low to stand for 10 minutes, filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. To 25 mL of the sample solution add water to make 50 mL, then add 1 mL of silver nitrate TS, and allow to stand for 5 minutes: the turbidity of the solution is not thicker than that of the following control solution.

Control solution: To 25 mL of the sample solution add 1 mL of silver nitrate TS, allow to stand for 10 minutes, and filter. Wash the precipitate with four 5-mL portions of water, and combine the washings with the filtrate. To this solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL, add 1 mL of water, and mix (not more than 0.021%).

(2) Heavy metals—Proceed with 2.0 g of Riboflavin Butyrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Free acid—To 1.0 g of Riboflavin Butyrate add 50 mL of freshly boiled and cooled water, shake, and filter. To 25 mL of the filtrate add 0.50 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: the solution shows a red color.

(4) Related substances—Dissolve 0.1 g of Riboflavin Butyrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 50 mL. Pipet 5 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and 2-propanol (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

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

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

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.04 g of Riboflavin Butyrate, previously dried, dissolve in ethanol (95) to make exactly 500 mL, and pipet 10 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of Riboflavin Reference Standard, previously dried at 105°C for 2 hours, dissolve in 150 mL of diluted acetic acid (100) (2 in 75) by warming, and after cooling, add water to make exactly 500 mL. Pipet 5 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 445 nm as directed under the Ultraviolet-visible Spectrophotometry.

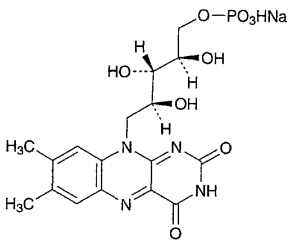
$$\text{Amount (mg) of } C_{33}H_{44}N_4O_{10} = \text{amount (mg) of Riboflavin Reference Standard} \times \frac{A_T}{A_S} \times 1.745 \times \frac{1}{2}$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Riboflavin Sodium Phosphate

Riboflavin Phosphate
Vitamin B_2 Phosphate Ester

リン酸リボフラビンナトリウム



$C_{17}H_{20}N_4NaO_9P$: 478.33
Monosodium (2*R*,3*S*,4*S*)-5-(3,4-dihydro-7,8-dimethyl-2,4-dioxobenzo[*g*]pteridin-10(2*H*)-yl)-2,3,4-trihydroxypentyl monohydrogenphosphate [*130-40-5*]

Riboflavin Sodium Phosphate contains not less than 92% of $C_{17}H_{20}N_4NaO_9P$, calculated on the anhydrous basis.

Description Riboflavin Sodium Phosphate is a yellow to orange-yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is soluble in water, and practically insoluble in ethanol (95), in chloroform and in diethyl ether.

It is decomposed on exposure to light.

It is very hygroscopic.

Identification **(1)** A solution of Riboflavin Sodium Phosphate (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the solution, and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(2) To 10 mL of a solution of Riboflavin Sodium Phosphate (1 in 100,000) placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, and after illumination with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

(3) Determine the absorption spectrum of a solution of Riboflavin Sodium Phosphate in phosphate buffer solution, pH 7.0, (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 0.05 g of Riboflavin Sodium Phosphate add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite. Boil the residue with 10 mL of nitric acid (1 in 50) for 5 minutes, after cooling, neutralize this solution with ammonia TS, and filter, if necessary: the solution responds to the Qualitative Tests for sodium salt and phosphate.

Optical rotation $[\alpha]_D^{20}$: +38 – +43° (0.3 g, calculated on

the anhydrous basis, 5 mol/L hydrochloric acid TS, 20 mL, 100 mm).

pH Dissolve 0.20 g of Riboflavin Sodium Phosphate in 20 mL of water: the pH of the solution is between 5.0 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Riboflavin Sodium Phosphate in 10 mL of water: the solution is clear and yellow to orange-yellow in color.

(2) Lumiflavin—To 0.035 g of Riboflavin Sodium Phosphate add 10 mL of ethanol-free chloroform, and shake for 5 minutes, then filter: the filtrate has no more color than the control solution.

