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LC–MS/MS for the simultaneous analysis of arachidonic acid and 32 related metabolites in human plasma: Basal plasma concentrations and aspirin-induced changes of eicosanoids

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ABSTRACT

Eicosanoids play an important role in various biological responses and can be used as biomarkers for specific diseases. Therefore, we developed a highly selective, sensitive, and robust liquid chromatography–tandem mass spectrometric method to measure arachidonic acid and its 32 metabolites in human plasma. Sample preparation involved solid phase extraction, which efficiently removed sources of interference present in human plasma. Chromatographic separation was performed using a Luna C_8-column with 0.5 mM ammonium formate buffer and acetonitrile as the mobile phase under gradient conditions. Detection was performed using tandem mass spectrometry equipped with an electrospray ionization interface in negative ion mode. The matrix did not affect the reproducibility and reliability of the assay. All analytes showed good linearity over the investigated concentration range ($r > 0.997$). The validated lower limit of quantitation for the analytes ranged from 10 to 400 pg/mL. Intra- and inter-day precision (RDS%) over the concentration ranges for all eicosanoids were within 16.8%, and accuracy ranged between 88.1 and 108.2%. This assay was suitable for the determination of basal plasma levels of eicosanoids and the evaluation of effect of aspirin on eicosanoid plasma levels in healthy subjects.

1. Introduction

Arachidonic acid (AA) is a polyunsaturated omega-6 fatty acid and mostly exists as a component of phospholipids. AA can be released by the enzyme phospholipase A2 (PLA2) and can also be generated from diacylglycerol by diacylglycerol lipase. AA is converted to biologically active compounds by cyclooxygenase (COX), lipoxygenase (LOX), cytochrome P450 (CYP), and reactive oxygen species (ROS) [1]. COX catalyzes the formation of prostaglandin H_2, which is used to produce prostacyclin, prostaglandins, and thromboxanes, whereas 5-lipoxygenase catalyzes the formation of 5-hydroperoxyeicosatetraenoic acid (5-HPETE), which is further converted to leukotrienes and lipoxins. CYPs convert AA into epoxyeicosatrienoic acids (EETs), which are further metabolized to dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH) [2] and hydroxyeicosatetraenoic acids (HETEs). ROS such as isoprostanes (isoP) are also produced from AA (Suppl. Fig. 1).

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Eicosanoids play an important role in physiological functions and homeostasis in the body [3]. Prostaglandin E2 (PGE2) regulates tumor angiogenesis in prostate cancer [4], whereas leukotrienes and lipoxins regulate vasoconstriction and vascular permeability [5,6]. Also, 20-hydroxyeicosatetraenoic acid (20-HETE) causes cerebral microvessel constriction [7], plays a role in cerebral blood flow autoregulation [8,9], regulates new blood vessel growth [10], and increases vascular remodeling [11]. Conversely, EET metabolites produce microvascular dilation [12,13], increase cerebral blood flow [2], and protect neurons and astrocytes from ischemic cell death *in vitro* [14,15]. A disruption in the level of eicosanoids is closely related to various diseases such as cardiovascular disease, stroke, myocardial infarction, asthma, Crohn's disease, hypertension, and cancer [16–20].

Given the clinical interest in eicosanoids and the complexity of their responses to biological stimuli, it is necessary to systematically evaluate their changes in a biological matrix.

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Table 1
Multiple reaction monitoring (MRM) parameters for AA and its metabolites.

Compound	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	DP (V)	CE (V)
Arachidonic acid	303	259	−100	−19
5-HETE	319	115	−100	−19
8-HETE	319	155	−110	−28
9-HETE	319	151	−130	−20
11-HETE	319	167	−130	−22
12-HETE	319	179	−100	−20
15-HETE	319	219	−110	−18
20-HETE	319	301	−100	−22
5(S)-HpETE	335	317	−55	−9
8,9-EET	319	123	−110	−20
11,12-EET	319	167	−130	−18
14,15-EET	319	219	−140	−16
5,6-DHET	337	145	−140	−24
8,9-DHET	337	127	−140	−28
11,12-DHET	337	167	−100	−25
14,15-DHET	337	207	−140	−25
LTB_4	335	195	−130	−22
LTC_4	624	272	−130	−34
LTD_4	495	177	−100	−27
LTE_4	438	333	−100	−25
LXA_4	351	115	−100	−19
PGD_2	351	271	−100	−23
PGE_2	351	271	−100	−23
$PGF_{2\alpha}$	353	309	−130	−26
PGJ_2	333	189	−100	−24
Tetranor-PGEM	327	309	−50	−16
Tetranor-PGFM	329	311	−55	−18
6-Keto$PGF_{1\alpha}$	369	163	−160	−35
13,14-Dihydro-15-keto$PGF_{1\alpha}$	353	113	−150	−36
8-IsoPGF2α	353	193	−137	−34
TXB_2	369	195	−100	−20
11-DehydroTXB_2	367	305	−100	−22
2,3-Dinor TXB_2	341	123	−100	−23

Various analytical tools have been developed and validated for the separation, detection, and quantification of these eicosanoids, including gas chromatography–mass spectrometry (GC–MS) [21,22], liquid chromatography–mass spectrometry (LC–MS) [23,24], LC–fluorescence detection [25], electrophoresis [26], and radio- and enzyme immunoassays [26–28]. However, quantification of highly similar isomeric eicosanoid metabolites in complex biological matrices has limitations, including high cost, low specificity, limited sensitivity, cross-over and cross-talk effects, and time-consuming analysis.

Although LC–MS/MS is the most common method used to analyze these compounds from biological matrices [29–34], its limitations include the small number of analytes detected simultaneously in a single run and the low sensitivity of the method. The highest number of analytes detected and quantified in a single run was 23 compounds [29], ultimately limiting its clinical application. Therefore, development of a more sensitive analytical method to quantitate wide ranges of eicosanoids over physiological concentrations is required to evaluate the role of eicosanoids in drug responses or specific diseases.

In the present study, we developed and validated a specific, highly sensitive, and robust LC–MS/MS method for the analysis of AA and its 32 metabolites in human plasma. This method was successfully applied to evaluate basal eicosanoid plasma levels and aspirin-derived changes in healthy volunteers.

