APPLICATION NOTE

![YMC logo]

# LC-MS/MS Analysis of Sphingophospholipids Using a Metal-free Column

Sphingolipids are major components forming biological membranes, and they serve as intracellular signalling molecules. It is important to measure the amount of these molecules in biological samples because they have massive influence on various metabolic diseases such as obesity, diabetes, and Alzheimer's disease. However, in LC analyses of sphingophospholipids, such as sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P), a phosphate group in these molecules causes significant peak tailing, and therefore loss of sensitivity and reproducibility. Recently, an improved method using a YMC-Triart C18 metal-free column was reported by Dr. Gowda et al. [1]

Adsorption of Compounds with Phosphate Groups

In LC analysis, compounds containing phosphate groups tend to be adsorbed on the metallic surfaces in the flow path of the LC system. This results in peak tailing, carryover, and insufficient sensitivity.

It is important to use a column packed with a packing material containing less metal impurities to prevent these problems. Material used for the column hardware is also important. Stainless steel-free column hardware is effective to improve peak shapes, especially for highly sensitive analyses such as LC/MS.
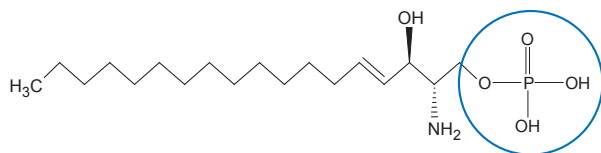
YMC-Triart C18 metal-free columns are ideal for highly sensitive analysis of coordination compounds, because their hardware consists of a PEEK-lined stainless steel tube and PEEK frits.
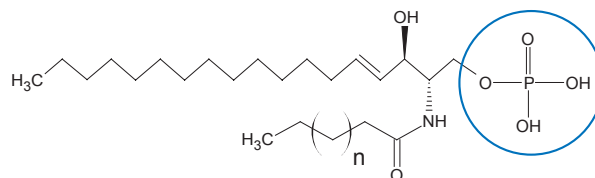

Frit: PEEK

Packing material

PEEK

Stainless steel
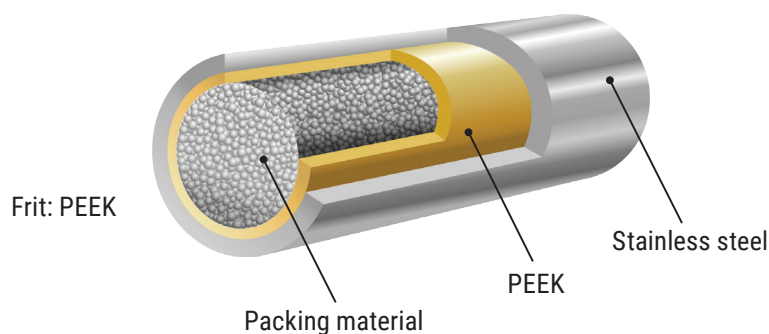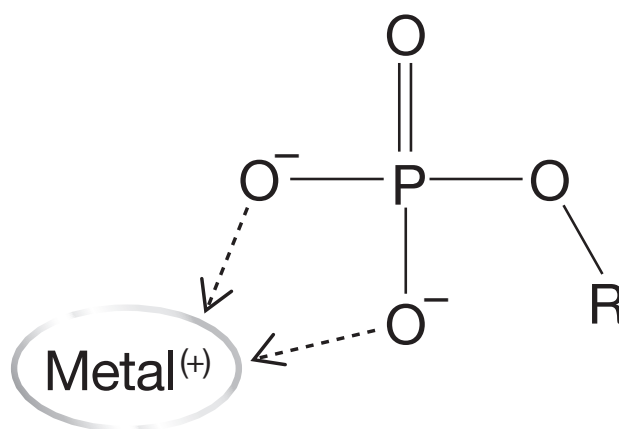
Reference [1]
Siddabasave Gowda B. Gowda, Kazutaka Ikeda, Makoto Arita,
Facile determination of sphingolipids under alkali condition using metal-free column by LC-MS/MS,
Analytical and Bioanalytical Chemistry, 410 (20): 4793-4803 AUG 2018
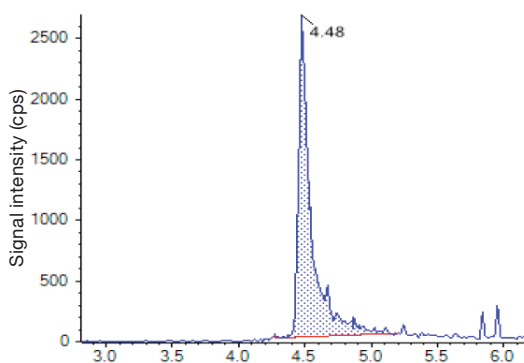
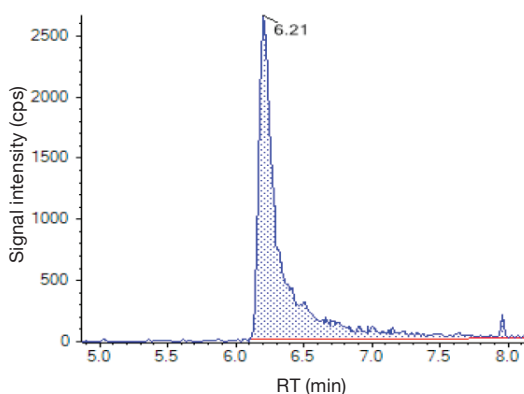# Analysis of Sphingophospholipids

