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Simultaneous eicosanoid profiling and identification by liquid chromatography and hybrid triple quadrupole-linear ion trap mass spectrometry for metabolomic studies in human plasma

Multiparametrische Bestimmung und Identifizierung von Eikosanoiden mit Flüssigchromatographie in Kombination mit Hybrid-Quadrupol/lineare Ionenfallen Massenspektrometrie für Metabolom-Studien in Humanplasma

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Abstract

Eicosanoids play a key role in many physiological and pathological processes and might therefore serve as interesting diagnostic targets. Methods for the analysis of arachidonic acid metabolites in cells and body fluids require high sensitivity and specificity because of the very low concentrations, similar chemical structures and short half-lives of these metabolites. We established a mass spectrometric method for the simultaneous identification and quantification of arachidonic acid metabolites in human plasma samples using solid phase extraction followed by liquid chromatography (LC) and hybrid triple quadrupole-linear ion trap (QqLIT) mass spectrometry. Quantitative analysis was performed using the 4000 QTrap tandem mass spectrometer in multiple reaction monitoring (MRM) mode. As part of an independent data acquisition experiment MRMs were used as survey scans, which dependently triggered enhanced product ion (EPI) scans. Compound identification was carried out by library search using a library based on EPI spectra of standard components (prostaglandins, thromboxanes, leukotrienes and isoprostanes). The newly developed compound library enables the verification of known and structural elucidation of unknown eicosanoid metabolites in human plasma. In conclusion, our mass spectrometric method allows the simultaneous identification and quantification of arachidonic acid metabolites in one single LC-MS/MS run.

Keywords: arachidonic acid metabolites; eicosanoids; fragment spectra library; linear ion trap; tandem-mass spectrometry.

Zusammenfassung

Eikosanoide sind Mediatoren vieler physiologischer und pathophysiologischer Prozesse im menschlichen Organismus. Analytische Methoden für die Bestimmung von Arachidonsäuremetaboliten müssen sensitiv und spezifisch sein, um die strukturell sehr ähnlichen Substanzklassen in den sehr geringen Konzentrationen, in denen Sie im Blut vorkommen, bestimmen zu können. Wir haben eine massenspektrometrische Methode, basierend auf der Kopplung zwischen Flüssigchromatographie und Quadrupol/lineare Ionenfalle entwickelt, die neben der multiparametrischen Quantifizierung von enzymatischen und nichtenzymatischen Metaboliten des Arachidonsäurestoffwechsels eine Identifizierung der Analyten über eine neu entwickelte Fragmentspektrenbibliothek ermöglicht. Die Untersuchungen erfolgten an einem API 4000 QTrap Tandem-Massenspektrometer. Selektive Massenübergänge wurden für 16 Eikosanoide und 6 interne Standards (Prostaglandine, Thromboxan, Leukotriene, Isoprostane, Hydroxyeikosatetraensäuren und Arachidonsäure) optimiert. Mit Experimenten bei unabhängiger Datenaufnahme wurde für Analyten über einer MRM-Intensitätsschwelle von 500 cps automatisch ein Fragmentspektrum über die lineare Ionenfalle aufgenommen. Eine neu entwickelte Spektrenbibliothek ermöglicht erstmals neben der Quantifizierung der Analyten über den MRM-Übergang auch eine eindeutige Identifizierung bekannter Metabolite über das Fragmentspektrum. Für unbekannte Metabolite können wichtige Strukturinfor-

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mationen aus dem Fragmentspektrum gewonnen werden. Unsere entwickelte QTrap-Methode erlaubt eine simultane Quantifizierung von multiplen Metaboliten des Arachidonsäurestoffwechsels. Die simultane Erzeugung eines Fragmentspektrums ermöglicht eine eindeutige Identifizierung bekannter Analyte und liefert Strukturhinweise für unbekannte Metabolite und ist deshalb für Metabolom-Untersuchungen gut geeignet.

Schlüsselwörter: Eikosanoide; lineare Ionenfalle; Tandem-Massenspektrometrie; Arachidonsäuremetaboliten; Fragmentspektrenbibliothek.

Introduction

Metabolites derived from arachidonic acid (AA) and related fatty acids are lipid signaling components representing central effectors of multiple physiological and pathophysiological processes in the human organism [1, 2]. Eicosanoid expression is tissue and cell specific and varies depending on the activation stimulus, leading to a vast variety of biological activities (inflammatory response, vascular activity, pain, and fever) [3].

