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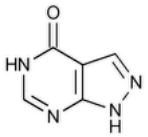
Allopurinol



$C_5H_4N_4O$ 136.11

4*H*-Pyrazolo[3,4-*d*]pyrimidin-4-one, 1,5-dihydro-;

1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one;

1*H*-Pyrazolo[3,4-*d*]pyrimidin-4-ol CAS RN®: 315-30-0; UNII: 63CZ7GJN5I.

DEFINITION

Allopurinol contains NLT 98.0% and NMT 102.0% of allopurinol ($C_5H_4N_4O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

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

ASSAY

• **Procedure**

[Note—Store and inject the *System suitability solution*, *Standard solution*, and *Sample solution* at 8°, using a cooled autosampler.]

Mobile phase: 1.25-g/L solution of monobasic potassium phosphate in water, filtered and degassed

System suitability solution: 0.5 µg/mL each of USP Allopurinol RS, USP Allopurinol Related Compound B RS, and USP Allopurinol Related Compound C RS, prepared as follows. Transfer weighed quantities of USP Allopurinol RS, USP Allopurinol Related Compound B RS, and USP Allopurinol Related Compound C RS to three separate suitable volumetric flasks, dissolve in a small volume of 0.1 N sodium hydroxide, and immediately dilute with *Mobile phase* to volume to obtain solutions containing 0.05 mg/mL each. Transfer 1.0 mL of each of these three solutions to a 100-mL volumetric flask and dilute with *Mobile phase* to volume.

Standard stock solution: 0.5 mg/mL of USP Allopurinol RS, prepared as follows. Transfer a weighed quantity of USP Allopurinol RS to a suitable volumetric flask, dissolve in a small volume of 0.1 N sodium hydroxide, and immediately dilute with *Mobile phase* to volume.

Standard solution: 0.08 mg/mL of USP Allopurinol RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: 0.5 mg/mL of Allopurinol, prepared as follows. Transfer 50 mg of Allopurinol to a 100-mL volumetric flask, dissolve in 5.0 mL of 0.1 N sodium hydroxide, and immediately dilute with *Mobile phase* to volume.

Sample solution: 0.08 mg/mL of Allopurinol in *Mobile phase* from the *Sample stock solution*

Chromatographic system

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

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.8 mL/min

Injection volume: 20 µL

System suitability

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

[Note—The relative retention times for allopurinol related compound B, allopurinol related compound C, and allopurinol are about 0.7, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.1 between allopurinol related compound B and allopurinol related compound C; NLT 6.0 between allopurinol related compound C and allopurinol, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of allopurinol ($C_5H_4N_4O$) in the portion of Allopurinol taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

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

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

IMPURITIES

• **Organic Impurities**

[Note—Store and inject the *Standard solution* and the *Sample solution* at 8°, using a cooled autosampler.]

Solution A: 1.25-g/L solution of monobasic potassium phosphate in water, filtered and degassed

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30	70	30
35	70	30
36	90	10
46	90	10

Diluent: *Solution A* and *Solution B* (90:10)

Standard stock solution: 0.05 mg/mL each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS, prepared as follows. Transfer 5 mg each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS to a 100-mL volumetric flask. Add 2.0 mL of 0.1 N sodium hydroxide, and promptly sonicate with swirling for NMT 1 min to dissolve. Add 80 mL of *Diluent*, and sonicate for an additional 5 min. Dilute with *Diluent* to volume. [Note—This solution is stable for 48 h when stored at 8°.]

Standard solution: 0.5 µg/mL each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS in *Diluent* from the *Standard stock solution*

Sample solution: 0.25 mg/mL of Allopurinol, prepared as follows. Transfer 25 mg of Allopurinol to a 100-mL volumetric flask. Add 5.0 mL of 0.1 N sodium hydroxide to dissolve, promptly sonicate with swirling for NMT 1 min, add 80 mL of *Diluent*, and sonicate for an additional 5 min. Dilute with *Diluent* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 40 µL

System suitability

Sample: *Standard solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 0.8 between allopurinol related compound C and allopurinol related compound B

Tailing factor: NMT 1.5 for the allopurinol peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of allopurinol related compounds A, B, C, D, and E in the portion of Allopurinol taken:

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

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

r_S = peak response of each individual impurity from the *Standard solution*

C_S = concentration of each individual impurity in the *Standard solution* (mg/mL)

