Application Note: 336

Ultra-sensitive LC/MS/MS Quantitation of Lipids in Biological Fluids Using Electron Capture APCI

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Key Words

- Finnigan™ TSQ Quantum Ultra™
- APCI
- Electron Capture
- Normal-phase chromatography

Introduction

Characterization of biologically active lipids and their metabolites, or lipidomics, is a growing area of research that is complementary to proteomics and metabonomics approaches. However, unlike most components in proteomics and metabonomics, understanding the biological activity of several lipids requires measurement of the different stereoisomers of those lipids. This application report describes the methodology and preliminary results, where normal-phase chiral liquid chromatography is employed to separate the enantiomers of hydroxy fatty acids produced from arachidonic acid and linoleic acid by lipoxygenases and cyclooxygenases.[1] High-sensitivity detection of these lipid metabolites is possible using derivatization with the pentafluorobenzyl (PFB) moiety[2] and employing electron capture atmospheric pressure chemical ionization (APCI), followed by analysis using selected reaction monitoring (SRM) on a triple quadrupole mass spectrometer.

Goal

- Develop an ultra-sensitive method for quantifying lipids in biological fluids
- Demonstrate the selectivity and sensitivity afforded by enhanced resolution SRM analysis
- Use PFB derivitization to improve analytical limits of quantitation (LOQs)

Experimental Conditions

Rat Intestinal Epithelial Cell Extraction and Preparation

Lipid extracts from rat intestinal epithelial (RIE) cells are derivatized to the corresponding pentafluorobenzyl-derivatized hydroxy fatty acids. Normal phase chiral LC was used to separate (R) and (S) enantiomers of lipid metabolites. Detection was achieved with a triple quadrupole mass spectrometer in SRM mode after ionization by electron capture APCI.

Three mL of cell media were extracted with diethyl-ether twice after adjusting to pH = 3. After reconstitution in acetonitrile, the solution was treated with PFB bromide and diisopropylethylamine and heated to 60 °C for one hour. The solution was allowed to cool, evaporated to dryness, and re-dissolved in hexane prior to analysis on the Finnigan TSQ Quantum Ultra.

HPLC

- Column: 4.6 × 250 mm CHIRALPAK® AD-H
- Pumps: Hitachi L-2100 and Finnigan Surveyor™
- Autosampler: Hitachi L-2200
- Mobile phase: (A) hexane, (B) 1:1 IPA/MeOH
- Flow rate = 1.0 mL/min
- Injection volume = 10 µL
- Post-column addition solution: 1:1 IPA/MeOH at 0.75 mL/min

Mass Spectrometer

- Finnigan TSQ Quantum Ultra
- APCI vaporizer temperature = 450 °C
- APCI discharge current = 30 µA
- Ion transfer tube temperature = 250 °C
- Source CID = 5 V

Selected Reaction Monitoring (SRM) Parameters

- Q2 pressure = 1.0 mTorr argon
- SRM transitions and collision energies: see Figures 1 and 2
- SRM scan time = 150 ms per transition
- Q1 resolution: 0.7 Da FWHM and 0.2 Da FWHM
- Q3 resolution: 0.7 Da FWHM

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Results and Discussion

Sensitivity of Electron Capture APCI for PFB-Derivatized Lipids

LOQs of 10–25 pg/mL (100-250 fg on column) were achieved for pentafluorobenzyl-derivatized hydroxy fatty acids (Figures 1 and 2). Post-column addition of 1:1 IPA/MeOH was used to suppress the formation of an electrically conductive "hair-like" filament on end of corona discharge needle at high discharge currents (>20 µA). The post-column addition did not decrease the sensitivity for the lipid metabolites (data not shown). Enhanced-resolution SRM show a two- to four-fold improvement in S/N with minimal loss in absolute signal for some lipid metabolites (Figure 3).

