Amifostine for Injection

Amifostine for Injection is a sterile, crystalline material suitable for parenteral use.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile 0.9 per cent w/v sodium chloride injection, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Amifostine for Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amifostine, $C_5H_{15}N_2O_3PS$.

The contents of the sealed containers comply with the requirements stated under Parenteral Preparations (powder for Injections) and with the following requirements.

Usual strength. 50 mg per ml.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amifostine IPRS* or with the reference spectrum of amifostine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24).6.5 to 7.5, in a solution constituted as directed in the labelling.

Related substances

NOTE — Prepare the solutions immediately before use.

A. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of injection containing 24 mg of amifostine in *water* and dilute to 10.0 ml with *water.*

Reference solution (a). A 0.007 per cent w/v solution of *amifostine thiol* (*Ethanethiol,2-[(3-aminopropyl)amino]-, dihydrochloride*) *IPRS* in *water*.

Reference solution (b). A solution containing 0.0015 per cent w/v of *sodium thiophosphate* and 0.0013 per cent w/v of *N,N-dimethylformamide* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Luna C_8),
- sample temperature: 4°,
- mobile phase: a mixture of 72 volumes of a buffer solution prepared by dissolving 0.94 g of *sodium 1-hexanesulphonate* in 1000 ml of *water,* adjusted to pH 3.0 with *orthophosphoric acid* and 28 volumes of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The retention times of sodium thiophosphate and *N,N*-dimethylformamide are about 2 minutes and about 3.6 minutes, respectively.

Inject reference solution (a) and (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (a) and not more than 4.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to sodium thiophosphate is not more than 0.16 times the area of sodium thiophosphate peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any peak corresponding to *N,N*-dimethylformamide is not more than 0.16 times the area of *N,N*-dimethylformamide peak in the chromatogram obtained with reference solution (b) (0.088 per cent) and the area of any other secondary peak is not more than 0.1 per cent, calculated by area normalisation.

Inject reference solution (a) and the test solution. Calculated the percentage of amifostine thiol in the test solution.

B. Determine byliquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve a suitable quantity of the injection in *water* to obtain a solution containing 1.0 per cent w/v of Amifostine.

Reference solution. A 0.0046 per cent w/v solution of *amifostine disulphide*(*1,3-Propanediamine, N,N-(dithiodi-2,1-ethanediyl)bis, tetrahydrochloride*) *IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Luna C_{18}),
- sample temperature: 4°,
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 0.4 g of *sodium 1-octanesulphonate* in 1000 ml of *water,* adjusted to pH 2.5 with *trifluoroacetic acid*, and 25 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 247 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 4.0 per cent.

Inject the reference solution and the test solution. Calculate the percentage of amifostine disulphide in the test solution.

Total impurities, including amifostine thiol and amifostinedisulphide is not more than 2.0 per cent.

Water (2.3.43). 18.0 per cent to 22.0 per cent, using Method 3, determined on 0.1 g contained in a stoppered centrifuge tube, add 10.0 ml of 4.0 per cent w/v solution of *N-ethylmaleimide* in *methanol* and sonicate for 15 minutes. Shake to disperse, and sonicate for an additional 15 minutes. Use 1.0 ml of the supernatant.

Bacterial endotoxins (2.2.3).Not more than 0.2 Endotoxin Unit per mg of amifostine.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances A with the following modifications.

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve a suitable quantity of injection in *water* to obtain a solution containing 0.3 per cent w/v of Amifostine.

Reference solution. A 0.3 per cent w/v solution of *amifostine IPRS* in *water*.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_5H_{15}N_2O_3PS$ in the injection.

Storage. Store protected from moisture, at a temperature not exceeding 30°.