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LCMS™-8060 High Performance Liquid Chromatograph Mass Spectrometer

Application News

Development of an Analytical Tool Using Metabolic Maps Corresponding to 196 Eicosanoids and Related Fatty Acid Metabolites

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User Benefits

- Can use the analytical tool with the metabolism map of 196 targets in "LC/MS/MS Method Package Lipid Mediator Ver. 3."
- By displaying quantitative differences in fatty acid metabolites on the metabolic map, the metabolic enzymes involved can be easily analyzed.

■ Introduction

Eicosanoids are lipid mediator species that are primarily part of the arachidonic acid cascade. The increasing sensitivity and high speed of LC/MS in recent years have led to the development of a method for comprehensively analyzing fatty acid metabolites of more than 100 targets, including not only arachidonic acid metabolites but also omega-3 fatty acid metabolites. The method is used to elucidate physiological functions and search for biomarkers.

We have developed a method for the analysis of 196 eicosanoids, the "LC/MS/MS Method Package Lipid Mediator Ver. 3." This article introduces an example of the analysis of metabolites detected in human plasma and serum by a newly developed analytical tool using the metabolic map display of the target 196 eicosanoids and related fatty acid metabolites. A total of 68 metabolites were detected in human plasma and serum by comprehensive analysis. Using this analytical tool, we were able to easily identify the enzymes involved in the detected metabolites.

■ Samples and Instruments

Standard samples were purchased from Cayman Chemical (Ann Arbor, MI). Human plasma and human serum were obtained from KOJIN BIO Co., Ltd. Two types of human plasma were used: EDTA plasma (EDTA Plasma) and heparin plasma (Heparin Plasma).

The LC-40 Series Nexera™ UHPLC system and the LCMS -8060 NX ultra-fast triple quadrupole mass spectrometer (Fig. 1) instruments were used for measurement. The analytical conditions for HPLC and mass spectrometry are shown in Table 1.

■ Sample Preparation

To 30 µL of human plasma and serum, 300 µL of methanol containing 0.1 % formic acid and 10 µL of 18 internal standards mixed solution were added and stirred for about 3 minutes. After centrifugation, the supernatant was diluted with 0.1% formic acid water and loaded into a solid-phase extraction cartridge. The extract was dried and dissolved in 30 µL of methanol, and 5 µL was used for LC/MS analysis. Each sample was analyzed three times.

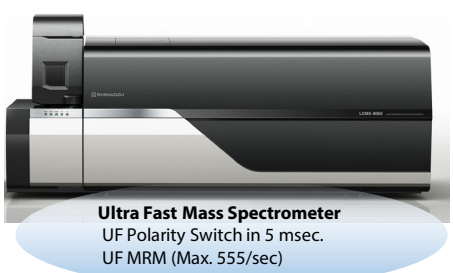

Fig. 1 Appearance of LCMS™-8060NX

■ Analytical Condition

Table 1 HPLC and Mass Spectrometry Conditions

[HPLC Conditions] (Nexera X3)	
Column:	Kinetex™ C8 (150 mm × 2.1 mm I.D., 2.6 µm)
Column Oven:	40 °C
Solvent A:	0.1 % Formic acid – water
Solvent B:	Acetonitrile
Flowrate:	0.4 mL/min
Injection Volume:	5 µL (co-injected with 15 µL of water)
[MS Conditions] (LCMS-8060NX)	
Ionization:	ESI, Positive/Negative
Mode:	MRM
Nebulizing Gas Flow:	2.5 L/min
Drying Gas Flow:	3.0 L/min
Heating Gas Flow:	15.0 L/min
DL Temp.:	200 °C
Block Heater Temp.:	450 °C
Interface Temp.:	300 °C
CID Gas Pressure:	230 kPa
Dwell Time/Pause Time:	7 msec./1 msec.

■ Results

A total of 68 eicosanoids were detected in 2 lots of 30 µL human plasma and an extract from serum by a comprehensive analysis of 196 eicosanoids. 67 targets excluding thromboxane B2 (TXB2) were detected in plasma, and 44 targets were detected in serum (Fig. 2).

Using the new analysis tool, quantitative profiles of 68 targets were shown on the metabolic map (Fig. 3). The area ratio obtained by dividing the peak area of each component by that of the corresponding internal standard is shown on the vertical axis of the graph.

