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Application News

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Supercritical Fluid Chromatography

Separation of Lipids Using the Nexera™ UC Supercritical Fluid Chromatograph

Lipids are often referred to as compounds derived from living organisms that dissolve in organic solvents, and there are many types including complex lipids that have polar groups such as phosphate groups and sugars in their structure, and simple lipids such as fatty acids and acylglycerol. They are classified into lipid classes with common functional groups in their structure, and the physical properties differ greatly depending on the class. There are also different molecular species of constituent fatty acids within the same lipid class. Therefore, a high separation capacity is required for lipid analysis, and with HPLC the normal phase mode and reversed phase mode are applied differently according to the purpose. Generally, the normal phase mode is used when separating into lipid classes and the reversed phase mode is used when separating molecular species. The mobile phases used in the two modes are completely different, and it is very troublesome to switch modes in an analysis. Here, we introduce separations by supercritical fluid chromatography (SFC) using various columns with different stationary phases.

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Target Components and Analysis Conditions

The different lipid components of the 10 lipid classes listed in Table 1 were separated using four different stationary phase columns--diol, silica gel, ODS, and embedded-polar-group ODS--and the retention behavior was checked. The structural formulae for each of the lipids are shown in Fig. 1. The MRM conditions of the mass spectrometer and the analysis conditions for SFC are summarized in Table 1 and Table 2. Fig. 2 through Fig. 5 show the chromatograms for standard samples of 1 µmol/L.

Table 1 Target Components and MRM Conditions

Standard	Conc. (µmol/L)	MRM Transition (Positive)	Mode
PC 17:0/17:0	1	762.60>184.00	+
PE 17:0/17:0	1	720.55>579.55	+
PI 14:1/17:0	0.5	811.95>534.95	-
PG 17:0/17:0	1	768.50>579.40	+
PS 17:0/17:0	1	764.50>579.40	+
SM d18:1/17:0	1	717.60>184.00	+
MG 17:0	1	362.30>327.30	+
DG 12:0/12:0	1	474.40>439.40	+
TG 17:0/17:0/17:0	1	866.80>579.50	+
Cer d18:1/17:0	1	552.50>264.30	+

Table 2 Analysis Conditions

Column :

No.	Column	Size
1	Shim-pack™ UC-Diol	150 mm L. 2.1 mm I.D. 3 µm
2	Shim-pack™ UC-Sil	
3	Shim-pack™ UC-GIS II (ODS)	
4	Shim-pack™ UC-RP (Embedded-polar-group ODS)	

Mobile phase : A; CO_2
B; 0.1 % (w/v) Ammonium formate in methanol
Gradient : B.conc. 5 % (0 min) - 40 % (12-14 min) – 5 % (14.1-17 min)
Flow rate : 0.8 mL/min
Column temp. : 40 °C
BPR pressure : 10 MPa
Detector : LCMS™-8050 (ESI, MRM mode)
Makeup : 0.1 % (w/v) Ammonium formate in methanol
Makeup flow rate : 0.05 mL/min
Injection vol. : 1 µL

