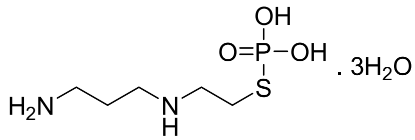
Amifostine



$C_5H_{15}N_2O_3PS,3H_2O$ Mol. Wt. 268.3

Amifostine is Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester), trihydrate.

Amifostine contains not less than 78.0 per cent and not more than 82.0 per cent of $C_5H_{15}N_2O_3PS,3H_2O$, calculated on as-is basis.

Category. Cytoprotective agent.

Description. A white crystalline powder.

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, determined in a 5.0 per cent w/v solution.

Related substances. Determine byliquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 150 mg of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution (a). A solution containing 0.007 per cent w/v of *amifostine thiol* (*ethanethiol,2-[(3-aminopropyl)amino]dihydrochloride*) *IPRS* and 0.0016 per cent w/v *amifostine IPRS* in *water*.

Reference solution (b). A 0.3 per cent w/v solution of *amifostine IPRS* 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.

Inject reference solution (a) and (b). 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 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 15.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to amifostine thiol is not more than 0.64 times the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than 0.94 times the area of amifostine peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 2.8 times the area of amifostine peak in the chromatogram obtained with reference solution (a) (0.3 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Water (2.3.43). 19.2 per cent to 21.2 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.

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

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 30 mg of the substance under examination in *water* and dilute to 10.0 ml with *water*.

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 $C_5H_{15}N_2O_3PS$.

Storage. Store protected from light and moisture, at a temperature 2° to 8°.

2.4.26 Solubility

Amifostine. Freely soluble in *water.*

4.2. General reagent

***N*-ethylmaleimide:** $C_6H_7NO_2 = 125.12$
General laboratory reagent grade of commerce. MP, about 43° to 46°.
