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Analysis Paracetamol and Critical Impurities Under European Pharmacopoeia Conditions and Utilizing the Kinetex® 2.7 µm C18 Core-Shell LC Column

Zeshan Aqeel, Heiko Behr, Philip J. Koerner, and Ryan Splitstone
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

N-(4-hydroxyphenyl) acetamide, commonly referred to as paracetamol in Europe and acetaminophen in the United States, is one of the most familiar analgesics and antipyretic therapeutics in today's drug market. In a previous technical note (TN-1244) we demonstrated that a column containing Kinetex 2.6 µm core-shell particles utilizing the European Pharmacopoeia (Ph. Eur.) HPLC conditions gave an improved separation of paracetamol and related impurities. However, in Pharmeuropa 28.1, a recent revision to the Ph. Eur. Method, an LC column 100 mm in length, inner diameter of 2.1 mm, and packed with superficially porous particles with diameter of 2.7 µm is referenced as a suitable analytical column for this analysis.1 Because the Ph. Eur. method for paracetamol is operated under gradient conditions, adjustments to the particle diameter, even as small as 0.1 µm are not allowed without full revalidation of the method.

In this technical note, utilizing a Kinetex 2.7 µm C18 100 x 2.1 mm column, we were able to provide good and reproducible resolution for paracetamol and the principal degradation product Impurity K by using the specific HPLC parameters (particle size, column dimension, and gradient mobile phase conditions) consistent with the Ph. Eur. impurities test for paracetamol published in Supplement 9.4.2-3 The Kinetex 2.7 µm C18 column we used is a direct equivalent to the specified suitable column in Pharmeuropa 28.1 revision

Experiment

We obtained the analytical reference standards for paracetamol and impurity 4-aminophenol (impurity K) from Sigma Aldrich® (St. Louis, MO). The additional critical paracetamol impurity J (4'-Chloroacetanilide) was obtained from TLC Pharmaceutical Standards Ltd. (Ontario, Canada). A Waters® ACQUITY® (Milford, MA) I-Class UPLC system equipped with a UV-Vis detector was used.

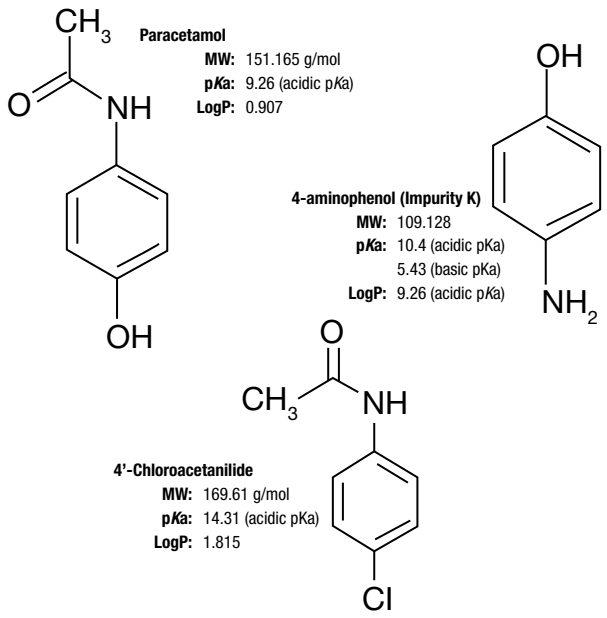
To ensure the suitability and repeatability of the Kinetex 2.7 µm C18 for analysis of paracetamol, three batches of Kinetex 2.7 µm C18 core-shell media were obtained. All columns used in this study were initially tested using the same isocratic test to confirm all were operating within expected efficiency and performance levels in accordance with the columns Certificates of Quality Assurance.

To demonstrate system suitability, the Ph. Eur. paracetamol monograph requires a minimum resolution of 5.0 between the peaks associated with paracetamol and the principal product degradant, impurity K, as determined using reference solution B. The preparation of reference solution B was conducted per Ph. Eur. 9.4, in which 5.0 mg of paracetamol and 5.0 mg of impurity K were dissolved in a mixture of water and methanol (15:85, v/v) in a 100-mL volumetric flask and diluted to volume. After this initial dilution, a 1 mL aliquot was further diluted to 100 mL in a mixture of water and methanol (15:85, v/v), which was prepared immediately before use. In addition, in line with Ph. Eur. 9.4 for paracetamol reference solution C containing impurity J was freshly prepared and analyzed. Reference solution C was prepared by dissolving 5.0 mg of impurity J in a mixture of water and methanol (15:85, v/v) and diluted to a final volume of 250 mL. After, a 1.0 mL aliquot of the solution was diluted to 200.0 mL for the final reference solution C concentration. The HPLC conditions are listed below and were used to generate all the chromatograms in this technical note.

