

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

Zeshan Aqeel, Dr. Bryan Tackett, and Anania Amenu
Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501 1430, USA

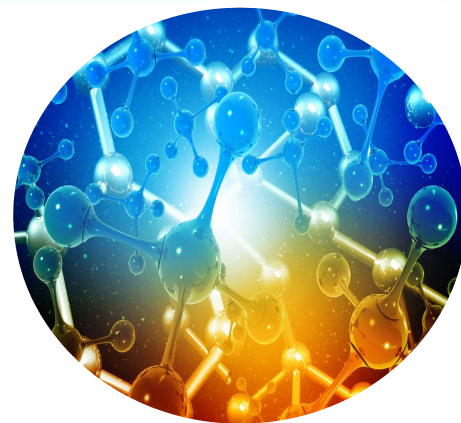
Overview

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 ± 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

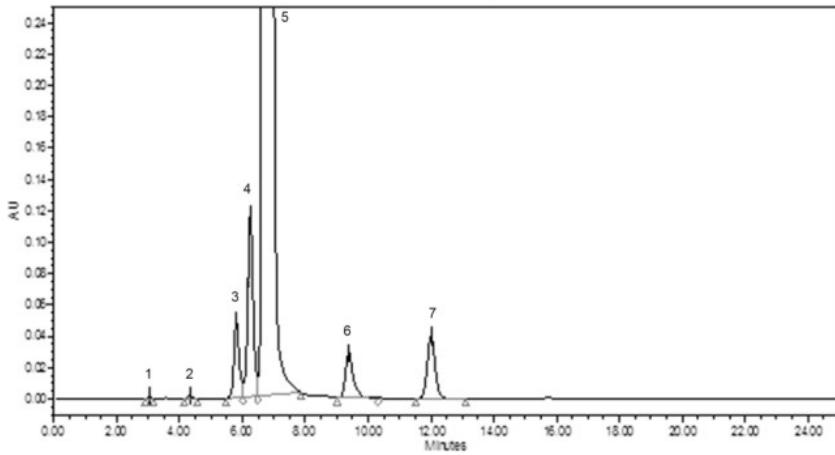
System: Agilent® 1260 Binary UHPLC

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.



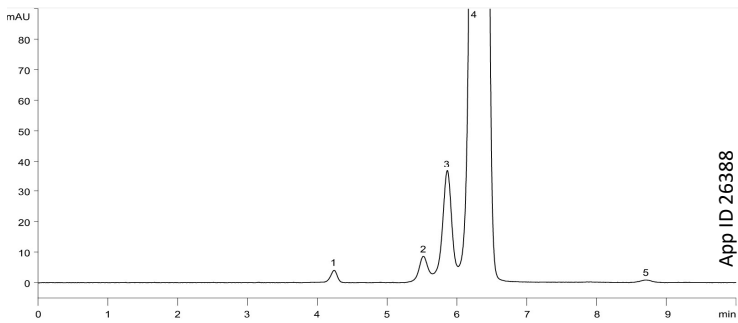
Figure 1. Original chromatogram published in EDQM for test related substances of Colchicine



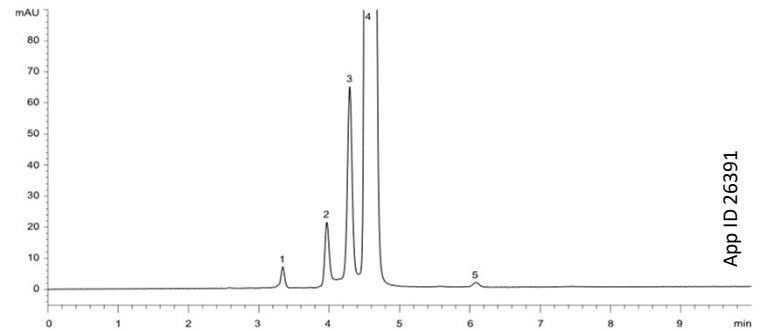
Peak No.	Analyte
1	Impurity D
2	Impurity E
3	Impurity B
4	Impurity A
5	Colchicine
6	Impurity G
7	Impurity C

Figure 2. Test Solution

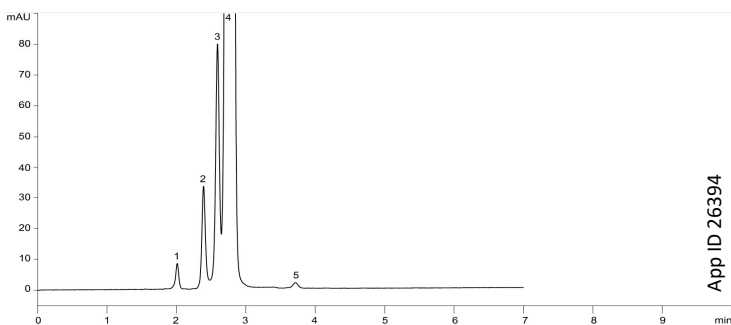
Luna® 5 µm C8(2) 250 x 4.6 mm



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)

