

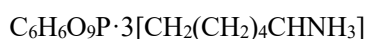
7 Regulations of reference standards, reagents, test solutions, standard solutions for volumetric analysis, Matching fluids for color, measuring instruments, appliances, filter papers, sterilization and Bertrand's saccharine assay table, which is used for standard of general tests for feed additives and compositional standards and standards for manufacturing method, etc. of feed additives.

(1) Reference standards

Reference standards are prepared to constant purity or biological activity, used for measuring biological and physicochemical characteristics of feed additives.

Ascorbic acid: $C_6H_8O_6$ [Japanese Pharmacopoeia reference standard]

L-Ascorbic acid 2-organophosphate tris cyclohexylammonium:



Content: It contains not less than 98.0 % of L-ascorbic acid 2-organophosphate tris cyclohexylammonium ($C_6H_6O_9P \cdot 3[CH_2(CH_2)_4CHNH_3]$).

Physical and chemical properties: White powder.

Identification: Determine as directed in the potassium bromine disk method under Infrared Spectrophotometry: it exhibits absorption at the wavenumbers of $2,939\text{ cm}^{-1}$, $2,859\text{ cm}^{-1}$, $1,719\text{ cm}^{-1}$, $1,586\text{ cm}^{-1}$, $1,448\text{ cm}^{-1}$, $1,389\text{ cm}^{-1}$ and 974 cm^{-1} .

Purity: Dissolve 0.01 g (0.005~0.014 g) of L-ascorbic acid 2-organophosphate tris cyclohexylammonium in 20 mL of water, and use this solution as the sample solution.

Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by automatic integration method: the total area of the peaks other than the peak of L-ascorbic acid 2-organophosphate tris cyclohexyl ammonium is not more than 2.0 %.

Operating conditions:

Detector: An ultraviolet absorption photometer (wavelength: 250 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 (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Dissolve 13.6 g (13.55~13.64 g) of potassium dihydrogen phosphate and 4.0 mL of tetrabutylammonium hydroxide test solution in 950 mL of water, adjust the pH at 6.0 with 2 mol/L sodium hydroxide test solution, and add water to make 1,000 mL. Add 50 mL of acetonitrile to 950 mL of this solution, and mix.

Flow rate: 1.0 mL/min

Time span of measurement: 6 times as long as the retention time of L-ascorbic acid 2-organophosphate tris cyclohexylammonium.

Loss on drying: Not more than 0.5 % (0.1 g, silica gel, 24 hours).

Assay: Weigh 11 mg of L-ascorbic acid 2-organophosphate tris cyclohexylammonium to one decimal place, record the value, add 0.1 mol/L of hydrochloric acid to dissolve, transfer to a 100 mL volumetric flask, add 0.1 mol/L hydrochloric acid to make exactly 100 mL.

Measure 2.0 mL of this solution with transfer pipet, transfer to a 100 mL volumetric flask, add 0.1 mol/L hydrochloric acid-sodium carbonate buffer solution (pH 10) to make exactly 100 mL, and use this solution as the sample solution. Determine the absorbance, A, of the sample solution of maximum absorption at about 263 nm, using 0.1 mol/L hydrochloric acid-sodium carbonate buffer solution (pH 10) as the blank.

Amount (mg) of L-ascorbic acid 2-organophosphate tris cyclohexylammonium =

$$\frac{A}{279.5} \times 50,000$$

L-ascorbic acid 2-phosphate ester magnesium decahydrate: $(C_6H_6O_9P)_2Mg_3 \cdot 10H_2O$

Content: It contains not less than 98.0 %.

Physical and chemical properties: White powder, and having a slightly characteristic odor.

Identification:

- A. To 2 mg (1.5~2.4 mg) of L-ascorbic acid 2-phosphate ester magnesium decahydrate add 0.1 mol/L hydrochloric acid to make 100 mL. Determine the absorption spectrum of this solution: it exhibits a maximum between 235 nm and 239 nm.
- B. To 5 mL of an aqueous solution of L-ascorbic acid 2-phosphate ester magnesium decahydrate (1 → 50) add 1 drop of ferric chloride test solution: a red-brown color is developed.
- C. To 0.1 g (0.05~0.14 g) of L-ascorbic acid 2-phosphate ester magnesium decahydrate add 2 mL of sulfuric acid and 15 mL of hydrogen peroxide water, heat until the volume of the solution becomes to 5 mL, cool, and add water to make 50 mL. To 1 mL of this solution add 1 mL of 3 mol/L sulfuric acid, 1 mL of ammonium molybdate test solution and 1 mL of amidol-sodium bisulfite: a blue color is developed.
- D. To 0.5 g (0.45~0.54 g) of L-ascorbic acid 2-phosphate ester magnesium decahydrate add 2 mL of sulfuric acid and 30 mL of hydrogen peroxide water, heat until the volume of this solution becomes to 5 mL, cool, and add water to make 20 mL. Neutralize the solution with 1 mol/L sodium hydroxide test solution: the solution responds to the Qualitative Test for magnesium salt.

Purity:

- A. Clarity and Color of Solution: Dissolve 1.0 g (0.95~1.04 g) of L-ascorbic acid 2-phosphate ester magnesium decahydrate in 10 mL of water: the solution is not more intensely colored than matching fluid J, and clear.
- B. Related substances: Dissolve 0.01 g (0.005~0.014 g) of L-ascorbic acid 2-phosphate ester magnesium decahydrate in 1 mL of water, and spot 10 μ L of this solution on the place 5 cm away from the bottom of No. 3 filter paper for chromatography, and air-dry the filter paper. Then develop with a mixture of 5 g (4.5~5.4 g) of trichloroacetic acid and 95 mL of a mixture of isopropanol and water (75:20) to a distance of about 30 cm, and air-dry the filter paper. Spray evenly the solution prepared by dissolving 0.5 g (0.45 ~0.54 g) of ferric chloride in ethanol to make 100 mL on the filter paper: only one red-brown spot with an R_f value of about 0.5 appears, and no spot other than the principal spot.

Water: Weigh 1.6 g of L-ascorbic acid 2-phosphate ester magnesium decahydrate to two decimal places, record the value, dissolve in a mixture of methanol and sulfuric acid (70:1), transfer to a 50 mL volumetric flask, and add the mixture of methanol and sulfuric acid to make exactly 50 mL, and use this solution as the sample solution. Weigh the mass of the sample solution to two decimal places, and record the value. Measure 1 mL of the sample solution with a transfer pipet, and determine water content as directed under Karl-Fisher method. Separately, determine water content with 1 mL of a mixture of methanol and sulfuric acid (70:1) weighed to two decimal places and recorded its value. Calculate the water content of L-ascorbic acid 2-phosphate ester magnesium decahydrate by the following equation: it is between 23.0 % and 24.5 %.

The amount of water of L-ascorbic acid 2-phosphate ester magnesium decahydrate (%) =

$$\frac{50 \times S - B \times (W_1 - W_2) / W_3}{W_1 \times 10}$$

S: Amount (mg) of water in 1 mL of sample solution.

B: Amount (mg) of water in 1 mL of a mixture of methanol and sulfuric acid (70:1) weighed to two decimal places and recorded the value.

W₁: Amount (g) of sample.

W₂: Mass (g) of 50 mL of sample solution.

W₃: Mass (g) of 1 mL of a mixture of methanol and sulfuric acid (70:1) used for determination of B.

Assay: Weigh 0.2 g of L-ascorbic acid 2-phosphate ester magnesium decahydrate to three decimal places and record the value, and dissolve in water. Transfer to a 100 mL volumetric flask, add water to make 100 mL, use this solution as the sample stock solution. Measure 25 mL of this solution with transfer pipet, transfer to a reaction flask, add 1 mL

of sulfuric acid (1 → 3) and 5 mL of potassium peroxodisulfate (2 → 25), stopper tightly, sterilize mixture in an autoclave at 121 °C for 30 minutes. Cool, transfer to a 100 mL volumetric flask, add water to make exactly 100 mL. Measure 2.5 mL of this solution with transfer pipet, add 1 mL of sodium hydrogen sulfite solution (1 → 20), shake, add 15 mL of ammonium molybdate-potassium antimonyl tartrate sesquihydrate-ascorbic acid test solution, shake, transfer to a 200 mL volumetric flask, and add water to make exactly 200 mL. Use this solution as the sample solution. To the sample solution add ammonium molybdate-potassium antimonyl tartrate sesquihydrate-ascorbic acid test solution, allow to stand exactly 30 minutes, determine the absorbance, A_{T1} , at 710 nm of this solution.

Measure 25 mL of the sample stock solution with transfer pipet, transfer to a 100 mL volumetric flask, add water to make exactly 100 mL, measure 2.5 mL of this solution with transfer pipet, add 1 mL of sodium bisulfite solution (1 → 20), shake, proceed in the same manner as the sample solution, determine the absorbance, A_{T2} , at 710 nm of this solution.

Separately, weigh about 0.1 g of potassium dihydrogen phosphate to three decimal places and record the value, add water to dissolve, transfer to a 500 mL volumetric flask, add water to make exactly 500 mL. Measure 2.5 mL of this solution with transfer pipet, add 1 mL of sodium hydrogen sulfite solution (1 → 20), and shake. Proceed in the same manner as the sample solution, and determine the absorbance, A_S , at 710 nm of this solution.

$$\text{Amount (mg) of L-ascorbic acid 2-phosphate ester magnesium} = W \times \frac{4}{5} \times \frac{A_{T1} - A_{T2}}{A_S} \times$$

$$\frac{379.61}{136.09} \times 1,000$$

W: Amount (g) of potassium dihydrogen phosphate

Methyl 4-acetamid-2-hydroxybenzonate: $C_{10}H_{11}NO_4$

Content: Methyl 4-acetamid-2-hydroxybenzonate, when dried at 105 °C for 2 hours, contains not less than 98.0 % of methyl 4-acetamid-2-hydroxybenzonate ($C_{10}H_{11}NO_4$).

Physical and chemical properties: A white crystalline powder, having no odor.

Identification:

- A. Determine the absorption spectrum of a solution of methyl 4-acetamid-2-hydroxybenzonate in methanol (1 → 125,000): it exhibits a maximum between 211 nm and 215 nm, between 272 nm and 276 nm and between 305 nm and 309 nm, and a minimum between 237 nm and 241 nm and between 291 nm and 295 nm.
- B. Determine the infrared absorption spectrum of methyl 4-acetamid-2-hydroxybenzonate dried at 105 °C for 2 hours as directed in the potassium bromine disk method under Infrared Spectrophotometry: it exhibits absorption at the wavenumbers of $3,300\text{ cm}^{-1}$, $1,680\text{ cm}^{-1}$, $1,615\text{ cm}^{-1}$, $1,320\text{ cm}^{-1}$ and $1,275\text{ cm}^{-1}$.

Purity:

- A. Melting point: Melting point of methyl 4-acetamid-2-hydroxybenzoate should be between 151 °C and 153 °C.
- B. Related substances: Weigh 0.01 g (0.005–0.014 g) of methyl 4-acetamid-2-hydroxybenzoate, dissolve in 0.1 mL of methanol, spot 10 µL of this solution on the plate prepared with silica gel for thin layer chromatography. Develop the plate with a mixture of diethyl ether, benzene and chloroform (50:35:15) to a distance of about 10 cm, and air-dry the plate. Spray evenly Drangendorff's test solution, then sulfuric acid (1 → 2): only one orange spot with an R_f value of about 0.3 appears, and no spot other than the principal spot.

Loss on drying: Not more than 1.0 % (1 g, 105 °C, 2 hours).

Residue on ignition: Not more than 0.10 % (1 g).

Assay: Dry methyl 4-acetamid-2-hydroxybenzoate at 105 °C for 2 hours, weigh 0.045 g to four decimal places, and record the value. Proceed as directed in Nitrogen Determination.

1 mL of 0.01 mol/L sulfuric acid = 4.184 mg of C₁₀H₁₁NO₄

Notes: Preserve in tight, light-resistant containers.

Methyl 4-amino-2-ethoxybenzoate: C₁₀H₁₃NO₃

Content: Methyl 4-amino-2-ethoxybenzoate, when dried in a desiccator (with silica gel) for 3 hours, contains not less than 98.0 % of methyl 4-amino-2-ethoxybenzoate (C₁₀H₁₃NO₃).

Physical and chemical properties:

- A. A grayish-brown crystalline powder, and having no odor.
- B. Melting point 99–101 °C (with decomposition)

Identification:

- A. Determine the absorption spectrum of methyl 4-amino-2-ethoxybenzoate in methanol (1 → 125,000): it exhibits a maximum between 208 nm and 212 nm, between 232 nm and 236 nm, between 277 nm and 281 nm and between 300 and 304 nm and a minimum between 222 nm and 226 nm, between 250 nm and 254 nm and between 287 nm and 291 nm.
- B. Dry methyl 4-amino-2-ethoxybenzoate in a desiccator (with silica gel) for 3 hours, determine the infrared absorption spectrum as directed in the potassium bromine disk method under Infrared Spectrophotometry: it exhibits absorption at the wavenumbers of 3,400 cm⁻¹, 1,695 cm⁻¹, 1,610 cm⁻¹ and 1,255 cm⁻¹.

Purity: Related substances; Weigh 0.01 g (0.0095–0.0104 g) of methyl 4-amino-2-ethoxybenzoate, dissolve in 1.0 mL of methanol, spot 10 µL of this solution on the plate prepared with silica gel for thin layer chromatography. Develop the plate with a mixture of diethyl ether, benzene and chloroform (50:35:15) to a distance of about 10 cm, and air-dry

the plate. Spray evenly Drangendorff's test solution, then sulfuric acid (1 → 2): only one orange spot with an Rf value of about 0.5 appears, and no spot other than the principal spot.

Loss on drying: Not more than 3.0 % (1 g, silica gel, 3 hours).

Residue on ignition: Not more than 0.10 % (1 g).

Assay: Dry methyl 4-amino-2-ethoxybenzoate in a desiccator with silica gel for 3 hours, weigh about 0.04 g to four decimal places and record the value. Proceed as directed in Nitrogen Determination.

1 mL of 0.01 mol/L sulfuric acid = 3.904 mg of C₁₀H₁₃NO₃

Notes: Preserve in tight, light-resistant containers.

Amprolium: C₁₄H₁₉ClN₄·HCl: Amprolium is prepared by weighing crude material for manufacturing amprolium, recrystallizing with glacial acetic acid, washing with acetone, dissolving in water with warming, adding isopropanol to recrystallize, and washing with acetone.

Physical and chemical properties:

A. A white crystalline powder, having no odor.

B. Melting Point 248 °C (with decomposition)

Identification:

A. Determine the absorption spectrum of amprolium in 0.1 mol/L hydrochloric acid (1 → 100,000): it exhibits a maximum between 244 nm and 248 nm and between 260 nm and 264 nm. Separately, determine the maximal absorbances, A₁ and A₂, between 244 nm and 248 nm and between 260 nm and 264 nm, respectively: the ratio of A₁/A₂ is between 1.04 and 1.06.

B. A solution of amprolium (1 → 50) responds to the Qualitative Tests for chloride.

Purity: Related substances: Weigh 0.10 g (0.095~0.104 g) of amprolium, dissolve in 10 mL of methanol. Spot 10 µL of this solution on the plate prepared with silica gel for thin layer chromatography. Develop the plate with a mixture of water, butanol and glacial acetic acid (5:4:1) to a distance of about 10 cm, air-dry the plate. Spray evenly Drangendorff's test solution, then sulfuric acid (1 → 2): only one orange spot with an Rf value of about 0.4 appears, and no spot other than the principal spot.

Loss on drying: Not more than 0.5 % (1 g, in vacuum, 100 °C, 2 hours).

Residue on ignition: Not more than 0.10 % (1 g).

Notes: Preserve in tight, light-resistant containers.

Ethopabate: C₁₂H₁₅NO₄ Ethopabate is prepared by weighing crude material for manufacturing ethopabate, recrystallizing with methanol.

Physical and chemical properties: A white to slightly yellowish white crystalline powder, having no odor.

Identification: Determine the absorption spectrum of ethopabate in methanol (1 → 125,000): it exhibits a maximum between 266 nm and 270 nm, between 297 nm and 301 nm, a minimum between 236 nm and 240 nm and between 285 nm and 289 nm.

Purity:

- A. Melting point Melting point of ethopabate should be between 147 and 151 °C.
- B. Related substances Weigh 0.010 g (0.0095~0.0104 g) of ethopabate, dissolve 1.0 mL of methanol, spot 10 µL of this solution on the plate prepared with silica gel for thin layer chromatography. Develop the plate with a mixture of diethyl ether, benzene and chloroform (10:9:1) to a distance of about 10 cm, air-dry the plate. To 10 g of sodium nitrate add 20 mL of hydrochloric chloride, evolve the nitrous acid vapors in a container, and allow the plate to stand in the container. Separately, dissolve 0.05 g (0.045~0.054 g) of chromotropic acid and 40 g (39.5~40.4 g) of sodium acetate in water to make 100 mL. Spray evenly this solution to the plate: only one red-purple spot with an R_f value of about 0.5 appears, and no spot other than the principal spot.

Loss on drying: Not more than 0.5 % (1 g, in vacuum, 100 °C, 2 hours).

Residue on ignition: Not more than 0.10 % (1 g).

Note: Preserve in tight, light-resistant containers.

Thiamine chloride hydrochloride: C₁₂H₁₇ClN₄OS·HCl [Japanese Pharmacopoeia reference standard]

Pyridoxine hydrochloride: C₈H₁₁NO₃·HCl [Japanese Pharmacopoeia reference standard]

Morantel citrate monohydrate: C₁₂H₁₆N₂S·C₆H₈O₇·H₂O Morantel citrate monohydrate is prepared by weighing crude material for manufacturing morantel citrate monohydrate, recrystallizing twice with water avoiding light.

Physical and chemical properties:

- A. Pale yellow crystalline powder, slightly bitter, and having characteristic odor.
- B. Soluble in Methanol, slightly soluble in water and in ethanol, practically insoluble in ethyl acetate and in benzene.
- C. The pH of aqueous solution (1 → 200) of morantel citrate monohydrate is between 3.5 and 4.3.
- D. Melting Point 117~120 °C

Identification:

- A. Dissolve 0.1 g (0.05~0.14 g) of morantel citrate monohydrate in 30 mL of water. To 0.5 mL of this solution add 3 mL of *p*-dimethylaminobenzaldehyde-ferric chloride test solution: a red-purple color is developed.

- B. Dissolve 0.01 g (0.005~0.014 g) of morantel citrate monohydrate in 2 mL of water, and add 1 drop of potassium permanganate test solution: the color of the solution disappears within 30 seconds.
- C. To 5 mg (4.5~5.4 mg) of morantel citrate monohydrate add 2 mL of citric acid in acetic anhydride (0.5 → 100), heat in a water bath: a red to red-purple color is developed.
- D. Dissolve 0.01 g (0.005~0.014 g) of morantel citrate monohydrate in 0.01 mol/L hydrochloric acid-methanol test solution to make 1,000 mL. Determine the absorption spectrum of this solution: it exhibits a maximum between 322 nm and 327 nm.
- E. Dissolve 0.02 g (0.015~0.024 g) of morantel citrate monohydrate in 4 mL of water, neutralize this solution with dilute sodium hydroxide: responds to the Qualitative Tests for citrate.

Purity:

- A. Clarity and color of solution: Dissolve 0.5 g (0.45~0.54 g) of morantel citrate monohydrate in 10 mL of methanol: the solution should be yellow, clear.
- B. cis-Isomer: Dissolve 0.2 g (0.15~0.24 g) of morantel citrate monohydrate in methanol, transfer to a 10 mL brown volumetric flask, add methanol to make exactly 10 mL, use this solution as the sample solution. Measure 1 mL of this solution with transfer pipet, transfer to a 10 mL brown volumetric flask, add methanol to make 10 mL, and use this solution as the control solution for cis-Isomer. Separately, weigh 0.1 g (0.05~0.14 g) of anhydrous citric acid, add methanol to dissolve, transfer to a 100 mL of volumetric flask, add methanol to make exactly 100 mL, use this solution as citric acid solution. Spot 5 µL each of the sample solution, the control solution for cis-isomer and citric acid solution on the plate prepared with silica gel for thin layer chromatography. Develop the plate in a dark place with a mixture of methyl isobutyl ketone, formic acid and water (2:1:1) to a distance of about 10 cm, dry the plate at 100 °C for 15 minutes. Place the plate in a chamber filled with iodine vapor, no spot other than morantel and citric acid from the sample solution, and are not more intense than the spot from the control solution for cis-isomer (not more than 1 %).

Water: 3.7~4.7 % (0.5 g)

Residue on ignition: Not more than 0.15 % (1.0 g).

Retinol Acetate, for thin-layer chromatography: $C_{22}H_{32}O_2$ [Retinol acetate for thin-layer chromatography, Japanese Pharmacopoeia reference standard]

Cyanocobamin: $C_{63}H_{88}CoN_{14}O_{14}P$ [Japanese Pharmacopoeia reference standard]

Sulfaquinoxaline: $C_{14}H_{12}N_4O_2S$ Sulfaquinoxaline is prepared by weighing crude material for manufacturing sulfaquinoxaline, recrystallizing with ethanol.

Physical and chemical properties:

- A. Pale yellow fine crystals, having no odor.
- B. Slightly soluble in acetone, very slightly soluble in ethanol, and practically insoluble in water.
- C. Sulfaquinoxaline dissolves in 1 mol/L sodium hydroxide test solution and sodium carbonate test solution.
- D. It is colored to dark color by light.

Identification: Weigh 0.02 g (0.015~0.024 g) of sulfaquinoxaline, add 5 mL of water, drop 1 mol/L sodium hydroxide test solution while stirring to dissolve, add 2 to 3 drops of copper sulfate test solution: a yellow-green precipitate is formed.

Purity:

- A. Melting point: Melting point of sulfaquinoxaline should be between 245 and 247 °C (with decomposition).
- B. Relate substances: Weigh 0.10 g (0.095~0.104 g) of sulfaquinoxaline, add 20 mL of acetone, warm and dissolve, spot 20 µL of this solution on the plate prepared with silica gel for thin layer chromatography. Develop the plate with a mixture of isopropanol, butyl acetate, water, and strong ammonium solution (10:6:3:1) to a distance of about 10 cm, air-dry the plate. To 10 g (9.5~10.4 g) of sodium nitrite and 20 mL of hydrochloric acid, evolve the nitrous acid vapors in a container, allow the plate to stand in the container. Separately, 0.05 g (0.045~0.054 g) of chromotropic acid and 40 g (39.5~40.4 g) of sodium acetate in water to make 100 mL. Spray evenly this solution to the plate: only one red-purple spot with an R_f value of about 0.5 appears, and no spot other than the principal spot.

Loss on drying: Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on ignition: Not more than 0.10 % (1 g).

Note: Preserve in tight, light-resistant containers.

Tyrosine: C₉H₁₁NO₃ [Japanese Pharmacopoeia reference standard]

Nicarbazin: C₁₃H₁₀N₄O₅·C₆H₈N₂O Nicarbazin is prepared by weighing crude material for manufacturing nicarbazin, recrystallizing with a mixture of dioxane, acetone and water (5:4:1).

Physical and chemical properties:

- A. A yellowish powder, odorless, or having a slightly characteristic odor.
- B. Melting point about 260 °C (with decomposition)

Identification:

- A. To 15 mL of nicarbazin in anhydrous ethanol (1 → 15,000) add 5 mL of sulfanilic acid test solution (2 → 125) and freshly prepared 5 mL of sodium nitrite test solution (1 → 100), stopper tightly, heat at 65 °C for 10 minutes: a red color is developed.

B. To 15 mL of nicarbazin in anhydrous ethanol (1 → 15,000) add 5 mL of potassium hydroxide in ethanol (1 → 100): a yellow color is developed.

Purity: Relate substances: Weigh 10 mg (9.5~10.4 mg) of nicarbazin, dissolve in 1.0 mL of dimethylformamide, spot 5 µL of this solution on the plate prepared with cellulose for thin-layer chromatography (with fluorescent). Develop the plate with a mixture of chloroform, diethyl ether and methanol (10:9:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): only one spot with an R_f value of about 0.3 appears, and no spot other than the principal spot.

Loss on drying: Not more than 0.5 % (1 g, in vacuum, phosphorus (V) oxide, 110 °C, 1 hour).

Residue on ignition: Not more than 0.10 % (1 g).

Note: Preserve in tight, light-resistant containers.

p-Aminobenzoyl glutamic acid: C₁₁H₁₄N₂O₃ [Japanese Pharmacopoeia reference standard]

Retinol palmitate, for thin-layer chromatography: C₃₆H₆₀O₂ [Retinol palmitate for thin layer chromatography, Japanese Pharmacopoeia reference standard]

Folic acid: C₁₉H₁₉N₇O₆ [Japanese Pharmacopoeia reference standard]

Riboflavin: C₁₇H₂₀N₄O₆ [Japanese Pharmacopoeia reference standard]

(2) Reagents · Test Solutions

Reagents are the substances used in the tests of feed additives. The reagents that are described as “Standard Reagent for volumetric analysis”, “Special grade”, “First grade”, “Arsenic-free”, etc. in square brackets meet the corresponding requirements of the Japan Industrial Standards (JIS).

The tests for them are performed according to the test methods of JIS. In the case where the reagent name in the “Ministerial Ordinance on the Specifications and Standards of Feeds and Feed Additives” differs from that of JIS, the JIS name is given in the brackets. The reagent for which a monograph’s title is given in the brackets meet the requirements of the corresponding monograph. In the case of the reagent that are described merely as test items, the corresponding test method of “Ministerial Ordinance on the Specifications and Standards of Feeds and Feed Additives” is applied.

Test solutions are the solutions prepared for use in the test of feed additives.

Absorbent cotton: [Japanese Pharmacopoeia]

Absorbing solution for hydrogen arsenide: Dissolve 0.50 g (0.495~0.504 g) of silver diethyldithiocarbamate in pyridine to make 100 mL. Preserve in a glass-stoppered bottle protected from light and in a cool place.

Acetic acid: CH₃COOH [Japanese Pharmacopoeia] (5 mol/L)

Acetic acid test solution, 6 mol/L: To 36 g (35.5~36.4 g) of glacial acetic acid add water to make 100 mL.

Acetic acid test solution, 0.2 mol/L: To 12 g (11.5~12.4 g) of glacial acetic acid add water to make 1,000 mL.

Acetic acid, anhydrous: Specified in an item of anhydrous acetic acid.

Acetic acid, dilute: Dilute 6 g (5.5~6.4 g) of glacial acetic acid to make 100 mL (1 mol/L).

Acetic acid, glacial: CH₃COOH [Acetic acid (Glacial acetic acid) (99~100 %), Special grade]

Acetic acid, glacial, for nonaqueous titration: Add 5 g (4.5~5.4 g) of chromium trioxide to 1 L of glacial acetic acid, and allow to stand for overnight. Filter and distill. Add 20 g (19.5~20.4 g) of acetic anhydride to the fraction distilling at more than 115 °C, and distill again. Collect the fraction distilling at 117~118 °C.

Acetic acid-ammonium acetate buffer solution, pH 4.8: Dissolve 77 g (76.5~77.4 g) of ammonium acetate in 200 mL of water, add 57 mL of glacial acetic acid, then add water to make 1,000 mL.

Acetic acid-ethanol test solution: To 1 mL of glacial acetic acid add 9 mL of water and 10 mL of ethanol, and mix.

Acetic acid-hydrochloric acid buffer solution, 0.1 mol/L, for digestion test: To 0.1 mol/L of sodium acetate add 0.1 mol/L of hydrochloric acid test solution to adjust the specified pH.

Acetic acid-hydrochloric acid buffer solution, 0.25 mol/L, for digestion test:
Dissolve 34.02 g (34.015~34.024 g) of sodium acetate in water, adjust the pH to 5.5 with hydrochloric acid, and add water to make 1,000 mL.

Acetic acid-lithium acetate buffer solution: To 407 g (406.5~407.4 g) of lithium acetate and 200 mL of acetic acid add water to make 2,000 mL.

Acetic acid-sodium acetate buffer solution, 1 mol/L, for digestion test: To sodium acetate test solution add dilute acetic acid to adjust the specified pH.

Acetic acid-sodium acetate buffer solution, 0.2 mol/L, for digestion test: To 0.2 mol/L sodium acetate test solution add 0.2 mol/L acetic acid test solution to adjust the specified pH.

Acetic acid-sodium acetate buffer solution (0.1 mol/L), for digestion test: To 0.2 mol/L sodium acetate test solution add 0.2 mol/L acetic acid test solution to adjust the specified pH, and add water to make exactly 2 times volumes.

Acetic acid-sodium acetate buffer solution, 0.01 mol/L, for digestion test: To 0.2 mol/L sodium acetate test solution add 0.2 mol/L acetic acid test solution to adjust the specified pH, and add water to make exactly 20 times volumes.

Acetic acid and sodium acetate buffer solution, 0.005 mol/L, for digestion test: To 0.2 mol/L sodium acetate test solution add 0.2 mol/L acetic acid test solution to adjust the specified pH, and add water to make exactly 40 times volumes.

Acetic acid-sodium acetate buffer solution, pH 5.0: To 140 mL of sodium acetate test solution add 60 mL of dilute acetic acid, and add water to make 1,000 mL.

Acetic anhydride: $(\text{CH}_3\text{CO})_2\text{O}$ [Special grade]

Acetic anhydride-pyridine test solution: To 25 g (24.5~25.4 g) of acetic anhydride add anhydride pyridine to make 100 mL. Prepare before use.

Acetone: CH_3COCH_3 [Special grade] Acetone for Vitamin D assay is specified in an item of Vitamin D assay in the paragraph of General tests.