In addition to the enzymatically derived metabolites a broad range of non-enzymatically oxidized isoprostanes (IsoPs) can be formed [4]. F(2)-IsoPs are a group of 64 compounds isomeric in structure to cyclooxygenase-derived prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). E(2)- and D(2)-IsoPs are other products of the IsoP pathway [5]. F(2)-IsoPs are the most reliable parameter to assess oxidative stress status in vivo [6]. Additionally, F(2)-IsoPs and other products of the IsoP pathway exert potent biological actions both via receptor-dependent and -independent mechanisms and therefore might be mediators of disease [7].

Methods for the analysis of AA metabolites in cells and body fluids require high sensitivity because of the very low concentrations and short half-lives of these metabolites. Additionally, specificity is required owing to the high amount of regio- and stereoisomers. Currently, enzyme immunoassays are primarily used for direct quantification of AA and its metabolites. However, immunoassays lack specificity and are, as single analyte tests, not applicable for the monitoring of AA pattern [8]. Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) are currently the methods of choice for metabolic profiling [9–11]. However, owing to time-consuming and laborious sample pretreatment and analysis, GC-MS is limited for high-throughput analysis [12]. Recently, different LC-MS/MS methods were developed for quantifying eicosanoid pattern in biological fluids (urine, plasma), cells, and tissue [13–18]. These methods are advantageous because of their high sensitivity and simple sample pretreatment. AA metabolites are very similar in their chemical structure. The multiple reaction monitoring (MRM) transitions are often only class specific and thus retention time of the metabolite is needed for correct analyte identification. Therefore, it would be advantageous to obtain additional structural information (fragment spectra from MS/MS experiments) for confirmation/identification of each eicosanoid. In Figure 1A, the characteristic workflow of an independent data acquisition (IDA) experiment, combining MRM and an enhanced product ion (EPI) scan, is presented. In a first step multiple MRMs are acquired. If a signal of a MRM transition exceeds a defined intensity threshold the Q3 linear ion trap scans for a product ion scan over a defined mass range. This takes only a few milliseconds and allows a combination of quantitative data/fragment spectra acquisition without loss in analytical sensitivity. The QTrap technology has been successfully applied for the simultaneous quantification and analyte verification for small molecules in both forensic (drug analysis) and environmental (pharmaceutical residues) applications as well as the MRM Initiated Detection and Sequencing (MIDAS) workflow for proteotypic peptides [19, 20]. The aim of our study was to develop a LC and hybrid triple quadrupole-linear ion trap (LC-QqLIT) based fragment spectra library for different eicosanoid classes and to evaluate whether it is advantageous to use the library for structural confirmation of AA metabolites in human plasma samples.

Materials and methods

Chemicals and reagents

Water and 2-propanol (both gradient grade) were purchased from Merck (Darmstadt, Germany). Acetonitrile gradient grade and LC-MS grade methanol were purchased from J.T. Baker (Deventer, Netherlands). Formic acid (p.A.) and glacial acetic acid (p.A.) were purchased from Merck (Darmstadt, Germany).

For method development a methanolic eicosanoid standard mixture (stock solution, $c = 5$ μg/mL) containing 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), thromboxane B_2 (TxB_2), prostaglandin F_{2x}, (PGF_{2x}), E_2 (PGE_2), prostaglandin D_2 (PGD_2), 5-S,6-S-lipoxin A_4 (LXA_4), leukotriene B_4 (LTB_4), ±5,6-dihydroxyeicosatrienoic acid (5,6-DHET), 11-S-hydroxyeicosatetraenoic acid (11-HETE), 12-S-hydroxyeicosa-tetraenoic acid (12-HETE), 5-S-hydroxyeicosatetraenoic acid (5-HETE), 5-S-hydroperoxyeicosatetraenoic acid (5-HpETE), 5-oxo-eicosatetraenoic acid (5-oxo-ETE), ±5,6-epoxyeicosatrienoic acid (5,6-EET), and AA was used and diluted as needed. Thromboxane B_2-d4 (TxB_2-d4), prostaglandin $F_{2\alpha}$-d4 ($PGF_{2\alpha}$-d4), prostaglandin E_2-d4 (PGE_2-d4), leukotriene B_4-d4 (LTB_4-d4), 5-S-hydroxyeicosatetraenoic acid-d8 (5-HETE-d8), and arachidonic acid (AA-d8) were used as internal standards for quantification of the eicosanoids. All analyte standards and deuterated standards were purchased from Cayman Chemical (Ann Arbor, MI, USA).

