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Comprehensive analysis of oxylipins in human plasma using reversed-phase liquid chromatography-triple quadrupole mass spectrometry with heatmap-assisted selection of transitions

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Abstract

Oxylipins, a subclass of lipid mediators, are metabolites of various polyunsaturated fatty acids with crucial functions in regulation of systemic inflammation. Elucidation of their roles in pathological conditions requires accurate quantification of their levels in biological samples. We refined an ultra-performance liquid chromatography-multiple reaction monitoring-mass spectrometry (UPLC-MRM-MS)-based workflow for comprehensive and specific quantification of 131 endogenous oxylipins in human plasma, in which we optimized LC mobile phase additives, column and gradient conditions. We employed heatmap-assisted strategy to identify unique transitions to improve the assay selectivity and optimized solid phase extraction procedures to achieve better analyte recovery. The method was validated according to FDA guidelines. Overall, 94.4% and 95.7% of analytes at tested concentrations were within acceptable accuracy (80–120%) and precision (CV<15%), respectively. Good linearity for most analytes was obtained with $R^2 > 0.99$. The method was also validated using a standard reference material – SRM 1950 frozen human plasma to demonstrate inter-lab compatibility.

Keywords

oxylipins; lipid mediators; LC-MRM-MS; heatmap; human plasma; SRM 1950

Introduction

Oxylipins, a subclass of lipid mediators [1], are originated from various polyunsaturated fatty acid (PUFA) precursors such as arachidonic acid (C20:4 n-6, AA), eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA), which are released by phospholipase A2 [2] from the membrane phospholipids and further metabolized by

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Compliance with Ethical Standards

The authors have no conflicts of interests to declare.

Deidentified, commercial human plasma were used in this work. Research conducted with unidentified samples is not human subjects research and is not regulated by the Federal Policy for the Protection of Human Subjects (45 CFR Part 46).

cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) as well as some non-enzymatic pathways [3]. As a result, hundreds of these mediators are released into circulation and involved in various physiological conditions [4] and pathogenesis, such as inflammation [3], vascular diseases [5–8], metabolic syndrome [9,1,10,11], neurological diseases [12–14] and cancers [15,16]. Elucidation of their roles in enhancing and resolving inflammation and disease progression requires an accurate quantification of their levels in human plasma and other biofluids.

Among the methods for analyzing oxylipins, LC coupled to triple quadrupole mass spectrometry (QQQ-MS) has gained popularity in recently years because of its selectivity and sensitivity. Recent developments of UPLC technology with sub-2 μ m columns have greatly improved chromatographic separation of eicosanoids and reduced analytical run time from 20–60 min [17–20] to 4–12 min [21,22], which in turn significantly increased the analytical throughput. When coupled with scheduled multiple reaction monitoring (MRM) MS, the number of oxylipins monitored within one single LC run can reach more than 180 [21]. Given these advancements, comprehensive analysis of oxylipins still remains a challenge because of the low concentration (<nM) in the real samples, wide dynamic ranges within sample and prevalence of structurally similar isomeric species [23], an example for the latter is that there are at least 14 HETEs (C₂₀H₃₂O₃), 4 EETs (C₂₀H₃₂O₃) and 10 hydroxydocosahexaenoic acids (HDoHEs, C₂₂H₃₂O₃) isomers reported in LIPID MAPS database.

Differentiation of isomeric lipids mainly relies on LC separation and specific MRM transitions. LC retention time resolves some of these isomers, but oftentimes the close structural similarity makes it incapable of separating all isomeric oxylipins even with the most recent advances in UPLC separation [21]. When chromatographic separation power is limited, specificity of selected MRM transitions is critical to differentiate co-eluting isomeric lipid mediators. Wang *et al.* used a 5-min LC gradient to separate 184 oxylipin metabolites, including 11 HETEs and 10 HDoHEs with average retention time differences (RT, RT_n-RT_{n-1}) of 0.059 and 0.046 min, respectively [21]. Slight RT differences are not sufficient to selectively identify these isomers, in this case some species can be considered as co-elutents, and if the MRM transitions are not unique, then the level of each isomer cannot be accurately determined. Therefore, it is critical to find the unique fragment ions as the surrogate of the particular oxylipin isomer. Considering the structural similarity between the isomers and the subsequent overlap of fragment ions, a strategy to identify selective MRM transitions for each isomer would facilitate their accurate quantification.

Another factor that affects the absolute quantification of oxylipins is extraction. Two major methods for sample clean-up, liquid-liquid extraction (LLE) and solid phase extraction (SPE) are widely used for analyzing these mediators. The LLE shows good recoveries for EETs, HETEs and PGs; however, it exhibited lower recoveries for hydrophilic analytes such as tetranor-PGEM and leukotrienes (LTs) [20]. Various SPE materials and protocols are employed and it is inconclusive which material and protocol is the best for extracting oxylipins from biological matrices [24].

Herein, we report a refined workflow for UPLC-MRM-MS based quantification of 131 endogenous oxylipins with 25 deuterium-labeled stable isotope standards, which incorporates optimized sample preparation and chromatographic separation conditions, and more importantly, utilization of specific MRM transitions for unambiguous identification and accurate quantification of isomeric eicosanoids. The method was validated according to the FDA guidelines and further validated using a standard reference material human plasma, which has been used in the past for interlaboratory comparisons of lipidomics analysis between different analytical platforms [25,26].

Materials and methods

Chemicals and reagents

All lipid mediator and deuterated internal standards were purchased from Cayman Chemical (Ann Arbor, MI). LC-MS grade acetonitrile (ACN), methanol (MeOH), water (H₂O) and formic acid (FA) were obtained from Fisher Scientific (Waltham, MA). The pooled plasma from healthy subjects and the standard reference material - metabolites in frozen human plasma (SRM 1950) were purchased from Bioreclamation IVT (Baltimore, MD) and NIST (Gaithersburg, MA), respectively.

Standards and sample preparation

All of the analytical standards were prepared in the concentration of 1000 µg/ml as stock solution. The actual concentration of each species in the standard mixture (Master Mix) was adjusted according to test trial after taking consideration of previously reported analytical ranges. The deuterated internal standard mixture (Master ISTD) was prepared at concentration 10 times of its non-deuterated analogs. They are stored at -20°C freezer until use.

Aliquots of 200 µl human plasma were mixed with 5 µl Master ISDT before loading onto 96-well SPE cartridges (30 mg, HLB, Waters, Milford, MA), which had been pre-conditioned with 1 ml MeOH and followed by 1 ml water. A 1.5 ml 5% MeOH was used to wash out the unbounded interferents. Elution was carried out by 1.2 ml of MeOH. The eluents were dried under stream nitrogen and reconstituted with 50 µl of 50% MeOH. SPE recoveries were used to assess the extraction efficiency by comparing pre-spike and post-spike of deuterium-labeled standards.

Optimization of selective ion monitoring transitions

All the analytical standards were diluted in MeOH at the final concentration of 10 µg/ml for direct infusion. Surrogate transitions for oxylipins were optimized by using TSQ Quantiva Tune 2.1 (Thermo Fisher Scientific, Haverhill, MA) via direct infusion of individual standard solution with a syringe pump at flow rate of 10 µl/min. Top 6 fragment ions were selected to optimize the collision energy. The following parameters were set for the mass spectrometer: 10 Arb, 2 Arb, 0 Arb, 275 °C and 250 °C for sheath gas, aux gas, sweep gas, ion transfer tube and vaporizer temperature, respectively. The ion source was operated using heated ESI with ion spray voltage set at 2,500 V in negative ion mode. MS/MS spectra were exported from MS raw files for heatmap analysis.

LC-MS analysis

A Vanquish UHPLC coupled with a Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific, Haverhill, MA) was used for LC-MS analysis. A HSS T3 column (100 × 2.1 mm, 1.8 μm, Waters, Milford, MA) with T3 VanGuard pre-column (5 × 2.1 mm, 1.8 μm, Waters) was employed for separation of analytes. The column was thermostated at 40°C. The mobile phase was composed of solvent A (0.1% FA in H₂O) and solvent B (0.1% FA in ACN). Gradient elution was used for 12 min at a flow rate of 0.3 ml/min. The gradient conditions were as follows: 0–0.5 min, 30% B; 0.5–1.0 min, 40% B; 1.0–2.5 min, 40% B; 2.5–4.5 min, 70% B; 4.5–6.5 min, 70% B; 6.5–9.0 min, 95% B; 9.0–12.0 min, 95% B. A 1.5 min equilibrium was used before the next injection. A 10 μl aliquot of each sample was injected onto column for analysis.

The following parameters were set for the mass spectrometer: 45 Arb, 13 Arb, 1 Arb, 350 °C and 350 °C for sheath gas, aux gas, sweep gas, ion transfer tube and vaporizer temperature, respectively. The ion source was operated using heated ESI with ion spray voltage set at 2,500 V in negative ion mode. Scheduled MRM was employed for analysis of all analytes and internal standards. The optimized SRM transitions and their respective collision energies were listed in Table 1.

Method validation

The validation procedures per the FDA draft guidelines on validation of bioanalytical methods were appropriately followed in this study [27]. The assay linearity, lower limit of quantification (LLOQ), lower limit of detection (LLOD), precision and accuracy were measured accordingly.

A serial dilution from the original stock was prepared to make the calibration curve. A total of 12 times serial dilution of highest point (Master Mix) with ISTD working solution was performed to construct the calibration curve and test the sensitivity. The LLOQ is generally defined as the lowest concentration of the standard curve that i) can be measured with acceptable accuracy (± 20%) and precision (< 20%) or ii) signal to noise ratio (S/N) is greater than 10. The LLOD is defined as an S/N ratio that is greater than 3.

