

Ph. Eur. Monograph 1002: Diclofenac Sodium Related Substances on Luna® C18(2) and Kinetex® C18 Columns

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Overview

Diclofenac sodium, chemically known as sodium {2-[(2,6-dichlorophenyl)-amino]phenyl}acetate, is a phenylacetic acid derivative non-steroidal anti-inflammatory drug.

In this application note we demonstrate the analytical applicability of three different columns to meet system suitability as set forth by the European Pharmacopoeia monograph for Diclofenac Sodium (1002), which requires system suitability for resolution between impurity F and diclofenac to be minimum of 4.0.

The first column is Luna 5 µm C18(2), which is a fully porous silica-based column. The other two columns are the core-shell Kinetex of varying particle size and column length; Kinetex 5 µm C18 250 x 4.6 mm and Kinetex 2.6 µm C18 100 x 4.6 mm. Regarding the column parameters Ph.Eur. General Chapter 2.2.46 permits some adjustments to the column dimensions. Specifically, the particle size may be reduced up to 50%, and column length may be adjusted ±70%. The Kinetex 2.6 µm C18, 100 x 4.6 mm column is within this allowable range.

All columns met system suitability. The Kinetex 2.6 µm C18, 100 x 4.6 mm column provided comparable resolution to the Luna 5 µm C18(2), 250 x 4.6 mm column with a significant reduction in total run time (**Figure 2**). The use of a smaller particle size primarily impacts efficiency, with increases in efficiency leading to narrower peaks and increase in resolution.

All reference solutions were prepared as indicated in Ph. Eur. Monograph 1002 for Diclofenac Sodium. Diclofenac Sodium Certified Reference Standard (CRS) (catalog no. S0765000), and Diclofenac for System Suitability CRS (catalog no. Y0001635) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) Council of Europe.

LC-UV Conditions

Column: Luna 5 µm C18(2) 250 x 4.6 mm ([00G-4252-E0](#))
Kinetex 5 µm C18 250 x 4.6 mm ([00G-4601-E0](#))
Kinetex 2.6 µm C18 100 x 4.6 mm ([00D-4462-E0](#))

Pressure (bar): 182 bar (Luna 5 µm C18(2) 250 x 4.6 mm)
212 bar (Kinetex 5 µm C18 250 x 4.6 mm)
368 bar (Kinetex 2.6 µm C18 100 x 4.6 mm)

Mobile Phase: Mixed 34 volumes of a solution containing 0.5 g/L of phosphoric acid R and 0.8 g/L of sodium dihydrogen phosphate R, previously adjusted to pH 2.5 with phosphoric acid R, and 66 volumes of methanol R.

Flow Rate: 1 mL/min

Injection Volume: 20 µL

Temperature: 25 °C

Detector: UV @ 254 nm

System: Agilent® HPLC (Quaternary)

Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Test Solution	Dissolved 50.0 mg of the substance to be examined in the mobile phase and diluted to 50.0 mL with the mobile phase.
Reference Solution (a)	Diluted 2.0 mL of the test solution to 100.0 mL with the mobile phase. Diluted 1.0 mL of this solution to 10.0 mL with the mobile phase.
Reference Solution (b)	Dissolved the contents of a vial of diclofenac for system suitability CRS (containing impurities A and F) in 1 mL of the mobile phase.



Figure 1. Original chromatogram published in EDQM for test related substances of diclofenac sodium