Plasma sample preparation

Aliquots of 1 mL EDTA-plasma of healthy volunteers were spiked with the eicosanoid standard mixture to final concentrations of 10 and 100 ng/mL. Sample clean-up via solid phase extraction was achieved using the following protocol: to 200 μL of sample (aqueous eicosanoid standard mixture or spiked plasma) 50 μL of deuterated standard mix [$c = 100$ ng/mL, AA $c = 1000$ ng/mL

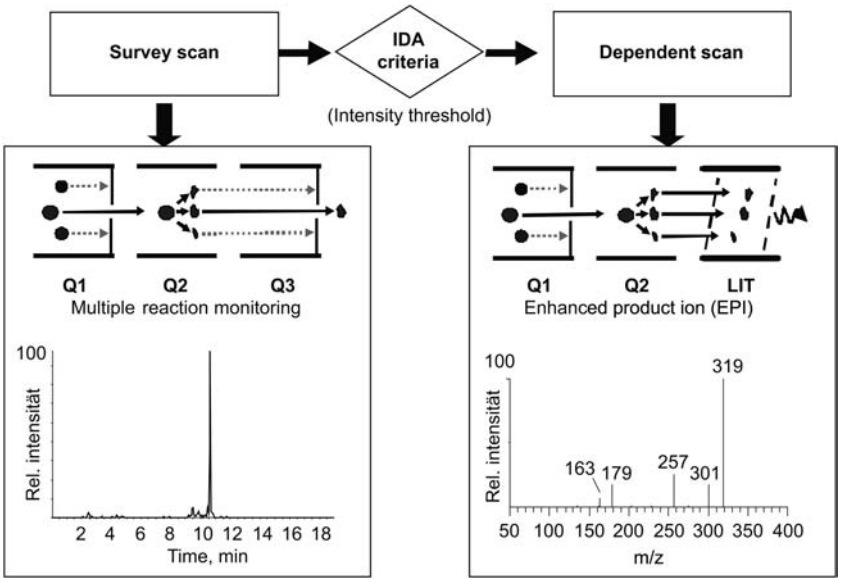


Figure 1 Characteristic workflow of a mass spectrometric independent data acquisition (IDA) experiment using a hybrid triple quadrupole-linear ion trap (QqLIT) mass spectrometer.

in methanol/water 50/50 (v/v)] was added, as well as 180 μL methanol for protein precipitation. The mixture was vortexed vigorously. Water (770 μL) was added, and the mixture was vortexed again and centrifuged at 10,000×*g* for 5 min. The supernatant (1000 μL) was transferred into a new vial, 100 μL 10% acetic acid was added and the mixture was vortexed again. SPE was performed using Strata-x 33 μm polymeric sorbent 60 mg/3 mL tubes (Phenomenex, Aschaffenburg, Germany). The sample was applied to the preconditioned SPE tubes (2 mL methanol, 2 mL water). The sample was washed with 2 mL methanol/water 10/90 (v/v). Elution of the eicosanoids was achieved with 1 mL methanol. The eluate was evaporated under a nitrogen stream, stored under nitrogen or reconstituted in 50 μL solvent A for direct analysis.

LC-MS/MS system and quantitative analysis

A QqLIT mass spectrometer (API 4000 QTrap, MDS Sciex, Toronto, ON, Canada) was applied for eicosanoid analysis using electrospray ionization (ESI) in negative ion mode. MRM transitions were created for the analytes and internal standards (Table 1). MS/MS parameters were tuned by direct infusion of the analytes into the ESI source. The source temperature was set at 600°C and the ionization voltage at −4500 V. The other parameters were set as follows: curtain gas, 10 psi; gas 1, 70 psi; gas 2, 50 psi; CAD gas, 5. Each MRM was performed with 25 ms dwell time. Analytes were quantified using the corresponding deuterated standards.

