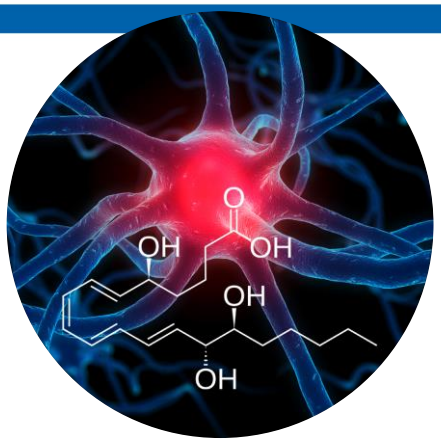

TN-1309

Nano-flow Lipidomics for Characterization of Lipid Mediators at Trace Levels Using a Biozen™ Peptide XB-C18 Column

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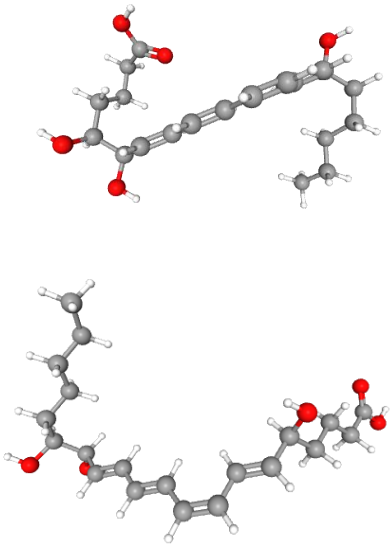
Introduction

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) that 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 (nano).

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



LC Conditions

Column: Biozen 2.6 µm Peptide XB-C18
Biozen 3 µm Peptide Polar C18

Dimension: 250 x 0.075 mm

Part No.: 00G-4768-AW-21 (XB-C18)
00G-4782-AW-21 (Polar C18)

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

Gradient:

Time (min)	%B
0.00	45
2.00	45
16.5	80
16.6	98
18.5	98
20.5	10
30.0	10

Flow Rate: 350 nL/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 Biozen™ 2.6 µm Peptide XB-C18 column to demonstrate the chromatographic separation performance of the core-shell particle.

The Biozen Peptide 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 5 classes of lipid mediators on the Biozen Peptide XB-C18 column. To better understand the effect and benefit of core-shell particle, peak widths and peak capacities were calculated. As expected, the Biozen Peptide 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 nanogram and picogram concentrations, as compared to the fully porous Luna™ Omega 3 µm Polar C18 column (**Table 2 & Figure 3**).

Interestingly, the Biozen Peptide XB-C18 column chemistry provided resolution of isobaric species of 3 Eicosanoids ((15)-S-HETE, (12)-S-HETE and 5(S)-HETE) as well as Lipoxins LXA_4 and LXB_4 (**Figures 4** and **5**). 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 Biozen 2.6 µm Peptide XB-C18 column.

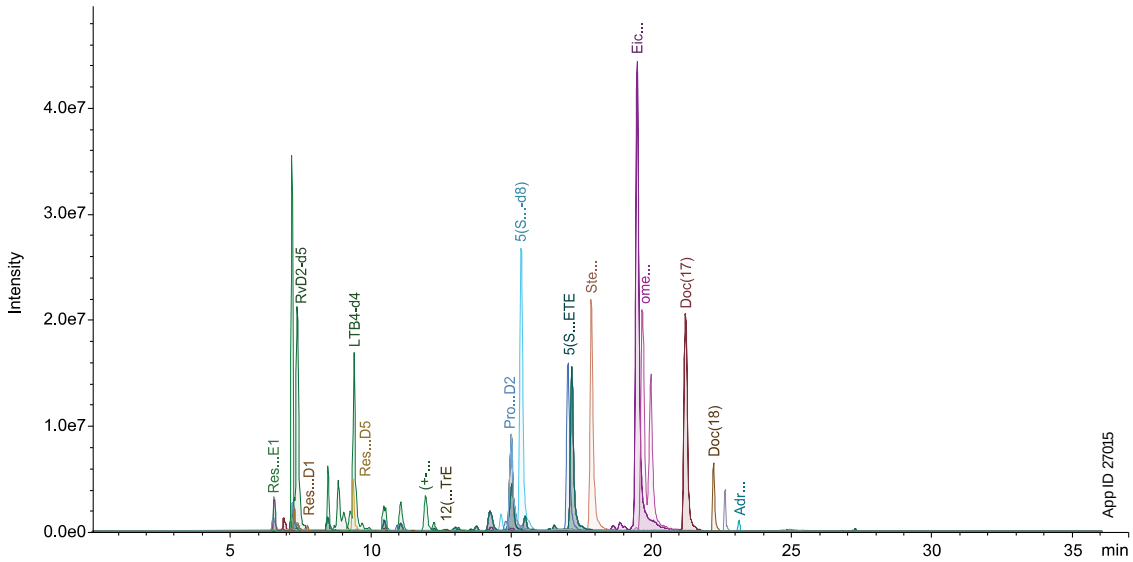


Table 2. Average Peak Widths and Peak Capacities for 35 Lipid Molecules.

