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The Innovation Solution of Eicosanoids and Related Oxylipins Profiles as Biomarkers by LC-MS/MS in Uterine Fibroids Disease

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Introduction

Certain lipids act as potent signaling molecules that regulate a multitude of cellular responses, including cell growth and death and inflammation/infection, via receptor-mediated pathways. These include oxylipins, which are small bioactive lipid mediators of human physiology and inflammation. Oxylipins involved in inflammation resolution are called specialized pro-resolving lipid mediators, SPMs, and oxylipins derived from 20-carbon fatty acid such as DGLA, AA, or EPA are known as eicosanoids[1,2] (Figure 1).

Developing a dynamic MRM method for oxylipin detection on an LC/TQ system is challenging when standards are not available (e.g., retention times (RTs) are unknown for target oxylipins). Using an innovative approach, and a few available standards, we were able to develop an LC/MS/MS-based method for over 100 oxylipins by predicting retention times based on those already published on a different LC system [1] and verified the approach with purchase and analysis of matching standards. This enabled the evaluation of lipids as potential mechanistic biomarkers in uterine fibroids disease and outlines an efficient approach to method development when standards are unavailable.

A targeted quantitative analysis of 45 oxylipin standards was undertaken to evaluate the reproducibility, recovery, and sensitivity of this method in biological fluid samples. The innovative approach enabled discovery of oxylipin profiles as functional characteristics of inflammatory responses in uterine fibroids pre- and post-treated with high-intensity focused ultrasound (HIFU).[3]

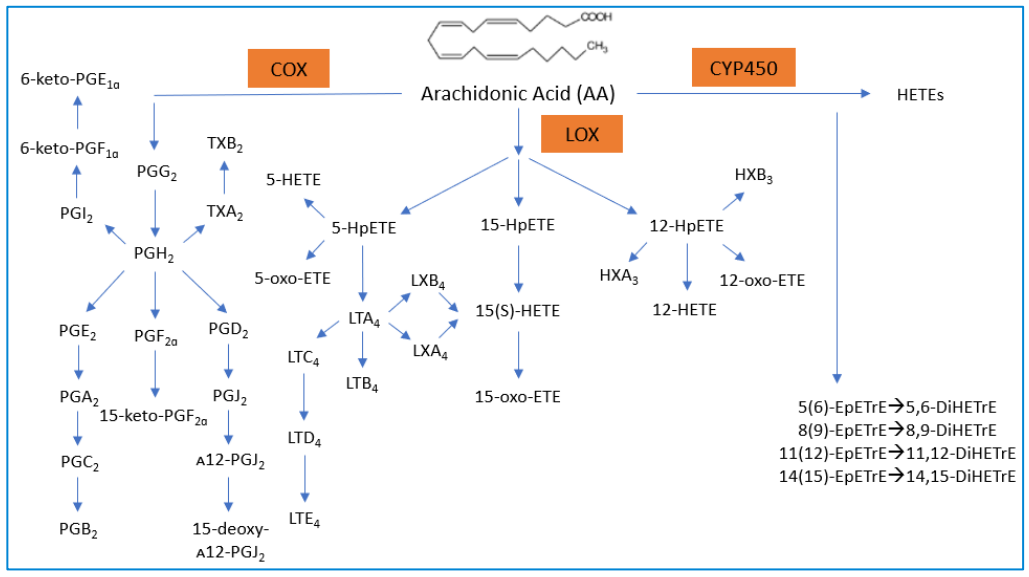

Figure 1. Schematic representation of oxylipin metabolic pathways. The figure shows oxylipins produced from AA in n-6 PUFA. It is noted that several oxylipins can be formed by enzymatic and auto-oxidation pathways. COX: cyclooxygenase; LOX: lipoxygenase; CYP450: cytochrome P450.

Experimental

A total of 125 oxylipins from key PUFA pathways were analyzed. Of these, 45 had available standards that allowed quantitative analysis; others were subjected to qualitative analysis (Figure 2).

