dissolves in water or ethanol.

iii. This product bubbles and dissolves in dilute hydrochloric acid.

iv. A saturated solution of this product is alkaline.

Confirmation test:

i. 1 g (0.5~1.4 g) of this product is dissolved with 10 mL of dilute hydrochloric acid, boiled, allowed to cool, and neutralized with 1 mol/L sodium hydroxide test solution (filtered, as appropriate). The resulting solution gives the qualitative reaction of magnesium salt.

ii. This product gives the qualitative reaction i. of carbonate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved in 10 mL of hydrochloric acid (2 → 3) and added with 10 mL of water. The resulting solution shall be almost clear.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, moistened with 4.0 mL of water, dissolved with 10 mL of dilute hydrochloric acid, and evaporated to dryness on a water bath. The residue is dissolved with 35 mL of water, 2 mL of diluted acetic acid, and a drop of ammonia test solution, and added with more water to make 50 mL. This is used as a sample solution. 10 mL of dilute hydrochloric acid is evaporated to dryness on a water bath and added with 2 mL of diluted acetic acid, 3.0 mL of lead standard solution and water to make 50 mL. This is used as a control solution. When these sample and control solutions are tested for heavy metal, the color of the sample solution shall not be darker than that of the control solution (30 mg/kg or less).

iii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed, moistened with 1.5 mL of water, dissolved with 3.5 mL of hydrochloric acid (1 → 4) to prepare a sample solution. When the arsenic test is performed by the method using device A for the sample solution, the color of absorbing solution shall not be darker than the standard color (5 mg/kg or less).

Loss on drying: 3.0 % or less (1 g, 105 °C, 2 hours)

Assay: Approximately 1 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with 30 mL of 0.5 mol/L sulfuric acid and excess sulfuric acid is titrated with 1 mol/L sodium hydroxide solution (indicator: 2~3 drops of bromphenol blue test solution). A blank test is performed in the same way.

0.5 mol/L sulfuric acid 1 mL = 20.15 mg MgO

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of magnesium carbonate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of magnesium carbonate is applied mutatis mutandis.

(58) Manganese Carbonate

A. Raw material for manufacturing

(a) Compositional standards

Content: This is basic manganese carbonate. When it is determined, it contains 42.8~44.7 % of manganese (Mn).

Physical and chemical properties:

i. This product is light brown powder with no odor.

ii. This product dissolves in hydrochloric acid and hardly dissolves in water.

Confirmation test:

i. 1.0 g (0.95~1.04 g) of this product is dissolved with a small amount of dilute hydrochloric acid, added with 1 mol/L sodium hydroxide test solution to make it neutral. The resulting solution gives the qualitative reaction of manganese salt.

ii. This product gives the qualitative reaction i. of carbonate.

Purity test:

i. Clarity and color of solution: When 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of water, 2 mL of concentrated hydrochloric acid, and a drop of hydrogen peroxide water (3 → 100), the resulting solution shall be clear.

ii. Lead: 2.0 g (1.95~2.04 g) of this product is weighed, dissolved with 40 mL of dilute hydrochloric acid, transferred to a 100 mL volumetric flask, added with water to the graduation line to make 100 mL. 25 mL of this solution is measured using a volumetric pipette and this is used as a sample solution. When this sample solution is tested for lead by the lead test method (dithizone method), the amount of lead shall be 20 mg/kg or less.

iii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed and dissolved with 5 mL of dilute hydrochloric acid to prepare a sample solution. For the sample solution, when the arsenic test is performed by the method using device B, the color of absorbing solution shall not be darker than the standard color (5 mg/kg or less).

Loss on drying: 3.0 % or less (1 g, 70 °C, 2 hours)

Assay: Approximately 0.1 g of this product is weighed to the digits of 1 mg and the value is recorded. It is added with 10 mL of dilute hydrochloric acid, dissolved by warming, allowed to cool, transferred to a 100 mL volumetric flask, and added with water to the

graduation line to make 100 mL. 20 mL of this solution is measured using a volumetric pipette, added with 80 mL of water, 5 mL of 0.1 mol/L magnesium-ethylenediaminetetraacetic acid disodium solution, and 0.1 g (0.05~0.14 g) of hydroxylamine hydrochloride, adjusted to pH 6.0~8.0 with dilute sodium hydroxide solution while shaking up well, added with 1 mL of potassium cyanide solution (1 → 20) and 2 mL of pH 10.7 ammonia-ammonium chloride buffer, and titrated with 0.01 mol/L ethylenediaminetetraacetic acid disodium solution (indicator: 0.04 g (0.035~0.044 g) of eriochrome black T-sodium chloride indicator). A blank test is performed in the same way and corrections are made.

0.01 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL = 0.5494 mg Mn

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of manganese carbonate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of manganese carbonate is applied mutatis mutandis.

(59) 2-deamino-2-hydroxymethionine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 88.0 % or more of 2-deamino-2-hydroxymethionine (2-hydroxy-4-metylthio butyric acid, $C_5H_{10}O_3S$).

Physical and chemical properties:

i. This product is brown, slightly viscous liquid with a specific odor.

ii. This product is miscible in water, ether or chloroform, and extremely easy to dissolve in ethanol.

iii. The pH of this product is 1.0 or less.

Confirmation test:

i. 25 mg (24.5~25.4 mg) of this product is added with 1 mL of sulfuric acid saturated with anhydrous copper sulfate. The resulting solution is yellow.

ii. 5 mg (4.5~5.4 mg) of this product is dissolved with 5 mL of water, added with 2 mL of 1 mol/L sodium hydroxide test solution, shaken up well, added with 0.3 mL of

sodium nitroprusside test solution, shaken up well again, allowed to stand at 35~40 °C for 10 minutes, cooled with ice for 2 minutes, added with 2 mL of dilute hydrochloric acid and mixed. The resulting solution is red.

iii. 25 mg (24.5~25.4 mg) of this product is added with 2 mL of a solution in which 1 mg (0.5~1.4 mg) of 2,7-dioxynaphthalene is dissolved in 10 mL of sulfuric acid, and is warmed on a water bath at 90 °C for 2 minutes. The resulting solution emits yellow-green fluorescence.

Purity test:

i. Sulfate: 0.025 g (0.0245~0.0254 g) of this product is weighed to prepare a sample solution by the sulfate test method. A control solution is prepared using 0.5 mL of 0.005 mol/L sulfate. When sulfate is tested using these sample and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.96 % or less).

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 1 g (0.5~1.4 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Content of water: 10.0~12.5 % (Direct titration).

Assay: Approximately 0.20 g of this product is weighed to the digits of 1 mg and the value is recorded. It is dissolved with 50 mL of a mixed solution of glacial acetic acid, hydrochloric acid, and water (40:10:3), and titrated with 0.05 mol/L bromine solution (Potentiometric titration).

A platinum electrode and a double junction electrode is used as a indicating electrode and a reference electrode, respectively.

0.05 mol/L bromine solution 1 mL = 7.510 mg $C_5H_{10}O_3S$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of 2-deamino-2-hydroxymethionine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of 2-deamino-2-hydroxymethionine is applied mutatis mutandis.

(c) Standard of labeling

The following words shall be described on the immediate container or the immediate wrapper of this product.

Precaution for use: This feed additive requires careful handling due to its low pH.

(60) Zn bis (2-hydroxy-4-metylthio butyrate)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 80.0~84.0 % of 2-deamino-2-hydroxymethionine (2-hydroxy-4-metylthio butyric acid, $C_5H_{10}O_3S$) and 16.0~20.0 % of zinc.

Physical and chemical properties: This product is gray powder with a specific odor.

Confirmation test:

i. 5 mg (4.5~5.4 mg) of this product is dissolved with 5 mL of water, added with 2 mL of 1 mol/L sodium hydroxide test solution, shaken up well, added with 0.3 mL of sodium nitroprusside test solution, shaken up well again, allowed to stand at 35~40 °C for 10 minutes, cooled with ice for 2 minutes, added with 2 mL of dilute hydrochloric acid and mixed. The resulting solution is red.

ii. 0.5 g (0.45~0.54 g) of this product is weighed, added with 25 mL of 0.5 mol/L hydrochloric acid test solution, and warmed in a water bath at 60 °C for 3 minutes while gently shaking. It is allowed to cool, added with 25 mL of 0.5 mol/L hydrochloric acid test solution, and shaken up. 10 mL of this solution is measured, added with 10 mL of trichloroacetic acid solution (1 → 10), shaken up, and allowed to stand for 10 minutes. This solution is filtered with filter paper and the filtrate is used as a sample solution. 1 mL of the sample solution is measured, and added with 1~2 drops of pyridine and 1 mL of potassium thiocyanate test solution. White precipitation is formed in the resulting solution.

iii. When the infrared absorption spectrum of this product is measured using the ATR method of the infrared absorption spectroscopy, absorptions are observed at wavelengths around 1,625 cm^{-1}, 1,577 cm^{-1}, and 1,370cm^{-1}.

Purity test:

i. Lead: 0.67 g (0.665~0.674 g) of this product is weighed, added with 3 mL of nitric acid and 5 mL of perchloric acid, evaporated to dryness, allowed to cool, added with 5 mL of dilute hydrochloric acid, and warmed on a water bath to dissolve. It is allowed to cool, added with 5 mL of water, mixed, and filtered using filter paper. The residue is washed with 5 mL of water. The filtrate and the washings are mixed, transferred to a 25 mL volumetric flask, and added with water to the graduation line to make 25 mL. This is used as a sample solution. Separately, 4 mL of lead standard solution for atomic absorption is measured using a volumetric pipette and put in a 50 mL volumetric flask, and added with water up to the capacity mark to make 50 mL. 10 mL of this solution is measured using a volumetric pipette and put in a 25 mL volumetric flask, and added with 5 mL of dilute hydrochloric acid and water up to the capacity mark to make 25 mL. This is used as a standard solution. When the sample and standard solutions are measured by the atomic absorption spectrophotometry (flame type) under the following conditions, the absorbance of the sample solution shall be lower than that of the standard solution (30 μg/g or less).

Gas to be used:

Flammable gas, acetylene

Combustion-supporting gas, air

Lamp: Lead hollow cathode lamp

Wavelength: 217.0 nm

ii. Arsenic: 0.20 g (0.195~0.204 g) of this product is weighed and the sample solution is prepared using method No. 3 of the arsenic test method. The arsenic test is performed in the method using device A. The color of the absorbing solution shall not be darker than the standard color (10 μg/g or less).

Assay:

i. 2-deamino-2-hydroxymethionine (2-hydroxy-4-metylthio butyric acid): Approximately 0.5 g of this product is weighed to the digit of 0.0001 g and the value is recorded. It is dissolved with 50 mL of a mixed solution of glacial acetic acid, water, and hydrochloric acid (50:10:3), and titrated with 0.05 mol/L bromine solution (potentiometric titration).

0.05 mol/L bromine solution 1 mL = 7.510 mg $C_5H_{10}O_3S$

ii. Zinc: Approximately 0.2 g of this product is measured to the digit of 0.001 g and the value is recorded. It is placed in a Kjeldahl flask, added with 5 mL of nitric acid and 10 mL of perchloric acid and heated until the amount of the residual liquid becomes approximately 2 mL. It is allowed to cool, added with 2 mL of hydrochloric acid and

heated on a water bath to dissolve. It is allowed to cool, transferred to a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This solution is filtered using filter paper. 10 mL of the first filtrate is discarded, 10 mL of the next filtrate is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. Then, 5 mL of this solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This is used as a sample solution. Separately, 5 mL of nitric acid and 10 mL of perchloric acid are placed in a Kjeldahl flask and heated until the amount of the residual liquid becomes approximately 2 mL. It is allowed to cool, added with 2 mL of hydrochloric acid, transferred to a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. 10 mL of this solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This is used as a blank test stock solution. Then, 5 mL of this solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This is used as a blank test solution. Separately, 5, 10, 15 and 20 mL each of zinc standard solutions is measured using a volumetric pipette and put in a 100 mL volumetric flask, 5 mL of the blank test stock solution is added using a volumetric pipette, and added with water up to the capacity mark to make 100 mL. The resulting solutions are called the standard solution 1, 2, 3 and 4, respectively. The sample solution, the standard solutions 1, 2, 3 and 4, and the blank test solution are determined by the atomic absorption spectrophotometry (flame type) under the following conditions. A calibration curve is prepared from the absorbances of the standard solutions 1, 2, 3 and 4, and the blank test solution, and the content of zinc in the sample is derived.

Gas to be used:

Flammable gas, acetylene

Combustion-supporting gas, air

Lamp: Zinc hollow cathode lamp

Wavelength: 213.9 nm

Content of zinc (%) =

$$\frac{\text{Concentration of zinc in the sample solution derived from the calibration curve (μg/mL)}}{\text{Collected amount of the sample (mg)}} \times 2000$$

(b) Standard of storage method

It shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Zn bis (2-hydroxy-4-methylthio butyrate) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Zn bis (2-hydroxy-4-methylthio butyrate) is applied mutatis mutandis.

(61) Cu bis (2-hydroxy-4-metylthio butyrate)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 78.0~83.0 % of 2-deamino-2-hydroxymethionine (2-hydroxy-4-metylthio butyric acid, $C_5H_{10}O_3S$) and 15.0~20.0 % of copper.

Physical and chemical properties: This product is gray to green powder with a specific odor.

Confirmation test:

i. 5 mg (4.5~5.4 mg) of this product is dissolved with 5 mL of water, added with 2 mL of 1 mol/L sodium hydroxide test solution, shaken up well, added with 0.3 mL of sodium nitroprusside test solution, shaken up well again, allowed to stand at 35~40 °C for 10 minutes, cooled with ice for 2 minutes, added with 2 mL of dilute hydrochloric acid and mixed. The resulting solution is red and precipitation is generated.

ii. 0.5 g (0.45~0.54 g) of this product is weighed, added with 25 mL of 0.5 mol/L hydrochloric acid test solution, and warmed in a water bath at 60 °C for 3 minutes while gently shaking. It is allowed to cool, added with 25 mL of 0.5 mol/L hydrochloric acid test solution, and shaken up. 10 mL of this solution is measured, added with 10 mL of trichloroacetic acid solution (1 → 10), shaken up, and allowed to stand for 10 minutes. This solution is filtered with filter paper and the filtrate is used as a sample solution. 1 mL of the sample solution is measured, and added with 1 mL of potassium ferrocyanide test solution. Red-brown precipitation is formed in the resulting solution.

iii. When the infrared absorption spectrum of this product is measured using the ATR method of the infrared absorption spectroscopy, absorptions are observed at wavelengths around 1,683 cm^{-1}, 1,591 cm^{-1}, 1,552 cm^{-1}, and 1,416cm^{-1}.

Purity test:

i. Lead: 0.67 g (0.665~0.674 g) of this product is weighed, added with 3 mL of nitric acid and 5 mL of perchloric acid, evaporated to dryness, allowed to cool, added with 5 mL of dilute hydrochloric acid, and warmed on a water bath to dissolve. It is allowed to cool, added with 5 mL of water, mixed, and filtered using filter paper. The residue is washed with 5 mL of water. The filtrate and the washings are mixed, transferred to a 25 mL volumetric flask, and added with water to the graduation line to make 25 mL. This is used as a sample solution. Separately, 4 mL of lead standard solution for atomic absorption is measured using a volumetric pipette and put in a 50 mL volumetric flask, and added with water up to the capacity mark to make 50 mL. 10 mL of this solution is measured using a volumetric pipette and put in a 25 mL volumetric flask, and added with 5 mL of dilute hydrochloric acid and water up to the capacity mark to make 25 mL. This is used as a standard solution. When the sample and standard solutions are measured by the atomic absorption spectrophotometry (flame type) under the following conditions, the absorbance of the sample solution shall be lower than that of the standard solution (30 μg/g or less).

Gas to be used:

Flammable gas, acetylene

Combustion-supporting gas, air

Lamp: Lead hollow cathode lamp

Wavelength: 217.0 nm

ii. Arsenic: 0.20 g (0.195~0.204 g) of this product is weighed and the sample solution is prepared using method No. 3 of the arsenic test method. The arsenic test is performed in the method using device A. The color of the absorbing solution shall not be darker than the standard color (10 μg/g or less)

Assay:

i. 2-deamino-2-hydroxymethionine (2-hydroxy-4-metylthio butyric acid): Approximately 0.5 g of this product is weighed to the digit of 0.0001 g and the value is recorded. It is dissolved with 50 mL of a mixed solution of glacial acetic acid, water, and hydrochloric acid (50:10:3), and titrated with 0.05 mol/L bromine solution (potentiometric titration).

0.05 mol/L bromine solution 1 mL = 7.510 mg $C_5H_{10}O_3S$

ii. Copper: Approximately 0.2 g of this product is measured to the digit of 0.001 g and the value is recorded. It is placed in a Kjeldahl flask, added with 5 mL of nitric acid and 10 mL of perchloric acid and heated until the amount of the residual liquid becomes approximately 2 mL. It is allowed to cool, added with 2 mL of hydrochloric

acid and heated on a water bath to dissolve. It is allowed to cool, transferred to a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This solution is filtered using filter paper. 10 mL of the first filtrate is discarded, 10 mL of the next filtrate is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. Then, 5 mL of this solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This is used as a sample solution. Separately, 5 mL of nitric acid and 10 mL of perchloric acid are placed in a Kjeldahl flask and heated until the amount of the residual liquid becomes approximately 2 mL. It is allowed to cool, transferred to a 100 mL volumetric flask, added with 2 mL of hydrochloric acid, and added with water up to the capacity mark to make 100 mL. 10 mL of this solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This is used as a blank test stock solution. Then, 5 mL of this solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This is used as a blank test solution. Separately, 2, 4, 6 and 8 mL each of copper standard solutions is measured using a volumetric pipette and put in a 100 mL volumetric flask, 5 mL of the blank test stock solution is added using a volumetric pipette, and added with water up to the capacity mark to make 100 mL. The resulting solutions are called the standard solution 1, 2, 3 and 4, respectively. The sample solution, the standard solutions 1, 2, 3 and 4, and the blank test solution are determined by the atomic absorption spectrophotometry (flame type) under the following conditions. A calibration curve is prepared from the absorbances of the standard solutions 1, 2, 3 and 4, and the blank test solution, and the content of copper in the sample is derived.

Gas to be used:

Flammable gas, acetylene

Combustion-supporting gas, air

Lamp: Copper hollow cathode lamp

Wavelength: 324.7 nm

$$\text{Content of copper (\%)} = \frac{\text{Concentration of copper in the sample solution derived from the calibration curve (μg/mL)}}{\text{Collected amount of the sample (mg)}} \times 2000$$

(b) Standard of storage method

It shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Cu bis (2-hydroxy-4-methylthio butyrate) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Cu bis (2-hydroxy-4-methylthio butyrate) is applied mutatis mutandis.

(62) Mn bis (2-hydroxy-4-metylthio butyrate)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 76.0~82.0 % of 2-deamino-2-hydroxymethionine (2-hydroxy-4-metylthio butyric acid, $C_5H_{10}O_3S$) and 13.0~19.0 % of manganese.

Physical and chemical properties: This product is gray to brown powder with a specific odor.

Confirmation test:

i. 5 mg (4.5~5.4 mg) of this product is dissolved with 5 mL of water, added with 2 mL of 1 mol/L sodium hydroxide test solution, shaken up well, added with 0.3 mL of sodium nitroprusside test solution, shaken up well again, allowed to stand at 35~40 °C for 10 minutes, cooled with ice for 2 minutes, added with 2 mL of dilute hydrochloric acid and mixed. The resulting solution is red.

ii. 0.5 g (0.45~0.54 g) of this product is weighed, added with 25 mL of 0.5 mol/L hydrochloric acid test solution, and warmed in a water bath at 60 °C for 3 minutes while gently shaking. It is allowed to cool, added with 25 mL of 0.5 mol/L hydrochloric acid test solution, and shaken up. 10 mL of this solution is measured, added with 10 mL of trichloroacetic acid solution (1 → 10), shaken up, and allowed to stand for 10 minutes. This solution is filtered with filter paper and the filtrate is used as a sample solution. 1 mL of the sample solution is measured, and added with ammonia test solution and 5 mL of silver nitrate test solution. White precipitation is formed in the resulting solution, and the generated precipitation gradually turns black.

iii. When the infrared absorption spectrum of this product is measured using the ATR method of the infrared absorption spectroscopy, absorptions are observed at wavelengths around 1,627 cm^{-1}, 1,574 cm^{-1}, and 1,435cm^{-1}.

Purity test:

i. Lead: 0.67 g (0.665~0.674 g) of this product is weighed, added with 3 mL of nitric acid and 5 mL of perchloric acid, evaporated to dryness, allowed to cool, added with 5 mL of dilute hydrochloric acid, and warmed on a water bath to dissolve. It is allowed to cool, added with 5 mL of water, mixed, and filtered using filter paper. The residue is washed with 5 mL of water. The filtrate and the washings are mixed, transferred to a 25 mL volumetric flask, and added with water to the graduation line to make 25 mL. This is used as a sample solution. Separately, 4 mL of lead standard solution for atomic absorption is measured using a volumetric pipette and put in a 50 mL volumetric flask, and added with water up to the capacity mark to make 50 mL. 10 mL of this solution is measured using a volumetric pipette and put in a 25 mL volumetric flask, and added with 5 mL of dilute hydrochloric acid and water up to the capacity mark to make 25 mL. This is used as a standard solution. When the sample and standard solutions are measured by the atomic absorption spectrophotometry (flame type) under the following conditions, the absorbance of the sample solution shall be lower than that of the standard solution (30 μg/g or less).

Gas to be used:

Flammable gas, acetylene

Combustion-supporting gas, air

Lamp: Lead hollow cathode lamp

Wavelength: 217.0 nm

ii. Arsenic: 0.10 g (0.095~0.104 g) of this product is weighed and the sample solution is prepared using method No. 3 of the arsenic test method. The arsenic test is performed in the method using device A. The color of the absorbing solution shall not be darker than the standard color (20 μg/g or less)

Assay:

i. 2-deamino-2-hydroxymethionine (2-hydroxy-4-metylthio butyric acid): Approximately 0.5 g of this product is weighed to the digit of 0.0001 g and the value is recorded. It is dissolved with 50 mL of a mixed solution of glacial acetic acid, water, and hydrochloric acid (50:10:3), and titrated with 0.05 mol/L bromine solution (potentiometric titration).

0.05 mol/L bromine solution 1 mL = 7.510 mg $C_5H_{10}O_3S$

ii. Manganese: Approximately 0.2 g of this product is measured to the digit of 0.001 g and the value is recorded. It is placed in a Kjeldahl flask, added with 5 mL of nitric acid and 10 mL of perchloric acid and heated until the amount of the residual liquid becomes approximately 2 mL. It is allowed to cool, added with 2 mL of hydrochloric

acid and heated on a water bath to dissolve. It is allowed to cool, transferred to a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This solution is filtered using filter paper. 10 mL of the first filtrate is discarded, 10 mL of the next filtrate is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. Then, 5 mL of this solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This is used as a sample solution. Separately, 5 mL of nitric acid and 10 mL of perchloric acid are placed in a Kjeldahl flask and heated until the amount of the residual liquid becomes approximately 2 mL. It is allowed to cool, transferred to a 100 mL volumetric flask, added with 2 mL of hydrochloric acid, and added with water up to the capacity mark to make 100 mL. 10 mL of this solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This is used as a blank test stock solution. Then, 5 mL of this solution is measured using a volumetric pipette and put in a 100 mL volumetric flask, and added with water up to the capacity mark to make 100 mL. This is used as a blank test solution. Separately, 5, 10, 15 and 20 mL each of manganese standard solutions is measured using a volumetric pipette and put in a 100 mL volumetric flask, 5 mL of the blank test stock solution is added using a volumetric pipette, and added with water up to the capacity mark to make 100 mL. The resulting solutions are called the standard solution 1, 2, 3 and 4, respectively. The sample solution, the standard solutions 1, 2, 3 and 4, and the blank test solution are determined by the atomic absorption spectrophotometry (flame type) under the following conditions. A calibration curve is prepared from the absorbances of the standard solutions 1, 2, 3 and 4, and the blank test solution, and the content of manganese in the sample is derived.

Gas to be used:

Flammable gas, acetylene

Combustion-supporting gas, air

Lamp: Manganese hollow cathode lamp

Wavelength: 279.5 nm

$$\text{Content of manganse (\%)} = \frac{\text{Concentration of manganese in the sample solution derived from the calibration curve (μg/mL)}}{\text{Collected amount of the sample (mg)}} \times 2000$$

(b) Standard of storage method

It shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Mn bis (2-hydroxy-4-methylthio butyrate) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Mn bis (2-hydroxy-4-methylthio butyrate) is applied mutatis mutandis.

(63) DL-Tryptophan

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 3 hours, it contains 98.5 % or more of DL-tryptophan ($C_{11}H_{12}N_2O_2$).

Physical and chemical properties:

i. This product is white to pale yellow powder with no odor or a slight specific odor.

ii. This product is hard to dissolve in water, extremely hard to dissolve in ethanol and hardly dissolves in ether.

iii. This product dissolves in dilute hydrochloric acid or 1 mol/L sodium hydroxide test solution.

iv. The pH of a solution (1 → 500) of this product is 5.5~7.0.

Confirmation test:

i. A solution of this product in water (1 → 500) does not have an optical rotation.

ii. 0.1 g (0.05~0.14 g) of this product is added with 50 mL of water, dissolved by heating and is allowed to cool. When 10 mL of the resulting solution is added with 5 mL of *p*-dimethylaminobenzaldehyde-ferric chloride test solution and 2 mL of dilute hydrochloric acid, heated in a water bath for 5 minutes, it is reddish violet to blue-violet.

Purity test:

i. Chloride: 0.09 g (0.085~0.094 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.5 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these samples and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.20 % or less).

ii. Ammonium salt: The purity test iii. for the raw material for manufacturing of aminoacetic acid is applied mutatis mutandis. In this case, “0.1 g” shall be replaced by “0.05 g” (0.04 % or less).

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 1.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 0.5 % or less (1 g)

Assay: This product is dried at 105 °C for 3 hours. Approximately 0.3 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolved in 3 mL of formic acid, added with 50 mL of glacial acetic acid for nonaqueous titration, and titrated with 0.1 mol/L perchloric acid (indicator: 10 drops of α-naphtholbenzein test solution). In this case, the end of titration is the time when the color of the solution changes from brown to green. A blank test is performed in the same way and corrections are made.

0.1 mol/L perchloric acid 1 mL = 20.42 mg $C_{11}H_{12}N_2O_2$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of DL-tryptophan are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of DL-tryptophan is applied mutatis mutandis.

(64) L-Tryptophan

L-Tryptophan (Part 1)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 3 hours, it contains 97.0 % or more of L-tryptophan ($C_{11}H_{12}N_2O_2$).

Physical and chemical properties:

i. This product is white to pale yellow powder with no odor or a slight specific odor.

ii. This product is hard to dissolve in water, extremely hard to dissolve in ethanol, and hardly dissolves in ether.

iii. This product dissolves in dilute hydrochloric acid or 1 mol/L sodium hydroxide test solution.

iv. The pH of a solution (1 → 500) of this product is 4.5~7.0.

Confirmation test:

i. A solution of this product in water (1 → 100) shows levorotation, which changes to dextrorotation when the solution is added with sodium hydroxide solution (1 → 5) to make it alkaline.

ii. The confirmation test ii, of the raw material for manufacturing of DL-tryptophan is applied mutatis mutandis.

Purity test:

i. Specific rotation: This product is dried at 105 °C for 3 hours. Approximately 0.5 g of it is weighed to the digits of 0.001 g and the value is recorded. It is added with approximately 40 mL of water, dissolved by heating, allowed to cool, added with water to make 50 mL, and if necessary filtered with a membrane filter (0.45 μm). The rotation of this solution shall be $[\alpha]_D^{20}$ = -28.4 ~ -33.0°.

ii. Chloride: The purity test i. of the raw material for manufacturing of DL-tryptophan is applied mutatis mutandis (0.20 % or less).

iii. Ammonium salt: The purity test ii. of the raw material for manufacturing of DL-tryptophan is applied mutatis mutandis (0.2 % or less).

iv. Heavy metal: The purity test iii. of the raw material for manufacturing of DL-tryptophan is applied mutatis mutandis (20 mg/kg or less).

v. Arsenic: The purity test iv. of the raw material for manufacturing of DL-tryptophan is applied mutatis mutandis (2 mg/kg or less).

Loss on drying: 1.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 1.0 % or less (1 g)

Assay: The assay of the raw material for manufacturing of DL-tryptophan is applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-tryptophan (part 1) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-tryptophan (part 1) is applied mutatis mutandis.

L-Tryptophan (Part 2)

A. Raw material for manufacturing

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-tryptophan (part 1) are applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the L-tryptophan producing strain of *Escherichia coli* is cultured. After the cultivation the bacterial cells are heat-treated to separate L-tryptophan, and dried.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-tryptophan (part 1) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-tryptophan (part 2) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-tryptophan (part 2) is applied mutatis mutandis.

(65) L-Threonine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 3 hours, it contains 98.5 % or more of L-threonine ($C_4H_9NO_3$).

Physical and chemical properties:

i. This product is white to light yellowish brown crystals or crystalline powder with no odor or a slight specific odor.

ii. The pH of a solution (1 → 20) of this product is 5.0~6.5.

Confirmation test:

i. 5 mL of a solution of this product in water (1 → 1,000) is added with 1 mL of ninhydrin test solution and heated for 3 minutes. The resulting solution is reddish violet to purple.

ii. When 5 mL of a solution of this product in water (1 → 10) is added with 5 mL of saturated potassium periodate and heated, a gas with an ammonia odor is produced. This gas changes moist red litmus paper to blue.

Purity test:

i. Specific rotation: This product is dried at 105 °C for 3 hours. Approximately 3 g of it is weighed to the digits of 0.01 g and the value is recorded. It is dissolved in water to make 50 mL, and filtered as appropriate. The rotation of this solution shall be $[\alpha]_D^{20}$ = -26.0 ~ -29.0°.

ii. Chloride: 0.09 g (0.085~0.094 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.5 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these samples and control solutions, the turbidity of the sample solution shall not be higher than that of the control solution (0.20 % or less).

iii. Ammonium salt: The purity test iii. for the raw material for manufacturing of aminoacetic acid is applied mutatis mutandis. In this case, “0.1 g” shall be replaced by “0.02 g” (0.10 % or less).

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

v. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 0.5 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 0.5 % or less (1 g)

Assay: This product is dried at 105 °C for 3 hours. Approximately 0.2 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolved in 3 mL of formic acid, added with 50 mL of glacial acetic acid for nonaqueous titration, and titrated with 0.1 mol/L perchloric acid (potentiometric titration). The end of titration using an indicator (1

mL of crystal violet-glacial acetic acid test solution) is the time when the color of the solution changes from purple through blue to green. A blank test is performed in the same way and corrections are made.

0.1 mol/L perchloric acid 1 mL = 11.91 mg $C_4H_9NO_3$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L- threonine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L- threonine is applied mutatis mutandis.

(66) Ferrous DL-Threonine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried in a desiccator (reduced pressure, silica gel) for 4 hours and determined, it contains 58.0~67.0 % of DL-threonine ($C_4H_9NO_3$) and 13.6~15.7 % of ferrous iron [Fe (II)].

Physical and chemical properties:

i. This product is pale yellow to light brown powder with a slight specific odor.

ii. This product dissolves in dilute hydrochloric acid, and hardly dissolves in water, ethanol or acetone.

Confirmation test:

i. A solution of 0.1 g (0.05~0.14 g) of this product in 10 mL of dilute hydrochloric acid gives the qualitative reaction of ferrous salt.

ii. 0.1 g (0.05~0.14 g) of this product is added with 5 ml of 1 mol/L hydrochloric acid test solution and boiled until aldehyde odor is eliminated. It is allowed to cool, added with ammonia test solution to make it alkaline, and filtered. Filter paper is immersed in the filtrate and then air-dried. When it is evenly sprayed with a solution of ninhydrin in water-saturated n-butanol (1 → 500) and heated at 100 °C for 10 minutes, it is purple.

Purity test:

i. Lead: 1.0 g (0.95~1.04 g) of this product is weighed and added with 20 mL of water and 5 mL of nitric acid (1 → 3) and gently boiled for 5 minutes. It is allowed to cool,

transferred to a 50 mL volumetric flask and added with water to make 50 mL. 25 mL of this solution is measured using a volumetric pipette to prepare a sample solution. When this sample solution is tested for lead by the lead test method (dithizone method), the amount of lead shall be 20 mg/kg or less.

ii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed and dissolved with 2 mL of dilute hydrochloric acid and 3 mL of water to prepare a sample solution. When this sample solution is tested for arsenic by the method using device A, the color of the absorbing solution shall not be darker than the standard color (5 mg/kg or less).

Loss on drying: 1.0 % or less (0.5 g, silica gel, reduced pressure, 4 hours)

Assay:

i. DL-threonine: This product is dried in a desiccator (reduced pressure, silica gel) for 4 hours, approximately 0.3 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is added with 50 mL of water and heated on a water bath for approximately 3 hours until the specific odor is eliminated. It is allowed to cool, added with 10 mL of formalin previously neutralized with 0.1 mol/L sodium hydroxide solution using phenolphthalein test solution by a volumetric pipette, and titrated with 0.1 mol/L sodium hydroxide solution (potentiometric titration). A blank test is performed in the same manner and corrections are made.

0.1 mol/L sodium hydroxide solution 1 mL = 11.91 mg $C_4H_9NO_3$

ii. Ferrous iron: This product is dried in a desiccator (reduced pressure, silica gel) for 4 hours, approximately 0.6 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 10 mL of 0.5 mol/L sulfuric acid, and titrated with 0.1 mol/L ceric ammonium sulfate solution (indicator: 3 drops of o-phenanthroline test solution). A blank test is performed in the same manner and corrections are made.

0.1 mol/L ceric ammonium sulfate solution 1 mL = 5.585 mg Fe

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of ferrous DL-threonine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of ferrous DL-threonine is applied mutatis mutandis.

(67) Nicotinic Acid

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 1 hour, it contains 99.0 % or more of nicotinic acid ($C_6H_5NO_2$).

Physical and chemical properties:

i. This product is white to pale yellow white crystals or crystalline powder with no odor or a slight specific odor and with a slight sour taste.

ii. This product is slightly hard to dissolve in water, hard to dissolve in ethanol, and extremely hard to dissolve in ether.

iii. This product dissolves in 1 mol/L sodium hydroxide test solution or sodium carbonate test solution.

iv. The pH of a solution (1 → 100) of this product is 3.0~4.0.

Confirmation test:

i. 5 mg (4.5~5.4 mg) of this product is mixed with 0.01 g (0.005~0.014 g) of 2,4-dinitrochlorobenzene, melted by gentle heating for 5~6 seconds, allowed to cool, and added with 4 mL of potassium hydroxide-ethanol test solution. The resulting solution is dark red.

ii. 2 mL of a solution of this product in water (1 → 1,000) is added with 1 mL of cyanogen bromide test solution and 1 mL of aniline solution (1 → 40). The resulting solution is yellow.

iii. 0.02 g (0.015~0.024 g) of this product is dissolved with water to make 1 L. In the measurement of the absorption spectrum of this solution, the absorption maximum and minimum are at the wavelengths of 261~263 nm and 235~239 nm, respectively. When the absorbances at the absorption maximum and minimum are A_1 and A_2, respectively, A_2/A_1 is 0.35~0.39.

Purity test:

i. Melting point: The melting point of this product shall be 234~238 °C

ii. Chloride: 0.5 g (0.45~0.54 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.30 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these sample and control solutions, the opacity of the sample solution shall not be higher than that of the control solution (0.021 % or less).

iii. Sulfate: 1.0 g (0.95~1.04 g) of this product is weighed, dissolved with 3 mL of dilute hydrochloric acid and water to make 50 mL. The resulting solution is used as a sample solution. 0.40 mL of 0.005 mol/L sulfuric acid is added with 3 mL of dilute

hydrochloric acid and water to make 50 mL. This is used as a control solution. When sulfate is tested using these solutions, the opacity of the sample solution shall not be higher than that of the control solution (0.019 % or less).

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

Loss on drying: 0.5 % or less (1 g, 105 °C, 1 hour)

Ignition residue: 0.10 % or less (1 g)

Assay: This product is dried at 105 °C for 1 hour, approximately 0.3 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 50 mL of water and titrated with 0.1 mol/L sodium hydroxide solution (indicator: 5 drops of phenolphthalein test solution).

0.1 mol/L sodium hydroxide solution 1 mL = 12.31 mg $C_6H_5NO_2$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of nicotinic acid are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of nicotinic acid is applied mutatis mutandis.

(68) Nicotinamide

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried in a desiccator (reduced pressure, silica gel) for 4 hours and determined, it contains 98.5 % or more of nicotinamide ($C_6H_6N_2O$).

Physical and chemical properties:

i. This product is white to pale yellow white crystals or crystalline powder with no odor or a slight specific odor and with a bitter taste.

ii. This product is easy to dissolve in water, ethanol or glycerin, and hard to dissolve in ether.

iii. The melting point of this product is 124~131 °C.

Confirmation test:

i. 5 mg (4.5~5.4 mg) of this product is mixed with 0.01 g (0.005~0.014 g) of 2,4-dinitrochlorobenzene, melted by gentle heating for 5~6 seconds, allowed to cool, and added with 4 mL of potassium hydroxide-ethanol test solution. The resulting solution is red.

ii. 1 mg (0.5~1.4 mg) of this product is dissolved with 100 mL of pH 7.0 phosphate buffer. 2 mL of this solution is added with 1 mL of cyanogen bromide test solution, heated at 80 °C for 7 minutes, rapidly cooled, added with 5 mL of 1 mol/L sodium hydroxide solution, and allowed to stand for 30 minutes. When the resulting solution is observed under ultraviolet light at a wavelength of 365 nm, it emits blue fluorescence.

iii. When 0.02 g (0.015~0.024 g) of this product is added with 5 mL of 1 mol/L sodium hydroxide solution and carefully boiled, the produced gas changes moist red litmus paper to blue.

iv. 0.02 g (0.015~0.024 g) of this product is dissolved with water to make 1 L. In the measurement of the absorption spectrum of this solution, the absorption maximum and minimum are at the wavelengths of 261~263 nm and 243~247 nm, respectively. When the absorbances at the absorption maximum and minimum are A_1 and A_2, respectively, A_2/A_1 is 0.63~0.67.

Purity test:

i. pH: The pH of a solution of this product in water (1 → 10) shall be 5.0~7.5.

ii. Chloride: 0.5 g (0.45~0.54 g) of this product is weighed to prepare a sample solution by the chloride test method. A control solution is prepared using 0.30 mL of 0.01 mol/L hydrochloric acid. When chloride is tested using these sample and control solutions, the opacity of the sample solution shall not be higher than that of the control solution (0.021 % or less).

iii. Sulfate: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by the sulfate test method. A control solution is prepared using 0.40 mL of 0.005 mol/L sulfuric acid. When sulfate is tested using these sample and control solutions, the opacity of the sample solution shall not be higher than that of the control solution (0.019 % or less).

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 3.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (30 mg/kg or less).

v. Readily carbonizable substances: When 0.2 g (0.15~0.24 g) of this product is weighed and conducted readily carbonizable substances test, its color shall not be darker than that of the control solution A.

Loss on drying: 0.5 % or less (1 g, reduced pressure, silica gel, 4 hours)

Ignition residue: 0.80 % or less (1 g)

Assay: This product is dried in a desiccator (reduced pressure, silica gel) for 4 hours, approximately 0.3 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is added with 20 mL of glacial acetic acid for nonaqueous titration, and heated if necessary to dissolve, allowed to cool, added with 100 mL of benzene, and titrated with 0.1 mol/L perchloric acid (indicator: 2 drops of methylrosanilinium chloride test solution). In this case, the end of titration is the time when the color of the solution changes from purple through blue to blue-green. A blank test is performed in the same manner and corrections are made.

0.1 mol/L perchloric acid 1 mL = 12.21 mg $C_6H_6N_2O$

(b) Standard of storage method

It shall be stored in an airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of nicotinamide are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of nicotinamide is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of nicotinamide and excipient.

Content: When this product is determined, it contains nicotinamide ($C_6H_6N_2O$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 5 mg of the raw material for manufacturing of nicotinamide is mixed with 0.01 g (0.005~0.014 g) of 2,4-dinitrochlorobenzene, and the confirmation test i. of the raw material for manufacturing of nicotinamide is hereinafter applied mutatis mutandis.

ii. According to the amount of this product on the label, the amount containing 1 mg of the raw material for manufacturing of nicotinamide is added with 100 mL of pH 7.0

phosphate buffer, allowed to stand for 15 minutes with occasional shaking, and filtered. 2 mL of this solution is added with 1 mL of cyanogen bromide test solution and the confirmation test ii. of the raw material for manufacturing of nicotinamide is hereinafter applied mutatis mutandis.

iii. According to the amount of this product on the label, the amount containing 0.02 g of the raw material for manufacturing of nicotinamide is added with 5 mL of 1 mol/L sodium hydroxide solution, and the confirmation test iii. of the raw material for manufacturing of nicotinamide is hereinafter applied mutatis mutandis.

iv. According to the amount of this product on the label, the amount containing 0.02 g of the raw material for manufacturing of nicotinamide is weighed, added with 1 L of water, allowed to stand for 15 minutes with occasional shaking, and filtered. To the resulting solution, the confirmation test iv. of the raw material for manufacturing of nicotinamide is hereinafter applied.

Assay: This product is determined using the device for the nitrogen determination method (Semimicro-Kjeldahl Method).

The amount of this product containing approximately 0.03 g of nicotinamide ($C_6H_6N_2O$) is weighed to three significant digits and the value is recorded. It is placed in a Kjeldahl flask, washed with 50 mL of water, and added with 20 mL of sodium hydroxide solution (35 → 100). The flask is connected to a distillation device. 15 mL of boric acid solution (1 → 25) and 3 drops of bromcresol green-methyl red test solution are put in a receiver and then an appropriate amount of water is added in it. The bottom edge of the condenser is immersed in this solution. It is distilled through steam until the volume of distillate liquid reaches 80~100 mL. The bottom edge of the condenser is lifted from the surface of the solution and washed with a little water. The resulting mixed solution is titrated with 0.005 mol/L sulfuric acid. In this case, the end of titration is the time when the color of the solution changes from green through light blue gray to light reddish violet gray. A blank test is performed in the same manner and corrections are made.

0.005 mol/L sulfuric acid 1 mL = 1.221 mg $C_6H_6N_2O$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of nicotinamide is applied mutatis mutandis.

(69) Calcium Lactate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 120 °C for 4 hours, it contains 98.0 % or more of calcium lactate ($C_6H_{10}CaO_6$).

Physical and chemical properties:

i. This product is white powder with no odor.

ii. This product dissolves in water and hardly dissolves in ethyl alcohol.

iii. This product is slightly weathered in air at room temperature and becomes anhydride at 120 °C.

iv. The pH of a solution (1 → 20) of this product is 6~7.

Confirmation test: A solution of this product in water (1 → 20) gives the qualitative reactions of calcium salt and lactate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is added with 20 mL of water and dissolved by warming. The resulting solution shall be colorless and clear.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed and added with 2 mL of diluted acetic acid and approximately 35 mL of water, dissolved by warming, allowed to cool, and added with water to make 50 mL. This is used as a sample solution. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg and less).

iii. Arsenic: 0.50 g (0.495~0.504 g) of this product is weighed and dissolved with 2 mL of water and 3 mL of hydrochloric acid to prepare a sample solution. When arsenic is tested using the sample solution by the method using device A, the color of the absorbing solution shall not be darker than the standard color (4 mg/kg or less).

iv. Volatile fatty acid: When 0.5 g (0.45~0.54 g) of this product is weighed, added with 1 mL of sulfuric acid, and warmed, the resulting solution shall not emit a fatty acid-like odor.

Loss on drying: 25.0~30.0 % (2 g, 120 °C, 4 hours)

Assay: This product is dried at 120 °C for 4 hours, approximately 1.0 g of it is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved in 20 mL of dilute hydrochloric acid, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 10 mL of the resulting solution is measured using a volumetric pipette. It is added with 15 mL of potassium hydroxide (1 → 10), 3 mL of potassium cyanide solution (1 → 20), and 100 mL of water, allowed to stand for approximately 1 minute, added with approximately 0.1 g of NN indicator, and titrated with 0.05 mol/L ethylenediaminetetraacetic acid disodium solution within 30 seconds. In this case, the end of titration is the time when the red color of the solution completely

disappears and turns to blue. A blank test is performed in the same manner and corrections are made.

0.05 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL

= 10.91 mg $C_6H_{10}CaO_6$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of calcium lactate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium lactate is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of calcium lactate and excipient.

Content: When this product is determined, it contains calcium lactate ($C_6H_{10}CaO_6$) corresponding to 90~110 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount containing 1.0 g of the raw material for manufacturing of calcium lactate is weighed, added with 20 mL of water, shaken up well, and filtered. To the filtrate, the confirmation test for the raw material for manufacturing of calcium lactate is applied mutatis mutandis.

Assay: The amount of this product containing approximately 1.0 g of calcium lactate ($C_6H_{10}CaO_6$) is weighed and the value is recorded. It is added with 20 mL of dilute hydrochloric acid, shaken up well, and filtered. The residue on the filter paper is washed three times with 20 mL of water each time. The mixture of the filtrate and the washings are transferred to a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. 10 mL of the solution is measured using a volumetric pipette, added with 15 mL of potassium hydroxide solution (1 → 10), 3 mL of potassium cyanide solution (1 → 20) and 100 mL of water, allowed to stand for approximately 1 minute, and added with approximately 0.1 g of NN indicator, and hereinafter the assay for the raw material for manufacturing of calcium lactate is applied mutatis mutandis.

0.05 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL

= 10.91 mg $C_6H_{10}CaO_6$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium lactate is applied mutatis mutandis.

(70) P-Aminobenzoic Acid

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 2 hours, it contains 98.5 % or more of *p*-aminobenzoic acid ($C_7H_7NO_2$).

Physical and chemical properties:

i. This product is white to pale yellow crystals or crystalline powder with no odor.

ii. This product is easy to dissolve in ethanol, easy to slightly dissolve in warm glycerin, slightly hard to dissolve in ether, and hard to dissolve in water or chloroform.

iii. This product is easy to dissolve in 1 mol/L sodium hydroxide test solution or sodium carbonate test solution and slightly hard to dissolve in dilute hydrochloric acid.

iv. This product gradually turns to dark color by light or air.

Confirmation test:

i. 0.01 g (0.005~0.014 g) of this product is dissolved with 2 mL of dilute hydrochloric acid and 3 mL of water. The resulting solution gives the qualitative reaction of aromatic primary amine.

ii. When 0.5 g (0.45~0.54 g) of this product is dissolved with 1 mL of 1 mol/L sodium hydroxide test solution and 1 mL of water and added with 0.5 mL of potassium iodide test solution, 0.5 mL of dilute hydrochloric acid and 0.5 mL of sodium hypochlorite test solution, brown precipitation is generated.

Purity test:

i. Melting point: The melting point of this product must be 186~189 °C

ii. Chloride: 0.5 g (0.45~0.54 g) of this product is weighed, added with 30 mL of water and 10 mL of dilute nitric acid, dissolved by warming, and is added with water to make 50 mL. This is used as a sample solution. By the chloride test method, a control solution is prepared using 0.7 mL of 0.01 mol/L hydrochloric acid. When chloride is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.05 % or less).

iii. Sulfate: 0.20 g (0.195~0.204 g) of this product is weighed, added with 30 mL of water, dissolved by warming, allowed to cool, and is added with 1 mL of dilute hydrochloric acid and water to make 50 mL. This is used as a sample solution. A

control solution is prepared using 1.0 mL of 0.005 mol/L sulfuric acid. When sulfate is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.24 % or less).

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

Loss on drying: 0.3 % or less (1 g, 105 °C, 2 hours)

Ignition residue: 0.2 % or less (1 g)

Assay: This product is dried at 105 °C for 2 hours, approximately 0.3 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 5 mL of ethanol and 60 mL of water and titrated with 0.1 mol/L sodium hydroxide solution (indicator: 2 drops of phenolphthalein test solution). A blank test is performed in the same manner and corrections are made.

0.1 mol/L sodium hydroxide solution 1 mL = 13.71 mg $C_7H_7NO_2$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of *p*-aminobenzoic acid are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of *p*-aminobenzoic acid is applied mutatis mutandis.

(71) L-Valine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 3 hours, it contains 98.0 % or more of L-valine ($C_5H_{11}NO_2$).

Physical and chemical properties:

i. This product is grayish white to grayish yellow brown crystals or crystalline powder with a specific odor.

ii. The pH of a solution or aqueous suspension of this product (1 → 30) is 5.0~7.0.

Confirmation test:

i. 5 mL of a solution of this product in water (1 → 5,000) is added with 1 mL of ninhydrin test solution and heated for 3 minutes. The resulting solution is blue-violet to purple.

ii. When 0.3 g (0.25~0.34 g) of this product is added with 10 mL of water, dissolved by warming, added with 10 drops of dilute hydrochloric acid and 2 mL of sodium nitrite test solution, it bubbles and generates colorless gas.

Purity test:

i. Specific rotation: This product is dried at 105 °C for 3 hours, approximately 4 g it is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved in 6 mol/L hydrochloric acid test solution to make 50 mL, and filtered with a membrane filter (0.45 μm). The rotation of this solution shall be $[\alpha]_D^{20} = +26.5 \sim +30.5°$.

ii. Ammonium salt: The purity test iii. of the raw material for manufacturing of aminoacetic acid is applied mutatis mutandis. In this case, “0.1 g” and “2 mL of ammonium standard solution” shall be replaced by “0.02 g” and “3 mL of ammonium standard solution,” respectively (0.15 % or less).

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Arsenic: The purity test of the raw material for manufacturing of L-isoleucine is applied mutatis mutandis.

Loss on drying: 1.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 1.0 % or less (1 g)

Assay: This product is dried at 105 °C for 3 hours, approximately 0.25 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved in 3 mL of formic acid, added with 50 mL of glacial acetic acid for nonaqueous titration, and titrated with 0.1 mol/L perchloric acid (indicator: 1 mL of α-naphtholbenzein test solution). In this case, the end of titration is the time when the color of the solution changes from orange-yellow through yellowish green to green. A blank test is performed in the same way and corrections are made.

0.1 mol/L perchloric acid 1 mL = 11.72 mg $C_5H_{11}NO_2$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-valine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-valine is applied mutatis mutandis.

(72) Calcium D-Pantothenate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried at 105 °C for 4 hours and determined, it contains 5.7~6.0 % of nitrogen (N) and 8.2~8.6 % of calcium (Ca).

Physical and chemical properties:

i. This product is white powder with no odor and with a bitter taste.

ii. This product is easy to dissolve in water, slightly hard to dissolve in glycerin, extremely hard to dissolve in ethanol, and hardly dissolves in ether and chloroform.

iii. The pH of a solution of this product in water (1 → 20) is 7.0~9.0.

iv. A solution of this product in water (1 → 20) has an optical rotation.

v. This product is hygroscopic.

Confirmation test:

i. 0.05 g (0.045~0.054 g) of this product is dissolved with 5 mL of 1 mol/L sodium hydroxide test solution and filtered. When the filtrate is added with a drop of copper sulfate test solution, the resulting solution is dark blue.

ii. 0.05 g (0.045~0.054 g) of this product is dissolved with 5 mL of 1 mol/L sodium hydroxide test solution, boiled for 1 minute, allowed to cool, added with hydrochloric acid (1 → 10) to make the solution pH 3 to 4, and added with 2 drops of ferric chloride test solution. The resulting solution is yellow.

iii. A solution of this product in water (1 → 10) gives the qualitative reactions of calcium salt.

Purity test:

i. Specific rotation: This product is dried at 105 °C for 4 hours, approximately 1 g of it is weighed to the digits of 0.1 g, and the value is recorded. It is dissolved in water to make 20 mL. The rotation of this solution shall be $[\alpha]_D^{20} = +25.0 \sim +28.5°$.

ii. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of water. The resulting solution shall be colorless and clear.

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 1 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Alkaloid: 0.05 g (0.045~0.054 g) of this product is dissolved with 5 mL of water, 1 mL of dilute hydrochloric acid and 2 drops of Mayer′s test solution, and allowed to stand for 1 minute. The resulting solution shall not produce opacity.

Loss on drying: 5.0 % or less (1 g, 105 °C, 4 hours)

Assay:

i. Nitrogen: This product is dried at 105 °C for 4 hours, approximately 0.05 g of it is weighed to the digits of 0.01 mg, and the value is recorded. The test is performed by the nitrogen determination method (Semimicro-Kjeldahl Method).

ii. Calcium: This product is dried at 105 °C for 4 hours, approximately 0.4 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is added with 30 mL of water, dissolved by warming, allowed to cool, added with 25 mL of 0.05 mol/L ethylenediaminetetraacetic acid disodium solution using a volumetric pipette, added with 10 mL of pH 10.7 ammonia-ammonium chloride buffer and 0.04 g (0.035~0.044 g) of eriochrome black T-sodium chloride indicator, and an excess amount of ethylenediaminetetraacetic acid disodium is titrated with 0.05 mol/L magnesium chloride solution. In this case, the end of titration is the time when the color of the solution changes from blue-violet to reddish violet. A blank test is performed in the same way.

0.05 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL = 2.004 mg Ca

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of calcium D-pantothenate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis.

(73) Calcium DL-Pantothenate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried at 105 °C for 4 hours and determined, it contains 5.7~6.0 % of nitrogen (N) and 8.2~8.6 % of calcium (Ca).

Physical and chemical properties:

i. This product is white powder with no odor and with a bitter taste.

ii. This product is easy to dissolve in water, slightly hard to dissolve in glycerin, extremely hard to dissolve in ethanol, and hardly dissolves in ether and chloroform.

iii. The pH of a solution of this product in water (1 → 20) is 7.0~9.0.

iv. A solution of this product in water (1 → 20) does not have an optical rotation.

v. This product is hygroscopic.

Confirmation test: The confirmation test of the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis.

Purity test:

i. Clarity and color of solution: The purity test ii, of the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis.

ii. Heavy metal: The purity test iii, of the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis (20 mg/kg or less).

Loss on drying: 5.0 % or less (1 g, 105 °C, 4 hours)

Assay: The assay of the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of calcium DL-pantothenate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium DL-pantothenate is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of calcium DL-pantothenate and excipient.

Content: When this product is determined, it contains nitrogen (N) and calcium (Ca) corresponding to calcium DL-pantothenate ($C_{18}H_{32}CaN_2O_5$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.05 g of the raw material for manufacturing of calcium DL-pantothenate is weighed and the confirmation test i. for the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis.

ii. According to the amount of this product on the label, the amount containing 0.05 g of the raw material for manufacturing of calcium DL-pantothenate is weighed, added with 5 mL of sodium hydroxide test solution, shaken up well, and filtered. To the filtrate, the confirmation test ii. for the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis.

iii. According to the amount of this product on the label, the amount containing 1.0 g of the raw material for manufacturing of calcium DL-pantothenate is weighed, added with water to make 10 mL, shaken up well, and filtered. To the filtrate, the confirmation test iii. for the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis.

Assay:

i. Nitrogen: The amount of this product containing approximately 0.05 g of calcium DL-pantothenate ($C_{18}H_{32}CaN_2O_5$) is weighed to three significant digits and the value is recorded. The determination of nitrogen for the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis.

ii. Calcium: The amount of this product containing approximately 0.4 g of calcium DL-pantothenate ($C_{18}H_{32}CaN_2O_5$) is weighed to three significant digits and the value is recorded. It is added with 10 mL of water, warmed, shaken up well, and filtered. The residue on the filter paper is washed twice with 10 mL of water each time. To the mixture of the filtrate and the washings, the determination of calcium of the raw material for manufacturing of calcium D-pantothenate is applied mutatis mutandis.

0.05 mol/L ethylenediaminetetraacetic acid disodium = 2.004 mg Ca

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium DL-pantothenate is applied mutatis mutandis.

(74) d-Biotin

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined after dried at 105 °C for 4 hours, it contains 98.0 % or more of d-biotin ($C_{10}H_{16}N_2O_3S$).

Physical and chemical properties:

i. This product is white to pale yellow powder with no odor.

ii. This product is hard to dissolve in water, extremely hard to dissolve in ethanol, and hardly dissolves in acetone, ether, and chloroform.

iii. Melting point: 228~232 °C (degradation)

Confirmation test:

i. 5 mg (4.5~5.4 mg) of this product is added with 5 mL of dilute sodium hydroxide test solution and then added with 3 drops of potassium permanganate test solution. The resulting solution changes from reddish violet through blue to verdigris color.

ii. 5 mL of a solution of this product in ethanol (1 → 10,000) is added with 1 mL of a solution of sulfuric acid in ethanol (2 → 100), then added with 1 mL of *p*-dimethylaminocinnamaldehyde, and is allowed to stand for an hour. The resulting solution is orange-red.

Purity test:

i. Specific rotation: 0.5 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved in dilute sodium hydroxide test solution to make 50 mL. The rotation of this solution measured at a layer length of 100 mm shall be $[\alpha]_D^{20}$ = +90 ~ +94°.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Loss on drying: 0.5 % or less (0.5 g, 105 °C, 4 hours)

Ignition residue: 0.1 % or less (0.5 g)

Assay: This product is dried at 105 °C for 4 hours, approximately 0.4 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is added with 10 mL of

dimethylformamide and heated on a water bath to dissolve. It is cooled down to room temperature, added with 30 mL of absolute ethanol, shaken up well, and titrated with 0.1 mol/L of sodium methoxide solution (indicator: 0.4 mL of thymol blue test solution). This time, the end of titration is the time when the color of the solution changes from yellow to verdigris color. A blank test is performed in the same manner and corrections are made.

0.1 mol/L of sodium methoxide solution 1 mL = 24.43 mg $C_{10}H_{16}N_2O_3S$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of d-biotin and excipient.

Content: When this product is determined, it contains d-biotin ($C_{10}H_{16}N_2O_3S$) corresponding to 90~120 % of the amount on the label.

Conformation test: This product is powdered. According to the amount on the label, the amount of the product containing 4 mg of the raw material for manufacturing of d-biotin is weighed, added with 1 mL of dimethylformamide, shaken up, added with 20 mL of ethanol, warmed for 10 minutes, and filtered. 5 mL of the filtrate is added with 1 mL of absolute ethanol solution (2 → 100), then added with 1 mL of a solution of *p*-dimethylaminocinnamaldehyde in absolute ethanol (2 → 1,000), and allowed to stand for an hour. The resulting solution is orange-red.

Assay: This product is powdered to prepare a sample. Method No. 2 is used for a preparation diluted with dextrin or those added with organic acids including sorbic acid as a preservative, and Method No. 1 is used for others.

Method No. 1: The amount of this product containing approximately 3 mg of d-biotin ($C_{10}H_{16}N_2O_3S$) is weighed to three significant digits and the value is recorded. It is placed in a flask and added with 30 mL of absolute ethanol. The flask is attached to a reflux condenser and heated in a bath for 45 minutes. Then, it is cooled down to room temperature and transferred to a 50 mL volumetric flask. The flask is washed with 10 mL of absolute ethanol and the washings are transferred to the volumetric flask. Absolute ethanol is added to the graduation line to make 50 mL and the resulting solution is filtered. 20 mL of the first filtrate is removed and the next filtrate is used as a sample solution. Separately, approximately 6 mg of d-biotin for assay is weighed to the digits of 0.01 mg and the value is recorded. It is dissolved with absolute ethanol, transferred to a 100 mL volumetric flask and added with more absolute ethanol to the

gradation line to make 100 mL. This is used as a standard solution. 2 mL each of the sample and standard solutions is measured using a volumetric pipette and placed in a 20 mL volumetric flask, and added with 2 mL of a solution of sulfuric acid in absolute ethanol (2 → 100) and 2 mL of a solution of *p*-dimethylaminocinnamaldehyde in absolute ethanol (2 → 1,000) using a volumetric pipette. It is added with more absolute ethanol to the graduation line to make 20 mL, shaken up, and allowed to stand for 1 hour. Separately, 2 mL of absolute ethanol is measured, and subjected to the same procedure to prepare a control solution. The absorbances of A_T and A_S at the maximum wavelength around 530 nm are measured.

Amount of d-biotin ($C_{10}H_{16}N_2O_3S$) (mg)

$$= \text{Amount of d-biotin for assay (mg)} \times \frac{A_T}{A_S} \times \frac{1}{2}$$

Method No. 2: The amount of this product containing approximately 1 mg of d-biotin ($C_{10}H_{16}N_2O_3S$) is weighed to three significant digits and the value is recorded. It is added with 30 mL of ammonia solution (8 → 40), added with 150 mL of sodium thiosulfate solution (2 → 1,000) within 30 seconds, allowed to stand for 30 minutes with occasional shaking. Then it is transferred to a 1 L volumetric flask, added with water to the graduation line to make 1 L, and filtered. 50 mL of the filtrate is measured using a volumetric pipette, adjusted with 0.1 mol/L hydrochloric acid test solution to make the solution pH 9.0, transferred to a 100 mL volumetric flask, and added with water to make 100 mL. This is used as a sample solution. Separately, approximately 10 mg of d-biotin for assay is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with 1 mL of ammonia solution (8 → 40), transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a standard stock solution. 1 mL of the standard stock solution is measured using a volumetric pipette, transferred to a 200 mL volumetric flask, and added with water to the graduation line to make 200 mL. This is used as a standard solution (prepared at time of use). The sample and standard solutions are tested by the microbioassay under the following conditions.