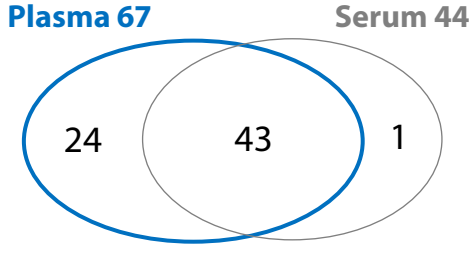

Fig. 2 Number of Compounds Detected in Human Plasma and Serum

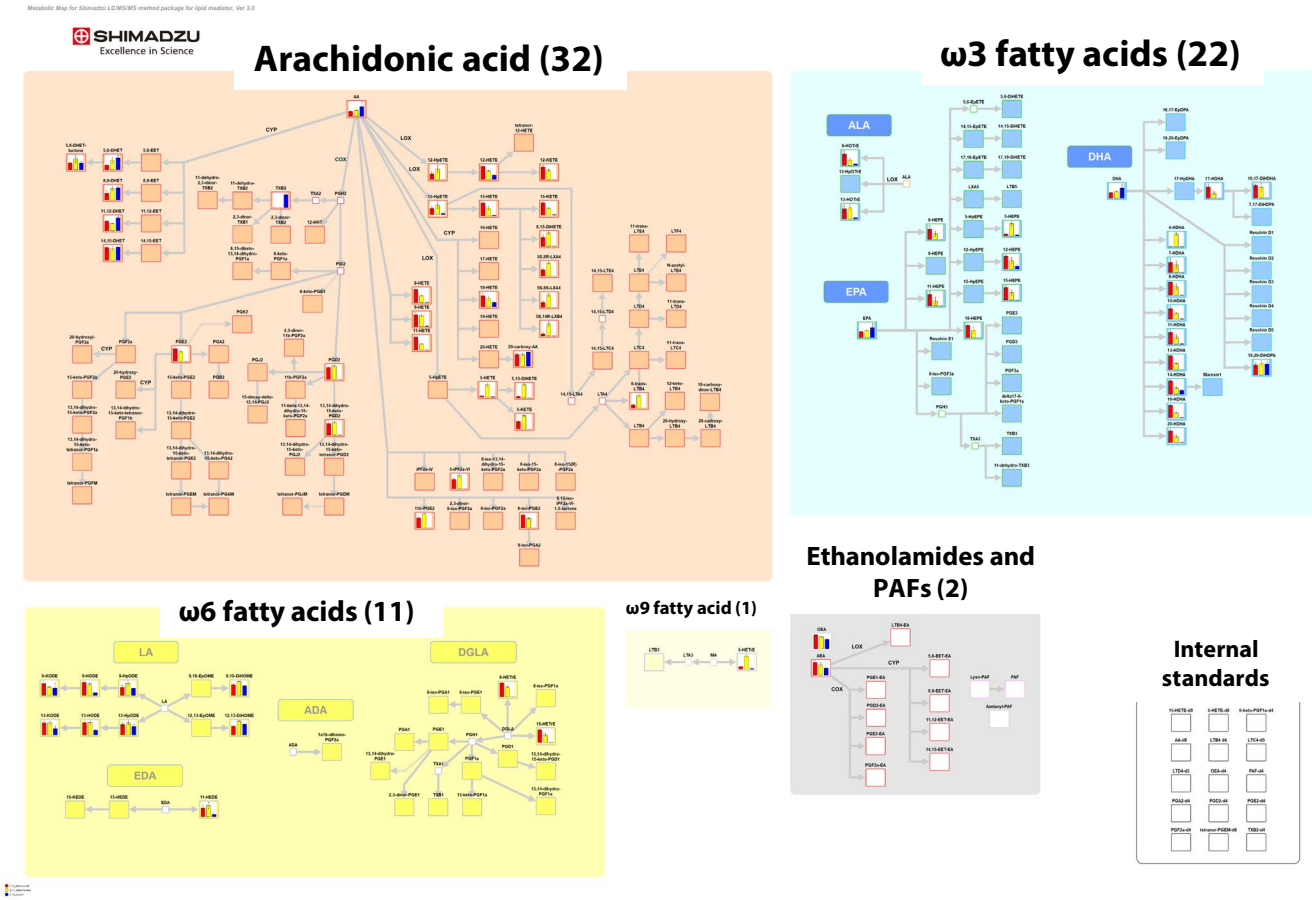

Fig. 3 Metabolic Maps and Quantitative Profiles of 68 Targets Detected in Human Plasma and Serum
Red: EDTA Plasma, Yellow: Heparin Plasma, Blue: Serum