2. Experimental

2.1. Materials

8,9-Epoxy-eicosatrienoic acid (8,9-EET), 11,12-epoxy-eicosatrienoic acid (11,12-EET), 14,15-epoxy-eicosatrienoic acid (14,15-EET), 5,6-dihydroxyeicosatrienoic acid (5,6-DHET), 8,9-dihydroxyeicosatrienoic acid (8,9-DHET), 11,12-dihydroxyeicosatrienoic acid (11,12-DHET), 14,15-dihydroxyeicosatrienoic acid (14,15-DHET), 5-hydroxyeicosatetraenoic acid (5-HETE), 8-hydroxyeicosatetraenoic acid (8-HETE), 9-hydroxyeicosatetraenoic acid (9-HETE), 11-hydroxyeicosatetraenoic acid (11-HETE), 12-hydroxyeicosatetraenoic acid (12-HETE), 15-hydroxyeicosatetraenoic acid (15-HETE), 20-hydroxyeicosatetraenoic acid (20-HETE), 5-hydroperoxyeicosatetraenoic (5(S)-HpETE), leukotriene B_4 (LTB_4), leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4), leukotriene E_4 (LTE_4), and 5(S),6(R)-lipoxin A_4 (LXA_4), prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin J_2 (PGJ_2), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF1α), 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (13,14-dihydro-15-keto-$PGF_{2\alpha}$), tetranor-prostaglandin E metabolite (tetranor-PGEM), tetranor-prostaglandin F metabolite (tetranor-PGFM), thromboxane B_2 (TXB_2), 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2), 2,3-dinor-thromboxane B_2 (2,3-dinor-TXB_2), 8-iso prostaglandin $F_{2\alpha}$ (8-iso$PGF_{2\alpha}$), arachidonic acid (AA), AA-d_8, 20-HETE-d_6, and PGE_2-d_4 were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Deuterated compounds (AA-d_8, 20-HETE-d_6, and PGE_2-d_4) were used as internal standards for quantification. HPLC-grade acetonitrile and methanol were purchased from J. T. Baker (Phillipsburg, NJ, USA). Solid-phase extraction (SPE) cartridges (Discovery DSC-C_8, 100 mg cartridge) were purchased from Supelco Co. (North Harrison Road, Bellefonte, USA). Lipid and protein-free blank serum (SeraSub™) were obtained from CST Technologies, Inc. (Great Neck, NY, USA). All other chemicals and solvents were of the highest analytical grade available.

2.2. Preparation of standards and quality control (QC) samples

Stock solutions of all analytes were prepared in ethanol at a concentration of 1 mg/mL for arachidonic acid and $PGF_{2\alpha}$, 500 μg/mL for tetranor-PGFM, 50 μg/mL for PGJ_2, 10 μg/mL for 8-iso $PGF_{2\alpha}$,

and 100 μg/mL for all other analytes. Standard solutions were prepared in lipid and protein-free blank plasma by spiking with an appropriate volume of the serially diluted stock solutions, resulting in six different concentrations required for the calibration curve (Table 2). Quality control (QC) samples were prepared daily at two different concentration levels including low and high concentrations as described in Table 3. The 20-HETE-d_6 (100 ng/mL), PGE_2-d_4 (50 ng/mL), and arachidonic acid-d_8 (2 μg/mL) were used as internal standards for the quantification of HETEs/EETs, prostaglandins/leukotrienes, and AA, respectively.

2.3. Sample preparation

Solid phase extraction was conducted according to the method described elsewhere [32] with minor modification. Briefly, the Discovery DSC-C8 cartridges (100 mg), connected to a Visiprep SPE vacuum manifold (Supelco Co., St. Louis, MO, USA), were washed with 1 mL of methanol followed by 1 mL of 0.1% formic acid solution. Plasma samples (1 mL) and internal standard solutions (10 μL) were applied to the column and subsequently washed with 1 mL of 0.1% formic acid solution. All analytes were eluted with 1 mL of methanol. The eluate was evaporated to dryness under nitrogen stream. The resulting residues were dissolved in 100 μL of 50% methanol, and the aliquots were injected into the LC–MS/MS system. Protein precipitation and solvent extraction were performed for comparison of sample preparation methods. For protein precipitation, two volumes of methanol were added to plasma samples (0.5 mL) and the supernatants were injected into the LC–MS/MS system after centrifugation. For solvent extraction, three volumes of organic solvents including ethyl acetate, dichloromethane and hexane/ethylacetate (3/2, v/v) were added to 1 mL of plasma samples. The organic phase was taken after centrifugation at 1000 × *g* for 10 min and dried under nitrogen stream. The residues were then dissolved in 100 μL of 50% methanol, and the aliquots were injected into the LC–MS/MS system.

2.4. Instrumentation

High-performance liquid chromatography (HPLC) was performed using an Agilent 1200 series HPLC equipped with an autosampler, a binary pump, and a column oven (Wilmington, DE, USA). The analytical column was a Luna C8 (150 mm × 2.0 mm, 5 μm; Phenomenex, Torrance, CA, USA) maintained at 40 °C. The mobile phases consisted of 0.5 mM ammonium formate (pH 3.3) (A) and acetonitrile containing 0.5 mM ammonium formate (B). The stepwise linear gradient was as follows: 5% B at 0 min, 35% B at 5 min, 65% B at 15 min, 75% at 20 min, 100% B at 24 min, 100% B at 28 min, and 5% B at 29 min. The flow rate was 0.3 mL/min, and the injection volume was 5 μL.

The HPLC system was coupled on-line to a QTrap 5500 mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo ion spray source. Electrospray ionization (ESI) was performed in the negative ion mode with nitrogen as the nebulizing, turbo spray, and curtain gases with the optimum values set at 50, 50, and 25 (arbitrary units). The turbo gas temperature was set at 550 °C and ESI needle voltage was adjusted to −4500 V. Multiple reaction monitoring (MRM) detection was employed using nitrogen as the collision gas with a dwell time of 200 ms for each transition. The collision gas pressure was set at 1.8×10^{-5} Torr. The optimal collision energies for each analyte were obtained using an automatic tuning process with continuous infusion of standard solution by the syringe pump. The mass transitions, declustering potential (DP), and collision energy (CE) for the analytes are described in Table 1 and the signals of all ions were collected at the same time. The mass spectrometer was operated with unit resolution for both the first (Q1) and third quadrupole (Q3). Data acquisition and processing was performed using Analyst software (ver. 1.4.1; Applied Biosystems).