LC Conditions

Column:	Kinetex® 2.7 µm C18
Dimensions:	100 x 2.1 mm
Part No.:	00B-4622-Y0
Mobile Phase:	A: A: Phosphate buffer (prepared by dissolving 1.7 g of potassium dihydrogen phosphate and 1.8 g of dipotassium hydrogen phosphate in HPLC grade water and diluting to 1000 mL with water) B: Methanol
Flow Rate:	0.3 mL/min
Temperature:	30 °C
Injection Volume:	5 µL
Detection:	UV-Vis @ 254 nm
HPLC System:	Waters® ACQUITY® I-Class UPLC (Milford, MA)

Figure 1.
Structures and Chemical Information for Paracetamol, 4-Aminophenol (Impurity K), and 4'-Chloroacetanilide (Impurity J)



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Results and Discussion

The Kinetex® 2.7 µm C18 produced values of resolution and peak performance which were in-line with Ph. Eur. requirements for system suitability and can be seen in Figure 2 and peak values summarized in **Table 1**.

Figure 2.
Reference solution B across three batches of Kinetex 2.7 µm C18

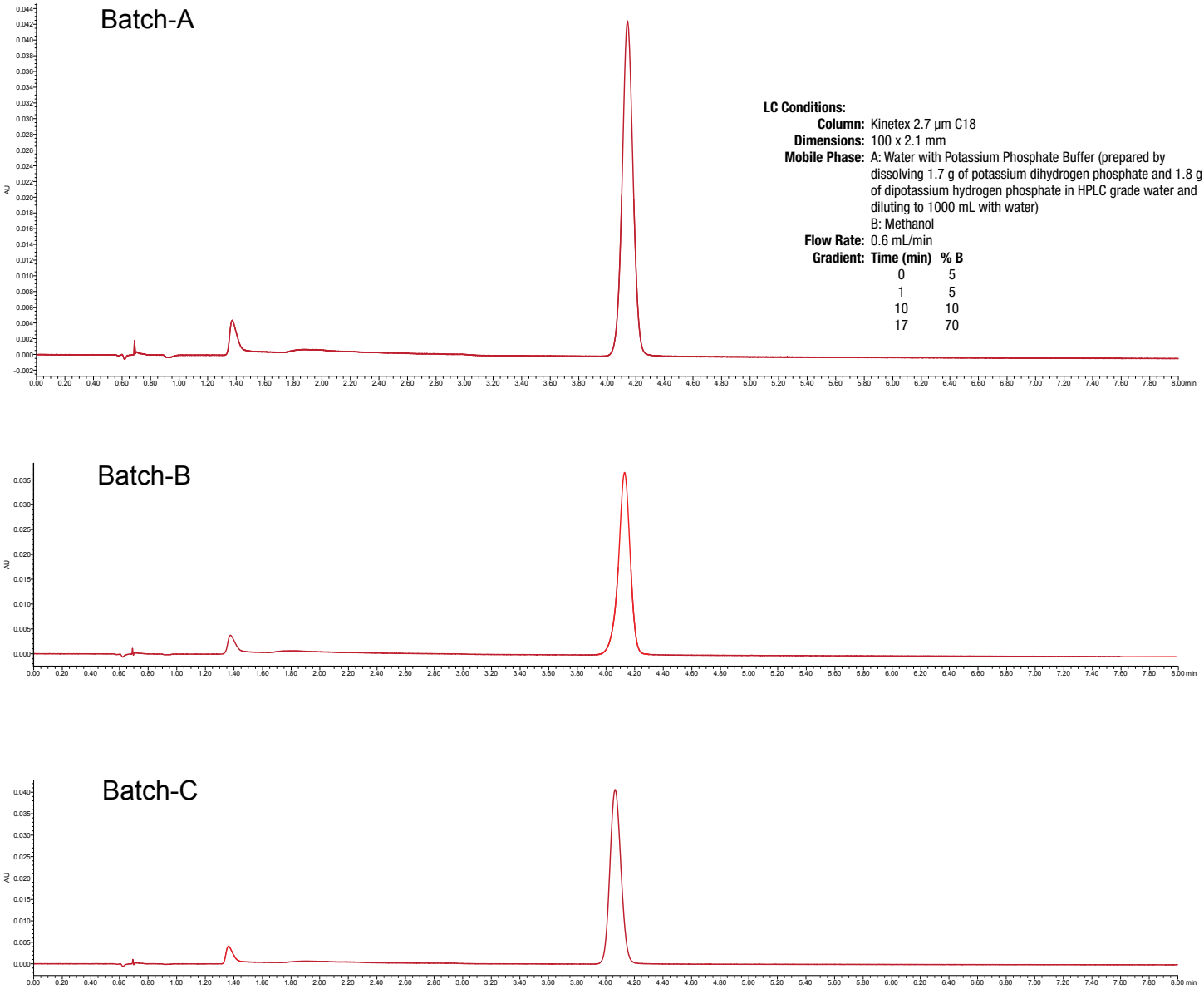

Table 1.
Three replicate injections of paracetamol and 4-aminophenol (Impurity K)

Batch	Paracetamol Retention Time	Impurity K Retention Time	Resolution	Paracetamol Symmetry Factor	Impurity K Symmetry Factor
Batch – A	4.14	1.37	23.0	1.0	1.7
Batch – B	4.13	1.38	21.8	0.9	1.4
Batch – C	4.07	1.36	22.8	1.1	1.6

Additionally, to ensure method reproducibility and batch-to-batch consistency, three different batches of Kinetex 2.7 C18 were tested separately and chromatographic overlays for replicate injections can be seen in **Figure 3**. **Table 2** summaries results and demonstrates the suitability of the Kinetex® 2.7 µm C18 in the analysis of paracetamol and its critical Impurity K.

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Figure 3.
Three replicate injections of paracetamol and 4-aminophenol (Impurity K)

