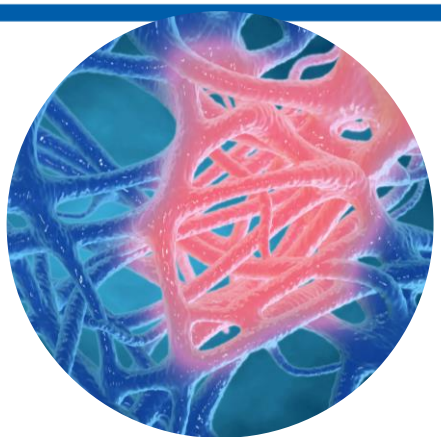


TN-1308

Micro-flow Lipidomics for Characterization of Lipid Mediators at Trace Levels Using a Kinetex™ XB-C18 Column

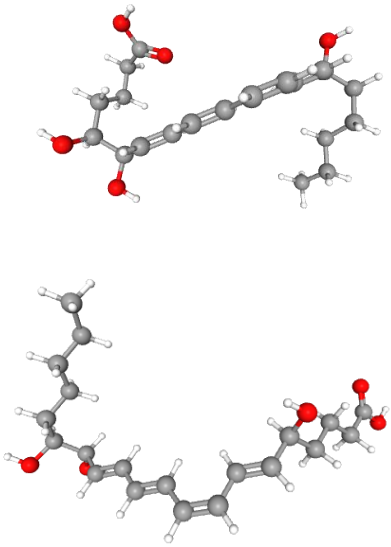
Roxana Eggleston-Rangel, Jason Anspach, PhD, Namrata Saxena, and Bryan Tackett, PhD
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

The initial signs of inflammation are triggered by specialized eicosanoids, such as prostaglandins and leukotrienes, which in turn stimulate recruitment of neutrophils. Arachidonic acid (AA) derived lipoxins (LX), and E & D-series resolvins (RvD) regulate the migration of immune cells, are responsible for the release of cytokines and are also involved in antibody generation. As a result of years of research involving metabolomics and lipidomics, it is now recognized that a novel class of lipid mediators, derived mainly from dietary polyunsaturated fatty acids (PUFAs), are involved in signal transduction pathways that are crucial for the regulation and termination of inflammation. These mediators are conjointly called as Specialized Pro-resolving lipid Mediators (SPMs), which include classes of compounds such as "resolvins," "lipoxins," "maresins," and "protectins."

LC-MS is commonly used to detect and quantify such lipid mediators. However, their detection is challenging due to their physical properties and their bioactive endogenous nanogram to picogram concentrations. In this technical note, we provide a possible solution to these known challenges by using a unique reversed phase core-shell based column chemistry in a miniaturized column format (micro).

Figure 1. Structures of Positional Isomers Lipoxin A4 (Top) and Lipoxin B4 (Bottom).



LC Conditions

Column: Kinetex 2.6 µm XB-C18
Luna™ Omega 3 µm Polar C18

Dimension: 150 x 0.3 mm

Part No.: 00F-4496-AC (XB-C18)
00F-4760-AC (Polar C18)

Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

Gradient:

Time (min)	%B
0	45
2	53
16.5	80
16.6	98
18.5	98
20.5	10
40	10

Flow Rate: 10 µL/min

Injection: 1 µL

Temperature: 40 °C

Detector: Q Exactive™ Plus Orbitrap™

System: NanoLC™ 425 (SCIEX®)

Detection: MS

MS Conditions

Scan Type: Full MS SIM

Polarity: Negative

Resolution: 140,000

Scan Range: 250 to 500 m/z

AGC Target: 3e6

Maximum IT: 200 ms

Results and Discussion

Typically, lipids are quite challenging to separate via liquid chromatography due to variations in their geometries, fatty acyl chains, linkages, modifications, and existence of isomeric and isobaric species. A panel of 35 lipid mediators from 5 different lipid mediator classes (**Table 1**), were assayed on a Kinetex™ 2.6 µm XB-C18 column to demonstrate the chromatographic separation performance of the core-shell particle.

