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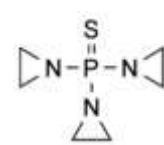
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Thiotepa

General Notices

Details for the public consultation of this monograph are as follows:

EAG/Panel/Working Party	Medicinal Chemicals 2
Contact Details	helen.corns@mhra.gov.uk rachael.feltham@mhra.gov.uk sophie.cherrington@mhra.gov.uk bpcom@mhra.gov.uk
Deadline for Comment	31 st December 2026
Target Publication Date (subject to change)	BP 2028
Notes	Revised monograph If limits are too restrictive, please provide batch/stability data to demonstrate that an increase is required. Identification tests B and C removed Related substances quantitative limits and reporting threshold introduced Impurities section added



C₆H₁₂N₃PS 189.2 52-24-4

Action and use

Antineoplastic alkylating agent.

Preparation

[Thiotepa Injection](#)

DEFINITION

Thiotepa is phosphorothioic tri(ethyleneamide). It contains not less than 97.0% and not more than 102.0% of $C_6H_{12}N_3PS$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

Fine, white crystalline flakes.

Freely soluble in [water](#), in [chloroform](#) and in [ethanol \(96%\)](#).

IDENTIFICATION

The [infrared absorption spectrum](#), [Appendix II A](#), is concordant with the *reference spectrum* of thiotepa ([RS 337](#)).

TESTS

Melting point

52° to 57°, [Appendix V A](#).

Clarity of solution

A 2.0% w/v 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) 0.350% w/v of the substance being examined in [water](#).
- (2) 0.00035% w/v of the substance being examined in [water](#).
- (3) Dissolve 10 mg of the substance being examined 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 methoxythiotepa). Allow the solution to cool and add 1 mL of [methanol](#).
- (4) Dissolve 15 mg of the substance being examined in 10 mL of [water](#), add 1 g of [sodium chloride](#), boil in a water bath for 10 minutes and cool (generation of impurity A).

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.

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](#) between thiotepa and methoxythiotepa is at least 3.0.

CALCULATION OF IMPURITIES

For each impurity, use the concentration of thiotepa in solution (2).

For the reporting threshold, use the concentration of thiotepa in solution (2).

For peak identification, use solution (4).

Thiotepa retention time: about 12 minutes.

Relative retentions: methoxythiotepa, about 1.3; impurity A, about 3.7.

LIMITS

- impurity A: not more than 0.15%;
- unspecified impurities: for each impurity, not more than 0.10%;
- total impurities: not more than 0.2%;
- reporting threshold: 0.05%.

[Water](#)

Not more than 0.5% w/w, [Appendix IX C](#). Cool the reagents and titration vessel in ice throughout the procedure and use 1.2 g. Complete the procedure as quickly as possible.

ASSAY

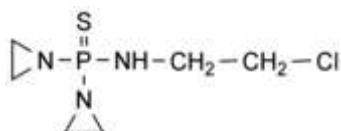
Transfer 0.2 g to an iodine flask with the aid of 50 mL of a 20% w/v solution of [sodium thiosulfate](#) and titrate immediately with [0.1M hydrochloric acid VS](#), using 0.05 mL of [methyl orange solution](#) as indicator, until a faint red colour persists for 10 seconds. Stopper the flask, allow to stand for 30 minutes and titrate with [0.1M sodium hydroxide VS](#) using [phenolphthalein solution R1](#) as indicator. Subtract the volume of [0.1M sodium hydroxide VS](#) used from the volume of [0.1M hydrochloric acid VS](#) used. Repeat the operation without the substance being examined. The difference between the

titrations represents the amount of hydrochloric acid required. Each mL of [0.1M hydrochloric acid VS](#) is equivalent to 6.307 mg of C₆H₁₂N₃PS.

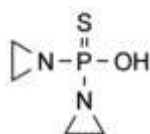
STORAGE

Thiotepa should be stored at a temperature of 2° to 8°. At higher temperatures it polymerises and becomes inactive.

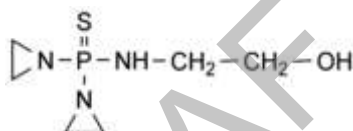
IMPURITIES



- A. bis(aziridine-1-yl)-N-(2-chloroethyl)sulfanylidene-λ⁵-phosphanamine (chloro-adduct),



- B. bis(aziridine-1-yl)sulfanylidene-λ⁵-phosphanol (hydroxythiotepa A),



- C. bis(aziridine-1-yl)-N-(2-hydroxyethyl)sulfanylidene-λ⁵-phosphanamine (hydroxythiotepa B).