

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).

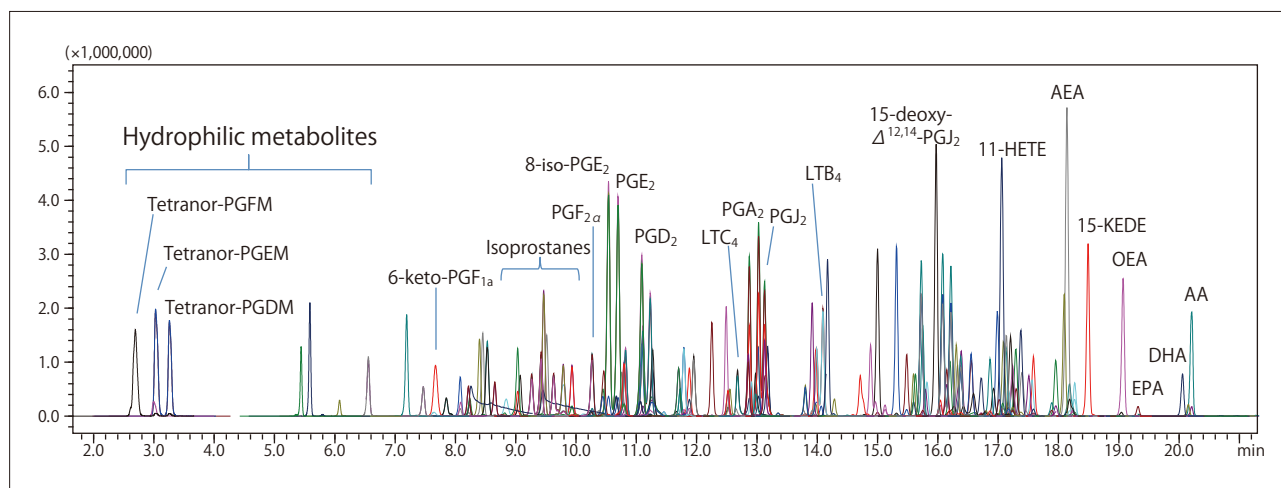


Fig. 1 MRM Chromatograms of Standard Mixture

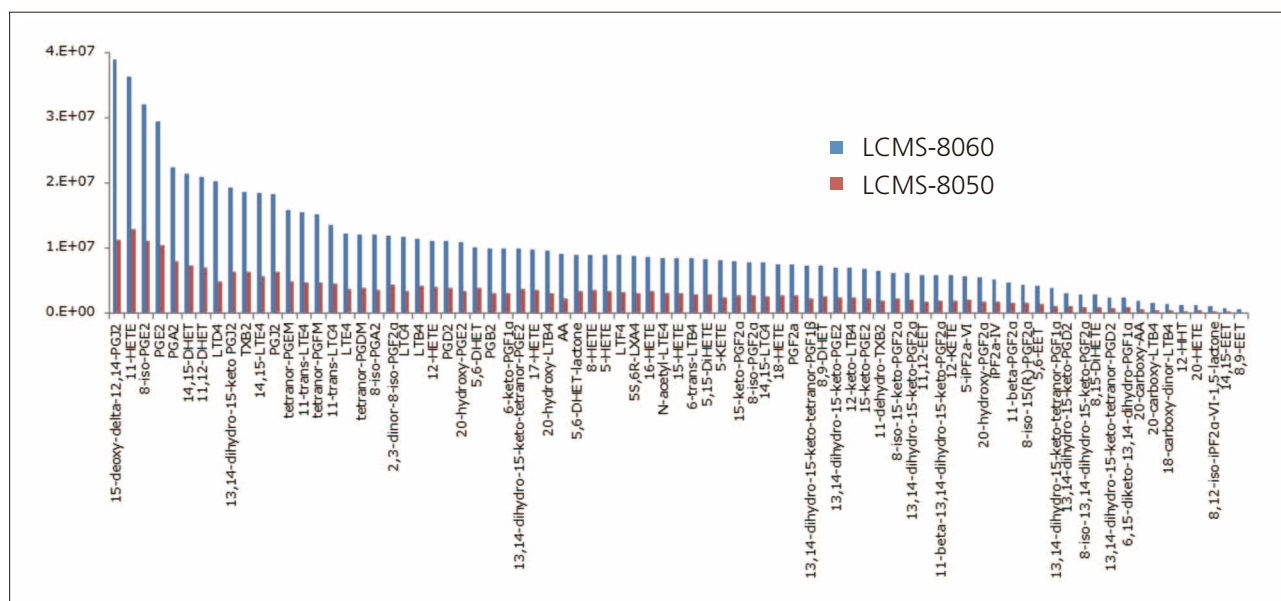


Fig. 2 Signal Intensity Comparison Abbreviations: AA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, AEA: arachidonylethanolamide, OEA: oleoylethanolamide, PG: prostaglandin, LT: leukotrien, TX: thromboxane, keto-eicosadienoic acid, DHET: dihydroxyeicosatrienoic acid, DiHETE: 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 μ L of internal standard mixture were added to 200 μ L of human serum and mixed for three minutes. After centrifugation, solid phase extraction (SPE) was conducted following elution with 300 μ L 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 μ M and 0.5 nM, respectively. 8-*iso*-PGF_{2 α} , 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 μ M. 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.