Culture media:

i. Agar medium for storing test strain

Yeast extract: 20 g

Glucose: 10 g

Peptone: 5 g

Polysorbate 80: 0.1 g

Potassium dihydrogenphosphate: 2 g

Agar: 10 g

The materials mentioned above are weighed, added with water to make 1 L, and sterilized. The pH shall be 6.7.

ii. Medium for enrichment of test strain

Yeast extract: 20 g

Glucose: 10 g

Peptone, casein: 5 g

Polysorbate 80: 0.1 g

Potassium dihydrogenphosphate: 2 g

The materials mentioned above are weighed, added with water to make 1 L, and sterilized. The pH shall be 6.7.

iii. Basal medium for determination

Glucose: 40 g

Sodium acetate: 20 g

Casamino acid: 14 g

L-cystine: 400 mg

DL-tryptophan: 200 mg

Uracil: 20 mg

Guanine hydrochloride: 20 mg

Adenine sulfate: 20 mg

Thiamine hydrochloride: 0.8 mg

Pyridoxine hydrochloride: 0.8 mg

Nicotinic acid: 0.4 mg

Calcium pantothenate: 0.4 mg

Riboflavin: 0.4 mg

p-aminobenzoate: 0.2 mg

Dibasic potassium phosphate: 1 g

Potassium dihydrogenphosphate: 1 g

Magnesium sulfate: 400 mg

Ferrous sulfate: 20 mg

Manganese sulfate: 20 mg

The materials mentioned above are weighed, added with water to make 500 mL and sterilized and the pH shall be 6.7 to 6.9. Separately, an agar solution (3 → 100) is prepared and sterilized. These solutions are mixed in equal amounts in heating, and stored in a water bath at 45 °C.

Preparation of test strain and test strain culture:

Lactobacillus plantarum ATCC 8014 is used as a test strain. 5 mL of agar medium for storing test strain is added in a test tube to prepare a slant medium. The test strain in the test tube is subcultured at about monthly intervals (37 °C, 18~24 hours) and stored at 4~8 °C. This stored test strain is added with 5 mL of sterile physiological saline to make the bacterial cells uniformly suspended. 0.5~1.0 mL of it is inoculated into 30 mL of a medium for enrichment of test strain and cultivated at 37 °C for 18~20 hours. After the cultivation, it is centrifuged to make the bacterial cells precipitated and the supernatant fluid is removed. The precipitated bacterial cells are added with 30 mL of sterile physiological saline, shaken up, and centrifuged again. This procedure is performed once again and then the bacterial cells are uniformly suspended in 5 mL of sterile physiological saline. This is used as a test strain culture.

Procedures:

80 mL of the basal medium for determination is added with 4 mL of the test strain culture to mix them. The mixture is poured into a sterile plate (20 × 20 cm) preheated at 50 °C to prepare a uniform plate medium. Six sets of discs, provided that a set has 8 discs with a diameter of 6 mm, are prepared and each of them is called S_L, S_M, S_H, U_L, U_M and U_H. 5, 10 and 20 μL of the standard solution are spotted on the discs S_L, S_M and S_H, respectively, and 5, 10 and 20 μL of the sample solution are spotted on the discs U_L, U_M and U_H, respectively. One each of the discs of each set is placed on the plate medium at intervals of 3 cm or more. A total of 8 plate media are prepared in this way, and cultured at 37 °C for 16~20 hours.

Calculation method:

The diameters of the growth obtained from the discs of the plate culture, S_L, S_M, S_H, U_L, U_M and U_H are measured to 0.1 mm. Each of them is called y_1, y_2, y_3, y_4, y_5 and y_6. y_1, y_2, y_3, y_4, y_5 and y_6 of each plate medium are summed, and each of them is called Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6.

Amount of d-biotin ($C_{10}H_{16}N_2O_3S$) (mg)

$$= \text{Amount of reference standard of d-biotin (mg)} \times \text{antilog}M \times \frac{1}{10}$$

$$M = -0.4013 \frac{Y_A}{Y_B}$$

$$Y_A = Y_1 + Y_2 + Y_3 - Y_4 - Y_5 - Y_6$$

$$Y_B = -Y_1 + Y_3 - Y_4 + Y_6$$

Where, V_A, V_B, V_C and S^2 are calculated using the following equation, $\frac{V_A}{S^2}$, $\frac{V_B}{S^2}$ and $\frac{V_C}{S^2}$, each of them shall be 4.05 or less. If the value is exceeded, the experiment shall be repeated under improved experimental conditions until the value is obtained.

$$V_A = \frac{(Y_1 - 2Y_2 + Y_3 + Y_4 - 2Y_5 + Y_6)^2}{96}$$

$$V_B = \frac{(-Y_1 + Y_3 + Y_4 - Y_6)^2}{32}$$

$$V_C = \frac{(Y_1 - 2Y_2 + Y_3 - Y_4 + 2Y_5 - Y_6)^2}{96}$$

$$S^2 = \frac{S_T - S_D}{42}$$

$S_T = \Sigma y^2 - \frac{(\Sigma y)^2}{42}$ (Σy^2: y_1, y_2, y_3, y_4, y_5 and y_6 each of each set is squared, and the obtained values are summed.)

$$S_D = \frac{(Y_A)^2}{48} + \frac{(Y_B)^2}{32} + V_A + V_B + V_C$$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(75) Vitamin A Powder

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it is 200,000 vitamin A international unit or more per 1 gram and contains vitamin A (retinol) corresponding to 90~130 % of the unit on the label.

Physical and chemical properties:

i. This product is pale yellow to red-brown powder with no odor or a specific odor.

ii. Degradation of this product is promoted by air or light.

Confirmation test: 2 mL of isopropanol solution obtained in the section of Assay is measured and isopropanol is distilled away under reduced pressure. The residue is dissolved with 1 mL of chloroform, and added with 3 mL of antimony trichloride test solution. The resulting solution is blue within 30 seconds but the color disappears within 30 seconds.

Purity test:

i. Rancidity: This product shall not emit an unpleasant odor.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed. The residue obtained according to the ignition residue test method is added with 1 mL of hydrochloric acid and 0.2 mL of nitric acid, evaporated to dryness on a water bath, added with 1 mL of dilute hydrochloric acid and 15 mL of water, heated to dissolve, allowed to cool, added with a drop of phenolphthalein test solution, added with ammonia test solution by dropping until the solution becomes slightly red, added with 2 mL of diluted acetic acid, filtered as appropriate, and washed with 10 mL of water. The filtrate and the washings are put in a Nessler tube and added with water to make 50 mL. This is used as a sample solution. The sample solution is added with 2 drops of sodium sulfide test solution and allowed to stand for 5 minutes. The color of this solution shall not be darker than that of a solution prepared in the procedure in which 5.0 mL of lead standard solution is added with 2 mL of diluted acetic acid and water to make 50 mL, added with 2 drops of sodium sulfide test solution and allowed to stand for 5 minutes (50 mg/kg or less).

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed into a decomposition flask, added with 20 mL of nitric acid, weakly heated until the content becomes fluid. It is allowed to cool, added with 5 mL of sulfuric acid, and heated until white smoke emerges. When the solution is still brown, it is allowed to cool and added with 5 mL of nitric acid and heated. This procedure is repeated until the solution is colorless to pale yellow. It is allowed to cool, added with 15 mL of saturated ammonium oxalate solution and heated until white smoke emerges. It is allowed to cool and added with water to make 25 mL. 5 mL of this solution is measured and used as a sample solution. When arsenic is tested using the sample solution by the method using device A, the color of the absorbing solution shall not be darker than the standard color. In this case, the standard color is prepared by way in which 4.0 mL of arsenic standard solution is measured into a decomposition flask, added with 20 mL of nitric acid, and hereinafter subjected to the same procedures for the sample solution (4 mg/kg or less).

Loss on drying: 5.0 % or less (1 g, reduced pressure, silica gel, 4 hours)

Assay: Approximately 0.1 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is placed in a flask, added with 2 mL of water, warmed for a while with shaking, and tested by Method No. 2 of the vitamin A assay.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of vitamin A oil, retinol acetate, or retinol palmitate is powdered or coated and granulated using appropriate base materials.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of vitamin A powder are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of mixture of the raw material for manufacturing of vitamin A powder and excipients.

Content: When this product is determined, it contains vitamin A (retinol) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

Assay: The assay of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

(76) Vitamin A Oil

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it is 30,000 vitamin A international unit or more per 1 gram and contains vitamin A (retinol) corresponding to 90~120 % of the unit on the label.

Physical and chemical properties:

i. This product is yellow to yellow-brown, clear or slightly opacified oil liquid with no odor or a slight specific odor.

ii. Degradation of this product is promoted by air or light.

Confirmation test: This product is dissolved in chloroform to prepare a solution containing 30 vitamin A international unit per 1 mL according to the unit on the label. When 1 mL

of this solution is measured, added with 3 mL of antimony trichloride test solution, the resulting solution is blue within 30 seconds but the color disappears within 30 seconds.

Purity test:

i. Ultraviolet absorption spectrum: This product shall be fit for the conditions which can be measured by Method No. 1 of the vitamin A assay. Or, the f value shall be 0.85 or more when it is measured by Method No. 2 of the vitamin A assay.

ii. Acid: 1.2 g (1.15~1.24 g) of this product is weighed and added with 30 mL of a mixed solution of neutralized ethanol and ether (1:1), gently boiled for 10 minutes with a reflux condenser, allowed to cool, added with 5 drops of phenolphthalein test solution and 0.60 mL of 0.1 mol/L sodium hydroxide solution. The resulting solution shall be red.

iii. Rancid: When this product is warmed, it shall not emit an unpleasant rancid odor.

Assay: Test is performed by Vitamin A quantification method.

(b) Standard of manufacturing method

It is manufactured from fatty oil from fresh livers or pyloric appendages of aquatic animals, or manufactured by the procedure in which the fatty oil, its concentrate, or fatty acid ester of vitamin A is added with cod liver oil or vegetable oil.

(c) Standard of storage method

This product shall be stored in a lightproof airtight container. The container shall be almost full of this product or the air in the container shall be replaced by N_2.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of vitamin A oil are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin A oil is applied mutatis mutandis.

C. Preparation (Part 2 Liquid)

(a) Compositional standards

This product is oil liquid or water-soluble liquid of the mixture of the raw material for manufacturing of vitamin A oil and hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats.

Content: When this product is determined, it contains vitamin A (retinol) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

Assay: The assay of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin A oil is applied mutatis mutandis.

D. Preparation (Part 3 Powder)

(a) Compositional standards

This product is powder or particles of mixture of the raw material for manufacturing of vitamin A oil and fillers.

Content: When this product is determined, it contains vitamin A (retinol) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

Assay: The assay of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

(77) Vitamin D Powder

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it is 100,000 vitamin D international unit or more per 1 gram and contains cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) corresponding to 90~130 % of the unit on the label.

Physical and chemical properties:

i. This product is grayish white to red-brown powder with little odor.

ii. Degradation of this product is promoted by air or light.

Confirmation test:

i. When 10 μL of the sample solution for thin-layer chromatography obtained by the vitamin D assay is measured, spotted in parallel with solutions containing vitamin D and previtamin D on the same thin-layer plate, developed, and irradiated with ultraviolet light (dominant wavelength: 254 nm), the spots of vitamin D and previtamin D are dark purple and their Rf values are equal.

ii. Peak retention times of vitamin D with low retention time and isopiro vitamin D and stigmasterol acetate, which are with high retention times, are determined from the chromatograms from gas chromatography of the sample and standard solutions obtained by the vitamin D assay. Their relative retentions to that of stigmasterol acetate are equal.

Purity test:

i. Rancid: This product shall not emit an unpleasant odor.

ii. Heavy metal: The purity test ii. for the raw material for manufacturing vitamin A powder is applied mutatis mutandis (50 mg/kg or less).

iii. Arsenic: The purity test iii. for the raw material for manufacturing vitamin A powder is applied mutatis mutandis (4 mg/kg or less).

Loss on drying: 7.0 % or less (1 g, reduced pressure, silica gel, 4 hours)

Assay: Test is performed by Vitamin D quantification method.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of cholecalciferol, the raw material for manufacturing of ergocalciferol or solutions of them in edible vegetable oil, or vitamin D_3 oil is powdered, or coated and granulated using appropriate base materials.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of vitamin D powder are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of mixture of the raw material for manufacturing of vitamin D powder and fillers.

Content: When this product is determined, it contains cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

Assay: Test is performed by Vitamin D quantification method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

(78) Vitamin D_3 Oil

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it is 200,000 vitamin D international unit or more per 1 gram and contains cholecalciferol ($C_{27}H_{44}O$) corresponding to 90~130 % of the unit on the label.

Physical and chemical properties:

i. This product is pale yellow to red-brown, clear or slightly opacified viscous oil liquid or waxy blocks with no odor or a slight specific odor.

ii. Degradation of this product is promoted by air or light.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

Purity test:

i. Acid: The purity test ii. for the raw material for manufacturing vitamin A oil is applied mutatis mutandis.

ii. Rancidity: When this product is warmed, it shall not emit an unpleasant rancid odor.

Assay: Test is performed by Vitamin D quantification method.

(b) Standard of manufacturing method

For manufacturing, vitamin D_3 is added with edible vegetable oil.

(c) Standard of storage method

This product shall be stored in a lightproof airtight container. The container shall be almost full of this product or the air in the container shall be replaced by N_2.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of vitamin D_3 oil are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin D_3 oil is applied mutatis mutandis.

C. Preparation (Part 2 Liquid)

(a) Compositional standards

This product is oil liquid or water-soluble liquid of the mixture of the raw material for manufacturing of vitamin D_3 oil and hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats.

Content: When this product is determined, it contains cholecalciferol ($C_{27}H_{44}O$) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

Assay: Test is performed by Vitamin D quantification method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin D_3 oil is applied mutatis mutandis.

D. Preparation (Part 3 Powder)

(a) Compositional standards

This product is powder or particles of mixture of the raw material for manufacturing of vitamin D_3 oil and excipients.

Content: When this product is determined, it contains cholecalciferol ($C_{27}H_{44}O$) corresponding to 90~130 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

Assay: Test is performed by Vitamin D quantification method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

(79) Vitamin E Powder

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 100 mg or more of *dl*-*α*-tocopheryl acetate per 1 gram and contains *dl*-*α*-tocopheryl acetate ($C_{31}H_{52}O_3$) corresponding to 95~120 % of the unit on the label.

Physical and chemical properties:

i. This product is yellowish white to brown powder with little odor.

ii. Degradation of this product is promoted by air or light.

Confirmation test: 10 mL of the sample solution obtained by the assay method is measured, added with 2 mL of nitric acid and heated at 75 °C for 15 minutes. The resulting solution is red to orange.

Purity test:

i. Rancid: This product shall not emit an unpleasant odor.

ii. Heavy metal:

Method No. 1: The purity test ii. for the raw material for manufacturing of vitamin A powder is applied mutatis mutandis. However, the residue on ignition of this product is added with 1 mL of hydrochloric acid and 0.2 mL of nitric acid, evaporated to dryness on a water bath. Then it is added with 1 mL of dilute hydrochloric acid and 15 mL of water, dissolved by heating. If there are insoluble matters, Method No. 2 shall be applied (50 mg/kg or less).

Method No. 2: 1.0 g (0.95~1.04 g) of this product is weighed into a platinum pot with a loose lid and weakly heated to be carbonized. It is allowed to cool, added with 2 mL of nitric acid and 5 drops of sulfuric acid, carefully heated until white smoke emerges, and ignited at 500~600 °C to be converted into ashes. After cooling, the residue is moisturized with water, added with 6 mL of hydrofluoric acid and 3 drops of sulfuric acid, evaporated to dryness, and ignited for 5 minutes. It is allowed to cool, added with 2 mL of hydrochloric acid, evaporated to dryness on a water bath, moisturized with 3 drops of hydrochloric acid, added with 10 mL of boiling water, and heated for 2 minutes to dissolve. It is allowed to cool, added with a drop of phenolphthalein test solution, added with ammonia test solution by dropping until the solution becomes slightly red, added with 2 mL of diluted acetic acid, filtered as appropriate, and washed with 10 mL of water. The filtrate and the washings are transferred to a Nessler tube and added with water to make 50 mL. This is used as a sample solution. When this sample solution is added with 2 drops of sodium sulfide test solution, and allowed to stand for 5 minutes, the color of the solution shall not be darker than that of the solution prepared by the following procedure: 2 mL of nitric acid, 8 drops of sulfuric acid, 2 mL of hydrochloric acid, 6 mL of hydrofluoric acid, and 2 mL of diluted acetic acid are evaporated on a water bath, and then evaporated to dryness on a sand bath. Then, the residue is moisturized with 3 drops of hydrochloric acid, added with 10 mL of boiling water, and hereinafter subjected to the preparation method of the sample solution, added with 5.0 mL of lead standard solution and water to make 50 mL, added with 2 drops of sodium sulfide test solution and allowed to stand for 5 minutes (50 mg/kg or less).

iii. Arsenic: The purity test iii. for the raw material for manufacturing of vitamin A powder is applied mutatis mutandis (4 mg/kg or less).

Loss on drying: 7.0 % or less (1 g, reduced pressure, silica gel, 4 hours)

Assay: The amount of this product containing approximately 0.02 g of *dl*-*α*-tocopheryl acetate is weighed to three significant digits and the value is recorded. It is saponified by the vitamin A assay, extracted, and dehydrated. The ether extract is evaporated with shaking in water at 45 °C using an aspirator. The residue is dissolved in ethanol within 30 seconds, transferred to a 100 mL volumetric flask, and added with ethanol to the graduation line to make 100 mL. 5 mL of this solution is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, and added with ethanol to the graduation line to make 50 mL. This is used as a sample solution. Separately, approximately 0.02 g of *dl*-*α*-tocopheryl acetate for assay is weighed to the digits of 0.1 mg, the value is recorded, and it is subjected to the preparation procedure of the sample solution to prepare a standard solution. 10 mL each of the sample and standard solutions is measured using a volumetric pipette, transferred to a 25 mL volumetric flask, added with 1 mL of dilute ferric chloride test solution and 1 mL of *α*-*α′*-dipyridyl test solution, and added with ethanol to the graduation line to make 25 mL. Separately, 10 mL of ethanol is measured using a volumetric pipette, and subjected to the same procedures. The resulting solution is used as a control solution, and the absorbance of A_T and A_S at a wavelength 520 nm is measured.

Amount of *dl*-*α*-tocopheryl acetate ($C_{31}H_{52}O_3$) (mg)

$$= \text{Amount of } dl\text{-}\alpha\text{-tocopheryl acetate for assay (mg)} \times \frac{A_T}{A_S}$$

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of *dl*-*α*-tocopheryl acetate is powdered or coated and granulated using appropriate base materials.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of vitamin E powder are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin E powder is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of mixture of the raw material for manufacturing of vitamin E powder and fillers.

Content: When this product is determined, it contains *dl*-α-tocopheryl acetate ($C_{31}H_{52}O_3$) corresponding to 90~120 % of the amount on the label.

Confirmation test: The confirmation test of the raw material for manufacturing of vitamin E powder is applied mutatis mutandis.

Assay: The assay of the raw material for manufacturing of vitamin E powder is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of vitamin E powder is applied mutatis mutandis.

(80) 25-Hydroxycholecalciferol

A. Raw material for manufacturing

(a) Compositional standards

Content: When quantified, this product contains 25-hydroxycholecalciferol ($C_{27}H_{44}O_2 \cdot H_2O$) at no less than 94.0 %.

Physical and chemical properties:

i. This product is white crystal.

ii. This product changes in air or light.

Confirmation test:

i. When 20 μL of a sample solution prepared by a quantification method and a standard solution is tested by liquid chromatography under the operating condition of the quantification method, the retention time pertaining to the peak of 25-hydroxycholecalciferol ($C_{27}H_{44}O_2 \cdot H_2O$) obtained from the sample solution is the same as that obtained from the standard solution.

ii. When this product and a 25-hydroxycholecalciferol reference standard are measured by infrared absorption spectroscopy using the potassium bromide pellet method and the spectra are compared, absorptions are observed at the same wavenumbers and the relative intensity of the absorptions is the same.

Purity test:

i. Erythrosine Weigh approximately 1.0 g of this product to the digit of 0.01 g, record the value, add methanol to dissolve, place in a 10 mL volumetric flask, and further add

methanol to the marked line (i.e. volume up to 10 mL) to prepare a sample solution. For this solution, measure the absorbance, A_1, at wavelength 530 nm using methanol as the reference. Next, for 10 mL of methanol, perform the same procedure as that for the sample solution, and measure the absorbance, A_0. When the content of ionized erythrosine is calculated by the equation below, the amount shall be no more than 5 μg/g.

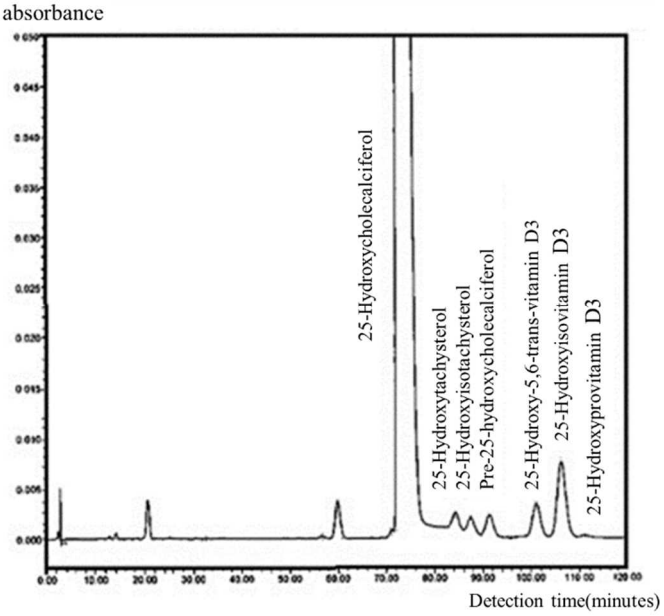
$$\text{Erythrosine content } (\mu g/g) = \frac{(A_1 - A_0) / \varepsilon \times 833.9 \times 10^6}{W_T}$$

W_T: Weight of this product (g)

ε : Molar absorption coefficient of erythrosine at 530 nm 110,000 (L/mol·cm)

ii. Analogous substances Measure 5 mL of the standard stock solution prepared by a quantification method using a volumetric pipette, place in a 100-mL volumetric flask, and add methanol to the marked line (i.e. volume up to 100 mL) to prepare the standard stock solution for purity test. Filter the standard stock solution for a purity test with a membrane filter (0.45 μm) to prepare a standard solution for the purity test. For 20 μL of a sample stock solution prepared by the quantification method and the standard solution for a purity test, perform testing by liquid chromatography under the conditions below. From the chromatograms obtained, identify the peak of each sterol that appears in the chromatogram of sample stock solution using a reference chromatogram of this product, and calculate the peak area, A_T. When the content of analogous substances of 25-hydroxycholecalciferol (which refer to sterols originating from manufacturing of 25-hydroxycholecalciferol other than 25-hydroxycholecalciferol; the same shall apply to ii. hereinafter) is calculated from this value and the peak area, A_S, of 25-hydroxycholecalciferol calculated from the chromatogram of the standard solution for the purity test using the equation below, the amount shall be no more than 1 % for each analogous substance.

Reference chromatogram of this product



$$\text{Content of each analogous substance (\%)} = p \times \frac{w_s}{w_T} \times \frac{A_T}{A_S} \times F \times 2$$

p: Purity of 25-hydroxycholecalciferol reference standard

w_T: Weight of this product (mg)

w_s: Weight of 25-hydroxycholecalciferol reference standard (mg)

A_T: Peak area of sterol in the sample solution

A_S: Peak area of 25-hydroxycholecalciferol in the standard solution

F: Absorption coefficient of each analogous substance specified in the table below

Table

Analogous substance	Absorption coefficient
25-Hydroxyisotachysterol	0.8300
25-Hydroxyisovitamin D_3	0.8300
25-Hydroxycholecalciferol	1.0000
25-Hydroxytachysterol	0.9109
25-Hydroxy-5,6-trans-vitamin D_3	0.8986
25-Hydroxyprovitamin D_3	1.7677
Pre-25-hydroxycholecalciferol	2.3863
Unknown sterol which shows a similar spectrum with 25-hydroxycholecalciferol	1.000

Operating condition

Detector: Photodiode array detector (measuring wavelength: 230~330 nm (measure at wavelength 270 nm for quantification))

Column: Fill a stainless steel tube of 4.6 mm in inner diameter and 250 mm in length with octadecylsilyl silica gel for liquid chromatography of no more than 5 μm in particle diameter.

Column temperature: A constant temperature around 28 °C

Mobile phase: Methanol-acetonitrile-water mixture solution (55:22:23)

Flow rate: Approximately 1.0 mL per minute

Measurement time: 120 minutes

Column selection: Heat 10 mL of the standard stock solution for purity test at 50~55°C for 2 hours to generate pre-25-hydroxycholecalciferol and filter with a membrane filter (0.45 μm). When the operation above is applied to 20 μL of this filtrate, the column to be used shall elute in the order of 25-hydroxycholecalciferol and then pre-25-hydroxycholecalciferol and the degree of separation shall be no less than 4.0.

iii. Lead Weigh 0.5 g (0.45~0.54 g) of this product and when a test for lead is performed by the limit test for lead (atomic absorption spectrometry No.1), the amount shall be no more than 20 μg/g.

iv. Aluminum Weigh 0.2 g (0.15~0.24 g) of this product, place in a platinum or quartz crucible, wet by adding a small amount of sulfuric acid, gradually heat to incinerate at as low a temperature as possible, allow to cool, further add 1 mL of sulfuric acid, gradually heat, and ignite until incinerated at 450~550 °C. Dissolve the residue by adding a small amount of nitric acid (1 → 150), place in a 50 mL, and add water to the marked line (i.e. volume up to 50 mL) to prepare the sample solution. Separately, measure 1 mL of an aluminum standard solution using a volumetric pipette, place in a 100 mL volumetric flask, and add water to the marked line (i.e. volume up to 100 mL). Measure 1 mL of this solution using a volumetric pipette, place in a 10-mL volumetric flask, and add water to the marked line (i.e. volume up to 10 mL). Measure 4 mL of this solution using a volumetric pipette, place in a 50 mL volumetric flask, add a small amount of nitric acid (1 → 150), and then add water to the marked line (i.e. volume up to 50 mL) to prepare the standard solution. When the sample solution and the standard solution are measured by atomic absorption spectrometry (flameless method (electric heating method)) under the conditions below, the absorbance of the sample solution shall be no more than the absorbance of the standard solution (no more than 20 μg/g).

Light source lamp: Aluminum hollow cathode lamp

Analysis line wavelength: 309.3 nm

Drying temperature: 140 °C

Incineration temperature: 900 °C

Atomization temperature: 2,600 °C

Water content No more than 5.0 % (direct titration)

Assay: Weigh approximately 0.20 g of this product to the digit of 0.001 g, record the value, add methanol to dissolve, place in a 200 mL volumetric flask, and further add methanol to the marked line (i.e. volume up to 10 mL) to prepare the sample stock solution. Measure 5 mL of this sample stock solution using a volumetric pipette, place in a 50 mL volumetric flask, add methanol to the marked line (i.e. volume up to 50 mL), filter with a membrane filter (0.45 μm), and serve the filtrate as the sample solution. Separately, weigh approximately 0.050 g of 25-hydroxycholecalciferol reference standard to the digit of 0.0001 g, record the value, add methanol to dissolve, place in a 50 mL volumetric flask, and further add methanol to the marked line (i.e. volume up to 50 mL). Measure 5 mL of this solution using a volumetric pipette, place in a 50 mL volumetric flask, and add methanol to the marked line (i.e. volume up to 50 mL) to prepare the standard stock solution. Filter this standard stock solution with a membrane filter (0.45 μm) to prepare a standard solution. For 20 μL of the sample solution and standard solution, perform testing by liquid chromatography under the conditions below. Calculate the peak area of 25-hydroxycholecalciferol from the chromatogram obtained.

$$\text{Content of 25-hydroxycholecalciferol } (C_{27}H_{44}O_2 \cdot H_2O)(\%) = \frac{A_T}{A_S} \times \frac{W_S}{W_T} \times p \times 400$$

P : Purity of 25-hydroxycholecalciferol reference standard

W_T: Weight of this product (mg)

W_S: Weight of 25-hydroxycholecalciferol reference standard (mg)

A_T: Peak area of 25-hydroxycholecalciferol in the sample solution

A_S: Peak area of 25-hydroxycholecalciferol in the standard solution

Operating condition

Detector: Ultraviolet spectrophotometer (measuring wavelength: 270 nm)

Column: Fill a stainless steel tube of 4.6 mm in inner diameter and 150 mm in length with octadecylsilyl silica gel for liquid chromatography of 5 μm in particle diameter.

Column temperature: A constant temperature around 28 °C

Mobile phase: Methanol-acetonitrile-water mixture solution (55:22:23)

Flow rate: Approximately 1.0 mL per minute

(b) Standard of manufacturing method

Shall be manufactured by aerobically culturing cholesta-5,7,24-trienol production recombinants hosted by bacterial strains belonging to Saccharomyces cerevisiae, heat

treating the cultured solution when the culturing is complete to separate cholesta-5,7,24-trienol, and by chemically treating (i.e. ultraviolet light irradiation).

(c) Standard of storage method

Shall be placed in a light-resistant sealed container, air replaced with nitrogen, and stored in a cool place.

B. Preparation (Part 1)

(a) Compositional standards

This product is powder in which excipient substances are mixed into source material for manufacturing 25-hydroxycholecalciferol.

Content: When quantified, this product contains 25-hydroxycholecalciferol ($C_{27}H_{44}O_2 \cdot H_2O$) at an amount equivalent to 90~120% of the declared content.

Confirmation test:

i. When 100 μL of a sample solution prepared by a quantification method and a standard solution is tested by liquid chromatography under the operating condition of the quantification method, the retention time pertaining to the peak of 25-hydroxycholecalciferol ($C_{27}H_{44}O_2 \cdot H_2O$) obtained from the sample solution is the same as that obtained from the standard solution.

ii. Spot 10 μL each of the sample stock solution prepared by a quantification method and the standard solution to a thin layer plate prepared using silica gel for thin layer chromatography containing fluorescent material. Develop for about 12 cm using an n-hexane - ethyl acetate mixed solution (1:1) as the developing solvent, and dry the thin layer plate in air. When an ultraviolet light (main wavelength 254 nm) is irradiated, the Rf value of the spot of 25-hydroxycholecalciferol obtained from the sample stock solution shall be the same as that obtained from the standard stock solution.

Loss on drying: No more than 8.0 % (1 g, 105 °C, 4 hours)

Assay: Weigh approximately 0.35 g of this product to the digit of 0.001 g, record the value, place in a 100 mL volumetric flask, add 15 mL of dimethyl sulfoxide, and sonicate until the powder becomes invisible. When the solution becomes semi-transparent, add ethyl acetate to the marked line (i.e. volume up to 100 mL), and allow to stand for 5 minutes to prepare the sample stock solution. Measure 3 mL of the supernatant of this sample stock solution using a volumetric pipette, place in a 100 mL volumetric flask, add isopropanol-ethyl acetate-isooctane mixed solution (1:30:69) to the marked line (i.e. volume up to 100 mL), and filter with a membrane filter (0.45 μm) to prepare the sample solution. Separately, weigh approximately 0.020 g of 25-hydroxycholecalciferol reference standard to the digit of 0.0001 g, record the value, place in a 2000 mL volumetric flask, add 15 mL of methanol, shake for 20 minutes to dissolve, and add ethyl acetate to the marked line (i.e.

volume up to 200 mL) to prepare the standard stock solution. Measure 3 mL of this standard stock solution using a volumetric pipette, place in a 200 mL volumetric flask, add isopropanol-ethyl acetate-isooctane mixed solution (1:30:69) to the marked line (i.e. volume up to 200 mL), filter with a membrane filter (0.45 μm), and serve the filtrate as a standard solution. Separately, allow 10 mL of the standard solution to stand at 40°C overnight or at room temperature for 3~4 days to generate pre-25-hydroxycholecalciferol, filter with a membrane filter (0.45 μm), and serve the filtrate as a separating solution for checking the retention time of pre-25-hydroxycholecalciferol and for column selection. For 100 μL of the sample solution, standard solution and separating solution, perform testing by liquid chromatography under the conditions below. Checking of 25-hydroxycholecalciferol and pre-25-hydroxycholecalciferol in a sample shall be carried out by checking the fact that the retention time of the standard solution is the same as that of the separating solution or the fact that the peak does not broaden when the standard solution or separating solution is added.

$$\frac{\text{25-hydroxycholecalciferol}}{\text{Content of } (C_{27}H_{44}O_2 \cdot H_2O)(\%)} = \frac{A_{T1}+A_{T2} \times 2.21}{A_S} \times \frac{W_S}{W_T} \times 25$$

W_T: Weight of this product (mg)

W_S: Weight of 25-hydroxycholecalciferol reference standard (mg)

A_{T1}: Peak area of 25-hydroxycholecalciferol in the sample solution

A_{T2}: Peak area of pre-25-hydroxycholecalciferol in the sample solution

A_S: Peak area of 25-hydroxycholecalciferol in the standard solution

Operating condition

Detector: Ultraviolet spectrophotometer (measuring wavelength: 260 nm)

Column: Fill a stainless steel tube of 4. 6 mm in inner diameter and 150 mm in length with silica gel for liquid chromatography of 5 μm in particle diameter.

Column temperature: Room temperature

Mobile phase: Isopropanol-ethyl acetate-isooctane mixed solution (1:10:89)

Flow rate: Approximately 1.5 mL per minute

Column selection: When the operation above is applied to 100 μL of this separating solution, the column to be used shall elute in the order of 25-hydroxycholecalciferol and then pre-25-hydroxycholecalciferol and the degree of separation shall be no less than 1.5.

(b) Standard of storage method

Shall be stored in a light-resistant airtight container.

(81) Ferrous Fumarate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 96.5 % or more of ferrous fumarate ($C_4H_2FeO_4$).

Physical and chemical properties:

i. This product is red-yellow or red-brown powder with no odor.

ii. This product is hard to dissolve in water and hardly dissolves in ethanol.

iii. This product is decomposed by hydrochloric acid to deposit fumaric acid.

Confirmation test:

i. 0.5 g (0.45~0.54 g) of this product is added with 100 mL of 0.1 mol/L hydrochloric acid test solution, dissolved by warming, and filtered. The filtrate gives the qualitative reaction i. of ferrous salt.

ii. 2.0 g (1.95~2.04 g) of this product is added with 25 mL of dilute hydrochloric acid, warmed on a water bath for 15 minutes, allowed to stand, and filtered. The residue is washed several times using hydrochloric acid (1 → 100), and dried at 100 °C for 30 minutes. 0.05 g (0.045~0.054 g) of it is weighed, added with 3 mg (2.5~3.4 mg) and 1 mL of sulfuric acid, shaken up, and heated at 120~130 °C for 5 minutes. It is allowed to cool, added with water to make 5 ml, gradually added with sodium hydroxide solution (2 → 5) by dropping while cooling to make it alkaline, and added with water to make 10 mL. When this solution is irradiated with ultraviolet light (365 nm), it emits green fluorescence.

Purity test:

i. Lead: When 1.0 g (0.95~1.04 g) of this product is weighed and subjected to the lead test method (Method No. 1 of atomic absorption spectrophotometry) its amount shall be 10 mg/kg or less.

ii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed, added with 15 mL of dilute sulfuric acid, gently heated. When the sample solution is tested for arsenic by the method using device A, the color of the absorbing solution shall not be darker than the standard color (5 mg/kg or less)

Loss on drying: Less than 1.0 % (1 g, 105 °C, 2 hours)

Assay: 0.5 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is placed in a 300 mL stoppered flask, added with 25 mL of hydrochloric acid (2 → 5), boiled, added with a solution of 5.6 g (5.55~5.64 g) of stannous chloride in 50 mL of hydrochloric acid (3 → 10) by dropping until the color of yellow disappears, and added with 2 more drops. The resulting solution is quenched down to room temperature, added

with 10 mL of mercuric chloride solution (1 → 20), allowed to stand for 5 minutes, added with 200 mL of water, 25 mL of sulfuric acid (1 → 2) and 4 mL of phosphoric acid, and titrated with 0.1 mol/L ceric ammonium sulfate solution (indicator: 2 drops of *o*-phenanthroline test solution).

0.1 mol/L ceric ammonium sulfate solution 1 mL = 16.99 mg $C_4H_2FeO_4$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of ferrous fumarate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of ferrous fumarate is applied mutatis mutandis.

(82) Peptide Zinc

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 5.5~8.5 % of nitrogen (N) and 10.0~13.0 % of zinc (Zn) at dry-matter conversion.

Physical and chemical properties:

i. This product is pale yellow to brown powder with a specific odor.

ii. This product hardly dissolves in water or ethanol.

Confirmation test:

i. 0.5 g (0.45~0.54 g) of this product is weighed, added with 25 mL of 0.5 mol/L hydrochloric acid test solution, and warmed in a water bath at 60 °C for 3 minutes while gently shaking. It is allowed to cool, added with 25 mL of 0.5 mol/L hydrochloric acid test solution and shaken up. 10 mL of this solution is measured, added with 10 mL of trichloroacetic acid solution (1 → 10), shaken up, and allowed to stand for 10 minutes. This solution is filtered using filter paper and the filtrate is used as a sample solution. 1 mL of the sample solution is measured, added with 1 mL of pyridine, 1 mL of ascorbic acid solution (1 → 2,000), 1 mL of ninhydrin solution (1 → 100), and heated on a water bath for 2 minutes. The resulting solution is blue-violet.

ii. 1 mL of the sample solution obtained in i. is measured and added with 1~2 drops of pyridine and 1 mL of potassium thiocyanate test solution. White precipitation is formed in the resulting solution.

iii. When the infrared absorption spectrum of this product is measured using the potassium bromide disk method of the infrared absorption spectroscopy, absorptions are observed at around wavelengths around 3,300~3,600 cm^{-1} and 1,640~1,660 cm^{-1}.

Purity test:

i. Lead: 1.0 g (0.95~1.04 g) of this product is weighed, added with 3 mL of nitric acid and 5 mL of perchloric acid, evaporated to dryness, allowed to cool, added with 5 mL of dilute hydrochloric acid, and warmed on a water bath to dissolve. It is allowed to cool, added with 5 mL of water, mixed, and filtered using filter paper. The residue is washed with 5 mL of water. The filtrate and the washings are mixed, transferred to a 25 mL volumetric flask, and added with water to the graduation line to make 25 mL. This is used as a sample solution. Separately, 4 mL of lead standard solution is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, and added with water to the graduation line to make 50 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 25 mL volumetric flask, and added with 5 mL of dilute hydrochloric acid and water to the graduation line to make 25 mL. This is used as a standard solution. When the sample and standard solutions are measured by the atomic absorption spectrophotometry (flame type) under the following conditions, the absorbance of the sample solution shall be lower than that of the standard solution (20 mg/kg or less).

Gas to be used: Flammable gas, acetylene
Combustion-supporting gas, air
Lamp: Lead hollow cathode lamp
Wavelength: 217.0 nm

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less)

Loss on drying: Less than 12.0 % (0.5 g, 110 °C, 1 hours)

Rate of hydrolysis: Approximately 1 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 30 mL of 0.5 mol/L hydrochloric acid test solution, warmed in a water bath at 60 °C for 30 minutes with occasional shaking. It is allowed to cool, and transferred to a 50 mL volumetric flask, added with 10 mL of trichloroacetic acid solution (1 → 10), vigorously shaken up, added with 0.5 mol/L hydrochloric acid

test solution to the graduation line to make 50 mL. Approximately 20 mL of this solution is measured, centrifuged for 10 minutes at 3,000 rpm. 5 mL of the supernatant fluid obtained is measured using a volumetric pipette, transferred to a 25 mL volumetric flask, and added with water to the graduation line to make 25 mL. 4 mL of this solution is measured using a volumetric pipette, transferred to a Kjeldahl flask, added with 10 mL of sulfuric acid along the sulfuric acid of the flask. The test is hereinafter performed by the nitrogen determination method (Semimicro-Kjeldahl Method). Based on the rate of hydrolysis obtained here (A%), and total nitrogen (B%) obtained in the section of Assay, the rate of hydrolysis determined by the following equation shall be 30 % or more.

$$\text{Rate of hydrolysis (\%)} = \frac{A}{B} \times 100$$

Assay:

i. Total nitrogen: Approximately 0.03 g of this product is weighed to the digits of 0.1 mg and the value is recorded. It is placed in a Kjeldahl flask and the test is hereinafter performed by the nitrogen determination method (Semimicro-Kjeldahl Method).

0.005 mol/L sulfuric acid 1 mL = 0.1401 mg N

ii. Zinc: Approximately 0.2 g of this product is measured to the digits of 0.001 g and the value is recorded. It is placed in a Kjeldahl flask, added with 5 mL of nitric acid and 10 mL of perchloric acid and heated until the residue reaches approximately 2 mL. It is allowed to cool, added with 2 mL of hydrochloric acid and heated on a water bath to dissolve. It is allowed to cool, transferred to a 100 mL volumetric flask, and added with more water to the graduation line to make 100 mL. This solution is filtered using filter paper. 10 mL of the first filtrate is discarded, 10 mL of the next filtrate is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to make 100 mL. Then, 5 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. This is used as a sample solution. Separately, 5 mL of nitric acid and 10 mL of perchloric acid are placed in a Kjeldahl flask and heated until the residue reaches approximately 2 mL. It is allowed to cool, added with 2 mL of hydrochloric acid, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a blank test stock solution. 5 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a blank test solution. Separately, 5, 10, 15 and 20 mL each of zinc standard

solutions is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, 5 mL of the blank test stock solution is added using a volumetric pipette, and added with water to the graduation line to make 100 mL. The resulting solutions are called the standard solution 1, 2, 3 and 4, respectively. The sample solution, the standard solutions 1, 2, 3 and 4, and the blank test solution are determined by the atomic absorption spectrophotometry (flame type) under the following conditions. A calibration curve is prepared from the absorbances of the standard solution 1, 2, 3 and 4, and the blank test solution and the content of zinc in the sample is calculated.

Gas to be used: Flammable gas, acetylene

Combustion-supporting gas, air

Lamp: Zinc hollow cathode lamp

Wavelength: 213.9 nm

Content of zinc (%)

$$= \frac{\text{Concentration of zine in the sample solution obtained from the calibration curve (μg/mL)}}{\text{collected amount of the sample (mg)}} \times 2{,}000$$

(b) Standard of storage method

It shall be stored in capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of peptide zinc are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of peptide zinc is applied mutatis mutandis.

(83) Peptide Iron

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 7.0~10.5 % of nitrogen (N) and 10.0~13.0 % of iron (Fe) at dry-matter conversion.

Physical and chemical properties:

i. This product is brown to greenish brown powder or particles with a specific odor.

ii. This product hardly dissolves in water and ethanol.

Confirmation test:

i. This product is powdered as appropriate. 0.05 g (0.045~0.054 g) of this product is weighed, added with 25 mL of 0.5 mol/L hydrochloric acid test solution, warmed in a water bath at 60 °C for 3 minutes while gently shaking. It is allowed to cool, added with 25 mL of 0.5 mol/L hydrochloric acid test solution, and shaken up. 10 mL of this solution is measured, added with 10 mL of trichloroacetic acid solution (1 → 10), shaken up, and allowed to stand for 10 minutes. This solution is filtered by filer paper and the filtrate is used as a sample solution. 1 mL of the sample solution is measured, added with 1 mL of pyridine, 1 mL of ascorbic acid solution (1 → 2,000) and 1 mL of ninhydrin solution (1 → 100), and heated on a water bath for 2 minutes. The resulting solution is blue-violet.

ii. 1 mL of the sample solution obtained in i. is measured, added with 1 mL of hydroxylamine hydrochloride solution (1 → 50), allowed to stand for 10 minutes, dissolved with 2 mL of a solution prepared by the way in which 0.25 g of *o*-phenanthroline is dissolved with pH 5.0 acetic acid-sodium acetate buffer to make 100 mL. The resulting solution is red to red-orange.

Purity test:

i. Lead: 1.0 g (0.95~1.04 g) of this product is weighed. When lead is tested by the lead test method (Method No. 1 of the atomic absorption spectrophotometry), the amount of lead shall be 10 mg/kg or less.

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less)

Loss on drying: Less than 13.0 % (0.5 g, 110 °C, 8 hours)

Rate of hydrolysis: This product is powdered as appropriate. Approximately 1 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 30 mL of 0.5 mol/L hydrochloric acid and warmed in a water bath at 60 °C for 30 minutes while gently shaking. It is allowed to cool, transferred to a 50 mL volumetric flask, added with 10 mL of trichloroacetic acid solution (1 → 10), vigorously shaken up, and added with 0.5 mol/L hydrochloric acid to the graduation line to make 50 mL. Approximately 20 mL of this solution is measured, and centrifuged for 5 minutes at 4,000 rpm. 5 mL of the supernatant fluid obtained is measured using a volumetric pipette, transferred to a 25 mL volumetric flask and added with water to the graduation line to make 25 mL. 4 mL of this solution is measured using a volumetric pipette, transferred to a Kjeldahl flask, and added with 7 mL of sulfuric acid along the inner wall of the flask. The test is hereinafter

performed by the nitrogen determination method. Based on the rate of hydrolysis of the dry-matter obtained here (A%), and total nitrogen (B%) obtain in the section of Assay, the rate of hydrolysis determined by the following equation shall be 30 % or more.

$$\text{Rate of hydrolysis (\%)} = \frac{A}{B} \times 100$$

Assay:

i. Total nitrogen: This product is powdered as appropriate. Approximately 0.03 g of this product is weighed to the digits of 0.1 mg and the value is recorded. It is placed in a Kjeldahl flask. The test is hereinafter performed by the nitrogen determination method.

0.005 mol/L sulfuric acid 1 mL = 0.1401 mg N

ii. Iron: This product is powdered as appropriate. Approximately 0.2 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is placed in a Kjeldahl flask, added with 3 mL of nitric acid and 5 mL of perchloric acid, and heated until the residual liquid reaches approximately 1 mL (about 2 hours). After cooling, the residual liquid is added with 2 mL of hydrochloric acid and heated on a water bath to dissolve. It is allowed to cool, transferred to a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. This solution is filtered by filter paper. 10 mL of the first filtrate is discarded, 10 mL of the next filtrate is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to make 100 mL. Then, 5 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. This is used as a sample solution. Separately, 3 mL of nitric acid and 5 mL of perchloric acid are placed in a Kjeldahl flask and heated until the residue reaches approximately 1 mL. It is allowed to cool, added with 2 mL of hydrochloric acid, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a blank test stock solution. 5 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a blank test solution. Separately, 5, 10, 15 and 20 mL each of iron standard solutions is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, 5 mL of the blank test stock solution is added using a volumetric pipette, and added with water to the graduation line to make 100 mL. The resulting solutions are called the standard solution 1, 2, 3 and 4, respectively. The sample solution, the standard solutions 1, 2, 3 and 4, and the blank test solution are determined by the atomic absorption

spectrophotometry (flame type) under the following conditions. A calibration curve is prepared from the absorbances of the standard solution 1, 2, 3 and 4 and the content of iron in the sample (mg/kg) is calculated.

Gas to be used: Flammable gas, acetylene

Combustion-supporting gas, air

Lamp: Iron hollow cathode lamp

Wavelength: 248.3 nm

Content of iron (%)

$$= \frac{\text{Amount of iron from the calibration curve (mg/kg)}}{\text{collected amount of the sample (mg)}} \times 2{,}000$$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of peptide iron are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of peptide iron is applied mutatis mutandis.

(84) Peptide Copper

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 5.5~8.5 % of nitrogen (N) and 10.0~13.0 % of copper (Cu) at dry-matter conversion.

Physical and chemical properties:

i. This product is blue-green powder with a specific odor.

ii. This product hardly dissolves in water or ethanol.

Confirmation test:

i. The confirmation test i. of the raw material for manufacturing of peptide zinc is applied mutatis mutandis.

ii. When 1 mL of the sample solution obtained in i. is measured, added with 1 mL of potassium ferrocyanide test solution, red-brown precipitation is formed in the resulting solution.

iii. When the infrared absorption spectrum of this product is measured using the potassium bromide disk method of the infrared absorption spectroscopy, absorptions are observed at around wavelengths around 3,300~3,600 cm^{-1} and 1,630~1,650 cm^{-1}.

Purity test:

i. Lead: The purity test i. of the raw material for manufacturing of peptide zinc is applied mutatis mutandis (20 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less)

Loss on drying: Less than 12.0 % (0.5 g, 110 °C, 1 hour)

Assay:

i. Total nitrogen: Approximately 0.03 g of this product is weighed to the digits of 0.1 mg and the value is recorded. It is placed in a Kjeldahl flask. The test is hereinafter performed by the nitrogen determination method.

0.005 mol/L sulfuric acid 1 mL = 0.1401 mg N

ii. Copper: Approximately 0.2 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is placed in a Kjeldahl flask, added with 5 mL of nitric acid and 10 mL of perchloric acid and heated until the residual liquid reaches approximately 2 mL. After cooling, it is added with 2 mL of hydrochloric acid, and is heated on a water bath to dissolve. It is allowed to cool, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This solution is filtered by filter paper. 10 mL of the first filtrate is discarded, 10 mL of the next filtrate is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to make 100 mL. Then, 5 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. This is used as a sample solution. Separately, 5 mL of nitric acid and 10 mL of perchloric acid are placed in a Kjeldahl flask and heated until the residue reaches approximately 2 mL. It is allowed to cool, transferred to a 100 mL volumetric flask, added with 2 mL of hydrochloric acid, and added with water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a blank test stock solution. 5 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a blank test solution. Separately, 2, 4, 6 and 8 mL

each of copper standard solutions is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, 5 mL of the blank test stock solution is added using a volumetric pipette, and added with water to the graduation line to make 100 mL. The resulting solutions are called the standard solution 1, 2, 3 and 4, respectively. The sample solution, the standard solutions 1, 2, 3 and 4, and the blank test solution are determined by the atomic absorption spectrophotometry (flame type) under the following conditions. A calibration curve is prepared from the absorbances of the standard solution 1, 2, 3 and 4 and the blank test solution and the content of copper in the sample is calculated.

Gas to be used: Flammable gas, acetylene
Combustion-supporting gas, air

Lamp: Copper hollow cathode lamp

Wavelength: 324.7 nm

Content of copper (%)

$$= \frac{\text{Concentration of copper in the sample solution obtained from the calibration curve (μg/mL)}}{\text{collected amount of the sample (mg)}} \times 2{,}000$$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of peptide copper are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of peptide copper is applied mutatis mutandis.

(85) Peptide Manganese

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 5.5~8.5 % of nitrogen (N) and 10.0~13.0 % of manganese (Mn) at dry-matter conversion.

Physical and chemical properties:

i. This product is pale yellow to brown powder with a specific odor.

ii. This product hardly dissolves in water or ethanol.

Confirmation test:

i. The confirmation test i. of the raw material for manufacturing of peptide zinc is applied mutatis mutandis.

ii. When 1 mL of the sample solution obtained in i. is measured, added with 5 mL of ammonia test solution and 5 mL of silver nitrate test solution, white precipitation is formed in the solution. The generated precipitation gradually turns black.

iii. When the infrared absorption spectrum of this product is measured using the potassium bromide disk method of the infrared absorption spectroscopy, absorptions are observed at around wavelengths around 3,300~3,600 cm^{-1} and 1,630~1,650 cm^{-1}.

Purity test:

i. Lead: The purity test i. of the raw material for manufacturing of peptide zinc is applied mutatis mutandis (20 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less)

Loss on drying: Less than 12.0 % (0.5 g, 110 °C, 1 hour)

Rate of hydrolysis: The rate of hydrolysis of the raw material for manufacturing of peptide zinc is applied mutatis mutandis

Assay:

i. Total nitrogen: Approximately 0.03 g of this product is weighed to the digits of 0.1 mg and the value is recorded. It is placed in a Kjeldahl flask. The test is hereinafter performed by the nitrogen determination method.

0.005 mol/L sulfuric acid 1 mL = 0.1401 mg N

ii. Manganese: Approximately 0.2 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is placed in a Kjeldahl flask, added with 5 mL of nitric acid and 10 mL of perchloric acid and heated until the residual liquid reaches approximately 2 mL. After cooling, it is added with 2 mL of hydrochloric acid, and heated on a water bath to dissolve. It is allowed to cool, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This solution is filtered by filter paper. 10 mL of the first filtrate is discarded, 10 mL of the next filtrate is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to make 100 mL. Then, 5 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. This is used as a sample solution. Separately, 5 mL of nitric acid and 10 mL of perchloric acid are placed in a Kjeldahl

flask and heated until the residue reaches approximately 2 mL. It is allowed to cool, transferred to a 100 mL volumetric flask, added with 2 mL of hydrochloric acid, and added with water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a blank test stock solution. 5 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a blank test solution. Separately, 5, 10, 15 and 20 mL each of manganese standard solutions is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, 5 mL of the blank test stock solution is added using a volumetric pipette, and added with water to the graduation line to make 100 mL. The resulting solutions are called the standard solution 1, 2, 3 and 4, respectively. The sample solution, the standard solutions 1, 2, 3 and 4, and the blank test solution are determined by the atomic absorption spectrophotometry (flame type) under the following conditions. A calibration curve is prepared from the absorbances of the standard solution 1, 2, 3 and 4 and the blank test solution and the content of manganese in the sample is calculated.

Gas to be used: Flammable gas, acetylene

Combustion-supporting gas, air

Lamp: Manganese hollow cathode lamp

Wavelength: 279.5 nm

Content of manganese (%)

$$= \frac{\text{Concentration of manganese in the sample solution obtained from the calibration curve (μg/mL)}}{\text{collected amount of the sample (mg)}} \times 2{,}000$$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of peptide manganese are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of peptide manganese is applied mutatis mutandis.

(86) DL-methionine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 4-hour drying at 105 °C, it contains 98.5 % or more of DL-methionine ($C_5H_{11}NO_2S$)

Physical and chemical properties:

i. This product is white to pale-yellow crystals or crystalline powder with a specific odor and with slight sweetness.

ii. This product is hard to lightly dissolve in water, hard to extremely dissolve in ethanol, and hardly dissolves in ether.

iii. This product dissolves in dilute hydrochloric acid or 1 mol/L sodium hydroxide test solution.

iv. The pH of a solution of this product in water (1 → 100) is 5.2 to 6.1

v. A solution of this product in water (1 → 100) does not have an optical rotation.

Confirmation test:

i. 25 mg (24.5~25.4 mg) of this product is added with 1 mL of sulfuric acid saturated with anhydrous copper sulfate. The resulting solution is yellow.

ii. 5 mg (4.5~5.4 mg) of this product is dissolved with 5 mL of water, added with 2 mL of 1 mol/L sodium hydroxide test solution, shaken up well, added with 0.3 mL of sodium nitroprusside test solution, shaken up well again, allowed to stand at 35~40 °C for 10 minutes, cooled with ice for 2 minutes, added with 2 mL of dilute hydrochloric acid and shaken up. The resulting solution is red.

Purity test:

i. Clarity and color of solution: 0.5 g (0.45~0.54 g) of this product is dissolved with 20 mL of water. The resulting solution shall be colorless and almost clear.

ii. Chloride: When chloride is tested using a sample solution prepared with 0.09 g (0.085~0.094 g) of this product by the chloride test method and a control solution prepared with 0.5 mL of 0.01 mol/L hydrochloric acid, the turbidity of the sample solution shall not be higher than that of the control solution (0.20 % or less).

iii. Sulfate: When sulfate is tested using a sample solution prepared with 0.08 g (0.075~0.084 g) of this product by the sulfate test method and a control solution prepared with 0.5 mL of 0.005 mol/L sulfuric acid, the turbidity of the sample solution shall not be higher than that of the control solution (0.30 % or less).

iv. Heavy metals: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using

these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

v. Arsenic: 1.0 g (0.95~1.04 g) of this product is placed in a 100 mL decomposition flask, and is added with 5 mL of nitric acid and 2 mL of sulfuric acid. A small funnel is placed on the mouth of the flask and the solution is carefully heated until white smoke emerges. It is allowed to cool, added with 2 mL each of nitric acid twice, heated, added with 2 mL each of hydrogen peroxide solution several times, and heated until the solution reaches colorless to slightly yellow. It is allowed to cool, added with 2 mL of saturated ammonium oxalate and again heated until white smoke emerges. It is allowed to cool and added with water to make 5 mL. This is used as a sample solution. When arsenic is tested by the method using device A, the color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: Less than 0.5 % (1 g 105 °C, 4 hours)

Ignition residue: Less than 0.5 % (1 g)

Assay: This product is dried at 105 °C for 4 hours, approximately 0.3 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is placed in a stoppered flask, added with 100 mL of water, 5 g (4.5~5.4 g) of dibasic potassium phosphate, 2 g (1.5~2.4 g) of potassium dihydrogenphosphate and 2 g (1.5~2.4 g) of potassium iodide, and shaken up to dissolve. It is added with 50 mL of 0.1 mol/L iodine solution using a volumetric pipetted, tightly stoppered, shaken up well, and allowed to stand for 30 minutes, and an excess amount of iodide is titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 1 mL of starch test solution). A blank test is performed in the same manner.

0.1 mol/L iodine solution 1 mL = 7.461 mg $C_5H_{11}NO_2S$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of DL-methionine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of DL-methionine is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is particles in which the raw material for manufacturing of DL-methionine is added with ethyl cellulose and sodium stearate as appropriate, and mixed with fillers.

Content: When this product is determined, it contains DL-methionine ($C_5H_{11}NO_2S$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 25 mg of the raw material for manufacturing of DL-methionine is weighed, and the confirmation test i. for the raw material for manufacturing of DL-methionine is hereinafter applied mutatis mutandis.

ii. According to the amount of this product on the label, the amount containing 5 mg of the raw material for manufacturing of DL-methionine is weighed, added with 1 mL of a mixed solution of n-hexane and isopropanol (1:1), and shaken up. This solution is added with 5 mL of water, shaken up, and allowed to stand. The aqueous layer is filtered and the confirmation test ii. for the raw material for manufacturing of DL-methionine is hereinafter applied to the filtrate mutatis mutandis.

A preparation containing fatty acid calcium is powdered. Then, according to the amount of this product on the label, the amount containing 5 mg of the raw material for manufacturing of DL-methionine is weighed, added with 5 mL of water, shaken up, and the confirmation test ii. for the raw material for manufacturing of DL-methionine is hereinafter applied mutatis mutandis.

Assay: Method No. 1 is used for a preparation containing fatty acid calcium and Method No. 2 is used for the others.

Method No. 1: This product is powdered, the amount of it containing approximately 0.03 g of DL-methionine ($C_5H_{11}NO_2S$) is weighed to three significant digits and the value is recorded. It is added with 70 mL of hydrochloric acid (9 → 250), heated at approximately 90 °C for 30 minutes while stirring to dissolve, allowed to cool, and filtered. 100 mL of the filtrate is transferred to a 100 mL volumetric flask, added with hydrochloric acid (9 → 250) to the graduation line to make 100 mL. 2 mL of this solution is measured using a volumetric pipette and added with 2 mL of hydrochloric acid (9 → 250) and 12 mL of water using a volumetric pipette. This is used as a sample solution. Separately, 0.030 g of dried DL-methionine for assay is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with hydrochloric acid (9 → 250), transferred to a 100 mL volumetric flask, and added with more hydrochloric acid (9 → 250) to the graduation line to make 100 mL. 2 mL of this solution is measured using a volumetric pipette and added with 2 mL of hydrochloric acid (9 → 250) and 12 mL of water using a volumetric pipette. This is used as a standard solution. Separately, 2 mL of hydrochloric acid (9 → 250) is measured using a volumetric pipette and added with 6 mL of water using a volumetric pipette. This is

used as a blank test solution. Then, 2 mL each of the sample, standard and blank test solutions is measured using a volumetric pipette, transferred to a 25 mL volumetric flask, added with 1 mL of newly prepared ninhydrin ascorbate test solution and 1 mL of pyridine using a volumetric pipette, and shaken up. Then, it is heated in a water bath for 25 minutes, allowed to cool, and added with water to the graduation line to make 25 ml. The absorbances, the sample and standard solutions, A_T and A_S respectively, at a wavelength 570 nm are measured using the blank test solution as a control solution.

Amount of DL-methionine ($C_5H_{11}NO_2S$) (mg)

$$= \text{Amount of DL-methionine for assay (mg)} \times \frac{A_T}{A_S}$$

Method No. 2: This product is powdered, the amount of it containing approximately 0.03 g of DL-methionine ($C_5H_{11}NO_2S$) is weighed to three significant digits, and the value is recorded. It is added with 15 mL of a mixed solution of n-hexane and isopropanol (1:1), stirred to dissolve, added with 70 mL of hydrochloric acid (9 → 250) and extracted while vigorously stirring. This solution is transferred to a separatory funnel while filtering with absorbent cotton and the container is washed with 5 mL of a mixed solution of n-hexane and isopropanol (1:1) and 15 mL of hydrochloric acid (9 → 250). The washings are poured into the absorbent cotton and these filtrates are mixed. The resulting solution is shaken up and the aqueous layer is transferred to a 100 mL volumetric flask and added with hydrochloric acid (9 → 250) to the graduation line to make 100 mL. 2 mL of this solution is measured using a volumetric pipette, added with 2 mL of hydrochloric acid (9 → 250) and 12 mL of water using a volumetric pipette. This is used as a sample solution and Method No. 1 is hereinafter applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of DL-methionine is applied mutatis mutandis.

