Quantitative Eicosanoids Profiling of Human Plasma and Serum: A New Data Processing Tool Using a Metabolic Map

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1. Introduction

Quantitative eicosanoids profiling is a key technology for finding biomarkers and searching pathophysiological mechanisms. MRM, multiple reaction monitoring enabled us to monitor hundreds of eicosanoids and related metabolites[1]. A data processing workflow developed using “Garuda platform” was shown in Fig. 1. Here we developed a novel metabolic map corresponding to 196 target eicosanoids and related metabolites. The processing tool has clearly shown where quantitative differences between human plasma and serum were in the eicosanoids metabolic map.

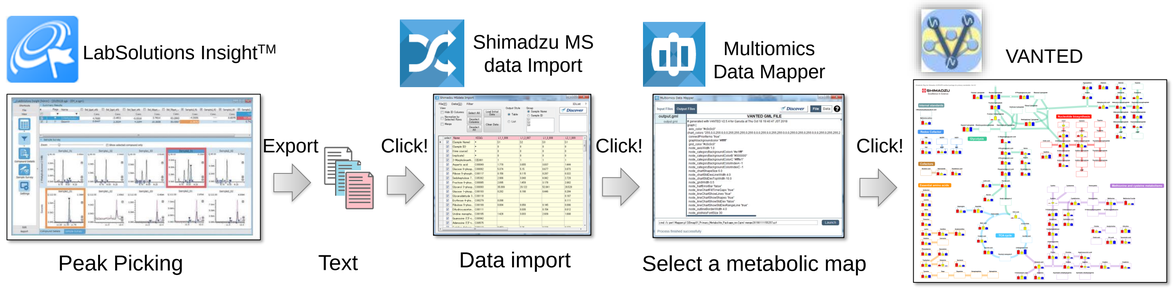


Fig. 1 Data processing workflow

2. Materials and Method

Two pooled human plasma, one is prepared by EDTA and the other was by heparin, and a human serum samples were obtained from Kojin-Bio Co. (Japan).

We have enlarged the targets to 196 eicosanoid related metabolites (Fig. 2). 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 and ethanolamides or some others were in positive ion mode with 5 msec polarity switching as reported previously[1].

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 a few minutes. After centrifugation, 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.