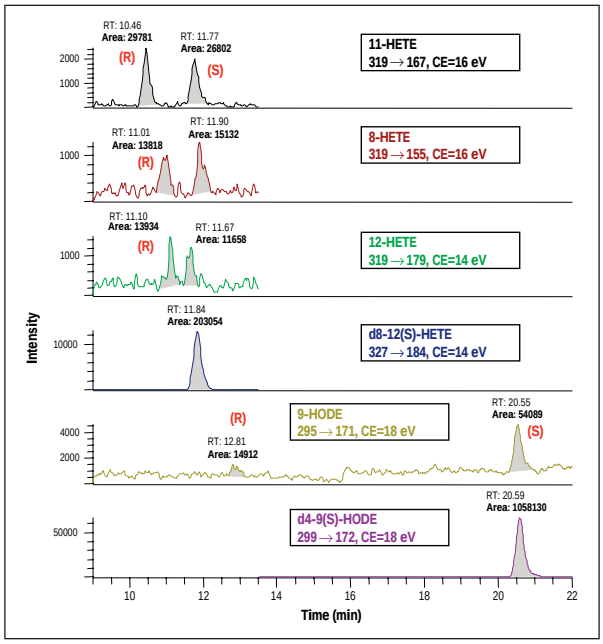

Figure 1: 10 pg/mL PFB-derivatized hydroxy fatty acid standards. Deuterated internal standards are at 500 pg/mL.

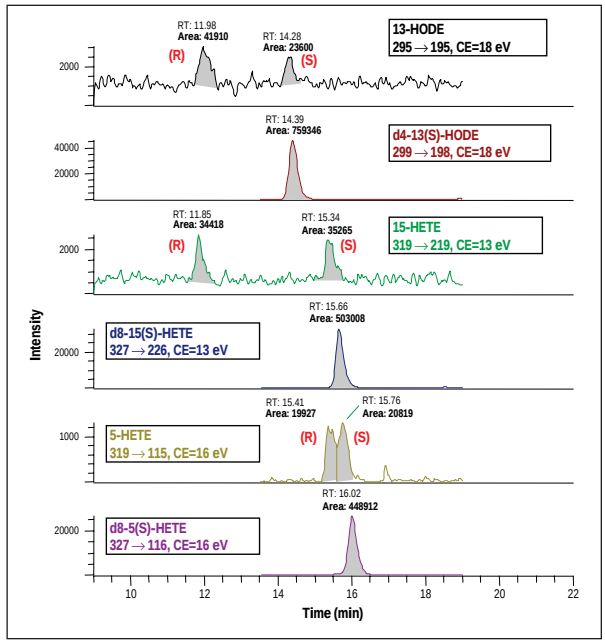

Figure 2: 25 pg/mL PFB-derivatized hydroxy fatty acid standards. Deuterated internal standards are at 500 pg/mL.