The precisions and accuracies were evaluated with QC standards at low (LQC), middle (MQC) and high (HQC) concentrations covering analytical ranges. Precisions were expressed as percent coefficient of variance (CV%) and calculated as dividing standard deviations by the means. Accuracies were denoted as trueness and calculated as observed values divided by expected values.

Data analysis

For selection of unique MRM transitions, MS spectra obtained from direct infusion ESI of isomeric lipids were averaged and exported into Microsoft Excel and normalized to the base peak. The normalized spectra of fragment ions from each isomer were combined into a single data matrix with respective LC retention time (RT) of each isomer. RT sorting was performed using Excel, and heatmap analysis of the resulting data matrix were performed

with R language (version 3.3.1) and pheatmap package (1.0.8), which is available from <https://cran.r-project.org/web/packages/pheatmap/>.

LC-MRM-MS datasets were processed with TraceFinder 4.1, and the auto-integrated peaks were inspected manually. The calibration curve of each analyte was constructed by normalizing to the selected ISTD followed by linear regression with 1/x weighting.

For comparing the measured concentrations of oxylipins in SRM1950 with values reported by other laboratories, measured values were converted to nmol/ml prior to importing into LipidQC software [28].

Result and discussion

Optimization of LC-MS condition

Mobile phase additives could affect ionization efficiency [29]. In order to obtain the optimal ionization efficiency, various mobile phase additives such as acetic acid (HOAc) [21,30], formic acid (FA) [31] and ammonium formate (AF) [20] were compared to see if enhancement of MS signals of standard mixtures can be observed, using the same mobile phases (50/50, ACN/H₂O) by flow injection analysis (FIA) with 20 replicates. The pH of tested mobile phase with 0.1% FA, 0.1% HOAc, 10mM AF and 20mM AF were 2.81, 3.51, 6.21 and 6.45, respectively. As shown in Figure S1, after normalizing to that of 0.1% FA, 0.1% FA and 10mM AF exhibited higher average responses compared to 0.1% HOAc and 20mM AF, with 0.1% FA showing less variation in comparison to 10 mM AF. Therefore, 0.1% FA was chosen as our mobile phase additive.

Most lipid mediator-related studies employ C18-based columns to perform the separation; however, C30 column also showed great potential in separation of lipid molecular species [32,33] and could be used as an alternative for analyzing oxylipins. We compared the chromatographic performances of core-shell C30 and HSS T3 C18 column. Overall, both columns were comparable in terms of elution order, peak shape and peak capacity for separation of these mediators, C18 column provided higher efficient separation with less retention for hydrophobic analytes and more retention for hydrophilic analytes. As a result, HSS T3 column was chosen as our analytical column.

To obtain better peak resolution, we further optimized the LC gradient. A linear gradient (0–10 min, 30–95%B) was used which resulted in two crowded chromatographic regions, 2–4 min and 5–7 min, as shown in Figure S2A. Slight adjustment of gradients can reduce the density of peaks to spread the peaks more evenly across the retention time window (Figure S2B–D). Finally, applying a four-stage gradient successfully separated all analytes with the least co-eluted species. The TIC overlay of all the mediators at HQC standard concentration using optimized LC gradient is shown in Figure 1. The obtained retention times were used to construct the scheduled MRM method as listed in Table 1.

Necessity of selecting unique transitions

Although some isomers can be easily resolved by the reversed phase LC, certain isomers share similar physicochemical properties and form poorly resolved critical pairs [34] in

terms of chromatographic separation. Selectivity of MRM transitions is crucial to distinguish these co-eluent. We focused on the selectivity of these critical pairs and employed different MRM transitions reported by others to examine the selectivity. Two critical pairs derived from AA, including HETEs (19-HETE and 20-HETE) and EETs (11,12-EET and 8,9-EET) were chosen to demonstrate the importance of selecting appropriate transitions (Figure 2).

Shinde *et al.* used transition 319 301 ($[M-H-H_2O]^-$) as a surrogate of 20-HETE [20]. However, the water-loss fragment is not unique among all HETE species. If the LC separation power was sufficient to separate isomers, non-unique fragment ions would not be a problem in terms of differentiating isomers. Unfortunately, even when we used a 12 min LC gradient, the 19-HETE cannot be fully resolved from 20-HETE. For this critical pair, we found fragments m/z 231 (**black**, Figure 2A) and m/z 245 (**green**, Figure 2A) employed by Wang *et al.* [21] were more selective to 19-HETE and 20-HETE, respectively, compared to the water-loss fragment, m/z 301 (**red**, Figure 2A), which was common in both HETEs. Even though fragment ion m/z 301 was more intensive than other fragments, poor selectivity prevented it from separating 19-HETE and 20-HETE in the mixture (**bottom**, Figure 2A). The EET pair displayed a slight difference in retention time ($RT=0.1$) and the reported fragment ion m/z 167 [20] (**red**, Figure 2B) can be found in both 8,9-EET and 11,12-EET.

The poor selectivity issue is not limited to oxylipins derived from AA, those derived from DHA, such as 14-HDoHE and 10-HDoHE also exhibited identical retention on LC. The fragment ion m/z 161 has been used as specific transition for 14-HDoHE in a previous study [31]; however, we noticed it can be generated by 10-HDoHE as well. Besides, previous study employed fragment ion m/z 281 ($[M-H-H_2O-CO_2]^-$) to represent 17-HDoHE [30], yet, this water and CO_2 loss is common for all HDoHEs. Given this, good quantitation of 17-HDoHE would not be possible if there was not enough LC resolving power.

Collectively, these evidences demonstrate that some common fragment ions cannot provide enough selectivity to differentiate isomeric lipid mediators. For an unbiased quantification, therefore, it is necessary to take into consideration the fragmentation spectra of neighboring eluting isomers when selecting appropriate MRM transitions.

Strategy for selecting unique transitions

Our strategy to select unique transitions for isomers is illustrated in Figure 3. We averaged the spectra collected under various collision energies during direct infusion-based optimization of collisional energies. The obtained spectra were then normalized to the base peak of the spectrum and imported to a data matrix. The resulting matrix was then sorted by the chromatographic retention times of each isomer, which was obtained by running each isomeric compound individually using LC-MS. On the basis of the chromatographic resolution and peak width, we can define the minimum difference of retention time (RT). Within an acceptable RT , for example, of 0.15 min, the critical pairs were grouped together. A heatmap was then generated and employed to find out the unique fragment ions to the isomer among a large array of ions. If the isomers were separated very well ($RT \gg 0.15$) and there was no co-elution, the same fragment ions can still be used for identification of each isomer.

We took 15 HETEs/EETs ($C_{20}H_{32}O_3$) as an example to demonstrate the process of selecting optimal MRM transitions for series of oxylipin isomers. The average product ion spectra from the same precursor ion (m/z 319) of each of the 15 HETEs/EETs were obtained in the negative ion mode and normalized to the base peak. The isomers were sorted based on their respective LC retention time in ascending order and denoted as “RT1, RT2....RT14, RT15”. Even after we optimized the LC gradient to obtain the maximum peak capacity in LC separation, there were still a large number of analytes with $RT < 0.15$ min and therefore co-eluted. Figure 4A showed the heatmap of those normalized spectra. The RT of isomers less than 0.15 min were grouped together with the same color. Among all the isomers studied, 5 critical pairs were found and m/z 301 ($[M-H-H_2O]^-$), m/z 275 ($[M-H-CO_2]^-$) and m/z 257 ($[M-H-H_2O-CO_2]^-$) were the common fragment ions. These common product ions were not suitable to be selected as specific MRM transitions for these critical pairs even though they usually were the most abundant among fragment ions. Consequently, we excluded these common ions and looked for unique fragments to each isomer within each critical pair. For example, 16-HETE, 17-HETE and 18-HETE did not resolve well with a RT of 0.07 min. Yet, through the heatmap analysis, we could easily identify m/z 233 (break between C-15 and C-16), m/z 247 (break between C-16 and C-17) and m/z 261 (break between C-17 and C-18) as unique fragment ion for 16-HETE, 17-HETE and 18 HETE, respectively (Figure 4B). Alternatively, Willenberg *et al.* mentioned product ion spectra of 9-HETE and 12-HETE were similar and these two compounds must be separated chromatographically for their identification [23]. Yet, we were able to identify unique fragment m/z 135 (beak at C11–12 and loss of CO_2) [35] and m/z 151 for 12-HETE and 9-HETE, respectively, from the heatmap. The optimal transitions and the retention times for each of the 15 HETEs/EETs were listed in Table 1. Similar strategy was applied to other isomeric species of lipid mediators. The most dominant fragment ion for three chromatographically unresolved HDoHEs (11-HDoHE, 10-HDoHE and 14-HDoHE) is m/z 281 ($[M-H-H_2O-CO_2]^-$). Sharing with other HDoHEs suggested it was not suitable as MRM transitions for the differentiation of HDoHEs. However, the unique fragment ions m/z 149, m/z 153 and m/z 205 for 11-HDoHE, 10-HDoHE and 14-HDoHE, respectively, can be easily identified by visually inspecting the heatmap. Once the fragment ions representing each isomer were chosen, we optimized the corresponding collision energies for each isomer and implemented those values in the following scheduled LC-MRM-MS analysis.

Accuracies of selected MRM transitions in quantification of isomeric critical pairs

To demonstrate the accuracies of selected transitions for analysis of critical pairs, we spiked three critical pairs at various ratios in both neat standard solution and human plasma. Physiologically, the concentrations between critical pairs might vary up to 10-fold [30]. Therefore, the ratios used in the accuracy test were 1:1 (20/20 ng, R1), 1:10 (20/200 ng, R2) and 10:1 (200/20 ng, R3). Figure 5 displayed the chromatograms for the critical pair 20-HETE/19-HETE at various ratios. We noticed that the peak areas of critical pairs were not equal at the ratio of 1:1 since they have different sensitivity. Herein, the accuracies were determined by comparing the peak area ratios at R1/R2 and R3/R1 between theoretical values ($TV=10$) and observed values. Table 2 listed the accuracies for the tested pairs of HETEs. The values for the tested pairs in neat solution and human plasma ranged from 90.0% to 118.0% suggested the transitions selected using the proposed strategy were reliable

and accurate. By using this strategy, MRM transitions for 140 analytes and 25 ISTD were optimized and listed in Table 1.

