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# Azathioprine Tablets

## General Notices

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

|                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| EAG/Panel/Working Party                     | Medicinal Chemicals 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
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| Deadline for Comment                        | 31st December 2026                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Target Publication Date (subject to change) | BP 2028                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Notes                                       | Revised monograph<br>If limits are too restrictive, please provide batch/stability data to demonstrate that an increase is required.<br><b>Content</b> Revised.<br><b>Identification</b> HPLC-DAD method introduced to replace test tests A and B.<br><b>Dissolution</b> New test added.<br><b>Related substances</b> Test added to replace previous impurities test. Quantitative limits introduced.<br><b>Assay</b> Test revised improved with methodology.<br><b>Impurities</b> section added. |

**Action and use**  
Immunosuppressant.

## DEFINITION

Azathioprine Tablets contain [Azathioprine](#).  
The tablets comply with the requirements stated under [Tablets](#) and with the following requirements.

**Content of azathioprine, C<sub>9</sub>H<sub>7</sub>N<sub>7</sub>O<sub>2</sub>S**  
95.0 to 105.0% of the stated amount.

## IDENTIFICATION

In the Assay, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 nm and 400 nm.

The UV spectrum of the principal peak in the chromatogram obtained with solution (1) is concordant with that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in chromatogram obtained with solution (2).

## TESTS

### Dissolution

Comply with the [dissolution test for tablets and capsules](#), [Appendix XII B1](#).

#### TEST CONDITIONS

- (a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.
- (b) Use 900 mL of 0.1M [hydrochloric acid](#), at a temperature of 37°, as the medium.

#### PROCEDURE

- (1) After 30 minutes withdraw a sample of the medium and filter (0.45-µm nylon syringe filter is suitable). Use the filtered medium, diluted, if necessary, to produce a solution expected to contain 0.003% w/v of Azathioprine.
- (2) 0.003% w/v solution of [azathioprine BPCRS](#) in dissolution medium.

#### CHROMATOGRAPHIC CONDITIONS

- (a) Use a stainless steel column (15cm × 3.9 mm) packed with [end-capped octadecylsilyl silica gel for chromatography](#) (5 µm) (Symmetry 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 a column temperature of 30°.
- (e) Use a chilled autosampler of 8°.
- (f) Use a detection wavelength of 285 nm.
- (g) Inject 10 µL of each solution.

#### MOBILE PHASE

46 volumes of [methanol](#) and 54 volumes of 0.05M [potassium dihydrogen orthophosphate](#), previously adjusted to pH 4.0 with [orthophosphoric acid](#).

When the chromatograms are recorded under the prescribed conditions the retention time of the azathioprine is about 2 minutes.

#### DETERMINATION OF CONTENT

Calculate the total content of azathioprine, C<sub>9</sub>H<sub>7</sub>N<sub>7</sub>O<sub>2</sub>S, in the medium from the absorbances obtained and using the declared content of C<sub>9</sub>H<sub>7</sub>N<sub>7</sub>O<sub>2</sub>S, in [azathioprine BPCRS](#).

## LIMITS

The amount of azathioprine released is not less than 75% (Q) of the stated amount.

## Related substances

Carry out the method for [liquid chromatography](#), [Appendix III D](#), using the following solutions immediately, protected from light.

**Solution A** 0.02M [sodium dihydrogen phosphate monohydrate](#)

- (1) Disperse a quantity of the powdered tablets containing 10 mg in 35 mL of 0.02M [sodium hydroxide](#). Dilute to 100 mL with solution A.
- (2) Dilute 1 volume of solution (1) to 100 volumes with solution A.
- (3) 0.015% w/v of [azathioprine BPCRS](#) and 0.002% w/v of [impurity G EPCRS](#) in 0.02M [sodium hydroxide](#). Dilute 1 volume to 100 volumes with solution A.
- (4) 0.004% w/v of [mercaptapurine BPCRS](#) (azathioprine impurity B) and 0.002% w/v of [5-chloro-1-methyl-4-nitroimidazole BPCRS](#) (azathioprine impurity C) in 0.02M [sodium hydroxide](#). Dilute 1 volume of this solution to 100 volumes with solution A.
- (5) Dilute 1 volume of solution (2) to 10 volumes with solution A.