Acetone for non-aqueous titration: To acetone add potassium phosphate permanganate in small portions, and shake. When the mixture keeps its purple color after standing for 2 to 3 days, distil, and dehydrate with freshly ignited anhydrous potassium carbonate. Distil by using a fractionating column under protein from moisture, and collect the fraction distilling at 56 °C.

Acetonitrile: CH_3CN [Special grade]

Acetonitrile for liquid chromatography : CH_3CN Clear, colorless liquid, miscible with water. Determine the absorbance at a layer length of 10 mm using water as the control solution: It shall be 0.07 or lower at a wavelength of 200 nm, 0.046 or lower at a wavelength of 210 nm, 0.027 or lower at a wavelength of 220 nm, 0.014 or lower at a wavelength of 230 nm, and 0.009 or lower at a wavelength of 240 nm.

Acetylene: C_2H_2 [Dissolved acetylene] Not less than 98 %.

Acidic stannous chloride test solution: Specified in an item of Stannous chloride test solution, acidic.

Acid-potassium chloride test solution: To 250 g (249.5~250.4 g) of potassium chloride add water to make 1,000 mL, and add 8.5 mL of hydrochloric acid.

Activated carbon: [Japanese Pharmacopoeia]

Adenine sulfate: $(\text{C}_5\text{H}_5\text{N}_5)_2\cdot\text{H}_2\text{SO}_4$ White crystals, slightly soluble in water and in ethanol. Clarity and color of solution: To 0.2 g (0.15~0.24 g) of adenine sulfate add 20 mL of water, warm to dissolve: the solution is clear and colorless.

Absorbances: Weigh accurately 0.1 g of adenine sulfate to three decimal places, record the value, dissolve in potassium chloride-hydrochloric acid buffer solution to make 10,000 mL. Determine the absorbances of this solution at 262 nm: $E_{1\text{cm}}^{1\%} = 638\sim 668$.

Water: 6.7~11.1 % (0.3 g)

Residue on ignition: Not more than 0.5 % (10 g).

Agar: [Japanese Pharmacopoeia]

Aldehyde-free ethanol: Specified in an item of Ethanol, aldehyde-free. Aldehyde-free ethanol for vitamin D assay is specified in an item of Vitamin D assay in the paragraph of General tests.

Alizarine red S: $C_{14}H_5O_2(OH)_2SO_3Na \cdot H_2O$ [Alizarine red S (Sodium Alizarin sulfonate), Special grade]

Color change: pH 3.7 (yellow) to pH 5.2 (orange red)

Alizarine red S test solution: Dissolve 0.1 g (0.05~0.14 g) of alizarine red S in water to make 100 mL. Filter if necessary.

Alizarine yellow GG: $C_{13}H_8N_3NaO_5$ [Special grade]

Color change: pH 10.0 (yellow) to pH 12.0 (brown)

Alizarine yellow GG test solution: Dissolve 0.1 g (0.05~0.14 g) of alizarine yellow GG in 100 mL of ethanol, filter if necessary.

Alizarine yellow GG-thymolphthalein test solution: To 10 mL of alizarine yellow GG add 20 mL of thymolphthalein test solution, and mix.

Alkaline blue tetrazolium test solution: Specified in an item of Blue tetrazolium test solution, alkaline.

Alkaline copper test solution A: Dissolve 71 g (70.5~71.4 g) of sodium hydrogen phosphate and 40 g (39.5~40.4 g) of potassium sodium tartrate in 650 mL of water, to this solution add 100 mL of 1 mol/L sodium hydroxide test solution, and add 80 mL of copper sulfate (10 → 100) in small portions while stirring gently. Dissolve 180 g (179.5~180.4 g) of anhydrous sodium carbonate in this solution, add 25 mL of potassium iodate solution (3.6 → 100), and add water to make 1,000 mL. Allow to stand for 2 to 3 days at 25 °C to 35 °C, filter and remove precipitate, preserve at 25 °C to 35 °C.

Alkaline copper test solution B: Dissolve 4.0 g (3.95~4.04 g) of copper sulfate, 24 g (23.5~24.4 g) of anhydrous sodium carbonate, 16 g (15.5~16.4 g) of sodium hydrogen carbonate, 180 g (179.5~180.4 g) of anhydrous sodium sulfate and 12 g (11.5~12.4 g) of potassium sodium tartrate in water to make 900 mL. Boil this solution for 10 minutes, cool, add water to make 1,000 mL, allow to stand for 1 week in tightly stoppered containers, and filter with glass filter (G3). Store protected from light.

Alkaline fuchsin: [Special grade]

Alkaline fuchsin test solution: Dissolve 10 g (9.5~10.4 g) of alkaline fuchsin to 100 mL of ethanol and allow to stand at 37 °C overnight.

Aluminium: Al [Special grade]

Amidol: $(\text{NH}_2)_2\text{C}_6\text{H}_3\text{OH}\cdot 2\text{HCl}$ Pale yellowish brown to greyish yellow-green crystalline powder.

Clarity and color of solution: Dissolve 1.0 g (0.95~1.04 g) of amidol in 20 mL of water: the solution is clear.

Content: Not less than 98 %.

Amidol-disodium bisulfite test solution: Dissolve 0.4 g (0.35~0.44 g) of amidol and 8 g (7.5~8.4 g) of disodium bisulfite in water to make 40 mL Prepared before use.

4-Amino antipyrine: $\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}$ [Special grade]

p-Amino benzoic acid: $\text{NH}_2\text{C}_6\text{H}_4\text{COOH}$ [Special grade]

Aminopyrine: $\text{C}_{13}\text{H}_{17}\text{N}_3\text{O}$ Colorless or white crystals, or white crystalline powder. Odorless, and having a slight bitter taste.

Melting point: 107~109 °C

Loss on drying: Not more than 0.5 % (1 g, silica gel, 4 hours).

Residue on ignition: Not more than 0.10 % (1 g).

Store protected from light.

Ammonia methanol test solution: To 10 mL of strong ammonia water add 190 mL of methanol.

Ammonia test solution: To 400 mL of strong ammonia water add water to make 1,000 mL (10 %).

Ammonia water: [Japanese Pharmacopoeia]

Ammonia water, 25 %: NH_4OH [Ammonia water, Special grade, Specific gravity 0.91]

Ammonia water, strong: NH_4OH [Ammonia water, Special grade, Specific gravity 0.90]

Ammonia-ammonium chloride buffer solution, pH 10.7: Dissolve 67.5 g (67.45~67.54 g) of ammonium chloride, add 570 mL of strong ammonia water, and add water to make 1,000 mL

Ammonium acetate: $\text{CH}_3\text{COONH}_4$ [Special grade]

Ammonium acetate test solution: Dissolve 10 g (9.5~10.4 g) of ammonium acetate in water to make 100 mL.

Ammonium amidosulfate: $\text{NH}_4\text{OSO}_2\text{NH}_2$ [First grade]

Ammonium amidosulfate test solution: Dissolve 1 g (0.5~1.4 g) of ammonium amidosulfate add water to make 40 mL.

Ammonium carbonate: [Special grade]

Ammonium carbonate test solution: To 20 g (19.5~20.4 g) of ammonium carbonate and 20 mL of ammonium test solution add water to make 100 mL

Ammonium chloride: NH_4Cl [Special grade]

Ammonium chloride test solution: Dissolve 10.5 g (10.45~10.54 g) of Ammonium Chloride in water to make 100 mL (2 mol/L).

Ammonium citrate: $C_6H_{14}N_2O_7$ [Diammonium hydrogen citrate, Special grade]

Ammonium iron (II) hexahydrate: $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ [Ferrous ammonium sulfate (Mohr's Salt), Special grade]

Ammonium iron (II) tetracosahydrate: $Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$ [Ferrous ammonium sulfate (iron alum), Special grade]

Ammonium molybdate test solution: Dissolve 21.2 g (21.15~21.24 g) of ammonium molybdate tetrahydrate in water to make 200 mL (10 %). Prepare before use.

Ammonium molybdate tetrahydrate: $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ [Special grade]

Ammonium molybdate-potassium antimonyl tartrate sesquihydrate-ascorbic acid test solution: Dissolve 6 g (5.5~6.4 g) of ammonium molybdate and 0.24 g (0.235~0.244 g) of potassium antimonyl titrate sesquihydrate in 300 mL of water, add 120 mL of sulfuric acid (2 → 3), add water to make 1,000 mL. Separately, dissolve 14.4 g (14.35~14.44 g) of ascorbic acid in 200 mL of water, mix both solutions. Prepare before use.

Ammonium oxalate: $(NH_4)_2C_2O_4 \cdot H_2O$ [Special grade]

Ammonium oxalate test solution: To 3.5 g (3.45~3.54 g) of ammonium oxalate add water to make 100 mL (0.25 mol/L).

Ammonium oxalate test solution, 0.07 mol/L: Dissolve 0.8 g (0.75~0.84 g) of ammonium oxalate in 80 mL of water.

Ammonium persulfate: $(NH_4)_2S_2O_8$ [Special grade]

Ammonium sulfate: $(NH_4)_2SO_4$ [Special grade]

Ammonium sulfate test solution: [Ammonium sulfate solution (colorless), First grade]

Filled full and keep it in small light-resistant containers in well-filled.

Ammonium thiocyanate: NH_4SCN [Special grade]

Ammonium thiocyanate test solution: Dissolve 8 g (7.5~8.4 g) of ammonium thiocyanate in water to make 100 mL (1 mol/L).

Ammonium thiocyanate-cobalt nitrate test solution: Dissolve 17.4 g (17.35~17.44 g) of ammonium thiocyanate and 2.8 g (2.75~2.84 g) of cobalt nitrate in water to make 100 mL

Ammonium vanadate (V): NH_4VO_3 [Special grade]

Amygdalin: $C_{20}H_{27}NO_{11}$ Soluble in water, slightly soluble in ethanol, particularly insoluble in diethyl ether.

pH: The pH of aqueous solution (1 → 100) of amygdalin should be 4.5 to 6.5.

Melting point: 210~222 °C

Optical rotation: $[\alpha]_D^{20} = -39^\circ \sim -43^\circ$

Loss on drying: Not more than 5 %.

Residue on ignition: Not more than 0.1 %.

Amyl alcohol, iso: $(\text{CH}_3)_2\text{CH}_2\text{CH}_2\text{CHOH}$ [Special grade]

Anhydrous citric acid: Specified in an item of Citric acid, anhydrous.

Anhydrous copper sulfate: Specified in an item of Copper sulfate, anhydrous.

Anhydrous disodium hydrogen phosphate: Specified in an item of Disodium hydrogen phosphate, anhydrous.

Anhydrous ethanol: Specified in an item of Ethanol, anhydrous.

Anhydrous potassium carbonate: Specified in an item of Potassium carbonate, anhydrous.

Anhydrous pyridine: Specified in an item of Pyridine, anhydrous.

Anhydrous silicic acid: SiO_2 [Special grade]

Anhydrous sodium acetate: Specified in an item of Sodium acetate, anhydrous.

Anhydrous sodium carbonate: Specified in an item of Sodium carbonate, anhydrous.

Anhydrous sodium hydrogen phosphate for pH determination: Specified in an item of sodium hydrogen phosphate, anhydrous, for pH determination.

Anhydrous sodium sulfate: Specified in an item of Sodium sulfate, anhydrous.

Anhydrous toluene: Specified in an item of Toluene, anhydrous.

Aniline: $\text{C}_6\text{H}_5\text{NH}_2$ [Special grade]

Anthrone: $\text{C}_{14}\text{H}_{10}\text{O}$ [Special grade]

Anthrone test solution: Dissolve 35 mg (34.5~35.4mg) of anthrone in 100 mL of sulfuric acid.

Antibiotic solution for probiotic test: Dissolve 0.025 g (0.0245~0.0254 g) of chloramphenicol in 1 mL of methanol, transfer this solution to 100 mL flask with sterilized water. Add 125,000 units of polymixin B sulfate, then add sterilized water to make 100 mL. Store not exceeding 5 °C, and use within 1 month.

Antimony (III) chloride: SbCl_3 [Special grade]

Antimony (III) chloride test solution: Wash chloroform with an equal volume of water 2 to 3 times, add freshly ignited and cooled potassium carbonate, and allow to stand overnight in a well-closed container protected from light. Separate chloroform layer, and distill it, preferably with protection from light. With this chloroform, wash the surface of Antimony (III) chloride until the rinsing solution becomes clear, add the chloroform to this antimony (III) chloride to make saturated solution, and place in light resistant, glass-stoppered bottles. Prepare before use.

Antimony (III) potassium tartrate: $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot 1/2\text{H}_2\text{O}$ [Potassium antimonyl tartarate, Special grade]

Aqua regia: Add 1 volume of nitric acid to 3 volumes of hydrochloric acid. Prepare before use.

Arabinose: $C_5H_{10}O_5$

Optical rotation: $[\alpha]_D^{20} = -103^\circ \sim -105^\circ$

Heavy metal: Not more than 10 $\mu\text{g/g}$.

Water: Not more than 0.5 %.

Arsenic trioxide (standard reagent): [Standard reagent for volumetric analysis]

Arsenic-free zinc: Specified in an item of Arsenic-free, zinc.

Arsenic-molybdate test solution: To 50 g (49.5~50.4 g) of ammonium molybdate add 900 mL of water, warm to dissolve, cool, pipet 42 mL of sulfuric acid. Then add 50 mL of sodium arsenate heptahydrate test solution and add water to make 1,000 mL. Allow to stand at 37 °C for 24 hours.

Ascorbic acid: $C_6H_8O_6$ [Japanese Pharmacopoeia]

Barbital buffer solution: Dissolve 15 g (14.5~15.4 g) of barbital sodium in 700 mL of water, adjust the pH to 7.6 with dilute hydrochloric acid, and filter.

Barbital sodium: $C_8H_{11}N_2NaO_3$ White, odorless, crystals or crystalline powder, having a bitter taste. Freely soluble in water, slightly soluble in ethanol, and practically insoluble in diethyl ether.

pH: The pH of an aqueous solution of barbital sodium (1 → 200) is between 9.9 and 10.3.

Loss on drying: Not more than 1.0 % (1 g, 105 °C, 4 hours).

Content: Not less than 98.5 %.

Assay: Weigh about 0.5 g of barbital sodium to three decimal places, record the value, previously dried at 105 °C for 4 hours, transfer to a separator, add 20 mL of water to dissolve, add 5 mL of ethanol and 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform. Then extract with three 25 mL portions of chloroform, combine the total extract, wash with two 5 mL portions of water, and extract the washings with 10 mL portions of chloroform. Combine the chloroform extracts, and filter into a conical flask. Wash the filter paper with three 5 mL portions of chloroform, combine the filtrate and the washings, add 10 mL of ethanol, and titrate with 0.1 mol/L potassium hydroxide-ethanol solution until the color of the solution changes from yellow to purple through light blue (indicator: 2 mL of alizarin yellow GG-thymolphthalein test solution). Perform a blank determination in same the manner.

1 mL of 0.1 mol/L potassium hydroxide-ethanol solution = 20.62 mg of $C_8H_{11}N_2NaO_3$

Barium chloride dihydrate: $BaCl_2 \cdot 2H_2O$ [Special grade]

Barium chloride test solution: To 12 g (11.5~12.4 g) of barium chloride dihydrate to make 100 mL (0.5 mol/L).

Barium hydroxide octahydrate: $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ [Special grade] Preserve in tightly stoppered bottles.

Barium oxide: BaO [For dry]

Benzalkonium chloride: Benzalkonium chloride [Japanese Pharmacopoeia]

Benzen for pesticide residue analysis: Benzen, for pesticide residue analysis

Benzene: C_6H_6 [Special grade] Benzene for vitamin D determination is specified in an item of Vitamin D assay in the paragraph of General tests.

Benzene, for pesticide residue testing

Propyl benzoate $\text{C}_6\text{H}_5\text{COOCH}_2\text{CH}_2\text{CH}_3$: Clear and colorless liquid. Contents not less than 98.0%

Propyl benzoate-dimethylformamide TS: 1.0 g (0.95 ~ 1.04 g) of propyl benzoate, add dimethylformamide to dissolve and make 100 mL.

Benzyl benzoate $\text{C}_6\text{H}_5\text{COOCH}_2\text{C}_6\text{H}_5$ [JP]

Neutralization of ethanol: Add two to three drops of phenolphthalein TS to a suitable amount of ethanol, and add a 0.01 mol/L sodium hydroxide solution or a 0.1 mol/L sodium hydroxide solution until a light red color develops. Prepare this solution before use.

Neutralized ethanol: As specified in the section on neutralization of ethanol.

Benzoic acid (standard reagent): $\text{C}_6\text{H}_5\text{COOH}$ [Standard reagent for volumetric analysis]

Bertrand's test solution A: Dissolve 40 g (39.5~40.4 g) of crystals of copper sulfate in water to make 1,000 mL Keep this solution in a glass-stoppered bottle in well-filled.

Bertrand's test solution B: Dissolve 200 g (199.5~200.4 g) of potassium sodium tartrate and 150 g (149.5~150.4 g) of sodium hydroxide in water to make 1,000 mL Preserve with rubber stoppers.

Bertrand's test solution C: Dissolve 50 g (49.5~50.4 g) ferric sulfate (not to reduce potassium permanganate) in an appreciate quantity of water, add 200 mL of sulfuric acid, and add water to make 1,000 mL.

Bertrand's test solution D: Dissolve 5 g (4.5~5.4 g) of potassium permanganate in water to make 1,000 mL.

Standardization: Dissolve 0.25 g (0.245~0.254 g) of ammonium oxalate in 100 mL water, add 2 mL of sulfuric acid, and warm between 60 °C and 70 °C. Titrate potassium permanganate solution. 1 mL of this solution correspond to $\text{Cu } 0.2238/a$ g as titration amount is a mL.

d-Biotin for assay: Specified in an item of d-Biotin, for assay.

d-Biotin, for assay: A crude material for manufacturing d-biotin. Contains not less than 99.0 % of d-biotin ($\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_3\text{S}$) for assay.

Biphenyl $\text{C}_{12}\text{H}_{10}$: White to almost white crystals to crystalline small pieces or powder.

Contents not less than 98.0 %

Sodium bicarbonate buffer solution (pH 9.3): To 21.0 g (20.95 ~ 21.04 g) of sodium bicarbonate, add water to dissolve, adjust the pH to 9.3 with 1 mol/L hydrochloric acid TS, and add water to make 500 mL.

Bismuth sodium trioxide: NaBiO_3 [Special grade]

Bismuth subnitrate: [Japanese Pharmacopoeia]

Blue tetrazolium: $\text{C}_{40}\text{H}_{32}\text{Cl}_2\text{N}_8\text{O}_2$ [3,3'-Dianisole-bis-[4,4'-(3,5-diphenyl)] tetrazorium chloride] Light yellow crystals. Freely soluble in methanol, in ethanol and in chloroform, slightly soluble in water, and practically insoluble in acetone and in diethyl ether.

Melting point: 245 °C (with decomposition)

Molar absorbance coefficient: Not less than 60,000 (252 nm, methanol).

Blue tetrazolium test solution, alkaline: To 1 volume of a solution of blue tetrazolium in methanol (1 → 200) add 3 volumes of a solution of sodium hydroxide in methanol (3 → 25). Prepare before use.

Borax: Specified in an item of Sodium borate decahydrate.

Boric acid: H_3BO_3 [Special grade]

Bovine serum albumen: White to light yellow-brown powder albumin obtained from bovine serum by fractionation by alcohol.

Purity: Not less than 96 %.

Bromine: Br_2 [Special grade] Bromine is a dark red-brown liquid. It has a strong irritating property and a caustic nature. It is slightly soluble in water, freely soluble in ethanol and in diethyl ether.

Bromine test solution: Prepare by saturating water with bromine as follows: transfer 2 to 3 mL of bromine to a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum, add 100 mL of cold water, insert the stopper and shake. Protect from light, preferably in a cold place.

Bromocresol green: $\text{C}_{21}\text{H}_{14}\text{Br}_4\text{O}_5\text{S}$ [Special grade] Color change: pH 3.8 (yellow) to pH 5.4 (blue).

Bromocresol green test solution: Dissolve 0.05 g (0.045~0.054 g) of bromocresol green in 100 mL of ethanol. Filter if necessary.

Bromocresol green-methyl orange test solution: To 80 mL of bromocresol green-ethanol solution (1 → 1,000) add 20 mL of methyl orange-ethanol solution (1 → 1,000).

Bromocresol green-methyl red test solution: Dissolve 0.15 g (0.145~0.154 g) of bromocresol green and 0.1 g (0.05~0.14 g) of methyl red in 180 mL of anhydrous ethanol, and add water to make 200 mL.

Bromocresol purple test solution: Dissolve 1.6 g (1.55~1.64 g) bromocresol purple in 100 mL of ethanol, and adjust the pH to 7.5 (blue-purple color) with 2 mol/L sodium hydroxide.

Bromophenol blue: $C_{19}H_{10}Br_4O_5S$ [Special grade] Color change: pH 3.0 (yellow) to 4.6 (blue purple).

Bromophenol blue test solution: Dissolve 0.1 g (0.05~0.14 g) of bromophenol blue in dilute ethanol to make 100 mL. Filter if necessary.

N-Bromosuccinimide: $(CH_2CO)_2NBr$ [Special grade] A white crystalline powder, soluble in acetone, slightly soluble in water and in glacial acetic acid, very slightly soluble in carbon tetrachloride.

Melting point: Around 175 °C.

Bromothymol blue: $C_{27}H_{28}Br_2O_5S$ [Special grade] Color change: pH 6.0 (yellow) to 7.6 (blue).

Bromothymol blue test solution: Dissolve 0.1 g (0.05~0.14 g) of bromothymol blue in 100 mL of dilute ethanol. Filter if necessary.

Bromothymol blue-sodium carbonate test solution: Dissolve 0.15 g (0.145~0.154 g) of bromothymol blue and 0.15 g (0.145~0.154 g) of anhydrous sodium carbonate in water to make 100 mL.

n-Butanol: $CH_3(CH_2)_2CH_2OH$ [n-Butyl alcohol (n-butanol), Special grade]

Butanol, iso: $(CH_3)_2CHCH_2OH$ [Isobutyl alcohol (isobutanol), Special grade]

Butanol, sec: $CH_3CH_2CH(OH)CH_3$ [Secondary butyl alcohol, First grade]

n-Butyl acetate: $CH_3COOCH_2CH_2CH_2CH_3$ [Special grade]

2-Butyl parahydroxybenzoate: $HOC_6H_4CO_2CH_2CH(CH_3)_2$ White to light yellowish white crystalline powder, odorless or slightly characteristic odor.

Clarity and color of solution: Dissolve 0.5 g (0.45~0.54 g) of butyl parahydroxybenzoate in 10 mL of acetonitrile: the solution is colorless to pale yellow and clear and no foreign inorganic matters.

Melting point: 59~61 °C

Butyl parahydroxybenzoate: $HOC_6H_4CO_2CH_2CH_2CH_2CH_3$ [Japanese Pharmacopoeia]

Butyric acid: $CH_3CH_2CH_2COOH$ [Special grade]

Caffeine: $C_8H_{10}N_4O_2 \cdot H_2O$ [Japanese Pharmacopoeia]

Calcium chloride dihydrate: $CaCl_2 \cdot 2H_2O$ [Calcium chloride (dihydrate), Special grade]

Calcium chloride test solution: To 7.5 g (7.45~7.54 g) of calcium chloride add water to make 100 mL (0.5 mol/L)

Calcium chloride, for water determination: $CaCl_2$ [For water determination, grade 1~3]

Calcium hydroxide: $Ca(OH)_2$ [First grade]

Calcium hydroxide for pH determination: [Calcium hydroxide, First grade] Use the saturated calcium hydroxide solution (pH 12.45 at 25 °C) obtained at 23 °C to 27 °C.

Calcium hydroxide for pH determination: Specified in an item of calcium hydroxide, for pH measurement.

Calcium hydroxide test solution: To 3 g (2.5~3.4 g) of calcium hydroxide add 1,000 mL of cold distilled water, and occasionally shake the mixture vigorously for 1 hour. Allow to stand, and use the supernatant liquid (0.04 mol/L).

Calcium pantothenate: $\text{Ca}(\text{C}_9\text{H}_{16}\text{NO}_3)_2$ [Japanese Pharmacopoeia]

Carbon dioxide: CO_2 [Japanese Pharmacopoeia]

Carbon disulfide: CS_2 [Special grade]

Carbon tetrachloride: CCl_4 [Special grade]

Casamino acid: Powder by proper treatment after hydrolyze vitamin-free casein after hydrolyzing by hydrochloric acid. This is white to light yellow powder, has specific odor.

Casein peptone: Specified in an item of Casein, peptone.

Casein test solution: To 0.1 g (0.05~0.14 g) of milk casein add 30 mL of water, disperse the casein well, add 1 mL of Sodium Hydroxide (1 → 10) to dissolve, and add water to make 50 mL. Prepare before use.

Casein, milk: Casein occurs as a white or almost white powder.

Clarity and color of solution: Add 100 mL of 0.05 mol/L lactic acid test solution to 0.6 g (0.55~0.64 g) of milk casein: the solution has a faint, or is clear.

Absorbance: Weigh 0.6 g of milk casein to three decimal places, record the value, and dissolve in 0.05 mol/L of lactic acid test solution to make 100 mL. Determine the absorbances of this solution in 400 nm $E_{1\text{ cm}}^{0.6\%} \leq 0.4$.

Catechol: $\text{C}_6\text{H}_4(\text{OH})_2$ [First grade]

Cellobiose: $\text{C}_{12}\text{H}_{22}\text{O}_{11}$

Optical rotation: $[\alpha]_D^{20} = +34^\circ \sim +35^\circ$

Heavy metal: Not more than 10 µg/g.

Water: Not more than 0.5 %.

Cellulose powder for thin-layer chromatography: Cellulose powder prepared for thin-layer chromatography, high quality.

Cellulose powder for thin-layer chromatography (with fluorescent indicator): To cellulose powder for thin-layer chromatography add fluorescent indicator.

Cellulose powder for thin-layer chromatography (with fluorescent indicator): Specified in an item of Cellulose powder (with fluorescent indicator), for thin-layer chromatography.

Ceric ammonium sulfate tetrahydrate: $\text{Ce}(\text{SO}_4)_2 \cdot 2(\text{NH}_4)_2\text{SO}_4 \cdot 4\text{H}_2\text{O}$ [Special grade]

Chloramine: $C_7H_7ClNNaO_2S \cdot 3H_2O$ [Chloramine T, Special grade]

Chloramine test solution: Dissolve 1 g (0.5~1.4 g) of chloramine in water to make 100 mL.

Prepare before use.

Chloramphenicol: $C_{11}H_{12}Cl_2N_2O_5$ [Japanese Pharmacopoeia]

Chloride: Proceed with 0.20 g (0.195~0.204 g) of iron (III) perchloride according to the Chloride, and perform the test: the amount of chloride is not more than that of equivalent to 0.40 mL of 0.001 mol/L hydrochloric acid (not more than 0.071 %).

Chlorine: Cl_2 A yellow green gas, having a suffocating odor. It is heavier than air and, dissolves in water. Prepared from chlorinated lime with hydrochloric Acid. Chlorine from a metal cylinder may be used.

Chloroform: $CHCl_3$ [Special grade]

Chloroform, ethanol-free: Mix 20 mL of chloroform and 20 mL of water, gently shake for 3 minutes, separate the chloroform layer, wash the layer again with two 20 mL of portions water, and filter it through dry filter paper. To the filtrate add 5 g (4.5~5.4 g) of anhydrous sodium sulfate, shake well for 5 minutes, allow the mixture to stand for 2 hours, and filter through dry paper. Prepare before use.

***p*-Chlorophenol:** ClC_6H_4OH *p*-Chlorophenol occurs as white or slightly red crystals or crystalline masses, has specific odor. It is very soluble in ethanol, in chloroform, in diethyl ether and in glycerol, sparingly soluble in water.

Melting Point: Around 43 °C.

Content: Not less than 99 %.

Assay: Weigh 0.2 g of *p*-chlorophenol to three decimal places, record the value, add water to dissolve, transfer to a 100 mL volumetric flask, and add water to make exactly 100 mL.

Measure exactly 25 mL of this solution with transfer pipet, transfer into an iodine pot, add exactly 20 mL of 0.05 mol/L bromine solution with transfer pipet, and then 5 mL of hydrochloric acid, stopper within 30 seconds, shake occasionally for 30 minutes, and allow to stand for 15 minutes. Add 5 mL of potassium iodide (1 → 5), stopper within 30 seconds, shake well, and titrate with 0.1 mol/L sodium thiosulfate. (Indicator: 1 mL of starch test solution). Perform a blank determination in the same manner.

1 mL of bromine solution (0.05 mol/L) = 3.214 mg of C_6H_5ClO

Chromium (VI) oxide: CrO_3 [Chromium trioxide (chromic anhydride), Special grade]

Chromotropic acid: $(HO)_2C_{10}H_4(SO_3Na)_2$ [Chromotropic acid (disodium salt), Special grade] Store protected from light.

Chromotropic acid test solution: Dissolve 0.05g (0.045~0.054 g) of Chromotropic acid in the solution prepared by cautiously adding 68 mL of sulfuric acid to 30 mL of water, cooling then adding water to make 100 mL. Store protected from light.

Citrate buffer solution: Dissolve 21.0 g (20.95~21.04 g) of citric acid and 8.4 g (8.35~8.44 g) of sodium hydroxide in 700 mL of water, adjust the pH to 1.3 with hydrochloric acid, add water to make 1,000 mL.

Citrate buffer solution, for digestion test: To 0.1 mol/L hydrochloric acid test solution add 0.1 mol/L disodium citrate to adjust the specified pH.

