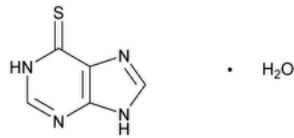


Status: Currently Official on 15-Feb-2025
Official Date: Official as of 01-Sep-2021
Document Type: USP Monographs
DocId: GUID-4B2D663D-5CC9-4A4B-A7E4-2A8ECB39043A_4_en-US
DOI: https://doi.org/10.31003/USPNF_M49330_04_01
DOI Ref: 1klx3

© 2025 USPC
Do not distribute

Mercaptopurine



$C_5H_4N_4S \cdot H_2O$ 170.19

$C_5H_4N_4S$ 152.18

6*H*-Purine-6-thione, 1,7-dihydro-, monohydrate;

Purine-6-thiol monohydrate CAS RN[®]: 6112-76-1; UNII: E7WED276I5.

Anhydrous CAS RN[®]: 50-44-2; UNII: PKK6MUZ20G.

DEFINITION

Mercaptopurine contains NLT 97.0% and NMT 102.0% of mercaptopurine ($C_5H_4N_4S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Infrared Spectroscopy](#):** ▲197A or ▲(IRA 1-Sep-2021) 197K
- **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

Change to read:

• **PROCEDURE**

Solution A: 0.77 g/L of [ammonium acetate](#) in [water](#)

Mobile phase: [Methanol](#) and *Solution A* (25:75)

Standard stock solution: 0.2 mg/mL of [USP Mercaptopurine RS](#) in a mixture of [methanol](#) and [water](#)▲(50:50).▲(IRA 1-Sep-2021) Transfer [USP Mercaptopurine RS](#) into a suitable volumetric flask, and add [methanol](#) equivalent to 50% of the final volume. Shake mechanically to dissolve, and dilute with [water](#) to volume.

Standard solution: 0.02 mg/mL of [USP Mercaptopurine RS](#) in *Mobile phase* from the *Standard stock solution*

Sample stock solution: ▲0.4 mg/mL of Mercaptopurine prepared as follows.▲(IRA 1-Sep-2021) Transfer 25 mg of Mercaptopurine into a 50-mL volumetric flask. Add 25 mL of [methanol](#), shake mechanically ▲to dissolve,▲(IRA 1-Sep-2021) and dilute with [water](#) to volume. Transfer 20 mL of this solution into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: 0.02 mg/mL of Mercaptopurine in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm × 15-cm; 5-μm packing [L68](#)

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mercaptopurine ($C_5H_4N_4S$) in the portion of Mercaptopurine taken:

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

r_U = peak response ▲ of mercaptopurine ▲ (IRA 1-Sep-2021) from the *Sample solution*

r_S = peak response ▲ of mercaptopurine ▲ (IRA 1-Sep-2021) from the *Standard solution*

C_S = concentration of [USP Mercaptopurine RS](#) in the *Standard solution* (mg/mL)

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

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Change to read:

• ORGANIC IMPURITIES

Solution A: 0.1% (v/v) [formic acid](#) in [water](#)

Solution B: [Methanol](#) and *Solution A* (2:98)

Solution C: [Methanol](#) and *Solution A* ▲ (50:50) ▲ (IRA 1-Sep-2021)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
8	100	0
20	0	100
25	0	100
27	100	0
30	100	0

Standard stock solution: 0.06 mg/mL of [USP Mercaptopurine RS](#) in *Solution A*. [NOTE—Use [methanol](#) equivalent to 2.5% of the final volume to help dissolve.]

Standard solution: 1.2 µg/mL of [USP Mercaptopurine RS](#) in *Solution B* from the *Standard stock solution*

Sensitivity solution: 0.06 µg/mL of [USP Mercaptopurine RS](#) in *Solution B* from the *Standard solution*

Sample stock solution: 0.5 mg/mL of Mercaptopurine in a mixture of [methanol](#) and *Solution A* ▲ (50:50) ▲ (IRA 1-Sep-2021) prepared as follows.

