TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 1.0 g in 25 mL of a 14 g/L solution of *tartaric acid R*.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 5.0 mg of *azaperone CRS* and 6.0 mg of *benperidol CRS* in *methanol R* and dilute to 200.0 mL with the same solvent.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 5.0 mL of the solution to 20.0 mL with *methanol R*.

Column:

- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 25 °C.

Mobile phase:

- *mobile phase A*: dissolve 1.4 g of *anhydrous sodium sulfate R* in 900 mL of *water R*, add 16.0 mL of *0.01 M sulfuric acid* and dilute to 1000 mL with *water R*;
- *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	95 → 20	5 → 80
15 - 20	20	80

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 230 nm.

Injection: 10 µL.

Relative retention with reference to azaperone (retention time = about 9 min): impurity A = about 0.9; impurity B = about 1.1; impurity C = about 1.15.

System suitability: reference solution (a):

- *resolution*: minimum 8.0 between the peaks due to azaperone and to benperidol.

Limits:

- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *unspecified impurities*: for each impurity, not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.20 per cent);
- *sum of impurities B and C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent);
- *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.130 g in 70 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, using 0.2 mL of *naphtholbenzein solution R* as indicator.

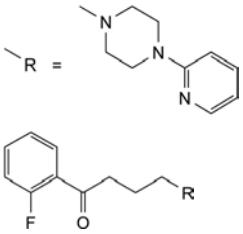
1 mL of *0.1 M perchloric acid* is equivalent to 16.37 mg of $C_{19}H_{22}FN_3O$.

STORAGE

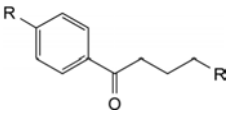
Protected from light.

IMPURITIES

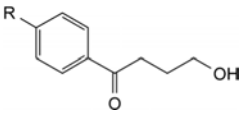
Specified impurities: A, B, C.



A. 1-(2-fluorophenyl)-4-[4-(pyridin-2-yl)piperazin-1-yl]butan-1-one,



B. 4-[4-(pyridin-2-yl)piperazin-1-yl]-1-[4-[4-(pyridin-2-yl)piperazin-1-yl]phenyl]butan-1-one,

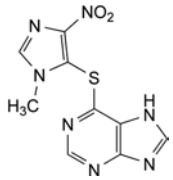


C. 4-hydroxy-1-[4-[4-(pyridin-2-yl)piperazin-1-yl]phenyl]butan-1-one.

07/2010:0369
corrected 7.0

AZATHIOPRINE

Azathioprinum



$C_9H_7N_7O_2S$ M_r 277.3
[446-86-6]

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 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 $C_9H_7N_7O_2S$.

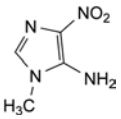
STORAGE

Protected from light.

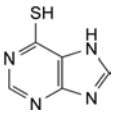
IMPURITIES

Specified impurities: A, B.

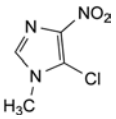
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical 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.*



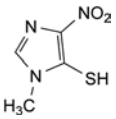
A. 1-methyl-4-nitro-1*H*-imidazol-5-amine,



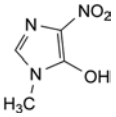
B. 7*H*-purine-6-thiol (mercaptopurine),



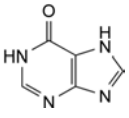
C. 5-chloro-1-methyl-4-nitro-1*H*-imidazole,



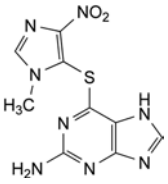
D. 1-methyl-4-nitro-1*H*-imidazole-5-thiol,



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).