RIBOFLAVIN 5'-PHOSPHATE SODIUM

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). Add 6. A group ADI 0-0.5 mg/kg bw for riboflavin from Bacillus subtilis, synthetic riboflavin and riboflavin-5-phosphate was established at the 51st JECFA (1998).

SYNONYMS Riboflavin 5'-phosphate ester monosodium salt, Vitamin B_2 phosphate ester monosodium salt; INS No. 101(ii)

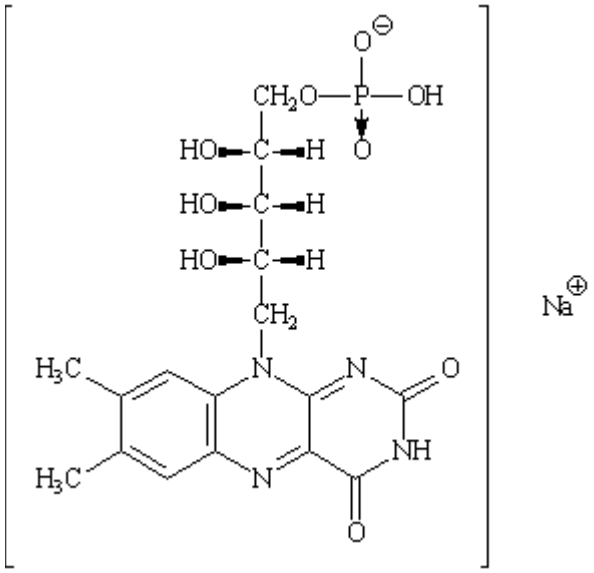
DEFINITION These specifications apply to riboflavin 5'-phosphate sodium together with minor amounts of free riboflavin and riboflavin diphosphate sodium.

Chemical names Monosodium (2R,3R,4S)-5-(3')10'-dihydro-7',8'-dimethyl-2',4'-dioxo-10'-benzo[g]pteridinyl)-2,3,4-trihydroxypentyl phosphate; monosodium salt of 5'-monophosphate ester of riboflavin.

C.A.S. number 130-40-5

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

Structural formula



Formula weight 514.36

Assay Not less than 95% of total colouring matters calculated as $C_{17}H_{20}N_4NaO_9P \cdot 2H_2O$

DESCRIPTION Yellow to orange crystalline hygroscopic powder, with slight odour

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; insoluble in ethanol
Spectrophotometry (Vol. 4)	Using the aqueous solution from the Assay, determine the absorbance (A) at 267 nm, 375 nm and 444 nm. The ratio A_{375}/A_{267} is between 0.30 and 0.34. The ratio A_{444}/A_{267} is between 0.35 and 0.40.
Specific rotation	[alpha] 20, D: Between +38° and +42° (1.5% w/v solution of dried sample in 20% w/v hydrochloric acid)
Test for sodium (Vol. 4)	Passes test Use the sulfated ash for the test

PURITY

Loss on drying (Vol. 4)	Not more than 8% (100°, 5 h in a vacuum over phosphorus pentoxide)
Sulfated ash (Vol. 4)	Not more than 25% Test 0.5 g of the sample
Inorganic phosphate	Not more than 1% calculated as PO_4 on a dried basis See description under TESTS
Subsidiary colouring matters	Not more than 6% of each of free riboflavin and riboflavine disphosphate See description under TESTS Passes test for absence of lumiflavin
Primary aromatic amines (Vol. 4)	Not more than 70 mg/kg calculated as aniline
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Inorganic phosphate	Standard preparation: Transfer 220.0 mg of monobasic potassium phosphate KH_2PO_4, to a 1000 ml volumetric flask, dissolve in and dilute to volume with water and mix. Transfer 20.0 ml of this solution to a 100 ml volumetric flask, dilute to volume with water and mix. Test preparation: Transfer 300.0 mg of the sample to a 100 ml volumetric flask, dissolve in

and dilute to volume with water, and mix.

Acid molybdate solution:

Dilute 25 ml of ammonium molybdate solution (7 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ in sufficient water to make 100 ml) to 200 ml with water, and then add slowly 25 ml of 7.5 N sulfuric acid.

