Targeted Lipidomic Analysis of Eicosanoids

Quantitative and Qualitative Data Acquisition using the SCIEX QTRAP® 4500 System

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Eicosanoids are lipid mediators generated from arachidonic acid and are involved in inflammatory and pro-inflammatory processes. Eicosanoids like prostaglandins and leukotrienes stimulate or inhibit the activity, migration and cytokine release of immune cells as well as the presentation of antigens and the generation of antibodies. In addition, eicosanoids are significantly produced and released during acute inflammatory conditions by neutrophilic granulocytes, macrophages as well as endothelial and epithelial cells to support the removal of infectious agents[1,2,3]. Therefore, eicosanoids regulate the pathophysiology of the immune system and adjust the tight balance between immune attack and immune tolerance even at very low concentrations. The ability to study eicosanoids and related molecules (Figure 1) is a key to understand many aspects of human physiology and disease response. The differentiation between many structurally related eicosanoids in biological samples at low concentrations is an important challenge that can be tackled by targeted lipidomic analysis using highly sensitive LC-MS/MS platforms[1,4,5].

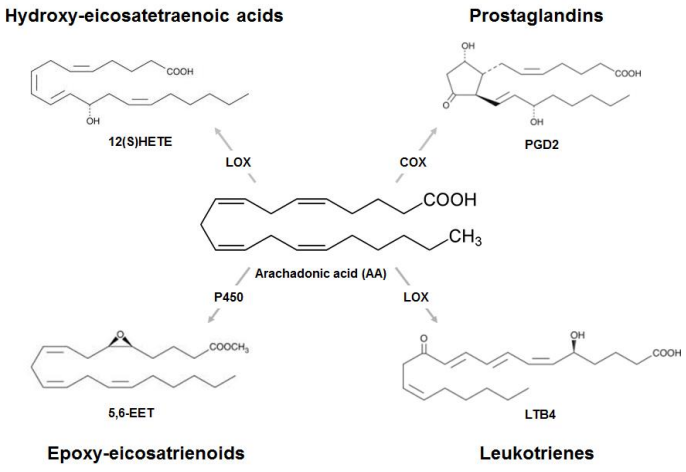

Figure 1. Schema of the Eicosanoid Pathway. The precursor of most of the eicosanoids is arachadonic acid which is released from phospholipid molecules by the enzyme phospholipase A2 (PLA2) and then metabolized by either the lipoxygenase (LOX), cyclooxygenase (COX) or cytochrome P450 classes of enzymes.

A quantitative and qualitative workflow is described here for the analysis of different eicosanoid species such as leukotrienes, prostaglandins, hydroxyl-eicosatetraenoic acids (HETEs) and epoxy-eicosatrienoids (EETs) using the QTRAP® 4500 system.

Key Features of QTRAP® System & DiscoveryQuant™ Software for the Analysis of Eicosanoids

- QTRAP® Systems offer both quantitative and qualitative data acquisition strategies.
- High-throughput quantitative analysis of eicosanoids using Multiple Reaction Monitoring (MRM).
- DiscoveryQuant™ Software uses a simple, flow injection-based approach for rapid compound optimization on every compound.
- Information Dependent Acquisition (IDA) workflow allows high quality MS/MS spectra to be triggered automatically from MRM data providing confidence in lipid species assignment through qualitative analysis.

Materials and Methods

***Sample* Preparation:** All samples comprised of a mixture of lipid standards (Cayman Chemical) dissolved in ethanol (Table 2). A dilution series of each lipid containing 30 pg/µL, 10 pg/µL, 3 pg/µL and 1 pg/µL were made to form a calibration curve. Such curves can be used for quantitative sample analysis and to evaluate the linearity response. Isotopically-labeled internal standards (Table 1) were added to the panel which is important to control efficient lipid isolation from biological samples for accurate quantification.

Table 1. List of Internal Standards Used for Accurate Quantitation.

Internal Standard
AA-D4
PGD2-D4
5(S)HETE -D8
LTB4-D5
PGE2-D4
LTC4-D5
13(S)HODE-D4

Chromatography: Replicate injections were performed on a Shimadzu XR Prominence ultra-high performance liquid chromatography (UHPLC) System, using a Waters Acquity BEH C18 column (1.7µm, 2.1 x 100mm). A gradient elution with mobile phase A (0.02% acetic Acid in 35% acetonitrile) and mobile phase B (50% acetonitrile and 50% isopropanol) was created at a flow rate of 0.5 mL/min over ten minutes.

