**Mobile phase:** Methanol and *Buffer* (7:18)
**Standard solution:** 3 mg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation.]
**Sample solution:** 3 mg/mL of Amifostine in water. [NOTE—Inject immediately after preparation.]
**Chromatographic system**
(See *Chromatography* ⟨621⟩, *System Suitability*.)
**Mode:** LC
**Detector:** UV 220 nm
**Column:** 4.6-mm × 25-cm; 5-µm packing L7
**Autosampler temperature:** 4°
**Flow rate:** 1.0 mL/min
**Injection size:** 10 µL
**System suitability**
**Sample:** *Standard solution*
**Suitability requirements**
**Tailing factor:** NMT 2.0
**Column efficiency:** NLT 100 theoretical plates
**Relative standard deviation:** NMT 2.0%
**Analysis**
**Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of $C_5H_{15}N_2O_3PS$ in the portion of Amifostine taken:

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

$r_U$ = peak response from the *Sample solution*
$r_S$ = peak response from the *Standard solution*
$C_S$ = concentration of USP Amifostine RS in the *Standard solution* (mg/mL)
$C_U$ = concentration of Amifostine in the *Sample solution* (mg/mL)
**Acceptance criteria:** 78.0%–82.0% on the as-is basis

**IMPURITIES**
**Inorganic Impurities**
• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm
**Organic Impurities**
• **PROCEDURE**
**Mobile phase** and **Chromatographic system:** Proceed as directed in the *Assay*.
**Standard solution:** 70 µg/mL of USP Amifostine Thiol RS and 16 µg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation.]
**System suitability solution:** Use the *Standard solution* as described in the *Assay*. [NOTE—Inject immediately after preparation.]
**Sample solution:** 15 mg/mL of Amifostine in water. [NOTE—Inject immediately after preparation.]
**System suitability**
**Samples:** *Standard solution* and *System suitability solution*
**Suitability requirements**
**Column efficiency:** NLT 1000 theoretical plates, *System suitability solution*
**Tailing factor:** NMT 2.0, *System suitability solution*
**Relative standard deviation:** NMT 15.0%, *Standard solution*
**Analysis**
**Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of amifostine thiol in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$ = peak response of amifostine thiol from the *Sample solution*
$r_S$ = peak response of amifostine thiol from the *Standard solution*
$C_S$ = concentration of USP Amifostine Thiol RS in the *Standard solution* (mg/mL)
$C_U$ = nominal concentration of amifostine in the *Sample solution* (mg/mL)
$M_{r1}$ = molecular weight of amifostine thiol, 134.24
$M_{r2}$ = molecular weight of amifostine thiol dihydrochloride, 207.17
Calculate the percentage of any other individual impurity in the portion of Amifostine taken:

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

$r_U$ = peak response of each individual impurity in the *Sample solution*
$r_S$ = peak response of amifostine in the *Standard solution*
$C_S$ = concentration of USP Amifostine RS in the *Standard solution* (µg/mL)
$C_U$ = concentration of the *Sample solution* (µg/mL)
**Acceptance criteria**
**Amifostine thiol:** NMT 0.3%
**Any individual impurity, excluding amifostine thiol:** NMT 0.1%
**Total impurities including amifostine thiol:** NMT 0.3%

**SPECIFIC TESTS**
• **pH** ⟨**791**⟩**:** 6.5–7.5, in a solution (5 in 100)
• **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩
**Sample solution:** To 100.0 mg of Amifostine, contained in a stoppered centrifuge tube, add 10.0 mL of the solution of *N*-ethylmaleimide in methanol (4 in 100), and sonicate for 15 min. Shake to disperse, and sonicate for an additional 15 min. Use 1.0 mL of the supernatant.
**Acceptance criteria:** 19.2%–21.2%

**ADDITIONAL REQUIREMENTS**
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a refrigerator.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Amifostine RS
USP Amifostine Thiol RS
Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrochloride.
$C_5H_{16}N_2SCl_2$ 207.17

## Amifostine for Injection

**DEFINITION**
Amifostine for Injection is a sterile, crystalline substance suitable for parenteral use. It contains NLT 90.0% and NMT 110.0% of the labeled amount of amifostine ($C_5H_{15}N_2O_3PS$).