2.5. Assay validation

Concentration ranges for the calibration span the plasma concentrations under normal physiological conditions. Calibration curves were obtained using six calibration standards and were fitted by a weighed ($1/x$) least-squares linear regressions. The accuracy and precision of the assay were assessed by analyzing QC samples under two different concentrations (Table 3) in six replicates on the same day and on six consecutive days for intra- and inter-day precision and accuracy, respectively. Precision was calculated as the relative standard deviation (RSD), and accuracy was determined from the percentage ratio of the measured concentration to the nominal concentration. The lower limit of quantitation (LLOQ) was set as the lowest concentration of each analyte satisfying the intra- and inter-day (six replication each) precision within 20% of RSD and accuracy of 80–120%. Recovery and matrix effects were assessed at two concentrations for five representative analytes (20-HETE, 8,9-DHET, LTB4, PGE_2, and TXB_2) by comparing the peak areas of triplicate runs at each concentration for the analyte standards in methanol and the standard spiked before and after extraction in human plasma. Absolute recovery was calculated as the ratio of the peak area of an analyte spiked before extraction to the peak area of the same analyte spiked in methanol at the same concentration multiplied by 100. Relative recovery was calculated as the ratio of the peak area of an analyte spiked before extraction to the peak area of the same analyte spiked post-extraction in the same matrix multiplied by 100. The matrix effect was evaluated by comparing the peak area of each analyte spiked in blank plasma extracts to the peak area of an equivalent concentration of the same analyte standard in methanol. To further evaluate whether plasma matrix causes ion suppression, each eicosanoid solution (10 ng/mL) was infused post-column at a flow rate of 10 μL/min, resulting in constant product ion intensity. Next, plasma extracts were injected onto the column and changes in ion intensity were monitored to evaluate ion suppression. The stability of the analytes in human plasma was assessed with plasma samples spiked with two QC levels of each analyte. Stability was assessed by analyzing three replicate samples after three different manipulations: (1) short-term storage of plasma samples (1 and 12 h at room temperature); (2) post-treatment storage (6 and 24 h at 4 °C); and (3) one freeze–thaw cycles (−20 °C to room temperature). Room temperature was defined to be 25 °C. The concentrations obtained were compared with the nominal values of the QC samples. The long-term stability was assessed after 6 month storage of QC samples at −80 °C by comparison with freshly prepared plasma samples of the same concentrations. The analyte was considered stable in the biological matrix when 85–115% of the initial concentrations were found.

2.6. Clinical applications

Six healthy male subjects who gave written informed consent took part in this study. The protocol of this study was approved by the Institutional Review Board of Busan Paik Hospital (Busan, Republic of Korea). Because eicosanoid levels are affected by the diet and administered drugs, medication and supplementary vitamins were strictly prohibited for 1 week prior to taking blood samples. After an overnight fast, all subjects were administered a single 1.0 g oral dose of aspirin. Blood samples (~9 mL each) were collected *via* the cannula at the following times: pre-dose, 0.5, 1, 1.5, 2, 3, 4, and 6 h after aspirin administration. Blood samples were collected into chilled heparin tubes and immediately centrifuged at 2000 × *g* for 10 min at 4 °C. Plasma was then taken and frozen at

Table 2
HPLC retention time, calibration range, day-to-day linear regression, and LLOQ for AA and its metabolites.

Compound name	Retention time (min)	Calibration range (pg/mL)	Correlation (r)	LLOQ (pg/mL)
Arachidonic acid	25.4	1–200 (ng/mL)	0.9983	1000
5-HETE	20.3	10–2000	0.9970	10
8-HETE	20.0	20–4000	0.9989	20
9-HETE	20.2	20–4000	0.9984	20
11-HETE	19.8	10–2000	0.9974	10
12-HETE	20.2	10–2000	0.9994	10
15-HETE	19.5	20–4000	0.9982	20
20-HETE	18.1	20–4000	0.9991	20
5(S)-HpETE	20.9	200–40,000	0.9980	200
8,9-EET	22.0	20–4000	0.9984	20
11,12-EET	21.9	20–4000	0.9979	20
14,15-EET	21.3	20–4000	0.9986	20
5,6-DHET	17.5	10–2000	0.9986	10
8,9-DHET	17.2	10–2000	0.9986	10
11,12-DHET	17.9	10–2000	0.9989	10
14,15-DHET	16.7	10–2000	0.9990	10
LTB_4	15.5	10–2000	0.9982	10
LTC_4	14.8	50–10,000	0.9980	50
LTD_4	12.0	50–10,000	0.9983	50
LTE_4	13.5	20–4000	0.9988	20
LXA_4	13.0	10–2000	0.9984	10
PGD_2	12.4	20–4000	0.9987	20
PGE_2	12.0	20–4000	0.9985	20
$PGF_2\alpha$	11.6	50–10,000	0.9992	50
PGJ_2	14.4	50–10,000	0.9989	50
Tetranor-PGEM	8.6	400–80,000	0.9967	400
Tetranor-PGFM	8.3	200–40,000	0.9980	200
6-Keto $PGF_1\alpha$	10.3	20–4000	0.9982	20
13,14-Dihydro 15-keto-$PGF_1\alpha$	12.9	50–10,000	0.9986	50
8-Iso$PGF_2\alpha$	11.5	20–4000	0.9991	20
TXB_2	11.2	20–4000	0.9990	20
11-DehydroTXB_2	12.3	20–4000	0.9985	20
2,3-Dinor TXB_2	10.3	100–20,000	0.9980	100

−80 °C prior to analysis. The results were expressed as mean ± SD and the concentrations below LLOQ were considered zero. Plasma concentrations of eicosanoids before and 2 h after aspirin administration were compared using the Wilcoxon rank sum test. Data were analyzed using the SAS software version 9.2. Differences were considered statistically significant when the *P*-value was <0.05.

3. Results and discussion

3.1. Optimization of the LC/MS/MS method

The structures of eicosanoids are presented in Fig. 1 and their product ion mass spectra are shown in Supplementary Fig. 2. Multiple reaction monitoring (MRM) conditions were selected from the spectra obtained by direct infusion of the individual standard solutions into the mass spectrometer. All analytes in this study generated a prominent, deprotonated molecular ion $[M-H]^-$ in negative-ion mode; most abundant fragment ions were chosen for MRM. Collision energies for each analyte were set according to their maximum intensities.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2012.11.004.

The precursor and product ions as well as the respective DP and CE values selected for the quantification of each analyte are given in Table 1.