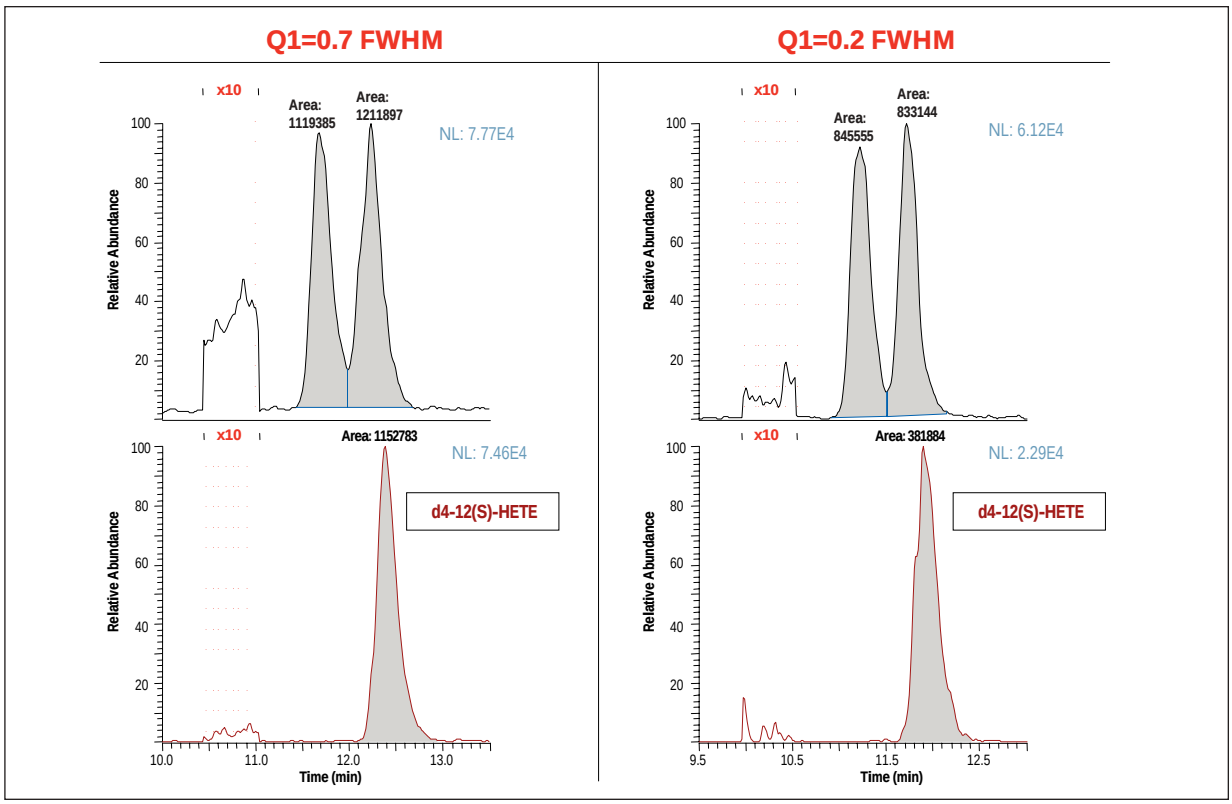

Figure 3: Chromatograms for 500 pg/mL 12-HETE and d4-12(S)-HETE using Unit-resolution SRM (left column) and Enhanced-resolution SRM (right column).

Quantitation of PFB-Derivatized Lipid Metabolites

Figures 4 and 5 illustrate the calibration curves for the PFB-derivatized hydroxy fatty acid, 5(S)-HETE, in pure HPLC-grade solvent and spiked into the cell incubation medium, respectively. Although 1-point calibrations were employed for the lipid metabolites, the linear regression values (i.e., R^2) were > 0.990 and the relative errors were < ±20% above the LOQs. LOQs for the lipid metabolites spiked into cell media were 100-200 pg/mL (1–2 pg on-column), which were 4- to 10-times higher than from standards diluted in HPLC-grade solvent (10–25 pg/mL). This is likely attributed to increased background and decreased ionization efficiency due to matrix interferences.