0.2 mol/L Citrate buffer solution, pH 5.2: Dissolve 59.6 g (59.55~59.64 g) of sodium citrate and 21.8 g (21.75~21.84 g) of citric acid in 750 mL of water, if necessary use citric acid solution (1 → 50) or sodium citrate solution (1 → 100) to adjust the pH to 5.2, then add water to make 1,000 mL.

Citrate test solution, 1 mol/L: Dissolve 12 g (11.5~12.4 g) of citrate in water to make 100 mL

Citric acid: $C_6H_8O_7 \cdot H_2O$ [Special grade]

Citric acid, anhydrous: $C_6H_8O_7$ [Japanese Pharmacopoeia]

Cobalt (II) nitrate hexahydrate: $Co(NO_3)_2 \cdot 6H_2O$ [Special grade]

Cobalt (II) sulfate heptahydrate: $CoSO_4 \cdot 7H_2O$ [Special grade]

Concentrated potassium iodide test solution: Specified in an item of Potassium iodide, concentrated.

Copper (II) chloride dihydrate: $CuCl_2 \cdot 2H_2O$ [Special grade]

Copper (II) sulfate pentahydrate: $CuSO_4 \cdot 5H_2O$ [Special grade]

Copper (II) sulfate, anhydrous: $CuSO_4$ [Copper sulfate (anhydrous), First grade]

Copper sulfate test solution: Dissolve 12.5 g (12.45~12.54 g) of copper sulfate in water to make 100 mL (0.5 mol/L).

Copper sulfate-ammonium test solution: Dissolve 0.4 g (0.35~0.44 g) of copper sulfate in 50 mL of a mixture (2:3) of ammonium test solution and citric acid solution (1 → 5).

m-Cresol: $CH_3C_6H_4OH$ [First grade]

p-Cresol: $C_6H_4(OH)CH_3$ [Special grade]

Crystal violet: [Special grade]

Crystal violet-glacial acetic acid test solution: Dissolve 50 mg (49.5~50.4 mg) of crystal violet in 100 mL of glacial acetic acid.

Cyanogen bromide test solution: To 100 mL of ice-cold water add 1 mL of bromine, shake vigorously, and add ice-cold potassium cyanide test solution dropwise until the color of bromine just disappears. Prepare this test solution in a draft chamber before use. On handling this solution, be careful not to inhale its vapors, which are very toxic.

Cyanogen bromide test solution, for dibenzoyl thiamine assay: To 100 mL of ice-cold water add 2 mL of bromine, shake vigorously, and add ice-cold potassium thiocyanate test solution dropwise until the color of bromine just disappears. Prepare this test solution in

a draft chamber before use and store in a cool place. Use within one month. On handling this solution, be careful not to inhale its vapors, which are very toxic.

Cyanogen bromide test solution for dibenzoyl thiamine assay: Specified in an item of Cyanogen bromide test solution, for dibenzoyl thiamine assay.

β -Cyclodextrin: (C₆H₁₀O₅)₇ β -Cyclodextrin occurs as white crystalline powder.

Loss on drying: Not more than 12.0 % (1 g, 105 °C, 3 hours).

Residue on ignition: Not more than 8.2 % (1 g).

β -Cyclodextrin buffer solution: To 5.5 g (5.45~5.54 g) of β -cyclodextrin add No. 3 buffer solution described in Microbial assay for antibiotics in the paragraph of General tests to make 1,000 mL.

Cyclohexane: C₆H₁₂ [Special grade]

Cyclohexanone: C₆H₁₀O [Special grade]

Cysteine hydrochloride: HSCH₂CH(NH₂)COOH·HCl [Special grade]

L-Cysteine hydrochloride monohydrate: HSCH₂CH(NH₂)COOH·HCl·H₂O [Special grade]

L-Cystine: HOOC(NH₂)CHCH₂SSCH₂CH(NH₂)COOH [Special grade]

DEAE-sephadex A-25 for chromatography: Specified in an item of DEAE-sephadex A-25, for chromatography.

DEAE-sephadex A-25, for chromatography: DEAE-sephadex for chromatography (water regain 2.5).

Diatomaceous earth for chromatography: Specified in an item of Diatomaceous earth and for chromatography.

Diazobenzenesulfonic acid test solution: Weigh 0.9 g (0.85~0.94 g) of sulfanilic acid, previously dried at 105 °C for 3 hours, dissolve it in 10 mL of diluted hydrochloric acid by heating, and add water to make 100 mL. Measure 3.0 mL of this solution, add 25 mL of sodium nitrite test solution, and allow to stand for 5 minutes while cooling with ice. Then add 5 mL of sodium nitrite test solution, and water to make 100 mL, allow to stand in ice water for 15 minutes. Prepare before use.

2,6-dibromoquinone chlorimide: O:C₆H₂Br₂:NCl [Special grade]

2,6-Dichlor phenol indophenol sodium test solution for titration: Specified in an item of 2,6-Dichlor phenol indophenol sodium test solution, for titration.

2,6-Dichloroindophenol sodium: C₁₂H₆Cl₂NNaO₂ [Special grade]

2,6-Dichloroindophenol sodium test solution: To 0.1 g (0.05~0.14 g) of 2,6-dichloroindophenol sodium add 100 mL of water, warm, and filter. Use within 3 days.

2,6-Dichloroindophenol sodium test solution, for titration: Dissolve 0.042 g (0.0415~0.0424 g) of sodium hydrogen carbonate in 50 mL of water, then dissolve 0.05 g

(0.045~0.054 g) of 2,6-dichloroindophenol sodium, add water to make 200 mL, and filter.

Prepare before use.

Dichloromethane: CH_2Cl_2 [Special grade]

Diethyl ether: $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ [Ethyl Ether, Special grade]

N,N-Diethyl-N'-1-naphthylethylenediamine oxalate: $\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_4 \cdot 1/2\text{H}_2\text{O}$ [N-(1-Naphthyl)-N'-diethylethylenediamine oxalate, Special grade] Store protected from light .

N,N-Diethyl-N'-1-naphthylethylenediamine oxalate test solution: Dissolve 1 g (0.5~1.4 g) of N,N-diethyl-N'-1-naphthylethylenediamine oxalate in water to make 1,000 mL.

Digest of serum: A yellow powder.

Loss on drying: Not more than 5 % (1 g, 85 °C, 1 hour).

Solubility: Adjust the pH at 7.0 of the solution (1 → 100) and sterilize in an autoclave at 121 °C for 15 minutes: no insoluble substance in the solution.

pH: Sterilize this solution (1 → 100) in an autoclave at 121 °C for 15 minutes: the pH of this solution should be 5.5 to 7.0.

Digitonin: $\text{C}_{55}\text{H}_{90}\text{O}_{29}$ Digitonin occurs as white crystalline powder and slightly soluble in water. It dissolves in warm ethanol and in glacial acetic acid, and does not dissolve in chloroform and in diethyl ether.

Clarity and color of solution: Dissolve 0.5 g (0.45~0.54 g) of digitonin in 20 mL warm ethanol: the solution is clear and colorless.

Melting point: Around 230 °C (with decomposition).

Optical rotation: $[\alpha]_D^{20} = -47^\circ$ to -49° (1 g, 75 % acetic acid, 10 mL, 100 mm).

Loss on drying: Not more than 6 % (105 °C).

Residue on ignition: Not more than 0.3 %.

Dilute 2,4-dinitrochlorobenzene test solution: Specified in an item of 2,4-Dinitrochlorobenzene test solution, dilute.

Dilute acetic acid: Specified in an item of Acetic acid, dilute.

Dilute ethanol: Specified in an item of Ethanol, dilute.

Dilute hydrochloric acid: Specified in an item of Hydrochloric acid, dilute.

Dilute iron (III) chloride test solution: Specified in an item of Iron (III) chloride, dilute.

Dilute nitric acid: Specified in an item of Nitric acid, dilute.

Dilute sodium hydroxide test solution: Specified in an item of Sodium hydroxide test solution, dilute.

Dilute sodium hydroxide-ethanol test solution: Specified in an item of Sodium hydroxide-ethanol test solution, dilute.

Dilute sulfuric acid: Specified in an item of Sulfuric acid, dilute.

Dimethylamine hydrochloride: $(\text{CH}_3)_2\text{NH}\cdot\text{HCl}$ White crystals, it is deliquescent, and is very soluble in water.

Melting point: 170~172 °C

***p*-Dimethylaminobenzaldehyde:** $(\text{CH}_3)_2\text{N}\cdot\text{C}_6\text{H}_4\text{CHO}$ [Special grade]

***p*-Dimethylaminobenzaldehyde-ethanol-sulfuric acid test solution:** Dissolve 1.5g (1.45~1.54 g) of *p*-dimethylaminobenzaldehyde in 50 mL of anhydrous ethanol, add 0.5 mL of sulfuric acid, and add anhydrous ethanol to make 100 mL Prepare before use.

***p*-Dimethylaminobenzaldehyde-ferric chloride test solution:** Dissolve 125 mg (124.5~125.4 mg) of *p*-dimethylaminobenzaldehyde in a cold mixture of 65 mL of sulfuric acid and 35 mL of water, then add 0.05 mL of ferric chloride test solution. Use within 7 days.

***p*-Dimethylaminocinnamaldehyde:** $\text{C}_{11}\text{H}_{13}\text{NO}$ Orange, crystals or crystalline powder, having a characteristic odor. Freely soluble in diluted hydrochloric acid, sparingly soluble in ethanol and diethyl ether, and practically insoluble in water.

Clarity and color of solution: Dissolve 0.2 g (0.15~0.24 g) of *p*-dimethylaminocinnamaldehyde in 20 mL of ethanol: the solution is clear.

Melting Point: 140~142 °C

Loss on drying: Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on ignition: Not more than 0.10 % (1 g).

Nitrogen content: 7.8~8.1 % (105 °C, 2 hours, After drying, Nitrogen determination).

***p*-Dimethylaminocinnamaldehyde test solution:** Before use, add 1 mL of glacial acetic acid to 10 mL of a solution of *p*-dimethylaminocinnamaldehyde (1 → 2,000) in ethanol.

Dimethylformamide: $\text{HCON}(\text{CH}_3)_2$ [N,N-dimethylformamide, Special grade]

Dimethylsulfoxide: CH_3SOCH_3 [Special grade]

3,5-Dinitrobenzyl chloride: $(\text{NO}_2)_2\text{C}_6\text{H}_3\text{COCl}$ [Special grade]

2,4-Dinitrochlorobenzene: $\text{C}_6\text{H}_3(\text{NO}_2)_2\text{Cl}$ [Special grade]

2,4-Dinitrochlorobenzene test solution: Dissolve 0.01 g (0.005~0.014 g) of 2,4-dinitrochlorobenzene in benzene for pesticide residue analysis, transfer to a 100 mL volumetric flask, add benzene for pesticide residue analysis to make exactly 100 mL Measure 1 mL of this solution with transfer pipet, place in a 100 mL volumetric flask, add benzene for pesticide residue analysis to make exactly 100 mL

2,4-Dinitrochlorobenzene test solution, dilute: Measure 10 mL of 2,4-dinitrochlorobenzene test solution with transfer pipet, transfer to a 100 mL volumetric flask, and add benzene for pesticide residue to make exactly 100 mL

3,5-Dinitrosalicylic acid: $(\text{NO}_2)_2\text{C}_6\text{H}_2(\text{OH})\text{COOH}$ 3,5-Dinitrosalicylic acid occurs as pale yellow to pale yellow-brown powder or crystalline powder. It is freely soluble in ethanol and in acetone, slightly soluble in water.

Clarity and color of solution: To 1 g (0.5~1.4 g) of 3,5-dinitrosalicylic acid add 20 mL of ethanol: the solution is clear.

Melting point: 171~175 °C

Identification: Weigh 1 mg (0.5~1.4 mg) of 3,5-dinitrosalicylic acid, determine the infrared absorption spectrum of it as directed in the potassium bromide disk method under Infrared spectrophotometry, it exhibits absorption at the wave numbers of about 3,100 cm^{-1} , 1,680 cm^{-1} , 1,600 cm^{-1} , 1,540 cm^{-1} , 1,340 cm^{-1} , 1,220 cm^{-1} , 1,160 cm^{-1} , 1,090 cm^{-1} , 900 cm^{-1} , 810 cm^{-1} , 740 cm^{-1} and 710 cm^{-1} .

Content: Not less than 98 %.

Assay: Weigh 0.4 g of 3,5-dinitrosalicylic acid to three decimal places, record the value, add 10 mL of ethanol and 20 mL of water, and titrate with 0.1 mol/L sodium hydroxide (the potentiometric titration).

1 mL of 0.1 mol/L sodium hydroxide = 0.02281 g of $(\text{NO}_2)_2\text{C}_6\text{H}_2(\text{OH})\text{COOH}$

3,5-Dinitrosalicylic acid test solution: To 20.0 g (19.95~20.04 g) of 3,5-dinitrosalicylic acid add 800 mL of water, and suspend. To this solution add 300 mL of sodium hydroxide test solution for digestion test in small portions while stirring. Warm this solution in a water bath at not exceeding 48 °C, and stir until it becomes clear. To this solution add 600 g (599.5~600.4 g) of sodium potassium tartrate in small portions, and stir until it becomes clear. If necessary, warm this solution in a water bath not exceeding 48 °C, and stir. Then, add water to make 2,000 mL, filter with glass filter (G3), and preserve in light-resistant containers. Use within 180 days.

1,4-Dioxane: $\text{C}_4\text{H}_8\text{O}_2$ [Special grade]

2,7-Dioxynaphthalene: $\text{C}_{10}\text{H}_6(\text{OH})_2$ (2,7-Dihydroxynaphthalene) 2,7-Dioxynaphthalene occurs white needle crystals or crystalline powder. It is freely soluble in ethanol and in diethyl ether, and slightly soluble in water.

Melting point: 190 °C

2,7-Dioxynaphthalene test solution: Dissolve 0.025 g (0.0245~0.0254 g) of 2,7-dihydroxynaphthalene in 1,000 mL of methanol, to 90 mL of this solution add 5 mL of potassium ferricyanide solution (1 → 500) and 5 mL of potassium cyanide solution (1 → 100), mix, and allow to stand for 30 minutes. Separately, to 15 mL of sodium hydroxide solution (2.25 → 200) add methanol to make 200 mL. Add 100 mL of this solution to the above. Filter before use, and use within 75 minutes.

Diphenylamine: $(\text{C}_6\text{H}_5)_2\text{NH}$ [Special grade]

Diphenylamine test solution: Dissolve 1 g (0.5~1.4 g) of diphenylamine in 100 mL of sulfuric acid. Use the colorless solution.

Diphenylcarbazone: $\text{C}_6\text{H}_5\text{NHNHCONC}_6\text{H}_5$ [Special grade]

Diphenylcarbazone test solution: Dissolve 1 g (0.5~1.4 g) of diphenylcarbazone in ethanol to make 1,000 mL.

Diphenylthiocarbazone: $C_6H_5NHNHCSN$: NC_6H_5 [Dithizone, Special grade]

Dipotassium hydrogen phosphate: K_2HPO_4 [Dibasic potassium phosphate, Special grade]

0.15 mol/L Dipotassium hydrogen phosphate test solution: Dissolve 26.13 g (26.125~26.134 g) of dipotassium hydrogen phosphate in water to make 1,000 mL.

α,α' -Dipyridyl: $C_{10}H_8N_2$ [Special grade]

α,α' -Dipyridyl test solution: To 0.25 g (0.245~0.254 g) of α,α' -dipyridyl to make 100 mL. Prepare before use.

Disodium 1-nitroso-2-naphthol-3,6-disulfonate: $C_{10}H_5NNa_2O_8S_2$ [Disodium 1-nitroso-2-naphthol-3,6-disulfonate (nitroso R salt), Special grade]

Disodium citrate: $C_6H_6Na_2O_7 \cdot 1 (1/2) H_2O$ [Special grade]

Disodium citrate test solution, 0.1 mol/L: To 26.3 g (26.25~26.34 g) of disodium citrate add water to make 1,000 mL.

Disodium dihydrogen ethylenediamine tetraacetate dihydrate: $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ [Special grade]

Disodium hydrogen phosphate dodecahydrate: $Na_2HPO_4 \cdot 12H_2O$ [Disodium phosphate (12 hydrate), Special grade]

Disodium hydrogen phosphate test solution: Dissolve 12 g (11.5~12.4 g) of disodium hydrogen phosphate in water to make 100 mL (1 mol/L).

Disodium hydrogen phosphate test solution, 0.1 mol/L: Dissolve 14.2 g (14.15~14.24 g) of anhydrous disodium hydrogen phosphate in water to make 1,000 mL

Disodium hydrogen phosphate test solution, 0.05 mol/L: Dissolve 7.1 g (7.05~7.14 g) of anhydrous disodium hydrogen phosphate in water to make 1,000 mL

Disodium hydrogen phosphate test solution, 0.02 mol/L: Dissolve 2.84 g (2.835~2.844 g) of anhydrous disodium hydrogen phosphate in water to make 1,000 mL

Disodium hydrogen phosphate, anhydrous: Na_2HPO_4 [Disodium phosphate (anhydrous), Special grade]

Disodium hydrogen phosphate, anhydrous, for pH determination: Na_2HPO_4 [Disodium phosphate (anhydrous), For pH determination]

Distilled water: Use purified water.

Dithizone: $C_6H_5NHNHCSN$: NC_6H_5 [Dithizone (diphenylthiocarbazone), Special grade]

Dithizone test solution: Dissolve 25 mg (24.5~25.4 mg) of dithizone in ethanol to make 100 mL. Prepare before use.

Dragendorff's test solution: Dissolve 0.85 g (0.845~0.854 g) of bismuth subnitrate in 10 mL of glacial acetate and 40 mL of water (Solution A). Dissolve 8 g (7.5~8.4 g) of potassium iodide in 20 mL of water (Solution B). Immediately before use, mix equal volumes of solution A and solution B and glacial acetic acid. Store solution A and solution B in light-resistant containers.

Dragendorff's test solution, for spraying: Add 20 mL of acetic acid (1 → 5) to 4 mL of mixture of equal volumes of solution A and solution B of Dragendorff's test solution. Prepare before use.

Drangendorff's test solution for spraying: Specified in an item of Drangendorff's test solution, for spraying.

Ephichlorohydrin: C_3H_5OCl [Special grade]

Eriochrome black T: $C_{20}H_{12}N_3NaO_7S$ [Eriochrome black T, (1-(1-hydroxy-2-naphthylazo)-5-nitro-4-naphtol-sodium sulfate), Special grade]

Eriochrome black T test solution: Dissolve 0.5 g (0.45~0.54 g) of eriochrome black T and 4.5 g (4.45~4.54 g) of hydroxylamine hydrochloride in 100 mL of ethanol. Preserve in light-resistance containers.

Eriochrome black T-sodium chloride indicator: Mix 0.1 g (0.05~0.14 g) of eriochrome black T with 10 g (9.5~10.4 g) of sodium chloride, and triturate until the mixture becomes homogenous.

Esterase suspension: Suspend the esterase which is separated from culture filtrate of *Streptomyces rochei* var. *volubilis* in ammonium sulfate solution (423 → 1,000). It contains not less than 1.0 unit/mL. One unit is equivalent to the amount of enzyme that produces 1 μ mol of 4-nitrophenol from 4-nitrophenyl acetate at 25 °C, pH 7.0 per minute. Store not exceeding 6 °C. Dilute with an appropriate amount of water, filter with membrane filter (0.45 μ m), and dilute with water to make 0.035 unit/mL of esterase suspension, prepare before use.

Ethanol: C_2H_5OH [Ethyl alcohol (95 v/v%), Special grade]

Ethanol, aldehyde-free: Transfer 1 L of ethanol to a glass-stoppered bottle, add the solution prepared by dissolving 2.5 g (2.45~2.54 g) of lead (II) acetate trihydrate in 5 mL of water, and mix well. Separately, dissolve 5 g (4.5~5.4 g) of potassium hydroxide in 25 mL of warm ethanol, cool, and add this solution gently, without stirring, to the first solution. After 1 hour, shake this mixture vigorously, allow to stand overnight, decant the supernatant liquid, and distill. For ethanol, aldehyde-free used to assay vitamin D, is defined in an item of Vitamin D assay in the paragraph of General tests.

Ethanol, anhydrous: C_2H_5OH [Ethyl alcohol (not less than 99.5 v/v%), Special grade]

Ethanol, chloroform-free: Specified in an item of Chloroform, ethanol-free.

Ethanol, dilute: Add water to an equal volume of ethanol to have C_2H_5OH 47.45~50.00 v/v% content.

Ethoxyquin for assay: Specified in an item of Ethoxyquin, for assay.

Ethoxyquin, for assay: A crude material for manufacturing ethoxyquin: It contains not less than 98.0 % ethoxyquin ($C_{14}H_{19}NO$).

Ethyl acetate: $CH_3COOC_2H_5$ [Special grade]

Ethyl cyanoacetate: $NCCH_2COOC_2H_5$ [First grade]

Ethylene dichloride: Specified in an item of Vitamin D assay in the paragraph of General tests.

Ethylene dichloride: $C_2H_2Cl_2$ [Special grade]

Ethylene glycol: $HOCH_2CH_2OH$ [Ethylene glycol (glycol), Special grade]

Ethylene glycol for Karl Fisher's Method: Specified in an item of Ethylene glycol, for Karl Fisher's Method.

Ethylene glycol, for Karl Fischer's Method: Distil ethylene glycol, and collect the fraction distilling at 195~198 °C. Water of 1 mL of this product is not more than 1.0 mg.

Ethylene glycol monomethyl ether: $HOCH_2CH_2OCH_3$ [Ethylene glycol monomethyl ether (methyl cellosolve), Special grade]

Extract liquid from bovine heart: Mince bovine cardiac muscle which is removed fat, tendon and blood vessel, add water, allow to stand for 24 hours at not exceeding 4 °C, warm in water bath at 50 °C for several hours, and boil, or allow to stand at 100 °C for several minutes with steam. Cool, filter with cloth then with filter paper.

Fehling's solution-reducing substance: Dissolve 0.5 g (0.45~0.54 g) of Fehling's solution-reducing substance in 10 mL of water, add 5 mL of Fehling's solution, boil for 3 minutes, allow to stand for 30 minutes: A color of the solution does not change.

Fehling's test solution

The copper solution: Dissolve 34.66 g (34.655~34.664 g) of copper (II) sulfate pentahydrate in water to make 500 mL Keep this solution in a glass-stoppered bottles in well-filled.

The alkaline tartrate solution: Dissolve 173 g (172.5~173.4 g) of potassium sodium tartrate tetrahydrate and 50 g (49.5~50.4 g) of sodium hydroxide in water to make 500 mL

Preserve this solution in a polyethylene container.

Before use, mix equal volumes of both solutions.

Ferrous ammonium sulfate test solution: Dissolve 8 g (7.5~8.4 g) of ferrous ammonium sulfate in water to make 100 mL

Fluorescein: $C_{20}H_{12}O_5$ [Special grade]

Fluorescein isothiocyanate (isomer I) $C_{21}H_{11}NO_5S$: A yellow-orange powder.

Fluorescein-labeled peptidoglycan TS, 0.5 mg/mL: Peptidoglycan derived from *Micrococcus lysodeikticus* labeled with fluorescein isothiocyanate (isomer I), a fluorescent material, by the following procedure. To 100 mg (99.5 ~ 100.4 mg) of peptidoglycan, add 35 mL of sodium bicarbonate buffer solution (pH 9.3), and shake thoroughly to make a suspension. Add this suspension to 800 mg (799.5 ~ 800.4 mg) of fluorescein isothiocyanate (isomer I), wash the container that contained the suspension with 10 mL of sodium bicarbonate buffer solution (pH 9.3), then add the washing liquid into the fluorescein isothiocyanate (isomer I) solution. After shaking at 37°C for four hours at 700 revolutions per minute, centrifuge at 1500 × g for 20 minutes, and discard the supernatant liquid. To the residue, add 35 mL of sodium bicarbonate buffer solution (pH 9.3), shake thoroughly, centrifuge at 1500 × g for 20 minutes, and discard the supernatant liquid. Repeat this procedure once more. To the residue, add 35 mL of water, shake thoroughly, centrifuge at 1500 × g for 20 minutes, and discard the supernatant liquid. Repeat this procedure once more. To the residue, add 35 mL of acetone, shake thoroughly, centrifuge at 1500 × g for 20 minutes, and discard the supernatant liquid. Repeat this procedure once more. To the residue, add 35 mL of ethanol, shake thoroughly, centrifuge at 1500 × g for 20 minutes, and discard the supernatant liquid. After repeating this procedure once more, lyophilize the solution, and store it at -20°C.

Fluorescein-ethanol test solution: Dissolve 50 mg (49.5~50.4 mg) of fluorescein in ethanol to make 100 mL

Folin's test solution: Place 20 g (19.5~20.4 g) of sodium tungstate (VI) dihydrate, 5 g (4.5~5.4 g) of disodium molybdate (VI) dihydrate and about 140 mL of water in a 300 mL volumetric flask, add 10 mL of diluted phosphoric acid (17 → 20) and 20 mL of hydrochloric acid, and boil gently using a reflux condenser with ground glass joints for 10 hours. To the mixture add 30 mL of lithium sulfate monohydrate and 10 mL of water, and then add a very small quantity of bromine to change the deep green color of the solution to yellow. Remove the excess bromine by boiling for 15 minutes without a condenser, and cool. Add water to make 200 mL, and filter through a glass filter. Store free from dust. Use this solution as the stock solution, and dilute with water to the directed concentration before use.

Formaldehyde solution: HCHO [Special grade]

Formalin: Specified in an item of Formaldehyde solution.

Formalin-magnesium carbonate test solution: Add magnesium carbonate to formalin, shake well, saturate and filter, add water to make 4 volumes.

Formic acid: HCOOH [Special grade]

Glacial acetic acid: Specified in an item of Acetic acid, glacial.

Glacial acetic acid for nonaqueous titration: Specified in an item of Acetic acid, glacial, for nonaqueous titration.

Glacial acetic acid for nonaqueous titration: Specified in an item of Acetic acid, glacial, for nonaqueous titration.

Glass Fiber: [Glass Wool, Special grade]

β -Glucan: β -Glucan is polysaccharide which exists on cell wall of barley, wheat and yeast, and produces *D*-glucose by hydrolysis. Use 200,000 molecular weight one which is obtained from barley.

Glucose: $C_6H_{12}O_6$ [Japanese Pharmacopoeia]

Glycerin: $C_3H_8O_3$ [Concentrated Glycerol, Japanese Pharmacopoeia]

Guanine hydrochloride: $C_5H_5N_5O \cdot HCl$ Guanine hydrochloride occurs as white crystals or a white crystalline powder. It is soluble in dilute hydrochloric acid, practically insoluble in water and in ethanol.

Clarity and Color of solution: Dissolve 0.2 g (0.15~0.24 g) of Guanine hydrochloride in 20 mL of hydrochloric by warming: the solution is clear and colorless.

Water: Not more than 5.0 % (1 g).

Content: Not less than 94 %.

Halofuginone hydrobromide: $C_{16}H_{18}Br_2ClN_3O_3$ Halofuginone hydrobromide occurs as a white to greyish white powder.

Content: Not less than 98.0 %.

Purity: Dissolve 0.020 g (0.0195~0.0204 g) of halofuginone hydrobromide in 10 mL of a mixture of chloroform, methanol and water (3:3:0.5). Spot 10 μ L of this solution on a plate of silica gel for thin-layer chromatography (with fluorescent indicator). Develop the plate with a mixture of chloroform, methanol and ammonium (75:25:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (DWL 254 nm): only one spot appears at an *R_f* value of about 0.3 and no spot other than the principal spot.

Assay: Dry halofuginone hydrobromide, weigh 0.3 g to three decimal places, record the value. Add 60 mL of methanol and 10 mL of mercuric acetate test solution, and titrate with 0.1 mol/L of perchloric acid (potentiometric titration). Perform a blank determination and make any necessary correction.

1 mL of 0.1 mol/L perchloric acid = 49.56 mg of $C_{16}H_{18}Br_2ClN_3O_3$

Halofuginone hydrobromide (cis-isomer): $C_{16}H_{18}Br_2ClN_3O_3$ Halofuginone hydrobromide (cis- isomer) occurs as a white to greyish white powder.

Content: Not less than 98 %.

Purity: Dissolve 0.020 g (0.0195~0.0204 g) of halofuginone hydrobromide (cis-isomer) in 10 mL of a mixture of chloroform, methanol and water (3:3:0.5). Spot 10 μ L of this solution

on a plate of silica gel for thin-layer chromatography (with fluorescent indicator). Develop the plate with a mixture of chloroform, methanol and ammonium (75:25:1) to a distance of 15 cm, and air-dry the plate. Examine under ultraviolet light (DWL 254 nm): only one spot appears at an *R_f* value of 0.5 and no spot other than the principal spot.

Assay: Dryhalofuginone hydrobromide (cis-isomer), weigh 0.3 g to three decimal places, record the value. Add 60 mL of methanol and 10 mL of mercuric acetate, and titrate with 0.1 mol/L of perchloric acid (Potentiometric titration). Perform a blank determination and make any necessary correction.

1 mL of 0.1 mol/L perchloric acid = 49.56 mg of C₁₆H₁₈Br₂ClN₃O₃

Hen's egg-york liquid: Separate egg-york from hen's egg aseptically, and dilute with the same amount of water. Prepare before use.

