Status: Currently Official on 13-Feb-2025
Official Date: Official as of 01-Nov-2020
Document Type: USP Monographs
DocId: GUID-1A643054-F701-45DD-AF21-B5486520DE72_2_en-US
DOI: https://doi.org/10.31003/USPNF_M6720_02_01
DOI Ref: 36hd1

Azathioprine Tablets

DEFINITION

Azathioprine Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of azathioprine ($C_9H_7N_7O_2S$).

IDENTIFICATION

Change to read:

• **A.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

Add the following:

▲• **B.** The UV spectrum of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **Procedure**

Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust the solution with 1 N hydrochloric acid to a pH of 3.5. ▲▲ (USP 1-May-2020)

Standard stock solution: 0.5 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add methanol equivalent to 30% of the flask volume and ammonium hydroxide equivalent to 1% of the flask volume, swirl, and sonicate for 2 min. Dilute with methanol to volume.

Standard solution: 0.1 mg/mL of USP Azathioprine RS in water prepared ▲from the *Standard stock solution*▲ (USP 1-May-2020)

Sample stock solution: Nominally 0.5 mg/mL of azathioprine prepared as follows. ▲Finely powder NLT 20 Tablets and transfer a portion of the powder to a suitable volumetric flask. Add methanol equivalent to 25% of the flask volume and ammonium hydroxide equivalent to 1% of the flask volume, swirl, and sonicate for 2 min. Dilute with methanol to volume. Allow the excipients to settle.▲ (USP 1-May-2020)

Sample solution: Nominally 0.1 mg/mL of azathioprine ▲from the *Sample stock solution* in water. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.▲ (USP 1-May-2020)

Chromatographic system

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

Mode: LC

Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2020)

Column: 4-mm × 30-cm; ▲10-µm▲ (USP 1-May-2020) packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

▲▲ (USP 1-May-2020)

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) in the portion of Tablets 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 = nominal concentration of azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Azathioprine RS in *Medium*

Sample solutions: ▲Pass portions of the solution under test through a suitable▲ (USP 1-May-2020) filter and dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 280 nm

▲Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the percentage of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of azathioprine in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = dilution factor for the *Sample solution*, if applicable

L = label claim (mg/Tablet)

▲ (USP 1-May-2020)

Tolerances: NLT 75% (Q) of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Add the following:

▲• Organic Impurities

Protect the solutions from light.

Mobile phase: Dissolve 1.2 g of sodium 1-heptanesulfonate in 750 mL of water, and add 250 mL of methanol. Adjust the solution with 1 N hydrochloric acid to a pH of 3.0.

System suitability stock solution: 0.25 mg/mL each of USP Mercaptopurine RS, USP Azathioprine Related Compound A RS, and chloromethylnitroimidazole in methanol

System suitability solution: 0.5 mg/mL of USP Azathioprine RS and 0.005 mg/mL each of USP Mercaptopurine RS, USP Azathioprine Related Compound A RS, and chloromethylnitroimidazole prepared as follows. Transfer a suitable amount of USP Azathioprine RS to a suitable volumetric flask. Add methanol equivalent to 20% of the flask volume and sonicate to dissolve. Add a suitable amount of the *System suitability stock solution* and dilute with *Mobile phase* to volume.

Standard stock solution: 0.25 mg/mL of USP Azathioprine RS prepared as follows. Transfer a suitable amount of USP Azathioprine RS to a suitable volumetric flask. Add methanol equivalent to 75% of the flask volume, sonicate to dissolve, and dilute with methanol to volume.

Standard solution: 0.005 mg/mL of USP Azathioprine RS in *Mobile phase* from the *Standard stock solution*

Sensitivity solution: 0.0005 mg/mL of USP Azathioprine RS in *Mobile phase* from the *Standard solution*

Sample solution: Nominally 0.5 mg/mL of azathioprine prepared as follows. Transfer a portion of the powder, from NLT 20 powdered Tablets, to a suitable volumetric flask. Add 20% of the flask volume of methanol, sonicate for 20 min with intermittent shaking, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

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

Column temperature: 30°

Flow rate: 1.1 mL/min

Injection volume: 25 µL

System suitability

Samples: *System suitability solution*, *Standard solution,* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between azathioprine related compound A and mercaptopurine; NLT 2.0 between chloromethylnitroimidazole and azathioprine, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

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

r_U = peak response of each degradation product 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 = nominal concentration of azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azathioprine related compound A[a]	0.47	—
Mercaptopurine	0.52	0.5
Dipurinyl sulfide[a,b]	0.75	—
Chloromethylnitroimidazole[a,c]	0.86	—
Azathioprine	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	2.0

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total degradation products for the drug product.

[b] Di(9*H*-purin-6-yl)sulfide.

[c] 5-Chloro-1-methyl-4-nitro-1*H*-imidazole.

▲ (USP 1-May-2020)

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Protect from light. ▲Store at controlled room temperature.▲ (USP 1-May-2020)

Change to read:

• **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 Mercaptopurine RS

9*H*-Purine-6-thiol monohydrate.

$C_5H_4N_4S \cdot H_2O$ 170.19▲ (USP 1-May-2020)

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

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

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 43(3)

Current DocID: GUID-1A643054-F701-45DD-AF21-B5486520DE72_2_en-US

DOI: https://doi.org/10.31003/USPNF_M6720_02_01

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