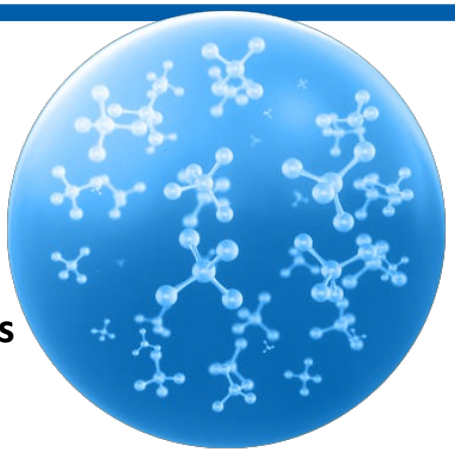
TN-1376

Alternative Column for USP Monograph-227: 4-Aminophenol in Acetaminophen-Containing Drug Products



Arunkumar D N[1], Rajesh Babu Dandamudi, PhD[1], and Sean Orlowicz [2]

[1]India Phenologix Lab, Phenomenex India, Hitech Defence and Aerospace Park Industrial Area, Mahadeva Kodigehalli, Holbi, Jala Taluka, Bengaluru 562149, India.

[2]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Acetaminophen, is a common non-opioid analgesic and antipyretic agent used to treat fever and mild to moderate pain. It is a widely available over-the-counter drug globally, sold under various brand names.

In this technical note we demonstrate that both Luna™ Omega PS C18, (5 µm 150 x 4.6 mm) and Kinetex™ EVO C18 (5 µm 150 x 4.6 mm) meet the acceptance criteria for USP -227: 4-Aminophenol in Acetaminophen containing drug products. The USP Chapter specifies the use of an L85 column, which is a mixed-mode phase column. However, in this work, we demonstrate the suitability of using a L1 columns that meet the SST requirements.

All reference solutions were prepared as indicated in USP monograph 227. The 4-aminophenol USP standard used for the analysis was from Lot No.R136K0.

Standard and System Suitability Solution Preparation

Standard stock solution: 25 µg/mL of USP 4-Aminophenol RS in Diluent. Prepare fresh.

System suitability solution: 2.5 µg/mL of USP 4-Aminophenol RS in Diluent, from the Standard stock solution.

Sample stock solution: Nominally 10 mg/mL of acetaminophen from a suitable quantity of drug product in Diluent

Standard solution: Add 25.0 mL of the Sample stock solution and 15.0 mL of the Standard stock solution to a 50-mL volumetric flask, a dilute with Diluent to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.

Sample Preparation

Sample stock preparation (Drug Product):

Transfer five acetaminophen tablets into a 250 mL volumetric flask. To this add 200 mL of diluent and the sonicate the mixture for 60 minutes with intermediate shaking. Make up to the mark with diluent, mix thoroughly, and centrifuge at 3500 RPM for 5 minutes.

Sample preparation (Drug Product):

Transfer 25 mL of the centrifuged sample stock (drug product) solution into a 50 mL volumetric flask and made up to the mark with diluent. Mix the solution thoroughly and filter through a 0.45 µm Verex PVDF hydrophilic filter. Discard initial 3 mL of filtrate before filtering.

Label claim of acetaminophen tablet is 500 mg.

Calculation

Formula for the calculation of the percentage of 4-aminophenol relative to acetaminophen in the portion of drug product.

$$= \left[\frac{r_U}{(r_S - r_U)}\right] \times \left(\frac{W_S}{W_U}\right) \times 100$$

r_U = peak response of 4 − aminophenol from Sample solution
r_S = peak response of 4 − aminophenol from Standard solution
W_S = amount of USP 4 − aminophenol RS added to the Standard Solution (mg)
W_U = amount of acetaminophen in the Sample solution (mg)

Acceptance criteria: NMT 0.15 % of 4-aminophenol relative to acetaminophen.

