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Application News

No.C113

Liquid Chromatography Mass Spectrometry

Lipid Mediator Profiling of Human Serum Using the Triple Quadrupole LC/MS/MS

Lipid mediator is a generic term for bioactive lipids, which play a role in many biological functions. Recent development of a high sensitivity ultra-fast mass spectrometer enables lower detection limits for lipid mediator species. A comprehensive and highly sensitive application for the analysis of lipid mediators and their metabolites using a triple quadrupole mass spectrometer LCMS-8060 is presented here. Conditions developed for "LC/MS/MS Method Package for Lipid Mediators Ver. 2", which can simultaneously analyze 158 lipid mediator-related compounds, were used as a basis for this application. Note that a single chromatographic analysis is capable of separating a wide range of species such as hydrophilic metabolites tetranor-PGs and hydrophobic arachidonic acid (Fig. 1) and both positive and negative ions are detected. Method sensitivity was compared between the LCMS-8060 and a conventional LCMS-8050 system. For the LCMS-8060, signal intensity was around 3 times higher than that of the LCMS-8050 (Fig. 2).

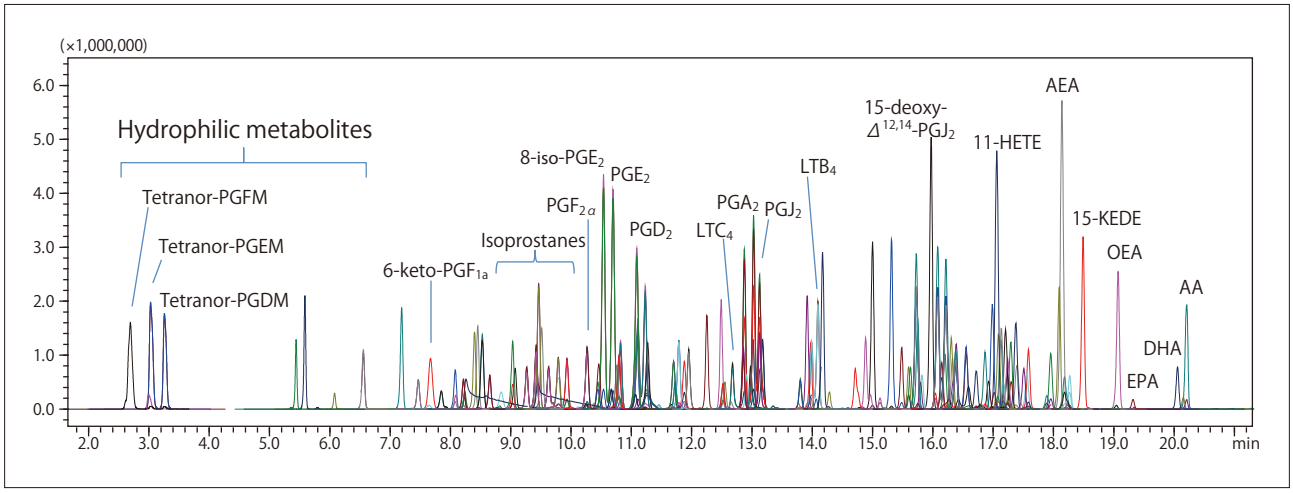

Fig. 1 MRM Chromatograms of Standard Mixture

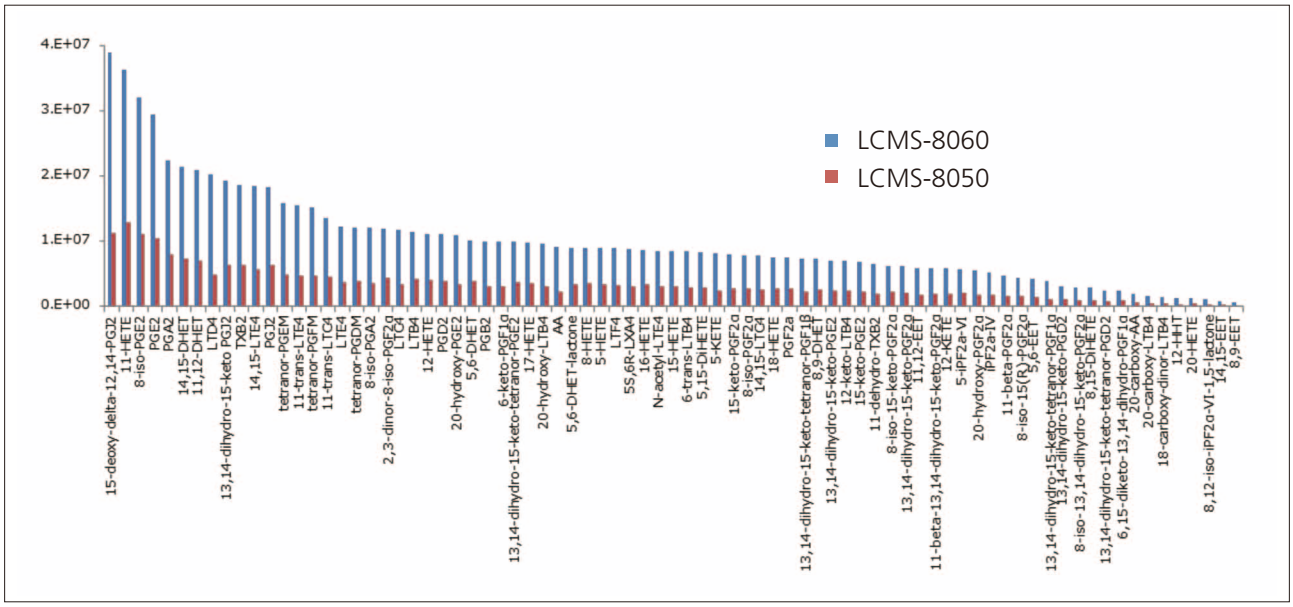

