

# Test for the Investigative Covid-19 Drug Colchicine on Fully Porous Luna® 5 µm C8(2) and Core-Shell Kinetex® 5 µm C8 Columns based on Ph. Eur. Monograph 0758

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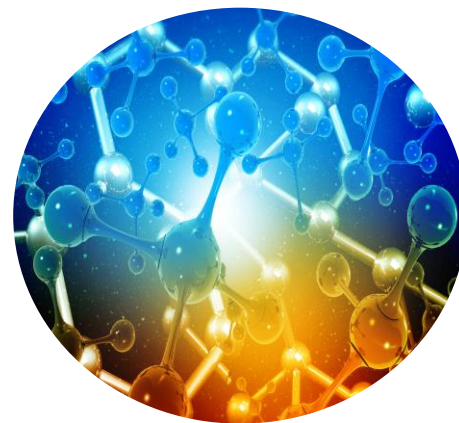
## Overview

Colchicine is an oral anti-inflammatory medication beneficial for treating gout, pericarditis, and coronary disease. There is an increase in interest recently in the effectiveness of Colchicine in mitigating inflammatory complications associated with COVID-19.

In this application note we demonstrate the effective identification and separation of Colchicine from its impurities and related substances according to Ph.Eur. Monograph 0758. To meet system suitability requirements, the peak-to-valley ratio must be a minimum of 2 between Colchicine and Impurity A for reference solution (a). The peak-to-valley ratio is defined as  $H_p/H_v$ , where  $H_p$  = height above the baseline of the peak due to impurity A and  $H_v$  = height above the baseline of the lowest point of the curve separating this peak from the peak due to colchicine. The peak-to-valley ratio between Impurity A and Impurity B must also be a minimum of 2 to meet system suitability with  $H_p$  is for Impurity B and  $H_v$  is for Impurity A.

We used the fully porous Luna 5 µm C8(2), and the core-shell Kinetex 5 µm C8 in two different column lengths with the shorter 150 mm column being an allowable adjustment per the Ph.Eur. General Chapter 2.2.46. All columns met system suitability and all peaks due to impurities A, B, E and G are identified by all three columns (**Figure 3**).

All reference solutions were prepared as indicated in Ph. Eur. Monograph 0758 for Colchicine. Colchicine (catalog no. C2580000), Colchicine Certified Reference Standard (CRS), and Colchicine for System Suitability A CRS (catalog no. Y0001958) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) Council of Europe.



## LC-UV Conditions

**Column:** 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](#))

**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 the pH to  $5.5 \pm 0.05$  with 0.5 M phosphoric acid, and filter through a 0.45 µm membrane filter.

**Pressure (bar):** 285 bar (Luna 5 µm C8(2) 250 x 4.6 mm)  
298 bar (Kinetex 5 µm C8 250 x 4.6 mm)  
233 bar (Kinetex 5 µm C8 150 x 4.6 mm)

**Flow Rate:** 1 mL/min

**Temperature:** 30 °C

**Detector:** UV @ 254 nm

**Injection:** 20 µL

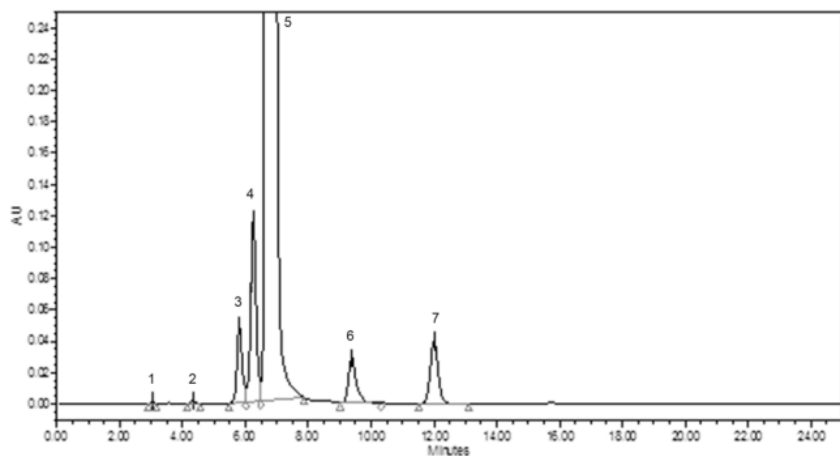
**System:** Agilent® 1260 Binary UHPLC

**Table 1.** Preparation of Test and Reference Solutions

| Solution               | Composition   |
|------------------------|---|
| Test Solution          | Dissolve 20.0 mg of the substance to be examined (Colchicine CRS) in the solvent mixture and dilute to 20.0 mL with the solvent mixture.                          |
| Reference Solution (a) | Dissolve 5 mg of Colchicine for system suitability A CRS (containing impurities A, E and G) in the solvent mixture and dilute to 5.0 mL with the solvent mixture. |
| Reference Solution (b) | Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.  |