## CHROMATOGRAPHIC CONDITIONS

- (a) Use a stainless steel column (15 cm × 4.6 mm) packed with [end-capped ethylene-bridged phenylsilyl silica gel for chromatography \(hybrid material\)](#) (3.5 µm) (XBridge BEH Phenyl is suitable).
- (b) Use gradient elution and the mobile phase described below.
- (c) Use a flow rate of 1.4 mL per minute.
- (d) Use a column temperature of 30°.
- (e) Use a chilled autosampler temperature of 8°.
- (f) Use a detection wavelength of 240 nm.
- (g) Inject 20 µL of each solution.

## MOBILE PHASE

**Mobile phase A** 5 volumes of [methanol](#) and 95 volumes of 0.02M [sodium dihydrogen phosphate monohydrate](#), previously adjusted to pH 2.4 with [orthophosphoric acid](#).

**Mobile phase B** 60 volumes of [methanol](#) and 40 volumes of 0.02M [sodium dihydrogen phosphate monohydrate](#), previously adjusted to pH 2.4 with [orthophosphoric acid](#).

| Time (Minutes) | Mobile phase A (% v/v) | Mobile phase B (% v/v) | Comment         |
|----------------|------------------------|------------------------|-----------------|
| 0-3.5          | 100                    | 0                      | isocratic       |
| 3.5-10.5       | 100→0                  | 0→100                  | linear gradient |
| 10.5-14.0      | 0                      | 100                    | isocratic       |
| 14.0-17.5      | 100→0                  | 100→0                  | linear gradient |

| Time (Minutes) | Mobile phase A (%<br>v/v) | Mobile phase B (%<br>v/v) | Comment          |
|----------------|---------------------------|---------------------------|------------------|
| 17.5-21.0      | 100                       | 0                         | re-equilibration |

#### SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3), the [resolution](#) between the peaks due to impurity G and azathioprine at least 2.0.

#### CALCULATION OF IMPURITIES

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

For peak identification, use solutions (3) and (4).

For the reporting threshold, use the concentration of Azathioprine in solution (5).

Azathioprine retention time: about 10 minutes.

Relative retention: impurity E, about 0.16; impurity F, about 0.18; impurity A, about 0.25; impurity D, about 0.27; impurity B, about 0.3 and impurity C, about 0.9.

Correction factors: impurity B, multiply by 0.8 and impurity G, multiply by 0.6.

#### LIMITS

- impurity B: not more than 1.0%;
- unspecified impurities, for each impurity: not more than 0.2%;
- total impurities: not more than 1.0%;
- reporting threshold: 0.1%.

## ASSAY

Carry out the method for [liquid chromatography, Appendix III D](#), using the following solutions.

(1) Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 25 mg in 25 mL of 0.02M [sodium hydroxide](#), with the use of ultrasound and intermittent shaking. Filter and dilute the filtrate to produce a solution expected to contain 0.005% w/v of Azathioprine in 0.02M [sodium hydroxide](#) (a 0.45-µm nylon filter is suitable).

(2) 0.005% w/v of [azathioprine impurity A EPCRS](#) in 0.02M [sodium hydroxide](#).

#### CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

#### MOBILE PHASE

46 volumes of [methanol](#) and 54 volumes of 0.05M [potassium dihydrogen orthophosphate](#), previously adjusted to pH 4.0 with [orthophosphoric acid](#).

#### DETERMINATION OF CONTENT

Calculate the content of azathioprine,  $C_9H_7N_7O_2S$ , in the tablets using the declared content of  $C_9H_7N_7O_2S$ , in [azathioprine BPCRS](#).

#### STORAGE

Azathioprine Tablets should be protected from light.

#### IMPURITIES

The impurities limited by the requirements of this monograph include impurities listed under [Azathioprine](#).

DRAFT MONOGRAPH  
SUBJECT TO CHANGE