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Comprehensive Ultra Performance Liquid Chromatographic Separation and Mass Spectrometric Analysis of Eicosanoid Metabolites Suitable for Human Materials

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Abstract

Over the past decade, the number of known eicosanoids has expanded immensely and we have now developed an ultra-performance liquid chromatography - electrospray ionization triple quadrupole mass spectrometric (UPLC-QTRAP/MS/MS) method to monitor and quantify numerous eicosanoids. The UPLC-QTRAP/MS/MS approach utilizes scheduled multiple reaction monitoring (MRM) to optimize sensitivity, number of metabolites that can be analyzed and the time requirement of the analysis. A total of 184 eicosanoids including 26 deuterated internal standards can be separated and monitored in a single 5 min UPLC run. To demonstrate a practical application, human plasma samples were analyzed following solid-phase extraction (SPE) and the recovery rate and matrix effects were determined for the 26 deuterated internal standards added to the plasma. The method was validated and shown to be sensitive with the limit of quantitation at pg levels for most compounds, accurate with recovery rates of 70-120%, and precise with a CV<30 for all compounds. Also, the method showed a linear response over a range spanning several orders of magnitude. In a QC human plasma sample, we identified and rigorously quantified over 120 eicosanoids.

Keywords

Eicosanoids; Ultra-performance liquid chromatography; Tandem mass spectrometry; Plasma; Lipidomics

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1. Introduction

Eicosanoids comprise a class of bioactive lipids derived from a unique group of polyunsaturated essential fatty acids that mediate a wide variety of important physiological functions [1]. They exert complex control over many physiological processes, including inflammation [2]. Also, many eicosanoids are associated with chronic disease conditions including heart disease [3], cancer [4] and arthritis [5].

Arachidonic acid (AA) and related polyunsaturated fatty acids serve as the metabolic precursors for eicosanoid synthesis. Biologically, these molecules are generally stored in the *sn*-2 position of the glycerol backbone of membrane phospholipids. To be used for biosynthesis, the arachidonic acid must first be released from phospholipids via phospholipase A_2 (PLA_2) [6], and which is then acted on by enzymes of the cyclooxygenase pathway (COX) or the lipoxygenase pathway (LOX) to form prostaglandins (PG) and thromboxanes (TX) or leukotrienes (LT), respectively. The COX site incorporates molecular oxygen at the 11- and 15-carbons on arachidonic acid to form PGG_2, followed by a peroxidase activity that reduces the peroxide to a hydroxyl to form PGH_2. PGH_2 is an intermediate for a number of different bioactive products through the action of PG synthases that can form a number of important signaling molecules, including PGI_2, TXA, PGE_2, PGD_2, and PGF_{2a}. Alternatively, the LOX pathway produces LTs including 5-HETE, 15-HETE, LTA_4, LTB_4, LTC_4 etc, which constitute a family of biologically active molecules formed in response to immunological and non-immunological stimuli. For example, 5-LOX produces 5-hydroperoxy-eicosatetraenoic acid (5-HpETE) by incorporating one molecular oxygen at the C-5 position of arachidonic acid that can be reduced to 5-HETE, or undergo a catalytic rearrangement in the 5-LOX active site to form LTA_4. An additional set of enzymes catalyzes the stereospecific rearrangement of LTA4 to produce LTB_4, LTC_4, and LTD_4.

This complete enzymatic system produces hundreds of eicosanoids derived from AA and related polyunsaturated fatty acids with very similar structures, chemistries and physical properties [7], which makes the analysis of eicosanoids a challenging task, especially in biological samples. The concentration of eicosanoids in plasma or serum is the lowest among all endogenous lipid metabolites [8]. However, under certain conditions the plasma level of eicosanoids may change considerably and thus, eicosanoids may serve as a useful readout reflecting disease progression. As a result, current research is focused on developing fast, sensitive, and reliable methods that accurately profile and quantify eicosanoid biomarkers [9,10].

In the past, eicosanoids were mainly analyzed by enzyme-linked immunosorbent assays (EIA) [11, 12], gas chromatography-mass spectrometry (GC-MS) [13] and liquid chromatography –mass spectrometry (LC-MS) [14]. The drawback of EIA is a lack of specificity and the ability to determine multiple analytes in a single set of analyses. GC-MS provides greater sensitivity and selectivity for eicosanoid analysis, but requires chemical derivatization steps that limit its application. The rapid progress of liquid-chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) has facilitated the use of this technology for accurate monitoring of eicosanoid metabolites in biological samples

[7]. Previous reports include liquid-liquid extraction for the determination of PGE_2 and LTB_4 in plasma using LC-MS [15], the analysis of four kinds of PGs and LTs in cell culture media by LC-MS [16], an on-line two dimensional reverse-phase LC-MS for the simultaneous determine of PGE_2, PGF_{2a} and 13,4-dihydro-15-keto PGF_{2a} [17], and a LC-MS method for the simultaneous determination of twenty-three eicosanoids [18], a UPLC-MS platform that enables profiling of 122 eicosanoids from human whole blood[19], a targeted HPLC-MS/MS analysis platform for 100 oxylipins and 36 oxylipins was detected from 250 uL human plasma in 26 min[20], a LC–MS/MS method for rapid and concomitant quantification of 26 PUFA metabolites from Caco-2 cells [21], a LC–MS/MS for the simultaneous analysis of arachidonic acid and 32 related metabolites in 1 mL human plasma [22], a online HPLC-MS/MS analyzed more than 20 different oxidized fatty acids and their precursors from 200 uL plasma or urine [23], a fast LC–MS/MS method including on-line SPE and LIT fragment confirmation for the profiling of 7 PUFAs and 94 oxidized metabolites from 200 uL plasma [24]. We previously developed a high-throughput lipidomic analysis of eicosanoids using LC-MS to monitor 141 unique species in a single 22 min analysis [25], and applied it to investigate the human plasma lipidome [26,27].

However, these methods may need much more biolgocial sample or longer analysis time. In order to address the need for large-scale high-throughput analysis of eicosanoids in small quantities of human plasma and tissues, we now report the development of a method utilizing an ultra-high performance liquid chromatography-QTRAP MS/MS (UPLC-MS/MS) for monitoring 184 eicosanoids in a 5 min run. The new methodology is validated by identification and quantitation of eicosanoids in 20 uL human plasma.

2. Experimental

2.1 Reagents

All eicosanoids and deuterated internal standards were purchased from Cayman Chemical. Optima LC-MS grade acetonitrile (ACN), methanol (MeOH), and water were obtained from Fisher Scientific. Isopropanol (IPA) was purchased from Sigma-Aldrich. Formic acid (FA) was obtained from EMD Technologies. Dulbecco's Phosphate Buffered Saline (DPBS) was obtained from Corning Life Science.