The Kinetex XB-C18 stationary phase employs core-shell technology that reduces the analyte's diffusion path, thus, delivers narrower peaks and in turn, significantly increases and improves the ion intensities for the analytes. This stationary phase also has protective Isobutyl side chains which reduce secondary interactions between analyte molecules and the residual silanol groups present on the stationary phase, thus giving improved peak shapes.

Figure 2 shows the Extracted Ion Chromatogram (XIC) for the 3 classes of lipid mediators on the Kinetex XB-C18 column. To better understand the effect and benefit of core-shell particles, peak widths and peak capacities were calculated. As expected, the Kinetex XB-C18 core-shell column delivered narrower peak widths, increased peak capacities, and peak ion intensities that assists in achieving the trace detection levels of picogram concentrations, as compared to the fully porous Luna™ Omega 3 µm Polar C18 column (**Table 2 & Figure 3**).

Interestingly, the Kinetex XB-C18 column chemistry provided resolution of Lipoxins LXA_4 and LXB_4, as well as Prostaglandins PGD_2 and PGE_2 (**Figure 4**). These two groups of lipid mediators belonging to the Lipoxin and COX & LOX lipid family, respectively, and share the same molecular formula and m/z making it difficult to separate by mass spectrometry alone. By using core-shell XB-C18 chemistry, these isobaric compounds were fully resolved.

Table 1. Lipid Mediator Analytes Used and their Respective M-H Species.

Lipid Type	Analyte - m/z
Polyunsaturated Fatty Acids	Arachidonic Acid - 303.2330[M-H]
	Dihomo-Ω-Linolenic Acid - 305.2486[M-H]
	Docosahexaenoic Acid - 327.2330[M-H]
	Docosapentaenoic Acid - 329.2486[M-H]
	Eicosapentaenoic Acid - 301.2173[M-H]
	Linoleic Acid - 279.2330[M-H]
	α-Linolenic Acid - 279.2330[M-H]
	Ω-Linolenic Acid - 277.2173[M-H]
	Stearidonic Acid - 275.2017[M-H]
COX & LOX	6-keto Prostaglandin F1a - 369.2283[M-H]
	Thromboxane B2 - 369.2283[M-H]
	Prostaglandin F2a - 353.2333[M-H]
	Prostaglandin E2 - 351.2177[M-H]
	Prostaglandin D2 - 351.2177[M-H]
	12(S)-HHTrE - 279.1966[M-H]
	15(S)-HETE - 319.2279[M-H]
	12(S)-HETE - 319.2279[M-H]
	5(S)-HETE - 319.2279[M-H]
SPM-E	Resolvin E1 - 349.2020[M-H]
	(±)18-HEPE - 317.2122[M-H]
	Eicosapentaenoic Acid - 301.2173[M-H]
Lipoxin	LXB4 - 351.2177[M-H]
	LXA4 - 351.2177[M-H]
	15(R)-Lipoxin A4 - 351.2177[M-H]
	Arachidonic Acid - 303.2330[M-H]
SPM-D	Resolvin D3 - 375.2177[M-H]
	17(R)-Resolvin D1 - 375.2177[M-H]
	Resolvin D1 - 375.2177[M-H]
	Resolvin D5 - 359.2228[M-H]
	Docosahexaenoic Acid - 327.2330[M-H]
Labeled Standards	(±)5(6)-EET - 319.2279[M-H]
	(±)11,12-EpETrE - 319.2279[M-H]
	(±)14,15-EET - 319.2279[M-H]
	(±)11,12-EET-d11 - 330.2969[M11D-H] (heavy)
	PGE2-d4 - 355.2428[M4D-H] (heavy)
	LTB4-d4 - 339.2479[M4D-H] (heavy)
	RvE1-d4 - 353.2272[M4D-H] (heavy)
	Lipoxin A4-d5 - 356.2491[M5D-H] (heavy)

Figure 2. Extracted Ion Chromatogram (XIC) of 35 Lipids Lipid Mediators and their Metabolites on a Kinetex 2.6 µm XB-C18 column.

