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MRM based phospholipid profiling of mouse tissues by an ultra-fast triple quadrupole mass spectrometer

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Introduction

Phospholipids (PLs) serve as cell membrane structure holding various fatty acids such as arachidonic acid (AA) or docosahexaenoic acid (DHA). Although multiple reaction monitoring (MRM) using triple quadrupole mass spectrometer (TQ-MS) is expected to be the better sensitivity than scan or Q-TOF based method, theoretical number of PLs reaches several thousands, which is hard to be covered by MRM based method. Hence we have narrowed down the target PLs to develop over 400 polar head monitoring MRM and over 800 MRM for fatty acid determination using an ultra-fast TQ-MS. The MRM based method is expected to be better for finding out the minor PLs. Here we report PLs profiling results of mouse tissues using the MRM based method.

Methods and Materials

An LC-MS system consisting of *Nexera* UHPLC and an LCMS-8060 mass spectrometer (Shimadzu Corp.) was used. Twenty mM ammonium formate and acetonitrile/isopropanol (1/1, v/v) were used for mobile phase A and B, respectively. Kinetex C8 (2.1 x 150 mm, 2.6 µm, Phenomenex, Torrance, CA) was used for chromatographic separation. Polarity switching time of the instrument was 5 ms. Dwell time was set at 2 ms or 3 ms for an MRM transition. Pause time was set at 1 ms. Lipid extracts with methanol from brain, lung, liver and spleen collected from C57BL/6J mice (CLEA, Japan) were gifted from department of lipidomics, The University of Tokyo. Ten µL of the extract 0.1 mg tissue /mL was injected to the LC-MS system.

MS conditions (LCMS-8060)	
Ionization	: ESI, Positive/Negative
Nebulizing Gas Flow	: 3.0L/min.
Drying Gas Flow	: 10.0L/min.
Heating Gas Flow	: 10.0L/min.
DL Temp.	: 250 °C
Block Heater Temp.	: 400 °C
Interface Temp.	: 300 °C
CID Gas Pressure	: 230 kPa

Ultra Fast Mass Spectrometer
UF Polarity Switch in 5 msec)
UF SRM (Max. 555/sec)

We developed two MRM-based phospholipid profiling methods which can analyze PLs and lyso-PLs being consist of PC, PE, PG, PI, PS and SM. One method was to monitor polar head group which contains >400 MRM transitions (1st method). Another was to determine fatty acid by monitoring fatty acid fragment ion in negative ion mode, which containing >800 MRM transitions (2nd method) where 17 kinds of fatty acids with carbon number from 14 to 22 were taken into account. Schematic view of the profiling method was shown below.

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- **First method** find MRM chromatographic peaks without fatty acid composition, PC (34:1).
- **Second method** is to determine fatty acid composition, PC (16:0/18:1)

Results

Profiling results

When analyzed mouse tissues by 1st method, 969 MRM transitions were required for fatty acid determination. Consequently we conducted 406 MRM method for PC and SM analysis and 563 MRM for PE, PG, PI and PS analysis to lipid extracts from mouse brain, lung, liver and spleen. The PCs and PEs method consisted of 3 ms and 2 ms dwell time for each MRM transition by time scheduling, respectively.
Totally 221 phospholipid species including 13 LPC and 88 PC, 6 LPE and 63 PE, one LPG and 12 PG, 19 PI, 10 PS, and 9 SM species were identified by detecting polar head fragmentation and fatty acid fragmentation in the same retention time.
Here we summarize the data focusing on arachidonic acid (AA, 20:4), eicosapentaenoic acid (EPA, 20:5) or docosahexaenoic acid (DHA, 22:6) containing phospholipid species.