2.2 Sample preparation

2.2.1 Preparation of primary standard and internal standard solutions—For the preparation of calibration curves, stock solutions were prepared in ethanol that contained all eicosanoid standards, each at a concentration of 0.25 ng/uL. Working standard solutions for all eicosanoids were prepared by serial dilution of the stock solutions to create the necessary concentrations. A solution containing 26 internal (deuterated) eicosanoid standards was prepared at 0.01 ng/uL in ethanol. All solutions were stored at -80°C when not in use.

2.2.2 Extraction of metabolites from plasma and tissue—Aliquots of 20 ul control plasma (Human Source Plasma, Gemini Bio-Products) were diluted to 1 mL with phosphate salt buffer spiked with 100 uL of the internal standard solution. The eicosanoids were extracted using Strata-X reversed-phase SPE columns (8B-S100-UBJ, Phenomenex).

Columns were washed with 3 mL MeOH and then equilibrated with 3 mL H_2O. After loading the sample, the columns were washed with 10% MeOH to remove impurities, and the metabolites were then eluted with 1 mL of MeOH and stored at -80°C to prevent metabolite degradation. Prior to analysis, the eluant was dried under vacuum and redissolved in 50 uL of the UPLC solvent A (water/acetonitrile/acetic acid (60:40:0.02; v/v/v)) for UPLC/MS/MS analysis.

Took tissue sample, then weighed, and transferred to 1 mL PBS buffer containing 10% MeOH tube. The tissue was homogenized using Beadbug Microtube Homogenizer (Benchmark Scientific). The eicosanoids extracted from homogenates with SPE followed the same protocol as plasma sample.

2.3 UPLC-MS/MS

An Acquity UPLC system (Waters Corp) was used. Reversed-phase separation was performed on an Acquity UPLC BEH shield RP18 column (2.1×100 mm 1.7 μm; Waters). The mobile phase consisted of (A) ACN/water/acetic acid (60/40/0.02, v/v) and (B) ACN/IPA(50/50, v/v). Gradient elution without splitting was carried out for 5 min at a flow rate of 0.5 mL/min. Gradient conditions were as follows: 0-4.0 min, 0.1-55% B; 4.0-4.5 min, 55-99% B; 4.5-5.0 min, 99% B; A 10 μL aliquot of each sample was injected into the column. The column temperature was kept at 40 °C. All samples were incubated at 4 °C during analysis.

Mass spectrometry was performed on an ABI/Sciex 6500 QTRAP hybrid, triple quadrupole, linear ion trap mass spectrometer equipped with a Turbo V ion source. Eicosanoids were detected in negative electrospray ion mode. Curtain gas (CUR), nebulizer gas (GS1) and turbo-gas (GS2) were set at 10 psi, 30 psi and 30 psi, respectively. The electrospray voltage was -4.5 kV, and the turbo ion spray source temperature was 525 °C. Nitrogen was employed as collision gas and the collisionally-activated dissociation (CID) was set at a high level.

Eicosanoids were analyzed using scheduled multiple reaction monitoring (MRM). Mass spectrometer parameters including the declustering potentials and collision energies were optimized for each analyte. Data acquisitions were performed using Analyst 1.6.2 software (Applied Biosystems). Multiquant software (Applied Biosystems) was used to quantify all metabolites.

2.4 Analytical validation

2.4.1 Linearity and LOD and LOQ—A typical standard curve was prepared by adding 1 ng of each internal (deuterated) eicosanoid standard to the following amounts of eicosanoid (nondeuterated) primary standards: 0.005, 0.015, 0.025, 0.035, 0.05, 0.15, 0.25, 0.35, 0.5, 1.5, 2.5, 3.5, and 5.0 ng. Quantitation of eicosanoid levels was performed using linear regression of the response ratios (peak area analyte/peak area internal standard) obtained from the calibration curve to calculate the corresponding eicosanoid amount. The limit of detection (LOD) was defined as the concentration that resulted in a peak with a signal-to-

noise ratio (S/N) greater than 3:1 (3 S/N) and the limit of quantitation (LOQ) was defined as (S/N) greater than 7:1 (7 S/N)

2.4.2 Recovery rate and matrix effect—Recovery from control plasma was determined by comparison of the analyte peak area of the internal standard spiked into a plasma sample compared with the corresponding peak area of standard solutions extracted in a similar manner. The determination was performed in triplicate. The matrix effect was calculated by dividing the peak area of the internal standard spiked into a plasma sample and extracted into mobile phase A by the area of the deuterated standard in the standard solution. This determination was also performed in triplicate.

2.4.3 Accuracy and precision—Accuracy and precision of the method was determined using quality control (QC) samples spiked at three levels of eicosanoids: low (LQC) 0.15 ng, medium (MQC) 1.5 ng, and high QC (HQC) 5 ng. QC samples were prepared by spiking blank plasma with three levels of eisosanoids and internal standards prior to plasma extraction as described. Intra-batch and inter-batch (three different batches) accuracy and precision were determined by analyzing five QC samples covering the calibration range. The precision of the quantitation was expressed as percent coefficient of variance (CV %), calculated by dividing the standard deviation by the mean and then multiplied by 100.

These QC amounts included the known fortified level added to the control plasma plus the endogenous amount of analytes. The endogenous amounts of analytes in plasma were determined in five replicate measurements. The accuracy of the analytic method was denoted by the relative error (RE %), calculated as percent of the mean deviation from the known amount plus endogenous amount, RE % = [(amount found − (known amount + endogenous amount) × 100/(known amount + endogenous amount)].

2.4.4 Stability—To determine the stability of the processed samples, they were kept at 4°C in the autosampler and injected three times at 0, 4 and 8 h with three levels of quality control samples, respectively. The peak area of the analytes at the initial point (0 h) was used as the reference to determine the relative stability at subsequent points.

3. Results and Discussion

3.1 Method development

SPE techniques were used to extract eicosanoid metabolites from plasma, which is more suitable for processing a large number of samples than a more efficient liquid/liquid extraction (LLE) technique [28]. The extraction efficiency of LLE is generally higher than SPE, but this method also extracts many endogenous impurities that can affect the separation and quantitation of target analytes. The ability of SPE to eliminate impurities is better than that of LLE, which improves the detection of eicosanoids in biological matrices, especially when present at low levels.

A crucial aspect of our method is the inclusion of 26 deuterated internal standards. All samples are spiked with a mixture of deuterated internal standards prior to lipid extraction. An internal standard is used to correct for run-to-run variation in extraction efficiency, for

monitoring the chromatographic response, and for normalization purpose which allows for accurate quantification. Also, eicosanoid quantitation was performed by the stable isotope dilution method. For each eicosanoid to be quantify, an internal standard was selected that had a different precursor ion mass than the target analyte, but was chemically and structurally as similar to the target analyte as possible. This is ideally achieved by using a deuterated analog of the analyte. We employed these standards whenever they were commercially available. For example, (d4) PGE_2 was employed as the internal standard for PGE_2. In other cases, we employed a deuterated analog that was the closest to the desired analog in characteristics. For example, (d4) 15d PGJ_2 was employed as the internal standard for PGJ_2, 15d- PGJ_2, and 15d-PGD_2. The 26 deuterated internal standards, which presently used to quantify, assigned to each of the 158 eicosanoids analyzed are listed in Table 1.

