Edition: BP 2025 (Ph. Eur. 11.6 update)

Thiotepa Injection

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Thiotepa Injection is a sterile solution of Thiotepa in Water for Injections. It is prepared by dissolving Thiotepa for Injection in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Thiotepa Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions. If solid matter separates, the solution should not be used.

THIOTEPA FOR INJECTION

DEFINITION

Thiotepa for Injection is a sterile material consisting of Thiotepa with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of thiotepa, $C_6H_{12}N_3PS$

95.0 to 110.0% of the stated amount.

CHARACTERISTICS

A white powder.

IDENTIFICATION

Burn 20 mg by the method for *oxygen-flask combustion*, Appendix VIII C, using 5 mL of 1.25M *sodium hydroxide* as the absorbing liquid. When the process is complete, dilute to 25 mL with *water*. The resulting solution complies with the following tests.

A. To 5 mL add 0.1 mL of *hydrogen peroxide solution (100 vol)* and 1 mL of 1M *hydrochloric acid*, mix and add 0.05 mL of *barium chloride solution*. The solution becomes turbid.
B. To 2 mL add 40 mL of *water* and 4 mL of *ammonium molybdate-sulfuric acid solution*, mix, add 0.1 g of L-*ascorbic acid* and boil for 1 minute. A blue colour is produced.

TESTS

Acidity or alkalinity

Dissolve a quantity containing 20 mg of Thiotepa in 2 mL of *carbon dioxide-free water*. The pH of the resulting solution is 5.5 to 7.5, Appendix V L.

Clarity of solution

Dissolve a quantity containing 15 mg of Thiotepa in 4 mL of *water*. The solution is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

(1) Dissolve, with shaking, a quantity of the contents of the sealed container containing 15 mg of Thiotepa in 4 mL of *water*, filter and use the filtrate.
(2) Dilute 1 volume of solution (1) to 100 volumes with *water* and further dilute 1 volume to 10 volumes with *water*.
(3) Dissolve 10 mg of *thiotepa BPCRS* in 2 mL of *methanol* in a ground-glass-stoppered tube, add 50 µL of a 0.1% v/v solution of *orthophosphoric acid*, stopper the tube and heat in a water bath at 65° for 50 seconds (generation of methoxy-thiotepa). Allow the solution to cool and add 1 mL of *methanol*.
(4) Dissolve 15 mg of *thiotepa BPCRS* in 10 mL of *water*, add 1 g of *sodium chloride*, boil in a water bath for 10 minutes and cool (generation of chloro-adduct impurity).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 215 nm.
(f) Inject 20 µL of each solution.
(g) For solution (1) allow the chromatography to proceed for 4 times the retention time of the principal peak.

The chromatogram obtained with solution (3) shows a peak corresponding to methoxy-thiotepa with a retention time relative to thiotepa of about 1.3 and the chromatogram obtained with solution (4) shows a peak corresponding to the chloro-adduct impurity with a retention time relative to thiotepa of about 3.1.

MOBILE PHASE

15 volumes of *acetonitrile* and 85 volumes of 0.1M *mixed phosphate buffer pH 7.0*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to the chloro-adduct impurity (identified from the peak in the chromatogram obtained with solution (4)) is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of not more than two such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing 0.15% w/v of Thiotepa.
(2) 0.15% w/v of *thiotepa BPCRS* in *water*.
(3) Dissolve 10 mg of *thiotepa BPCRS* in 2 mL of *methanol* in a ground-glass-stoppered tube, add 50 µL of a 0.1% v/v solution of *orthophosphoric acid*, stopper the tube and heat in a water bath at 65° for 50 seconds (to produce a sufficient quantity of methoxy-thiotepa). Allow the solution to cool and add 1 mL of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 215 nm.
(f) Inject 20 µL of each solution.

The chromatogram obtained with solution (3) shows a peak corresponding to methoxy-thiotepa with a retention time relative to thiotepa of about 1.3.

MOBILE PHASE

15 volumes of *acetonitrile* and 85 volumes of *0.1M mixed phosphate buffer pH 7.0*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.

DETERMINATION OF CONTENT

Calculate the amount of $C_6H_{12}N_3PS$ in the sealed container using the declared content of $C_6H_{12}N_3PS$ in *thiotepa BPCRS*.

Repeat the procedure with a further nine sealed containers. Calculate the average content of $C_6H_{12}N_3PS$ per container from the 10 individual results thus obtained.

STORAGE

The sealed container should be stored at a temperature of 2° to 8°.