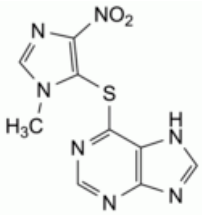
Edition: BP 2025 (Ph. Eur. 11.6 update)

Azathioprine

General Notices

(Ph. Eur. monograph 0369)



$C_9H_7N_7O_2S$ 277.3 *446-86-6*

Action and use

Immunosuppressant.

Preparations

Azathioprine Oral Suspension

Azathioprine Tablets

Ph Eur

DEFINITION

6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)sulfanyl]-7*H*-purine.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Pale-yellow powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It is soluble in dilute solutions of alkali hydroxides and sparingly soluble in dilute mineral acids.

IDENTIFICATION

Infrared absorption spectrophotometry (2.2.24).

Comparison azathioprine CRS.

TESTS

Related substances

Liquid chromatography (2.2.29).

Solution A 2.76 g/L solution of sodium dihydrogen phosphate monohydrate R adjusted to pH 2.5 with phosphoric acid R.

Test solution Dissolve 10 mg of the substance to be examined in 35 mL of a 0.8 g/L solution of sodium hydroxide R and dilute to 100.0 mL with solution A.

Reference solution (a) Dissolve 5 mg of azathioprine impurity A CRS and 5 mg of mercaptopurine monohydrate R (impurity B) in 8.75 mL of a 0.8 g/L solution of sodium hydroxide R and dilute to 25.0 mL with solution A. To 1.0 mL of this solution, add 35 mL of a 0.8 g/L solution of sodium hydroxide R and dilute to 100.0 mL with solution A.

Reference solution (b) Dissolve 2.5 mg of azathioprine impurity G CRS and 2.5 mg of the substance to be examined in 8.8 mL of a 0.8 g/L solution of sodium hydroxide R and dilute to 25.0 mL with solution A. To 1.0 mL of this solution, add 17.5 mL of a 0.8 g/L solution of sodium hydroxide R and dilute to 50.0 mL with solution A.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: phenylsilyl silica gel for chromatography R (5 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: methanol R, solution A (5:95 *V/V*);

— *mobile phase B*: solution A, methanol R (40:60 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 15	100 → 0	0 → 100
15 - 20	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B. Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity G.

Relative retention With reference to azathioprine (retention time = about 15 min): impurity A = about 0.3; impurity B = about 0.4; impurity G = about 0.97.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to impurities A and B in the chromatogram obtained with reference solution (a); minimum 2.0 between the peaks due to impurity G and azathioprine in the chromatogram obtained with reference solution (b).

Limits:

— *impurities A, B*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 25 mL of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 27.73 mg of $C_9H_7N_7O_2S$.

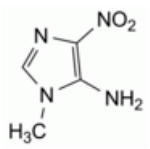
STORAGE

Protected from light.

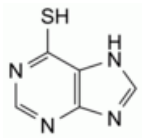
IMPURITIES

Specified impurities A, B.

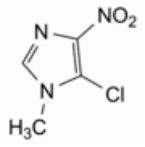
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) C, D, E, F, G.



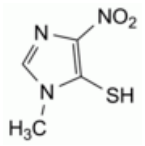
A. 1-methyl-4-nitro-1*H*-imidazol-5-amine,



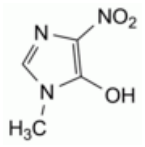
B. 7*H*-purine-6-thiol (mercaptopurine),



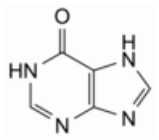
C. 5-chloro-1-methyl-4-nitro-1*H*-imidazole,



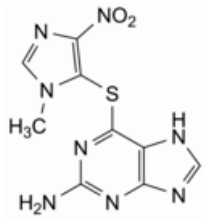
D. 1-methyl-4-nitro-1*H*-imidazole-5-thiol,



E. 1-methyl-4-nitro-1*H*-imidazol-5-ol,



F. 1,7-dihydro-6*H*-purin-6-one (hypoxanthine),



G. 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)sulfanyl]-7*H*-purin-2-amine (thiamiprine).

Ph Eur