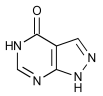
Allopurinol



$C_5H_4N_4O$ 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*].

» Allopurinol contains not less than 98.0 percent and not more than 102.0 percent of $C_5H_4N_4O$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at room temperature.

USP Reference standards ⟨11⟩**—***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. USP Allopurinol Related Compound E RS. USP Allopurinol Related Compound F RS.*

Identification, *Infrared Absorption* ⟨197K⟩.

Loss on drying ⟨731⟩**—**Dry it in vacuum at 105° for 5 hours: it loses not more than 0.5% of its weight.

Organic volatile impurities, *Method V* ⟨467⟩: meets the requirements.

Solvent—Use dimethyl sulfoxide.

(Official until July 1, 2008)

Related compounds—[NOTE—Store and inject the *Standard solution* and the *Test solution* at 8°, using a cooled autosampler.]

Solution A—Dissolve 1.25 g of monobasic potassium phosphate in 1000 mL of water, filter, and degas.

Solution B—Use methanol.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of *Solution A* and *Solution B* (90 : 10).

Standard stock solution—Accurately weigh about 5 mg of 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, USP Allopurinol Related Compound E RS, and USP Allopurinol Related Compound F RS, and transfer to a 100-mL volumetric flask. Add 2.0 mL of 0.1 N sodium hydroxide to dissolve, promptly sonicate with swirling for not more than 1 minute, add 80 mL of *Diluent*, and sonicate for an additional 5 minutes. Dilute with *Diluent* to volume. [NOTE—This solution is stable for 48 hours when stored at 8°.]

Standard solution—Quantitatively dilute an accurately measured volume of the *Standard stock solution* with *Diluent* to obtain a solution having known concentrations of about 0.5 µg of allopurinol and about 0.5 µg of each of allopurinol related compounds A, B C, D, E, and F per mL.

Test solution—Transfer about 25 mg of Allopurinol, accurately weighed, to a 100-mL volumetric flask, add 5.0 mL of 0.1 N sodium hydroxide to dissolve, promptly sonicate with swirling for not more than 1 minute, add 80 mL of *Diluent*, and sonicate for an additional 5 minutes. Dilute with *Diluent* to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The column temperature is maintained at 30°. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–30	90→70	10→30	linear gradient
30–35	70	30	isocratic
35–36	70→90	30→10	linear gradient
36–46	90	10	re-equilibration

Chromatograph the *Standard solution*, identify the peaks (see *Table 1*), and record the peak responses as directed for *Procedure:* the resolution, *R*, between allopurinol related compounds C and B is not less than 0.8; and the tailing factor for the allopurinol peak is not more than 1.5.

Procedure—Separately inject equal volumes (about 40 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and identify the allopurinol peak and the peaks due to impurities listed in *Table 1*.

Table 1

Name	Relative Retention Time	Limit (%)
Allopurinol related compound A[1]	0.62	0.2
Allopurinol related compound C[3]	0.79	0.2
Allopurinol related compound B[2]	0.81	0.2
Allopurinol	1.0	—
Allopurinol related compound D[4]	4.4	0.2
Allopurinol related compound E[5]	4.8	0.2
Allopurinol related compound F[6]	6.5	0.2

[1]3-Amino-1*H*-pyrazole-4-carboxamide
[2]5-(Formylamino)-1*H*-pyrazole-4-carboxamide
[3]5-(4*H*-1,2,4-Triazol-4-yl)-1*H*-pyrazole-4-carboxamide
[4]Ethyl-5-amino-1*H*-pyrazole-4-carboxylate
[5]Ethyl-5-(formylamino)-1*H*-pyrazole-4-carboxylate
[6]Ethyl-(*E*/*Z*)-3-(2-carbethoxy-2-cyanoethenyl)amino-1*H*-pyrazole-4-carboxylate

Calculate the percentage of each impurity in the portion of Allopurinol taken by the formula:

$$10(C/W)(r_i / r_S)$$

in which C is the concentration, in µg per mL, of each individual impurity in the *Standard solution; W* is the weight, in mg, of Allopurinol taken to prepare the *Test solution;* and r_i and r_S are the peak responses for each individual impurity obtained from the *Test solution* and the *Standard solution*, respectively. [NOTE—For unspecified impurities, r_S is the peak response for the allopurinol peak obtained from the *Standard solution.*] In addition to not exceeding the limits for each impurity in *Table 1*, not more than 0.1% of any individual unspecified impurity is found; and not more than 1.0% of total impurities is found.

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

Mobile phase—Dissolve 1.25 g of monobasic potassium phosphate in 1000 mL of water, filter, and degas.

System suitability solution—Transfer accurately weighed quantities of USP Allopurinol RS, USP Allopurinol Related Compound B RS, and USP Allopurinol Related Compound C RS, each to a suitable volumetric flask, dissolve in a small volume of 0.1 N sodium hydroxide, and immediately and quantitatively dilute with *Mobile phase* to obtain solutions having known concentrations of about 0.05 mg per mL. Transfer 1.0 mL of each of these three solutions to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Allopurinol RS in a small volume of 0.1 N sodium hydroxide, and immediately and quantitatively dilute with *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per

mL. Quantitatively dilute an accurately measured volume of this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.08 mg of allopurinol per mL.

Assay preparation—Transfer about 50 mg of Allopurinol, accurately weighed, to a 100-mL volumetric flask, dissolve in 5.0 mL of 0.1 N sodium hydroxide, immediately dilute with *Mobile phase* to volume, and mix. Quantitatively dilute an accurately measured volume of this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.08 mg of allopurinol per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.8 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between allopurinol related compound B and allopurinol related compound C is not less than 1.1, and that between allopurinol related compound C and allopurinol is not less than 6.0. [NOTE—For the purpose of identification, the relative retention times are about 0.7 for allopurinol related compound B, 0.8 for allopurinol related compound C, and 1.0 for allopurinol.] Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_5H_4N_4O$ in the portion of Allopurinol taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_U and C_S are the concentrations, in mg per mL, of allopurinol in the *Assay preparation* and the *Standard preparation*, respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.