

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Boil 20 mg with a mixture of 1 mL of *dilute sodium hydroxide solution R* and 1 mL of *water R*. Allow to cool. Add 1 mL of *dilute hydrochloric acid R*. To 0.1 mL of the solution add 0.1 mL of a 100 g/L solution of *potassium bromide R*, 0.1 mL of a 20 g/L solution of *resorcinol R* and 3 mL of *sulfuric acid R*. Heat for 5 min to 10 min on a water-bath. A dark blue colour develops, which becomes red after cooling and pouring into about 10 mL of *water R*.

D. Heat about 0.5 g. Ammonia vapour is evolved, which turns *red litmus paper R* blue.

#### TESTS

**Solution S.** Dissolve 0.5 g in *carbon dioxide-free water R*, with heating if necessary, and dilute to 100 mL with the same solvent.

**Acidity or alkalinity.** To 5 mL of solution S add 5 mL of *carbon dioxide-free water R*, 0.1 mL of *methyl red solution R* and 0.2 mL of 0.01 M *sodium hydroxide*. The solution is yellow. Add 0.4 mL of 0.01 M *hydrochloric acid*. The solution is red.

**Optical rotation** (2.2.7). The angle of optical rotation, determined on solution S, is  $-0.10^\circ$  to  $+0.10^\circ$ .

**Reducing substances.** Shake 1.0 g with 10 mL of *water R* for 2 min. Filter. Add 1.5 mL of 0.02 M *potassium permanganate*. The solution must remain violet for at least 10 min.

**Related substances.** Examine by thin-layer chromatography (2.2.27), using a suitable *cellulose for chromatography R* as the coating substance.

**Test solution (a).** Dissolve 0.10 g of the substance to be examined in 5.0 mL of *water R* with heating. Allow to cool. Dilute to 10 mL with *methanol R*. *Use the solution immediately after preparation.*

**Test solution (b).** Dilute 1 mL of test solution (a) to 10 mL with a mixture of 1 volume of *methanol R* and 1 volume of *water R*.

**Reference solution (a).** Dissolve 10 mg of *allantoin CRS* in a mixture of 1 volume of *methanol R* and 1 volume of *water R* and dilute to 10 mL with the same mixture of solvents.

**Reference solution (b).** Dissolve 10 mg of *urea R* in 10 mL of *water R*. Dilute 1 mL of this solution to 10 mL with *methanol R*.

**Reference solution (c).** Mix 1 mL of reference solution (a) and 1 mL of reference solution (b).

Apply to the plate 10  $\mu$ L of test solution (a) and 5  $\mu$ L each of test solution (b), reference solution (a), reference solution (b) and reference solution (c). Develop over a path of 10 cm using a mixture of 15 volumes of *glacial acetic acid R*, 25 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air. Spray the plate with a 5 g/L solution of *dimethylaminobenzaldehyde R* in a mixture of 1 volume of *hydrochloric acid R* and 3 volumes of *methanol R*. Dry the plate in a current of hot air. Examine in daylight after 30 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

**Loss on drying** (2.2.32). Not more than 0.1 per cent, determined on 1.000 g by drying in an oven at 105 °C.

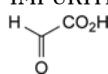
**Sulfated ash** (2.4.14). Not more than 0.1 per cent, determined on 1.0 g.

#### ASSAY

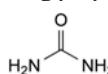
Dissolve 120.0 mg in 40 mL of *water R*. Titrate with 0.1 M *sodium hydroxide*, determining the end-point potentiometrically (2.2.20).

1 mL of 0.1 M *sodium hydroxide* is equivalent to 15.81 mg of  $\text{C}_4\text{H}_6\text{N}_4\text{O}_3$ .

#### IMPURITIES



A. glyoxylic acid,

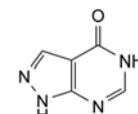


B. carbamide (urea).

01/2008:0576  
corrected 6.8

## ALLOPURINOL

### Allopurinolum



$M_r$  136.1

$\text{C}_5\text{H}_4\text{N}_4\text{O}$   
[315-30-0]

#### DEFINITION

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

**Content:** 97.0 per cent to 102.0 per cent (dried substance).

#### CHARACTERS

**Appearance:** white or almost white powder.

**Solubility:** very slightly soluble in water and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

#### IDENTIFICATION

**First identification:** B.

**Second identification:** A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (2.2.25).

**Test solution.** Dissolve 10 mg in 1 mL of a 4 g/L solution of *sodium hydroxide R* and dilute to 100.0 mL with a 10.3 g/L solution of *hydrochloric acid R*. Dilute 10.0 mL of this solution to 100.0 mL with a 10.3 g/L solution of *hydrochloric acid R*.

