Fast lipidomic analysis with high resolution of the molecular species

The lipid signature of biological samples, or lipidome, is remarkably different between health and disease states. Therefore, lipids are good candidates to produce potent biomarkers. From an analytical point of view analysis of lipidomes deals with a large number of isomeric compounds, making comprehensive separation essential to generate a biologically informative datasets.

In order to achieve sufficient separation, the analysis times with standard methods, commonly using C18 stationary phases, tend to be longer and typically feature runtimes of >20 min. However, measurements of large cohorts of clinical samples (>200 per batch) require shorter runtimes in order to maintain overall reliability and improve cost-efficiency of the analysis. This example uses human plasma samples to demonstrate that separations under 10 minutes, are also possible with real samples using a less hydrophobic YMC Accura Triart C8 column with bioinert coating to facilitate higher levels of sensitivity and recovery.

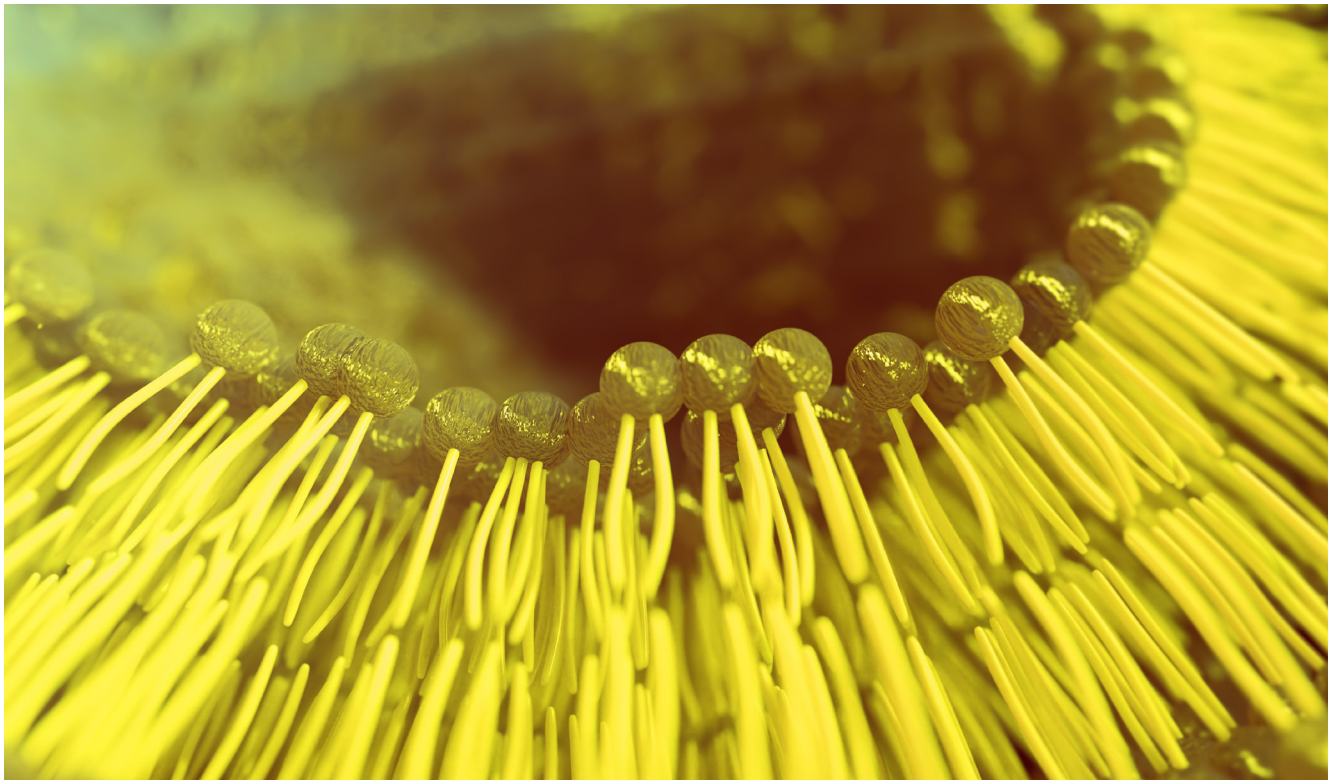

Table 1: Chromatographic conditions.