Mass Spectrometry: A QTRAP® 4500 system with a Turbo V™ Source (electrospray ionization probe) was used for data acquisition in negative mode. Multiple Reaction Monitoring (MRM) using Information Dependent Acquisition (IDA) to trigger Enhanced Product Ion (EPI) spectra were used to confirm the individual eicosanoids. The MRM method used is described in Table 3. DiscoveryQuant™ Software was used to automatically optimize MRMs for the final method.

Data Processing: Data were extracted from the MS and MS/MS spectra using PeakView® Software 2.1. All data processing for quantitation was performed using MultiQuant™ Software 3.0, including computation of calibration curves, percent CVs and standard deviations.

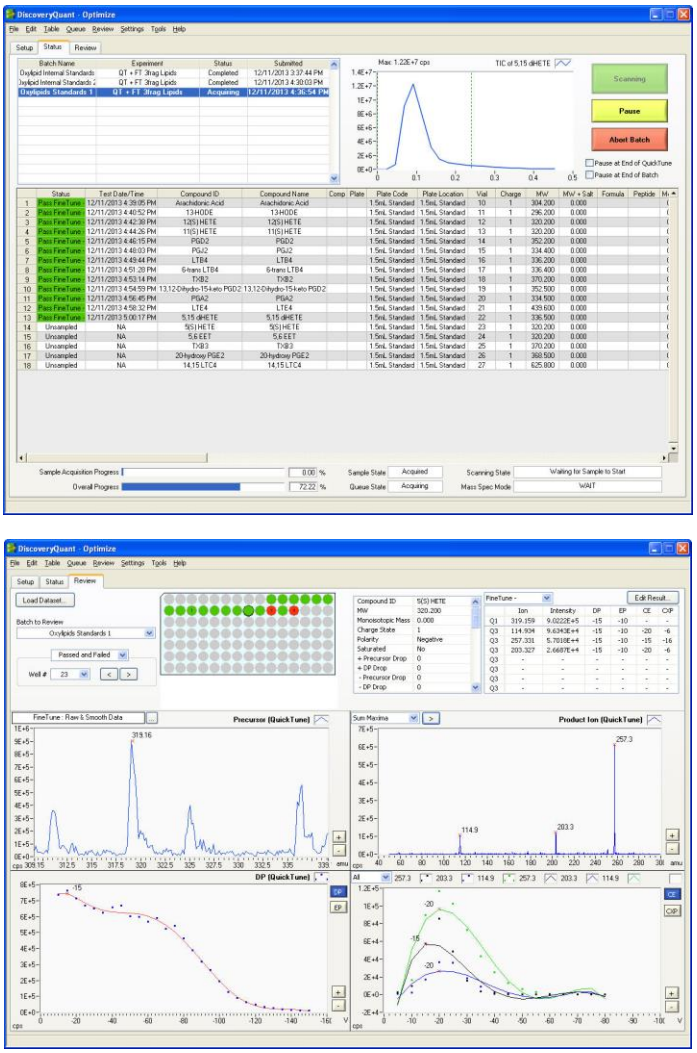

Figure 2. DiscoveryQuant™ Software Workflow. The upper screenshot shows the queue from DiscoveryQuant Software. The flow injection analysis (FIA) profile for the current compound being optimized is displayed (top left) with the dotted green lines showing the optimization region. For the optimization of the eicosanoids the FIA profile was 0.1-0.15 minutes with a total runtime of 30 seconds. The lower section shows the review pane with each of the spectra and voltage ramp profiles. This shows the speed of the QTRAP® system which is able to collect high quality spectra and ramps to optimize the compound in less than 10 seconds.

MRM Optimization with DiscoveryQuant™ Software

Using a QTRAP® 4500 System, MRMs were optimized automatically by using DiscoveryQuant™ Software. This software uses a simple, flow-injection based approach to perform rapid and accurate compound optimization on every compound. DiscoveryQuant™ software performs two injection sequences to optimize a compound. The first injection is used to confirm the precursor m/z from the molecular weight or formula entered in the batch setup. It then optimizes the Declustering Potential (DP) and collects an initial product ion spectrum to find the product ion

masses. In the method used to optimize the eicosanoids, QTRAP® Enhanced Product Ion (EPI) spectra were used due to the extra speed and sensitivity of this ion trap scan mode. Where the precursor ion is only 3-5 times the baseline noise due to lower concentration or less efficient ionization, the greater sensitivity of the EPI scan will provide better quality spectra. An improved spectra quality is especially important for the identification of highly similar compounds like eicosanoids and their metabolites.