Sample preparation and chromatographic conditions were optimized for simple, rapid, and practical quantitative analysis. Given the complexity and variety of the matrices, several clean-up strategies were evaluated such as protein precipitation, liquid–liquid extraction, and solid-phase extraction. The protein precipitation by adding two volumes of methanol showed high extraction efficiencies for all analytes (>90%). However, this precipitation method was not suitable for the detection of eicosanoids at low concentrations in biological samples. Liquid extraction with ethyl acetate, dichloromethane and hexane/ethylacetate showed reasonable recovery for EETs, HETEs, and PGs. However, organic solvents poorly extracted hydrophilic compounds such as tetranor-PGEM, tetranor-PGFM, and leukotrienes. Solid-phase extraction was most suitable for extracting all target analytes from plasma. Solid-phase extraction has been widely used for the analysis of eicosanoids in various tissues [22,25,32] and the Discovery DSC-C8 cartridge (100 mg, Supelco, USA) was selected because it showed relatively high extraction recoveries for all analytes among the cartridges tested (Supelclean LC-8, Sep-Pak C18, Oasis HLB, and Discovery DSC-18).

To optimize the resolution, different buffers of varying pH and organic modifiers (methanol and acetonitrile) were tested, and 0.5 mM ammonium formate (pH 3.3) and acetonitrile were selected as the mobile phase. The Phenomenex Luna C_8 column (2.1 mm × 150 mm) with a 5-μm particle size using ammonium formate/acetonitrile as the mobile phase in gradient elution mode achieved the highest resolution and sensitivity. The representative selected reaction monitoring (SRM) mass chromatograms of all the analytes spiked in synthetic plasma are shown in Fig. 2. The separation of analytes with the same molecular mass and similar product ion patterns, such as PGD_2 and PGE_2 ($[M-H]^-$, *m/z* 351), and 8-, 9-, 11-, 12-HETE and 11(12)-EET ($[M-H]^-$, *m/z* 319), were also optimized to minimize crosstalk.

3.2. Validation of the optimized LC–MS/MS method

Calibration ranges for the analytes were selected by considering previously reported human plasma concentrations of eicosanoids [35,36] and sensitivity of the present analytical method. Calibration curves for each analyte over the designated concentration

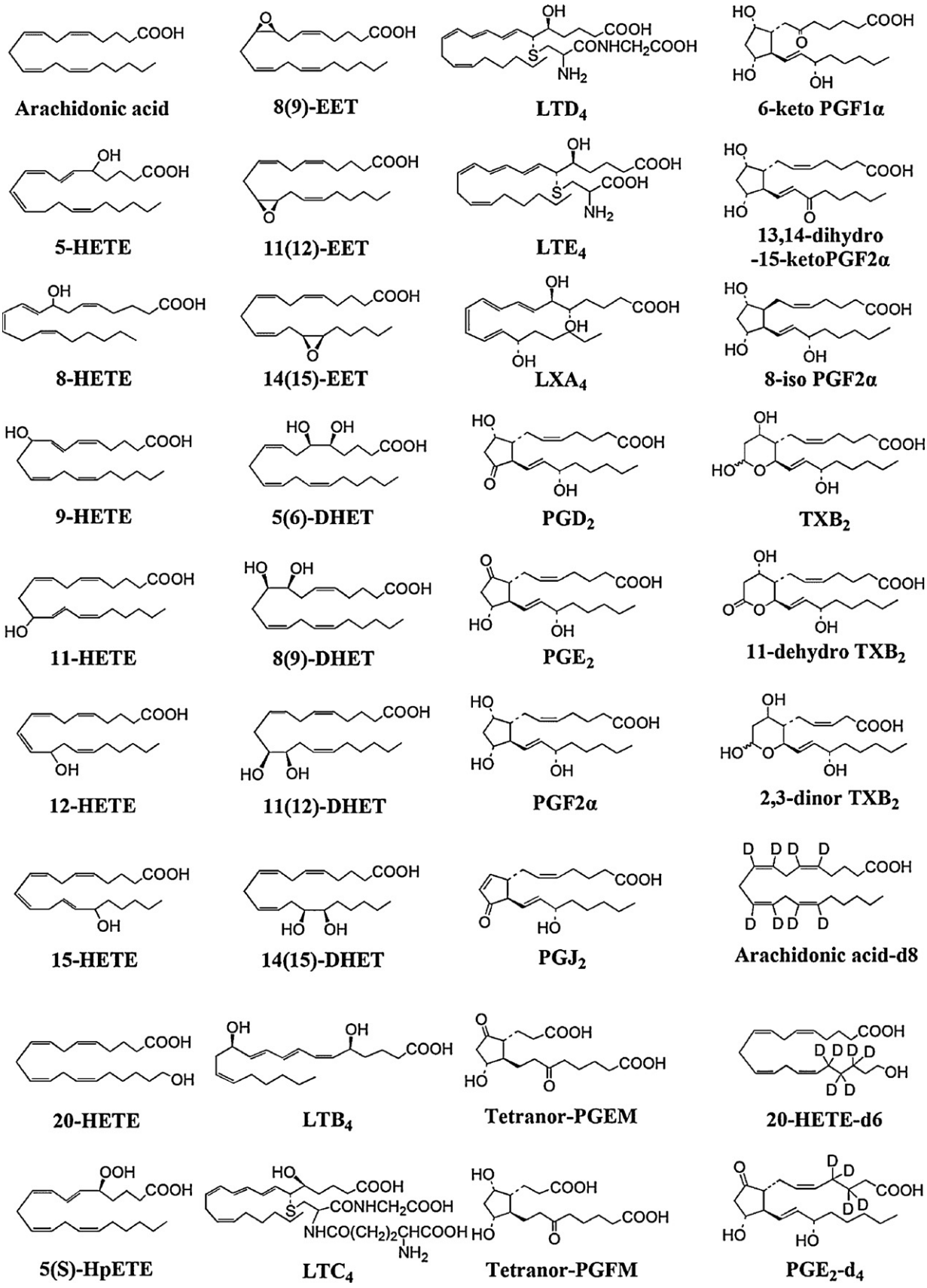

Fig. 1. Chemical structures of AA and its metabolites.

