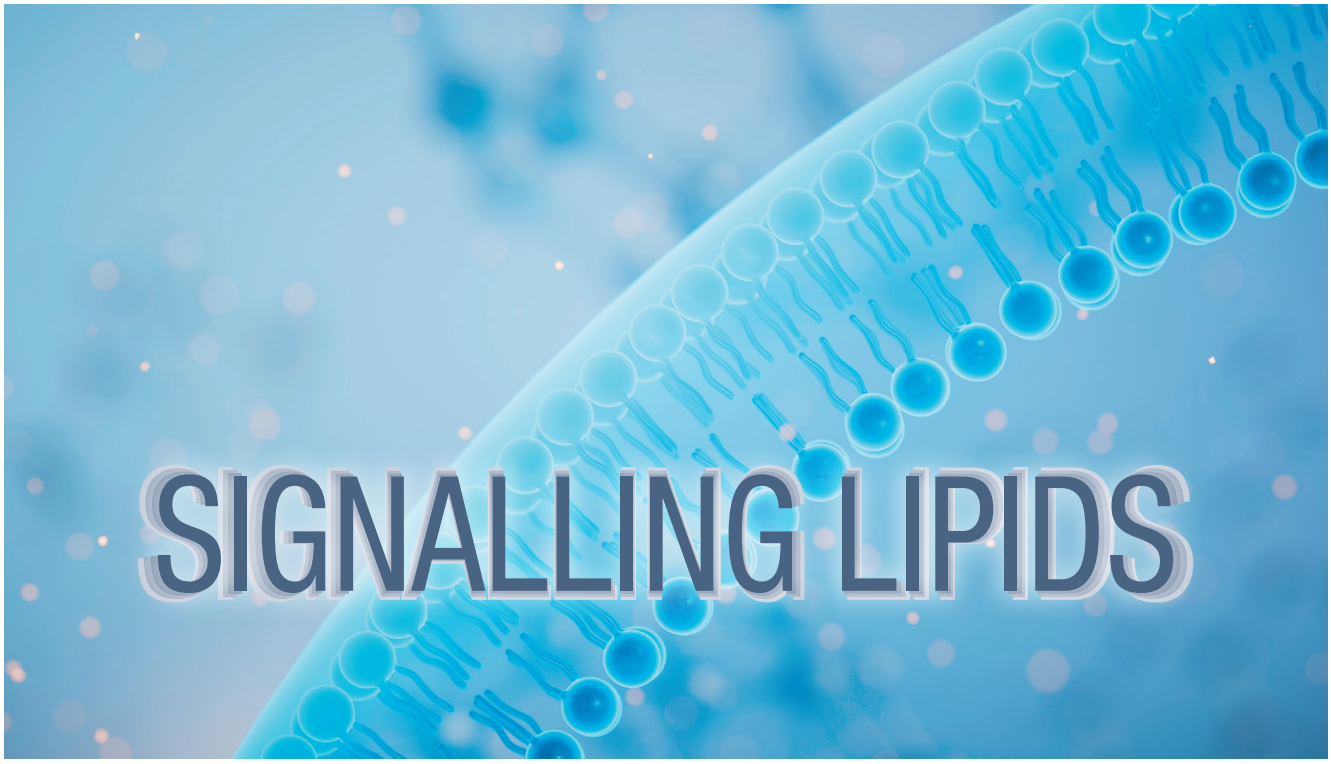
Comprehensive high resolution targeted LC-MS/MS analysis of challenging signalling lipids

Liquid chromatography coupled to mass spectrometry (LC-MS) became the gold standard for analysing signalling lipids. However, the analysis poses special challenges such as peak tailing and loss of recovery for phosphate group containing lipids.



This application note shows an optimised LC-MS/MS method covering 388 signalling lipids from 17 lipid classes. The game changer is the bioinert coated YMC Accura Triart C18 column, that combines high sensitivity for phosphate group containing lipids with high resolution, making it the first-choice column for signalling lipid analysis.

Table 1: Chromatographic conditions [1].

