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Comparing Kinetex™ Biphenyl and Luna™ Omega Polar C18 Columns for Analysis of an 11 Analyte Anticonvulsant Panel

Shahana Wahab Huq, Stephanie Marin, PhD, and Bryan Tackett, PhD
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods are considered the gold standard for therapeutic drug monitoring (TDM) of medications. LC-MS/MS provides several advantages for TDM, including high sensitivity, selectivity, and the ability to analyze multiple drugs in a single sample. Typically, serum or whole blood is used as the standard matrix for therapeutic drug monitoring, with serum preferred due to its ease of handling compared to whole blood. However, serum is a complex biological matrix containing various endogenous compounds that can interfere with analysis. These matrix effects can affect the accuracy and precision of drug concentration measurements, often requiring the use of complex sample preparation methods.

In this technical note, we demonstrate a fast and accurate method for the LC-MS/MS analysis of 11 Anticonvulsants using a Strata™-X Method Development 96-well plate that is packed with 4 different polymeric Solid Phase Extraction (SPE) sorbents. This is combined with a fast LC method using a Kinetex 2.6 µm Biphenyl LC column and a Luna Omega 3 µm Polar C18 LC column to resolve all target analytes.

Sample Preparation

A 1 ng/mL standard mix was used for initial LC-MS/MS analysis. A more detailed explanation of the use of the Strata-X Method Development 96-well plate (Part No.: KS0-8209) to determine the best sample extraction protocol for the Anticonvulsants drug class can be found in TN-0163. Briefly, 500 µL of human serum was spiked with an Anticonvulsants standard mix (1 ng/mL) and extracted using the Strata-X Method Development plate under Neutral, Basic, or Acid loading buffer, with subsequent Neutral, Basic, or Acidic elution. The best results were obtained using the Strata-X-CW sorbent chemistry and the Acid load/Basic elution extraction conditions. After dry-down, samples were reconstituted in 500 µL of initial mobile phase and spiked with 5 ng/mL internal standards. 5 µL of sample was injected onto columns for analysis.

LC Conditions

Column:	Kinetex 2.6 µm Biphenyl	Luna Omega 3.0 µm Polar C18
Dimensions:	50 x 3.0 mm	50 x 3.0 mm
Part No.:	00B-4622-Y0	00B-4760-Y0
Mobile Phase:	A: 0.1 % Formic acid in Water B: 0.1 % Formic acid in Methanol	A: 2 mM Ammonium Acetate B: 2 mM Ammonium Acetate in Methanol

Gradient:	**Time (min)**	**% B**	**Time (min)**	**% B**
	0	20	0	20
	1.5	80	0.5	20
	3	80	1.5	40
	3.1	95	2.5	80
	3.5	95	3	95
	3.51	20	3.5	95
	5	20	3.51	20
			5	20

Flow Rate:	0.5 mL/min	0.8 mL/min
Injection Volume:	5 µL	
Temperature:	40° C	
LC System:	Agilent® 1260 Infinity	
Detection:	MS/MS	
Detector:	SCIEX® 6500 Triple Quad™	

MS/MS Conditions

Ion Source:	ESI
Polarity:	Positive/Negative
Source Temperature:	450° C
GS1:	55 psi
GS2:	60 psi
CUR:	35 psi
IS:	+2500 V or -2500V

Table 1. MS Transitions.

Analyte	Q1 Mass (Da)	Q3 Mass (Da)
Pregabalin	160.1	55
Gabapentin	172	137
Levetiracetam	171	126.1
Lamotrigine	256.1	211.1
Felbamate	178.1	117.1
Lacosemide	251.1	108
Zonisamide	212.9	132
Topiramate	338	78
Oxcarbazapine	253	236
Carbamazepine Epoxide	253.1	180.1
Carbamazepine	237.1	194.1

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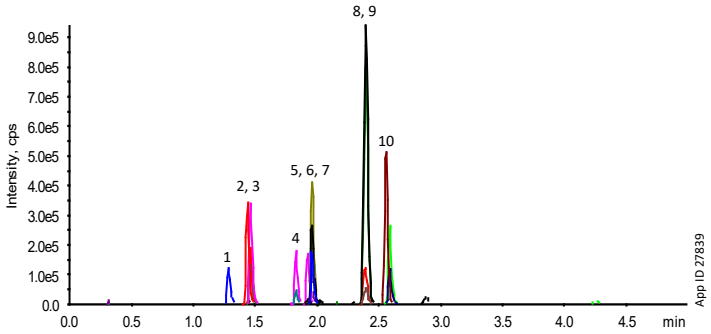
Results and Discussion