(c) Standard of the label

As for a preparation for cattle, which is coated for degradation less in the rumen but more in the abomasum, the following words shall be written on the immediate container or the immediate wrapper of this product.

Precautions for use: Since this product is covered for degradation less in the rumen of the cattle but more in the abomasum, use it with attention to the amino-acid balance.

(87) L-methionine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 4-hour drying at 105 °C, it contains 98.5 % or more of L-methionine ($C_5H_{11}NO_2S$)

Physical and chemical properties:

i. This product is white to pale-yellow crystals or crystalline powder.

ii. This product is easy to lightly dissolve in water, and hardly dissolves in ethanol and ether.

iii. This product dissolves in dilute hydrochloric acid or 1 mol/L sodium hydroxide test solution.

iv. The pH of a solution of this product in water (1 → 100) is 5.2 to 6.1

Confirmation test:

Confirmation test of the raw material for manufacturing of DL-methionine are applied mutatis mutandis.

Purity test:

i. Specific rotation: This product is dried at 105 °C for 4 hours. Approximately 1 g of it is weighed to the digits of 0.01 g and the value is recorded. It is dissolved in hydrochloric acid test solution, 6 mol/L to make 50 mL and as appropriate filtered. The rotation of this solution measured shall be $[\alpha]_D^{25} = +21.1 \sim +25.1°$.

ii. Clarity and color of solution: Purity test i of the raw material for manufacturing of DL-methionine are applied mutatis mutandis.

iii. Chloride: Purity test ii of the raw material for manufacturing of DL-methionine are applied mutatis mutandis.

iv. Sulfate: Purity test iii of the raw material for manufacturing of DL-methionine are applied mutatis mutandis.

v. Lead: When 0.5 g (0.45~0.54 g) of this product is weighed and subjected to the lead test method (Method No. 1 of atomic absorption spectrophotometry), the content of lead shall be 20 mg/kg or less.

vi. Arsenic: Purity test v of the raw material for manufacturing of DL-methionine are applied mutatis mutandis.

Loss on drying: Less than 0.5 % (1 g 105 °C, 4 hours)

Ignition residue: Less than 0.5 % (1 g)

Assay: This product is dried at 105 °C for 4 hours, approximately 0.3 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is placed in a stoppered flask, added with 100 mL of water, 5 g (4.5~5.4 g) of dibasic potassium phosphate, 2 g (1.5~2.4 g) of

potassium dihydrogenphosphate and 2 g (1.5~2.4 g) of potassium iodide, and shaken up to dissolve. It is added with 50 mL of 0.1 mol/L iodine solution using a volumetric pipetted, tightly stoppered, shaken up well, and allowed to stand for 30 minutes, and an excess amount of iodide is titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 1 mL of starch test solution). A blank test is performed in the same manner.

0.1 mol/L iodine solution 1 mL = 7.461 mg $C_5H_{11}NO_2S$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-methionine are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-methionine is applied mutatis mutandis.

(88) Menadione dimethylpyrimidinol bisulfite

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 94.0 % or more of menadione dimethylpymidinol bisulfite ($C_{17}H_{18}N_2O_6S$) at dehydration product conversion.

Physical and chemical properties:

i. This product is white to pale yellow-brown crystalline powder with no odor and with a slightly bitter taste.

ii. This product is hard to lightly dissolve in water and hard to extremely dissolve in ethanol, acetone, and ether.

Confirmation test:

i. 1 mL of a solution of this product in water (1 → 500) is added with 10 mL of ammonia test solution and 1 mL of isopropanol, shaken up, added with a drop of ethyl cyanoacetate, and shaken up. The resulting solution is blue-violet.

ii. 5 mL of a solution of this product in water (1 → 500) is added with 5 mL of ethanol and 5 mL of a solution of sulfanilic acid in 0.2 mol/L sulfuric acid (1.6 → 100), shaken up, added with 2 mL of sodium nitrite solution (5 → 100), shaken up, and heated on a water bath for 5 minutes. The resulting solution is reddish violet.

Purity test:

i. Heavy metals 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. 2-methyl-1, 4-naphthohydroquinone-3-sulfonate: When 0.10 g (0.095~0.104 g) of this product is dissolved with 5 mL of water, and added with 2 drops of *o*-phenanthroline test solution, precipitation shall not be generated.

Water content: Less than 1.0 % (1 g)

Ignition residue: Less than 0.20 % (1 g)

Assay: Approximately 0.3 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 30 mL of water, added with 30 mL of chloroform, and then added with 2 mL of 1 mol/L of sodium hydroxide test solution, and within 30 seconds vigorous shaking of the resulting solution is started and then maintained for 15 seconds. The solution is added with 2 mL more of 1 mol/L sodium hydroxide test solution, within 30 seconds vigorous shaking is started and maintained for 15 seconds, and again 2 mL of 1 mol/L sodium hydroxide test solution is added, and within 30 seconds vigorous shaking is started and maintained for 90 seconds. Then it is allowed to stand and the chloroform layer is fractionated. The aqueous layer is extracted twice with 30 mL of chloroform each time. The mixture of all chloroform extracts is washed with 20 mL of water, filtered with pre-chloroform-moisturized filter paper, and washed with 5 mL of chloroform. The filtrate and the washings are mixed and transferred to a 200 mL flask, and the chloroform is evaporated on a water bath. Then, it is evaporated to dryness while injecting nitrogen at room temperature. The residue is dissolved with 20 mL of glacial acetic acid and 15 mL of dilute hydrochloric acid, added with 3 g (2.5~3.4 g) of zinc powder, attached with a Bunsen valve, frequently shaken up, allowed to stand in a dark place for an hour, and filtered using a cotton plug. The flask is washed five times with 20 mL each time with newly boiled and cooled water. The filtrate and the washings are mixed and titrated with 0.1 mol/L ceric ammonium sulfate solution within 30 seconds (indicator: 2 drops of *o*-phenanthroline test solution). A blank test is performed in the same manner and corrections are made.

0.1 mol/L ceric ammonium sulfate solution 1 mL = 18.92 mg $C_{17}H_{18}N_2O_6S$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of menadione dimethylpymidinol bisulfite are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of menadione dimethylpymidinol bisulfite is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of menadione dimethylpymidinol bisulfite and fillers.

Content: When this product is determined, it contains menadione dimethylpymidinol bisulfite ($C_{17}H_{18}N_2O_6S$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.03 g of the raw material for manufacturing of menadione dimethylpyrimidinol bisulfite is weighed, added with 15 mL of water, and for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats added with 5 mL of chloroform, shaken up, and allowed to stand. The aqueous layer is filtered to prepare a sample solution and 1 mL of this solution is measured. The confirmation test i. of the raw material for manufacturing of menadione dimethylpyrimidinol bisulfite is hereinafter applied mutatis mutandis.

ii. 5 mL of the sample solution in i. is measured and confirmation test ii. of the raw material for manufacturing of menadione dimethylpyrimidinol bisulfite is hereinafter applied mutatis mutandis.

Assay: Method No. 1 is used for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats and Method No. 2 is used for the others.

Method No. 1: The amount of this product containing approximately 0.1 g of menadione dimethylpyrimidinol bisulfite ($C_{17}H_{18}N_2O_6S$) is weighed to three significant digits and the value is recorded. It is added with 50 mL of chloroform, shaken up, and filtered using a glass filter (G4). The residue on the filter is washed three times with 20 mL of chloroform each time. When the chloroform odor on the residue disappears, the

residue is filtered by extracting with approximately 80 mL of warm water. The filtrate is placed in a 100 mL volumetric flask, allowed to cool, and added with water to the graduation line to make 100 mL. This is used as a sample solution. Separately, approximately 0.1 g of menadione dimethylpyrimidinol bisulfite for assay is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask, added with more water to the graduation line and dissolved to make 100 mL. This is used as a standard solution. 5 mL each of the sample solution is measured into 100 mL brown volumetric flasks T and T′ using a volumetric pipette. Each of them is added with 80 mL of water, mixed well, added with 2 mL of a mixed solution of strong ammonia solution and isopropanol (1:1) and shaken up well. Within 30 seconds, the resulting solution in T is added with 3 mL of a solution of ethyl cyanoacetate in isopropanol (3 → 20), shaken up, and then within 30 seconds, added with water to the graduation line to make 100 mL. The resulting solution in T′ is added with water to the graduation line to make 100 mL. Separately, 5 mL each of the standard solution is measured into 100 mL brown volumetric flasks S and S′ using a volumetric pipette, and the same procedure as for the sample solution is performed. They are allowed to stand for 5 minutes. For each solution, absorbances, A_T, $A_{T'}$, As and $A_{S'}$ at the maximum wavelength around 565 nm are measured using water as a control.

Amount of menadione dimethylpyrimidinol bisulfite ($C_{17}H_{18}N_2O_6S$) (mg)
= Amount of menadione dimethylpyrimidinol bisulfite for assay ($C_{17}H_{18}N_2O_6S$) (mg)
$\times \dfrac{A_T - A_{T'}}{A_S - A_{S'}} \times \dfrac{1}{2}$

Method No. 2: The amount of this product containing approximately 0.1 g of menadione dimethylpyrimidinol bisulfite ($C_{17}H_{18}N_2O_6S$) is weighed to three significant digits and the value is recorded. It is added with 60 mL of water, vigorously shaken up, and filtered. The residue on the paper filter is washed twice with 10 mL of water each time. The filtrate and the washings are mixed and transferred to a 100 mL volumetric flask to make 100 mL. This is used as a sample solution and Method No. 1 is hereinafter applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(89) Menadione Sodium Bisulfite

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 93.0 % or more of menadione sodium bisulfite ($C_{11}H_8O_2 \cdot NaHSO_3$) at dehydration product conversion.

Physical and chemical properties:

i. This product is white to slight brown crystalline powder with no odor.

ii. This product is easy to dissolve in water, hard to dissolve in ethanol, and hardly dissolves in ether or benzene.

Confirmation test:

i. 0.1 g (0.05~0.14 g) of this product in placed in a separatory funnel, dissolved with 10 mL of water, added with 3 mL of 1 mol/L sodium hydroxide test solution, and extracted twice with 5 mL of chloroform each time. The chloroform extracts are mixed, filtered with chloroform moisturized filter paper, and evaporated to dryness while injecting air at room temperature. When the residue is dissolved with 2 mL of ethanol, and again evaporated to dryness, the melting point is 104~107 °C.

ii. When 2 mL of a solution of this product in water (1 → 1,000) is added with 1 mL of ethanol and 1 mL of ammonia test solution, shaken up, and added with 3 drops of ethyl cyanoacetate by dropping, the solution is purple-blue. When it is added with 1 mL of sodium hydroxide solution (1 → 3), the color of the solution turns green and then brownish yellow.

iii. When 2 mL of a solution of this product in water (1 → 25) is added with 2~3 drops of dilute hydrochloric acid and heated, the generated gas changes water-moisturized potassium iodide-starch paper to blue.

Purity test:

i. Heavy metals 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

ii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

iii. 2-methyl-1, 4-naphthohydroquinone-3-sulfonate: When 0.10 g (0.095~0.104 g) of this product is dissolved with 5 mL of water, and added with 2 drops of *o*-phenanthroline test solution, precipitation shall not be generated.

Water content: 10.0~16.5 % (0.2 g)

Assay: Approximately 0.3 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 20 mL of water, added with 20 mL of chloroform, shaken up by adding 5 mL of sodium carbonate solution (1 → 2) little by little, and then more vigorously shaken up. The chloroform layer is fractionated. The aqueous layer is extracted twice with 20 mL of chloroform each time. The mixture of all chloroform extracts is washed with 10 mL of water, filtered with pre-chloroform-moisturized filter paper, and washed with chloroform. The filtrate and the washings are mixed and transferred to a 150 mL flask, and the chloroform is evaporated on a water bath. Then, it is evaporated to dryness while injecting nitrogen at room temperature. The residue is dissolved with 20 mL of glacial acetic acid and 15 mL of dilute hydrochloric acid, added with 3 g (2.5~3.4 g) of zinc powder, attached with a Bunsen valve, repeatedly shaken up, allowed to stand in a dark place for an hour, and filtered using a cotton plug. The flask is washed three times with 20 mL of newly boiled and cooled water each time. The filtrate and the washings are mixed and titrated with 0.1 mol/L ceric ammonium sulfate solution within 30 seconds (indicator: 2 drops of *o*-phenanthroline test solution). A blank test is performed in the same manner and corrections are made.

0.1 mol/L ceric ammonium sulfate solution 1 mL = 13.81 mg $C_{11}H_8O_2 \cdot NaHSO_3$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of menadione sodium bisulfite are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of menadione sodium bisulfite is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of menadione sodium bisulfite and sodium bisulfite, fillers.

Content: When this product is determined, it contains menadione dimethylpymidinol bisulfite ($C_{11}H_8O_2 \cdot NaHSO_3 \cdot 3H_2O$) corresponding to 90~130 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount containing 0.1 g of the raw material for manufacturing of menadione sodium bisulfite is weighed, added with 100 mL of water, shaken up, and filtered. 2 mL of the filtrate is added with 1 mL of ethanol and 1 mL of ammonia test solution, and shaken up. The confirmation test ii. for the raw material for manufacturing of menadione sodium bisulfite is hereinafter applied mutatis mutandis.

Assay: Method No. 1 is used for a preparation containing hydrogenated oils and Method No. 2 is used for the others.

Method No. 1: The amount of this product containing approximately 0.1 g of menadione sodium bisulfite ($C_{11}H_8O_2 \cdot NaHSO_3 \cdot 3H_2O$) is weighed to three significant digits and the value is recorded. It is placed in a separatory funnel, added with 30 mL of water and 30 mL of n-hexane, and vigorously shaken up, and then the aqueous layer is filtered. The residue on the separatory funnel and the filter paper is washed twice with 10 mL of water each time, and the filtrate and the washings are mixed. The rest of the n-hexane layer is added with 20 mL of water and shaken up, and the aqueous layer is filtered. The residue on the filter paper is washed with 3 mL of water. The filtrate and the washings are mixed in the previously mixture of the filtrated and washings. The resulting solution is transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a sample solution. Separately, approximately 0.1 g of menadione sodium bisulfite is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask, and added with more water to the graduation line to make 100 mL. This is used as a standard solution. 5 mL each of the sample solutions is measured into 100 mL brown volumetric flasks T and T′, added with 80 mL of water each, mixed well, added with 2 mL of a mixed solution of ammonia and isopropanol (1:1), and shaken up. The resulting solution in T is, within 30 seconds, added with 4 mL of a solution of ethyl cyanoacetate in isopropanol (3 → 20), shaken up, and then within 30 seconds, added with water to the graduation line to make 100 mL. The resulting solution in T′ is, within 30 seconds, added with water to the graduation line to make 100 mL. Separately, 5 mL each of the standard solutions is measured into 100 mL brown volumetric flasks S and S′ using a volumetric pipette, and the same procedure as for the sample solution is performed. They are allowed to stand for 5 minutes. For

each solution, absorbances, A_T, $A_{T'}$, A_S, and $A_{S'}$ at the maximum wavelength around 565 nm are measured using water as a control.

Amount of menadione sodium bisulfite ($C_{11}H_8O_2 \cdot NaHSO_3 \cdot 3H_2O$) (mg)

= Amount of menadione sodium bisulfite for assay ($C_{11}H_8O_2 \cdot NaHSO_3$)

$$\times \frac{A_T - A_{T'}}{A_S - A_{S'}} \times 1.1957$$

Method No. 2: The amount of this product containing approximately 0.1 g of menadione sodium bisulfite ($C_{11}H_8O_2 \cdot NaHSO_3 \cdot 3H_2O$) is weighed to three significant digits and the value is recorded. It is added with 60 mL of water, vigorously shaken up, and filtered. The residue on the filter paper is washed twice with 10 mL of water each time. The filtrate and the washings are mixed, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a sample solution and Method No. 1 is hereinafter applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(90) Potassium Iodide

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 4-hour drying at 105 °C, it contains 99.0 % or more of potassium iodide (KI).

Physical and chemical properties:

i. This product is colorless or white crystals or white crystalline powder with no odor.

ii. This product is easy to extremely dissolve in water, easy to dissolve in glycerin, and easy to slightly dissolve in ethanol.

iii. The pH of a solution of this product in water (1 → 10) is 7.0~9.0.

iv. This slightly deliquesces in moist air.

Confirmation test: A solution of this product in water (1 → 20) gives the qualitative reactions of potassium salt and iodide.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 2 mL of water. The resulting solution shall be colorless and clear.

ii. Heavy metals: 2.0 g (1.95~2.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using

these solutions, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

iii. Arsenic: 0.40 g of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (5 mg/kg or less).

Loss on drying: Less than 1.0 % (2 g, 105 °C, 4 hours)

Assay: This product is dried at 105 °C for 4 hours, approximately 0.5 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is placed in a stoppered flask, dissolved with 10 mL of water, added with 35 mL of hydrochloric acid and 5 mL of chloroform, and titrated with 0.05 mol/L potassium iodate solution while shaking until the reddish violet color of the chloroform layer disappears. In this case, the end of titration is the time when the reddish violet color does not appear again within 5 minutes after the chloroform layer is decolorized.

0.05 mol/L potassium iodate solution 1 mL = 16.60 mg KI

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of potassium iodide are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of potassium iodide is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of potassium iodide and fillers.

Content: When this product is determined, it contains potassium iodide (KI) corresponding to 90~110 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of potassium iodide is weighed, added with 10 mL of water, shaken up well, and filtered. The confirmation test for the raw materials for manufacturing of potassium iodide is applied to the filtrate mutatis mutandis.

Assay: The amount of this product containing approximately 0.5 g of potassium iodide (KI) is weighed to three significant digits and the value is recorded. It is added with 30 mL of water, shaken up well, and filtered. The residue on the filter paper is washed twice with 10 mL of water each time. The filtrate and the washings are mixed and Assay for the raw material for manufacturing of potassium iodide is applied mutatis mutandis.

0.05 mol/L potassium iodide 1 mL = 16.60 mg KI

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of potassium iodide is applied mutatis mutandis.

(91) Folic Acid

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 98.0~102.0 % or more of folic acid ($C_{19}H_{19}N_7O_6$) at dehydration product conversion.

Physical and chemical properties:

i. This product is yellow to orange-yellow crystalline powder with no odor.

ii. This product hardly dissolves in water, ethanol, and other organic solvents.

iii. This product dissolves in dilute sodium hydroxide test solution, sodium carbonate solution (1 → 100), hydrochloric acid, and sulfuric acid. The solution is yellow.

iv. This product gradually changes by light.

Confirmation test:

i. 1.5 mg (1.45~1.54 mg) of this product is dissolved with dilute sodium hydroxide test solution to make 100 mL. This is used as a sample solution. In the measurement of the absorption spectrum of this solution, the absorption maximum is at the wavelengths of 255~257 nm, 281~285 nm, and 361~369 nm. When the absorbances at the absorption maximum of 255~257 nm and 361~369 nm are A_1 and A_2, respectively, A_1/A_2 is 2.80~3.00.

ii. When 10 mL of the sample solution in i. is added with a drop of potassium permanganate test solution, shaken up until the solution turns blue, and observed under ultraviolet light within 30 seconds, it emits blue fluorescence.

Purity test:

i. Clarity and color of solution: 0.10 g (0.095~0.104 g) of this product is dissolved with 10 mL of dilute sodium hydroxide test solution. The resulting solution shall be yellow and clear.

ii. Free amine: *p*-aminobenzoyl glutamic acid reference standard is dried in a desiccator (reduced pressure, silica gel) for 4 hours, approximately 0.05 g of it is weighed to the digits of 0.1 mg, and the value is recorded. It is dissolved with absolute ethanol (2 → 5), transferred to a 100 mL volumetric flask, and added with more absolute ethanol (2 → 5) to the graduation line to make 100 mL. Then 3 mL of this solution is measured using a volumetric pipette, transferred to a 1 L volumetric flask, added with water to the graduation line to make 1 L. 4 mL of this solution is measured using a volumetric pipette, and the absorbance $A_{S'}$ of this solution is measured in the same procedures as for S_2 solution in the assay. Based on $A_{S'}$ and A_C obtained by the assay when the amount of free amine is calculated by the following equation, it shall be 1.0 % or less.

$$\text{Amount of free amine } (\%) = \frac{A_C}{A_{S'}} \times \frac{W'}{W}$$

W: Amount of the sample collected by the assay, which is converted into dehydration product (mg)

W′: Amount of *p*-aminobenzoyl glutamic acid reference standard (mg)

Water content: 5 mL of pyridine for Karl Fischer and 20 mL of methanol for Karl Fischer are put in a dry titration flask, and titrated with Karl Fischer test solution by the end. Then, approximately 0.2 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is placed in a titration flask within 30 seconds, added with a certain volume of the excess Karl Fischer test solution, stirred for 30 minutes, and tested. The water content shall be 8.5 % or less.

Ignition residue: Less than 0.5 % (1 g)

Assay: Approximately 0.05 each of this product and folic acid reference standard (whose water content was previously measured in the same manner for this product) is weighed to the digits of 0.1 mg and the value is recorded. Each of them is added with 50 mL of dilute sodium hydroxide test solution, dissolved by shaking well, transferred to a 100 mL volumetric flask, added with more dilute sodium hydroxide test solution to the graduation line to make 100 mL, and called T_1 and S_1 solutions, respectively. 30 mL each of T_1 and S_1 solutions is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with 20 mL of dilute hydrochloric acid and water to the graduation line to make 100 mL. 60 mL each is added with 0.5 g (0.45~0.54 g) of zinc powder, repeatedly shaken up, and allowed to stand for 20 minutes. Then, each solution is filtered with dry filter paper. 10 ml of the first filtrate is removed, and 10 mL of the next filtrate is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. They are called T_2 and S_2 solutions, respectively. 4 mL each of T_2 and S_2 solutions, is measured using a volumetric pipette,

added with 1 mL of water, 1 mL of dilute hydrochloric acid and 1 mL of sodium nitrite solution (1 → 1,000), mixed well, allowed to stand for 2 minutes, added with 1 mL of ammonium sulfamate (1 → 200), shaken up well, and allowed to stand for 2 minutes. Each solution is transferred to a 20 mL volumetric flask, added with 1 mL of N-(1-naphthyl)-N′-diethylethylenediamine oxalate (1 → 1,000), shaken up, allowed to stand for 10 minutes, added with water to the graduation line to make 20 mL, and called T_3 and S_3 solutions, respectively. Separately, 30 mL of the T_1 solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with 20 mL of dilute hydrochloric acid and water to the graduation line to make 100 mL. 4 mL of this solution is measured using a volumetric pipette and subjected to the same procedure as for preparing T_3 solution from T_2 solution. The resulting solution is called C solution. Separately, 4 mL of water is measured and subjected to the same procedure as for preparing T_3 solution from T_2 solution. This is used as a control solution. The absorbances of A_T, A_S, and A_C at a wavelength 550 nm are measured for T_3, S_3, and C solutions. $A_{T'}$ is one-tenth of A_C reduced from A_T.

Amount of folic acid ($C_{19}H_{19}N_7O_6$) (mg)

$$= \text{Amount of folic acid converted into dehydration product (mg)} \times \frac{A_{T'}}{A_S}$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of folic acid are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of folic acid is applied mutatis mutandis.

C. Preparation (part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of folic acid and fillers.

Content: When this product is determined, it contains folic acid ($C_{19}H_{19}N_7O_6$) corresponding to 90~115 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount containing 1.5 mg of the raw material for manufacturing of folic acid is weighed, added with 100 mL of dilute sodium hydroxide test solution for a preparation containing

hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats added with 30 mL of chloroform, shaken up, and allowed to stand. The aqueous layer is filtered. The first filtrate of 10 mL is discarded, and the next filtrate is used as a sample solution. The confirmation tests i. and ii. for the raw material for manufacturing of folic acid are hereinafter applied mutatis mutandis.

Assay: Method No. 1 is used for a preparation containing hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats and Method No. 2 is used for the others.

Method No. 1: The amount of this product containing approximately 0.05 g of folic acid ($C_{19}H_{19}N_7O_6$) is weighed to three significant digits and the value is recorded. It is added with 50 mL of chloroform, shaken up, and filtered using a glass filter (G4). The residue on the filter is washed three times with 20 mL of chloroform each time. When the chloroform odor on the residue disappears, the residue is filtered by extracting with approximately 90 mL of dilute sodium hydroxide test solution and the filtrate is added with dilute sodium hydroxide test solution to make 100 mL. This is used as a T_1 solution. Separately, approximately 0.05 g of folic acid reference standard (whose water content was previously measured in the same manner for the raw material for manufacturing of folic acid) is weighed to the digits of 0.1 mg, and the value is recorded. It is dissolved with dilute sodium hydroxide test solution, transferred to a 100 mL volumetric flask, and added with more dilute sodium hydroxide test solution to the graduation line to make 100 mL. This is used as a S_1 solution. 30 mL each of T_1 and S_1 solutions is measured using a volumetric pipette. The assay for the raw material for manufacturing of folic acid is hereinafter applied mutatis mutandis.

Method No. 2: The amount of this product containing approximately 0.05 g of folic acid ($C_{19}H_{19}N_7O_6$) is weighed to three significant digits and the value is recorded. It is added with 50 mL of dilute sodium hydroxide test solution, frequently shaken up, filtered into a 100 mL volumetric flask, and washed with dilute sodium hydroxide test solution. The filtrate and the washings are mixed and added with more dilute sodium hydroxide test solution to make 100 mL. This is used as a T_1 solution and Method No. 1 is hereinafter applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(92) Potassium Iodate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 99.0 % or more of potassium iodate (KIO_3).

Physical and chemical properties:

i. This product is white crystals or crystalline powder with no odor.

ii. This product is easy to dissolve in water but hard to dissolve in ethanol.

iii. The pH of a solution of this product in water (1 → 20) is 7.0~9.0

Confirmation test:

i. When 1 mL of a solution of this product in water (1 → 20) is added with a drop of starch test solution and 2 drops of hypophosphoric acid test solution (1 → 5), the resulting solution is blue.

ii. A solution of this product in water (1 → 20) gives the qualitative reactions i. of potassium salt.

Purity test:

i. Clarity and color of solution: When 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of water, the resulting solution shall be colorless and clear.

ii. Chlorate: When 1.0 g (0.95~1.04 g) of this product is placed in a porcelain dish, added with 2 mL of sulfuric acid, and allowed to stand for 10 minutes, chlorine odor or gas shall not be generated.

iii. Heavy metal: 2.0 g (1.95~2.04 g) of this product is placed in a porcelain pot, added with 3 mL of hydrochloric acid (1 → 2), evaporated to dryness on a sand bath, added with 3 mL of hydrochloric acid (1 → 2), and again evaporated to dryness on a sand bath. Then, the same procedure is performed three times using 3 mL of hydrochloric acid each time (1 → 2). The residue is dissolved with 10 mL of water, added with a drop of phenolphthalein test solution, added with ammonia test solution by dropping until the solution turns slightly red, added with 2 mL of diluted acetic acid, and added with water to make 50 mL. This is used as a sample solution. 2.0 mL of lead standard solution is added with 3 mL of hydrochloric acid (1 → 2), evaporated to dryness on a sand bath, and subjected to the same procedure for preparation of the sample solution. This is used as a control solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg and less).

iv. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed and a sample solution is prepared by Method No. 2 of the arsenic test method. The arsenic test is performed by

the method using device A. The color of absorbing solution shall not be darker than the standard color (5 mg/kg or less).

Assay: Approximately 1.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with 50 mL of water, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a stoppered flask, added with 30 mL of water, 3 g (2.5~3.4 g) of potassium iodide and 5 mL of sulfuric acid (1 → 5), tightly stoppered within 30 seconds, allowed to stand in a dark place for 5 minutes, and titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 1 mL of starch test solution). A blank test is performed in the same manner and corrections are made.

0.1 mol/L sodium thiosulfate solution 1 mL = 3.567 mg KIO_3

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of potassium iodate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of potassium iodate is applied mutatis mutandis.

(93) Calcium Iodate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 95.0 % or more of calcium iodate [$Ca(IO_3)_2$].

Physical and chemical properties:

i. This product is white crystals or crystalline powder with no odor or a slightly specific odor.

ii. This product is hard to dissolve in water and ethanol.

Confirmation test:

i. When 5 mL of a saturated solution of this product in water is added with a drop of starch test solution and 2 drops of hypophosphoric acid test solution (1 → 5), the resulting solution is blue.

ii. Flame coloration test for this product moisturized with hydrochloric gives red color.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is added with 50 mL of 1 mol/L hydrochloric acid test solution, and dissolved on a water bath by warming. The resulting solution shall be colorless to pale yellow and clear.

ii. Chlorate: The purity test ii. for the raw material for manufacturing of potassium iodate is applied mutatis mutandis.

iii. Heavy metal: The purity test iii. for the raw material for manufacturing of potassium iodate is applied mutatis mutandis (10 mg/kg or less).

iv. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed, added with 5 mL of chlorine (2 → 3), evaporated to dryness on a water bath, added with 5 ml of hydrochloric acid (2 → 3), and again evaporated to dryness on a water bath. This procedure is repeated until the color of iodine disappears, and then it is added with 5 mL of water, and dissolved by heating on a water bath to prepare a sample solution. Arsenic is tested by the method using device A, the color of this sample solution shall not be darker than that obtained when the same procedure is performed without using this product, and 2.0 mL of arsenic standard solution is added and thereinafter the same procedure as for the sample solution is performed (5 mg/kg or less).

Assay: 0.1 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 30 mL of water, 3 g (2.5~3.4 g) of potassium iodide, and 5 mL of hydrochloric acid (1 → 9), and titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 1 mL of starch test solution). A blank test is performed in the same manner and corrections are made.

0.1 mol/L sodium thiosulfate solution 1 mL = 3.249 mg Ca $(IO_3)_2$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of calcium iodate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium iodate is applied mutatis mutandis.

(94) Riboflavin

Riboflavin (part 1)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 2-hour drying at 105 °C, it contains 96.0 % or more of riboflavin ($C_{17}H_{20}N_4O_6$).

Physical and chemical properties:

i. This product is yellow to orange-yellow crystals with a slightly specific odor.

ii. This product is hard to extremely dissolve in water and hardly dissolves in ethanol, ether, or chloroform.

iii. This product dissolves in 1 mol/L sodium hydroxide test solution.

iv. A saturated solution of this product is neutral.

v. This product decomposes by light.

vi. Melting point: Approximately 280 °C (dissolution).

Confirmation test:

i. A solution of this product in water (1 → 100,000) is pale yellow-green and emits strong yellow-green fluorescence. When 5 mL of this product is added with 0.02 g (0.015~0.024 g) of hydrosulfite sodium, the color of the solution and the fluorescence disappear, but when it is shaken up in air they gradually reappear. The fluorescence of the solution disappears when dilute hydrochloric acid or 1 mol/L sodium hydroxide test solution is added by dropping.

ii. When 10 mL of a solution of this product in water (1 → 100,000) is put in a stoppered test tube, added with 1 mL of 1 mol/L sodium hydroxide test solution, irradiated with 10~30 watts of fluorescent light at a distance of 20 cm at 20~40 °C for 30 minutes, added with 0.5 mL of acetic acid to make it acidic, added with 5 mL of chloroform, and shaken up well, the chloroform layer emits yellow-green fluorescence.

iii. In the measurement of the absorption spectrum of a solution of this product in pH 7.0 phosphate buffer (1 → 100,000), the absorption maximum is at the wavelengths of 265~267 nm, 372~374 nm, and 444~446 nm. When the absorbances are called A_1, A_2, and A_3, respectively, A_2/A_1 and A_3/A_1 are 0.314~0.333 and 0.364~0.388, respectively.

Purity test:

i. Specific rotation: This product is dried at 105 °C for 2 hours, approximately 0.1 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is added with 4 mL of dilute sodium hydroxide test solution using a volumetric pipette to dissolve, added with 10 mL of newly boiled and cooled water, added with 4 mL of non-aldehyde ethanol using a volumetric pipette while shaking well, transferred to a 20 mL

volumetric flask, and added with newly boiled and cooled water to the graduation line to make 20 mL. When rotation of this solution is measured within 30 minutes, it shall be $[\alpha]_D^{20} = -120 \sim -140°$.

ii. Lumiflavin: 0.025 g (0.0245~0.0254 g) of this product is added with 10 mL of chloroform without ethanol, shaken up for 5 minutes, and filtered. The color of this filtrate shall not be darker than that of a solution in which 4.0 mL of 0.017 mol/L potassium dichromate is added with water to make 1 L.

Loss on drying: Less than 1.5 % (0.5 g, 105 °C, 2 hours)

Ignition residue: Less than 0.30 % (1 g)

Assay: The procedure is performed using a lightproof container away from direct sunlight. This product is dried at 105 °C for 2 hours, approximately 0.015 g of it is weighed to the digits of 0.1 mg, and the value is recorded. It is added with 800 mL of glacial acetic acid (1 → 400), dissolved by warming, allowed to stand, transferred to a 1 L volumetric flask, and added with water to the graduation line to make 1 L. This is used as a sample solution. Separately, riboflavin reference standard is dried at 105 °C for 2 hours, approximately 0.015 of it is weighed to the digits of 0.1 mg, and the value is recorded. It is transferred to a 1 L volumetric flask, added with 800 mL of glacial acetic acid (1 → 400), dissolved by warming, allowed to stand, and added with water to the graduation line to 1 L. This is used as a standard solution. The absorbances of A_T and A_S of the sample and standard solutions, respectively, at a wavelength of 445 nm are measured using water as a control solution. Then, 5 mL each of these solutions is added with 0.02 g (0.015~0.024 g) of sodium hydrosulfite and shaken up to be decolorized, and the absorbances of $A_{T'}$ and $A_{S'}$ of these solutions are measured within 30 seconds.

Amount of riboflavin ($C_{17}H_{20}N_4O_6$) (mg)

$$= \text{Amount of riboflavin reference standard (mg)} \times \frac{A_T - A_{T'}}{A_S - A_{S'}}$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of riboflavin (part 1) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of riboflavin (part 1) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of riboflavin (part 1) and fillers.

Content: When this product is determined, it contains riboflavin ($C_{17}H_{20}N_4O_6$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 1 mg of the raw material for manufacturing of riboflavin (part 1) is weighed, added with 100 mL of water, shaken up, and filtered. To the filtrate, the confirmation test i. for the raw material for manufacturing of riboflavin (part 1) is hereinafter applied mutatis mutandis.

ii. 10 mL of the filtrate in i. is put in a stoppered test tube, the confirmation test ii. for the raw material for manufacturing of riboflavin (part 1) is hereinafter applied mutatis mutandis.

Assay: The procedure is performed using a lightproof container away from direct sunlight. The amount of this product containing approximately 0.015 g of riboflavin ($C_{17}H_{20}N_4O_6$) is weighed to three significant digits and the value is recorded. It is added with 800 mL of glacial acetic acid (1 → 400), dissolved by warming, allowed to stand, transferred to a 1 L volumetric flask, added with water to the graduation line to make 1 L, and filtered with dry filter paper. This is used as a sample solution. Separately, riboflavin reference standard is dried at 105 °C for 2 hours, and the assay for the raw material for manufacturing of riboflavin (part 1) is hereinafter applied mutatis mutandis.

Amount of riboflavin ($C_{17}H_{20}N_4O_6$) (mg)

$$= \text{Amount of riboflavin reference standard (mg)} \times \frac{A_T - A_{T'}}{A_S - A_{S'}}$$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

Riboflavin (part 2)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 3-hour drying at 105 °C, it contains 80.0 % or more of riboflavin ($C_{17}H_{20}N_4O_6$).

Physical and chemical properties:

i. This product is orange-yellow to yellow-brown fine particles with a specific odor.

ii. This product is hard to extremely dissolve in water and hardly dissolves in ethanol, ether, or chloroform.

iii. This product decomposes by light.

Confirmation test:

i. The confirmation test i. for the raw material for manufacturing of riboflavin (part 1) is applied mutatis mutandis.

ii. The confirmation test ii. for the raw material for manufacturing of riboflavin (part 1) is applied mutatis mutandis.

iii. In the measurement of the absorption spectrum of a solution of this product in pH 7.0 phosphate buffer (1 → 100,000), the absorption maximum is at the wavelengths of 264~268 nm, 371~375 nm, and 443~447 nm. When the absorbances are called A_1, A_2, and A_3, respectively, A_2/A_1 and A_3/A_1 are 0.310~0.333 and 0.364~0.388, respectively.

Purity test:

i. Specific rotation: This product is dried at 105 °C for 3 hours, approximately 0.1 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is added with 4 mL of dilute sodium hydroxide test solution using a volumetric pipette to dissolve, added with 10 mL of newly boiled and cooled water, added with 4 mL of non-aldehyde ethanol using a volumetric pipette while shaking well, transferred to a 20 mL volumetric flask, added with newly boiled and cooled water to the graduation line to make 20 mL, and centrifuged. When rotation of the supernatant liquid is measured within 30 minutes, it shall be $[\alpha]_D^{20} = -100 \sim -120°$.

ii. Lumiflavin: The purity test ii. for the raw material for manufacturing of riboflavin (part 1) is applied mutatis mutandis.

iii. Heavy metals: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: Less than 5.0 % (1 g, 105 °C, 3 hours)

Ignition residue: Less than 3.0 % (1 g)

Assay: The procedure is performed using a lightproof container away from direct sunlight. This product is dried at 105 °C for 3 hours, approximately 0.05 g of it is weighed to the

digits of 0.1 mg, and the value is recorded. It is added with 500 mL of 10 w/v% saccharin sodium solution, stirred for 30 minutes, and centrifuges as appropriate. This is used as a sample stock solution. 10 mL of the sample stock solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, added with water to the graduation line to make 100 mL, and filtered with a 0.45 μm membrane filter. This is used as a sample solution. 20 μL of this solution is tested by the liquid chromatography under the following conditions. The peak height or area of riboflavin is measured from the obtained chromatogram, the concentration of riboflavin is determined from a calibration curve separately obtained, and the content is calculated.

Operating conditions:

Detector: Fluorescence detector (excitation wavelength: 445 nm, fluorescence wavelength: 530 nm)

Column: A stainless tube (inner diameter: approx. 4.0 mm, length: approx. 300 mm) is filled with 10 μm or less of octadecyl-silylated silica gel.

Column temperature: 40 °C

Mobile phase: Water / methanol (7:3)

Flow rate: 0.8 mL/min.

Preparation of calibration curve:Riboflavin reference standard is dried at 105 °C for 3 hours, approximately 25 mg of it is weighed to the digits of 0.1 mg, and the value is recorded. It is dissolved with 10 w/v% saccharin sodium solution, transferred to a 250 mL brown stoppered Erlenmeyer flask, and added with the same solution to the graduation line to prepare a standard stock solution of riboflavin (1 mL of this solution contains 0.1 mg of riboflavin ($C_{17}H_{20}N_4O_6$)). In the use, a certain volume of the standard stock solution is accurately diluted with water to prepare the solutions containing 5, 7.5, and 10 μg/mL. Each solution is filtered with a 0.45 μm membrane filter to prepare a standard solution. 20 μL each of the standard solutions is hereinafter tested by the liquid chromatography as is the case in the sample solution. The peak height or area of riboflavin is measured from the obtained chromatogram and a calibration curve is prepared.

(b) Standard of manufacturing method

For manufacturing, the riboflavin producing strain of *Ashbya gossypii* is aerobically cultured.After the cultivation, the bacterial cells are heat-treated to separate riboflavin, and dried.

(c) Standard of storage method

It shall be stored in a lightproof airtight container.

(d) Standard of the label

The following words shall be described on the immediate container or the immediate wrapper of this product.

Riboflavin (Feed grade)

B. Preparation

(a) Compositional standards

The compositional standard of the raw material for manufacturing of riboflavin (part 2) is applied mutatis mutandis.

(b) Standard of manufacturing method

The standard of manufacturing method of the raw material for manufacturing of riboflavin (part 2) is applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of riboflavin (part 2) is applied mutatis mutandis.

(d) Standard of the label

The standard of the label of the raw material for manufacturing of riboflavin (part 2) is applied mutatis mutandis.

Riboflavin (part 3)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 2-hour drying at 105 °C, it contains 96.0 % or more of riboflavin ($C_{17}H_{20}N_4O_6$).

Physical and chemical properties:

i. This product is yellow to orange-yellow crystals with a slightly specific odor.

ii. This product is hard to extremely dissolve in water and hardly dissolves in ethanol, ether, or chloroform.

iii. This product dissolves in 1 mol/L sodium hydroxide test solution.

iv. A saturated solution of this product is neutral.

v. This product decomposes by light.

vi. Melting point: Approximately 290 °C (dissolution)

Confirmation test: The confirmation test of the raw material for manufacturing of riboflavin (part 1) is applied mutatis mutandis.

Purity test: The purity test of the raw material for manufacturing of riboflavin (part 1) is applied mutatis mutandis.

Loss on drying: Less than 1.5 % (0.5 g, 105 °C, 2 hours)

Ignition residue: Less than 0.30 % (1 g)

Assay: The assay of the raw material for manufacturing of riboflavin (part 1) is applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the riboflavin producing recombinant, whose host is the strain belonging to *Bacillus subtilis*, is aerobically cultured. After the cultivation, the bacterial cells are heat-treated to separate crude crystal fractions of riboflavin. Then the crude crystals are purified and the obtained solid is dried.

(c) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation (part 1)

(a) Compositional standards

The compositional standard of the raw material for manufacturing of riboflavin (part 3) is applied mutatis mutandis.

(b) Standard of manufacturing method

The standard of manufacturing method of the raw material for manufacturing of riboflavin (part 3) is applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of riboflavin (part 3) is applied mutatis mutandis.

C. Preparation (part 2)

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of riboflavin (part 3) and fillers.

Content: When this product is determined, it contains riboflavin ($C_{17}H_{20}N_4O_6$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 1 mg of the raw material for manufacturing of riboflavin (part 3) is weighed, added with 100 mL of water, shaken up, and filtered. To the filtrate, the confirmation test i. for the raw material for manufacturing of riboflavin (part 1) is applied mutatis mutandis.

ii. 10 mL of the filtrate in i. is put in a stoppered test tube, and the confirmation test ii. for the raw material for manufacturing of riboflavin (part 1) is applied mutatis mutandis.

Assay: The procedure is performed using a lightproof container away from direct sunlight. The amount of this product containing approximately 0.015 g of riboflavin ($C_{17}H_{20}N_4O_6$) is weighed to three significant digits and the value is recorded. It is added with 800 mL of

glacial acetic acid (1 → 400), dissolved by warming, allowed to cool, transferred to a 1 L volumetric flask, added with water to the graduation line to make 1 L, and filtered with dry filter paper. This is used as a sample solution. Separately, riboflavin reference standard is dried at 105 °C for 2 hours, and the assay for the raw material for manufacturing of riboflavin (part 1) is hereinafter applied mutatis mutandis.

Amount of riboflavin ($C_{17}H_{20}N_4O_6$) (mg)

$$= \text{Amount of riboflavin reference standard (mg)} \times \frac{A_T - A_{T'}}{A_S - A_{S'}}$$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(95) Riboflavin Butyrate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried in a desiccator (reduced pressure, silica gel) for 4 hours and determined, it contains 97.0~102.0 % of riboflavin butyrate ($C_{33}H_{44}N_4O_{10}$).

Physical and chemical properties:

i. This product is yellow-orange crystals or crystalline powder with a slight specific odor.

ii. This product is easy to extremely dissolve in ethanol and chloroform and hardly dissolves in water.

iii. Melting point: Approximately 148 °C.

Confirmation test:

i. A solution of this product in ethanol (1 → 100,000) is pale yellow-green and emits strong yellow-green fluorescence. This fluorescence disappears when dilute hydrochloric acid or 1 mol/L sodium hydroxide test solution is added.

ii. 0.01 g (0.005~0.014 g) of this product is weighed, dissolved with 5 mL of ethanol, added with 2 mL of a mixed solution of equivalent volume of hydroxylamine hydrochloride solution (3 → 20) and sodium hydroxide solution (3 → 20), shaken up well, and added with 0.8 mL of hydrochloric acid, 0.5 mL of ferric chloride test solution, and 5 mL of ethanol. The resulting solution is dark red-brown.

Purity test:

i. Clarity and color of solution: 0.10 g (0.095~0.104 g) of this product is dissolved with 10 mL of chloroform. The resulting solution shall be clear.

ii. Ratio of absorbance: In the measurement of the absorption spectrum of a solution of this solution in ethanol (1.7 → 100,000), the absorption maximum is at the

wavelengths of 269~271 nm, 349~351 nm, and 444~446 nm. When the absorbances at the absorption maximums are called A_1, A_2, and A_3, respectively, A_1/A_2, A_1/A_3, and, A_2/A_3 shall be 3.50~3.90, 2.47~2.77, and 0.65~0.75, respectively.

Loss on drying: Less than 1.0 % (1 g, reduced pressure, silica gel, 4 hours)

Ignition residue: Less than 0.5 % (1 g)

Assay: This product is dried for 4 hours in a desiccator (reduced pressure, silica gel), approximately 0.04 g of it is weighed to the digits of 0.1 mg, and the value is recorded. It is dissolved with ethanol, transferred to a 500 mL volumetric flask, and added with ethanol to the graduation line to make 500 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, and added with ethanol to the graduation line to make 50 mL. This is used as a sample solution. Separately, riboflavin reference standard is dried at 105 °C for 3 hours, approximately 0.05 g of it is weighed to the digits of 0.1 mg, and the value is recorded. It is added with 4 mL of glacial acetic acid and 150 mL of water, dissolved by warming, allowed to cool, transferred to a 500 mL volumetric flask, and is added with water to the graduation line to make 500 mL. 5 mL of this solution is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, and added with ethanol to the graduation line to make 50 mL. This is used as a standard solution. The absorbances of A_T and A_S of the sample and standard solutions at a wavelength of 445 nm are measured, using ethanol as a control solution.

Amount of riboflavin butyrate ($C_{33}H_{44}N_4O_{10}$) (mg)

$$= \text{Amount of riboflavin reference standard (mg)} \times \frac{A_T}{A_S} \times 0.8725$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of riboflavin butyrate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of riboflavin butyrate is applied mutatis mutandis.

(96) Zinc Sulfate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 80.0 % or more of zinc sulfate (dry) ($ZnSO_4$).

Physical and chemical properties:

i. This product is white powder with no odor.

ii. This product is easy to dissolve in water, easy to slightly dissolve in glycerin, and hardly dissolves in ethanol.

iii. The pH of a solution of this product in water (1 → 20) is 4.5~6.5

iv. This product is hygroscopic.

Confirmation test: A solution of this product in water (1 → 20) gives the qualitative reactions of zinc salt and sulfate.

Purity test:

i. Clarity and color of solution: 0.25 g (0.245~0.254 g) of this product is dissolved with 5 mL of water and 1 mL of sulfuric acid (1 → 20). The resulting solution shall be clear.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is placed in a Nessler tube, dissolved with 10 mL of water, added with 20 mL of potassium cyanide test solution, shaken up well, added with 2 drops of sodium sulfide test solution, and after 5 minutes is observed from above using a white paper background. Then, the color of this solution shall not be darker than that of a solution prepared by the procedure in which 2.0 mL of lead standard solution is added with 8.0 mL of water and 20 mL of potassium cyanide test solution, shaken up well, and hereinafter subjected to the same procedures as for the sample solution (20 mg/kg or less).

iii. Arsenic: 0.20 g (0.195~0.204 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (10 mg/kg or less).

Assay: Approximately 0.2 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 25 mL of this solution is measured using a volumetric pipette, added with 100 mL of water and 2 mL of pH 10.7 ammonia-ammonium chloride buffer and titrated with 0.01 mol/L ethylene-diamine-tetraacetic acid disodium solution (indicator: 0.04 g (0.035~0.044 g) of eriochrome black T-sodium chloride indicator).

0.01 mol/L ethylene-diamine-tetraacetic acid disodium solution 1 mL

=1.614 mg $ZnSO_4$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of zinc sulfate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of zinc sulfate (dry) is applied mutatis mutandis.

C. Preparation (part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of zinc sulfate (dry) and fillers.

Content: When this product is determined, it contains zinc sulfate ($ZnSO_4$) corresponding to 90~110 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of zinc sulfate (dry) is weighed, added with 10 mL of water, shaken up well, and filtered. To the filtrate the confirmation test for the raw material for manufacturing of zinc sulfate (dry) is hereinafter applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.2 g of zinc sulfate ($ZnSO_4$) is weighed to three significant digits and the value is recorded. The assay for the raw material for manufacturing of zinc sulfate (dry) is hereinafter applied mutatis mutandis.

0.01 mol/L ethylene-diamine-tetraacetic acid disodium solution 1 mL

= 1.614 mg $ZnSO_4$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of zinc sulfate (dry) is applied mutatis mutandis.

(97) Zinc Sulfate (crystal)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 99.0~102.0 % of zinc sulfate ($ZnSO_4 \cdot 7H_2O$).

Physical and chemical properties:

i. This product is colorless crystals or white crystalline powder with no odor.

ii. This product is easy to extremely dissolve in water, easy to dissolve in glycerin, and hardly dissolves in ethanol.

iii. The pH of a solution of this product in water (1 → 20) is 3.5~6.0.

iv. This product effloresces in dry air.

Confirmation test: A solution of this product in water (1 → 20) gives the qualitative reactions of zinc salt and sulfate.

Purity test:

i. Clarity and color of solution: 0.25 g (0.245~0.254 g) of this product is dissolved with 5 mL of water. The resulting solution shall be clear.

ii. Heavy metal: The purity test ii. for the raw material for manufacturing of zinc sulfate (dry) is applied mutatis mutandis. In this case, “2.0 mL” and “8.0 mL” shall be replaced by “1.0 mL” and “9.0 mL” respectively (10 mg/kg or less).

iii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (5 mg/kg or less).

Assay: Approximately 0.4 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 25 mL of this solution is measured using a volumetric pipette, added with 100 mL of water and 2 mL of pH 10.7 ammonia-ammonium chloride buffer and titrated with 0.01 mol/L ethylene-diamine-tetraacetic acid disodium solution (indicator: 0.04 g (0.035~0.044 g) of eriochrome black T-sodium chloride indicator).

0.01 mol/L ethylene-diamine-tetraacetic acid disodium solution 1 mL

= 2.876 mg $ZnSO_4 \cdot 7H_2O$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of zinc sulfate (crystal) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of zinc sulfate (crystal) is applied mutatis mutandis.

(98) Zinc Sulfate Methionine

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 2-hour drying at 110 °C, it contains 20.0~23.5 % of zinc (Zn) and 38.0~44.0 % of methionine ($C_5H_{11}NO_2S$).

Physical and chemical properties:

i. This product is pale purple powder with a specific odor.

ii. This product is hard to dissolve in water and hardly dissolves in ethanol and .methanol

iii. The pH of a solution of this product in water (1 → 100) is 4.2~4.7.

Confirmation test:

i. When 10 mL of a solution of this product in dilute hydrochloric acid (1 → 10) is added with several drops of ammonium sulfide test solution or sodium sulfide test solution, gray-brown precipitation is generated. The precipitation is fractionated. It does not dissolve by adding diluted acetic acid but dissolves by adding dilute hydrochloric acid.

ii. When 10 mL of a solution of this product in dilute hydrochloric acid (1 → 10) is added with several drops of potassium ferrocyanide test solution, blue-white precipitation is generated. It does not dissolve when dilute hydrochloric acid is added to a part of it, but with the addition of 1 mol/L sodium hydroxide test solution, another part of it dissolves.

iii. A solution of this product in water (1 → 100) gives the qualitative reaction i. of sulfate.

iv. 0.5 g (0.45~0.54 g) of this product and 0.1 g (0.05~0.14 g) of the raw material for manufacturing of DL-methionine are weighed. Each of them is dissolved with 10 mL of water to prepare sample and standard solutions. 5 μL each of the sample and standard solutions is spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then it is developed approximately 10~15 cm with the developing solvent, a mixed solution of ethanol and water (63:37), and the thin layer is dried at 105 °C for 30 minutes. When it is evenly sprayed with a solution of ninhydrin in ethanol (1 → 200) and heated at 105 °C for 5 minutes, the spots obtained from the sample and standard solutions are purple, and their Rf values are equal.

v. 1 mg (0.5~1.4 mg) of this product is weighed. When the infrared absorption spectrum of this product is measured using the potassium bromide disk method of the infrared absorption spectroscopy, absorptions are observed at around 1,640 cm^{-1}, 1,490 cm^{-1}, 1,420 cm^{-1}, 1,340 cm^{-1}, 1,120 cm^{-1}, 1,060 cm^{-1}, 980 cm^{-1} and 610 cm^{-1}, but not observed at around 2,100 cm^{-1}.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and ashed by ignition at 500~600 °C. After cooling, it is added with 5 mL of hydrochloric acid (1 → 2) and evaporated to dryness on a water bath. The residue is added with 15 mL of water and 2 drops of hydrochloric acid (1 → 2) and heated on a water bath for 30 minutes. Then, it is added with a drop of phenolphthalein test solution, added with ammonia test solution by dropping until the solution turns slightly red, and added with 2 mL of acetic acid. It is added with 30 mL of potassium cyanide test solution, stirred, and filtered. The filtrate and the washings are transferred to a Nessler tube and added with water to make 50 mL. When it is added with 2 drops of sodium sulfide test solution and allowed to stand for 5 minutes, the color of this solution shall not be darker than that of a solution prepared by the procedure in which 3.0 mL of lead standard solution is added with 15 mL of water and 30 mL of potassium cyanide test solution, stirred well, added with 2 drops of sodium sulfide test solution and allowed to stand for 5 minutes (30 mg/kg or less).

ii. Arsenic: 0.67 g (0.665~0.674 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (3 mg/kg or less).

Loss on drying: Less than 15.0 % (1 g, 110 °C, 2 hours)

Assay

i. Zinc: This product is dried at 110 °C for 2 hours, approximately 0.2 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is placed in a 100 mL volumetric flask, dissolved with water, and added with more water to the graduation line to make 100 mL. 25 ml of this solution is measured using a volumetric pipette, added with 100 mL of water and 2 mL of pH 10.7 ammonia-ammonium chloride buffer and titrated with 0.05 mol/L ethylene-diamine-tetraacetic acid disodium solution (indicator: 0.04 g (0.035~0.044 g) of eriochrome black T-sodium chloride indicator).

0.05 mol/L ethylene-diamine-tetraacetic acid disodium solution 1 mL = 3.269 mg Zn

ii. Methionine: This product is dried at 110 °C for 2 hours, approximately 0.6 g of it is weighed to the digits of 0.001 g, and the value is recorded. The assay for the raw material for manufacturing of DL-methionine is hereinafter applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of zinc methionine sulfate and fillers.

Content: When this product is determined, it contains zinc (Zn) and methionine ($C_5H_{11}NO_2S$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.4 g of zinc (Zn) is weighed, added with 10 mL of dilute hydrochloric acid, shaken up well, and filtered. When the filtrate is added with several drops of ammonium sulfide or sodium sulfide test solution, gray precipitation is generated. The fractionated precipitation does not dissolve by adding diluted acetic acid but dissolves by adding dilute hydrochloric acid.

ii. According to the amount of this product on the label, the amount containing 0.4 g of zinc (Zn) is weighed, added with 10 mL of dilute hydrochloric acid, shaken up well, and filtered. When the filtrate is added with several drops of potassium ferrocyanide test solution, pale yellow-white precipitation is generated. The precipitation does not dissolve when dilute hydrochloric acid is added to a part of it, but with the addition of 1 mol/L sodium hydroxide test solution, another part of it dissolves.

iii. According to the amount of this product on the label, the amount containing 0.4 g of zinc (Zn) is weighed, added with 100 mL of water, stirred, and filtered. The filtrate gives the qualitative reaction i. of sulfate.

iv. According to the amount of this product on the label, the amount containing 0.1 g of methionine ($C_5H_{11}NO_2S$) is weighed. Separately, 0.1 g (0.05~0.14 g) of the raw material for manufacturing of methionine is weighed. Each of them is dissolved with 10 mL of water and filtered. Both filtrates are used as sample and standard solutions. 5 μL each of the sample and standard solutions is spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then it is developed approximately 10~15 cm with the developing solvent, a mixed solution of ethanol and water (63:37), and the thin layer is dried at 105 °C for approximately 30 minutes. When it is evenly sprayed with a solution of ninhydrin in ethanol (1 → 200) and heated at 105 °C for 5 minutes, the spots obtained from the sample and standard solutions are purple, and their Rf values are equal.

v. According to the amount of this product on the label, the amount containing 1 mg of the raw material for manufacturing of zinc sulfate methionine. When the infrared absorption spectrum of it is measured using the potassium bromide disk method of the

infrared absorption spectroscopy, absorptions are observed at around 1,640 cm^{-1}, but not observed at around 2,100 cm^{-1}.

Assay:

i. Zinc: The amount of this product containing approximately 0.02 g of zinc sulfate methionine is weighed to three significant digits and the value is recorded. It is added with hydrochloric acid (1 → 11), stirred for 30 minutes, filtered, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 5 mL of the filtrate is measured using a volumetric pipette, and added with 100 mL of hydrochloric acid (1 → 100) to make 100 mL. This is used as a sample solution. Separately, the same procedure is performed without adding this product to prepare a blank test solution. Then 10, 20, 30, 40 and 50 mL of lead standard solution are measured by a volumetric pipette and added with hydrochloric acid (1 → 100) to make 100 mL, and they are called the standard solutions 1, 2, 3, 4 and 5. The sample solution, the standard solutions 1, 2, 3, 4 and 5, and the blank test solution are determined by the atomic absorption spectrophotometry. A calibration curve is prepared from the absorbances of the standard solutions 1, 2, 3, 4 and 5, and the content of zinc in the sample is calculated.

Gas to be used: Flammable gas, acetylene
Combustion-supporting gas, air

Lamp: Zinc hollow cathode lamp

Wavelength: 213.9 nm

Content of zinc (%)

$$= \frac{\text{Concentration of zinc obtained from the calibration curve (μg/mL)} \times 200}{\text{Collected amount of the sample (mg)}}$$

ii. Methionine: The amount of this product containing approximately 0.03 g of methionine ($C_5H_{11}NO_2S$) is weighed to three significant digits and the value is recorded. It is added with 15 mL of a mixed solution of n-hexane and isopropanol (1:1), stirred to dissolve, added with 70 mL of hydrochloric acid (9 → 250), vigorously stirred and extracted. This solution is transferred to a separatory funnel while filtering with absorbent cotton and the container is washed with 5 mL of a mixed solution of n-hexane and isopropanol (1:1) and 15 mL of hydrochloric acid (9 → 250). The washings are poured into the absorbent cotton and these filtrates are mixed. The resulting solution is vigorously shaken up and the aqueous layer is transferred to a 100 mL volumetric flask and added with hydrochloric acid (9 → 250) to the graduation line to make 100 mL. 2 mL of this solution is measured using a volumetric pipette, added with 2 mL of hydrochloric acid (9 → 250) and 12 mL of water using a

volumetric pipette. This is used as a sample solution. Separately, 0.030 g of dry DL-methionine for assay is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with hydrochloric acid (9 → 250), transferred to a 100 mL volumetric flask, added with hydrochloric acid (9 → 250) to the graduation line to make 100 mL. 2 mL of this solution is measured using a volumetric pipette, added with 2 mL of hydrochloric acid (9 → 250) and 12 mL of water using a volumetric pipette. This is used as a standard solution. Separately, 2 mL of hydrochloric acid (9 → 250) is measured using a volumetric pipette, added with 6 mL of water using a volumetric pipette to prepare a blank test solution. Then, 2 mL each of the sample, standard, and blank test solutions is measured using a volumetric pipette, transferred to a 25 mL volumetric flask, added with 1 mL of newly prepared ninhydrin-ascorbic acid test solution and 1 mL of pyridine using a volumetric pipette, shaken up, heated in a water bath for 25 minutes, cooled with water, and added with water to the graduation line to make 25 mL. The blank test solution is used as a control solution, and the absorbances of A_T and A_S of sample and standard solutions at a wavelength 570 nm are measured.

Amount of methionine ($C_5H_{11}NO_2S$) (mg)

$$= \text{Amount of DL-methionine for assay (mg)} \times \frac{A_T}{A_S}$$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of zinc sulfate methionine is applied mutatis mutandis.

(99) Cobalt Sulfate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 87.0 % or more of cobalt sulfate ($CoSO_4$).

Physical and chemical properties:

i. This product is pale pink powder with no odor.

ii. This product gradually dissolves in water and is hard to slightly dissolve in ethanol.

Confirmation test:

i. When 5 mL of a solution of this product in water (1 → 200) is added with 0.5 mL of 1 mol/L sodium hydroxide test solution, blue precipitation is generated. It changes to brown by heating.

ii. A solution of this product in water (1 → 20) gives the qualitative reaction of sulfate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of dilute hydrochloric acid. The resulting solution shall be red and clear.

ii. Lead: 1.0 g (0.95~1.04 g) of this product is weighed, dissolved with nitric acid (1 → 150), transferred to a 20 mL volumetric flask, and added with more nitric acid (1 → 150) to the graduation line to make 20 mL. When lead is tested, using this solution as a sample solution, by the lead test method (Method No. 1 of the atomic absorption spectrophotometry), the amount of lead shall be 20 mg/kg or less.

iii. Arsenic: 2.0 g (1.95~2.04 g) of this product is weighed, dissolved with 50 mL of water and 5 mL of dilute hydrochloric acid, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette to prepare a sample solution. When arsenic is tested using the sample solution by the method using device B, the color of mercuric bromide paper shall not be darker than the standard color (10 mg/kg or less).