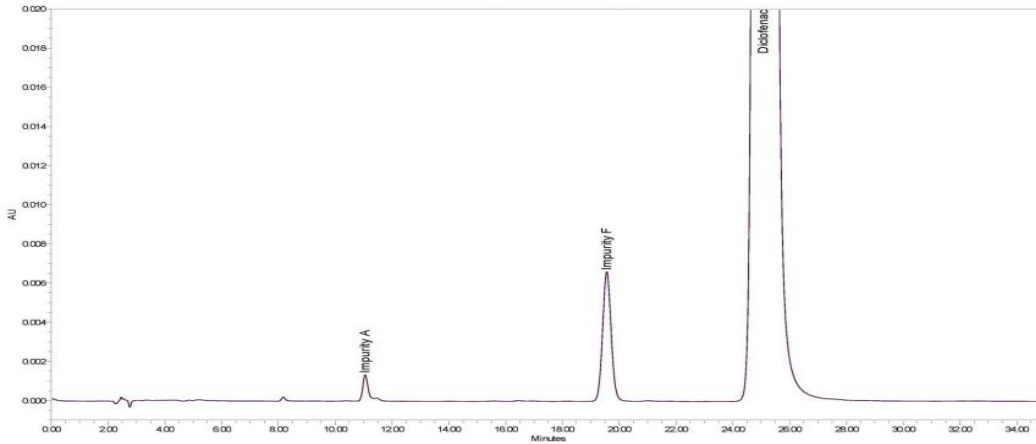
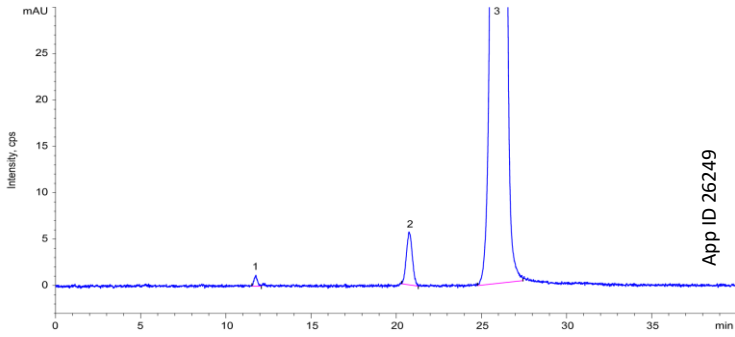
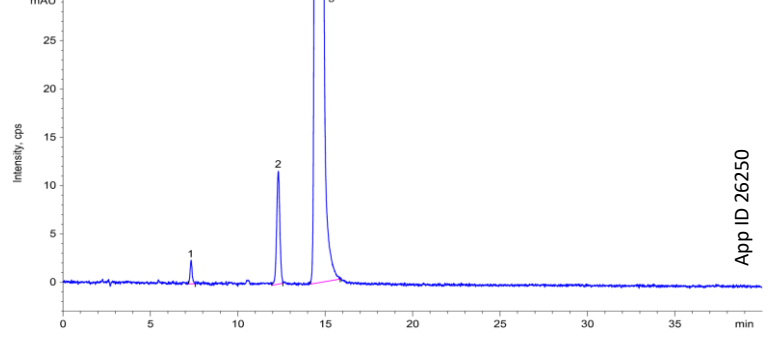


Figure 2. Reference Soutlution (b)

Luna® 5 µm C18(2) 250 x 4.6 mm



Kinetex® 5 µm C18 250 x 4.6 mm



Kinetex 2.6 µm C18 100 x 4.6 mm

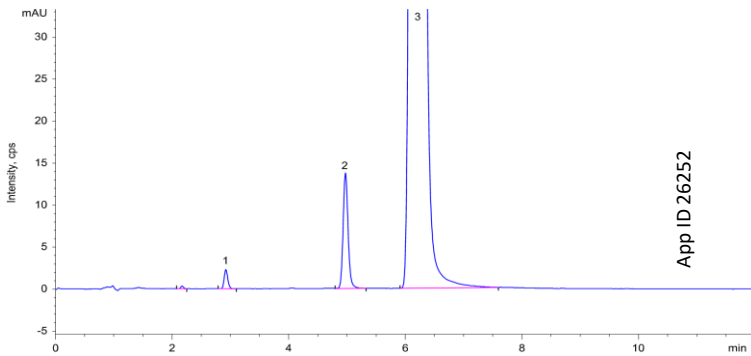


Table 2. Peak information for (1) impurity A, (2) impurity F, and (3) diclofenac; and resolution between impurity F and diclofenac peaks for the three columns

Column	Peaks	RT (min)	Peak Height	Peak Width	Resolution
Luna 5 µm C18(2) 250 x 4.6 mm	1	11.7	1.3	0.2	7.0
	2	20.8	5.8	0.3	
	3	26.1	622	0.5	
Kinetex 5 µm C18 250 x 4.6 mm	1	7.3	2.3	0.1	6.3
	2	12.3	11.6	0.2	
	3	14.6	1300	0.2	
Kinetex 2.6 µm C18 100 x 4.6 mm	1	2.9	2.3	0.1	6.9
	2	5.0	13.7	0.1	
	3	6.2	1435	0.1	



Figure 3. Reference Solution (a)

