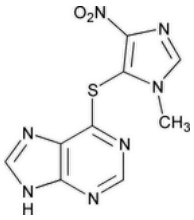
Status: Currently Official on 13-Feb-2025
Official Date: Official as of 01-May-2020
Document Type: USP Monographs
DocId: GUID-12D459B6-F188-43C9-A983-D1AAA2D82FEA_2_en-US
DOI: https://doi.org/10.31003/USPNF_M6690_02_01
DOI Ref: 3x82c

Azathioprine



$C_9H_7N_7O_2S$ 277.26

1*H*-Purine, 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-;

6-[(1-Methyl-4-nitroimidazol-5-yl)thio]purine CAS RN®: 446-86-6; UNII: MRK240IY2L.

DEFINITION

Azathioprine contains NLT 98.0% and NMT 102.0% of azathioprine ($C_9H_7N_7O_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Solution A: 1.6 g/L of sodium 1-heptanesulfonate in water

Mobile phase: Methanol and *Solution A* (30:70). Adjust with 1 N hydrochloric acid to a pH of 3.5 ± 0.1.

Standard stock solution: 0.5 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add 25% of the flask volume of methanol and 1% of ammonium hydroxide to the flask. Swirl, and sonicate for 2 min or until dissolved. Dilute with methanol to volume.

Standard solution: 0.1 mg/mL of USP Azathioprine RS in water from the *Standard stock solution*

Sample solution: 0.1 mg/mL of Azathioprine prepared as follows. Transfer 50 mg of sample to a 100-mL volumetric flask. Add 25 mL of methanol and 1.0 mL of ammonium hydroxide to the flask, swirl, and sonicate for 2 min or until dissolved. Dilute with methanol to volume. Transfer 10.0 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1.8 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of azathioprine ($C_9H_7N_7O_2S$) in the portion of Azathioprine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of azathioprine from the *Sample solution*

r_S = peak response of azathioprine from the *Standard solution*

C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)

C_U = concentration of Azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Organic Impurities**

Buffer: 2.76 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.

Solution A: Methanol and *Buffer* (5:95)

Solution B: Methanol and *Buffer* (60:40)

Mobile phase: See *Table 1*. Return to original conditions, and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
15	0	100
20	0	100

Diluent: 0.8 g/L of sodium hydroxide in water

System suitability stock solution A: 0.2 mg/mL each of USP Azathioprine Related Compound A RS and USP Mercaptopurine RS prepared as follows. Transfer USP Azathioprine Related Compound A RS and USP Mercaptopurine RS to a suitable volumetric flask. Add 35% of the flask volume of *Diluent*, and dilute with *Buffer* to volume.

System suitability stock solution B: 0.1 mg/mL each of USP Azathioprine Related Compound G RS and USP Azathioprine RS prepared as follows. Transfer USP Azathioprine Related Compound G RS and USP Azathioprine RS to a suitable volumetric flask. Add 35% of the flask volume of *Diluent*, and dilute with *Buffer* to volume.

System suitability solution: 0.002 mg/mL each of USP Azathioprine Related Compound A RS, USP Mercaptopurine RS, USP Azathioprine Related Compound G RS, and USP Azathioprine RS prepared as follows. Transfer 1 mL of *System suitability stock solution A* and 2 mL of *System suitability stock solution B* to a 100-mL volumetric flask. Add 35 mL of *Diluent*, and dilute with *Buffer* to volume.

Standard stock solution: 0.1 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add 35% of the flask volume of *Diluent*, and dilute with *Buffer* to volume.

Standard solution: 0.1 µg/mL of USP Azathioprine RS in *Buffer* from *Standard stock solution*

Sample solution: 0.1 mg/mL of Azathioprine prepared as follows. Transfer Azathioprine to a suitable volumetric flask. Add 35% of the flask volume with *Diluent*, and dilute with *Buffer* to volume.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 5-µm packing L11

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2 between azathioprine related compound A and mercaptopurine; NLT 2 between azathioprine related compound G and azathioprine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Azathioprine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of azathioprine from the *Standard solution*

C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)

C_U = concentration of Azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azathioprine related compound A[a]	0.3	0.15
Mercaptopurine[b]	0.4	0.15
Azathioprine related compound G[c]	0.97	0.10
Azathioprine	1.0	—
Any other unspecified impurity	—	0.10
Total impurities	—	0.5

[a] 1-Methyl-4-nitro-1*H*-imidazol-5-amine.

[b] 9*H*-Purine-6-thiol.

[c] 6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9*H*-purin-2-amine.

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩

Analysis: Dry under vacuum at 105° for 5 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP Reference Standards** ⟨11⟩

USP Azathioprine RS

USP Azathioprine Related Compound A RS

1-Methyl-4-nitro-1*H*-imidazol-5-amine.

$C_4H_6N_4O_2$ 142.12

USP Azathioprine Related Compound G RS

6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9*H*-purin-2-amine.

$C_9H_8N_8O_2S$ 292.28

USP Mercaptopurine RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
AZATHIOPRINE	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 39(6)

Current DocID: GUID-12D459B6-F188-43C9-A983-D1AAA2D82FEA_2_en-US

DOI: https://doi.org/10.31003/USPNF_M6690_02_01

DOI ref: 3x82c