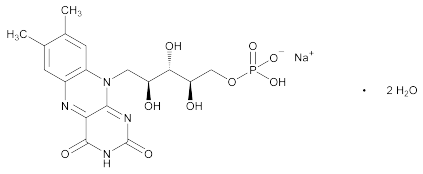
Riboflavin 5′-Phosphate Sodium



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

$C_{17}H_{20}N_4NaO_9P$ 478.33

Riboflavin 5′-(dihydrogen phosphate), monosodium salt, dihydrate;
Riboflavine 5′-(sodium hydrogen phosphate), dihydrate
Anhydrous [130-40-5].

DEFINITION

Riboflavin 5′-Phosphate Sodium contains NLT the equivalent of 73.0% and NMT the equivalent of 79.0% of riboflavin ($C_{17}H_{20}N_4O_6$), calculated on the dried basis.

IDENTIFICATION

- **A. Color and Fluorescence of Solution**
  Sample solution: 0.01 mg/mL in water
  Analysis: Alternately expose to transmitted light and long-wavelength UV light.
  Acceptance criteria: The solution is pale greenish yellow by transmitted light, and it exhibits an intense yellowish green fluorescence by reflected light under long-wavelength UV light that disappears upon the addition of mineral acids or alkalies.
- **B. Identification Tests—General,** *Sodium* ⟨**191**⟩ and *Phosphate* ⟨**191**⟩
  Sample solution: To 0.5 g add 10 mL of nitric acid. Evaporate the mixture on a water bath to dryness, and ignite the residue until the carbon is removed. Dissolve the residue in 5 mL of water, and filter.
  Acceptance criteria: The *Sample solution* meets the requirements.

ASSAY

- **Procedure**
  [Note—Conduct the assay so that all solutions are protected from actinic light at all stages, preferably by using low-actinic glassware.]
  Standard solution: 0.35 µg/mL prepared as follows. Transfer 35 mg of USP Riboflavin RS 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, and dilute with water to volume. 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, and dilute with water to volume.
  Sample solution: Transfer 50 mg of Riboflavin 5′-Phosphate Sodium 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, and dilute with water to volume. 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, and dilute with water to volume.
  Blank: Proceed as directed in the *Analysis*, omitting the test specimen.
  Instrumental conditions
  (See *Fluorescence Spectroscopy* ⟨853⟩.)
  Mode: Fluorescence
  Excitation wavelength: 440 nm
  Emission wavelength: 530 nm
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Blank*
  Determine the maximum fluorescence intensities of the solutions against the *Blank*.
  Calculate the percentage of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of Riboflavin 5′-Phosphate Sodium taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence intensity from the *Sample solution*
I_S = fluorescence intensity from the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)
C_U = concentration of Riboflavin 5′-Phosphate Sodium in the *Sample solution* (µg/mL)

Acceptance criteria: 73.0%–79.0% on the dried basis

IMPURITIES

- **Free Phosphate**
  Acid molybdate solution: Prepare a 70-mg/mL solution of ammonium molybdate in water. Dilute 25 mL of this solution with water to 200 mL. To this dilution slowly add 25 mL of 7.5 N sulfuric acid.
  Ferrous sulfate solution: 100 mg/mL of ferrous sulfate in 0.15 N sulfuric acid, prepared just before use
  Standard solution: 44.0 µg/mL of monobasic potassium phosphate in water.
  Sample solution: 3 mg/mL of Riboflavin 5′-Phosphate Sodium in water
  Blank: Water
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Mode: UV-Vis
  Analytical wavelength: 700 nm
  Cell: 1 cm
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Blank*
  Transfer 10.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* 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. Determine the absorbances of the solutions against that of the *Blank*.
  Acceptance criteria: NMT 1% as PO_4. The absorbance of the *Sample solution* is NMT that of the *Standard solution*.
- **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: Methanol and 0.054 M monobasic potassium phosphate (15:85)
  System suitability solution: Prepare a 2-mg/mL solution of USP Phosphated Riboflavin RS in water. Dilute this solution with *Mobile phase* to 160 µg/mL.
  Standard solution: Transfer 60 mg of USP Riboflavin RS to a 250-mL volumetric flask. Dissolve carefully in 1 mL of hydrochloric acid, and dilute with water to volume. Dilute with *Mobile phase* to 9.6 µg/mL.
  Sample solution: Prepare a 2-mg/mL solution of Riboflavin 5′-Phosphate Sodium in water. Dilute this solution with *Mobile phase* to 160 µg/mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: Fluorometer
  Excitation wavelength: 440 nm
  Emission wavelength: 530 nm (monochromator-based detector) or 470 nm (filtered-type detector)

USP Monographs

Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection size: 100 µL
System suitability
Sample: *System suitability solution*
The retention time of riboflavin 5′-monophosphate is 20–25 min. The approximate relative retention times for the components are listed in *Table 1*.