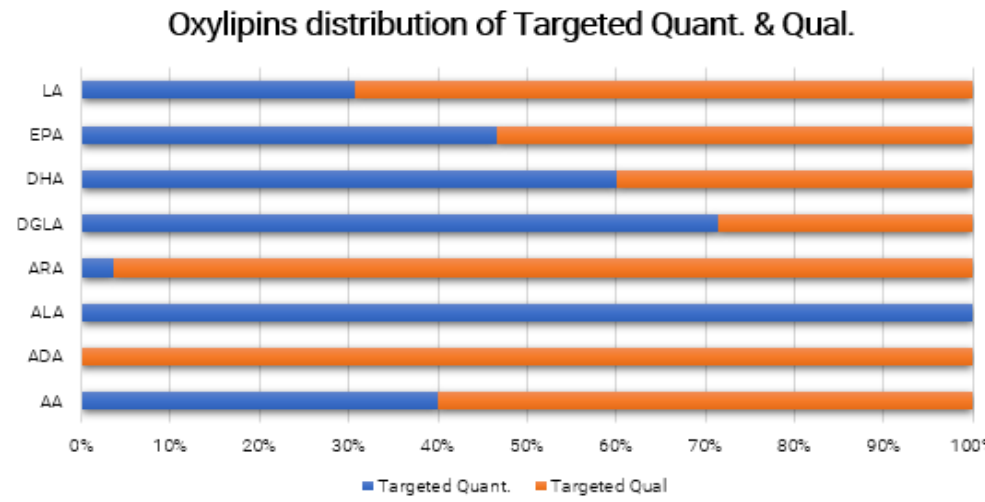

Figure 2. Percentage of the metabolites for which targeted quant or qual was performed with respect to those metabolites analyzed per pathway: AA-50, ADA-1, ALA-2, ARA-27, DGLA-7, DHA-10, EPA-15, LA-13.

1290 Infinity II UHPLC Method 1

Column: Agilent Poroshell EC-C18, 3.0 x 150 mm, 1.8 µm

Mobile phase A (MPA): 0.1% acetic acid in water

Mobile phase B (MPB): 10% isopropanol/90% acetonitrile (v/v)

Column oven: 40°C

The linear gradient was used with flow rate of 0.5 mL min^{-1}; the total run time was 30 min.

6495 LC/TQ System

Ion source: AJS

Nebulizer gas: 35 psi

Dry gas: 11 L min^{-1}

Dry gas temperature: 230°C

Sheath gas: 12 L min^{-1}

Sheath gas temperature: 350°C

Nozzle voltage: 1250 V

Capillary voltage: 3500 V

iFunnel voltage: low RF-60V; high RF-90V

Scan mode: dynamic MRM scan

Polarity scan mode: negative mode

Results and discussion

- 10 oxylipins, for which standards were available, were run on the 1290 UHPLC and RTs plotted *versus* published RTs measured with the 1260 RRLC [1] and a correlation (simulation) curve fitted (Figure 3).
- The RT of a 125 oxylipins measured empirically on the 1260 RRLC can be predicted for the 1290 UHPLC, and respective LC conditions, using the fitted correlation curve and equation in the absence of standards (Figure 3).
- To verify the approach matching oxylipin standards were purchased and ran on the 1290 UPLC to verify the accuracy of the RT predictions.

This enabled simultaneous detection of 125 oxylipins, including 45 targeted for quantitative analysis, with the remainder analyzed qualitatively in plasma samples.