Assay: Appropriately 0.4 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is transferred to a 100 mL volumetric flask, dissolved with 5 mL of dilute hydrochloric acid and 20 mL of water, and added with more water to the graduation line to make 100 mL. 10 mL of this product is measured using a volumetric pipette, added with 100 mL of water, and titrated with 0.01 mol/L ethylenediaminetetraacetic acid disodium solution, while adjusting to pH 8.0 with ammonia test solution (indicator: 0.2 g (0.15~0.24 g) of murexide indicator).

0.01 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL

= 1.550 mg $CoSO_4$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of cobalt sulfate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of cobalt sulfate (dry) is applied mutatis mutandis.

(100) Cobalt Sulfate (crystal)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 98.0~103.0 % of cobalt sulfate ($CoSO_4 \cdot 7H_2O$).

Physical and chemical properties:

i. This product is glossy, dark red, transparent crystals or pink-white sandy crystals with no odor.

ii. This product is easy to dissolve in water and hardly dissolves in ethanol.

Confirmation test:

i. When 5 mL of a solution of this product in water (1 → 100) is added with 0.5 mL of 1 mol/L sodium hydroxide test solution, blue precipitation is generated. It changes to brown by heating.

ii. A solution of this product in water (1 → 10) gives the qualitative reaction of sulfate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) is dissolved with 20 mL of water. The resulting solution shall be red and clear.

ii. Lead: 2.0 g (1.95~2.04 g) of this product is weighed and dissolved with 50 mL of water and 5 mL of dilute hydrochloric acid, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 50 mL of this solution is measured using a volumetric pipette to prepare a sample solution. When lead is tested using the sample solution by the lead test method (dithizone method), the amount of lead shall be 10 mg/kg or less.

iii. Arsenic: 4.0 g (3.95~4.04 g) of this product is weighed, dissolved with 50 mL of water and 5 mL of dilute hydrochloric acid, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette to make a sample solution. When arsenic is tested using the sample solution by the method using device B, the color of mercuric bromide paper shall not be darker than the standard color (5 mg/kg or less).

Assay: Appropriately 0.5 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is transferred to a 100 mL volumetric flask, dissolved with 50 mL of water, and added with more water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette, added with 100 mL of water, and titrated with 0.01 mol/L ethylenediaminetetraacetic acid disodium solution, while adjusting to pH 8.0 with ammonia test solution (indicator: 0.2 g of murexide indicator).

0.01 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL

= 2.811 mg $CoSO_4 \cdot 7H_2O$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of cobalt sulfate (crystal) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of cobalt sulfate (crystal) is applied mutatis mutandis.

C. Preparation (part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of and cobalt sulfate (crystal) fillers.

Content: When this product is determined, it contains cobalt sulfate ($CoSO_4 \cdot 7H_2O$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of cobalt sulfate (crystal) is weighed, added with 100 mL of water, shaken up well, and filtered. To the filtrate the confirmation test i. for the raw material for manufacturing of cobalt sulfate (crystal) is applied mutatis mutandis.

ii. According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of cobalt sulfate (crystal) is weighed, added with 10 mL of water, shaken up well, and filtered. To the filtrate the confirmation test ii. for the raw material for manufacturing of cobalt sulfate (crystal) is applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.5 g of cobalt sulfate (crystal) ($CoSO_4 \cdot 7H_2O$) is weighed to three significant digits and the value is recorded. It is added with 20 mL of water, shaken up well, and filtered. The residue on the filter paper is washed four times with 10 mL of water each time. The filtrate and the washings are mixed, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. The assay for the raw material for manufacturing of cobalt sulfate (crystal) is applied mutatis mutandis.

0.01 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL

= 2.811 mg $CoSO_4 \cdot 7H_2O$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of cobalt sulfate (crystal) is applied mutatis mutandis.

(101) Ferrous Sulfate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 80.0 % or more of ferrous sulfate ($FeSO_4$).

Physical and chemical properties:

i. This product is grayish white powder with no odor.

ii. This product is easy to dissolve in newly boiled and cooled water, and hardly dissolves in ethanol.

iii. This product is hygroscopic.

Confirmation test: A solution of this product in water (1 → 50) gives the qualitative reactions of ferrous salt and sulfate.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is placed in a porcelain dish, dissolved with 5 mL of aqua regia, and evaporated to dryness on a water bath. The residue is dissolved with 5 mL of 6 mol/L hydrochloric acid test solution and transferred to a separatory funnel. The porcelain dish is washed twice with 5 mL each of 6 mol/L hydrochloric acid test solution and the washings are put in the separatory funnel. The mixed solution is shaken up twice with 40 mL of ether, then shaken up with 20 mL of ether, and allowed to stand and the separated ether layer is eliminated. The aqueous layer is dissolved with 0.08 g (0.075~0.084 g) of hydroxylamine hydrochloride, heated on a water bath for 10 minutes, allowed to cool, added with strong ammonia solution by adding to adjust the pH of the solution to 3 to 4, and added with water to make 50 mL. This is used as a sample solution. 4.0 mL of the lead standard solution is put in a porcelain dish, added with 5 mL of aqua regia, and subjected to the same procedure as for the sample solution. This is used as a control solution. When heavy metal is tested using the control solution, the color of the sample solution shall not be darker than that of the standard solution. However, if precipitation is generated when the pH of the sample solution is adjusted to 3~4, the pH is adjusted to 1.8~2.0, and the same test is hereinafter performed (40 mg/kg or less).

ii. Arsenic: 0.6 g (0.55~0.64 g) of this product is weighed and the sample solution is prepared by method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (3.3 mg/kg or less)

Loss on drying: Less than 2.0 % (5 g, 105 °C, 2 hours)

Assay: Approximately 0.4 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 20 mL of water and 20 mL of dilute sulfuric acid, added with 2 mL of phosphoric acid, and titrated with 0.02 mol/L potassium permanganate solution within 30 seconds.

0.02 mol/L potassium permanganate solution 1 mL = 15.19 mg $FeSO_4$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation (part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of ferrous sulfate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of ferrous sulfate (dry) is applied mutatis mutandis.

C. Preparation (part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of and ferrous sulfate (dry) fillers.

Content: When this product is determined, it contains ferrous sulfate ($FeSO_4$) corresponding to 90~110 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of ferrous sulfate (dry) is weighed, added with 20 mL of acetone and 30 mL of water, shaken up well, and filtered. To the filtrate the confirmation test for the raw material for manufacturing of ferrous sulfate (dry) is applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.4 g of ferrous sulfate ($FeSO_4$) is weighed to three significant digits and the value is recorded. It is added with 10 mL of acetone, 20 mL of water and 20 mL of dilute sulfuric acid, shaken up well, and filtered. The residue is shaken up three times with 10 mL of water each time, and filtered using the same filter paper. The filtrates are mixed, added with 2 mL of phosphoric acid,

and subjected to the assay for the raw material for manufacturing of ferrous sulfate (dry) is applied mutatis mutandis.

0.02 mol/L potassium permanganate solution 1 mL = 15.19 mg $FeSO_4$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of ferrous sulfate (dry) is applied mutatis mutandis.

(102) Copper Sulfate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 85.0 % or more of copper sulfate ($CuSO_4$).

Physical and chemical properties:

i. This product is blue-white crystalline powder with no odor.

ii. This product is easy to dissolve in water, hard to slightly dissolve in glycerin, and hardly dissolves in ethanol.

iii. The pH of a solution of this product in water (1 → 20) is 3.0~4.5.

iv. This product is hygroscopic.

Confirmation test: A solution of this product in water (1 → 10) gives the qualitative reactions of cuprate and sulfate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of water. The resulting solution shall be blue and clear.

ii. Lead: 1.0 g (0.95~1.04 g) of this product is weighed and added with water to make 20 mL. This is used as a sample solution. 1.0 mL of the lead standard solution is measured, added with water to make 20 mL. This is used as a standard solution. When lead is tested using these sample and standard solutions by the lead test method (Method No. 1 of the atomic absorption spectrophotometry), the amount of lead shall not be 10 mg/kg or less.

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed, dissolved with 20 mL of water, added with 4 mL of glacial acetic acid and 3 g (2.5~3.4 g) of potassium iodide, added with 20 mL of water, shaken up, allowed to stand for 5 minutes, filtered, and washed with 5 mL of water. The filtrate and the washings are mixed and concentrated by heating until the mixed solution reaches approximately 10 mL. After cooling, it is dissolved with 0.4 g (0.35~0.44 g) of ascorbic acid to prepare a sample solution. When

arsenic is tested using the sample solution by the method using device B, the color of the sample solution shall not be darker than that of a solution prepared in the procedure in which 30 mL of water is added with 4 mL of glacial acetic acid, 3 g (2.5~3.4 g) of potassium iodide and 5.0 mL of arsenic standard solution, allowed to stand for 5 minutes, and hereinafter subjected to the same procedure as for the sample solution (10 mg/kg or less).

Assay: Approximately 0.4 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 50 mL of water, and added with 4 mL of acetic acid and 3 g (2.5~3.4 g) of potassium iodide. Then, the released iodine is titrated with 0.1 mol/L sodium thiosulfate solution (indicator: 1 mL of starch test solution). A blank test is performed in the same manner and corrections are made.

0.1 mol/L sodium thiosulfate solution 1 mL = 15.96 mg $CuSO_4$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of copper sulfate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of copper sulfate (dry) is applied mutatis mutandis.

C. Preparation (part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of and copper sulfate (dry) fillers.

Content: When this product is determined, it contains copper sulfate ($CuSO_4$) corresponding to 90~110 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of copper sulfate (dry) is weighed, added with 10 mL of water, shaken up well, and filtered. To the filtrate the confirmation test for the raw material for manufacturing of copper sulfate (dry) is applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.4 g of copper sulfate ($CuSO_4$) is weighed to three significant digits and the value is recorded. It is dissolved with 50 mL of water, or dissolved with 20 mL of water as appropriate, then filtered and washed with water. The filtrate and the washings are mixed to make 50 mL, and the assay

for the raw material for manufacturing copper sulfate (dry) is hereinafter applied mutatis mutandis.

0.1 mol/L sodium thiosulfate solution 1 mL = 15.96 mg $CuSO_4$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of copper sulfate (dry) is applied mutatis mutandis.

(103) Copper Sulfate (crystal)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 98.5 % or more of copper sulfate ($CuSO_4 \cdot 5H_2O$).

Physical and chemical properties:

i. This product is blue crystals, blocks or powder with no odor.

ii. This product is easy to dissolve in water and glycerin and hard to dissolve in ethanol.

iii. The pH of a solution of this product in water (1 → 20) is 2.5~4.0.

iv. This product is efflorescent.

Confirmation test: A solution of this product in water (1 → 10) gives the qualitative reactions of cuprate and sulfate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of water. The resulting solution shall be blue and clear.

ii. Lead: 1.0 g (0.95~1.04 g) of this product is weighed and added with water to make 20 mL. This is used as a sample solution. 1.0 mL of the lead standard solution is measured, added with water to make 20 mL. This is used as a standard solution. When lead is tested using these sample and standard solutions by the lead test method (Method No. 1 of the atomic absorption spectrophotometry), the amount of lead shall not be 10 mg/kg or less.

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and dissolved with water to make 25 mL. The purity test (3) for the raw material for manufacturing of copper sulfate (dry) is applied mutatis mutandis (5 mg/kg or less).

Assay: Approximately 0.5 g of this product is weighed to the digits of 0.001 g and the value is recorded, and the assay for the raw material for manufacturing of copper sulfate (dry) is hereinafter applied mutatis mutandis.

0.1 mol/L sodium thiosulfate 1 mL = 24.97 mg $CuSO_4 \cdot 5H_2O$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation (part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of copper sulfate (crystal) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of copper sulfate (crystal) is applied mutatis mutandis.

C. Preparation (part 2)

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of and copper sulfate (crystal) fillers.

Content: When this product is determined, it contains copper sulfate ($CuSO_4 \cdot 5H_2O$) corresponding to 90~110 % of the amount on the label.

Confirmation test: According to the amount of this product on the label, the amount containing 1 g of the raw material for manufacturing of copper sulfate (crystal) is weighed, added with 10 mL of water, shaken up well, and filtered. To the filtrate the confirmation test for the raw material for manufacturing of copper sulfate (crystal) is applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.5 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) is weighed to three significant digits and the value is recorded. It is dissolved with 50 mL of water, or dissolved with 20 mL of water as appropriate, filtered, and washed with water. The filtrate and the washings are mixed to make 50 mL and the assay for the raw material for manufacturing of copper sulfate (crystal) is hereinafter applied mutatis mutandis.

0.1 mol/L sodium thiosulfate 1 mL = 24.97 mg $CuSO_4 \cdot 5H_2O$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of copper sulfate (crystal) is applied mutatis mutandis.

(104) Sodium Sulfate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 4-hour drying at 105 °C, it contains 99.0 % or more of sodium sulfate (Na_2SO_4).

Physical and chemical properties:

i. This product is white powder with no odor.

ii. This product is easy to dissolve in water and hardly dissolves in ethanol.

Confirmation test: A solution of this product in water (1 → 20) gives the qualitative reactions of sodium salt and sulfate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 10 mL of newly boiled and cooled water. The resulting solution shall be colorless and its turbidity shall be almost clear or clear.

ii. Heavy metals: 2.0 g (1.95~2.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: Less than 11.4 % (2 g, 105 °C, 4 hours)

Assay: This product is dried at 105 °C for 4 hours, approximately 0.4 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 200 mL of water, added with 1 mL of hydrochloric acid, boiled, and gradually added with 8 mL of hot barium chloride test solution. The resulting solution is heated on a water bath for an hour and allowed to cool. The precipitation is collected by filtration, washed with water until the washings produce no opacity when silver nitrate test solution is added to the washings, dried, and ignited to the constant weight. Then the mass is weighed, which is regarded as the amount of barium sulfate ($BaSO_4$).

Amount of sodium sulfate (Na_2SO_4) (mg)

= Amount of barium sulfate ($BaSO_4$) (mg) × 0.6086

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium sulfate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium sulfate (dry) is applied mutatis mutandis.

(105) Magnesium Sulfate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 3-hour igniting at 450 °C, it contains 99.0 % or more of sodium sulfate ($MgSO_4$).

Physical and chemical properties:

i. This product is white powder or crystalline powder with salty and bitter tastes.

ii. This product is easy to dissolve in water, hard to dissolve in glycerin and hardly dissolves in ethanol.

Confirmation test: A solution of this product in water (1 → 20) gives the qualitative reactions of magnesium salt and sulfate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 10 mL of water. The resulting solution shall be colorless and its turbidity shall be delicately slight cloudiness or less.

ii. Chloride: 1.0 g (0.95~1.04 g) of this products is weighted and a sample solution is prepared by the chloride test method. A control solution is prepared using 0.4 mL of 0.01 mol/L hydrochloric acid. When chloride is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.014 % or less).

iii. Heavy metals: 2.0 g (1.95~2.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

iv. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Ignition residue: Less than 35.0 % (1 g, 450 °C, 3 hours)

Assay: This product is ignited at 450 °C for 3 hours, approximately 0.6 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 2 mL of dilute hydrochloric acid and water, transferred to a 100 volumetric flask, and added with more water to the graduation line to make 100 mL. 25 mL of this solution is measured using a volumetric pipette, added with 50 mL of water, 5 mL of pH 10.7 ammonia-ammonium chloride buffer, and titrated with 0.05 mol/L ethylenediaminetetraacetic acid disodium solution (indicator: 0.04 g (0.035~0.044 g) of eriochrome black T-sodium chloride indicator). In this case, the end of titration is the time when the color of the solution changes from reddish violet to blue. A blank test is performed in the same way and corrections are made.

0.05 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL = 6.019 mg $MgSO_4$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of magnesium sulfate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of magnesium sulfate (dry) is applied mutatis mutandis.

(106) Magnesium Sulfate (crystal)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 2-hour drying at 105 °C and 3-hour igniting at 450 °C, it contains 99.0 % or more of sodium sulfate ($MgSO_4$).

Physical and chemical properties:

i. This product is colorless pillar or needle crystals with salty and bitter tastes.

ii. This product is easy to dissolve in water or glycerin and hard to slightly dissolve in ethanol.

Confirmation test: A solution of this product in water (1 → 20) gives the qualitative reactions of magnesium salt and sulfate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 10 mL of water. The resulting solution shall be colorless and its turbidity shall be almost clear or clear.

ii. Chloride: 1.0 g (0.95~1.04 g) of this products is weighted and a sample solution is prepared by the chloride test method. A control solution is prepared using 0.4 mL of 0.01 mol/L hydrochloric acid. When chloride is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.014 % or less).

iii. Heavy metals: 2.0 g (1.95~2.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

iv. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Ignition residue: 40.0~52.0 % (1 g, igniting at 450 °C for 3 hours after drying at 105 °C for 2 hours)

Assay: The compositional standards of the raw material for manufacturing of magnesium sulfate (dry) are applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of magnesium sulfate (crystal) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of magnesium sulfate (crystal) is applied mutatis mutandis.

(107) Manganese Sulfate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following 2-hour igniting at 450 °C, it contains 95.0 % or more of manganese sulfate ($MnSO_4$).

Physical and chemical properties:

i. This product is pink crystals or reddish white power with no odor.

ii. This product is easy to dissolve in water and hardly dissolves in ethanol.

iii. The pH of a solution of this product in water (1 → 20) is 4.5~6.5.

Confirmation test: A solution of this product in water (1 → 20) gives the qualitative reactions of manganese salt and sulfate.

Purity test:

i. Clarity and color of solution: 2.0 g (1.95~2.04 g) of this product is dissolved with 20 mL of water and 0.5 mL of dilute hydrochloric acid. The resulting solution shall be clear.

ii. Lead: 1.0 g (0.95~1.04 g) of this product is weighed, dissolved with nitric acid (1 → 150), transferred to a 20 mL volumetric flask, and added with more nitric acid (1 → 150) to the graduation line to make 20 mL. When lead is tested, using this solution as a sample solution, by the lead test method (Method No. 1 of the atomic absorption spectrophotometry), the amount of lead shall be 10 mg/kg or less.

iii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed and the sample solution is prepared by method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (5 mg/kg or less)

Ignition residue: Less than 42.0 % (1 g, 450 °C, 2 hours)

Assay: This product is ignited at 450 °C for 2 hours, approximately 0.1 g of it is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with 20 mL of water, transferred to a 100 mL volumetric flask, and added with more water to the graduation line to make 100 mL. 30 mL of this solution is measured using a volumetric pipette, and added with 70 mL of water, 5 mL of 0.1 mol/L magnesium-ethylenediaminetetraacetic acid disodium solution and 0.1 g (0.05~0.14 g) of hydroxylamine hydrochloride. Then the assay for manganese carbonate is hereinafter applied mutatis mutandis.

0.01 mol/L ethylenediaminetetraacetic acid disodium solution 1 mL = 1.510 mg $MnSO_4$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of manganese sulfate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of manganese sulfate is applied mutatis mutandis.

(108) L-lysine Sulfate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 62.5 % or more of L-lysine sulfate ($(C_6H_{14}N_2O_2)_2 \cdot H_2SO_4$).

Physical and chemical properties:

i. This product is pale brown to pale brownish-red granules with a specific odor.

ii. A suspension of this product in water (1 → 10) is centrifuged. The pH of the obtained supernatant is 4.5~7.5.

Confirmation test:

i. A suspension of this product in water (1 → 500) is centrifuged. 5 mL of the obtained supernatant is added with 1 mL of ninhydrin test solution, heated for 3 minutes, added with 20 mL of water, and allowed to stand for 15 minutes. The resulting solution is reddish violet.

ii. A suspension of this product in water (1 → 50) is centrifuged. The obtained supernatant gives the qualitative reaction i. of sulfate.

Purity test:

i. Heavy metals: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is placed in a decomposition flask, added with 10 mL of nitric acid and 5 mL of sulfuric acid, and gently heated. If the solution is still brown, it is allowed to cool, then is heated with 1~2 mL of additional nitric acid. This procedure is repeated until the solution becomes colorless to slightly yellow. It is allowed to cool, added with 0.5 mL of perchloric acid, and heated until white smoke emerges. It is allowed to cool, added with 15 mL of saturated ammonium oxalate solution and again heated until white smoke emerges. It is allowed to cool and added with water to make approximately 10 mL. This is used as a sample solution. When arsenic is tested by the method using device A, the color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Water content: Less than 5.0 % (direct titration)

Ignition residue: Less than 5.0 % (1 g)

Assay: Approximately 45 mg of this product is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with 1 mol/L hydrochloric acid, transferred to a 50 mL volumetric flask, and added with another 1 mol/L hydrochloric acid to the graduation line to make 50 mL. 2.5 mL of this solution is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, added with 2.5 mL of L-norleucine test solution using a volumetric pipette, dissolved with sodium citrate buffer (pH 2.2), added with more sodium citrate buffer (pH 2.2) to the graduation line to make 50 mL, and filtered with a membrane filter (0.45 μm). The filtrated is used as a sample solution. Separately, 24 mg of lysine hydrochloride is weighed to the digits of 0.01 mg and the value is recorded. It is dissolved with 1 mol/L hydrochloric acid, transferred to a 50 mL volumetric flask, and added with more 1 mol/L hydrochloric acid to the graduation line to make 50 mL. 2.5 mL of this solution is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, added with 2.5 mL of L-norleucine test solution using a volumetric pipette, dissolved with sodium citrate buffer (pH 2.2), and added with more sodium citrate buffer (pH 2.2) to the graduation line to make 50 mL. This is used as a standard solution. As for 100 μL each of the sample and standard solutions, an amino acid automatic analyzer is operated under the following conditions, and a test is performed by the internal standard method of the liquid chromatography.

$$\text{Content of L-lysine sulfate (\%)} = \frac{W_S}{W_T} \times \frac{A_T}{A_{iT}} \times \frac{A_{iS}}{A_S} \times 106.89$$

W_T: Collected amount of the sample (mg)

W_S: Collected amount of the reference standard (mg)

A_T: Peak area of L-lysine in the sample solution

A_{iT}: Peak area of L-norleucine in the sample solution

A_S: Peak area of L-lysine in the standard solution

A_{iS}: Peak area of L-norleucine in the standard solution

Operating conditions:

Detector: Visible absorption spectrometer (measurement wavelength: 570 nm)

Column: A stainless tube (inner diameter: approx. 4.0 mm, length: approx. 120 mm) is filled with strong acid cation exchange resin.

Column temperature: 35 °C (0 → 15.3 min.) retention, 64 °C (15.3 → 31.0 min.) retention, 44 °C (31.0 → 44.4 min.) retention, 72 °C (44.4 → 80.0 min.) retention

Reaction vessel temperature: Constant temperature at around 135 °C

Mobile phase: Lithium citrate buffer

Reaction solution: Ninhydrin test solution for amino acid analysis

Flow rate of mobile phase: 0.50 mL/min.

Flow rate of reaction solution: 0.30 mL/min.

Column selection: A column to be used is selected when 100 µl of the standard solution is operated under the above conditions, the internal standard solution and L-lysine are eluted in that order, and the separation degree is 5 or over.

(b) Standard of manufacturing method

For manufacturing, the lysine producing strain of *Corynebacterium glutamicum* is aerobically cultured. After the cultivation, the bacterial cells in the culture solution are heat-treated, dried, and made into granules.

(c) Standard of storage method

It shall be put in a capped container and stored in a dry cold place.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of L-lysine sulfate are applied mutatis mutandis.

(b) Standard of manufacturing method

The standard of manudacturing method of the raw material for manufacturing of L-lysine sulfate is applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of L-lysine sulfate is applied mutatis mutandis.

(109) Dipotassium hydrogen phosphate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 4-hour drying at 105 °C, it contains 98.0 % or more of dibasic potassium phosphate (K_2HPO_4).

Physical and chemical properties:

i. This product is white crystals or blocks.

ii. This product is easy to extremely dissolve in water and hard to slightly dissolve in ethanol.

Confirmation test:

i. When a solution of this product in water (1 → 20) is added with a drop of phenolphthalein test solution, it is red.

ii. A solution of this product in water (1 → 20) gives the qualitative reactions of potassium salt and phosphate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of water. The resulting solution shall be colorless and its turbidity shall be delicate slight cloudiness or less.

ii. Primary salt and tertiary salt: When 2.0 g (1.95~2.04 g) of this product is weighed, added with 50 mL of water, boiled for 5 minutes, cooled down to approximately 25 °C, and added with 3 drops of phenolphthalein test solution, it shall be red. When it is added with 1.8 mL of 1 mol/L hydrochloric acid, it shall be colorless.

iii. Chloride: 1.0 g (0.95~1.04 g) of this products is weighted and a sample solution is prepared by the chloride test method. A control solution is prepared using 0.3 mL of 0.01 mol/L hydrochloric acid. When chloride is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.01 % or less).

iv. Sulfate: 1.0 g (0.95~1.04 g) of this products is weighted and a sample solution is prepared by the sulfate test method. A control solution is prepared using 0.4 mL of 0.005 mol/L sulfuric acid. When sulfate is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.02 % or less).

v. Carbonate: When 2.0 g (1.95~2.04 g) of this product is weighed, dissolved in 10 mL of newly boiled and cooled water, and added with 5 mL of dilute hydrochloric acid, it shall not bubble.

vi. Heavy metals: 1.0 g (0.95~1.04 g) of this product is weighed and neutralized with diluted acetic acid and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

vii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: Less than 5.0 % (1 g, 105 °C, 4 hours)

Assay: This product is dried at 105 °C for 4 hours, approximately 3.0 g of it is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved with 50 mL of water and titrated with 1 mol/L hydrochloric acid, maintaining it at approximately 15 °C (indicator:

3 to 4 drops of methyl orange-xylene cyanol FF test solution). In this case, the end of titration is the time when the color of the solution changes from green to dark greenish reddish violet. A blank test is performed in the same way and corrections are made.

1 mol/L hydrochloric acid 1 mL = 174.2 mg K_2HPO_4

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of dipotassium hydrogen phosphate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of dipotassium hydrogen phosphate (dry) is applied mutatis mutandis.

(110) Sodium Hydrogen Phosphate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 18.0~22.0 % of phosphorus (P) and 27.0~32.5 % of sodium (Na).

Physical and chemical properties:

i. This product is white or gray crystalline powder with no odor.

ii. This product is easy to extremely dissolve in water and hardly dissolves in ethanol.

Confirmation test:

i. When a solution of this product in water (1 → 20) is added with a drop of phenolphthalein test solution, it is red.

ii. A solution of this product in water (1 → 20) gives the qualitative reactions i. and ii. of sodium salt and the qualitative reaction of phosphate.

Purity test:

i. Arsenic: 0.166 g (0.1655~0.1664 g) of this product is weighed and dissolved with 5mL of dilute hydrochloric acid and the sample solution is prepared by method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (12 mg/kg or less).

ii. Heavy metals: 1.0 g (0.95~1.04 g) of this product is weighed, added with dilute hydrochloric acid heated to dissolve, allowed to cool, and added with water to make 50

mL. This is used as a sample solution. A control solution is prepared using 5.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

iii. Fluorine: 1.0 g (0.95~1.04 g) of this product is weighed and added with approximately 0.2~0.3 g of silicic acid powder, and steam distillation is performed. The distillate liquid is neutralized with sodium hydroxide solution (1 → 100) (indicator: methyl red test solution), transferred to a 250 mL volumetric flask, and is added with water to the graduation line to make 250 mL. 5 mL of this solution is measured using a volumetric pipette, transferred to a 25 mL volumetric flask, added with 5 mL of water, 3 mL of lanthanum-alizarin complexone test solution and 10 mL of acetone, allowed to cool, and is added with water to the graduation line to make 25 mL. This is used as a sample solution. Separately, 5 mL of fluorine standard solution is measured using a volumetric pipette, added with 5 ml of water, and hereinafter subjected to the same procedure as for the sample solution. This is used as a standard solution. Separately, 5 mL of water is measured and the same procedure is performed to prepare a blank test solution. The sample and standard solutions are separately shaken up and allowed to stand for 90 minutes. When using the blank test solution as a control solution, the absorbance is measured at the absorption maximum at a wavelength around 620 nm, the absorbance of the sample solution shall be that or less of the standard solution (0.125 % or less).

Loss on drying: Less than 2.0 % (1 g, 105 °C, 2 hours)

Assay:

i. Phosphorus: Approximately 2.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 50 mL of dilute hydrochloric acid, dissolved by warming, allowed to cool, transferred to a 250 mL volumetric flask, added with water to the graduation line to make 250 mL, and filtered with dry filter paper. 20 mL of the first filtrate is eliminated and 10 mL of the next filtrate is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric flask, and transferred to a 100 mL volumetric flask. When it is strongly acidic, it is neutralized with ammonia test solution using phenolphthalein as an indicator and make it slightly acid by the addition of several drops of nitric acid, added with water to make 70 mL, and added with 20 mL of phosphorus coloring test solution and water to the graduation line to make 100 mL. This is used as a sample solution. Separately, 10 mL and 20 mL of phosphorus standard solution are measured using a

volumetric pipette. Each of them is transferred to each 100 mL volumetric flask, added with water to make approximately 70 mL, added with 20 mL of phosphorus coloring test solution and water to the graduation line to make 100 mL, and used as the S_1 and S_2 solutions, respectively. The absorbances A_T and A_S for the sample solution and S_2 solution, respectively, are measured at the absorption maximum at a wavelength around 400~420 nm, using S_1 solution as a control solution.

$$\text{Amount of phosphorus (P) (mg)} = 1\ (\text{mg}) + \frac{A_T}{A_S}\ (\text{mg})$$

ii. Sodium: Approximately 2.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 50 mL of dilute hydrochloric acid, dissolved by warming, allowed to cool, transferred to a 250 mL volumetric flask, added with water to the graduation line to make 250 mL, and filtered with dry filter paper. 20 mL of the first filtrate is eliminated. 5 mL of the next filtrate is measured using a volumetric pipette, transferred to a 500 mL volumetric flask, and is added with water to the graduation line to make 500 mL. This is used as a sample solution. Separately, 20, 25 and 30 mL of sodium standard solutions are measured and each of them is transferred to a 1 L volumetric flask and is added with water to the graduation line to make 1 L. They are used a standard solution. For the sample and standard solutions, the intensity of light at a wavelength of 589 nm is measured using a flame spectrophotometer, and the amount of sodium (Na) in the sample solution is calculated from the calibration curve obtained from the standard solutions. Propane and air are used as fuel gas and supporting gas, respectively, of a flame spectrophotometer.

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium hydrogen phosphate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Sodium hydrogen phosphate (dry) is applied mutatis mutandis.

(111) Potassium Dihydrogen Phosphate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following a 4-hour drying at 105 °C, it contains 98.0 % or more of potassium dihydrogen phosphate (KH_2PO_4).

Physical and chemical properties:

i. This product is colorless crystals or white crystalline powder.

ii. This product is easy to dissolve in water and hardly dissolves in ethanol.

Confirmation test:

i. A solution of this product in water (1 → 20) is acidic.

ii. A solution of this product in water (1 → 20) gives the qualitative reactions of potassium salt and phosphate.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 20 mL of water. The resulting solution shall be colorless and its turbidity shall be delicate slight cloudiness or less.

ii. pH: The pH of a solution of this product in water (2.7 → 100) shall be 4.2~4.7.

iii. Chloride: 1.0 g (0.95~1.04 g) of this products is weighted and a sample solution is prepared by the chloride test method. A control solution is prepared using 0.3 mL of 0.01 mol/L hydrochloric acid. When chloride is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.01 % or less).

iv. Sulfate: 1.0 g (0.95~1.04 g) of this products is weighted and a sample solution is prepared by the sulfate test method. A control solution is prepared using 0.4 mL of 0.005 mol/L sulfuric acid. When sulfate is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.02 % or less).

v. Heavy metals: 0.5 g (0.45~0.54 g) of this product is weighed and neutralized with diluted acetic acid and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 1.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 ppm or less).

vi. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 ppm or less).Loss on drying: Less than 0.5 % (1 g, 105 °C, 4 hours)

Assay: This product is dried at 105 °C for 4 hours, approximately 3.0 g of it is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved with 30 mL of water, added with 5 g (4.5~5.4 g) of sodium chloride, dissolved by shaking well, and titrated with 1 mol/L sodium hydroxide solution, maintaining it at approximately 15 °C (indicator: 3~4

drops of thymol blue test solution). In this case, the end of titration is the time when the color of the solution changes from yellow to blue. A blank test is performed in the same manner and corrections are made.

1 mol/L sodium hydroxide solution 1 mL = 136.1 mg KH_2PO_4

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of potassium dihydrogen phosphate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of potassium dihydrogen phosphate (dry) is applied mutatis mutandis.

(112) Sodium Dihydrogen Phosphate (dry)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried and determined, it contains 98.0~102.0 % of sodium dihydrogen phosphate (NaH_2PO_4).

Physical and chemical properties:

i. This product is white powder or particles.

ii. This product is easy to extremely dissolve in water and hardly dissolves in ethanol.

Confirmation test:

i. A solution of this product in water (1 → 20) is weak acid.

ii. A solution of this product in water (1 → 20) gives the qualitative reactions i. and ii. of sodium salt and the qualitative reaction of phosphate.

Purity test:

i. Clarity and color of solution: 2.0 g (1.95~2.04 g) of this product is dissolved with 20 mL of water. The resulting solution shall be colorless and its turbidity shall be delicate slight cloudiness or less.

ii. Chloride: 0.2 g (0.15~0.24 g) of this products is weighted and a sample solution is prepared by the chloride test method. A control solution is prepared using 0.6 mL of 0.01 mol/L hydrochloric acid. When chloride is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.1 % or less).

iii. Sulfate: 0.5 g (0.45~0.54 g) of this products is weighted and a sample solution is prepared by the sulfate test method. A control solution is prepared using 0.5 mL of 0.005 mol/L sulfuric acid. When sulfate is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.048 % or less).

iv. Heavy metals: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

v. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: Less than 2.0 % (1 g, 105 °C, 5 hours)

Assay: This product is dried, approximately 3.0 g of it is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved in 30 mL of water, added with 5 g (4.5~5.4 g) of sodium chloride, shaken up well to dissolve, and titrated with 1 mol/L sodium hydroxide solution maintaining it at approximately 15 °C (indicator: 3~4 drops of thymol blue test solution). In this case, the end of titration is the time when the color of the solution changes from yellow to blue. A blank test is performed in the same manner and corrections are made.

1 mol/L sodium hydroxide solution 1 mL = 120.0 mg NaH_2PO_4

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium dihydrogen phosphate (dry) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium dihydrogen phosphate (dry) is applied mutatis mutandis.

(113) Sodium Dihydrogen Phosphate (crystal)

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 98.0 % or more of sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$).

Physical and chemical properties:

i. This product is colorless to white crystals or white crystalline powder.

ii. This product is easy to dissolve in water and hardly dissolves in ethanol.

Confirmation test:

i. A solution of this product in water (1 → 20) is weak acid.

ii. A solution of this product in water (1 → 20) gives the qualitative reactions i. and ii. of sodium salt and the qualitative reaction of phosphate.

Purity test:

i. Clarity and color of solution: 2.0 g (1.95~2.04 g) of this product is dissolved with 20 mL of water. The resulting solution shall be colorless and its turbidity shall be delicate slight cloudiness or less.

ii. Chloride: 1.0 g (0.95~1.04 g) of this products is weighted and a sample solution is prepared by the chloride test method. A control solution is prepared using 0.3 mL of 0.01 mol/L hydrochloric acid. When chloride is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.01 % or less).

iii. Sulfate: 0.5 g (0.45~0.54 g) of this products is weighted and a sample solution is prepared by the sulfate test method. A control solution is prepared using 0.4 mL of 0.005 mol/L sulfuric acid. When sulfate is tested, the opacity of the sample solution shall not be higher than that of the control solution (0.038 % or less).

iv. Heavy metals: 1.0 g (0.95~1.04 g) of this product is weighed and a sample solution is prepared by Method No. 1 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution. When heavy metal is tested using these solutions, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

v. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 1 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 22.0~24.0 % (1 g, 40 °C, 16 hours and 105 °C, 5 hours)

Assay: This product is dried, approximately 3.0 g of it is weighed to the digits of 0.01 g, and the value is recorded. It is dissolved in 70 mL of water, added with 5 g (4.5~5.4 g) of sodium chloride, shaken up well to dissolve, and the assay for the raw material for manufacturing of sodium dihydrogen phosphate (dry) is hereinafter applied mutatis mutandis.

1 mol/L sodium hydroxide solution 1 mL = 156.0 mg $NaH_2PO_4 \cdot 2H_2O$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium dihydrogen phosphate (crystal) are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium dihydrogen phosphate (crystal) is applied mutatis mutandis.

(114) Zinc Bacitracin

A. Raw material for manufacturing

(a) Compositional standards

Potency: The potency test shows that 4.2 units of this product are contained in 1 mg.

Physical and chemical properties: This product is yellowish-gray-brown to brown powder with a specific odor.

Confirmation test:

i. The amount of this product containing approximately 4,200 units of bacitracin is weighed, added with 100 mL of buffer No. 3, stirred for approximately 15 minutes and then allowed to stand. The supernatant of the mixture is a sample solution. Separately, the amount of working standard bacitracin containing approximately 4,200 units of bacitracin is weighed, added with 1 g (0.5~1.4 g) of peptone, and dissolved in 100 mL of No. 3 buffer. This solution is used as a standard solution. 5 μl each of the sample and standard solutions are spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then, they are developed with the developing solvent, acetone (3 → 10), by the ascending method and the thin layer plate is air dried. The thin layer plate is placed on a large flat plate medium consisting of a base layer (approximately 3 mm thick) and a inoculated layer (approximately 1 mm thick), allowed to stand for approximately 1 hour at room temperature, taken off, cultured at 37 °C for 16 hours, and then observed. The Rf values of the inhibition zones of the sample and standard solutions are equal.

Agar plate: Medium No. 1 is used for both the media of base and inoculated layers, and 1 % of bacterial culture is added in the inoculated layer medium.

Preparation of bacterial culture: The bacterial culture is prepared using *Micrococcus luteus* ATCC 10240 as a test strain by the potency test method specified in General Tests.

ii. 5 g (4.5~5.4 g) of this product is weighed, incinerated at 550 °C for 4 hours, added with 10 mL of 3 mol/L hydrochloric acid test solution, covered with a watch glass, and then mildly heated for 10 minutes. It is allowed to cool, filtered, added with water to make 50 mL, and neutralized with sodium hydroxide solution (1 → 5) (it is filtered if necessary). This solution gives the qualitative reaction iii. of zinc salt.

Purity test:

i. pH: The pH of water suspension (1 → 10) of this product shall be 5.5~7.5.

ii. Lead: Approximately 10.0 of this product is weighed to the digit of 0.1 g and the value is recorded. It is incinerated at 550 °C for 4 hours, allowed to cool, added with 10 mL of hydrochloric acid (1 → 10), covered with a watch glass, and mildly heated for 10 minutes. It is allowed to cool and filtered. The filtrate is transferred to a 10 mL volumetric flask and added with water to the marked line to make 10 mL. This is used as a sample solution. Separately, 5 mL of lead standard solution for dithizone is measured using a volumetric pipette, transferred to a 10 mL volumetric flask, and added with water to the marked line to make 10 mL. This is used as a standard solution. The absorbance A_T and A_S of the sample and standard solutions are measured at the wavelength of 283.3 nm using an atomic absorption spectrophotometer with a lead hollow cathode lamp and the amount of lead in the sample is calculated by the following formula. The amount shall be 10 mg/kg or less.

$$\text{The amount of lead (mg/kg)} = 0.5 \times \frac{A_T}{A_S}$$

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 12.0 % or less (1 g, 0.67 kPa or less, 60 °C, 3 hours)

Ignition residue: 35.0 % or less (1 g)

Nitrogen: 5.0~10.0 % (Kjeldahl method)

Crude fat: 10.0 % or less

Crude fiber: 7.0 % or less

Potency test:

Agar plate: No. 1 medium is used for both the media of base and inoculated layers.

Test strain: *Micrococcus luteus* ATCC 10240 is used.

Preparation of the working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is added with buffer No. 3 to make a constant volume at a concentration of approximately 100 units/mL. This is used as a diluted stock solution. The volume of the diluted stock solution required for testing is measured using a volumetric pipette and accurately diluted by adding buffer No. 3 to prepare the working standard diluent at a high concentration of 2 units/mL and the working standard diluent at a low concentration of 0.5 units/mL.

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with a mixed solution of buffer No. 3 and pyridine (31:9), shaken or pretreated with approximately5 mL of 1 mol/L hydrochloric acid test solution to adjust pH to 2.0 or less, and stirred for approximately 3 minutes. Then, it is added with a mixed solution of buffer No. 3 and pyridine (31:9) to make a constant volume with the concentration 100 units/mL (estimated value) and stirred for 15~30 minutes without foaming. The solution is centrifuged or allowed to stand and the supernatant is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette and accurately diluted by adding buffer No. 3 to prepare the sample solution at a high concentration of 2 units/mL (estimated value) and the sample solution at a low concentration of 0.5 units/mL (estimated value).

(b) Standard of manufacturing method

For manufacturing, the bacitracin producing strain of *Bacillus licheniformis* is aerobically cultured. Bacitracin in the culture solution after the cultivation is used as a zinc complex salt, and it is added with calcium carbonate as appropriate, and the culture solution is dried.

(c) Standard of storage method

It shall be stored in a light resistant sealed container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of zinc bacitracin are applied mutatis mutandis.

(b) Standard of manufacturing method

The standard of manufacturing method of the raw material for manufacturing of zinc bacitracin is applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of zinc bacitracin is applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“有効期間 製造の翌月から２年”

Valid period: 2 years from the month following the manufacture

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces or powder of the mixture of the raw material for manufacturing of zinc bacitracin and carriers.

Potency: The potency test shows that it contains 85~125 % of the label potency.

Physical and chemical properties:

i. This product is pale yellow grayish white to brown small pieces or powder with a specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test:

i. The Confirmation test i. of the raw material for manufacturing of zinc bacitracin are applied mutatis mutandis.

ii. According to the label potency of this product, the amount containing approximately 21,000 units of bacitracin is weighed and incinerated at 550 °C for 4 hours. The confirmation test ii. of the raw material for manufacturing of zinc bacitracin is hereinafter applied mutatis mutandis.

Loss on drying: 15.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate: The provisions of the raw materials for manufacturing of zinc bacitracin are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of zinc bacitracin are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of zinc bacitracin are applied mutatis mutandis.

Preparation of sample solution: According to the label potency of this product, the amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with a mixed solution of buffer No. 3 and pyridine (31:9), shaken or pretreated with approximately 5 mL of 1 mol/L hydrochloric acid test solution to adjust pH to 2.0 or less, and stirred for approximately 3 minutes. Then, it is added with a mixed solution of buffer No. 3 and pyridine (31:9) to make a

constant volume at a concentration 100 units/mL or less, and the provisions of the raw materials for manufacturing of zinc bacitracin are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

It shall be manufactured by mixing the raw materials for manufacturing of zinc bacitracin with carriers.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of zinc bacitracin is applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

"有効期限 製造の翌月から２年"

Valid period: 2 years from the month following the manufacture

(115) Avilamycin

A. Raw material for manufacturing

(a) Compositional standards

Potency: The potency test shows that 100 μg (potency) of this produce are contained in 1 mg.

Physical and chemical properties: This product is gray to dark brown powder or particles with a specific odor.

Confirmation test: The amount of this product containing approximately 100 mg (potency) of avilamycin is weighed, added with 10 mL of acetone, shaken for 10 minutes, and filtered. This filtrate is used as a sample solution. Separately, the amount of the working standard avilamycin containing approximately 10 mg (potency) of avilamycin is weighed and dissolved with 1 mL of acetone. This is used as a standard solution. 10 μL each of the sample and standard solutions are spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Then, they are developed to approximately 10 cm with the developing solvent, a mixed solution of ethyl acetate, cyclohexane, and methanol (85:15:4), and the thin layer plate is air dried. It is sprayed with a mixed solution of sulfuric acid and methanol (1:1) and heated at 100 °C for 5 minutes. Then, the Rf values of the main spots obtained from the sample and standard solutions are equal.

Purity test:

i. pH: This product 1.0 g (0.95~1.04 g) is added with 20 mL of water, shaken for 10 minutes, and filtered. This pH of this filtrate shall be 5.0~8.5.

ii. Heavy metal: 1.0 g of this product (0.95~1.04 g) is weighed. In the test using the sample solution prepared by method NO.3 of the heavy metal test method and the control solution using 3.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (30 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (4 mg/kg or less).

iv. Content ratio of avilamycin A: This product 1.0 g (0.95~1.04 g) is weighed, added with 100 mL of acetone, and stirred for 1 hour. The supernatant 4 mL is measured, the solvent is distilled away under reduced pressure in a water bath at 50 °C, and the residue is dissolved with 5 mL of acetonitrile and then added with 5 mL of pH 7.0 phosphate buffer. The solution is filtered with a membrane filter (0.45 μm) and the filtrate is used as a sample solution. When the sample solution 50 μL is tested by the liquid chromatography under the following conditions, the content ratio of avilamycin A shall be 60 % or greater.

$$\text{The content ratio of avilamaycin A in this product (\%)} = \frac{A_{T1}}{A_T} \times 100$$

A_{T1}: Peak area of avilamaycin A in the sample solution

A_T: Sum of peak areas of the sample solution

Operating condition:

Detector: Ultraviolet absorptiometer (measurement wavelength: 286 nm)

Column: A stainless tube (internal diameter: 4.0~4.6 mm, length: 200~250 mm) is filled with 5 μm of octadecyl-silylated silica gel.

Column temperature: Constant temperature around 25 °C

Mobile phase A: Mixed solution of methanol and pH 7.0 phosphate buffer (11:9)

Mobile phase B: Mixed solution of methanol and pH 7.0 phosphate buffer (4:1)

Gradient method: The mixing ratio of mobile phases A and B is 78:22. After 5 minute elution, the mixing ratio is changed to 35:65 in the linear gradient for 30 minutes, and then the mixing ratio is maintained.

Flow rate: The retention time of avilamaycin A is adjusted to approximately 25 minutes.

Column selection: 5 mg each of Sec-butyl-*p*-hydroxybenzoate and butyl-*p*-hydroxybenzoate (4.5~5.4 mg) are dissolved in 50 mL of acetonitrile and added with water to make 100 mL. The column to be used: when 10 µL of this solution is operated under the above conditions with the mixing ratio of mobile phases A and B, 78:22, and sec-butyl-*p*-hydroxybenzoate and butyl-*p*-hydroxybenzoate are eluted in that order, the separation degree shall be 3.0 or greater.

Area measurement range: About twice as much as the retention time of avilamaycin A.

Loss on drying: 10.0 % or less (1 g, decompression, 100 ℃, 3 hours)

Ignition residue: 70.0 % or less (1 g)

Nitrogen: 6.0 % or less (kjeldahl method)

Crude fat: 20.0 % or less

Crude fiber: 20.0 % or less

Potency test:

Agar plate (single layer): 10 mL of buffer No. 4 mixed with the test strain (11 mL in a Petri dish with internal diameter of 100 mm) is used.

The strain: *Micrococcus luteus* ATCC 10240 is used.

Preparation of working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is dissolved with acetone, and is further added with acetone to make an accurate constant volume at a concentration of approximately 1 mg/mL (potency). This is used as a diluted stock solution. The volume of the diluted stock solution required for testing is measured using a volumetric pipette and accurately diluted by adding a mixed solution of acetone and buffer No. 7 (1:4) to prepare the working standard diluent at a high concentration of 2 µg/mL (potency) and the working standard diluent at a low concentration of 0.5 µg/mL (potency).

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with approximately 10 hold buffer No. 7 using a volumetric pipette and mildly shaken. Next, it is added with acetone four times as much as buffer No. 7 using a volumetric pipette, stirred for 20 minutes, filtered or centrifuged. This is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette and accurately diluted by adding a mixed solution of acetone and buffer No. 7 (1:4) to prepare the sample solution at a high concentration of 2 µg/mL (estimated value) (potency) and the sample solution at a low concentration of 0.5 µg/mL (estimated value) (potency).

(b) Standard of manufacturing method

The avilamaycin producing strain of *Streptomyces viridochromogenes* is aerobically cultured, and after the cultivation, where appropriate added with calcium hydroxide and others as a coagulating agent to separate solids. The solids are dried.

(c) Standard of storage method

It shall be stored in a sealed container

B. Preparation

(a) Compositional standards

This product is powder or particles of the mixture of the raw material for manufacturing of avilamycin and carriers.

Potency: The potency test shows that it contains 85~125 % of the label potency.

Physical and chemical properties:

i. This product is pale orange-yellow to brown or blackish brown power or particles with a specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test: The confirmation test of the raw material for manufacturing of avilamycin are applied mutatis mutandis.

Loss on drying: 10.0 % or less (1 g, decompression, 100 ℃, 3 hours)

Potency test:

Agar plate (single layer): The provisions of the raw materials for manufacturing of the avilamycin are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of the avilamycin are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of the avilamycin are applied mutatis mutandis.

Preparation of sample solution: According to the label potency of this product, the amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with approximately 10 hold buffer No. 7 using a volumetric pipette and mildly shaken. Then, it is added with acetone four times as much as buffer No. 7 using a volumetric pipette, stirred for 20 minutes, and the provisions of the raw materials for manufacturing of avilamaycin are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

It shall be manufactured by mixing the raw materials for manufacturing of avilamycin with carriers.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of avilamycin is applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

"有効期間 製造の翌月から２年"

Valid period: 2 years from the month following the manufacture

(116) Enramycin

A. Raw material for manufacturing

(a) Compositional standards

Potency: The potency test shows that 10.0 μg (potency) or more of this product are contained in 1 mg.

Physical and chemical properties: This product is grayish brown to brown powder or particles with a slightly specific odor.

Confirmation test:

i. The amount of this product containing approximately 10 mg (potency) of enramycin is weighed, and dissolved with 50 mL of a mixed solution of methanol and 0.1 mol/L hydrochloric acid test solution (1:1), shaken for approximately 20 minutes, and then filtered. The filtrate is added with ammonia test solution to make it neutral to slightly alkaline and filtered as appropriate to prepare a sample solution. The sample solution 20 mL is added with approximately 1 mL of porous styrene divinylbenzene copolymer refined resin immersed in methanol (1 → 2), shaken for 10 minutes, and filtered with a glass filter. The resin is washed twice with approximately 20 mL of water, added with 20 mL of a mixed solution of methanol and 0.2 mol/L hydrochloric acid test solution (7:3), vigorously shaken, and filtered. In the measurement of the absorption spectrum of this solution, the absorption maximum and the absorption minimum are at the wavelengths of 269~275 nm and 250~256 nm, respectively.

ii. The amount of this product containing approximately 100 mg (potency) of enramycin is weighed, added with 50 mL of a mixed solution of acetone, 1 mol/L hydrochloric acid test solution and water (35:12:56), stirred, neutralized with ammonia test solution, and filtered or centrifuged. The volume required for testing of the filtrate or supernatant is measured, added with acetone (7 → 20) to make its concentration approximately 1 mg/mL (potency) to prepare a sample solution. 5 μL each of this

sample solution and the working standard diluted stock solution obtained by the potency test are spotted on a thin layer plate prepared using cellulose powder for thin-layer chromatography. They are developed to approximately 10 cm using the upper layer solution of a mixed solution of n-butanol, pyridine and water (8:3:9) as the developing solvent and the thin layer plate is air-dried. When the thin layer plate is uniformly sprayed with the acetone solution of ninhydrin (1 → 50) and heated at 105 °C for 10 minutes, the main spot of the sample solution and the spot of the working standard diluted stock solution are purple and their Rf values are equal.

Purity test:

i. pH: The pH of solution (1 → 10) of this product shall be 5.0~8.0.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by Method No. 3 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 3.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (30 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Loss on drying: 6.0 % or less (1 g, 0.67 kPa or less, 60 °C, 3 hours)

Acid soluble ash: Approximately 1 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is added with 20 mL of dilute hydrochloric acid, heated at 50 °C for 15 minutes while stirring, allowed to cool, placed in a 50 mL volumetric flask, added with water to the marked line to make 50 mL, and filtered. This solution is centrifuged until clear as appropriate and 25 mL of it is measured, added with 1 mL of dilute sulfuric acid, evaporated to dryness, ignited to constant weight at 800 ± 25 °C. The resulting weight shall be 20.0 % or less.

Nitrogen: 1.0~8.0 % (Kjeldahl method)

Crude fat: 20.0 % or less

Crude fiber: 5.0 % or less

Potency test:

Agar plate (single layer): 10 mL of the No. 13 medium mixed with the test strain is used (11 mL in a Petri dish with internal diameter of 100 mm).

Test strain: *Bacillus subtilis* ATCC 6633 is used.

Preparation of the working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is

recorded. It is dissolved with methanol (4 → 5), and is further added with methanol (4 → 5) to make a constant volume at a concentration of approximately 1 mg/mL (potency). This is used as a diluted stock solution. The volume required for testing of the diluted stock solution is measured using a volumetric pipette and accurately diluted by adding buffer No. 8 to prepare the working standard diluent at a high concentration of 40 μg/mL (potency) and the working standard diluent at a low concentration of 10 μg/mL (potency).

Preparation of the sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of a mixed solution of acetone, 1 mol/L hydrochloric acid test solution and water (35:12:56) using a volumetric pipette to make its concentration (estimated value) approximately 1 mg/mL (potency) and stirred. It is confirmed to be pH 2.8~3.2, as appropriate the pH is adjusted with 1 mol/L hydrochloric acid test solution or 1 mol/L sodium hydroxide test solution, and then filtered or centrifuged to prepare a sample stock solution. The volume required for testing of the sample stock solution is measured using a volumetric pipette and accurately diluted by adding buffer No. 8 to prepare the sample solution at a high concentration (estimated value) of 40 μg/mL (potency) and the sample solution at a low concentration (estimated value) of 10 μg/mL (potency).

(b) Standard of manufacturing method

The enramycin producing strain of *Streptomyces fungicidicus* is aerobically cultured, and after cultivation the pH of the culture solution is adjusted. Then, the solution is filtered, where appropriate using a filter aid, and the obtained solids are dried.

(c) Standard of storage method

It shall be stored in a light resistant sealed container.

B. Preparation

(a) Compositional standards

This product is pieces or powder of the mixture of the raw material for manufacturing of enramycin and carriers.

Potency: The potency test shows that it contains 85~125 % of the label potency.

Physical and chemical properties:

i. This product is grayish brown to brown or yellow brown small pieces or powder with a slightly specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test: The confirmation test of the raw materials for manufacturing of enramycin are applied mutatis mutandis.

Loss on drying: 10 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate (single layer): The provisions of the raw materials for manufacturing of enramycin are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of enramycin are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of enramycin are applied mutatis mutandis.

Preparation of the sample solution: Where appropriate, this product is ground, according to the label potency, the amount required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of a mixed solution of acetone, 1 mol/L hydrochloric acid test solution and water (35: 12: 56) using a volumetric pipette to make its concentration approximately 1 mg/mL (potency), stirred, and the provisions of the raw materials for manufacturing of enramycin are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of enramycin is mixed with carriers, and as appropriate particle-seized or sieved.

(c) Standard of storage method

The standard storage method of the raw materials for manufacturing of enramycin are applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“有効期間 製造の翌月から２年”

Valid period: 2 years from the month following the manufacture

(117) Salinomycin Sodium

Salinomycin sodium (part 1)

A. Raw material for manufacturing

(a) Compositional standards

Potency: This product is sodium salt of salinomycin. The potency test shows that 800 μg (potency) or more is contained in 1 mg.

Physical and chemical properties:

i. This product is white to pale yellow-white crystalline powder with a slightly specific odor.

ii. This product is extremely easy to dissolve in ethyl acetate, easy to dissolves in acetone, ethylether, chloroform and methanol, hard to lightly dissolve in n-hexane, and hardly dissolve in water.

Confirmation test:

i. This product in methanol solution (3 → 1,000) 2 mL is added with 2 mL of vanillin-hydrochloric acid test solution and gently shaken. The resulting solution is red.

ii. This product in methanol solution (1 → 10,000) 9 mL is added with 1 mL of 1 mol/L hydrochloric acid test solution, shaken well, allowed to stand at room temperature for 5 minutes. In the measurement of the ultraviolet absorption spectrum of this solution, the absorption maximum is at the wavelengths of 285~300 nm.

iii. The solution obtained when 3 g (2.5~3.4 g) of this product is ashed (incinerated), shaken with 10 mL of water and filtered gives the qualitative reaction of sodium salt.

Purity test: The potency test shows that it contains 85~125 % of the label potency.

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is weighed, dissolved with 20 mL of methanol. The resulting solution shall be pale yellow and clear or almost clear.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by method No. 3 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Loss on drying: 7.0 % or less (1 g, 0.67 kPa or less, 60 °C, 3 hours)

Ignition residue: 7.0~12.0 % (1 g)

Potency test:

Agar plate: Medium No. 14 is used for both the media of base and inoculated layers. However, the medium of base layer is 10 mL (11 mL in a Petri dish with internal diameter of 100 mm).

Test strain: *Bacillus subtilis* ATCC 6633 is used.

Preparation of the working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is dissolved with methanol, and is further added with methanol to make a constant volume at a concentration of approximately 400 μg/mL (potency). This is used as a diluted stock solution. The volume required for testing of the diluted stock solution is measured using a volumetric pipette and accurately diluted by adding water and methanol to prepare the working standard diluent at a high concentration of 40 μg/mL (potency) with 10 % methanol and the working standard diluent at a low concentration of 10 μg/mL (potency) with 10 % methanol.

Preparation of the sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is dissolved with methanol to make an accurate constant volume with the concentration (estimated value) of approximately 400 μg/mL (potency). This is used as a sample stock solution. The volume required for testing of the sample stock solution is measured using a volumetric pipette and accurately diluted by adding water and methanol to prepare the sample solution at a high concentration (estimated value) of 40 μg/mL (potency) and 10 % methanol and the sample solution at a low concentration (estimated value) of 10 μg/mL (potency) and 10 % methanol.

(b) Standard of manufacturing method

The salinomycin producing strain of *Streptomyces albus* is aerobically cultured, after the cultivation solids are separated by filtration, and salinomycin in the solids are extracted with an organic solvent. This solution is concentrated, filtered and crystallized as sodium salt under alkaline conditions. The obtained crystals are washed and dried.

(c) Standard of storage method

It shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

This product is pieces, particles, powder or in which the raw material for manufacturing salinomycin sodium (part 1) is mixed with carriers or granulated.

Potency: The potency test shows that 100 μg (potency) or less is contained in 1 mg, and that it contains 85~125 % of the label potency.

Physical and chemical properties:

i. This product is pale yellow-white to pale brown small powder or particles with a specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test: According to the label potency of this product, the amount containing approximately 30 mg (potency) of salinomycin is weighed, shaken with 10 mL of methanol, and filtered. This solution 2 mL is measured, and the confirmation test i. for the raw material for manufacturing of salinomycin sodium (part 1) is hereinafter applied mutatis mutandis.

Loss on drying: 12.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate (single layer): The provisions of the raw materials for manufacturing of salinomycin sodium (part 1) are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of sample solution: According to the label potency of this product, the amount required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of methanol using a volumetric pipette to make its concentration approximately 400 μg/mL (potency), stirred or shaken, and filtered or centrifuged. The resulting filtrate or supernatant is used as a sample stock solution. The volume required for testing of the sample stock solution is measured by a volumetric pipette, and the provisions of the raw material for manufacturing of salinomycin sodium (part 1) are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of salinomycin sodium (part 1) is mixed with carriers, and as appropriate particle-seized or sieved.

(c) Standard of storage method

The storage method of the raw materials for manufacturing of salinomycin sodium (part 1) are applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“有効期間　製造の翌月から２年”

Valid period: 2 years from the month following the manufacture

“使用上の注意　この飼料添加物は、鶏又は牛に過剰投与した場合発育障害がおこるので、定められた添加量を厳守するとともに、均一に配合するよう注意すること。”

Precautions: Overdose of this feed additive causes developmental disorders in chicken or cattle, so strictly follow the specified amount to be added and make sure to mix it uniformly.

Salinomycin sodium (part 2)

A. Raw material for manufacturing (Part 1)

(a) Compositional standards

Potency: The potency test of this product shows that 300 μg (potency) or more is contained in 1 mg.

Physical and chemical properties: This product is pale brown powder with a specific odor.

Confirmation test:

i. 0.1 g (0.05~0.14 g) of this product is added with 10 mL of methanol, shaken well, and filtered. 2 mL of the filtrate is added with 2 mL of vanillin-hydrochloric acid test solution, shaken, and warmed for 5 minutes. The resulting solution is red.

ii. 30 mg (29.5~30.4 mg) of this product is added with 100 mL of methanol, shaken well, and filtered. 10 mL of the filtrate is added with 1 mL of 1 mol/L hydrochloric acid test solution, shaken well, and allowed to stand at room temperature for 5 minutes. In the measurement of the ultraviolet absorption spectrum of this solution, the absorption maximum is at the wavelengths of 285~300 nm.

iii. 1 g (0.5~1.4 g) of this product is added with 10 mL of methanol, shaken well, and filtered. The filtrate is evaporated to dryness on a water bath. The resulting residue gives the qualitative reaction i. of sodium salt.

Purity test:

i. pH: The pH of a suspension of this product in water (1 → 100) shall be 8.0 to 10.0.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution using method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 μg/g or less).