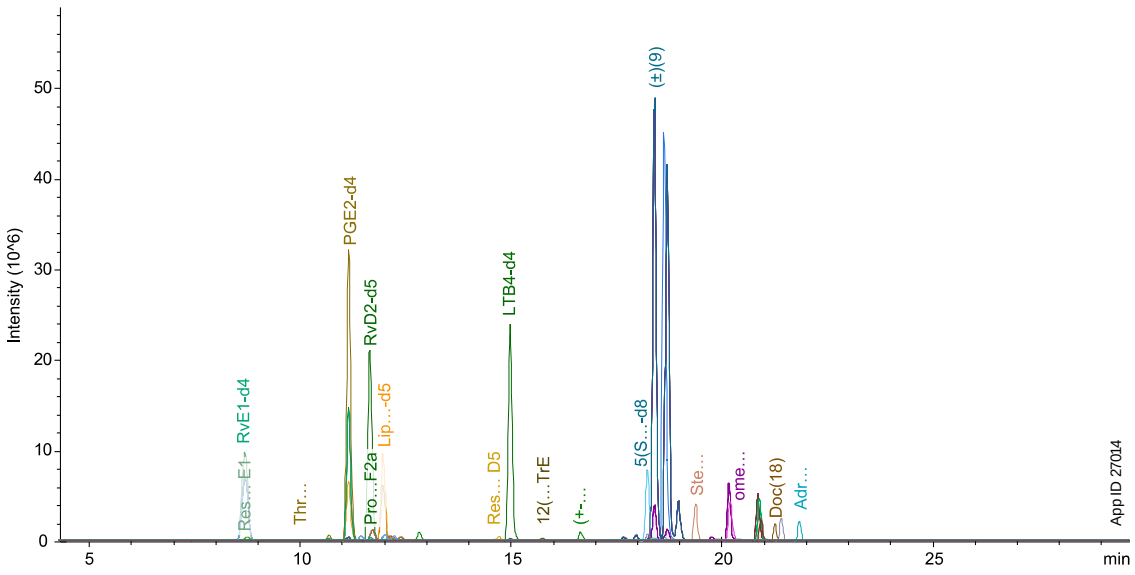


Table 2. Average Peak Widths and Peak Capacities for 35 Lipid Molecules.

Column	Avg Peak Width	Peak Capacity
Kinetex 2.6 µm XB-C18	0.35	39
Luna Omega 3 µm Polar C18	0.37	34

Figure 3. Ion Intensity of 8 Lipid Mediators from the COX & LOX Family when Injecting 1µL of Sample.