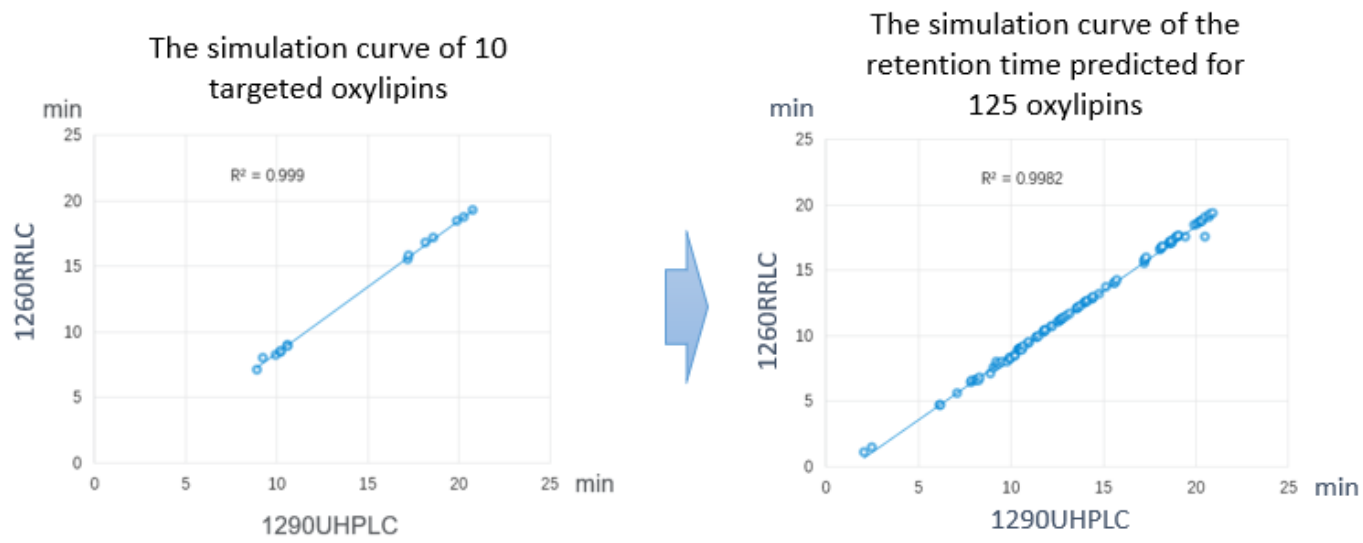

Figure 3. The simulation curve was used to predict the behavior of oxylipin separation with C18 column analysis and correlation with the retention times of 1260RRLC from reference article [1].

The resulting easy, quick, and convenient solution demonstrated excellent chromatographic separation and peak shape for groups of isomeric oxylipins (e.g., HETE) (Figure 4).

