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Comparison of quantitative eicosanoids profiling in human plasma and serum by ultra-fast multiple reaction monitoring

FP-506

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1. Overview

A total of 68 eicosanoids and related metabolites were detected in human blood samples by a comprehensive MRM analysis. By showing quantitative profiles on the metabolic map using the newly developed tool, we could easily discriminate the enzymes related to target metabolite or the quantitative differences between serum and plasma.

2. Introduction

Eicosanoids profiling by MRM, multiple reaction monitoring, is widely used for understanding physiological functions and finding disease biomarker. However, it is not fully understood what eicosanoids can be detected in plasma and serum and how accurately they are quantified. Previously we developed an MRM based method, which can monitor 196 eicosanoids and related fatty acid metabolites. The method enabled to detect totally 68 targets in human plasma and serum. All the metabolites except for Thromboxane B2 (TXB_2) were detected in plasma sample. The difference of eicosanoid profiling between heparin plasma and EDTA plasma was also revealed by displaying quantitative profiles on the metabolic map using newly developed analysis tool.

3. Methods

Chemical standards were purchased from Cayman Chemical (Ann Arbor, MI). Pooled plasma and serum from human volunteer were obtained from Kojin-Bio Co. (Japan). Two types of human plasma, heparin plasma and EDTA plasma, were used. STRATA-X 10 mg (Phenomenex, Torrance, CA) cartridge was used for solid phase extraction (SPE). A Kinetex™ C8, 2.1 x 150 mm, 2.6 µm (Phenomenex, Torrance, CA) was used as analytical column. An LC/MS system consisting of *Nexera*™ UHPLC system and ultra-fast triple quadrupole mass spectrometer LCMS™-8060NX (Shimadzu Corporation) was used. Most of fatty acids were monitored in negative ion mode but ethanolamides or some others were in positive ion mode with 5 msec polarity switching.

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 to a solid-phase extraction cartridge. The extract was dried and dissolved with 30 µL of methanol and 5 µL was subjected to LC/MS analysis. Each sample analysis was triplicated.

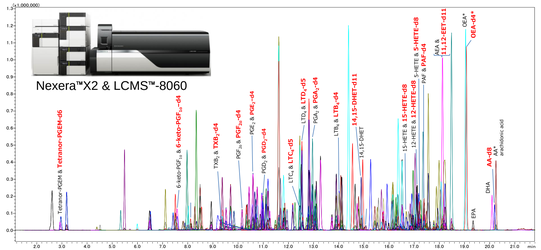

Fig. 1 Overlaid MRM chromatograms. A mixture of 190 standards and 18 internal standards mixture was analysed. Internal standards were shown in red.

4. Results

Previously, we developed the method for monitoring 196 target eicosanoids, related fatty acid metabolites and 18 internal standards in 20 minutes reversed phase chromatographic condition (Fig. 1).

A total of 68 eicosanoids were detected in EDTA plasma, heparin plasma and serum. 67 targets except TXB_2 were detected in plasma, and 44 targets were detected in serum (Fig. 2).

Fig. 3 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.

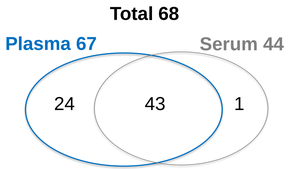

Fig. 2 Number of compounds detected in human plasma and serum

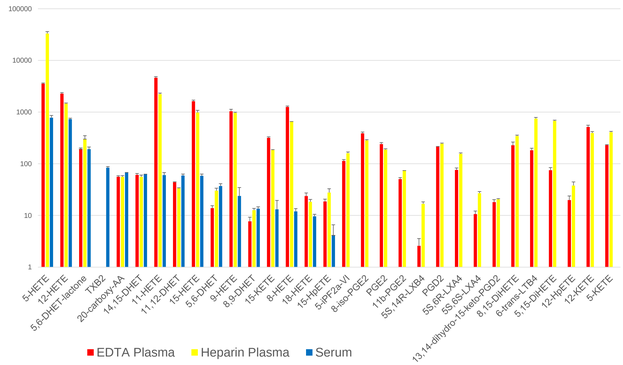

Fig. 3 Quantitative profiling of 31 arachidonic acid metabolites detected from human plasma and serum. The vertical axis shows the area ratio of the internal standard multiplied by 1000.