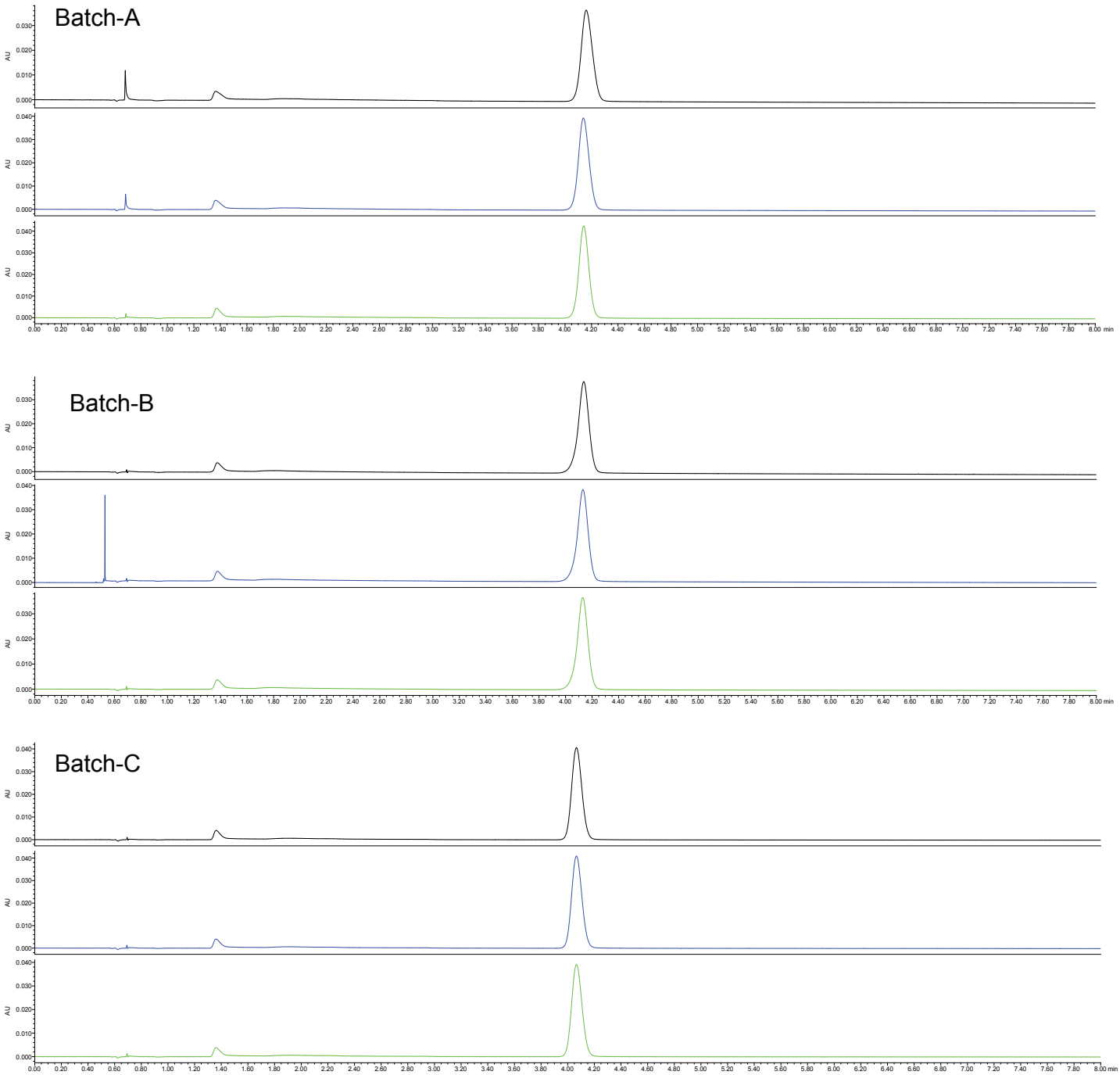

Table 2.
Reference B replicate injection batch summary

Kinetex 2.7 µm C18	Impurity K	Impurity K Retention Time (Mean)	Resolution
Batch – A	1.37	4.22	20
Batch – B	1.38	4.14	22
Batch – C	1.36	4.07	22

The European Pharmacopeia method for the analysis of paracetamol and related impurities includes a reference solution C which contains the critical impurity J. In **Figure 4**, is the batch-to-batch comparison of reference solution C across three batches of media. Results are summarized in **Table 3**.

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Figure 4.
Reference solution C Impurity J across three batches of Kinetex 2.7 µm C18