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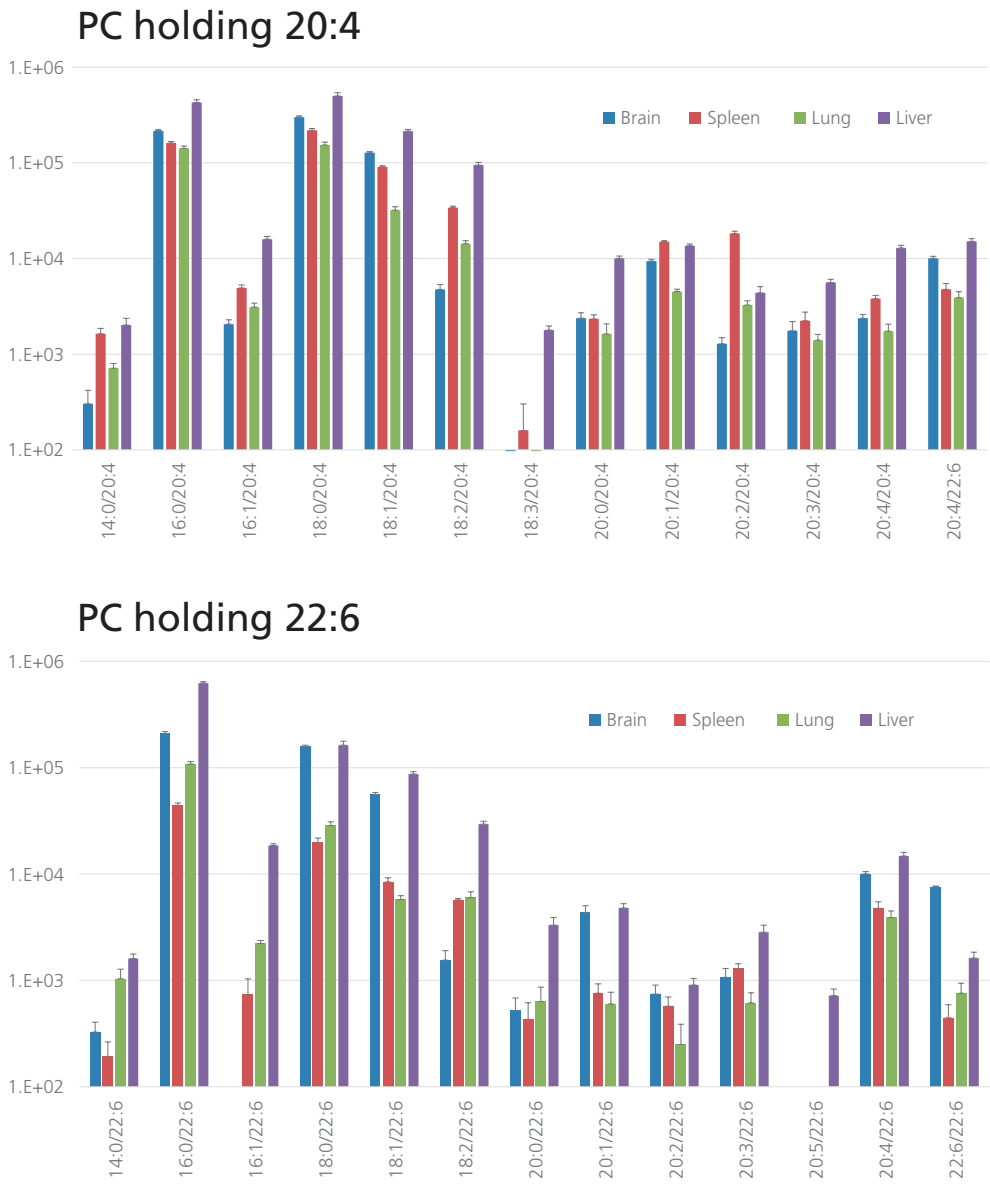

Figure 1. Profiling result of PC species containing arachidonic acid (AA, 20:4) upper, and that of containing docosahexaenoic acid (DHA, 22:6) lower. Chromatographic peak heights were used. Error bar indicated standard error of biological replicates (n = 5).