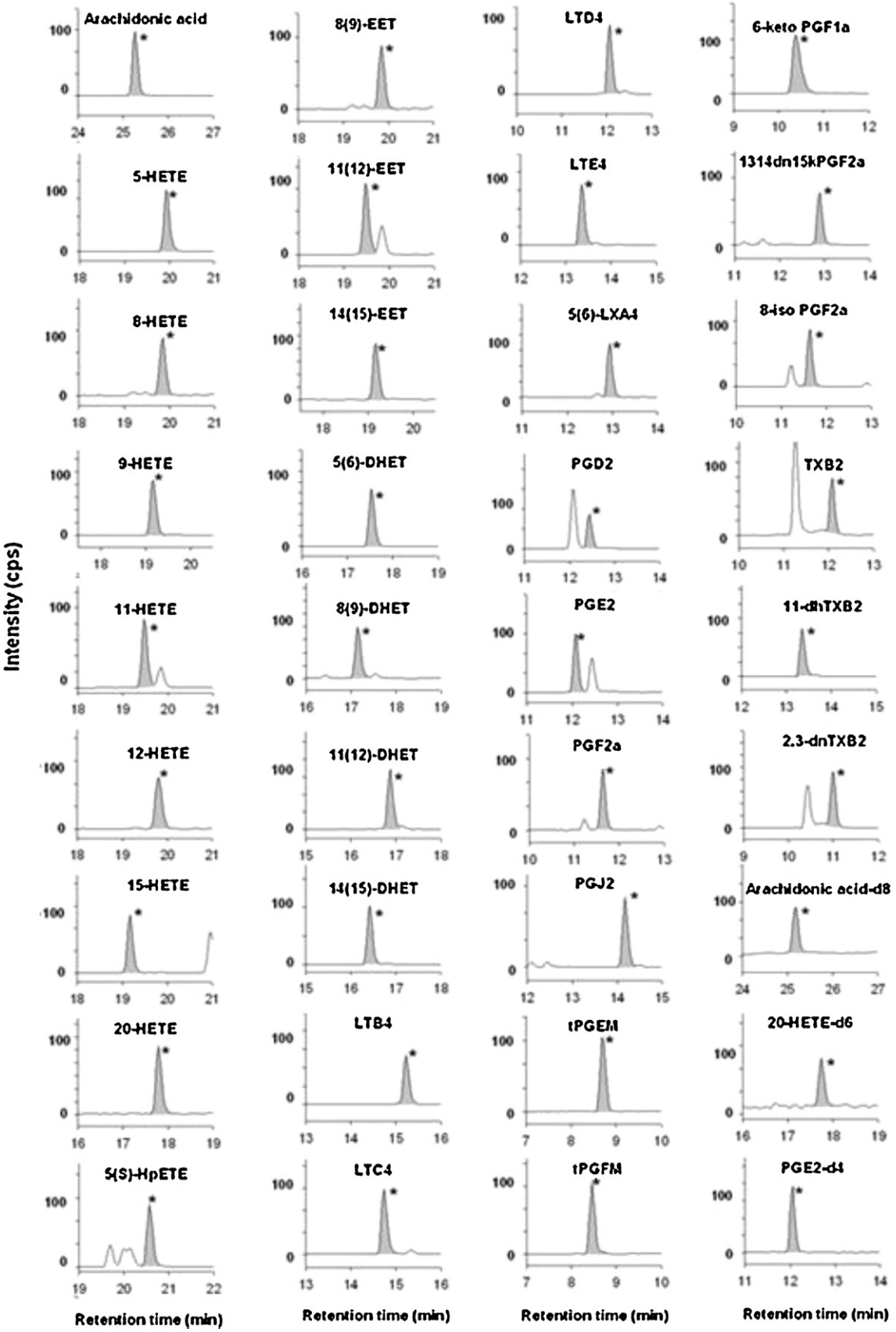

Fig. 2. Representative MRM chromatograms of AA and its metabolites including internal standards.

Table 3
Intra-day and inter-day coefficients of variation and accuracies for the determination of AA and 32 eicosanoids ($n = 6$).

