chromatography, and dry the spots completely. Develop the plate with a mixture of 1,2-dichloroethane, acetic acid (100), methanol and water (10:5:3:2) to a distance of about 15 cm, and dry the plate with a current of warm air. Repeat the development of the plate immediately in the same manner with the developing solvent newly replaced, and dry the plate with a current of warm air. Spray evenly on the plate a solution of 0.5 g of thymol in 100 mL of a mixture of ethanol (95) and sulfuric acid (19:1), and heat the plate at 130°C for 10 minutes: the principal spot from the sample solution is similar in position, color and size to the principal spot from the standard solution (1), and the four spots from the standard solution (2) are clearly separated.

(3) Dissolve 0.25 g of Lactose in 5 mL of water, add 3 mL of ammonia solution (28), and heat in a water bath at 80°C for 10 minutes: a red color develops.

Optical rotation $[\alpha]_D^{20}$: +54.4 - +55.9°. Weigh accurately about 10 g of Lactose, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Lactose in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank: not more than 0.04.

(2) Acid or alkali—Dissolve 6 g of Lactose in 25 mL of freshly boiled and cooled water by heating, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless. To this solution add 0.4 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals—Dissolve 4.0 g of Lactose in 20 mL of warm water, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 1 mL of 0.1 mol/L hydrochloric acid TS and 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(4) Light absorbing substances—Dissolve 1.0 g of Lactose in water to make 100 mL, and determine the absorbances as directed under the Ultraviolet-visible Spectrophotometry: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

Loss on drying Not more than 0.5% (1 g, 80°C, 2 hours). (For the granulated powder, not more than 1.0%.)

Water 4.5 - 5.5% (1 g, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method). (For the granulated powder, 4.0 - 5.5%.)

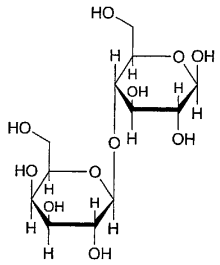
Residue on ignition Not more than 0.10% (1 g).

Microbial limit Proceed with Lactose as directed under the Microbial Limit Test: the total viable aerobic microbial count is not more than 100 per g, and the total count of fungi and yeast is not more than 50 per g. *Salmonella* and *Escherichia coli* should not be observed.

Containers and storage Containers—Well-closed containers.

Anhydrous Lactose

無水乳糖



$C_{12}H_{22}O_{11}$: 342.30

4-*O*-*β*-D-Galactopyranosyl-*β*-D-glucopyranose [*63-42-3*]

Anhydrous Lactose is *β*-lactose or a mixture of *β*-lactose and *α*-lactose.

The relative quantities of *β*-lactose in Anhydrous Lactose is indicated as the isomer ratio.

Description Anhydrous Lactose occurs as white crystals or powder. It is odorless.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification **(1)** Determine the infrared absorption spectrum of Anhydrous Lactose, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Anhydrous Lactose Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 25 mg each of Anhydrous Lactose and anhydrous lactose add diluted methanol (3 in 5) to make 50 mL each, and use these solutions as the sample solution and the standard solution (1), respectively. Separately, dissolve 25 mg each of glucose, anhydrous lactose, fructose and sucrose in diluted methanol and water (3 in 5) to make 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 2 *μ*L each of the sample solution, the standard solution (1) and the standard solution (2) on a plate of silica gel for thin-layer chromatography, and dry the spots completely. Develop the plate with a mixture of 1,2-dichloroethane, acetic acid (100), methanol and water (10:5:3:2) to a distance of about 15 cm, and dry the plate with a current of warm air. Repeat the development of the plate immediately in the same manner with the developing solvent newly replaced, and dry the plate with a current of warm air. Spray evenly on the plate a solution of 0.5 g of thymol in 100 mL of a mixture of ethanol (95) and sulfuric acid (19:1), and heat the plate at 130°C for 10 minutes: the principal spot from the sample solution is similar in position, color and size to the principal spot from the standard solution (1), and the four spots from the standard solution (2) are clearly separated.