Cluster analysis

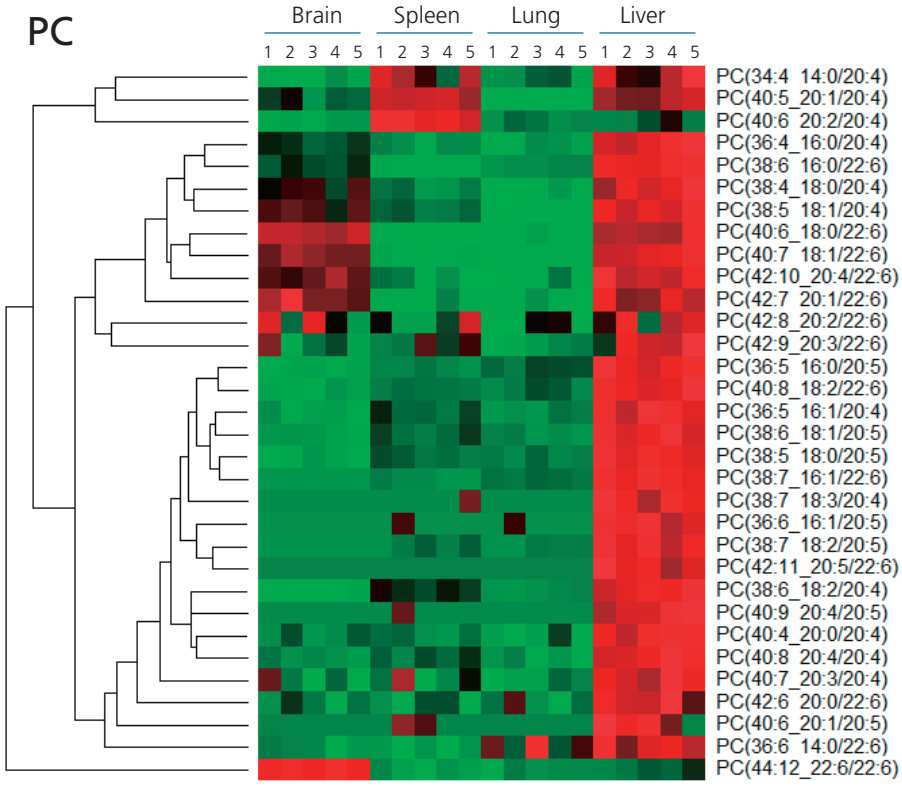

Figure 2. Cluster analysis result of PC species including AA, 20:4, EPA 20:5 and DHA, 22:6.

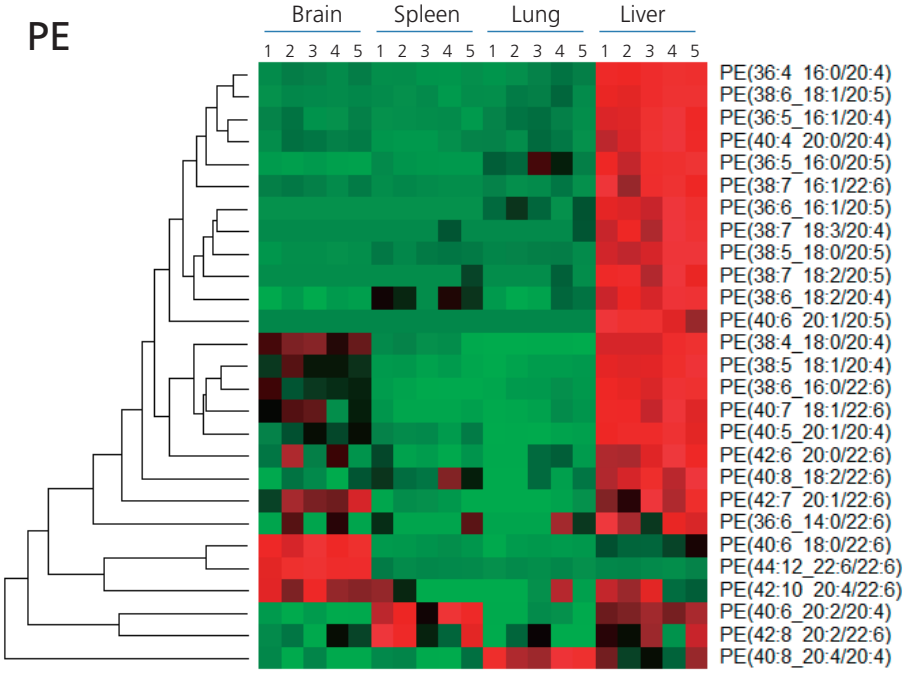

Figure 3. Cluster analysis result of PE species including AA, 20:4, EPA 20:5 and DHA, 22:6.