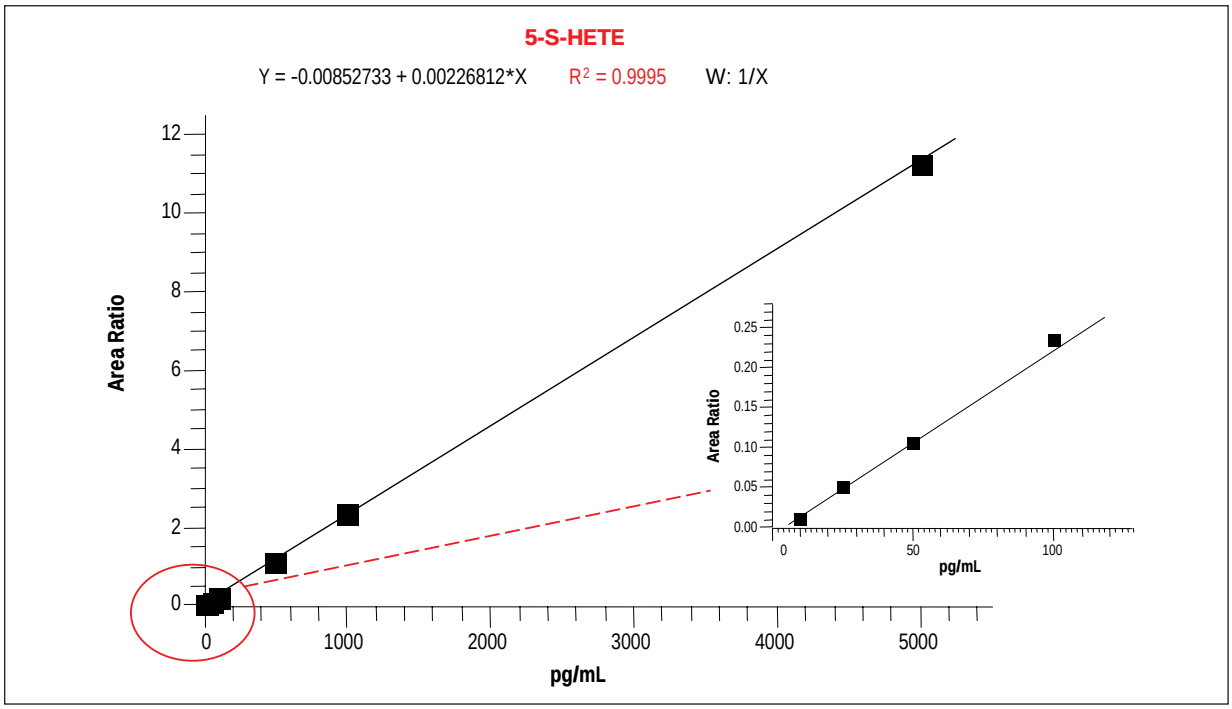

Figure 4: Calibration curve for 5(S)-HETE prepared in HPLC-grade solvent using 500 pg/mL d8-5(S)-HETE as the internal standard.

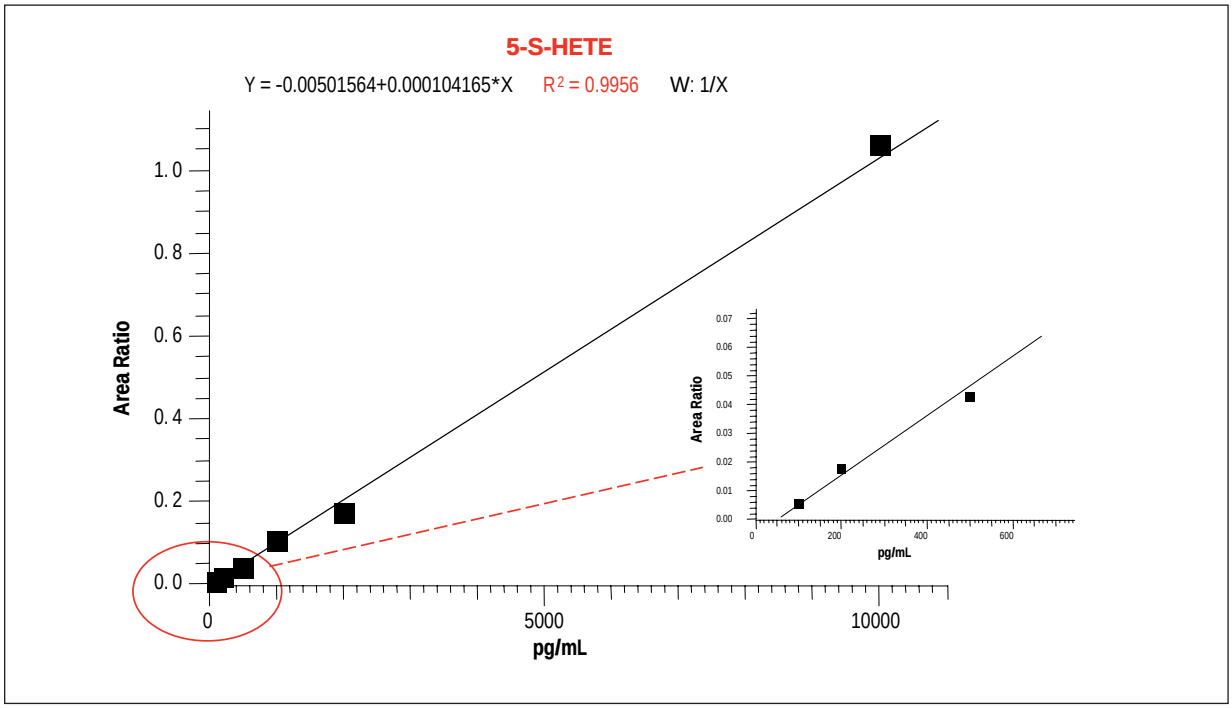

Figure 5: Calibration curve for 5(S)-HETE spiked into cell culture media using 10 ng/mL d8-5(S)-HETE as the internal standard.

