Tobramycin Inhalation Solution

DEFINITION

Tobramycin Inhalation Solution is a sterile, nonpyrogenic, preservative-free solution of Tobramycin in Water for Injection containing Sodium Chloride. It is prepared with the aid of Sulfuric Acid or Sodium Hydroxide and contains, in each mL, NLT 90.0% and NMT 110.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Derivatized sample solution* corresponds to that of the *Derivatized standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 2.0 g of tris(hydroxymethyl) aminomethane in about 800 mL of water. To this solution add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Allow to cool, and pass through a filter of 0.2-µm or finer pore size.

Blank: Water

2,4-Dinitrofluorobenzene reagent: 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.

Tris(hydroxymethyl)aminomethane stock reagent: 15 mg/mL of tris(hydroxymethyl)aminomethane in water. This reagent may be used for 1 month if refrigerated when not in use.

Tris(hydroxymethyl)aminomethane reagent: 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Tris(hydroxymethyl)aminomethane stock solution* to a 200-mL volumetric flask, add dimethyl sulfoxide with mixing, and dilute with dimethyl sulfoxide to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

Standard stock solution: 1.1 mg/mL of USP Tobramycin RS prepared as follows. Weigh a suitable amount of USP Tobramycin RS into a suitable volumetric flask. Add 1 N sulfuric acid, using 2% of the final volume, and enough water to dissolve the tobramycin, and dilute with water to volume.

Standard solution: 0.22 mg/mL of USP Tobramycin RS from the *Standard stock solution* in water

Sample solution: Nominally 0.192 mg/mL of tobramycin from the Inhalation Solution in water

Derivatization procedure: Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.

To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard solution*, 4.0 mL of the *Sample solution*, and 4.0 mL of the *Blank*. To each flask add 10 mL of *2,4-Dinitrofluorobenzene reagent* and 10 mL of *Tris(hydroxymethyl)aminomethane reagent*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume. The solutions thus obtained are the *Derivatized standard solution*, *Derivatized sample solution*, and *Derivatized blank solution*, respectively.

System suitability solution: 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Transfer 2 mL of this solution to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL

System suitability
Samples: *Derivatized standard solution*, *Derivatized blank solution*, and *System suitability solution*
[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]
Identify the solvent and reagent peaks using the *Derivatized blank solution*.
Suitability requirements
Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis
Samples: *Derivatized standard solution* and *Derivatized sample solution*
Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Inhalation Solution taken:

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

r_U = peak response from the *Derivatized sample solution*
r_S = peak response from the *Derivatized standard solution*
C_S = concentration of USP Tobramycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tobramycin in the *Sample solution* (mg/mL)
P = potency of tobramycin in USP Tobramycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF SODIUM CHLORIDE**

Solution A: 2 g of gelatin and 50 mL of nitric acid in 1000 mL of water

Sample solution: Pipet 25 mL of Inhalation Solution into a suitable container. Add 70–100 mL of water and 10 mL of *Solution A*.

Analysis: Titrate potentiometrically with 0.1 N silver nitrate VS using a suitable silver electrode.

Acceptance criteria: 90.0%–110.0% of the labeled amount of sodium chloride

PERFORMANCE TESTS

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

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Acetonitrile, water, and phosphoric acid (5: 95: 0.08)

Solution B: Acetonitrile, water, and phosphoric acid (75: 25: 0.08)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	79	21
14	66	34
25	30	70
35	30	70
40	20	80
50	5	95

Blank: Water

2,4-Dinitrofluorobenzene reagent and **Tris(hydroxymethyl)aminomethane reagent:** Prepare as directed in the *Assay*.

System suitability stock solution: Dissolve USP Tobramycin RS in water, and adjust with 1 N sulfuric acid to a pH of 6.0. Dilute with water to obtain a solution having a known concentration of 1.1 mg/mL.

System suitability solution 1: 0.22 mg/mL of tobramycin from the *System suitability stock solution* in water

System suitability solution 2: Heat a portion of the *System suitability stock solution* in a suitable sealed glass container at 100° for 8–9 h. Cool to room temperature, and dilute with water to obtain a solution containing nominally 0.22 mg/mL of tobramycin.