**IDENTIFICATION**
• **A. INFRARED ABSORPTION** ⟨**197K**⟩
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**
• **PROCEDURE**
**Buffer:** 0.94 g/L of sodium 1-hexanesulfonate. Adjust with phosphoric acid to a pH of 3.0.
**Mobile phase:** Methanol and *Buffer* (7:18)
**Standard solution:** 3 mg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation, or refrigerate until use.]
**Sample solution:** 3 mg/mL of amifostine from Amifostine for Injection, in water. [NOTE—Inject immediately after preparation, or refrigerate until use.]
**Chromatographic system**
(See *Chromatography* ⟨621⟩, *System Suitability*.)

**Mode:** LC
**Detector:** UV 220 nm
**Column:** 4.6-mm × 25-cm; 5-µm packing L7
**Autosampler temperature:** 4°
**Flow rate:** 1.0 mL/min
**Injection size:** 10 µL
**System suitability**
**Sample:** *Standard solution*
**Suitability requirements**
**Column efficiency:** NLT 1000 theoretical plates
**Tailing factor:** NMT 2.0
**Relative standard deviation:** NMT 2.0%
**Analysis**
**Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of $C_5H_{15}N_2O_3PS$ in the portion of Amifostine for Injection taken:

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

$r_U$ = peak responses from the *Sample solution*
$r_S$ = peak responses from the *Standard solution*
$C_S$ = concentration of USP Amifostine RS in the *Standard solution* (mg/mL)
$C_U$ = nominal concentration of amifostine in the *Sample solution* (mg/mL)
**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

• **Uniformity of Dosage Units ⟨905⟩:** Meets the requirements

## IMPURITIES

### Organic Impurities

• **Procedure 1**
**Mobile phase** and **Chromatographic system:** Proceed as directed in the *Assay*.
**Standard solution 1:** 70 µg/mL of USP Amifostine Thiol RS in water
**Standard solution 2:** 15 µg/mL of sodium thiophosphate and 13 µg/mL of *N,N*-dimethylformamide in water. [NOTE—The retention times of sodium thiophosphate and *N,N*-dimethylformamide are about 2 min and about 3.6 min, respectively.]
**Sample solution:** 2.4 mg/mL of amifostine from Amifostine for Injection in water. [NOTE—Inject immediately after preparation.]
**System suitability**
**Samples:** *Standard solution 1* and *Standard solution 2*
**Suitability requirements**
**Relative standard deviation:** NMT 10.0%, *Standard solution 1*; NMT 4.0%, *Standard solution 2*
**Analysis**
**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Calculate the percentage of amifostine thiol in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$ = peak response of amifostine thiol from the *Sample solution*
$r_S$ = peak response of amifostine thiol from *Standard solution 1*
$C_S$ = concentration of USP Amifostine Thiol RS in *Standard solution 1* (mg/mL)
$C_U$ = concentration of amifostine in the *Sample solution* (mg/mL)
$M_{r1}$ = molecular weight of amifostine thiol, 134.24
$M_{r2}$ = molecular weight of amifostine thiol dihydrochloride, 207.17
Calculate the percentage of sodium thiophosphate or *N,N*-dimethylformamide in the portion of sample taken, if present:

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

$r_U$ = peak response of sodium thiophosphate or *N,N*-dimethylformamide from the *Sample solution*
$r_S$ = peak response of sodium thiophosphate or *N,N*-dimethylformamide from *Standard solution 2*
$C_S$ = concentration of sodium thiophosphate or *N,N*-dimethylformamide in *Standard solution 2* (mg/mL)
$C_U$ = concentration of amifostine in the *Sample solution* (mg/mL)
Calculate the percentage of any other individual, unspecified impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$ = peak response of each individual impurity in the *Sample solution*
$r_T$ = total of all peak responses in the *Sample solution*
**Acceptance criteria:** NMT 0.1% of sodium thiophosphate; NMT 0.088% of *N,N*-dimethylformamide; NMT 0.1% of any other individual unspecified impurity