A Shimadzu LC-20A Prominence HPLC System with LC-20AD binary high pressure gradient system, DGU-20A5 degasser, CTO-20AC column oven, and SIL-20AC auto-sampler (Shimadzu Deutschland GmbH, Duisburg, Germany) was used for LC separation. The chromatographic conditions were chosen based on the protocols by Deems et al. [18]. A Vydac C18 250×2.1 mm column (Grace Vydac, Hesperia, CA, USA) with solvent A: acetonitrile/water 63/37 (v/v), 0.2% formic acid, and solvent B: acetonitrile/2-propanol 50/50 (v/v) was run with the following LC-gradient: linear gradient 0–20% solvent B between 0 and 6 min; solvent B was increased to 55% from 6 to 6.5 min; solvent B was increased to 72% from 6.5 to 11 min and held until 16 min; then solvent B was dropped to 0% by 16.1 min and held until 19 min. The LC flow rate was 0.3 mL/min at 35°C. The injection volume was 10 μL for standards and human plasma samples.

QTrap library development

For library development, IDA experiments were performed with the MRM scan as a survey scan and one EPI scan as a dependent scan using the chromatographic conditions described

Table 1 MRM transitions and retention times of arachidonic acid metabolites.

	Q1/Q3, m/z, Da	Retention time, min
6-keto-$PGF_{1\alpha}$	369.1/245.2	2.70
8-iso-$PGF_{2\alpha}$	353.2/193.1	3.34
TxB_2	369.1/169.3	3.42
TxB_2-d4	373.3/173.3	3.42
$PGF_{2\alpha}$	353.2/193.1	3.82
$PGF_{2\alpha}$-d4	357.1/313.1	3.82
PGE_2	351.3/271.2	4.14
PGE_2-d4	355.3/275.2	4.14
PGD_2	351.3/271.2	4.45
LXA_4	351.2/217.2	5.34
LTB_4	335.4/195.2	8.36
LTB_4-d4	339.1/197.2	8.36
5,6-DHET	337.2/145.2	9.83
11-HETE	319.2/167.2	10.09
12-HETE	319.2/179.2	10.15
5-HETE	319.2/115.2	10.37
5-HETE-d8	327.2/116.2	10.37
5-HpETE	317.2/203.2	10.38
5-oxo-ETE	317.2/203.2	10.50
5,6-EET	319.2/163.2	10.72
Arachidonic acid	303.2/259.2	12.10
Arachidonic acid-d8	311.3/267.2	12.10

above. The survey scan contained the MRM transitions for the AA metabolites and internal standards as described above. Parameters of the EPI scan were set as follows: source parameters as described for the MRM scan; CAD gas, 8; declustering potential, –60 V; scan rate, 4000 Da/s; LIT fill time, dynamic; intensity threshold, 500 counts per second (cps); no dynamic exclusion; exclusion list includes MRMs of deuterated standards. Collision energy (CE) was set to –15, –30, –45 eV, or CE –30 eV combined with collision energy spread (CES) ±15 eV, depending on the experiment. The maximum cycle time (MRM and EPI scans) was 1.6 s.

Methanolic standard solutions of each eicosanoid (1 μg/mL) were injected separately and EPI spectra were acquired for the CEs: –15, –30, –45 eV, and CE –30 V/CES ±15 eV. Two separate libraries were built: one set up with the CE –30 eV spectra, the other consisting of the spectra acquired with CE –30 eV/CES ±15 eV.

For library evaluation, an aqueous standard mixture containing 16 AA metabolites and human EDTA-plasma spiked with the same standard mixture (both c = 10 and 100 ng/mL) were subjected to the SPE protocol and then analyzed by LC-QqLIT. The dependent scan was an EPI scan, which was carried out at