Optimization of sample preparation procedures for better analyte recovery

Previous studies employed various strategies to clean up samples. One of the most used approaches was solid phase extraction (SPE). We briefly compared different SPE cartridges on the recoveries of spiked ISTD in plasma. As shown in Figure S3A, Waters HLB provided higher recoveries compared to Biotage isolate C18. In addition, we examined factors that might affect recoveries such as solvents used for elution and temperature for sample drying. We found high temperature (40 °C) might decompose the cysteinyl leukotrienes (i.e. LTC₄ and LTE₄) in the drying process as shown in Figure S3B which is consistent with previous work [36]. Although ethyl acetate (EA) had been used as eluting solvent in previous reports [17,30], we noticed that EA caused poor responses of cysteinyl leukotrienes as well which might be due to decomposition of the thiol group. Although others have demonstrated that using EA as eluting solvent can provide better recoveries for deuterium-labeled mediators than MeOH with the same SPE cartridge [26], they did not consider cysteinyl leukotrienes. Given that EA could cause breakdown of cysteinyl leukotrienes, we chose MeOH as eluting solvent. The effect of eluting volume was also examined and there were no significant changes from 1000 to 2000 µl of eluent (Figure S3C). In the end, we selected Waters HLB column to perform sample cleanup and oxylipins were eluted with 1200 µl MeOH following dryness at room temperature. The SPE recoveries were calculated as peak area of pre-spiked ISTD divided by peak area of post-spiked ISTD. Figure 6 displayed the overall recoveries of SPE under optimized sample preparation conditions. 70% of ISTD were within 80–120% whilst 7 species were at 65–80%, which was comparable to previous studies [30,26,21].

Method validation

The developed method was validated and the validation results including linearity, sensitivity, precision and accuracy are listed in Table 3. A total of 131 oxylipins were validated. 94.4% and 95.7% of analytes at tested concentrations were within acceptable accuracy (80–120%) and precision (CV<15%), respectively. The best-fit line of the calibration curve for each analyte was obtained by using a weighting factor of 1/x. Good linearity for most analytes was obtained with $R^2 > 0.99$. LLOQs are listed in Table 3 as well. The sensitivities of the present method on detecting oxylipins are comparable to other studies [21].

Lipid mediator levels in human plasma

The developed method was applied to analyze two sources of human plasma, standard reference material metabolites in frozen human plasma (SRM 1950) from NIST and healthy donor plasma from Bioreclamation IVT (BR). A total of 77 and 32 oxylipins can be detected in BR and SRM 1950 plasma, respectively and they are listed in Table 4. Although both samples were from healthy individuals, large variations in the concentration of oxylipins were observed, which is in agreement with previous work [37]. SRM 1950 has been employed in an inter laboratory comparison study for lipid profiling, we compared our results to the concentrations of oxylipins reported by Bowden *et al.*, [25] using LipidQC software [28]. A total of 11 mediators in our result were matched by LipidQC and 10 of

them were within 99% expanded uncertainty as shown in Figure S4, which suggests that our results were consistent to the NIST-ILCE summary report [25].

Conclusion

The MRM transitions can be easily obtained from mass spectrometry vendor's software such as Compound Optimizer from Agilent or Optimization in TSQ Tune from Thermo via either direct infusion with a syringe pump or flow injection with an autosampler. These tools provide great convenience for researchers to effectively acquire dominant fragment ions at optimal collisional energies for individual compound; however, they are not designed to determine unique fragments to distinguish isomeric compounds. Biased interpretation of lipid mediator networks could be made based on inaccurate quantification resulted from using inappropriate MRM transitions.

Herein, we proposed a heatmap-assisted strategy to effectively select the unique fragment ions as specific MRM transitions to a large number of isomers. This greatly facilitated our development of an unbiased and reliable MRM-based LC-MS method to determine the concentrations of oxylipins in human plasma, which monitors 131 endogenous oxylipins and 25 deuterium-labeled internal standards in a single LC run. Whilst the selection of MRM transitions is based on unique fragment ions and not the top abundant ions may result in reduction in assay's sensitivity; the optimization of mobile phase composition and LC gradient, together with improvements in SPE sample preparation helped to improve the method's sensitivity, as demonstrated by quantification of 77 oxylipins in human plasma. The method was rigorously validated according to the FDA guidelines, and further validated by the consistent results obtained when measuring a standard reference human plasma, as compared to the NIST inter-laboratory reported values.

Although most oxylipins are highly expressed under inflammatory conditions, and it is reported by others and also demonstrated in this work that oxylipin levels have high inter individual variation, it is still worthwhile to investigate the basal levels of oxylipins in healthy individuals, in particular for longitudinal follow-up of a person's health. Temporal changes of lipid mediator levels, together with other measurable clinical parameters are integral components of precision diagnosis and personalized medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Overlaid total ion chromatograms obtained from scheduled LC-MRM-MS analysis of analytes at HQC. All MRM transitions of all analytes listed in Table 1 were included.

Figure 2. Issues of inappropriate ion transitions on distinction of isomeric lipids. Extracted ion chromatograms from LC-MRM-MS analysis of 19-HETE and 20-HETE (A), 11,12-EET and 8,9-EET (B) monitored at different transitions.

Figure 3.
Workflow depicting the strategy to select optimal MRM transitions for each isomer.

Figure 4.

Identification of unique fragment ions within chromatographically challenged critical pairs. (A) Heatmap analysis of normalized fragment ion spectra of HETEs/EETs isomers, and (B) structure and annotation of common (m/z 301) and unique fragment ions of 16-HETE, 17-HETE and 18-HETE.

Figure 5.
LC-MRM-MS Chromatograms for 19-HETE and 20-HETE at three different ratios in neat solution. Ratio of 19-HETE/20-HETE: A, 1:1; B, 10:1; C, 1:10.

Figure 6.

SPE recoveries assessed by ISTDs on Waters HLC column, calculated as peak area of pre-spiked divided by that of post-spiked (mean $\pm$ RSD, $n=3$). ISTDs-spiked BR human plasma samples were processed with SPE columns before eluting with 1200 μ L MeOH and dried at room temperature and reconstituted for LC-MRM-MS analysis.

Table 1.

List of LC retention time and MRM transitions for each of the oxylipins included in the assay.

Name	Formula	Type	RT	Precursor	Product1	CE1	Product2	CE2
<i>tetranor 12-HETE</i>	C ₁₆ H ₂₆ O ₃	Analyte	6.33	265	109	10	165	12
<i>12-HHTrE</i>	C ₁₇ H ₂₈ O ₃	Analyte	6.41	279	179	11	217	10
<i>9-HOTrE</i>	C ₁₈ H ₃₀ O ₃	Analyte	6.68	293	171	14		
<i>13-HOTrE</i>	C ₁₈ H ₃₀ O ₃	Analyte	6.78	293	195	12		
<i>13-HOTrE(y)</i>	C ₁₈ H ₃₀ O ₃	Analyte	6.86	293	113	19		
<i>13-oxoODE</i>	C ₁₈ H ₃₀ O ₃	Analyte	7.73	293	113	22	179	10
<i>9-oxoODE</i>	C ₁₈ H ₃₀ O ₃	Analyte	7.93	293	185	18	197	21
<i>12,13-EpOME</i>	C ₁₈ H ₃₂ O ₃	Analyte	6.15	295	195	15		
<i>9,10-EpOME</i>	C ₁₈ H ₃₂ O ₃	Analyte	6.23	295	171	15		
<i>13-HODE</i>	C ₁₈ H ₃₂ O ₃	Analyte	7.33	295	195	17		
<i>9-HODE</i>	C ₁₈ H ₃₂ O ₃	Analyte	7.34	295	171	16		
<i>(d4) 13-HODE</i>	C ₁₈ H ₂₈ D ₄ O ₃	IS	7.29	299	198	17		
<i>(d4) 9-HODE</i>	C ₁₈ H ₂₈ D ₄ O ₃	IS	7.3	299	172	18		
<i>Eicosapentaenoic acid</i>	C ₂₀ H ₃₀ O ₂	Analyte	9.76	301	257	10	203	13
<i>Arachidonic acid</i>	C ₂₀ H ₃₂ O ₂	Analyte	10.21	303	259	13	205	15
<i>(d8) Arachidonic acid</i>	C ₂₀ H ₂₄ D ₈ O ₂	IS	10.21	311	267	10		
<i>12,13-diHOME</i>	C ₁₈ H ₃₄ O ₄	Analyte	6.15	313	183	19		
<i>9,10-diHOME</i>	C ₁₈ H ₃₄ O ₄	Analyte	6.23	313	171	27		
<i>15d PGA₂</i>	C ₂₀ H ₂₈ O ₃	Analyte	5.07	315	187	20		
<i>15d PGJ₂</i>	C ₂₀ H ₂₈ O ₃	Analyte	6.93	315	203	20		
<i>(d4) 12,13-diHOME</i>	C ₁₈ H ₃₀ D ₄ O ₄	IS	6.15	317	185	21		
<i>(d4) 9,10-diHOME</i>	C ₁₈ H ₃₀ D ₄ O ₄	IS	6.2	317	203	18		
<i>18-HEPE</i>	C ₂₀ H ₃₀ O ₃	Analyte	6.78	317	215	10	259	13
<i>11-HEPE</i>	C ₂₀ H ₃₀ O ₃	Analyte	6.95	317	167	11	195	15
<i>15-HEPE</i>	C ₂₀ H ₃₀ O ₃	Analyte	6.95	317	175	15	247	13
<i>8-HEPE</i>	C ₂₀ H ₃₀ O ₃	Analyte	7.03	317	155	11		
<i>12-HEPE</i>	C ₂₀ H ₃₀ O ₃	Analyte	7.09	317	179	10		
<i>9-HEPE</i>	C ₂₀ H ₃₀ O ₃	Analyte	7.13	317	149	12		
<i>5-HEPE</i>	C ₂₀ H ₃₀ O ₃	Analyte	7.19	317	115	10		
<i>17(18)-EpETE</i>	C ₂₀ H ₃₀ O ₃	Analyte	7.68	317	215	10		
<i>15-oxoETE</i>	C ₂₀ H ₃₀ O ₃	Analyte	7.95	317	113	15	139	18
<i>14(15)-EpETE</i>	C ₂₀ H ₃₀ O ₃	Analyte	7.95	317	207	12		
<i>12-oxoETE</i>	C ₂₀ H ₃₀ O ₃	Analyte	8.26	317	153	16		
<i>5-oxoETE</i>	C ₂₀ H ₃₀ O ₃	Analyte	8.82	317	203	17		
<i>(d4) 15d PGJ₂</i>	C ₂₀ H ₂₄ D ₄ O ₃	IS	4.11	319	275	14		
<i>19-HETE</i>	C ₂₀ H ₃₂ O ₃	Analyte	6.89	319	231	10	177	15