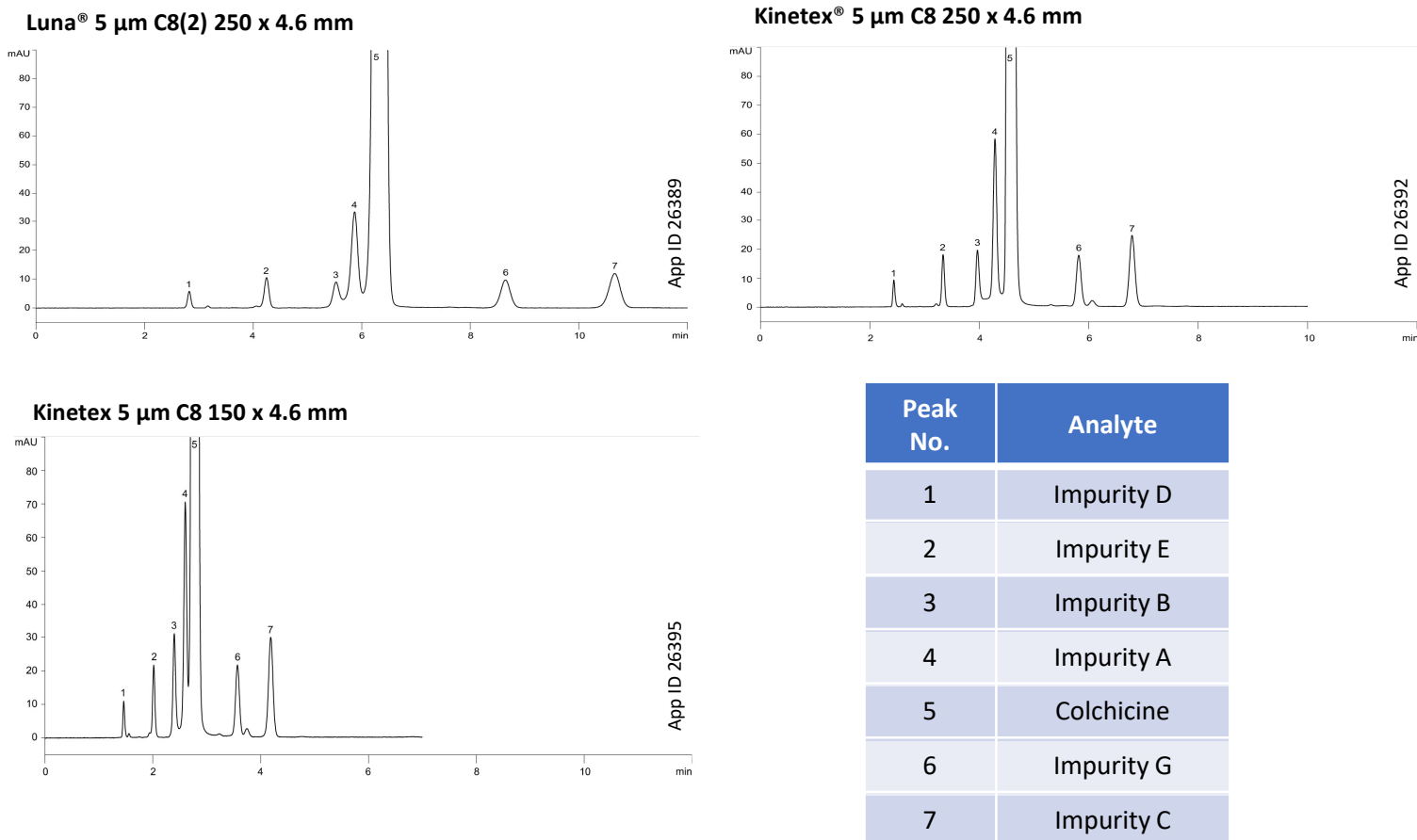


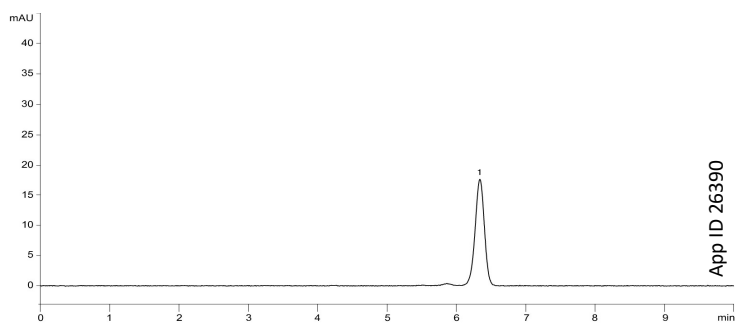
Table 2. Calculated peak-to-valley ratios for Reference Solution (a)