Column	Avg Peak Width	Peak Capacity
Biozen 2.6 µm Peptide XB-C18	0.43	39
Biozen 3 µm Peptide Polar C18	0.59	29

Figure 3. Ion Intensity of 10 Polyunsaturated Fatty Acids when Injecting 1 µL of Sample.

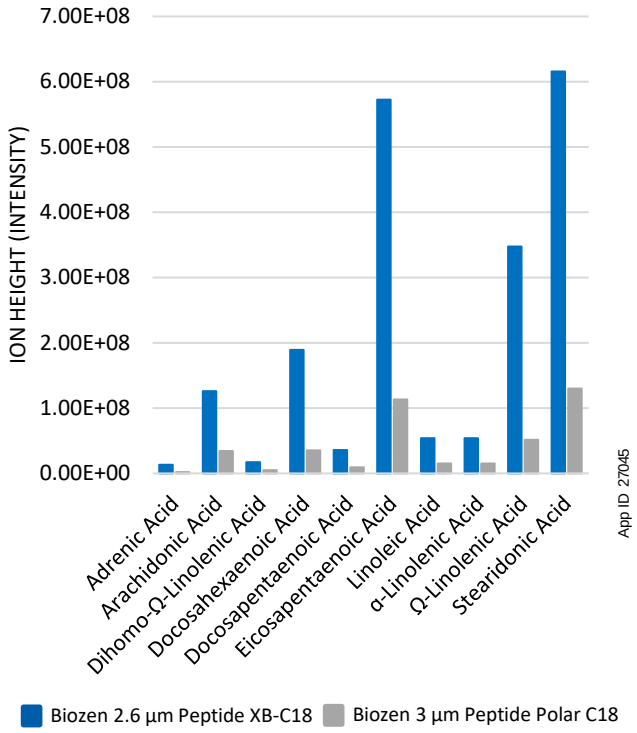


Figure 4. XIC of Fully Resolved Isobaric Species 3 Eicosanoids: (15)-S-HETE (Left) , (12)-S-HETE (Middle) and (5(S)-HETE (Right) on a Biozen 2.6 µm Peptide XB-C18 Column. Retention Times and Mass Errors are Shown.

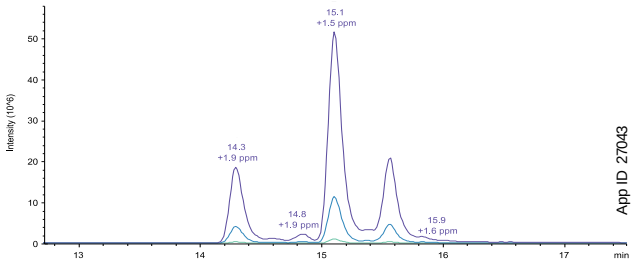
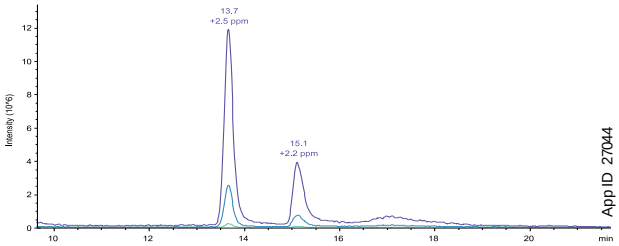


Figure 5. XIC of Two Lipoxin Isobaric Species, LXB_4 (Left) and LXA_4 (Right) on a Biozen 2.6 µm Peptide XB-C18 Column. Respective Retention Times and Mass Errors are shown.



Conclusions

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

Biozen™ Nano LC Columns with Integrated SecurityLINK™ Fitting

1.6 µm Minibore Columns (mm)			
Phases	**150 x 0.075 mm**	**250 x 0.075 mm**	**500 x 0.075 mm**
Biozen 3 µm Peptide PS C18	00F-4771-AW-21	00G-4771-AW-21	–
Biozen 2.6 µm Peptide XB-C18	00F-4768-AW-21	00G-4768-AW-21	–
Biozen 3 µm Polar C18	00F-4782-AW-21	00G-4782-AW-21	–
Biozen 5 µm Peptide XB-C18	–	–	00J-4792-AW-21

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.

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