• **Procedure 2**
**Buffer:** 0.4 g/L of sodium 1-octanesulfonate. Adjust with trifluoroacetic acid to a pH of 2.5 ± 0.1.
**Mobile phase:** Acetonitrile and *Buffer* (1:3)
**Standard solution:** 46 µg/mL of USP Amifostine Disulfide RS in water
**Sample solution:** Dilute a quantity of Amifostine for Injection in water to prepare a solution equivalent to 10 mg/mL. [NOTE—Inject immediately after preparation.]
**Chromatographic system**
(See *Chromatography ⟨621⟩, System Suitability*.)
**Mode:** LC
**Detector:** UV 247 nm
**Column:** 4.6-mm × 25-cm; 5-µm packing L1
**Autosampler temperature:** 4°
**Flow rate:** 1.0 mL/min
**Injection size:** 10 µL
**System suitability**
**Sample:** *Standard solution*
**Suitability requirements**
**Tailing factor:** NMT 2.5
**Relative standard deviation:** NMT 4.0%
**Analysis**
**Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of amifostine disulfide in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$ = peak response of amifostine disulfide from the *Sample solution*
$r_S$ = peak response of amifostine disulfide from the *Standard solution*
$C_S$ = concentration of USP Amifostine Disulfide RS in the *Standard solution* (mg/mL)
$C_U$ = concentration of amifostine in the *Sample solution* (mg/mL)
$M_{r1}$ = molecular weight of amifostine disulfide, 266.47
$M_{r2}$ = molecular weight of amifostine disulfide tetrahydrochloride, 412.31
**Acceptance criteria:** NMT 2.0% of total impurities, including amifostine thiol and amifostine disulfide

## SPECIFIC TESTS

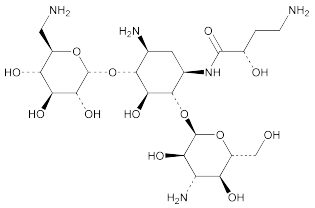
• **Constituted Solution:** At the time of use, it meets the requirements for *Injections ⟨1⟩, Constituted Solutions*. When constituted with 0.9% *Sodium Chloride Injection*, the solution must completely dissolve in 45 s.
• **X-Ray Diffraction ⟨941⟩:** Its X-ray diffraction pattern conforms to that of USP Amifostine RS, similarly determined.
• **Sterility Tests ⟨71⟩:** It meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined, Membrane Filtration*.
• **pH ⟨791⟩:** 6.5–7.5, in a solution constituted as directed in the labeling

- **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩
  **Sample solution:** To 100.0 mg of Amifostine for Injection, contained in a stoppered centrifuge tube, add 10.0 mL of a solution of *N*-ethylmaleimide in methanol (4 in 100), and sonicate for 15 min. Shake to disperse, and sonicate for an additional 15 min. Use 1.0 mL of the supernatant.
  **Acceptance criteria:** 18.0%–22.0%
- **PARTICULATE MATTER IN INJECTIONS** ⟨**788**⟩**:** Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Contains NMT 0.2 USP Endotoxin Unit/mg of amifostine
- **OTHER REQUIREMENTS:** Meets the requirements for *Injections* ⟨1⟩*, Labeling.*

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight *Containers for Sterile Solids* as described under *Injections* ⟨1⟩, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Amifostine RS
  USP Amifostine Disulfide RS
  1,3-Propanediamine, *N,N*-(dithiodi-2,1-ethanediyl)bis, tetrahydrochloride.
  $C_{10}H_{30}N_4S_2Cl_4$ 412.32
  USP Amifostine Thiol RS
  Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrochloride.
  $C_5H_{16}N_2SCl_2$ 207.17
  USP Endotoxin RS

## Amikacin



$C_{22}H_{43}N_5O_{13}$ 585.60

D-Streptamine, *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl(1→4)]-*N*$^1$-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-, (*S*)-.