Fig. 2 Signal Intensity Comparison Abbreviations:. AA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, AEA: arachidonoylethanolamide, OEA: oleoylethanolamide, PG: prostaglandin, LT: leukotrien, TX: thromboxane, keto-eicosadienoic acid, DHET: dihydroxyeicosatrienoic acid, DiHETE: dihydroxyeicosatetraenoic acid, HETE: hydroxyeicosatetraenoic acid, EET: epoxyeicosatrienoic acid, HHT: heptadecatrienoic acid, EDE: eicosadienoic acid

The method was applied to analysis of lipid extracts from human serum. One mL of methanol and 10 µL of internal standard mixture were added to 200 µL of human serum and mixed for three minutes. After centrifugation, solid phase extraction (SPE) was conducted following elution with 300 µL of methanol. An analysis of a 10-fold concentrated SPE eluent resulted in the detection of 85 peaks with signal-to-noise ratio (S/N) of greater than 3 and a retention time difference of less than 1 second from primary standards. Internal standard quantitation showed that the concentrations of 5-HETE, the most abundant of all metabolites, and 12-HHT, the least abundant, were 1 µM and 0.5 nM, respectively. 8-*iso*-$PGF_{2\alpha}$, an oxidative stress marker, was 0.1 nM. Fig. 3 shows replicate analysis (N = 5) in human serum for three example analytes, %CV results are also shown. Analysis results indicate that the LCMS-8060 is capable of quantitative profiling in a wide dynamic range from sub-nM to µM. Because of the potential for interfering peaks, MRM analysis often requires confirmation ions. Fig. 4 shows consistent reference ion ratios in the analysis of 20-carboxy arachidonic acid. The LCMS-8060 offers ultra-fast MRM analysis, allowing the acquisition of multiple MRM transitions while maintaining high data quality.

