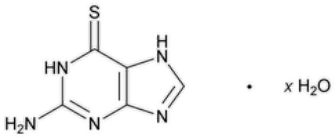
Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-Dec-2021
Document Type: USP Monographs
DocId: GUID-0B521B01-51DD-4FDA-BAA2-EAC835BE511D_3_en-US
DOI: https://doi.org/10.31003/USPNF_M82810_03_01
DOI Ref: a2tdv

Thioguanine

Change to read:



$C_5H_5N_5S$▲ ▲ (USP 1-Dec-2021) (anhydrous) 167.19
$C_5H_5N_5S \cdot ½H_2O$ 176.20
6*H*-Purine-6-thione, 2-amino-1,7-dihydro-;
2-Aminopurine-6(1*H*)-thione CAS RN®: 154-42-7; UNII: WIX31ZPX66.
Hemihydrate CAS RN®: 5580-03-0; UNII: FTK8U1GZNX.

DEFINITION
Thioguanine is anhydrous or contains one-half molecule of water of hydration. It contains NLT 96.0% and NMT 100.5% of thioguanine ($C_5H_5N_5S$), calculated on the dried basis.

IDENTIFICATION
- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K
- **B. Spectroscopic Identification Tests ⟨197⟩, *Ultraviolet-Visible Spectroscopy*:** 197U

Sample solution: Transfer 100 mg of Thioguanine, previously dried, to a 100-mL volumetric flask. Dissolve in a mixture of 15 mL of water and 1.5 mL of 1 N sodium hydroxide, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, add 1% hydrochloric acid to volume, and mix. Finally, transfer 5.0 mL of the last solution to a third 100-mL volumetric flask, then add 1% hydrochloric acid to volume, and mix.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Thioguanine RS.

ASSAY
- **Procedure**

Phosphoric acid solution: Add 1 mL of phosphoric acid to 99 mL of water.
Mobile phase: 0.05 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Standard stock solution: 0.4 mg/mL of USP Thioguanine RS in 0.01 N sodium hydroxide
Standard solution: 0.04 mg/mL of USP Thioguanine RS in *Phosphoric acid solution* from *Standard stock solution*
Sample stock solution: 0.4 mg/mL of Thioguanine in 0.01 N sodium hydroxide
Sample solution: 0.04 mg/mL of Thioguanine in *Phosphoric acid solution* from *Sample stock solution*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 248 nm
Column: 4.6-mm × 5-cm; packing L1
Flow rate: 2.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of thioguanine ($C_5H_5N_5S$) in the portion of Thioguanine taken:

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

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

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

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

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

Acceptance criteria: 96.0%–100.5% on the dried basis

OTHER COMPONENTS

• **Nitrogen Determination ⟨461⟩, *Method II***

Sample: 100 mg

Analysis: Each milliliter of 0.1 N sulfuric acid is equivalent to 1.401 mg of nitrogen (N).

Acceptance criteria: 40.2%–43.1% on the dried basis

IMPURITIES

Delete the following:

▲• **Selenium ⟨291⟩**

Sample: 200 mg

Acceptance criteria: NMT 30 ppm▲ (USP 1-Dec-2021)

• **Free Sulfur**

Sample solution: Dissolve 50 mg of Thioguanine in 5 mL of 1 N sodium hydroxide.

Acceptance criteria: The *Sample solution* is clear.

• **Phosphorous-Containing Substances**

Solution A: Dissolve 8.3 g of ammonium molybdate in 40 mL of water, add 33 mL of dilute sulfuric acid (2 in 7), and dilute with water to 100.0 mL. [Note—This solution is stable for about 2 weeks.]

Instrumental conditions

Mode: UV-Vis

Cell: 1 cm

Analytical wavelength: 620 nm

Analysis: Transfer 50.0 mg of Thioguanine to a large test tube, add 1 mL of dilute sulfuric acid (2 in 7), and heat in a boiling water bath for 5 min. Cautiously add nitric acid, dropwise, continue heating until the mixture becomes colorless, and then heat for 1 min longer. Cool, dilute with water to about 10 mL, and transfer the solution to a 25-mL volumetric flask with the aid of a few milliliters of water. To the flask add 0.75 mL of *Solution A* and 1.0 mL of aminonaphtholsulfonic acid TS, dilute with water to volume, and mix.

Acceptance criteria: NMT 0.03% as phosphate; the absorbance is not greater than that produced by 1.5 mL of a similar solution of monobasic potassium phosphate in water having a known concentration of 10 µg/mL of phosphate (PO_4^{-3}), concomitantly measured.

Change to read:

• **Organic Impurities**

Mobile phase, Sample stock solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.04 mg/mL of USP Guanine RS in 0.01 N sodium hydroxide

Standard solution: 0.4 µg/mL of USP Guanine RS in *Mobile phase* from *Standard stock solution*

Sample solution: 0.04 mg/mL of Thioguanine in *Mobile phase* from *Sample stock solution*

System suitability solution: Transfer 1.0 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with the *Sample solution* to volume.

System suitability

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

[Note—The relative retention times for guanine and thioguanine are about 0.60 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between guanine and thioguanine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of guanine in the portion of Thioguanine taken:

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

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

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

C_S = concentration of ▲USP Guanine RS▲ (USP 1-Dec-2021) in the *Standard solution* (mg/mL)

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

Acceptance criteria: NMT 2.5%

SPECIFIC TESTS

• **Loss on Drying ⟨731⟩**

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

Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **Labeling:** Label it to indicate its state of hydration.

• **USP Reference Standards ⟨11⟩**

USP Guanine RS

USP Thioguanine RS

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

Topic/Question	Contact	Expert Committee
THIOGUANINE	Documentary Standards Support	SM32020 Small Molecules 3
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 46(4)

Current DocID: GUID-0B521B01-51DD-4FDA-BAA2-EAC835BE511D_3_en-US

DOI: https://doi.org/10.31003/USPNF_M82810_03_01

DOI ref: a2tdv