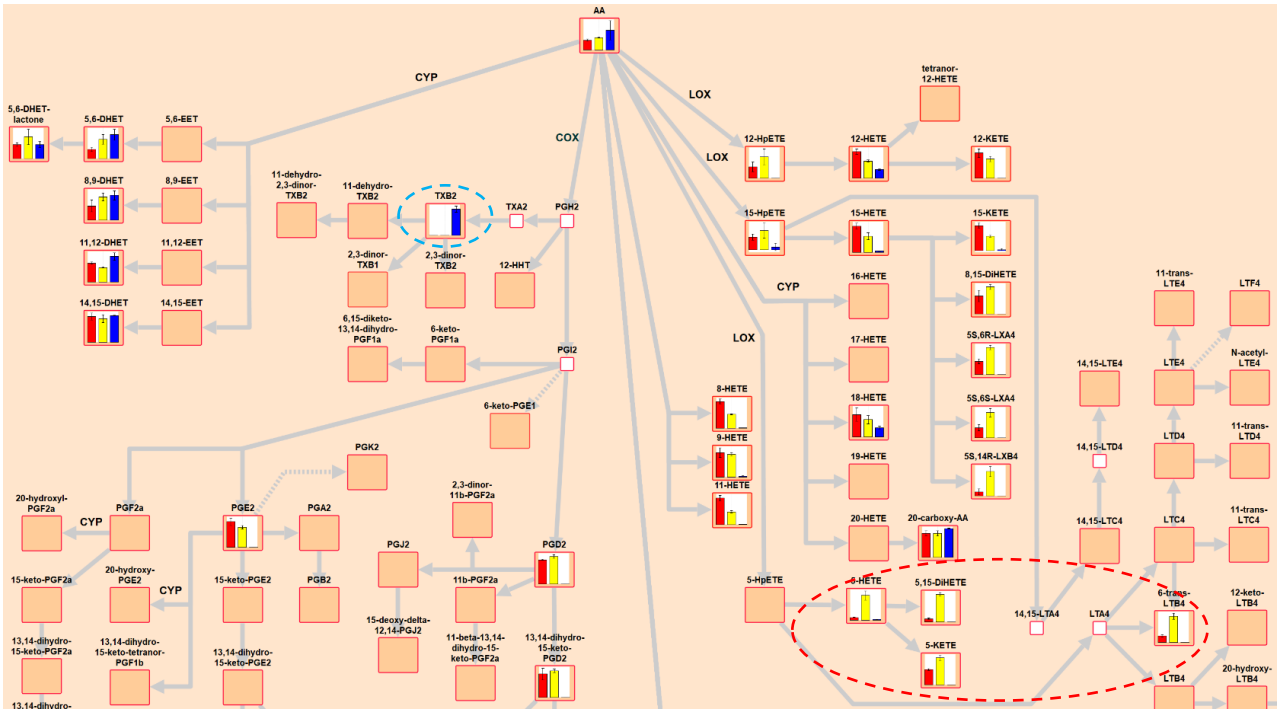

Fig. 4 Metabolic Maps and Quantitative Profiles of Arachidonic Acid Metabolites Detected from Human Plasma and Serum
Red: EDTA Plasma, Yellow: Heparin Plasma, Blue: Serum
TXB2 detected in serum is surrounded by a blue dashed line, and 5-LOX-induced metabolites significantly detected in heparin plasma are indicated by a red dashed line.

Abbreviations: CYP: Cytochrome P450, COX: Cyclooxygenase, LOX: Lipoxygenase, AA: Arachidonic acid, ADA: Adrenic acid, ALA: alpha-Linolenic acid, DGLA: Dihomo-gamma-linolenic acid, DHA: Docosahexaenoic acid, EA: Ethanolamide, EDA: Eicosadienoic acid, EPA: Eicosapentaenoic acid, LA: Linoleic acid, LPC: Lyso-phosphatidylcholine, MA: Mead acid, PAF: Platelet activation factor, DHET: Dihydroxyeicosatrienoic acid, EET: Epoxyeicosatrienoic acid, TX: Thromboxane, PG: Prostaglandin, HETE: Hydroxyeicosatetraenoic acid, HpETE: Hydroperoxyeicosatetraenoic acid, LX: Lipoxin, LT: Leukotriene, KETE: Keto-eicosatetraeinoic acid

