Allopurinol

Type of Posting	**Revision Bulletin**
Posting Date	**29–May–2015**
Official Date	**01–Jun–2015**
Expert Committee	**Monographs—Small Molecules 3**
Reason for Revision	**Compliance**

In accordance with the Rules and Procedures of the 2010-2015 Council of Experts, the Monographs—Small Molecules 3 Expert Committee has revised the Allopurinol monograph. The purpose for the revision is to eliminate the use of USP Allopurinol Related Compound F RS under the test for Organic Impurities. Recent evaluation of the current lot of USP Allopurinol Related Compound F RS by USP scientific staff has determined that it is not suitable for its compendial usage as described in the currently official monograph.

The test for Organic Impurities is revised as follows:

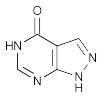
- Allopurinol related compound F solution is deleted from the monograph. Standard stock solution and Standard solution are revised to delete the use of USP Allopurinol Related Compound F RS.
- In the Table 2, the specified impurity eluting at the relative retention time of 6.5 is listed under its chemical name. The percentage of this impurity is calculated against the peak response for allopurinol peak from the Standard solution. The limit of this impurity remains unchanged at NMT 0.2%.

Additionally, minor editorial changes have been made to update the monograph to current USP style.

The Allopurinol Revision Bulletin supersedes the currently official monograph. The Revision Bulletin will be incorporated in the *USP 39–NF 34*.

Should you have any questions, please contact Elena Gonikberg, PhD (301–816-8251 or eg@usp.org.)

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 [315-30-0].

DEFINITION

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

IDENTIFICATION

• **INFRARED ABSORPTION** ⟨197K⟩

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

Change to read:

• **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)
•• (RB 1-Jun-2015)

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, •• (RB 1-Jun-2015) 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• (RB 1-Jun-2015) 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 •• (RB 1-Jun-2015) 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• (RB 1-Jun-2015) 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• (RB 1-Jun-2015) 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 •• (RB 1-Jun-2015) 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• (RB 1-Jun-2015)	6.5	0.2
Unspecified impurity	—	0.1
Total impurities	—	1.0

•• (RB 1-Jun-2015)

• **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.
  $(C_5H_6N_4O)_2 \cdot H_2SO_4$ 350.32
  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.• (ERR 1-Aug-2014)
  $C_6H_6N_6O$ 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
• • (RB 1-Jun-2015)