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Short communication

Fast liquid chromatography–quadrupole linear ion trap-mass spectrometry analysis of polyunsaturated fatty acids and eicosanoids in human plasma☆

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ABSTRACT

Profiling of polyunsaturated fatty acids (PUFAs) and their oxidized metabolites, mainly eicosanoids, in human plasma by fast liquid chromatography–mass spectrometry is described. Sample preparation involved protein precipitation of 200 μL plasma followed by on-line solid-phase extraction. 7 PUFAs and 94 oxidized metabolites were separated utilizing a C-18 column packed with 2.6 μm core–shell particles in 7 min. The analytes and deuterium-labeled standards were detected via scheduled multiple reaction monitoring transitions (123 sMRM). Simultaneously, linear ion trap fragment spectra were acquired for confirmation, if necessary. The lower limit of quantitation ranged between 200 and 1000 ng/mL for the PUFAs and 10–1000 pg/mL for the metabolites. The method was applied to a study on plasma samples from 50 healthy subjects.

1. Introduction

Eicosanoids are a class of over 100 lipid mediators derived from the twenty-carbon polyunsaturated fatty acids (PUFAs) arachidonic acid (AA), eicosapentaenoic acid (EPA) and dihomo-γ-linolenic acid (DHGLA) [1,2]. They are generated via the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP450) enzymes. Prostaglandins and thromboxanes are COX products, hydroxyeicosatetraenoic acids (HETEs) and leukotrienes derive mainly from LOX, whereas epoxyeicosatrienoic acids (EETs) and several HETEs are CYP450 products [1]. Isoprostanes are formed non-enzymatically from AA [3]. Beside eicosanoids there exist LOX-derived metabolites from docosahexaenoic acid (DHA) called protectins and resolvins [4] and LOX-derived metabolites from linoleic acid (LA) called hydroxyoctadecadienoic acids (HODEs) [5].

The analysis of these bioactive metabolites in cells, tissues and body fluids is of growing interest but challenging due to low endogenous concentrations (ng/L-range), isomeric and isobaric structures, and *in vitro* generation. In the past, eicosanoids were mainly analyzed by gas chromatography–mass spectrometry [6–8] or by immunoassays [3]. Recently, liquid chromatography–mass spectrometry (LC–MS) became more important for eicosanoid analysis due to its less laborious sample pretreatment (no derivatization) and the potential for multi-analyte testing. LC–MS methods are either focused on selected eicosanoids [9,10] or on profiling up to 50 and more metabolites with typical analysis times of 20 min [11–15].

For chromatographic separation of eicosanoid isobars and regioisomers high chromatographic resolution power is required, which usually results in long analysis times. For example, 8-iso-$PGF_{2\alpha}$ and isomers were separated in 12 min [16], whereas PGE_2 isomers

Abbreviations: AA, arachidonic acid; CE, collision energy; COX, cyclooxygenase; Cytochrome P450, CYP450; DHET, dihydroxyeicosatrienoic acid; DHA, docosahexaenoic acid; DHGLA, dihomo-γ-linolenic acid; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid; EPI, enhanced product ion; ESI, electrospray ionization; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; LA, linoleic acid; LC–MS, liquid chromatography–mass spectrometry; LLOQ, lower limit of quantitation; LOD, limit of detection; LOX, lipoxygenase; LC-QqLIT-MS, liquid chromatography–quadrupole linear ion trap-mass spectrometry; MRM, multiple reaction monitoring; PUFA, polyunsaturated fatty acid; sMRM, scheduled multiple reaction monitoring; S/N, signal-to-noise ratio; SPE, solid-phase extraction; UHPLC, ultra high performance liquid chromatography; ULOQ, upper limit of quantitation.

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Table 1
Limits of detection (LODs) and linear ranges of the PUFA/PUFA-metabolites selected for validation as well as intra-assay (n = 10) and inter-assay variability (n = 15 over 5 days).