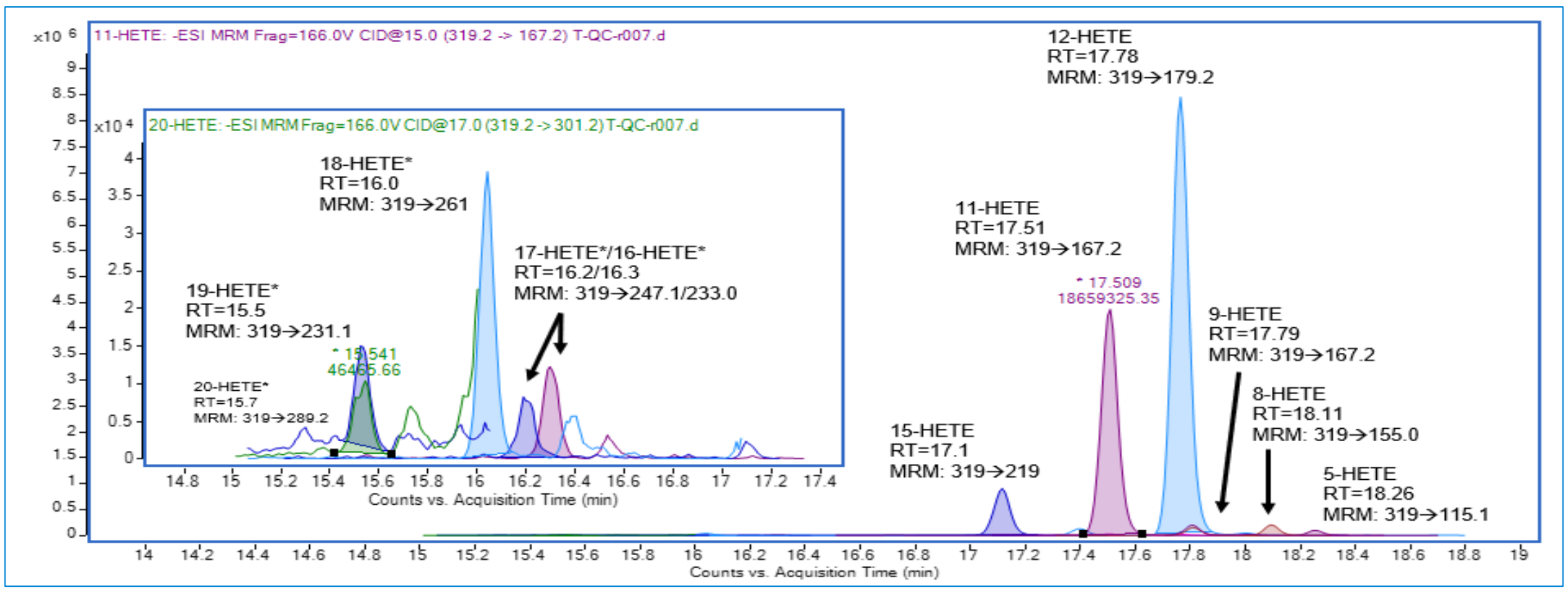

Figure 4. A mixture containing multiple HETEs was well separated into key oxylipins, including 5-HETE, 8-HETE, 9-HETE, 12-HETE, 11-HETE, 15-HETE, 16-HETE, 17-HETE, 18-HETE, 19-HETE, and 20-HETE.

Results and discussion

The sample preparation utilized HLB SPE. The recovery of 41 quantified oxylipins were found to be in the range of 50–120%, and the detection limit (DL) was 0.01–0.05 ng ml^{-1} for quantitative analysis (Figure 5).

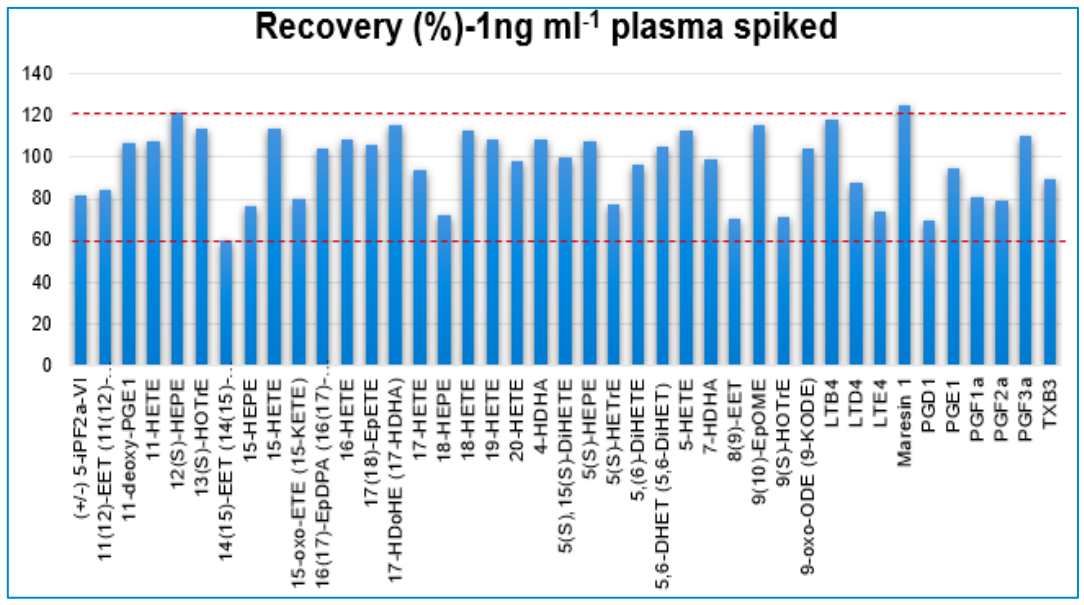

Figure 5. The recovery of 41 oxylipin standards spiked into a plasma sample (spiked conc. = 1 ng ml^{-1}).