Name	Formula	Type	RT	Precursor	Product1	CE1	Product2	CE2
20-HETE	C ₂₀ H ₃₂ O ₃	Analyte	6.92	319	289	15		
5,6-EET	C ₂₀ H ₃₂ O ₃	Analyte	6.92	319	191	14		
18-HETE	C ₂₀ H ₃₂ O ₃	Analyte	7.12	319	261	18		
17-HETE	C ₂₀ H ₃₂ O ₃	Analyte	7.18	319	247	10		
16-HETE	C ₂₀ H ₃₂ O ₃	Analyte	7.19	319	233	12	189	11
15-HETE	C ₂₀ H ₃₂ O ₃	Analyte	7.56	319	175	13	219	10
11-HETE	C ₂₀ H ₃₂ O ₃	Analyte	7.7	319	167	16		
8-HETE	C ₂₀ H ₃₂ O ₃	Analyte	7.83	319	155	10		
12-HETE	C ₂₀ H ₃₂ O ₃	Analyte	7.88	319	135	13		
9-HETE	C ₂₀ H ₃₂ O ₃	Analyte	8	319	151	12	123	12
5-HETE	C ₂₀ H ₃₂ O ₃	Analyte	8.11	319	115	15		
14,15-EET	C ₂₀ H ₃₂ O ₃	Analyte	8.7	319	219	10	175	12
11,12-EET	C ₂₀ H ₃₂ O ₃	Analyte	8.94	319	208	10		
8,9-EET	C ₂₀ H ₃₂ O ₃	Analyte	9.03	319	155	12	151	10
15-HETrE	C ₂₀ H ₃₄ O ₃	Analyte	8.06	321	221	16		
8-HETrE	C ₂₀ H ₃₄ O ₃	Analyte	8.21	321	157	16	163	18
5-HETrE	C ₂₀ H ₃₄ O ₃	Analyte	9.12	321	115	13		
15-oxoEDE	C ₂₀ H ₃₄ O ₃	Analyte	9.21	321	223	22	195	20
(d7) 5-oxoETE	C ₂₀ H ₂₃ D ₇ O ₃	IS	8.78	323	279	11	130	15
2,3-dinor 8-iso PGF_{2a}	C ₁₈ H ₃₀ O ₅	Analyte	2.44	325	237	10		
2,3-dinor 11b PGF_{2a}	C ₁₈ H ₃₀ O ₅	Analyte	2.62	325	145	15	163	10
(d6) 20-HETE	C ₂₀ H ₂₆ D ₆ O ₃	IS	6.91	325	307	15	281	17
10-Nitrooleate	C ₁₈ H ₃₃ NO ₄	Analyte	9.74	326	181	15	279	14
9-Nitrooleate	C ₁₈ H ₃₃ NO ₄	Analyte	9.75	326	195	27		
tetranor-PGDM	C ₁₆ H ₂₄ O ₇	Analyte	1.13	327	309	10		
(d8) 15-HETE	C ₂₀ H ₂₄ D ₈ O ₃	IS	7.47	327	226	11	182	14
(d8) 12-HETE	C ₂₀ H ₂₄ D ₈ O ₃	IS	7.82	327	184	14	214	14
(d8) 5-HETE	C ₂₀ H ₂₄ D ₈ O ₃	IS	8.05	327	116	14	210	16
Docosahexaenoic acid	C ₂₂ H ₃₂ O ₂	Analyte	10.07	327	283	15	229	15
(d11) 14,15-EET	C ₂₀ H ₂₁ D ₁₁ O ₃	IS	8.65	330	219	10	175	13
(d11) 11,12-EET	C ₂₀ H ₂₁ D ₁₁ O ₃	IS	8.9	330	179	11		
(d11) 8,9-EET	C ₂₀ H ₂₁ D ₁₁ O ₃	IS	8.98	330	155	12	190	15
Adrenic acid	C ₂₂ H ₃₆ O ₂	Analyte	4.7	331	287	14	233	15
PGA₂	C ₂₀ H ₃₀ O ₄	Analyte	5.5	333	315	10	271	14
PGJ₂	C ₂₀ H ₃₀ O ₄	Analyte	5.53	333	233	10		
PGB₂	C ₂₀ H ₃₀ O ₄	Analyte	5.68	333	175	15	235	15
bicyclo PGE₂	C ₂₀ H ₃₀ O ₄	Analyte	5.85	333	235	20	204	20
15d PGD₂	C ₂₀ H ₃₀ O ₄	Analyte	6.01	333	271	14	315	10

Name	Formula	Type	RT	Precursor	Product1	CE1	Product2	CE2
<i>12oxo LTB₄</i>	C ₂₀ H ₃₀ O ₄	Analyte	6.25	333	179	15	153	15
<i>20cooh AA</i>	C ₂₀ H ₃₀ O ₄	Analyte	6.56	333	289	16	297	18
<i>8,15-diHETE</i>	C ₂₀ H ₃₂ O ₄	Analyte	5.81	335	155	15	127	15
<i>5,15-diHETE</i>	C ₂₀ H ₃₂ O ₄	Analyte	5.9	335	173	14		
<i>LTB₄</i>	C ₂₀ H ₃₂ O ₄	Analyte	5.98	335	195	14	317	13
<i>5,6-diHETE</i>	C ₂₀ H ₃₂ O ₄	Analyte	6.33	335	317	19	317	10
<i>14,15-diHETrE</i>	C ₂₀ H ₃₄ O ₄	Analyte	6.38	337	207	15		
<i>11,12-diHETrE</i>	C ₂₀ H ₃₄ O ₄	Analyte	6.54	337	167	17	169	16
<i>8,9-diHETrE</i>	C ₂₀ H ₃₄ O ₄	Analyte	6.68	337	127	19	185	15
<i>5,6-diHETrE</i>	C ₂₀ H ₃₄ O ₄	Analyte	6.87	337	145	16	319	15
<i>(d4) LTB₄</i>	C ₂₀ H ₂₈ D ₄ O ₄	IS	5.96	339	321	14	153	16
<i>2,3-dinor TXB₂</i>	C ₁₈ H ₃₀ O ₆	Analyte	2.49	341	167	10	141	14
<i>2,3-dinor-6k PGF_{1a}</i>	C ₁₈ H ₃₀ O ₆	Analyte	3.39	341	323	14	161	20
<i>20-HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.38	343	241	10		
<i>16-HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.59	343	233	10	189	13
<i>17 HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.59	343	245	10		
<i>19(20)-EpDPE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.59	343	241	10		
<i>10-HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.73	343	153	14	181	10
<i>14-HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.73	343	205	10	234	10
<i>11-HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.85	343	149	10	165	11
<i>13-HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.87	343	193	10	221	10
<i>7-HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.91	343	141	11	201	15
<i>8-HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	7.99	343	189	10	109	13
<i>4-HDoHE</i>	C ₂₂ H ₃₂ O ₃	Analyte	8.35	343	101	12		
<i>17k DPA</i>	C ₂₂ H ₃₂ O ₃	Analyte	8.51	343	247	16		
<i>16(17)-EpDPE</i>	C ₂₂ H ₃₂ O ₃	Analyte	8.79	343	233	10	201	10
<i>Resolvin E₁</i>	C ₂₀ H ₃₀ O ₅	Analyte	2.77	349	161	16	195	15
<i>PGE₃</i>	C ₂₀ H ₃₀ O ₅	Analyte	3.41	349	313	10		
<i>PGD₃</i>	C ₂₀ H ₃₀ O ₅	Analyte	3.7	349	233	10		
<i>LXA₅</i>	C ₂₀ H ₃₀ O ₅	Analyte	4.16	349	115	14	233	12
<i>PGK₂</i>	C ₂₀ H ₃₀ O ₅	Analyte	4.19	349	249	14	287	16
<i>15k PGE₂</i>	C ₂₀ H ₃₀ O ₅	Analyte	4.7	349	331	10	287	12
<i>8-iso PGF_{3a}</i>	C ₂₀ H ₃₂ O ₅	Analyte	2.79	351	307	17	245	18
<i>20oh LTB₄</i>	C ₂₀ H ₃₂ O ₅	Analyte	2.84	351	195	16		
<i>PGF_{3a}</i>	C ₂₀ H ₃₂ O ₅	Analyte	3.14	351	307	16		
<i>dhk PGE₂</i>	C ₂₀ H ₃₂ O ₅	Analyte	3.75	351	333	10		
<i>PGE₂</i>	C ₂₀ H ₃₂ O ₅	Analyte	4.15	351	271	14	315	10
<i>11βPGE₂</i>	C ₂₀ H ₃₂ O ₅	Analyte	4.32	351	315	10	271	16