A mix of Anticonvulsants standards was analyzed with the Kinetex™ 2.6 µm Biphenyl column showing good peak shape and separation in both positive (**Figure 1a**) and negative mode (**Figure 1b**). However, the isobaric analytes Oxcarbazapine and Carbazepine Epoxide failed to show complete separation (**Figure 1c**). Because of the lack of separation of this isobaric pair, spiked serum was not analyzed on the Kinetex 2.6 µm Biphenyl column.

The Antipsychotics standards mix was also analyzed with the Luna™ Omega 3 µm Polar C18 column. Analytes were separated with good peak shape (**Figures 2a** and **2b**) and demonstrated great selectivity towards the separation of the critical isobaric pair of Oxcarbazapine and Carbazepine Epoxide (**Figure 2c**). To evaluate the chromatography from a matrix-matched sample, 11 Anticonvulsants were spiked into serum and extracted using the Strata™-X Method Development Plate before being analyzed on a Luna Omega 3 µm Polar C18 column. The Strata-X-C sorbent with the acidic load and basic elution solvents were found to be the most successful extraction method for these analytes. For more information on the SPE protocol, please see TN-0163. The same degree of separation of analytes was observed between the extracted serum sample and the neat standard (**Figures 3a**, **3b**, and **3c**).

Figure 1a. Analysis of Anticonvulsants Standards on a Kinetex 2.6 µm Biphenyl Column, Positive Polarity.



Peak No.	Analyte	Retention Time (min)
1	Pregabalin	1.3
2	Gabapentin	1.4
3	Levetiracetam	1.4
4	Lamotrigine	1.8
5	Felbamate	1.9
6	Lacosemide	1.9
7	Zonisamide	1.9
8	Oxcarbazapine	2.4
9	Carbamazepine Epoxide	2.4
10	Carbamazepine	2.6

Figure 1b. Analysis of Anticonvulsant Standard Topiramate on a Kinetex 2.6 µm Biphenyl Column, Negative Polarity.

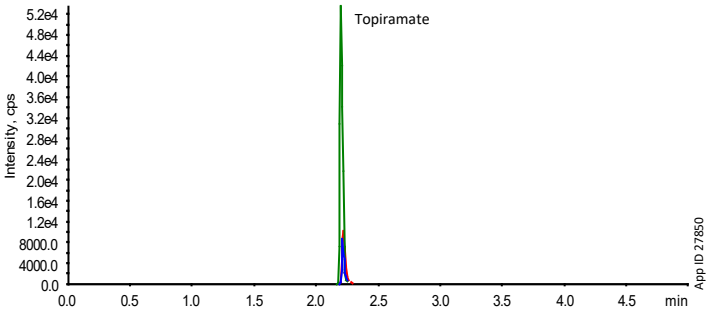


Figure 1c. Incomplete Separation of Isobaric Oxcarbazapine and Carbazepine Epoxide Anticonvulsants on a Kinetex 2.6 µm Biphenyl Column.

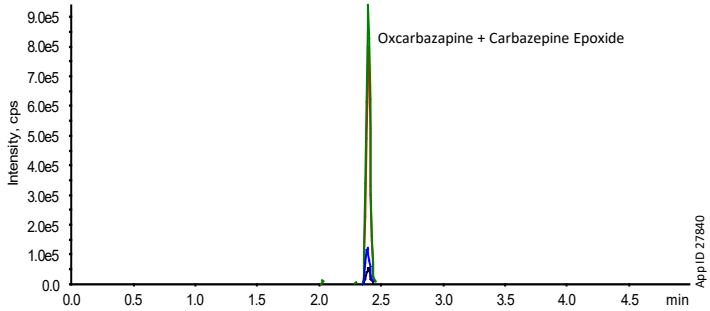
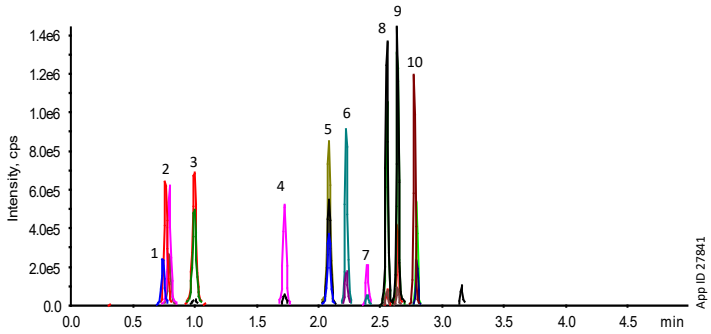


Figure 2a. Analysis of Anticonvulsants Standards on a Luna™ Omega 3 µm Polar C18 Column, Positive Polarity.



