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Application News

No. C237

High Performance Liquid Chromatograph Mass Spectrometer LCMS™-8060

Development of Profiling Method for Major Lipids in Blood by Triple Quadrupole LC/MS/MS

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User Benefits

- Profiling analysis of the major lipids in blood is possible.
- The LCMS-8060 can acquire high quality data, even in positive/negative simultaneous analysis (polarity switching time: 5 msec).
- Fatty acid composition analysis of the major lipids is possible using small amount of human blood.

■ Introduction

The major lipids in blood are phospholipids (PL), triacylglycerols (TG), and cholesteryl esters (CE) (Fig. 1). The fine structures of these major lipids have attracted attention in connection with various types of diseases and in research on pathophysiology.

The authors developed a phospholipid profiling method based on multi-reaction monitoring (MRM) using the LCMS-8060, as reported in Application News No. C137.

This article introduces the development of a profiling method which widens the range of targets to the major lipids in blood, including not only the phospholipids, but also CE, TG, free fatty acids, and cholesterol. Approximately 360 MRM transitions were prepared in this method.

It was possible to identify approximately 100 types of lipids in blood, including 51 PLs, 26 TGs, 11 CEs, CE hydroperoxides, free fatty acids, and free cholesterol. It was also possible to apply this method to blood plasma and blood serum, and the compositions of the fatty acids of various classes of lipids were clarified.

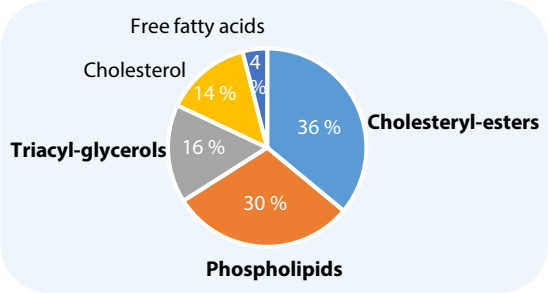

Fig. 1 Major Lipids in Blood Plasma

■ Analysis Conditions

A Nexera UHPLC system and LCMS-8060 (Fig. 2) were used as the measurement instruments. The LCMS-8060 is an ultra-fast quadrupole mass spectrometer with a positive/negative polarity switching time of 5 msec. Table 1 shows the HPLC and MS analysis conditions.

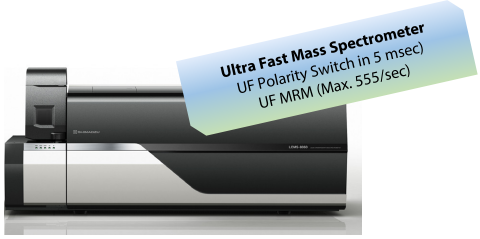

Fig. 2 Appearance of LCMS-8060

Table 1 Analysis Conditions

[HPLC conditions] (Nexera™)	
Column	: Phenomenex Kinetex® C8 (150 mm x 2.1 mmI.D., 2.6 µm)
Column oven	: 50 ˚C
Solvent A	: 20 mM Ammonium formate - water
Solvent B	: Acetonitrile/2-Propanol (50/50, v/v)
Flow rate	: 0.4 mL/min
Injection volume	: 1 µL
[MS conditions] (LCMS-8060)	
Ionization	: ESI , Positive/Negative
Mode	: MRM
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
DL temp.	: 250 ˚C
Block heater temp.	: 400 ˚C
Interface temp.	: 150 ˚C
CID Gas Pressure	: 270 kPa
Dwell time/Pause time	: 3 msec./1 msec.

■ Sample Preparation

Human blood was purchased from BioIVT (US). 500 µL of methanol containing 0.1 % of formic acid was added to 5 µL of blood and mixed well. After centrifugal separation, 1 µL of the supernatant was used in the LC/MS/MS analysis (Fig. 3). Fig. 4 shows the MRM chromatograms of LPL (lysophospholipids), PL, CE, and TG.