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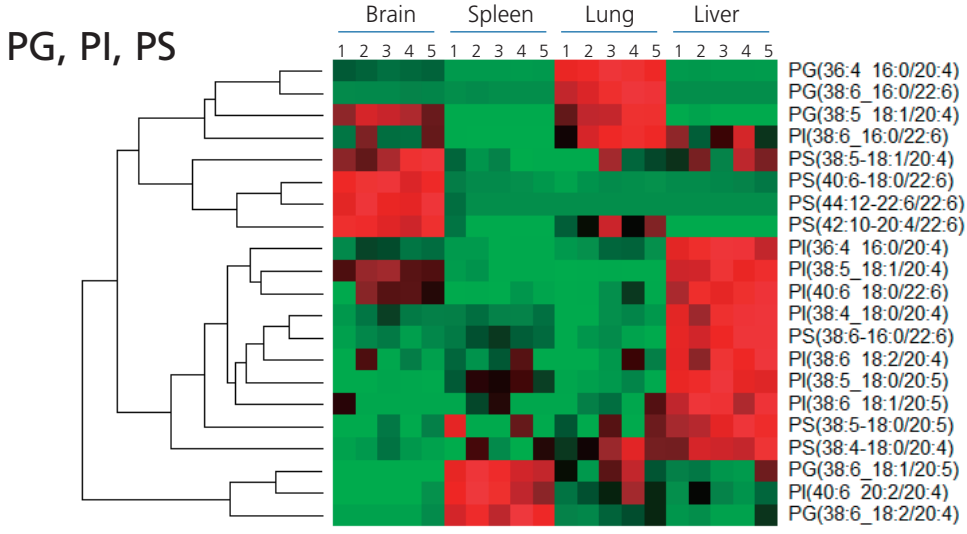

Figure 4. Cluster analysis result of PG, PI and PS species including AA, 20:4, EPA 20:5 and DHA, 22:6.

MRM chromatograms and profiling of PE 36:5

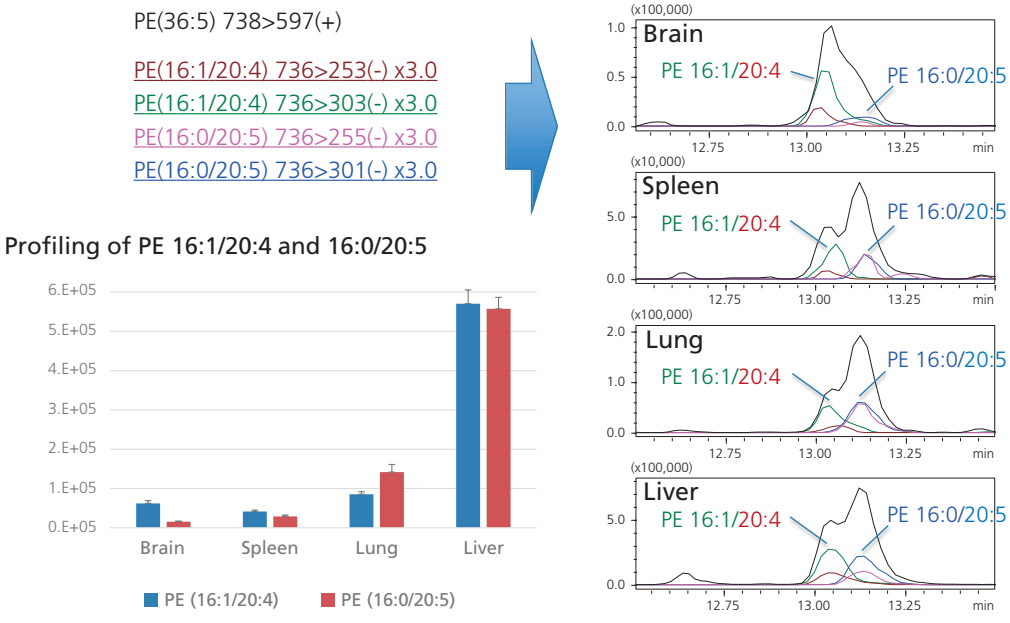

Figure 5. Profiling of PE 16:1/20:4 and PE 16:0/20:5.
Peak heights were used for the profiling and SEM (biological replicates n = 5) was shown.
MRM chromatograms were shown. Both PEs were well separated by retention time.

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Conclusions

- We have applied the MRM based phospholipid profiling method to mouse tissues.
- At least 221 phospholipid species were identified.
- We believe that the straightforward method will be available for pathophysiological study and disease biomarker analysis.

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