Name	Formula	Type	RT	Precursor	Product1	CE1	Product2	CE2
<i>LXB₄</i>	C ₂₀ H ₃₂ O ₅	Analyte	4.39	351	221	14	163	16
<i>PGD₂</i>	C ₂₀ H ₃₂ O ₅	Analyte	4.54	351	271	16	315	10
<i>15R-LXA₄</i>	C ₂₀ H ₃₂ O ₅	Analyte	4.93	351	115	10	217	17
<i>6S-LXA₄</i>	C ₂₀ H ₃₂ O ₅	Analyte	5.04	351	115	13	217	18
<i>8-iso-15k PGF_{2b}</i>	C ₂₀ H ₃₂ O ₅	Analyte	5.05	351	219	14		
<i>PGEM</i>	C ₂₀ H ₃₂ O ₅	Analyte	5.06	351	333	10	315	18
<i>dhk PGD₂</i>	C ₂₀ H ₃₂ O ₅	Analyte	5.37	351	333	10	315	12
<i>8-iso PGF_{2a}III</i>	C ₂₀ H ₃₄ O ₅	Analyte	3.23	353	309	19	291	20
<i>11β PGF_{2a}</i>	C ₂₀ H ₃₄ O ₅	Analyte	3.38	353	309	18	193	24
<i>5-iso PGF_{2a}VI</i>	C ₂₀ H ₃₄ O ₅	Analyte	3.53	353	115	18		
<i>PGF_{2a}</i>	C ₂₀ H ₃₄ O ₅	Analyte	3.75	353	309	17	193	18
<i>PGE₁</i>	C ₂₀ H ₃₄ O ₅	Analyte	4.32	353	317	10	273	19
<i>PGD₁</i>	C ₂₀ H ₃₄ O ₅	Analyte	4.51	353	235	13		
<i>15k PGF_{1a}</i>	C ₂₀ H ₃₄ O ₅	Analyte	4.55	353	193	25		
<i>dhk PGF_{2a}</i>	C ₁₈ H ₃₀ O ₅	Analyte	4.78	353	195	15	113	22
<i>PGFM</i>	C ₂₀ H ₃₄ O ₅	Analyte	4.97	353	183	24	223	20
<i>PGF_{1a}</i>	C ₂₀ H ₃₆ O ₅	Analyte	3.78	355	311	19	293	22
<i>(d4) PGE₂</i>	C ₂₀ H ₂₈ D ₄ O ₅	IS	4.11	355	275	16	319	10
<i>dh PGF_{2a}</i>	C ₂₀ H ₃₆ O ₅	Analyte	4.53	355	311	22	337	20
<i>(d4) PGD₂</i>	C ₂₀ H ₂₈ D ₄ O ₅	IS	4.58	355	319	10	275	16
<i>(d4) dhk PGD₂</i>	C ₂₀ H ₂₈ D ₄ O ₅	IS	5.38	355	337	10	319	11
<i>(d4) 8-iso PGF_{2a}VI</i>	C ₂₀ H ₃₀ D ₄ O ₅	IS	3.23	357	197	24	295	20
<i>(d4) PGF_{2a}</i>	C ₂₀ H ₃₀ D ₄ O ₅	IS	3.75	357	313	17		
<i>(d4) dhk PGF_{2a}</i>	C ₂₀ H ₃₀ D ₄ O ₅	IS	4.98	357	187	22	199	22
<i>Protectin D₁</i>	C ₂₂ H ₃₂ O ₄	Analyte	5.92	359	153	15	206	15
<i>7(R) Maresin-1</i>	C ₂₂ H ₃₂ O ₄	Analyte	5.92	359	177	15	341	10
<i>19,20-DiHDP A</i>	C ₂₂ H ₃₄ O ₄	Analyte	6.36	361	273	15	229	15
<i>20cooh LTB₄</i>	C ₂₀ H ₃₀ O ₆	Analyte	2.72	365	347	16	169	20
<i>20oh PGE₂</i>	C ₂₀ H ₃₂ O ₆	Analyte	1.5	367	331	10	349	10
<i>d17 6k PGF_{1a}</i>	C ₂₀ H ₃₂ O ₆	Analyte	2.14	367	163	24	243	22
<i>6k PGE₁</i>	C ₂₀ H ₃₂ O ₆	Analyte	2.8	367	143	18		
<i>TXB₃</i>	C ₂₀ H ₃₂ O ₆	Analyte	2.81	367	169	14	195	11
<i>11d-TXB₂</i>	C ₂₀ H ₃₂ O ₆	Analyte	4.28	367	305	14	161	17
<i>20oh PGF_{2a}</i>	C ₂₀ H ₃₄ O ₆	Analyte	1.37	369	325	19	193	26
<i>6k PGF_{1a}</i>	C ₂₀ H ₃₄ O ₆	Analyte	2.6	369	163	25	245	24
<i>TXB₂</i>	C ₂₀ H ₃₄ O ₆	Analyte	3.27	369	169	15	195	12
<i>6,15-dk-,dh-PGF_{1a}</i>	C ₂₀ H ₃₄ O ₆	Analyte	3.32	369	267	20	223	20
<i>TXB₁</i>	C ₂₀ H ₃₆ O ₆	Analyte	3.12	371	171	17	197	14

Name	Formula	Type	RT	Precursor	Product1	CE1	Product2	CE2
<i>(d4) 6k PGF_{1α}</i>	C ₂₀ H ₃₀ D ₄ O ₆	IS	2.61	373	167	25	249	24
<i>(d4) TXB₂</i>	C ₂₀ H ₃₀ D ₄ O ₆	IS	3.27	373	173	15	199	12
<i>Resolvin D₁</i>	C ₂₂ H ₃₂ O ₅	Analyte	4.98	375	141	13	215	17
<i>dihomo PGE₂</i>	C ₂₂ H ₃₆ O ₅	Analyte	5.28	379	343	12	361	10
<i>dihomo PGF_{2α}</i>	C ₂₂ H ₃₈ O ₅	Analyte	5.05	381	337	20	319	21
<i>LTE₄</i>	C ₂₃ H ₃₇ NO ₅ S	Analyte	5.2	438	333	17	351	14
<i>11t LTE₄</i>	C ₂₃ H ₃₇ NO ₅ S	Analyte	5.33	438	333	16	351	14
<i>(d5) LTE₄</i>	C ₂₃ H ₃₂ D ₅ NO ₅ S	IS	5.2	443	338	17	356	15
<i>14,15-LTD₄</i>	C ₂₅ H ₄₀ N ₂ O ₆ S	Analyte	4.31	495	177	18	143	22
<i>LTD₄</i>	C ₂₅ H ₄₀ N ₂ O ₆ S	Analyte	5.01	495	177	18	143	23
<i>11t LTD₄</i>	C ₂₅ H ₄₀ N ₂ O ₆ S	Analyte	5.15	495	177	18	143	22
<i>14,15-LTC₄</i>	C ₃₀ H ₄₇ N ₃ O ₉ S	Analyte	4.28	624	272	21	254	23
<i>LTC₄</i>	C ₃₀ H ₄₇ N ₃ O ₉ S	Analyte	5.06	624	272	21	254	22
<i>11t LTC₄</i>	C ₃₀ H ₄₇ N ₃ O ₉ S	Analyte	5.2	624	272	21	254	22
<i>(d5) LTC₄</i>	C ₃₀ H ₄₂ D ₅ N ₃ O ₉ S	IS	5.06	629	272	21	254	23

Table 2.

Accuracies (%) for selected MRM transitions in critical pairs analysis.

Critical pairs ratio	Pair 1	Pair 2	Pair 3
	8-HETE/ 12-HETE	20-HETE/ 19-HETE	17-HETE/ 16-HETE
R1/R2 (S)	90.1	98.3	90.0
R3/R1 (S)	95.2	103.0	105.0
R1/R2 (P)	118.0	98.7	97.0
R3/R1 (P)	116.0	110.0	97.2

S: standards in neat solution; P: standards spiked in human plasma.

Amount of spike-in: R1, 20/20 ng; R2, 200/20 ng; R3, 20/200 ng

Table 3.

Validation of LC-MRM-MS based oxylipin assay.