A targeted approach was used to identify and quantify lipids using mass spectrometry (MS) coupled with ultra-high performance liquid chromatography (UPLC-MS). A targeted MRM approach provided for higher sensitivity than an unbiased full scan MS analysis. UPLC also provided enhanced chromatographic resolution, sensitivity, reproducibility and fast separation. Additionally, a QTRAP 6500 was used in the present study. The advantages of the QTRAP 6500 are scan speeds of up to 20,000 Da/second for optimized UPLC strategies, a twenty-fold increase in the detector dynamic range, and LOQ improvement of up to five fold. In summary, the UPLC-QTRAP 6500 system provides a sensitive, accurate and fast separation and monitoring platform.

All eicosanoids are detected in the negative ion mode to take advantage of their conserved terminal carboxyl moiety. Fig. 1 shows a representative extracted ion chromatogram for the separation of all 184 eicosanoids including the 26 internal standards, which are monitored in a single 5 min LC-MS/MS analytical run. The optimal declustering potential (DP) and the collision energy (CE) has been determined for each MRM pair (Table 2). These values were optimized by directly infusing commercial standards into the mass spectrometer.

Peak selectivity was demonstrated by comparison with individual MRM transitions and the retention time of each analyte, as shown in Table 2. Scheduled MRM is an improvement over traditional MRM allowing for better data collection and more analytes to be monitored in a single analysis. We found a 30 sec retention time window for each MRM pair sufficient to allow for potential small shifts in retention time. In addition to providing good sensitivity, MRM approaches are highly selective, reducing the need for extensive sample cleanup. As a proof of principle, control plasma was analyzed and we found no discernable distortions.

The MS/MS spectra of most eicosanoids show numerous product ions. Various product ions from the precursor were also listed In Table 2, and the product ions for MRM detection was marked with underline. The product ions employed here for the MRM detection were selected to yield the best discrimination from other eicosanoids that co-elute in the vicinity of the analyte and to yield the highest signal. By balancing LC retention time and product ion selection, we were able to successfully distinguish the large majority of the eicosanoids listed. For example, owing to the silimar structure, (d4) PGE_2 and PGE_2 have the same retention 7.1 min from LC, but the precursor and product of (d4) PGE_2 and PGE_2 was different, which was 355→275 and 351→271, respectively. In the other case, the precursor

and product of PGE_2 and PGD_2 was the same, 351→271, but the retention time of PGE_2 and PGD_2 was different.

While a similar eicosanoid may have the same product ions, their relative intensities usually vary. The ratio of intensities of these product ions can be used to distinguish these species. In this case, multiple MRM transitions can then be analyzed, and the ratio of product ions found in the unknown can be compared with either an MS/MS library spectra or a pure standard run under the same conditions. This would aid in confirming the identity of a chromatographic peak.

3.2 Different precursor for eicosanoids

The key precursor polyunsaturated fatty acid (PUFA) for eicosanoids is arachidonic acid (AA, 20:4, *n*-6). Other eicosanoids and related compounds are formed from eicosapentaenoic acid (EPA, 20:5, n-3), docosahexaenoic acid (DHA, 22:6, *n*-3), and dihomo-γ-linolenic acid (DGLA, 20:3, *n*-6). Arachidonic acid is the main precursor for a wide spectrum of unique eicosanoids produced by COX, LOX, and CYP. Biochemical characterizations of EPA and DHA have generally suggested that these fatty acids are less prone to metabolism by eicosanoid pathway enzymes [29]. In the present studies, we probed for 88 eicosanoids derived from AA, 22 from DHA, 17 from EPA, 13 from DGLA, and 18 from other fatty acids, that were identified as outlined in Fig 2. The solid black lines around circles showed how many metabolites from the different PUFA. The comprehensive and simultaneous analysis of all eicosanoids is important because eicosanoids derived from different PUFA sources may have different physiological effects. For example, in neural trauma and neurodegenerative diseases, there is a dramatic rise in the levels of AA-derived eicosanoids and in contrast, DHA-derived metabolytes can prevent neuroinflammation [30].

3.3 Method validation

The assay was validated for analyte recovery rate, matrix effect, linearity, inter-batch and intra-batch precision and accuracy for the major analytes. This procedure was performed on five replicates and on three consecutive days. Analyte stability was performed on processed samples maintained at 4°C for 4h and 8h.

3.3.1 Recovery rate and matrix effect—(line 254)Matrix effect occurring between different matrics and adding to the complexity of a measurement and can lead to a bias. In the case of eicosanoid analysis, there is no true “blank” plasma available because of endogenous analytes present in all human plasma at various levels. Since a deuterated internal standard is either an analogous lipid metabolite or a molecule with similar chemical characteristics (chemical structure of the eicocanoids can be found in Ref. 2, 21 and 23), both lipid metabolites and internal standards will have similar ion suppression and extraction efficiencies. Thus, internal standards were used to evaluate the matrix effect and the recovery rate of the method.

The matrix effect was determined by spiking the internal standard mixture into a blank plasma sample after exaction. The results are shown in Table 3. Except for (d4) PGF2a, (d8) 5-HETE and (d8) arachidonic acid, the matrix effect of the other 23 internal standards are all

(line 264) above 80 %, indicating that ion suppression affects during analysis are minimal for most analytes. For the three exceptions, it is possible that co-eluting matrix components may reduce the ion intensity of the analytes. Phospholipids, and in particular glycerophosphocholines and lysophosphatidylcholines, represent the major class of endogenous compounds causing significant matrix effects, which will suppress the signal of other co-eluting lipids. So the removal of phospholipids is preferable for analysis of other kinds of lipids in lipidomics study. Because Phospholipids are amphipathic compounds which generally consist of a phosphate-containing polar head group and one or two long hydrophobic fatty acid ester chains. So the absorption of phospholipids on the C18 column is much stronger than eicosanoids. If using C18 material, phospholipids generally can be eluted with isopropanol as mobile phase. In our experiment condition, phospholipids generally cannot be eluted from SPE column. So the interference from phospholipids may be neglected. However, lysophospholipids may be coeluted from SPE in our experiment conditions. So part of matrix effect may be caused from lysophospholipids. To minimize the matrix effect, a more efficient and selective extraction method or an efficient chromatographic separation of the analytes from matrix interferences could be developed.

Absolute recovery was determined by spiking the internal standards into plasma samples before exaction, as shown in Table 3. Recovery of 21 of the internal standards was above 70%. The lower recovery for (d4) PGF2a, (d8) 5-HETE and (d8) arachidonic acid may be caused by the matrix effect noted above. The lower recovery for (d4) 14d PGJ_2 and (d4) Resolvin E_1 may be due to non-specific binding by the SPE absorbent. The error margin for the matrix effect and absolute recovery rate was ±10%.