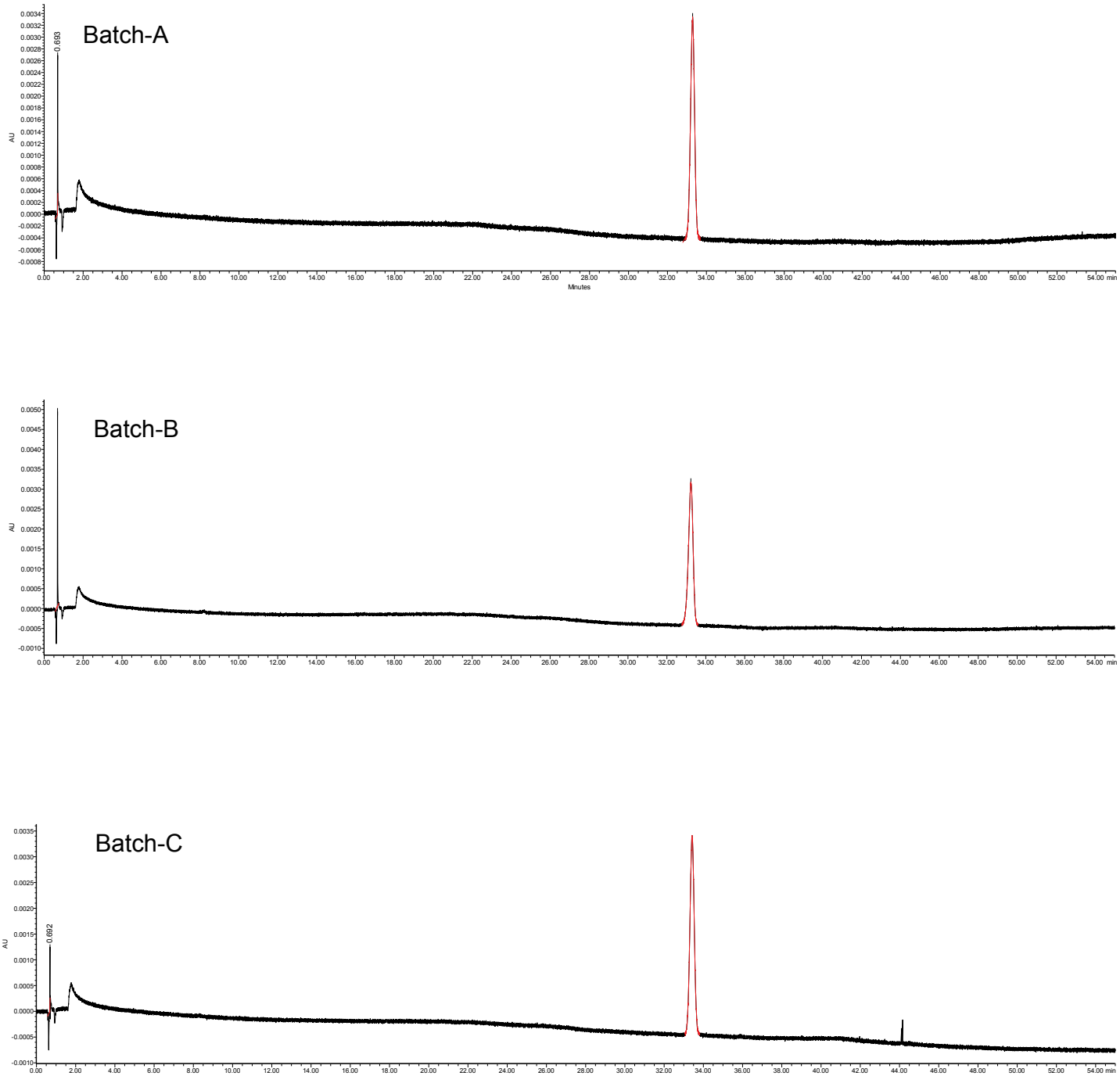

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Table 4.
Kinetex 2.7 µm C18 Batch-to-Batch and Reference Solution C (Impurity J)

Batch	Solvent Peak	Impurity K Retention Time (Mean)	Resolution	Impurity J Symmetry Factor
Batch – A	0.069	33.29	157.1	0.9
Batch – B	0.069	33.23	143.7	0.9
Batch – C	0.069	33.43	152.0	1.0

Conclusions

A recent revision to the Ph. Eur. Method for paracetamol and related impurities references the use of an LC column packed with superficially porous particles of 2.7 µm diameter and 100 x 2.1 mm dimension. Therefore, for this technical note we investigated the new Kinetex 2.7 µm C18 applied to the analysis of paracetamol. The Kinetex 2.7 µm column was able to provide reliable and reproducible results for reference solutions A, B, and C which easily met the system suitability requirement over multiple column batches.

References

1. EDQM Knowledge Database; Figure 0049-1: chromatogram for paracetamol and related substances; https://extranet.edqm.eu/4DLink1-pdfs/chromatos/0049.pdf.
2. European Pharmacopeia, Supplement 9.4, 2017, pp. 5429 – 5430.
3. Hanyšová, L & Kastner, P & Klimeš, J. (2004). Study of stability of 4-aminophenol as dominant decomposition product of paracetamol. Chemicke Listy. 98. 152-156.

Ordering Information

Kinetex® HPLC/UHPLC Columns

2.6 µm Analytical Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
EVO C18	—	00B-4725-E0	—	00D-4725-E0	00F-4725-E0	AJ0-9296
Polar C18	—	00B-4759-E0	—	00D-4759-E0	00F-4759-E0	AJ0-9532
F5	—	00B-4723-E0	—	00D-4723-E0	00F-4723-E0	AJ0-9320
Biphenyl	—	00B-4622-E0	—	00D-4622-E0	00F-4622-E0	AJ0-9207
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJ0-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AJ0-8770
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AJ0-8772
Phenyl-Hexyl	—	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	AJ0-8774
						for 4.6 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000.

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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

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

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@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 (12) 881 0121
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@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

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

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