The second injection parameter settings are based on the results from the first injection and fine tunes the Collision Energy (CE) and Cell Exit Potential (CXP) for each product ion. In the method used for optimized eicosanoid identification, the FIA peak profile was approximately 0.1-0.15 minutes wide (Figure 2, top). The total flow injection analysis was 30 seconds. Due to the speed of the QTRAP System, it can generate and optimize all of the parameters to a high quality and accuracy within 6-10 seconds (across the FIA peak profile) (Figure 2, bottom). Once both injections have been performed, a compound is fully optimized and all data are saved in the DiscoveryQuant™ Software database.

One of the main issues with infusion-based optimization is choosing the correct concentration for all compounds to be analyzed. Some compounds will require a dilution and some will need a higher concentration. This problem is solved within DiscoveryQuant™ Software due to its built-in saturation control detector. The general approach is to use the same concentration for all compounds. For compounds that ionize very efficiently the software will attenuate the signal to avoid detector saturation and still generate accurate optimized values. For the lower abundant compounds, the concentration will be sufficient for optimization.

The final MRM method can be exported for use in quantitative acquisition, either through Analyst® or DiscoveryQuant™ Software.

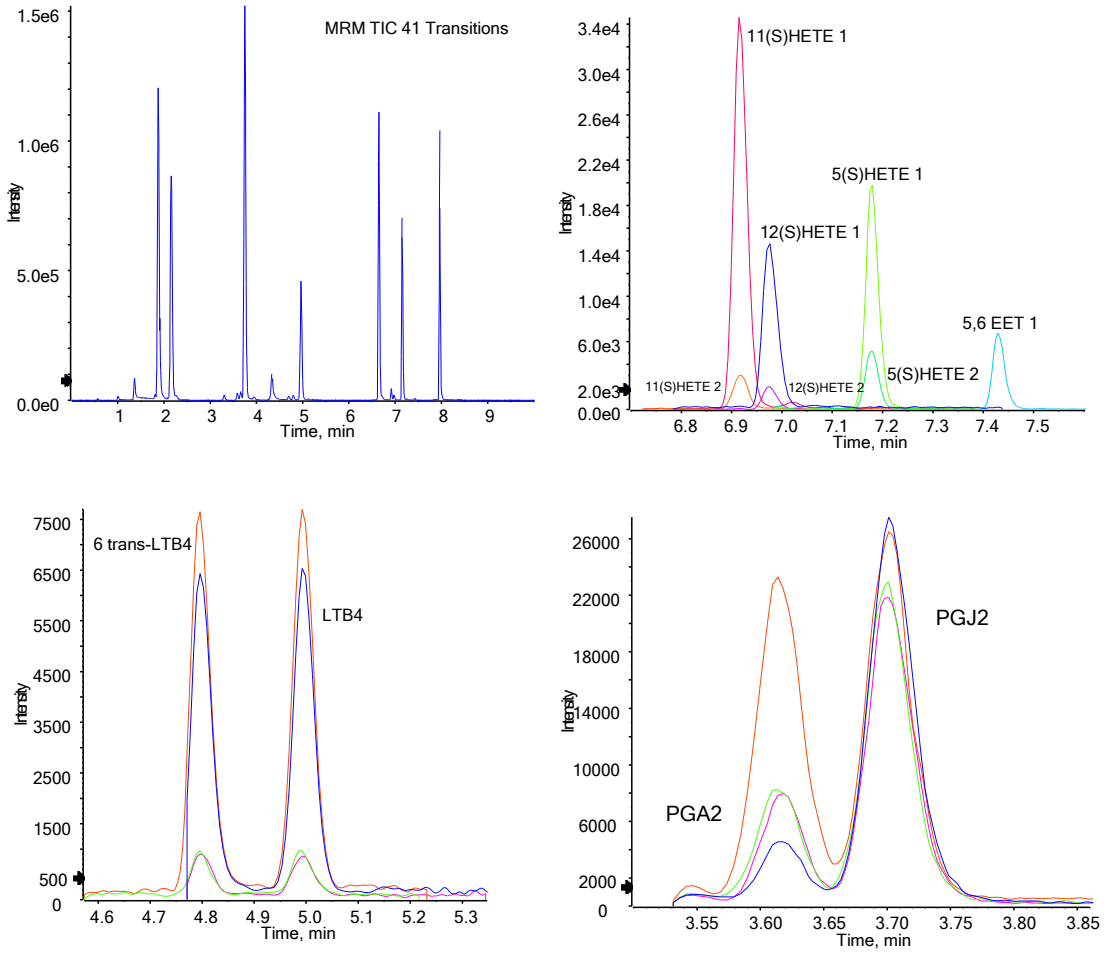

Figure 3. Representative Total Ion Chromatograms. MRM TIC of the 41 transitions during a 10-minute chromatographic run (top left) demonstrates the high quality separations obtained. Specifically, the separation of hydroxyeicosatatraenoic acids (HETE) with same precursor mass of 319.2 is shown in the top right. In addition, nice separation of leukotrienes LTB4 and 6 trans-LTB4 (precursor mass of 333, bottom left) and the prostaglandins PGA2 and PGJ2 (precursor mass of 335, bottom right) are shown.

High Quality Quantitative Lipid Data

Using UHPLC chromatography, a high quality lipid separation method was developed to separate isomeric and isobaric eicosanoid species in a fast 10-minute chromatographic run. For example, separations of isomeric/isobaric species of HETEs, leukotrienes as well as prostaglandins are shown in Figure 3.

Calibration curves were generated in MultiQuant™ Software 3.0 for all compounds shown in Table 2. These were calculated by using the relevant isotopically-labeled internal standards, which were added at a fixed amount for concentration correction between different sample preparations.

To demonstrate reproducibility and robustness of this assay, 5 repeat injections were made of each standard at all concentrations and then the linearity plotted as in Figure 4. Linearity of all compounds was more than an r of 0.99 demonstrating robust reproducibility.

Coefficients of variance (CVs) for the computed concentration of the standards at the 1 pg/µL level are displayed in Table 2. Tight CVs demonstrate the robustness of this high throughput assay.