Hexane: C₆H₁₄ [n-Hexane, Special grade] n-Hexane for vitamin D assay is specified in an item of Vitamin D assay in the paragraph of General tests.

n-Hexane, for ultraviolet-visible spectrophotometry: [n-Hexane, Special grade] Read absorbance of hexane for ultraviolet-visible spectrophotometry, using as the blank as directed under Ultraviolet-visible Spectrophotometry: its value is not more than 0.10 at 220 nm, not more than 0.02 at 260 nm. It exhibits no characteristic absorption between 260 nm and 350 nm.

L-histidine hydrochloride for assay : When dried, it contains not less than 98.5% of L-histidine hydrochloride (C₆H₉N₃O₂.HCl.H₂O).

L-Histidine hydrochloride for assay: As specified in the section on L-histidine hydrochloride, for assay.

Hooker's staining solution: Mix 0.037 mol/L methylrosaniline chloride-ethanol test solution and 0.07 mol/L ammonium oxalate test solution, allow to stand for one night, and filter. Preserve in light-resistant containers.

Horse defibernated blood: Collect the blood from horse by applying aseptic manipulation, defibernate with sterile glass beads within 30 seconds.

Storage temperature: 2~6 °C

Expiry date: 2 weeks after the production date.

Hydrochloric acid: HCl [Special grade] Not less than 35 %.

Hydrochloric acid test solution, 8 mol/L: To 720 mL of hydrochloric acid add water to make 1,000 mL.

Hydrochloric acid test solution, 6 mol/L: To 540 mL of hydrochloric acid add water to make 1,000 mL.

Hydrochloric acid test solution, 2.5 mol/L: To 225 mL of hydrochloric acid add water to make 1,000 mL.

Hydrochloric acid test solution, 2 mol/L: To 180 mL of hydrochloric acid add water to make 1,000 mL.

Hydrochloric acid test solution, 1 mol/L: To 90 mL of hydrochloric acid add water to make 1,000 mL.

Hydrochloric acid test solution, 0.5 mol/L: To 45 mL of hydrochloric acid add to make 1,000 mL.

Hydrochloric acid test solution, 0.2 mol/L: To 200 mL of 1 mol/L hydrochloric acid add water to make 1,000 mL.

Hydrochloric acid test solution, 0.1 mol/L: To 100 mL of 1 mol/L hydrochloric acid add water to make 1,000 mL.

Hydrochloric acid test solution, 0.001 mol/L: To 10 mL of 0.1 mol/L hydrochloric acid add water to make 1,000 mL.

Hydrochloric acid, dilute: To 23.6 mL of hydrochloric acid add water to make 100 mL (10 %).

0.1 mol/L Hydrochloric acid-methanol test solution: To 9.0 mL of hydrochloric acid add methanol to make 1,000 mL.

0.01 mol/L Hydrochloric acid-methanol test solution: To 10 mL of 1 mol/L hydrochloric acid add methanol to make 1,000 mL.

Hydrofluoric acid: HF [Special grade] It contains not less than 46.0 % of HF.

Hydrogen: H₂ [Reference materials, Third grade]

Hydrogen peroxide test solution: Add 9 volumes of water to 1 volume of strong hydrogen peroxide (3 %). Prepare before use.

Hydrogen peroxide water, strong: H₂O₂ [Hydrogen peroxide solution (30 %), Special grade] Contains not less than 30 w/v% of H₂O₂. Protect from light and store at a cool place.

Hydrogen sulfide: H₂S Colorless poisonous gas, heavier than air. It dissolves in water. Prepare by treating iron (II) sulfide with diluted sulfuric acid or diluted hydrochloric acid. Other sulfides yielding hydrogen sulfides with dilute acids may be used.

Hydroxy semduramicin sodium: C₄₅H₇₅O₁₇Na A white crystalline powder.

Purity: Dissolve 1 g (0.5~1.4 g) of hydroxy semduramicin sodium in 1 mL of methanol, spot 5 µL of this solution on a plate of silica gel for thin-layer chromatography. Develop the plate 10 cm with a mixture of ethyl acetate and glacial acetic acid (4:1) as developing solvent, and air-dry the plate. To this plate spray vanillin-sulfuric acid-ethanol test solution, heat at 105 °C for 10 minutes: only one red-brown spot at an *R_f* value of 0.5, no spots other than the principal spot.

25-Hydroxycholecalciferol: Contains not less than 97.0 % of C₂₇H₄₄O₂·H₂O.

Hydroxylammonium chloride: NH₂OH·HCl [Special grade]

2-Hydroxy-*m*-toluic acid: $\text{CH}_3\text{C}_6\text{H}_3(\text{OH})\text{COOH}$ A white to slightly red crystalline powder.

It is freely soluble in ethanol, slightly soluble in water.

Melting point: 163~170 °C

Hypophosphorous acid: H_3PO_2 [First grade] It contains 30 % to 32 % of H_3PO_2 .

Iodine: I_2 [Special grade]

Iodine test solution: Dissolve 14 g (13.5~14.4 g) of iodine in 100 mL of potassium iodide solution (2 → 5), add 1 mL of dilute hydrochloric acid, and dilute with water to make 1,000 mL (0.05 mol/L). Preserve protected from light.

Iodine-acetone test solution: Triturate 10 g (9.5~10.4 g) of iodine and 6 g (5.5~6.4 g) of potassium iodide, dissolve in 10 mL of water, add 90 % ethanol to make 100 mL. Pipet 3.5 mL of this solution, add acetone to make 100 mL.

Iodine-Lugol's solution: Triturate 5 g (4.5~5.4 g) of iodine and 10 g (9.5~10.4 g) of potassium iodide, and dissolve in water to make 100 mL. Dilute 5 times with water before use.

Iron (II) sulfate heptahydrate: $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ [Special grade]

Iron (II) sulfate test solution: Dissolve 8 g (7.5~8.4 g) of iron (II) sulfate heptahydrate in 100 mL of freshly boiled and cooled water. Prepare before use.

Iron (II) sulfide: FeS [First grade]

Iron (III) chloride hexahydrate: $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ [Special grade]

Iron (III) chloride -hydrochloric acid test solution: Dissolve 10 g (9.5~10.4 g) of iron (III) chloride in 0.1 mol/L of hydrochloric acid to make 100 mL.

Iron (III) chloride test solution: Dissolve 9 g (8.5~9.4 g) of iron (III) chloride in water to make 100 mL (0.5 mol/L).

Iron (III) chloride test solution, dilute: To 2 mL of ferric chloride test solution add water to make 100 mL. Prepare before use.

Iron (III) perchloride: $\text{Fe}(\text{ClO}_4)_3$ Iron (III) perchloride occurs as light grey to light brown crystals, deliquescent. It is very soluble in water.

Clarity and color of solution: The solution (1 → 20) is clear and colorless.

Iron (III) perchloride test solution: Dissolve 3 g (2.5~3.4 g) of Iron (III) perchloride in water to make 500 mL, filter.

Iron (III) sulfate n-hydrate: $\text{Fe}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$ [Special grade]

Isoamyl acetate: $\text{CH}_3\text{COOCH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$ [Special grade]

Isoamyl alcohol: Specified in an item of Amyl alcohol, iso.

Isobutanol: Specified in an item of butanol, iso.

Isooctane: $(\text{CH}_3)_3\text{CCH}_2\text{CH}(\text{CH}_3)_2$ [Japanese Pharmacopoeia]

Isopropanol: Specified in an item of propanol, iso.

L-isoleucine for assay: Specified in an item of L-isoleucine, for assay.

L-isoleucine for assay: When dried, it contains not less than 99.0% of L-isoleucine ($C_6H_{13}NO_2$).

Karl Fisher's test solution: Specified in an item of Water Determination in the paragraph of General tests.

Lactic acid: $C_3H_6O_3$ [Special grade]

Lactic acid buffer solution, 0.1 mol/L, for digestion test: Dissolve 9.01 g (9.005~9.014 g) of lactic acid in water to make 900 mL. To this solution add dilute sodium hydroxide test solution to adjust the specific pH, and add water to make 1,000 mL.

Lactic acid test solution, 1 mol/L: To 90.1 g (90.05~90.14 g) of lactic acid add water to make 1,000 mL.

Lactic acid test solution, 0.05 mol/L: To 4.5 g (4.45~4.54 g) of lactic acid add water to make 1,000 mL.

Lactose monohydrate: $C_{12}H_{22}O_{11} \cdot H_2O$ [Japanese Pharmacopoeia]

Lanthanum-alizarin complexone test solution: 10 % aqueous solution prepared by diluting hexamethylenetetramine-hydrogen phthalate buffer solution in which dissolved alizarin complexone and lanthanum salts.

Lead (II) nitrate: $Pb(NO_3)_2$ [Special grade]

Lead (II) oxide: PbO [Special grade]

Lead acetate: $Pb(CH_3COO)_2 \cdot 3H_2O$ [Special grade]

Lead acetate test solution: Dissolve 9.5 g (9.45~9.54 g) of lead acetate in freshly boiled and cooled water to make 100 mL. Preserve in tightly stoppered bottles (0.25 mol/L).

Lead subacetate test solution: Place the yellowish mixture obtained by triturating 3 g (2.5~3.4 g) of lead (II) acetate and 1 g (0.5~1.4 g) of lead (II) oxide with 0.5 mL of water in a beaker, and heat on a water bath, covering with a watch glass, until it shows a homogeneous, white to reddish white color. Then add 9.5 mL of hot water in a small portions, cover it again with a watch glass, and set it aside. Decant the supernatant liquid, and adjust the specific gravity to 1.23 to 1.24 (15 °C) by adding water. Preserve in tightly stoppered bottles.

Lily's staining solution: Mix 20 mL of 0.25 mol/L methylrosaniline chloride-ethanol test solution and 80 mL of ammonium oxalate solution (1 → 100), and dissolve. Prepare before use.

Liquid paraffin: Specified in an item of Paraffin, liquid.

Lithium acetate dihydrate: $CH_3COOLi \cdot 2H_2O$ [For amino acid analysis]

Lithium chloride monohydrate: $LiCl \cdot H_2O$ [For amino acid analysis]

Lithium citrate buffer solution: To 6.9 g (6.85~6.94 g) of lithium citrate, 1.3 g (1.25~1.34 g) of lithium chloride, 8.8 g (8.75~8.84 g) of citric acid, 4.0 mL of hydrochloric acid, 40.0

mL of ethanol, 2.5 mL of thiodiethylene glycol and 0.1 mL of octanoic acid add water to make 1,000 mL. Adjust the pH to 2.98 with hydrochloric acid and 5 mol/L of lithium hydroxide.

Lithium citrate tetrahydrate: $\text{Li}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 4\text{H}_2\text{O}$ [For amino acid analysis]

Lithium hydroxide: LiOH [Special grade]

Lithium sulfate monohydrate: $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O}$ [Special grade]

Litmus paper, red: [Litmus paper, Red litmus paper]

Liver extract: Liver extract occurs a dark brown powder or particles.

Loss on drying: Not more than 6 % (1 g, 85 °C, 1 hour).

Solubility: Adjust the pH of liver extract solution (1 → 1,000) to 7.0, sterilization this

solution in an autoclave at 121 °C for 15 minutes: no insoluble substance in the solution.

Lugol's solution: Triturate 1 g (0.5~1.4 g) of iodine and 2 g (1.5~2.4 g) of potassium iodide, add 300 mL of water in small portions while stirring, and dissolve.

Lysine hydrochloride: $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2 \cdot \text{HCl}$ [Japanese Pharmacopoeia]

Magnesia test solution: Dissolve 5.5 g (5.45~5.54 g) of magnesium chloride hexahydrate and 7 g (6.5~7.4 g) of ammonium chloride in 65 mL of water, add 35 mL of ammonia test solution, allow the mixture to stand for a few days in tightly stoppered bottles, and filter. If the solution is not clear, filter before use.

Magnesium carbonate: [Japanese Pharmacopoeia]

Magnesium chloride hexahydrate: $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ [Special grade]

Magnesium chloride test solution: To 3.75 g (3.745~3.754 g) of magnesium oxide add 8 mol/L of hydrochloric acid in a small portions to make 100 mL.

Magnesium nitrate hexahydrate: $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ [Special grade]

Magnesium nitrate test solution: To 3.75 g (3.745~3.754 g) of magnesium nitrate add 30 mL of water, add 10 mL of nitric acid in small portions and dissolve. Allow to cool, add water to make 50 mL.

Magnesium oxide: MgO [Special grade]

Magnesium powder: Mg [Special grade]

Magnesium sulfate heptahydrate: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ [Special grade]

Magnesium sulfate test solution: Dissolve 12 g (11.5~12.4 g) of magnesium sulfate in water to make 100 mL (0.5 mol/L).

Malachite green oxalate: $\text{C}_{52}\text{H}_{54}\text{N}_4\text{O}_{12}$ [Japanese Pharmacopoeia]

Maleic acid: $(\text{CHCOOH})_2$ [Special grade]

Manganese (II) sulfate n-hydrate: $\text{MnSO}_4 \cdot n\text{H}_2\text{O}$ [Special grade]

Mercury(II) acetate test solution for nonaqueous titration: Specified in an item of mercury (II) acetate test solution, for nonaqueous titration.

Mcilvaine buffer solution, for digestion test: Dissolve 21.02 g (21.015~21.024 g) of citric acid in water to make 1,000 mL. Adjust specific pH with dibasic sodium phosphoric test solution.

Meat extract: Concentrated extract manufactured from low fat meat. Boil or warm low fat meat in water, and concentrate the obtained meat soup until it has become thick paste under reduced pressure. It is possible to use similar commercial products which have following chemical and physical properties or confirm the standards, if these products does not give problem for test.

Physical and chemical property: Meat extract occurs as a yellow-brown to dark brown paste, slightly acidic, and having a meat-like odor.

Clarity and color of solution: Mix well, to 25 g (24.5~25.4 g) of meat extract add water to make 250 mL, shake well, and use this solution as the sample solution: It dissolves in water, forming a clear solution, or with a turbidity, and no precipitate is observable.

Total solid material: Heat 10 mL of the sample solution in a porcelain dish at 100 °C to 110 °C for 16 hours: the mass of the residue is not less than 650 mg.

Residue on ignition: Ignite the above residue by the Residue on Ignition Test: not more than 30 % of the mass of total solid material.

Salt equivalent: Add about 50 mL of water to above obtained residue on ignition, and shake well. Transfer to a 100 mL volumetric flask, add 2 to 4 drops of nitric acid and 10 mL of 0.1 mol/L silver nitrate with transfer pipet, and add water to make 100 mL. Shake well and filter, and remove the first of 10 mL of filtrate, measure next 50 mL of filtrate, add 1 mL of ferric ammonium sulfate test solution, and titrate with 0.1 mol/L ammonium thiocyanate: the amount of sodium chloride is not exceeding 6 % of that of total solid material.

1 mL of 0.1 mol/L silver nitrate solution = 5.844 mg of NaCl

Nitrate: Measure 10 mL of test solution, add 1.5 g (1.45~1.54 g) of activated carbon. Boil the solution for 1 minute, add water to make 10 mL, and filter. Add three drops of diphenylamine to filtrate: it does not exhibits blue color.

Ammonia nitrogen: To 100 mL of test solution add 5 g (4.5~5.4 g) of barium carbonate and 100 mL of water. Then begin the distillation with steam, and continue until the distillate measures 100 mL in the flask filled with 50 mL of 0.05 mol/L sulfuric acid. Add 1 to 3 drops of methyl red to the distillate and titrate with 0.1 mol/L sodium hydroxide solution: The total amount of ammonia nitrogen is not exceeding 0.35 % of total solid material.

1 mL of 0.05 mol/L sulfuric acid = 1.703 mg of NH₃

Melezitose monohydrate: C₁₈H₃₂O₁₆·H₂O

Clarity and color of solution: Dissolve 1 g (0.5~1.4 g) of melezitose in 20 mL of water: the solution is clear, or having a faint.

Residue on ignition: Not more than 0.2 %.

Water 2.5~4.5 %

Melibiose: $C_{12}H_{22}O_{11} \cdot H_2O$

Optical rotation: $[\alpha]_D^{20} = +141.2^\circ \sim +141.8^\circ$

Water: 3.5~5.5 %

Membrane filter (0.45 μm): Porous film filter with 0.45 μm in pore size, consists of cellulose derivatives.

Membrane filter (0.8 μm): Porous film filter with 0.8 μm in pore size, consists of cellulose derivatives.

Menadione dimethylpyrimidinol bisulfite for assay: Specified in an item of Menadione dimethylpyrimidinol bisulfite, for assay.

Menadione dimethylpyrimidinol bisulfite, for assay: A crude material for manufacturing menadione dimethylpyrimidinol bisulfite. It contains not less than 94.5 % of menadione dimethylpyrimidinol bisulfite ($C_{17}H_{18}N_2O_6S$).

Menadione sodium bisulfite for assay: Specified in an item of Menadione sodium bisulfite, for assay.

Menadione sodium hydrogen sulfite, for assay: A crude material for manufacturing menadione sodium hydrogen sulfite. It contains not less than 93.5 % of menadione sodium hydrogensulfite ($C_{11}H_8O_2 \cdot NaHSO_3$).

2-mercaptoethanol $HSCH_2CH_2OH$: It is a clear, colorless liquid.

Specific gravity: $d_{420} = 1.112 \sim 1.117$

Mercuric acetate: $Hg(CH_3COO)_2$ [Special grade]

Mercuric acetate test solution, for nonaqueous titration: To 5 g (4.5~5.4 g) of mercuric acetate add glacial acetic acid for nonaqueous titration to make 100 mL.

Mercuric bromide: $HgBr_2$ [Special grade]

Mercuric bromide paper: To 5 g (4.5~5.4 g) of mercuric bromide add 100 mL of ethanol, and melt by gentle heating. Immerse a filter paper for chromatography (W: 4 cm \times L: 10 cm) in this solution for 1 hour in a dark place. Hang a piece of filter paper taking care not to touch the places using for the test with fingers. Allow to dry spontaneously while the paper is suspended on a rod. After dry, cut off the upper side and lower side of the paper to make 20 mm², then cut off the corners. Protect from light and store in a dark place.

Mercuric nitrate test solution: Dissolve 40 g (39.5~40.4 g) of mercuric oxide yellow in a mixture of 32 mL of nitric acid and 15 mL of water (2 mol/L). Preserve in a glass-stoppered bottle protected from light.

Mercury (II) ammonium thiocyanate test solution: Dissolve 30 g (29.5~30.4 g) of ammonium thiocyanate and 27 g (26.5~27.4 g) of mercury (II) chloride in water to make 1,000 mL.

Mercury (II) chloride: HgCl₂ [Special grade]

Mercury (II) chloride test solution: Dissolve 6.5 g (6.45~6.54 g) of mercury (II) chloride in water to make 100 mL (0.25 mol/L).

Mercury (II) oxide, yellow: HgO [Mercury (II) oxide (yellow), Special grade] Store protected from light.

Mercury (II) sulfate test solution: To 5 g (4.5~5.4 g) of mercury (II) oxide yellow add 40 mL of water, add 20 mL of sulfuric acid slowly while stirring, add 40 mL of water, and stir until mercury (II) oxide dissolves.

Metallic sodium: Specified in an item of Sodium, metal.

Metaphosphate acid-acetic acid test solution: To 15 g (14.5~15.4 g) of metaphosphate acid and 40 mL of glacial acetic acid add water to make 500 mL. Store in a cool place, use within 2 days.

Metaphosphoric acid: HPO₃ [Special grade]

Methanol: CH₃OH [Methyl alcohol (methanol), Special grade]

Methanol for Karl Fisher's Method: Specified in an item of Water Determination in the paragraph of General tests.

2-Methylbiphenyl C₁₃H₁₂: Colorless to pale yellow liquid, its content not less than 95.0 %

3-Methylbiphenyl C₁₃H₁₂: Colorless to pale yellow liquid, its content not less than 95.0 %

4-Methylbiphenyl C₁₃H₁₂: White to yellow-red or green powder or crystals, its content not less than 97.5 %

DL-Methionine for assay: Specified in an item of DL-Methionine, for assay.

DL-Methionine, for assay: A crude material for manufacturing DL-methionine. Previously dried, it contains not less than 99 % of DL-methionine (C₅H₁₁NO₂S).

Methyl cellosolve: Specified in an item of Ethyleneglycol monomethyl ether.

Methyl ethyl ketone: CH₃COC₂H₅ [Special grade]

Methyl isobutyl ketone: CH₃COCH₂CH(CH₃)₂ [Special grade]

Methyl orange: C₁₄H₁₄N₃NaO₃S [Special grade] Color change: pH 3.1 (red) to pH 4.4 (orange-yellow).

Methyl orange test solution: Dissolve 0.1 g (0.05~0.14 g) of methyl orange in 100 mL of water. Filter if necessary.

Methyl orange-xylenecyanol FF test solution: Dissolve 1 g (0.5~1.4 g) of methyl orange and 1.4 g (1.35~1.44 g) of xylene cyanol FF in 500 mL of dilute ethanol.

Methyl red: C₁₅H₁₅N₃O₂ [Special grade] Color change: pH 4.2 (red) to pH 6.2 (yellow).

Methyl red test solution: Dissolve 0.1 g (0.05~0.14 g) of methyl red in 100 mL of ethanol. Filter if necessary.

Methylene blue n-hydrate: $C_{15}H_{18}ClN_3S \cdot nH_2O$ [Methylene blue (dihydrate, trihydrate, tetrahydrate), Special grade]

Methylrosanilinium chloride: $C_{25}H_{30}ClN_3$ [Japanese Pharmacopoeia]

Methylrosanilinium chloride test solution: Dissolve 0.1 g (0.05~0.14 g) of methylrosanilinium chloride in 10 mL of glacial acetic acid.

Methylrosanilinium chloride-ethanol test solution, 0.25 mol/L: Dissolve 10 g (9.5~10.4 g) of methylrosanilinium chloride in ethanol to make 100 mL.

Methylrosanilinium chloride-ethanol test solution, 0.037 mol/L: Dissolve 0.3 g (0.25~0.34 g) of methylrosanilinium chloride in ethanol to make 20 mL.

Meyer's test solution: Dissolve 1.358 g (1.3575~1.3584 g) of mercury (II) chloride in 60 mL of water. Separately, dissolve 5 g (4.5~5.4 g) of potassium iodide in 10 mL of water. Mix both solutions, and add water to make 100 mL.

Milk casein: Specified in an item of Casein, milk.

Sodium monohydrogen phosphate dihydrate $Na_2HPO_4 \cdot 2H_2O$

Murexide: $C_8H_8N_6O_6$ Red-purple crystals, having a metallic luster. The wavelength of absorption maximum is 520 nm. A red-purple color develops in aqueous solutions, a dark blue color develops in alkali solutions.

Murexide indicator: Prepared by mixing 0.2~0.4 g of murexide and 100 g (99.5~100.4 g) of potassium sulfate [Special grade] and grinding to get homogenous.

N-1-Naphthylethylenediamine dihydrochloride: [Special grade]

α -Naphthol: $C_{10}H_7OH$ [Special grade] Store protected from light.

α -Naphtholbenzein: $C_{27}H_{20}O_3$ [Special grade] α -Naphtholbenzein occurs as a red-brown powder. It is insoluble in water, and dissolves in ethanol, in benzene, in diethyl ether and in glacial acetic acid. The solution shows yellow-red color under acidic conditions and shows green color under alkaline conditions.

α -Naphtholbenzein test solution: Dissolve 0.2 g (0.15~0.24 g) of α -naphtholbenzein in glacial acetic acid to make 100 mL.

Clarity and color of solution: Dissolve 0.1 g (0.05~0.14 g) of α -naphtholbenzein in 100 mL of ethanol: the solution is red in color and clear.

Sensitivity: Add 100 mL of freshly boiled and cooled water cooled to 0.2 mL of α -naphtholbenzein in ethanol (1 \rightarrow 1,000), and add 0.1 mL of 0.1 mol/L sodium hydroxide: a green color develops. Add subsequently 0.2 mL of 0.1 mol/L hydrochloric acid: the color of the solution changes to yellow-red.

Nessler's test solution: Dissolve 10 g (9.5~10.4 g) of potassium iodide in 10 mL of water, and add mercury (II) chloride saturated solution dropwise while stirring until the red precipitant remains. Dissolve 30 g (29.5~30.4 g) of potassium hydroxide in water to make 60 mL. Add this solution to the above solution, then add 1 mL of mercury (II) chloride saturated solution and water to make 200 mL. After sedimentation of precipitant, use the supernatant. To 100 mL of ammonium chloride solution (1 → 300,000) add 2 mL of this solution: the solution: yellow-brown color develops within 30 seconds.

Nicotinic acid: $C_6H_5NO_2$ [Japanese Pharmacopoeia]

Ninhydrin for amino acid analysis: $C_9H_6O_4$ [For amino acid analysis]

Ninhydrin for analyzing amino acid: Specified in Ninhydrin, for amino acid analysis.

Ninhydrin monohydrate: $C_9H_4O_3 \cdot H_2O$ [Ninhydrin (triketohydrindene hydrate), Special grade]

Ninhydrin test solution: Dissolve 0.2 g (0.15~0.24 g) of ninhydrin in water to make 10 mL. Prepare before use.

Ninhydrin test solution for amino acid analysis: Specified in an item of Ninhydrin reagent, for amino acid analysis.

Ninhydrin test solution, for amino acid analysis: Mix 30 g (29.5~30.4 g) of ninhydrin for amino acid analysis, 134 mg (133.5~134.4 mg) of boron sodium hydroxide, 800 mL of acetic acid buffer solution and 1,200 mL of methylcellosolve, and dissolve under nitrogen gas. Preserve in refrigerator.

Ninhydrin test solution for assay: Specified in an item of Ninhydrin, for assay.

Ninhydrin test solution, for assay: To 5 g (4.5~5.4 g) of ninhydrin, 6.7 g (6.65~6.74 g) of cupric chloride, 125 mL of citric acid and 375 mL of methylcellosolve add water to make 1,000 mL. It is stable for 2 weeks after preparation.

Ninhydrin-ascorbic acid test solution: To 0.25 g (0.245~0.254 g) of ninhydrin and 0.01 g (0.005~0.014 g) of ascorbic acid add water to make 50 mL. Prepare before use.

Ninhydrin-butanol test solution: Dissolve 0.3 g (0.25~0.34 g) of ninhydrin in 100 mL of n-butanol, and add 3 mL of glacial acetic acid.

Nitric acid: HNO_3 [Special grade, Specific gravity: around 1.42]

Nitric acid, dilute: To 10.5 mL of nitric acid add water to make 100 mL (10 %).

p-Nitroaniline: $O_2NC_6H_4NH_2$ [First grade]

Nitrobenzene: $C_6H_5NO_2$ [Special grade]

Nitrogen gas: N_2 [Nitrogen, Japanese Pharmacopoeia]

4-Nitrophenyl acetate: $CH_3COOC_6H_4NO_2$ 4-Nitrophenyl acetate occurs as a white to light yellow crystalline powder.

Melting point: 77~80 °C

Molar absorbance coefficient: Weigh 200 mg of 4-nitrophenyl acetate, the value shall be recorded to 3 significant digits, dissolve in methanol, transfer into a 100 mL volumetric flask, add methanol to make exactly 100 mL. Transfer exactly 10 mL of this solution into a 100 mL of volumetric flask with transfer pipet, add methanol to make exactly 100 mL. Then, transfer exactly 5 mL of this solution into a volumetric flask with transfer pipet, add methanol to make exactly 100 mL. Determine the absorbances at 268 nm of this solution using methanol as the blank: the molar absorbance coefficient of 4-nitrophenyl acetate is 9,200~9,600.

o-Nitrophenyl- β -D-galactopyranoside: $C_{12}H_{15}NO_8$ White crystalline powder, odorless. It is sparingly soluble in water, slightly soluble in ethanol.

Melting point: 193~194 °C

Optical rotation: Weigh 1.0 g of disodium 1-nitroso-2-naphthol-3,6-disulfonate to two decimal places, record the value, and add water to dissolve. Transfer to a 100 mL volumetric flask, add water to make exactly 100 mL. Optical rotation of this solution is $[\alpha]_D^{18} = -51.9^\circ$ (100 mm).

Residue on ignition: Not more than 0.05 % (2 g).

5-Nitroso-8-oxyquinoline: C_9H_5NOHNO 5-Nitroso-8-oxyquinoline occurs as a dark greyish green crystalline powder.

Melting point: 245 °C (with decomposition)

Clarity and color of solution: Dissolve 0.1 g (0.05~0.14 g) of 5-nitroso-8-oxyquinoline in 100 mL of sulfuric acid: the solution is clear.

Sensitivity: Evaporate to dryness 0.05 mL of resorcin-ethanol solution (1 \rightarrow 1,000) in a crucible on the water bath. After cooling, add 0.05 mL of 5-nitroso-8-oxyquinoline-sulfuric acid solution (1 \rightarrow 1,000), and heat: the solution becomes red-violet.

α -Nitroso- β -naphthol: $C_{10}H_7NO_2$ [Special grade]

α -Nitroso- β -naphthol test solution, strong: To 1 g (0.5~1.4 g) of α -nitroso- β -naphthol add 25 mL of glacial acetic acid, warm to dissolve. Add 25 mL of water and warm to dissolve again. Then, add 25 mL of water, allow to stand for 1 hour, and filter.

NN Indicator: Mix 0.5 g (0.45~0.54 g) of 2-oxy-1-(2'-oxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid with 50 g (49.5~50.4 g) of anhydrous sodium sulfate, and triturate until the mixture becomes homogenous.

L-Norleucine: $C_6H_{13}NO_2$ [For amino acid analysis]

L-Norleucine test solution: To 16 mg (15.5~16.4 mg) of L-norleucine add 1 mol/L of hydrochloric acid to make 50 mL.