3.3.2 Linear and lower limit of quantitation—The limit of quantitation for each analyte was defined as the lowest signal obtained with S/N ≥7. Based on these criteria, the LOQ of 86% of the analytes was less than 10 pg, as shown in Table 2. For target analytes, if the concentration of endogenous is much higher and separation interference is much lower, the LOQ is lower. Otherwise, the LOQ for target eicosanoids was much high.

Eicosanoid quantitation was performed by the stable isotope dilution method. For each eicosanoid to be quantified an internal standard was selected and a linear standard curve was generated where the ratio of analyte standard peak area to internal standard peak area was plotted versus the amount of analyte standard. The method offers good linearity for all analytes, the R^2 value is above 0.97 and the typical standard eicosanoid curves are shown in Fig. 3.

3.3.3 Accuracy and Precision—The results of intra- and inter-day precision and accuracy testing using QC standard samples prepared in plasma as a blank matrix are summarized in Table 4. Human plasma contains a number of endogenous eicosanoids and we found that we could identify and quantify 60 endogenous metabolites in 20 ul of standard plasma.

For QC samples, the nominal amount of the QC sample was expressed as the sum of the endogenous amount and the spiked amount. In all, 147 eicosanoids from plasma can be

monitored and 121 eicosanoids can be monitored and also quantified using QC samples (Supplementary Table S1, Supporting Information).

Precision (CV %) values for 95% of the analytes were within 20%, 100% of the analytes were within 30%. Accuracy (RE %) for 87% of the analytes was within 20%, 100% analytes were within 30%. These results indicate a good reproducibility for the determination of 121 analytes in human plasma using QC samples.

For the bioanalytical method validation and sample analysis, calibration standards and QC samples should ideally be prepared in the same matrix as the intended sample. This is especially true when electrospray ionization mass spectrometry is used because, as outlined above, components in the biological sample can lead to matrix effects [31]. Typically, suppression or enhancement of analyte response is accompanied by diminished precision and the accuracy of subsequent measurements [32]. Due to the endogenous presence of eicosanoids at various levels, it is difficult to prepare a calibration curve in plasma; thus, we had to use calibration curves prepared in ethanol that are devoid of interfering matrices and impurities. This may contribute to analytical difficulties in detecting and accurately quantitating some of the eicosanoid molecular species for plasma sample. For example, the matrix effect may contribute to the deviation of the measurements of arachidonic acid for spiked plasma samples. To analyze the free fatty acids themselves, we have found that GC-MS provides an optimal approach [33].

3.3.4 Stability—To test the stability of the processed samples, they were kept at 4°C in the loading tray for various times prior to injection. Post-preparative stability of resuspended extracts showed a precision (CV %) and accuracy (RE %) for 100% of the analytes were within 20% for 0, 4 and 8h of pre-loading. Therefore it is concluded that eicosanoids in resuspended plasma extracts are stable for at least 8h when stored at 4°C.

3.3.5 Application of method to other tissues—The method was also applied to detect eicosanoids in mouse and human tissues, including adipose tissues, liver tissues and muscle tissues. Complete eicosanoid profiles similar to these from plasma were obtained from sample sizes as small as 2 mg of tissue. Of course, homogenization of the tissue samples before SPE extraction may be needed. These results demonstrate that this method is broadly applicable and can be used to measure eicosanoids in various biological sources.

4. Conclusion

Eicosanoids have wide ranging physiological functions, thus it is important to be able to accurately quantify and profile them in biological materials. A targeted UPLC-MS/MS method to globally monitor and quantify eicosanoids from human plasma and other tissues was developed and validated. Based on this new separation and detection platform, 184 unique metabolites were simultaneous monitored in a single 5 min analytical run. This makes it possible to analyze a large number of samples on a routine basis, including clinical samples. Target eicosanoid profiling should help the identification of potential biomarkers and understanding their role in specific diseases. Moreover, due to the sensitivity of the

method only small tissue samples (line 329) are needed (i.e., 20 uL of human plasma or 2 mg of tissue) to comprehensive analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Funk CD. Science. 2001; 294:1871. [PubMed: 11729303]
2. Buczynski MW, Dumlao DS, Dennis EA. J Lipid Res. 2009; 50:1015. [PubMed: 19244215]
3. Jenkins CM, Cedars A, Gross RW. Cardiovascular Research. 2009; 82:240. [PubMed: 19074824]
4. Antona CR, Sundberg MI. Oncogene. 2006; 25:1679. [PubMed: 16550168]
5. Calder PC, Zurier RB. Curr Opin Clin Nutr Metab Care. 2001; 4:115. [PubMed: 11224655]
6. Dennis EA, Cao J, YH, Magrioti V, Kokotos G. Chem Rev. 2011; 111:6130–6185. [PubMed: 21910409]
7. Deems R, Buczynski MW, Gentry RB, Harkewicz R, Dennis EA. Methods in Enzymology. 2007; 432:59. [PubMed: 17954213]
8. Kortz BS, Helmschrodt L, Thiery C, J, Ceglarek U. J Chromatogr B. 2012; 883-884:68–75.
9. Ferreiro-Vera C, Mata-Granados JM, Priego-Capote F, Quesada-Gómez JM, Luque de Castro MD. Anal Bioanal Chem. 2011; 399:1093. [PubMed: 21079925]
10. Hofseth LJ, Ying L. Biochim Biophys Acta. 2006; 1765:74. [PubMed: 16169156]
11. Reinke M. Am J Physiol. 1992; 262:E658. [PubMed: 1590375]
12. Shono F, Yokota K, Horie K, Yamamoto S, Yamashita K, Watanabe K, Miyazaki H. Anal Biochem. 1988; 168:284. [PubMed: 3129960]
13. Baranowski R, Pacha K. Mini Rev Med Chem. 2002; 2:135. [PubMed: 12370075]
14. Margalit A, Duffin KL, Isakson PC. Anal Biochem. 1996; 235:73. [PubMed: 8850549]
15. Araujo P, Mengesha Z, Lucena E, Grung B. J Chromatogr A. 2013 online.
16. Araujo P, Janagap S, Holen E. J Chromatogr A. 2012; 1260:102. [PubMed: 22959773]
17. Komaba JJ, Matsuda D, Shibakawa K, Nakade S, Hashimoto Y, Miyataa Y, Ogawaa M. Biomed Chromatogr. 2009; 23:315. [PubMed: 18800333]
18. Blewett AJ, Varma D, Gilles T, Libonati JR, Jansen Susan A. J Pharmaceut Biomed Anal. 2008; 46:653.
19. Song J, Liu XJ, Wu JJ, Meehan MJ, Blevitt JM, Dorrestein PC, Milla ME. Anal Biochem. 2013; 433:181. [PubMed: 23103340]
20. Strassburg K, Huijbrechts AML, Kortekaas KA, Lindeman JH, Pedersen TL, Dane A, Berger R, Brenkman A, Hankemeier T, van Duynhoven J, Kalkhoven E, Newman JW, Vreeken RJ. Anal Bioanal Chem. 2012; 404:1413–1426. [PubMed: 22814969]
21. Faouder PL, Baillif V, Spreadburyd I, Mottae JP, Roussete P, Chênec G, Guignéc C, Tercéa F, Vannerd S, Vergnollee N, Bertrand-Michel J, Dubourdeauc M, Cenace N. J Chromatogr B. 2013; 932:123.
22. Shindea DD, Kima KB, Oha KS, Abdallaa N, Liub KH, Baec SK, Shona JH, Kima HS, Kima DH, Shina JG. J Chromatogr B. 2012; 911:113–121.
23. Levison BS, Zhang RL, Wang ZN, Fu XM, Di Donato JA, Hazen SL. Free Radical Biology and Medicine. 2013; 59:2–13. [PubMed: 23499838]