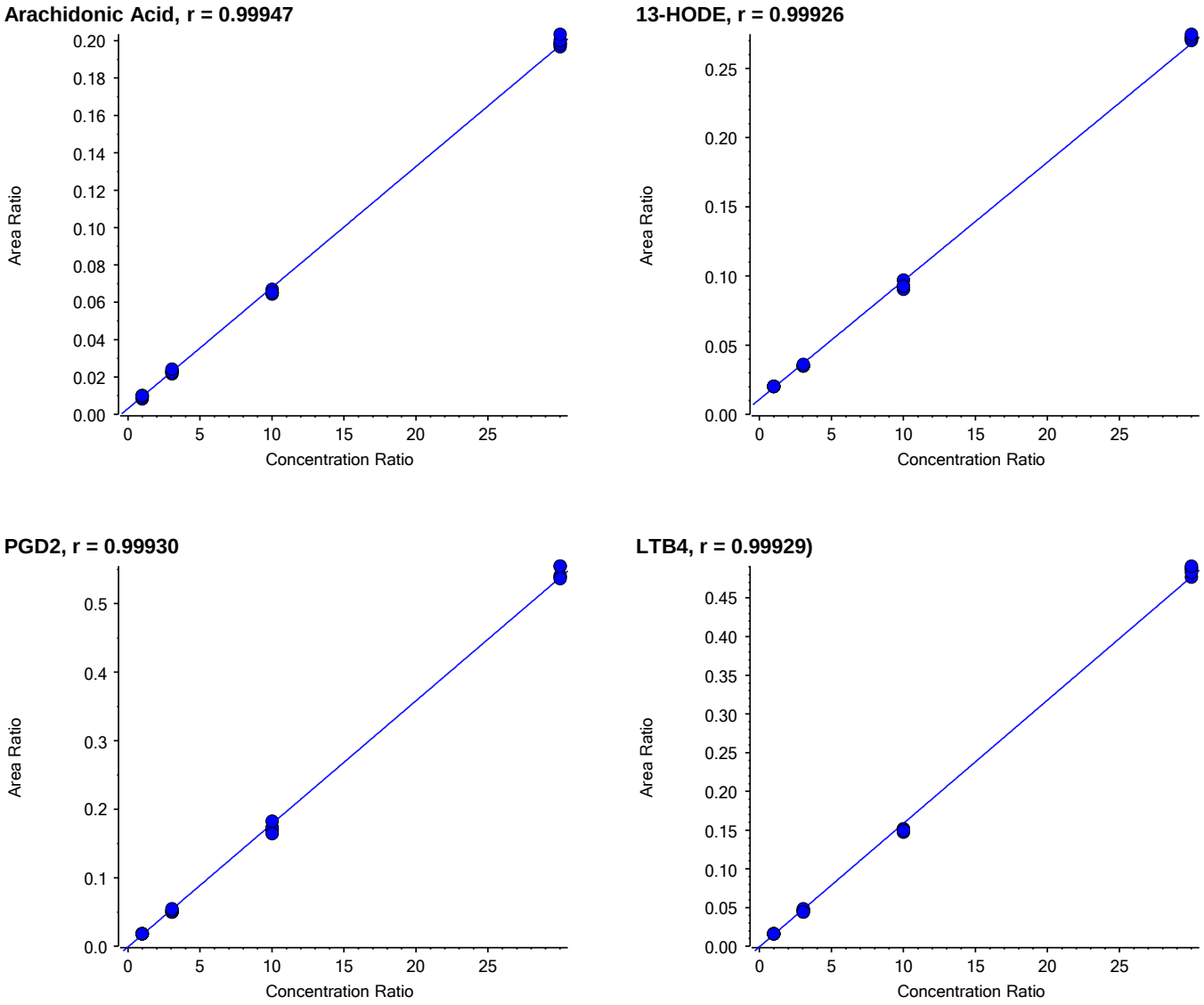

Figure 4. Representative Concentration Curves for Selected Eicosanoids. Five replicate injections of each lipid species at 4 different concentrations were performed and concentration curves were generated. Linearity and reproducibility was assessed for the each compound.

Table 2. Five Replicate Injections of Mixture of Oxidized Lipids. Data for each lipid was processed using MulitQuant™ Software and the coefficients of variance were calculated. Excellent reproducibility was obtained across 5 replicates, the average calculated CVs was 4.3 % with all lipids showing less than 11%.

Compound	Mean	Std Dev	% CV	Conc 1 (pg/µL)	Conc 2 (pg/µL)	Conc 3 (pg/µL)	Conc 4 (pg/µL)	Conc 5 (pg/µL)
AA	*0.996*	*0.110*	*11.0*	*0.850*	*0.937*	*1.130*	*0.989*	*1.072*
13-HODE	*1.079*	*0.023*	*2.16*	*1.097*	*1.055*	*1.068*	*1.110*	*1.066*
12(S)HETE	*1.036*	*0.021*	*2.06*	*1.041*	*1.066*	*1.017*	*1.045*	*1.014*
11(S)HETE	*1.048*	*0.037*	*3.57*	*1.070*	*1.100*	*1.028*	*1.037*	*1.004*
