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Specification Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 31st Meeting 1987

RIBOFLAVIN

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RIBOFLAVIN

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). A group ADI of 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 Vitamin B_2, lactoflavin; INS No. 101(i)

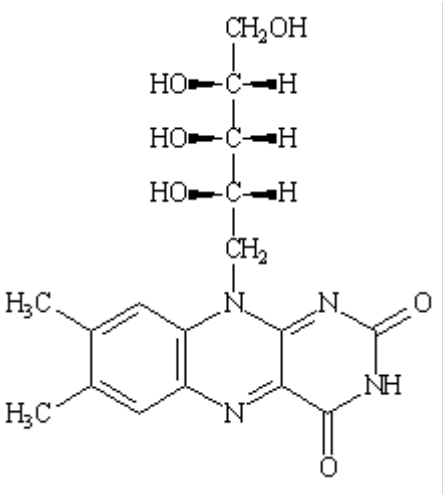
DEFINITION

Chemical names Riboflavin; 3,10-dihydro-7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]benzo-[g]pteridine-2,4-dione; 7,8-dimethyl-10-(1'-D-ribityl)isoalloxazine,

C.A.S. number 83-88-5

Chemical formula $C_{17}H_{20}N_4O_6$

Structural formula



Formula weight 376.37

Assay Not less than 98%

DESCRIPTION Yellow to orange-yellow crystalline powder, with slight odour

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very slightly soluble in water; practically insoluble in alcohol, chloroform, acetone and ether; very soluble in dilute alkali solutions

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 0.31 and 0.33. The ratio A_{444}/A_{267} is between 0.36 and 0.39.

Specific rotation

[alpha] 20, D: Between -115° and -140°
Dry the sample at 100° for 4 h. Dissolve 50.0 mg in 0.05 N sodium hydroxide free from carbonate and dilute to 10.0 ml with the same solvent. Measure the optical rotation within 30 min of dissolution.

Colour reaction

Dissolve about 1 mg of sample in 100 ml of water. The solution has a pale greenish-yellow colour by transmitted light, and by reflected light has an intense yellowish-green fluorescence which disappears on the addition of mineral acids and alkalis.

PURITY

Loss on drying (Vol. 4)

Not more than 1.5% (105°, 4 h)

Sulfated ash (Vol. 4)

Not more than 0.1%
Test 2 g of the sample (Method I)

Subsidiary colouring matters

Prepare the standard for this 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.

Primary aromatic amines (Vol. 4)

Not more than 100 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.”

METHOD OF ASSAY

Carry out the assay in subdued light. In a brown glass 500 ml volumetric flask, suspend 65.0 mg of the sample in 5 ml of water, ensuring that it is completely wetted, and dissolve in 5 ml of 2 N sodium hydroxide solution. As soon as dissolution is complete, add 100 ml of water and 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.

$$\% \text{ Riboflavin} = (A \times 5000)/(328 \times W)$$

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