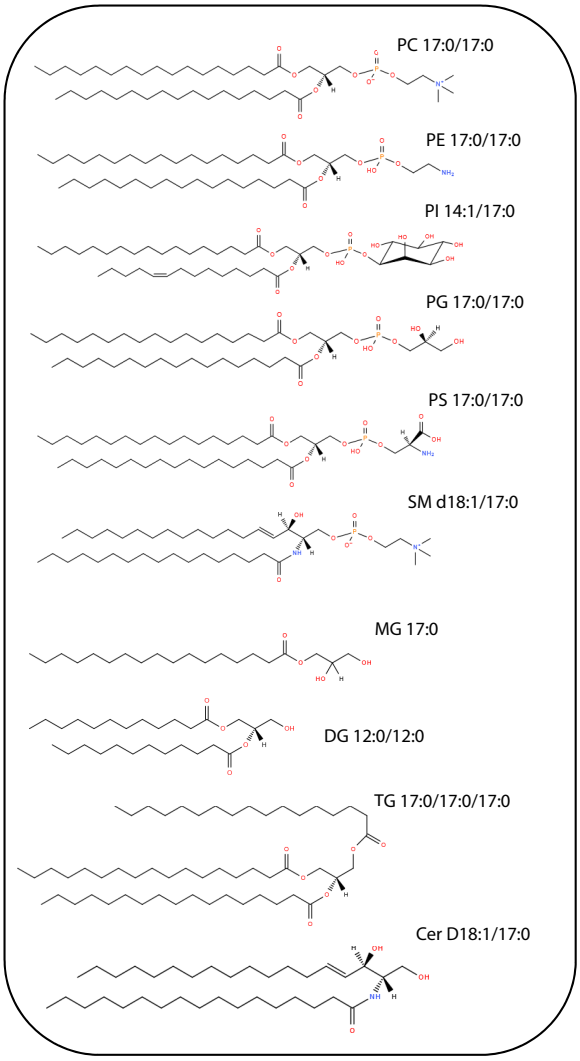

Fig. 1 Structural Formulae of Lipids

Chromatograms

From Fig. 2 through Fig. 5, we can see that the retention behavior and peak shape differ greatly according to differences in the stationary phase. Diol and silica gel are used in the normal phase mode in HPLC, and their retention behaviors are fairly similar in SFC, but silica gel may be specifically adsorbed on the stationary phase. ODS and embedded-polar-group ODS are used in the reversed phase mode in HPLC, and the retention time tends to increase when the target component has strong hydrophobic properties. There is a similar trend in SFC, but when an embedded-polar-group ODS is used, retention by polar bases sometimes may take place as in normal phase, rather than the retention as in reversed phase using ODS groups. In the stationary phase for reversed phase such as ODS, acylglycerols are eluted in the order MG > DG > TG, but in the stationary phase for normal phase such as diol, they are eluted in the order TG > DG > MG; the order of elution is reversed. So with SFC, while using the same mobile phase, different stationary phases for normal phase and reversed phase can be used to achieve a variety of separations.

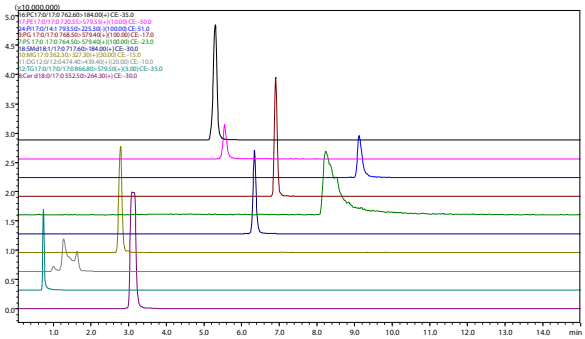

Fig. 2 Chromatogram with a Diol Stationary Phase

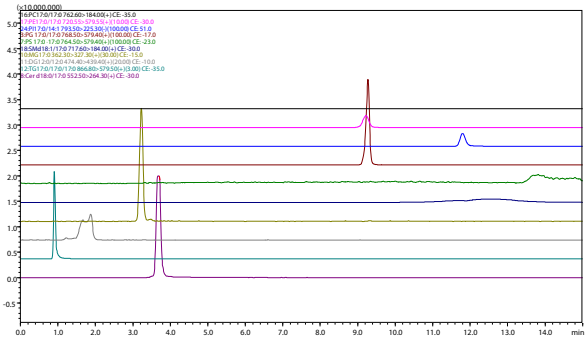

Fig. 3 Chromatogram with a Silica Gel Stationary Phase

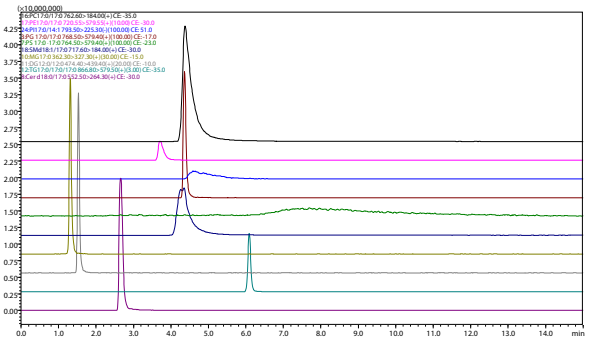

Fig. 4 Chromatogram with an ODS Stationary Phase

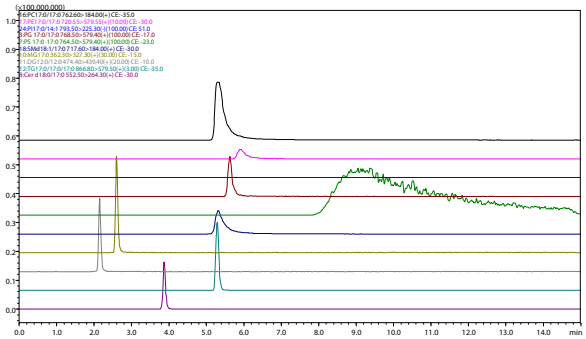

Fig. 5 Chromatogram with an Embedded-polar-group ODS Stationary Phase

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