Sphingosine-1-phosphate (S1P)

Ceramide-1-phosphate (C1P)

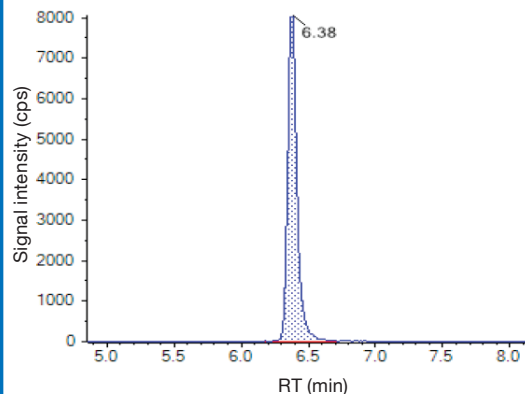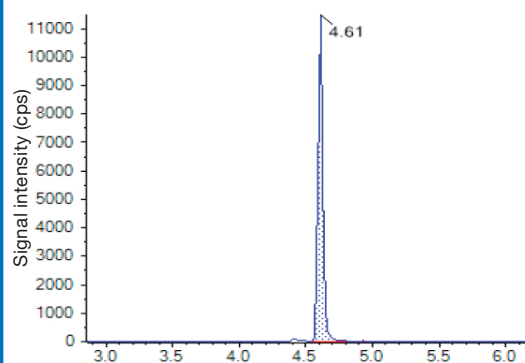**HSST3 C18 column with conventional stainless steel hardware (1.8 µm, 50 x 2.1 mm ID)**

YMC-Triart C 18 metal-free column (1.9 µm, 50 x 2.1 mm ID)

S1P (d17:1)

C1P (d18:1/12.0)

Part No: TA12SP9-05Q1PTP
Eluent: A) methanol/acetonitrile/water (1/1/3) containing X
             B) 2-propanol containing X
               X: 5 mM ammonium acetate, 500 nM EDTA and 0.025% NH_3 water
Gradient: 0%B (0–1 min), 0–50%B (1–5 min), 50–64%B (5–11 min), 64–95%B (11–13 min), 95%B (13–15 min), 0%B (15–20 min)
Flow rate: 0.25 mL/min
Temperature: 40 °C
Detection: ESI, positive
Injection: 1 µL
Instrument: LC) Waters ACQUITY UPLC H-class system
             MS) AB Sciex QTRAP 6500

**Significant peak tailing was observed for the conventional stainless steel column.
On the other hand, peak shape and intensity were improved using the YMC-Triart C18 metal-free column.**