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared using method No. 3 of the arsenic test method. The arsenic test is performed in the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 μg/g or less)

Loss on drying: 7.0 % or less (1 g, 0.67 kPa or less, 60 °C, 3 hours)

Ignition residue: 45.0 % or less (1 g)

Nitrogen: 0.2~2.0 % (Kjeldahl method)

Crude fat: 47.0~85.0 %

Crude fiber: 2.0 % or less

Potency test:

Agar plate: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Test strain: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of working standard diluent: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with a certain volume of methanol using a volumetric pipette to make its concentration (estimated value) approximately 400 μg/mL (potency), stirred or shaken, and filtered or centrifuged. The filtrate or supernatant is used as a sample stock solution. The volume required for testing of the sample stock solution is measured using a volumetric pipette, and thereafter the provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

(b) Standard of manufacturing method

The Salinomycin producing strain of *Streptomyces albus* is aerobically cultured. After the cultivation, the solids are separated through filtration with adding a filter aid where necessary. The solids are dried, added with silicic acid, light anhydrous silicic acid, or silicic acid anhydride at a ratio within 12% of the mass of the dried solids, and crushed and mixed.

(c) Standard of storage method

It shall be stored in a sealed container.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“サリノマイシンナトリウム（飼料級）”

Salinomycin sodium (feed grade)

B. Raw material for manufacturing (Part 2)

(a) Compositional standards

Potency: The potency test of this product shows that 200 μg (potency) or more is contained in 1 mg.

Physical and chemical properties: This product is pale brown powder or particles with a specific odor.

Confirmation test:

i. 0.1 g (0.05~0.14 g) of this product is added with 10 mL of methanol, shaken well, and filtered. 2 mL of the filtrate is added with 2 mL of vanillin-hydrochloric acid test solution, shaken, and warmed for 5 minutes. The resulting solution is red.

ii. 30 mg (29.5~30.4 mg) of this product is added with 100 mL of methanol, shaken well, and filtered. 10 mL of the filtrate is added with 1 mL of 1 mol/L hydrochloric acid test solution, shaken well, and allowed to stand at room temperature for 5 minutes. In the measurement of the ultraviolet absorption spectrum of this solution, the absorption maximum is at the wavelengths of 285~300 nm.

Purity test:

i. pH: The pH of a suspension of this product in water (1 → 100) shall be 8.0 to 10.0.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution using method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 μg/g or less).

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared using method No. 3 of the arsenic test method. The arsenic test is performed in the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 μg/g or less)

Loss on drying: 7.0 % or less (1 g, 0.67 kPa or less, 60 °C, 3 hours)

Ignition residue: 60.0 % or less (1 g)

Nitrogen: 0.2~2.0 % (Kjeldahl method)

Crude fat: 30.0~40.0 %

Crude fiber: 2.0 % or less

Potency test:

Agar plate: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Test strain: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of working standard diluent: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with a certain volume of methanol using a volumetric pipette to make its concentration (estimated value) approximately 400 μg/mL (potency), stirred or shaken, and filtered or centrifuged. The filtrate or supernatant is used as a sample stock solution. The volume

required for testing of the sample stock solution is measured using a volumetric pipette, and thereafter the provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the Salinomycin producing strain of *Streptomyces albus* is aerobically cultured, and after the cultivation the culture solution is added with sodium carboxymethyl cellulose and calcium carbonate, concentrated, added with light anhydrous silicic acid, and dried.

(c) Standard of storage method

It shall be stored in a sealed container.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

"サリノマイシンナトリウム（飼料級）"

Salinomycin sodium (feed grade)

C. Preparation (Part 1)

(a) Compositional standards

This product is pieces or powder in which fillers are mixed into the raw material for manufacturing (Part 1) of Salinomycin sodium (part 2).

Potency: The potency test of this product shows that 100 μg (potency) or less is contained in 1 mg, and that it contains 85~125 % of potency on the label.

Physical and chemical properties:

i. This product is pale yellowish white to pale brown pieces or powder with a specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. No molding is observed in this product.

Confirmation test:

i. According to the labeled potency of this product, the amount containing approximately 30 mg (potency) of Salinomycin is weighed, added with 10 mL of methanol, shaken, and filtered. 2 mL of the filtrate is measured, and thereafter the confirmation test i. of the raw material for manufacturing of Salinomycin sodium (part 2) is applied mutatis mutandis.

ii. According to the labeled potency of this product, the amount containing approximately 10 mg (potency) of Salinomycin is weighed, added with 100 mL of methanol, shaken, and filtered. 10 mL of the filtrate is measured, and thereafter the confirmation test ii. of

the raw material for manufacturing of Salinomycin sodium (part 2) is applied mutatis mutandis.

iii. 1 g (0.5~1.4 g) of this product is weighed, added with 10 mL of methanol, shaken for 5 minutes, and filtered. The filtrate is pale yellow to yellow.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Test strain: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of working standard diluent: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of sample solution: According to the labeled potency of this product, the amount required for testing is weighed to three significant digits and the value is recorded. It is added with a certain volume of methanol using a volumetric pipette to make its concentration approximately 400 μg/mL (potency), and thereafter the provisions of the raw material for manufacturing of Salinomycin sodium (part 2) are applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing (Part 1) of Salinomycin sodium (part 2) is mixed with carriers.

(c) Standard of storage method

The storage method of the raw material for manufacturing (Part 1) of Salinomycin sodium (part 2) is applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“サリノマイシンナトリウム（飼料級）”

Salinomycin sodium (feed grade)

“有効期間　製造の翌月から２年”

Valid period: 2 years from the month following the manufacture

“使用上の注意　この飼料添加物は、鶏に過剰投与した場合発育障害がおこるので、定められた添加量を厳守するとともに、均一に配合するよう注意すること。”

Precautions: Overdose of this feed additive causes developmental disorders in chicken, so strictly follow the specified amount to be added and make sure to mix it uniformly.

D. Preparation (Part 2)

(a) Compositional standards

This product is pieces, particles or powder in which fillers are mixed into the raw material for manufacturing (Part 2) of Salinomycin sodium (part 2).

Potency: The potency test of this product shows that 200 μg (potency) or less is contained in 1 mg, and that it contains 85~125 % of potency on the label.

Physical and chemical properties:

i. This product is pale yellowish white to pale brown pieces, particles or powder with a specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. No molding is observed in this product.

Confirmation test: The confirmation test of the preparation (Part 1) of Salinomycin sodium (part 2) is applied mutatis mutandis.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Test strain: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of working standard diluent: The provisions of the raw material for manufacturing of Salinomycin sodium (part 1) are applied mutatis mutandis.

Preparation of sample solution: The provisions of the preparation (Part 1) of Salinomycin sodium (part 2) are applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing (Part 2) of Salinomycin sodium (part 2) is mixed with carriers.

(c) Standard of storage method

The storage method of the raw material for manufacturing (Part 2) of Salinomycin sodium (part 2) is applied mutatis mutandis.

(d) Standards of the label

The standards of the label of the preparation (Part 1) of Salinomycin sodium (part 2) are applied mutatis mutandis.

(118) Semduramicin Sodium

A. Raw material for manufacturing

(a) Compositional standards

Potency: This product is sodium salt of semduramicin. The potency test shows that 860 μg (potency) or more is contained in 1 mg.

Physical and chemical properties:

i. This product is white to grayish white crystalline powder.

ii. This product is easy to lightly dissolve in methanol, hard to lightly dissolve in ethanol, hard to dissolves in dichloromethane and ether and hardly dissolves in water and isooctane.

Confirmation test:

i. This product and working reference standard are measured by the potassium bromide disk method of the infrared absorption spectroscopy and their spectra are compared, and then absorption is observed at approximately 3,450 cm^{-1}, 2,972 cm^{-1}, 2,935 cm^{-1}, 1,595 cm^{-1}, 1,460 cm^{-1}, 1,381 cm^{-1}, and 1,063 cm^{-1}.

ii. 5 mg (4.5~5.4 mg) of this product and working reference standard are each weighed, and both are dissolved with 5 mL of methanol to prepare a sample solution and a standard solution, respectively. 5 μL each of the sample and standard solutions are spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Next, they are developed to approximately 10 cm with developing solvent, a mixed solution of ethyl acetate and glacial acetic acid (4:1), and the thin layer plate is air dried. It is then sprayed with a vanillin-sulfuric acid-ethanol test solution and heated at 105 °C for approximately 10 minutes. Then, the main spots obtained from the sample and standard solutions are red-brown and the Rf values of them are equal.

Purity test:

i. Specific rotation: 0.25 g of this product is weighed to the digit of 0.001 g and the value is recorded. It is dissolved with methanol to make 25 mL. The optical rotation of this solution shall be $[\alpha]_D^{20} = +19.0 \sim +23.0°$

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by

the method using device A. The color of the absorbing solution shall not be darker than the standard color (4 mg/kg or less).

iv. Hydroxysemduramicin: Approximately 25 mg of this product of is weighed and dissolved with 25 mL of a mixed solution of ethyl acetate, isooctane, glacial acetic acid and triethylamine (375:125:2:1). This is used as a sample solution. When the sample solution of 50 μL is tested by the liquid chromatography under the following conditions, the peak (the peak appearing when the time 1.5 fold longer than the retention time of the peak of semduramicin has passed) area of hydroxysemduramicin shall be 2.5 % or less of the total area of the peaks obtained from the sample solution.

Operating condition:

Device: Consisting of a mobile phase delivery pump, coloring solution delivery pump, sample injector, column, reaction vessel, detector, and recorder. The column and the reaction vessel shall be maintained at constant temperature for use.

Detector: Ultraviolet absorptiometer (measurement wavelength: 522 nm)

Column: A stainless tube (internal diameter: 4.0 mm, length: about 250 mm) is filled with 5 μm of silica gel.

Column temperature: Constant temperature around 25 °C

Reaction vessel (reaction coil): Tube with internal diameter of approximately 0.3 mm and length of approximately 7.6 m

Mobile phase: Mixed solution of ethyl acetate, isooctane, glacial acetic acid and triethylamine (375:125:2:1)

Coloring solution: A mixture 250 mL of absolute ethanol and 10 mL of sulfuric acid is added in a solution of 15 g (14.5~15.4 g) of vanillin dissolved in 250 mL of absolute ethanol.

Mobile phase flow rate: 0.6 mL/min

Coloring solution flow rate: 0.3 mL/min

Column selection: A column to be used is selected when the solution, which is prepared by the dissolving of the working reference standard and sodium hydroxysemduramicin in the mobile phase and adjusting its concentration to 0.02 mg/mL, is operated under the above conditions and both substances are completely separated.

Detection sensitivity: The peak of semduramicin obtained 50 μL of the sample solution (1 → 50) in the mobile phase is adjusted to 10~40 mm.

Area measurement range: Three times as much as the retention time of semduramichin.

Loss on drying: 2.5 % or less (1 g, decompression, 100 °C, 3 hours)

Ignition residue: 9.0 % or less (1 g)

Potency test:

Agar plate (single layer): 20 mL of buffer No. 19 mixed with the test strain is used (21 mL in a Petri dish with internal diameter of 100 mm). It shall be a perforated agar plate.

Test strain: *Bacillus subtilis* ATCC 6633 is used.

Preparation of working standard diluent: The amount of working standard diluent required for testing is weighed to three significant digits and the value is recorded. It is dissolved with an adequate volume of methanol to make an accurate constant volume at a concentration of approximately 1 mg/mL (potency). This is used as a diluted stock solution. The volume of the diluted stock solution required for testing is measured using a volumetric pipette, accurately diluted with water and methanol to prepare the working standard diluent at a high concentration of 20 μg/mL (potency) with 25 % methanol and the working standard diluent at a low concentration of 5 μg/mL (potency) with 25 % methanol.

Preparation of the sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is dissolved with methanol and is further added with methanol to make an accurate constant volume at a concentration approximately 1 mg/mL (estimated value) (potency). This is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette and accurately diluted with water and methanol to prepare the sample solution at a high concentration of 20 μg/mL (potency) with 25 % methanol and the sample solution at a low concentration of 5 μg/mL (potency) with 25 % methanol.

(b) Standard of manufacturing method

The semduramicin producing strain of *Actinomadura roseorufa* is aerobically cultured, after the cultivation, semduramicin sodium is diluted from concentrated slurry of the culture solution using an organic solvent under alkaline conditions. The crystals obtained from the extract are dried.

(c) Standard of storage method

It shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

This product is pieces or powder of the mixture of the raw material for manufacturing of semduramicin sodium and carriers.

Potency: The potency test shows that 50 μg (potency) or less is contained in 1 mg, and that it contains 85~125 % of the label potency.

Physical and chemical properties:

i. This product is grayish yellow to pale brown small pieces or powder.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test: According to the label potency of this product, the amount containing approximately 50 mg (potency) of semduramycin sodium is weighed, added with 50 mL methanol, vigorous stirred for 30 minutes and centrifuged. The supernatant is used as a sample stock solution. The confirmation test ii. of the raw materials for manufacturing of semduramycin sodium are hereinafter applied mutatis mutandis.

Loss on drying: 12.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate (single layer): The provisions of the raw materials for manufacturing of semduramicin sodium are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of senduramicin sodium are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of senduramicin sodium are applied mutatis mutandis.

Preparation of the sample solution: Where appropriate, this product is ground, according to the label potency, the amount required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of methanol using a volumetric pipette to make its concentration approximately 1 mg/mL (potency), vigorous stirred for 20 minutes and centrifuged. The volume required for testing of the sample stock solution is measured using a volumetric pipette, and the provisions of the raw material for manufacturing of monensin sodium are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

The raw material for manufacturing of semduramicin sodium is mixed with carriers.

(c) Standard of storage method

The storage method of the raw materials for manufacturing of semduramicin sodeium are applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“有効期間 製造の翌月から２年”

Valid period: 2 years from the month following the manufacture

“使用上の注意 この飼料添加物は、鶏に過剰投与した場合発育障害がおこるので、定められた添加量を厳守するとともに、均一に配合するよう注意すること。”

Precautions: Overdose of this feed additive causes developmental disorders in chicken, so strictly follow the specified amount to be added and make sure to mix it uniformly.

(119) Narasin

A. Raw material for manufacturing

(a) Compositional standards

Potency: When this product is conducted potency test, it contains 100 μg (potency) or more in 1 mg.

Physical and chemical properties: This product is grayish brown to dark brown particles.

Confirmation test:

ⅰ. 0.8 g (0.75~0.84 g) of this product is weighed, added with 100 mL of methanol, stirred for 20 minutes, and filtered. The filtrate is used as a sample solution. Separately, the amount of working standard narasin containing approximately 10 mg (potency) of narasin is weighed and dissolved with 10 mL of methanol. This is used as a standard solution. 2 μL each of the sample and standard solutions are spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Next, they are developed to approximately 15 cm with developing solvent, a mixed solution of ethyl acetate and strong ammonia solution (90:1), and the thin layer plate is air dried and is further dried at 105 °C for 10 minutes. It is uniformly sprayed with a vanillin-sulfuric acid-ethanol coloring reagent and heated at 105 °C for approximately 10 minutes. Then the main spots obtained from the sample and standard solutions are bluish purple and the Rf values of them are equal.

ii. 10 mg (9.5~10.4 mg) of this product is weighed, added with 50 mL of methanol (9 → 10), stirred for 20 minutes, and filtered. This solution is further filtered using a membrane filter (0.45 μm) and the filtrate is used as a sample solution. Separately, 5 mg (4.5~5.4 mg) of working standard narasin is weighed and dissolved with 250 mL of methanol (9 → 10). This is used as a standard solution. 200 μL each of the sample and standard solutions are tested by the liquid chromatography under the following operating conditions, and the retention times of the peaks of narasin A obtained from the sample and standard solutions are consistent.

Operating condition:

Device: Consisting of a mobile phase delivery pump, coloring solution delivery pump, sample injector, column, reaction vessel, detector, and recorder. The column and the reaction vessel shall be maintained at constant temperature for use.

Detector: Visible spectrophotometer (measurement wavelength: 520 nm)

Column: A stainless tube internal) diameter: 4.6 mm, length: about 250 mm) is filled with 5 μm of octadecylsilylated silica gel for liquid chromatograph.

Column temperature: Constant temperature around 25 °C

Reaction vessel (reaction coil): Tube with internal diameter approximately 0.5 mm and length of approximately 5.6 m

Mobile phase: Mixed solution of methanol, water and acetic acid (940:60:1)

Coloring solution: Mix 950 mL of methanol and 20 mL of acetic acid, dissolved 30 g (29.5~30.4 g) of vanillin.

Mobile phase flow rate: 0.65 mL/min

Coloring solution flow rate: 0.65 mL/min

Column selection: 5 mg (4.5~5.4 mg) of each working standard monensin and narasin is weighed, added with methanol (9 → 10) up to 250mL. When each 250mL of this solution is operated under the above operating conditions, elution is performed in the order of monensinA, narasinB. Using a solution having a degree of separation of 3.0 or more.

Area measurement range: Two times as much as the retention time of narasinA.

Purity test:

i. Lead: 1.0 g (0.95~1.04 g) of this product is weighed, carbonized at approximately 200 °C for 2 hours, heated from 250 °C up to approximately 450 °C at the rate of temperature increase of 50 °C/hour, and incinerated for two hours with this temperature maintained. After cooling, it is added with 5 mL of nitric acid, covered with a watch glass, and heated on a sand bath and then the watch glass is removed and the product is almost dried. After cooling, it is added with approximately 25 mL of hydrochloric acid (1 → 6) and gently heated for 10 minutes. After cooling, it is transferred to a 100 mL volumetric flask, added with water to the marked line to make 100 mL, and filtered within 30 seconds. This is used as a sample solution. Separately, 1.0 mL of lead standard solution for atomic absorption is measured, transferred to a 100 mL volumetric flask, added with approximately 25 mL of hydrochloric acid (1 → 6), and is further added with water to the marked line to make 100 mL. This is used as a standard solution. 30 mL each of the sample and standard solutions are measured, added with 8 mL of phosphate, 2 mL of potassium iodide solution (6 → 10), and 10 mL of methyl isobutyl ketone using a volumetric pipette, vigorously shaken for

approximately 1 minute, and allowed to stand for a short time. Within 30 seconds, away from direct sunlight, the methyl isobutyl ketone layers obtained from the sample and standard solutions are tested by the atomic absorption spectrophotometry (flame type) at the wavelength of 283.3 nm, using a lead hollow cathode lamp as the light source, acetylene or hydrogen as a flammable gas, and air as a combustion-supporting gas. The absorbance of the methyl isobutyl ketone layer of the sample solution shall be equal to or lower than that of the standard solution (25 mg/kg or lower).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (26 mg/kg or less).However, the volume of arsenic standard solution for preparation of the standard color shall be 13 mL. The color is corresponding to 0.026 mg of arsenic trioxide.

Arsenic standard solution: 20 mL of the arsenic standard solution is measured using a volumetric pipette, transferred to a 1,000 mL volumetric flask, added with 5 mL of dilute sulfuric acid, and then added with newly boiled and cooled water to the 1,000 mL volumetric flask up to the marked line. 1 mL of this solution contains arsenic trioxide 0.002 mg. This solution is prepared when needed and stored in a ground stopper bottle.

iii. Content ratio of narasin A: When 200 μL each of the sample and standard solutions in the confirmation test (2) are tested by the liquid chromatography, under the operating conditions of the confirmation test (2), mutatis mutandis, the content ratio of narasin A shall be 85 % or more.

$$\text{Content ratio of narasin A in this product (\%)} = \frac{A_{T1}}{A_T} \times 100$$

A_{T1}: Peak area of nalasinA in sample solution

A_T: Sum of peak areas of sample solution

Loss on drying: 10.0 % or less (1 g, 0.67kPa or less, 60 °C, 3 hours)

Nitrogen: 2.0 % or less (Kjeldahl method)

Crude fat: 27.0 % or less (2 g)

Crude fiber: 2.0 % or less (2 g)

Potency test:

Agar plate (single layer): 10 mL of the No. 15 medium mixed with the test strain is used (11 mL in a Petri dish with internal diameter of 100 mm).

Test strain: *Bacillus subtilis* ATCC 6633 is used.

Preparation of the working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is dissolved with sodium bicarbonate-methanol reagent, and is further added with sodium bicarbonate-methanol reagent to make a constant volume at a concentration of approximately 1 mg/mL (potency) (estimated value). This is used as a diluted stock solution. The volume required for testing of the diluted stock solution is measured using a volumetric pipette, accurately diluted with water and methanol to prepare the working standard diluent at a high concentration of 5 μg/mL (potency) (estimated value) with 30 % methanol and the working standard diluent at a low concentration of 1.25 μg/mL (potency) (estimated value) with 30 % methanol.

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of methanol (9 → 10) using a volumetric pipette to make its concentration (estimated value) approximately 1 mg/mL (potency), stirred for 20 minutes and filtered. The filtrate is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette, accurately diluted with water and methanol to prepare the sample solution at a high concentration of 5 μg/mL (potency) (estimated value) with 30 % methanol and the sample solution at a low concentration of 1.25 μg/mL (potency) (estimated value) with 30 % methanol.

(b) Standard of manufacturing method

For manufacturing, the narasin producing strain of *Streptomyces aureofaciens* is aerobically cultured, after the cultivation the culture solution is dried, added with bentonite and calcium carbonate, and granulated.

(c) Standard of storage method

It shall be stored in a light resistant sealed container.

B. Preparation (Part 1)

(a) Compositional standards

This product is powder or particles of mixture of the raw material for manufacturing of narasin and carriers.

Potency: When this product is conducted potency test, it contains 100 μg (potency) or less in 1 mg including 85~125 % of the indicated potency.

Physical and chemical properties:

i. This product is grayish brown to brown or yellowish white to yellow powder or particles.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test:

ⅰ. 1.0 g (0.95~1.04 g) of this product is weighed, added with 100 mL of methanol, stirred for 20 minutes, and filtered. The filtrate is used as a sample solution. The confirmation test i. for the raw material for manufacturing of Narasin is applied mutatis mutandis.

ii. The confirmation test ii. for the raw material for manufacturing of Narasin is applied mutatis mutandis.

Loss on drying: 12.0 % or less (1 g, 0.67 kPa or less, 60 °C, 3 hours)

Potency test: The provisions of the raw materials for manufacturing of narasin are applied mutatis mutandis.

Agar plate (single layer): The provisions of the raw materials for manufacturing of narasin are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of narasin are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of narasin are applied mutatis mutandis.

Preparation of sample solution: According to the label potency, the amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of a mixed solution of methanol (9 → 10), using a volumetric pipette to make its concentration approximately 1 mg/mL (potency) (estimated value), stirred for 20 minutes and filtered. The filtrate is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette, then the provisions of the raw materials for manufacturing of narasin are applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of narasin is added with carriers.

(c) Standard of storage method

The standard storage method of the raw materials for manufacturing of narasin are applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“有効期間　製造の翌月から２年”

Valid period: 2 years from the month following the manufacture

“使用上の注意　この飼料添加物は、鶏に過剰投与した場合発育障害がおこるので、定められた添加量を厳守するとともに、均一に配合するよう注意すること。”

Precautions: Overdose of this feed additive causes developmental disorders in chicken, so strictly follow the specified amount to be added and make sure to mix it uniformly.

(120) Nosiheptide

Nosiheptide (part 1)

A. Raw material for manufacturing

(a) Compositional standards

Potency: The potency test shows that 800 μg (potency) or more is contained in 1 mg.

Physical and chemical properties:

i. This product is pale yellow-gray-white to greenish pale yellow-brown crystals or powder without odor or with a slightly specific odor.

ii. This product is easy to slightly dissolve in cyclohexanone, hard to lightly dissolve in tetrahydrofuran, hard to dissolve in acetone and chloroform, is extremely hard to dissolve in ethanol, and hardly dissolves in water.

Confirmation test:

ⅰ. 50 mg (49.5~50.4 mg) of this product is weighed, added with 50 mL of tetrahydrofuran, used as a sample solution. Measure 1 mL of this solution, added with 2 mL of a mixed solution of methanol and ammonia solution (87:13) to 20 mL. In the measurement of the absorption spectrum of this solution, the absorption maximum is at the wavelengths of 405~411 nm.

ii. 10 mg (9.5~10.4 mg) of this product is measured, added with 0.1 g (0.05~0.14 g) of sodium hydroxide, gradually heated to melt. After cooling, it is dissolved with 1 mL of distilled water, and added with 0.3 g (0.25~0.34 g) of zinc powder and 5 mL of hydrochloric acid (1 → 2), and the produced gas discolors wet lead acetate paper to black.

Purity test:

ⅰ. pH: The pH of solution (1 → 1000) of this product shall be 4.5~8.0.

ii. Lead: 2.0 g (1.95~2.04 g) of this product is weighed and heated at 500 °C for 4 hours. After cooling, it is added with 5 mL each of nitric acid and perchloric acid, covered with a watch glass, and heated on a sand bath to transparent, and the watch glass is removed to make it almost dry. After cooling, it is added with approximately 10 mL of dilute nitric acid (1 → 3) and gently heated for 10 minutes. After cooling, it is

transferred to a 20 mL volumetric flask and added with dilute nitric acid (1 → 3) to the marked line to make 20 mL. This is used as a sample solution. Separately, 2.0 mL of lead standard solution for atomic absorption is measured, transferred to a 50 mL volumetric flask, and added with dilute nitric acid (1 → 3) to the marked line to make 50 mL. This is used as a standard solution. When the sample and standard solutions are tested by atomic absorption spectrophotometry (flame type) at the wavelength of 283.3 nm, using a lead hollow cathode lamp as the light source, acetylene or hydrogen as a flammable gas, and air as a combustion-supporting gas, the absorbance of the sample solution shall be equal to or lower than that of the standard solution (10 mg/kg or lower).

iii. Copper: The sample solution obtained in ii. 5 mL is measured by a volumetric pipette, transferred to a 50 mL volumetric flask, and added with dilute nitric acid (1 → 3) to the marked line to make 50 mL. This is used as a sample solution. Separately, 2.0 mL of copper standard solution is measured, transferred to a 50 mL volumetric flask and added with dilute nitric acid (1 → 3) to the marked line to make 50 mL. This is used as a standard solution. When the sample and standard solutions are tested by the atomic absorption spectrophotometry (flame type) at the wavelength of 324.7 nm, using a copper hollow cathode lamp as the light source, acetylene or hydrogen as a flammable gas, and air as a combustion-supporting gas, the absorbance of the sample solution shall be equal to or lower than that of the standard solution (100 mg/kg or lower).

iv. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 7.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 5.0 % or less (1 g)

Potency test:

Agar plate (single layer): 10 mL of the No. 7 medium mixed with the test strain is used (11 mL in a Petri dish with internal diameter of 100 mm).

Test strain: *Micrococcus luteus* ATCC 9341 is used.

Preparation of the working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is dissolved with dimethylformamide, and is further added with dimethylformamide to make a constant volume at a concentration of approximately 1 mg/mL (potency) (estimated value). This is used as a diluted stock solution. The volume required for testing of the diluted stock solution is measured using a

volumetric pipette, accurately diluted with a mixed solution of buffer No. 4 and etanol (85:15) to prepare the working standard diluent at a high concentration of 2 μg/mL (potency) (estimated value) and the working standard diluent at a low concentration of 0.5 μg/mL (potency) (estimated value).

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is dissolved with dimethylformamide and is further added with dimethylformamide to make an accurate constant volume at a concentration of approximately 1 mg/mL (potency) (estimated value). This is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette and accurately diluted with a mixed solution of buffer No. 4 and ethanol (85:15) to prepare the sample solution at a high concentration of 2 μg/mL (potency) (estimated value) and the sample solution at a low concentration of 0.5 μg/mL (potency) (estimated value).

(b) Standard of manufacturing method

For manufacturing, the nosiheptide producing strain of *Streptomyces actuosus* is aerobically cultured, after the cultivation, solids are separated by filtration. Nosiheptide in the solids is extracted with an organic solvent, the extract is concentrated, the concentrate is added with isopropanol, and the resulting precipitation is washed with isopropanol once or twice and then dried.

(c) Standard of storage method

It shall be stored in a light resistant sealed container.

B. Preparation

(a) Compositional standards

This product is powder or particles of mixture of the raw material for manufacturing of nosiheptide (part 1) and carriers.

Potency: When this product is conducted potency test, it contains 85~125 % of the indicated potency.

Physical and chemical properties:

i. This product is pale yellow to light brown powder or particles without odor, or with a slightly specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test:

ⅰ. According to the label potency of this product, the amount containing approximately 50 mg (potency) of nosiheptide is weighed, added 50 mL of tetrahydrofuran, hardly

shaken for 20 minutes, and filtered. The confirmation test i. for the raw material for manufacturing of nosiheptide (part 1) is hereinafter applied mutatis mutandis.

ii. According to the label potency of this product, the amount containing approximately 20 mg (potency) of nosiheptide is weighed, added 50 mL of tetrahydrofuran, hardly shaken for 20 minutes, and filtered. Measure 10 mL of this solution, evaporated to dryness, the confirmation test ii. for the raw material for manufacturing of nosiheptide (part 1) is hereinafter applied mutatis mutandis.

Loss on drying: 15.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate (single layer): The provisions of the raw materials for manufacturing of nosiheptide (part 1) are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of nosiheptide (part 1) are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of nosiheptide (part 1) are applied mutatis mutandis.

Preparation of sample solution: According to the label potency, the amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of a mixed solution of dimethylformamide, using a volumetric pipette to make its concentration approximately 1 mg/mL (potency), vigorously stirred for 20 minutes and filtered or centrifuged. The filtrate is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette, then the provisions of the raw materials for manufacturing of nosiheptide (part 1) are applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of nosiheptide is added with carriers.

(c) Standard of storage method

The standard storage method of the raw materials for manufacturing of nosiheptide are applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“有効期間　製造の翌月から２年”

Validity period: 2 years from the month following production

Nosiheptide (part 2)

A. Raw material for manufacturing

(a) Compositional standards

Potency: The potency test shows that 65 μg (potency) or more is contained in 1 mg.

Physical and chemical properties: This product is yellowish-brown to dark brown powder or particle with a specific odor.

Confirmation test:

i. 0.5 g (0.45~0.54 g) of this product is weighed, added with 50 mL of a mixed solution of tetrahydrofuran and water (80:20), vigorously shaken for 20 minutes, and filtered. This filtrate is used as a sample solution. 1 mL of this sample solution is measured and added with a mixed solution of methanol and ammonia (87:13) to make 20 mL. In the measurement of the absorption spectrum, the absorption maximum of this solution is at the wavelengths of 405~411 nm.

ii. 0.1 g (0.05~0.14 g) of this product is measured, added with 0.1 g (0.05~0.14 g) of sodium hydroxide, gradually heated to melt. After cooling, it is dissolved with 1 mL of distilled water, and added with 0.3 g (0.25~0.34 g) of zinc powder and 5 mL of hydrochloric acid (1 → 2), and the produced gas discolors wet lead acetate paper to black.

Purity test:

i. pH: The pH of solution (1 → 20) of this product shall be 4.5~8.0.

ii. Lead: 2.0 g (1.95~2.04 g) of this product is weighed and heated at 500 °C for 4 hours. After cooling, it is added with 5 mL of nitric acid and 5 mL of perchloric acid, covered with a watch glass, and heated on a sand bath to transparent, and removed the watch glass to make it almost dry. After cooling, it is added with appropriately 10 mL of dilute nitric acid (1 → 3) and gently heated for 10 minutes. After cooling, it is transferred to a 20 mL volumetric flask and added with dilute nitric acid (1 → 3) to the 20 mL volumetric flask up to the marked line. This is used as a sample solution. Separately, 1.0 mL of lead standard solution for atomic absorption is measured, transferred to a 50 mL volumetric flask, and added with dilute nitric acid (1 → 3) to the 50 mL volumetric flask up to the marked line. This is used as a standard solution. When the sample and standard solutions are tested by the atomic absorption spectrophotometry (flame type) at the wavelength of 283.3 nm, using a lead hollow cathode lamp as the light source, acetylene or hydrogen as a flammable gas, and air as a combustion-supporting gas, the absorbance of the sample solution shall be equal to or lower than that of the standard solution (5 mg/kg or lower).

iii. Copper: The sample solution obtained in ii. 5 mL is measured by a volumetric pipette, transferred to a 50 mL volumetric flask, and added with dilute nitric acid (1 → 3) to the 50 mL volumetric flask up to the marked line. This is used as a sample solution. Separately, 2.0 mL of copper standard solution is measured, transferred to a 50 mL volumetric flask and added with dilute nitric acid (1 → 3) to the 50 mL volumetric flask up to the marked line. This is used as a standard solution. When the sample and standard solutions are tested by the atomic absorption spectrophotometry (flame type) at the wavelength of 324.7 nm, using a copper hollow cathode lamp as the light source, acetylene or hydrogen as a flammable gas, and air as a combustion-supporting gas, the absorbance of the sample solution shall be equal to or lower than that of the standard solution (10 mg/kg or lower).

iv. Arsenic: 1.0 g (0.95~1.04 g) of this product is transferred to a dissolution flask, added with 10 mL of nitric acid and 5 mL of sulfuric acid, and gently heated. If the solution remains brown, it is allowed to cool, is added with 1~2 mL of nitric acid, and heated. This procedure is repeated until the solution is colorless to pale yellow. After cooling, it is added with 0.5 mL of perchloric acid and heated until white smoke emerges. After cooling, it is added with 15 mL of saturated ammonium oxalate and heated until white smoke emerges. After cooling, it is added with water to make approximately 10 mL and this is used as a sample solution. When the sample solution is tested for arsenic using device A, the color of the absorbing solution shall not be darker than standard color (2 mg/kg or lower).

Loss on drying: 13.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 20.0 % or less (0.5 g)

Nitrogen: 8.0 % or less (Kjeldahl method)

Crude fat: 22.0 % or less

Crude fiber: 6.0 % or less

Potency test:

Agar plate (single layer): The provisions of the raw materials for manufacturing of nosiheptide (part 1) are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of nosiheptide (part1) are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of nosiheptide (part 1) are applied mutatis mutandis.

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is dissolved with a mixed solution of dimethylformamide and buffer No. 4 (80:20) and is further added

with a mixed solution of dimethylformamide and buffer No. 4 (80:20) to make an accurate constant volume at a concentration of approximately 1 mg/mL (potency) (estimated value), is vigorously shaken for 20 minutes, and filtered or centrifuged. This is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette. The provisions of the raw material for manufacturing of nosiheptide (part 1) are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

For manufactuaring, the nosiheptide producing strain of *Streptomyces actuosus* is aerobically cultured, and after the cultivation, dried and crushed.

(c) Standard of storage method

It shall be stored in a light resistant sealed container.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“ノシヘプタイド（飼料級）”

Nosiheptide (feed grade)

B. Preparation

(a) Compositional standards

This product is powder or particles of mixture of the raw material for manufacturing of nosiheptide (part 2) and carriers.

Potency: When this product is conducted potency test, it contains 85~125 % of the indicated potency.

Physical and chemical properties:

i. This product is light brown to brown powder or particles with a slightly specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test:

i. According to the label potency of this product, the amount containing approximately 50 mg (potency) of nosiheptide is weighed, and the confirmation test i. for the raw material for manufacturing of nosiheptide (part 2) is hereinafter applied mutatis mutandis.

ii. According to the label potency of this product, the amount containing approximately 20 mg (potency) of nosiheptide is weighed, added 20 mL of a mixed solution of tetrahydrofuran and water (80:20), vigorously shaken for 20 minutes, and filtered. The filterer is used as a sample solution. Measure 10mL of this solution, evaporated to

dryness, the confirmation test ii. for the raw material for manufacturing of nosiheptide (part 2) is hereinafter applied mutatis mutandis.

Loss on drying: 15.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate (single layer): The provisions of the raw materials for manufacturing of nosiheptide (part 2) are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of nosiheptide (part 2) are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of nosiheptide (part 2) are applied mutatis mutandis.

Preparation of sample solution: According to the label potency of this product, the amount required for testing is measured to three significant digits and the value is recorded. It is added with a constant volume of a mixed solution of dimethylformamide and buffer No. 4 (80:20) using a volumetric pipette to make its concentration approximately 1 mg/mL (potency), vigorously shaken for 20 minutes, and filtered or centrifuged. This is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette. The provisions of the raw material for manufacturing of nosiheptide (part 2) are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of nosiheptide (part2) is added with carriers.

(c) Standard of storage method

The standard storage method of the raw materials for manufacturing of nosiheptide (part 2) are applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“ノシヘプタイド（飼料級）”

Nosiheptide (feed grade)

“有効期間　製造の翌月から２年”

Validity period: 2 years from the month following production

(121) Bicozamycin

A. Raw material for manufacturing

(a) Compositional standards

Potency: When this product is conducted potency test, it contains 850μg (potency) or more in 1mg.

Physical and chemical properties:

i. This product is white to brownish white crystals or powder without odor or with a slightly specific odor.

ii. This product is easy to dissolve in water, slightly easy to dissolve in methanol, hard to dissolve in acetone or etanol, and hardly dissolves in ether or chloroform.

Confirmation test:

i. This product in the solution (1 → 100) 10 mL is added with 1 mL of ninhydrin reagent and gently shaken in a water bath for 15 minutes. The resulting solution is purple.

ii. The solution of this product (1 → 10) of 10 mL is gently added with anthrone test solution, and the color of interface is yellow-brown.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Loss on drying: 6.0 % or less (1 g, reduced pressures, 60 °C, 3 hours)

Ignition residue: 0.5 % or less (1 g)

Potency test:

Agar plate: Medium No. 14 is used for both the media of base and inoculated layers.

Test strain: *Escherichia coli* ATCC 27166 is used.

Preparation of working standard diluent: The amount of the working reference standard required for testing is measured to three significant digits and the value is recorded. It is dissolved with buffer No. 3 and is further added with buffer No. 3 to make an accurate constant volume at a concentration of appropriately 1 mg/mL (potency). This is used as a diluted stock solution. The volume required for testing of the diluted stock solution is measured using a volumetric pipette and accurately diluted with buffer No.

3 to prepare the working standard diluent at a high concentration of 120 μg/mL (potency) and the working standard diluent at a low concentration of 30 μg/mL (potency).

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is dissolved with buffer No. 3 and is further added with buffer No. 3 to make an accurate constant volume at a concentration of approximately 1 mg/mL (potency) (estimated value). This is used as a sample stock solution. The volume of the sample stock solution required for testing is measured using a volumetric pipette, accurately diluted with buffer No. 3 to prepare the sample solution at a high concentration of 120 μg/mL (potency) (estimated value) and the sample solution at a low concentration of 30 μg/mL (potency) (estimated value).

(b) Standard of manufacturing method

For manufacturing, the bicozamycin producing strain of *Streptomyces griseoflavus* is aerobically cultured, after the cultivation the bacterial cells are removed. Next, bicozamycin in this culture solution is adsorbed on a resin, eluted using a hydrous organic solvent, and the eluate is concentrated to crystallize, or the culture filtrate is purified using ion exchange resin, the purified solution is concentrated to crystallize.

(c) Standard of storage method

It shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

This product is powder or particles of mixture of the raw material for manufacturing of bicozamycin and carriers.

Potency: When this product is conducted potency test, it contains 85~125 % of the indicated potency.

Physical and chemical properties:

i. This product is white to light-brown powder or particles without odor, or with a slightly specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test:

i. According to the label potency of this product, the volume containing approximately 100 mg (potency) of bicozamycin is weighed, added with 40 mL of a mixed solution of chloroform and methanol (2:1), shaken well for 30 minutes, and filtered. The insoluble matter on the filter is washed twice with 5 mL of a mixed solution of chloroform and

methanol (2:1) each, the filtrate and the washings are mixed, and the solvent is distilled off under reduced pressure. The residue is added with 10 mL of water and 5 mL of chloroform, shaken well for 1 minute, and if necessary centrifuged. The aqueous layer is used as a sample solution. This sample solution of 5 mL is measured, added with 0.5 mL of ninhydrin test solution, heated in a water bath for 15 minutes, and then the resulting solution is purple.

ii. The amount of the working standard bicozamycin containing approximately 50 mg (potency) of bicozamycin is weighed and dissolved with 5 mL of water. This is used as a standard solution. 5 μg each of the sample and standard solutions of (i) are spotted on a thin layer plate prepared using silica gel for thin-layer chromatography. Next, they are developed to approximately 10 cm with the developing solvent, a mixed solution of ethyl acetate, methyl ethyl ketone, formic acid and water (5:3:1:1), and the thin layer plate is air dried. It is heated at 80 °C for 30 minutes, allowed to cool, and evenly sprayed with alkaline blue tetrazolium test solution, the spots obtained from the sample and standard solutions are purple, and the Rf values of them are the equal.

Loss on drying: 15.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate (single layer): The provisions of the raw materials for manufacturing of bicozamycin are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of bicozamycin are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of bicozamycin are applied mutatis mutandis.

Preparation of sample solution: According to the label potency of this product, the amount required for testing is weighed to three significant digits and the value is recorded. It is added with the adequate amount of chloroform, added with a constant volume of buffer No. 3 using a volumetric pipette to make its concentration 1~10 mg/mL (potency), vigorously shaken, and if necessary filtered or centrifuged. The aqueous layer is used as a sample stock solution. The volume required for testing of the sample stock solution is measured using a volumetric pipette, and the provisions of the raw material for manufacturing of bicozamycin are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of bicozamycin is added with carriers.

(c) Standard of storage method

It shall be stored in a sealed container.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“有効期間　製造の翌月から２年”

Validity period: 2 years from the month following production

(122) Flavophospholipol

A. Raw material for manufacturing (Part 1)

(a) Compositional standards

Potency: When this product is conducted potency test, it contains 40 μg (potency) or more in 1 mg.

Physical and chemical properties: This product is brown to dark brown powder with specific odor.

Confirmation test: 0.1 g (0.05~0.14 g) of this product is weighed, added with 1 mL of methanol (1 → 2), shaken well, and centrifuged. The supernatant is used as a sample solution. Separately, 10 mL (9.5~10.4 mg) of the working standard flavophospholipol is weighed and dissolved with 5 mL of methanol. This is used as a standard solution. 0.1 mL each of the sample and standard solutions are spotted on a thin layer plate prepared using silica gel with a fluorescent agent for thin-layer chromatography. Next, they are developed with the developing solvent, a mixed solution of isopropanol and strong ammonia solution (65:35), for 4 hours and the thin layer plate is air dried. When it is irradiated with ultraviolet light (dominant wavelength: 254 nm), the Rf values of spots of the sample and standard solutions are equal.

Purity test:

ⅰ. pH: The pH of solution (1 → 1000) of this product shall be 4.5~8.0.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (30 mg/kg or less).

iii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by

the method using device A. The color of the absorbing solution shall not be darker than the standard color (5 mg/kg or less).

Loss on drying: 4.0 % or less (1 g, 0.67 kPa or less, 3 hours)

Ignition residue: 20.0 % or less (1 g)

Nitrogen: 3.5~4.5 % (Kjeldahl method)

Crude fat: 22.0 % or less

Crude fiber: 4.0 % or less

Potency test:

Agar plate: Medium No. 12 is used for both the media of base and inoculated layers. However, the medium of inoculated layer is dissolved and mixed with 1 mg of methylene blue and 40 mg of boric acid per 100 mL of medium.

Test strain: *Bacillus cereus* ATCC 19637 is used.

Preparation of working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is dissolved with methanol (1 → 2), and is further added with methanol (1 → 2) to make a constant volume at a concentration of approximately 1 mg/mL (potency). This is used as a diluted stock solution. The volume required for testing of the diluted stock solution is measured using a volumetric pipette. After diluting this solution 10 times with methanol (1 → 2), the volume of this solution is measured using a volumetric pipette, accurately diluted with buffer No.7 to prepare the working standard diluent at a high concentration and at a low concentration.

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of methanol (1 → 2) using a volumetric pipette to make its concentration (estimated value) approximately 100 μg/mL (potency), and stirred well. It is adjusted to pH 8.0 with 1 mol/L sodium hydroxide solution, attached a reflux condenser, and heated at 85 °C for 15 minutes. After cooling, it is adjusted to pH 7.0 with hydrochloric acid solution. It is added small amount of methanol (1 → 2) to make exactly the constant volume using a volumetric flask. The supernatant solution is used as the sample stock solution.The volume of the sample stock solution required for testing is measured using a volumetric pipette, accurately diluted with buffer No.7 to prepare the sample solution at a high concentration of 6 μg/mL (potency) (estimated value) and the sample solution at a low concentration of 1.5 μg/mL (potency) (estimated value).

(b) Standard of manufacturing method

For manufacturing, the flavophospholipol producing strain of *Streptomyces bambergiensis, Streptomyces ghanaensis, Streptomyces geyseriensis* or *Streptomyces ederensis* is aerobically cultured, after the cultivation, the culture solution is dried

(c) Standard of storage method

It shall be stored in a light resistant sealed container.

B. Preparation (Part 2)

(a) Compositional standards

Potency: When this product is conducted potency test, it contains 100 μg (potency) or more in 1 mg.

Physical and chemical properties: This product is brown to dark brown powder with specific odor.

Confirmation test: 0.1 g (0.05~0.14 g) of this product is weighed, added with 1 mL of methanol (1 → 2), shaken well, and centrifuged. The supernatant is used as a sample solution. Separately, 10 mL (9.5~10.4 mg) of the working standard flavophospholipol is weighed and dissolved with 5 mL of methanol. This is used as a standard solution. 0.1 mL each of the sample and standard solutions are spotted on a thin layer plate prepared using silica gel with a fluorescent agent for thin-layer chromatography. Next, they are developed with the developing solvent, a mixed solution of isopropanol and strong ammonia solution (65:35), for 4 hours or more and the thin layer plate is air dried. When it is irradiated with ultraviolet light (dominant wavelength: 254 nm), the Rf values of spots of the sample and standard solutions are equal.

Purity test:

i. pH: The pH of solution (1 → 50) of this product shall be 8.0~9.5.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 3.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (30 mg/kg or less).

iii. Arsenic: 0.40 g (0.395~0.404 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (5 mg/kg or less).

Loss on drying: 10.0 % or less (1 g, 0.67 kPa or less, 60 °C, 3 hours)

Ignition residue: 40.0 % or less (1 g)

Nitrogen: 2.5~4.5 % (Kjeldahl method)

Crude fat: 22.0 % or less

Crude fiber: 4.0 % or less

Potency test:

Agar plate: Medium No. 12 is used for both the media of base and inoculated layers. However, the medium of inoculated layer is dissolved and mixed with 1 mg of methylene blue and 40 mg of boric acid per 100 mL of medium.

Test strain: *Bacillus cereus* ATCC 19637 is used.

Preparation of working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is dissolved with methanol (1 → 2), and is further added with methanol (1 → 2) to make a constant volume at a concentration of approximately 1 mg/mL (potency). This is used as a diluted stock solution. The volume required for testing of the diluted stock solution is measured using a volumetric pipette. After diluting this solution 10 times with methanol (1 → 2), the volume of this solution is measured using a volumetric pipette, accurately diluted with buffer No.7 to prepare the working standard diluent at a high concentration of 6 μg/mL (potency) and the working standard diluent at a low concentration of 1.5 μg/mL (potency).

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of methanol (1 → 2) using a volumetric pipette to make its concentration (estimated value) approximately 100 μg/mL (potency), and stirred well. It is adjusted to pH 8.0 with 1 mol/L hydrochloric acid solution, attached a reflux condenser, and heated at 85 °C for 15 minutes. After cooling, it is adjusted to pH 7.0 with hydrochloric acid solution. It is added small amount of methanol (1 → 2) to make exactly the constant volume using a volumetric flask. The supernatant solution is used as the sample stock solution.The volume of the sample stock solution required for testing is measured using a volumetric pipette, accurately diluted with buffer No.7 to prepare the sample solution at a high concentration of 6 μg/mL (potency) (estimated value) and the sample solution at a low concentration of 1.5 μg/mL (potency) (estimated value).

(b) Standard of manufacturing method

For manufacturing, the flavophospholipol producing strain of *Streptomyces ghanaensis* is aerobically cultured, after the cultivation the culture solution is concentrated, added with light anhydrous silicic acid, and dried.

(c) Standard of storage method

It shall be stored in a light resistant sealed container.

C. Preparation

(a) Compositional standards

This product is powder of mixture of the raw material for manufacturing of flavophospholipol and carriers.

Potency: When this product is conducted potency test, it contains 85~125 % of the indicated potency.

Physical and chemical properties:

i. This product is brown powder with a slightly specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test: According to the label potency of this product, the volume containing approximately 5 mg (potency) of flavophospholipol is weighed, added with 1 mL of methanol (1 → 2), and the provisions of the raw material for manufacturing of bicozamycin are hereinafter applied mutatis mutandis.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate: The provisions of the raw materials for manufacturing of flavophospholipol are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of flavophospholipol are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of flavophospholipol are applied mutatis mutandis.

Preparation of sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of methanol (1 → 2) to make its concentration (estimated value) approximately 100 μg/mL (potency), and stirred well. The provisions of the raw materials for manufacturing of flavophospholipol are applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of flavophospholipol is mixed with carriers, and as appropriate particle-seized and sieved.

(c) Standard of storage method

It shall be stored in a light resistant sealed container.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“有効期間　製造の翌月から２年”

Validity period: 2 years from the month following production

(123) Monensin Sodium

A. Raw material for manufacturing

(a) Compositional standards

Potency: This product is sodium salt of monensin. The potency test shows that 800 μg (potency) or more is contained in 1 mg.

Physical and chemical properties:

i. This product is slightly pale brown-white to pale orange-yellow powder or crystalline powder with a slightly specific odor.

ii. This product is easy to dissolve in ethanol and chloroform, hard to lightly dissolve in acetone and hardly dissolves in water.

Confirmation test:

i. This product in methanol solution (1 → 1,000) 5 mL is added with 5 mL of vanillin-hydrochloric acid test solution and gently shaken. The resulting solution is red.

ii. This product in methanol solution (1 → 1,000) 5 mL is added with 5 mL of *p*-dimethylaminobenzaldehyde-ethanol-sulfuric acid test solution and heated in water bath at 70 ℃ for 5 minutes. The resulting solution is blue.

iii. This product 3 g (2.5~3.4 g) is added with 20 mL of methanol and 2 mL of diluted hydrochloric acid, gently boiled in water bath for 5 minutes, and added with 3 mL of water, cooled and filtered. After almost evaporating the filtrate, added with a small amount of ethanol, and evaporate to dryness. A solution obtained by adding 10 mL of water to the residue and dissolving it shows a qualitative reaction of sodium salt.

Purity test:

i. pH: 0.1 g (0.05~0.14 g) of this product is weighed, added with 100 mL of methanol (9 → 10). The pH of this solution shall be 6.5~9.5.

ii. Potency ratio of monensin A: According to the results of the potency test, the amount of this product containing approximately 50 mg (potency) of monensin is weighed to three significant digits and the value is recorded. It is dissolved with methanol, and is further added with methanol to make an accurate constant volume at a concentration of approximately 1 mg/mL (potency). This is used as a sample stock solution. The volume required for testing of the sample stock solution using a volumetric pipette and added with methanol to an accurate constant volume to prepare the sample solution at a high concentration of approximately 100 μg/mL (potency) and the sample solution at a low concentration of approximately 10 μg/mL (potency). Separately, in the same

manner, working standard monensin is weighed to prepare the solutions of 100 μg/mL (potency), 10 μg/mL (potency), 5 μg/mL (potency), and 2.5 μg/mL (potency). They are used as standard solutions S_1, S_2, S_3, and S_4.

The base line is set at the height of approximately 3 cm from the lower end of a layer plate prepared using silica gel for thin-layer chromatography. The sample solution at a high concentration is spotted on the base line approximately 3 cm or more away from the edge, the sample solution at low concentration is spotted on the place 2.5 cm or more away from the place of the high concentration solution, and 20 μg each of the standard solutions are spotted at 2 cm or more intervals, and they are air dried. Next, they are developed using ethyl acetate as the developing solvent at room temperature. When the tip of the developing solvent reaches approximately 15 cm from the base line, the thin layer plate is taken out and air dried, and it is developed and air dried well again in the same way.

The surface of this thin layer plate is uniformly sprayed with the medium dissolved in advance by heating in a water bath. Within 30 seconds, the thin layer plate agar side up is put in a culture box, gently added with an adequate volume of the medium with spore fluid maintained at 50~60 °C, and then uniformly spread. Then, it is solidified in the horizontal culture box at room temperature, and cultured at 35~37 °C for 16~18 hours. The Rf values of the maximum inhibition zone (monensin A) and the second maximum inhibition zone (monensin B) obtained from the sample solution are equal to those obtained from the standard solution, and shall be 90 % or over when the potency ratio of monensin A is determined.

Medium: 2.5 g (2.45~2.54 g) of yeast extract, 10 g (9.5~10.4 g) of glucose, 10 g (9.5~10.4 g) of magnesium chloride, and 15 g (14.5~15.4 g) of agar are weighed, added with water to make 1,000 mL, and sterilized (pH 4.8~5.2).

Preparation of spore fluid: *Bacillus subtilis* ATCC 6633 is used as a test strain. The spore fluid is prepared by the potency test specified in General Tests.

Measurement: As for monensin A in the standard solution S_1, S_2, S_3, and S_4, monensin A in the sample solution at a low concentration, monensin B in the sample solution at a high concentration, and other trace components, C_1, C_2… and C_n, diameters in the long and short axial directions of the inhibition zones of all these components are measured to the 0.1 mm with a caliper. The mean of the values is recognized as a diameter of the inhibition zone. To prepare the standard curve, the potencies of monensin in the standard solution S_2, S_3, and S_4, are plotted on the logarithmic axis and the diameters of the inhibition zones of monensin A in the standard solutions are plotted on the

integral axis. The potency of monensin A is measured using the standard curve as for monensin A, B, C_1, C_2… and C_n, in the sample solutions.

Calculation: The potency ratio of monensin A is calculated by the following formula.

$$\text{Potency ratio of monensin A (\%)} = \frac{10P_A}{10P_A + P_B + P_{C1} \ldots\ldots + P_{Cn}} \times 100$$

P_A: Potency of monensin A in the sample solution at a low concentration

P_B: Potency of monensin B in the sample solution at a high concentration as monensin A

P_{C1} to C_n: Potency of trace components C_1~C_n in the sample solution at a high concentration as monensin A

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by method No. 3 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Loss on drying: 4.0 % or less (1 g, 0.67 kPa or less, 60 °C, 3 hours)

Ignition residue: 8.0~13.0 % (1 g)

Potency test:

Agar plate (single layer): 10 mL of the No. 14 medium mixed with the test strain is used (11 mL in a Petri dish with internal diameter of 100 mm).

Test strain: *Bacillus subtilis* ATCC 6633 is used.

Preparation of the working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is dissolved with methanol, and is further added with methanol to make a constant volume at a concentration of approximately 1 mg/mL (potency). This is used as a diluted stock solution. The volume required for testing of the diluted stock solution is measured using a volumetric pipette, accurately diluted with water and methanol to prepare the working standard diluent at a high concentration of 40 µg/mL (potency) with 10 % methanol and the working standard diluent at a low concentration of 10 µg/mL (potency) with 10 % methanol.

Preparation of sample solution: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is

dissolved with methanol, and is further added with methanol to make a constant volume at a concentration (estimated value) of approximately 1 mg/mL (potency). This is used as a sample stock solution. The volume required for testing of the sample stock solution is measured using a volumetric pipette, accurately diluted with water and methanol to prepare the sample solution at a high concentration of 40 μg/mL (potency) with 10 % methanol and the sample solution at a low concentration of 10 μg/mL (potency) with 10 % methanol.

(b) Standard of manufacturing method

For manufacturing: The monensin producing strain of *Streptomyces cinnamonensis* is aerobically cultured, after the cultivation the pH of the culture solution is adjusted and monensin is extracted with an organic solvent. The solution is concentrated and filtered and crystallized as sodium salt under alkaline conditions. The obtained crystals are washed and dried.

(c) Standard of storage method

It shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

This product is small pieces, powder or particles of mixture of the raw material for manufacturing of monensin sodium and carriers.

Potency: When this product is conducted potency test, it contains 240 μg (potency) or less in 1 mg including 85~125 % of the indicated potency.

Physical and chemical properties:

i. This product is light grayish brown to brown or yellowish white to yellow small pieces, powder or particles with a slightly specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test:

i. According to the label potency of this product, the amount containing approximately 10 mg (potency) of monensin is weighed, added with 10 mL of methanol, shaken and filtered. This solution 5 mL is measured, and the confirmation test i. of the raw material for manufacturing of monensin sodium is hereinafter applied mutatis mutandis.

ii. According to the label potency of this product, the amount containing approximately 10 mg (potency) of monensin is weighed, added with 10 mL of ethanol, shaken and filtered. This solution 5 mL is measured, and the confirmation test ii. of the raw

material for manufacturing of monensin sodium is hereinafter applied mutatis mutandis.

Loss on drying: 12.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate: The provisions of the raw materials for manufacturing of monensin sodium are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of monensin sodium are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of monensin sodium are applied mutatis mutandis.

Preparation of sample solution: According to the label potency of this product, the amount required for testing is weighed to three significant digits and the value is recorded. It is added with methanol using a volumetric pipette to make its concentration of 1~2 mg/mL (potency), stirred or shaken, and then filtered or centrifuged. The filtrate or supernatant is used as a sample stock solution. The volume required for testing of the sample stock solution is measured using a volumetric pipette, and the provisions of the raw material for manufacturing of monensin sodium are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the raw material for manufacturing of monensin sodium is mixed with carriers, and as appropriate granulated.

(c) Standard of storage method

The provisions of the raw materials for manufacturing of monensin sodium are applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“有効期間　製造の翌月から２年”

Valid period: 2 years from the month following the manufacture

“使用上の注意　この飼料添加物は、鶏又は牛に過剰投与した場合発育障害がおこるので、定められた添加量を厳守するとともに、均一に配合するよう注意すること。”

Precautions: Overdose of this feed additive causes developmental disorders in chicken or cattle, so strictly follow the specified amount to be added and make sure to mix it uniformly.

(124) Lasalocid Sodium

A. Raw material for manufacturing

(a) Compositional standards

Potency: This product is sodium salt of lasalocid. The potency test shows that 800 μg (potency) or more is contained in 1 mg.

Physical and chemical properties:

i. This product is white to band brown white powder with a specific odor.

ii. This product is hard to dissolve in acetone, ethanol, choloroform, ethyl acetate or methanol and hardly dissolves in water.

Confirmation test:

i. 10 mL of absolute ethanol solution of this product (1 → 500) is shaken with 0.5 mL of absolute ethanol solution of ferric chloride (1 → 50). The resulting solution is reddish violet.

ii. 75 mg (74.5~75.4 mg) of this product is weighed and dissolved with a mixed solution of sulfuric acid and absolute ethanol (3: 1,000) to make 50 mL. 5 mL of this solution is measured and added with a mixed solution of sulfuric acid and absolute ethanol (3: 1,000) to make 100 mL. In the measurement of the absorption spectrum of the solution, the absorption maximum is at the wavelengths of 245~249 nm and 315~319 nm, the absorption minimum is at the wavelengths of 269~273 nm.

iii. The ethyl acetate solution of this product (1 → 200) irradiated with ultraviolet light (dominant wavelength: 365 nm) exhibits blue fluorescence.

iv. 3 g (2.5~3.4 g) of this product is ashed, shaken with 10 mL of water, and filtered. The resulting solution gives the qualitative reaction of sodium salt.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed to prepare a sample solution by method No. 2 of the heavy metals test method. When heavy metal is tested using the sample solution and a control solution prepared using 2.0 mL of lead standard solution, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

ii. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed and the sample solution is prepared by method No. 3 of the arsenic test method. The arsenic test is performed by the method using device A. The color of the absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Loss on drying: 3.0 % or less (1 g, 0.67 kPa or less, 60 °C, 3 hours)

Ignition residue: 10.0~15.0 % (1 g)

Potency test:

Agar plate: Medium No. 14 is used for both the media of base and inoculated layers.

Test strain: *Bacillus subtilis* ATCC 6633 is used.

Preparation of the working standard diluent: The amount of the working reference standard required for testing is weighed to three significant digits and the value is recorded. It is dissolved with methanol, and is further added with methanol to make a constant volume at a concentration of 1 mg/mL (potency). This is used as a diluted stock solution. The volume required for testing of the diluted stock solution is measured using a volumetric pipette and accurately diluted by adding water and methanol to prepare the working standard diluent at a high concentration of 40 μg/mL (potency) with 10 % methanol and the working standard diluent at a low concentration of 10 μg/mL (potency) with 10 % methanol.

Preparation of the sample solution: The amount of this product required for testing is weighed to three significant digits and the value is recorded. It is dissolved with methanol to make an accurate constant volume with the concentration (estimated value) of approximately 1 mg/mL (potency). This is used as a sample stock solution. The volume required for testing of the sample stock solution is measured using a volumetric pipette and accurately diluted by adding water and methanol to prepare the sample solution at a high concentration (estimated value) of 40 μg/mL (potency) with 10 % methanol and the sample solution at a low concentration (estimated value) of 10 μg/mL (potency) with 10 % methanol.

(b) Standard of manufacturing method

The lasalocid producing strain of *Streptomyces lasaliensis* is aerobically cultured, after the cultivation, the culture solution is added with a small amount of a filter aid, and the solids are separated by filtration. Lasalocid in the solids is extracted using an organic solvent, and the extract is concentrated, dissolved with a different organic solvent, washed, and filtered and crystallized as a sodium salt under alkaline conditions. The resulting crystals are washed and dried.