Octadecylsilanized silica gel for liquid chromatography: [Japanese Pharmacopoeia]

Octanoic acid: $CH_3(CH_2)_6COOH$ [For amino acid analysis]

Sodium 1-octanesulfonate $C_8H_{17}NaO_3S$: It occurs as a white powder.

Clarity and color of solution: Weigh 1.1 g (1.05~ 1.14 g) of sodium 1-octanesulfonate, add 50 mL of water to dissolve: the solution is clear. its contents not less than 98.0%

Assay: Weigh about 0.4 g of L-isoleucine, previously dried at 105 °C for two hours, to the order of 0.0001 g, record the value, add 25 mL of water, and titrate with a 0.1 mol/L sodium hydroxide solution (indicator: 2 ~ 3 drops of phenolphthalein solution). Keep titrating until the color of the solution remains pale red for 15 seconds.

Each mL of 0.1 mol/L sodium hydroxide solution = 21.672 mg of $CH_3(CH_2)_7SO_3Na$

Octylphenol ethoxylate $C_{14}H_{22}O(C_2H_4O)_n$

Octylphenol ethoxylate TS: To 25 g (24.5 ~ 25.4 g) of octylphenol ethoxylate, add water to dissolve and make 250 mL.

Olive oil: [Japanese Pharmacopoeia]

2-Oxy-1-(2'-oxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid: $C_{21}H_{14}N_2O_7S$ [2-Hydroxy-1-(2'-hydroxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid, Special grade]

8-Oxyquinoline: C_9H_6NOH [8-Quinolinol, Special grade]

8-Oxyquinoline test solution: Dissolve 20 mg (19.5~20.4 mg) of 8-oxyquinoline in 100 mL of sodium hydroxide (13 → 100).

Pancreatic digest of casein: Obtained by enzymatic hydrolysis of casein with pancreatin.

A greyish yellow powder, and having a characteristic odor. It is soluble in water, and develops pale yellow.

pH: 9.5~7.0 (2 % aqueous solution)

Nitrogen content: Not more than 10 %.

Amino nitrogen values / total nitrogen content determined by Van Slyke method (%):

0.25~0.50

Tryptophan content: Not more than 1.5 % (Determined by microbiological assay.).

Loss on drying: Not more than 7 %.

Residue on ignition: Not more than 15 %.

Papaic digest of soybean: The protein of soybean digested by enzymic hydrolysis with papain. Use appropriate commercial products.

Papaic digest of liver: The protein of liver of bull digested by enzymic hydrolysis with papain. Use appropriate commercial products.

Paraffin, liquid: [Light masses liquid paraffin, Japanese Pharmacopoeia]

Pectin: Pectin is acidic polysaccharide characteristic of non-lignified tissue on plant body. The parts of the structure of pectin acid is methyl-esterified. Colorless, odorless, no taste, amorphous material. Preserve 5 °C or below.

Peptone, animal tissue: Reddish yellow to brown powder, having a characteristic odor but not putrescent odor. It dissolves in water, and develops yellow-brown color, weakly acidic solution. It is insoluble in ethanol and in diethyl ether.

Nitrogen content: 14.0~16.5 %

Loss on drying: Not more than 7 %.

Residue on Ignition: Not more than 5 % (0.5 g).

Coagulable protein: On heating a solution of animal tissue peptone (1 → 70) to boiling: no precipitate is produced.

Protease: To 5 mL of the aqueous of animal tissue peptone add 20 mL of zinc sulfate solution (50 → 35): light precipitate is produced.

Peptidoglycan: A polysaccharide present in the cell wall of bacteria, etc. In this procedure, peptidoglycan derived from *Micrococcus lysodeikticus* is used.

Peptone, casein: Grayish yellow powder, and having a characteristic odor but not putrescent odor. It dissolves in water, but not in ethanol and in diethyl ether.

Degree of digestion:

A. Dissolve 1 g (0.5~1.4 g) of casein peptone in 10 mL of water, use this solution as the sample solution. Overlay 1 mL of the sample solution with 0.5 mL of a mixture of 10 mL of dilute ethanol and 1 mL of glacial acetic acid: no ring or precipitate forms at the junction of the two liquids, and on shaking, no turbidity results.

B. Mix 1 mL of the sample solution A with 4 mL of saturated solutions of zinc sulfate heptahydrate: a small quantity of precipitate is produced (proteoses).

C. Filter the mixture of A, and to 1 mL of the filtrate add 3 mL of water and 4 drops of bromine test solution: a red-purple color is produced.

Loss on drying: Not more than 7.0 % (0.5 g, 105 °C, constant mass).

Residue on ignition: Not more than 15.0 % (0.5 g).

Nitrogen content: Not less than 10.0 % (105 °C, constant mass, determine nitrogen content following nitrogen determination after drying at 105 °C).

Perchloric acid: HClO₄ [Special grade, Specific gravity about 1.67] Contains 70~72 % of HClO₄.

Periodic acid: HIO₄ [Metaperiodate, Special grade]

Periodic acid test solution: Dissolve 2.5 g (2.45~2.54 g) of periodic acid in water to make 100 mL, then add 400 mL of glacial acetic acid. Store protected from light.

Petroleum benzine: [Special grade]

Petroleum ether: [Special class]

o-Phenanthroline monohydrate: C₁₂H₈N₂·H₂O [Special grade]

***o*-Phenanthroline test solution:** Dissolve 0.15 g (0.145~0.154 g) of *o*-phenanthroline in 10 mL of a freshly prepared iron (II) sulfate heptahydrate solution (37 → 2,500) and 1 mL of dilute sulfuric acid. Preserve in tightly stoppered containers.

Phenol: C₆H₅OH [Japanese Pharmacopoeia]

Phenolphthalein: C₂₀H₁₄O₄ [Special grade] Color change: pH 8.3 (colorless) to pH 10.0 (pale red)

Phenolphthalein test solution: Dissolve 1 g (0.5~1.4 g) of phenolphthalein in 100 mL of ethanol.

Phenylhydrazine: C₆H₅NHNH₂ [Special grade]

Phloroglucin dihydrate: C₆H₃(OH)₃·2H₂O [Special grade]

Phloroglucin-hydrochloric acid test solution: Dissolve 0.1 g (0.05~0.14 g) of phloroglucin in 1 mL of ethanol, and add 9 mL of hydrochloric acid, mix well. Storage in a dark place.

Phosphate buffer solution, 0.1 mol/L, for digestion test: To 0.1 mol/L potassium dihydrogen phosphate test solution add 0.1 mol/L dipotassium hydrogen phosphate, and adjust specified pH.

Phosphate buffer solution, 0.02 mol/L, for digestion test: To 0.02 mol/L potassium dihydrogen phosphate test solution add 0.02 mol/L dipotassium hydrogen phosphate, and adjust the specified pH.

Phosphate buffer solution, pH 2.0: Dissolve 31.2 g (31.15~31.24 g) of disodium hydrogen phosphate in water to make 1,000 mL, adjust the pH with phosphoric acid (1 → 50) to 2.0.

Phosphate buffer solution, pH 3.5: Dissolve 7.8 g (7.75~7.84 g) of disodium hydrogen phosphate in water to make 1,000 mL, and adjust the pH with phosphoric acid (3 → 500) to 3.5.

Phosphate buffer solution, pH 7.0: Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate test solution and 29.54 mL of sodium hydroxide test solution, add water to make 200 mL.

Phosphate buffer solution, pH 7.5: Dissolve 22.2 g (22.15~22.24 g) of potassium dihydrogen phosphate and 177.8 g (177.75~177.84 g) of dipotassium hydrogen phosphate in water to make 1,000 mL.

Phosphate buffer solution, pH 8.0: Dissolve 3.06 g (3.055~3.064 g) of potassium dihydrogen phosphate in 450 mL of water, adjust the pH with 1 mol/L sodium hydroxide to 8.0, then add water to make 500 mL.

Phosphate-sodium hydrogen carbonate buffer solution: Dissolve 16.73 g (16.725~16.734 g) of dipotassium hydrogen phosphate, 0.523 g (0.5225~0.5234 g) of

potassium dihydrogen phosphate and 20.0g (19.95~20.04 g) sodium hydrogen carbonate in water to make 1,000 mL.

Phosphomolybdic acid n-hydrate: $P_2O_5 \cdot 24MoO_3 \cdot H_2O$ [Special grade]

Phosphoric acid: H_3PO_4 [Special grade]

Phosphoric acid-acetic acid-boric acid-sodium hydroxide buffer solution:

Dissolve 3.92 g (3.915~3.924 g) of phosphoric acid, 2.4 g (2.35~2.44 g) of acetic acid and 2.48 g (2.475~2.484 g) of boric acid in 1,000 mL of water. To 40 mL of this solution add 28 mL of 0.2 mol/L sodium hydroxide test solution, adjust the pH to 9.2. To 30 mL of this solution add 70 mL of ethanol, and mix well.

Phosphorus pentoxide: P_2O_5 [Special grade]

Phosphotungstic acid n-hydrate: $P_2O_5 \cdot 24WO_3 \cdot nH_2O$ [Special grade]

Phosphotungstic acid test solution: Dissolve 1 g (0.5~1.4 g) of phosphotungstic acid in water to make 100 mL.

Phosphorus staining test solution: Dissolve 1.12 g (1.115~1.124 g) ammonium vanadate in 300 mL of water, add 250 mL of nitric acid. To this solution add the aqueous solution in which is dissolved 27 g (26.5~27.4 g) of ammonium molybdate, and add water to make 1,000 mL.

o-phthalaldehyde $C_8H_4(CHO)_2$: It occurs as light yellow to yellow crystals.

Purity, related substances: Dissolve 1 g of L-isoleucine in 10 mL of ethanol, and use this solution as the test solution. Pipet 1 mL of the test solution exactly, add ethanol to make exactly 100 mL, and use this solution as the control solution. Pipet 10 μ L of each of the test solution and the control solution, perform gas chromatography under the following operating conditions, and determine the peak areas: the total area of the peaks other than the principal peak of the test solution is not larger than the principal peak area of the control solution.

However, time span of measurement shall be seven times as long as the retention time of the principal peak, beginning after the solvent peak.

Operating conditions

Detector: Thermal conductivity detector

Column filler:

Liquid phase: Methyl silicone polymer at a ratio of 10% against a carrier

Carrier: Acidized and silanized siliceous earth for gas chromatography (177 ~ 250 μ m in particle diameter)

Column tube: Glass column 3 mm in inside diameter and 2 m in length

Column temperature: A constant temperature of about 180 °C.

Carrier gas: Helium

Flow rate: Adjust so that the retention time of o-phthalaldehyde is three to four minutes at a constant volume of about 50 mL per minute.

Physiological saline: [Japanese Pharmacopoeia]

Phytic acid sodium salt hydrate: $C_6H_6O_{24}P_6Na_{12} \cdot xH_2O$ [Content: not less than 90 %.]

Picric acid: $HOC_6H_2(NO_2)_3$ [2,4,6-Trinitrophenol (picric acid), Special grade] Preserve in tightly stoppered bottle and remote from fire, in a cool place.

Picric acid test solution: Dissolve 1 g (0.5~1.4 g) of picric acid in 100 mL of hot water, cool, and filter if necessary.

Picric acid-toluene test solution: Dissolve 0.20 g (0.195~0.204 g) of picric acid in toluene to make 1,000 mL.

Polymixin B sulfate: [Japanese Pharmacopoeia]

Polysorbate 20: $C_{58}H_{114}O_{26}$ A mixture of sorbitol and sorbitol anhydride, partially esterified with lauric acid, and condensation polymer of 20 molecules of ethyleneoxide. Pale yellow to yellow liquid, having a characteristic odor. Specific gravity: 1.1. Viscosity (25 °C): 300~500 mPa·S.

Polysorbate 80: [Japanese Pharmacopoeia]

Polyvinyl alcohol: $(-CH_2CHOH-)_n$ White to pale yellow, granules or powder. It is freely soluble in water, slightly soluble in hot water, practically insoluble in ethanol and in ethyl ether.

Clarity and color of solution: Add 100 mL of water to 0.5 g (0.45~0.54 g) of polyvinyl alcohol, and shake well: the solution is clear.

Saponification value: 78~82 mol%

Weigh 0.5 g of polyvinyl alcohol to three decimal places, record the value, transfer to a 300 mL glass-stoppered conical flask, add 100 mL of water, allow to stand not less than 12 hours to dissolve. Add exactly 25 mL of 0.2 mol/L sodium hydroxide test solution with transfer pipet, allow to stand at ordinary temperature for 2 hours. Add exactly 25 mL of 0.1 mol/L sulfuric acid test solution with transfer pipet, shake well, titrate with 0.1 mol/L sodium hydroxide test solution (indicator: 2 drops of phenolphthalein test solution) until a red color of the solution disappears, the amount of 0.1 mol/L sodium hydroxide test solution consumed in the reaction is designated as *a* mL. Perform a blank determination in the same manner, the amount of 0.1 mol/L sodium hydroxide test solution consumed in the blank test is designated as *b* mL.

$$\text{saponification value (mol\%)} = 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A (\%) = \frac{0.6005 \times (a-b)f}{\text{amount (g) of polyvinyl alcohol taken}}$$

f: Molarity factor of 0.1 mol/L sodium hydroxide test solution

Polyvinyl alcohol test solution: Suspend 20.0 g (19.95~20.04 g) of polyvinyl alcohol in 800 mL of water, heat for 1 hour at 75~80 °C while stirring. Cool, filter if necessary, add water to make 1,000 mL.

Porous styrene-divinylbenzene copolymer: Porous styrene-divinylbenzene copolymer prepared for liquid chromatography, high quality.

Potassium Bromate: KBrO_3 [Special grade]

Potassium bromate-potassium bromide test solution: Dissolve 1.4 g (1.35~1.44 g) of potassium bromate and 8.1 g (8.05~8.14 g) of potassium bromide in water to make 100 mL.

Potassium bromide: KBr [Special grade]

Potassium bromide for infrared spectroscopy: Specified in an item of Potassium bromide, for infrared spectroscopy.

Potassium bromide, for infrared spectrophotometry: Crush homocrystals of potassium bromide or potassium bromide [Special grade], collect a powder passed through a No. 200 (74 μm) sieve, and dry at 120 °C for 10 hours or at 500 °C for 5 hours. Prepare tablets with this powder, and determine the infrared absorption spectrum: any abnormal absorption does not appear.

Potassium carbonate: K_2CO_3 [Potassium carbonate (anhydrous), Special grade]

Potassium chromate: K_2CrO_4 [Special grade]

Potassium chromate test solution: To 10 g (9.5~10.4 g) of potassium chromate add water to make 100 mL.

Potassium cyanide: KCN [Special grade]

Potassium cyanide test solution: Dissolve 1 g (0.5~1.4 g) of potassium cyanide in water to make 10 mL. Prepare before use.

Potassium dichromate: $\text{K}_2\text{Cr}_2\text{O}_7$ [Special grade]

Potassium dichromate (standard reagent): $\text{K}_2\text{Cr}_2\text{O}_7$ [Standard reagent for volumetric analysis]

Potassium dihydrogen phosphate: KH_2PO_4 [Monobasic potassium phosphate, Special grade]

0.2 mol/L Potassium dihydrogen phosphate for buffer solution: Specified in an item of Potassium dihydrogen phosphate, for buffer solution.

Potassium dihydrogen phosphate for pH determination: Specified in an item of Potassium dihydrogen phosphate, for pH determination.

Potassium dihydrogen phosphate test solution, 0.1 mol/L: Dissolve 13.61 g (13.605~13.614 g) of potassium dihydrogen phosphate in water to make 1,000 mL.

- Potassium dihydrogen phosphate test solution, 0.02 mol/L:** Dissolve 2.72 g (2.715~2.724 g) of potassium dihydrogen phosphate in water to make 1,000 mL.
- 0.2 mol/L Potassium dihydrogen phosphate test solution, for buffer solution:**
[Potassium dihydrogen phosphate, For pH determination] Dissolve 27.22 g (27.215~27.224 g) of potassium dihydrogen phosphate in water to make 1,000 mL.
- Potassium dihydrogen phosphate, for pH determination:** KH_2PO_4 [Monobasic potassium phosphate, For pH determination]
- Potassium hexacyanoferrate (II) test solution:** Dissolve 1 g (0.5~1.4 g) of potassium ferrocyanide in 10 mL of water (0.25 mol/L). Prepare before use.
- Potassium hexacyanoferrate (II) trihydrate:** $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ [Potassium ferrocyanide (yellow prussiate of potash), Special grade]
- Potassium hexacyanoferrate (III):** $\text{K}_3\text{Fe}(\text{CN})_6$ [Potassium hexacyanoferrate (red prussiate of potash), Special grade]
- Potassium hexacyanoferrate test solution:** Dissolve 1 g (0.5~1.4 g) of potassium hexacyanoferrate in 10 mL of water (0.33 mol/L). Prepare before use.
- Potassium hydrogen phthalate for pH determination:** Specified in an item of potassium hydrogen phthalate, for pH determination.
- Potassium hydrogen phthalate, (standard reagent):** $\text{C}_6\text{H}_4(\text{COOK})(\text{COOH})$ [Standard reagent for volumetric analysis]
- Potassium hydrogen phthalate, for pH determination:** $\text{C}_6\text{H}_4(\text{COOK})(\text{COOH})$ [For pH determination]
- Potassium hydrogen sulfate:** KHSO_4 [Potassium hydrogen sulfate (acidic potassium sulfate), Special grade]
- Potassium hydroxide:** KOH [Special grade] Potassium hydroxide contains not less than 85.0 % of KOH .
- Potassium hydroxide test solution:** Dissolve 6.5 g (6.45~6.54 g) of potassium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.
- Potassium hydroxide-ethanol test solution:** Dissolve 10 g (9.5~10.4 g) of potassium hydroxide in ethanol to make 100 mL. Prepare before use.
- Potassium hydroxide-ethanol test solution, dilute:** Dissolve 35 g (34.5~35.4 g) of potassium hydroxide in 20 mL of water, and add ethanol to make 1,000 mL (0.5 mol/L). Preserve in tightly stoppered bottles.
- Potassium iodate:** KIO_3 [Special grade]
- Potassium iodate (standard reagent):** KIO_3 [Standard reagent for volumetric analysis]
- Potassium iodide:** KI [Special grade]

Potassium iodide test solution: Dissolve 16.5 g (16.45~16.54 g) of potassium iodide in water to make 100 mL (1 mol/L). Store protected from light. Prepare before use.

Potassium iodide test solution, concentrated: Dissolve 30 g (29.5~30.4 g) of potassium iodide in 70 mL of water. Store protected from light. Prepare before use.

Potassium iodide-starch paper: Impregnate filter paper with freshly prepared potassium iodide-starch test solution, and dry in a clean room. Store in a glass-stoppered bottle, protect from light and moisture.

Potassium iodide-starch tests solution: Dissolve 0.5 g (0.45~0.54 g) of potassium iodide in 100 mL of freshly prepared starch test solution. Prepare before use.

Potassium periodate: KIO_4 [Special grade]

Potassium permanganate: KMnO_4 [Special grade]

Potassium permanganate test solution: Dissolve 3.3 g (3.25~3.34 g) of potassium permanganate in water to make 1,000 mL (0.02 mol/L).

Potassium peroxodisulfate: $\text{K}_2\text{S}_2\text{O}_8$ [Special grade]

Potassium sodium tartrate tetrahydrate: $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ [Potassium sodium tartrate (Rochelle salt (Seignette salt)), Special grade]

Potassium sulfate: K_2SO_4 [Special grade]

Potassium tetraoxalate for pH determination: Specified in an item of Potassium tetraoxalate, for pH determination.

Potassium tetraoxalate, for pH determination: $\text{KH}_3(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$ [For pH determination]

Potassium thiocyanate: KSCN [Special grade]

Potassium thiocyanate test solution: Dissolve 1 g (0.5~1.4 g) of potassium thiocyanate in water to make 10 mL.

Potassium iodate-starch paper: Impregnate filter paper for assay in the mixture of equal volumes of 0.2 % KIO_3 solution and starch test solution, and air-dry in a dark place. Protect from light, and preserve in tightly stoppered containers.

Potassium chloride: KCl [Special grade]

Potassium chloride-hydrochloric acid buffer solution: To a mixture of 250 mL of potassium chloride solution (3 → 20) and 53 mL of hydrochloric acid (2 mol/L) add water to make 1,000 mL.

Potassium pyroantimonate: $\text{K}_2\text{H}_2\text{Sb}_2\text{O}_7 \cdot 4\text{H}_2\text{O}$ [First grade]

Potato starch: [Japanese Pharmacopoeia]

Potassium pyroantimonate test solution: To 2 g (1.5~2.4 g) of pyroantimonate add 100 mL of water, boil for 5 minutes, cool within 30 seconds. To this solution add 10 mL of potassium hydroxide solution (3 → 20). Allow to stand for 1 day, filter.

n-Propanol: $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$ [n-Propyl alcohol (n-propanol), Special grade]

Propanol, iso: $(\text{CH}_3)_2\text{CHOH}$ [Isopropyl alcohol (isopropanol), Special grade]

Proteose peptone: Light yellow powder or particles.

Loss on drying: Not more than 3 % (1 g, 85 °C, 1 hour).

Solubility: Sterilize the solution (1 → 100) in an autoclave at 121 °C for 15 minutes: no insoluble substance in the solution.

pH: Sterilize the solution (1 → 100) in a autoclave at 121 °C for 15 minutes: the pH of the solution should be between 6.5 and 7.5.

Purified porous styrene-divinylbenzene copolymer resin: Immerse porous styrene-divinyl benzene copolymer to 20 times amount of methanol for 30 minutes, stirring, and discard supernatant, then treat by 20 times amount of water, 10 times amount of acetone, 10 times amount of mixture of methanol-0.2 mol/L hydrochloric acid test solution (7:3), 20 times of water and methanol (1 → 2) in a sequential order, and immerse into methanol (1 → 2) before use.

Purified water: Purified water is prepared from Water by distillation or ion exchange.

Physical and chemical property: Purified water is clear and colorless liquid, having no odor and taste.

Acidity or alkalinity: pH 5~7.

Chloride: To 50 mL of purified water add 3 drops of nitric acid and 0.5 mL of silver nitrate test solution: the solution remains unchanged.

Sulfate: To 50 mL of purified water add 0.5 mL barium chloride test solution: the solution remains unchanged.

Ammonium salt: To 50 mL of purified water add 0.5 mL of Nessler's reagent: the solution remains unchanged.

Heavy metal: To 40 mL of purified water add 1 mL of diluted acetic acid and 10 mL of freshly prepared hydrogen sulfide test solution, and allow to stand for 10 minutes: the color of the solution should not be deeper than that of 50 mL of purified water added 1 mL of diluted acetic acid.

Potassium permanganate-reducing substances: To 100 mL of purified water add 10 mL of dilute sulfuric acid, and boiled, add 0.1 mL of 0.02 mol/L potassium permanganate, and boiled for 10 minutes again: the color of the solution does not disappear.

Residue on evaporation: Evaporate to dryness 100 mL of purified water in water bath, and dry at 100 °C until its weight becomes constant: the residue is not more than 1 mg.

Pyridine: $\text{C}_5\text{H}_5\text{N}$ [Special grade]

Pyridine for Karl Fisher's Method: Specified in an item of Water Determination in the paragraph of General tests.

Pyridine, anhydrous: C_5H_5N To 100 mL of pyridine add 10 g (9.5~10.4 g) of sodium hydroxide, allow to stand for 24 hours, decant the supernatant liquid, and distillate.

Pyridine, for Karl-fischer's Method: Specified in an item of Water determination in the paragraph of General tests.

Pyridoxine hydrochloride: $C_8H_{11}NO_3 \cdot HCl$ [Japanese Pharmacopoeia]

Pyrogallol: $C_6H_3(OH)_3$ [Special grade]

Pyrole: C_4H_5N [Special grade]

Quinaldine red: ($C_{21}H_{23}N_2I$) Quinaldine red occurs as a crystalline powder. It is soluble in ethanol. The wavelength of absorption maximum of this ethanol solution (0.005 → 1,000) appears in 526 nm. Absorbance on the wavelength of absorption maximum is not less than 0.5.

Quinaldine red test solution: Dissolve 0.1 g (0.05~0.14 g) of quinaldine red in acetic acid to make 100 mL. Prepare before use.

Raffinose pentahydrate: $C_{18}H_{32}O_{16} \cdot 5H_2O$

Optical rotation: $[\alpha]_D^{20} = +122^\circ \sim +124^\circ$

Heavy metal: Not more than 10 µg/g.

Water: 14~16 %

Red litmus paper: Specified in an item of Litmus paper, red.

Reinecke salt: $NH_4 [Cr(NH_3)_2(SCN)_4] H_2O$ [First grade]

Reinecke salt test solution: To 0.5 g (0.45~0.54 g) of reinecke salt add 20 mL of water, shake frequently for 1 hour, then filter. Use within 48 hours.

Resorcin: $C_6H_4(OH)_2$ [Special grade]

Resorcinol $C_6H_4(OH)_2$: [Special grade]

Resorcinol-sulfuric acid TS: Dissolve 0.1 g of resorcinol in 10 mL of sulfuric acid (1 ~ 10).

Resorcin: As specified in the section on resorcinol.

Riboflavin: $C_{17}H_{20}N_4O_6$ [Japanese Pharmacopoeia]

Safranine: Safranine occurs as a nearly black powder or small masses, and sparingly soluble in water and in ethanol.

Clarity and color of solution: To 0.1 g (0.05~0.14 g) of safranine add 100 mL of water, heat in a water bath to dissolve: the solution is nearly clear.

Absorbance: Weigh 0.1 g of safranine to three decimal places, record the value, add water to dissolve, transfer to a 100 mL volumetric flask, add water to make exactly 100 mL, and use this solution as the sample stock solution. Dilute the sample stock solution with water and prepare exactly 200 times diluted solution, and use this solution as sample solution. Determine the absorbances at 517 nm: the measuring result is given in 0.45~0.65.

Loss on drying: Not more than 5 % (1 g, 105 °C, 4 hours).

Residue on ignition: Not more than 1.0 %.

Salt: NaCl [Sodium chloride, White]

Silica gel: An amorphous, partly hydrated silicic acid occurring in glassy granules of various sizes. When used as a desiccant, it is frequently coated with a substance that changes color when the capacity to absorb water is exhausted. Such colored products may be regenerated by being heated at 110 °C until the gel assumes the original color.

Loss on ignition: Not more than 6 % (2 g, 950 ± 50 °C).

Water absorption: Not less than 31 %.

Weigh 10 g of silica gel to three decimal places, record the value. Allow to stand for 24 hours in a closed container in which the atmosphere is maintained at 80 % relative humidity with sulfuric acid having a specific gravity of 1.19. Weigh again, and calculate the increase in mass.

Silica gel for liquid chromatography: Specified in an item of Silica gel for liquid chromatography.

Silica gel for thin-layer chromatography: Specified in an item of Silica gel, for thin-layer chromatography.

Silica gel for thin-layer chromatography (with fluorescent indicator): Specified in an item of Silica gel (with fluorescent indicator), for thin-layer chromatography.

Silica gel with fluorescent indicator, for thin-layer chromatography: To Silica gel for thin-layer chromatography add fluorescent indicator.

Silica gel, for liquid chromatography: High quality porous silica gel prepared for liquid chromatography.

Silica gel, for thin-layer chromatography: High quality silica gel prepared for thin-layer chromatography.

Siliceous earth, for chromatography: White or grey white color, use high quality one.

Silicone emulsions: Silicone emulsions are milky white liquid which disperse the silicone compounds in water with emulsifier. It is miscible with water.

pH: 2~5 (25 °C)

Residue on evaporation residue: Not less than 11.5 % (1 g, 105 °C, 2 hours).

Silicone oil: Colorless clear liquid, having no odor. Use silicone oil having a viscosity in the range of 50 to 100 mm²/s at an ordinary temperature.

Silver N,N-diethyldithiocarbamate: C₅H₁₀AgNS₂ [Japanese Pharmacopoeia]

Silver nitrate: AgNO₃ [Special grade]

Silver nitrate test solution: To 17.5 g (17.45~17.54 g) of silver nitrate add water to make 1,000 mL (0.1 mol/L). Preserve in light-resistant containers.

Sodium acetate test solution: To 13.6 g (13.55~13.64 g) of sodium acetate add water to make 100 mL (1 mol/L)

Sodium acetate test solution, 0.2 mol/L: Dissolve 27.2 g (27.15~27.24 g) of sodium acetate in water to make 1,000 mL.

Sodium acetate test solution, 0.1 mol/L: To 100 mL of sodium acetate test solution add water to make 1,000 mL.

Sodium acetate trihydrate: $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$ [Special grade]

Sodium acetate, anhydrous: CH_3COONa [Sodium acetate (anhydrous), Special grade]

Sodium Alizarinesulfonate: $\text{C}_{14}\text{H}_5\text{O}_2(\text{OH})_2\text{SO}_3\text{Na}\cdot \text{H}_2\text{O}$ [Special grade]

Sodium arsenate heptahydrate: $\text{Na}_2\text{HAsO}_4\cdot 7\text{H}_2\text{O}$ [Special grade]

Sodium arsenate heptahydrate test solution: Dissolve 6.0 g (5.95~6.04 g) of sodium arsenate heptahydrate in water to make 50 mL.

Sodium ascorbate: $\text{C}_6\text{H}_7\text{NaO}_6$ [Special grade]

Sodium borate decahydrate: $\text{Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}$ [Sodium borate (borax), Special grade]

Sodium borate for pH determination: Specified in at the item of sodium borate, for pH determination.