*O*-3-Amino-3-deoxy-α-D-glucopyranosyl(1→4)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl(1→6)]-*N*$^3$-(4-amino-L-2-hydroxybutyryl)-2-deoxy-L-streptamine [37517-28-5].

» Amikacin has a potency of not less than 900 µg of $C_{22}H_{43}N_5O_{13}$ per mg, calculated on the anhydrous basis.

**Packaging and storage—**Preserve in tight containers.

**USP Reference standards** ⟨11⟩**—**
USP Amikacin RS
USP Kanamycin Sulfate RS

**Identification—**

**A:** *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

*Test solution:* 6 mg per mL, in water. Apply 3 µL.

*Standard solution:* 6 mg per mL, in water. Apply 3 µL.

*Mixed solution:* a mixture of the *Test solution* and the *Standard solution* (1:1). Apply 3 µL.

*Developing solvent system:* a mixture of methanol, ammonium hydroxide, and chloroform (60:35:25).

*Spray reagent:* a 1 in 100 solution of ninhydrin in a mixture of butyl alcohol and pyridine (100:1).

*Procedure*—Proceed as directed in the chapter, except to develop the chromatogram by continuous flow for 5.5 hours. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110 ° for 15 minutes. Spray the plate with *Spray reagent*, and immediately locate the spots: amikacin appears as a pink spot, and the spots obtained from the *Test solution* and the *Mixed solution* correspond in distance from the origin to that obtained from the *Standard solution.*

**B:** The retention time of the peak for amikacin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay.*

**Specific rotation** ⟨781S⟩**:** between +97° and +105°.

*Test solution:* 20 mg per mL, in water.

**Crystallinity** ⟨695⟩**:** meets the requirements.

**pH** ⟨791⟩**:** between 9.5 and 11.5, in a solution containing 10 mg per mL.

**Water,** *Method I* ⟨921⟩**:** not more than 8.5%.

**Residue on ignition** ⟨281⟩**:** not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Assay—**

*Mobile phase*—Use 0.115 N sodium hydroxide. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

*System suitability solution*—Prepare a solution in water containing about 0.02 mg of USP Amikacin RS per mL and 0.008 mg of USP Kanamycin Sulfate RS per mL.

*Standard preparation*—Quantitatively dissolve an accurately weighed quantity of USP Amikacin RS in water to obtain a solution having a known concentration of about 0.02 mg per mL.

*Assay preparation*—Transfer about 50 mg of Amikacin, accurately weighed, to a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with an electrochemical detector, a gold working electrode, and a pH silver-silver chloride reference electrode, a guard column that contains packing L47, and a 4-mm × 25-cm analytical column that contains packing L47. The electrochemical detector is used in the integrated amperometric mode with a range of 300 nC, an output of 1 V full scale, a rise time of 0.5 second, positive polarity, potential E = 0.04 V; t1 = 200 ms; E2 = 0.8 V; t2 = 190 ms; E3 = −0.8 V; t3 = 190 ms. The flow rate is about 0.5 mL per minute. Chromatograph the *System suitability solution*, and measure the peak areas as directed for *Procedure:* the relative retention times are about 0.8 for kanamycin and 1.0 for amikacin; and the resolution, *R,* between kanamycin and amikacin is not less than 3. Chromatograph the *Standard preparation*, and measure the peak areas as directed for *Procedure:* the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 3%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of $C_{22}H_{43}N_5O_{13}$ in each mg of Amikacin taken by the formula:

$$2500(CE / W)(r_U / r_S)$$

in which $C$ is the concentration, in mg per mL, of USP Amikacin RS in the *Standard preparation; E* is the designated amikacin content, in µg per mg, of USP Amikacin RS ; $W$ is the weight, in mg, of Amikacin taken to prepare the *Assay preparation;* and $r_U$ and $r_S$ are the amikacin peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.