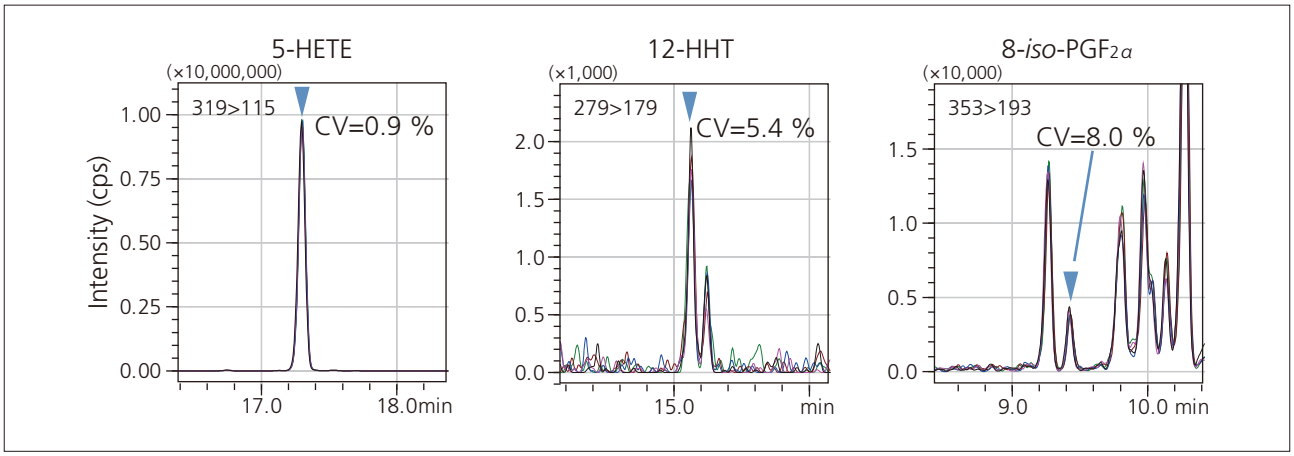

Fig. 3 Replicate MRMs of 5-HETE, 12-HHT and 8-*iso*-$PGF_{2\alpha}$ ▼: assigned peak

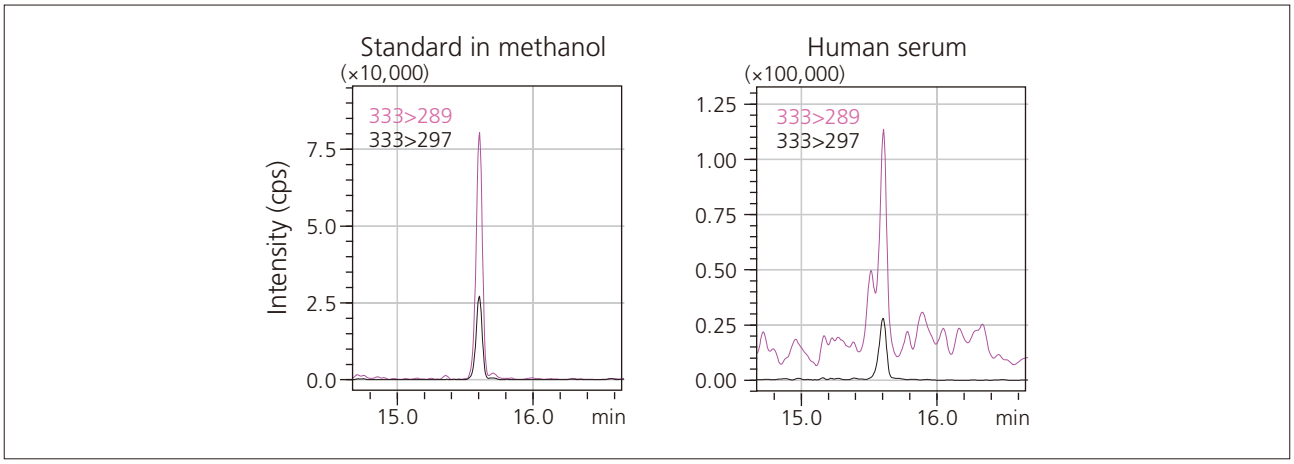

Fig. 4 Comparison of MRM Chromatograms of 20-Carboxy Arachidonic Acid in Standard and Human Serum

HPLC Conditions

Column	: Phenomenex Kinetex C8 (150 mm L. × 2.1 mm I.D., 2.6 µm)
Mobile Phase A	: 0.1 % Formic acid – Water
Mobile Phase B	: Acetonitrile
Gradient Program	: 10 %B. (0 min) → 25 %B. (5.0 min) → 35 %B. (10.0 min) → 75 %B. (20.0 min) → 95 %B. (20.1 - 25.0 min)
Flowrate	: 0.4 mL/min.
Injection Volume	: 5 µL
Column Oven Temp.	: 40 °C

MS Conditions

Ionization	: ESI (Positive/Negative)
Nebulizing Gas Flowrate	: 3.0 L/min.
Drying Gas Flowrate	: 10.0 L/min
Heating Gas Flowrate	: 10.0 L/min
DL Temp.	: 250 °C
Block Heater Temp.	: 400 °C
Interface Temp.	: 300 °C
CID Gas Pressure	: 230 kPa

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