LC Conditions

Column: Luna Omega 5 µm PS C18 (**Part No:** 00F-4753-E0)
Kinetex EVO 5 µm C18 (**Part No:** 00F-4633-E0)

Dimensions: 150 x 4.6 mm

Mobile Phase: **A**: 10 mM phosphate buffer (0.60 g of monobasic potassium phosphate and 0.82 g of anhydrous dibasic sodium phosphate to a 1 L volumetric flask. Dissolve and dilute with water to volume to a pH of 7).
B: Water
C: Acetonitrile

Buffer 4.0 g/L of sodium citrate dihydrate and 1.5 g/L of anhydrous citric acid, in water

Diluent Acetonitrile and Buffer (10:90 V/V)

Gradient:

Time (min)	% B	% C
0	5	5
5	5	5
7	10	80
7.1	5	5
10	5	5

Flow Rate: 1 mL/min

Injection Volume: 10 µL

Temperature: 20 °C

LC System: Waters® Arc HPLC

Detection: UV @ 300 nm

Detector: (2998 PDA / UV / VWD-Detector)

Results and Discussion

The system suitability solution and standard solution were run on both the Luna Omega PS C18, 5 µm 150 x 4.6 mm and Kinetex EVO C18, 5 µm 150 x 4.6 mm columns. **Figures 2** and **8** show the system suitability solution chromatograms and **Tables 1** and **5** show system suitability criteria S/N ratio. **Figures 3** and **9** show overlayed chromatogram of diluent and System suitability solution chromatogram, respectively. **Figures 4**, **5**, **10**, and **11** show standard solution chromatograms and **Tables 2**, **3**, **6**, and 7 show peak tailing and resolution and relative standard deviation (% RSD) of the standard solution. **Figures 6** and **12** show sample solution chromatograms and **Tables 4** and **7** show 4-aminophenol peak area in sample.

The results demonstrated that the system suitability requirements were met for both columns.

Figure 1. Diluent on Luna Omega PS C18, 5 µm 150 x 4.6 mm 100 Å.

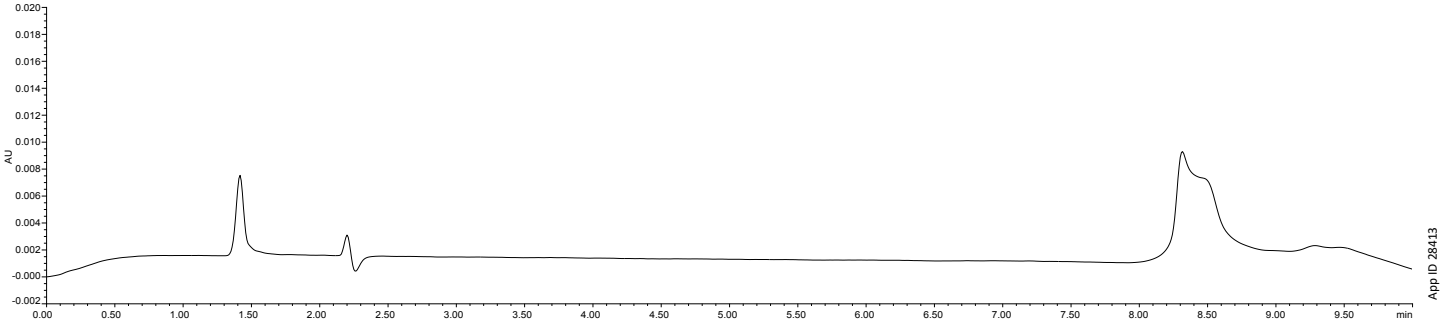


Figure 2 . System suitability solution on Luna Omega PS C18, 5 µm 150 x 4.6 mm 100 Å.