Ferrous sulfate solution:

Just before use, prepare a 10% aqueous ferrous sulfate solution containing 2 ml of 7.5 N sulfuric acid per 100 ml of final solution.

Procedure:

Transfer 10.0 ml each of the Standard preparations and of the Test preparation into separate 50 ml Erlenmeyer flasks, add 10.0 ml of Acid molybdate solution and 5.0 ml of Ferrous sulfate solution to each flask, and mix. Determine the absorbance of each solution in a 1 cm cell at 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 of the Standard preparation.

Subsidiary colouring matters

Free riboflavin and riboflavine disphosphate

Standard preparation:

Transfer 35.0 mg of Riboflavin reference standard into a 250 ml Erlenmeyer 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 to volume with water, and mix. Transfer 20.0 ml of this solution to a second 1000 ml volumetric flask, adjust the pH to 6.0 by the addition of 8 ml of 0.1 N sulfuric acid, dilute to volume with water, and mix. Finally, transfer 25.0 ml of the last solution into a 100 ml volumetric flask, dilute to volume with dioxane-water mixture (1:3), and mix. This solution contains 0.175 µg of riboflavin per ml.

pH Buffer solution:

Dissolve 15.6 g of monobasic phosphate ($NaH_2PO_4.2H_20$) in about 100 ml of water, add 59.3 ml of 1 N sodium hydroxide TS, and dilute to 2000 ml with water. Check the pH with a pH meter, and adjust to 7.0 if necessary.

Test preparation:

Dissolve 100.0 g of the sample in 10.0 ml of pH 7 Buffer solution. Prepare a strip of Whatman chromatography paper, Type 3 mm, medium flow rate, or other equivalent paper suitable for electrophoresis, and saturate the paper with pH 7 Buffer solution. Using a micropipette, apply 0.01 ml of the sample solution along a narrow line of the cathode side of the paper strip contained in a suitable paper electrophoresis chamber. Apply a potential of approximately 250 V, allow electrophoresis to continue for 6 h, and then remove the paper from the chamber. Detect any free riboflavin and/or riboflavin diphosphate by observing the strip in daylight or under ultraviolet light. Free riboflavin, if present, will appear as a band nearest to the starting line, and riboflavin diphosphate will appear farthest from the starting line.

CAUTION:

The riboflavin will be destroyed if exposed to the ultraviolet light for more

than a few sec.
Cut off the respective bands, place them in separate 250 ml Erlenmeyer flasks containing 35.0 ml of dioxane-water mixture (1:3), and allow to stand until the spots are completely eluted from the strips.

Procedure:
Using a suitable fluorometer, determine the intensity of the fluorescence of each sample solution and of the Standard preparation at about 530 nm, using an excitation wavelength of about 460 nm. The fluorescence of the sample solution containing the eluted riboflavin band and riboflavin diphosphate band, respectively, is not greater than that produced by the Standard preparation.

Lumiflavin
Prepare the standard for this limit test for the absence of lumiflavin by diluting 3 ml of 0.1 N potassium dichromate with water to 1000 ml. Pour some chloroform through an alumina column to remove any ethanol. To 10 ml of this chloroform add 35 mg of the sample, shake for 5 min and filter. The colour of the filtrate should be no more intense than that of 10 ml of the standard when viewed in identical containers.

METHOD OF ASSAY

Carry out the assay in subdued light. In a brown glass 500 ml volumetric flask, dissolve 100 mg of the sample in 100 ml of water and add 2.5 ml of glacial acetic acid and dilute to 500.0 ml with water. Place 20.0 ml of this solution in a brown glass 200 ml volumetric flask, add 3.5 ml of a 1.4% w/v solution of sodium acetate and dilute to 200.0 ml with water. Measure the absorbance (A) at the maximum at 444 nm.

$$\% \ Total \ colouring \ matters = \frac{A \times 5000}{328 \times W} \times 1.367$$

where
A = absorbance of the sample solution at 444 nm.
W = weight of sample in g