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Application News

No.L462

High Performance Liquid Chromatography

Glycerophospholipids Analysis by Comprehensive HPLC Coupled with a Triple Quadrupole Mass Spectrometer

Glycerophospholipids (GPLs) are the major component of biological membranes. They can not only act as a barrier from the external environment, but can also play a key role in a variety of biological processes including membrane trafficking and signal transduction. Thus, analysis of GPLs is one of the most important studies in the metabolomics field. Although reversed phase (RP) HPLC coupled with electrospray ionization (ESI) MS/MS is an effective strategy for lipidomics, there is still room for further improvement of the analytical methods. One drawback to performing determination of GPLs is ion suppression caused by co-eluting compounds. To obtain reliable results, complete separation of target GPLs by comprehensive HPLC with ESI-MS/MS is an effective strategy.

Flow Diagram of Comprehensive HPLC

Fig. 1 shows the flow diagram of the comprehensive HPLC-ESI-MS/MS system. The system comprises 2 flow lines: one for the first dimension separation with a normal phase column and the second dimension separation with a reversed phase column. A mixture of GPLs was roughly classified by normal phase chromatography in the first dimension. All the eluents are trapped into two loops alternatively. Then the entire eluents are introduced into second dimensional reversed phase UHPLC without any risk of sample-loss. The GPLs of interest are separated according to the orthogonal retention selectivity and detected with ESI-MS/MS quantitatively.

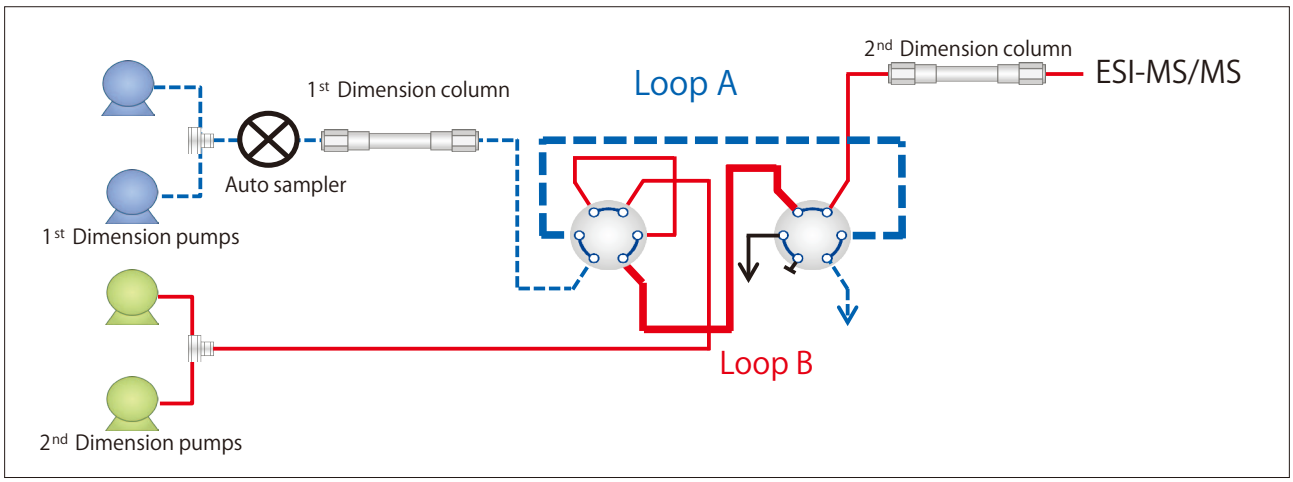

Fig. 1 Flow Diagram of the Comprehensive HPLC-ESI-MS/MS System

Table 1 Analytical Conditions

1D Column	: Nucleosil SIL (150 mm L. × 1.0 mm I.D., 3 µm)
Mobile Phase	: A: Isooctane / Acetone / Ethyl Acetate / Acetic acid = 40/20/20/0.03 (v/v/v/v) B: Isooctane / 2-propanol / Water / Acetic acid / 28 % Ammonia aq.sol. = 40/51/9/0.03/0.03 (v/v/v/v/v)
Flowrate	: 0.02 mL/min
Time Program	: B Conc. 30 % (0 min) → 40 % (25 min) → 100 % (40 min) → 100 % (55 min) → 30 % (55.1 min) → STOP (70 min)
Column Temp.	: 40 °C
Injection Vol.	: 5 µL
Loop Vol.	: 20 µL
2D Column	: Phenomenex Kinetex C18 (50 mm L. × 4.6 mm I.D., 2.6 µm)
Mobile Phase	: A: Methanol / Water / Acetic acid / 28 % Ammonia aq.sol. = 90/10/0.05/0.05 (v/v/v/v) B: 2-propanol / Acetic acid / 28 % Ammonium hydroxide = 100/0.05/0.05 (V/V/V)
Flowrate	: 3.5 mL/min (50 % split to MS)
Time Program	: B Conc. 10 % (0 min) → 50 % (0.75 min) → 10 % (0.76 min) → STOP (1 min) The initial B Conc. has been changed by a stepwise method
Detector	: Shimadzu LCMS-8050 (ESI positive, MRM mode)

Comprehensive Separation of Glycerophospholipids

Comprehensive Separation of GPLs in ESI-positive MRM mode are shown in Fig. 2.