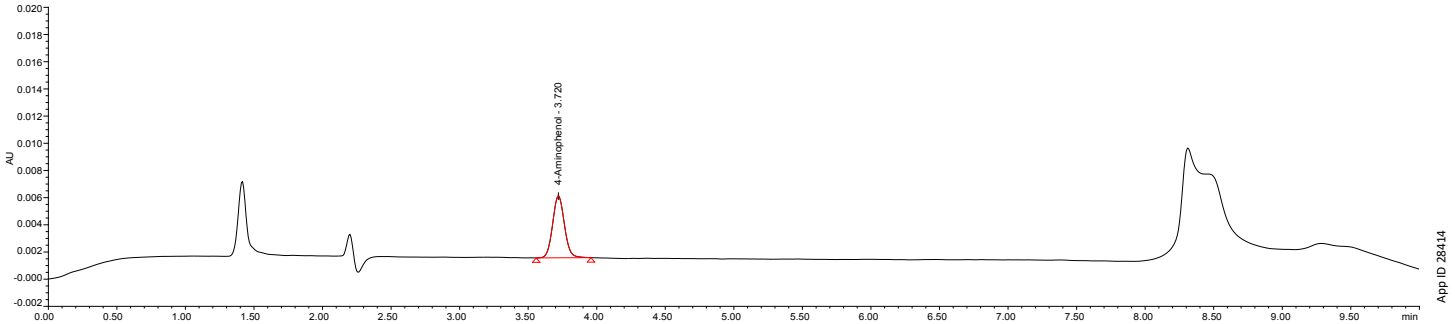


Table 1.

Peak No.	Name	Retention Time	Area	% Area	Height	s/n
1	4-Aminophenol	3.720	27140	100.00	4559	147

Figure 3 . Overlayed chromatogram of diluent and System suitability solution on Luna Omega PS C18, 5 µm 150 x 4.6 mm 100 Å.

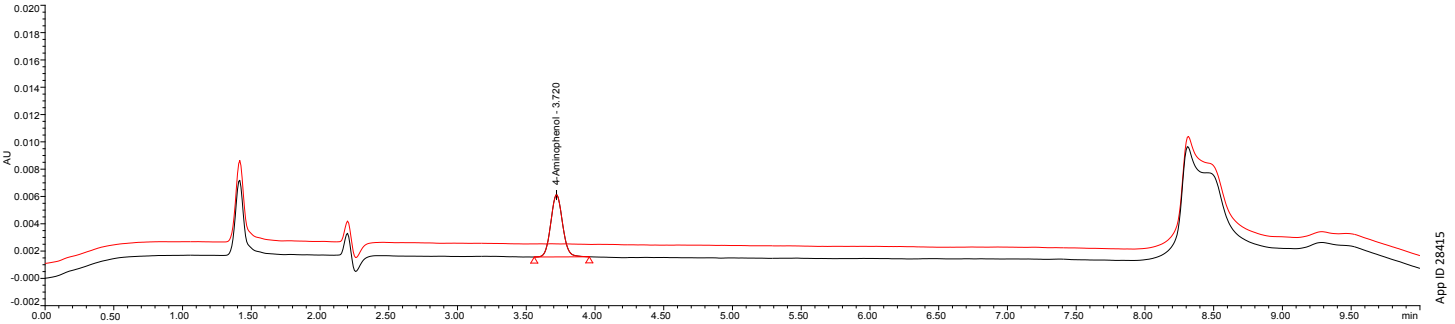


Figure 4 . Standard solution on Luna Omega PS C18, 5 µm 150 x 4.6 mm 100 Å.