Sodium borate, for pH determination: [Sodium borate (borax), for pH determination]

Sodium borohydride: NaBH_4 White to greyish white, crystals, powder or masses. It is freely soluble in water, and very slightly soluble in diethyl ether. Decomposed at 400 °C, having an inflammability and a hygroscopicity. Should be stored remote from fire.

Sodium borohydride test solution: Dissolve 4.0 g (3.95~4.04 g) of sodium borohydride in sodium hydroxide test solution to make 100 mL.

Sodium bromide: NaBr [Special grade]

n-Sodium butyrate: $\text{CH}_3\text{CH}_2\text{CH}_2\text{COONa}$ White to practically white powder.

Clarity and color of solution: The solution (1 → 20) is clear or having a faint.

Content: Not less than 90 %.

Sodium carbonate (standard reagent): Na_2CO_3 [Standard reagent for volumetric analysis]

Sodium carbonate decahydrate: $\text{Na}_2\text{CO}_3\cdot 10\text{H}_2\text{O}$ [Special grade]

Sodium carbonate for pH determination: Specified in an item of Sodium carbonate, for pH determination.

Sodium carbonate test solution: Dissolve 10.5 g (10.45~10.54 g) of anhydrous sodium carbonate in water to make 100 mL (1 mol/L).

Sodium carbonate test solution, 0.55 mol/L: Dissolve 58.3 g (58.25~58.34 g) of anhydrous sodium carbonate in water to make 1,000 mL.

Sodium carbonate, anhydrous: Na_2CO_3 [Sodium carbonate (anhydrous), Special grade]

Sodium carbonate, for pH determination: Na_2CO_3 [Sodium carbonate (anhydrous), for pH determination]

Sodium carboxymethyl cellulose: [Japanese Pharmacopoeia] Degree of substitution should be 0.62 to 0.68.

Sodium chloride: NaCl [Special grade]

Sodium chloride (standard reagent): NaCl [Standard reagent for volumetric analysis]

Sodium chloride test solution: Dissolve 10 g (9.5~10.4 g) of sodium chloride in water to make 100 mL.

Sodium chloride-sodium hydroxide test solution: To 100 g (99.5~100.4 g) of sodium chloride add 40 mL of 2.5 mol/L sodium hydroxide and water to make 1,000 mL.

Sodium citrate buffer solution: To 980 g (979.5~980.4 g) of sodium citrate add 3,500 mL of water, 700 mL of hydrochloric acid and 5 mL of octanoic acid. Adjust the pH to 2.2 with 1 mol/L hydrochloric acid, add water to make 5,000 mL. To 500 mL of this solution add 500 mL of 20 v/v% thiodiethylene solution and 3,500 mL water, adjust the pH to 2.2 with 1 mol/L hydrochloric acid, add water to make 5,000 mL.

Sodium citrate dihydrate: $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ [Special grade]

Sodium dihydrogen phosphate test solution, pH 4.5: Dissolve 15.6 g (15.55~15.64 g) of sodium dihydrogen phosphate in water to make 1,000 mL. If necessary, add 1 mol/L sodium hydroxide test solution or phosphoric acid (1 → 10) to adjust the pH to 4.5.

Sodium fluoride: NaF [Special grade]

Sodium fluoride (standard reagent): NaF [Standard reagent for volumetric analysis]

Sodium formate for assay: Specified in an item of Sodium formate, for assay.

Sodium formate, for assay: HCOONa [Special grade]

Sodium hexanitrocobaltate (III): $\text{Na}_3\text{Co}(\text{NO}_2)_6$ [Special grade]

Sodium hexanitrocobaltate (III) test solution: To 10 g (9.5~10.4 g) of sodium hexanitrocobaltate (III) add water to make 50 mL, and filter if necessary. Prepare before use.

Sodium hydrogen carbonate: NaHCO_3 [Sodium hydrogen carbonate (sodium bicarbonate), Special grade]

Sodium hydrogen carbonate for pH determination: Specified in an item of Sodium hydrogen carbonate, for pH determination.

Sodium hydrogen carbonate, for pH determination: NaHCO_3 [Sodium hydrogen carbonate (sodium bicarbonate), for pH determination]

Sodium hydrogen carbonate-methanol test solution: Dissolve 0.25 g (0.245~0.254 g) of sodium hydrogen carbonate in methanol to make 1,000 mL, and filter.

Sodium hydrogen carbonate-sodium hydroxide buffer solution (pH 9.0): Dissolve 84.0 g (83.95~84.04 g) of sodium hydrogen carbonate in water to make 900 mL. To this

solution add sodium hydroxide solution (3 → 10) to adjust pH at 9.0, and add water to make 1,000 mL.

Sodium hydrogen sulfite: NaHSO₃ [Special grade]

Sodium hydrogen tartrate monohydrate: NaHC₄H₄O₆·H₂O [Special grade]

Sodium hydrogen tartrate monohydrate test solution: Dissolve 1 g (0.5~1.4 g) of sodium bitartrate in water to make 10 mL (0.5 mol/L). Prepare before use.

Sodium hydrosulfite: Na₂S₂O₄ [Sodium hydrosulfite (hydrosulfite), First grade]

Sodium hydroxide: NaOH [Special grade] Sodium hydroxide contains not less than 95.0 % of NaOH.

5 % Sodium hydroxide in methanol test solution: Dissolve 5 g (4.5~5.4 g) of sodium hydroxide in 5 mL of water, add methanol to make 100 mL. Allow to stand, and use the supernatant.

Sodium hydroxide test solution, 2.5 mol/L: Dissolve 107 g (106.5~107.4 g) of sodium hydroxide add water to make 1,000 mL. Preserve in polyethylene bottles.

Sodium hydroxide test solution, 2 mol/L: Dissolve 86 g (85.5~86.4 g) of sodium hydroxide add water to make 1,000 mL. Preserve in polyethylene bottles.

Sodium hydroxide test solution, 1 mol/L: Dissolve 4.3 g (4.25~4.34 g) of sodium hydroxide add water to make 100 mL. Preserve in polyethylene bottles.

Sodium hydroxide test solution, 0.5 mol/L: Dissolve 22 g (21.5~22.4 g) of sodium hydroxide add water to make 1,000 mL. Preserve in polyethylene bottles.

Sodium hydroxide test solution, 0.2 mol/L: To 200 mL of 1 mol/L sodium hydroxide test solution add water to make 1,000 mL. Preserve in polyethylene bottles.

Sodium hydroxide test solution, 0.05 mol/L: To 50 mL of 1 mol/L sodium hydroxide test solution add water to make 1,000 mL. Preserve in polyethylene bottles.

Sodium hydroxide test solution, dilute: Dissolve 4.3 g (4.25~4.34 g) of sodium hydroxide in freshly boiled and cooled water to make 1,000 mL (0.1 mol/L). Prepare before use.

Sodium hydroxide test solution, for digestion test: Dissolve 32.0 g (31.95~32.04 g) of sodium hydroxide in water to make 300 mL. Preserve in polyethylene bottles.

0.1 mol/L Sodium hydroxide-methanol test solution: Dissolve 4.5 g (4.45~4.54 g) of sodium hydroxide in methanol to make 1,000 mL. Filter with glass filter.

0.05 mol/L Sodium hydroxide-methanol test solution: To 50 mL of 0.1 mol/L sodium hydroxide-methanol test solution add methanol to make 100 mL.

Sodium hypochlorite test solution: Prepare the solution by passing chlorine into 1 mol/L sodium hydroxide while cooling with ice, so as to contain 5 % of sodium hypochlorite (NaClO: 74.44). Prepare before use.

Sodium hypochlorite test solution, for ammonium limit test: Clear, colorless or pale green-yellow solution prepared by passing chlorine into sodium hydroxide or sodium carbonate decahydrate solution.

Content: Not less than 4.2 g/dL as sodium hypochlorite (NaClO: 74.44)

Assay: Measure 10 mL of sodium hypochlorite test solution for ammonium limit test with transfer pipet, transfer into a 100 mL volumetric flask, and add water to make exactly 100 mL. Transfer exactly 10 mL of this solution into a stoppered flask with transfer pipet, add 90 mL of water, then add 2 g (1.5~2.4 g) of potassium iodide and 6 mL of acetic acid (1 → 2), stopper tightly, shake well, and allow to stand in a dark place for 5 minutes. Titrate the free iodine with 0.1 mol/L sodium thiosulfate solution (Indicator, 3 mL of starch test solution). Perform a blank determination in the same manner, and make any necessary correction.

1 mL of 0.1 mol/L sodium thiosulfate solution = 3.722 mg of NaClO

Sodium molybdate (VI) dihydrate: $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ [Special grade]

Sodium nitrite: NaNO_2 [Special grade]

Sodium nitrite test solution: Dissolve 10 g (9.5~10.4 g) of sodium nitrite in water to make 100 mL. Prepare before use.

Sodium nitroprusside: $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ [Special grade]

Sodium nitroprusside test solution: Dissolve 1 g (0.5~1.4 g) of sodium nitroprusside in 20 mL of water. Prepare before use.

Sodium oxalate (standard reagent): $\text{C}_2\text{O}_4\text{Na}_2$ [Standard reagent for volumetric analysis]

Sodium pentacyanoammine ferroate (II): Specified in an item of Sodium pentacyanoammine ferroate (II) n-hydrate.

Sodium pentacyanoammine ferroate (II) n-hydrate: $\text{Na}_3[\text{Fe}(\text{CN})_5\text{NH}_3] \cdot n\text{H}_2\text{O}$ [First grade]

Sodium periodate: NaIO_4 [Special grade]

Sodium periodate test solution: Dissolve 25 g (24.5~25.4 g) of sodium metaperiodate in water to make 100 mL.

Sodium picrate monohydrate: $(\text{NO}_2)_3\text{C}_6\text{H}_2\text{ONa} \cdot \text{H}_2\text{O}$ [Special grade]

Sodium picrate test solution: To 0.4 g (0.35~0.44 g) of sodium picrate monohydrate and 4 g (3.5~4.4 g) of sodium hydroxide add water to make 1,000 mL.

Sodium propionate for assay: $\text{C}_2\text{H}_5\text{COONa}$ White crystals or a crystalline powder or particles. Odorless or slightly characteristic odor.

Clarity and color of solution: To 1.0 g (0.5~1.4 g) of sodium propionate add 20 mL of water: the solution is colorless, and having a faint.

Loss on drying: Not more than 0.5 % (1 g, 110 °C, 3 hours).

Content: Not less than 99 %.

Sodium propionate for assay: Specified in an item of Sodium propionate, for assay.

Sodium sulfate, anhydrous: Na_2SO_4 [Sodium sulfate (anhydrous), Special grade]

Sodium sulfide ennehydrate: $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ [Special grade]

Sodium sulfide test solution: Prepare according to either of the following methods.

A. Dissolve 5 g (4.5~5.4 g) of sodium sulfide in a mixture of 10 mL of water and 30 mL of glycerin.

B. Dissolve 5 g (4.5~5.4 g) of sodium hydroxide in a mixture of 30 mL of water and 90 mL of glycerin, saturate a half volume of this solution with hydrogen sulfide, while cooling, and mix with the remaining half. Preserve in well-filled, light-resistant bottles. Use within 3 months.

Sodium sulfite heptahydrate: $\text{Na}_2\text{SO}_3\cdot 7\text{H}_2\text{O}$ [Special grade]

Sodium thioglycolate: $\text{HSCH}_2\text{COONa}$ [Japanese Pharmacopoeia]

Sodium thiosulfate pentahydrate: $\text{Na}_2\text{S}_2\text{O}_3\cdot 5\text{H}_2\text{O}$ [Special grade]

Sodium tungstate (VI) dihydrate: $\text{Na}_2\text{WO}_4\cdot 2\text{H}_2\text{O}$ [Special grade]

Sodium, metal: Na [Sodium, Special grade]

Soluble starch: [Special grade]

Soluble starch test solution: Triturate 1 g (0.5~1.4 g) of soluble starch in 10 mL of cooled water, pour gradually into 90 mL of boiled water while constantly stirring, boil gently for 3 minutes, and cool. Prepare before use.

Soy peptone: Soy peptone occurs as a pale yellow powder.

Loss on drying: Not more than 5 % (1 g, 85 °C, 1 hour).

Solubility: Sterilize soy peptone solution (1 → 100) in an autoclave at 121 °C for 15 minutes: no insoluble substance in the solution.

pH: Sterilize soy peptone solution (1 → 100) in an autoclave at 121 °C for 15 minutes: the pH of this solution should be 7.0 to 7.5.

Starch: [Special grade]

Starch test solution: Triturate 1 g (0.5~1.4 g) of starch in 10 mL of cold water, pour gradually into 200 mL of boiled water while constantly stirring, boil until the solution has become translucent, allow to stand, use supernatant liquid. Prepare before use.

Stigmasterol: $\text{C}_{29}\text{H}_{48}\text{O}$ [Special grade]

Stigmasterol acetate: Specified in an item of Vitamin D assay in the paragraph of General tests.

Strong ammonia water: Specified in an item of Ammonia water, strong.

Strong hydrogen peroxide water: Specified in at the item of Hydrogen peroxide water, strong.

Strongly acidic cation exchange resins: Prepared for high performance liquid chromatography composed with styrene-divinylbenzene copolymer which is introduced sulfonate group (degree of cross-linking: 8 %).

Strontium nitrate: $\text{Sr}(\text{NO}_3)_2$ [Strontium nitrate (anhydrous), Special grade]

Sulfamic acid (standard reagent): HOSO_2NH_2 [Standard reagent for volumetric analysis]

Sulfanilamide: $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2$ [Special grade]

Sulfanilamide test solution: Dissolve 1 g (0.5~1.4 g) of sulfanilamide add ethanol to make 100 mL. To this solution add 100 mL of 0.5 mol/L sulfuric acid to make 200 mL. Prepare before use.

Sulfanilic acid: $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_3\text{H}$ [Special grade]

Sulfanilic acid test solution: To 4 g (3.5~4.4 g) of sulfanilic acid add dilute sulfuric acid to make 250 mL.

Sulfate acid, for readily carbonizable substances: To sulfuric acid, the content of which previously been determined by the following method, add water cautiously, and adjust the final concentration to 94.5~95.5 % of sulfuric acid (H_2SO_4). When the concentration is changed owing to absorption of water during storage, prepare freshly.

Assay: Weigh about 2 g of sulfuric acid to two decimals places, record the value, put in a glass-stoppered flask rapidly, add 30 mL of water, cool, and titrate the solution with 1 mol/L sodium hydroxide solution (indicator: 2~3 drops of bromthymol blue test solution)
1 mL of 1 mol/L sodium hydroxide solution = 49.04 mg of H_2SO_4

Sulfuric acid: H_2SO_4 [Special grade] It contains not less than 95.0 % of H_2SO_4 .

Sulfuric acid for readily carbonizable substances: Specified in an item of Sulfate acid, for readily carbonizable substances.

Sulfuric acid test solution, 3 mol/L: Add 180 mL of sulfuric acid in 1,000 mL of water slowly while stirring, then allow to cool.

Sulfuric acid test solution, 2.5 mol/L: Add 150 mL of sulfuric acid in 1,000 mL of water slowly while stirring, then allow to cool.

Sulfuric acid test solution, 2 mol/L: Add 120 mL of sulfuric acid in 1,000 mL of water slowly while stirring, then allow to cool.

Sulfuric acid test solution, 0.5 mol/L: Add 30 mL of sulfuric acid in 1,000 mL of water slowly while stirring, then allow to cool.

Sulfuric acid test solution, 0.2 mol/L: Add 12 mL of sulfuric acid in 1,000 mL of water slowly while stirring, then allow to cool.

Sulfuric acid test solution, 0.1 mol/L: Add 6 mL of sulfuric acid in 1,000 mL of water slowly while stirring, then allow to cool.

Sulfuric acid, dilute: Cautiously add 5.7 mL of sulfuric acid to 10 mL of water, cool, and dilute with water to make 100 mL (10 %).

Tetrabutylammonium hydroxide test solution: [Japanese Pharmacopoeia]

Tetrahydrofuran: C₄H₈O [Special grade]

Thiamine chloride hydrochloride: C₁₂H₁₇ClN₄OS·HCl [Japanese Pharmacopoeia]

Thiodiethylene glycol: S(CH₂CH₂OH)₂ [β -Thiodiglycol, Japanese Pharmacopoeia]

Thionyl chloride: SOCl₂ [Special grade]

Thymol: CH₃C₆H₃(OH)CH(CH₃)₂ [Japanese Pharmacopoeia]

Thymol blue: C₂₇H₃₀O₅S [Special grade] Color change: Acidic side pH 1.2 (red) to 2.8 (yellow), Alkali side pH 8.0 (yellow) to pH 9.6 (blue)

Thymol blue test solution: Dissolve 0.1 g (0.05~0.14 g) of thymol blue in ethanol to make 100 mL. Filter if necessary.

Thymol blue-dimethylformamide test solution: Dissolve 0.1 g (0.05~0.14 g) of thymol blue in dimethylformamide to make 100 mL.

Thymolphthalein: C₂₈H₃₀O₄ [Special grade] Color change: pH 9.3 (colorless) to pH 10.5 (blue).

Thymolphthalein test solution: Dissolve 0.1 g (0.05~0.14 g) of thymolphthalein in ethanol to make 100 mL. Filter if necessary.

Thymol-sulfuric acid test solution: Dissolve 0.5 g (0.45~0.54 g) of thymol in 5 mL of sulfuric acid, and add ethanol to make 100 mL.

Tin (II) chloride dihydrate: SnCl₂·2H₂O [Special grade]

Tin (II) chloride test solution, acidic: Dissolve 8 g (7.5~8.4 g) of tin (II) chloride dihydrate in 500 mL of hydrochloric acid. Storage in a glass-stoppered bottle and use within 3 months.

***dl*- α -Tocopherol acetate for assay:** Specified in an item of *dl*- α -Tocopherol acetate, for assay.

***dl*- α -Tocopherol acetate, for assay:** A crude material for manufacturing *dl*- α -tocopherol acetate. Not less than 98 % of *dl*- α -tocopherol acetate for assay.

Toluene: C₆H₅CH₃ [Special grade]

***o*-Toluene sulfonamide:** C₇H₉NO₂S *o*-Toluene sulfonamide occurs as colorless crystals or a white crystalline powder.

Melting point: 157~160 °C

Purity: *p*-Toluene sulfonamide Proceed with *o*-toluene sulfonamide-ethyl acetate (1 → 5,000) solution according to gas chromatography method under operation condition of purify test No. 6 of a crude material for manufacturing saccharin sodium: no other peak than *o*-toluene sulfonamide appears.

Toluene, anhydrous: $C_6H_5CH_3$ To 500 mL of toluene add 10 g (9.5~10.4 g) of anhydrous sodium sulfate, allow to stand for 12 hours, remove sodium sulfate.

Trichloroacetic acid: CCl_3COOH [Special grade]

Trichloroacetic acid test solution A: Dissolve 7.20 g (7.195~7.204 g) of trichloroacetic acid in water to make 100 mL.

Trichloroacetic acid test solution B: Dissolve 1.80 g (1.795~1.804 g) of trichloroacetic acid and 1.80 g (1.795~1.804 g) of anhydrous sodium acetate in 5.5 mL of 6 mol/L acetic acid and water, add water to make 100 mL.

Triethylamine: $(C_2H_5)_3N$ [Japanese Pharmacopoeia]

Trimethylamine hydrochloride: $(CH_3)_3N \cdot HCl$ [Special grade]

Triphenylchloromethane: $(C_6H_5)_3CCl$ [Triphenylchloromethane (trityl chloride), Special grade]

Trypsin: Prepare with pancreas of bovine.

Degree of digestion: To 0.01 g (0.005~0.014 g) of trypsin add water to make 500 mL, and use this solution as the sample solution. To 2 mL of this solution add 5 mL of casein test solution and 3 mL of water, mix and allow to stand at 40 °C for 1 hour, and add three drops of acetic acid-ethanol test solution: no precipitate in the solution.

DL-Tryptophan: $C_{11}H_{12}N_2O_2$ DL-Tryptophan occurs as a white to slightly brown crystalline powder. It is freely soluble in diluted hydrochloric acid, slightly soluble in water, and very slightly soluble in ethanol.

Clarity and color of solution: To 1 g (0.5~1.4 g) of DL-tryptophan add 20 mL of water, dissolve with the aid of heat: the solution is clear.

Chloride: Not more than 0.02 %.

Sulfate: Not more than 0.03 %.

Loss on drying: Not more than 0.3 % (1 g, 105 °C, 3 hours).

Residue on ignition: Not more than 0.05 % (1 g).

Nitrogen content: 13.4~13.8 % (Determined by Nitrogen determination)

Tryptose: Mixture of casein peptone and proteose peptone.

Uracil: $C_4H_4N_2O_2$ [First grade]

Vanillin: $C_6H_3CHO(OCH_3)OH$ White crystals, having characteristic odor and taste.

Melting point: 81~83 °C

Loss on drying: Not more than 1.0 % (1 g, Silica gel, for 4 hours).

Residue on ignition: Not more than 0.05 % (1 g).

Store protected from light.

Vanillin-hydrochloric acid test solution: Dissolve 10 mg (9.5~10.4 mg) of vanillin in 1 mL of ethanol, add 1 mL of water and 6 mL of hydrochloric acid. Prepare before use.

Vanillin-sulfuric acid-ethanol color developing test solution: Dissolve 3 g (2.5~3.4 g) of vanillin in anhydride ethanol to make 100 mL, to this solution add 3 mL of sulfuric acid.

Vanillin-sulfuric acid-ethanol test solution: Dissolve 3 g (2.5~3.4 g) of vanillin in anhydride ethanol to make 100 mL, to this solution add 0.5 mL sulfuric acid.

Weak carbol-fuchsin solution: To 100 mL of phenol (5 → 100) add 10 mL of basic fuchsin solution, and dilute with water 10 to 20 times. Prepare before use.

Weakly acidic cation exchange resins: Prepared for liquid chromatography composed with methacrylic acid divinylbenzene copolymer, high quality.

Weakly acidic cation exchange resins column: Disperse weakly acid cation exchange resins in 0.5 mol/L of sulfuric acid and allow to stand for 3 hours. Wash with water until the washings become neutral, and add lithium hydroxide to keep the pH between 7 and 8 while stirring. After the pH value has become stable, allow to stand overnight. Wash with water not less than 5 times, and adjust the pH at 7.0 with phosphoric acid (1 → 25), then preserve. Packed with weakly acidic cation exchange resins to a column 1 cm in inside diameter until 10 cm in height. Wash with not less than 25 mL of water just before loading test solution.

Xylan: Xylan is a complicated polysaccharide obtained by alkaline extraction of vegetable fiber, and produces xylose by hydrolysis. Preserve not exceeding 5 °C.

Xylene cyanol FF: C₂₅H₂₇N₂NaO₇S₂ [First grade]

Xylose: C₅H₁₀O₅ Xylose occurs as white to light brown color crystalline powder or powder.

Clarity and color of solution: Dissolve 1 g (0.5~1.4 g) of xylose in 20 mL of water: the solution is clear and colorless.

Optical rotation: Weigh 20 g of xylose to one decimal place, record the value, add water to dissolve, transfer to a 100 mL volumetric, add water to make exactly 100 mL: the specific optical rotation $[\alpha]_D^{20}$ of this solution in a 100 mm cell is between +18.0 and +20.0°.

Loss on drying: Not more than 0.3 % (1 g, 105 °C, 2 hours).

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

Yeast extract: A peptone-like substance which represents all the soluble product of yeast cells (*Saccharomyces*) prepared under optimum conditions, clarified, and dried by evaporation to a powder. 1 g of yeast extract represents not less than 7.5 g of yeast. A reddish yellow to brown powder, having a characteristic but not putrescent odor. Soluble in water, forming a yellow to brown solution, having a slight acidic reaction. It contains no added carbohydrate. Coagulable protein: On heating a solution of yeast extract (1 → 20) to boiling, no precipitate is produced.

Chloride: (calculated as NaCl) Not more than 5 %.

Loss on drying: Not more than 5.0 % (0.5 g, 105 °C, Constant mass).

Residue on ignition: Not more than 15.0 % (0.5 g).

Nitrogen content: 7.2~9.5 % (105 °C, Constant mass, After drying, Determine nitrogen content following nitrogen determination.).

Yellow mercury (II) oxide: Specified in an item of Mercury (II) oxide, yellow.

Zinc (standard reagent): Zn [Standard reagent for volumetric analysis]

Zinc acetate dihydrate: $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ [Special grade]

Zinc dust: Zn [Special grade]

Zinc iodide-starch paper: Impregnate the filter paper for volumetric analysis with freshly prepared zinc iodide-starch test solution, and dry it in the clean room. Preserve in a glass-stoppered bottle, protected from light and moisture.

Zinc iodide-starch test solution: To 100 mL of boiling water add a solution of 0.75 g (0.745~0.754 g) of potassium iodide in 5 mL of water, a solution of 2 g (1.5~2.4 g) of zinc chloride in 10 mL of water and a smooth suspension of 5 g (4.5~5.4 g) of starch in 30 mL of water, with stirring. Continue to boil for 2 minutes, then cool. Preserve in tightly stoppered bottles, in a cool place.

Sensitivity: Dip a glass rod into a mixture of 1 mL of 0.1 mol/L sodium nitride solution, 500 mL of water and 10 mL of hydrochloric acid, and touch on zinc iodide-starch test solution: an apparently blue color appears.

Zinc sulfate heptahydrate: $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ [Special grade]

Zinc, Arsenic-free: Zn [Arsenic-free] Use granules of about 800 μm .

(3) Standard Solution for Capacity Analysis

Standard Solution for Capacity Analysis is reagent solution which is known as concentration is fine.

We use molar solution as standard solution for capacity analysis. 1 mol solution means the solution which contain 1 g molecular weight of effective substance in 1,000 mL solution. It is described as 1 mol/L. Or Use dilute solution at fixed rate according to required. Standard solution for capacity analysis should be kept in shaded or colorless ground stopper bottle except defined case.

Preparation

Standard solution for capacity analysis is prepared by one of following methods and fix molar concentration coefficient.

A. Measure about 1 g molecular weight of pure substance till 3 digit effective number and record the number. Add solvent and dissolve it and make it 1,000 mL. Prepare 1 mol/L solution of approximate concentration. Use substance which purity is clear in case you cannot use pure substance.

B. Measure 1 g molecular weight of substance and add solvent and make it 1,000 mL and prepare 1 mol/L solution of approximate concentration and use after setting molar concentration coefficient by standardization. Standardization is the operation to fix molar concentration coefficient.

Measure standard substance till 3 digit effective number and record the number and add solvent and dissolve and titrate it by non standardization molar concentration and find f molar concentration coefficient.

$$f = \frac{1000a}{V \times E \times c}$$

E: molecular weight of standard substance (g)

a: collection quantity of standard substance (g)

V: consumption of non standardization molar solution (mL)

c: molar concentration

In case we do not know use standard substance, standardize non standardization molar solution by using molar solution that we know molar concentration coefficient.

$$f_2 = \frac{V_1 \times f_1}{V_2}$$

f_1 = molar concentration coefficient that we know the molar concentration coefficient of molar solution

f_2 = molar concentration coefficient of non standardization molar solution

V_1 = molar solution quantity (mL) of that we know the molar concentration coefficient.

V_2 = molar solution quantity (mL) of non standardization molar solution

C. Dilute accurately fixed capacity of molar solution that we know the molar concentration coefficient.

0.1 mol/L sodium nitrite solution

It contains (NaNO₂: 69.00) 6.900 g of Sodium nitrite in 1,000 mL.

Preparation: Add water to 7.2 g (7.15~7.24 g) sodium nitrite and make 1,000 mL and standardize next.

Standardization: Dry sulfamic acid (standard reagent) by desiccators (reduction of pressure, silica gel) for 48 hours and measure about 0.25 g of that till 0.001 g digit and record the number. Add 5 mL hydrochloric acid and 50 mL water and dissolve it. Add 25g (24.5~25.4 g) broken ice after cooling less than 15 °C and titrate it by prepared sodium nitrite solution while stirring and calculate molar concentration coefficient. In this case, we finish titration when color changes blue within 30 seconds by touching zinc iodide starch test paper at the top of glass rods with after 1 minutes of titrating sodium nitrite solution.
0.1 mol/L sodium nitrite solution 1 mL = 9.709 mg HOSO₂NH₂

Note: Shade and keep it. Standardize again and use after keeping for long time.

0.05 mol/L disodium ethylenediaminetetraacetate solution

It contains disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24) 18.61 g in 1,000 mL.

Preparation: Add water to 19 g (18.5~19.4 g) disodium ethylenediaminetetraacetate and make 1,000 mL and standardize next.

Standardization: Wash zinc (standard reagent) with dilute hydrochloric acid. Next wash with water and more wash with acetone and dry it at 110 °C for 5 minutes. After cooling by desiccators (silica gel) and measure about 0.8 g of it till 0.001 g digit and record the number. Add 12 mL dilute hydrochloric acid and 5 drops of bromine and heat gently and dissolve it. After removing extra bromine by boiling pour it into 200 mL measuring flask and add water till capacity mark to make 200 mL. Measure 20 mL this solution by transfer volumetric pipette and add sodium hydroxide solution (1 → 50) to make neuter. Add 5 mL ammonium-ammonium chloride buffer solution (pH 10.7) and 0.04 g (0.035~0.044 g) eriochrome black T-sodium chloride indicator. Perform titrate the solution with prepared disodium ethylenediaminetetraacetate solution until reddish purple color of solution changes to blue purple and calculate molar concentration coefficient.

0.05 mol/L disodium ethylenediaminetetraacetate solution 1 mL = 3.269 mg Zn

Note: Keep it in polyether pot.

0.02 mol/L disodium ethylenediaminetetraacetate solution

It contains ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24) 7.445 g disodium ethylenediaminetetraacetate in 1,000 mL.