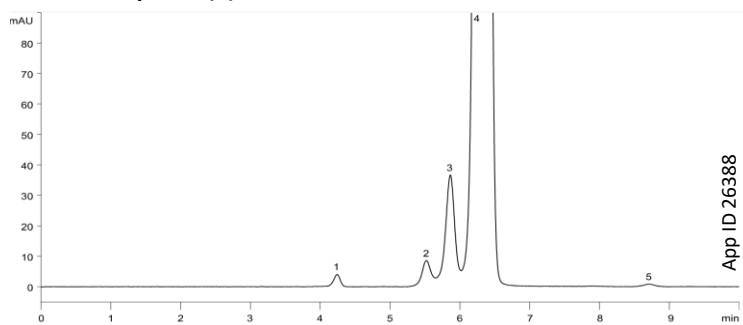
**Figure 1.** Original chromatogram published in EDQM for test related substances of Colchicine



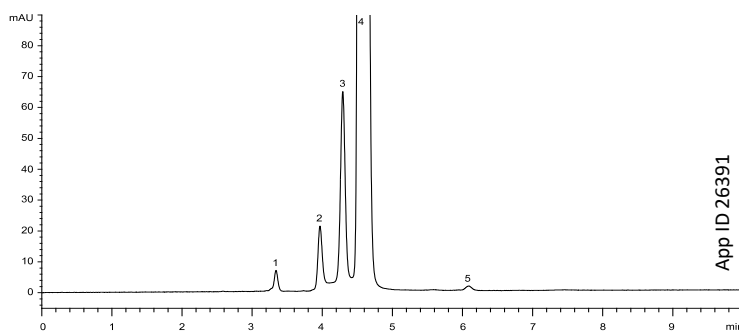
| Peak No. | Analyte    |
|----------|------------|
| 1        | Impurity D |
| 2        | Impurity E |
| 3        | Impurity B |
| 4        | Impurity A |
| 5        | Colchicine |
| 6        | Impurity G |
| 7        | Impurity C |

