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

Mercaptopurine Tablets

General Notices

Action and use

Thiopurine cytotoxic.

DEFINITION

Mercaptopurine Tablets contain Mercaptopurine Monohydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mercaptopurine monohydrate, $C_5H_4N_4S,H_2O$

90.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Mercaptopurine Monohydrate with 20 mL of *absolute ethanol*, filter and evaporate to dryness under reduced pressure. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mercaptopurine *(RS 426)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using the following conditions.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.
(b) Use 0.1M *hydrochloric acid* as the dissolution medium at a temperature of 37°.

Withdraw a sample of 10 mL of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium, if necessary, at the maximum at 325 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *mercaptopurine BPCRS* in the dissolution medium using the declared content of $C_5H_4N_4S,H_2O$ in *mercaptopurine BPCRS*.

Related substances

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

(1) Add a quantity of the powdered tablets containing 0.25 g of Mercaptopurine Monohydrate to 80 mL of 0.02M *methanolic sodium hydroxide* in a 100 mL volumetric flask, mix with the aid of ultrasound for 10 minutes, dilute to 100 mL and filter through a GF/F filter (Whatman Autotop is suitable). Dilute 1 volume of the resulting solution to 20 volumes with 0.1% v/v of *anhydrous formic acid*.
(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1% w/v of *anhydrous formic acid* and further dilute 1 volume of the resulting solution to 10 volumes with 0.1% w/v of *anhydrous formic acid*.
(3) 0.025% w/v of *hypoxanthine* in 0.02M *methanolic sodium hydroxide* diluted to 0.00025% w/v with 0.1% v/v of *anhydrous formic acid*.
(4) 0.03% w/v of *bis-(1,7-dihydro-6H-purine)-6,6-disulfide BPCRS* in 0.02M *methanolic sodium hydroxide* and dilute to 0.0003% w/v with 0.1% v/v of *anhydrous formic acid*.
(5) Dissolve 25 mg of *mercaptopurine impurity standard BPCRS* in 10 mL of 0.02M *methanolic sodium hydroxide*. Dilute 1 volume to 50 volumes with 0.1% v/v of *anhydrous formic acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped phenylethyl silica gel for chromatography* (4 µm) (Phenomenex Synergi Polar RP is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1.5 mL per minute.
(d) Use ambient column temperature.
(e) Use a detection wavelength of 254 nm.
(f) Inject 50 µL of each solution and prepare the samples immediately before injection.

MOBILE PHASE

Mobile phase A 2 volumes of *methanol* and 98 volumes of 0.1% v/v of *anhydrous formic acid*.

Mobile phase B 50 volumes of *methanol* and 50 volumes of 0.1% v/v of *anhydrous formic acid*.

Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0 -8	100	0	isocratic
8 - 20	100→0	0→100	linear gradient
20 - 25	0	100	isocratic
25 - 27	0→100	100→0	linear gradient
27 - 30	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless there is baseline resolution between the principal peaks corresponding to thioguanine, hypoxanthine and adenine in the chromatogram obtained with solution (5).

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to hypoxanthine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2.0%);

the area of any peak corresponding to bis-(1,7-dihydro-6*H*-purine)-6,6-disulfide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2.5%);

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 sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a quantity of the powdered tablets, containing 50 mg of Mercaptopurine Monohydrate in 100 mL of 0.02M *methanolic sodium hydroxide*, mix with the aid of ultrasound for 10 minutes and filter through a GF/F filter (Whatman Autotop is suitable). Dilute 1 volume to 20 volumes with 0.1% v/v *anhydrous formic acid*.
(2) 0.50% w/v of *mercaptopurine BPCRS* in 0.02M *methanolic sodium hydroxide* diluted to 0.0025% w/v with 0.1% v/v of *anhydrous formic acid*.
(3) Dissolve 25 mg of *mercaptopurine impurity standard BPCRS* in 10 mL of 0.02M *methanolic sodium hydroxide*. Dilute 1 volume to 50 volumes with 0.1% v/v of *anhydrous formic acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but with isocratic elution and the mobile phase described below.

MOBILE PHASE

2 volumes of *methanol* and 98 volumes of 0.1% v/v of *anhydrous formic acid*.

SYSTEM SUITABILITY

The test is not valid unless there is baseline resolution between the principal peaks corresponding to thioguanine, hypoxanthine and adenine in the chromatogram obtained with solution (3).

DETERMINATION OF CONTENT

Calculate the total content of mercaptopurine monohydrate, $C_5H_4N_4S,H_2O$, in the tablets using the chromatogram obtained and the declared content of $C_5H_4N_4S,H_2O$ in *mercaptopurine BPCRS*.

STORAGE

Mercaptopurine Tablets should be protected from light.