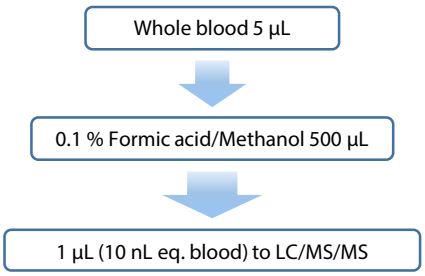

Fig. 3 Sample Pretreatment Protocol

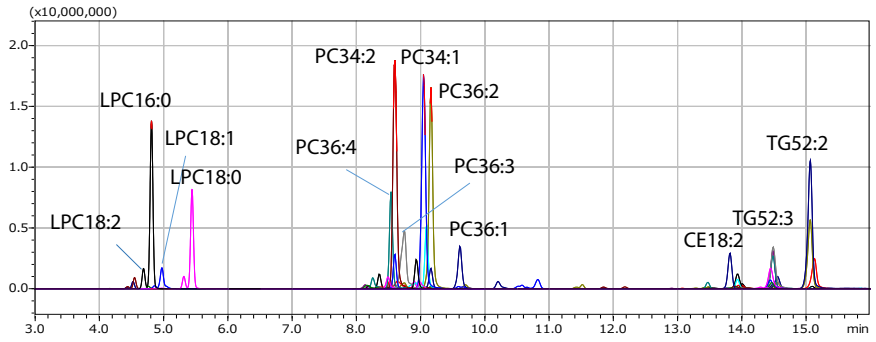

Fig. 4 MRM Chromatograms of LPL, PL, CE, and TG

■ Results

The MRM-based phospholipid profiling method developed by the authors includes approximately 2000 MRM transitions of phospholipids and lysophospholipids targeting phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI), and sphingomyelin (SM). The product ions formed by separation of the polar groups of PC, SM, PE, and PS are detected by the positive mode, while PI-derived polar groups and fatty acid-derived product ions are detected by the negative mode. As noted above, the positive/negative polarity switching time of the LCMS-8060 is only 5 msec.

In addition to CE and TG, the profiling method for major lipids developed in this work narrowed the number of MRM transitions to phospholipids and lysophospholipids which can be detected in human blood. About 360 MRM transitions were set so as to enable detection in 20 min. More than 100 lipids could be identified in an analysis in which 1 µL of human blood extract (equivalent to 10 nL of blood) was injected into the HPLC. Of these lipids, analysis of the combinations of fatty acids was possible for 51 PLs and 26 TGs.

<Phospholipids: PL>

It was possible to detect 51 phospholipids from a trace sample of human blood. Fig. 5 shows the MRM chromatograms of PE and the assignment results.

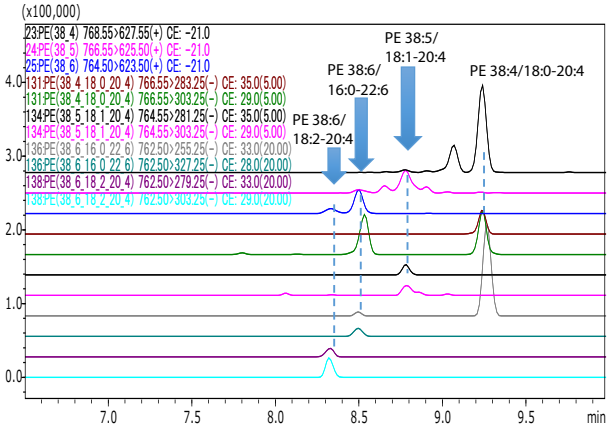

Fig. 5 MRM Chromatograms of PE38 : 4, PE38 : 5, and PE38 : 6

<Cholesteryl Esters: CE>

CEs were detected in the positive mode by 12 MRM transitions, in which *m/z* 369 was set as the product ion. CE hydroperoxide (CE-OOH) was also monitored. The strongest CE peak detected here was CE18 : 2, and the CE hydroperoxide CE-OOH18 : 2 was detected in the blood and blood serum samples.

<Triacylglycerols: TG>

In monitoring of TGs, more than 90 MRM transitions were applied with neutral loss product ions from target TG precursors. Fig. 6 shows the MRM chromatograms of TG 54 : 6 and the assignment results.

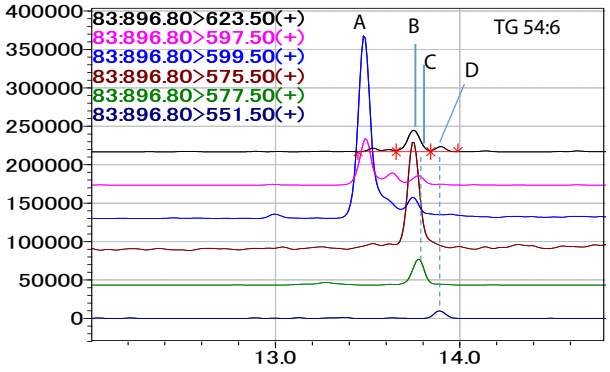

Fig. 6 MRM Chromatograms of TG 54 : 6
(A) TG 18 : 2-18 : 2-18 : 2 (B) TG 16 : 0-18 : 2-20 : 4
(C) TG 16 : 0-18 : 1-20 : 5 (D) TG 16 : 0-18 : 0-22 : 6 *1

＊1 The product ion with neutral loss of 18:0 was not monitored.

■ Conclusion

An MRM based profiling method of major lipids in blood was developed. By using this method, it was possible to detect more than 100 lipids from a trace sample of human blood and determine the combinations of fatty acids of the phospholipids and triacylglycerols.

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