Compound name	Nominal conc. (ng/mL)	Intra-day			Inter-day		
		Measured	RSD (%)	Accuracy (%)	Measured	RSD (%)	Accuracy (%)
Arachidonic acid	3.2	3.07 ± 0.37	12.2	95.8	3.11 ± 0.29	9.2	97.2
	32	32.1 ± 1.78	5.6	100.3	31.7 ± 2.83	8.9	99.0
5-HETE	0.032	0.030 ± 0.002	7.0	91.3	0.034 ± 0.002	4.6	106.8
	0.32	0.312 ± 0.019	6.0	97.3	0.317 ± 0.028	8.8	99.2
8-HETE	0.064	0.060 ± 0.004	6.1	93.1	0.066 ± 0.006	8.7	102.7
	0.64	0.597 ± 0.050	8.4	93.3	0.638 ± 0.047	7.3	99.9
9-HETE	0.064	0.061 ± 0.006	10.2	94.5	0.069 ± 0.007	10.4	107.0
	0.64	0.599 ± 0.039	6.4	93.6	0.666 ± 0.046	6.9	104.1
11-HETE	0.032	0.034 ± 0.004	13.1	105.8	0.033 ± 0.003	8.2	102.9
	0.32	0.296 ± 0.017	5.8	92.5	0.331 ± 0.025	7.7	103.3
12-HETE	0.032	0.030 ± 0.002	7.9	92.3	0.034 ± 0.003	7.6	105.9
	0.32	0.289 ± 0.010	3.5	90.2	0.327 ± 0.028	8.6	102.1
15-HETE	0.064	0.062 ± 0.008	13.3	96.1	0.067 ± 0.005	8.0	104.1
	0.64	0.597 ± 0.029	4.8	93.3	0.654 ± 0.049	7.4	102.2
20-HETE	0.064	0.076 ± 0.010	13.3	101.2	0.067 ± 0.006	8.9	104.2
	0.64	0.575 ± 0.028	4.9	89.9	0.637 ± 0.046	7.2	99.4
5(S)-HpETE	0.64	0.604 ± 0.048	8.0	94.4	0.693 ± 0.037	5.4	108.2
	6.4	6.226 ± 0.480	7.7	97.3	6.497 ± 1.047	16.1	101.5
8,9-EET	0.064	0.066 ± 0.012	16.8	98.1	0.065 ± 0.007	10.1	102.2
	0.64	0.582 ± 0.033	5.7	90.9	0.630 ± 0.035	5.6	98.5
11,12-EET	0.064	0.065 ± 0.007	10.5	101.5	0.068 ± 0.003	4.4	103.3
	0.64	0.596 ± 0.025	4.2	93.1	0.636 ± 0.046	7.3	99.5
14,15-EET	0.064	0.066 ± 0.007	10.3	99.2	0.064 ± 0.009	14.6	99.1
	0.64	0.628 ± 0.059	9.5	98.1	0.663 ± 0.042	6.3	103.5
5,6-DHET	0.032	0.033 ± 0.003	8.8	102.4	0.034 ± 0.002	7.0	106.0
	0.32	0.319 ± 0.022	6.8	99.8	0.316 ± 0.025	7.9	98.6
8,9-DHET	0.032	0.031 ± 0.002	7.1	96.2	0.032 ± 0.003	8.3	101.2
	0.32	0.339 ± 0.017	4.9	105.8	0.316 ± 0.030	9.4	98.6
11,12-DHET	0.032	0.032 ± 0.003	10.2	98.8	0.033 ± 0.003	8.2	103.0
	0.32	0.323 ± 0.029	8.8	100.9	0.316 ± 0.027	8.6	98.8
14,15-DHET	0.032	0.031 ± 0.002	5.9	98.2	0.033 ± 0.002	7.3	103.7
	0.32	0.336 ± 0.023	7.0	104.8	0.318 ± 0.034	10.8	99.5
LTB_4	0.032	0.030 ± 0.004	11.5	94.1	0.034 ± 0.002	4.5	106.0
	0.32	0.312 ± 0.023	7.3	97.5	0.319 ± 0.028	8.7	99.6
LTC_4	0.16	0.152 ± 0.016	10.3	94.9	0.153 ± 0.014	8.9	102.8
	1.6	1.520 ± 0.085	5.6	95.0	1.618 ± 0.122	7.5	101.1
LTD_4	0.16	0.148 ± 0.010	6.6	92.7	0.170 ± 0.011	6.4	106.0
	1.6	1.410 ± 0.043	3.1	88.1	1.593 ± 0.155	9.7	99.5
LTE_4	0.064	0.065 ± 0.006	9.4	101.0	0.068 ± 0.004	5.6	105.8
	0.64	0.619 ± 0.047	7.6	96.6	0.632 ± 0.047	7.4	98.7
LXA_4	0.032	0.033 ± 0.003	9.0	104.7	0.032 ± 0.003	9.2	101.1
	0.32	0.307 ± 0.010	3.1	95.8	0.312 ± 0.023	7.4	97.5
PGD_2	0.064	0.063 ± 0.003	5.4	98.2	0.067 ± 0.004	6.3	105.0
	0.64	0.611 ± 0.025	4.1	95.5	0.626 ± 0.038	6.0	97.8
PGE_2	0.064	0.059 ± 0.003	5.2	92.7	0.066 ± 0.005	6.9	103.7
	0.64	0.600 ± 0.028	4.7	93.7	0.627 ± 0.050	7.9	98.0
$PGF_2\alpha$	0.16	0.152 ± 0.010	6.8	95.0	0.167 ± 0.012	7.4	104.6
	1.6	1.485 ± 0.075	5.1	92.8	1.537 ± 0.113	7.4	96.0
PGJ_2	0.16	0.155 ± 0.007	4.5	97.0	0.168 ± 0.016	9.8	105.0
	1.6	1.478 ± 0.060	4.1	92.3	1.508 ± 0.104	6.9	94.2
Tetranor-PGEM	1.28	1.295 ± 0.111	8.6	101.2	1.364 ± 0.110	8.1	106.6
	12.8	12.48 ± 1.07	8.6	97.5	12.51 ± 0.96	7.7	97.7
Tetranor-PGFM	0.64	0.658 ± 0.047	7.2	102.9	0.647 ± 0.054	8.4	101.1
	6.4	6.051 ± 0.204	3.4	94.5	6.498 ± 0.647	10.0	101.5
6-Keto $PGF_1\alpha$	0.064	0.063 ± 0.004	6.8	98.8	0.068 ± 0.004	6.3	105.5
	0.64	0.603 ± 0.015	2.4	94.3	0.627 ± 0.057	9.1	97.9
13,14-Dihydro 15-keto$PGF_1\alpha$	0.16	0.151 ± 0.015	9.8	94.3	0.168 ± 0.014	8.4	104.9
	1.6	1.495 ± 0.057	3.8	93.4	1.539 ± 0.110	7.1	96.2

Table 3 (*Continued*)

Compound name	Nominal conc. (ng/mL)	Intra-day			Inter-day		
		Measured	RSD (%)	Accuracy (%)	Measured	RSD (%)	Accuracy (%)
8-IsoPGF$_2\alpha$	0.064	0.061 ± 0.006	9.4	94.8	0.067 ± 0.006	9.3	102.5
	0.64	0.628 ± 0.017	2.8	98.1	0.630 ± 0.042	6.6	98.4
TXB$_2$	0.064	0.065 ± 0.003	4.1	95.8	0.066 ± 0.004	5.9	103.3
	0.64	0.629 ± 0.035	5.6	98.3	0.624 ± 0.027	4.4	97.5
11-DehydroTXB$_2$	0.064	0.064 ± 0.010	16.3	95.2	0.066 ± 0.006	8.6	103.1
	0.64	0.601 ± 0.033	5.5	93.9	0.620 ± 0.049	8.0	96.9
2,3-Dinor TXB$_2$	0.32	0.326 ± 0.029	8.9	101.8	0.320 ± 0.024	7.6	100.0
	3.2	3.188 ± 0.215	6.8	99.6	3.178 ± 0.232	7.3	99.3

Table 4
Recovery and matrix effect of 20-HETE, 8,9-DHET, LTB$_4$, PGE$_2$, and TXB$_2$ (n = 3).

Compound name	Added (ng/mL)	Absolute recovery (%)	Relative recovery (%)	Matrix effect (%)
20-HETE	0.064	115 ± 26.3	107 ± 30.3	107 ± 4.9
	0.64	102 ± 7.8	96 ± 8.7	107 ± 2.2
8,9-DHET	0.032	97 ± 20.5	102 ± 21.6	96 ± 14.2
	0.32	99 ± 1.8	92 ± 5.9	107 ± 7.7
LTB4	0.032	97 ± 17.9	103 ± 5.0	95 ± 13.7
	0.32	90 ± 7.2	94 ± 7.4	96 ± 4.0
PGE2	0.064	96 ± 9.0	105 ± 15.7	91 ± 17.9
	0.64	98 ± 12.9	97 ± 14.1	101 ± 4.2
TXB2	0.064	85 ± 2.0	92 ± 11.4	92 ± 13.1
	0.64	89 ± 17.7	103 ± 19.5	87 ± 4.6

ranges were linear with correlation coefficient (r) greater than 0.997 (Table 2). The best-fit line of the calibration curve for each analyte was obtained by using a weighting factor of 1/x for all analytes. Lower limits of quantitation (LLOQ) were found to be in the range of 10–50 pg/mL depending on the compound except for 2,3-dinor TXB$_2$ (100 pg/mL), 5(S)-HpETE and tetranor-PGFM (200 pg/mL), tetranor-PGEM (400 pg/mL), and AA (1.0 ng/mL).