Columns:	YMC Accura Triart C18 (1.9 µm) 150 x 2.1 mm ID (bioinert coated) YMC-Triart C18 metal-free (1.9 µm) 150 x 2.1 mm ID (PEEK-lined) YMC-Triart C18 (1.9 µm) 150 x 2.1 mm ID (regular stainless-steel)
Part Nos.:	TA12SP9-15Q1PTC TA12SP9-15Q1PTP TA12SP9-15Q1PT
Eluents:	A) water/acetonitrile (80/20) containing 0.5 mM ammonium acetate and 0.2% acetic acid B) 2-propanol/acetonitrile/water (60/35/5) containing 0.5 mM ammonium acetate and 0.2% acetic acid
Gradient:	30%B (0–1 min), 30–100%B (1–11 min), 100%B (11–16 min), 30%B (16.1–20 min)
Flow rate:	0.4 mL/min
Temperature:	45 °C
Injection:	5 µL
Detection:	ESI-MS, negative
Sample:	Mixture of 17 lipid standards Extracted plasma and platelet samples

Table 2: List of considered signalling lipids [1].

Lipid class	Analyte	Abbreviation
Lysoglycerophospholipids	Lysophosphatidic acid 17:1	LPA 17:1
	Lysophosphatidylinositol 18:1	LPI 18:1
	Lysophosphatidylserine 18:1	LPS 18:1
	Lysophosphatidylglycerol 18:1	LPG 18:1
	Lysophosphatidic acid 18:1	LPA 18:1
	Cyclic glycerophosphatidic acid 18:1	CPA 18:1
	Lysophosphatidylcholine 18:1	LPC 18:1
	Lysophosphatidylethanolamine 18:1	LPE 18:1
	Lysophosphatidylinositol 13:0	LPI 13:0
Sphingolipids	Sphingosine-1-phosphate 18:1,O2	S1P 18:1;O2
	Ceramide phosphate 26:1;O2	CerP 26:1;O2
	Ceramide phosphate 30:1;O2	CerP 30:1;O2
Oxylipins & Fatty Acyls	Prostaglandin E2	PGE_2
	Prostaglandin D2	PGD_2
	Thromboxane B2-d4	TXB_2-d4
Glycerolipids	Diacylglycerol 33:1-d7	DG 33:1-d7

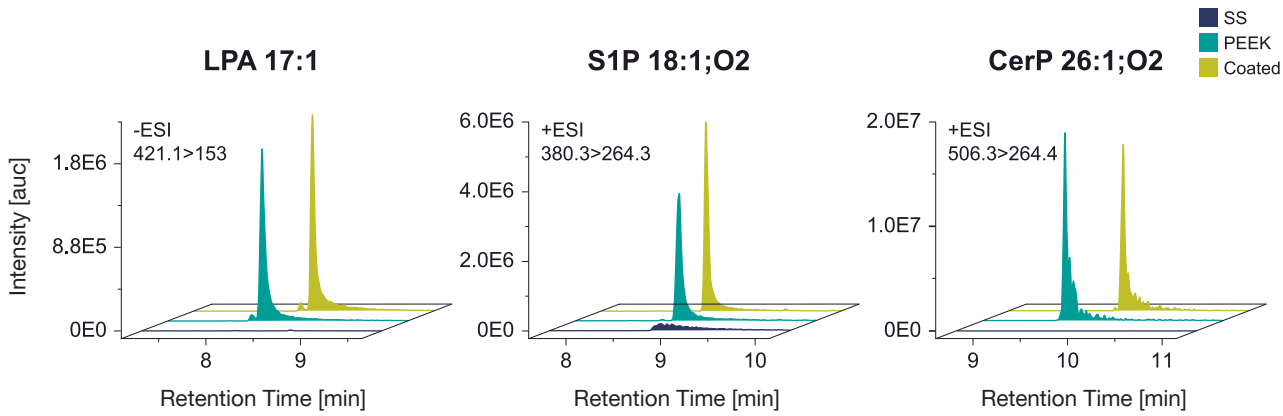


Figure 1: Comparison of the analysis of challenging signalling lipids using regular stainless-steel column hardware (SS), PEEK-lined (PEEK) and bioinert coated (Coated) columns. As eluent additive 0.1% acetic acid is used [1].

Figure 1 shows the analysis of phosphate group containing and therefore challenging lipids. These lipids tend to interact with metal surfaces and as a result show strong peak tailing and low to no recovery when using stainless-steel hardware. Furthermore, strong carry-over can be observed. Using bioinert column hardware such as the bioinert coated YMC Accura Triart C18 or the PEEK-lined YMC-Triart C18 metal-free column improves recovery and peak shape. The comparison of the intensities shows the supremacy of the bioinert coated YMC Accura Triart C18 column for most lipids. An optimisation of the eluent to 0.5 mM ammonium acetate and 0.2% acetic acid as additive led to highly improved peak shapes (see Figure 2).