Analyte	Regression Formula	R ²	Sensitivity ^a			Accuracy (%)			Precision (%)		
			LOD(pg)	LOQ(pg)	LQC	MQC	HQC	LQC	MQC	HQC	HQC
10-HDoHE	$Y = -0.0109037 + 0.0272432 * X$	0.9974	2.44	4.88	86.44	101.49	99	2.44	5.15	2.36	2.36
10-Nitrooleate	$Y = -0.00703 + 0.00136237 * X$	0.9947	78.13	156.25	-	95.22	98.91	-	10.33	3.79	3.79
11,12-diHETE	$Y = -0.009776 + 0.013691 * X$	0.9965	0.49	0.98	86.02	92.5	98.13	4.34	3.73	0.31	0.31
11,12-EET	$Y = -0.00816802 + 0.00600753 * X$	0.9953	9.77	19.53	98.96	90.08	102.24	15.55	7.62	4.71	4.71
11d-TXB ₂	$Y = -0.0491238 + 0.0219417 * X$	0.9878	9.77	19.53	99.64	85.96	102.36	9.63	15.22	5.61	5.61
11-HDoHE	$Y = -0.0202172 + 0.0176525 * X$	0.9966	4.88	9.77	94.71	100.33	99.53	6.45	3.45	0.91	0.91
11-HEPE	$Y = -0.00235828 + 0.00203465 * X$	0.9936	3.91	7.81	131.61	99.04	98.37	3.85	12.65	4.46	4.46
11-HETE	$Y = -0.0108311 + 0.0132479 * X$	0.9972	0.98	1.95	106.5	99.54	100.03	8.14	4.22	1.36	1.36
11t LTC ₄	$Y = 0.00217414 + 0.000563321 * X$	0.9924	3.91	7.81	-	95.45	110.27	-	8.4	3.42	3.42
11t LTD ₄	$Y = 0.000139865 + 0.00209018 * X$	0.9957	0.98	1.95	126.91	97.24	103.19	10.08	7.62	6.17	6.17
11t LTE ₄	$Y = 0.00529497 + 0.000698434 * X$	0.9921	15.63	31.25	-	93.17	106.29	-	8.84	2.93	2.93
11β PGF _{2α}	$Y = -0.0204599 + 0.0155092 * X$	0.9938	0.98	1.95	117.85	93.67	105.42	3.63	10.83	3.22	3.22
12,13-diHOME	$Y = -0.000903627 + 0.0123171 * X$	0.9988	0.49	0.98	97.8	95.44	99.08	11.48	1.64	2.82	2.82
12-HEPE	$Y = -0.00801699 + 0.00382348 * X$	0.9982	1.95	3.91	102.31	96.19	99.88	4.11	6.34	1.65	1.65
12-HETE	$Y = 0.00575988 + 0.00219425 * X$	0.9939	1.95	3.91	90.97	110.35	97.74	12.14	2.81	2.89	2.89
12-HHTE	$Y = -0.012523 + 0.00485223 * X$	0.9943	9.77	19.53	99.51	88.87	96.62	9.36	9.8	1.19	1.19
12oxo LTB ₄	$Y = -0.0407613 + 0.00390706 * X$	0.9909	7.81	15.63	-	75.3	100.27	-	2.7	4.44	4.44
12-oxoETE	$Y = -0.00494085 + 0.00459273 * X$	0.9921	7.81	15.63	-	121.17	97.96	-	6.8	1.17	1.17
13-HDoHE	$Y = -0.00261673 + 0.00247811 * X$	0.9922	9.77	19.53	100.84	87.04	107.78	20.1	3.63	3.09	3.09
13-HODE	$Y = -0.00433048 + 0.00785322 * X$	0.9977	0.98	1.95	103.94	96.19	98.49	4.26	4.28	1.41	1.41
13-HOTE	$Y = -0.011928 + 0.00375363 * X$	0.9972	3.91	7.81	95.48	98.32	99.28	10.83	10.65	1.65	1.65
13-HOTE(y)	$Y = -0.0133511 + 0.00167496 * X$	0.9927	15.63	31.25	-	90.33	98.2	-	9.55	2.36	2.36
13-oxoODE	$Y = -0.00671703 + 0.00995266 * X$	0.9957	4.88	9.77	111.53	91.69	102.02	5.84	4.49	2.52	2.52
14(15)-EpETE	$Y = -0.0276261 + 0.0197225 * X$	0.9949	9.77	19.53	94.07	99.05	95.45	15.19	13.81	2.31	2.31
14,15-diHETE	$Y = -0.0299797 + 0.0250185 * X$	0.9945	0.49	0.98	78.02	91.9	96.62	3.05	1.18	0.75	0.75
14,15-EET	$Y = -0.0520585 + 0.0288804 * X$	0.9976	9.77	19.53	115.74	95.26	95.55	5.92	4.59	1.43	1.43
14,15-LTD ₄	$Y = -0.0165509 + 0.000486832 * X$	0.9914	15.63	31.25	-	107.83	100.53	-	7.26	5.58	5.58

Analyte	Regression Formula	R ²	Sensitivity ^a		Accuracy (%)			Precision (%)		
			LOD(µg)	LOQ(µg)	LQC	MQC	HQC	LQC	MQC	HQC
14-HDoHE	$Y = -0.0175391 + 0.0140231 * X$	0.9958	4.88	9.77	91.17	94.73	99.77	20.82	6.26	1.45
15d PGD ₂	$Y = -0.047299 + 0.0181792 * X$	0.9938	1.95	3.91	107.57	79.93	101.09	3.27	12.67	3.45
15d PGJ ₂	$Y = -0.00518629 + 0.00906777 * X$	0.9983	0.98	1.95	96.63	106.97	97.31	2.92	2.96	2.43
15-HEPE	$Y = -0.00497533 + 0.0036944 * X$	0.9975	1.95	3.91	109.91	96.54	97.29	9.67	5.62	2.33
15-HETE	$Y = -0.00123146 + 0.00436834 * X$	0.9975	3.91	7.81	114.25	101.31	98.74	17.03	3.21	0.63
15-HETE	$Y = -0.0592895 + 0.0293608 * X$	0.9962	1.95	3.91	102.65	98.38	96.1	6.9	5.02	0.99
15k PGE ₂	$Y = 0.011296 + 0.0650588 * X$	0.9954	0.98	1.95	100.22	110.83	98.27	6.83	5.22	4.35
15k PGF _{1α}	$Y = 0.0162384 + 0.0079837 * X$	0.9909	1.95	3.91	95.37	120.26	99.76	1.97	11	6.92
15-oxoEDE	$Y = -0.058245 + 0.119673 * X$	0.995	9.77	19.53	109.94	95.89	100.51	8.61	2.96	6.22
15-oxoETE	$Y = -0.0103203 + 0.00404482 * X$	0.9902	7.81	15.63	-	101.34	95.92	-	9.32	4.72
15R-LXA ₄	$Y = 2.54549e-005 + 0.00445501 * X$	0.9968	2.44	4.88	98.26	101.97	100.04	13.5	2.34	3.22
16(17)-EpDPE	$Y = -0.0119976 + 0.0200056 * X$	0.996	19.53	39.06	79.81	96.46	100.9	3.72	2.75	3.54
16-HDoHE	$Y = -0.0301663 + 0.0483778 * X$	0.9979	2.44	4.88	90.02	95.71	98.84	5.23	2.55	1.64
16-HETE	$Y = -0.00439201 + 0.00738173 * X$	0.9969	1.95	3.91	110.91	91.78	98.73	2.9	6.66	1.7
17(18)-EpETE	$Y = -0.0069115 + 0.00400072 * X$	0.9964	9.77	19.53	105.5	92.45	98.49	10.91	2.76	4.89
17 HDoHE	$Y = -0.00371709 + 0.00633553 * X$	0.9955	9.77	19.53	105.91	97.53	96.04	4.91	7.15	0.56
17-HETE	$Y = -0.00216451 + 0.0057664 * X$	0.9967	0.98	1.95	114.01	90.05	98.42	2.21	5.87	1.22
17k DPA	$Y = -0.040765 + 0.00973775 * X$	0.9963	3.91	7.81	109.2	93.29	94.75	8.44	5.72	1.03
18-HEPE	$Y = -0.00558342 + 0.00344369 * X$	0.998	1.95	3.91	107.7	99.16	98.81	11.29	9.28	3.42
18-HETE	$Y = -0.00504199 + 0.00721175 * X$	0.9964	0.98	1.95	118.94	92.89	100.23	1.12	2.25	2.47
19(20)-EpDPE	$Y = -0.0198214 + 0.0200159 * X$	0.9973	2.44	4.88	84.55	96.84	97.84	3.97	3.3	1.23
19,20-DHDPA	$Y = -0.154147 + 0.0170745 * X$	0.9913	1.95	3.91	-	84.73	95.98	3.67	1.31	2.8
19-HETE	$Y = -0.00308487 + 0.00206641 * X$	0.9963	3.91	7.81	112.94	82.43	98.58	11.8	2.84	1.54
2,3-dinor 11b PGF _{2α}	$Y = -0.00573246 + 0.00446358 * X$	0.9961	3.91	7.81	116.02	93.04	105.17	8.38	2.07	2.54
2,3-dinor 8-iso PGF _{2α}	$Y = -0.00681802 + 0.00386933 * X$	0.9968	1.95	3.91	117.36	97.01	103.24	1.54	2.43	7.23
2,3-dinor TXB ₂	$Y = -0.0159749 + 0.00203039 * X$	0.9944	19.53	39.06	-	111.54	99.89	-	17.82	2.16
2,3-dinor-6k PGF _{1α}	$Y = 0.4589 + 0.00829456 * X$	0.9802	39.06	78.13	-	112.19	108.8	-	14.26	6.11
20cooh AA	$Y = -0.0779621 + 0.0897709 * X$	0.9981	4.88	9.77	84.93	97.8	98.72	7.89	3.41	1.46
20cooh LTB ₄	$Y = -0.0217471 + 0.00785531 * X$	0.9951	3.91	7.81	-	85.1	107.89	0.19	4.94	4.3