PGD2	*1.052*	*0.026*	*2.50*	*1.039*	*1.026*	*1.071*	*1.087*	*1.034*
PGJ2	*0.981*	*0.012*	*1.26*	*0.991*	*0.984*	*0.973*	*0.994*	*0.964*
LTB4	*1.057*	*0.011*	*1.06*	*1.075*	*1.062*	*1.053*	*1.049*	*1.048*
6-trans LTB4	*1.046*	*0.050*	*4.80*	*1.052*	*0.976*	*1.113*	*1.025*	*1.062*
TXB2	*1.106*	*0.056*	*5.05*	*1.100*	*1.017*	*1.117*	*1.170*	*1.124*
13,14-Dihydro-15-keto PGD2	*1.035*	*0.068*	*6.61*	*0.955*	*0.976*	*1.106*	*1.038*	*1.097*
PGA2	*1.042*	*0.030*	*2.87*	*1.079*	*1.008*	*1.045*	*1.061*	*1.016*
LTE4	*0.969*	*0.093*	*9.58*	*1.119*	*0.987*	*0.937*	*0.928*	*0.874*
5,15-DiHETE	*1.072*	*0.033*	*3.05*	*1.113*	*1.085*	*1.086*	*1.033*	*1.044*
5(S)HETE	*1.037*	*0.034*	*3.23*	*1.044*	*1.092*	*1.006*	*1.026*	*1.019*
5,6-EET	*1.016*	*0.016*	*1.61*	*1.012*	*1.008*	*0.996*	*1.030*	*1.036*
TXB3	*1.134*	*0.090*	*7.94*	*1.193*	*1.033*	*1.042*	*1.226*	*1.176*
20-OH-PGE2	*1.065*	*0.035*	*3.25*	*1.093*	*1.071*	*1.054*	*1.094*	*1.011*
14,15-LCT4	*1.059*	*0.060*	*5.62*	*1.042*	*0.983*	*1.037*	*1.097*	*1.137*