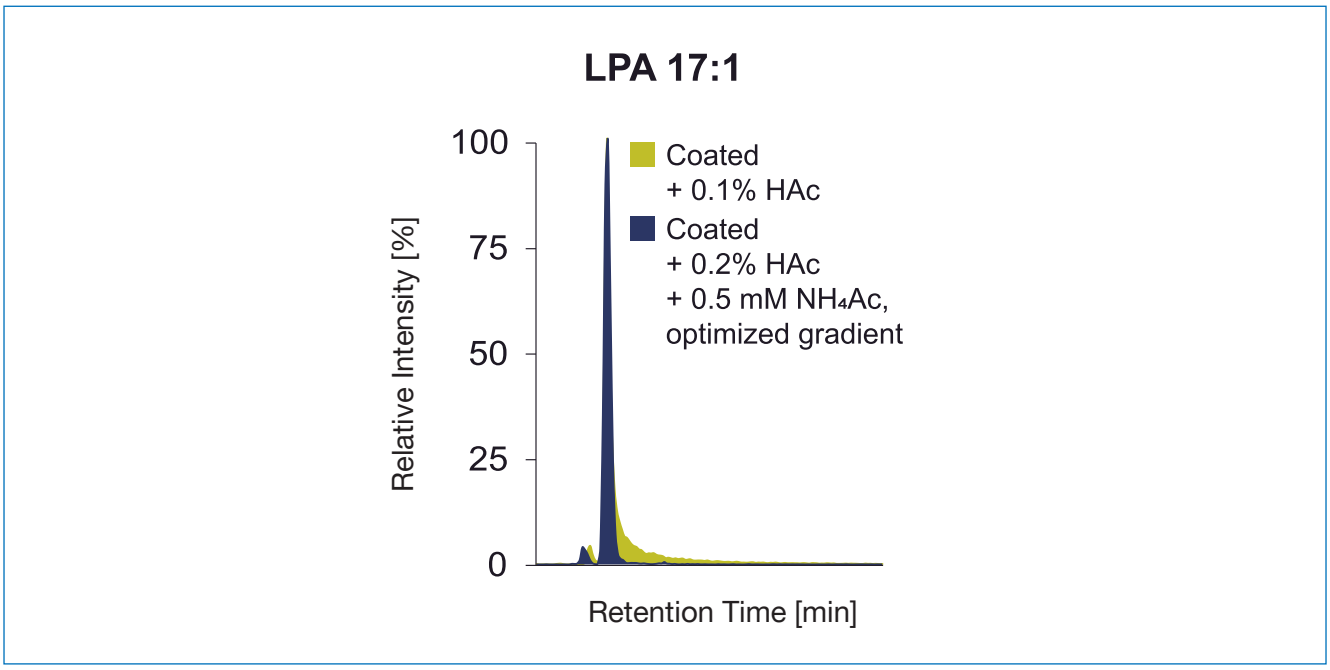


Figure 2: The analysis of LPA 17:1 using the bioinert coated YMC Accura Triart C18 column and optimised eluents provides ideal peak shapes [1].

Having overcome the chromatographic challenges, in-source fragmentation and isomeric species pose further difficulties. Therefore, separating these critical analytes is crucial.

The isomeric species PGE_2 and PGD_2 represent such a critical peak pair as they do not have unique MS/MS fragments.

Figure 3 shows the separation of this peak pair using both bioinert column hardware types. Higher intensities, as well as sharper peaks and thus higher resolution, are provided by the bioinert coated YMC Accura C18 column. Therefore, YMC Accura Triart C18 is the column of choice for this analysis.