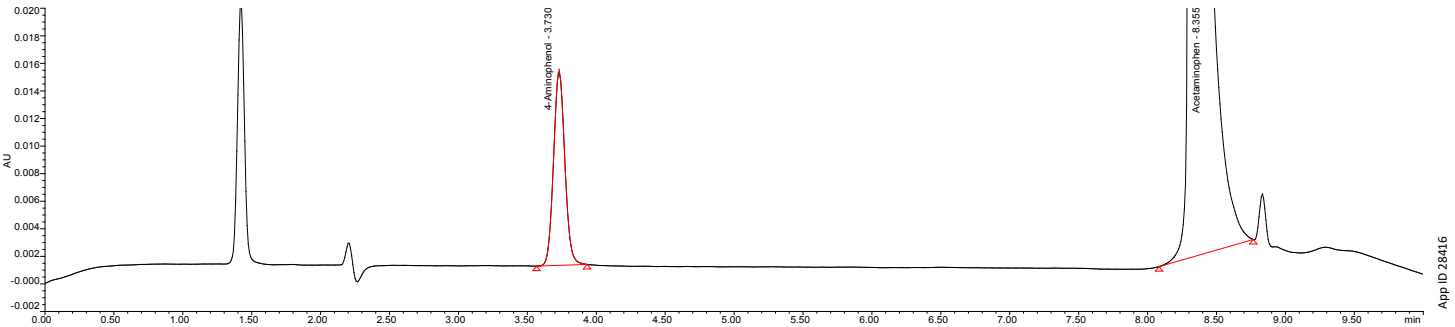


Table 2.

Peak No.	Name	Retention Time	Area	RT Ratio	Resolution	USP Tailing
1	4-Aminophenol	3.730	79979	0.446		1.1
2	Acetaminophen	8.355	6919019	1.000	37.1	1.1

Figure 5 . Six replicate injections of Standard solution on Luna Omega PS C18, 5 µm 150 x 4.6 mm 100 Å.

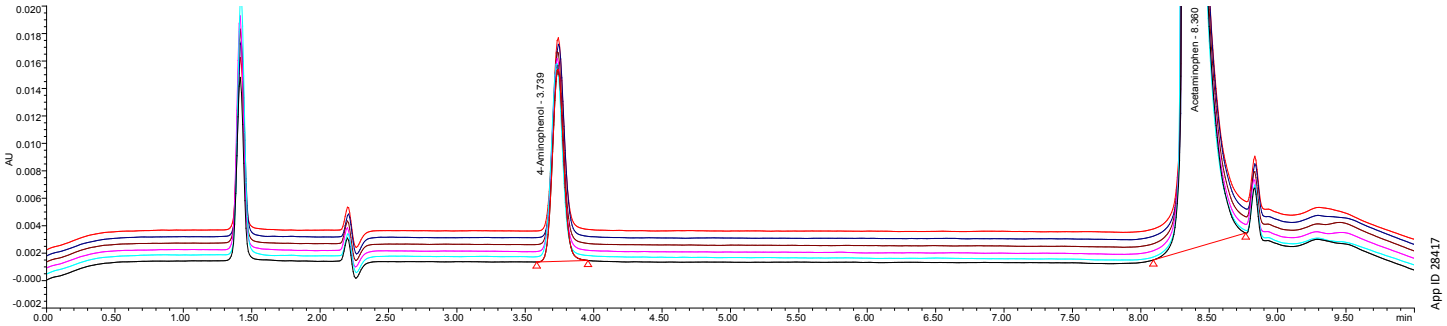


Table 3.

Injection	Retention Time	Area
Injection 1	3.730	79979
Injection 2	3.734	80223
Injection 3	3.737	80246
Injection 4	3.744	80119
Injection 5	3.739	80264
Injection 6	3.740	80288
Mean	3.737	80187
SD	0.005	117
%RSD	0.1	0.1

Figure 6 . Sample solution on Luna Omega PS C18, 5 µm 150 x 4.6 mm 100 Å.