Standard stock solution: Prepare as directed in the *Assay*.

Standard solution: 1.10 µg/mL of tobramycin from the *Standard stock solution* in water

Sample solution: Prepare as directed in the *Assay*.

Derivatization procedure: Heat all solutions at the same temperature and for the same duration as indicated. Move all flasks to and from the 60° constant-temperature bath at the same time.

To separate 50-mL flasks transfer 15.0 mL of *System suitability solution 1*, 15.0 mL of *System suitability solution 2*, 15.0 mL of *Standard solution*, 15.0 mL of *Sample solution*, and 15.0 mL of *Blank*. To each flask add 10 mL of *2,4-Dinitrofluorobenzene reagent* and 10 mL of *Tris(hydroxymethyl)aminomethane reagent*, shake, and insert the stopper. Place the flasks in a constant-temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, and dilute with acetonitrile to volume. Allow the solutions to stand for 16 h. The solutions thus obtained are *Derivatized system suitability solution 1*, *Derivatized system suitability solution 2*, *Derivatized standard solution*, *Derivatized sample solution*, and *Derivatized blank solution*.

Chromatographic system

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

Mode: LC

Detector: UV 365 nm

Column: 4.6-mm × 25-cm; packing L11

Flow rate: 1.2 mL/min

Injection volume: 45 µL

System suitability

Samples: *Derivatized system suitability solution 1*, *Derivatized system suitability solution 2*, and *Derivatized standard solution*

[NOTE—See *Table 2* for relative retention times.]

Compare *Derivatized system suitability solutions 1* and *2* chromatograms to identify degradation peaks. Deoxystreptamine kanosaminide and nebramine will increase in response in *Derivatized system suitability solution 2*.

Suitability requirements

Capacity factor (*k'*): NLT 15.5 for tobramycin, *Derivatized system suitability solution 2*

Resolution: NLT 1.0 between the nebramine and kanamycin peaks, *Derivatized system suitability solution 2*

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

Analysis

Samples: *Derivatized system suitability solution 1*, *Derivatized standard solution*, *Derivatized sample solution*, and *Derivatized blank solution*

Disregard any peak corresponding to those from the *Derivatized blank solution*, and subtract the quantities of any such peaks found at the relative retention times of 0.36, 0.66, and 0.94 from those found in the *Derivatized sample solution*. For unknown peak determinations, disregard any peaks found in the chromatogram of the *Derivatized sample solution* that correspond to those in the chromatogram of *Derivatized system suitability solution 1*.

Calculate the percentage of each impurity in the portion of Inhalation Solution taken:

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

r_U = peak area of each impurity from the *Derivatized sample solution*

r_S = peak area of tobramycin from the *Derivatized standard solution*

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

C_U = nominal concentration of tobramycin in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Specified unidentified impurity	0.36	0.25
Deoxystreptamine kanosaminide	0.66	0.3
Nebramine	0.94	0.4
Kanamycin B	0.96	—
Tobramycin	1.0	—
Any individual unspecified impurity	—	0.1
Total unspecified impurities	—	0.2
Total impurities	—	1.0

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains NMT 60 USP Endotoxin Units/mL.
- **STERILITY TESTS ⟨71⟩:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

Change to read:

- **ABSORBANCE**
  Sample: Inhalation Solution
  Analysis: Determine the absorbance of the *Sample* at 410 nm in a 1-cm cell.
  Acceptance criteria: NMT •0.30• (RB 1-Apr-2013)

Change to read:

- **pH** ⟨**791**⟩**:** •4.5–6.5• (RB 1-Apr-2013)
- **PARTICULATE MATTER IN INJECTIONS** ⟨**788**⟩**:** Meets the requirements for small-volume injections

Change to read:

- **OSMOLALITY AND OSMOLARITY** ⟨**785**⟩**:** The osmolality is •135–285• (RB 1-Apr-2013) mOsmol/kg.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in low-density, polyethylene, single-use ampules stored in light-resistant foil over-wrapped packaging, in a refrigerator.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Endotoxin RS
  USP Tobramycin RS