Analyte	Regression Formula	R ²	Sensitivity ^a		Accuracy (%)			Precision (%)		
			LOD(µg)	LOQ(µg)	LQC	MQC	HQC	LQC	MQC	HQC
20-HDoHE	$Y = -0.0297813 + 0.0307731 * X$	0.9946	2.44	4.88	85.53	95.67	94.64	4.83	0.31	4.05
20-HETE	$Y = 0.0153093 + 0.00260037 * X$	0.9967	1.95	3.91	93.09	110.3	95.55	33.11	3.9	2.05
20oh LTB ₄	$Y = -0.0259249 + 0.012246 * X$	0.9947	0.98	1.95	83.87	81.55	102.15	8.32	6.88	4.57
20oh PGE ₂	$Y = 0.000253512 + 0.00896078 * X$	0.9964	1.95	3.91	-	89.54	99.64	10.44	3.57	3.23
20oh PGF _{2α}	$Y = -0.0202579 + 0.0225324 * X$	0.9981	0.98	1.95	90.96	98.94	100.82	6.86	2.06	3.05
4-HDoHE	$Y = -0.0608019 + 0.0445828 * X$	0.9967	2.44	4.88	90.04	96.04	94.27	6.56	1.64	2.35
5,15-diHETE	$Y = -0.0868644 + 0.0407013 * X$	0.9931	3.91	7.81	112.95	88.45	97.47	7.47	2.12	1.3
5,6-diHETE	$Y = -0.0138686 + 0.00851191 * X$	0.9974	0.98	1.95	88.74	97.17	98.73	5.2	1.21	1.34
5,6-EET	$Y = 0.00280349 + 0.000532731 * X$	0.9854	156.25	312.5	-	119.98	98.49	-	11.64	4.68
5-HEPE	$Y = 0.00140558 + 0.00294769 * X$	0.9957	0.98	1.95	106.41	95.11	98.73	10.74	2.98	4.8
5-HETE	$Y = -0.0376006 + 0.00756184 * X$	0.9962	3.91	7.81	-	102.15	96.9	-	8.32	1.83
5-HETE	$Y = -0.0106957 + 0.0304337 * X$	0.9954	1.95	3.91	111.16	89.77	98.28	2.61	6.3	4.78
5-iso PGF _{2α} , VI	$Y = 0.000736148 + 0.00603093 * X$	0.9969	0.98	1.95	83.39	114.65	99.12	9.47	9.73	4.76
5-oxoETE	$Y = -0.0669815 + 0.0213273 * X$	0.9971	1.95	3.91	105.94	101.01	99.96	24.35	4.24	3.21
6,15-dk-, db-, PGF _{1α}	$Y = 0.00134409 + 0.00533188 * X$	0.9966	1.95	3.91	92.21	111.79	98.56	21.14	7.28	0.9
6k PGE ₁	$Y = -0.00561738 + 0.00486436 * X$	0.995	0.98	1.95	86.03	93.54	105.51	4.26	6.35	4.42
6S-LXA ₄	$Y = 0.0106072 + 0.00345495 * X$	0.9914	19.53	39.06	-	109	92.95	-	7.63	4.19
7(R) Maresin-1	$Y = -0.00996824 + 0.00777101 * X$	0.9945	1.95	3.91	84.51	78.9	98.63	5.57	2.68	5.6
7-HDoHE	$Y = -0.00925436 + 0.013752 * X$	0.9974	2.44	4.88	87.09	99.4	99.81	13.49	5.75	1.77
8,15-diHETE	$Y = 0.00898316 + 0.00250198 * X$	0.9886	7.81	15.63	-	85.19	110.95	15.18	2.16	5.1
8,9-diHETE	$Y = -0.00808737 + 0.00410411 * X$	0.9969	0.98	1.95	94.08	96.58	98.96	1.38	0.58	0.92
8,9-EET	$Y = -0.0248726 + 0.00708235 * X$	0.997	19.53	39.06	107.54	105.75	106.53	3.76	1.95	2.39
8-HDoHE	$Y = -0.0256942 + 0.0235549 * X$	0.9969	4.88	9.77	105.07	97.32	94.51	2.94	2.97	1.71
8-HEPE	$Y = -0.00242834 + 0.00341065 * X$	0.9984	0.98	1.95	92.79	100.35	101.1	8.93	3.94	2.04
8-HETE	$Y = -0.173772 + 0.0356632 * X$	0.9937	7.81	15.63	126.15	84.92	98.15	6.67	3.83	6.87
8-HETE	$Y = -0.0261272 + 0.00770905 * X$	0.9948	1.95	3.91	86.76	96.41	96.04	4.55	7.37	1.68
8-iso PGF _{2α} , III	$Y = -0.0103351 + 0.0121181 * X$	0.9968	0.98	1.95	99.22	95.28	96.99	3.82	2.65	2.12
8-iso PGF _{3α}	$Y = -0.0456402 + 0.00699653 * X$	0.9953	7.81	15.63	-	93.1	105.06	-	5.9	4.3
9,10-diHOME	$Y = -0.00765706 + 0.00337784 * X$	0.9899	1.95	3.91	108.51	80	97.01	1.37	2.88	2.43

Analyte	Regression Formula	R ²	Sensitivity ^a		Accuracy (%)			Precision (%)		
			LOD(µg)	LOQ(µg)	LQC	MQC	HQC	LQC	MQC	HQC
9,10-EpOME	$Y = -0.00494373 + 0.000335517 * X$	0.9924	78.13	156.25	-	91.62	101.28	-	18.78	2.3
9-HEPE	$Y = -0.0230474 + 0.00424271 * X$	0.9961	7.81	15.63	130.38	95.03	99.88	3.59	20.2	2.57
9-HETE	$Y = -0.0176336 + 0.00714602 * X$	0.9973	1.95	3.91	104.79	96.23	100.36	2.84	4.67	1.21
9-HODE	$Y = 0.00844371 + 0.00729797 * X$	0.9952	0.98	1.95	113.38	94.89	94.96	10.68	6.18	4.2
9-HOTe	$Y = -0.000376633 + 0.00659175 * X$	0.9981	0.98	0.98	99.72	92.79	100.46	5.31	7.77	0.88
9-Nitrooleate	$Y = 0.017519 + 0.0757139 * X$	0.9988	4.88	4.88	104.89	107.14	102.21	10	2.07	1.99
9-oxoODE	$Y = -0.0169786 + 0.0239847 * X$	0.996	9.77	19.53	97.05	98.28	95.82	2.94	8.59	3.87
Adrenic acid	$Y = -0.394973 + 0.0223128 * X$	0.9953	1562.5	3125	-	108.49	96.09	-	4.82	4.14
Arachidonic acid	$Y = -0.00556938 + 0.0146891 * X$	0.9981	97.66	195.31	102.61	95.07	100.05	5.01	1.9	0.49
bicyclo PGE ₂	$Y = -0.00289952 + 0.000906674 * X$	0.9908	7.81	15.63	-	117.2	96.1	5.76	10.17	2.62
d17 6k PGF _{1α}	$Y = -0.00882442 + 0.0146 * X$	0.9976	0.49	0.49	108.3	96.21	99.28	6.68	2.27	4.56
dh PGF _{2α}	$Y = -0.00208584 + 0.00850272 * X$	0.9957	1.95	3.91	90.34	101.07	96.52	8.02	3.04	4
Docosahexaenoic acid	$Y = -0.0852456 + 0.163768 * X$	0.9982	48.83	48.83	98.36	108.66	103.38	5.48	2.49	1.73
dhk PGD ₂	$Y = -0.00243871 + 0.00447801 * X$	0.9934	1.95	3.91	107.96	85.64	101.43	5.39	4.59	2.72
dhk PGE ₂	$Y = -0.00490411 + 0.00440119 * X$	0.9966	1.95	3.91	101.26	96.04	95.1	3.38	8.42	3.99
dhk PGF _{2α}	$Y = -0.0118497 + 0.00538533 * X$	0.9959	3.91	7.81	108.54	100.62	98.8	5.39	9.34	3.42
dihomo PGE ₂	$Y = -0.0282368 + 0.0385317 * X$	0.9979	0.49	0.98	83.68	100.44	103.78	10.37	1.72	6.08
dihomo PGF _{2α}	$Y = -0.0227349 + 0.00528333 * X$	0.9955	3.91	7.81	-	94.16	102.45	-	16.02	6.08
Eicosapentaenoic acid	$Y = -0.00726793 + 0.044985 * X$	0.9977	48.83	97.66	101.86	110.94	100.45	8.22	3.55	4.74
LTB ₄	$Y = -0.0317264 + 0.107824 * X$	0.997	0.49	0.49	82.34	84.36	101.99	9.44	2.61	6.7
LTC ₄	$Y = 0.00172598 + 0.000680304 * X$	0.9924	3.91	7.81	-	87.83	101.18	-	8.94	2.18
LTD ₄	$Y = -0.0118855 + 0.00133122 * X$	0.9953	7.81	15.63	-	99.93	100.68	-	6.31	1.05
LTE ₄	$Y = 0.000709438 + 0.000835493 * X$	0.9904	7.81	15.63	-	87	106.72	-	5.21	4.86
LXB ₄	$Y = -0.0102955 + 0.00539339 * X$	0.9946	9.77	19.53	84.9	106.04	95.97	12.7	4.84	7.91
PGA ₂	$Y = -0.0393577 + 0.0211829 * X$	0.9918	0.98	1.95	92.8	84.98	98.34	3.93	4.61	0.55
PGB ₂	$Y = -0.00996138 + 0.0113116 * X$	0.9957	2.44	4.88	86.27	92.54	103.24	5.02	3.12	1.67
PGD ₁	$Y = 0.0134629 + 0.00660275 * X$	0.9939	0.98	1.95	94.68	125.3	96.6	6.21	1.71	5.41
PGD ₂	$Y = 0.069616 + 0.00794019 * X$	0.9939	3.91	7.81	-	116.43	100.2	22.59	9.56	2.31
PGD ₃	$Y = -0.0252769 + 0.0132198 * X$	0.9966	4.88	9.77	97.96	112.46	98.86	5.99	6.88	6.19

