• **USP REFERENCE STANDARDS** ⟨11⟩
USP Tobramycin RS

## 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 |

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**Table 1** *(Continued)*

| Time (min) | Solution A (%) | Solution B (%) |
|---|---|---|
| 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.

USP Monographs

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**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

## Tobramycin Ophthalmic Solution

**DEFINITION**

Tobramycin Ophthalmic Solution contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$). It may contain one or more suitable buffers, dispersants, preservatives, and tonicity agents.

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHY**
  **Diluent:** Butyl alcohol and pyridine (100:1)
  **Standard solution:** 3 mg/mL of USP Tobramycin RS in water
  **Sample solution:** Ophthalmic Solution
  **Solution A:** *Standard solution* and *Sample solution* (1:1)
  **Chromatographic system**
  (See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)
  **Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture
  **Application volume:** 6 µL
  **Developing solvent system:** Methanol, chloroform, and ammonium hydroxide (60:25:30)
  **Spray reagent:** 10 mg/mL of ninhydrin in *Diluent*
  **Analysis**
  **Samples:** *Standard solution*, *Sample solution*, and *Solution A*
  Apply the *Standard solution*, the *Sample solution*, and *Solution A* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 min. Immediately locate the spots on the plate by spraying it with *Spray reagent*.
  **Acceptance criteria:** Tobramycin appears as a pink spot, and the $R_F$ values of the spots of the *Sample solution* and of *Solution A*, respectively, correspond to those of the *Standard solution*.
- **B.** The retention time of the major peak of the *Derivatized sample solution* corresponds to that of the *Derivatized standard solution* obtained as directed in the *Assay*.

**ASSAY**

- **PROCEDURE**
  **Mobile phase:** Dissolve 2.0 g of tris(hydroxymethyl)aminomethane in 800 mL of water. Add 20 mL of 1 N sulfuric acid, and dilute with acetonitrile to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-µm or finer pore size.
  **Solution A:** 10 mg/mL of 2,4-dinitrofluorobenzene in alcohol. This solution may be used for 5 days if refrigerated when not in use.
  **Solution B:** 15 mg/mL of tris(hydroxymethyl)aminomethane in water. This solution may be used for 1 month if refrigerated when not in use.
  **Solution C:** 3 mg/mL of tris(hydroxymethyl)aminomethane prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask. Add dimethyl sulfoxide while 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:** 0.66 mg of USP Tobramycin RS prepared as follows. Transfer 33 mg of USP Tobramycin RS into a 50-mL volumetric flask. Add 20 mL of water, 1 mL of 1 N sulfuric acid, and mix. Dilute with water to volume.
  **Standard solution:** 0.132 mg/mL of USP Tobramycin RS from *Standard stock solution* in water
  **Sample solution:** Nominally 0.12 mg/mL of tobramycin from Ophthalmic Solution in water
  **Derivatized standard solution, Derivatized sample solution,** and **Blank solution:** Proceed as follows. 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 5.0 mL of the *Standard solution*, 5.0 mL of the *Sample solution*, and 5.0 mL of water. To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, 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*, the *Derivatized sample solution*, and the *Blank solution*, respectively.
  **System suitability stock solution:** 0.24 mg/mL of *p*-naphtholbenzein in acetonitrile. Prepare freshly.
  **System suitability solution:** Transfer 2 mL of the *System suitability stock 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:** 4-mm × 15-cm; packing L1
  **Column temperature:** 40°
  **Flow rate:** 1.2 mL/min
  **Injection volume:** 20 µL
  **System suitability**
  **Samples:** *Derivatized standard solution* and *System suitability solution*
  [NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]
  **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*, *Derivatized sample solution*, and *Blank solution*
  Use the *Blank solution* to identify the solvent and reagent peaks.
  Calculate the percentage of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$) in the portion of Ophthalmic Solution taken:

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

$r_U$ = peak area of tobramycin 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* (mg/mL)
$C_U$ = nominal concentration of tobramycin in the *Sample solution* (mg/mL)
$P$ = potency of tobramycin in USP Tobramycin RS (µg/mg)



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