Preparation: Add water to 7.5 g (7.45~7.54 g) disodium ethylenediaminetetraacetate and make it 1,000 mL and standardize next.

Standardization: Wash zinc (standard reagent) with dilute hydrochloric acid. Next wash with water and more wash with acetone and dry it at 110 °C for 5 minutes. After cooling by desiccators (silica gel) and measure about 0.3 g of it till 0.001 g digit and record the number. Add 5 mL dilute hydrochloric acid and 5 drops of bromine and heat gently and dissolve it. After removing extra bromine by boiling pour it into 200 mL measuring flask and add water till capacity mark to make 200 mL. Measure 20 mL this solution by transfer volumetric pipette and add sodium hydroxide solution (1 → 50) to make neuter. Add 5 mL ammonium-ammonium chloride buffer solution (pH 10.7) and 0.04 g (0.035~0.044 g) eriochrome black T-sodium chloride indicator. Perform titrate the solution with prepared disodium ethylenediaminetetraacetate solution until reddish purple color of solution changes to blue purple and calculate molar concentration coefficient.

0.02 mol/L disodium ethylenediaminetetraacetate solution 1 mL = 1.308 mg Zn

Note: Keep it in polyether pot.

0.01 mol/L disodium ethylenediaminetetraacetate solution

It contains disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24) 3.722 g.

Preparation: Add water to 0.02 mol/L disodium ethylenediaminetetraacetate solution and make exactly double amount when you use.

0.05 mol/L magnesium chloride solution

It contains ($MgCl_2 \cdot 6H_2O$: 203.30) 10.17 g magnesium chloride in 1,000 mL.

Preparation: Add fresh cooled water after boiling to 10.2 g (10.15~10.24 g) magnesium chloride and make it 1,000 mL.

Standardization: Measure 25 mL magnesium chloride solution by volumetric pipette and add 50 mL water, 3 mL ammonium-ammonium chloride buffer solution (pH 10.7) and 0.04 g (0.035~0.044 g), Perform titrate the solution with 0.02 mol/L disodium ethylenediaminetetraacetate solution and calculate molar concentration coefficient. In this case, the endpoint of titration shall be the time when the color of the solution turns red purple to blue purple. Further, titrate slowly near the endpoint.

1 mol/L hydrochloric acid

Containing 36.46 g of hydrochloric acid in 1,000 mL (HCl: 36.46).

Preparation: Add water to 9.0 mL of hydrochloric acid in order to make the total amount 1,000 mL. Then, perform the following standardization.

Standardization: After heating sodium carbonate (Na_2CO_3) (standard reagent) at the temperature of 500 to 650 °C for 40 to 50 minutes, cool it in desiccator (silica gel), weigh approximately 1.3 g to the digit of 0.01 g, dissolve it by adding 50 mL of water, add 3 drops of methyl red reagent titrate by prepared hydrochloric acid and calculate molar concentration coefficient. In this case, the endpoint of titration is the time when the solution is boiled carefully, cooled down by plugging loosely and turns orange or orange-red continuously.

$$1 \text{ mol/L hydrochloric acid } 1 \text{ mL} = 5.300 \text{ mg } Na_2CO_3$$

0.1 mol/L hydrochloric acid

Containing 3.646 g of hydrochloric acid in 1,000 mL (HCl: 36.46).

Preparation: Add water to 9.0 mL of hydrochloric acid in order to make the total amount 1,000 mL. Then, perform the following standardization.

Standardization: After heating sodium carbonate (Na_2CO_3) (standard reagent) at the temperature of 500 to 650 °C for 40 to 50 minutes, cool it in desiccator (silica gel), weigh approximately 0.15 g to the digit of 0.001 g, dissolve it by adding 30 mL of water, add 3 drops of methyl red reagent titrate by prepared hydrochloric acid and calculate molar concentration coefficient. In this case, the endpoint of titration is the time when the

solution is boiled carefully, cooled down by plugging loosely and turns orange or orange-red continuously.

0.1 mol/L hydrochloric acid 1 mL = 5.300 mg Na₂CO₃

0.05 mol/L hydrochloric acid

Containing 1.823 g of hydrochloric acid in 1,000 mL (HCl: 36.46).

Preparation: Before use, add water to 0.1 mol/L of hydrochloric acid to double in capacity accurately.

0.01 mol/L hydrochloric acid

Containing 0.3646 g of hydrochloric acid in 1,000 mL (HCl: 36.46).

Preparation: Before use, add water 0.1 mol/L of hydrochloric acid to decuple in capacity accurately.

0.001 mol/L hydrochloric acid

Containing 0.3646 g of hydrochloric acid in 1,000 mL (HCl: 36.46).

Preparation: Before use, add water 0.1 mol/L of hydrochloric acid to centuple in capacity accurately.

0.1 mol/L perchloric acid

Containing 10.05 g of perchloric acid in 1,000 mL (HClO₄: 100.46).

Preparation: Add 8.5 mL of perchloric acid gradually to 800 mL of glacial acetic acid for titration performed in nonaqueous solvent by keeping the temperature under 30 °C. After left for several hours, add 22.2 mL of acetic anhydride, mix by shaking, add glacial acetic acid for titration performed in nonaqueous solvent to the total amount of 1,000 mL and leave for 48 hours and perform the following standardization.

Standardization: After drying potassium hydrogen phthalate (standard reagent) at the temperature of 105 °C for four hours, cool it in desiccator (silica gel), weigh approximately 0.5 g to the digit of 0.001 g, record the numerical value, solve it by adding 80 mL of glacial acetic acid for titration performed in nonaqueous solvent, add 3 drops of methylrosanilinium chloride, titrate until it turns blue by prepared perchloric acid and calculate molar concentration coefficient. In the same method, conduct blank test and correct it.

0.1 mol/L perchloric acid 1 mL = 20.42 mg KHC₆H₄(COO)₂

Notice: Conserve by avoiding the humidity.

0.1 mol/L perchloric acid-dioxane solution

Containing 10.05 g of perchloric acid (HClO₄: 100.46) in 1,000 mL (HClO₄: 100.46).

Preparation: Add dioxane to 8.5 mL of perchloric acid to the total amount of 1,000 mL.

Then, perform the following standardization.

Standardization: After drying potassium hydrogen phthalate (standard reagent) at the temperature of 105 °C for four hours, cool it in

desiccator (silica gel), weigh approximately 0.5 g to the digit of 0.001 g, solve it by adding 80 mL of glacial acetic acid for titration performed in nonaqueous solvent, add 3 drops of methylrosanilinium chloride, titrate until it turns blue by prepared perchloric acid-dioxane solution, and calculate molar concentration coefficient. In the same method, conduct blank test and correct it.

0.1 mol/L perchloric acid-dioxane solution 1 mL = 20.42 mg $\text{KHC}_6\text{H}_4(\text{COO})_2$

Notice: Conserve by avoiding the humidity.

0.02 mol/L potassium permanganate solution

Containing 3.161 g of permanganate of potassium (KMnO_4 : 158.03) in 1,000 mL (KMnO_4 :158.03).

Preparation: Solve 3.2 g (3.15~3.24 g) of permanganate of potassium by adding water in order to make the total amount 1000 mL, boil for 15 minutes and cap tightly. After leaving for more than 48 hours, filter it by using glass filtering (G3 or G4) implement and conduct the following standardization.

Standardization: After drying sodium oxalate (standard reagent) at the temperature of 150 to 200 °C for 1 to 1.5 hours, cool it in desiccator (silica gel), weigh approximately 0.3 g to the digit of 0.001g, record the numerical value, put it into erlenmeyer flask of 500 mL and solve it by adding 30 mL of water. Next, add 250 mL of sulfuric acid (1 → 20) 250 mL, set the solution temperature as 30 to 35 °C, put prepared permanganate of potassium into burettes, mixing softly, add 40 mL of the solution within 30 seconds and leave it until the red of the solution disappears. After that, continue to titrate by humidifying to the temperature of 55 to 60 °C, titrate for 30 seconds continuously until it turns pink and calculate molar concentration coefficient. In this case, 0.5~1 mL of the solution shall be titrated before the endpoint, add the next drop after the color of permanganate of potassium disappears.

0.02 mol/L permanganate of potassium solution 1 mL = 6.700 mg $\text{Na}_2\text{C}_2\text{O}_4$

Notice: Conserve by shading. In case it is conserved for a long time, use it after re-standardization.

0.05 mol/L zinc acetate solution

Containing 10.98 g of zinc acetate solution ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$: 219.50) in 1,000 mL.

Preparation: Solve 11.1 g (11.05~11.14 g) of zinc acetate by adding 40 mL of water and 4 mL of acetic acid, add water to the total amount of 1,000 mL and conduct the following standardization.

Standardization: Weigh 20 mL of the solution of 0.05 mol/L of disodium edetate by using volumetric pipette, add 50 mL of water, 3 mL of ammonia of pH 10.7 and ammonium chloride and 0.04 g (0.035~0.044 g) of eriochrome black T and sodium chloride indicator, titrate by prepared zinc acetate solution and calculate molar concentration coefficient. The

endpoint of titration shall be the time when the color of the solution turns blue to blue purple.

0.005 mol/L acetic acid mercuric solution

Containing 1.593 g of acetic acid mercuric solution ($\text{Hg}(\text{CH}_3\text{COO})_2$: 318.68) in 1,000 mL.

Preparation: Solve by adding 60 mL of dilute nitric acid (1 → 10) to 1.6 g (1.55~1.64 g) of acetic acid mercuric, add water to the total amount of 1,000 mL and conduct the following standardization.

Standardization: After drying sodium chloride (standard reagent) at the temperature of 500 to 650 °C for 40 to 50 minutes, cool it in desiccator (silica gel), weigh approximately 0.58 g of the solution to the digit to 0.001 g, record the numerical value, solve it by adding water, put the measuring flask of 1,000 mL and add water to the solution to the capacity mark to the total amount of 1,000 mL. Weigh the total amount of 20 mL by using the pipette, add a drop of bromophenol blue reagent, after titrating dilute nitric acid until the solution turns yellow, add 5 mL of dilute nitric acid, 100 mL of methanol and 1 mL of diphenylcarbazone reagent, mixing well, titrate it by prepared acetic acid mercuric solution until light-yellow of solution turns to reddish purple and calculate molar concentration coefficient.

0.005 mol/L acetic acid mercuric solution 1 mL = 0.5844 mg NaCl

0.017 mol/L potassium dichromate

Containing 4.903 g of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$: 294.18) in 1000 mL.

Preparation: Powder potassium dichromate (standard reagent), after drying it at the temperature of 100 to 110 °C for 3 to 4 hours, cool it in desiccator (silica gel), weigh approximately 4.903 g of the solution to the digit of 0.001 g, record the numerical value, solve it by adding water, put into the measuring flask of 1,000 mL, Further, add water to the solution to the capacity mark to the total amount of 1,000 mL and calculate molar concentration coefficient.

0.05 mol/L solution of bromine

Containing 7.990 g of bromine (Br_2 : 159.80) in 1,000 mL.

Preparation: Solve 2.8 g (2.75~2.84 g) of potassium bromate and 15 g (14.5~15.4 g) of potassium bromide by adding water to the total amount of 1,000 mL and conduct the following standardization.

Standardization: Weigh the total amount of 25 mL of prepared solution of bromine in iodine bottle by using the volumetric pipette, add 120 mL of water. Next, add 5 mL of hydrochloric acid within 30 minutes. Within 30 minutes after adding hydrochloric acid, cap tightly, mix softly by shaking, after leaving for 5 minutes, titrate free iodine by 0.1 mol/L of thiosulfate and calculate molar concentration coefficient. In this case, the

endpoint of the titration shall be the time when the solution turns yellow at the close endpoint and the blue appeared by adding 3 mL of starch test solution is removed. In the same method, conduct blank test and correct.

0.1 mol/L silver nitrate solution

Containing 16.99 g of silver nitrate solution in 1,000 mL.

Preparation: Solve 17.0g (16.95~17.04 g) of silver nitrate by adding water to the total amount of 1,000 mL and conduct the following standardization.

Standardization: After drying sodium chloride (standard reagent) at the temperature of 500 to 650 °C for 40 to 50 minutes, cool it in desiccator (silica gel), weigh approximately 0.15 g of the solution to the digit of 0.001 g, record numerical value, solve it by adding 50 mL of water, add 1 mL of potassium chromate, with shaking, titrate by prepared silver nitrate solution continuously until it turns light red brown, calculate molar concentration coefficient.

0.1 mol/L silver nitrate solution 1 mL = 5.844 mg NaCl

Notice: Conserve by shading

0.1 mol/L potassium hydroxide solution

Containing 5.611 g of potassium hydroxide (KOH: 56.11) in 1,000 mL.

Preparation: Solve 6.5g (6.45~6.54 g) of potassium hydroxide by adding 950 mL of water, add newly prepared saturated barium hydroxide solution until there is no precipitation, mix the solution well, cap tightly, after left for 24 hours, descend supernatant liquid, or filter by using glass filtering equipment (G3 or G4) and conduct the following standardization.

Standardization: Dry sulphamic acid (standard reagent) by desiccator (decompression, silicagel) for approximately 48 hours, weigh approximately 0.25 g of the solution to the digit of 0.001 g, record the numerical value, solve it by adding newly boiled and cooled water, add 2 drops of bromothymol blue, titrate prepared potassium hydroxide solution until it turns green and calculate molar concentration coefficient.

0.1 mol/L potassium hydroxide solution 1mL = 9.709 mg HOSO₂NH₂

Notice: Conserve in the capped bottle or the bottle with carbon dioxide absorption tube (soda-lime tube). In case it is conserved for a long time, use it after re-standardization.

0.5 mol/L potassium hydroxide-ethanol solution

Containing 28.06 g of potassium (KOH: 56.11) in 1,000 mL.

Preparation: Solve 35g (34.5~35.4 g) of potassium hydroxide by adding 20 mL of water, add ethanol without aldehyde to the total amount of 1,000 mL, capped tightly, after left for 24 hours, extract supernatant liquid within 30 seconds by descending and conduct the following standardization.

Standardization: Weigh the total amount of 25 mL of 0.25 mol/L sulfuric acid by using volumetric pipette, add 50 mL of water and 2 drops of phenolphthalein test reagent, titrate by prepared potassium hydroxide-ethanol solution until it turns pink and calculate molar concentration coefficient.

Notice: Conserve in shaded bottle by capping. Standardize before use.

0.1 mol/L potassium hydroxide-ethanol solution

Containing 5.611 g of potassium hydroxide (KOH: 56.11) in 1,000 mL.

Preparation: Solve 7g (6.5~7.4 g) of potassium hydroxide by adding 20 mL of water, add ethanol without aldehyde to the total amount of 1,000 mL, capped tightly, after left for 24 hours, extract supernatant liquid within 30 seconds by descending and conduct the following standardization.

Standardization: Weigh the total amount of 25 mL of 0.25 mol/L sulfuric acid by using volumetric pipette, add 50 mL of water and 2 drops of phenolphthalein test reagent, titrate by prepared potassium hydroxide-ethanol solution until it turns pink and calculate molar concentration coefficient.

Notice: Conserve in shaded bottle by capping. Standardize before use.

1 mol/L sodium hydroxide solution

Containing 40.00 g of sodium hydroxide (NaOH: 40.00) in 1,000 mL.

Preparation: Solve 42 g (41.5~42.4 g) of sodium hydroxide by adding 950 mL of water, Add newly prepared barium hydroxide saturated solution to this by titrating until there is no precipitation, mix the solution well, cap tightly, after leaving for 24 hours, descend supernatant liquid, or filter by using glass filtering equipment (G3 or G4) and conduct the following standardization.

Standardization: Dry sulfamic acid (standard reagent) in desiccator (decompression, silica gel) for approximately 48 hours, weigh approximately 2.5 g of the solution to the digit of 0.01 g, record the numerical value, solve it by adding 25 mL of newly boiled and cooled water, add 2 drops of bromthymol blue test reagent, titrate it by prepared sodium hydroxide solution until it turns green and calculate molar concentration coefficient.

1 mol/L sodium hydroxide solution 1mL = 97.09 mg HOSO₂NH₂

Notice: Conserve in tightly capped bottle or the bottle with carbon dioxide absorption tube (soda lime). In case it is conserved for a long time, use it after re-standardization.

0.1 mol/L sodium hydroxide solution

Containing 4.000 g of sodium hydroxide in 1,000 mL.

Preparation: Weigh 4.5 g (4.45~4.54 g) of sodium hydroxide, 1 mol/L, prepare according to sodium hydroxide solution and conduct the following standardization.

Standardization: Dry sulphamic acid (standard reagent) in desiccator (decompression, silica gel) for approximately 48 hours, weigh approximately 0.25 g of the solution to the digit of 0.001 g and record the numerical value, solve by adding newly boiled and cooled 25 mL of water, add 2 drops of bromthymol blue test reagent, titrate it by prepared sodium hydroxide solution until it turns green and calculate molar concentration coefficient.

0.1 mol/L sodium hydroxide solution 1 mL = 9.709 mg HOSO₂NH₂

Notice: Conserve according to 1 mol/L sodium hydroxide solution. In case it is conserved for a long time, use it after re-standardization.

0.1 mol/L ammonium thiocyanate solution

Containing 7.612 g of ammonium thiocyanate (NH₄SCN: 76.12) in 1,000 mL.

Preparation: Solve 8 g (7.5~8.4 g) of ammonium thiocyanate by adding water to the total amount of 1,000 mL and conduct the following standardization.

Standardization: Weigh 25 mL of 0.1 mol/L silver nitrate solution by using volumetric pipette, add 50 mL of water, 2 mL of nitric acid and 2 mL of ferric nitrate test reagent, shaking, titrate by prepared ammonium thiocyanate solution continuously until it turns red brown and calculate molar concentration coefficient.

Notice: Conserve by shading.

0.1 mol/L sodium thiosulfate

Containing 24.82 g of sodium thiosulfate (Na₂S₂O₃·5H₂O: 248.18) in 1000 mL.

Preparation: Solve 25 g (24.5~25.4 g) of sodium thiosulfate and 0.2 g (0.15~0.24 g) of soda ash by adding newly boiled and cooled water to the total amount of 1,000 mL. After leaving for 24 hours, conduct the following standardization. Standardization: After drying potassium iodate (standard test reagent) at the temperature of 120~140 °C for 1.5~2 hours, cool it in desiccator (silica gel), weigh approximately 0.1 g of the solution to the digit of 0.0001 g in iodate bottle, record the numerical value, solve 25 mL of water, add 2 g (1.5~2.4 g) potassium iodide and 10 mL of dilute sulfuric acid and cap tightly. After leaving for 10 minutes, add 100 mL of water, titrate isolated iodate by prepared sodium thiosulfate solution and molar concentration coefficient. In this case, the endpoint of titration is the time when the solution turns light yellow, add 3 mL of starch test reagent and the blue appeared in the solution is removed. In the same method, conduct blank test and correct it.

0.1 mol/L prepared sodium thiosulfate solution 1 mL = 3.567 mg KIO₃

Notice: In case it is conserved for a long time, use it after re-standardization.

0.05 mol/L sodium thiosulfate solution

Containing 12.41 g of sodium thiosulfate (Na₂S₂O₃·5H₂O: 248.18) in 1,000 mL.

Preparation: Before use, by adding newly boiled and cooled water to 0.1 mol/L sodium thiosulfate the solution shall be doubled accurately.

0.01 mol/L sodium thiosulfate solution

Containing 2.482 g of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 248.18) in 1,000 mL.

Preparation: Before use, by adding newly boiled and cooled water to 0.1 mol/L sodium thiosulfate, the solution shall be decupled accurately.

0.005 mol/L sodium thiosulfate solution

Containing 1.241 g of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 248.18) in 1,000 mL.

Preparation: Before use, by adding newly boiled and cooled water to 0.1 mol/L sodium thiosulfate, the solution shall be 20 times accurately.

0.1 mol/L sodium methoxide solution

Containing 5.402 g of sodium methoxide in 1,000 mL.

Preparation: After solving 2.5 g (2.45~2.54 g) of a new piece of metallic sodium by adding in small portions into 150 mL of cooled methanol, add benzene to the total amount of 1,000 mL and conduct the following standardization.

Standardization: Dry benzoic acid by desiccator (silica gel) for 24 hours, weigh approximately 0.3 g of the solution to the digit of 0.001 g, record the numerical value, solve by adding 80 mL of dimethylformamide, add 3 drops of thymol blue·dimethylformamide test reagent, titrate by prepared methoxide solution until it turns blue and calculate molar concentration coefficient. In the same method, conduct blank test and correct.

0.1 mol/L sodium methoxide solution 1 mL = 12.21 mg $\text{C}_6\text{H}_5\text{COOH}$

Notice: Avoid the humidity and conserve in a cold place. Standardization shall be conducted before use.

0.1 mol/L magnesium disodium edetate solution

Solve 20.33 g (20.325~20.334 g) of magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) by adding 300 mL of newly boiled and cooled water. Further, mix the solution where 37.22 g (37.215~37.224 g) of disodium edetate ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$) is solved by adding 300 mL of water, adjust it to 1 mol/L pH 7.0 to 8.0 by 1 mol/L of sodium hydroxide test solution and add water to the total amount of 1,000 mL accurately.

0.05 mol/L iodine solution

Containing 12.69 g of iodine (I: 126.90) in 1,000 mL.

Preparation: Solve 13 g (12.5~13.4 g) of iodine by adding 100 mL of potassium iodide Solution (2 → 5), add 1 mL of dilute hydrochloric acid and water to the total amount of 1,000 mL and conduct the following standardization.

Standardization: Powder arsenic trioxide (standard reagent), after drying at the temperature of 105 °C for 3~4 hours, cool it in desiccator (silica gel), weigh approximately 0.15 g of the solution to the digit of 0.001 g, record the numerical value, add 20 mL of sodium hydroxide solution (1 → 25) and, if necessary, solve by heating. Add 40 mL of water and 2 drops of methyl orange reagent test solution, after adding dilute hydrochloric acid until the solution turns pink, add 2 g (1.5~2.4 g) of sodium hydrogen carbonate, 50 mL of water and 3 mL of starch test solution, titrate by adding prepared iodine solution gradually and titrate until the solution turns blue continuously and calculate molar concentration coefficient.

0.05 mol/L iodine solution 1 mL = 4.946 mg As₂O₃

Notice: Conserve by shading. In case it is conserved for a long time, use it after re-standardization.

0.05 mol/L potassium iodate solution

Containing 10.70 g of potassium iodate (KIO₃: 214.00) in 1000 mL.

Preparation: After drying potassium iodate (standard reagent) at the temperature of 120~140 °C for 1.5~2 hours, cool it in desiccator (silica gel), weigh approximately 10.70 g of the solution to the digit of 0.001 g, record the numerical value, put all into measuring flask of 1,000 mL, solve it by adding water. Further, add water to the capacity mark to the total amount of 1,000 mL and calculate molar concentration coefficient.

0.5 mol/L sulfuric acid

Containing 49.04 g of sulfuric acid (H₂SO₄: 98.08) in 1000 mL.

Preparation: Add 30 mL of sulfuric acid gradually into 1,000 mL of water by mixing, cool it and conduct the following standardization.

Standardization: After heating sodium carbonate (standard reagent) at the temperature of 500~650 °C for 40~50 minutes, cool it in desiccator (silica gel), weigh approximately 1.3 g of the solution to the digit of 0.01 g, record the numerical value, solve it by 50 mL of water, add 3 droops of methyl red test solution, titrate by prepared sulfuric acid and calculate molar concentration coefficient. In this case, the endpoint of titration is the time when the solution is carefully boiled and cooled by capping loosely and it turns orange to reddish orange continuously.

0.5 mol/L sulfuric acid 1mL = 53.00 mg Na₂CO₃

0.05 mol/L sulfuric acid

Containing 4.904 g of sulfuric acid (H₂SO₄: 98.08) in 1,000 mL.

Preparation: Add 3 mL of sodium carbonate gradually into 1,000 mL of water by mixing, cool it and conduct the following standardization. Standardization: After heating sulfuric acid (standard reagent) at the temperature of 500~650 °C for 40~50 minutes, cool it in

desiccator (silica gel), weigh approximately 0.15 g of the solution to the digit of 0.001 g, record the numerical value, solve it by 30 mL of water, add 3 droops of methyl red test solution, titrate by prepared sulfuric acid and calculate molar concentration coefficient. In this case, the endpoint of titration is the time when the solution is carefully boiled and cooled by capping loosely and it turns orange to reddish orange continuously.

0.05 mol/L sulfuric acid 1 mL = 5.300 mg Na₂CO₃

0.01 mol/L sulfuric acid

Containing 0.9808 g of sulfuric acid (H₂SO₄: 98.08) in 1,000 mL.

Preparation: Before use, quintuple accurately by adding water to 0.05 mol/L sulfuric acid.

0.005 mol/L sulfuric acid

Containing 0.4904 g of sulfuric acid in 1,000 mL.

Preparation: Before use, decuple accurately by adding water to 0.05 mol/L sulfuric acid.

0.1 mol/L cerium sulfate ammonium

Containing 86 g of cerium sulfate ammonium (Ce(SO₄)₂·2 (NH₄)₂SO₄·4H₂O: 668.56) in 1,000 mL.

Preparation: Solve 68g (67.5~68.4 g) of cerium sulfate ammonium by adding 0.5 mol/L sulfate acid to the total amount of 1,000 mL, after leaving for 24 hour, if necessary, filter by using glacial filter (G3 of G4) and conduct the following standardization.

Standardization: Weigh 25 mL of prepared cerium sulfate ammonium in iodate bottle by using volumetric pipette, add 20 mL of water and 20 mL of dilute sulfuric acid. Next, solve by adding 1 g (0.5~1.4 g) of potassium iodide, titrate 3 by 0.1 mol/L of sodium thiosulfate solution within 30 seconds and calculate molar concentration coefficient. In this case, the endpoint of titration is the time when the solution turns light yellow at the close endpoint, by adding 3 mL of starch test solution, the blue appeared is remove. In the same method, conduct blank test and correct.

Notice: Conserve by shading. In case it is conserved for a long time, use it after re-standardization.

0.01 mol/L cerium sulfate ammonium solution

Containing 6.686 g of cerium sulfate ammonium (Ce(SO₄)₂·2 (NH₄)₂SO₄·4H₂O: 668.58) in 1,000 mL.

Preparation: Before use, decuple accurately by adding 0.5 mol/L of sulfuric acid to 0.1 mol/L cerium sulfate ammonium solution.

(4) Standard solution

Standard solution is the liquid that is used as the basis for the comparison of tests in the tests for feed additive.

Zinc standard solution: Solve 4.40 g (4.395~4.404 g) of zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) by adding water, put into flask of 1,000 mL to the total amount of 1,000 mL to the capacity mark by adding water. Weigh 10 mL of this solution all by using pipette, put into flask of 1,000 mL to the total amount of 1,000 mL to the capacity mark by adding water. 1 mL of this solution contains 0.01 mg of zinc (Zn).

Ammonium standard solution: Solve 2.97 g (2.965~2.974 g) of ammonium chloride by adding water, put into flask of 1,000 mL to the total amount of 1,000 mL to the benchmark by adding water. Weigh 10 mL of this solution by using volumetric pipette, put into flask of 1,000 mL to the total amount of 1,000 mL to the capacity mark by adding water. 1 mL of this solution contains 0.01 mg of ammonium.

Hydrochloric acid dimethylamine standard solution: After solving 1.116 g (1.1155~1.1164 g) of hydrochloric acid dimethylamine into water to the total amount of 1,000 mL, extract 1 mL of the solution and add water to the total amount of 1,000 mL. 1 mL of this solution is correspondent to 1 μg of dimethylformamide.

Potassium standard solution: After drying potassium chloride [high quality] at the temperature of 400 to 500 °C for 40 to 50 minutes, cool it in desiccator (silica gel), weigh 1.907 g (1.9065~1.9074 g) of the solution, solve by adding water, put it into measuring flask of 1,000 mL all and add water to the total amount of 1,000 mL to the capacity mark. 1 mL of this solution contains 1 mg of sodium (K).

Calcium standard solution: Dry calcium carbonate [high quality] at the temperature of 180 °C for 4 hours, weigh 0.2500 g (0.24995~0.25004 g) of the solution, solve by adding 2 mL of dilute hydrochloric acid and 60 mL of water, adjust pH to 6.0 to 7.0 by ammonia test solution, put into measuring flask of 100 mL and add water to the total amount of 100 mL to the capacity mark.

Lead standard solution for atomic absorption photometry: It shall be provided in the items for lead standard solution and atomic absorption photometry.

Lead standard solution for dithizone: It shall be provided as limit test for lead as general test procedures.

Oxalate pH standard solution: It shall be provided in the items for pH standard solution and oxalate.

Calcium hydroxide pH standard solution: It shall be provided in the items of pH standard solution and calcium hydroxide.

Carbonate pH standard solution: It shall be provided in the items for pH standard solution and carbonate.

Trimethylamine standard solution: Dry trimethylamine [high quality] at the temperature of 105 °C for 4 hours, weigh 80.84 mg of the solution to the digit of 0.001 mg, record the numerical value, solve by adding water, put into 100 mL of total amount measuring flask. Further, add water to the total amount of 100 mL to the capacity mark. Weigh 10 mL of the solution all by pipette, put into 100 mL of flask all, add water to the total amount of 100 mL to the capacity mark. Further, weigh 10 mL of the solution all by pipette put into 50 mL of total amount flask, add water to the total amount of 50 mL to the capacity mark. 1 mL of this solution contains 10.0 µg of trimethylamin.