Peak No.	Analyte	Retention Time (min)
1	Pregabalin	0.7
2	Gabapentin	0.8
3	Levetiracetam	1.0
4	Zonisamide	1.7
5	Lacosemide	2.1
6	Felbamate	2.2
7	Lamotrigine	2.4
8	Carbamazepine Epoxide	2.5
9	Oxcarbazapine	2.6
10	Carbamazepine	2.8

Figure 2b. Analysis of Anticonvulsant Standard Topiramate on a Luna Omega 3 µm Polar C18 Column, Negative Polarity.

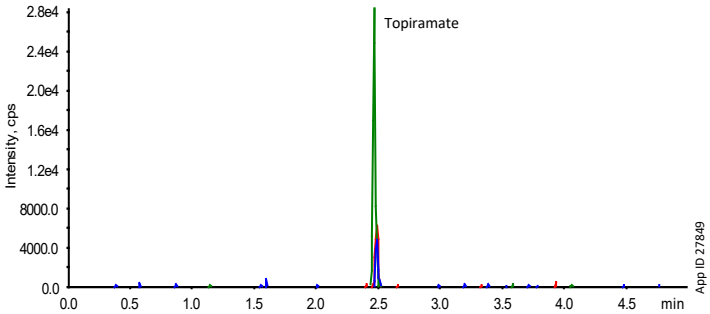


Figure 2c. Separation of Isobaric Oxcarbazapine and Carbazepine Epoxide Anticonvulsants on a Luna Omega 3 µm Polar C18 Column.

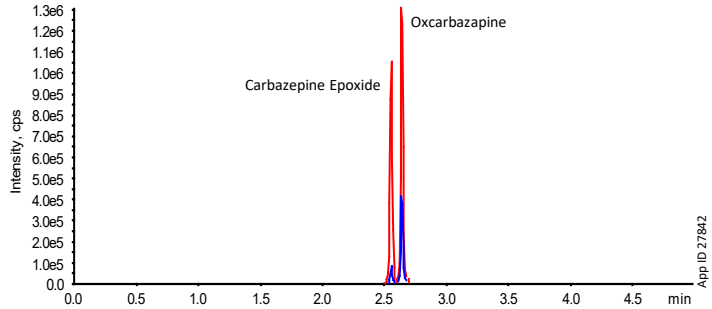
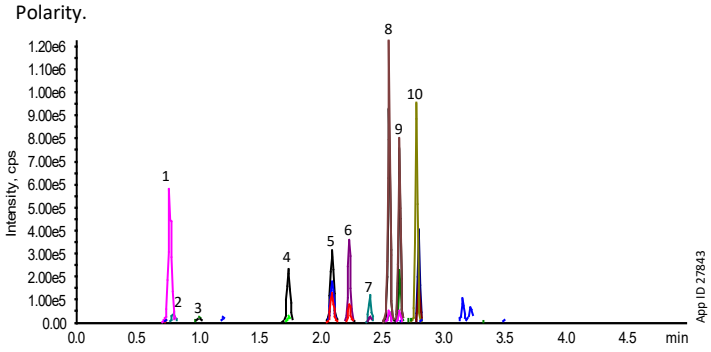


Figure 3a. Analysis of Anticonvulsants Extracted from Serum Using Strata™-X-C, Under Acidic Load and Basic Elution, on a Luna Omega 3 µm Polar C18 Column, Positive Polarity.



Peak No.	Analyte	Retention Time (min)
1	Pregabalin	0.7
2	Gabapentin	0.8
3	Levetiracetam	1.0
4	Zonisamide	1.7
5	Lacosemide	2.1
6	Felbamate	2.2
7	Lamotrigine	2.4
8	Carbamazepine Epoxide	2.5
9	Oxcarbazapine	2.6
10	Carbamazepine	2.8

Figure 3b. Analysis of Anticonvulsant Topiramate Extracted from Serum Using Strata-X-C, Under Acidic Load and Basic Elution, on a Luna Omega 3 µm Polar C18 Column, Negative Polarity.