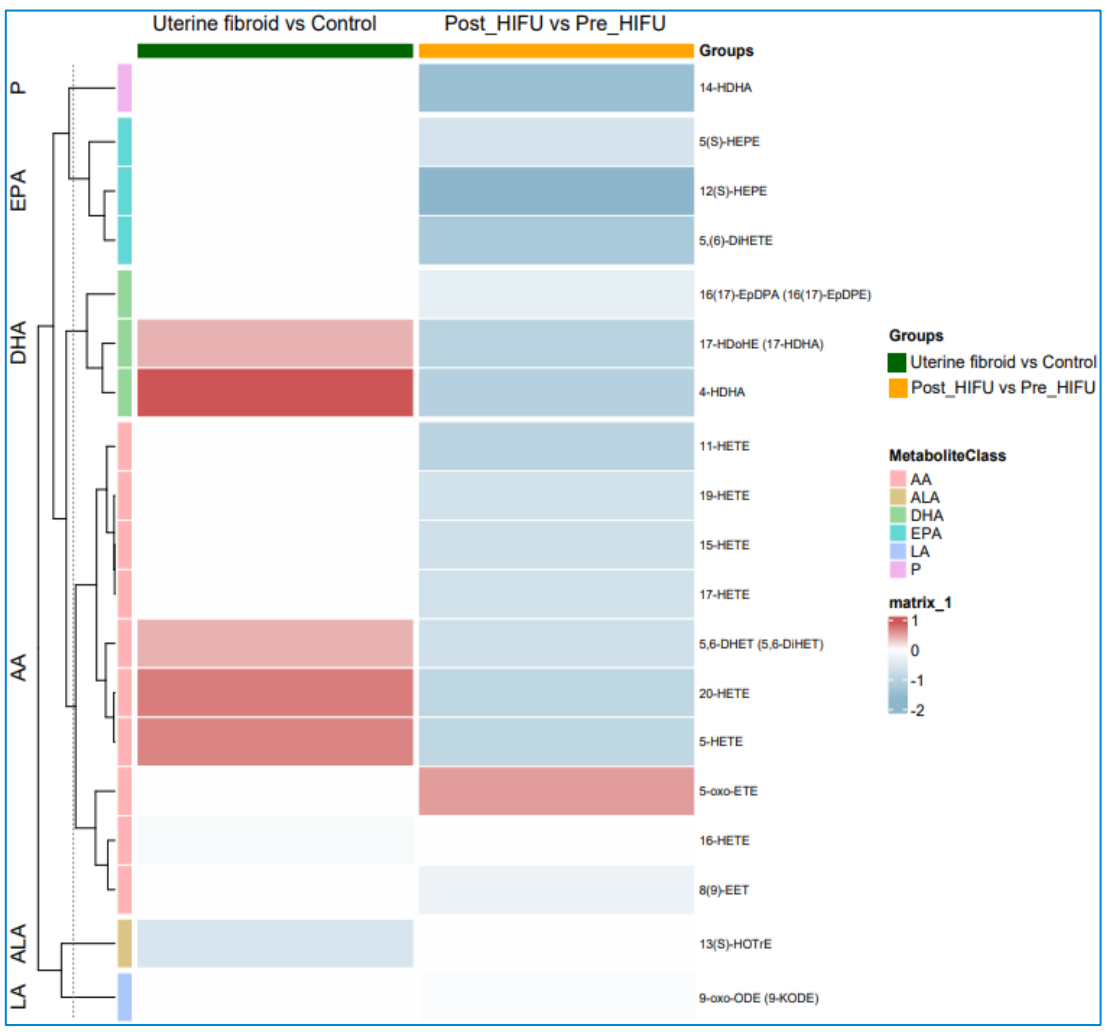

Figure 6. The resulting heatmap showed significant changes for specific oxylipins following HIFU treatment.

Among the 45 quantified oxylipins, 17 exhibited significant alterations between pre- and post-HIFU states. In comparison to healthy controls, specific oxylipins, including 4-HDHA, 17-HDHA, 5-HETE, 20-HETE, and 5,6-DHET, were elevated significantly in uterine fibroid (UF) individuals. Conversely, post-high intensity focused ultrasound (post-HIFU) treatment individuals displayed a remarkable reduction in oxylipins such as 14-HDHA, 4-HDHA, 17-HDHA, 8(9)-EET, 9-oxo-ODE, 5-HEPE, and 12-HEPE compared to pre-treatment levels (Figure 6). Time-series results revealed a general reduction in plasma oxylipin concentration post-HIFU, gradually elevating at three months (still lower than pre-HIFU, except for 13(S)-HOTrE). Significantly changed oxylipins were consistently categorized as inflammatory mediators associated with tumor proliferation.

Conclusions

In this study, we demonstrate use of an RT simulation curve for predicting retention times of oxylipins lacking standards using a previously published article. The method offered highly selective separation and sensitive detection in qualitative and quantitative analysis of oxylipins. In this study, HIFU has emerged as a promising noninvasive surgical intervention for UF.

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