– *impurities B, C, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
– *disregard limit for peaks other than those due to impurity A*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 1.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Carry out the assay protected from light.

In a brown-glass 500 mL volumetric flask, suspend 65.0 mg in 5 mL of *water R* ensuring that it is completely wetted and dissolve in 5 mL of *dilute sodium hydroxide solution R*. As soon as dissolution is complete, add 100 mL of *water R* and 2.5 mL of *glacial acetic acid R* and dilute to 500.0 mL with *water R*. Place 20.0 mL of this solution in a 200 mL brown-glass volumetric flask, add 3.5 mL of a 14 g/L solution of *sodium acetate R* and dilute to 200.0 mL with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 444 nm.

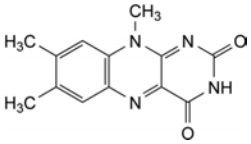
Calculate the content of $C_{17}H_{20}N_4O_6$ taking the specific absorbance to be 328.

STORAGE

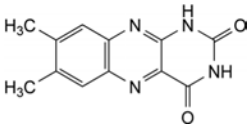
In an airtight container, protected from light.

IMPURITIES

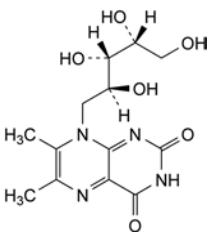
Specified impurities: *A, B, C, D.*



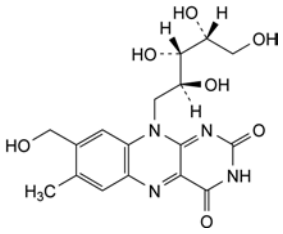
A. 7,8,10-trimethylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (lumiflavine),



B. 7,8-dimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione,



C. 6,7-dimethyl-8-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]-pteridine-2,4(3*H*,8*H*)-dione,

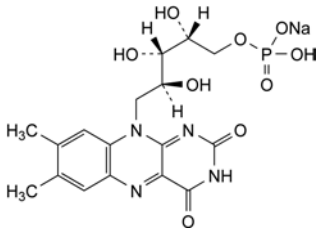


D. 8-(hydroxymethyl)-7-methyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione.

01/2008:0786
corrected 6.0

RIBOFLAVIN SODIUM PHOSPHATE

Riboflavini natrii phosphas



$C_{17}H_{20}N_4NaO_9P$ M_r 478.3
[130-40-5]

DEFINITION

Mixture containing riboflavin 5′-(sodium hydrogen phosphate) as the main component and other riboflavin sodium monophosphates.

Content: 73.0 per cent to 79.0 per cent of riboflavin ($C_{17}H_{20}N_4O_6$; M_r 376.4) (dried substance).

It contains a variable amount of water.

CHARACTERS

Appearance: yellow or orange-yellow, crystalline, hygroscopic powder.

Solubility: soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 50.0 mg in *phosphate buffer solution pH 7.0 R* and dilute to 100.0 mL with the same buffer solution. Dilute 2.0 mL of this solution to 100.0 mL with *phosphate buffer solution pH 7.0 R*.

Spectral range: 230-350 nm.

Absorption maximum: at 266 nm.

Specific absorbance at the absorption maximum: 580 to 640.

B. Examine the chromatograms obtained in the test for related substances.

Results: the principal peak in the chromatogram obtained with the test solution is similar in position and approximate size to the principal peak in the chromatogram obtained with reference solution (b).

C. Dissolve about 10 mg in *dilute sodium hydroxide solution R* and dilute to 100 mL with the same solution. Expose 1 mL of this solution to ultraviolet light at 254 nm for 5 min, add sufficient *acetic acid R* to make the solution acidic to *blue litmus paper R* and shake with 2 mL of *methylene chloride R*. The lower layer shows yellow fluorescence.

D. To 0.5 g add 10 mL of *nitric acid R* and evaporate the mixture to dryness on a water-bath. Ignite the residue until it becomes white, dissolve the residue in 5 mL of *water R* and filter. The filtrate gives reaction (a) of sodium and reaction (b) of phosphates (*2.3.1*).

TESTS

pH (*2.2.3*): 5.0 to 6.5.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Specific optical rotation (*2.2.7*): + 38.0 to + 43.0 (dried substance).

Dissolve 0.300 g in 18.2 mL of *hydrochloric acid R1* and dilute to 25.0 mL with *water R*.