**Spectral range:** 220-350 nm.

**Absorption maximum:** at 250 nm.

**Absorption minimum:** at 231 nm.

**Absorbance ratio:**  $A_{231}/A_{250} = 0.52$  to 0.62.

B. Infrared absorption spectrophotometry (2.2.24).

**Comparison:** *allopurinol CRS*.

C. Dissolve 0.3 g in 2.5 mL of *dilute sodium hydroxide solution R* and add 50 mL of *water R*. Add slowly and with shaking 5 mL of *silver nitrate solution R1*. A white precipitate is formed which does not dissolve on the addition of 5 mL of *ammonia R*.

D. Thin-layer chromatography (2.2.27).

**Test solution.** Dissolve 20 mg of the substance to be examined in *concentrated ammonia R* and dilute to 10 mL with the same solvent.

**Reference solution.** Dissolve 20 mg of *allopurinol CRS* in *concentrated ammonia R* and dilute to 10 mL with the same solvent.

**Plate:** TLC silica gel  $F_{254}$  plate R.

**Mobile phase:** *anhydrous ethanol R*, *methylene chloride R* (40:60 V/V).

**Application:** 10  $\mu$ L.

**Development:** over 2/3 of the plate.

**Drying:** in air.

**Detection:** examine in ultraviolet light at 254 nm.

**Results:** the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

## TESTS

**Related substances.** Liquid chromatography (2.2.29). *Use freshly prepared solutions. Store and inject them at 8 °C, using a cooled autosampler.*

**Test solution (a).** Dissolve 25.0 mg of the substance to be examined in 2.5 mL of a 4 g/L solution of *sodium hydroxide R* and dilute immediately to 50.0 mL with the mobile phase.

**Test solution (b).** Dissolve 20.0 mg of the substance to be examined in 5.0 mL of a 4 g/L solution of *sodium hydroxide R* and dilute immediately to 250.0 mL with the mobile phase.

**Reference solution (a).** Dilute 2.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 100.0 mL with the mobile phase.

**Reference solution (b).** Dissolve 5 mg of *allopurinol impurity A CRS*, 5 mg of *allopurinol impurity B CRS* and 5.0 mg of *allopurinol impurity C CRS* in 5.0 mL of a 4 g/L solution of *sodium hydroxide R* and dilute immediately to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

**Reference solution (c).** Dissolve 20.0 mg of *allopurinol CRS* in 5.0 mL of a 4 g/L solution of *sodium hydroxide R* and dilute immediately to 250.0 mL with the mobile phase.

**Column:**

- *size: l = 0.25 m, Ø = 4.6 mm;*
- *stationary phase: octadecylsilyl silica gel for chromatography R (5 µm).*

**Mobile phase:** 1.25 g/L solution of *potassium dihydrogen phosphate R*.

**Flow rate:** 1.4 mL/min.

**Detection:** spectrophotometer at 230 nm.

**Injection:** 20 µL of test solution (a) and reference solutions (a) and (b).

**Run time:** twice the retention time of allopurinol.

**Elution order:** impurity A, impurity B, impurity C, allopurinol.

**Retention time:** allopurinol = about 10 min.

**System suitability:** reference solution (b):

- *resolution: minimum 1.1 between the peaks due to impurities B and C.*

**Limits:**

- *impurity A:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity B:* not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *impurity C:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *sum of impurities other than A, B and C:* not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Impurities D and E.** Liquid chromatography (2.2.29). *Use freshly prepared solutions. Store and inject them at 8 °C, using a cooled autosampler.*

**Solution A:** 1.25 g/L solution of *potassium dihydrogen phosphate R*.

**Test solution.** Dissolve 50.0 mg of the substance to be examined in 5.0 mL of a 4 g/L solution of *sodium hydroxide R* and dilute immediately to 100.0 mL with solution A.