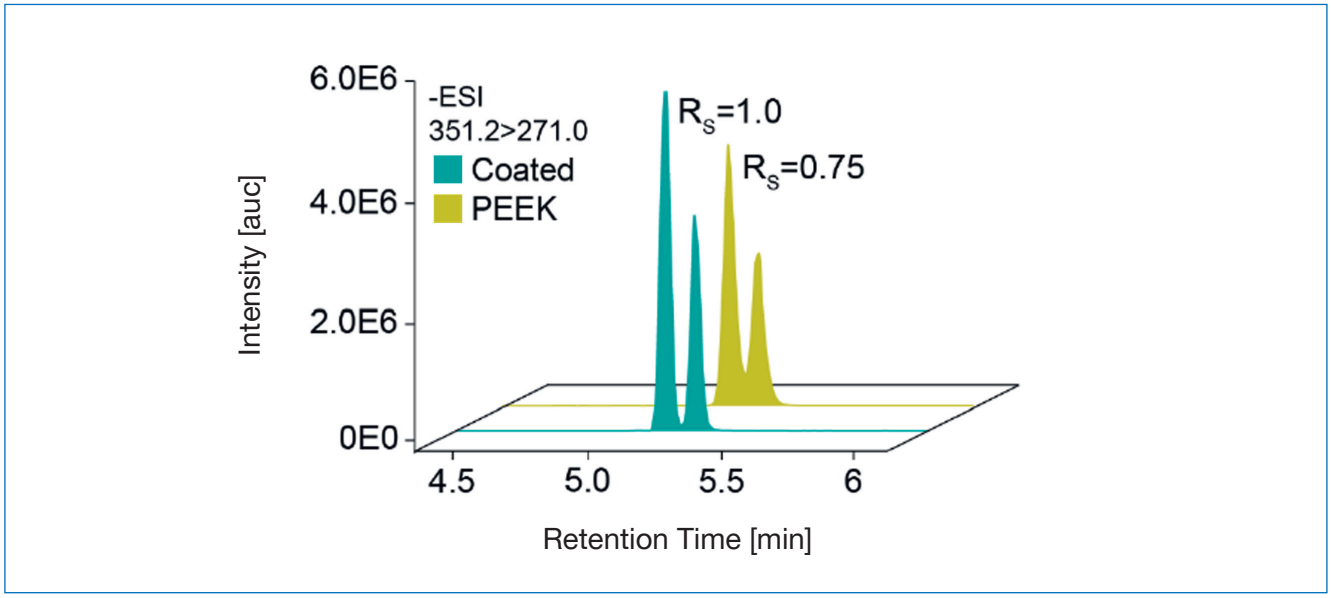


Figure 3: Comparison of the separation of PGE_2 and PGD_2 using the bioinert coated YMC Accura Triart C18 column (Coated) and the PEEK-lined YMC-Triart C18 metal-free column (PEEK) [1].

The separation of the lysoglycerophospholipids LPA, LPS and CPA is also crucial due to possible in-source fragmentation. Figure 4 shows that all three lipids can be separated with this method using the YMC Accura Triart C18 column.

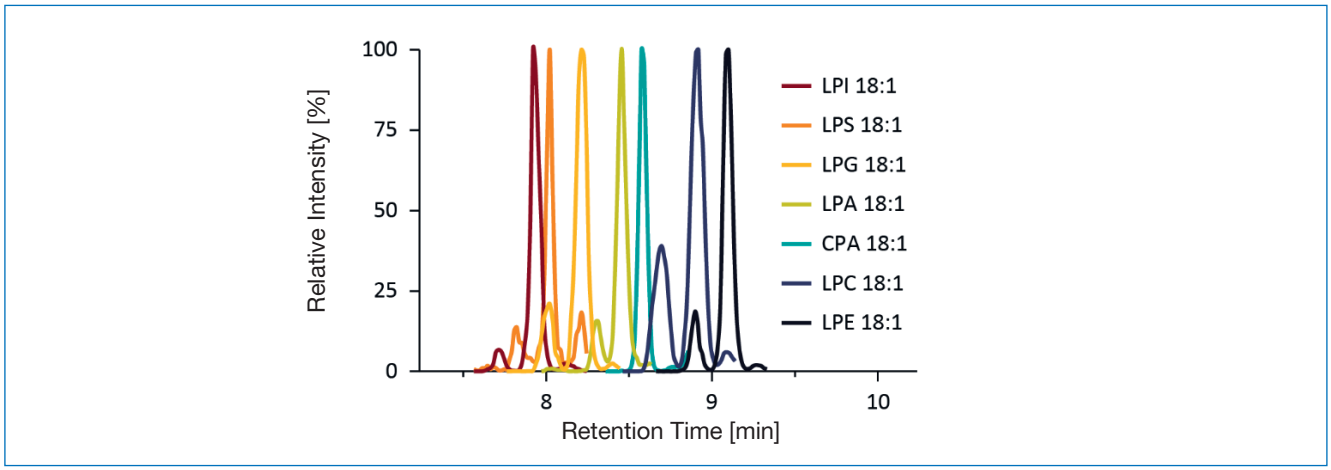


Figure 4: Analysis of lysoglycerophospholipids using the bioinert YMC Accura Triart C18 column [1].