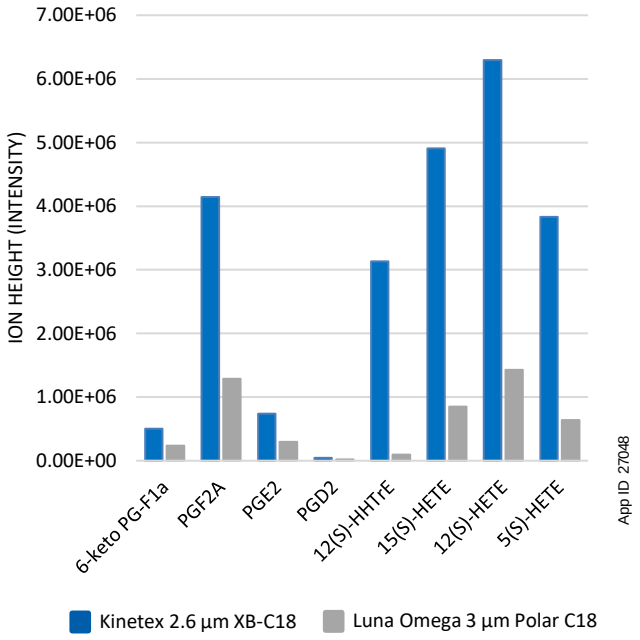
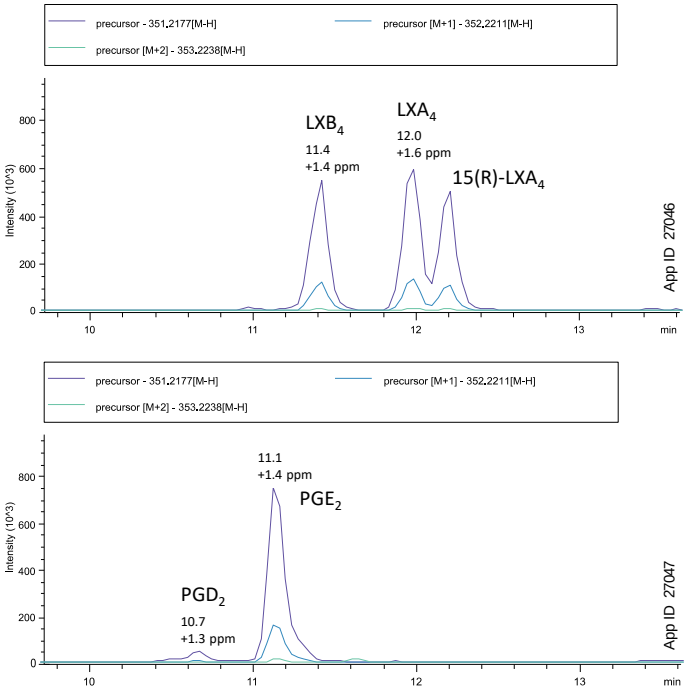


Figure 4. XIC of 5 Resolved Isobaric Species PGE_2, PGD_2, LXB_4, 15(R)-LXA_4, and LXA_4. Respective Retention Times and Mass Errors are shown.



Conclusions

The selected core-shell column chemistry of the Kinetex XB-C18, with its ability to reduce analyte diffusion path and silanol activity, resulted in improved peak shapes and significantly increased intensities at low picogram concentration levels. Additionally, its ability to fully resolve lipid isobaric species makes Kinetex XB-C18 an appropriate selection for analyte characterization for lipidomics studies.

Kinetex™ Micro LC Columns Ordering Information

2.6 µm Micro LC Columns (mm)						
Phases	**30 x 0.3**	**50 x 0.3**	**100 x 0.3**	**150 x 0.3**	**50 x 0.5**	**150 x 0.5**
XB-C18	00A-4496-AC	00B-4496-AC	00D-4496-AC	00F-4496-AC	00B-4496-AF	00F-4496-AF
Biphenyl	—	00B-4622-AC	—	00F-4622-AC	00B-4622-AF	—
C18	00A-4462-AC	00B-4462-AC	—	00F-4462-AC	00B-4462-AF	—
EVO C18	—	00B-4725-AC	—	00F-4725-AC	00B-4725-AF	—
F5	—	00B-4723-AC	00D-4723-AC	00F-4723-AC	00B-4723-AF	—

Luna ™ Omega Micro LC Columns Ordering Information

3 µm Micro LC Columns (mm)							Trap Column
Phases	**50 x 0.30**	**100 x 0.30**	**150 x 0.30**	**50 x 0.50**	**100 x 0.50**	**150 x 0.50**	**20 x 0.3**
Polar C18	00B-4760-AC	00D-4760-AC	00F-4760-AC	00B-4760-AF	00D-4760-AF	00F-4760-AF	—
PS C18	00B-4758-AC	00D-4758-AC	00F-4758-AC	00B-4758-AF	00D-4758-AF	00F-4758-AF	05M-4758-AC

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 5019 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 6559 4364
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
www.phenomenex.com/chat

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

TN45730322_W

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*