Control solution: To 3.0 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

(3) Free phosphoric acid—Weigh accurately about 0.4 g of Riboflavin Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Measure exactly 5 mL each of the sample solution and Phosphoric Acid Standard Solution, transfer to separate 25-mL volumetric flasks, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS to each of these flasks, mix, and add water to make 25 mL. Allow to stand for 30 minutes at 20 ± 1°C, and perform the test with these solutions as directed under the Ultraviolet-visible Spectrophotometry, using a solution prepared with 5 mL of water in the same manner as a blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard phosphoric acid solution at 740 nm: the free phosphoric acid content is not more than 1.5%.

$$\text{Content (\%) of free phosphoric acid } (H_3PO_4) = \frac{A_T}{A_S} \times \frac{1}{W} \times 257.8$$

W: Amount (mg) of Riboflavin Sodium Phosphate calculated on the anhydrous basis.

Water Place 25 mL of a mixture of methanol for Karl Fischer method and ethylene glycol for Karl Fischer method (1:1) in a dry flask for titration, and titrate with water determination TS to the end point. Weigh accurately about 0.1 g of Riboflavin Sodium Phosphate, place quickly into the flask, add a known excess volume of Karl Fischer TS, mix for 10 minutes, and perform the test: the water content is not more than 10.0%.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. To about 0.1 g of Riboflavin Sodium Phosphate, accurately weighed, dissolve in diluted acetic acid (100) (1 in 500) to make exactly 1000 mL, then pipet 10 mL of this solution, and add diluted acetic acid (100) (1 in 500) to make exactly 50 mL. Use this solution as the sample solution. Separately, dry Riboflavin Reference Standard at 105°C for 2 hours, weigh accurately about 0.015 g, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank, and determine the absorbances, A_T and A_S, at 445 nm. Add 0.02 g of sodium hydrosulfite to 5 mL of each solution, shake until decolorized, and immediately measure the absorbances, A_T' and A_S', of the solutions.

$$\text{Amount (mg) of } C_{17}H_{20}N_4NaO_9P = \text{amount (mg) of Riboflavin Reference Standard} \times \frac{A_T - A_{T'}}{A_S - A_{S'}} \times 1.2709 \times 5$$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Sodium Phosphate Injection

Riboflavin Phosphate Injection
Vitamin B_2 Phosphate Ester Injection

リン酸リボフラビンナトリウム注射液

Riboflavin Sodium Phosphate Injection is an aqueous solution for injection. It contains not less than 95% and not more than 120% of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$: 376.36).

The concentration of Riboflavin Sodium Phosphate Injection should be stated as the amount of riboflavin ($C_{17}H_{20}N_4O_6$).

Method of preparation Prepare as directed under Injections, with Riboflavin Sodium Phosphate.

Description Riboflavin Sodium Phosphate Injection is a clear, yellow to orange-yellow liquid.

pH: 5.0 - 7.0

Identification **(1)** To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 1 mg of Riboflavin according to the labeled amount, add water to make 100 mL, and proceed with this solution as directed in the Identification (1) and (2) under Riboflavin Sodium Phosphate.

(2) To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 0.05 g of Riboflavin according to the labeled amount, and evaporate on a water bath to dryness. Proceed with this residue as directed in the Identification (4) under Riboflavin Sodium Phosphate.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. To an accurately measured volume of Riboflavin Sodium Phosphate Injection, equivalent to about 0.015 g of riboflavin ($C_{17}H_{20}N_4O_6$), add diluted acetic acid (100) (1 in 500) to make exactly 1000 mL, and use this solution as the sample solution. Proceed as directed in the Assay under Riboflavin Sodium Phosphate.

$$\text{Amount (mg) of Riboflavin } (C_{17}H_{20}N_4O_6) = \text{amount (mg) of Riboflavin Reference Standard} \times \frac{A_T - A_{T'}}{A_S - A_{S'}}$$

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.