The intra- and inter-day variations of target eicosanoid determinations in plasma at two concentrations are listed in Table 3. The intra-day coefficients of variation ranged from 2.4 to 16.8%, and accuracies ranged from 88.1 to 105.8%. The inter-day coefficients of variation were between 4.4 and 16.1%, and the accuracies were between 94.2 and 108.2%.

The LC–MS response of an analyte is influenced by the nature of the co-eluting sample matrix, especially when the matrix is complex. The use of stable isotope-labeled compounds as internal standards is one of the best approaches to correct for matrix effects. Although we used a suite of stable isotope-labeled compounds as internal standards, we evaluated ion suppression and enhancement. The recovery and matrix effects of five representative eicosanoids are presented in Table 4. Plasma matrix did not cause any significant ion suppression or enhancement in our analytical system. The absolute recovery of representative eicosanoids was between 85 and 115%. Furthermore, no significant matrix interference was also observed near the retention times of all analytes when blank plasma extracts were injected into the LC–MS/MS system maintaining constant ion signal of the individual analyte by post-column infusion.

All eicosanoids were stable in plasma at room temperature for 1 h within a 17% standard deviation. But the intensities of AA, 5(S)-HpETE, LTD$_4$, LTE$_4$, tetranor-PGEM, and 11-dehydroTXB$_2$ were dramatically decreased in plasma samples leaved for 12 h at room temperature. The extracted samples were also stable at 4 °C for 24 h without significant loss (83–126%). All eicosanoids were stable after single freeze–thaw cycle. Long-term storage of plasma samples at −80 °C demonstrated that all analytes were stable for 6 months except AA (Suppl. Table 1).

Table 5
Eicosanoid profiles in human plasma before and 2 h after an oral dose of aspirin to healthy volunteers (n = 6).

Compound name	Plasma concentration (ng/mL)		P-value
	Before	After	
Arachidonic acid	1143 ± 468	1304 ± 702	0.844
5-HETE	0.399 ± 0.148	0.487 ± 0.130	0.313
8-HETE	0.280 ± 0.092	0.326 ± 0.130	0.219
9-HETE	0.328 ± 0.122	0.372 ± 0.143	0.250
11-HETE	0.316 ± 0.104	0.301 ± 0.102	0.844
12-HETE	3.12 ± 2.502	3.781 ± 2.067	0.563
15-HETE	1.307 ± 0.356	1.717 ± 1.048	0.438
20-HETE	1.308 ± 0.212	1.318 ± 0.424	0.844
5(S)-HpETE	0.619 ± 0.448	0.338 ± 0.285	0.063
8,9-EET	0.147 ± 0.104	0.082 ± 0.063	0.094
11,12-EET	0.165 ± 0.225	0.183 ± 0.289	1.000
14,15-EET	0.125 ± 0.136	0.070 ± 0.046	0.438
5,6-DHET	0.387 ± 0.225	0.414 ± 0.132	0.750
8,9-DHET	0.329 ± 0.153	0.351 ± 0.050	0.688
11,12-DHET	0.678 ± 0.280	0.713 ± 0.385	0.999
14,15-DHET	1.456 ± 0.600	1.431 ± 0.443	0.688
LTB$_4$	<0.01	<0.01	–
LTC$_4$	<.02	<0.02	–
LTD$_4$	0.041 ± 0.024	0.051 ± 0.018	0.125
LTE$_4$	0.011 ± 0.013	0.024 ± 0.019	0.438
LXA$_4$	0.016 ± 0.019	0.044 ± 0.065	0.188
PGD$_2$	0.042 ± 0.032	0.085 ± 0.116	0.813
PGE$_2$	0.029 ± 0.013	0.018 ± 0.009	0.188
PGF2α	<0.05	<0.05	–
PGJ$_2$	<0.05	<0.05	–
Tetranor-PGEM	2.642 ± 2.390	2.094 ± 2.741	0.820
Tetranor-PGFM	<0.20	<0.20	–
6-KetoPGF$_1\alpha$	0.051 ± 0.124	0.039 ± 0.096	1.000
13,14-Dihydro-15-ketoPGF$_1\alpha$	0.049 ± 0.041	0.018 ± 0.027	0.125
8-IsoPGF$_2\alpha$	0.052 ± 0.026	0.024 ± 0.027	0.219
TXB$_2$	0.179 ± 0.065	0.025 ± 0.024	0.031
11-DehydroTXB$_2$	0.020 ± 0.041	0.042 ± 0.055	0.875
2,3-Dinor TXB$_2$	<0.10	<0.10	–

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2012.11.004.

3.3. Applications to clinical studies

The developed method was used to analyze the basal eicosanoid profile in six young healthy volunteers as well as changes in their plasma profile 2 h after oral administration of 1.0 g aspirin. Estimated basal eicosanoid levels in plasma were comparable to those described in the human metabolome database [35,36]. Tetranor-PGEM, a well-known urinary marker of endogenous PGE_2, was also detected in the plasma using our analytical system. Administration of aspirin significantly decreased plasma concentrations of COX-catalyzed metabolite TXB_2 ($p = 0.031$) (Table 5). The plasma levels of TXB_2 rapidly decreased to less than 10% of the basal level 2 h after aspirin administration. These decreased levels were maintained until the last sampling time (6 h after administration). 13,14-Dihydro-15-keto$PGF_{1\alpha}$ decreased by more than 50% although no statistical significance was observed due to limited sample number. The concentrations of AA and LOX-mediated metabolites such as LTD_4 and LTE_4 and CYP-mediated metabolites such as HETEs and DHETs were not significantly changed.