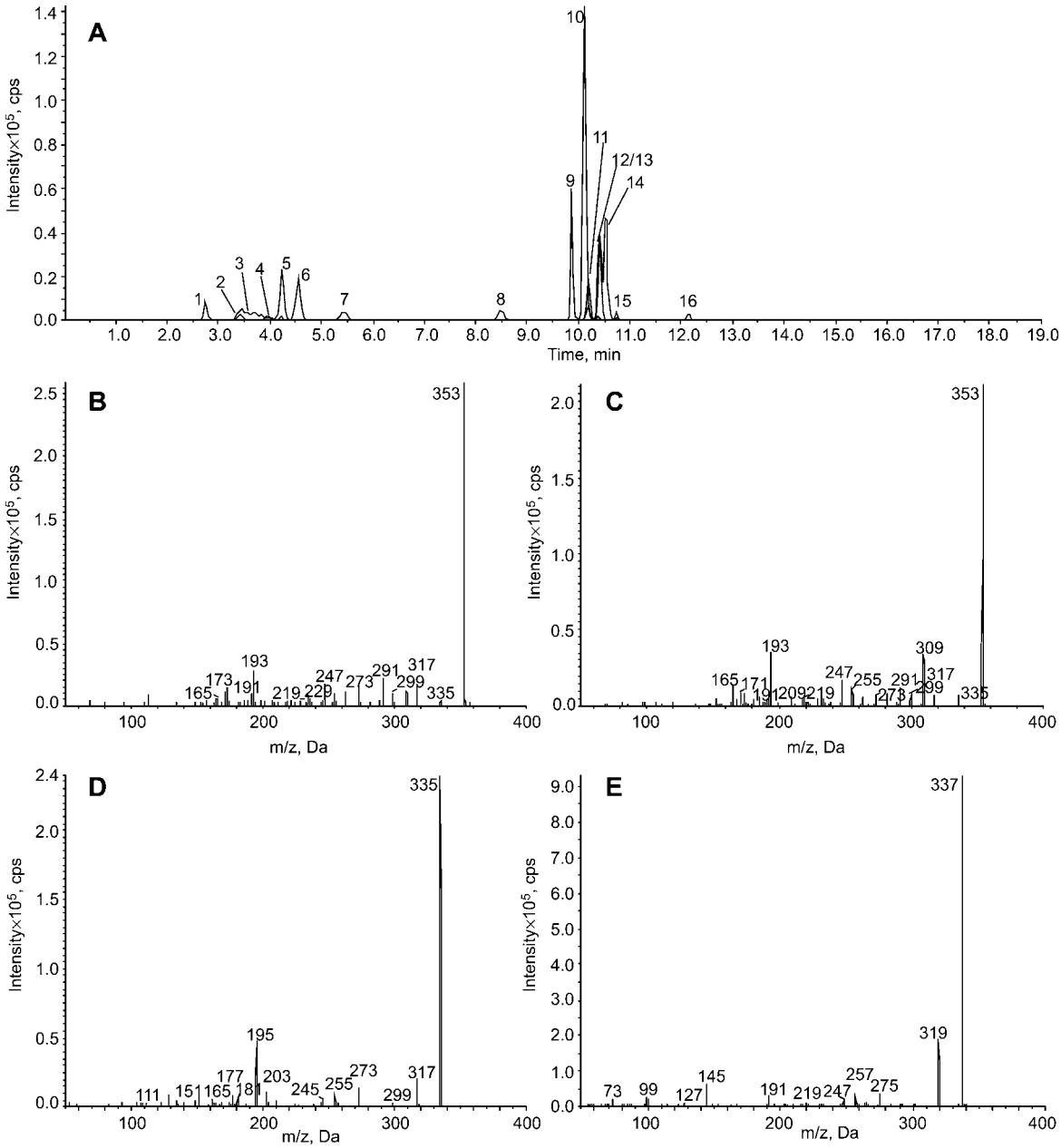


Figure 2 Chromatographic separation of a 100 ng/mL standard mixture.
(A) (1) 6-ketoPGF$_{2\alpha}$, (2) 8-iso-PGF$_{2\alpha}$, (3) TxB$_2$, (4) PGF$_{2\alpha}$, (5) PGE$_2$, (6) PGD$_2$, (7) LTB$_4$, (8) LXA$_4$, (9) 5,6-DHET, (10) 11-HETE, (11) 12-HETE, (12) 5-HETE, (13) 5-HpETE, (14) 5-oxo-ETE, (15) 5,6-EET, (16) arachidonic acid; EPI spectra acquired with CE –30 eV/CES ±15 eV of (B) 8-iso-PGF$_{2\alpha}$ (autoxidation product); (C) PGF$_{2\alpha}$ (COX product); (D) LTB$_4$ (LOX product); (E) 5,6-DHET (P450 product).