24. Kortz L, Dorow JL, Becker S, Thiery J, Ceglareka U. J Chromatogr B. 2013; 927:209–213.
25. Dumlao DS, Buczynski MW, Norris PC, Harkewicz R, Dennis EA. Biochim Biophy Acta. 2011; 1811:7240.
26. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, Bandyopadhyay S, Jones KN, Kelly S, Shaner RL, Sullards CM, Wang E, Murphy RC, Barkley RM, Leiker TJ, Raetz CRH, Guan ZQ, Laird GM, Six DA, Russell DW, McDonald JG, Subramaniam S, Fahy E, Dennis EA. J Lip Res. 2010; 51:3299.
27. Quehenberger O, Dennis EA. New Engl J Med. 2013; 365:1812. [PubMed: 22070478]
28. Golovko MY, Murphy EJ. J Lipid Res. 2008; 49:893. [PubMed: 18187404]
29. Wada M, De Long CJ, Hong YH, Rieke CJ, Song I, Sidhu RS, Yuan C, Warnock M, Schmaier AH, Yokoyama C, Smyth CEM, Wilson SJ, Fitz Gerald GA, Garavito RM, Sui DX, Regan JW, Smith WL. J Biol Chem. 2007; 282:22254. [PubMed: 17519235]
30. Tassoni D, Kaur G, Weisinger RS, Sinclair AJ. Asia Pac J Clin Nutr. 2008; 17:220. [PubMed: 18296342]
31. Jian W, Edom R, Weng N, Zannikos P, Zhang Z, Wang ZH. J Chromatogr B. 2010; 878:1687.
32. Dams R, Huestis MA, Lambert WE, Murphy CM. J Am Soc Mass Spectrom. 2003; 14:1290. [PubMed: 14597119]
33. Quehenberger O, Armando A, Dumlao D, Stephens DL, Dennis EA. Prostaglandins Leukot Essent Fatty Acids. 2008; 79:123. [PubMed: 18996688]

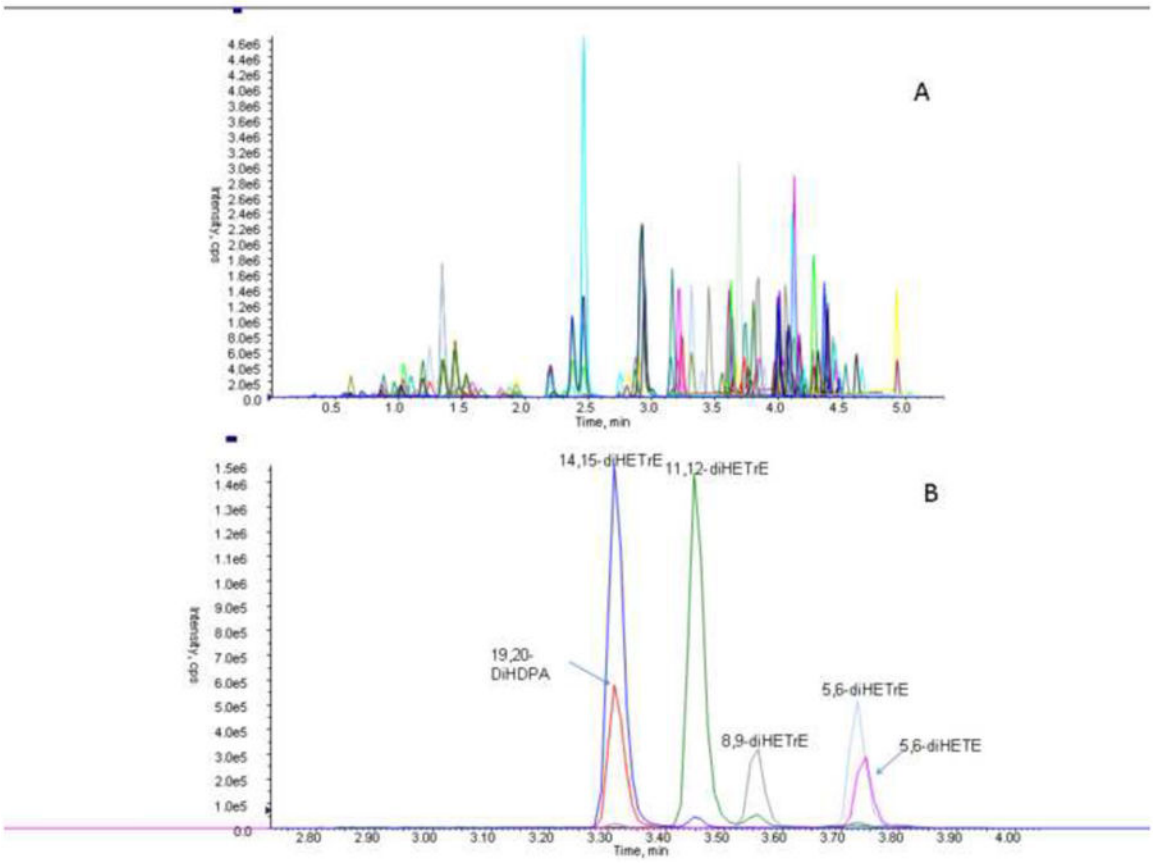

Fig. 1. Extracted Ion Chromatograms of a mixture 187 Eicosanoid Standard (A) and a Magnified View of the Chromatograms of HETE Metabolites