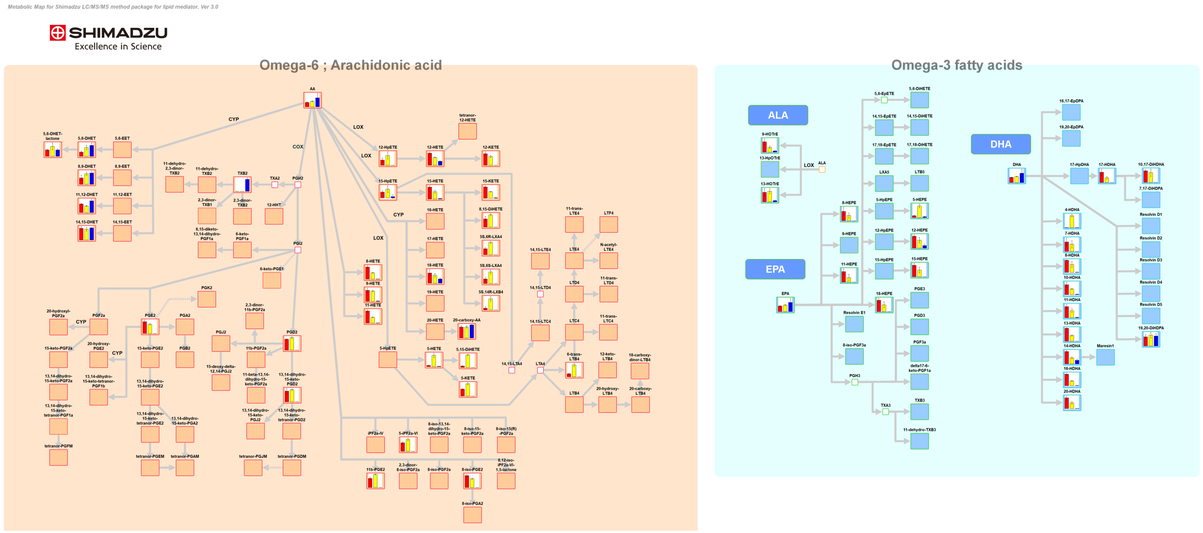

Fig. 4 Metabolic maps and quantitative profiles of the metabolites detected from human plasma and serum. Arachidonic acid metabolites were shown in left and omega-3 fatty acid metabolites were shown in right. Red: EDTA Plasma, Yellow: Heparin Plasma, Blue: Serum. Abbreviations: Cytochrome P450, COX: Cyclooxygenase, LOX: Lipoxygenase, AA: Arachidonic acid, ALA: alpha-linolenic acid, DHA: Docosahexaenoic acid, , EPA: Eicosapentaenoic acid, DHET: dihydroxyeicosatrienoic acid, EET: epoxyeicosatrienoic acid, TX: thromboxane, PG: Prostaglandin, HETE: hydroxyeicosatetraenoic acid, HpETE: hydroperoxyeicosatetraenoic acid, LX: lipoxin, LT: leukotriene, KETE: keto-eicosatetraeinoic acid.

Arachidonic acid (AA) and its 31 metabolites, EPA, DHA and 20 metabolites of ω3 fatty acids, 11 targets of ω6 fatty acid metabolites and 3 other targets including one mead acid metabolite and two ethanolamides were detected. Using the new analysis tool, quantitative profiles of 68 targets were shown on the metabolic map. The area ratio obtained by dividing peak area of each component by that of corresponding internal standard is shown on the vertical axis of the graph. Fig. 4 shows AA metabolites and omega-3 fatty acids metabolites on their map.

As shown in Fig. 4 the fatty acids upstream of the detected metabolites were easily identified. All free fatty acids, AA, EPA and DHA, were relatively high concentration in serum, and fatty acid metabolites were high in plasma. TXB_2 (thromboxane B2), a stable metabolite of the blood clotting factor TXA_2, was not detected in plasma, which clearly shown that blood clotting factor was suppressed by EDTA and heparin. On the other hand, COX metabolites such as PGE_2 and PGD_2 were detected in plasma. The CYP metabolites DHETs and 20 carboxy-AA were not significantly different between plasma and serum. HETEs, LOX metabolites, were highly detected in plasma. 5-LOX metabolites, 5-HETE, 5, 15 DiHETE, and 6-trans-LTB4, were detected at significantly higher concentrations in heparin plasma.

5. Conclusion

A total of 68 eicosanoids and related fatty acids and metabolites were detected in human blood samples by a comprehensive MRM analysis. We have newly developed a data analysis tool using the metabolic maps corresponding to target 196 fatty acid metabolites. This analytical tool enabled us to quantitative analysis of target metabolites detected in human plasma and serum by visualizing quantitative profiling on the metabolic map.

Reference

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