Table 1

Name	Relative Retention Time
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

Suitability requirements
Resolution: NLT 1.0 between riboflavin 4′-monophosphate and riboflavin 5′-monophosphate
Relative standard deviation: NMT 1.5% for riboflavin 5′-monophosphate
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the peak areas of the solutions. Identify the peaks to be measured in the *Sample solution* by comparison with the chromatogram obtained from the *System suitability solution*.
Calculate the percentage of free riboflavin in the portion of Riboflavin 5′-Phosphate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of free riboflavin from the *Sample solution*
r_S = peak area of riboflavin from the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)
C_U = concentration of Riboflavin 5′-Phosphate Sodium in the *Sample solution* (µg/mL)

Calculate the percentage of riboflavin diphosphates in the portion of Riboflavin 5′-Phosphate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak areas for any of the 3 riboflavin diphosphates from the *Sample solution*
r_S = peak area of riboflavin from the *Standard solution*
C_S = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)
C_U = concentration of Riboflavin 5′-Phosphate Sodium in the *Sample solution* (µg/mL)

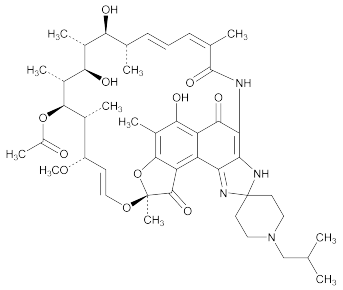
Acceptance criteria
Free riboflavin: NMT 6.0% on the dried basis
Riboflavin diphosphates, as riboflavin: NMT 6.0% on the dried basis

- **LIMIT OF LUMIFLAVIN**
Alcohol-free chloroform: Shake 20 mL of chloroform gently but thoroughly with 20 mL of water for 3 min, draw off the chloroform layer, and wash twice more with 20-mL portions of water. Finally, pass the chloroform through a dry filter paper, shake it for 5 min with 5 g of powdered anhydrous sodium sulfate, allow the mixture to stand for 2 h, and decant or filter the clear chloroform.
Sample solution: Shake 35 mg of Riboflavin 5′-Phosphate Sodium with 10 mL of *Alcohol-free chloroform* for 5 min, and filter.
Blank: Alcohol-free chloroform
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Analytical wavelength: 440 nm
Cell: 1 cm
Analysis
Samples: *Sample solution* and *Blank*
Measure the absorbances of the *Sample solution* and *Blank*. Correct the absorbance of the *Sample solution* with the *Blank*.
Acceptance criteria: The absorbance is NMT 0.025.

SPECIFIC TESTS
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 25.0%
- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩
Sample solution: 15 mg/mL in 5 N hydrochloric acid. Use the solution within 15 min.
Acceptance criteria: +37.0° to +42.0°
- **pH** ⟨**791**⟩
Sample solution: 10 mg/mL solution
Acceptance criteria: 5.0–6.5
- **LOSS ON DRYING ⟨731⟩:** Dry a sample under vacuum over phosphorus pentoxide at 100° for 5 h: it loses NMT 7.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Phosphated Riboflavin RS
USP Riboflavin RS

Rifabutin



$C_{46}H_{62}N_4O_{11}$ 847.00
(9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,16,18,20-Tetrahydroxy-1′-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2′,3′:7,8]naphth[1,2-*d*]imidazole-2,4′-piperidine]-5,10,26-(3*H*,9*H*)-trione-16-acetate [72559-06-9].

» Rifabutin contains not less than 950 µg and not more than 1020 µg of $C_{46}H_{62}N_4O_{11}$ per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers, protected from light and from excessive heat.

USP Reference standards ⟨11⟩**—**
USP Rifabutin RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the