Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 4.0 and 6.5, in the solution constituted as directed in the labeling, to each 50 mL of which has been added 0.2 mL of a saturated potassium chloride solution.

Chromatographic purity—

Mobile phase, Standard preparation, Test solution, and *Chromatographic system*—Prepare as directed in the *Assay*.

Procedure—Inject about 10 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses of all the peaks, except that of the solvent peak. Calculate the percentage of each peak, other than that of the ribavirin peak, in the chromatogram of the *Test solution* by the formula:

$$100 r_i / r_t$$

in which r_i is the response of the individual peak, and r_t is the sum of the responses of all the peaks in the chromatogram: not more than 0.25% of any individual peak is found, and the sum of all such peaks does not exceed 1.0%.

Other requirements—It meets the requirements for *Specific rotation*, *Loss on drying*, *Residue on ignition*, and *Heavy metals* under *Ribavirin.*

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Ribavirin.*

Test solution—Constitute Ribavirin for Inhalation Solution as directed in the labeling, using an accurately measured volume of diluent. Transfer an accurately measured volume of the constituted inhalation solution, equivalent to about 100 mg of ribavirin, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer 5.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Ribavirin.* Calculate the quantity, in mg, of ribavirin ($C_8H_{12}N_4O_5$) in each mL of the constituted Inhalation Solution taken by the formula:

$$4000(C / V)(r_U / r_S)$$

in which V is the volume, in mL, of constituted Inhalation Solution taken, and the other terms are as defined therein.

Ribavirin Tablets

DEFINITION

Ribavirin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ribavirin ($C_8H_{12}N_4O_5$).

IDENTIFICATION

- The retention time of the ribavirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 4.0 g/L of sodium dihydrogen phosphate dihydrate in water. Adjust with 5% sodium hydroxide solution to a pH of 5.0 ± 0.05. Pass the solution through a suitable filter of 0.45-µm pore size.

Mobile phase: Acetonitrile and *Buffer* (1:49)

Diluent: Acetonitrile and water (3:7)

Standard stock solution: 0.6 mg/mL of USP Ribavirin RS in *Diluent*

Standard solution: 0.03 mg/mL of USP Ribavirin RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: Transfer a portion of ribavirin, equivalent to 1000 mg of ribavirin from finely powdered Tablets (NLT 10), to a 1000-mL volumetric flask. Add about 750 mL of *Diluent*, and sonicate with occasional shaking for 30 min. Cool to room temperature, dilute with *Diluent* to volume, and mix. Centrifuge and decant the supernatant.

Sample solution: 0.03 mg/mL of ribavirin in *Mobile phase* from the *Sample stock solution*. Pass the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 207 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
Run time: 10 min

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NLT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_8H_{12}N_4O_5$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ribavirin from the *Sample solution*
r_S = peak response of ribavirin from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ribavirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨**711**⟩

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
Standard solution: 0.22 mg/mL of USP Ribavirin RS in *Medium*
Sample solution: Pass the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Calculate the percentage of $C_8H_{12}N_4O_5$ dissolved:

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

r_U = peak response of ribavirin from the *Sample solution*
r_S = peak response of ribavirin from the *Standard solution*
C_S = concentration of USP Ribavirin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of ribavirin is dissolved.

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

Organic Impurities

[NOTE—If uracil and/or uridine are known impurities, *Procedure 2* is recommended.]

• **PROCEDURE 1**

Solution A: 3.4 g/L of potassium dihydrogen phosphate in water. Adjust with 5% potassium hydroxide solution to a pH of 5.00 ± 0.05. Pass the solution through a suitable filter of 0.45-µm pore size.

Solution B: Acetonitrile

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	90	10
40	75	25
50	50	50
55	50	50
56	100	0
70	100	0

Standard stock solution: 0.4 mg/mL of USP Ribavirin RS in *Solution A*

Standard solution: 1 µg/mL of USP Ribavirin RS in *Solution A* from the *Standard stock solution*

Sample solution: Transfer a portion of ribavirin, equivalent to 100 mg of ribavirin from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask. Add about 150 mL of *Solution A*, and sonicate with occasional shaking for 15 min. Cool to room temperature, dilute with *Solution A* to volume, and mix. Pass the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

Run time: 70 min. [NOTE—Data collection is only for the first 55 min. The remaining gradient steps re-equilibrate the column.]

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Impurities are listed in *Impurity Table 1*.]

Calculate the percentage of any unknown impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unknown impurity from the *Sample solution*

r_S = peak response of ribavirin from the *Standard solution*

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

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

Acceptance criteria

Individual unknown impurity: NMT 0.10%. [NOTE—Disregard any peak area less than 0.05%.]

Total impurities: NMT 0.30%

Impurity Table 1

Name	Relative Retention Time
Triazole acid[a]	0.35
Ribavirin acid[b]	0.40
Triazole amide[c]	0.64
Ribavirin	1.0
Ribavirin 5-isomer[d]	1.37
Ribavirin methyl ester[e]	2.09
Ribavirin 5′-acetyl[f]	2.43
Ribavirin 5′-benzoyl[g]	4.83
[NOTE—These are process impurities listed for information only.]	

[a] 1*H*-1,2,4-Triazole-3-carboxylic acid.

[b] 1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid.

[c] 1*H*-1,2,4-Triazole-3-carboxamide.

[d] 1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide.

[e] Methyl 1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylate.

[f] 1-(5-*O*-Acetyl-β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide.

[g] 1-(5-*O*-Benzoyl-β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide.

• **PROCEDURE 2**

Buffer: 3.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0 ± 0.1. Pass the solution through a suitable filter of 0.45-µm pore size.

Mobile phase: Methanol and *Buffer* (1:39)

Standard stock solution: 1 mg/mL of USP Ribavirin RS in water. [NOTE—Sonicate with occasional shaking to dissolve the solids.]

Standard solution: 0.01 mg/mL of USP Ribavirin RS in water from the *Standard stock solution*

Sensitivity solution: 0.5 µg/mL of USP Ribavirin RS from the *Standard solution* in water

Sample solution: 1.0 mg/mL. Transfer a portion of ribavirin, equivalent to 1000 mg of ribavirin from finely powdered Tablets (NLT 20), to a 1000-mL volumetric flask. Add about 500 mL of water, and sonicate with occasional shaking for 15 min. Shake the solution for 15 min, and cool to room temperature. Dilute with water to volume, and centrifuge the solution for 10 min.

Chromatographic system

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

Mode: LC

Detector: UV 207 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 10 µL

Run time: NLT 4.3 times the retention time of the ribavirin peak

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Impurities are listed in *Impurity Table 2*.]

Calculate the percentage of any impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*

r_S = peak response of ribavirin from the *Standard solution*

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

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

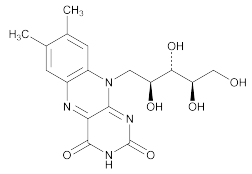
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ribavirin acid[a]	0.55	0.98	0.25
Triazole amide[b]	0.73	1.1	0.25
Uracil[c]	0.89	1.6	0.25
Ribavirin	1.00	—	—
Uridine[d]	1.71	1.0	0.25
Any other individual impurity[e]	—	1.0	0.17

[a] 1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid.
[b] 1*H*-1,2,4-Triazole-3-carboxamide.
[c] Pyrimidine-2,4(1*H*,3*H*)-dione.
[d] 1-β-D-Ribofuranosylpyrimidine-2,4(1*H*,3*H*)-dione.
[e] [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 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 *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