Impurity E. To about 35 mg add 10 mL of *methylene chloride R*, shake for 5 min and filter. The filtrate is not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from actinic light.*

Test solution. Dissolve 0.100 g of the substance to be examined in 50 mL of *water R* and dilute to 100.0 mL with the mobile phase. Dilute 8.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (a). Dissolve 60 mg of *riboflavin CRS* (impurity D) in 1 mL of *hydrochloric acid R* and dilute to 250.0 mL with *water R*. Dilute 4.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b). Dissolve 0.100 g of *riboflavin sodium phosphate CRS* in 50 mL of *water R* and dilute to 100.0 mL with the mobile phase. Dilute 8.0 mL of this solution to 50.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *methanol R*, 7.35 g/L solution of *potassium dihydrogen phosphate R* (150:850 *V/V*).

Flow rate: 2 mL/min.

Detection: spectrophotometer at 266 nm.

Injection: 100 µL.

Run time: until the peak due to riboflavin can be clearly evaluated.

Relative retention with reference to riboflavin 5′-monophosphate (retention time = about 20 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.5; riboflavin 3′-monophosphate = about 0.7; riboflavin 4′-monophosphate = about 0.9; impurity D = about 2.

System suitability: reference solution (b):

– *resolution*: minimum 1.5 between the peaks due to riboflavin 4′-monophosphate and riboflavin 5′-monophosphate.

Calculate the percentage content of free riboflavin (impurity D) and of riboflavin in the form of the diphosphates of riboflavin (impurities A, B, C) from the areas of the peaks in the chromatogram obtained with the test solution and the amount of free riboflavin in reference solution (a).

Limits:

– *impurity D*: maximum 6.0 per cent (dried substance);
– *sum of impurities A, B and C*: maximum 6.0 per cent (dried substance).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Inorganic phosphate: maximum 1.5 per cent.

Dissolve 0.10 g in *water R* and dilute to 100 mL with the same solvent. To 5 mL of this solution, add 10 mL of *water R*, 5 mL of *buffered copper sulfate solution pH 4.0 R*, 2 mL of a 30 g/L solution of *ammonium molybdate R*, 1 mL of a freshly prepared solution containing 20 g/L of *4-methylaminophenol sulfate R* and 50 g/L of *sodium metabisulfite R*, and 1 mL of a 3 per cent *V/V* solution of *perchloric acid R*. Dilute to 25.0 mL with *water R* and measure, within 15 min of its preparation, the absorbance (*2.2.25*) of the solution at 800 nm, using as the compensation liquid a solution prepared in the same manner but without the substance to be examined. The absorbance is not greater than that of a solution prepared as follows: to 15 mL of *phosphate standard solution (5 ppm PO_4) R*, add 5 mL of *buffered copper sulfate solution pH 4.0 R*, 2 mL of a 30 g/L solution of *ammonium molybdate R*, 1 mL of a freshly prepared solution containing 20 g/L of *4-methylaminophenol sulfate R* and 50 g/L of *sodium metabisulfite R*, and 1 mL of a 3 per cent *V/V* solution of *perchloric acid R*; dilute to 25.0 mL with *water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

To 2.0 g in a silica crucible add 2 mL of *nitric acid R*, dropwise, followed by 0.25 mL of *sulfuric acid R*. Heat cautiously until white fumes are evolved and ignite. Extract the cooled residue with 2 quantities, each of 2 mL, of *hydrochloric acid R* and evaporate the extracts to dryness. Dissolve the residue in 2 mL of *dilute acetic acid R* and dilute to 20 mL with *water R*. 12 mL of the solution complies with test A. Prepare the reference solution using 10 mL of *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 8.0 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa for 5 h.

ASSAY

Carry out the assay protected from light.

Dissolve 0.100 g in 150 mL of *water R*, add 2 mL of *glacial acetic acid R* and dilute to 1000.0 mL with *water R*. To 10.0 mL of this solution add 3.5 mL of a 14 g/L solution of *sodium acetate R* and dilute to 50.0 mL with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 444 nm.