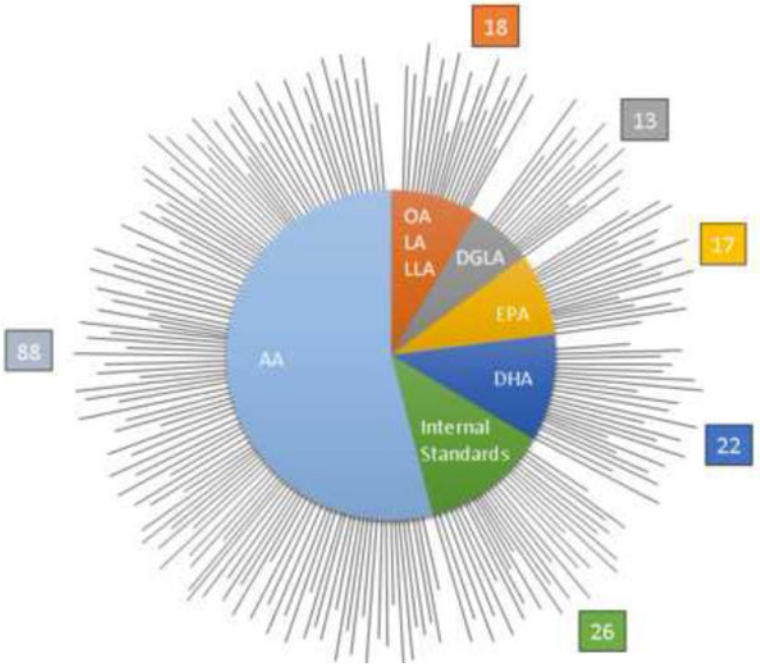

Fig. 2. The Relationship of the Member Eicosanoid Analytes to PUFA Precursors

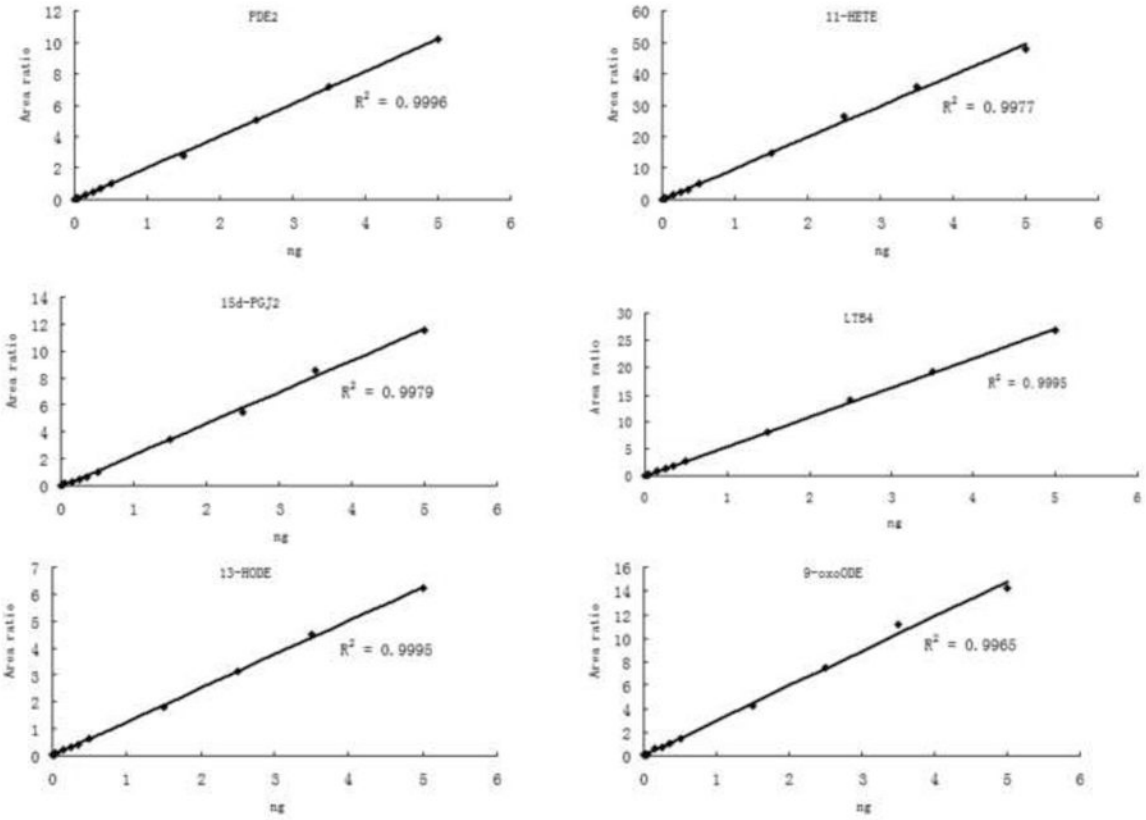

Fig. 3. Linearity of Representative Standards

Table 1

Internal Standard Assigned for Analytes

No.	Internal Standard	Analytes Assigned	No.	Internal Standard	Analytes Assigned
1	**(d4) 6k $PGF_{1\alpha}$**	6	**14**	**(d6) 20-HETE**	10
2	**(d4) TXB_2**	5	**15**	**(d4) 9-HODE**	2
3	**(d4) $PGF_{2\alpha}$**	12	**16**	**(d4) 13-HODE**	4
4	**(d4) PGE_2**	10	**17**	**(d7) 5-oxoETE**	6
5	**(d4) PGD_2**	11	**18**	**(d4) Resolvin E_1**	6
6	**(d4) 15d PGJ_2**	6	**19**	**(d11) 8,9 EET**	1
7	**(d4) dhk $PGF_{2\alpha}$**	1	**20**	**(d11) 11,12 EET**	5
8	**(d4) dhk PGD_2**	2	**21**	**(d11) 14,15 EET**	1
9	**(d11) 5-iso $PGF_{2\alpha}$VI**	4	**22**	**(d4) 9,10 diHOME**	1
10	**(d4) LTB_4**	18	**23**	**(d4) 12,13 diHOME**	1
11	**(d8) 5-HETE**	13	**24**	**(d5) LTC_4**	1
12	**(d8) 12-HETE**	13	**25**	**(d5) LTE_4**	2
13	**(d8) 15-HETE**	9	**26**	**(d8) Arachidonic Acid**	8

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Table 2

Optimized MRM Pairs and Parameters for Eicosanoids

No.	Common name	Retention Time (min)	m/z		DP (V)	CE (V)	LOQ (pg)
			Parent	Daughter			
001	(d4) 6k $PGF_{1\alpha}$	0.76	373	167	-60	-34	-
002	(d4) TXB_2	0.98	373	173	-50	-22	-
003	(d4) $PGF_{2\alpha}$	1.14	357	197	-50	-35	-
004	(d4) PGE_2	1.29	355	275	-50	-23	-
005	(d4) PGD_2	1.47	355	275	-50	-23	-
006	(d4) 15d PGJ_2	3.54	319	203	-30	-20	-
007	(d4) dhk $PGF_{2\alpha}$	1.74	357	295	-80	-28	-
008	(d4) dhk PGD_2	2.13	355	297	-40	-26	-
009	(d4) 8-iso $PGF_{2\alpha}$ VI	0.99	357	197	-20	-22	-
010	(d4) LTB_4	2.85	339	197	-45	-23	-
011	(d8) 5-HETE	4.17	327	116	-40	-20	-
012	(d8) 12-HETE	4.05	327	184	-50	-19	-
013	(d8) 15-HETE	3.92	327	226	-40	-19	-
014	(d6) 20-HETE	3.66	325	281	-50	-24	-
015	(d4) 9-HODE	3.89	299	172	-60	-23	-
016	(d4) 13-HODE	3.89	299	198	-60	-23	-
017	(d7) 5-oxoETE	4.31	323	279	-60	-22	-
018	(d4) Resolvin E_1	0.80	353	197	-40	-20	-
019	(d11) 8,9 EET	4.37	330	155	-50	-19	-
020	(d11) 11,12 EET	4.34	330	167	-50	-19	-
021	(d11) 14,15 EET	4.23	330	175	-40	-19	-
022	(d4) 9,10 diHOME	3.12	317	203	-50	-29	-
023	(d4) 12,13 diHOME	3.07	317	185	-50	-29	-
024	(d5) LTC_4	2.00	629	272	-50	-34	-
025	(d5) LTE_4	2.25	443	338	-30	-26	-
026	(d8) Arachiconic Acid	4.87	311	267	-55	-20	-