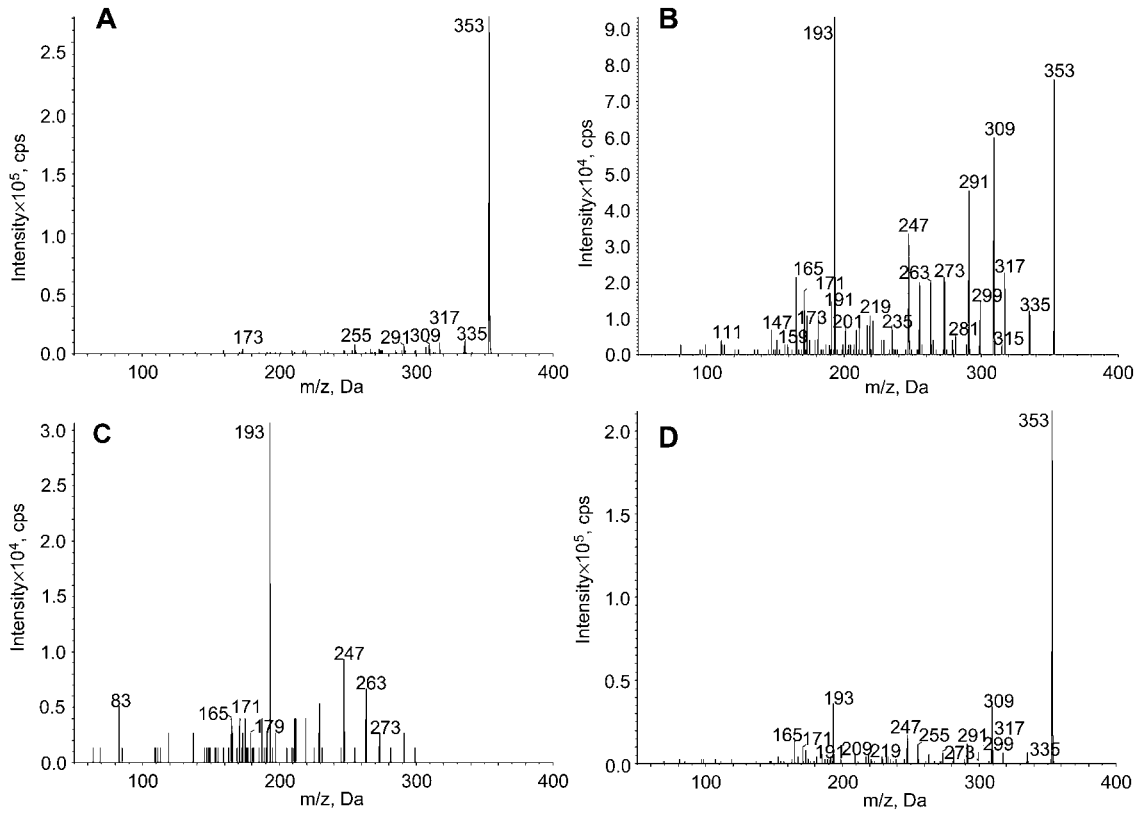


Figure 3 EPI spectra of $PGF_{2\alpha}$ at different CEs.
(A) CE −15 eV, (B) CE −30 eV, (C) CE −45 eV, (D) CE −30 eV/CES ±15 eV.

CE −30 eV and CE −30 eV/CES ±15 eV, respectively. The resulting EPI spectra were then searched against the mass spectral library built with the corresponding CE.

Results and discussion

Step 1: Development of the mass spectrometric method

Applying a QqLIT mass spectrometer, the MRM survey scan can trigger dependent EPI scans and thus generate fragment spectra in addition to quantitative data. As a first step, we developed a method combining MRM and EPI scans in regard to both quantitation via MRMs and maximum fragment spectra information. As scan time is an issue in combined MRM and EPI experiments, we chose to acquire only one EPI spectrum at a given CE to reduce duty cycle time. However, we refrained from using the dynamic exclusion function (time for which a transition is excluded after acquiring an EPI scan) to allow the detection of isomeric substances. Using our combined MRM-EPI method setup we did not observe any significant loss in analytical sensitivity for the detection of the eicosanoids. For example, $PGF_{2\alpha}$ showed an analyte peak area of 1.84×10^3 counts in MRM mode combined with a LIT experiment compared to 2.07×10^3 counts using a MRM scan (data not shown). In Figure 2A, a representative chromatogram of the MRM transitions used for quantification of the eicosanoids (standard mixture of 16 enzymatic and non-enzymatic AA metabolites c = 100 ng/mL) is shown. In Figure 2B–D, the fragment pattern of characteristic metabolites from each enzymatic pathway $PGF_{2\alpha}$, LTB_4, and 5,6-DHET (derived through COX, LOX, and P450, respectively) and the isoprostane 8-iso-$PGF_{2\alpha}$ as product of free radical-mediated