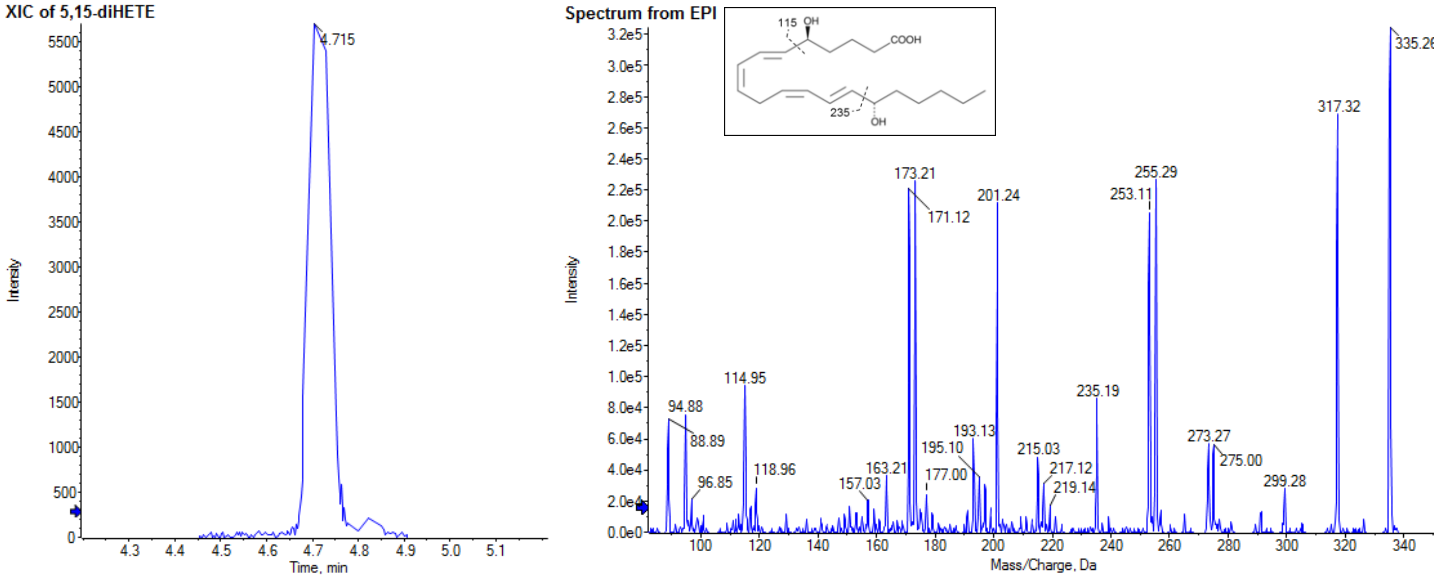

Figure 5. QTRAP® System Data from MRM triggered EPI Experiment. Extracted ion chromatogram (XIC) from the MRM transition for 5,15-diHETE at a concentration of 3 pg/mL is shown on the left. This MRM signal triggers an Enhanced Product Ion (EPI) scan using information dependent criteria (right) to provide qualitative information. The fragmentation pattern observed in the MS/MS data provides confirmation on the identity of each lipid species.

Table 3. Table of MRM and Retention Times for All Eicosanoid Lipid Species Measured. MRM 1 denotes the transition used for quantitation and MRM 2 denotes transition used for qualification of eicosanoid species. CE = -45, CES = 25, EP = -10