Column	Peak-to-valley ratio Impurity A and Colchicine	Peak-to-valley ratio 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

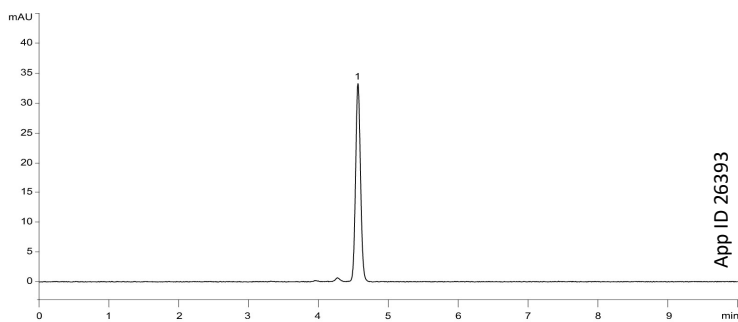


Figure 4. Reference Solution (b)

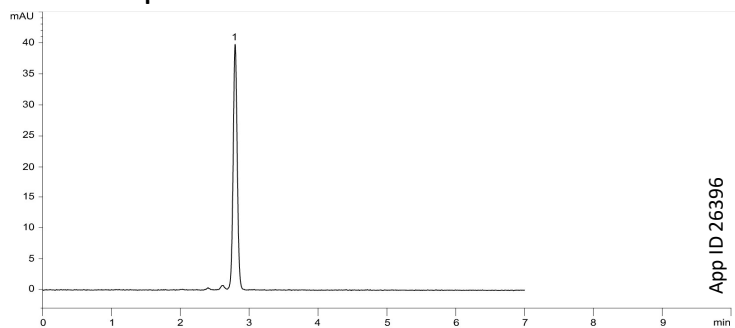
Luna® 5 µm C8(2) 250 x 4.6 mm



Kinetex® 5 µm C8 250 x 4.6 mm



Kinetex 5 µm C8 150 x 4.6 mm



Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/LiveChat.

- Australia**
t: +61 (0)2-9428-6444
auinfo@phenomenex.com
- Austria**
t: +43 (0)1-319-1301
anfrage@phenomenex.com
- Belgium**
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com
- Canada**
t: +1 (800) 543-3681
info@phenomenex.com
- China**
t: +86 400-606-8099
cninfo@phenomenex.com
- Czech Republic**
t: +420 272 017 077
cz-info@phenomenex.com
- Denmark**
t: +45 4824 8048
nordicinfo@phenomenex.com
- Finland**
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com
- France**
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com
- Germany**
t: +49 (0)6021-58830-0
anfrage@phenomenex.com
- Hong Kong**
t: +852 6012 8162
hkinfo@phenomenex.com
- India**
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com
- Indonesia**
t: +62 21 5010 9707
indoinfo@phenomenex.com
- Ireland**
t: +353 (0)1 247 5405
eireinfo@phenomenex.com
- Italy**
t: +39 051 6327511
italiainfo@phenomenex.com
- Japan**
t: +81 (0) 120-149-262
jpinfo@phenomenex.com
- Luxembourg**
t: +31 (0)30-2418700
nlinfo@phenomenex.com
- Mexico**
t: 01-800-844-5226
tecnicomx@phenomenex.com
- The Netherlands**
t: +31 (0)30-2418700
nlinfo@phenomenex.com
- New Zealand**
t: +64 (0)9-4780951
nzinfo@phenomenex.com
- Norway**
t: +47 810 02 005
nordicinfo@phenomenex.com
- Poland**
t: +48 22 104 21 72
pl-info@phenomenex.com
- Portugal**
t: +351 221 450 488
ptinfo@phenomenex.com
- Singapore**
t: +65 800-852-3944
sginfo@phenomenex.com
- Slovakia**
t: +420 272 017 077
sk-info@phenomenex.com
- Spain**
t: +34 91-413-8613
espinfo@phenomenex.com
- Sweden**
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com
- Switzerland**
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com
- Taiwan**
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com
- Thailand**
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com
- United Kingdom**
t: +44 (0)1625-501367
ukinfo@phenomenex.com
- USA**
t: +1 (310) 212-0555
info@phenomenex.com
- 🌐 All other countries/regions
Corporate Office USA**
t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

BE-HAPPY™

GUARANTEE

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.

www.phenomenex.com/behappy

Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions, which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks
Luna and Kinetex are registered trademarks and BE-HAPPY is a trademark of Phenomenex.
Agilent is a registered trademark of Agilent Technologies, Inc.

Disclaimer
Comparative separations may not be representative of all applications.
Phenomenex is in no way affiliated with Agilent Technologies, Inc.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.
© 2021 Phenomenex, Inc. All rights reserved.

AN40030621_W