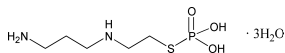


Amifostine



$C_5H_{15}N_2O_3PS \cdot 3H_2O$ 268.27

Ethanedithiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester), trihydrate.

S-[2-(3-Aminopropyl)amino]ethyl]dihydrogen phosphorothioate, trihydrate [112901-68-5].

» Amifostine contains not less than 78.0 percent and not more than 82.0 percent of $C_5H_{15}N_2O_3PS$, calculated on the as-is basis.

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*.

Identification—

A: *Infrared Absorption* (197K).

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

X-ray diffraction (941)—Its X-ray diffraction pattern conforms to that of USP Amifostine RS, similarly determined.

pH (791): between 6.5 and 7.5, in a solution (5 in 100).

Water, Method Ic (921): between 19.2% and 21.2%, the *Test Preparation* being prepared as follows. To about 100.0 mg of Amifostine, accurately weighed, 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 minutes. Shake to disperse, and sonicate for an additional 15 minutes. Use 1.0 mL of the supernatant for *Procedure*.

Heavy metals, Method II (231): 0.002%.

Related compounds—

Mobile phase—Dissolve 1.0 mL of nonafluorobutane sulfonic acid in 1200 mL of HPLC grade water, add 400 μ L of trifluoroacetic acid, and adjust with triethylamine to a pH of 2.5. Prepare a degassed mixture of this solution and acetonitrile (68 : 32).

Blank solution—Use water.

Standard thiol solution—Transfer about 12.4 mg of USP Amifostine Thiol RS, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and mix. [NOTE—Inject immediately after preparation, or refrigerate until use. The solution is stable for 48 hours if maintained at about 5°.]

System suitability solution—Dissolve about 5.0 mg of USP Amifostine RS, accurately weighed, in 1 mL of *Standard thiol solution*, and mix. [NOTE—Inject immediately after preparation, or refrigerate until use. The solution is stable for 12 hours if maintained at about 5°.]

Test solution—Transfer about 50 mg of Amifostine, accurately weighed, to a 1-mL volumetric flask. Dissolve in and dilute with water to volume, and mix. [NOTE—Inject immediately after preparation, or refrigerate until use. The solution is stable for 48 hours if maintained at about 5°.]

Chromatographic system (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm \times 25-cm column that contains packing L1. The column temperature is maintained at 30°, and the temperature of the solutions to be injected is maintained at 2° to 8°. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution* and the *Standard thiol solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between amifostine and amifostine thiol is not less than 2.0; the column efficiency calculated for the

amifostine thiol peak is not less than 2300 theoretical plates; the tailing factor is not more than 4.0; the capacity factor, *k'*, is more than 0.5; and the relative standard deviation for replicate injections is not more than 4.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard thiol solution*, the *Test solution*, and the *Blank solution* into the chromatograph, record the chromatograms, and measure the responses of all the peaks, excluding the peaks corresponding to those obtained from the *Blank solution*. Calculate the percentage of amifostine thiol in the portion of Amifostine taken by the formula:

$$(134.24/207.17)100(C/W)(r_U / r_S)$$

in which 134.24 and 207.17 are the molecular weights of amifostine thiol and amifostine thiol dihydrochloride, respectively; *C* is the concentration, in mg per mL, of amifostine thiol dihydrochloride in the *Standard thiol solution*; *W* is the weight, in mg, of Amifostine taken to prepare the *Test solution*; and *r_U* and *r_S* are the amifostine thiol peak responses obtained from the *Test solution* and the *Standard thiol solution*, respectively. Calculate the percentage of each of the other impurities in the portion of Amifostine taken by the formula:

$$100(r_i / r_A)$$

in which *r_i* and *r_A* are the peak responses for each impurity and amifostine, respectively, obtained from the *Test solution*: not more than 0.1% of any individual impurity, excluding amifostine thiol, is found; and not more than 0.3% of total impurities, including amifostine thiol, is found.

Organic volatile impurities, Method V (467): meets the requirements.

(Official until July 1, 2008)

Assay—

Mobile phase—Dissolve 1.0 mL of nonafluorobutane sulfonic acid in 1200 mL of HPLC grade water. Prepare a degassed mixture of this solution and acetonitrile (90 : 10).

Standard preparation—Transfer about 30 mg of USP Amifostine RS, accurately weighed, to a 10-mL volumetric flask. Dissolve in and dilute with water to volume, and mix. [NOTE—Inject immediately after preparation, or refrigerate until use. The solution is stable for 48 hours if maintained at about 5°.]

Assay preparation—Transfer about 30 mg of Amifostine, accurately weighed, to a 10-mL volumetric flask. Dissolve in and dilute with water to volume, and mix. [NOTE—Inject immediately after preparation, or refrigerate until use. The solution is stable for 48 hours if maintained at about 5°.]

Chromatographic system (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L1. The column temperature is maintained at 30°, and the temperature of the solutions to be injected is maintained at 2° – 8°. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation* and the *Assay preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 7500 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_5H_{15}N_2O_3PS$ in the portion of Amifostine taken by the formula:

$$10C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Amifostine RS in the *Standard preparation*; and *r_U* and *r_S* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.