(3) Dissolve 0.25 g of Anhydrous Lactose in 5 mL of water, add 3 mL of ammonia solution (28), and heat in a water bath at 80°C for 10 minutes: a red color develops.

Optical rotation $[\alpha]_D^{20}$: +54.4 - +55.9°. Weigh accurately

about 10 g of Anhydrous Lactose, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Anhydrous Lactose in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank: not more than 0.04.

(2) Acid or alkali—Dissolve 6 g of Anhydrous Lactose in 25 mL of freshly boiled and cooled water by heating, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless. To this solution add 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals—Proceed with 4.0 g of Anhydrous Lactose according to Method 2, and perform the test. Prepare the control solution with 2 mL of Standard Lead Solution (not more than 5 ppm).

(4) Light absorbing substances—Dissolve 1.0 g of Anhydrous Lactose in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

Loss on drying Not more than 0.5% (1 g, 80°C, 2 hours).

Water Not more than 1.0% (1 g, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

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

Microbial limit Proceed with Anhydrous Lactose as directed under the Microbial Limit Test: the total viable aerobic microbial count is not more than 100 per g, and the total count of fungi and yeasts is not more than 50 per g, and *Salmonella* and *Escherichia coli* should not be observed.

Isomer ratio Place 100 μg of Anhydrous Lactose in an about 3-mL glass-stoppered reaction vial for gas chromatography, add 225 μL of a mixture of pyridine, trimethylsilyl imidazole and dimethylsulfoxide (117:44:39), stopper the bottle, shake well, and allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under the Gas Chromatography according to the following conditions, and determine peak areas of α-lactose and β-lactose, A_a and A_b, and calculate the content (%) of β-lactose in Anhydrous Lactose by the following equation.

$$\text{Content (\%) of } \beta\text{-lactose} = \frac{A_b}{A_a + A_b} \times 100$$

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Sample injection port: about 275°C

Column: A column about 4 mm in inside diameter and about 0.9 m in length, packed with siliceous earth for gas chromatography coated at the ratio of 3% with 25% phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography.

Column temperature: A constant temperature of about 215°C.

Carrier gas: Helium

Flow rate: A constant flow rate of about 40 mL per minute.

Selection of column: Prepare a solution with 100 μg of α-lactose and β-lactose mixture (1:1) in the same manner as for preparing the sample solution, and proceed with 2 μL of this solution under the above operating conditions, and determine the retention times of the peaks of α-lactose and β-lactose. Use a column giving a ratio of the retention time of α-lactose to that of β-lactose is about 0.7 with the resolution between these peaks being not less than 3.0.

Containers and storage Containers—Well-closed containers.

Hydrous Lanolin

加水ラノリン

Hydrous Lanolin is Purified Lanolin to which water is added. It contains not less than 70% and not more than 75% of Purified Lanolin (as determined by the test for Residue on evaporation).

Description Hydrous Lanolin is a yellowish white, ointment-like substance, and has a slight, characteristic odor, which is not rancid.

It is soluble in diethyl ether and in cyclohexane, with the separation of water.

When melted by heating on a water bath, it separates into a clear oily layer and a clear water layer.

Melting point: about 39°C

Identification Dissolve 1 g of Hydrous Lanolin in 50 mL of cyclohexane, and remove the separated water. Superimpose carefully 1 mL of the cyclohexane solution on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact, and sulfuric acid layer shows a green fluorescence.

Acid value Not more than 1.0.

Iodine value 18 - 36 Heat a suitable amount of Hydrous Lanolin on a water bath to remove its almost moisture, then weigh accurately about 0.8 g of the treated Hydrous Lanolin in a glass-stoppered 500-mL flask, and add 10 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus's TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in a light-resistant, well-closed container while occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

$$\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{amount (g) of sample}}$$

a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination.

b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the titration.