(c) Standard of storage method

It shall be stored in a sealed container.

B. Preparation

(a) Compositional standards

This product is powder or particles in which the raw material for manufacturing lasalocid sodium is mixed with carriers.

Potency: The potency test shows that 150 μg (potency) or less is contained in 1 mg, and that it contains 85~125 % of the label potency.

Physical and chemical properties:

i. This product is pale brown to brown small powder or particles with a specific odor.

ii. This product passes through a standard 2.00 mm mesh sieve.

iii. There is no mold in this product.

Confirmation test:

i. According to the label potency of this product, the amount containing approximately 100 mg (potency) of lasalocid sodium is weighed, added with 50 mL of absolute ethanol, shaken well for 10 minutes, and filtered. The filtrate 10 mL is shaken with a drop of absolute ethanol solution of ferric chloride (1 → 50), and the resulting solution is reddish violet.

ii. According to the label potency of this product, the amount containing approximately 20 mg (potency) of lasalocid sodium is weighed, added with 10 mL of methanol, shaken well for 10 minutes, and filtered. The filtrate is used as a sample solution. Separately, the amount of working standard lasalocid containing approximately 20 mg (potency) of lasalocid sodium is weighed, dissolved with 10 mL of methanol. This is used as a standard solution. 10 μL each of the sample and standard solutions are spotted on a thin layer plate prepared using silica gel with a fluorescent agent for thin-layer chromatography. Next, they are developed to approximately 10 cm using a mixed solution of chloroform and methanol (9:1) as the developing solvent and the thin layer plate is air-dried. When it is irradiated with ultraviolet light (dominant wavelength: 254 nm), the spots of the sample and standard solutions are dark-blue and the Rf values are equal.

Loss on drying: 15.0 % or less (1 g, 105 °C, 3 hours)

Potency test:

Agar plate: The provisions of the raw materials for manufacturing of lasalocid sodium are applied mutatis mutandis.

Test strain: The provisions of the raw materials for manufacturing of lasalocid sodium are applied mutatis mutandis.

Preparation of the working standard diluent: The provisions of the raw materials for manufacturing of lasalocid sodium are applied mutatis mutandis.

Preparation of sample solution: According to the label potency of this product, the amount required for testing is weighed to three significant digits and the value is recorded. It is added with a constant volume of methanol (9 → 10) using a volumetric pipette to make its concentration approximately 1 mg/mL (potency), vigorously shaken, and if necessary filtered or centrifuged. The resulting filtrate or supernatant is used as a sample stock solution. The volume required for testing of the sample stock

solution is measured by a volumetric pipette, and the provisions of the raw material for manufacturing of lasalocid sodium are hereinafter applied mutatis mutandis.

(b) Standard of manufacturing method

The raw material for manufacturing of lasalocid sodium is mixed with carriers.

(c) Standard of storage method

The storage method of the raw materials for manufacturing of lasalocid sodium are applied mutatis mutandis.

(d) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

"有効期間 製造の翌月から２年"

Valid period: 2 years from the month following the manufacture

"使用上の注意　この飼料添加物は、鶏又は牛に過剰投与した場合発育障害がおこるので、定められた添加量を厳守するとともに、均一に配合するよう注意すること。"

Precautions: Overdose of this feed additive causes developmental disorders in chicken or cattle, so strictly follow the specified amount to be added and make sure to mix it uniformly.

(125) Amprolium plus Ethopabate

A. Raw material for manufacturing

(a) Compositional standards

Amprolium

Content: When this product is determined, it contains 95.0 % or over of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$).

Physical and chemical properties:

i. This product is white to pale yellow powder with no odor or a slight specific odor.

ii. This product is easy to dissolve in water, easy to slightly dissolve in methanol, hard to dissolve in ethanol, and hardly dissolves in ether or chloroform.

Confirmation test:

i. In the measurement of the absorption spectrum of 0.1 mol/L hydrochloric acid solution of this product (1 → 100,000), the absorption maximum is at the wavelengths of 244~248 nm and 260~264 nm. The absorbances at both wavelengths are referred to as A1 and A2 respectively, and the A1 / A2 is 1.04~1.06.

ii. The solution of this product in water (1 → 50) gives the qualitative reaction of chloride.

Purity test:

i. Picoline:

Device: To the side-arm of a 100 mL side-arm distilling flask, a 200 mL receiver is connected so that the tip almost contacts with the bottom. The mouth of the distilling flask is connected to a dropping funnel and an air tube so that the lower end is near the bottom of the flask. The other end of the air tube is sequentially connected to two scrubbing bottles, and glass fibers and sulfuric acid are placed so that the one directly connects to the air tube and the other one, respectively.

Procedure: Approximately 1.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is placed in a side-arm distilling flask and dissolved with 10 mL of water. Boric acid solution (1 → 50) 75 mL and 0.5 mL of bromcresol green, methyl orange test solution are transferred to a receiver and the tip of the connected side-arm is dipped in the solution. A dropping funnel and air tube are attached. With adding 25 mL of saturated solution of potassium carbonate by dropping from the dropping funnel for approximately 10 minutes and aeration through the scrubbing bottles for approximately 1 hour, picoline present in the solution is collected in the solution in the receiver. Then, the aeration is stopped, the receiver is replaced with another receiver with 75 mL of boric acid solution (1 → 50) and 0.5 mL of bromcresol green, methyl orange test solution, and again aeration is performed for 30 minutes. The color of solution in the receiver is changed, the receiver is also replaced. This procedure is repeated, and stopped when there is no change in color of the solution in the receiver. When total solution in all receivers is titrated with 0.1 mol/L hydrochloric acid to determine the amount of picoline (C_6H_7N), the amount shall be 1.0 % or less. In this case, the end of titration is the time when green-blue changes to yellow-green to yellow.

1 mL of 0.1 mol/L hydrochloric acid = 9.313 mg C_6H_7N

ii. Chlorine: Approximately 0.15 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 50 mL of water, added with 5 mL of nitric acid, added with 25 mL of 0.1 mol/L of silver nitrate solution using a volumetric pipette, mixed, added with 3 mL of nitrobenzene, vigorously shaken, and added with 2 mL of ferric ammonium sulfate test solution. The excess silver nitrate is titrated with 0.1 mol/L of ammonium thiocyanate to determine the amount of chlorine (Cl), and the amount shall be 21.5~23.5 %.

1 mL of 0.1 mol/L silver nitrate solution = 3.545 mg Cl

Loss on drying: 1.0 % or less (1 g, reduced pressure, 100 °C, 3 hours)

Ignition residue: 0.20 % or less (1 g)

Assay: 0.05 g of this product is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved in methanol (2 → 3), transferred to a 100 mL volumetric flask, is further added with methanol (2 → 3) to the graduation linegraduation line to make 100 mL. This solution 5 mL is measured with a volumetric pipette, transferred to a 100 mL volumetric flask, added with methanol (2 → 3) to the graduation linegraduation line to make 100 mL. This is used as a sample solution. Separately, appropriately 0.05 g of dried amprolium reference standard is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved in methanol (2 → 3), transferred to a 100 mL volumetric flask, is further added with methanol (2 → 3) to the graduation linegraduation line to make 100 mL. 5 mL of this solution is measured with a volumetric pipette, transferred to a 100 mL volumetric flask, added with methanol (2 → 3) to the graduation line to make 100 mL. This is used as a standard solution. 4 mL each of the sample and standard solutions are measured using a volumetric pipette, transferred to test tubes (1) and (2), and separately, 4 mL of methanol (2 → 3) is measured using a volumetric pipette and transferred to a test tube (3). Each of the test tubes is added with 10 mL of 2,7-dioxynaphthalene using a volumetric pipette, tightly stoppered, mixed well, and allowed to stand for 20 minutes. Then, each tube is centrifuged for 2~3 minutes, and the supernatants of (1) and (2) are added with 2,7-dioxynaphthalene test solution, in 20~45 minutes, the absorbances AT and AS at the maximum wavelength near 530 nm are measured using the supernatant of (3) as a control.

Amount of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) (mg)

$$= \text{Amount of amprolium standard (mg)} \frac{A_T}{A_S}$$

Ethopabate

Content: When this product is determined, it contains 95.0~103.0 % of ethopabate ($C_{12}H_{15}NO_4$).

Physical and chemical properties:

i. This product is white to pale red-white powder and with a slight or no odor.

ii. This product is easy to dissolve in chloroform, easy to slightly dissolve in methanol or ethanol, hard to extreamly dissolve in ether, and hardly dissolves in water.

Confirmation test:

i. In the measurement of the absorption spectrum of the methanol solution (1 → 125,000) of this product, the absorption maximum is at the wavelengths of 266~270 nm and

297~301 nm, and the absorption minimum is at the wavelengths of 236~240 nm and 285~289 nm.

ii. This product and ethopabate reference standard are dried, measured by the potassium bromide disk method of the infrared absorption spectroscopy. When their spectra are compared, both absorptions are observed at the same wavenumber and the relative intensities of these absorptions are equal.

Purity test:

i. Melting point: The melting point of this product shall be 146~151 °C.

ii. Diazotized substance: Approximately 1.0 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with chloroform, transferred to a 100 mL volumetric flask, and is further added with chloroform to the graduation line to make 100 mL. This solution 10 mL is measured using a volumetric pipette, added with 100 mL of 0.1 mol/L hydrochloric acid using a volumetric pipette and shaken, and the aqueous layer is sufficiently separated to prepare the first extract. The chloroform layer is added with 100 mL of 0.1 mol/L hydrochloric acid using a volumetric pipette and shaken, and the aqueous layer is separated to prepare the second extract. 40 mL each of the first and second extracts are centrifuged and 20 mL each of the supernatants is measured using a volumetric pipette are mixed to prepare a sample solution. Separately, 4-amino-2-ethoxybenzoic acid methyl reference standard is dried under reduced-pressure at 100 °C for 2 hours and approximately 0.05 g of it is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with chloroform, transferred to a 100 mL volumetric flask, and is further added with chloroform to the graduation line to make 100 mL. 2 mL of this solution is measured using a volumetric pipette, added with 8 mL of chloroform, added with 100 mL of 0.1 mol/L hydrochloric acid using a volumetric pipette and shaken, and the same procedure as that for the sample solution is hereinafter taken to prepare a standard solution. Five stoppered test tubes (1) to (5) are prepared. 5 mL of the sample solution, standard solution 1 mL and 0.1 mol/L hydrochloric acid 4 mL, standard solution 3 mL and 0.1 mol/L hydrochloric acid 2 mL, standard solution 5 mL, 0.1 mol/L hydrochloric acid 5 mL are measured using a volumetric pipette and transferred to the stoppered test tubes (1), (2), (3), (4), and (5), respectively. Each stoppered test tube is subjected to the following procedure. Into each tube, 6 mL of 1 mol/L hydrochloric acid and 1 mL of newly prepared sodium nitrite solution (1 → 1,000) are added using a volumetric pipette, shaken, and allowed to stand for 4 minutes. Then it is added with 1 mL of newly prepared ammonium sulfamate solution (1 → 200) measured using a volumetric pipette, shaken, and allowed to stand for 3 minutes. Next, it is added with 1 mL of newly prepared N-(1-

naphthyl)-ethylenediamine dihydrochloride solution (1 → 1,000) using a volumetric pipette, shaken, and allowed to stand for 30 minutes. The absorbances A_1, A_2, A_3 and A_4 of the solutions of the tubes (1) to (4) are measured at the maximum wavelength near 530 nm, using the solution of the tube (5) as a control. The calibration curves are prepared from the absorbances A_2, A_3 and A_4 and the amounts (mg) of the reference standard in the corresponding 1 mL, 3 mL and 5 mL of standard solutions. The amount (mg) of the diazotized substance [as 4-amino-2-ethoxybenzoic acid methyl ($C_{10}H_{13}NO_3$)] in the sample solution 5 mL corresponding to the absorbance A_1 shall be 1.0 % or less.

iii. Phenolic substance: Approximately 0.4 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with methanol, transferred to a 20 mL volumetric flask, and is further added with methanol to the graduation line to make 20 mL. This is used as a sample solution. Separately, 4-acetamide-2-hydroxybenzoic acid methyl reference standard is dried and approximately 0.028 g of it is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with methanol, transferred to a 100 mL volumetric flask, and is further added with methanol to the graduation line to make 100 mL. This is used as a standard solution. Nine centrifugal precipitation tubes (1) to (9) are prepared. 1 mL of the sample solution and 4 mL of methanol, 3 mL of sample solution and 2 mL of methanol, 5 mL of sample solution, 1 mL of standard solution and 4 mL of methanol, 2 mL of standard solution and 3 mL of methanol, 3 mL of standard solution and 2 mL of methanol, 4 mL of standard solution and 1 mL of methanol, 5 mL of standard solution, and 5 mL of methanol are measured using a volumetric pipette and placed in the centrifugal precipitation tubes (1) to (9), respectively. Each centrifugal precipitation tube is added with 5 mL of ferric perchloric acid test solution using a volumetric pipette, shaken, allowed to stand for 10 minutes. The centrifugal precipitation tubes (1), (2), and (3) are centrifuge to separate the supernatant. The absorbances A_1 to A_8 of the solutions of (1) to (8) are measured at the maximum wavelength near 525 nm, using the solution of (9) as a control. The calibration curves are prepared from the absorbances A_4 to A_8 and the amounts (mg) of the reference standard in the corresponding 1 to 5 mL of standard solutions. The amount (mg) of the phenolic substance [as 4-acetamide-2-hydroxybenzoic acid methyl ($C_{10}H_{11}NO_4$)] in the sample solution 1, 3 and 5 mL corresponding to the absorbance A_1 A_2 and A_3 is determined. The average of the amounts of phenolic substance determined by respective sample solutions shall be 2.0 % or less.

Loss on drying: 1.0 % or less (1 g, reduced pressure, 100 °C, 2 hours)

Ignition residue: 0.5 % or less (1 g)

Assay: Approximately 0.075 g of this product is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved in methanol, transferred to a 250 mL volumetric flask, and is further added with methanol to the graduation line to make 250 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. 10 mL of this solution is transferred to a beaker using a volumetric pipette, added with 10 mL of 1 mol/L sodium hydroxide test solution, and evaporated to dryness in a water bath. It is added with 10 mL of boiling water while washing the inside wall, and is further heated for 15 minutes. After cooling, it is added with water to make 20 mL and transferred to a 100 mL volumetric flask. The beaker is washed three times with 20 mL of water each. The washings are put in the volumetric flask and more water is added to the graduation line to make 100 mL. This is used as a sample solution. Separately, ethopabate reference standard is dried, approximately 0.03 g of it is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved in methanol, transferred to a 100 mL volumetric flask, and is further added with methanol to the graduation line to make 100 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL volumetric flask and added with water to the graduation line to make 100 mL. 10 mL of this solution is transferred to a beaker using a volumetric pipette, added with 10 mL of 1 mol/L sodium hydroxide test solution, and the same procedure as that for the sample solution is hereinafter performed to prepare a standard solution. Separately, 10 mL of water is measured using a volumetric pipette and transferred to a beaker, added with 10 mL of 1 mol/L sodium hydroxide test solution, and the same procedure as that for the sample solution is hereinafter performed to prepare a blank test solution. Three centrifugal precipitation tubes (1) to (3) are prepared. 10 mL of the sample solution, 10 mL of the standard solution and 10 mL of the blank test solution are measured using a volumetric pipette and transferred to the centrifugal precipitation tubes (1), (2) and (3), respectively. Each precipitation tube is subjected to the following procedure. Each precipitation tube is added with 2 mL of 1 mol/L hydrochloric acid and 2 mL of newly prepared sodium nitrite solution (1 → 1,000) using a volumetric pipette, shaken and allowed to stand for 2 minutes. Then, it is added with 2 mL of newly prepared sulfamic acid ammonium solution (1 → 200) measured using a volumetric pipette, shaken and allowed to stand for 2 minutes. It is added with 2 mL of newly prepared N-(1-naphthyl)-ethylenediamine dihydrochloride (1 → 1,000) measured using a volumetric pipette, shaken and allowed to stand for 10 minutes. It is added with 4 g (3.5~4.4 g) of sodium chloride, and added with 10 mL of n-butanol measured using a volumetric pipette. It is vigorously shaken for 2 minutes and centrifuged to separate the

supernatant. The absorbances A_T and A_S of the solution (1) and (2) at the maximum wavelength near 555 nm are measured, using the solution (3) as a control solution.

Amount of ethopabate ($C_{12}H_{15}NO_4$) (mg)

$$= \text{Amount of ethopabate reference standard (mg)} \times \frac{A_T}{A_S} \times 2.5$$

- Amount of corresponding diazotized substance (mg) × 1.215
- Amount of corresponding phenolic substance (mg) × 1.134

(b) Standard of storage method

Amprolium

It shall be stored in a capped container.

Ethopabate

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

This product is solid or powder, in which the raw material for manufacturing of formic acid or its preparation and the raw material for manufacturing of propionic acid or its preparation are mixed and if necessary added with ammonia (limited to that meeting the standards of food additives), caprylic acid or food dye.

Content: This product is determined to contain amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) and ethopabate ($C_{12}H_{15}NO_4$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. The sample solution obtained by the assay of amprolium is reddish violet and the absorption maximum is at the wavelengths of 528~532 nm by measuring the absorption spectrum.

ii. The sample solution obtained by the assay of ethopabate is reddish violet and the absorption maximum is at the wavelengths of 553~557 nm by measuring the absorption spectrum.

Assay:

Amprolium: The amount of this product containing approximately 0.05 g of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) is weighed to three significant digits and the value is recorded. It is added with 100 mL of methanol (2 → 3) using a volumetric pipette and shaken for 20 minutes. This solution is filtered, the first filtrate 20 mL is removed, and the next filtrate 5 mL is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, added with methanol (2 → 3) to the graduation line to make 100 mL, and the assay of the raw material for manufacturing of amprolium is hereinafter applied mutatis mutandis.

Amount of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) (mg)

$$= \text{Amount of amprolium standard (mg)} \times \frac{A_T}{A_S}$$

Ethopabate: The amount of this product containing approximately 3 mg of ethopabate ($C_{12}H_{15}NO_4$) is weighed to three significant digits and the value is recorded. It is placed in a 100 mL volumetric flask, added with 75 mL of methanol, shaken for 20 minutes, is further added with methanol to the graduation line to make 100 mL, and centrifuged. The supernatant 10 mL is transferred to a beaker using a volumetric pipette, added with 10 mL of sodium hydroxide test solution, and the assay of the raw material for manufacturing of ethopabate is hereinafter applied mutatis mutandis.

Amount of ethopabsate ($C_{12}H_{15}NO_4$) (mg)

$$= \text{Amount of ethopabsate standard (mg)} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

(b) Standard of storage method

It shall be stored in a capped container.

(126) Amprolium/ethopabate/sulfaquinoxaline

A. Raw material for manufacturing

(a) Compositional standards

Amprolium

The compositional standards of the raw material for manufacturing of Amprolium in Amprolium plus Ethopabate are applied mutatis mutandis.

Ethopabate

The compositional standards of the raw material for manufacturing of Ethopabate in Amprolium plus Ethopabate are applied mutatis mutandis.

Sulfaquinoxaline

Content: When this product is determined after dried, it contains 98.0 % or over of sulfaquinoxaline ($C_{14}H_{12}N_4O_2S$).

Physical and chemical properties:

i. This product is pale yellow to yellowish-brown crystalline powder with little odor.

ii. This product is hard to dissolve in acetone, extremely hard to dissolve in ethanol and hardly dissolves in water.

iii. This product is dissolved in sodium carbonate test solution or 1 mol/L sodium hydroxide test solution.

iv. This product gradually becomes dark in color by light.

Confirmation test:

i. 0.05 g (0.045~0.054 g) of this product is dissolved with 4 mL of dilute hydrochloric by heating. The resulting solution gives the qualitative reaction of aromatic primary amine, however, its color is orange-red.

ii. 0.02 g (0.015~0.024 g) of this product is weighed, added with 5 mL of water, added with 1 mol/L sodium hydroxide by dropping while stirring to dissolve, and added with 2~3 drops of copper sulfate test solution, and then yellow-green precipitation develops.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved with 5 mL of 1 mol/L sodium hydroxide test solution, and is further added with 20 mL of water. The resulting solution shall be orange-yellow and clear.

ii. Melting point: The melting point of this product shall be 244~247 °C (Disassembly)

iii. Acid: 1.0 g (0.95~1.04 g) of this product is weighed, added with 50 mL of water, heated at 70 °C for 5 minutes, quenched to room temperature, and filtered. The filtrate 25 mL is added with 2 drops of methyl red test solution and 0.50 mL of 0.1 mol/L sodium hydroxide solution. The resulting solution shall be yellow.

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

Loss on drying: 1.0 % or less (1 g, 105 °C, 4hours)

Ignition residue: After drying, 0.10% or less (1 g)

Assay: This product is dried and approximately 0.5 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 75 mL of glacial acetic acid, 8 mL of hydrochloric acid, and 25 mL of water. It is cooled to 15 °C, added with 25 g (24.5~25.4 g) of crushed ice, titrated with 0.1 mol/L sodium nitrite solution with constant stirring. In this case, the end of titration is the time when the titrated solution is put on a glass rod 1 minute after dropping 0.1 mol/L sodium nitrite solution, the tip of the glass rod attaches to zinc iodide-starch test paper, and it turns blue within 30 seconds. In the same manner, a blank test is performed and corrections are made.

(b) Standard of storage method

Amprolium: The standard of storage method of the raw material for manufacturing of Amprolium in Amprolium plus Ethopabate are applied mutatis mutandis.

Ethopabate: The standard of storage method of the raw material for manufacturing of Ethopabate in Amprolium plus Ethopabate are applied mutatis mutandis.

Sulfaquinoxaline: It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of Amprolium,ethopabate and sulfaquinoxaline and fillers.

Content: This product is determined to contain amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) , ethopabate ($C_{12}H_{15}NO_4$) and sulfaquinoxaline ($C_{14}H_{12}N_4O_2S$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. The sample solution obtained by the assay of amprolium is reddish violet and the absorption maximum is at the wavelengths of 528~532 nm by measuring the absorption spectrum.

ii. The sample solution obtained by the assay of ethopabate is reddish violet and the absorption maximum is at the wavelengths of 538~542 nm by measuring the absorption spectrum.

iii. The sample solution obtained by the assay of sulfaquinoxaline is reddish violet and the absorption maximum is at the wavelengths of 543~547 nm by measuring the absorption spectrum.

Assay: Amprolium: The amount of this product containing approximately 0.05 g of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) is weighed to three significant digits and the value is recorded. It is added with 100 mL of methanol (2 → 3) using a volumetric pipette and shaken for 20 minutes. This solution is filtered, the first filtrate 20 mL is removed, and the second filtrate 5 mL is measured using a volumetric pipette, transferred to a 100 mL volumetric flask, added with methanol (2 → 3) to the graduation line to make 100 mL, and the assay of the raw material for manufacturing of amprolium is hereinafter applied mutatis mutandis.

Amount of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) (mg)

$$= \text{Amount of amprolium standard (mg)} \frac{A_T}{A_S}$$

Ethopabate: The amount of this product containing approximately 6 mg of ethopabate ($C_{12}H_{15}NO_4$) is weighed to three significant digits and the value is recorded. It is placed in a saponification flask, added with 100 mL of chloroform using a volumetric pipette. The flask is attached with a reflux condenser and the reflux is performed in a water bath for 15 minutes, avoiding chloroform evaporation. After cooling, it is filtered. The filtrate

50 mL is measured using a volumetric pipette, placed in a separatory funnel, washed three times with 25 mL each of sodium carbonate solution (1 → 20), and then washed twice with 10 mL of water, and the washings are discarded. The chloroform layer is filtered and 10 mL of the filtrate is measured using a volumetric pipette, almost evaporated in a water bath, added with 10 mL of methanol and 10 mL of 1 mol/L sodium hydroxide test solution and evaporated to dryness in a water bath. It is added with 10 mL of boiling water, heated for 15 minutes, allowed to cool, transferred to a 100 mL volumetric flask, added with 20 mL of 1 mol/L hydrochloric acid and water to the graduation line to make 100 mL, and filtered. This is used as a sample solution. Separately, ethopabate reference standard is dried, and approximately 0.03 g of it is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with methanol, transferred to a 100 mL volumetric flask, and is further added with methanol to the graduation line to make 100 mL. 10 mL of this solution is measured by a volumetric pipette, transferred to a 50 mL volumetric flask, and added with methanol to the graduation line to make 50 mL. 10 mL of this solution is measured using a volumetric pipette, added with 10 mL of 1 mol/L sodium hydroxide test solution, and the same procedure as that for the sample solution is hereinafter performed. This is used as a standard solution. Separately, 10 mL of methanol is measured, added with 10 mL of 1 mol/L sodium hydroxide test solution, and hereinafter subjected to the same procedure as that for the sample solution to prepare a blank test solution.

Three 50 mL volumetric flasks (1) to (3) are prepared, and 25 mL each of the sample solution, the standard solution, and the blank test solution are measured using a volumetric pipette and transferred to the 50 mL volumetric flasks (1), (2), and (3), respectively. Then each of them is subjected to the following procedure. Each solution is added with 5 mL of 1 mol/L hydrochloric acid and 5 mL of newly prepared sodium nitrite solution (1 → 1,000) using a volumetric pipette, shaken, and allowed to stand for 2 minutes. It is added with 5 mL of newly prepared sulfamic acid ammonium solution (1 → 200) using a volumetric pipette, shaken, allowed to stand for 3 minutes, added with 5 mL of newly prepared N-(1-naphthyl)-ethylenediamine dihydrochloride solution (1 → 1,000) using a volumetric pipette, shaken, allowed to stand for 10 minutes, and then added with water to the graduation line to make 50 mL. The absorbances A_T and A_S of the solution (1) and (2) at the maximum wavelength near 540 nm are measured, using the solution (3) as a control solution.

Amount of ethopabate ($C_{12}H_{15}NO_4$) (mg)

$$= \text{Amount of amprolium standard (mg)} \ \frac{A_T}{A_S} \times \frac{1}{5}$$

Sulfaquinoxaline: The amount of this product containing approximately 0.05 g of sulfaquinoxaline ($C_{14}H_{12}N_4O_2S$) is weighed to three significant digits and the value is recorded. It is added with 70 mL of water and 10 mL of 0.5 mol/L sodium hydroxide test solution, shaken for 10 minutes, transferred to a 250 mL volumetric flask, and added with water to the graduation line to make 250 mL. It is allowed to stand for 2 minutes, and if necessary centrifuged. 10 mL of the supernatant is measured using a volumetric pipette, transferred to a 200 mL volumetric flask with 10 mL of 1 mol/L hydrochloric acid, and added with water to the graduation line to make 200 mL. This is used as a sample solution. Separately, sulfaquinoxaline reference standard is dried, and approximately 0.05 g of it is weighed to the digits of 0.1 mg and the value is recorded. It is dissolved with 70 mL of water and 10 mL of 0.5 mol/L sodium hydroxide test solution, transferred to a 250 mL volumetric flask, and added with water to the graduation line to make 250 mL. This solution 10 mL is measured using a volumetric pipette, transferred to a 200 mL volumetric flask with 10 mL of 1 mol/L hydrochloric acid, and added water to the graduation line to make 200 mL. This is used as a standard solution. Separately, 10 mL of 0.5 mol/L sodium hydroxide test solution is added with water to make 250 mL and 10 mL of this solution is transferred to a 200 mL volumetric flask and added with 10 mL of 1 mol/L hydrochloric acid and water to the graduation line to make 200 mL. This is used as a blank test solution. Three 50 mL volumetric flasks (1) to (3) are prepared, and 10 mL each of the sample solution, the standard solution, and the blank test solution are measured using a volumetric pipette and transferred to the 50 mL volumetric flasks (1), (2), and (3), respectively. Then each of them is subjected to the following procedure.

Each solution is added with 5 mL of 1 mol/L hydrochloric acid and 5 mL of newly prepared sodium nitrite solution (1 → 1,000) using a volumetric pipette, shaken, and allowed to stand for 2 minutes. It is added with 5 mL of newly prepared sulfamic acid ammonium solution (1 → 200) using a volumetric pipette, shaken, allowed to stand for 3 minutes, added with 5 mL of newly prepared N-(1-naphthyl)-ethylenediamine dihydrochloride solution (1 → 1,000) using a volumetric pipette, shaken, allowed to stand for 10 minutes, and then added with water to the graduation line to make 50 mL. The absorbances A_T and A_S of the solution (1) and (2) at the maximum wavelength near 545 nm are measured, using the solution (3) as a control solution.

Amount of sulfaquinoxaline ($C_{14}H_{12}N_4O_2S$) (mg)

$$= \text{Amount of amprolium standard (mg)} \times \frac{A_T}{A_S}$$

(b) Standard of storage method

It shall be stored in a capped container.

(127) Morantel citrate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 98.0 % or over of Morantel citrate ($C_{12}H_{16}N_2S \cdot C_6H_8O_7 \cdot H_2O$).

Physical and chemical properties:

i. This product is pale-yellow to yellow crystalline powder, with a slightly bitter taste and a specific odor.

ii. This product is easy to slightly dissolve in methanol, hard to dissolve in ethanol and water, and hardly dissolves in ether or chloroform.

iii. The pH of solution (1 → 200) of this product is 3.3~4.5.

iv. Melting point is 116~120°C

Confirmation test:

i. 0.1 g (0.05~0.14 g) of this product is dissolved with 30 mL of water. 0.5 mL of this solution is added with 3 mL of *p*-dimethylaminobenzaldehyde-ferric chloride test solution. The resulting solution is reddish violet.

ii. 0.01 g (0.005~0.014 g) of this product is dissolved with 2 mL of water and added with 1 drop of potassium permanganate test solution. The color of the test solution disappears within 30 minutes.

iii. 5 mg (4.5~5.4 mg) of this product is added with 2 mL of citric acid-acetic anhydride solution (0.5 → 100) and heated in a water bath, and then the solution is red to reddish violet.

iv. 0.01 g (0.005~0.014 g) of this product is dissolved with 0.01 mol/L hydrochloric acid-methanol test solution to make 1,000 mL. This solution shows the absorption maximum at the wavelengths of 322~327 nm by measuring the absorption spectrum.

v. 0.02 g (0.015~0.024 g) of this product is dissolved with 4 mL of water and added with dilute sodium hydroxide to be neutral. The resulting solution gives the qualitative reaction iii. of citrate.

Purity test:

i. Clarity and color of solution: 0.5 g (0.45~0.54 g) of this product is dissolved with 10 mL of methanol. The resulting solution shall be yellow and clear.

ii. Chloride: 0.5 g (0.45~0.54 g) of this product is added with 40 mL of water and dissolved by heating. It is added with 6 mL of dilute nitric acid and water to make 50 mL. This is used as a sample solution. The chloride is tested using the control solution prepared using 0.25 mL of 0.01 mol/L hydrochloric acid by the chloride test method.

The opacity of the sample solution shall not be darker than that of the control solution (0.018 % or less).

iii. Sulfate: 0.5 g (0.45~0.54 g) of this product is added with 40 mL of water and dissolved by heating. It is added with 1 mL of dilute hydrochloric acid and water to make 50 mL. This is used as a sample solution. The sulfate is tested using the control solution prepared using 0.50 mL of 0.005 mol/L sulfuric acid by the sulfate test method. The opacity of the sample solution shall not be darker than that of the control solution (0.048 % or less).

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 3.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (30 mg/kg or less).

v. Arsenic: 2.0 g (1.95~2.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (1 mg/kg or less).

vi. Cis isomer: 0.2 g (0.15~0.24 g) of this product is dissolved with methanol, transferred to a 10 mL brown volumetric flask, and is further added with methanol to the graduation line to make 10 mL. This is used as a sample solution. 1 mL of this solution is measured using a volumetric pipette, transferred to a 100 mL brown volumetric flask, and added with methanol to the graduation line to make 100 mL. This is used as a control solution for cis isomer. Separately, 0.1 g (0.05~0.14 g) of anhydrous citric acid is weighed, dissolved with methanol, transferred to a 100 mL volumetric flask, and is further added with methanol to the graduation line to make 100 mL. This is used as a citric acid solution. 5 μL each of the sample, the control solution for cis isomer, and the citric acid solution are spotted on a thin layer plate prepared using silica gel for thin-layer chromatography in the dark place. Then, they are developed to approximately 10 cm using the upper layer of a mixed solution of methyl isobutyl ketone, formic acid, and water (2:1:1) as the developing solvent in a dark place, and the thin layer plate is dried at 100 °C for 15 minutes. The thin layer is placed in a bath filled with iodine vapor, and then spots except those of morantel and citric acid obtained from the sample solution are not observed, or shall not be darker than that obtained from the control solution for cis isomer (1 % or less).

Moisture: 3.5~5.0 % (0.5 g)

Ignition residue: 0.20 % or less (1 g)

Assay: Approximately 0.1 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 0.01 mol/L hydrochloric acid-methanol test solution, transferred to a 200 mL brown volumetric flask, and is further added with the same test solution to the graduation line to make 200 mL. This solution 2 mL is measured using a volumetric pipette, transferred to a 100 mL brown volumetric flask, and added with 0.01 mol/L hydrochloric acid-methanol test solution to the graduation line to make 100 mL. This is used as a sample solution. Separately, approximately 0.1 g of morantel citrate reference standard is weighed to the digits of 0.001 g, and the value is recorded. It is subjected to the same preparation procedure as that of the sample solution to prepare the standard solution. The absorbances A_T and A_S of them at the wavelength 323 nm are measured, using 0.01 mol/L hydrochloric acid/methanol test solution as a control solution.

Amount of Morantel citrate ($C_{13}H_{16}N_2S \cdot C_6H_8O_7 \cdot H_2O$) (mg)

$$= \text{Amount of Morantel citrate standard (mg)} \times \frac{A_T}{A_S}$$

(c) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation

(a) Compositional standards

This product is powder or particle in which the raw material for manufacturing of Morantel citrate and fillers.

Content: This product is determined to contain Morantel citrate ($C_{12}H_{16}N_2S \cdot C_6H_8O_7 \cdot H_2O$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.1 g of the raw material for manufacturing of morantel citrate is weighed, added with 30 mL of water, shaken for 5 minutes, and centrifuged. The supernatant 0.5 mL is added with 3 mL of *p*-dimethylaminobenzaldehyde-ferric chloride test solution. The resulting solution is reddish violet.

ii. According to the amount of this product on the label, the amount containing 0.01 g of the raw material for manufacturing of morantel citrate is weighed, added with 0.01 mol/L hydrochloric acid/methanol test solution, shaken and filtered. The filtrate shows the absorption maximum at the wavelengths of 322~327 nm by measuring the absorption spectrum.

Assay: The amount of this product containing approximately 0.06 g of morantel citrate ($C_{12}H_{16}N_2S \cdot C_6H_8O_7 \cdot H_2O$) is weighed to three significant digits and the value is recorded. It is placed in a brown volumetric flask, added with 150 mL of 0.01 mol/L hydrochloric acid/methanol test solution, shaken for 30 minutes, added with 0.01 mol/L hydrochloric acid/methanol test solution to accurately make 250 mL, and centrifuged. 5 mL of the supernatant is measured using a volumetric pipette, transferred to a brown stoppered flask, added with 5 mL of the solution, in which 0.12 g (0.115~0.124 g) of 2-hydroxy-*m*-toluic acid is dissolved with 100 mL of 0.01 mol/L hydrochloric acid/methanol test solution, using a volumetric pipette. This is used as a sample solution. Separately, approximately 0.06 g of morantel citrate reference standard is weighed to the digits of 0.1 mg, and the value is recorded. It is dissolved with 0.01 mol/L hydrochloric acid/methanol test solution, transferred to a 250 mL volumetric flask, and added with 0.01 mol/L hydrochloric acid/methanol test solution to the graduation line to make 250 mL. 5 mL of this solution is measured using a volumetric pipette and subjected to the same preparation procedure as that of the sample solution to prepare the standard solution. 5 μL each of the sample and standard solutions are tested by the liquid chromatography under the following conditions. Based on the obtained chromatograms, the peak heights of morantel citrate and the internal standard solution of each solution are measured, and the ratio of the peak height of morantel citrate to the peak height of the internal standard solution, H_T and H_S are determined.

Amount of Morantel citrate ($C_{12}H_{16}N_2S \cdot C_6H_8O_7 \cdot H_2O$) (mg)

$$= \text{Amount of Morantel citrate standard (mg)} \times \frac{H_T}{H_S}$$

Operating conditions:

Detector: Ultraviolet absorptiometer (measurement wavelength: 320 nm,sensitivity : 0.08AUFS)

Column: A stainless tube (inner diameter: 4.6 mm, length: 150 mm) is filled with 10 μm of octadecyl silylation silica gel.

Column temperature: room temperature.

Mobile phase: Mixed solution of the solution, for which 1,000 mL of 0.05 mol/L potassium dihydrogenphosphate is added with phosphoric acid (1 → 10) and adjusted to pH 3.3, and acetonitrile (3:1).

Flow rate: 1.5 mL/min.

Column selection: A column to be used is selected when the standard solution 5 μL is operated under the above conditions, morantel and the internal standard solution are eluted in that order, and the separation degree is 20 or greater.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Morantel citrate is applied mutatis mutandis.

(128) Nicarbazin

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried and determined, it contains 67.4~73.0 % of 4,4′-dinitrocarbanilide ($C_{13}H_{10}N_4O_5$) and 27.7~30.0 % of 2-hydroxy-4, 6-dimethylpyrimidine ($C_6H_8N_2O$).

Physical and chemical properties:

i. This product is yellow-brown to green-yellow powder without odor or with a slightly specific odor.

ii. This product is hard to dissolve in dimethylformamide and is extremely hard to dissolve in water, ethanol, ether, and chloroform.

iii. Melting point: Approximately 260 °C (dissolution)

Confirmation test:

i. The absolute ethanol solution of this product (1 → 15,000) 15 mL is added with 5 mL of sulfanilic acid test solution and 5 mL of newly prepared sodium nitrite solution (1 → 100), tightly stoppered, heated in a water bath at 65 °C for 10 minutes. The resulting solution is red.

ii. The absolute ethanol solution (1 → 15,000) 15 mL is added with 5 mL of potassium hydroxide/ethanol solution (1 → 100), and the resulting solution is yellow.

iii. The sample solution obtained by the assay of 4,4′-dinitrocarbanilide of the absorption maximum is at the wavelengths of 428~432 nm by measuring the absorption spectrum.

iv. The sample solution obtained by the assay of 2-hydroxy-4, 6-dimethylpyrimidine of the absorption maximum is at the wavelengths of 538~542 nm by measuring the absorption spectrum.

Purity test:

i. pH: 0.20 g (0.195~0.204 g) of this product is weighed, added with 20 mL of water and stirred. The resulting solution shall be pH 5.0 to 7.0.

ii. Chloride: 0.20 g (0.195~0.204 g) of this product is weighed, added with 20 mL of water, boiled for 2 minutes, allowed to cool, and filtered. The filtrate 5 mL is measured and added with 6 mL of dilute nitric acid and water to make 50 mL to prepare the sample solution. The chloride is tested using the control solution prepared using 0.4

mL of 0.01 mol/L hydrochloric acid by the chloride test method. The opacity of the sample solution shall not be darker than that of the control solution (0.28 % or less).

iii. Sulfate: 0.20 g (0.195~0.204 g) of this product is weighed, added with 20 mL of water, boiled for 2 minutes, allowed to cool, and filtered. The filtrate 2 mL is measured and added with 1 mL of dilute hydrochloric acid and water to make 50 mL to prepare the sample solution. The sulfate is tested using the control solution prepared using 0.6 mL of 0.005 mol/L sulfuric acid by the sulfate test method. The opacity of the sample solution shall not be darker than that of the control solution (1.44 % or less).

iv. Ammonium salt: 0.30 g (0.295~0.304 g) of this product is placed in a flask and added with 1 g (0.5~1.4 g) of magnesium oxide and 70 mL of water, and the flask is connected to a distillation device. A 100 mL measuring cylinder with 2 mL of 0.1 mol/L hydrochloric acid is used as a receiver and the distillation is continued until 40 mL of solution is collected. The collected solution is added with water to make 100 mL and 10 mL of this solution is measured using a volumetric pipette, transferred to a Nessler tube, added with water to make 40 mL, and added with 2 mL of sodium hydroxide solution (1 → 10) and 2 mL of Nessler reagent. The color of the resulting solution shall not be darker than that of the solution prepared by that 15 mL of ammonia standard solution in a Nessler tube added with water to make 40 mL subjected to the same procedure for the sample (0.5 % or less).

v. Free 2-hydroxy-4, 6-dimethylpyrimidine: 0.5 g of this product is weighed to the digits of 0.001 g and the value is recorded. It is mixed with 25 mL of pH 7.0 phosphate buffer, shaken for 10 minutes, and filtered within 30 seconds. A few mL of the first filtrate is removed and 5 mL of the clear filtrate is measured and added with pH 7.0 phosphate buffer to make 100 mL. When the absorbance A_T of this solution at the maximum wavelength near 295 nm within 30 seconds using water as a control solution, the amount of free 2-hydroxy-4, 6-dimethylpyrimidine ($C_6H_8N_2O$) shall be 3.0 % or less.

$$\text{Amount of 4,4′-dinitrocarbanilide } (C_{13}H_{10}N_4O_5)\ (\text{mg}) = \frac{A_T}{118} \times 1{,}000$$

Loss on drying: 1.0 % or less (1 g, reduced pressure, 110 °C, 1 hour)

Ignition residue: 0.3% or less (1 g)

Assay: This product is dried and approximately 0.05 g of it is weighed to the digits of 0.1 mg and the value is recorded. It is added with dimethylformamide, dissolved by heating, allowed to cool, transferred to a 100 mL volumetric flak, is further added with dimethylformamide to the graduation line to make 100 mL, mixed well. This is used as a sample stock solution. Separately, nicarbazin reference standard is dried in the same way

as the sample, and approximately 0.05 g of it is weighed to the digits of 0.1 mg and the value is recorded. It is added with dimethylformamide, dissolved by heating, allowed to cool, transferred to a 100 mL volumetric flak, is further added with dimethylformamide to the graduation line to make 100 mL. This is used as a standard stock solution.

4,4′-dinitrocarbanilide: 4 mL of the sample stock solution is measured using a volumetric pipette, transferred to a 200 mL volumetric flask, added with ethanol to make 200 mL, and mixed well. This solution 15 mL is measured using a volumetric pipette, transferred to a 25 mL volumetric flask, added with 5 mL of potassium hydroxide/ethanol solution (1 → 100), and is further added with ethanol to the graduation line to make 25 mL. This is used as a sample solution. The standard stock solution 4 mL is measured using a volumetric pipette, transferred to a 200 mL volumetric flask, added with ethanol to make 200 mL, and hereinafter subjected to the same procedure as the sample solution to prepare the standard solution. When the absorbances A_S and A_T of the standard and sample solutions at the maximum wavelength near 430 nm are measured using potassium hydroxide/ethanol solution (1 → 500) as a control solution.

Amount of 4,4′-dinitrocarbanilide ($C_{13}H_{10}N_4O_5$) (mg)

$$= \text{Nicarbazin reference standard (mg)} \times \frac{A_T}{A_S} \times 0.7089$$

2-hydroxy-4, 6-dimethylpyrimidine: 4 mL of the sample stock solution is measured using a volumetric pipette, transferred to a 200 mL volumetric flask, added with a mixed solution of dimethylformamide and ethanol (1:1) to the graduation line to make 200 mL, and mixed well. 10 mL of this solution is measured using a volumetric pipette, transferred to a 50 mL stoppered test tube, added with 10 mL of newly prepared sulfanilamide test solution and mixed. It is added with 2 mL of newly prepared sodium nitrite solution (1 → 50), tightly stoppered, mixed, heated in a water bath at 65 °C for 15 minutes, cooled with running water, allowed to stand at room temperature for 20 minutes. This is used as a sample solution. Separately, 4 mL of the standard stock solution is measured using a volumetric pipette, transferred to a 200 mL volumetric flask, added with ethanol to the graduation line to make 200 mL, and from here is subjected to the same procedure as the sample solution to prepare the standard solution. Separately, 10 mL of a mixed solution of dimethylformamide and ethanol (1:1) is measured with a volumetric pipette, transferred to a 50 mL stoppered test tube, and hereinafter subjected to the same procedure as the sample solution to prepare the blank test solution. The absorbances A_S and A_T of the standard and sample solutions at the maximum wavelength near 540 nm are measured using the blank test solution as a control solution.

Amount of 2-hydroxy-4,6-dimethylpyrimidine ($C_6H_8N_2O$) (mg)

$$= \text{Nicarbazin reference standard (mg)} \times \frac{A_T}{A_S} \times 0.2912$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container.

B. Preparation

(a) Compositional standards

This product is solid and powder in which the raw material for manufacturing of nicarbazin and fillers.

Content: This product is determined to contain nicarbazin ($C_{19}H_{18}N_6O_6$) corresponding to 98~106 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing 5 mg of the raw material for manufacturing of nicarbazin is weighed, added with 60 mL of absolute ethanol and shaken well while heating. It is allowed to cool and filtered, and the filtrate 15 mL is added with 5 mL of sulfanilic acid test solution and 5 mL of newly prepared sodium nitrite solution (1 → 100), tightly stoppered, and heated in a water bath at 65 °C for 10 minutes. The resulting solution is red.

ii. According to the amount of this product on the label, the amount containing 5 mg of the raw material for manufacturing of nicarbazin is weighed, added with 60 mL of absolute ethanol and shaken well while heating. It is allowed to cool and filtered, and the filtrate 15 mL is added with 5 mL of potassium hydroxide/ethanol solution (1 → 100). The resulting solution is yellow.

iii. In the measurement of the absorption spectrum of the sample solution prepared by the assay for 4,4′-dinitrocarbanilide, the absorption maximum is at the wavelengths of 428~432 nm.

iv. In the measurement of the absorption spectrum of the sample solution prepared by the assay for 2-hydroxy-4, 6-dimethylpyrimidine, the absorption maximum is at the wavelengths of 538~542 nm.

Assay: The amount of this product containing approximately 0.125 g of nicarbazin ($C_{19}H_{18}N_6O_6$) is weighed to three significant digits and the value is recorded. It is placed in a 200 mL volumetric flask, added with 150 mL of dimethylformamide, heated in a water bath for 15 minutes, and shaken for 15 minutes. It is allowed cool, added with dimethylformamide to the graduation line to make 200 mL, mixed well and centrifuged for 5 minutes. This supernatant is collected to use as the sample stock solution. Separately, nicarbazin reference standard is dried, and approximately 0.05 g of it is weighed to the digits of 0.01 mg and the value is recorded. It is added with

dimethylformamide, dissolved by heating, allowed to cool, transferred to a 100 mL volumetric flask, added with dimethylformamide to the graduation line to 100 mL. This is used as a standard stock solution.

4,4′-dinitrocarbanilide: The sample stock solution 4 mL is measured using a volumetric pipette, transferred to a 250 mL volumetric flask, added with ethanol to make 250 mL. 15 mL of this solution is measured using a volumetric pipette, transferred to a 25 mL volumetric flask, added with 5 mL of potassium hydroxide/ethanol solution (1 → 100) using a volumetric pipette, and added with ethanol to the graduation line to make 25 mL, and mixed well. This is used as a sample solution. Separately, 5 mL of the standard stock solution is measured using a volumetric pipette, transferred to a 250 mL volumetric flask, and added with ethanol to the graduation line to make 250 mL. 15 mL of this solution is measured using a volumetric pipette, and from here subjected to the same procedure as that for the sample solution to prepare the standard solution. The absorbances A_T and A_S of the sample and standard solutions at the maximum wavelength near 430 nm are measured using potassium hydroxide/ethanol solution (1 → 500) as a control solution.

Amount of nicarbazin ($C_{19}H_{18}N_6O_6$) (mg)

$$= \text{Nicarbazin reference standard (mg)} \times \frac{A_T}{A_S} \times 2.5$$

2-hydroxy-4, 6-dimethylpyrimidine: 4 mL of the sample stock solution is measured using a volumetric pipette, transferred to a 250 mL volumetric flask, added with a mixed solution of dimethylformamide and ethanol (1:1) to the graduation line to make 250 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 50 mL stoppered test tube, added with 10 mL of newly prepared sulfanilamide test solution and mixed. It is added with 2 mL of newly prepared sodium nitrite solution (1 → 50), tightly stoppered, mixed, heated in a water bath at 65 °C for 15 minutes, cooled with running water, allowed to stand for 20 minutes. This is used as a sample solution. Separately, 5 mL of the standard stock solution is measured using a volumetric pipette, transferred to a 250 mL volumetric flask, and added with a mixed solution of dimethylformamide and ethanol (1:1) to the graduation line to make 250 mL, and from here subjected to the same procedure as that for the sample solution to prepare a standard solution. Separately, 10 mL of a mixed solution of dimethylformamide and ethanol (1:1) is subjected to the same procedure as that for the sample solution to prepare a blank test solution. The absorbances A_S and A_T of the standard and sample solutions at the maximum wavelength near 540 nm are measured using the blank test solution as a control solution.

Amount of nicarbazin ($C_{19}H_{18}N_6O_6$) (mg)

$$= \text{Nicarbazin reference standard (mg)} \times \frac{A_T}{A_S} \times 2.5$$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(129) Calcium Halofuginone Polystyrene-sulfonate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried and determined, it contains 95.0 % or more of calcium halofuginone polystyrene-sulfonate ($C_{16}H_{17}BrClN_3O_3$·Calcium halofuginone Polystyrene sulfonate).

Physical and chemical properties: This product is pale yellow-brown to brown powder with little odor.

Confirmation test:

i. 0.2 g (0.15~0.24 g) of this product is weighed, added with 20 mL of 6 mol/L hydrochloric acid test solution, stirred for 30 minutes, and centrifuged. 10 mL of the supernatant is measured and added with 10 mL of methanol to prepare a sample solution. Separately, 20 mg (19.5~20.4 mg) of halofuginone hydrobromide is weighed and dissolved with 100 mL of a mixed solution of 6 mol/L hydrochloric acid test solution and methanol (1:1) to prepare a standard solution. 10 μL each of the sample and standard solutions are measured, and spotted on a thin layer plate prepared using silica gel for thin-layer chromatography (with a fluorescent agent). Then, they are developed to approximately 15 cm with the developing solvent, a mixed solution of chloroform, methanol and ammonia solution (90:10:1), and the thin layer plate is air dried. When it is irradiated with ultraviolet light (dominant wavelength: 254 nm), the spots obtained from the sample and standard solutions are dark purple and their Rf values are equal.

ii. 0.2 g (0.15~0.24 g) of this product is weighed, added with 5 mL of 6 mol/L hydrochloric acid test solution, stirred for 5 minutes, filtered, and neutralized with ammonia test solution. The resulting solution gives the qualitative reaction iii. of calcium salt.

iii. 20 mg (19.5~20.4 mg) of this product is weighed, added with 5 mL of 6 mol/L hydrochloric acid test solution, stirred for 5 minutes, and centrifuged, and the supernatant is removed. Then, the residue is added with 10 mL of water, shaken, and centrifuged, and the supernatant is removed. This residue is added to 2 mL of copper

sulfate solution (1 → 10), shaken well, and centrifuged, and the supernatant is removed. Then, this residue is added with 10 mL of water, shaken, and centrifuged, and the supernatant is removed. This residue is added with 2 mL of ammonia test solution and shaken for 1 minute, and the resulting solution is dark blue.

iv. 0.5 g (0.45~0.54 g) of this product is weighed, added with 10 mL of 6 mol/L hydrochloric acid test solution, stirred for 15 minutes, and filtered. In the measurement of the absorption spectrum of the solution of 1 mL of the filtrate and 25 mL of water, the absorption maximum is at the wavelengths of 241~245 nm.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Halofuginone: This product is dried, and 0.5 g (0.45~0.54 g) of it is weighed, added with 50 mL of dimethyl sulfoxide using a volumetric pipette, stirred for 30 minutes, and centrifuged. 1 mL of this supernatant is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, added with hydrochloric acid (1 → 100) to the graduation line to make 50 mL. This is used as a sample solution. Separately, 17 mg (16.5~17.4 mg) of halofuginone hydrobromide is weighed, dissolved with hydrochloric acid (1 → 100), transferred to a 500 mL volumetric flak, and is further added with hydrochloric acid (1 → 100) to the graduation line to make 500 mL. The solution 1 mL is measured using a volumetric pipette, transferred to a 200 mL volumetric flask, added with hydrochloric acid (1 → 100) to the graduation line to make 200 mL. This is used as a standard solution. When 100 μL each of the sample and standard solutions are measured, and tested by the liquid chromatography under the following conditions, the peak area of halofuginone in the sample solution shall be equal to or lower than that of halofuginone in the standard solution (0.1 % or less).

Operating conditions:

Detector: Ultraviolet absorptiometer (measurement wavelength: 243 nm)

Column: A stainless tube (inner diameter: 4~6 mm, length: 250 mm) is filled with approximately 5 μm of octadecyl silylation silica gel.

Column temperature: Constant temperature at around 40 °C

Mobile phase: Ammonium acetate 4.2 g (4.15~4.24 g) is dissolved with approximately 200 mL of water, added with 6.4 mL of acetic acid, and added with water to make 1,000 mL. This solution 700 mL is measured and added with 300 mL of acetonitrile.

Flow rate: 1.5 mL/min.

iv. Cis isomer: 17 mg (16.5~17.4 mg) of Halofuginone hydrobromide (cis isomer) is weighed, dissolved with hydrochloric acid (1 → 100), transferred to a 500 mL volumetric flask, and is further added with hydrochloric acid (1 → 100) to the graduation line to make 500 mL. 1 mL of this solution is measured using a volumetric pipette, transferred to a 500 mL volumetric flask, and added with 500 mL of hydrochloric acid (1 → 100) to the graduation line to prepare the standard solution. When 100 μL each of the sample and standard solutions obtained by the assay are measured and tested by the liquid chromatography under the same conditions as iii., the peak area of halofuginone in the sample solution shall be equal to or lower than that of halofuginone in the standard solution (0.2 % or less).

Loss on drying: 6.0 % or less (1 g, 105 °C, 4 hours)

Ignition residue: After drying, 29.0 % or less (1 g)

Polystyrene sulfonate: This product is dried, 0.5 g of it is weighed to the digits of 0.1 mg, and the value is recorded. It is added with 50 mL of 6 mol/L hydrochloric acid test solution, shaken for 15 minutes, and centrifuged, and the supernatant is removed. This procedure is repeated four times. The residue is added with 40 mL of water and shaken. The washing is repeated until opacity does not occur even if silver nitrate test solution is added in the washings, and the residue is dried at 105 °C for 12 hours. The amount of the residue weighed to the digits of 0.1 mg shall be 87.0 % or less.

$$\text{Amount of polystyrene sulfonate (\%)} = \frac{\text{Amount after drying (mg)}}{\text{Collected amount of this product (mg)}} \times 100$$

Assay: This product is dried and approximately 0.5 g of it is weighed to the digits of 0.001 g, and the valued is recorded. It is added with approximately 10 mL of 6 mol/L hydrochloric acid test solution, dispersed, washed with 6 mol/L hydrochloric acid test solution into a chromatographic tube with glass wool at the bottom (inner diameter 10 mm, height 150 mm). A 250 mL volumetric flask is placed as a receiver under the chromatographic tube. Then, it is eluted using 6 mol/L hydrochloric acid test solution until the flow rate is approximately 225 mL. Then it is added with 6 mol/L hydrochloric acid test solution to the graduation line to make 250 mL. 1 mL of the solution is measured

using a volumetric pipette, transferred to a 50 mL volumetric flask, added with water to the graduation line to make 50 mL. This is used as a sample solution. The absorbance A of the sample solution at the maximum wavelength near 243 nm is measured using hydrochloric acid (1 → 100) as a control solution.

Amount of Calcium halofuginone Polystyrene sulfonate ($C_{16}H_{17}BrClN_3O_3$) (mg)

$$= \frac{A}{86.3} \times 125{,}000$$

(b) Standard of storage method

It shall be stored in an airtight container.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of Calcium Polystyrene sulfonate and fillers.

Content: When this product is determined, it contains 80 μg/mg or less or the amount corresponding to 90~110 % of the amount on the label of halofuginone calcium polystyrene-sulfonate ($C_{16}H_{17}BrClN_3O_3$·Calcium halofuginonePolystyrene sulfonate)

Confirmation test:

i. According to the amount of this product on the label, the amount containing 0.02 g of the raw material for manufacturing of calcium halofuginone polystyrene-sulfonate is weighed, added with 50 mL of 6 mol/L hydrochloric acid test solution, stirred for approximately 15 minutes, and filtered. Approximately 2 mL of porous styrene divinylbenzene copolymer refined resin immersed in methanol is washed twice with water, and it is added in the above filtrate, stirred for 15 minutes, filtered with a glass filter. This resin is washed twice with 20 mL of water, added with 5 mL of methanol, stirred, and filtered. This methanol solution is used as a sample solution. Separately, 0.02 g (0.015~0.024 g) of the raw material for manufacturing of calcium halofuginone polystyrene-sulfonate is weighed, and subjected to the same procedure to prepare the solution. This is used as a standard solution. 10 μL each of the sample and standard solutions are weighed, and the confirmation test i. of the raw material for manufacturing of calcium halofuginone polystyrene-sulfonate is hereinafter applied mutatis mutandis.

ii. The sample solution obtained by the assay of calcium halofuginone polystyrene sulfonate *of the absorption maximum is* at the wavelengths of 243~247 nm by measuring the *absorption spectrum.*

Assay: The amount of this product containing 0.05 g of the raw material for manufacturing of calcium halofuginone polystyrene-sulfonate is weighed to three significant digits and

the value is recorded. It is gradually added with 20 mL of 6 mol/L hydrochloric acid test solution and shaken well. When the bubble release stops, it is washed with a little 6 mol/L hydrochloric acid test solution into a chromatographic tube with glass wool at the bottom (inner diameter 10 mm, height 150 mm). A 200 mL volumetric flask is placed as a receiver under the chromatographic tube. Then, it is eluted using 6 mol/L hydrochloric acid test solution until the flow rate is approximately 180 mL. Then it is added with 6 mol/L hydrochloric acid test solution to the graduation line to make 200 mL. 10 mL of this solution is measured using a volumetric pipette, transferred to a 50 mL volumetric flask, added with water to the graduation line to make 50 mL. This is used as a sample solution. The absorbance A of the sample solution at the maximum wavelength near 245 nm is measured using hydrochloric acid (10 → 100) as a control solution.

Amount of Calcium halofuginone Polystyrene sulfonate ($C_{16}H_{17}BrClN_3O_3$) (mg)

$$= \frac{A}{78.5} \times 10{,}000$$

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Calcium halofuginone polystyrene sulfonate is applied mutatis mutandis.

(c) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“使用上の注意　この飼料添加物は、鶏に過剰投与した場合発育障害が起こるので、定められた添加量を厳守するとともに、均一に配合するよう注意すること。”

Precautions: Overdose of this feed additive causes developmental disorder in chicken, so strictly follow the specified amount to be added and make sure to mix it uniformly.

(130) Saccharin Sodium

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is dried at 120 °C for 4 hours and then determined, it contains 99.0 % or greater saccharin sodium ($C_7H_4NNaO_3S$).

Physical and chemical properties: This product is colorless to white crystals, white crystalline powder or white powder with an extremely sweet taste. Even a 10,000-fold solution is sweet.

Confirmation test:

i. 0.5 g (0.45~0.54 g) of this product is dissolved with 10 mL of water, added with 1 mL of dilute hydrochloric acid, allowed to stand for 1 hour, and the white crystalline precipitation is collected by filtration, washed well, dried at 105 °C for 2 hours, and the melting point is 226~230 °C.

ii. 20 mg (19.5~20.4 mg) of this product is mixed with 40 mg (39.5~40.4 mg) of resorcin, added with 10 drops of sulfuric acid, and gently heated. When the mixture turns dark green, it is allowed to cool and dissolved with 10 mL of water and 10 mL of 1 mol/L sodium hydroxide test solution. The resulting solution emits green fluorescence.

iii. 0.1 g (0.05~0.14 g) of this product is dissolved with 5 mL of 1 mol/L sodium hydroxide test solution, gently heated, evaporated to dryness, then melted avoiding carbonization, and allowed to cool when ammonia odor is not produced. The residue is dissolved with approximately 20 mL of water, neutralized with dilute hydrochloric acid, and filtered. The filtrate with 1 drop of ferric chloride test solution is purple to reddish violet.

iv. The residue obtained by ashing of this product gives the qualitative reaction of sodium salt.