Transfer a suitable quantity of Mercaptopurine to an appropriate volumetric flask, add [methanol](#) equivalent to ▲50% ▲ (IRA 1-Sep-2021) of the final volume, and shake to dissolve. Dilute with *Solution A* to volume.

Sample solution: 0.12 mg/mL of Mercaptopurine in *Solution A* from the *Sample stock solution*. [NOTE—Inject the *Sample solution* within 1 h of preparation.]

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 10-cm; 3-µm packing [L1](#)

Temperatures

Column: 30°

Sample: 4°

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

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

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

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

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

C_S = concentration of [USP Mercaptopurine RS](#) in the *Standard solution* (mg/mL)

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

F = relative response factor for each individual impurity (see [Table 2](#))

Acceptance criteria: See [Table 2](#). ▲The reporting threshold is ▲ (IRA 1-Sep-2021) 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Didanosine related compound A ^a	0.54	6.3	0.15
Mercaptopurine	1.00	—	—
Mercaptopurine disulfide ^b	2.90	4.4	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

^a Hypoxanthine.

^b 1,2-Di(9H-purin-6-yl)disulfane.

SPECIFIC TESTS

• PHOSPHORUS

Standard phosphate solution: 43.96 µg/mL of dried [monobasic potassium phosphate](#) (equivalent to 10 µg of phosphorus)

Standard solution: Transfer 2 mL of *Standard phosphate solution* to a 25-mL volumetric flask. Add 1 mL of 15 N [sulfuric acid](#), 0.5 mL of [nitric acid](#), 0.75 mL of [ammonium molybdate TS](#), and 1 mL of [aminonaphtholsulfonic acid TS](#); then dilute with [water](#) to volume; and mix. Allow to stand for 5 min.

Sample solution: Digest 200 mg with 2 mL of 15 N [sulfuric acid](#) in a large test tube, periodically adding [nitric acid](#), dropwise and with caution. Continue heating until practically all of the liquid has evaporated and the residue is colorless. Transfer the residue, with the aid of small portions of [water](#), to a 25-mL volumetric flask. Add 1 mL of 15 N [sulfuric acid](#), 0.5 mL of [nitric acid](#), 0.75 mL of [ammonium molybdate TS](#), and 1 mL of [aminonaphtholsulfonic acid TS](#), and then dilute with [water](#) to volume. Allow to stand for 5 min.

Blank: Transfer 2 mL of 15 N [sulfuric acid](#) to a large test tube, periodically adding [nitric acid](#), dropwise and with caution. Continue heating until practically all of the liquid has evaporated and the residue is colorless. Transfer the residue, with the aid of small portions of [water](#), to a 25-mL volumetric flask. Add 1 mL of 15 N [sulfuric acid](#), 0.5 mL of [nitric acid](#), 0.75 mL of [ammonium molybdate TS](#), and 1 mL of [aminonaphtholsulfonic acid TS](#), and then dilute with [water](#) to volume. Allow to stand for 5 min.

Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

Mode: UV-Vis

Analytical wavelength: 750 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 100 ppm).

• WATER DETERMINATION (921), Method I

Medium: 30 mL of [methanol](#) and 5 g of [salicylic acid](#) in the titration vessel

Sample: 0.3 g of Mercaptopurine

Acceptance criteria: NMT 12.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.

- [USP REFERENCE STANDARDS \(11\)](#).
[USP Mercaptopurine RS](#)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

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

Chromatographic Database Information: [Chromatographic Database](#)

Most Recently Appeared In:

Pharmacopeial Forum: Volume No. 47(2)

Current DocID: GUID-4B2D663D-5CC9-4A4B-A7E4-2A8ECB39043A_4_en-US

DOI: https://doi.org/10.31003/USPNF_M49330_04_01

DOI ref: [1klx3](#)

OFFICIAL