

$C_U$  = nominal concentration of ribavirin in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 2*)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 2* below.

**Total impurities:** NMT 1.0%

**Impurity Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ribavirin acid <sup>a</sup>	0.55	0.98	0.25
Triazole amide <sup>b</sup>	0.73	1.1	0.25
Uracil <sup>c</sup>	0.89	1.6	0.25
Ribavirin	1.00	—	—
Uridine <sup>d</sup>	1.71	1.0	0.25
Any other individual impurity <sup>e</sup>	—	1.0	0.17

<sup>a</sup> 1-β-D-Ribofuranosyl-1H-1,2,4-triazole-3-carboxylic acid.

<sup>b</sup> 1H-1,2,4-Triazole-3-carboxamide.

<sup>c</sup> Pyrimidine-2,4(1H,3H)-dione.

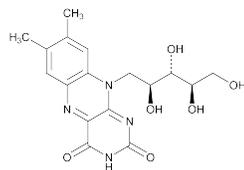
<sup>d</sup> 1-β-D-Ribofuranosylpyrimidine-2,4(1H,3H)-dione.

<sup>e</sup> [NOTE—Disregard any peak area less than 0.05%.]

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 30°.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS** <11>  
USP Ribavirin RS

## Riboflavin



$C_{17}H_{20}N_4O_6$  376.36  
Riboflavine [83-88-5].

#### 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 alkalis.

#### 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 *Spectrophotometry and Light-Scattering* <851>.)

**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*

$C_S$  = 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 *Spectrophotometry and Light-Scattering* <851>.)

**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:**  $-115^{\circ}$  to  $-135^{\circ}$

- **Loss on Drying (731):** Dry 500 mg at  $105^{\circ}$  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

## Riboflavin Injection

» Riboflavin Injection is a sterile solution of Riboflavin in Water for Injection. It contains not less than 95.0 percent and not more than 120.0 percent of the labeled amount of  $C_{17}H_{20}N_4O_6$ . It may contain niacinamide or other suitable solubilizers.

**Packaging and storage**—Preserve in light-resistant, in single-dose or in multiple-dose containers, preferably of Type I glass.

#### USP Reference standards (11)—

USP Riboflavin RS  
USP Endotoxin RS

**Identification**—It responds to the *Identification* test under *Riboflavin*.

**Bacterial endotoxins (85)**—It contains not more than 7.1 USP Endotoxin Units per mg of riboflavin.

**pH (791):** between 4.5 and 7.0.

**Other requirements**—It meets the requirements under *Injections (1)*.

**Assay**—Dilute an accurately measured volume of not less than 1 mL of Injection to make a solution containing approximately 0.1  $\mu$ g of riboflavin per mL. Using this as the *Assay Preparation*, proceed as directed under *Riboflavin Assay (481)*. Calculate the quantity, in mg, of  $C_{17}H_{20}N_4O_6$  in each mL of the Injection taken by the formula:

$$C(A/B)$$

in which *C* is the concentration, in mg per mL, of  $C_{17}H_{20}N_4O_6$  obtained for the *Assay Preparation*, *A* is the test specimen dilution volume, in mL, and *B* is the volume, in mL, of Injection taken.

## Riboflavin Tablets

#### DEFINITION

Riboflavin Tablets contains NLT 95.0% and NMT 115.0% of the labeled amount of riboflavin ( $C_{17}H_{20}N_4O_6$ ).

#### ASSAY

##### • RIBOFLAVIN ASSAY (481)

**Assay preparation:** Transfer a portion of the powder from NLT 20 finely powdered Tablets, equivalent to 20 mg of riboflavin, to a 250-mL flask, and add 150 mL of 0.1 N hydrochloric acid. Shake vigorously, and wash down the sides of the flask with sufficient 0.1 N hydrochloric acid to ensure that the pH remains below 1.5 during the subse-

quent period of heating. Heat the mixture on a steam bath, with frequent agitation, until the riboflavin has dissolved, or in an autoclave at  $121^{\circ}$  for 30 min. Cool, and with vigorous agitation, adjust the mixture with 1 N sodium hydroxide to a pH of 5–6. Transfer to a 1000-mL volumetric flask, and dilute with water to volume. If the solution is not clear, filter through paper known not to adsorb riboflavin. Dilute an aliquot of the clear solution with water to a final volume that contains 0.1  $\mu$ g/mL of riboflavin.

**Analysis:** Proceed as directed in the chapter.

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

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

$I_U$  = corrected fluorescence value from the *Assay preparation*

$I_S$  = corrected fluorescence value from the *Standard Preparation*

$C_S$  = concentration of USP Riboflavin RS in the *Standard Preparation* ( $\mu$ g/mL)

$C_U$  = nominal concentration of riboflavin in the *Assay preparation* ( $\mu$ g/mL)

**Acceptance criteria:** 95.0%–115.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** Known concentration of USP Riboflavin RS in *Medium*

**Sample solution:** Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

**Mobile phase:** A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 1.40 mg/mL of sodium 1-hexanesulfonate

##### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

##### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Relative standard deviation:** NMT 3.0%

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of riboflavin ( $C_{17}H_{20}N_4O_6$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

$r_U$  = peak area of riboflavin from the *Sample solution*

$r_S$  = peak area of riboflavin from the *Standard solution*

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

$D$  = dilution factor for the *Sample solution*

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (*Q*) of the labeled amount of riboflavin ( $C_{17}H_{20}N_4O_6$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.