Purity test:

i. Clarity and color of solution: This product is powdered, and 1 g (0.5~1.4 g) each of the powder is dissolved in 1.5 mL of water and in 70 mL of ethanol. These solutions shall be colorless and clear.

ii. Free acid and free alkali: 1 g (0.5~1.4 g) of this product is dissolved in 10 mL of newly boiled and cooled, added with 1 drop of phenolphthalein test solution. The resulting solution shall not be red. Furthermore, the solution added with an additional 1 drop of 0.1 mol/L sodium hydroxide shall be red.

iii. Benzoate and salicylate: 0.5 g (0.45~0.54 g) of this product is dissolved in 10 mL of water and added with 5 drops of acetic acid and 3 drops of ferric chloride test solution. In the resulting solution there shall be no precipitation. Also, the solution shall not be purple to reddish violet.

iv. Heavy metal: 1.0 g (0.95~1.04 g) of this product is dissolved in 45 mL of water and added with 2 mL of diluted acetic acid. The solution is used as a sample solution to perform the test on heavy metal, the amount of heavy metal shall be 10 mg/kg or less.

v. Arsenic: 2.5 g (2.45~2.54 g) of this product is placed in a dissolution flask, added with 10 mL of nitric acid and 5 mL of sulfuric acid and heated. When the solution remains brown, it is allowed to cool, added with an additional 1 mL of nitric acid and heated. The procedure is repeated until the solution becomes colorless to pale yellow, and then

the solution is heated until white smoke emerges. It is allowed to cool, added with 10 mL of water and 15 mL of saturated ammonium oxalate solution and again heated until white smoke emerges. It is allowed to cool and added with 25 mL of water, and 5 mL of this solution is measured and used as a sample solution. When the sample solution is tested on arsenic by the method using device A, the color of absorbing solution shall not be darker than the standard color. However, the standard color is made by the procedure so that 10 mL of arsenic standard solution is transferred to a dissolution flask, added with 10 mL of nitric acid and 5 mL of sulfuric acid and hereinafter subjected to the same procedure as that for the sample solution (4 mg/kg or less).

vi. *o*-toluenesulfonamide: 10 g (9.5~10.4 g) of this product is dissolved in 50 mL of water and extracted three times with 30 mL each of ethyl acetate. The ethyl acetate layer is collected, washed with 30 mL of 25 w/v% sodium chloride solution, and dehydrated with anhydrous sodium sulfate, ethyl acetate is distilled away, and the residue is dissolved with 5 mL of internal standard solution . This is a sample solution. Separately, *o*-Toluenesulfonamide-ethyl acetate solution (1 → 1,000) 1 mL is measured, heated in a water bath to remove ethyl acetate, and the residue is dissolved with 5 mL of internal standard solution to prepare a standard solution. When the sample and standard solutions are tested by the gas chromatography under the following conditions, the ratio H/H_S of the peak height of caffeine (H_S) and *o*-toluenesulfonamide (H) in the sample solution shall not exceed the ratio H'/H'_S of the peak height of caffeine (H'_S) and *o*-toluenesulfonamide (H′) in the standard solution (100 mg/kg or less). However, as the internal standard solution solution, caffeine-ethyl acetate solution (1 → 5,000) is used.

Operating conditions:

Column packings: Diatomaceous earth carrier for gas chromatograph (177~250 μm) is added with chloroform solution containing succinate diethylene glycol polyester, corresponding to 3 % of such carrier to evaporate chloroform, and dried.

Column tube: Glass or stainless tube with inner diameter of 3~4 mm and length of 1 m

Column temperature: Constant temperature at around 195~205 °C

Detector: Hydrogen flame ionization detector

Carrier gas: Nitrogen gas is used. The column temperature and the flow of carrier gas are adjusted so that caffeine appears approximately 6 minutes after.

Loss on drying: 15 % or less (1 g, 120 °C, 4 hours)

Assay: This product is dried at 120 °C for 4 hours and approximately 0.3 g of it is weighted to the digits of 0.001 g, and the value is recorded. It is dissolved with 20 mL of glacial

acetic acid for nonaqueous titration, and titrated with 0.1 mol/L perchloric acid (indicator: 2 drops of crystal violet-glacial acetic acid test solution). The end point of titration is the time when the color of the solution changes from purple through blue to green. Separately, a blank test is performed in the same way and corrections are made.

1 mL of 0.1 mol/L perchloric acid = 20.52 mg $C_7H_4NNaO_3S$

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of saccharin sodium are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of saccharin sodium is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or crystalline powder of a mixture of the raw material for manufacturing of saccharin sodium and fillers.

Content: This product is determined to contain saccharin sodium ($C_7H_4NNaO_3S$) corresponding to 90~110 % of the amount on the label.

Confirmation test:

i. According to the amount of this product on the label, the amount containing approximately 0.5 g of the raw material for manufacturing of saccharin sodium is weighed, added with 30 mL of water, shaken well, and if necessary filtered. This solution is added with 1 mL of dilute hydrochloric acid and allowed to stand for 1 hour, and the resulting white crystalline precipitation is filtered, washed well, and dried at 105 °C for 2 hours. This is used as a sample. The sample 0.5 g (0.45~0.54 g) is weighed, and the confirmation test i. of the raw material for manufacturing of saccharin sodium is hereinafter applied mutatis mutandis.

ii. 20 mg (19.5~20.4 mg) of the sample i. is weighed, and the confirmation test ii. of raw material for manufacturing of saccharin sodium is hereinafter applied mutatis mutandis.

iii. 0.1 g (0.05~0.14 g) of the sample i. is dissolved in 5 mL of 1 mol/L sodium hydroxide test solution, gently heated, evaporated to dryness, and allowed to cool. The residue is dissolved in approximately 20 mL of water, neutralized with dilute hydrochloric acid,

and filtered. The filtrate added with 1 drop of ferric chloride test solution is purple to reddish violet.

iv. For the sample of i., the confirmation test iv. of the raw material for manufacturing of saccharin sodium id applied mutatis mutandis.

Assay: The amount of this product containing approximately 0.3 g of saccharin sodium ($C_7H_4NNaO_3S$) is weighed to three significant digits and the value is recorded. It is dissolved with 20 mL of glacial acetic acid for nonaqueous titration and titrated with 0.1 mol/L perchloric acid (indicator: 2 drops of crystal violet-glacial acetic acid test solution). In this case, the end point of titration is the time when the color of the solution changes from purple to blue. A blank test is performed in the same manner, and corrections are made.

1 mL of 0.1 mol/L perchloric acid = 20.52 mg $C_7H_4NNaO_3S$

(b) Standard of storage method

It shall be stored in a capped container.

(131) Flavour

A. Raw material for manufacturing

(a) Compositional standards

This product is liquid including one or not less than two of the following active ingredients: esters, ethers, ketone, fatty acids, aliphatic higher alcohols, aliphatic higher aldehydes, aliphatic higher hydrocarbons, terpene hydrocarbons, phenol ethers, phenol, aromatic alcohols, aromatic aldehydes, and lactone.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of raw materials for manufacturing are applied mutatis mutandis. However, no adverse effects are observed in the amount used for feed.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is powder or particles of a mixture of the raw material for manufacturing and fillers. No adverse effects are observed in the amount used for feed.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing is applied mutatis mutandis.

D. Preparation (Part 3 oil liquid)

(a) Compositional standards

This product is oil liquid of a mixture of the raw material for manufacturing and hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil, or animal fats. No adverse effects are observed in the amount used for feed.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing is applied mutatis mutandis.

(132) Amylase

Amylase (Part 1)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 3,500 starch saccharification power units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale yellow to brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 5.0~7.5.

iii. This product produces maximum enzyme activity at pH 4.5~5.5.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 25.0 % or less (1 g)

Enzymatic activity test: It is tested by the starch saccharification power test method.

(b)Standard of manufacturing method

For manufacturing, the amylase producing strain of *Aspergillus oryzae* or *Rhizopus delemar* is cultured, after the cultivation, the culture is filtered, or extracted with water, and then filtered to remove bacterial cells, and then the filtrate is dried or the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Amylase (Part 1) are applied mutatis mutandis.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Amylase (Part 1) are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Amylase (Part 1) is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Amylase (Part 1) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces or powder of the mixture of the raw material for manufacturing of s Amylase (Part 1) and fillers.

Enzymatic activity unit: This product contains 85~170 % of starch saccharification power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the starch saccharification power test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Amylase (Part 1) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Amylase (Part 1) is applied mutatis mutandis.

Amylase (Part 2)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 2,000 starch saccharification power units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is dark brown liquid with a specific odor.

ii. pH: The pH of this product is 5.0~7.5.

iii. This product has maximum enzyme activity at pH 5.0~7.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less)

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Ignition residue: After drying, 20.0 % or less (1 g)

Enzymatic activity test: It is tested by the starch saccharification power test method.

(b) Standard of manufacturing method

For manufacturing: The amylase producing strain of *Bacillus subtilis* or *Bacillus amyloliquefaciens* is cultured, after the cultivation, the culture is filtered, or extracted with water, then filtered to remove bacterial cells, and then the filtrate is concentrated.

(c) Standard of storage method

It shall be stored in a capped container and in the freezer.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Amylase (Part 1) is applied mutatis mutandis.

B. Preparation (Part 1)

(a) Compositional standards

This product is pieces, powder, or particles in which the raw material for manufacturing of of s Amylase (Part 2) and fillers.

Enzymatic activity unit: This product contains 85~170 % of starch saccharification power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the starch saccharification power test method.

(b) Standard of storage method

It shall be stored in a capped container.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Amylase (Part 1) is applied mutatis mutandis.

C. Preparation (Part 2 liquid)

(a) Compositional standards

This product is semiliquid, oil liquid, or water-soluble liquid of the mixture of the raw material for manufacturing of amylase (Part 2) and hydrated silicon dioxide, hydrated amorphous silicon oxide, silicic acid, calcium silicate, light anhydrous silicic acid, hydrogenated oils, higher saturated fatty acids, glycerin, fatty acids, salt, vegetable oil, animal fats, lactose, maltose, white sugar, glucose, silicic anhydride or anhydrous tricalcium silicates.

Enzymatic activity unit: This product contains 85~170 % of starch saccharification power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the starch saccharification power test method.

(b) Standard of storage method

The standards of storage method of amylase (Part 2) Preparation (Part 1) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Amylase (Part 1) is applied mutatis mutandis.

(133) Alkaline Protease

Alkaline protease (Part 1)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 100,000 protein digestive capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is white to pale brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 5.5~8.5.

iii. This product produces maximum enzyme activity at pH 7.0~9.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 25.0 % or less (1 g)

Enzymatic activity test: It is tested by method No. 1 in the protein digestive capacity test method.

(b)Standard of manufacturing method

For manufacturing, alkaline protease producing strain of *Aspergillus melleus, Bacillus licheniformis*, or *Streptomyces caespitosus* is cultured, after the cultivation, the culture is filtered, or extracted with water and filtered to remove bacterial cells, and then the filtrate is dried or the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Alkaline protease (Part 1) are applied mutatis mutandis.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Alkaline protease (Part 1) are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Alkaline protease (Part 1) is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Alkaline protease (Part 1) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces, powder, or particles in which the raw material for manufacturing of Alkaline protease (Part 1) is mixed with calcium sulfate as appropriate, and sodium sulfate and fillers.

Enzymatic activity unit: This product contains 85~170 % of protein digestive power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 1 in the protein digestive capacity test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Alkaline protease (Part 1) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Alkaline protease (Part 1) is applied mutatis mutandis.

D. Preparation (Part 3 liquid)

(a) Compositional standards

This product is water-soluble liquid of a mixture of the raw material for manufacturing of Alkaline protease (Part 1) and glycerin.

Enzymatic activity unit: This product contains 85~170 % of protein digestive power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 1 in the protein digestive capacity test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Alkaline protease (Part 1) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Alkaline protease (Part 1) is applied mutatis mutandis.

Alkaline Protease (Part 2)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 80,000 protein digestive capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale brown liquid with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 5.5~8.5.

iii. This product produces maximum enzyme activity at pH 7.0~9.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Ignition residue: After drying, 10.0% or less (1 g)

Enzymatic activity test: It is tested by method No. 1 in the protein digestive capacity test method.

(b)Standard of manufacturing method

For manufacturing, alkaline protease producing strain of *Bacillus subtilis* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is concentrated.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

This product is pieces, powder, or particles in which the raw material for manufacturing of Alkaline protease (Part 2) is mixed with fillers.

Enzymatic activity unit: This product contains 85~170 % of protein digestive power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the protein digestive power test method.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Alkaline protease (Part 2) is applied mutatis mutandis.

C. Preparation (Part 2 liquid)

(a) Compositional standards

This product is water-soluble liquid of a mixture of the raw material for manufacturing of Alkaline protease (Part 2) and propylene glycol.

Enzymatic activity unit: This product contains 85~170 % of protein digestive power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the protein digestive power test method.

(b) Standard of storage method

It shall be stored in a capped container and under 25 degrees.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Alkaline protease (Part 2) is applied mutatis mutandis.

Alkaline protease (Part 3)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 200,000 protein digestive capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale brown to rich brown liquid with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 4.0~7.0.

iii. This product produces maximum enzyme activity at pH 9.0~11.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Ignition residue: After drying, 5.0 % or less (1 g)

Enzymatic activity test: It is tested by method No. 1 in the protein digestive capacity test method.

(b)Standard of manufacturing method

For manufacturing, alkaline protease producing recombinant, whose host is the strain belonging to *Bacillus subtilis* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is concentrated.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

This product is pieces, powder, or particles in which the raw material for manufacturing of Alkaline protease (Part 3) is mixed with sodium sulfate as appropriate, and sucrose and fillers.

Enzymatic activity unit: This product contains 85~170 % of protein digestive power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 1 in the protein digestive capacity test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Alkaline protease (Part 3) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Alkaline protease (Part 3) is applied mutatis mutandis.

C. Preparation (Part 2 liquid)

(a) Compositional standards

This product is water-soluble liquid in which the raw material for manufacturing of Alkaline protease (Part 3) is mixed with sodium benzoate and potassium sorbate as appropriate, and sorbitol, glycerin.

Enzymatic activity unit: This product contains 85~170 % of protein digestive power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 1 in the protein digestive capacity test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Alkaline protease (Part 3) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Alkaline protease (Part 3) is applied mutatis mutandis.

(134) Xylanase

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 4,000 xylan saccharification power units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale brown liquid with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 4.0~7.0.

iii. This product produces maximum enzyme activity at pH 5.0~6.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Ignition residue: After drying, 10.0 % or less (1 g)

Enzymatic activity test: It is tested by the xylan saccharification power test method.

(b)Standard of manufacturing method

For manufacturing, Xylanase producing strain of *Trichoderma longibrachiatum* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is concentrated.

(c) Standard of storage method

It shall be stored in a lightproof and airtight container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

This product is pieces, powder, or particles in which the raw material for manufacturing of Xylanase is mixed with fillers.

Enzymatic activity unit: This product contains 85~170 % of xylan saccharification power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the xylan saccharification power test method.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of xylanase is applied mutatis mutandis.

C. Preparation (Part 2 liquid)

(a) Compositional standards

This product is water-soluble liquid of a mixture of the raw material for manufacturing of xylanase and D-sorbitol.

Enzymatic activity unit: This product contains 85~170 % of starch saccharification power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the starch saccharification power test method.

(b) Standard of storage method

It shall be stored in a capped container and 25 degrees or less.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of xylanase is applied mutatis mutandis.

(135) Xylanase/Pectinase complexing enzyme

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 400 xylan saccharification power units or more and 12,000 pectin saccharification power units or more per 1 g by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale yellow and pale brown pawder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 5.0~7.0.

iii. This product has xylanase maximum enzyme activity at pH 3.0~5.0 and pectinase maximum enzyme activity at pH 3.5~4.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is placed in a quartz or porcelain pot, loosely covered with a lid, and weakly heated to be carbonized. It is allowed to cool, added with 2 mL of nitric acid and 5 drops of sulfuric acid, heated carefully until white smoke emerges, and ignited at 500~600 °C to be ashed. It is allowed to cool, added

with 2 mL of hydrochloric acid, and evaporated to dryness in a water bath, and the resides is moisturized with 3 drops of hydrochloric acid, added with 10 mL of boiling water, and heated for 2 minutes. Then, it is adjusted pH 5.0~6.0 with ammonia test solution, added with 2 mL of diluted acetic acid, if necessary filtered, and washed with 10 mL of water. The filtrate and the washings are transferred to a Nessler tube and added with 50 mL of water. This is used as a sample solution. By the heavy metals test using a control solution prepared with 5.0 mL of lead standard solution by method No. 2 of the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 10 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 30 % or less (1 g)

Enzymatic activity test: It is tested by the xylan saccharification power test method and the pectin saccharification power test method.

(b)Standard of manufacturing method

For manufacturing, xylanase-pectinase complexing enzyme producing strain of *Aspergillus usamii mut. shiro-usamii* is cultured, after the cultivation, the culture is extracted with water and filtered to remove bacterial cells, and then the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation

(a) Compositional standards

This product is powder of the mixture of the raw material for manufacturing of Xylanase/pectinase complexing enzyme and fillers.

Enzymatic activity unit: This product contains 85~200 % of xylan saccharification power units and pectin saccharification power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the Xylane saccharification power test method and pectin saccharification power test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Xylanase/pectinase complexing enzyme is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Xylanase/pectinase complexing enzyme is applied mutatis mutandis.

(136) β-glucanase

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 4,000 beta-glucan saccharification power units or more/g by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale brown liquid with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 3.5~6.0.

iii. This product has maximum enzyme activity at pH 3.5~5.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Ignition residue: 10.0 % or less (1 g)

Enzymatic activity test: It is tested by the beta-glucan saccharification power test method.

(b)Standard of manufacturing method

For manufacturing, *β*-glucanase producing strain of *Trichoderma longibrachiatum* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is concentrated.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

This product is pieces, powder, or particles in which the raw material for manufacturing of *β*-glucanase complexing enzyme and fillers.

Enzymatic activity unit: This product contains 85~170 % of *β*-glucanase saccharification power units and pectin saccharification power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the *β*-glucanase saccharification power test method.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of *β*-glucanase complexing enzyme is applied mutatis mutandis.

C. Preparation (Part 2 liquid)

(a) Compositional standards

This product is water-soluble liquid of a mixture of the raw material for manufacturing of *β*-glucanase and water.

Enzymatic activity unit: This product contains 85~170 % of *β*-glucanase saccharification power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the *β*-glucanase saccharification power test method.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of *β*-glucanase is applied mutatis mutandis.

(137) Acid Protease

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 60,000 protein digestive capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale white to brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 3.5~7.0.

iii. This product has maximum enzyme activity at pH 2.0~4.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 15.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 25.0 % or less (1 g)

Enzymatic activity test: It is tested by method No. 2 of the protein digestive power test method.

(b)Standard of manufacturing method

For manufacturing, acid protease producing strain of *Aspergillus niger, Aspergillus saito, Rhizopus delemar,* or *Rhizopus niveus* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is dried or the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Acid protease are applied mutatis mutandis.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Acid protease are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Acid protease is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Acid protease is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of Acid protease complexing enzyme and fillers.

Enzymatic activity unit: This product contains 85~170 % of protein digestive power units and pectin saccharification power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 2 of the protein digestive power test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Acid protease is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Acid protease is applied mutatis mutandis.

(138) Cellulase

Cellulase(Part 1)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 1,500 fiber disruption capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale yellow to brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 4.0~7.5.

iii. This product produces maximum enzyme activity at pH 4.0~5.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 25.0 % or less (1 g)

Enzymatic activity test: It is tested by the fiber disruption capacity test method.

(b)Standard of manufacturing method

For manufacturing, cellulase producing strain of *Trichoderma reesei,* or *Trichoderma viride* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is dried or the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Cellulase (Part 1) are applied mutatis mutandis.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Cellulase (Part 1) are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Cellulase (Part 1) is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Cellulase (Part 1) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces, powder, or particles in which the raw material for manufacturing of Cellulase (Part 1) is mixed with fillers.

Enzymatic activity unit: This product contains 85~170 % of fiber disruption power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the fiber disruption capacity test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Cellulase (Part 1) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Cellulase (Part 1) is applied mutatis mutandis.

Cellulase (Part 2)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 250 fiber saccharification power units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale yellow to brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 4.0~7.5.

iii. This product produces maximum enzyme activity at pH 4.0~5.5.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 25.0 % or less (1 g)

Enzymatic activity test: It is tested by the fiber saccharification power test method.

(b)Standard of manufacturing method

For manufacturing, Cellulase producing strain of *Acremonium cellulolyticus, Aspergillus aculeatus, Humicola insolens,* or *Trichoderma viride* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is dried or the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Cellulase (Part 2) are applied mutatis mutandis.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Cellulase (Part 2) are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Cellulase (Part 2) is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Cellulase (Part 2) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This is pieces, powder or particles of the granulated mixture with the raw material for manufacturing Cellulase (Part 2) and as appropriate polyvinyl alcohol solution, or of the mixture added with sodium sulfate and fillers.

Enzymatic activity unit: This product contains 85~170 % fiber saccharification power capacity units/g or more by an enzymatic activity test.

Enzymatic activity test: It is tested by the fiber saccharification power test method.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Cellulase (Part 2) are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Cellulase (Part 2) is applied mutatis mutandis.

D. Preparation (Part 3 liquid)

(a) Compositional standards

This product is water-soluble liquid of the mixture of the raw material for manufacturing of Cellulase (Part 2) and, as appropriate sorbitol, glycerin, salt, glucose, lactose, maltose, and white sugar.

Enzymatic activity unit: This product contains 85~170 % fiber saccharification power capacity units/g or more by an enzymatic activity test.

Enzymatic activity test: It is tested by the fiber saccharification power test method.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Cellulase (Part 2) are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Cellulase (Part2) is applied mutatis mutandis.

(139) Cellulase/Protease/Pectinase Complexing enzyme

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 800 fiber disruption capacity units or more, 300 protein digestive capacity units or more and 200 pectin liquefaction capacity units or more per 1 g by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale brown to yellow brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 4.0~7.0.

iii. This product has cellulose, protease and pectinase maximum enzyme activities at pH 3.5~5.0, pH 3.0~4.0 and pH 4.5~5.5, respectively.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 25.0 % or less (1 g)

Enzymatic activity test: It is tested by the fiber disruption capacity test method, method No. 2 of the protein digestive capacity test method and the pectin liquefaction capacity test method.

(b)Standard of manufacturing method

For manufacturing, cellulase/protease/pectinase complexing enzyme producing strain of *Irpex lacteus* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is dried or the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity of each cellulose, cellulose, and pectinase shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Cellulase/protease/pectinase complexing enzyme are applied mutatis mutandis.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Cellulase/protease/pectinase complexing enzyme are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Cellulase/protease/pectinase complexing enzyme is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Cellulase/protease/pectinase complexing enzyme is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This is pieces, powder or particles of the mixture with the raw material for manufacturing Cellulase/protease/pectinase complexing enzyme and fillers.

Enzymatic activity unit: This product contains 85~200 % of fiber disruption power units, protein digestive power units and pectin liquefaction power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by the fiber disruption capacity test method, method No. 2 of the protein digestive capacity test method and the pectin liquefaction capacity test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Cellulase/protease/pectinase complexing enzyme is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Cellulase/protease/pectinase complexing enzyme is applied mutatis mutandis.

(140) Neutral Protease

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 60,000 protein digestive capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale brown to brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 5.5~7.5.

iii. This product produces maximum enzyme activity at pH 6.0~7.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 25.0 % or less (1 g)

Enzymatic activity test: It is tested by method No. 3 of the protein digestive capacity test method.

(b)Standard of manufacturing method

For manufacturing, Neutral protease enzyme producing strain of *Aspergillus oryzac* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is dried or the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Neutral protease are applied mutatis mutandis.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Neutral protease are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Neutral protease is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Neutral protease is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces, powder, or particles in which the raw material for manufacturing of Neutral Protease is mixed with fillers.

Enzymatic activity unit: This product contains 85~170 % of protein digestive power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 3 of the protein digestive power test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Neutral protease is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Neutral protease is applied mutatis mutandis.

(141) Phytase

Phytase (Part 1)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 1,500 phytic acid degradation capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is white to pale brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 4.5~7.5.

iii. This product produces maximum enzyme activity at pH 5.0~6.0.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample

solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is placed in a dissolution flask, added with 10 mL of nitric acid and 5 mL of sulfuric acid, and gently heated. When the solution remains brown, it is allowed to cool, added with an additional 1~2 mL of nitric acid and heated. The procedure is repeated until the solution becomes colorless to slightly yellow. After cooling, it is added with 0.5 mL of perchloric acid and heated until white smoke emerges. After cooling, it is added with 15 mL of saturated ammonium oxalate and again heated until white smoke emerges. It is allowed to cool and added with water to make approximately 10 mL. This is used as a sample solution. When the sample solution is tested on arsenic by the method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 12.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 25.0 % or less (0.5 g)

Enzymatic activity test: It is tested by method No. 1 of the phytic acid degradation capacity test method.

(b) Standard of manufacturing method

For manufacturing, Neutral protease enzyme producing strain of *Aspergillus niger* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is dried or the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Phytase (Part 1) are applied mutatis mutandis.

(b) Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Phytase (Part 1) are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase (Part 1) is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Phytase (Part 1) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This is pieces, powder or particles of the mixture with the raw material for manufacturing Phytase (Part 1) and fillers.

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 1 of the phytic acid degradation capacity test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase (Part 1) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Phytase (Part 1) is applied mutatis mutandis.

Phytase ((1) of Part 2)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 5,000 phytic acid degradation capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale brown liquid with a slightly specific odor.

ii. The solution (1 → 100) of this product is pH 3.5~6.5.

iii. This product produces maximum enzyme activity at pH 4.0~6.0.

Purity test: The purity test of the raw material for manufacturing of Phytase (Part 1) are applied mutatis mutandis.

Ignition residue: 5.0 % or less (0.5 g)

Enzymatic activity test: It is tested by method No. 2 of the phytic acid degradation capacity test method.

Preparation of sample solution: Polysorbate 20 0.1 g (0.05~0.14 g) is dissolved with 0.25 mol/L acetic acid/hydrochloric acid buffer, and is further added with the same buffer to make 1 liter. This is used as a diluent. The amount required for testing of the sample is weighed to three significant digits and the value is recorded. It is added with the diluent to make its concentration 50 phytic acid degradation capacity units/mL, vigorously stirred to be dissolved. This is used as a sample stock solution. Adequate volume of this stock solution is measured using a volumetric pipette, added with the diluent to make its concentration 0.5 phytic acid degradation capacity units/mL to prepare a sample solution.

(b) Standard of manufacturing method

For manufacturing, the phytase producing recombinant, whose host is the strain belonging to *Aspergillus oryzae*, is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells and then the filtrate is concentrated.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1 liquid)

(a) Compositional standards

This product is water-soluble liquid of a mixture of the raw material for manufacturing of phytase ((1) of part 2), as appropriate sorbitol, and glycerin.

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 2 of the phytic acid digestive power test method.

Preparation of sample solution: The preparation of sample solution of the raw material for manufacturing of phytase ((1) of part 2) is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((1) of part 2) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Phytase ((1) of Part 2) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces or particles of a mixture of the raw material for manufacturing of phytase ((1) of part 2), as appropriate sodium sulfate, and fillers.

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 2 of the phytic acid digestive power test method.

Preparation of sample solution: 5 g (4.5~5.4 g) of polysorbate 20 and 0.6 g (0.55~0.64 g) of bovine serum albumin is dissolved with 0.25 mol/L acetic acid/hydrochloric acid buffer, and is further added with the same buffer to make 1 liter. This is used as an extract. For the diluent, the raw material for manufacturing of phytase ((1) of part 2) is applied mutatis mutandis. The amount required for testing of the sample is weighed to three significant digits and the value is recorded. It is added with the extract to make its concentration of 50 phytic acid degradation capacity units/mL, and sonicated for 20 minutes using a triangular rotor while vigorously stirring, is further vigorously stirred for 20 minutes to be dissolved, and then centrifuged for 3 minutes at 14,000 rpm. The supernatant is used as a sample stock solution. The adequate volume of the stock solution is measured using a volumetric pipette, and added with the diluent to make its concentration of 0.25 phytic acid degradation capacity units/mL. This is used as a sample solution.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((1) of part 2) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Phytase ((1) of Part 2) is applied mutatis mutandis.

Phytase ((2) of Part 2)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 5,000 phytic acid degradation capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale yellow brown liquid with a slightly specific odor.

ii. The solution (1 → 100) of this product is pH 3.5~6.5.

iii. This product produces maximum enzyme activity at pH 5.0~6.0.

Purity test: The purity test of the raw material for manufacturing of Phytase (Part 1) are applied mutatis mutandis.

Ignition residue: 5.0 % or less (0.5 g)

Enzymatic activity test: It is tested by method No. 1 of the phytic acid degradation capacity test method. However, the words in the Procedures “0.005 mol/L acetic acid/sodium acetate buffer adjusted to pH showing maximum enzyme activity of the sample” shall be replaced by the words “buffer containing 0.1 g of polysorbate 20 per 1 liter of 0.005 mol/L acetic acid/sodium acetate adjusted to pH showing maximum enzyme activity of the sample”.

(b) Standard of manufacturing method

For manufacturing, the phytase producing recombinant, whose host is the strain belonging to *Aspergillus niger*, is cultured, and after the cultivation bacterial cells are disinfected and filtered, or extracted with water and filtered to be removed, and then the filtrate is concentrated.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1 liquid)

(a) Compositional standards

This product is water-soluble liquid of a mixture of the raw material for manufacturing of phytase ((2) of part 2), as appropriate sorbitol.

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 1 of the phytic acid degradation capacity test method. However, the words in the Procedures “0.005 mol/L acetic acid/sodium acetate buffer adjusted to pH showing maximum enzyme activity of the sample” shall be replaced by the words “buffer containing 0.1 g of polysorbate 20 per 1 liter of 0.005 mol/L acetic acid/sodium acetate adjusted to pH showing maximum enzyme activity of the sample”.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((2) of part 2) is applied mutatis mutandis.

(c) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity and the following words shall be described on the immediate container or the immediate wrapper of this product.

Valid period: 6 months from the day manufacture

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces or particles of a granulated mixture of the raw material for manufacturing of phytase ((2) of part 2) and corn starch and magnesium sulfate.

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 1 of the phytic acid degradation capacity test method. However, the words in the Procedures "0.005 mol/L acetic acid/sodium acetate buffer adjusted to pH showing maximum enzyme activity of the sample" shall be replaced by the words "buffer containing 0.1 g of polysorbate 20 per 1 liter of 0.005 mol/L acetic acid/sodium acetate adjusted to pH showing maximum enzyme activity of the sample".

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((2) of part 2) is applied mutatis mutandis.

(c) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity and the following words shall be described on the immediate container or the immediate wrapper of this product.

Valid period: 9 months from the day manufacture

Phytase ((3) of Part 2)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 5,000 phytic acid degradation capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale yellow brown liquid with a slight characteristic odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 3.5~6.5.

iii. This product produces maximum enzyme activity at pH 4.5~6.0.

Purity test: The purity test of the raw material for manufacturing of Phytase (Part 1) are applied mutatis mutandis.

Ignition residue: 5.0 % or less (0.5 g)

Enzymatic activity test: It is tested by method No. 1 of the phytic acid degradation capacity test method. However, the words in the Procedures “0.005 mol/L acetic acid/sodium acetate buffer adjusted to pH showing maximum enzyme activity of the sample” shall be replaced by the words “buffer containing 0.1 g of polysorbate 20 per 1 liter of 0.2 mol/L acetic acid/sodium acetate adjusted to pH showing maximum enzyme activity of the sample”.

(b) Standard of manufacturing method

Shall be manufactured by culturing phytase production recombinants hosted by bacterial strains belonging to *Schizosaccharomyces pombe*, filtering the culture product ,or filtrating the culture product after extracting with water, to remove bacterial cells when the culturing is complete, and by concentrating the filtrate.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1 liquid)

(a) Compositional standards

This product is a water-soluble liquid in which sodium chloride, citric acid and sorbitol are mixed into the raw material for manufacturing of Phytase ((3) of part 2).

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 1 of the phytic acid degradation capacity test method. However, the words in the Procedures “0.005 mol/L acetic acid/sodium acetate buffer adjusted to pH showing maximum enzyme activity of the sample” shall be replaced by the words “buffer containing 0.1 g of polysorbate 20 per 1 liter of 0.2 mol/L acetic acid/sodium acetate adjusted to pH showing maximum enzyme activity of the sample”.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((3) of part 2) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Phytase ((3) of Part 2) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This is pieces, powder or particles of the granulated mixture with the raw material for manufacturing Phytase ((3) of Part 2) and as appropriate polyvinyl alcohol solution, and then of the mixture added and mixed or granulated with sodium sulfate and fillers, or citric acid, wheat flour and calcium propionate are added into.

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method No. 1 of the phytic acid degradation capacity test method. However, the words in the Procedures "add and melt 0.005 mol/L acetic acid/sodium acetate buffer adjusted to pH showing maximum enzyme activity of the sample" shall be replaced by the words "add buffer containing 0.1 g of polysorbate 20 per 1 liter of 0.2 mol/L acetic acid/sodium acetate adjusted to pH showing maximum enzyme activity of the sample and stir in ice for 60 minutes to dissolve".

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((3) of part 2) is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Phytase ((3) of Part 2) is applied mutatis mutandis.

Phytase ((4) of Part 2)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 5,000 phytic acid degradation capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale brown liquid.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 3.5~6.5.

iii. This product produces maximum enzyme activity at pH 3.5~4.5.

Purity test:

i. Lead: Weigh 0.5 g (0.45~0.54 g) of this product and when a test for lead is performed by the limit test for lead (atomic absorption spectrometry No.1), the amount shall be no more than 20 μg/g.

ii. Arsenic: Apply mutatis mutandis the purity test ii. For source material for manufacturing Phytase (Part 1).

iii. Antibacterial activity: Apply mutatis mutandis the purity test iii for source material for manufacturing Phytase (Part 1).

Ignition residue: After drying, 5.0 % or less (0.5 g)

Enzymatic activity test: It is tested by method 1 of Phytic acid decomposition power test method. However, in the section of "Preparation of substrate solution", "dissolve by adding 50 mL of 0.2 mol/L acetate-sodium acetate buffer solution adjusted to pH showing the maximum enzymatic activity of the sample, adjust to pH 5.5 by adding 0.2 mol/L acetic acid test solution" shall read as "dissolve by adding 50 mL of 0.2 mol/L acetate-sodium acetate buffer solution adjusted to pH 5.5, adjust to pH 5.5 by adding 0.2 mol/L acetic acid test solution" Also, in the section of "Procedure", "Weigh up to three significant digits of the amount of sample required to perform the test,record the number, dissolve by adding 0.005 mol/L acetate-sodium acetate buffer adjusted topH showing the maximum enzymatic activity of the sample so that the concentration per 1mL becomes 0.04~0.06 phytic acid decomposition activity units. If necessary, perform filtration, use this solution as the sample solution" shall read as "To 1.0 g (0.995~1.004 g) of a sample, add a buffer which contains polysorbate 20 at a rate of 0.1 g per 1 L of 0.2-mol/L acetic acid-sodium acetate buffer whose pH is adjusted for the sample to show the maximum enzyme activity to dissolve, filter if necessary, and dilute using the same buffer so that the phytate degradation capacity unit per mL becomes 0.04~0.06 to prepare the sample solution."

(b) Standard of manufacturing method

Shall be manufactured by culturing phytase production recombinants hosted by bacterial strains belonging to *Trichoderma reesei*, filtering the culture product (or filtrating the culture product after extracting with water) to remove bacterial cells when the culturing is complete, and by concentrating the filtrate.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

C. Preparation (Part 1 Liquid)

(a) Compositional standards

This product is aqueous liquid in which sodium chloride and sorbitol are mixed into raw material for manufacturing phytase ((4) of Part 2).

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation capacity units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method 1 of the Phytic acid decomposition power test method. However, in the section of "Preparation of substrate solution", "dissolve by adding 50 mL of 0.2 mol/L acetate-sodium acetate buffer solution adjusted to pH showing the maximum enzymatic activity of the sample, adjust to pH 5.5 by adding 0.2 mol/L acetic acid test solution" shall read as "dissolve by adding 50 mL of 0.2 mol/L acetate-sodium acetate buffer solution adjusted to pH 5.5, adjust to pH 5.5 by adding 0.2 mol/L acetic acid test solution." Also, in the section of "Procedure", "Weigh up to three significant digits of the amount of sample required to perform the test, record the number, dissolve by adding 0.005 mol/L acetate-sodium acetate buffer adjusted to pH showing the maximum enzymatic activity of the sample so that the concentration per 1 mL becomes 0.04~0.06 phytic acid decomposition activity units. If necessary, perform filtration, use this solution as the sample solution." shall read as "To 1.0 g (0.995~1.004 g) of a sample, add a buffer which contains polysorbate 20 at a rate of 0.1 g per 1 L of 0.2 mol/L acetic acid-sodium acetate buffer whose pH is adjusted for the sample to show the maximum enzyme activity to dissolve, filter if necessary, and dilute using the same buffer so that the phytate degradation capacity unit per mL becomes 0.04~0.06 to prepare the sample solution."

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((4) of part 2) is applied mutatis mutandis.

(c) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

C. Preparation (Part 2)

(a) Compositional standards

This product is small piece, powder or granule in which an aqueous solution where polyvinyl alcohol, sodium phytate, sodium monohydrogen phosphate, sodium dihydrogen phosphate, potassium monohydrogen phosphate, potassium dihydrogen phosphate, inositol, vegetable fats and oils, white sugar, and starch selected as necessary are mixed into and added to raw material for manufacturing Phytase ((4) of Part 2), sodium sulfate and carrier are further added if necessary, and mixed or granulated.

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation capacity units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by method 1 of the Phytic acid decomposition power test method. However, in the section of "Preparation of substrate solution", "dissolve by adding 50 mL of 0.2 mol/L acetate-sodium acetate buffer solution adjusted to pH showing the maximum enzymatic activity of the sample, adjust to pH 5.5 by adding 0.2 mol/L acetic acid test solution" shall read as "dissolve by adding 50 mL of 0.2 mol/L acetate-sodium acetate buffer solution adjusted to pH 5.5, adjust to pH 5.5 by adding 0.2 mol/L acetic acid test solution" Also, in the section of "Procedure", "Weigh up to three significant digits of the amount of sample required to perform the test,record the number, dissolve by adding 0.005 mol/L acetate-sodium acetate buffer adjusted topH showing the maximum enzymatic activity of the sample so that the concentration per 1mL becomes 0.04~0.06 phytic acid decomposition activity units. If necessary, perform filtration, use this solution as the sample solution." shall read as "To 1.0 g (0.995~1.004 g) of a sample, add a buffer which contains polysorbate 20 at a rate of 0.1 g per 1 L of 0.2-mol/L acetic acid-sodium acetate buffer whose pH is adjusted for the sample to show the maximum enzyme activity and stir in ice for 60 minutes to dissolve, filter if necessary, and dilute using the same buffer so that the phytate degrading power unit per mL becomes 0.04~0.06 to prepare the sample solution."

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((4) of part 2) is applied mutatis mutandis.

(c) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

Phytase ((5) of Part 2)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 5,000 phytic acid degradation capacity units/g or more using an enzymatic activity test.

Physical and chemical properties:

i. This product is yellow brown liquid.

ii. The pH of a solution of this product in water (1 → 100) is 3.5 to 6.0.

iii. This product produces maximum enzyme activity at pH 3.5~5.5.

Purity test:

i. Lead: 2.0 g (1.95~2.04 g) of this product is weighed. When lead is tested using the lead test method (Method No. 1 of the atomic absorption spectrophotometry), the amount of lead shall be 5 μg/g or less.

ii. Arsenic: The purity test ii. of the raw material for manufacturing of Phytase (Part 1) is applied mutatis mutandis.

iii. Antibacterial activity: The purity test iii. of the raw material for manufacturing of Phytase (Part 1) is applied mutatis mutandis.

Ignition residue: 5.0 % or less (0.5 g)

Enzymatic activity test: It is tested using method No. 2 of the phytic acid degradation capacity test method.

Preparation of sample solution: The preparation of sample solution of the raw material for manufacturing of Phytase ((1) of Part 2) is applied mutatis mutandis.

(b) Standard of manufacturing method

For manufacturing, the Phytase producing recombinant, whose host is the strain belonging to *Aspergillus niger*, is cultured, and after the cultivation bacterial cells are disinfected and filtered to be removed, the filtrate is concentrated.

(c) Standard of storage method

It shall be stored in a lightproof sealed container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1 liquid)

(a) Compositional standards

This product is a water-soluble liquid in which fillers are mixed into the raw material for manufacturing of Phytase ((5) of Part 2).

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label using an enzymatic activity test.

Enzymatic activity test: It is tested using method No. 2 of the phytic acid degradation capacity test method.

Preparation of sample solution: The preparation of sample solution of the raw material for manufacturing of Phytase ((1) of Part 2) is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((5) of Part 2) is applied mutatis mutandis.

(c) Standards of the label

The standards of the label of the raw material for manufacturing of Phytase ((5) of Part 2) is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is granules, pieces, or particles in which fillers and an aqueous solution of polyvinyl alcohol are mixed into the raw material for manufacturing of Phytase ((5) of Part 2), which is granulated, dried, and coated with oxidized polyethylene wax, oleic acid, and ammonium hydroxide.

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label using an enzymatic activity test.

Enzymatic activity test: It is tested using method No. 2 of the phytic acid degradation capacity test method.

Preparation of sample solution: The preparation of sample solution of the raw material for manufacturing of Phytase ((1) of Part 2) is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((5) of Part 2) is applied mutatis mutandis.

(c) Standards of the label

The standards of the label of the raw material for manufacturing of Phytase ((5) of Part 2) is applied mutatis mutandis.

Phytase ((6) of Part 2)

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 200,000 phytic acid degradation capacity units/g or more using an enzymatic activity test.

Physical and chemical properties:

i. This product is yellow to brown powder or particles.

ii. The pH of a solution or suspension of this product in water (1 → 100) is 4.0 to 5.0.

iii. This product produces maximum enzyme activity at pH 2.5~5.0.

Purity test:

i. Lead: 1.0 g (0.95~1.04 g) of this product is weighed. When lead is tested using the lead test method (Method No. 1 of the atomic absorption spectrophotometry), the amount of lead shall be 5 μg/g or less.

ii. Arsenic: The purity test ii. of the raw material for manufacturing of Phytase (Part 1) is applied mutatis mutandis.

iii. Antibacterial activity: The purity test iii. of the raw material for manufacturing of Phytase (Part 1) is applied mutatis mutandis.

Loss on drying: 12.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: 25.0 % or less (0.5 g)

Enzymatic activity test: It is tested using method No. 3 of the phytic acid degradation capacity test method.

Preparation of sample solution: 2.00 g (1.995~2.004 g) of this product is weighed in a 100 mL volumetric flask, and the value is recorded. To which 0.2 mol/L citric acid-sodium citrate buffer (pH 5.5) is added up to the capacity mark to make 100 mL. This solution is stirred at room temperature for 1 hour, and centrifuged at 3,400×g for 10 minutes. The supernatant is used as a sample stock solution. An appropriate amount of the sample stock solution is measured using a volumetric pipette, and added with the said buffer to make its concentration 0.1 phytic acid degradation capacity units/mL. This solution is used as a sample solution.

(b) Standard of manufacturing method

For manufacturing, the Phytase producing recombinant, whose host is the strain belonging to *Komagataella pastoris*, is cultured, and after the cultivation the culture is filtered to remove bacterial cells, the filtrate is spray dried.

(c) Standard of storage method

It shall be stored in a lightproof sealed container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

B. Preparation (Part 1 liquid)

(a) Compositional standards

This product is a water-soluble liquid in which an aqueous sucrose solution is mixed into the raw material for manufacturing of Phytase ((6) of Part 2).

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label using an enzymatic activity test.

Enzymatic activity test: It is tested using method No. 3 of the phytic acid degradation capacity test method.

Preparation of sample solution: 5 mL of this product is measured using a volumetric pipette, put in a 50 mL volumetric flask, and added with 0.2 mol/L citric acid-sodium citrate buffer (pH 5.5) up to the capacity mark to make 50 mL. This is used as a sample stock solution. An appropriate amount of the sample stock solution is measured using a

volumetric pipette, and added with the said buffer to make its concentration 0.1 phytic acid degradation capacity units/mL. This solution is used as a sample solution.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((6) of Part 2) is applied mutatis mutandis.

(c) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces, powder or particles in which wheat flour is added to the raw material for manufacturing of Phytase ((6) of Part 2), mixed with an aqueous solution of α-starch, granulated, dried, and mixed with fillers as appropriate.

Enzymatic activity unit: This product contains 85~170 % of phytic acid degradation power units on the label using an enzymatic activity test.

Enzymatic activity test: It is tested using method No. 3 of the phytic acid degradation capacity test method.

Preparation of sample solution: The enzymatic activity test of the raw material for manufacturing of Phytase ((6) of Part 2) is applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Phytase ((6) of Part 2) is applied mutatis mutandis.

(c) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product.

(142) Muramidase

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: Perform the enzymatic activity test: 1 g of muramidase contains not less than 30,000 peptidoglycan units.

Physical and chemical properties

i. Muramidase is a light brown to deep brown liquid.

ii. The pH of an aqueous solution of muramidase or a suspension of muramidase in water (1 → 100) is between 3.0 ~ 5.0.

iii. Muramidase has maximum enzymatic activity at a pH of between 4.0 ~ 7.5.

Purity

i. Lead: Perform the test for lead with 0.5 g (0.45 ~ 0.54 g) of muramidase as directed under Lead Test (Method 1 of Atomic Absorption Spectrophotometry): the amount of lead shall be not more than 20 μg/g.

ii. Arsenic: Weigh 1.0 g (0.95 ~ 1.04 g) of muramidase, prepare the sample solution according to Method 3 of the Arsenic Test, and perform the test for arsenic by the method using Apparatus A: the color of the absorbing liquid shall have no more color than the standard color (not more than 2 μg/g).

iii. Antibacterial activity: Perform the test with 1 g (0.5 ~1.4 g) of muramidase as directed under Antibacterial Activity Assay for Micrococcus luteus ATCC 9341 and Escherichia coli ATCC 27166: the result should show no antibacterial activity.

Residue on ignition: 10.0 % or less (1 g)

Enzymatic activity test: Perform the test as directed under the Peptidoglycan Degradation Test.

(b)Standard of manufacturing method

Culture a muramidase-producing recombinant using a strain belonging to Trichodermareesei as a host. After culturing, filter the culture or filter upon extracting with water to remove bacterial bodies, and concentrate the filtrate to produce muramidase.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product .

B. Preparation (Part 1)

(a) Compositional standards

Muramidase is a small piece, powder, or particle obtained by adding sodium sulfate as necessary to the active ingredients for muramidase production and mixing the excipient.

Enzymatic activity unit: Perform the enzymatic activity test: muramidase contains not less than 85 % and not more than 170 % of the labeled peptidoglycan units.

Enzymatic activity test: Perform the test as directed under the Peptidoglycan Degradation Test.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Muramidase is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Muramidase is applied mutatis mutandis.

C. Preparation (Part 1)

(a) Compositional standards

Muramidase is a water-soluble liquid obtained by mixing sorbitol with the active ingredients for muramidase production.

Enzymatic activity unit: Perform the enzymatic activity test: muramidase contains not less than 85 % and not more than 170 % of the labeled peptidoglycan units.

Enzymatic activity test: Perform the test as directed under the Peptidoglycan Degradation Test.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Muramidase is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Muramidase is applied mutatis mutandis.

(143) Lactase

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 25,000 lactase units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale yellow to brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 5.5~7.5.

iii. This product produces maximum enzyme activity at pH 4.0~5.5.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by

the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 30.0 % or less (1 g)

Enzymatic activity test: It is tested by the lactase test method.

(b)Standard of manufacturing method

For manufacturing, Lactase enzyme producing strain of *Aspergillus oryzac* is cultured, after the cultivation, the culture is filtered or extracted with water and filtered to remove bacterial cells, and then the filtrate is dried or the precipitate produced by adding a solvent in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Lactase are applied mutatis mutandis.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Lactase are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Lactase is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Lactase is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces, powder, or particles in which the raw material for manufacturing of Lactase is mixed with fillers.

Enzymatic activity unit: This product contains 85~170 % of Lactase units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by Lactase test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Lactase is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Lactase is applied mutatis mutandis.

(144) Lipase

A. Raw material for manufacturing

(a) Compositional standards

Enzymatic activity unit: This product contains 5,000 lipid digestive capacity units/g or more by an enzymatic activity test.

Physical and chemical properties:

i. This product is pale yellow to pale brown powder with a slightly specific odor.

ii. The solution or aqueous suspension (1 → 100) of this product is pH 5.0~6.5.

iii. This product produces maximum enzyme activity at pH 6.0~7.5.

Purity test:

i. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 5.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (50 mg/kg or less).

ii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

iii. Antibacterial activity: When 1 g (0.5~1.4 g) of this product is weighed and tested by the antibacterial activity test method, it shall not exhibit antibacterial activity.

Loss on drying: 10.0 % or less (1 g, 105 °C, 3 hours)

Ignition residue: After drying, 25.0 % or less (1 g)

Enzymatic activity test: It is tested by the lipid digestive capacity test method.

(b)Standard of manufacturing method

For manufacturing, the lipase producing strain, *Alcaligenes eutrophus, Candida cylindracea* or *Rhizopus japonicus* is cultured, and after the cultivation, the culture is extracted with weak alkaline solution and filtered to be removed, and then the filtrate is dried, or the precipitate produced by adding a solvent or salt in the filtrate is dried.

(c) Standard of storage method

It shall be stored in a lightproof capped container.

(d) Standards of the label

The pH value (to one place of decimals) showing maximum enzyme activity shall be described on the immediate container or the immediate wrapper of this product .

B. Preparation (Part 1)

(a) Compositional standards

The compositional standards of the raw material for manufacturing of Lipase are applied mutatis mutandis.

(b)Standard of manufacturing method

The Standard of manufacturing method of the raw material for manufacturing of Lipase are applied mutatis mutandis.

(c) Standard of storage method

The standard of storage method of the raw material for manufacturing of Lipase is applied mutatis mutandis.

(d) Standards of the label

The Standards of the label of the raw material for manufacturing of Lipase is applied mutatis mutandis.

C. Preparation (Part 2)

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of Lipase is mixed with fillers.

Enzymatic activity unit: This product contains 85~170 % of lipid digestive power units on the label by an enzymatic activity test.

Enzymatic activity test: It is tested by lipid digestive power test method.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of Lipase is applied mutatis mutandis.

(c) Standards of the label

The Standards of the label of the raw material for manufacturing of Lipase is applied mutatis mutandis.

(145) *Enterococcus faecalis*

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze-dried *Enterococcus faecalis* NT strain.

Origin: The original strain is *Enterococcus faecalis* NT strain which was separated from human feces in 1983.

Physical and chemical properties: This product is a facultative anaerobic Gram-positive coccus. It breaks down lactose to produce lactate, breaks down melezitose, but does not break down arabinose or melibiose.

Confirmation test:

i. This product is applied on medium No. 3, cultured at 36~38 °C for 2 days. The colony forming on the medium is round, raised, with smooth surface, and milky.

ii. This product is applied on medium No. 3 and cultured at 36~38 °C for 2 days. A platinum-loop amount of water is placed on a slide glass. The colony is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, cocci stained blue-violet to dark purple are observed.

iii. Medium No. 3 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2 days. In the test using the colony forming on the medium by the lactic acid producing capacity test method, production of lactic acid is observed.

iv. Medium No. 9 is used as a test agar medium. This product is applied on the medium and anaerobically cultured or aerobically at 36~38 °C for 2 days. The growth of the bacteria is observed under both conditions.

v. Medium No. 3 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2 days. The results of the test on arabinose, melibiose, and melezitose using the colony forming on the medium by method No. 2 of the glycolytic capacity test method shows that arabinose and melibiose are negative and melezitose is positive.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing sodium acetate, glucose, peptone and others, freeze-dried, and stored at 4 °C. This product, the subdivided original strain grown in the same medium, is freeze-dried and then stored at 4 °C. The subculture of the original strain is only one passage. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of *Enterococcus faecalis* is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. The colony forming by the procedure of the assay is round, raised, with smooth surface, and milky.

ii. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, cocci stained blue-violet to dark purple are observed.

iii. In the test using the sample stock solution 0.1 mL prepared by the assay or the colony obtained by the procedure of the assay by the lactic acid producing capacity test method, production of lactic acid is observed.

iv. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. In this procedure, medium No. 9 is used as a test agar medium. The sample solution is anaerobically cultured or aerobically at 36~38 °C for 2 days, and then the growth of the bacteria is observed under both conditions.

v. In the test using the colony obtained by the procedure of the assay, the results of the test on arabinose, melibiose, and melezitose using the colony obtained by the procedure of the assay by method No. 2 of the glycolytic capacity test method shows that arabinose and melibiose are negative and melezitose is positive.

Assay:

Preparation of sample solution: The diluent No. 1 is used as a diluent. The sample solution is prepared at a concentration of 300~3,000 viable cell counts/mL according to the preparation of the sample solution specified in the viable bacteria agent quantification method.

Procedure: Medium No. 3 is used as a test agar medium. The culture medium is subjected to the procedures of method No. 2 of viable bacteria agent quantification method, and cultured at 36~38 °C for 2 days.

(b) For manufacturing

The raw material for manufacturing of *Enterococcus faecalis* is cultured, and the bacterial cells are collected, dried, and mixed with fillers. However, a mixture of *Bacillus subtilis* (part 4) preparation and *Clostridium butyricum* (part 2) preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

(146) *Enterococcus faecium*

Enterococcus faecium (Part 1)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze-dried *Enterococcus faecium* ATCC19434 strain.

Origin: The original strain is *Enterococcus faecium* ATCC 19434 strain which was separated from bovine intestine in 1979.

Physical and chemical properties: This product is a facultative anaerobic Gram-positive coccus. It breaks down lactose to produce lactate, breaks down arabinose or melibiose, but does not break down melezitose.

Confirmation test:

i. This product is applied on medium No. 3, cultured at 36~38 °C for 2 days. The colony forming on the medium is round, raised, with smooth surface, and milky.

ii. This product is applied on medium No. 3, cultured at 36~38 °C for 2 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, cocci stained blue-violet to dark purple are observed.

iii. Medium No. 3 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2 days. In the test using the colony forming on the medium by the lactic acid producing capacity test method, production of lactic acid is observed.

iv. Medium No. 9 is used as a test agar medium. This product is applied on the medium and anaerobically cultured or aerobically at 36~38 °C for 2 days. The growth of the bacteria is observed under both conditions.

v. Medium No. 3 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2 days. The results of the test on arabinose, melibiose, and melezitose using the colony forming on the medium by method No. 2 of the glycolytic capacity test method shows that arabinose and melibiose are positive and melezitose is negative.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing agar, glucose, beef extract, peptone and others, freeze-dried, and stored at 4 °C. This product is grown in a medium containing glucose, beef extract, peptone and others, subdivided, freeze-dried, and stored at 4 °C. The subculture of the original strain is five passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Enterococcus faecium* (part 1) is cultured, and the bacterial cells are collected, added with beta-glucan, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. The colony forming by the procedure of the assay is round, raised, with smooth surface, and milky.

ii. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, cocci stained blue-violet to dark purple are observed.

iii. In the test using the sample stock solution 0.1 mL prepared by the assay or the colony obtained by the procedure of the assay by the lactic acid producing capacity test method, production of lactic acid is observed.

iv. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. In this procedure, medium No. 9 is used as a test agar medium. The sample solution is anaerobically cultured or aerobically at 36~38 °C for 2 days, and then the growth of the bacteria is observed under both conditions.

v. In the test using the colony obtained by the procedure of the assay, the results of the test on arabinose, melibiose, and melezitose using the colony obtained by the procedure of the assay by method No. 2 of the glycolytic capacity test method shows that arabinose and melibiose are positive and melezitose is negative.

Assay: The assay of preparation of *Enterococcus faecium* (part 1) is applied mutatis mutandis.

(b) For manufacturing,

The raw material for manufacturing of *Enterococcus faecium* (part 1) is cultured, and the bacterial cells are collected, added with beta-glucan, dried, and mixed with fillers.

However, a mixture of *Lactobacillus acidophilus* (part 1) preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

Enterococcus faecium (Part 2)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze-dried *Enterococcus faecium* 129 BIO 3B strain.

Origin: The original strain is *Enterococcus faecium* 129 BIO 3B strain which was separated from yogurt in 1949.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Enterococcus faecium* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Enterococcus faecium* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing beef, bovine liver, glucose and others, and cryopreserved at −80 °C. For this product, the subdivided original strain grown in the same medium, is cryopreserved at −20 °C. The subculture of the original strain is two passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Enterococcus faecium* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Enterococcus faecium* (part 1) is applied mutatis mutandis

Assay: The assay of preparation of *Enterococcus faecium* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Enterococcus faecium* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers. However, a mixture of *Lactobacillus acidophilus* (part 6) preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

Enterococcus faecium (Part 3)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze-dried *Enterococcus faecium* BIO-4R strain.

Origin: The original strain is *Enterococcus faecium* BIO-4R strain, which is *Enterococcus faecium* BIO strain separated from human intestine in 1929 with the added resistance to antibacterial substances.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Enterococcus faecium* (part 1).

Confirmation test:

i. This product is applied on medium No. 3, cultured at 36~38 °C for 5 days. The colony forming on the medium is round, raised, with smooth surface, and milky.

ii. This product is applied on medium No. 3, cultured at 36~38 °C for 5 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, cocci stained blue-violet to dark purple are observed.

iii. Medium No. 3 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 5 days. In the test using the colony forming on the medium by the lactic acid producing capacity test method, production of lactic acid is observed.

iv. Medium No. 9 is used as a test agar medium. This product is applied on the medium and anaerobically cultured or aerobically at 36~38 °C for 5 days. The growth of the bacteria is observed under both conditions.

v. Medium No. 3 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 5 days. The results of the test on arabinose, melibiose, and melezitose using the colony forming on the medium by method No. 2 of the glycolytic capacity test method shows that arabinose and melibiose are positive and melezitose is negative.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing precipitated calcium carbonate, lactose, peptone and others, freeze-dried, and stored at −80 °C. This product is grown in the same medium, subdivided, and stored at 4 °C. The subculture of the original strain is two passages or less. This product shall not be subcultured.

B. Preparation (Part 1)

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Enterococcus faecium* (part 3) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. The colony forming by the procedure of the assay is round, raised, with smooth surface, and milky.

ii. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, cocci stained blue-violet to dark purple are observed.

iii. In the test using the sample stock solution 0.1 mL prepared by the assay or the colony obtained by the procedure of the assay by the lactic acid producing capacity test method, production of lactic acid is observed.

iv. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. In this procedure, medium No. 9 is used as a test agar medium. The sample solution is anaerobically cultured or

aerobically at 36~38 °C for 5 days, and then the growth of the bacteria is observed under both conditions.

v. In the test using the colony obtained by the procedure of the assay, the results of the test on arabinose, melibiose, and melezitose using the colony obtained by the procedure of the assay by method No. 2 of the glycolytic capacity test method shows that arabinose and melibiose are positive and melezitose is negative.

Assay:

Preparation of sample solution: The assay of the raw material for manufacturing of *Enterococcus faecalis* is applied mutatis mutandis.

Procedure: Medium No. 3 is used as a test agar medium. The culture medium is subjected to the procedures of method No. 2 of viable bacteria agent quantification method, and cultured at 36~38 °C for 5 days.

(b) For manufacturing,

The raw material for manufacturing of *Enterococcus faecium* (part 3) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

B. Preparation (Part 2)

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Enterococcus faecium* (part 3) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation (part 1) of *Enterococcus faecium* (part 3) is applied mutatis mutandis.

Assay: The assay of preparation (part 1) of *Enterococcus faecium* (part 3) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Enterococcus faecium* (part 3) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Enterococcus faecium (Part 4)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze-dried *Enterococcus faecium* FA-5 strain.

Origin: The original strain is *Enterococcus faecium* FA-5 strain which was separated from human feces in 1975.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Enterococcus faecium* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Enterococcus faecium* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing yeast extract, glucose pepton and others, and cryopreserved at −80 °C. For this product, the subdivided original strain grown in the same medium, is cryopreserved at −80 °C. The subculture of the original strain is two passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Enterococcus faecium* (part 4) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Enterococcus faecium* (part 1) is applied mutatis mutandis.

Assay: The assay of preparation of *Enterococcus faecalis* is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Enterococcus faecium* (part 4) is cultured, and the bacterial cells are collected, dried, and mixed with fillers. However, a mixture of *Bifidobacterium thermophilum* (part 2) and *Lactobacillus acidophilus* (part 5) preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

(147) *Clostridium butyricum*

Clostridium butyricum (part 1)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze or freeze-dried *Clostridium butyricum* MIYAIRI strain.

Origin: The original strain is *Clostridium butyricum* MIYAIRI strain which was separated from human intestine in 1933.

Physical and chemical properties: This product is an obligate anaerobic Gram-positive bacillus. It forms spores and breaks down glucose to produce butyric acid.

Confirmation test:

i. This product is applied on medium No. 1, anaerobically cultured at 36~38 °C for 1~2 days. A platinumLoop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. This product is applied on medium No. 1, anaerobically cultured at 36~38 °C for 2~7 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. Medium No. 1 is used as a test agar medium. This product is applied on the medium and anaerobically cultured at 36~38 °C for 1~2 days. In the test using the colony forming on the medium by the butyric acid producing capacity test method, production of butyric acid is observed.

iv. Medium No. 1 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 1~2 days, and then the growth of the bacteria is not observed.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing agar, beef extract, glucose, and others, cryopreserved at −80 °C, or freeze-dried and then stored at 4 °C. This product is grown in the same medium, subdivided, and cryopreserved at −80 °C, or freeze-dried and

then stored at 4 °C. The original strain is subcultured every five years, and this product shall not be subcultured

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Clostridium butyricum* (part 1) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. A platinum-loop amount of water is placed on a slide glass. The sample stock solution prepared by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. In the test using the sample stock solution 0.1 mL prepared by the assay or the colony obtained by the procedure of the assay by the butyric acid producing capacity test method, production of butyric acid is observed.

iv. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. The sample solution is cultured at 36~38 °C for 1~2 days, and then the growth of the bacteria is not observed.