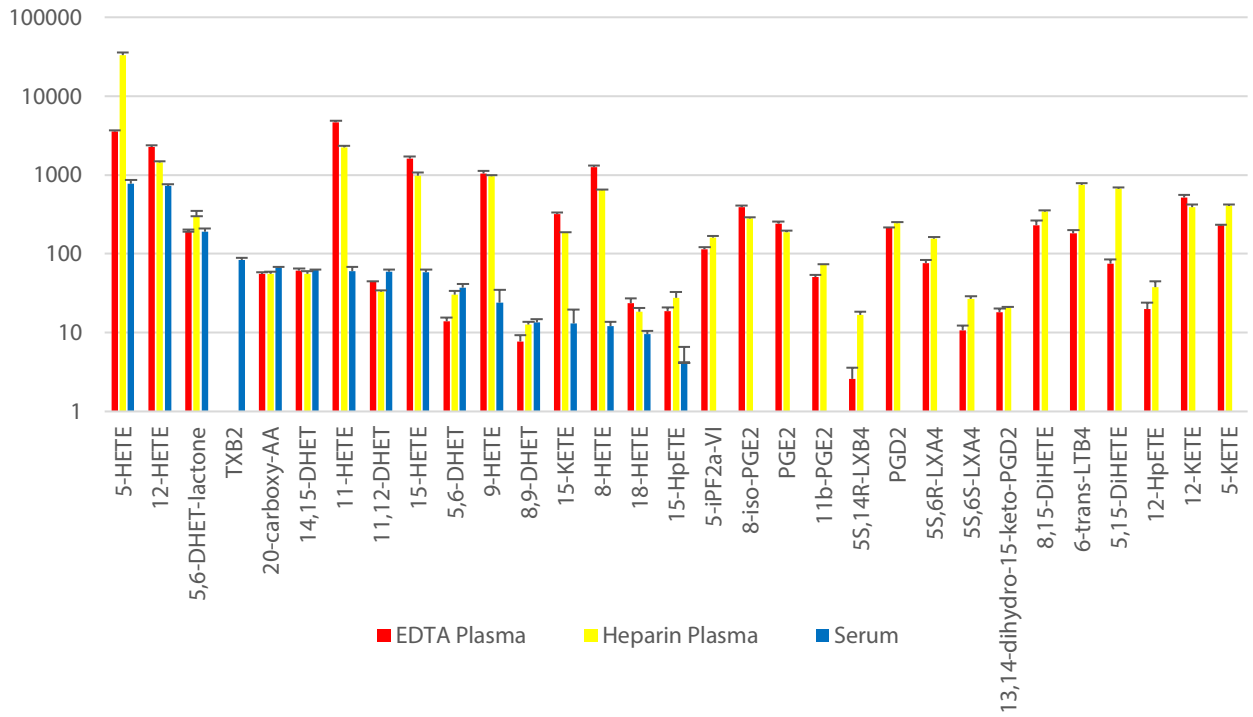

Fig. 5 Quantitative Profiling of 31 Arachidonic Acid Metabolites Detected from Human Plasma and Serum
The vertical axis shows the area ratio of the internal standard multiplied by 1000.

■ Results

In total, arachidonic acid and its 31 metabolites (32), EPA and DHA and 20 ω3 fatty acid metabolites (22), 11 targets of ω6 fatty acid metabolites, and three other targets including one mead acid metabolite and two ethanolamides were detected. As shown in Fig. 3, the fatty acids upstream of the detected metabolites were easily identified. This assay measures three free fatty acids, arachidonic acid, EPA, and DHA. All free fatty acids were in relatively high concentrations in serum, and fatty acid metabolites were generally abundant in plasma.

Fig. 4 shows an enlarged view of the arachidonic acid cascade. TXB2 (thromboxane B2), a stable metabolite of the blood clotting factor TXA2, was not detected in plasma, which clearly showed that the blood clotting factor was suppressed by EDTA and heparin. On the other hand, COX metabolites such as PGE2 and PGD2 were detected in plasma. The CYP metabolites DHETs and 20 carboxy- AA were not significantly different between plasma and serum. HETEs, LOX metabolites, were detected at significantly higher concentrations in plasma. 5-LOX metabolites, 5-HETE, 5, 15 DiHETE, and 6-trans-LTB4 were detected at significantly higher concentrations in heparin plasma.

Fig. 5 shows a quantitative profiling of 31 arachidonic acid metabolites. 1000 times the area ratio to the internal standard was shown on the vertical axis. The internal standard dosage for 31 targets is approximately 20 nmol/L, and the blood concentration of 20 nmol/L corresponds to approximately 1000 on the vertical axis.

The 5-HETE level in heparin plasma was approximately 660 nmol/L, which was the highest among the metabolites. The concentration of 5,6-DHET and 8,9-DHET, which were approximately 0.2 nmol/L, were measured with good reproducibility.

■ Conclusion

We have developed a data analysis tool using a metabolic map for the comprehensive analysis of 196 fatty acid metabolites, including eicosanoids. Using this analytical tool to analyze metabolites detected in human plasma and serum, we easily determined which fatty acids each metabolite was derived from and by which enzymes. Please use the LC/MS/MS Method Package Lipid Mediator Ver. 3. for your data analysis.

■ Acknowledgments

The metabolism map corresponding to the 196 targets analyzed in the lipid mediator method package ver. 3 was developed based on the metabolism map published in the paper (1) co-authored with Dr. Yoshihiro Kita and Dr. Suzumi Tokuoka of the department of Lipidomics, Graduate School of Medicine, The University of Tokyo.

■ References

1. Yamada M. et al., *J. Chromatography B*, 995, 74-84 (2015).

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