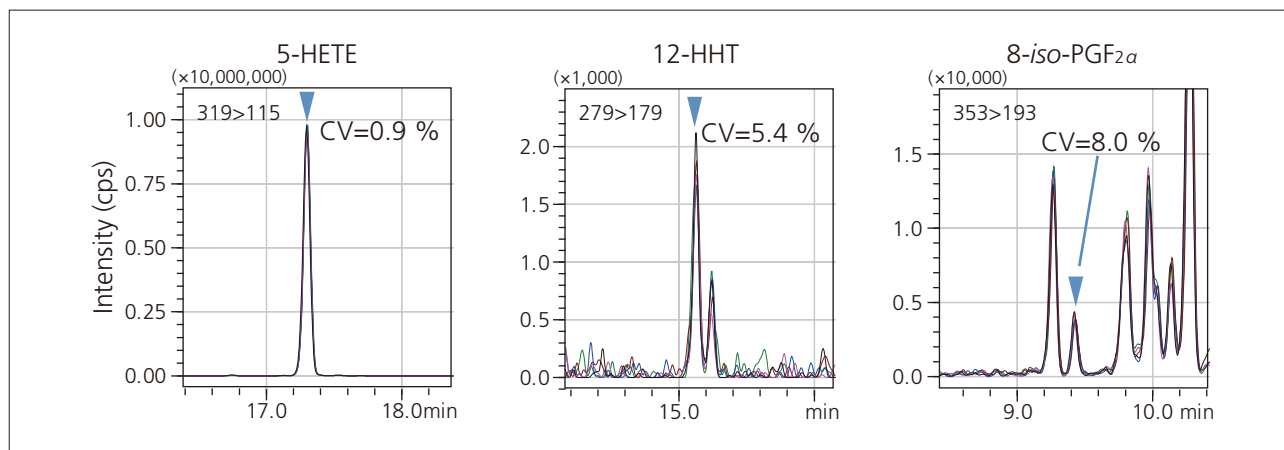


Fig. 3 Replicate MRMs of 5-HETE, 12-HHT and 8-*iso*-PGF_{2 α} ▼: assigned peak

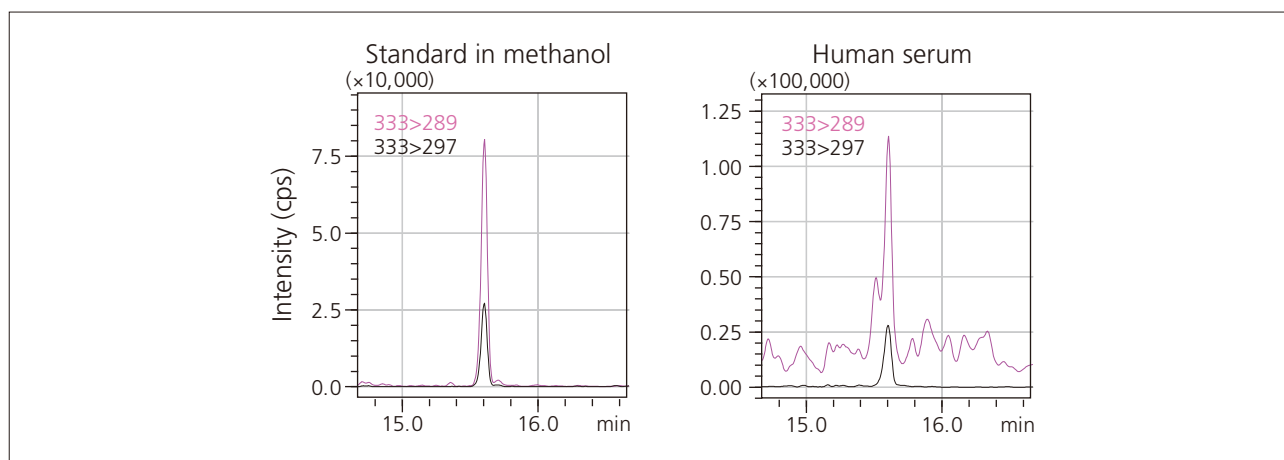


Fig. 4 Comparison of MRM Chromatograms of 20-Carboxy Arachidonic Acid in Standard and Human Serum

HPLC Conditions

Column	: Phenomenex Kinetex C8 (150 mm L. × 2.1 mm I.D., 2.6 μ m)
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 μ L
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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