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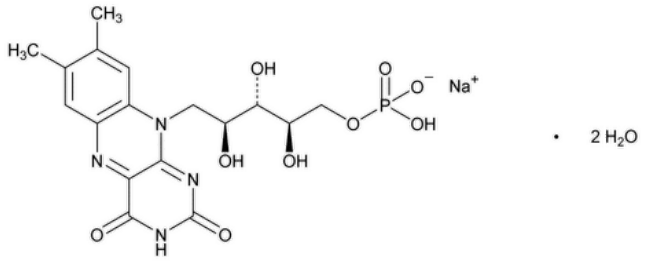
Riboflavin 5'-Phosphate Sodium



$C_{17}H_{20}N_4NaO_9P \cdot 2H_2O$ 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 CAS RN®: 130-40-5; UNII: 957E53WV42.

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)

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

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
RIBOFLAVIN 5'-PHOSPHATE SODIUM	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. Information currently unavailable

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