Riboflavin Powder

Vitamin B₂ Powder

Riboflavin Powder contains not less than 95% and not more than 115% of the labeled amount of riboflavin (C₁₇H₂₀N₄O₅): 376.36.

Method of preparation Prepare as directed under Powders, with Riboflavin.

Identification Shake a portion of Riboflavin Powder, equivalent to 1 mg of Riboflavin according to the labeled amount, with 100 mL of water, filter, and proceed with the filtrate as directed in the Identification (1) and (2) under Riboflavin.

Purity Rancidity—Riboflavin Powder is free from any unpleasant or rancid odor or taste.

Assay The procedure should be performed under protection from direct sunlight and in light-resistant vessels. Weigh accurately Riboflavin Powder equivalent to about 0.015 g of riboflavin (C₁₇H₂₀N₄O₅), add 800 mL of diluted acetic acid (100) (1 in 400), and extract by warming for 30 minutes with occasional shaking. Cool, dilute with water to make exactly 1000 mL., and filter through a glass filter (G4). Use this filtrate as the sample solution, and proceed as directed in the Assay under Riboflavin.

Amount (mg) of riboflavin (C₁₇H₂₀N₄O₅) = amount (mg) of Riboflavin Reference Standard \( \times \frac{A_T - A_T^e}{A_S - A_S^e} \)

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

Riboflavin Butyrate

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\text{C}_{33}\text{H}_{44}\text{N}_{10} \text{O}_{10} : 656.72
\]
\[(2R,3S,4S)-5-(3,4-Dihydro-7,8-dimethyl-2,4-dioxobenzophenon-\[g\]pteridin-10(9H)-yl)-2,3,4-tris(butyryloxy)pentyl butyrate \ [752-56-7] \]

Riboflavin Butyrate, when dried, contains not less than 98.5% of C₃₃H₄₄N₁₀O₁₀.

Description Riboflavin Butyrate occurs as orange-yellow crystals or crystalline powder. It has a slight, characteristic odor and a slightly bitter taste.

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

It is decomposed by light.

Identification (1) A solution of Riboflavin Butyrate in ethanol (95) (1 in 100,000) shows a light yellow-green color with a strong yellowish green fluorescence. To the solution add dilute hydrochloric acid or sodium hydroxide TS: the fluorescence disappears.

(2) Dissolve 0.01 g of Riboflavin Butyrate in 5 mL of ethanol (95), add 2 mL of a mixture of solutions of hydroxylammonium chloride (3 in 20) and a solution of sodium hydroxide (3 in 20) (1:1), and shake well. To this solution add 0.8 mL of hydrochloric acid and 0.5 mL of iron (III) chloride TS, and add 8 mL of ethanol (95): a deep red-brown color develops.

(3) Determine the absorption spectrum of the sample solution obtained in the Assay 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.

Melting point 146 – 150°C

Purity (1) Chloride—Dissolve 2.0 g of Riboflavin Butyrate in 10 mL of methanol, and add 24 mL of dilute nitric acid and water to make 100 mL. After shaking well, al-
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 50 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, A₁ and A₅, of the sample solution and the standard solution at 445 nm as directed under the Ultraviolet-visible Spectrophotometry.

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\text{Amount (mg) of } C_{17}H_{38}N_4Na_2P = \frac{A_1 \times 1.745 \times 1}{2} \\
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Containers and storageContainers—Tight containers. Storage—Light-resistant.

### Riboflavin Sodium Phosphate

**Vitamin B₂ Phosphate Ester**

![Structure of Riboflavin Sodium Phosphate](image)

**C₁₇H₃₈N₄Na₂P: 478.33**

Monosodium (2R,3S,4S)-5-(3,4-dihydro-7,8-dimethyl-2,4-dioxobenzolg)pteridin-10(2H)-yl)-2,3,4-trihydroxypentyl monohydrogenophosphate [130-40-3]

Riboflavin Sodium Phosphate contains not less than 92% of C₁₇H₃₈N₄Na₂P, 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 hydrosulfit 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** \([\alpha]_{D}^{20} + 38° \pm 43° \) (0.3 g, calculated on