Column:	YMC Accura Triart C8 (12 nm, 1.9 µm) 100 x 2.1 mm ID
Part No.:	TO12SP9-10Q1PTC
Eluents:	A) 10 mM ammonium acetate in water/acetonitrile (50/50)
	B) acetonitrile/2-propanol (50/50)
Gradient:	10%B (0–0.5 min), 10–50%B (0.5–1.5 min), 50–99%B (1.5–7.5 min), 99%B (7.5–8.5 min), 99–10%B (8.5–8.6 min), 10%B (8.6–9.5 min)
Flow rate:	0.6 mL/min
Temperature:	50 °C
Injection:	2 µL
Sample:	Human plasma extracted with 2-propanol (solvent to sample ratio of 4:1)
Detection:	ESI-MS

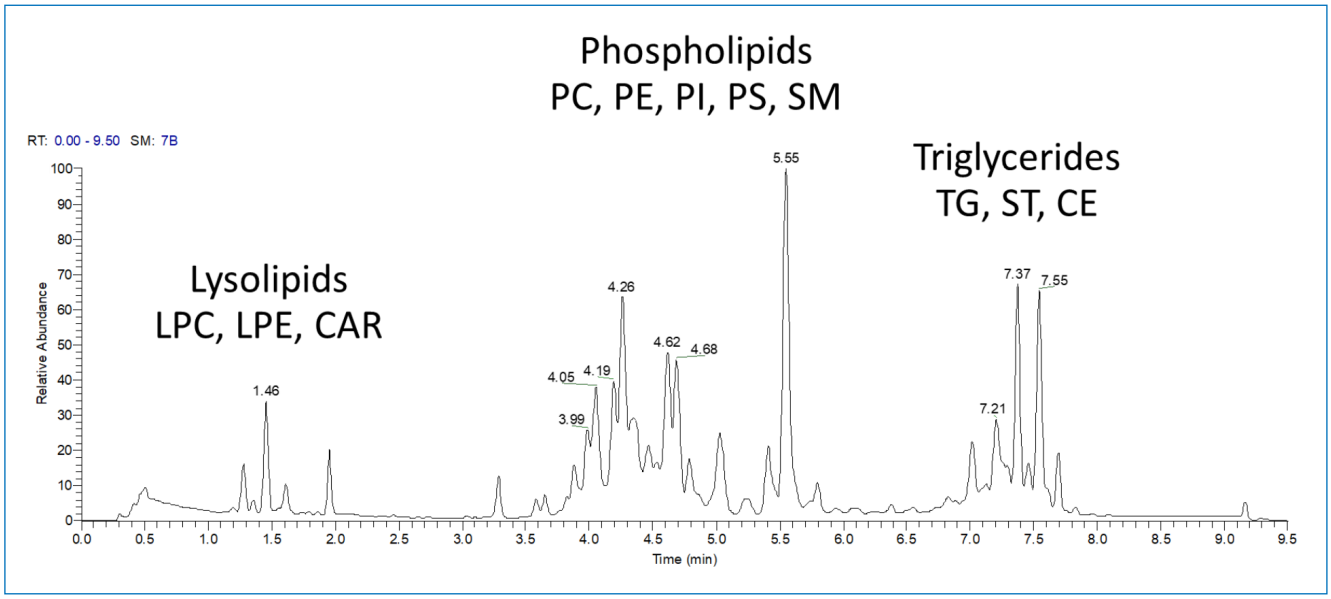

Figure 1: Successful lipidomic analysis of a human plasma sample using a YMC Accura Triart C8 column.

Figure 1 shows a well-defined separation between classes of lysolipids, phospholipids and triglycerides, typical for reversed-phase lipidomic analysis. Even with the less hydrophobic YMC-Triart C8 modification, molecular species of complex lipids can be feasibly separated in a fast run, as already visible from a TIC chromatogram. Hence, around 700 distinct molecules may be reliably determined during this fast analysis depending on the MS/MS performance of the spectrometer. The YMC-Triart C8 stationary phase has a very high specific surface area of 360 m^2/g, providing a sufficient loading capacity to accomodate the samples with a very high content of the analytes, such as plasma lipid extracts.

Table 2: Evaluated lipids.

Lipid class	Lipid	Abbreviation
Lysolipids	Lysophosphatidylcholine	LPC
	Lysophosphatidylethanolamine	LPE
Carnitins	Acylcarnitine	CAR
Phospholipids	Phosphatidylcholine	PC
	Phosphatidylethanolamine	PE
	Phosphatidylinositol	PI
	Phosphatidylserin	PS
	Sphingomyelin	SM
Triglycerides	Triglyceride	TG
	Sterols	ST
	Cholesterol esters	CE

Figure 2 shows, as an example, the chromatographic resolution of some early, mid-gradient and late eluting lipids. The early eluting LPC and LPE and the mid-gradient eluting PC and PE can be separated with appropriate resolution. This LC-MS method is also suitable for the characterisation of late eluting triglycerides with similar retention.

