# HPLC DATA SHEET

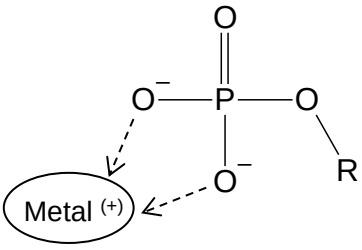
## LC-MS/MS analysis of sphingophospholipids using metal free column

U181204AE

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 it greatly influences on various metabolic diseases such as obesity, diabetes, and Alzheimer's disease. However, on the 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, the improved method using YMC-Triart C18 metal free column was reported by Dr. Gowda et al. [1)]

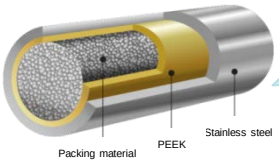
## Adsorption of compounds with phosphate group

On the 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.



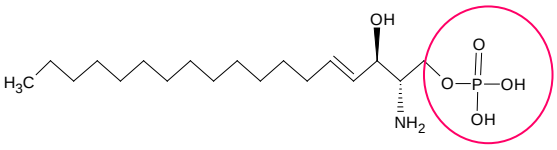

It is important to use a column packed with packing material containing less metal impurities to prevent these problems. Material of column hardware is also important and stainless steel-free column hardware is effective to improve peak shapes, especially for highly sensitive analysis like on LC/MS.

YMC-Triart C18 metal free column is ideal for highly sensitive analysis of coordination compounds, because its hardware consists of a PEEK-lined stainless steel tube and PEEK frits.

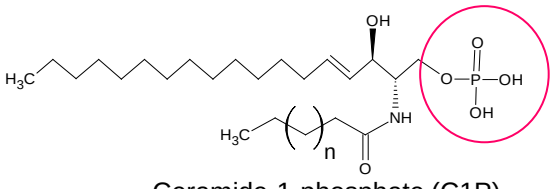



Wetted part (liner) : PEEK
Outer tube : Stainless steel
Frit : PEEK

## Analysis of sphingophospholipids




Sphingosine-1-phosphate (S1P)

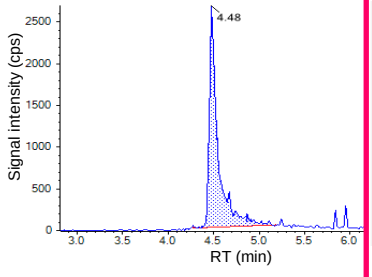


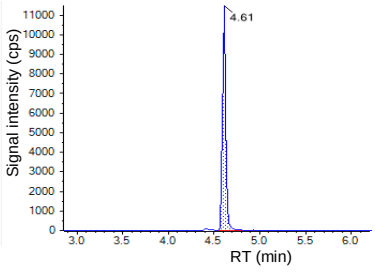

Ceramide-1-phosphate (C1P)

C18 column with conventional stainless steel hardware (1.8 µm, 50 X 2.1 mmI.D.)

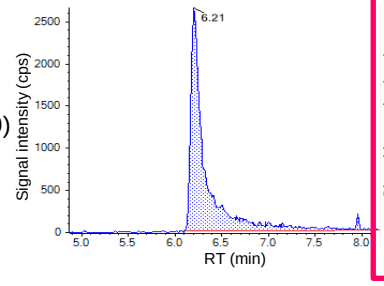
**Triart C18 metal free column** (1.9 µm, 50 X 2.1 mmI.D.)

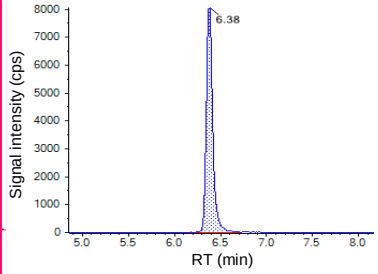
S1P(d17:1)




C1P(d18:1/12:0)




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

| | |
|---|---|
| Eluent | : A) methanol/acetonitrile/water (1/1/3) containing X<br>B) 2-propanol containing X<br>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℃ |
| Detection | : ESI, positive |
| Injection | : 1 µL |
| Instrument | : LC) Waters ACQUITY UPLC H-class system<br>MS) AB Sciex QTRAP 6500 |


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