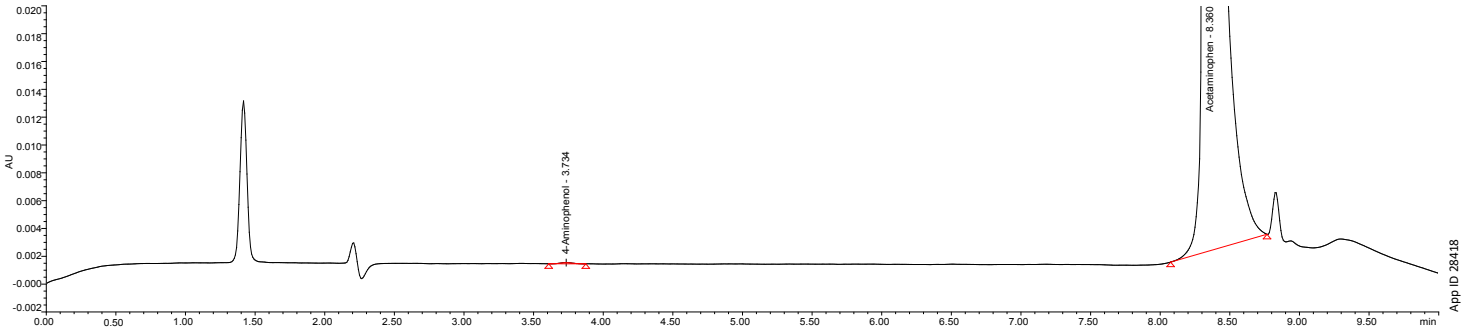


Table 4.

Peak No.	Name	Retention Time	Area	% Area	RT Ratio
1	4-Aminophenol	3.734	528	0.01	0.447
2	Acetaminophen	8.360	6686079	99.99	1.000

$$\%\ of\ 4-aminophenol = \left[\frac{r_U}{(r_S - r_U)}\right] \times \left(\frac{W_S}{W_U}\right) \times 100$$

$$= \left[\frac{r_U}{(r_S - r_U)}\right] \times \left(\frac{W_S}{W_U}\right) \times 100$$

$$= \left[\frac{528}{(80187 - 528)}\right] \times \left(\frac{0.007605}{5}\right) \times 100$$

$$= 0.001\ \%$$

Figure 7. Diluent on Kinetex EVO C18, 5 µm 150 x 4.6 mm 100 Å.

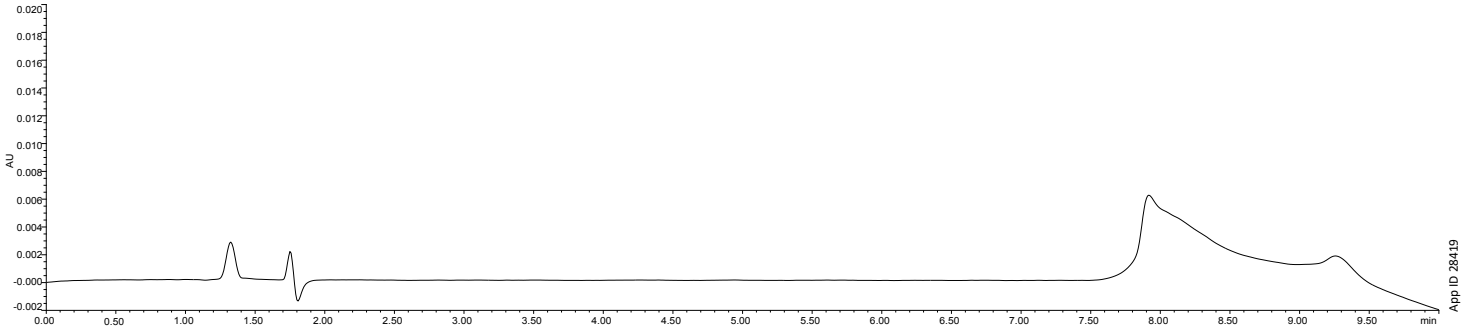


Figure 8. System suitability solution on Kinetex EVO C18, 5 µm 150 x 4.6 mm 100 Å.