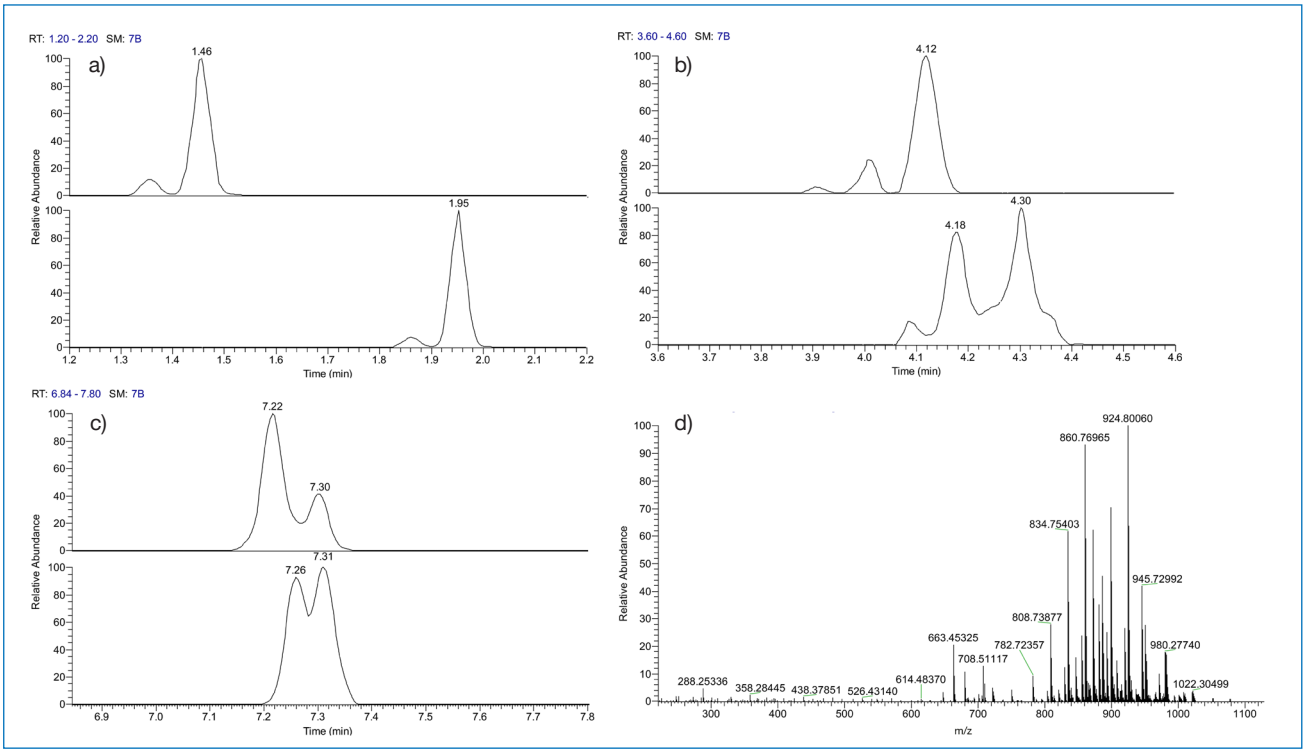

Figure 2: Chromatographic resolution of a) early eluting LPC/LPE (1.20–2.20 min), b) mid-gradient eluting PC/PE (3.60–4.60 min) and c) late eluting triglycerides (6.84–7.80 min) with d) a typical full scan spectrum at 7.27 min.

The YMC Accura Triart C8 column used is equipped with a bioinert coating on the column body and frits. This ensures high recovery from the first injection (see Figure 3) since the lipids containing phosphate groups do not come into contact with metal surfaces, preventing adsorption. Twenty consecutive injections prove that the column provides reliable results from the first injection.

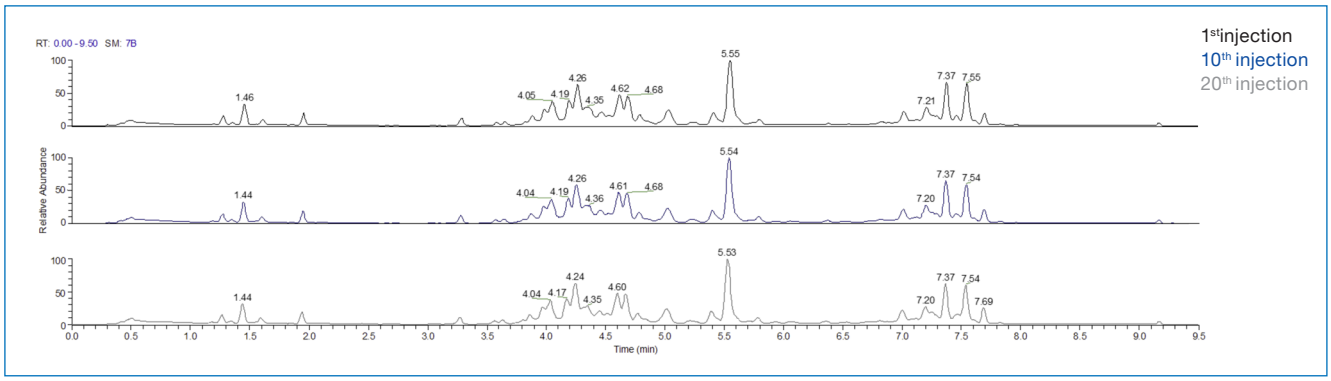

Figure 3: 20 consecutive injections show that high recoveries and good peak shapes are achieved from the first injection.

** Application data by courtesy of Sergey Girel, Institute of Pharmaceutical Sciences of Western Switzerland (University of Geneva), Geneva, Switzerland.*