

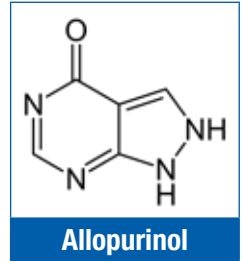
# APPLICATION

## Allopurinol and Related Substances

Ph. Eur. monograph 0576

### Overview

The related substances test of the Ph. Eur. Monograph 0576 outlines the separation of all relevant impurities from Allopurinol. This method was studied and improvements were made to provide higher resolution (Rs) and a faster separation time within allowable adjustments.



### Ph. Eur. Monograph 0576 Details

<b>Test Solution (a)</b>	Dissolve 25.0 mg of Allopurinol CRS* 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
<b>Reference Solution</b>	<b>(a)</b> Dilute 2.0 mL of the test solution (a) to 100.0 mL with the mobile phase. Dilute 5 mL of this solution to 100.0 mL with the mobile phase. <b>(b)</b> Dissolve 5.0 mg of Allopurinol Impurity A CRS*, 5.0 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.

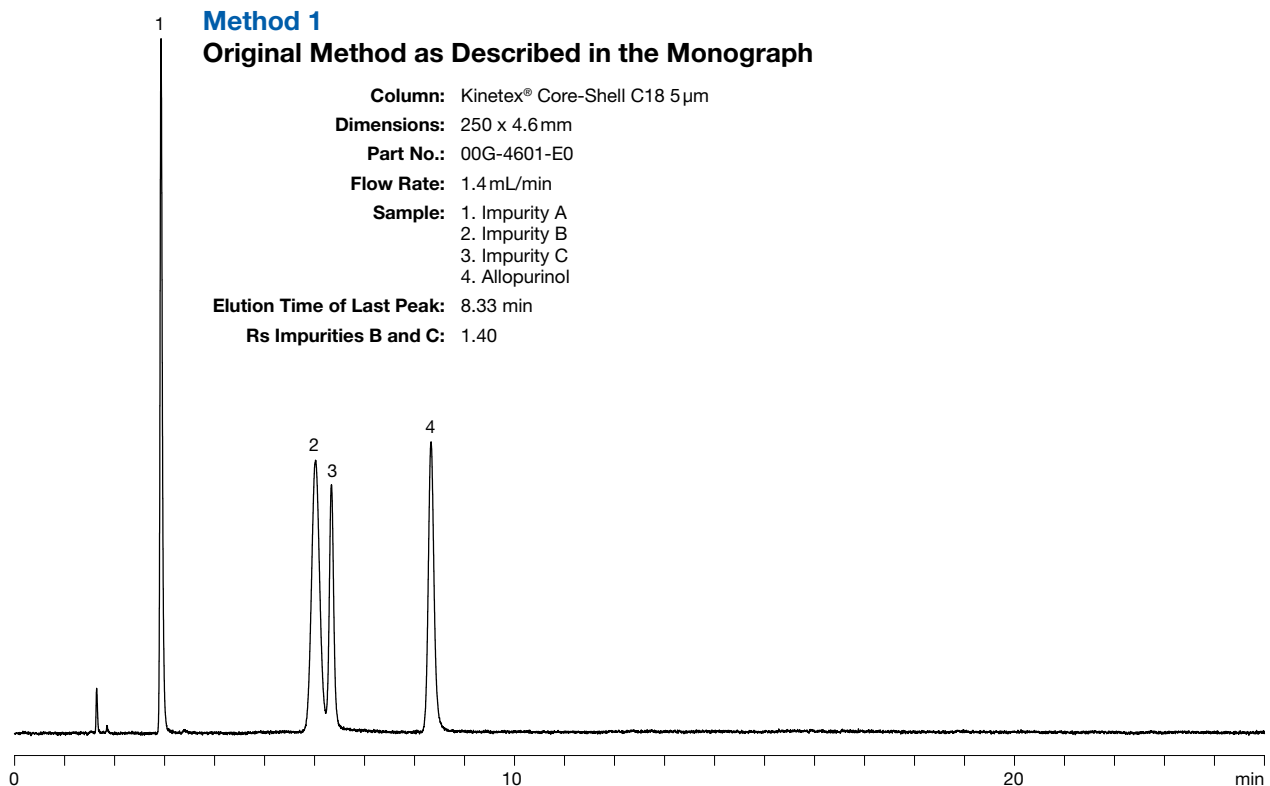
### Column

<b>Size</b>	250 x 4.6 mm
<b>Stationary Phase</b>	Octadecylsilyl silica gel for chromatography R (5 µm)
<b>Mobile Phase</b>	1.25 g/L solution of potassium dihydrogen phosphate R
<b>Flow Rate</b>	1.4 mL/min
<b>Detection</b>	Spectrophotometer @ 230 nm
<b>Injection</b>	20 µL (reference solution (a) and (b))
<b>Run Time</b>	Twice the retention time of Allopurinol
<b>Elution Order</b>	1. Impurity A 2. Impurity B 3. Impurity C 4. Allopurinol (about 10 min)

### System Suitability

**Reference Solution (b)** Minimum resolution of 1.1 between peaks due to Impurities B and C

\* Allopurinol CRS (A0350000), Allopurinol Impurity A CRS (A0350010), Allopurinol Impurity B CRS (A0350020) and Allopurinol Impurity C CRS (A0350030) were purchased from European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: 7 Allée Kastner CS 30026F - 67081 STRASBOURG (France).



## Adjustments for Meeting System Suitability

(European Pharmacopeia 9.0, Chapter 2.2.46. Chromatographic separation techniques)

Method Parameter	Allowed Adjustments (isocratic elution)	Method 1
Mobile Phase pH	± 0.2 units	As specified
Concentration of Salts in Buffer	± 10 %	As specified in Monograph 0576 Details Table
Composition of the Mobile Phase	± 30 % of the minor solvent component relative or 2 % absolute, whichever is the larger. No other component is altered by more than 10 % absolute.	As specified in Monograph 0576 Details Table
Wavelength of Detector	No deviations permitted	230 nm (as specified)
Injection Volume	May be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory.	20 µL (as specified)
Column Temperature	± 10 °C	Ambient (as specified)
Stationary Phase	No change of the identity of the substituent permitted (e.g. no replacement of C18 by C8)	Octadecylsilyl silica gel for chromatography (as specified)
Column Length	± 70 %	250 mm (as specified)
Column Internal Diameter	± 25 %	4.6 mm (as specified)
Particle Size	-50 %	5 µm (as specified)
Flow Rate	± 50 %	1.4 mL/min (as specified)

## Kinetex® Ordering Information

5 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges <sup>†</sup>
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4601-AN	00B-4601-AN	00D-4601-AN	00F-4601-AN	AJ0-8782 for 2.1 mm ID

5 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges <sup>†</sup>
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
C18	00B-4601-Y0	00D-4601-Y0	00F-4601-Y0	AJ0-8775 for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges <sup>†</sup>
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768 for 4.6 mm ID

5 µm Semi-Preparative Columns (mm)			SecurityGuard SemiPrep Cartridges <sup>***</sup>
Phases	150 x 10	250 x 10	3/pk
C18	00F-4601-N0	00G-4601-N0	AJ0-9278 for 9-16 mm ID

<sup>†</sup>SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

<sup>\*\*\*</sup>SemiPrep SecurityGuard Cartridges require holder, Part No.: AJ0-9281



If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to a competing product of the same particle size, similar phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

# APPLICATION

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