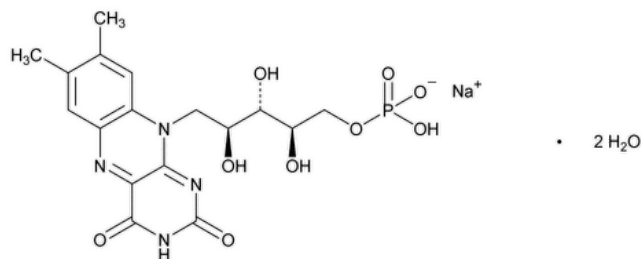


Status: Currently Official on 16-Feb-2025
 Official Date: Official Prior to 2013
 Document Type: USP Monographs
 DocId: GUID-FDC010A2-F2C8-4431-AF61-D37522E5AB74_1_en-US
 DOI: https://doi.org/10.31003/USPNF_M73550_01_01
 DOI Ref: 457j2

© 2025 USPC
 Do not distribute

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*