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

Calculate the percentage of any other individual impurity in the portion of Allopurinol 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 allopurinol from the *Standard solution*

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

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

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Allopurinol related compound A	0.62	0.2
Allopurinol related compound C	0.79	0.2
Allopurinol related compound B	0.81	0.2
Allopurinol	1.0	—
Allopurinol related compound D	4.4	0.2
Allopurinol related compound E	4.8	0.2
Ethyl-(*E*/*Z*)-3-(2-carbethoxy-2-cyanoethenyl)amino-1*H*-pyrazole-4-carboxylate	6.5	0.2
Unspecified impurity	—	0.1
Total impurities	—	1.0

• **Limit of Hydrazine**

[Note—Under the following conditions, any hydrazine present in the sample will react with benzaldehyde to form benzalazine.]

Mobile phase: Hexane and isopropyl alcohol (95:5)

2 N sodium hydroxide solution: Dissolve 8.5 g of sodium hydroxide in water, and dilute with the same solvent to 100 mL. Alternatively, a commercially available 2 N sodium hydroxide solution can be used.

Diluent: Methanol and *2 N sodium hydroxide solution* (1:1)

Benzaldehyde solution: 40 mg/mL of benzaldehyde in *Diluent*. [Note—Prepare immediately before use.]

Hydrazine solution: 2.0 µg/mL of hydrazine sulfate in *Diluent*. Use sonication if necessary.

Standard solution: Transfer 5.0 mL of *Hydrazine solution* to a suitable flask and add 4 mL of *Benzaldehyde solution*. Mix and allow to stand for 2.5 h at room temperature. Add 5.0 mL of hexane, and shake for 1 min. Allow the layers to separate, and use the upper (hexane) layer.

Allopurinol solution: Dissolve 250 mg of Allopurinol in 5 mL of *Diluent*.

Sample solution: Transfer the *Allopurinol solution* to a suitable flask, and add 4 mL of *Benzaldehyde solution*. Mix, and allow to stand for 2.5 h at room temperature. Add 5.0 mL of hexane, and shake for 1 min. Allow the layers to separate, and use the upper (hexane) layer.

Blank solution: Mix 5.0 mL of *Diluent* and 4 mL of *Benzaldehyde solution*, and allow to stand for 2.5 h at room temperature. Add 5.0 mL of hexane, and shake for 1 min. Allow the layers to separate, and use the upper (hexane) layer.

Chromatographic system

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

Mode: LC

Detector: UV 310 nm

Column: 4.0-mm × 25-cm; 5-µm packing L10

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for benzalazine and benzaldehyde are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between benzalazine and benzaldehyde

Relative standard deviation: NMT 15.0% for the benzalazine peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount, in ppm, of hydrazine in the portion of Allopurinol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F$$

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

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

C_S = concentration of hydrazine sulfate in the *Hydrazine solution* (µg/mL)

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

M_{r1} = molecular weight of hydrazine, 32.05

M_{r2} = molecular weight of hydrazine sulfate, 130.12

F = unit conversion factor (from µg/mg to ppm), 1000

Acceptance criteria: NMT 10 ppm of hydrazine

SPECIFIC TESTS

• **LOSS ON DRYING ⟨731⟩**

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

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

Change to read:

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Allopurinol RS

USP Allopurinol Related Compound A RS

3-Amino-4-carboxamidopyrazole hemisulfate.

▲$(C_4H_6N_4O)_2 \cdot H_2SO_4$ 350.31▲ (ERR 1-Dec-2018)

USP Allopurinol Related Compound B RS

5-(Formylamino)-1*H*-pyrazole-4-carboxamide.

$C_5H_6N_4O_2$ 154.13

USP Allopurinol Related Compound C RS

5-(4*H*-1,2,4-Triazol-4-yl)-1*H*-pyrazole-4-carboxamide.

$C_6H_6N_6O$ 178.15

USP Allopurinol Related Compound D RS

Ethyl 5-amino-1*H*-pyrazole-4-carboxylate.

$C_6H_9N_3O_2$ 155.15

USP Allopurinol Related Compound E RS

Ethyl 5-(formylamino)-1*H*-pyrazole-4-carboxylate.

$C_7H_9N_3O_3$ 183.16

Topic/Question	Contact	Expert Committee
ALLOPURINOL	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

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