The GPLs mixture was comprised of 500 ppb each of Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), Phosphatidylinositol (PI), Phosphatidylserine (PS) and Phosphatidylcholine (PC). The 2D plot of ESI-positive MRM shows the separation of PG, PE, PI, PS and PC. The repeatability (n=5) of retention times and blob areas, which correspond to peak areas in ordinary quantitation and linearity for 50-5000 µg/L of each 3 PC compounds are is shown in Table 2.
Necessary information for compound-identification is shown in Fig. 2.

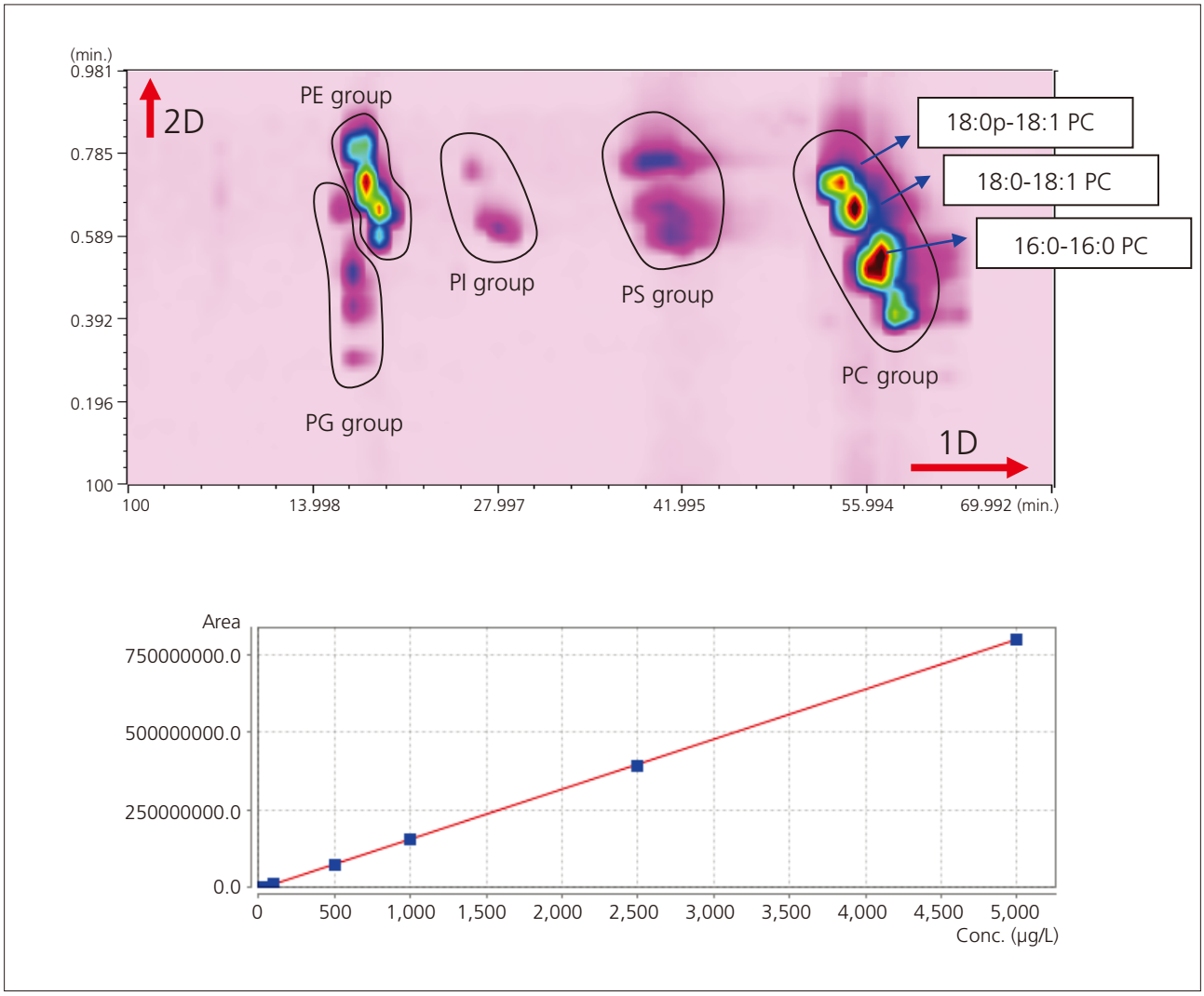

Fig. 2 Comprehensive Separation and Calibration Curve of GPLs

Table 2 Repeatability of 5 Analyses in %RSD and Linearity of 50-5000 µg/L for 3 PC Compounds

Compound	MRM transition	Total retention time	Retention time (2D)	Blob Area	Correlation coefficient (R)
16 : 0 -16 : 0 PC	*m/z* 734.6 > 184.1	0.0072	0.9	6.8	0.999799
18 : 0 -18 : 1 PC	*m/z* 788.6 > 184.1	0.013	1.1	8.9	0.999947
18 : 0p -18 : 1 PC	*m/z* 772.6 > 184.1	0.013	1.2	6.4	0.999656

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