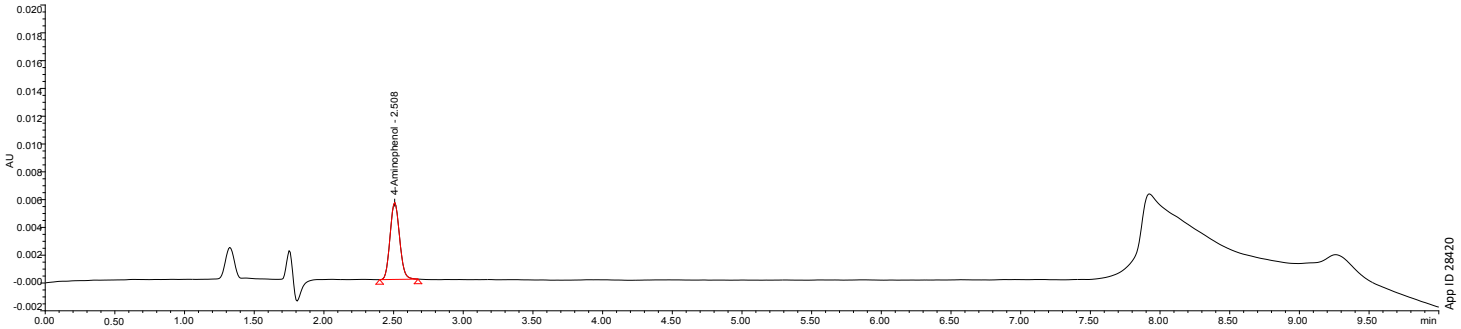


Table 5.

Peak No.	Name	Retention Time	Area	% Area	Height	s/n
1	4-Aminophenol	2.508	26413	100.00	5533	242

Figure 9. Overlayed chromatogram of diluent and System suitability solution on Kinetex EVO C18, 5 µm 150 x 4.6 mm 100 Å.

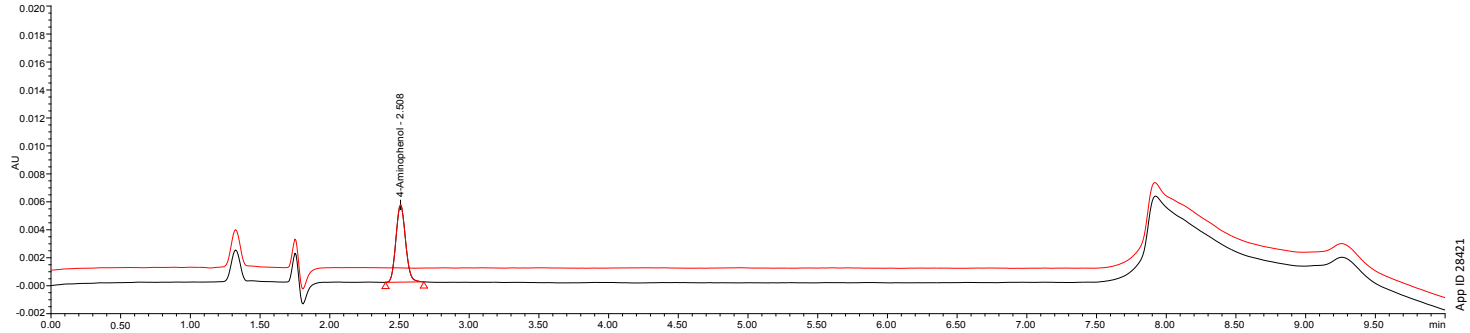


Figure 10. Standard solution on Kinetex EVO C18, 5 µm 150 x 4.6 mm 100 Å.