4. Conclusions

A highly sensitive and specific LC–MS/MS method was developed for the simultaneous determination of AA and 32 eicosanoids in human plasma. To the best of our knowledge, this is the most sensitive (pg/mL) and rapid method that can detect up to 32 eicosanoids in biological samples available to date. The present method is highly sensitive, accurate, and has the precision necessary for determining eicosanoid profiles in plasma. This method was successfully applied to the study of aspirin-related changes in eicosanoid profiles in humans

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References

[1] R.J. Roman, Physiol. Rev. 82 (2002) 131.
[2] A.A. Spector, X. Fang, G.D. Snyder, N.L. Weintraub, Prog. Lipid Res. 43 (2004) 55.
[3] C.D. Funk, Science 294 (2001) 1871.
[4] S. Jain, G. Chakraborty, R. Raja, S. Kale, G.C. Kundu, Cancer Res. 68 (2008) 7750.
[5] A.H. Stephenson, A.J. Lonigro, T.M. Hyers, R.O. Webster, A.A. Fowler, Am. Rev. Respir. Dis. 138 (1988) 714.
[6] J.W. Weiss, J.M. Drazen, E.R. McFadden, P. Weller, E.J. Corey, R.A. Lewis, K.F. Austen, JAMA 249 (1983) 2814.
[7] N. Miyata, R.J. Roman, J. Smooth Muscle Res. 41 (2005) 175.
[8] L. Cambj-Sapunar, M. Yu, D.R. Harder, R.J. Roman, Stroke 34 (2003) 1269.
[9] D. Gebremedhin, A.R. Lange, T.F. Lowry, M.R. Taheri, E.K. Birks, A.G. Hudetz, J. Narayanan, J.R. Falck, H. Okamoto, R.J. Roman, K. Nithipatikom, W.B. Campbell, D.R. Harder, Circ. Res. 87 (2000) 60.
[10] S.L. Amaral, K.G. Maier, D.N. Schippers, R.J. Roman, A.S. Greene, Am. J. Physiol. Heart. Circ. Physiol. 284 (2003) H1528.
[11] D.E. Stec, K.P. Gannon, J.S. Beaird, H.A. Drummond, Cell. Physiol. Biochem. 19 (2007) 121.
[12] S.C. Amruthesh, J.R. Falck, E.F. Ellis, J. Neurochem. 58 (1992) 503.
[13] D.R. Harder, N.J. Alkayed, A.R. Lange, D. Gebremedhin, R.J. Roman, Stroke 29 (1998) 229.
[14] J.J. Iliff, L.N. Close, N.R. Selden, N.J. Alkayed, Exp. Physiol. 92 (2007) 653.
[15] M. Liu, N.J. Alkayed, J. Cereb. Blood Flow Metab. 25 (2005) 939.
[16] L.M. Dong, X.O. Shu, Y.T. Gao, G. Milne, B.T. Ji, G. Yang, H.L. Li, N. Rothman, W. Zheng, W.H. Chow, C.C. Abnet, Cancer Epidemiol. Biomarkers Prev. 18 (2009) 3075.
[17] J.W. Eikelboom, J. Hirsh, J.I. Weitz, M. Johnston, Q. Yi, S. Yusuf, Circulation 105 (2002) 1650.
[18] J.V. Gainer, A. Bellamine, E.P. Dawson, K.E. Womble, S.W. Grant, Y. Wang, L.A. Cupples, C.Y. Guo, S. Demissie, C.J. O'Donnell, N.J. Brown, M.R. Waterman, J.H. Capdevila, Circulation 111 (2005) 63.
[19] J.C. Johnson, C.R. Schmidt, M.J. Shrubsole, D.D. Billheimer, P.R. Joshi, J.D. Morrow, M.J. Heslin, M.K. Washington, R.M. Ness, W. Zheng, D.A. Schwartz, R.J. Coffey, R.D. Beauchamp, N.B. Merchant, Clin. Gastroenterol. Hepatol. 4 (2006) 1358.
[20] S.L. Ong, Y. Zhang, J.A. Whitworth, Clin. Exp. Pharmacol. Physiol. 35 (2008) 477.
[21] K. Nithipatikom, A.J. Grall, B.B. Holmes, D.R. Harder, J.R. Falck, W.B. Campbell, Anal. Biochem. 298 (2001) 327.
[22] J. Rivera, N. Ward, J. Hodgson, I.B. Puddey, J.R. Falck, K.D. Croft, Clin. Chem. 50 (2004) 224.
[23] C.A. Bolcato, R.F. Frye, M.A. Zemaitis, S.M. Poloyac, J. Chromatogr. B 794 (2003) 363.
[24] R. Deems, M.W. Buczynski, R. Bowers-Gentry, R. Harkewicz, E.A. Dennis, Methods Enzymol. 432 (2007) 59.
[25] H. Yue, K.I. Strauss, M.R. Borenstein, M.F. Barbe, L.J. Rossi, S.A. Jansen, J. Chromatogr. B 803 (2004) 267.
[26] V.A. VanderNoot, M. VanRollins, Anal. Chem. 74 (2002) 5859.
[27] J.A. Salmon, Br. Med. Bull. 39 (1983) 227.
[28] H.E. Grates, R.M. McGowen, S.V. Gupta, J.R. Falck, T.R. Brown, D.M. Callewaert, D.M. Sasaki, J. Biosci. 28 (2003) 109.
[29] E.C. Kempen, P. Yang, E. Felix, T. Madden, R.A. Newman, Anal. Biochem. 297 (2001) 183.
[30] V. Zelinski, J. Borlak, Xenobiotica 35 (2005) 811.
[31] Y. Mu, M.M. Klamerus, T.M. Miller, L.C. Rohan, S.H. Graham, S.M. Poloyac, Drug Metab. Dispos. 36 (2008) 2324.
[32] H. Yue, S.A. Jansen, K.I. Strauss, M.R. Borenstein, M.F. Barbe, L.J. Rossi, E. Murphy, J. Pharm. Biomed. Anal. 43 (2007) 1122.
[33] J.H. Zhang, T. Pearson, B. Matharoo-Ball, C.A. Ortori, A.Y. Warren, R. Khan, D.A. Barrett, Anal. Biochem. 365 (2007) 40.
[34] C. Chavis, L. Fraissinet, P. Chanez, E. Thomas, J. Bousquet, Anal. Biochem. 271 (1999) 105.
[35] N. Psychogios, D. Hau, J. Peng, A.C. Guo, R. Mandal, S. Bouatra, I. Sinelnikov, R. Krishnamurthy, R. Eisner, B. Gautam, N. Young, J. Xia, C. Knox, E. Dong, P. Huang, Z. Hollander, T. Pedersen, S. Smith, F. Bamforth, R. Greiner, B. McManus, J. Newman, T. Goodfriend, D. Wishart, PLoS ONE 6 (2011) e16957.
[36] O. Quehenberger, A.M. Armando, A.H. Brown, S.B. Milne, D.S. Myers, A.H. Merrill, S. Bandyopadhyay, J. Lipid Res. 51 (2010) 3299.