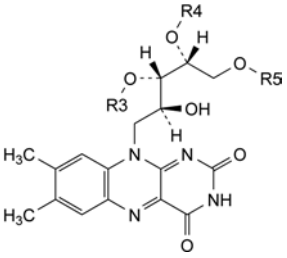
Calculate the content of $C_{17}H_{20}N_4O_6$ taking the specific absorbance to be 328.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities: *A, B, C, D, E.*

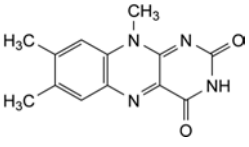


A. R3 = R4 = PO_3H_2, R5 = H: riboflavin 3′,4′-diphosphate,

B. R3 = R5 = PO_3H_2, R4 = H: riboflavin 3′,5′-diphosphate,

C. R3 = H, R4 = R5 = PO_3H_2: riboflavin 4′,5′-diphosphate,

D. R3 = R4 = R5 = H: riboflavin,



E. 7,8,10-trimethylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (lumiflavin).

01/2009:0349

RICE STARCH

Oryzae amylum

DEFINITION

Rice starch is obtained from the caryopsis of *Oryza sativa* L.

CHARACTERS

Appearance: very fine, white or almost white powder, which creaks when pressed between the fingers.

Solubility: practically insoluble in cold water and in ethanol (96 per cent).

Rice starch does not contain starch grains of any other origin. It may contain traces of, if any, fragments of the endosperm tissue of the fruit.

IDENTIFICATION

A. Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R*, it presents polyhedral, simple grains 1-10 µm, mostly 4-6 µm, in size. These simple grains often gather in ellipsoidal, compound grains 50-100 µm in diameter. The grains have a poorly visible central hilum and there are no concentric striations. Between orthogonally orientated polarising plates or prisms, the starch grains show a distinct black cross intersecting at the hilum.

B. Suspend 1 g in 50 mL of *water R*, boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 mL of the mucilage obtained in identification test B add 0.05 mL of *iodine solution R1*. An orange-red to dark blue colour is produced, which disappears on heating.

TESTS

pH (*2.2.3*): 5.0 to 8.0.

Shake 5.0 g with 25.0 mL of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

Iron (*2.4.9*): maximum 10 ppm for the filtrate.

Shake 1.5 g with 15 mL of *dilute hydrochloric acid R*. Filter.

Foreign matter. Examine under a microscope using a mixture of equal volumes of *glycerol R* and *water R*. Not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.

Loss on drying (*2.2.32*): maximum 15.0 per cent, determined on 1.00 g by drying in an oven at 130 °C for 90 min.

Sulfated ash (*2.4.14*): maximum 0.6 per cent, determined on 1.0 g.

Oxidising substances (*2.5.30*): maximum 0.002 per cent, calculated as H_2O_2.

Sulfur dioxide (*2.5.29*): maximum 50 ppm.

Microbial contamination

TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

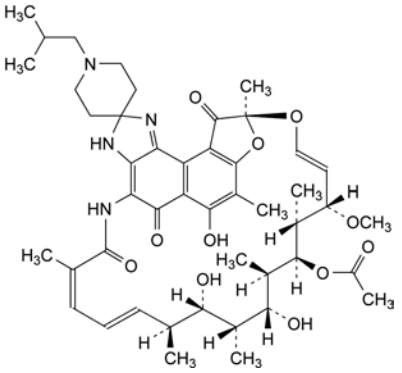
Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

01/2008:1657
corrected 6.0

RIFABUTIN

Rifabutinum



$C_{46}H_{62}N_4O_{11}$ M_r 847
[72559-06-9]

DEFINITION

(9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,18,20-trihydroxy-14-methoxy-7,9,15,17,19,21,25-heptamethyl-1′-(2-methylpropyl)-5,10,26-trioxo-3,5,9,10-tetrahydrospiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2′,3′:7,8]naphtho[1,2-*d*]imidazole-2,4′-piperidine]-16-yl acetate.

Semi-synthetic product derived from a fermentation product.

Content: 96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: reddish-violet amorphous powder.

Solubility: slightly soluble in water, soluble in methanol, slightly soluble in alcohol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *rifabutin CRS*.

B. Examine the chromatograms obtained in the test for related substances.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Impurity A. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 0.100 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.

Reference solution. Dissolve 10 mg of *rifabutin impurity A CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 mL with the same mixture of solvents. Dilute 3 mL of the solution to 100 mL with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Plate: *TLC silica gel F_{254} plate R*.

Mobile phase: *acetone R, light petroleum R* (23:77 *V/V*).

Application: 10 µL.

Development: over 2/3 of the plate.

Drying: in air.

Detection: expose the plate to iodine vapour for about 5 min, then spray with *potassium iodide and starch solution R* and allow to stand for 5 min.