**Reference solution.** Dissolve 5.0 mg of *allopurinol impurity D CRS* and 5.0 mg of *allopurinol impurity E CRS* in 5.0 mL of a 4 g/L solution of *sodium hydroxide R* and dilute immediately to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

**Column:**

- *size: l = 0.05 m, Ø = 4.6 mm;*
- *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R (3 µm).*

**Mobile phase:** *methanol R, 1.25 g/L solution of potassium dihydrogen phosphate R (10:90 V/V).*

**Flow rate:** 2 mL/min.

**Detection:** spectrophotometer at 230 nm.

**Injection:** 20 µL.

**Run time:** 1.5 times the retention time of impurity E.

**Retention times:** impurity D = about 3.6 min; impurity E = about 4.5 min.

**System suitability:** reference solution:

- *resolution: minimum 2.0 between the peaks due to impurities D and E.*

**Limits:**

- *impurity D:* not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent);
- *impurity E:* not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

**Impurity F.** Liquid chromatography (2.2.29).

Under the following conditions, any hydrazine in the sample reacts with benzaldehyde to give benzaldehyde azine.

**Solvent mixture.** Mix equal volumes of *dilute sodium hydroxide solution R* and *methanol R*.

**Solution A.** Dissolve 2.0 g of *benzaldehyde R* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Prepare immediately before use.

**Test solution.** Dissolve 250.0 mg of the substance to be examined in 5 mL of the solvent mixture. Add 4 mL of solution A, mix and allow to stand for 2.5 h at room temperature. Add 5.0 mL of *hexane R* and shake for 1 min. Allow the layers to separate and use the upper layer.

**Reference solution.** Dissolve 10.0 mg of *hydrazine sulfate R* in the solvent mixture by sonicating for about 2 min and dilute to 50.0 mL with the solvent mixture. Dilute 1.0 mL to 20.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture. To 5.0 mL of the solution obtained, add 4 mL of solution A, mix and allow to stand for 2.5 h at room temperature. Add 5.0 mL of *hexane R* and shake for 1 min. Allow the layers to separate and use the upper layer.

**Blank solution.** To 5 mL of the solvent mixture add 4 mL of solution A, mix and allow to stand for 2.5 h at room temperature. Add 5.0 mL of *hexane R* and shake for 1 min. Allow the layers to separate and use the upper layer.

**Column:**

- *size: l = 0.25 m, Ø = 4.0 mm;*
- *stationary phase: cyanosilyl silica gel for chromatography R (5 µm) with a pore size of 10 nm;*
- *temperature: 30 °C.*

**Mobile phase:** *2-propanol R, hexane R (5:95 V/V).*

**Flow rate:** 1.5 mL/min.

**Detection:** spectrophotometer at 310 nm.

**Injection:** 20 µL.

**Relative retention** with reference to benzaldehyde (retention time = about 2.8 min): benzaldehyde azine = about 0.8.

**System suitability:** reference solution:

- **resolution:** minimum 2 between the peaks due to benzaldehyde azine and benzaldehyde;
- **signal-to-noise ratio:** minimum 20 for the peak due to benzaldehyde azine.

**Limit:**

- **impurity F:** the area of the peak due to benzaldehyde azine in the chromatogram obtained with the test solution is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (10 ppm of hydrazine sulfate equivalent to 2.5 ppm of hydrazine).

**Heavy metals (2.4.8):** maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 mL of *lead standard solution (10 ppm Pb) R*.

**Loss on drying (2.2.32):** maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

**Sulfated ash (2.4.14):** maximum 0.1 per cent, determined on 1.0 g.

## ASSAY

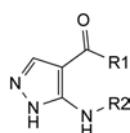
Liquid chromatography (2.2.29) as described in the test for related substances with the following modification.

**Injection:** test solution (b) and reference solution (c).

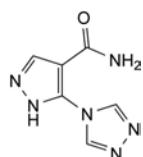
Calculate the percentage content of C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O from the declared content of *allopurinol CRS*.

## IMPURITIES

**Specified impurities:** A, B, C, D, E, F.



- A. R1 = NH<sub>2</sub>, R2 = H: 5-amino-1H-pyrazole-4-carboxamide,
- B. R1 = NH<sub>2</sub>, R2 = CHO: 5-(formylamino)-1H-pyrazole-4-carboxamide,
- D. R1 = O-C<sub>2</sub>H<sub>5</sub>, R2 = H: ethyl 5-amino-1H-pyrazole-4-carboxylate,
- E. R1 = O-C<sub>2</sub>H<sub>5</sub>, R2 = CHO: ethyl 5-(formylamino)-1H-pyrazole-4-carboxylate,



- C. 5-(4H-1,2,4-triazol-4-yl)-1H-pyrazole-4-carboxamide,
- F. H<sub>2</sub>N-NH<sub>2</sub>: diazane (hydrazine).