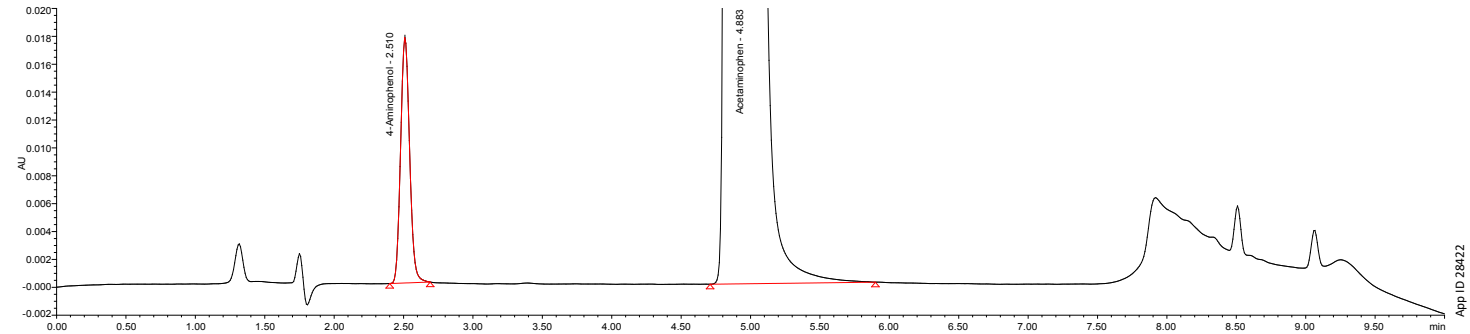


Table 6.

Peak No.	Name	Retention Time	Area	RT Ratio	Resolution	USP Tailing
1	4-Aminophenol	2.510	79607	0.514		1.1
2	Acetaminophen	4.883	6453330	1.000	13.0	1.9

Figure 11. Six replicate injections of Standard solution on Kinetex EVO C18, 5 µm 150 x 4.6 mm 100 Å.

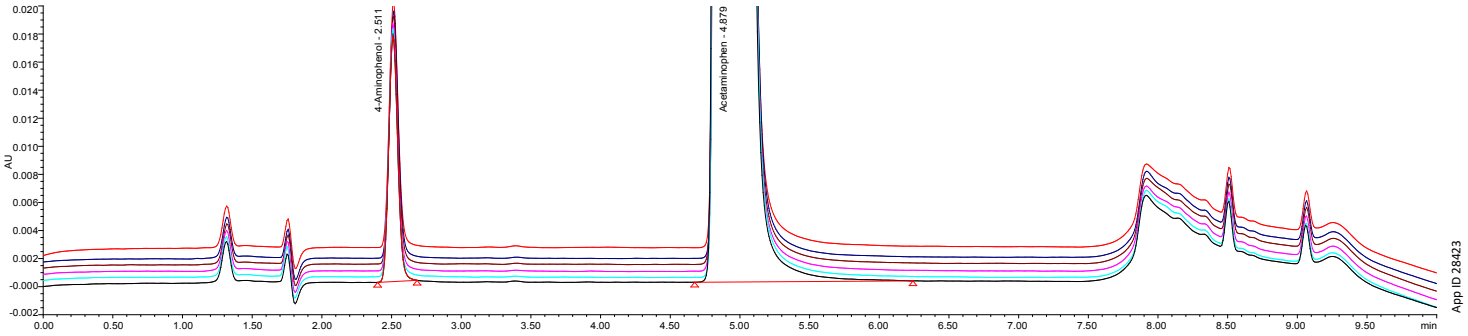


Table 7.

Injection	Retention Time	Area
Injection 1	2.510	79607
Injection 2	2.511	79491
Injection 3	2.514	79533
Injection 4	2.516	79451
Injection 5	2.511	79492
Injection 6	2.514	79338
Mean	2.513	79485
SD	0.002	89
%RSD	0.1	0.1

Figure 12. Sample solution on Kinetex EVO C18, 5 µm 150 x 4.6 mm 100 Å.

