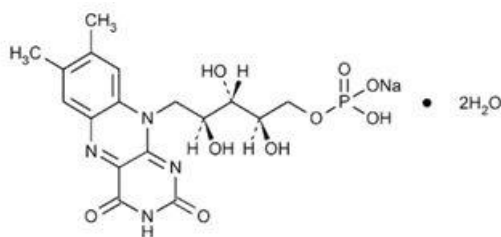


## Riboflavin 5'-Phosphate Sodium



$C_{17}H_{20}N_4NaO_9P \cdot 2H_2O$  514.36

Riboflavin 5'-(dihydrogen phosphate), monosodium salt, dihydrate.

Riboflavine 5'-(sodium hydrogen phosphate), dihydrate

Anhydrous 478.33 [130-40-5].

Riboflavin 5'-Phosphate Sodium contains not less than the equivalent of 73.0 percent and not more than the equivalent of 79.0 percent of riboflavin ( $C_{17}H_{20}N_4O_6$ ), calculated on the dried basis.

Packaging and storage— Preserve in tight, light-resistant containers.

### Identification—

A: Dissolve 1 mg in 100 mL of water: the solution is pale greenish yellow by transmitted light, and it exhibits an intense yellowish green fluorescence under long-wavelength UV light that disappears upon the addition of mineral acids or alkalies.

B: To 0.5 g add 10 mL of nitric acid, evaporate the mixture on a water bath to dryness, ignite the residue until the carbon is removed, dissolve the residue in 5 mL of water, and filter: the filtrate so obtained responds to the tests for [Sodium](#) [191](#) and for [Phosphate](#) [191](#).

[Specific rotation](#) [781S](#) : between +37.0°C and +42.0°C, determined within 15 minutes.

Test solution: 15 mg per mL, in 5 N hydrochloric acid.

[pH](#) [791](#) : between 5.0 and 6.5, in a solution (1 in 100).

[Loss on drying](#) [731](#) — Dry it in vacuum over phosphorus pentoxide at 100°C for 5 hours: it loses not more than 7.5% of its weight.

[Residue on ignition](#) [281](#) : not more than 25.0%.

### Free phosphate—

Acid molybdate solution— Dilute 25 mL of ammonium molybdate solution (7 in 100) with water to 200 mL. To this dilution add slowly 25 mL of 7.5 N sulfuric acid, and mix.

Ferrous sulfate solution— Just prior to use, prepare a 1 in 10 solution of ferrous sulfate in 0.15 N sulfuric acid.

Standard preparation— Prepare a solution in water containing 44.0 µg of monobasic potassium phosphate in each mL.

Test preparation— Transfer 300 mg of Riboflavin 5`-Phosphate Sodium to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Procedure**— Transfer 10.0 mL each of the Standard preparation and the Test preparation to separate 50-mL conical flasks, add 10.0 mL of Acid molybdate solution and 5.0 mL of Ferrous sulfate solution to each flask, and mix. Concomitantly determine the absorbances of the solutions, in 1-cm cells, at the wavelength of maximum absorbance at about 700 nm, with a suitable spectrophotometer, using as the blank a mixture of 10.0 mL of water, 10.0 mL of Acid molybdate solution, and 5.0 mL of Ferrous sulfate solution: the absorbance of the solution from the Test preparation is not greater than that from the Standard preparation (1% as PO<sub>4</sub>).

**Free riboflavin and riboflavin diphosphates**— [note—Conduct this test so that all solutions are protected from actinic light at all stages, preferably by using low-actinic glassware.]

**Mobile phase**— Mix 850 mL of 0.054 M monobasic potassium phosphate with 150 mL of methanol, filter, and degas the solution. Make adjustments if necessary.

**Standard preparation**— Transfer 60 mg of [USP Riboflavin RS](#), accurately weighed, to a 250-mL volumetric flask, dissolve carefully in 1 mL of hydrochloric acid, dilute with water to volume, and mix. Pipet a 4-mL aliquot into a 100-mL volumetric flask, dilute with Mobile phase to volume, and mix.

**Test preparation**— Transfer 100.0 mg of Riboflavin 5`-Phosphate Sodium to a 100-mL volumetric flask, dissolve in 50 mL of water, dilute with Mobile phase to volume, and mix. Pipet 8 mL of this solution into a 50-mL volumetric flask, dilute with Mobile phase to volume, and mix.

**System suitability preparation**— Dissolve [USP Phosphated Riboflavin RS](#) in water to obtain a solution containing 2 mg per mL. Add an equal volume of Mobile phase, and mix. Dilute 8 mL of this solution with Mobile phase to 50 mL, and mix.