Luna® 5 µm C18(2) 250 x 4.6 mm

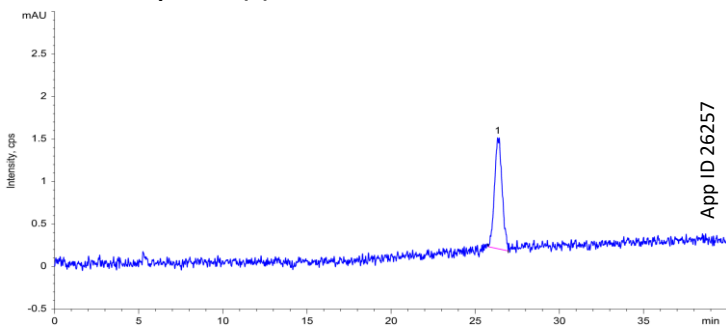
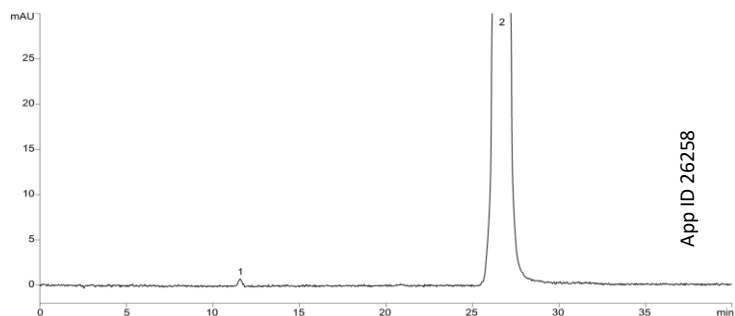
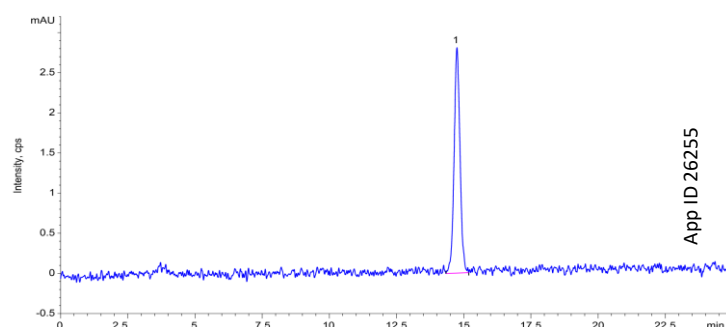


Figure 4. Test Solution

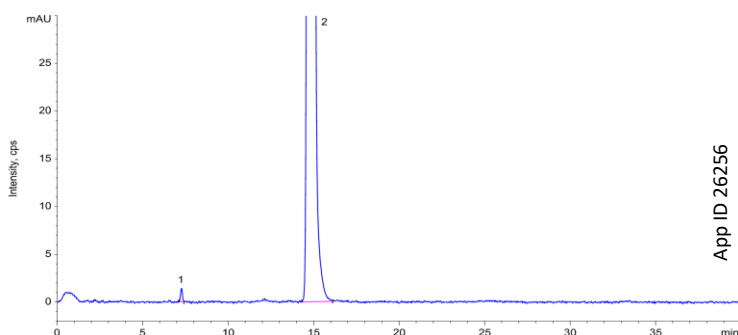
Luna 5 µm C18(2) 250 x 4.6 mm



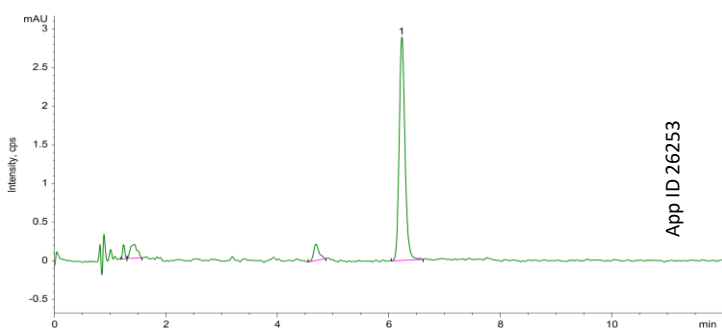
Kinetex® 5 µm C18 250 x 4.6 mm



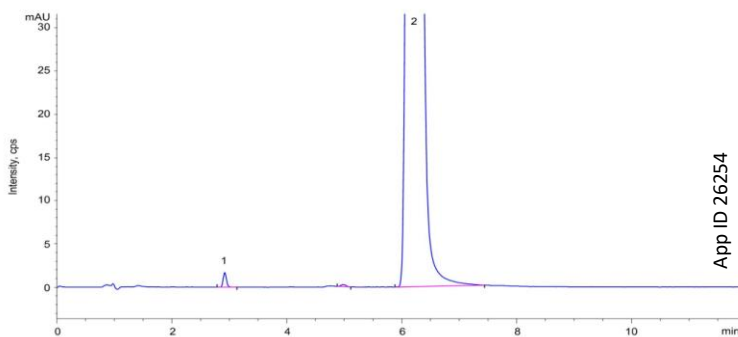
Kinetex 5 µm C18 250 x 4.6 mm



Kinetex 2.6 µm C18 100 x 4.6 mm



Kinetex 2.6 µm C18 100 x 4.6 mm



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