

USP Colchicine Assay on Luna® 5 µm C8(2) and Kinetex® 5 µm C8

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Overview

Colchicine is an anti-inflammatory used to treat gout and Behçet's disease. This application note for the LC-UV assay for Colchicine is based on the official USP assay method which calls for an L7 (C8) 5 µm, 250 x 4.6 mm column under isocratic conditions. The mobile phase and standard solution for the assay was prepared in accordance with the USP monograph, using the USP reference standard for Colchicine (catalog no. 1146006).

We utilized fully porous C8 and core-shell C8 columns, Luna C8(2) and Kinetex C8, respectively. In addition, a shorter 150 mm Kinetex 5 µm C8 column was utilized to demonstrate an allowable adjustment per USP General Chapter <621>. While the shorter 150 mm length Kinetex 5 µm C8 column has a $L/dp = 30,000$, which is outside the allowable -25% to +50% range allowed per USP <621>, the theoretical plates for this column are within the allowable range (in this case equivalent) relative to the original 250 mm length 5 µm C8 column.

All columns met the system suitability requirements for the assay: column efficiency NLT 4500 theoretical plates, and RSD NMT 2 % (**Table 1**). The shorter 150 mm Kinetex C8 column provided a much faster analysis while meeting all system suitability requirements.

Table 1. Summary of Results

Column	Retention Time, min	Theoretical Plates (NLT 4500)	% RSD (n = 6) (NMT 2 %)	L/dp
Luna 5 µm C8(2)	6.437	11134	0.49	50,000
Kinetex 5 µm C8	4.618	19406	0.56	50,000
Kinetex 5 µm C8 (150 x 4.6 mm)	2.761	11100	0.73	30,000

LC-UV Conditions

Column: Luna 5 µm C8(2) ([00G-4249-E0](#))
Kinetex 5 µm C8 ([00G-4608-E0](#))
Kinetex 5 µm C8, 150 x 4.6 mm ([00F-4608-E0](#))

Dimension: 250 x 4.6 mm

Mobile Phase: Dilute 45 mL of 0.5 M monobasic potassium phosphate with water to 450 mL. Add 530 mL of methanol, cool to room temperature, and add methanol to bring the volume to 1000 mL. Adjust with 0.5 M phosphoric acid to a pH of 5.5 ± 0.05 , and pass through a 0.45 µm membrane filter.

Flow Rate: 1.0 mL/min

Temperature: 30 °C

Detector: UV @ 254 nm

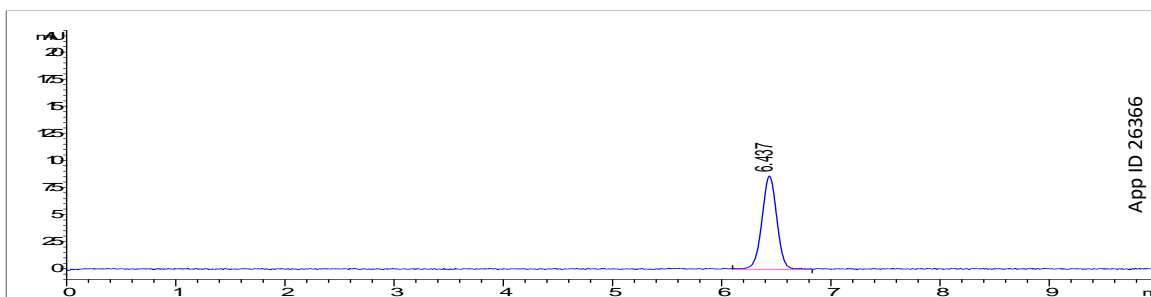
Injection: 20 µL

System: Agilent® 1260 (binary)

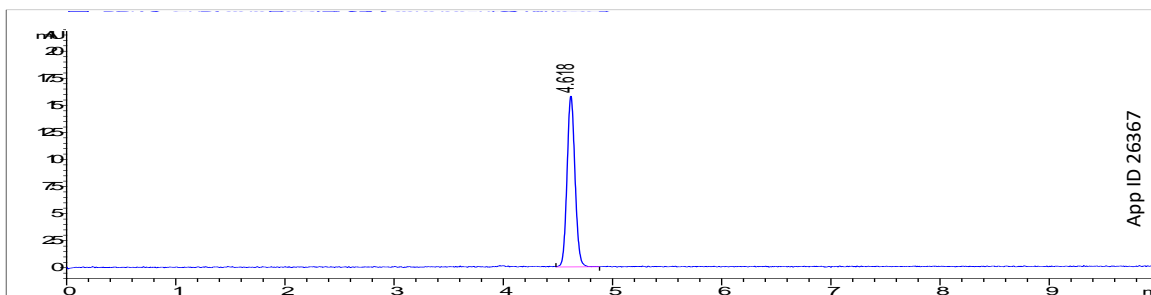
Sample: Colchicine (6 µg/mL, USP Colchicine RS)



Luna 5 µm C8(2) 250 x 4.6 mm ([00G-4249-E0](#))



Kinetex 5 µm C8 250 x 4.6 mm ([00G-4608-E0](#))



Kinetex 5 µm C8 150 x 4.6 mm ([00F-4608-E0](#))

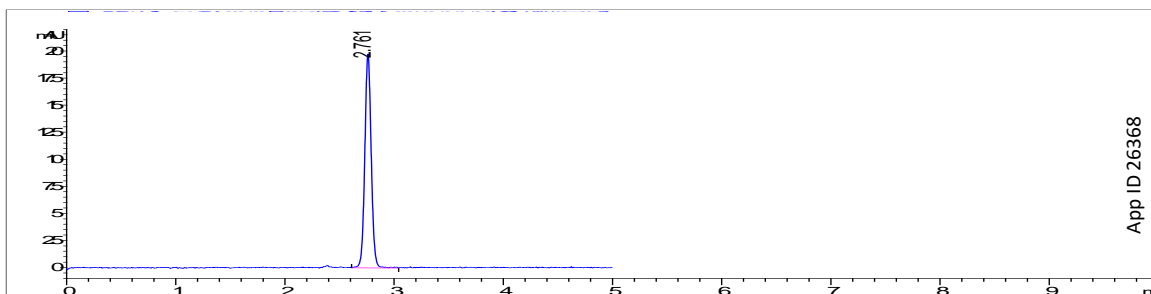


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