Compound	LOD	Linear range		Intra-assay precision		Inter-assay precision	
	pg/mL (ng/mL)[a]	pg/mL (ng/mL)[a]		Mean, pg/mL (ng/mL)[a]	CV, %	Mean, pg/mL (ng/mL)[a]	CV, %
Tetranor-PGEM/Tetranor-PGDM	10	25	– 10,000	542	7.0	574	10.0
$iPF_{2\alpha}$-IV	50	100	– 10,000	267	14.1	259	30.7
TxB_2	50	100	– 10,000	527	7.2	565	9.0
$PGF_{2\alpha}$	50	100	– 10,000	395	11.8	361	24.6
15-keto-PGE_2/8-iso-15-keto-PGE_2	50	100	– 10,000	176.8	12.1	168	17.8
5(S),6(R)-LXA_4	50	100	– 10,000	207.7	9.7	193	18.2
LTE_4	100	250	– 10,000	1394	12.4	1439	13.9
PGB_2	50	100	– 10,000	440	12.7	467	33.4
7(S)-Maresin 1	50	100	– 10,000	1157	10.7	1105	12.3
10(S),17(S)-DiHDoHe	50	100	– 10,000	1483	9.3	1448	14.7
LTB_4	50	250	– 5000	370	4.4	361	6.3
5,6-DHET	50	250	– 10,000	555	3.3	508	10.3
5-HEPE	50	250	– 10,000	816	7.7	701	15.4
13-HODE	50	100	– 10,000	11,011	6.5	12,315	9.7
15-oxo-ETE	100	500	– 10,000	343	6.9	347	12.7
11-HETE	50	100	– 10,000	1915	5.6	1921	12.4
12-HETE	100	500	– 10,000	3249	11.1	3631	20.1
5-HETE	100	250	– 5000	4777	10.8	5022	15.7
11,12-EET	100	500	– 10,000	383	5.5	361	12.7
AA[a]	100	500	– 10,000	1006	3.5	922	9.6

needed 65 min [17] using 4 and 3 μm particles, respectively. Recent developments in chromatographic supports and instrumentation allow rapid and efficient separations based on columns filled with sub-2 μm particles (ultra high performance liquid chromatography, UHPLC) or core–shell material [18]. The resulting peak widths <10 s are challenging for MS-detection if multiple reaction monitoring (MRM) experiments with 5–20 ms/MRM are applied. The simultaneous generation of a characteristic fragment spectrum for analyte confirmation using a hybrid quadrupole linear ion trap instrument (QqLIT) [19] is also demanding in combination with fast chromatography due to prolonged scan times.

The aim of the current study was the development of a rapid profiling method combining on-line solid-phase extraction for sample-clean up and preconcentration with fast LC and QqLIT-MS for PUFAs and their oxidized metabolites.

2. Experimental

2.1. Standards and solutions

Unlabeled and deuterium-labeled standards were obtained from Cayman Chemical (Ann Arbor, MI, USA) (see Supplement Tables 1 and 2). Standards were diluted in methanol and stored at −80 °C. Acetonitrile, 2-propanol, methanol and formic acid, all ULC–MS grade, were purchased from Biosolve (Valkenswaard, Netherlands). Water was obtained in-house from a nanopure water purification system (Thermo Scientific, Germany). Precipitation solution consisting of methanol–zinc sulfate 4:1 v/v was prepared with a solution of 89 g/L zinc sulfate heptahydrate (p.A. Merck Darmstadt, Germany) in water. Calibrators were prepared by diluting the stock solutions with isotonic saline solution to final concentrations of 5, 10, 25, 50, 100, 250, 500, 1000, 2500, 5000, 10,000 ng/mL for PUFAs and 5, 10, 25, 50, 100, 250, 500, 1000, 2500, 5000, 10,000 pg/mL for the metabolites. Calibration curves were performed with $1/x$ weighted linear regression to account for the smaller concentration values. Quality controls were prepared using pooled plasma spiked with appropriate volumes of PUFA and metabolite stock solutions. Labeled standard solutions were diluted from stock to a final concentration of 5 ng/mL in methanol–water, 50:50 (v/v) (50 ng/mL for AA-d_8).

2.2. Collection of samples

EDTA-K_3-blood samples were collected from 50 healthy subjects (ethical approval 082-10-190-42010) and stored approximately 0.5 h at room temperature after blood taking. After centrifugation at 3220 × g for 10 min the plasma was stored at −80 °C in safe-lock tubes (Eppendorf, Hamburg, Germany) until analysis.

2.3. Sample preparation

200 μL EDTA-plasma were transferred in 1.5 mL polypropylene tubes and mixed with 50 μL of labeled standard solution and 400 μL precipitation solution for 2 min. After 5 min of centrifugation at 10,000 × g, 250 μL of clear supernatant were transferred into an autosampler vial and stored at 10 °C in a temperature controlled autosampler until injection of 200 μL to the online-SPE-LC–MS/MS system.

2.4. On-line SPE-LC–MS/MS analysis

For MS/MS analysis a 5500 QTrap mass spectrometer (AB Sciex, Darmstadt) with electrospray ionization (ESI) in negative ion mode was applied (for mass spectrometric parameters see Supplement Table 1). Quantitative analysis was performed using sMRM scans