

UHPLC-MS analysis of phospholipids using a bioinert coated **YMC-Accura Triart C18** column

Phospholipids are a key component of cell membranes. Commercially produced phospholipids can be applied e.g., in nanotechnology. The phosphate group which is contained in the hydrophilic head of the lipid poses a major challenge for chromatographic analyses. Phosphate containing compounds tend to be adsorbed on metallic surfaces such as the column body, frits, and tubing made from stainless steel due

to ionic interactions with the electron-rich phosphate group. This effect is enhanced when working at low to neutral pH as metals become more electropositive. To overcome these challenges bioinert systems and columns such as the recently introduced YMC-Accura Triart columns are beneficial. YMC-Accura Triart columns have a bioinert coating on all surfaces, including the frits, to prevent any unwanted ionic interactions.

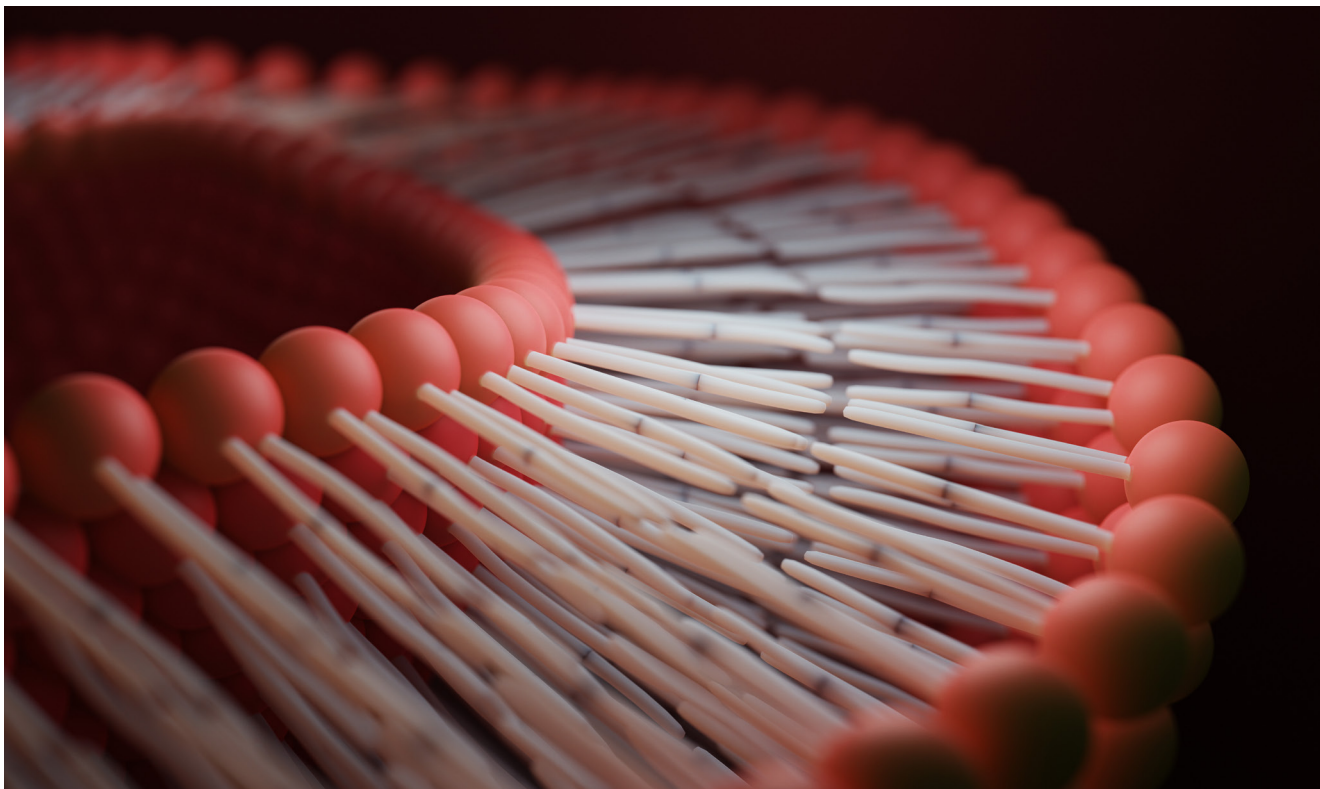
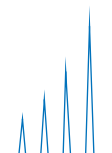


Figure 1: Lipid bilayer.

This application shows the reversed phase chromatography-mass spectrometry analysis of two phospholipids: LPC (16:0) and PC (34:2) using a bioinert coated YMC-Accura Triart C18 UHPLC column in comparison with a regular stainless steel column. Figure 2 shows that the bioinert coated YMC-Accura Triart C18 column provides excellent peak shapes while using a regular

stainless steel column results in massive peak tailing. The two phospholipids are further analysed in precipitated plasma, which requires a quite matrix tolerant column. YMC-Triart columns are also ideal for such purposes, due to their large surface area of 360 m²/g and their high robustness.