Analyte	Regression Formula	R ²	Sensitivity ^a		Accuracy (%)			Precision (%)		
			LOD(pg)	LOQ(pg)	LQC	MQC	HQC	LQC	MQC	HQC
PGE ₁	Y = -0.00880038+0.0122419*X	0.9969	0.24	0.49	104.29	95.83	102.16	5.4	7.17	3.9
PGE ₂	Y = -0.0388571+0.0229049*X	0.9928	0.98	1.95	92.22	89.61	96.12	5.14	1.31	4.28
PGE ₃	Y = -0.017804+0.00304922*X	0.994	3.91	7.81	-	82.06	100.13	-	3.5	2.22
PGF _{1α}	Y = -0.0422301+0.0331548*X	0.9939	0.49	0.98	90.4	75.38	102.09	4.93	5.48	4.11
PGF _{2α}	Y = -0.0443765+0.0205826*X	0.9951	1.95	3.91	94.47	84.18	98.81	5.39	5.41	0.65
PGF _{3α}	Y = -0.00530456+0.00644135*X	0.9971	1.95	3.91	119.46	96.83	104.21	6.9	5.94	2.3
PGFM	Y = -0.0128176+0.00680921*X	0.9963	1.95	3.91	104.87	85.51	97.88	13.82	9.1	0.99
PGJ ₂	Y = -0.0117915+0.00657162*X	0.9956	4.88	9.77	95.32	88.66	99.5	6.32	6.87	2.15
PGK ₂	Y = -0.0342431+0.00407618*X	0.9845	7.81	15.63	-	103.14	92.45	-	2.27	10.67
Protectin D1	Y = -0.0217126+0.0247917*X	0.9953	0.98	1.95	72.08	80.67	99.74	8.95	1.67	4.32
Resolvin D1	Y = -0.00442084+0.00313776*X	0.9948	3.91	7.81	102.28	91.76	101.84	5.27	9.57	2.99
Resolvin E1	Y = -0.0136544+0.00695023*X	0.996	3.91	7.81	93.01	95.95	106.05	11.48	9.71	5.18
tetranor 12-HETE	Y = 0.00424854+0.00430041*X	0.9972	0.98	1.95	89.3	103.16	99.83	19.21	4.32	1.29
tetranor-PGDM	Y = -0.0324347+0.00971906*X	0.9987	1.95	3.91	102.57	97.85	99.99	3.1	2.95	3.26
TXB ₁	Y = -0.0295277+0.0196896*X	0.994	9.77	19.53	99.15	110.99	93.52	11.92	11.37	3.72
TXB ₂	Y = -0.044364+0.048052*X	0.9941	2.44	4.88	105.04	103.38	102.75	7.51	6.3	2.98
TXB ₃	Y = -0.0386312+0.00743354*X	0.9938	9.77	19.53	-	99.94	99.76	0.5	3.35	1.09

^athe LOQ and LOD were determined in actual amount on column.

Table 4. Concentrations of oxylipins in human blood specimen measured in this work and reported in literature.

Lipid mediators	Concentrations measured with our assay in plasma		Concentrations reported in literature		
	BR (ng/ml)	SRM1950 (ng/ml)	SRM1950 Ref [25] (ng/ml) ^a	Pooled plasma Ref [20] (ng/ml)	Serum from 27 healthy subjects Ref [37] (ng/ml)
Sources					
10-HDoHE	3.067 ± 0.138				
11,12-diHETrE	0.053 ± 0.01	0.261 ± 0.04	0.278 ± 0.09		
11-HDoHE	3.924 ± 0.15		0.217 ± 0.03		
11-HEPE	0.74 ± 0.138				
11-HETE	15.068 ± 1.029	0.216 ± 0.031		0.3	0.4–13.6
12,13-diHOME	0.709 ± 0.028	1.481 ± 0.107	1.601 ± 0.119		
12-HEPE	0.971 ± 0.045	0.164 ± 0.038			
12-HETE	13.795 ± 0.429	2.296 ± 0.521	2.176 ± 0.48	3.12	6.8–167.3
12-HHTrE	0.417 ± 0.057		0.064 ± 0.01		
13-HDoHE	30.203 ± 18.077				
13-HODE	101.506 ± 0.982	4.069 ± 0.306			
13-HOTrE	4.534 ± 0.383		0.159 ± 0.02		
13-HOTE(y)	2.118 ± 0.181				
13-oxoODE	3.266 ± 0.452				
14,15-diHETrE	0.078 ± 0.014	0.375 ± 0.078			
14-HDoHE	7.245 ± 0.282	0.411 ± 0.065	0.447 ± 0.038		
15d PGA ₂	67.743 ± 4.316				
15d PGD ₂	1.839 ± 0.479	0.17 ± 0.03			
15-HEPE	0.629 ± 0.05				
15-HETE	31.087 ± 2.052	0.648 ± 0.093	0.768 ± 0.205	1.3	0.5–14.1
15-HETrE	4.652 ± 0.044	0.155 ± 0.031			
15k PGE ₂	0.129 ± 0.024				
15-oxoETE	0.897 ± 0.123				
16-HDoHE	3.972 ± 0.084				
16-HETE	0.14 ± 0				0.018–0.18

Lipid mediators	Concentrations measured with our assay in plasma		Concentrations reported in literature		
	BR (ng/ml)	SRM1950 (ng/ml)	SRM1950 Ref [25] (ng/ml) ^a	Pooled plasma Ref [20] (ng/ml)	Serum from 27 healthy subjects Ref [37] (ng/ml)
Sources					
17-HDoHE	17.137 ± 0.634		0.282 ± 0.01		
18-HEPE	1.309 ± 0.227		0.089 ± 0.02		
18-HETE	0.157 ± 0.029				0.065–0.2
19(20)-EpDPE	4.405 ± 0.181				
19,20-DiHDDPA	0.071 ± 0.002	0.107 ± 0.016			
20cooh AA	0.419 ± 0.007	0.695 ± 0.118			0.5–7.1
20-HDoHE	4.293 ± 0.166				
20-HETE		0.504 ± 0.049	0.672 ± 0.17		
20oh PGF _{2α}		0.407 ± 0.021			
4-HDoHE	7.61 ± 0.599	0.852 ± 0.158			
5,15-diHETE	1.241 ± 0.261				
5,6-diHETE	27.351 ± 5.131	0.5 ± 0.042			
5,6-diHETe	1.053 ± 0.046				
5,6-EET	7.663 ± 0		0.263 ± 0.09		
5-HEPE	5.034 ± 0.376		0.271 ± 0.01		
5-HETE	127.093 ± 1.311	2.037 ± 0.161	3.2 ± 0.416	0.3	0.3–3.2
5-HETe	1.808 ± 0.023				
5-iso PGF _{2α} VI	0.794 ± 0.171	0.102 ± 0.016			
5-oxoETE	2.201 ± 0.082				
6S-LXA4	1.491 ± 0.379				
6t LTB4	0.465 ± 0.142	0.029 ± 0.005			
7(R) Maresin-1	0.231 ± 0.011				
7-HDoHE	1.401 ± 0.068				
8,15-diHETE	2.152 ± 0.135	1.803 ± 0.264			
8,9-diHETe	0.164 ± 0.018				0.005–0.09
8,9-EET	32.835 ± 2.316			0.15	
8-HDoHE	7.845 ± 0.216				
8-HEPE	0.444 ± 0.052				

Lipid mediators	Concentrations measured with our assay in plasma		Concentrations reported in literature		
	BR (ng/ml)	SRM1950 (ng/ml)	SRM1950 Ref [25] (ng/ml) ^a	Pooled plasma Ref [20] (ng/ml)	Serum from 27 healthy subjects Ref [37] (ng/ml)
Sources					
8-HETrE	4.881 ± 0.064		0.123 ± 0.03		
8-iso PGF _{2α} III		0.074 ± 0.017		0.052	
8-iso PGF _{3α}		6.136 ± 0.584			
9,10-dHOME	0.797 ± 0.006	1.775 ± 0.422	2.104 ± 0.138		
9-HEPE	0.655 ± 0		0.137 ± 0.03		
9-HETE	18.637 ± 1.524		0.272 ± 0.03	0.3	0.2–4.7
9-HODE	95.31 ± 1.605	2.864 ± 0.3	2.96 ± 0.77		
9-HOTrE	3.722 ± 0.038	0.129 ± 0.021			
9-oxoODE	1.861 ± 0.137	0.712 ± 0.183	2.146 ± 0.382		
Arachidonic acid	927.891 ± 52.743	862.171 ± 130.811	1428.8 ± 456	1143	800–2500
bicyclo PGE ₂	2.781 ± 0.222				
DHA	280.291 ± 17.21	538.442 ± 67.604	492 ± 55.76		
dhk PGD ₂	0.471 ± 0.041				
dhk PGE ₂	1.18 ± 0.3				
dhk PGF _{2α}	0.287 ± 0.01				
dihomo PGE ₂	0.057 ± 0.009				
EPA	56.159 ± 3.895	81.197 ± 13.999	126.84 ± 16.912		
LTB ₄	0.259 ± 0.08			<0.01	0.002–0.9
PGA ₂	0.341 ± 0.04				0.013–0.365
PGB ₂	1.115 ± 0.086				0.012–0.124
PGD ₁	0.437 ± 0.027				
PGD ₂	1.315 ± 0.383			0.042	
PGE ₂	0.097 ± 0.016		0.012 ± 0.005	0.029	
PGF _{2α}	0.096 ± 0			<0.05	
PGFM	0.111 ± 0.01				
PGJ ₂	0.262 ± 0.001			<0.05	0.03–1.054
Protectin D1	0.743 ± 0.226				

Lipid mediators	Concentrations measured with our assay in plasma		Concentrations reported in literature		
	BR (ng/ml)	SRM1950 (ng/ml)	SRM1950 Ref [25] (ng/ml) ^a	Pooled plasma Ref [20] (ng/ml)	Serum from 27 healthy subjects Ref [37] (ng/ml)
tetranor-PGDM	0.129 ± 0.006				

^a Concentrations were converted from nmol/ml to ng/ml from the original source.