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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. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 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 and Implanted Drug Products ⟨1⟩, Specific Tests, Completeness and clarity of solutions*. When constituted with 0.9% *Sodium Chloride Injection*, the solution must completely dissolve in 45 s.

Change to read:

• **▲X-Ray Powder Diffraction ⟨941⟩▲** (CN 1-May-2022)**:** 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 *Labeling ⟨7⟩, Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging*, 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

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
AMIFOSTINE FOR INJECTION	Documentary Standards Support	SM22020 Small Molecules 2

Chromatographic Database Information: Chromatographic Database

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