Iron standard solution: Weigh 86.34 mg (86.335~86.344 g) of ferric ammonium sulfate, solve it by adding 100 mL of water, put into measuring flask of 1,000 mL all, add 5 mL of dilute hydrochloric acid and water to the total amount of 1,000 mL to the capacity mark. 1 mL of this solution contains 0.01 mg of iron (Fe).

Copper standard undiluted solution: Weigh 3.929 g (3.9285~3.9294 g) of copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), solve it by dilute nitric acid (3 → 5), put it into total amount measuring flask of 1,000 mL and add dilute nitric acid (3 → 5) to the total amount of 1,000 mL to the capacity mark.

Copper standard solution: Weigh 5 mL of copper standard undiluted solution by volumetric pipette, put it into measuring flask of 200 mL and add dilute nitric acid (1 → 3) to the total amount of 200 mL to the capacity mark. Prepare before use. 1 mL of this solution contains 0.025 mg of copper (Cu).

Sodium standard solution: After drying sodium chloride at the temperature of 500 to 650 °C for 40 to 50, cool it in desiccator (silica gel), weigh 2.542 g (2.5415~2.5424 g) of the solution, solve by adding water, put it into measuring flask of 1,000 mL all and add water to the total amount of 1,000 mL to the capacity mark. 1 mL of this solution contains 1 mg of sodium (Na).

Lead standard undiluted solution: Weigh 159.9 mg (159.85~159.94 mg) of lead nitrate, solve by adding 10 mL of dilute nitric acid, put into total amount measuring flask of 1,000 mL and add water to the total amount of 1,000 mL to the capacity mark. For preparation and conservation of this solution, the glass container that contains no soluble lead salt shall be used.

Lead standard solution: Weigh 10 mL of lead standard undiluted solution by using total amount volumetric pipette, put it into measuring flask of 100 mL all and add water to the total amount of 100 mL to the capacity mark. Prepare before use. 1 mL of this solution contains 0.01 mg of lead (Pb).

Lead standard undiluted solution, for atomic absorption photometry: Weigh 25 mL of lead standard undiluted solution by volumetric pipette, put into 100 mL of measuring flask, add dilute nitric acid (1 → 3) to the total amount of 100 mL to the capacity mark.

Prepare before use. 1 mL of this solution contains 0.025 mg of lead (Pb).

Lead standard solution, for dithizone: It shall be provided as lead test method of general test procedures.

pH standard solution, oxalate: Powder potassium tetroxalate dihydrate for pH measurement after drying in desiccator (silica gel), weigh 12.71 g (12.705~12.714 g) (0.05 g of molecular amount) of the solution, solve by adding water, put into 1,000 mL of measuring flask and add water to the total amount of 1,000 mL to the capacity mark.

pH standard solution, calcium hydroxide: Powder calcium hydroxide for pH measurement, put 5 g (4.5~5.4 g) of the solution into measuring flask, add 1,000 mL of water, shake well and set the temperature as 23~27 °C. After saturated enough, filter the supernatant fluid at the temperature and use clear filtrate (ca. 0.02 mol/L).

pH standard solution, carbonate: Dry sodium hydrogen carbonate for pH measurement in desiccator (silica gel) until it becomes constant weight, dry 2.100 g (0.025 g molecular weight) and sodium carbonate for pH measurement at the temperature of 300 to 500 °C until it becomes constant weight, weigh 2.650 g (2.6495~2.6504 g) (0.025 g molecular weight) of the solution, solve by adding water, put into total amount measuring flask of 1,000 mL and add water to the total amount of 1,000 mL to the capacity mark.

pH standard solution, phthalate: Powder phthalic acid oxalate for pH measurement, dry at the temperature of 110 °C until it becomes constant weight, weigh 10.21 g (10.205~10.214 g) (0.05 g molecular weight) of the solution, solve by adding water, put into total amount 1,000 mL of measuring flask and add water to the total amount of 1,000 mL to the capacity mark.

pH standard solution, borate salt: Leave sodium borate for pH measurement in desiccator (moistened by sodium bromide), after setting as constant weight, weigh 3.814 g (3.8135~3.8144 g) (0.01 g molecular weight) of the solution, solve by water, put into measuring flask of 1,000 mL and add water further to the total amount to the capacity mark.

pH standard solution, phosphate: Potassium dihydrogen phosphate for pH measurement and powder disodium phosphate for pH measurement at the temperature of 110 °C, dry it until it becomes constant weight, weigh 3.402 g (3.4015~3.4024 g) (0.025 g molecular weight) of potassium dihydrogen phosphate and 3.549 g (3.5485~3.5494 g) (0.025 g molecular weight) of disodium phosphate, solve it by adding water, put into measuring flask of total amount 1,000 mL and add water further to the total amount to the capacity mark.

Arsenic standard undiluted solution: It shall be as provided in arsenic method of test for general test procedures.

Arsenic standard solution: It shall be provided as arsenic test method of general test procedures.

Phthalate pH standard solution, pH standard solution: It shall be provided in the item of phthalate.

Fluorine standard solution: After drying sodium fluoride (standard reagent) at the temperature of 500~550 °C for 40~50 minutes, cool it in desiccator (silica gel), weigh 1.105 g (1.1045~1.1054 g) of the solution, solve by adding water, put into measuring flask of 1,000 mL and add water to the total amount of 1,000 mL to the capacity mark. 1 mL of this solution contains 0.005 mg of fluorine.

Borate pH standard solution: It shall be provided in the item of pH standard solution, borate.

Formaldehyde standard solution: Weigh 0.54 g (0.535~0.544 g) of formalin (equivalent to 37 %), solve by putting water, put into total amount measuring flask, add water to the total amount of 1,000 mL to the benchmark further. Weigh 10 ml of this solution all by using pipette, put into total amount flask of 1,000 mL and add water to the total amount of 1,000 mL to the benchmark. 1 mL of this solution contain 2 µg of formaldehyde (HCHO). Prepared at time of use.

Manganese standard solution: Weigh 0.2876 g (0.28755~0.28764 g) of potassium permanganate, solve by adding 100 mL of water and 1 mL of sulfuric acid, add 0.5 g (0.45~0.54 g) of sodium acid sulfite, after boiling and cooling, put 200 mL of measuring flask, add water to the total amount of 200 mL to the capacity mark, weigh 20 mL of this solution by using volumetric pipette, put into 1,000 mL of measuring flask, and add water to the total amount of 1,000 mL to the capacity mark. This solution of 1 mL contains 0.01 mg of manganese (Mn).

Water • Methanol standard solution: It shall be provide as moisture assay system of general test procedures.

Phosphate pH standard solution: It is provided in the items of pH standard solution, phosphate.

Phosphate standard solution: Dry monobasic potassium phosphate in desiccator (silica gel) until it becomes constant weight, weigh 0.3582 g (0.35815~0.35824 g) of the solution, add 10 mL of sulfuric acid (3 → 10), put into 1,000 mL of measuring flask, add water to the total amount of 1,000 mL to the capacity mark, weigh 10 mL of this solution by volumetric pipette and add water to the total amount of 100 mL to the capacity mark. 1 mL of this solution contains 0.025 mg of phosphate (as PO₄).

Phosphorous standard solution: Dry monobasic potassium phosphate in desiccator (silica gel) until it becomes constant weight, weigh 10.99 g (10.985~10.994 g) of the solution, solve

by adding water, put into 1,000 mL of measuring flask and add water to the total amount of 1,000 mL to the capacity mark. Weigh 20 mL of this solution by volumetric pipette, put into measuring flask of 500 mL and complete as phosphorous standard solution by adding water to the total amount of 500 mL to the capacity mark. 1 mL of this solution contains 0.1 mg of phosphorous (P).

(5) Color control solution

Color control solution shall be used as a reference of color comparison for feed additive test.

Color control solution shall be prepared by the following control stock solution.

Control stock solution if prepared by the following method and conserved in bottle with ground-in stopper. In order to compare the color of the solution by using color control solution, put into Nessler tube and observe from the side by using a white background, unless otherwise specified.

Color control stock solution for cobalt chloride

Weigh 65 g (64.5~65.4 g) of cobalt chloride, solve it by adding 25 mL of hydrochloric acid and water to the total amount of 1,000 mL. Weigh 5 mL of this solution by volumetric pipet, put into iodine flask, add 5 mL of hydrogen peroxide test solution and add 15 mL of sodium hydroxide solution (1 → 5) and boil for 10 minutes. After cooling, add 2 g (1.5~2.4 g) of potassium iodide and 20 mL of sulfuric acid solution (1 → 4). After the precipitation is dissolved, titrate liberated iodine by 0.1 mol/L sodium thiosulfate solution (indicator: starch test solution 1 mL).

0.1 mol/L sodium thiosulfate solution 1 mL = 23.79 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$

From the value gained by titration, add hydrochloric acid (1 → 40) so as it contains 59.48 mg of cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$: 237.93) in 1 mL as control stock solution.

Color control stock solution for ferric chloride

Weigh 55 g (54.5~55.4 g) of ferric chloride, add 25 mL of hydrochloric acid and water to the total amount of 1,000 mL. Weigh 10 mL of this solution by volumetric pipet, put into iodine flask, add 15 mL of water and 3 g (2.5~3.4 g) of potassium iodide, cap tightly. After leaving for 15 minutes in the dark, add 100 mL of water, titrate liberated iodine by 0.1 mol/L sodium thiosulfate (indicator: starch test solution 1 mL).

0.1 mol/L sodium thiosulfate solution 1 mL = 27.03 mg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$

From the value gained by titration, add hydrochloric acid (1 → 40) so as it contains 45.05 mg ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$: 270.30) in 1 mL as control stock solution.

Color control stock solution for copper sulfate

Weigh 65 g (64.5~65.4 g) of copper sulfate, add 25 mL of hydrochloric acid and water to the total amount of 1,000 mL. Weigh 10 mL of this solution by volumetric pipette, put into iodine flask, add 4 mL of acetic acid and 3 g (2.5~3.4 g) of potassium iodide and titrate liberated iodine by 0.1 mol/L sodium thiosulfate (indicator: starch test solution 1 mL).

0.1 mol/L sodium thiosulfate solution 1 mL = 24.97 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

From the value gained by titration, add hydrochloric acid (1 → 40) so as it contains 62.42 mg of copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$: 249.69) in 1 mL as control stock solution.

Color control solution

Weigh each color control solution and definite quantity of water in the following chart by burette or pipette with scale that is equal to or lower than 0.1 mL, then mix and prepare.

Code of color control solution	Color control undiluted solution of cobalt chloride (mL)	Color control undiluted solution of ferric (mL)	Color control undiluted solution of copper sulfate (mL)	Water (mL)
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	3.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0.4	1.2	0.3	3.1
F	0.3	1.2	—	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	—	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	—	—
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	—	4.9	0.1	—
O	0.1	4.8	0.1	—
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	—	4.7
T	0.5	0.5	0.4	3.6

(6) Measuring apparatus • instrument

Measuring apparatus is instrument or machine that is used for measurement in feed additive test.

Instrument is the one that is provided in order to make the conditions as consistent as possible in feed additive test.

Thermometer

Generally, the thermometer for which instrumental error test between partial immersion thermometer (cylinder) and full immersion mercury thermometer (cylinder) by the Japanese Industrial Standards is conducted shall be used. However, partial immersion thermometer (cylinder) shall be used for measurement method of freezing point, measurement method for melting point (Method 1), measurement method for boiling point and distillation measurement method, partial immersion thermometer (cylinder) is used.

Partial immersion thermometers (cylinder) are shown as follows.

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Liquid	Mercury	Mercury	Mercury	Mercury	Mercury	Mercury
Gas filled on liquid	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen
Temperature range	-17~50 °C	40~100 °C	90~150 °C	140~200 °C	190~250 °C	240~320 °C
Minimum scale value	0.2 °C	0.2 °C	0.2 °C	0.2 °C	0.2 °C	0.2 °C
Long gridline	Per 1 °C	Per 1 °C	Per 1 °C	Per 1 °C	Per 1 °C	Per 1 °C
Scale patterns	Per 2 °C	Per 2 °C	Per 2 °C	Per 2 °C	Per 2 °C	Per 2 °C
Full length (mm)	280~300	280~300	280~300	280~300	280~300	280~300
Diameter of cylinder (mm)	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1
Length of mercury bulb (mm)	12~15	12~15	12~15	12~15	12~15	12~15
Distance from the bottom edge of mercury bulb to the lowest gridline (mm)	75~90	75~90	75~90	75~90	75~90	75~90
Distance from the upper edge of thermometer to the top gridline (mm)	35~50	35~50	35~50	35~50	35~50	35~50
Distance from the	60	60	60	60	60	60

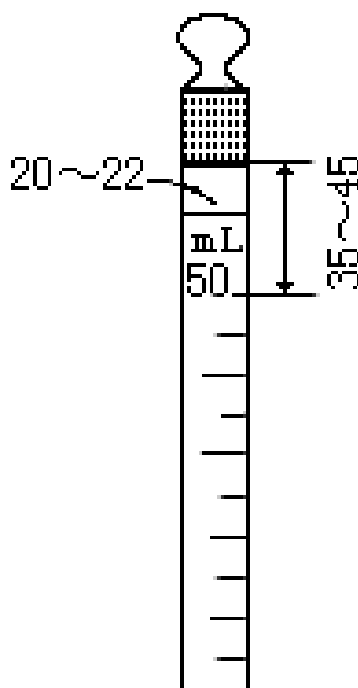
bottom edge of mercury bulb to the immersion line (mm)						
Form of the top	Circular	Circular	Circular	Circular	Circular	Circular
Tolerance	0.2 °C	0.2 °C	0.2 °C	0.2 °C	0.2 °C	0.4 °C

Volumemeter for science

Regarding total amount flask, pipette, burette and measuring cylinder, the ones tested for temperature shall be used.

Nessler tube

Nessler tube shall be colorless and a cylinder with stopper made of hard glass with a thickness of 1.0~1.5 mm as shown in the figure below. The one where the difference of the height of 50 mL gridline of each tube is under 2 mm shall be used.



The numbers are mm

Balance and weight

- Chemical balance: The one that can be read to 0.1 mg shall be used.
- Semimicro balance: The one that can be read to 0.01 mg shall be used.
- Micro balance: The one that can be read to 0.001 mg shall be used.
- Weight: The one where instrumental error test was conducted shall be used.

Sieve

The standard sieve by the Japanese Industrial Standards shall be used.

(7) Filter paper

Filter paper is the paper that is produced especially carefully in order to meet the purpose for filtering.

The filter paper with the standards shown as follows shall be used. Further, it shall be mentioned as “filter” and the one not specified regarding the kind means the filter for qualitative analysis. The filter paper shall be conserved not to be polluted by gas etc.

Filter paper for qualitative analysis: It shall be the one that meets the standard for qualitative analysis of the Japanese Industrial Standards for filter paper (for chemical analysis).

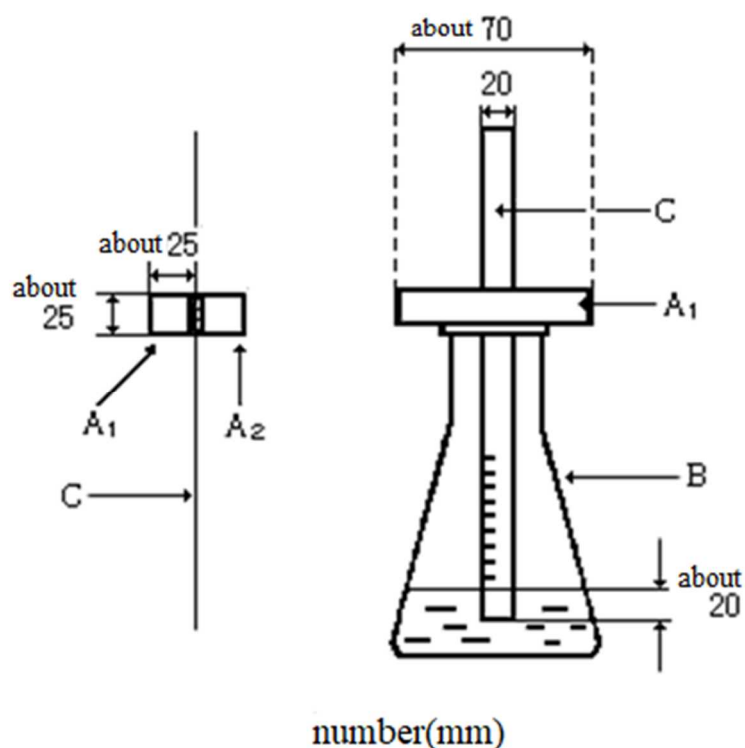
Filter paper for quantitative analysis: It shall be the one that meets the standard for quantitative analysis of the Japanese Industrial Standards for filter paper (for chemical analysis).

Filter for chromatography: It shall be the one that meets the standard for quantitative analysis and the standards shown as follows. However, regarding the tests for α cellulose content, copper number, pH, ash content, time of filtered water and strength of wet burst, they shall be conducted according to the methods provided by the Japan Industrial Standards. As for the test for water absorption elevation, it shall be conducted by the method shown below.

Test for water absorption elevation

Apparatus

Use as shown in the figure.



A₁ and A₂: Glass block for keeping filter paper

B: Conical flask (the content is approximately 1,000 mL)

C: Filter paper sample

Method for operation

Pour distilled water of approximately 300 mL of conical B and set glass block A₁ and A₂ for keeping filter paper side by side on the flask. Clip filter paper sample calibrated per 1 cm by pencil in advance between glass blocks, slide softly first. After the edge of filter paper arrived on the surface, then slide it quickly, fix by making 0 point of the gridline correspond to the surface and measure the height of distilled water moving up for 10 minutes.

(8) Sterilization method

Sterilization method means to kill or remove all the microorganisms in materials. Sterilization method is usually conducted by the method shown as follows independently or adjunctively, generally depending on kinds of microorganisms, the conditions of pollution, the property and condition of the material to be sterilized.

Suitability of sterilization is usually determined by sterility test.

Operation for sterilization shall be conducted by confirming that the temperature or pressure etc. is suitable to the conditions of sterilization as the purpose thoroughly. Further, when

conducting the choice of the conditions of sterilization or the confirmation of the effect of sterilization, biological indicator suitable to each sterilization condition can be used.

Sterilization method by heating

In conducting sterilization method by heating, heating time before the temperature or pressure etc. correspond to the regulated sterilization condition differs according to the character of the material to be sterilized, capacity of the container and the condition of storage. Further, time for sterilization shall be calculated from the time when all the parts of the material to be sterilized reach the regulated temperature.

A. Direct flame sterilization

It means the method to sterilize microorganism by heating in the flame. This method shall be mainly used for the materials that are not destroyed by the flame, such as the ones made of glass, porcelain or made from metal etc. Usually, it shall be heated in the flame of a Bunsen burner or alcohol lamp for more than 20 seconds.

B. Dry-heat sterilization

It means the method to sterilize microorganism by heating in dry-heat air. This method shall be mainly used for the materials that bear dried high temperature, such as the materials made of glass, porcelain or made from metal, or the goods made of fiber, mineral oil, fat, grease, test reagent or solid feed additive etc. It is the method to heat directly by gas or electricity or the one to keep the condition of dried high temperature by circulating heated air. Usually, sterilization shall be conducted under any of the following conditions.

135 ~ 145 °C	3 ~ 5 hours
160 ~ 170 °C	2 ~ 4 hours
180 ~ 200 °C	0.5 ~ 1 hour
over 200 °C	over 0.5 hours

In addition, for the solution of feed additive put in capped container which bears high temperature and the like, the method to sterilize in dried heat at the temperature of 134~138 °C for more than 3 minutes can be used.

C. High pressure steam sterilization method

It means the method to sterilize microorganism by heating in saturated steam of appropriate temperature and pressure.

This method shall be mainly used for the materials that bear high temperature, high pressure steam, such as the materials made of glass, porcelain or made from metal, rubber, paper or the goods made of fiber, water, medium, test reagent·test solution or liquid feed additive etc. In order to make sterilization sure, air in sterilizer shall be removed from outlet in operation as much as possible, and the materials to be sterilized shall be filled

with saturated steam. Usually, sterilization shall be conducted under any of the following conditions.

115 °C (0.7 kg/cm²) for 30 minutes

121 °C (1.0 kg/cm²) for 20 minutes

126 °C (1.4 kg/cm²) for 15 minutes

D. Free-flowing steam sterilization

It means the method to sterilize microorganism by making heated steam flow directly.

This method shall be mainly used for the materials that is at risk of changing in quality by dry-heat sterilization method or high pressure steam sterilization method, such as the materials made of glass, porcelain or made from metal, rubber or the goods made of fiber, water, medium, test reagent-test solution or liquid feed additive etc. Usually, sterilization shall be conducted in free-flowing steam at the temperature of 100 °C for 30~60 minutes.

E. Boiling sterilization method

It means the method to sterilize microorganism by sinking into boiling water and heating.

This method shall be mainly used for the materials that is at risk of changing in quality by dry-heat sterilization method or high pressure steam sterilization method, such as the materials made of glass, porcelain or made from metal, rubber or the goods made of fiber, medium, test reagent-test solution or liquid feed additive etc. Besides, in order to enhance the effect of sterilization, 1~2 % of sodium carbonate can be added in boiling water. Usually, sterilization shall be conducted in by sinking into boiling water by boiling for more than 15 minutes.

F. Fractional sterilization

It means the method to sterilize microorganism by repeating heating three to five times in the water of 80~100 °C or in free-flowing steam once in every 24 hours for 30~60 minutes. Besides, there is another fractional sterilization at low temperature where humidification is repeated in the same way at the temperature of 60~80 °C.

This method shall be mainly used for the goods made of rubber, medium, test reagent-test solution or liquid feed additive etc. and that is at risk of changing in quality by dry-heat sterilization method or high pressure steam sterilization method.

Filtering sterilization method

It means the method to remove microorganism by using appropriate filter. This method shall be mainly used for gas, water and medium that are soluble and contains the materials vulnerable to heat, test reagent or liquid feed additive etc. Usually, membrane filter, porcelain filter or glass filter etc. shall be used for filter.

Irradiation sterilization method

A. Radiogenic sterilization method

It means the method to sterilize microorganism by gamma rays from radiation source that contains radioactive isotope.

This method shall be mainly used for the goods made of glass, porcelain, metal, rubber, plastic or fiber and that bear radiogenic irradiation. Usually radiogenic radiation source that contains ^{60}Co or ^{137}Cs etc. shall be used. Sterilization shall be conducted by adjusting according to the quality of materials to be sterilized, physical-chemical properties or the conditions for pollution.

B. Ultraviolet sterilization method

It means the method to sterilize microorganism by irradiating ultraviolet.

This method shall be mainly used for the goods, made of glass, metal, rubber, plastic or fiber, facilities, equipment, water or feed additive etc. and the materials that bear ultraviolet irradiation. Usually, ultraviolet of 200 to 300 nm shall be used.

C. High-frequency sterilization

It means the method to sterilize microorganism by heat given off from direct irradiation.

This method shall be used for water, medium, test reagent or liquid feed additive and the materials that bear high-frequency irradiation. Usually, high-frequency of 915 or 2,450 MHz shall be used.

Chemical sterilization method

A. Gas sterilization method

It means the method to sterilize microorganism by using germicidal gas, such as ethylene oxide or formaldehyde etc.

This method shall be mainly used for the materials made of glass, porcelain, metal, rubber, plastic or the goods made of fiber, facilities, equipment, or powdered feed additive etc. and the materials that do not change in quality by the gas. Further, gas sterilizers shall be mainly used in order to adjust temperature, gas sterilizers shall be mainly used in order to adjust humidity, concentration of gas or time. After sterilization, pay special attention to residue of used gas or its by-product.

B. Medicinal solution sterilization method

It means the method to sterilize microorganism by using medicinal solution.

This method shall be mainly used for the materials made of glass, porcelain, metal, rubber, plastic or the goods made of fiber, hands and fingers, in aseptic box or aseptic facility etc., and the materials that do not change in quality by the medicinal solution. Usually, ethanol for disinfection, benzalkonium chloride of 0.1 %~1 w/v%, cresol water, phenol water or formalin water etc. shall be used.

Aseptic manipulation

After sterilizing all the equipment and materials for use by any of the above-mentioned methods, manipulate aseptically in aseptic box or aseptic facility etc.

This method shall be mainly used for manipulation for preparation, filling or sealing etc. of the sterilized feed additive etc. by each item of the above-mentioned sterilization.

Manipulation shall be conducted as quickly as possible.

(9) Bertrand sugar determinate quantity

Sugar (mg)	Weight of copper equivalent to each sugar (mg)				
	Invert sugar	Glucose	Galactose	Maltose	Lactose
10	20.6	20.4	19.3	11.2	14.4
11	22.6	22.4	21.2	12.3	15.8
12	24.6	24.3	23.0	13.4	17.2
13	26.5	26.3	24.9	14.5	18.6
14	28.5	28.3	26.7	15.6	20.0
15	30.5	30.2	28.6	16.7	21.4
16	32.5	32.2	30.5	17.8	22.8
17	34.5	34.2	32.3	18.9	24.2
18	36.4	36.2	34.2	20.0	25.6
19	38.4	38.1	36.0	21.1	27.0
20	40.4	40.1	37.9	22.2	28.4
21	42.3	42.0	39.7	23.3	29.8
22	44.2	43.9	41.6	24.4	31.1
23	46.1	45.8	43.4	25.5	32.5
24	48.0	47.7	45.2	26.6	33.9
25	49.8	49.6	47.0	27.7	35.2
26	51.7	51.5	48.9	28.9	36.6
27	53.6	53.4	50.7	30.0	38.0
28	55.5	55.3	52.5	31.1	39.4
29	57.4	57.2	54.4	32.2	40.7
30	59.3	59.1	56.2	33.3	42.1
31	61.1	60.9	58.0	34.4	43.4
32	63.0	62.8	59.7	35.5	44.8
33	64.8	64.6	61.5	36.5	46.1
34	66.7	66.5	63.3	37.6	47.4
35	68.5	68.3	65.0	38.7	48.7
36	70.3	70.1	66.8	39.8	50.1
37	72.2	72.0	68.6	40.9	51.4
38	74.0	73.8	70.4	41.9	52.7
39	75.9	75.7	72.1	43.0	54.1
40	77.7	77.5	73.9	44.1	55.4
41	79.5	79.3	75.6	45.2	56.7
42	81.2	81.1	77.4	46.3	58.0
43	83.0	82.9	79.1	47.4	59.3
44	84.8	84.7	80.8	48.5	60.6
45	86.5	86.4	82.5	49.5	61.9
46	88.3	88.2	84.3	50.6	63.3

Sugar (mg)	Weight of copper equivalent to each sugar (mg)				
	Invert sugar	Glucose	Galactose	Maltose	Lactose
47	90.1	90.0	86.0	51.7	64.6
48	91.9	91.8	87.7	52.8	65.9
49	93.6	93.6	89.5	53.9	67.2
50	95.4	95.4	91.2	55.0	68.5
51	97.1	97.1	92.9	56.1	69.8
52	98.8	98.9	94.6	57.1	71.1
53	100.6	100.6	96.3	58.2	72.4
54	102.2	102.3	98.0	59.3	73.7
55	104.0	104.1	99.7	60.3	74.9
56	105.7	105.8	101.5	61.4	76.2
57	107.4	107.6	103.2	62.5	77.5
58	109.2	109.3	104.9	63.5	78.8
59	110.9	111.1	106.6	64.6	80.1
60	112.6	112.8	108.3	65.7	81.4
61	114.3	114.5	110.0	66.8	82.7
62	115.9	116.2	111.6	67.9	83.9
63	117.6	117.9	113.3	68.9	85.2
64	119.2	119.6	115.0	70.0	86.5
65	120.9	121.3	116.6	71.1	87.7
66	122.6	123.0	118.3	72.2	89.0
67	124.2	124.7	120.0	73.3	90.3
68	125.9	126.4	121.7	74.3	91.6
69	127.5	128.1	123.3	75.4	92.8
70	129.2	129.8	125.0	76.5	94.1
71	130.8	131.4	126.6	77.6	95.4
72	132.4	133.1	128.3	78.6	96.9
73	134.0	134.7	130.0	79.7	98.0
74	135.6	136.3	131.5	80.8	99.1
75	137.2	137.9	133.1	81.8	100.4
76	138.9	139.6	134.8	82.9	101.7
77	140.5	141.2	136.4	84.0	102.9
78	142.1	142.8	138.0	85.1	104.2
79	143.7	144.5	139.7	86.1	105.4
80	145.3	146.1	141.3	87.2	106.7
81	146.9	147.7	142.9	88.3	107.9
82	148.5	149.3	144.6	89.4	109.2
83	150.0	150.9	46.2	90.4	110.4

Sugar (mg)	Weight of copper equivalent to each sugar (mg)				
	Invert sugar	Glucose	Galactose	Maltose	Lactose
84	151.6	152.5	147.8	91.5	111.7
85	153.2	154.0	149.4	92.6	112.9
86	154.8	155.6	151.1	93.7	114.1
87	156.4	157.2	152.7	94.8	115.4
88	157.9	158.8	154.3	95.8	116.6
89	159.5	160.4	156.0	96.9	117.9
90	161.1	162.0	157.6	98.0	119.1
91	162.6	163.6	159.2	99.0	120.3
92	164.2	165.2	160.8	100.1	121.6
93	165.7	166.7	162.4	101.1	122.8
94	167.3	168.3	164.0	102.2	124.0
95	168.8	169.9	165.6	103.2	125.2
96	170.3	171.5	167.2	104.2	126.5
97	171.9	173.1	168.8	105.3	127.7
98	173.4	174.6	170.4	106.3	128.9
99	175.0	176.2	172.0	107.4	130.2
100	176.5	177.8	173.6	108.4	131.4