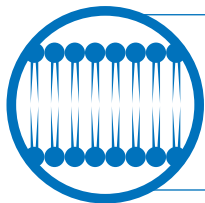
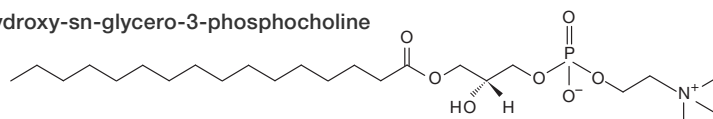


Table 1: Chromatographic conditions

Column:	YMC-Accura Triart C18 (1.9 μm , 12 nm) 100 x 2.1 mm ID
Part No.:	TA12SP9-10Q1PTC
Eluent:	A) 10 mM ammonium formate / acetonitrile + 0.1 % formic acid (40/60) B) 10 mM ammonium formate in 2-propanol/acetonitrile + 0.1% formic acid (90/10)
Gradient:	20–55% B (0–3.5 min), 55–95%B (3.5–15 min), 95%B (15–17 min)
Flow rate:	0.4 mL/min
Temperature:	50°C
Detection:	ESI positive mode, Orbitrap QExactive Focus (FS 70k at mz200, AGC 1e6 charges, IT 50ms; DDA top4 17.5k at mz200, IT 50ms)
Injection:	2 μL
Sample:	100 μL pooled human plasma precipitated with 2-propanol (Sample-to-solvent ratio 1:6; reconstituted with 100 μL of 2-propanol)

LPC (16:0): 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine



PC (34:2): 1-vaccenoyl-2-palmitoleoyl-sn-glycero-3-phosphocholine

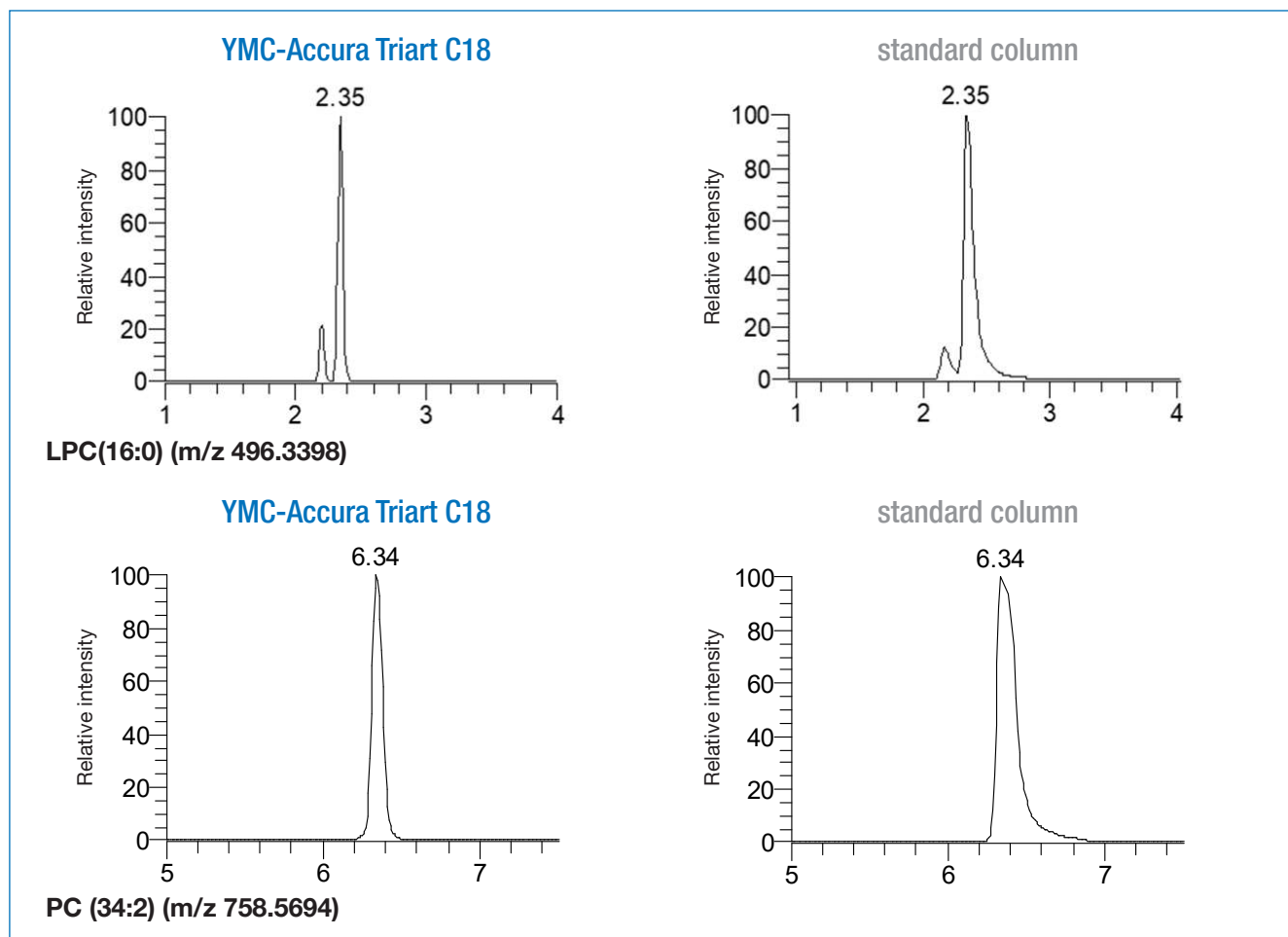
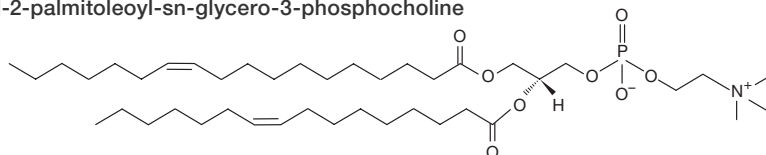


Figure 2: Selected ion chromatograms (SIC) of m/z 496.3398 (LPC 16:0) and m/z 758.5694 (PC 34:2) using the bioinert coated YMC-Accura Triart C18 column (left) and a regular C18 stainless steel column (right).

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