## Content:

- aluminium: 15.0 per cent to 17.0 per cent (calculated as Al<sub>2</sub>O<sub>3</sub>),
- magnesium: 36.0 per cent to 40.0 per cent (calculated as MgO),
- carbonic acid: 12.5 per cent to 14.5 per cent (calculated as CO<sub>2</sub>).

## CHARACTERS

**Appearance:** white or almost white, fine, crystalline powder.

**Solubility:** practically insoluble in water, in ethanol (96 per cent) and in methylene chloride. It dissolves with effervescence and heating in dilute mineral acids.

## IDENTIFICATION

A. Infrared absorption spectrophotometry (2.2.24).

*Comparison:* *Ph. Eur. reference spectrum of almagate.*

B. Dissolve 0.15 g in *dilute hydrochloric acid R* and dilute to 20 mL with the same acid. 2 mL of the solution gives the reaction of aluminium (2.3.1).

C. 2 mL of the solution prepared under identification test B gives the reaction of magnesium (2.3.1).

## TESTS

**pH (2.2.3):** 9.1 to 9.7.

Disperse 4.0 g in 100 mL of *carbon dioxide-free water R*, stir for 2 min and filter.

**Neutralising capacity.** Carry out the test at 37 °C. Disperse 0.5 g in 100 mL of *water R*, heat, add 100.0 mL of 0.1 M *hydrochloric acid*, previously heated and stir continuously; the pH (2.2.3) of the solution between 5 min and 20 min is not less than 3.0 and not greater than 4.5. Add 10.0 mL of 0.5 M *hydrochloric acid*, previously heated, stir continuously for 1 h and titrate with 0.1 M *sodium hydroxide* to pH 3.5; not more than 20.0 mL of 0.1 M *sodium hydroxide* is required.

**Chlorides (2.4.4):** maximum 0.1 per cent.

Dissolve 0.33 g in 5 mL of *dilute nitric acid R* and dilute to 100 mL with *water R*. 15 mL of the solution complies with the limit test for chlorides. Prepare simultaneously the standard by diluting 0.7 mL of *dilute nitric acid R* to 5 mL with *water R* and adding 10 mL of *chloride standard solution (5 ppm Cl) R*.

**Sulfates (2.4.13):** maximum 0.4 per cent.

Dissolve 0.25 g in 5 mL of *dilute hydrochloric acid R* and dilute to 100 mL with *distilled water R*. 15 mL of the solution complies with the limit test for sulfates. Prepare simultaneously the standard by adding 0.8 mL of *dilute hydrochloric acid R* to 15 mL of *sulfate standard solution (10 ppm SO<sub>4</sub>) R*.

**Sodium:** maximum 150 ppm.

Atomic absorption spectrometry (2.2.23, *Method I*).

**Test solution.** Dissolve 0.25 g in 50 mL of a 103 g/L solution of *hydrochloric acid R*.

**Reference solutions.** Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with a 103 g/L solution of *hydrochloric acid R*.

**Heavy metals (2.4.8):** maximum 20 ppm.

Dissolve 1.0 g in *dilute hydrochloric acid R* and dilute to 20.0 mL with the same acid. 12 mL of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

**Loss on ignition:** 43.0 per cent to 49.0 per cent, determined on 1.000 g by ignition at 900 ± 50 °C.

## Microbial contamination

TAMC: acceptance criterion 10<sup>3</sup> CFU/g (2.6.12).

TYMC: acceptance criterion 10<sup>2</sup> CFU/g (2.6.12).

Absence of *Escherichia coli* (2.6.13).

Absence of *Pseudomonas aeruginosa* (2.6.13).

Al<sub>2</sub>Mg<sub>6</sub>C<sub>2</sub>O<sub>20</sub>H<sub>14</sub>·4H<sub>2</sub>O  
[66827-12-1]

M<sub>r</sub> 630

## DEFINITION

Hydrated aluminium magnesium hydroxycarbonate.