Compound	Q1 (m/z)	Q3 (m/z)	Retention Time (min)
Arachidonic Acid 1	*303.1*	*259.4*	*8.0*
Arachidonic Acid 2	*303.1*	*205.3*	*8.0*
13-HODE	*294.8*	*195.2*	*6.6*
12(S)HETE 1	*319.2*	*179.2*	*7.0*
12(S)HETE 2	*319.2*	*135.1*	*7.0*
11(S)HETE 1	*319.2*	*167.2*	*6.9*
11(S)HETE 2	*319.2*	*149.2*	*6.9*
PGD2 1	*351.1*	*271.3*	*2.1*
PGD2 2	*351.1*	*233.3*	*2.1*
PGJ2 1	*333.2*	*233.2*	*3.7*
PGJ2 2	*333.2*	*189.2*	*3.7*
LTB4 1	*335.2*	*195.2*	*4.9*
LTB4 2	*335.2*	*151.2*	*4.9*
6-trans LTB4 1	*335.2*	*195.2*	*4.7*
6-trans LTB4 2	*335.2*	*151.1*	*4.7*
TXB2 1	*369.2*	*169.0*	*1.3*
TXB2 2	*369.2*	*195.2*	*1.3*
13,14-Dihydro-15-keto PGD2 1	*351.3*	*175.2*	*3.3*
13,14-Dihydro-15-keto PGD2 2	*351.3*	*207.2*	*3.3*
PGA2 1	*333.2*	*271.3*	*3.7*
PGA2 2	*333.2*	*189.2*	*3.7*
LTE4 1	*438.3*	*333.3*	*4.3*
LTE4 2	*438.3*	*351.3*	*4.3*
5,15-diHETE 1	*335.2*	*201.2*	*4.6*
5,15-diHETE 2	*335.2*	*255.3*	*4.6*
5(S)HETE 1	*319.2*	*114.9*	*7.2*
5(S)HETE 2	*319.2*	*203.3*	*7.2*
5,6 EET 1	*319.2*	*191.2*	*7.4*
TXB3 1	*367.2*	*169.2*	*1.0*
TXB3 2	*367.2*	*195.2*	*1.0*
20-hydroxy PGE2 1	*367.2*	*331.3*	*0.6*
20-hydroxy PGE2 2	*367.2*	*189.2*	*0.6*
14,15 LTC4 1	*624.3*	*272.2*	*3.0*
14,15 LTC4 2	*624.3*	*143.1*	*3.0*

Qualitative Confirmation of Lipid Identity

Figure 5 highlights the unique capability of the QTRAP® System, to construct powerful workflows leveraging both the ion trap and triple quadrupole functionality of the system. Here, the MRM triggered EPI workflow was used for simultaneously collection of both quantitative MRM data as well as qualitative full scan MS/MS data. For isobaric lipid species like eicosanoids and its metabolites, informative, high quality MS/MS spectra will allow greater confidence in lipid species identification rather than MS alone.

Conclusions

In this work, a robust, reproducible and high-throughput method for the analysis of eicosanoids has been developed on the QTRAP® 4500 System. The method can accurately quantitate concentrations of these lipid species in a wide linear range and also allow the collection of qualitative information for confident lipid identification. This method can be easily transferred and implemented on any QTRAP System.

Table 3 Continued. Table of MRM and Retention Times for All Eicosanoid Lipid Species Measured. IS denotes the transition used as an internal standard.

Compound	Q1 (m/z)	Q3 (m/z)	Retention Time (min)
AA-D8 (IS)	*311.1*	*267.5*	*8.0*
PGD2-D4 (IS)	*355.2*	*319.3*	*1.8*
5(S) HETE-D8 (IS)	*327.1*	*265.4*	*7.1*
LTB4-D4 (IS)	*339.2*	*197.2*	*5.0*
PGE2-D4 (IS)	*355.2*	*275.4*	*2.1*
LTC4-D5 (IS)	*629.3*	*272.2*	*4.4*
13-(S)HODE-D4 (IS)	*299.1*	*198.3*	*6.6*

References

1. Ekroos, K. Lipidomics; Technologies and Applications. Wiley VCH, 2012.
2. Funk, C. D. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 294, 1871-1875, doi:10.1126/science.294.5548.1871294/5548/1871 (2001).
3. Harizi, H., Corcuff J. B.; Gualde, N. Arachidonic Acid derived eicosanoids: roles in biology and immunopathology. Trends Mol Med 14, 461-469, (2008).
4. Benjamim, C. F., Canetti, C., Cunha, F. Q., Kunkel, S. L. & Peters-Golden, M. Opposing and hierarchical roles of leukotrienes in local innate immune versus vascular responses in a model of sepsis. J Immunol 174, 1616-1620, doi:174/3/1616 (2005).
5. Harkewicz, R. & Dennis, E. A. Applications of mass spectrometry to lipids and membranes. Annu Rev Biochem 80, 301-325, doi:10.1146/annurev-biochem-060409-092612 (2011).

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