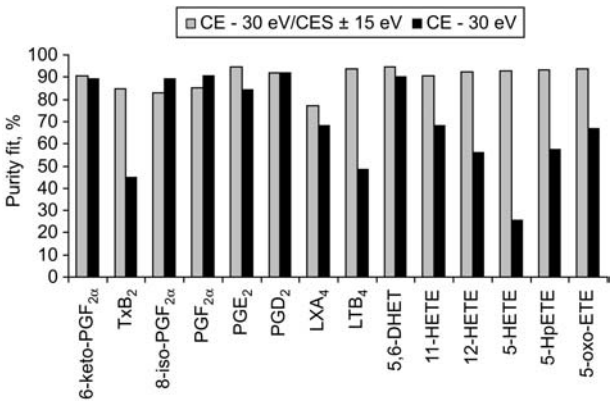


Figure 4 Peak purity fit of a methanolic eicosanoid standard mixture (100 ng/mL).
EPI scans were generated using CE −30 eV/CES ±15 eV and CE −30 eV, respectively.

lipid peroxidation are presented. The different eicosanoid classes are characterized by differentiating fragment patterns, making them suitable targets for identification by a library search. Even stereoisomers such as $PGF_{2\alpha}$ (trans-conformation) and 8-iso$PGF_{2\alpha}$ (cis-conformation) differ in their characteristic fragment pattern (Figure 2B, C).

Step 2: Influence of CE on library performance

In a next step, a library of fragment spectra from each of the 16 eicosanoids was created. For acquisition of these spectra, the chromatographic gradient program and MRM-EPI experiment used for sample analysis was applied instead of direct infusion into the source to avoid

Table 2 Fit, RevFit, and purity fit values for 16 eicosanoids as methanolic standard solution and spiked in human EDTA-plasma at two concentration levels (10 and 100 ng/mL); the library spectra were created using collision energy spread CE –30 eV CES ±15 eV.

	Library search results CE –30 eV CES ±15 eV											
	CES 1											
	c = 10 ng/mL						c = 100 ng/mL					
	Standard			Spiked plasma			Standard			Spiked plasma		
	Fit	RevFit	Purity	Fit	RevFit	Purity	Fit	RevFit	Purity	Fit	RevFit	Purity
6-keto-$PGF_{2\alpha}$	62.8	100.0	62.8	64.5	97.8	63.1	90.8	96.5	90.6	85.5	92.1	84.0
TxB_2	39.8	100.0	39.8	9.6	96.7	9.2	84.7	97.7	84.7	31.9	37.1	31.4
8-iso-$PGF_{2\alpha}$	79.5	94.5	75.1	79.2	95.4	76.3	84.8	88.2	83.1	93.4	94.1	90.8
$PGF_{2\alpha}$	68.9	98.8	68.9	69.2	99.4	69.2	90.6	88.5	85.4	73.6	82.4	63.2
PGE_2	73.0	88.9	73.0	48.4	63.2	35.9	95.6	97.5	94.6	95.1	91.8	89.5
PGD_2	51.6	81.1	42.6	50.9	96.9	50.9	92.8	95.2	91.9	95.0	97.5	94.1
LXA_4	67.7	100.0	67.7	67.7	100.0	67.7	80.4	95.5	77.3	78.2	93.5	73.4
LTB_4	81.5	99.4	81.5	77.4	100.0	77.4	93.7	96.4	93.7	93.1	95.8	93.1
5,6-DHET	94.6	96.3	92.7	94.5	96.6	92.9	96.2	97.8	94.7	94.6	97.5	93.7
11-HETE	72.6	96.0	69.7	81.1	97.7	81.1	91.3	97.3	90.7	95.7	95.4	94.8
12-HETE	64.8	100.0	64.8	78.9	99.9	78.9	94.5	95.1	92.3	89.9	94.1	87.3
5-HETE	67.3	81.0	58.9	93.6	99.5	93.6	93.0	96.5	92.6	94.3	98.3	94.3
5-HpETE	79.7	99.9	79.7	n/a	n/a	n/a	93.7	98.3	93.3	n/a	n/a	n/a
5-oxo-ETE	70.8	95.4	68.8	74.9	96.3	73.5	94.7	97.0	93.8	86.6	95.2	86.6
5,6-EET	n/a	n/a	n/a	n/a	n/a	n/a	64.7	90.2	58.3	94.6	99.4	94.6
Arachidonic acid	n/a	n/a	n/a	95.1	98.5	95.1	n/a	n/a	n/a	97.6	98.8	97.6