Assay:

Preparation of sample solution: Approximately 1 g of this product is weighed to the digits of 0.01 g, and the value is recorded. It is placed in a 100 mL homogenizer vessel, added with 50 mL of diluent No. 2, and stirred at 10,000 rpm for 5 minutes. This is used as a sample stock solution. Diluent No. 2 is hereinafter used as a diluent, and the sample solution is prepared at a concentration of 300~3,000 viable cell counts/mL according to the preparation of the sample solution specified in the viable

bacteria agent quantification method. If necessary, the sample solution, which is heated in a water bath at 75 °C for 20 minutes and quenched in running water.

Procedure: Medium No. 1 is used as a test agar medium. The culture medium is subjected to the procedures of method No. 2 of viable bacteria agent quantification method, and anaerobically cultured at 36~38 °C for 1~2 days.

(b) For manufacturing

The raw material for manufacturing of *Clostridium butyricum* (part 1) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Clostridium butyricum (part 2)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze-dried *Clostridium butyricum* NT strain.

Origin: The original strain is *Clostridium butyricum* NT strain which was separated from human feces in 1955.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Clostridium butyricum (*part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of Clostridium butyricum (part 1) is applied mutatis mutandis.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing lactose, casein peptone, precipitated calcium carbonate and others, freeze-dried, and stored at 4 °C. This product is grown in the same medium, subdivided, freeze-dried, and stored at 4 °C. The subculture of the original strain is one passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of *Clostridium butyricum* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of the preparation of *Clostridium butyricum* (part 1) is applied mutatis mutandis

Assay: The assay of the preparation of *Clostridium butyricum* (part 1) is applied mutatis mutandis

(b) Standard of storage method

The raw material for manufacturing of *Clostridium butyricum* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers. However, a mixture of *Bacillus subtilis* (part 4) and Enterococcus faecalis preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

(148) *Bacillus coagulans*

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated *Bacillus coagulans* P-22 strain.

Origin: The original strain is *Bacillus coagulans* P-22 strain separated from green malt in 1949.

Physical and chemical properties: This product is a facultative anaerobic Gram-positive bacillus. It forms spores and breaks down lactose to produce lactic acid.

Confirmation test:

i. This product is applied on medium No. 5 cultured at 36~38 °C for 2~3 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. This product is applied on medium No. 5 cultured at 36~38 °C for 3~7 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. Medium No. 5 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2~3 days. In the test using the colony forming on the

medium by the lactic acid producing capacity test method, production of lactic acid is observed. In addition, the culture by the lactic acid producing capacity test method is performed for 3 days.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing agar, yeast extract, casein peptone, and others, freeze-dried, and stored at 4 °C. This product is grown in the same medium, subdivided, and stored at 4 °C. The subculture of the original strain is one passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of *Bacillus coagulans* is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. A platinum-loop amount of water is placed on a slide glass. The sample stock solution prepared by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. In the test using the sample stock solution 0.1 mL prepared by the assay or the colony obtained by the procedure of the assay by the lactic acid producing capacity test method, production of lactic acid is observed. In addition, culturing by the lactic acid producing capacity test method is carried out for 3 days.

Assay:

Preparation of sample solution: Approximately 1 g of this product is weighed to the digits of 0.01 g, and the value is recorded. It is placed in a 100 mL homogenizer vessel, added with 50 mL of diluent No. 1, and stirred at 15,000 rpm for 5 minutes. This is used as a sample stock solution. Diluent No. 1 is hereinafter used as a diluent,

and the sample solution is prepared at a concentration of 30~300 viable cell counts/mL according to the preparation of the sample solution specified in the viable bacteria agent quantification method. In addition, the sample solution, which is heated in a water bath at 75 °C for 20 minutes and quenched in running water.

Procedure: Medium No. 5 is used as a test agar medium. The culture medium is subjected to the procedures of method No. 1 of viable bacteria agent quantification method, and cultured at 36~38 °C for 2~3 days.

(b) For manufacturing,

The raw material for manufacturing of bacillus coagulans is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

(149) *Bacillus subtilis*

Bacillus subtilis (part 1)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated *Bacillus subtilis* BN strain.

Origin: The original strain is *Bacillus subtilis* BN strain separated from natto in 1928.

Physical and chemical properties: This product is a Gram-positive bacillus. It forms spores and does not grow under anaerobic conditions.

Confirmation test:

i. This product is applied on medium No. 4 cultured at 36~38 °C for 1~2 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. This product is applied on medium No. 4 cultured at 36~38 °C for 3~7 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a

sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. This product is applied on medium No. 4, cultured at 36~38 °C for 1~2 days. The colony forming on the medium is collected, inoculated in a 10 mL of medium No. 7, and anaerobically cultured at 36~38 °C for 2~3 days, and then the growth of the bacteria is not observed.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing heat treated koji, soy flour, gelatin, and others, and cryopreserved at −80 °C. This product is grown in the same medium, subdivided, and cryopreserved at −80 °C. The subculture of the original strain is ten passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of *Bacillus subtilis* (Part 1) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. A platinum-loop amount of water is placed on a slide glass. The sample stock solution prepared by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. In the test using the the colony obtained by the procedure of the assay, inoculated into 10 ml of No. 7 medium, and cultured anaerobically at 36~38 °C for 2~3 days, and then the growth of the bacteria is not observed.

Assay:

Preparation of sample solution: The diluent No. 1 is used as a diluent. The sample solution is prepared at a concentration of 30~300 viable cell counts/mL according to

the preparation of the sample solution specified in the viable bacteria agent quantification method. If necessary, the sample solution, which is heated in a water bath at 75 °C for 20 minutes and quenched in running water.

Procedure: Medium No. 4 is used as a test agar medium. The culture medium is subjected to the procedures of method No. 1 of viable bacteria agent quantification method, and cultured for 1~2 days.

(b) For manufacturing,

The raw material for manufacturing of *Bacillus subtilis* (part 1) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Bacillus subtilis (part 2)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated *Bacillus subtilis* C-3102 strain.

Origin: The original strain is *Bacillus subtilis* C-3102 strain which was separated from pig feces in 1984.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Bacillus subtilis* (part 1).

Confirmation test:

i. This product is applied on medium No. 4 cultured at 36~38 °C for 1~2 days. A platinumLoop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. This product is applied on medium No. 4 cultured at 36~38 °C for 3~7 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. This product is applied on medium No. 4, cultured at 36~38 °C for 1~2 days. The colony forming on the medium is collected, inoculated in a 10 mL of medium No. 7, and anaerobically cultured at 36~38 °C for 2~3 days, and then the growth of the bacteria is not observed.

iv. This product is applied on medium No. 6, anaerobically cultured at 36~38 °C for 1 day. The grayish white, conical, and unique colony is detected 80 % or greater.

(b) Storage method and standards of subculture

The original strain is subcultured on medium No. 6 and cryopreserved at −80 °C. This product is grown in the same medium, subdivided, and cryopreserved at −80 °C. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of *Bacillus subtilis* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. A platinum-loop amount of water is placed on a slide glass. The sample stock solution prepared by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. In the test using the the colony obtained by the procedure of the assay, inoculated into 10 ml of No. 7 medium, and cultured anaerobically at 36~38 °C for 2~3 days, and then the growth of the bacteria is not observed.

iv. The sample stock solution prepared by the assay is measured, and diluent No. 1 is hereinafter used as a diluent, and the sample solution is prepared at a concentration of 30~300 viable cell counts/mL according to the preparation of the sample solution specified in the viable bacteria agent quantification method. The sample solution is

applied on medium No. 6 and cultured at 36~38 °C for 1 day. The grayish white, conical, and specific colony is detected at 80 % or greater.

Assay:

Preparation of sample solution: The preparation of sample solution of *Bacillus subtilis* (part 1) is applied mutatis mutandis.

Procedure: The procedure of the *Bacillus subtilis* (part 1) preparation is applied mutatis mutandis. However, medium No. 4 added with glucose 1 % is used as a test agar medium.

(b) For manufacturing

The raw material for manufacturing of *Bacillus subtilis* (part 2) is cultured, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Bacillus subtilis (part 3)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated *Bacillus subtilis* DB 9011 strain.

Origin: The original strain is *Bacillus subtilis* DB 9011 strain which was separated from soil in 1990.

Physical and chemical properties: The Property are the same as those of the raw material for manufacturing of *Bacillus subtilis* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Bacillus subtilis* (part 1) is applied mutatis mutandis.

(b) Storage method and standards of subculture

The original strain is subcultured on medium No. 4 and cryopreserved at −80 °C. This product is grown in the same medium, subdivided, and cryopreserved at −80 °C. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of *Bacillus subtilis* (part 3) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Bacillus subtilis* (part 1) is applied mutatis mutandis.

Assay: The assay of preparation of *Bacillus subtilis* (part 1) is applied mutatis mutandis.

(b) For manufacturing,

The raw material for manufacturing of *Bacillus subtilis* (part 3) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Bacillus subtilis (part 4)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated *Bacillus subtilis* NT strain.

Origin: The original strain is *Bacillus subtilis* NT strain which was separated from natto in 1985.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Bacillus subtilis* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Bacillus subtilis* (part 1) is applied mutatis mutandis.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium No. 4, freeze-dried, and stored at 4 °C. This product is grown in the same medium, subdivided, and stored at 4 °C. The subculture of the original strain is one passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of *Bacillus subtilis* (part 4) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Bacillus subtilis* (part 1) is applied mutatis mutandis.

Assay: The assay of preparation of *Bacillus subtilis* (part 1) is applied mutatis mutandis.

(b) For manufacturing,

The raw material for manufacturing of *Bacillus subtilis* (part 4) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

However, a mixture of *Clostridium butyricum* (part 2) and *Enterococcus faecalis* preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

***Bacillus subtilis* (part 5)**

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze or freeze-dried *Bacillus subtilis* JA-ZK strain.

Origin: The original strain is *Bacillus subtilis* JA-ZK strain which was separated from soil in 2000.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Bacillus subtilis* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Bacillus subtilis* (part 1) is applied mutatis mutandis.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing yeast extract, and cryopreserved at −70 °C or less, or freeze-dried and then stored at 2~8 °C. This product is grown in the same medium, subdivided, and cryopreserved at −70 °C or less, or freeze-dried and then stored at 2~8 °C. The subculture of the original strain is ten passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of *Bacillus subtilis* (part 5) is cultured, and the bacterial cells are collected, added with starch, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis

Assay: The confirmation test of preparation of *Bacillus subtilis* (part 1) is applied mutatis mutandis.

(b) For manufacturing,

Preparation of sample solution: The diluent No. 1 is used as a diluent. The sample solution is prepared at a concentration of 300~3,000 viable cell counts/mL according to the preparation of the sample solution specified in the viable bacteria agent quantification

method. In addition, the sample solution, which is heated in a water bath at 75 °C for 20 minutes and cooled.

Procedure: Medium No. 9 not containing the horse defibrinated blood is used as a test agar medium. The culture medium is subjected to the procedures of method No. 2 of viable bacteria agent quantification method, and cultured at 28~30 °C for 1~2 days.

(c) Standard of storage method

It shall be stored in a capped container.

(150) *Bacillus cereus*

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated *Bacillus cereus* TOYOI strain.

Origin: The original strain is *Bacillus cereus* TOYOI strain which was separated from soil in 1968.

Physical and chemical properties: This product is a Gram-positive bacillus. It forms spores and grows in the medium with chloramphenicol and polymyxin B sulfate.

Confirmation test:

i. This product is applied on medium No. 4 cultured at 36~38 °C for 1~2 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. This product is applied on medium No. 4 cultured at 36~38 °C for 3~7 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. 100 mL of medium No. 4 and 1 mL of the antibiotic solution for viable bacteria agent test is used as a test agar medium, cultured at 36~38 °C for 1~2 days, and then the growth of the bacteria is observed.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing agar, pepton, beef extract, and others, freeze-dried, and stored at 4 °C. This product is grown in the same medium, subdivided, and cryopreserved at −80 °C. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is powder in which the raw material for manufacturing of *Bacillus cereus* is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. A platinum-loop amount of water is placed on a slide glass. The sample stock solution prepared by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. Then 100 mL of medium No. 4 and 1 mL of the antibiotic solution for viable bacteria agent test is used as a test agar medium, cultured at 36~38 °C for 1~2 days, and then the growth of the bacteria is observed.

Assay:

Preparation of sample solution: Approximately 1 g of this product is weighed to the digits of 0.01 g, and the value is recorded. It is placed in a 100 mL homogenizer vessel, added with 50 mL of diluent No. 1, and stirred at 10,000 rpm for 5 minutes. This is used as a sample stock solution. Diluent No. 1 is hereinafter used as a diluent, and the sample solution is prepared at a concentration of 30~300 viable cell counts/mL according to the preparation of the sample solution specified in the viable bacteria

agent quantification method. If necessary the sample solution, which is heated in a water bath at 75 °C for 20 minutes and quenched in running water.

Procedure: The procedure of preparation of *Bacillus subtilis* is applied mutatis mutandis.

(b) For manufacturing,

The raw material for manufacturing of *Bacillus cereus* is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a lightproof airtight container.

(151) *Bacillus badius*

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated *Bacillus badius* MA001 strain.

Origin: The original strain is *Bacillus badius* MA 001 strain separated from the soil in Shizuoka prefecture in 1991.

Physical and chemical properties: This product is a Gram-positive bacillus. It forms spores and does not grow under anaerobic conditions.

Confirmation test:

i. This product is applied on the medium, in which 0.5 g (0.45~0.54 g) of yeast extract, 0.5 g (0.45~0.54 g) of sodium chloride, 0.1 g (0.05~0.14 g) of n-sodium butyrate, and 1 g (0.5~1.4 g) of agar are added with water to make 100 mL and adjusted to pH 7.7~7.9 (from (2) to (4), in B. (a). confirmation test (3) and (4), and in the procedure of the assay, referred to as “test agar medium”), cultured at 36~38 °C for 1~2 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. This product is applied on a test agar medium and cultured at 36~38 °C for 3~7 days. A platinum-loop amount of water is placed on a slide glass. The colony is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the

flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. When this product is applied on the test agar medium, cultured at 36~38 °C for 1~2 days, the white to pale yellow-white, irregular shaped, specific colony is detected.

iv. This product is applied on the test agar medium, anaerobically cultured at 36~38 °C for 2~3 days, and then the growth of the bacteria is not observed. This product is applied on the medium, in which 0.5 g (0.45~0.54 g) of sodium chloride, 50 g (49.5~50.4 g) of bovine heart extract, 1.0 g (0.95~1.04 g) of tryptose, and 1.5 g (1.45~1.54 g) of agar are added with water to make 70 mL, adjusted to pH 7.2~7.6, and added with 30 mL of chicken egg yolk liquid (in B. (a). confirmation test (4), referred to as “egg yolk agar medium”), cultured aerobically at 36~38 °C for 1 day, and then the growth of the bacteria is not observed.

(b) Storage method and standards of subculture

The original strain is subcultured on a medium containing agar, bovine heart extract, and others, freeze-dried, and stored at 4 °C. This product is grown in the same medium, subdivided, and cryopreserved at −80 °C. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Bacillus badius* is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by Gram stain, bacilli stained blue-violet to dark purple are observed.

ii. A platinum-loop amount of water is placed on a slide glass. The sample stock solution prepared by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the spore stain method, spores stained pale-green to green are observed.

iii. The sample stock solution prepared by the assay is measured, and the diluent No. 1 added with 0.01 v/v% of polysorbate 80 is hereinafter used as a diluent to prepare the sample solution according to the preparation of sample solution by the viable bacteria agent quantification method. This sample solution is applied on the test agar medium, cultured at 36~38 °C for 1~2 days, and then the white to pale yellow-white, irregular shaped, specific colony is detected.

iv. In the test using the colony obtained by the procedure of the assay, inoculated into the test agar medium, and cultured anaerobically at 36~38 °C for 2~3 days, and then the growth of the bacteria is not observed. And in the test using the colony obtained by the procedure of the assay, inoculated into the egg yolk agar medium, and cultured aerobically at 36~38 °C for 1 day, and then the growth of the bacteria is not observed.

Assay:

Preparation of sample solution: Approximately 1 g of this product is weighed to the digits of 0.01 g, and the value is recorded. It is placed in a 100 mL homogenizer vessel, added with 50 mL of 0.1 w/v% of potassium hydroxide solution, and stirred at 10,000 rpm for 5 minutes. This is used as a sample stock solution. The diluent No. 1 added with 0.01v/v% of polysorbate 80 is used as a diluent to prepare a sample solution at a concentration of 30~300 viable cell counts/mL according to the preparation of sample solution by the viable bacteria agent quantification method.

Procedure: The test agar medium is used as a test agar medium. The culture medium is subjected to the procedures of method No. 1 of viable bacteria agent quantification method, and cultured for 1~2 days.

(b) For manufacturing

The raw material for manufacturing of *Bacillus badius* is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

(152) *Bifidobacterium thermophilum*

Bifidobacterium thermophilum (Part 1)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze-dried *Bifidobacterium thermophilum* chN-118 strain.

Origin: The original strain is *Bifidobacterium thermophilum* chN-118 strain which was separated from chicken intestine in 1966.

Physical and chemical properties: This product is an obligate anaerobic Gram-positive bacillus. It breaks down raffinose but does not break down xylose.

Confirmation test:

i. This product is applied on medium No. 1, anaerobically cultured at 36~38 °C for 2~3 days and then the colony growing in the medium is milky brown to brown, a precise circle, hemispherically raised, with a smooth surface and circumference.

ii. This product is applied on medium No. 1 and anaerobically cultured at 36~38 °C for 2~3 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli, which are stained blue-violet to dark purple, claviform, curved, V-shaped and Y-shaped, are observed.

iii. Medium No. 1 is used as a test agar medium. This product is applied on the medium and anaerobically cultured at 36~38 °C for 2~3 days. The results of the test on xylose and raffinose using the colony forming on the medium by method No. 1 of the glycolytic capacity test method shows that xylose are negative and raffinose is positive.

iv. Medium No. 1 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2~3 days, and then the growth of the bacteria is not observed.

(b) Storage method and standards of subculture

The original strain is subcultured on medium No. 1 and cryopreserved at −80 °C, or freeze-dried and then stored at 4 °C. This product is grown in the same medium, subdivided, freeze-dried and then stored at 4 °C. The subculture of the original strain is three passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Bifidobacterium thermophilum* (part 1) is cultured, and the bacterial cells are collected, dried, added with corn starch, shellac and lactose, granulated and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. The colony forming by the procedure of the assay is a precise circle, hemispherically raised, with a smooth surface and circumference, and milky brown to brown.

ii. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli, which are stained blue-violet to dark purple, claviform, curved, V-shaped and Y-shaped, are observed.

iii. In the test using the colony obtained by the procedure of the assay, the results of the test on xylose and raffinose using the colony forming on the medium by method No. 1 of the glycolytic capacity test method shows that xylose are negative and raffinose is positive.

iv. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. The sample solution is cultured at 36~38 °C for 2~3 days, and then the growth of the bacteria is not observed.

Assay:

Preparation of sample solution: The diluent No. 2 is used as a diluent. The sample solution is prepared at a concentration of 300~3,000 viable cell counts/mL according to the preparation of the sample solution specified in the viable bacteria agent quantification method

Procedure: Medium No. 1 is used as a test agar medium. The culture medium is subjected to the procedures of method No. 2 of viable bacteria agent quantification method, and cultured at 36~38 °C for 2~3 days.

(b) For manufacturing,

The raw material for manufacturing of *Bifidobacterium thermophilum* (Part 1) is cultured, and the bacterial cells are collected, dried, added with corn starch, shellac and lactose, granulated and mixed with fillers.

However, a mixture of *Lactobacillus sarivalius* preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

Bifidobacterium thermophilum (Part 2)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freezed *Bifidobacterium thermophilum* S-501 strain.

Origin: The original strain is *Bifidobacterium thermophilum* S-501 strain which was separated from pig feces in 1985.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Bifidobacterium thermophilum* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The storage method and standards of subculture of the raw material for manufacturing of *Enterococcus faecium* (Part 4) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Bifidobacterium thermophilum* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis

Assay: The assay of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Bifidobacterium thermophilum* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

However, a mixture of *Enterococcus faecium* (part 4) preparation and *Lactobacillus acidophilus* (part 5) preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

***Bifidobacterium thermophilum* (Part 3)**

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freezed *Bifidobacterium thermophilum* SS-4 strain.

Origin: The original strain is *Bifidobacterium thermophilum* SS-4 strain which was separated from pig intestine in 1966.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Bifidobacterium thermophilum* (Part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Bifidobacterium thermophilum* (Part 1) is applied mutatis mutandis.

(b) Storage method and standards of subculture

The storage method and standards of subculture of the raw material for manufacturing of *Bifidobacterium thermophilum* (Part1) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Bifidobacterium thermophilum* (part 3) is cultured, and the bacterial cells are collected, dried, added with corn starch, shellac and lactose, granulated and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis.

Assay: The assay of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Bifidobacterium thermophilum* (Part 3) is cultured, and the bacterial cells are collected, dried, added with corn starch, shellac and lactose, granulated and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

***Bifidobacterium thermophilum* (Part 4)**

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze or freeze-dried *Bifidobacterium thermophilum*WBL-4R strain.

Origin: The original strain is *Bifidobacterium thermophilum*WBL-4R strain which was separated from bovine feces in 1979.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Bifidobacterium thermophilum* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The original strain is subcultured on medium No. 1 and cryopreserved at −80 °C, or freeze-dried and then stored at 4 °C. This product is grown in the same medium, subdivided, and cryopreserved at −80 °C, or freeze-dried and then stored at 4 °C.The subculture of the original strain is three passages or less. This product shall not be subcultured.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Bifidobacterium thermophilum* (part 4) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis

Assay: The assay of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Bifidobacterium thermophilum* (part 4) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

(153) *Bifidobacterium pseudolongum*

Bifidobacterium pseudolongum (Part 1)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze or freeze-dried *Bifidobacterium pseudolongum* GSL-3 strain.

Origin: The original strain is *Bifidobacterium pseudolongum* GSL-3 strain which was separated from pig feces in 1979.

Physical and chemical properties: This product is an obligate anaerobic Gram-positive bacillus. It breaks down xylose and raffinose.

Confirmation test:

i. This product is applied on medium No. 1, anaerobically cultured at 36~38 °C for 2~3 days and then the colony growing in the medium is milky brown to brown, a precise circle, hemispherically raised, with a smooth surface and circumference.

ii. This product is applied on medium No. 1 and anaerobically cultured at 36~38 °C for 2~3 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli, which are stained blue-violet to dark purple, claviform, are observed.

iii. Medium No. 1 is used as a test agar medium. This product is applied on the medium and anaerobically cultured at 36~38 °C for 2~3 days. The results of the test on xylose and raffinose using the colony forming on the medium by method No. 1 of the glycolytic capacity test method shows that both are positive.

iv. Medium No. 1 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2~3 days, and then the growth of the bacteria is not observed.

(b) Storage method and standards of subculture

The storage method and standards of subculture of the raw material for manufacturing of *Bifidobacterium thermophilum* (Part 4) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Bifidobacterium pseudolongum* (part 1) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1} to 10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. The colony forming by the procedure of the assay is a precise circle, hemispherically raised, with a smooth surface and circumference, and milky brown to brown.

ii. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli, which are stained blue-violet to dark purple, claviform, are observed.

iii. In the test using the colony obtained by the procedure of the assay, the results of the test on arabinose, melibiose, and melezitose using the colony obtained by the

procedure of the assay by method No. 1 of the glycolytic capacity test method shows that both are positive.

iv. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. The sample solution is cultured at 36~38 ℃ for 2~3 days, and then the growth of the bacteria is not observed.

Assay: The assay of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Bifidobacterium pseudolongum* (part 1) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Bifidobacterium pseudolongum (Part 2)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freezed *Bifidobacterium pseudolongum* M-602 strain.

Origin: The original strain is *Bifidobacterium pseudolongum* M-602 strain which was separated from chicken feces in 1980.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Bifidobacterium pseudolongum* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Bifidobacterium pseudolongum* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The storage method and standards of subculture of the raw material for manufacturing of *Enterococcus faecium* (Part 4) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Bifidobacterium pseudolongum* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Bifidobacterium pseudolongum* (part 1) is applied mutatis mutandis

Assay: The assay of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Bifidobacterium pseudolongum* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

(154) *Lactobacillus acidophilus*

Lactobacillus acidophilus (Part 1)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze-dried *Lactobacillus acidophilus* ATCC 33199 strain.

Origin: The original strain is *Lactobacillus acidophilus* ATCC 33199 strain separated from the avian crop (chicken) in 1980.

Physical and chemical properties: This product is a facultative anaerobic Gram-positive bacillus. It breaks down lactose to produce lactic acid and breaks down amygdalin and cellobiose.

Confirmation test:

i. This product is applied on medium No. 1, anaerobically cultured at 36~38 °C for 2 days and then the colony growing in the medium is milky brown to brown, slightly irregular or precise circle, hemispherically or slightly raised, with a smooth or rough surface and circumference.

ii. This product is applied on medium No. 1 and anaerobically cultured at 36~38 °C for 2 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli or long bacilli, which are stained blue-violet to dark purple are observed.

iii. Medium No. 9 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2 days, and then the growth of the bacteria is poor.

iv. Medium No. 1 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2 days, and then the growth of the bacteria is observed.

v. Medium No. 1 is used as a test agar medium. This product is applied on the medium and anaerobically cultured at 36~38 °C for 2 days. In the test using the colony forming

on the medium by the lactic acid producing capacity test method, production of lactic acid is observed.

vi. Medium No. 1 is used as a test agar medium. This product is applied on the medium and anaerobically cultured at 36~38 °C for 2 days. The results of the test using the colony growing in the medium on amygdalin and cellobiose by method No. 1 of the glycolytic capacity test method shows that both are positive.

(b) Storage method and standards of subculture

The storage method and standards of subculture of the raw material for manufacturing of Enterococcus faecium (Part 1) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Lactobacillus acidophilus* (part 1) is cultured, and the bacterial cells are collected, added with beta-glucan, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1} to 10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. The colony forming by the procedure of the assay is milky brown to brown, slightly irregular or precise circle, hemispherically or slightly raised, with a smooth or rough surface and circumference.

ii. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli or long bacilli, which are stained blue-violet to dark purple, are observed.

iii. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. In this procedure, medium No. 9 is used as a test agar medium. The sample solution is cultured at 36~38 °C for 2 days, and then the growth of the bacteria is poor.

iv. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. In this procedure, medium No.1 is used as a test agar medium. The sample solution is cultured at 36~38 °C for 2 days, and then the growth of the bacteria is observed.

v. In the test using the sample stock solution 0.1 mL prepared by the assay or the colony obtained by the procedure of the assay by the lactic acid producing capacity test method, production of lactic acid is observed

vi. In the test using the colony obtained by the procedure of the assay, the results of the test using the colony growing in the medium on amygdalin and cellobiose by method No. 1 of the glycolytic capacity test method shows that both are positive.

Assay:

Preparation of sample solution: The preparation of sample solution of *Bifidobacterium thermophilum* (part 1) is applied mutatis mutandis.

Procedure: Procedure: Medium No. 1 is used as a test agar medium. The culture medium is subjected to the procedures of method No. 2 of viable bacteria agent quantification method, and cultured at 36~38 ℃ for 2days.

(b) For manufacturing

The raw material for manufacturing of *Lactobacillus acidophilus* (part 1) is cultured, and the bacterial cells are collected, added with beta-glucan, dried, and mixed with fillers. However, a mixture of Enterococcus faecium (part 1) preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

Lactobacillus acidophilus (Part 2)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze or freeze-dried *Lactobacillus acidophilus* GAL-2 strain.

Origin: The original strain is *Lactobacillus acidophilus* GAL-2strain which was separated from chicken feces in 1979.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The storage method and standards of subculture of *Bifidobacterium thermophilum* (part 4) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Lactobacillus acidophilus* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains $10|^{-1}$ to 10^2 times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

Assay: The assay of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Lactobacillus acidophilus* (part 2) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Lactobacillus acidophilus (Part 3)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze or freeze-dried *Lactobacillus acidophilus* GBL-2 strain.

Origin: The original strain is *Lactobacillus acidophilus* GBL-2 strain which was separated from bovine feces in 1979.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The storage method and standards of subculture of *Bifidobacterium thermophilum* (part 4) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Lactobacillus acidophilus* (part 3) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^2 times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

Assay: The assay of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Lactobacillus acidophilus* (part 3) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Lactobacillus acidophilus (Part 4)

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze or freeze-dried *Lactobacillus acidophilus* GSL-2 strain.

Origin: The original strain is *Lactobacillus acidophilus* GSL-2 strain which was separated from pig feces in 1979.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The storage method and standards of subculture of *Bifidobacterium thermophilum* (part 4) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Lactobacillus acidophilus* (part 4) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

Assay: The assay of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Lactobacillus acidophilus* (part 4) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Lactobacillus acidophilus **(Part 5)**

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freezed *Lactobacillus acidophilus* LAC-300 strain.

Origin: The original strain is *Lactobacillus acidophilus* LAC-300 strain which was separated from human feces in 1977.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The storage method and standards of subculture of *Enterococcus faecium* (part 4) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Lactobacillus acidophilus (*part 5) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

Assay: The assay of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Lactobacillus acidophilus (*part 5) is cultured, and the bacterial cells are collected, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

Lactobacillus acidophilus **(Part 6)**

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freezed *Lactobacillus acidophilus* M-13 strain.

Origin: The original strain is *Lactobacillus acidophilus* M-13 strain which was separated from pig feces in 1970.

Physical and chemical properties: The physical and chemical properties are the same as those of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1).

Confirmation test: The confirmation test of the raw material for manufacturing of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

(b) Storage method and standards of subculture

The storage method and standards of subculture of *Enterococcus faecium* (part 2) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Lactobacillus acidophilus* (part 6) is cultured and the bacterial cells are collected, added with L-arginine hydrochloride, dried, and added with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test: The confirmation test of preparation of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis

Assay: The assay of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis.

(b) For manufacturing

For manufacturing, the raw material for manufacturing of *Lactobacillus acidophilus* (part 6) is cultured and the bacterial cells are collected, added with L-arginine hydrochloride, dried, and mixed with fillers.

(c) Standard of storage method

It shall be stored in a capped container.

(155) *Lactobacillus salivarius*

A. Raw material for manufacturing

(a) Compositional standards

This product is an inoculum for manufacturing proliferated and freeze-dried *Lactobacillus salivarius* chN-426 strain.

Origin: The original strain is *Lactobacillus salivarius* chN-426 strain which was separated from chicken intestine in 1966.

Physical and chemical properties: This product is a facultative anaerobic Gram-positive bacillus. It breaks down lactose to produce lactic acid and not breaks down amygdalin and cellobiose.

Confirmation test:

i. This product is applied on medium No. 1, anaerobically cultured at 36~38 °C for 2 days and then the colony growing in the medium is milky brown to brown, slightly irregular or precise circle, hemispherically or slightly raised, with a smooth or rough surface and circumference.

ii. This product is applied on medium No. 1 and anaerobically cultured at 36~38 °C for 2 days. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli or long bacilli, which are stained blue-violet to dark purple, are observed.

iii. Medium No. 9 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2 days, and then the growth of the bacteria is poor.

iv. Medium No. 1 is used as a test agar medium. This product is applied on the medium and cultured at 36~38 °C for 2 days, and then the growth of the bacteria is observed.

v. Medium No. 1 is used as a test agar medium. This product is applied on the medium and anaerobically cultured at 36~38 °C for 2 days. In the test using the colony forming on the medium by the lactic acid producing capacity test method, production of lactic acid is observed.

vi. Medium No. 1 is used as a test agar medium. This product is applied on the medium and anaerobically cultured at 36~38 °C for 2 days. The results of the test using the colony growing in the medium on amygdalin and cellobiose by method No. 1 of the glycolytic capacity test method shows that both are negative.

(b) Storage method and standards of subculture

The storage method and standards of subculture of the raw material for manufacturing of *Bifidobacterium thermophilum* (Part 1) is applied mutatis mutandis.

B. Preparation

(a) Compositional standards

This product is pieces or powder in which the raw material for manufacturing of *Lactobacillus salivarius* is cultured, and the bacterial cells are collected, dried, added with corn starch, shellac and lactose, granulated and mixed with fillers.

Content: When this product is determined, it contains 10^{-1}~10^{2} times/g as much as the viable cell count on the label.

Confirmation test:

i. The colony forming by the procedure of the assay is milky brown to brown, slightly irregular or precise circle, hemispherically or slightly raised, with a smooth or rough surface and circumference.

ii. A platinum-loop amount of water is placed on a slide glass. The colony obtained by the procedure of the assay is placed on the slide glass using a platinum wire, stirred to be suspended, spread to the proper size, and dried at room temperature or by fire from a distance. Then, it is passed through the flame 2~3 times to be fixed. This is used as a sample. In the test of the sample by the Gram stain, bacilli or long bacilli, which are stained blue-violet to dark purple, claviform, are observed.

iii. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. In this procedure, medium No. 9 is used as a test agar medium. The sample solution is cultured at 36~38 ℃ for 2 days, and then the growth of the bacteria is poor.

iv. The sample solution prepared by the assay is subjected to the procedure of method No. 2 of the viable bacteria agent quantification method. In this procedure, medium No.1 is used as a test agar medium. The sample solution is cultured at 36~38 ℃ for 2 days, and then the growth of the bacteria is observed.

v. In the test using the sample stock solution 0.1 mL prepared by the assay or the colony obtained by the procedure of the assay by the lactic acid producing capacity test method, production of lactic acid is observed

vi. In the test using the colony obtained by the procedure of the assay, the results of the test using the colony growing in the medium on amygdalin and cellobiose by method No. 1 of the glycolytic capacity test method shows that both are negative.

Assay: The assay of *Lactobacillus acidophilus* (part 1) is applied mutatis mutandis.

(b) For manufacturing

The raw material for manufacturing of *Lactobacillus salivarius* is cultured, and the bacterial cells are collected, dried, added with corn starch, shellac and lactose, granulated and mixed with fillers.

However, a mixture of *Bifidobacterium thermophilum* (part 1) preparation shall be used.

(c) Standard of storage method

It shall be stored in a capped container.

(156) Benzoic acid

A.Active ingredients for production

(a)Compositional standards

Content: Benzoic acid, when dried, contains not less than 99.5% of benzoic acid (C7H6O2).

Physical and chemical properties

i. Benzoic acid occurs as white crystals or crystalline powder.

ii. Melting point: 121 ~ 123°C

Identification: Dissolve 1.0 g (0.95 ~ 1.04 g) of benzoic acid in 8 mL of 1 mol/L sodium hydroxide TS, add water to make 100 mL, and add ferric chloride TS. A light yellow-red precipitate is formed, which changes to a white precipitate upon the addition of dilute hydrochloric acid.

Purity

i. Lead: Weigh 2.0 g (1.95 ~ 2.04 g) of benzoic acid, place it in a platinum, quartz, or porcelain crucible, and heat gradually. Before carbonization begins, stop the heating, add 1 mL of sulfuric acid, and gradually raise the temperature until the sample is carbonized to generate no white fumes of sulfuric acid.If necessary, add more sulfuric acid and heat it until most of the sample is carbonized. Put the lid on the container loosely, heat it gradually, and ignite to incinerate at 450 ~ 600 °C. If carbides remain, crush with a glass rod where necessary, moisten with 1 mL of sulfuric acid (1 → 4) and 1 mL of nitric acid, heat until no white fumes are evolved, and ignite to incinerate completely. To the residue, add 10 mL of hydrochloric acid (1 → 4), heat on a water bath, and evaporate to dryness. To the residue, add a small amount of nitric acid (1 → 100), and warm to dissolve it. After cooling, add nitric acid (1 → 100) to make exactly 10 mL, and use this solution as the sample solution. Measure 0.4 mL of standard lead solution using a volumetric pipette, transfer to a 10-mL volumetric flask, add nitric acid (1 → 100) to the marked line to make 10 mL, and use this solution as the standard solution. Perform the test for lead with the sample solution and standard solution as directed under Lead Test (Method 1 of Atomic Absorption Spectrophotometry): the amount of lead shall be not more than 2μg/g.

ii. Arsenic: Prepare the sample solution with 0.5 g (0.45 ~ 0.54 g) of benzoic acid according to Method 3 of the Arsenic Test, and perform the test for arsenic by the method using Apparatus A: the color of the absorbing liquid shall have no more color than the standard color (not more than 3 μg/g).

iii. Phthalic acid: To 0.10 g (0.095 ~ 0.104 g) of benzoic acid, add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, heat in an oil bath at 120 ~125 °C to evaporate water, and heat for another 90 minutes. After cooling, dissolve the residue in 5 mL of water, add 10 mL of a sodium hydroxide solution (43 → 500) to 1 mL of this solution, shake, and use

this solution as the sample solution.Separately, dissolve 61 mg of potassium hydrogen phthalate (standard reagent) in water to make exactly 1000 mL. Pipet 1 mL of this solution exactly, add 1 mL of resorcinol-sulfuric acid TS, proceed in the same manner as for the sample solution, and use this solution as the control solution. Examine the sample solution and the control solution under 470 ~ 490 nm light: the green fluorescence of the sample solution shall have no more color than the control solution (not more than 500 μg/g).

iv. Related substances: Weigh 5.0 g (4.95 ~5.04 g) of benzoic acid, transfer it into a 25-mL volumetric flask, dissolve it with 1 mL of propyl benzoate-dimethylformamide TS, add dimethylformamide to make 25 mL, and use this solution as the sample solution. Separately, weigh 40 mg each of propyl benzoate, biphenyl, 2-methylbiphenyl, 3-methylbiphenyl, 4-methylbiphenyl, and benzyl benzoate, and 20 g of benzoic acid, transfer them into a 100-mL volumetric flask, add about 50 mL of dimethylformamide to dissolve them, add dimethylformamide to make 100 mL, and use this solution as the standard solution. Perform the test with 1μL each of the sample solution and standard solution as directed under Gas Chromatography according to the following conditions. Based on the chromatogram obtained, identify the peaks of biphenyl, 2-methylbiphenyl, 3-methylbiphenyl, 4-methylbiphenyl, and benzyl benzoate appearing on the chromatogram of the sample solution by using the chromatogram of the standard solution, and determine the peak area Ac1. Treat the unidentified peak that appears between the peaks of 2-methylbiphenyl and benzoic acid as the peak of the dimethylbiphenyl isomer, and determine the content Ac1 of the peak area. Based on these values and the peak area Ai1 of propyl benzoate determined from the chromatogram of the sample solution, calculate the content of the related substances of benzoic acid (biphenyl, 2-methylbiphenyl, 3-methylbiphenyl, 4-methylbiphenyl, benzyl benzoate, and dimethylbiphenyl isomer) in the sample using the following equation: the total content shall not exceed 100 μg/g.

Ai1: Peak area of propyl benzoate in the sample solution

Ac1: Peak area of each related substance in the sample solution

Ms1: Amount (g) of sample in the sample solution

Mi1: Amount (mg) of propyl benzoate in the sample solution

RFc: Response factor of each related substance

The response factor (RFc) of each related substance shall be determined using the following equation:

As for the RFc of the dimethylbiphenyl isomer, use the RFc of 3-methylbiphenyl.

Ai2: Peak area of propyl benzoate in the standard solution

Ac2: Peak area of each related substance in the standard solution

Mc2: Amount (mg) of each related substance in the standard solution

Mi2: Amount (mg) of propyl benzoate in the standard solution

Operating conditions

Detector: Hydrogen flame ionization detector

Column: A fused silica column 0.32 mm in inside diameter and 15 to 30 m in length, coated with polyethylene glycol modified with nitroterephthalic acid to a thickness of 1 μm.

Column temperature: Hold the temperature at 100 °C for one minute, then raise it to 210 °C at a rate of 3 °C per minute.

Injection port temperature: 240 °C (if a temperature increase program can be set, raise the temperature to 270°C at a rate of 12°C per second with an initial temperature of 80 °C)

Detector temperature: 240 ~ 250 °C

Carrier gas: Helium

Flow rate: About 3 mL per minute (If pressure control is possible, hold the pressure at 47 kPa for 4.35 minutes and increase it to 58 kPa at a rate of 0.28 kPa per minute.)

Injection method: Split

Split ratio: 1:10

Loss on drying: Not more than 0.5% (1 g, silica gel, 3 hours)

Residue on ignition: Not more than 0.05% (1 g)

Assay: Weigh about 0.5 g of benzoic acid, previously dried, to the order of 0.001 g, record the value, add 25 mL of neutralized ethanol and 25 mL of water to dissolve, and titrate with a 0.1 mol/L sodium hydroxide solution (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide solution = 12.21 mg $C_7H_6O_2$

(b)Standards for manufacturing method

Manufacture by air oxidation of toluene.

(c)Standards for preservation method

Store in an airtight container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of benzoic acid are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of benzoic acid is applied mutatis mutandis.

(157) Calcium formate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following drying, it contains 98.0 % or over of calcium formate ($Ca(HCOO)_2$).

Physical and chemical properties:

i. This product is white to pale yellow crystals or powder.

ii. This product is easy to dissolve in water and hardly dissolves in methanol.

Confirmation test:

i. The solution of this product in water (1 → 10) gives the qualitative reactions i. to iii. of calcium salt.

ii. 1 mL of the solution, in which approximately 0.5 g of this product is dissolved in 5 mL of water, is measured, added with 0.5 mL of hydrochloric acid, and added with 20 mg (19.5~20.4 mg) of magnesium powder in several times. When bubbles disappear, it is added with 3 mL of sulfuric acid (3 → 5) and 10 mg (9.5~10.4 mg) of chromotropic acid and vigorously shaken. It is heated in a water bath for approximately 10 minutes, and then it is red to purple.

Purity test:

i. Clarity and color of solution: 1.0 g (0.95~1.04 g) of this product is dissolved in 100 mL of water. The resulting solution shall be colorless to pale yellow and transparent.

ii. pH: The pH of solution in water (1→100) of this product shall be 7.0~8.5.

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 1 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Water content: 0.2 % or less (Water metering method).

Assay: Approximately 2.0 g of this product is weighed to the digits of 0.001 g, and the value is recorded. It is dissolved with water 50 mL and titrated with 1 mol/L hydrochloric acid (potentiometric titration). A blank test is performed by the same method and corrections are made.

1 mL of 1 mol/L hydrochloric acid = 65.01 mg $Ca(HCOO)_2$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of calcium formate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of calcium formate is applied mutatis mutandis.

(158) Sodium gluconate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following drying, it contains 98.0~102.0 % of sodium gluconate ($C_6H_{11}O_7Na$).

Physical and chemical properties: This product is white to yellowish-white crystalline powder or particles.

Confirmation test:

i. The solution of this product in water (1 → 20) gives the qualitative reaction i. and iii. of sodium salt.

ii. When an infrared absorption spectrum of this product is measured by the paste method of the infrared absorption spectroscopy, the absorption is observed at near 3,520~3,560 cm^{-1}, 3,410~3,450 cm^{-1}, 3,290~3,330 cm^{-1}, 1,580~1,660 cm^{-1}, and 1,080~1,100 cm^{-1}.

Purity test:

i. Clarity and color of solution: This product 1.0 g (0.95~1.04 g) is dissolved in 10 mL of water. The resulting solution shall be colorless and almost clear.

ii. pH: The pH the solution of this product in water (1 → 10) shall be 6.2~7.8.

iii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iv. Arsenic: 0.5 g (0.45~0.54 g) of this product is weighed, and a sample solution is prepared by Method No. 1 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (4 mg/kg or less).

Loss on drying: 0.30 % or less (2 g, 105 °C, 2 hours)

Assay: This product is dried and appropriately 0.15 g of it is weighed to the digits of 0.001 g, and the values is recorded. It is added with 75 mL of acetic acid and titrated with 0.1 mol/L perchloric acid test solution (indicator: 10 drops of quinaldine red). In this case, the end point of titration is the time when the red color of the solution disappears. A blank test is performed by the same method and corrections are made.

1 mL of 0.1 mol/L perchloric acid = 21.81 mg $C_6H_{11}O_7Na$

(b) Standard of storage method

It shall be stored in a capped container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of sodium gluconate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of sodium gluconate is applied mutatis mutandis.

(158) Potassium diformate

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined following drying, it contains 95.0 % or over of potassium diformate ($C_2H_3O_4K$).

Physical and chemical properties: This product is white crystalline powder with no odor or a slight specific odor.

Confirmation test:

i. The pH of the solution of this product in water (1 → 100) shall be 3.3~4.3.

ii. 1 g (0.95~1.04 g) of this product is added with 10 mL of water and filtered. This solution 1 mL is added with 1 mL of ethanol and 3 drops of sulfuric acid, heated in a water bath, and then the odor of ethyl formate occurs.

iii. 1 g (0.95~1.04 g) of this product is added with 10 mL of water and filtered. This solution is added with lead acetate solution and then white crystalline precipitation is

generated. In addition, when it is added with silver nitrate test solution and heated, it becomes cloudy within 30 seconds.

iv. This product gives the qualitative reaction i. of potassium salt.

Purity test:

i. Melting point: The melting point of this product shall be 108~109 °C.

ii. Heavy metal: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 2 of the heavy metals test method. A control solution is prepared using 2.0 mL of lead standard solution by the method. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (20 mg/kg or less).

iii. Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed, and a sample solution is prepared by Method No. 3 of the arsenic test method. When the solution is tested by the arsenic test method using device A, the color of absorbing solution shall not be darker than the standard color (2 mg/kg or less).

Water content: 1.0 % or less (Direct titration method).

Assay: Approximately 2 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with water, transferred to a 500 mL volumetric flask, added with water to the graduation line to make 500 mL. 20 mL of this solution measured using a volumetric pipette is transferred to a stoppered Erlenmeyer flask, and while shaking added with 18~19 mL of sodium carbonate test solution and 50 mL of 0.02 mol/L potassium permanganate using a volumetric pipette. It is heated at 80 °C for 5 minutes with occasionally shaking, quenched, while shaking added with 1 g (0.5~1.4 g) of potassium iodide and 15 mL of 2 mol/L sulfuric acid test solution, and titrated with 0.1 mol/L sodium thiosulfate solution (indicator: starch test solution 1 mL). A blank test is performed by the same method and corrections are made.

Amount of potassium diformate (mg) = Titer of 0.1 mol/L sodium thiosulfate solution (mL) × 3.254

(b) Standard of manufacturing method

For manufacturing, potassium diformate crystals produced by reaction of formic acid and potassium formate are added with vegetable oil, silica gel and others.

(c) Standard of storage method

It shall be stored in an air tight container.

B. Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of potassium diformate are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of potassium diformate is applied mutatis mutandis.

(160) Fumaric acid

A. Raw material for manufacturing

(a) Compositional standards

Content: When this product is determined, it contains 99.0 % or over of fumaric acid ($C_4H_4O_4$).

Physical and chemical properties: This product is white crystalline powder without odor, and with a specific sourness.

Confirmation test:

i. This product sublimes by heating.

ii. When this product is dried at 105 °C for 3 hours and its melting point is measured, that is 287~302 °C (degradation).

iii. When 0.5 g (0.45~0.54 g) of this product is added with 10 mL of water, dissolved by boiling, and then added with 2~3 drops of thermal bromine test solution, the color of the solution disappears.

iv. 50 mg (49.5~50.4 mg) of this product is placed in a test tube, added with 2~3 mg of resorcin and 1 mL of sulfuric acid, shaken, heated at 120~130 °C for 5 minutes, allowed to cool, and added with water to make 5 mL. While cooling, this solution is added with drops of sodium hydroxide solution (2 → 7) to make it alkaline, and added with water to make 10 mL. The resulting solution emits green-blue fluorescence under ultraviolet light.

Purity test:

i. Clarity and color of solution: 0.5 g (0.45~0.54 g) of this product is weighed and dissolved with 10 mL of sodium hydroxide solution (1 → 25). The resulting solution shall be colorless and clear.

ii. Sulfate: 1.0 g (0.95~1.04 g) of this product is weighed, added with 30 mL of water, shaken, added with 1 drop of phenolphthalein test solution, and added with drops of ammonia test solution until the solution exhibits slightly red. This is used as a sample solution. A control solution is prepared using 0.2 mL of 0.005 mol/L sulfuric acid. When the sample solution and the control solution are tested by the qualitative reaction

test method of sulfate, the opacity of the sample solution is shall not be darker than that of the control solution (0.01 % or less).

iii. Heavy metal: 2.0 g (1.95~2.04 g) of this product is weighed, added with 30 mL of water, shaken, added with 1 drop of phenolphthalein test solution, added with drops of ammonia test solution until the solution exhibits slightly red, and added with 2 mL of acetic acid (1 → 20) and water to make 50 mL. This is used as a sample solution. A control solution is prepared using 2.0 mL of lead standard solution. When the sample solution and the control solution are tested by the heavy metals test method, the color of the sample solution shall not be darker than that of the control solution (10 mg/kg or less).

iv. Arsenic: 0.5 g (0.45~0.54 g) of the product is weighed, added with 10 mL of water, dissolved by heating, and allowed to cool. This is used as a sample solution. When the sample solution is tested by the arsenic test method using device A, except, 10 mL of acidic stannous chloride test solution and 3 g (2.5~3.4 g) of arsenic-free zinc are used, the color of the absorbing solution shall not darker than that of standard, (4 mg/kg or less).

Ignition residue: 0.05 % or less (5 g)

Assay: Approximately 1 g of this product is weighed to the digits of 0.01 g and the value is recorded. It is dissolved with water, transferred to a 250 mL volumetric flask, and added with water to the graduation line to make 250 mL. This solution 25 mL is measured using a volumetric pipette and titrated with 0.1 mol/L sodium hydroxide solution (indicator: 2 drops of phenolphthalein test solution).

0.1 mol/L sodium hydroxide solution = 5.804 mg of $C_4H_4O_4$

(b) Standard of storage method

It shall be stored in a capped container.

B.Preparation

(a) Compositional standards

The compositional standards of the raw material for manufacturing of fumaric acid are applied mutatis mutandis.

(b) Standard of storage method

The standard of storage method of the raw material for manufacturing of fumaric acid is applied mutatis mutandis.

(161) Formic Acid/Propionic Acid

Preparation

(a) Compositional standards

This product is liquid, in which the raw material for manufacturing of formic acid or its preparation and the raw material for manufacturing of propionic acid or its preparation are mixed and if necessary added with ammonia (limited to that meeting the standards of food additives), caprylic acid or food dye.

Content: This product is determined to contain formic acid (CH_2O_2) corresponding to 97~103 % of the amount on the label and propionic acid ($C_3H_6O_2$) corresponding to 95~105 % of the amount on the label.

Confirmation test:

i. The solution of this product in water (1 → 10) is acidic.

ii. This product 1 mL is added with 1 mL of ethanol and 3 drops of sulfuric acid, heated in a water bath, and then the odor of carboxylate occurs.

iii. This product is added with lead acetate test solution and then while crystalline precipitation occurs. In addition, when it is added with silver nitrate test solution and heated, it becomes cloudy within 30 seconds.

Assay:

i. Formic acid: The amount of this product containing approximately 100 mg of formic acid (CH_2O_2) is weighed to three significant digits and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask, added with water to the graduation line to make 100 mL. This is used as a sample solution. Separately, approximately 150 mg of sodium formate for assay is measured to the digits of 1 mg and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask, added with water to the graduation line to make 100 mL. This is used as a standard solution. 20 μL each of the sample and standard solution are tested by the liquid chromatography under the following conditions. The peak areas are measured from the obtained chromatograms.

Amount of formic acid (CH_2O_2) (mg)

$$= \text{Collected amount of sodium formate for assay (mg)} \times \frac{A_T}{1{,}478 \times A_S}$$

A_T: Peak area of the sample solution

A_S: Peak area of the standard solution

Operating condition

Detector: Ultraviolet absorptiometer (measurement wavelength: 210 nm)

Column: A stainless tube (inner diameter: 8 mm, length: 300 mm) is filled with 8~11 μm of strong-acid cation exchange resin.

Column temperature: 50 °C

Mobile phase: Perchloric acid 0.85 mL is added with 1,000 mL of water.

Flow rate: Approx. 1.0 mL/min.

Column selection: A column is used when: 150 mg (149.5~150.4 mg) of sodium formate for assay and 32 mg (31.5~32.4 mg) of sodium propionate for assay are weighed, dissolved with water, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. When 20 μL of this solution is subjected to the procedure under the above conditions, formic acid and propionic acid are eluted in this order, and the separation degree is 2.0 or greater.

ii. Propionic acid: The amount of this product containing approximately 25 mg of propionic acid ($C_3H_6O_2$) is weighed to three significant digits and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a sample solution. Separately, approximately 32 mg of sodium propionate for assay is weighed to the digits of 0.01 mg and the value is recorded. It is dissolved with water, transferred to a 100 mL volumetric flask, and added with water to the graduation line to make 100 mL. This is used as a standard solution. 20 μL each of the sample and standard solutions are tested by the liquid chromatography under the conditions of i. The peak areas are measured from the obtained chromatograms.

Amount of formic acid ($C_3H_6O_2$) (mg)

$$= \text{Collected amount of sodium formate for assay (mg)} \times \frac{A_T}{1{,}297 \times A_S}$$

A_T: Peak area of the sample solution

A_S: Peak area of the standard solution

(b) Standard of storage method

It shall be stored in an acid resistant airtight capped container.

(c) Standards of the label

The following words shall be written on the immediate container or the immediate wrapper of this product.

“使用上の注意　この飼料添加物は、pH が低いことから取扱いに注意すること。”

Precaution: This feed additive requires careful handling due to its low pH.

(162) Vitamin AD

A. Preparation (Part 1: liquid)

(a) Compositional standards

This is oil liquid or water-soluble liquid, which is a mixture of the raw material for manufacturing or preparations of vitamin A oil or vitamin A powder and the raw material for manufacturing or preparations of cholecalciferol, ergocalciferol, vitamin D_3 oil or vitamin D powder in hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats.

Content: The assay shows that this product contains vitamin A (retinol) corresponding to 90~130 % of the unit on the label and cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) corresponding to 90~130 % of the unit on the label.

Confirmation test:

i. Vitamin A: The confirmation test of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

ii. Cholecalciferol or ergocalciferol: The confirmation test of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

Assay:

i. Vitamin A: It tested by Method No. 2 of the vitamin A assay.

ii. Cholecalciferol or ergocalciferol: It tested by method of the vitamin D assay.

(b) Standard of storage method

It shall be stored in a lightproof airtight container making it almost full or with nitrogen replaced with air.

B. Preparation (Part 2: powder)

(a) Compositional standards

This is powder or particles, which is a mixture of the raw material for manufacturing or preparations of vitamin A oil or vitamin A powder and the raw material for manufacturing or preparations of cholecalciferol, ergocalciferol, vitamin D_3 oil or vitamin D powder in fillers.

Content: The assay shows that this product contains vitamin A (retinol) corresponding to 90~130 % of the unit on the label and cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) corresponding to 90~130 % of the unit on the label.

Confirmation test:

i. Vitamin A: The confirmation test of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

ii. Cholecalciferol or ergocalciferol: The confirmation test of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

Assay:

i. Vitamin A: The amount of this product corresponding to 500 units or more of Vitamin A and containing 1 or less of fat and oil is weighed to three significant digits and the value is recorded. It is placed in a flask, added with 2 mL of water, heated for a while with shaking, and added with 30 mL of aldehyde-free ethanol and 1 mL of ethanol solution of pyrogallol (1 → 10), and from here the test by Method No. 2 of the vitamin A assay is performed.

ii. Cholecalciferol or ergocalciferol: It tested by method of the vitamin D assay.

(b) Standard of storage method

It shall be stored in a lightproof capped container.

(163) Vitamin ADE

A. Preparation (Part 1: liquid)

(a) Compositional standards

This is oil liquid or water-soluble liquid, which is a mixture of the raw material for manufacturing or preparations of vitamin A oil or vitamin A powder, the raw material for manufacturing or preparations of cholecalciferol, ergocalciferol, vitamin D_3 oil or vitamin D powder, and the raw material for manufacturing or preparations of *dl*-*α*-tocopherol acetate or vitamin E powder in hydrogenated oils, higher saturated fatty acids, fatty acids, vegetable oil or animal fats.

Content: The assay shows that this product contains vitamin A (retinol) corresponding to 90~130 % of the unit on the label, cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) corresponding to 90~130 % of the unit on the label, and *dl*-*α*-tocopherol acetate ($C_{31}H_{52}O_3$) corresponding to 90~120 % of the unit on the label.

Confirmation test:

i. Vitamin A: The confirmation test of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

ii. Cholecalciferol or ergocalciferol: The confirmation test of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

iii. *dl*-*α*-tocopherol acetate: 5 μL of the sample solution for thin layer chromatograph obtained by the assay is measured and spotted on a thin layer plate. 5 μL of the standard solution for thin layer chromatograph is spotted in parallel on the same thin layer plate. Then, they are developed to approximately 10 cm with the developing solvent, a mixed solution of n-hexane and ethyl acetate (4:1), sprayed with dilute ferric chloride test solution, and then sprayed with *α*,*α*′-dipyridyl test solution, and the spots

of tocopherol obtained from the sample and standard solutions are both red and their Rf values are equal.

Assay:

i. Vitamin A: It tested by Method No. 2 of the vitamin A assay.

ii. Cholecalciferol or ergocalciferol: It tested by method of the vitamin D assay.

iii. *dl*-*α*-tocopherol acetate: The amount of this product containing approximately 0.02 g of *dl*-*α*-tocopherol acetate ($C_{31}H_{52}O_3$) is weighted to three significant digits and the value is recorded. It is saponified, extracted, and dehydrated by the vitamin A assay. The ether extract is distilled off under reduced pressure in water at 45 °C, 2 mL of acetone is measured using a volumetric pipette, and it is added in the residue and dissolved. This is used as a sample solution for thin layer chromatograph. Separately, approximately 0.02 g of *dl*-*α*-tocopherol acetate for assay is weighed to the digits of 0.1 mg and the value is recorded. It is subjected to the same procedure of the preparation of a sample solution and used as a standard solution of thin layer chromatograph. 0.2 mL each of the sample and standard solutions of thin layer chromatograph are measured using a volumetric pipette and spotted on a thin layer plate prepared using silica gel for thin-layer chromatography (with a fluorescent agent). Then, they are developed to approximately 15 cm with the developing solvent, a mixed solution of n-hexane and ethyl acetate (4:1), and the thin layer plate is air dried and irradiated with ultraviolet light (dominant wavelength: 254 nm). The parts of tocopherol obtained from the sample and standard solutions are collected by scratching with a stainless microspatula, and placed in a 50 mL beaker. It is added with a little ethanol, shaken, and allowed to stand, and the supernatant is filtered with a glass filter. Extraction is performed by repeating the same procedure. The extract is transferred to a 100 mL volumetric flask and added with 100 mL of ethanol to the graduation line to make 100 mL. This is used as a sample and standard solutions. 10 mL each of the sample and standard solutions are measured using a volumetric pipette, transferred to 25 mL volumetric flasks, added with 1 mL of dilute ferric chloride test solution and 1 mL of *α*,*α*′-dipyridyl test solution, and added with ethanol to the graduation line to make 25 mL. Separately, 10 mL of ethanol is measured using a volumetric pipette and subjected to the same procedure to prepare a control solution. Then, the absorbances A_T and A_S at the wavelength 520 nm are measured.

$$\text{Amount of } dl\text{-}\alpha\text{-tocopherol acetate (mg)} = \text{Amount of } dl\text{-}\alpha\text{-tocopherol acetate for assay (mg)} \times \frac{A_T}{A_S}$$

(b) Standard of storage method

It shall be stored in a lightproof airtight container making it almost full or with nitrogen replaced with air.

B. Preparation (Part 2: powder)

(a) Compositional standards

This is powder or particles, which is a mixture of the raw material for manufacturing or preparations of vitamin A oil or vitamin A powder, the raw material for manufacturing or preparations of cholecalciferol, ergocalciferol, vitamin D_3 oil or vitamin D powder, and the raw material for manufacturing or preparations of *dl*-α-tocopherol acetate or vitamin E powder in fillers.

Content: The assay shows that this product contains vitamin A (retinol) corresponding to 90~130 % of the unit on the label, cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) corresponding to 90~130 % of the unit on the label, and *dl*-α-tocopherol acetate ($C_{31}H_{52}O_3$) corresponding to 90~120 % of the unit on the label.

Confirmation test:

i. Vitamin A: The confirmation test of the raw material for manufacturing of vitamin A powder is applied mutatis mutandis.

ii. Cholecalciferol or ergocalciferol: The confirmation test of the raw material for manufacturing of vitamin D powder is applied mutatis mutandis.

iii. *dl*-α-tocopherol acetate: The confirmation test iii. of the preparation (Part 1: liquid) of vitamin ADE is applied mutatis mutandis.

Assay:

i. Vitamin A: The amount of this product containing vitamin A 500 units or more and 1 g or less of fat and oil is weighed to three significant digits and the value is recorded. It is placed in a flask, added with 2 mL of water, heated for a while with shaking, then added with 30 ml of aldehyde-free ethanol and 1 ml of ethanol solution of pyrogallol (1 → 10), and from here the test by Method No. 2 of the vitamin A assay is performed.

ii. Cholecalciferol or ergocalciferol: It tested by method of the vitamin D assay.

iii. *dl*-α-tocopherol acetate: The assay of Vitamin ADE preparation (Part 1: liquid) is applied mutatis mutandis.

(b) Standard of storage method

It shall be stored in a lightproof capped container.