No.	Common name	Retention Time (min)	m/z		DP (V)	CE (V)	LOQ (pg)
			Parent	Daughter			
027	6k $PGF_{1\alpha}$	0.76	369	245	-60	-34	5
028	TxB_2	0.98	369	169	-50	-22	3
029	$PGF_{2\alpha}$	1.12	353	197	-50	-35	70
030	PGE_2	1.27	351	271	-50	-23	3
031	PGD_2	1.45	351	27	-50	-23	3
032	11b $PGF_{2\alpha}$	1.08	353	335	-50	-35	100
033	TXB_1	0.95	371	171	-30	-27	5
034	$PGF_{1\alpha}$	1.18	355	293	-60	-33	7
035	PGE_1	1.40	353	235	-40	-29	30
036	PGD_1	1.51	353	235	-40	-29	30
037	d17 6k $PGF_{1\alpha}$	0.66	367	163	-60	-34	7
038	TXB_3	0.81	367	169	-40	-27	7
039	$PGF_{3\alpha}$	0.94	351	193	-50	-30	50
040	PGE_3	1.03	349	269	-30	-24	3
041	PGD_3	1.14	349	269	-30	-24	7
042	dihomo $PGF_{2\alpha}$	1.85	381	337	-40	-37	3
043	dihomo PGE_2	2.20	379	299	-40	-37	-
044	dihomo PGD_2	2.30	379	299	-40	-37	-
045	dihomo PGJ_2	3.00	361	299	-40	-37	-
046	dihomo 15d PGD_2	3.30	361	299	-40	-37	-
047	6k PGE_1	0.83	367	331	-40	-25	7
048	6,15 dk-,dh- $PGF_{1\alpha}$	1.01	369	267	-40	-37	300
049	15k $PGF_{1\alpha}$	1.59	353	221	-50	-38	30
050	15k $PGF_{2\alpha}$	1.42	351	219	-50	-32	10
051	15k PGE_2	1.56	349	235	-30	-26	7
052	15k PGD_2	1.65	349	235	-30	-40	70
053	dh $PGF_{2\alpha}$	1.42	355	311	-60	-29	7

No.	Common name	Retention Time (min)	m/z		DP (V)	CE (V)	LOQ (pg)
			Parent	Daughter			
054	dhk $PGF_{2\alpha}$	1.71	353	291	-80	-28	5
055	dhk PGE_2	1.82	351	207	-40	-26	7
056	dhk PGD_2	2.10	351	207	-40	-26	3
057	bicyclo PGE_2	2.28	333	175	-40	-30	7
058	11b dhk $PGF_{2\alpha}$	1.50	353	221	-60	-37	50
059	9oh $PGF_{2\alpha}$	0.55	369	192	-60	-35	300
060	20oh $PGF_{2\alpha}$	0.54	369	165	-40	-39	7
061	19oh PGE_2	0.57	367	243	-20	-31	10
062	20oh PGE_2	0.57	367	175	-30	-27	5
063	2,3 dinor 11b $PGF_{2\alpha}$	0.83	325	227	-30	-22	5
064	PGFM	0.58	329	293	-40	-25	1
065	PGEM	0.60	327	291	-30	-23	7
066	tetranor 12-HETE	3.12	265	109	-20	-18	1
067	11b PGE_2	1.37	351	271	-40	-23	3
068	PGK_2	2.02	349	249	-40	-31	10
069	12-HHTrE	3.28	279	217	-30	-21	5
070	11-HETE	4.00	319	167	-40	-23	1
071	11-HEPE	3.58	317	215	-40	-20	3
072	13 HDoHE	3.98	343	221	-30	-19	5
073	PGA_2	2.36	333	271	-20	-20	3
074	PGB_2	3.13	333	271	-40	-25	3
075	15d PGA_2	3.64	315	255	-40	-20	-
076	PGJ_2	2.28	333	189	-40	-22	3
077	15d PGD_2	2.83	333	271	-30	-22	3
078	15d PGJ_2	3.52	315	203	-30	-20	3
079	5-iso $PGF_{2\alpha}$VI	1.08	353	115	-60	-28	10
080	8-iso $PGF_{2\alpha}$III	0.96	353	193	-40	-33	7
081	9-HETE	4.09	319	123	-40	-20	3

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No.	Common name	Retention Time (min)	m/z		DP (V)	CE (V)	LOQ (pg)
			Parent	Daughter			
082	9-HEPE	3.70	317	149	-40	-20	3
083	8 HDoHE	4.11	343	109	-40	-20	5
084	16 HDoHE	3.94	343	233	-50	-19	3
085	20 HDoHE	3.86	343	241	-30	-18	3
086	LTB_4	2.82	335	195	-45	-23	3
087	20oh LTB_4	0.83	351	195	-40	-23	5
088	20cooh LTB_4	0.84	365	303	-40	-26	30
089	5,6-diHETE	3.64	335	115	-50	-29	3
090	6t LTB_4	2.90	335	195	-45	-22	3
091	12epi LTB_4	2.83	335	195	-45	-22	3
092	6t,12epi LTB_4	2.82	335	195	-45	-22	3
093	12oxo LTB_4	2.84	335	253	-50	-22	3
094	LTC_4	1.80	624	272	-50	-33	-
095	LTD_4	1.47	495	177	-50	-29	5
096	LTE_4	2.18	438	333	-30	-25	3
097	11t LTC_4	2.30	624	272	-50	-34	-
098	11t LTD_4	1.77	495	177	-50	-29	5
099	11t LTE_4	2.42	438	333	-50	-33	7
100	5-HETE	4.16	319	115	-40	-20	3
101	5-HEPE	3.77	317	115	-30	-22	3
102	7 HDoHE	4.07	343	141	-40	-19	5
103	4 HDoHE	4.28	343	101	-60	-18	3
104	9-HOTrE	3.48	293	171	-40	-22	0.1
105	5-HETrE	4.56	321	205	-30	-19	3
106	5,15-diHETE	2.73	335	201	-40	-26	3
107	6R-LXA_4	1.81	351	167	-20	-21	-
108	6S-LXA_4	1.89	351	217	-20	-18	-
109	15R-LXA_4	1.82	351	165	-20	-23	50

