

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

Sphingolipids are major components forming biological membranes, and they serve as intracellular signaling 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 analysis of

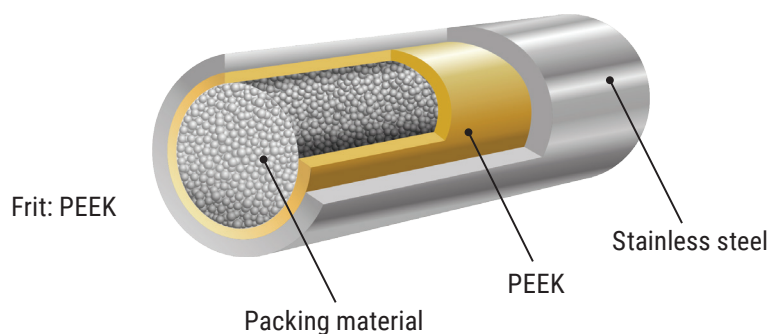
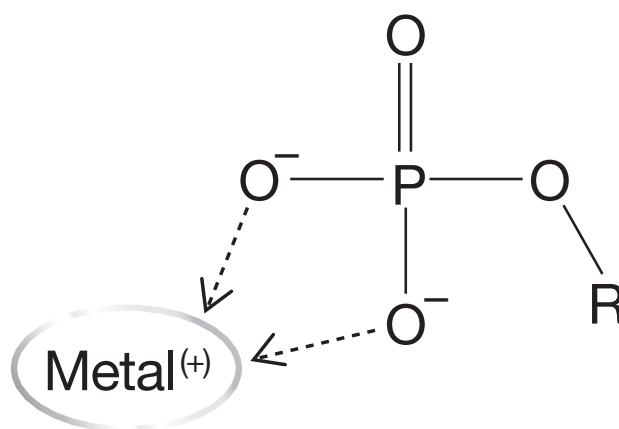
sphingophospholipids, such as sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P), a phosphate group in those molecules causes significant peak tailing, and thus loss of sensitivity and reproducibility. Recently, an improved method using a YMC-Triart C18 metal-free column was reported by Dr. Gowda et al.<sup>1)</sup>

## Adsorption of Compounds with Phosphate Groups

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

It is important to use a column packed with packing material containing less metal impurities to prevent these problems. Material of the column hardware is also important. Stainless steel-free column hardware is effective to improve peak shapes, especially for highly sensitive analysis like LC/MS. YMC-Triart C18

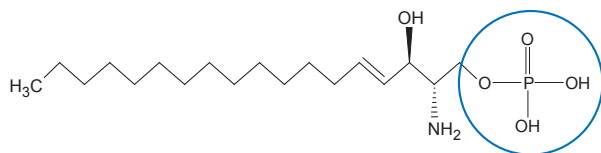
metal-free columns are ideal for highly sensitive analysis of coordination compounds, because their hardware consists of a PEEK-lined stainless steel tube and PEEK frits.



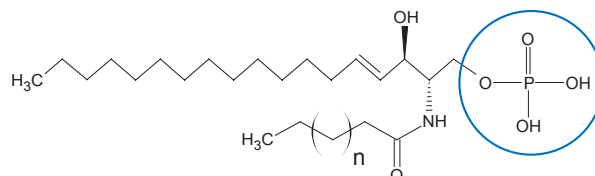
### Reference <sup>1)</sup>

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



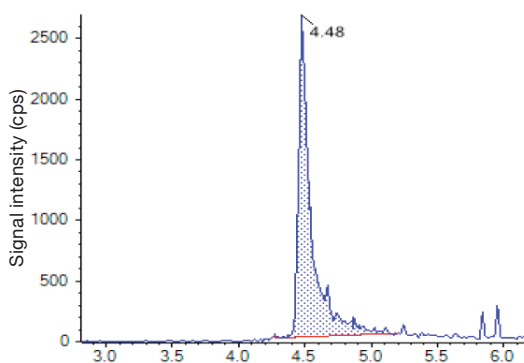
**Sphingosine-1-phosphate (S1P)**



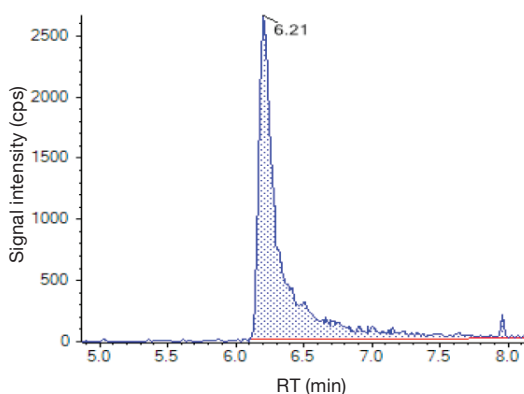
**Ceramide-1-phosphate (C1P)**

HSST3 C18 column with  
conventional stainless steel hardware  
(1.8  $\mu\text{m}$ , 50 x 2.1 mm ID)

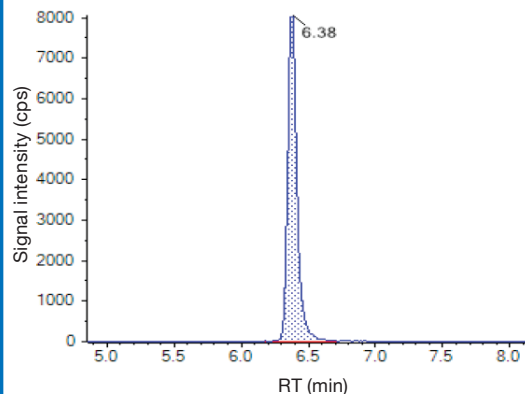
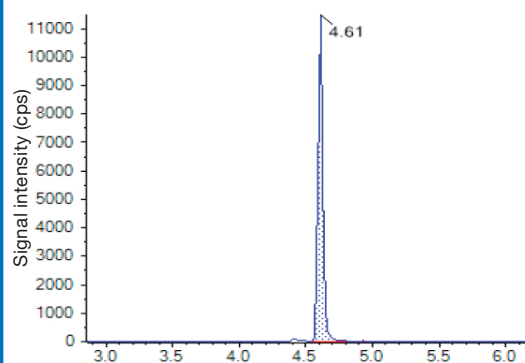
**S1P (d17:1)**



**C1P  
(d18:1/12.0)**



YMC-Triart C 18 metal-free column  
(1.9  $\mu\text{m}$ , 50 x 2.1 mm ID)



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%  $\text{NH}_3$  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  $\mu\text{L}$   
 Instrument: LC) Waters ACQUITY UPLC H-class system  
 MS) AB Sciex QTRAP 6500

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