Concentration of 5-HETE in Aspirin-incubated RIE Cells

RIE cells incubated with aspirin show that a near equivalent amount of 5(R)- and 5(S)-HETE are produced (see Figure 6 and Chart 1). Analysis of a control RIE cell extract did not show the presence of 5(R)- or 5(S)-HETE (data not shown). The near racemic mixture of (R) and (S) isomers suggests that 5-HETE is formed by non-enzymatic means in aspirin-incubated RIE cells.

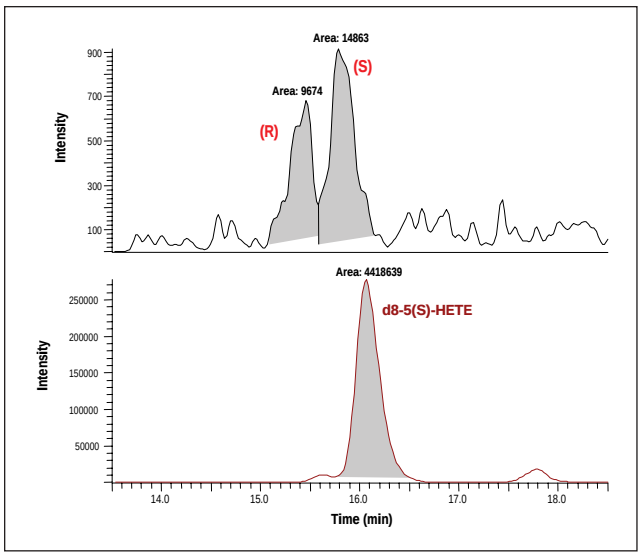

Figure 6: Chromatograms for 5-HETE and d8-5(S)HETE from Aspirin-incubated rat intestinal epithelial cell extracts.

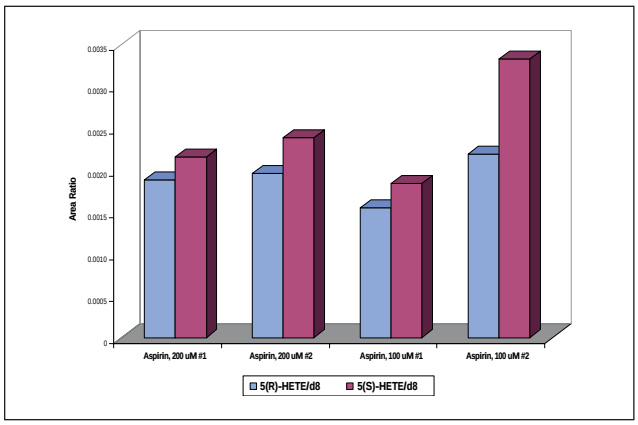

Chart 1: Measurement of (R) and (S) enantiomers of 5-HETE in Aspirin-incubated rat intestinal epithelial cells.

Conclusions

Derivatization of hydroxy fatty acids with pentafluorobenzyl bromide affords low-level detection using electron capture APCI and selected reaction monitoring. The unique enhanced-resolution SRM capability of the Finnigan TSQ Quantum Ultra increased the selectivity and sensitivity of certain PFB-derivatized lipid metabolites, leading to improved LOQs compared with unit-resolution SRM. In addition, enhanced-resolution SRM provides higher confidence in the LOQs when analyzing target analytes in samples prepared from complex biological matrices. The LOQs for the lipid metabolites were 4–10 times higher in the biological cell media versus pure solvent solutions. Incubation of RIE cells with aspirin showed a near equivalent concentration of 5(R)- and 5(S)-HETE, suggesting that 5-HETE is formed by a non-enzymatic pathway under these conditions.

References

(1) Lee, S.H.; Williams, M.V.; DuBois, R.N.; Blair, I.A. *Rapid Commun. Mass Spectrom.*, **2003**, *17*, 2168-2176.

(2) Singh, G.; Gutierrez, A.; Xu, K.; Blair, I.A. *Anal. Chem.*, **2000**, 72, 3007-3013.

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