**Chromatographic system**—The liquid chromatograph is equipped with a fluorometric detector set at 440-nm excitation wavelength and provided with a 470-nm emission filter or set at about 530 nm for a fluorescence detector that uses a monochromator for emission wavelength selection, and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the System suitability preparation, and record the peak responses. The retention time for riboflavin 5'-monophosphate is about 20 to 25 minutes, and the approximate relative retention times for the components are as follows:

Riboflavin 3`4`-diphosphate:	0.23
Riboflavin 3`5`-diphosphate:	0.39
Riboflavin 4`5`-diphosphate:	0.58
Riboflavin 3`-monophosphate:	0.70
Riboflavin 4`-monophosphate:	0.87
Riboflavin 5`-monophosphate:	1.00
Riboflavin:	1.63

The resolution, R, between the peaks for riboflavin 4'-monophosphate and riboflavin 5'-monophosphate is not less than 1.0, and the relative standard deviation of the response for riboflavin 5'-monophosphate in replicate injections is not more than 1.5%.

**Procedure**— Separately inject equal volumes (about 100 µL) of the Standard preparation, the Test preparation, and the System suitability preparation into the chromatograph. Measure the peak responses obtained from the Standard preparation and the Test preparation, identifying the peaks to be measured in the chromatogram of the Test preparation by comparison of retention times with those of the peaks in the chromatogram of the System suitability preparation. Calculate the percentage of free riboflavin taken by the formula:

$$625C(r_F / r_S)$$

and calculate the percentage of riboflavin in the form of riboflavin diphosphates taken by the formula:

$$625C(r_D / r_S)$$

in which C is the concentration, in mg per mL, of [USP Riboflavin RS](#) in the Standard preparation,  $r_F$  is the riboflavin peak response, if any, obtained from the Test preparation,  $r_D$  is the sum of the responses for any of the 3 riboflavin diphosphate peaks obtained from the Test preparation, and  $r_S$  is the riboflavin peak response obtained from the Standard preparation. Not more than 6.0% of

free riboflavin and not more than 6.0% of riboflavin diphosphates, as riboflavin, calculated on the dried basis, are found.

**Limit of lumiflavin**— Prepare alcohol-free chloroform just prior to use, as follows. Shake 20 mL of chloroform gently but thoroughly with 20 mL of water for 3 minutes, draw off the chloroform layer, and wash twice more with 20-mL portions of water. Finally filter the chloroform through a dry filter paper, shake it for 5 minutes with 5 g of powdered anhydrous sodium sulfate, allow the mixture to stand for 2 hours, and decant or filter the clear chloroform. Shake 35 mg of Riboflavin 5'-Phosphate Sodium with 10 mL of the alcohol-free chloroform for 5 minutes, and filter: the absorbance of the filtrate so obtained, determined in 1-cm cells at a wavelength of 440 nm, with a suitable spectrophotometer, alcohol-free chloroform being used as the blank, does not exceed 0.025.

**Assay**— [note—Conduct the assay so that all solutions are protected from actinic light at all stages, preferably by using low-actinic glassware.]

**Standard preparation**— Transfer about 35 mg of [USP Riboflavin RS](#), accurately weighed, to a 250-mL conical flask, add 20 mL of pyridine and 75 mL of water, and dissolve the riboflavin by frequent shaking. Transfer the solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 1000-mL volumetric flask, add sufficient 0.1 N sulfuric acid (about 4 mL) so that the final pH of the solution is between 5.9 and 6.1, dilute with water to volume, and mix. The Standard preparation so obtained contains about 0.35 µg of riboflavin per mL.

**Assay preparation**— Transfer about 50 mg of Riboflavin 5'-Phosphate Sodium, accurately weighed, to a 250-mL conical flask, add 20 mL of pyridine and 75 mL of water, and dissolve by frequent shaking. Transfer the solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 1000-mL volumetric flask, add sufficient 0.1 N sulfuric acid (about 4 mL) so that the final pH of the solution is between 5.9 and 6.1, dilute with water to volume, and mix.

**Procedure**— With a suitable fluorometer, determine the maximum fluorescence intensities, IS and IU, of the Standard preparation and the Assay preparation, respectively, at about 530 nm, using an excitation wavelength of about 440 nm. Calculate the quantity, in mg, of C<sub>17</sub>H<sub>20</sub>N<sub>4</sub>O<sub>6</sub> in the portion of Riboflavin 5'-Phosphate Sodium taken by the formula:

$$100C(I_U / I_S)$$

in which C is the concentration, in µg per mL, of [USP Riboflavin RS](#) in the Standard preparation.