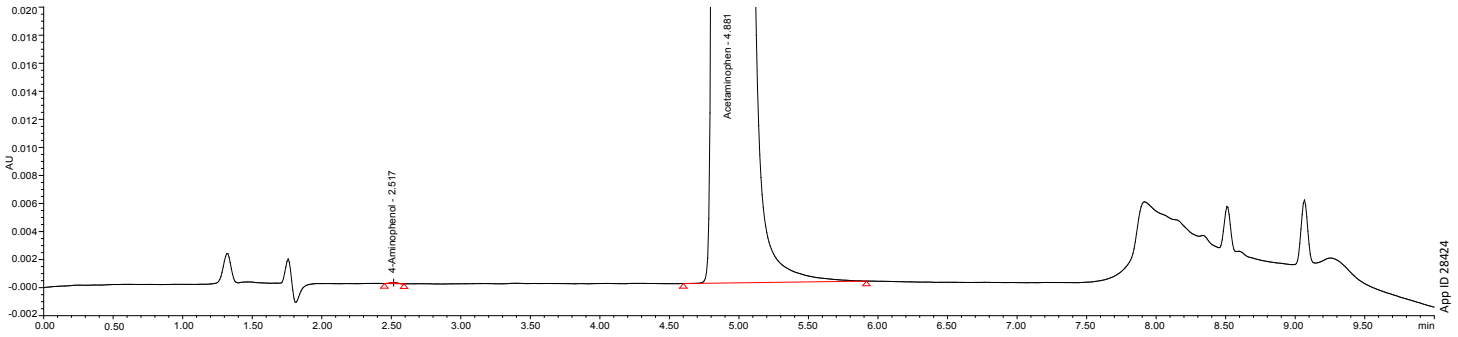


Table 7.

Peak No.	Name	Retention Time	Area	% Area	RT Ratio
1	4-Aminophenol	2.517	288	0.00	0.516
2	Acetaminophen	4.881	6250705	100.00	1.000

$$\%\ of\ 4-aminophenol = \left[\frac{r_U}{(r_S - r_U)}\right] \times \left(\frac{W_S}{W_U}\right) \times 100$$

$$= \left[\frac{r_U}{(r_S - r_U)}\right] \times \left(\frac{W_S}{W_U}\right) \times 100$$

$$= \left[\frac{288}{(79485 - 288)}\right] \times \left(\frac{0.007605}{5}\right) \times 100$$

$$= 0.001\ \%$$

Conclusions

The USP monograph 227 describes the method for analyzing 4-aminophenol in acetaminophen Drug Products. The monograph mentions the use of an L85 column. We have evaluated two L1 columns (Luna Omega PS C18 (5 µm, 150 x 4.6 mm) and Kinetex EVO C18 (5 µm, 150 x 4.6 mm).) to verify their suitability for the same monograph. Other method parameters were left unchanged.

The SST requirement of resolution NLT 1.0 between 4-aminophenol and the nearest peak in standard solution, the tailing factor NMT 1.5 for the 4-aminophenol peak in standard solution, the relative standard deviation of NMT 5.0 in standard solution and the signal-to-noise ratio NLT 20 for the 4-aminophenol peak in system suitability solution were all achieved utilizing both the Luna Omega PS C18 (5 µm, 150 x 4.6 mm) and the Kinetex EVO C18 (5 µm, 150 x 4.6 mm) columns.

This study demonstrates that both Luna Omega PS C18, 5 µm 150 x 4.6 mm and Kinetex EVO C18, 5 µm 150 x 4.6 mm columns meet the acceptance criteria for USP- 227: 4-Aminophenol in Acetaminophen- containing Drug Products and may be considered as an alternative to the recommended L85 phase column.

Luna™ Omega Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard™ Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0*/10pk**
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJ0-7601
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606
C18	00B-4785-E0	00D-4785-E0	00F-4785-E0	00G-4785-E0	AJ0-7612

Kinetex™ Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**
EVO C18	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJ0-9296
F5	00B-4724-E0	00D-4724-E0	00F-4724-E0	00G-4724-E0	AJ0-9320
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207
XB-C18	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJ0-8768
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
C8	00B-4608-E0	00D-4608-E0	00F-4608-E0	00G-4608-E0	AJ0-8770
Phenyl-Hexyl	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774
HILIC	-	00D-4606-E0	00F-4606-E0	00G-4606-E0	AJ0-8772

*SecurityGuard Analytical Cartridges require holder, Part No.:KJ0-4282
‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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/Chat.

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 3952 5747
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 51 02 180
pl-info@phenomenex.com

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

Singapore
t: 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
www.phenomenex.com/chat

www.phenomenex.com

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