**Figure 2.** Test Solution

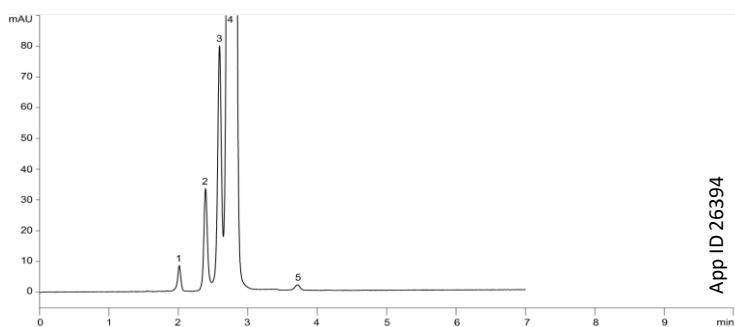
**Luna® 5 µm C8(2) 250 x 4.6 mm**



**Kinetex® 5 µm C8 250 x 4.6 mm**



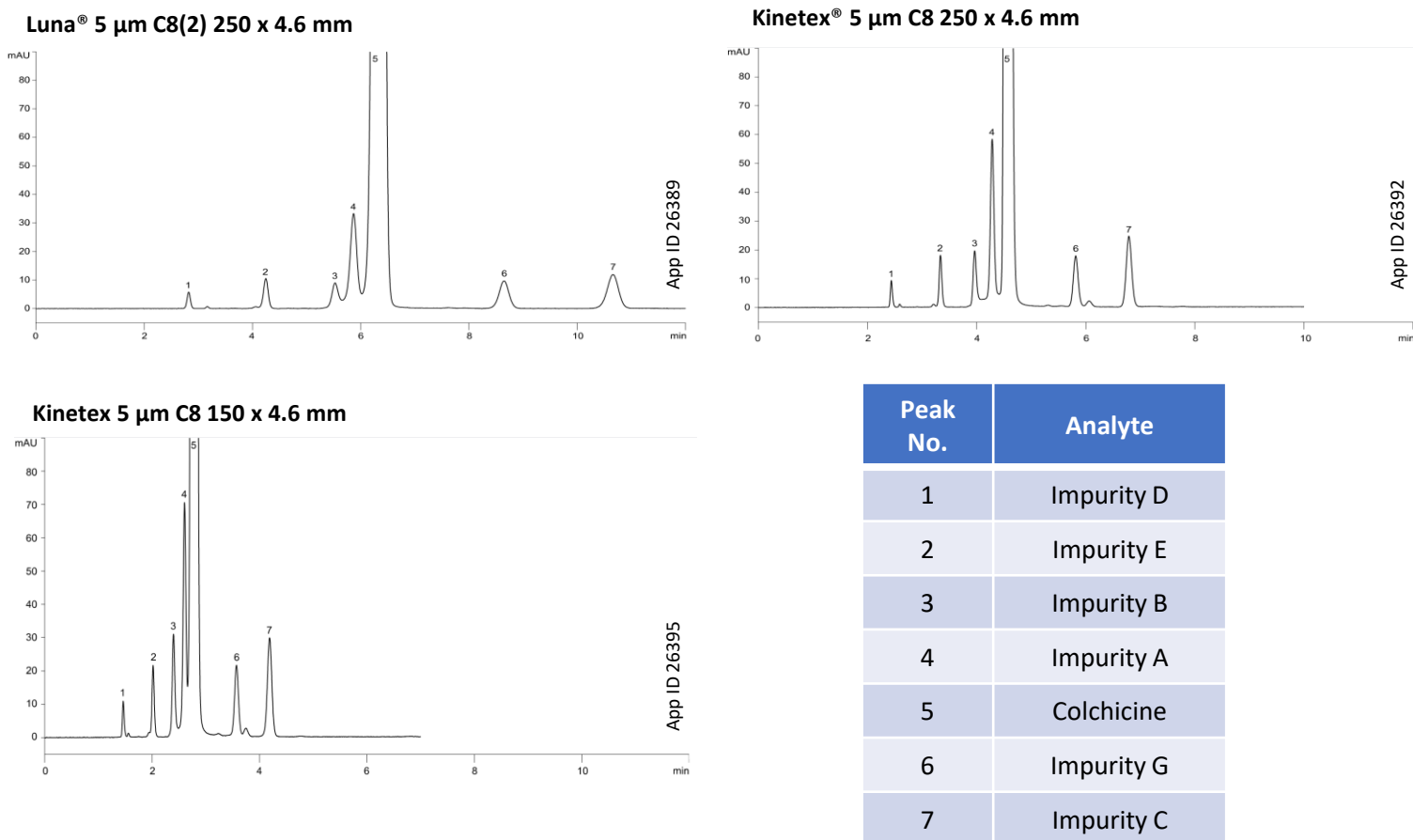
**Kinetex 5 µm C8 150 x 4.6 mm**



| Peak No. | Analyte    |
|----------|------------|
| 1        | Impurity E |
| 2        | Impurity B |
| 3        | Impurity A |
| 4        | Colchicine |
| 5        | Impurity G |



**Figure 3.** Reference Solution (a)



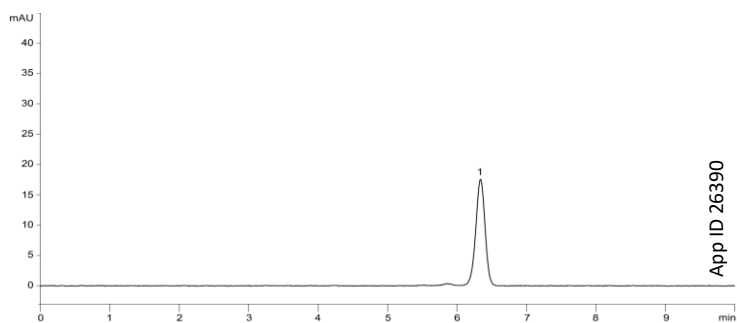
**Table 2.** Calculated peak-to-valley ratios for Reference Solution (a)

| Column                       | Peak-to-valley ratio<br>Impurity A and Colchicine | Peak-to-valley ratio<br>Impurity B and Impurity A |
|------------------------------|---|---|
| Luna 5 µm C8(2) 250 x 4.6 mm | 6.45  | 3.11  |
| Kinetex 5 µm C8 250 x 4.6 mm | 12.07   | 6.31  |
| Kinetex 5 µm C8 150 x 4.6 mm | 4.55  | 11.08   |

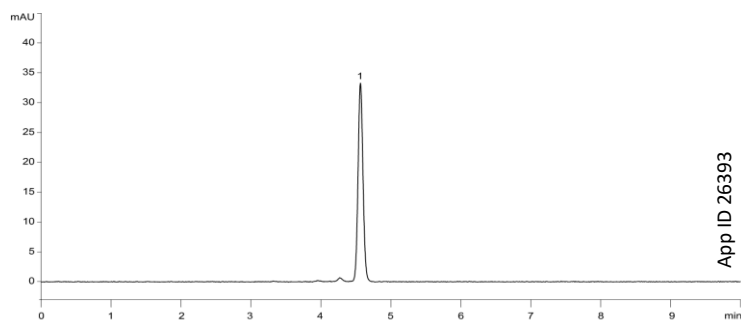


**Figure 4.** Reference Solution (b)

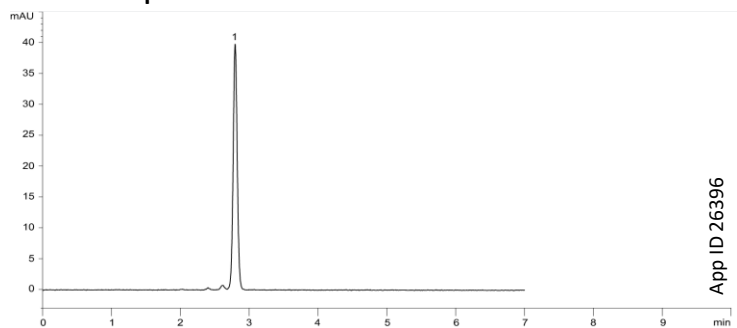
**Luna® 5 µm C8(2) 250 x 4.6 mm**



**Kinetex® 5 µm C8 250 x 4.6 mm**



**Kinetex 5 µm C8 150 x 4.6 mm**



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