In addition to high resolution, sharp peaks and high recovery, the YMC Accura Triart C18 column provides reliable, stable results even for varying matrices (extracted plasma and platelet, see Figure 5). The retention time of distinct analytes does not significantly change over weeks and across different matrices, proving the robustness of the column and method.

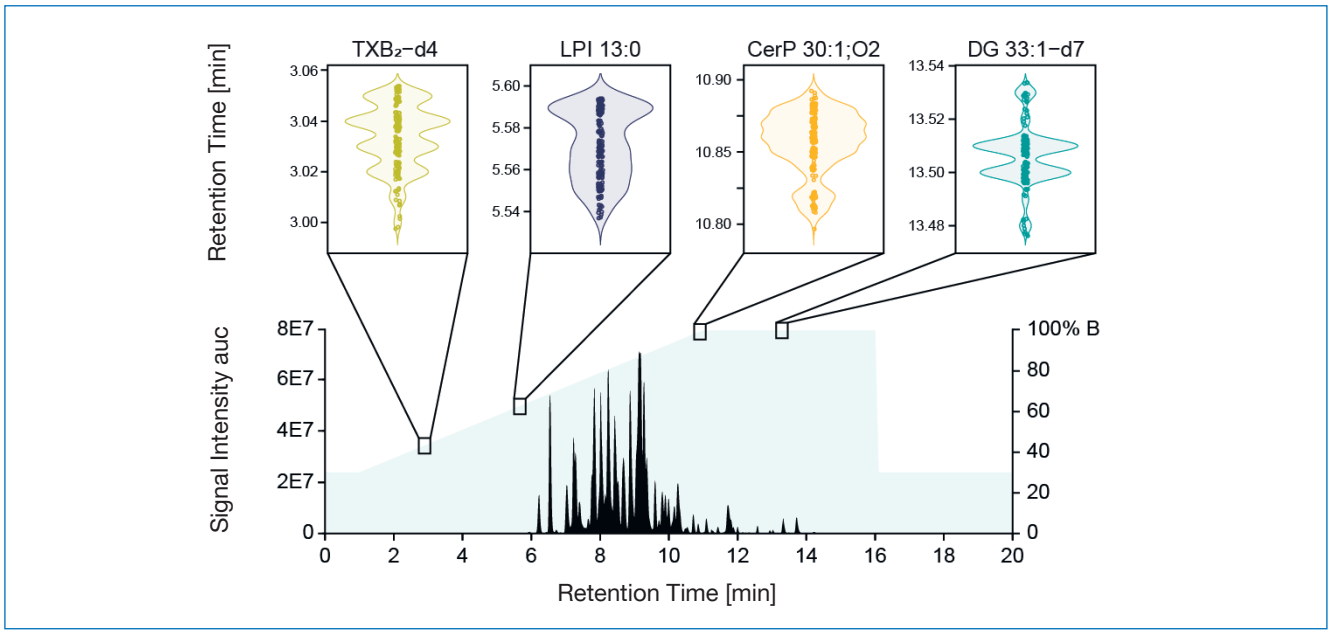


Figure 5: YMC Accura Triart C18 providing high retention time stability over multiple weeks and different matrices (extracted plasma and platelet) for selected analytes (n=188) [1].

Conclusion

Since a number of lipids contain phosphate groups, it is essential to use bioinert column hardware. The bioinert coated YMC Accura Triart C18 column combines high sensitivity for phosphate group containing lipids with high resolution, making it the first-choice column for signalling lipid analysis.

References:

[1] Stefanie Rubenzucker, Mailin-Christin Manke, Rainer Lehmann, Alice Assinger, Oliver Borst, and Robert Ahrends, Analytical Chemistry 2024 96 (23), 9643-9652, DOI: 10.1021/acs.analchem.4c01388