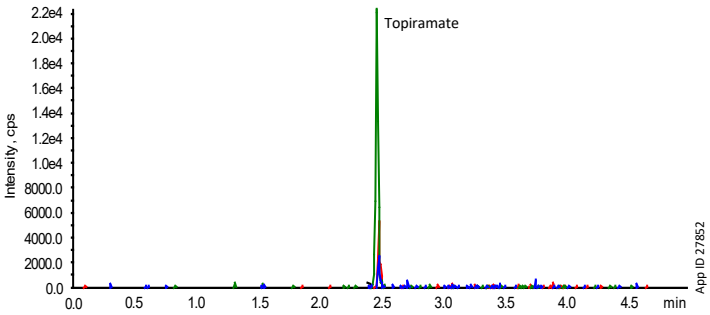
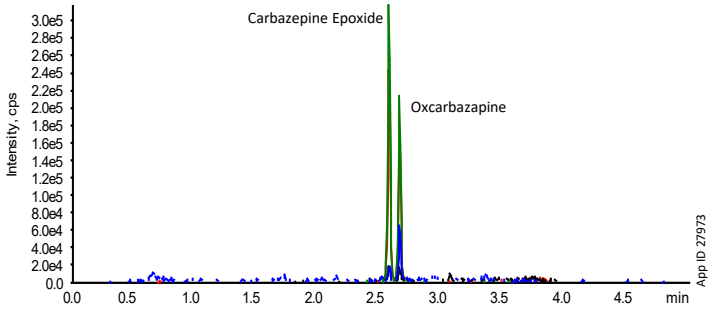


Figure 3c. Separation of Isobaric Oxcarbazapine and Carbazepine Epoxide Anticonvulsants Extracted from Serum Using Strata-X-C, Under Acidic Load and Basic Elution, on a Luna Omega 3 µm Polar C18 Column.



Have questions or want more details on implementing this method? We would love to help!
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Conclusions

The developed LC-MS/MS method utilizing the Luna™ Omega 3 µm Polar C18 column has been found to be the optimal chromatography conditions for analysis of the TDM panel of Anticonvulsants. The Method Development plate failed to identify a single optimal protocol for extraction of the Anticonvulsants from serum. Further work is needed to develop an SPE method. A Kinetex™ 2.6 µm Biphenyl column was compared to the Luna Omega 3 µm Polar C18 column but was unable to separate the isobaric pair of Oxcarbazapine and Carbazepine Epoxide. This suggests that the Luna Omega column is a better option for separating Anticonvulsant analytes when compared to the Kinetex Biphenyl column.

SPE Ordering Information

Strata™-X Method Development 96-Well Plate

Part No.	Description	Unit
KS0-8209	Strata-X, -X-C, -X-CW, and -X-AW 30 mg/well each	ea

Kinetex Ordering Information

2.6 µm Midbore™ Columns (mm)						SecurityGuard™ ULTRA Cartridges (mm)‡
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
EVO C18	00A-4725-Y0	00B-4725-Y0	—	00D-4725-Y0	00F-4725-Y0	AJ0-9297
PS C18	00A-4780-Y0	00B-4780-Y0	—	00D-4780-Y0	00F-4780-Y0	AJ0-8950
Polar C18	—	00B-4759-Y0	—	00D-4759-Y0	00F-4759-Y0	AJ0-9531
Biphenyl	—	00B-4622-Y0	—	00D-4622-Y0	00F-4622-Y0	AJ0-9208
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJ0-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	00F-4497-Y0	AJ0-8777
HILIC	00A-4461-Y0	—	—	00D-4461-Y0	00F-4461-Y0	AJ0-8779
Phenyl-Hexyl	—	00B-4495-Y0	—	00D-4495-Y0	00F-4495-Y0	AJ0-8781
F5	—	00B-4723-Y0	—	00D-4723-Y0	00F-4723-Y0	AJ0-9321

for 3.0 mm ID

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

Luna Omega Ordering Information

3 µm MidBore Columns (mm)				SecurityGuard Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*/10pk
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJ0-7605
C18	00B-4784-Y0	00D-4784-Y0	00F-4784-Y0	AJ0-7611
SUGAR	—	—	00F-4775-Y0	AJ0-4496

for ID: 2.0 – 3.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

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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

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
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