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No.	Common name	Retention Time (min)	m/z		DP (V)	CE (V)	LOQ (pg)
			Parent	Daughter			
110	LXA_5	3.29	349	215	-30	-25	-
111	LXB_4	1.44	351	221	-50	-21	5
112	Resolvin E_1	0.80	349	195	-40	-20	70
113	Resolvin D_1	1.70	375	141	-20	-20	5
114	Protectin D_1	1.80	359	153	-20	-20	5
115	15t-Protectin D_1	2.75	359	153	-20	-27	5
116	10S-Protectin D_1	2.78	359	153	-20	-21	3
117	8,15-diHETE	2.64	335	235	-40	-26	100
118	15-HETE	3.91	319	175	-40	-19	3
119	15-HEPE	3.61	317	219	-40	-18	3
120	17 HDoHE	3.94	343	229	-20	-19	30
121	13-HODE	3.89	295	195	-60	-23	3
122	13-HOTrE	3.65	293	195	-40	-28	10
123	13-HOTrE(y)	3.63	293	193	-40	-19	5
124	15-HETrE	4.15	321	221	-30	-21	3
125	8-HETE	4.05	319	155	-40	-19	5
126	8-HEPE	3.74	317	155	-50	-29	7
127	10 HDoHE	4.06	343	153	-50	-19	3
128	8-HETrE	4.20	321	157	-20	-22	5
129	14,15 LTC4	3.29	624	272	-30	-32	-
130	14,15 LTD4	1.10	495	177	-60	-25	300
131	14,15 LTE4	1.61	438	333	-40	-22	7
132	12-HETE	4.03	319	135	-50	-19	3
133	12-HEPE	3.67	317	179	-30	-19	3
134	14 HDoHE	4.00	343	205	-30	-18	5
135	11 HDoHE	4.04	343	149	-20	-19	5
136	9-HODE	3.88	295	171	-60	-23	3
137	HXA3	3.48	335	195	-60	-26	70
138	HXB3	3.46	335	183	-40	-21	5

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No.	Common name	Retention Time (min)	m/z		DP (V)	CE (V)	LOQ (pg)
			Parent	Daughter			
139	5-oxoETE	4.30	317	203	-60	-22	3
140	12-oxoETE	4.06	317	153	-50	-23	5
141	15-oxoETE	3.94	317	113	-20	-25	3
142	9-oxoODE	3.97	293	185	-50	-28	3
143	13-oxoODE	3.91	293	167	-50	-29	10
144	15 oxoEDE	4.49	321	223	-80	-32	3
145	20-HETE	3.64	319	245	-50	-24	3
146	19-HETE	3.57	319	231	-40	-23	30
147	18-HETE	3.67	319	261	-60	-20	3
148	17-HETE	3.71	319	247	-50	-20	3
149	16-HETE	3.73	319	189	-30	-21	3
150	18-HEPE	3.52	317	215	-50	-20	3
151	5,6-EET	4.42	319	191	-30	-17	3
152	8,9-EET	4.37	319	155	-30	-18	7
153	11,12-EET	4.34	319	167	-30	-17	3
154	14,15-EET	4.23	319	175	-30	-17	3
155	14(15) EpETE	3.99	317	207	-30	-19	3
156	17(18) EpETE	3.85	317	259	-40	-18	10
157	16(17) EpDPE	4.27	343	193	-40	-19	10
158	19(20) EpDPE	4.16	343	241	-50	-18	5
159	19,20 DiHDPA	3.20	361	229	-40	-22	5
160	9,10 EpOME	4.25	295	171	-60	-21	3
161	12,13 EpOME	4.23	295	195	-50	-23	3
162	5,6-diHETrE	3.62	337	145	-40	-22	3
163	8,9-diHETrE	3.44	337	127	-30	-27	3
164	11,12-diHETrE	3.33	337	167	-40	-25	3
165	14,15-diHETrE	3.19	337	207	-30	-24	3
166	9,10 diHOME	3.09	313	201	-50	-29	3
167	12,13 diHOME	3.04	313	183	-50	-29	3
168	Arachidonic Acid	4.85	303	259	-55	-20	0.1

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No.	Common name	Retention Time (min)	m/z		DP (V)	CE (V)	LOQ (pg)
			Parent	Daughter			
169	Adrenic Acid	4.97	331	287	-70	-20	0.1
170	EPA	4.76	301	257	-40	-16	0.1
171	DHA	4.88	327	283	-40	-19	0.1
172	20cooh AA	3.66	333	271	-60	-23	5
173	17k DPA	4.21	343	247	-40	-23	3
174	2,3 dinor TXB2	0.84	341	137	-20	-31	50
175	11d-TXB2	1.38	367	305	-20	-26	7
176	2,3 dinor 8-iso PGF2a	0.77	325	237	-30	-19	5
177	2,3 dinor-6k PGF1a	4.32	363	281	-30	-23	-
178	PGK1	2.02	351	251	-40	-26	5
179	8-iso PGF3a	0.82	351	307	-30	-28	5
180	8-iso-15k PGF2b	1.20	351	219	-50	-22	5
181	9-Nitrooleate	4.74	326	168	-40	-20	3
182	10-Nitrooleate	4.73	326	169	-40	-19	7
183	tetranor-PGDM	0.70	327	247	-20	-20	50
184	7(R) Maresin-1	2.75	359	177	-30	-22	30

Table 3

Recovery Rate and Matrix Effect of Internal Standards

No	Internal Standard	Recovery rate(%)	Matrix effect (%)
1	(d4) 6k PGF1a	94.1	86.6
2	(d4) TXB2	87.2	89.0
3	(d4) PGF2a	62.1	76.6
4	(d4) PGE2	89.5	84.8
5	(d4) PGD2	82.2	99.5
6	(d4) 15d PGJ2	58.2	91.4
7	(d4) dhk PGF2a	94.7	97.3
8	(d4) dhk PGD2	84.4	96.8
9	(d11) 8-iso PGF2a III	81.3	97.0
10	(d4) LTB4	82.9	90.8
11	(d8) 5-HETE	67.9	68.1
12	(d8) 12-HETE	80.8	87.4
13	(d8) 15-HETE	82.5	88.4
14	(d6) 20-HETE	71.0	85.8
15	(d4) 9-HODE	76.2	82.1
16	(d4) 13-HODE	86.71	85.9
17	(d7) 5-oxoETE	92.8	85.2
18	(d4) Resolvin E1	63.0	85.8
19	(d11) 8,9 EET	78.7	87.5
20	(d11) 11,12 EET	74.4	89.9
21	(d11) 14,15 EET	78.1	93.9
22	(d4) 9,10 diHOME	70.9	91.1
23	(d4) 12,13 diHOME	78.0	90.0
24	(d5) LTC4	112.6	119.0
25	(d5) LTE4	112.7	118.3
26	(d8) Arachiconic Acid	54.3	70.4