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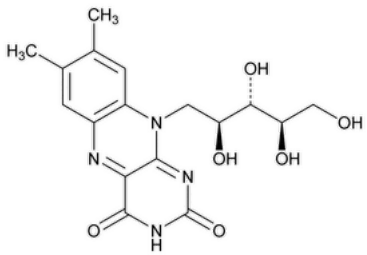
# Riboflavin



$C_{17}H_{20}N_4O_6$ 376.36

Riboflavine CAS RN®: 83-88-5; UNII: TLM2976OFR.

## DEFINITION

Riboflavin contains NLT 98.0% and NMT 102.0% 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 *Sample solution* is pale greenish yellow by transmitted light. By reflected light, it exhibits an intense yellowish-green fluorescence that disappears upon the addition of mineral acids or alkalies.

## ASSAY

• **Procedure**

[Note—Conduct the entire *Analysis* without exposure to direct sunlight.]

**Standard solution:** Transfer 50 mg of USP Riboflavin RS to a 1000-mL volumetric flask containing 50 mL of water. Add 5 mL of 6 N acetic acid and sufficient water to make 800 mL. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to 25°, and dilute with water to volume. Dilute this solution with water to bring it within the operating sensitivity of the fluorometer used.

**Sample solution:** Transfer 50 mg of Riboflavin to a 1000-mL volumetric flask containing 50 mL of water. Add 5 mL of 6 N acetic acid and sufficient water to make 800 mL. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to 25°, and dilute with water to volume. Dilute this solution with water to bring it to the same concentration as that of the *Standard solution*.

**Blank:** Prepare as directed for the *Sample solution*, except omit the test specimen.

**Instrumental conditions**

(See *Fluorescence Spectroscopy* ⟨853⟩.)

**Mode:** Fluorescence

**Excitation wavelength:** 444 nm

**Emission wavelength:** 530 nm

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank*

Measure the fluorescence intensity of the *Standard solution*. Immediately after the reading, add to the solution 10 mg of sodium hydrosulfite, stirring with a glass rod until dissolved, and at once measure the fluorescence again. [Note—Depending on the final concentration of riboflavin in the solution, it may be necessary to increase the amount of sodium hydrosulfite to suppress the fluorescence activity completely.] The difference between the two readings represents the fluorescence intensity ($I_S$) due to the *Standard solution*. Similarly, measure the fluorescence intensity ($I_U$) due to the *Sample solution*. Perform the *Blank* determination, and make any necessary correction.

Calculate the percentage of riboflavin ($C_{17}H_{20}N_4O_6$) in the portion of Riboflavin taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

$I_U$ = fluorescence of the *Sample solution*

$I_S$ = fluorescence of the *Standard solution*

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$C_S$ = concentration of USP Riboflavin RS in the *Standard solution* (µg/mL)

$C_U$ = concentration of Riboflavin in the *Sample solution* (µg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

## IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.3%

• **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, and 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 25 mg of Riboflavin 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 that of the *Blank*.

**Acceptance criteria:** Absorbance is NMT 0.025.

## SPECIFIC TESTS

• **Optical Rotation,** *Specific Rotation*⟨781S⟩

**Sample solution:** 5 mg/mL in 0.05 M carbonate-free sodium hydroxide

**Analysis:** Measure the specific rotation within 30 min of preparation.

**Acceptance criteria:** −115° to −135°

• **Loss on Drying ⟨731⟩:** Dry 500 mg at 105° for 2 h. It loses NMT 1.5% of its weight.

## ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP Reference Standards ⟨11⟩**

USP Riboflavin RS

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

| Topic/Question | Contact | Expert Committee |
|---|---|---|
| RIBOFLAVIN | Natalia Davydova<br>Scientific Liaison | NBDS2020 Non-botanical Dietary Supplements |
| REFERENCE STANDARD SUPPORT | RS Technical Services<br>RSTECH@usp.org | NBDS2020 Non-botanical Dietary Supplements |

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