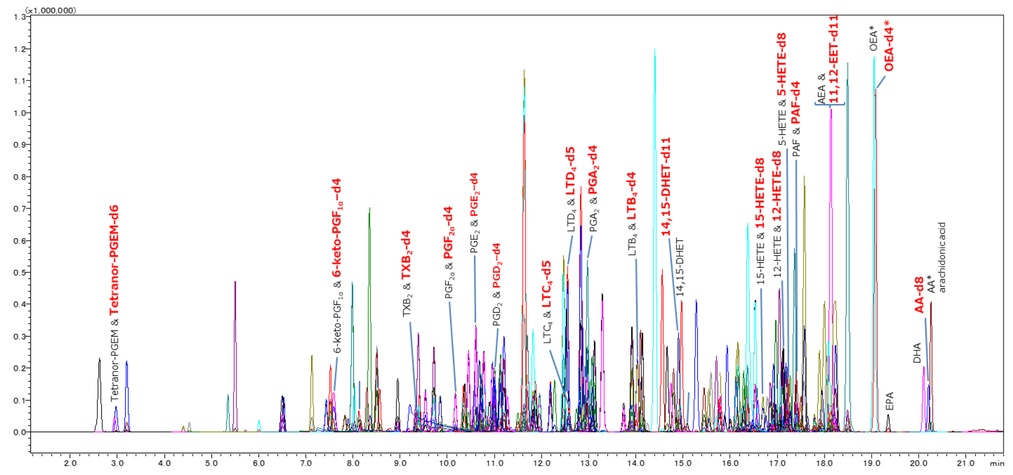
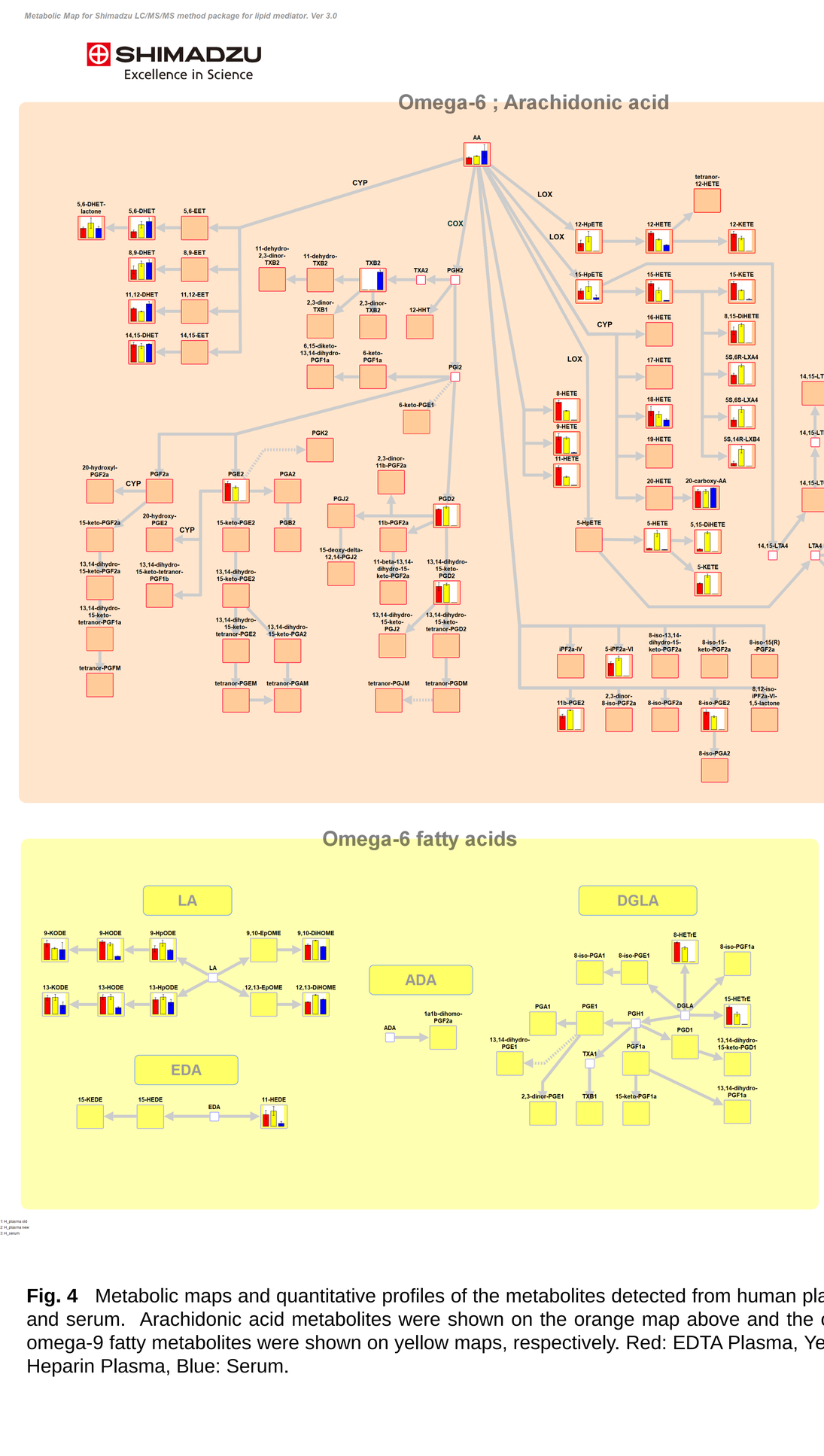


Fig. 2 Overlaid MRM chromatograms. A mixture of 190 standards and 18 internal standards mixture was analysed. Internal standards were annotated in red. The MRM based method consisted of over 300 MRM transitions for 196 target eicosanoid related metabolites.

3. Results

Totally 68 eicosanoid related metabolites were detected from methanol extracts of plasma and serum. 67 targets except for TXB_2 were detected in plasma, and 44 targets were detected in serum. 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. Quantitative profiles of 68 targets were shown on the metabolic map (Fig. 3). The area ratio obtained by dividing peak area of each component by that of corresponding internal standard was shown on the vertical axis of the graph.

As shown in Fig. 3 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-LTB_4, were detected at significantly higher concentrations in heparin plasma.



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.

Fig. 4 Metabolic maps and quantitative profiles of the metabolites detected from human plasma and serum. Arachidonic acid metabolites were shown on the orange map above and the other omega-9 fatty metabolites were shown on yellow maps, respectively. Red: EDTA Plasma, Yellow: Heparin Plasma, Blue: Serum.

Fig. 4 continued, Omega-3 fatty acid metabolites were shown on blue map above. Ethanolamides were shown on the gray map in left.

Red: EDTA Plasma, Yellow: Heparin Plasma, Blue: Serum.

4. Conclusion

A total of 68 eicosanoids and related fatty acid metabolites were detected in human blood samples by a comprehensive MRM analysis. We have newly developed a data processing tool using the metabolic maps corresponding to target 196 fatty acid metabolites. This analytical tool has clearly shown where were the quantitative differences between human plasma and serum.

Reference

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