Table 3 Fit, RevFit and purity fit values for 16 eicosanoids as methanolic standard solution and spiked in human EDTA-plasma at two concentration levels (10 and 100 ng/mL); the library spectra were created using collision energy CE –30 eV.

	Library search results CE –30 eV											
	c = 10 ng/mL						c = 100 ng/mL					
	Standard			Spiked plasma			Standard			Spiked plasma		
	Fit	RevFit	Purity	Fit	RevFit	Purity	Fit	RevFit	Purity	Fit	RevFit	Purity
6-keto-$PGF_{2\alpha}$	33.1	92.0	33.1	41.2	66.6	28.7	93.0	93.1	89.1	87.1	78.2	69.3
TxB_2	6.7	33.7	2.3	30.0	70.3	30.0	45.0	91.2	45.0	76.2	9.9	8.3
8-iso-$PGF_{2\alpha}$	52.3	99.1	52.3	27.2	67.8	21.2	92.2	91.6	89.1	88.1	92.0	86.8
$PGF_{2\alpha}$	26.3	73.7	19.8	29.5	94.8	29.5	91.3	93.0	90.6	84.6	87.7	77.2
PGE_2	58.9	99.5	58.9	66.6	99.1	66.6	86.0	95.8	84.2	88.3	91.4	83.9
PGD_2	73.1	89.2	73.1	79.2	96.7	79.2	93.3	97.1	91.7	89.9	93.4	86.5
LXA_4	6.8	100.0	6.8	47.4	4.2	2.0	74.2	87.3	68.2	74.3	88.4	69.1
LTB_4	39.1	61.8	29.1	9.9	100.0	9.9	49.5	87.0	48.3	46.4	88.2	44.3
5,6-DHET	68.2	100.0	68.2	76.2	86.5	72.2	93.1	92.6	90.3	88.9	88.1	85.1
11-HETE	33.6	33.9	11.4	33.6	100.0	33.6	73.6	88.9	68.1	59.0	95.4	59.0
12-HETE	44.3	73.6	44.3	52.0	68.3	44.5	56.0	87.5	56.0	72.1	87.7	67.7
5-HETE	49.0	65.9	49.0	56.7	99.5	56.7	44.0	49.1	25.6	82.6	96.5	82.6
5-HpETE	n/a	n/a	n/a	n/a	n/a	n/a	67.4	62.7	57.6	n/a	n/a	n/a
5-oxo-ETE	43.8	79.6	35.6	39.5	82.4	32.8	68.4	91.7	67.0	71.2	92.0	69.3
5,6-EET	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Arachidonic acid	n/a	n/a	n/a	67.5	100.0	67.5	n/a	n/a	n/a	84.4	88.7	79.8

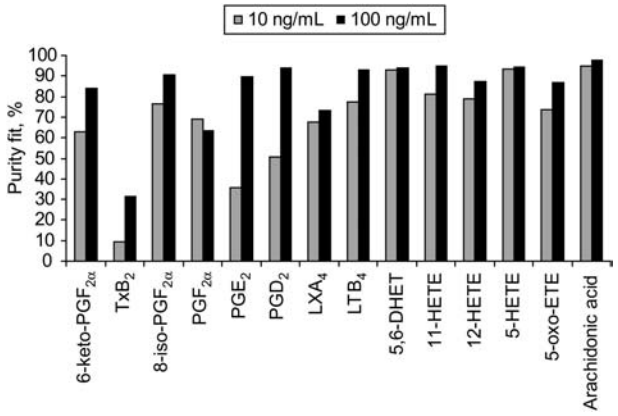
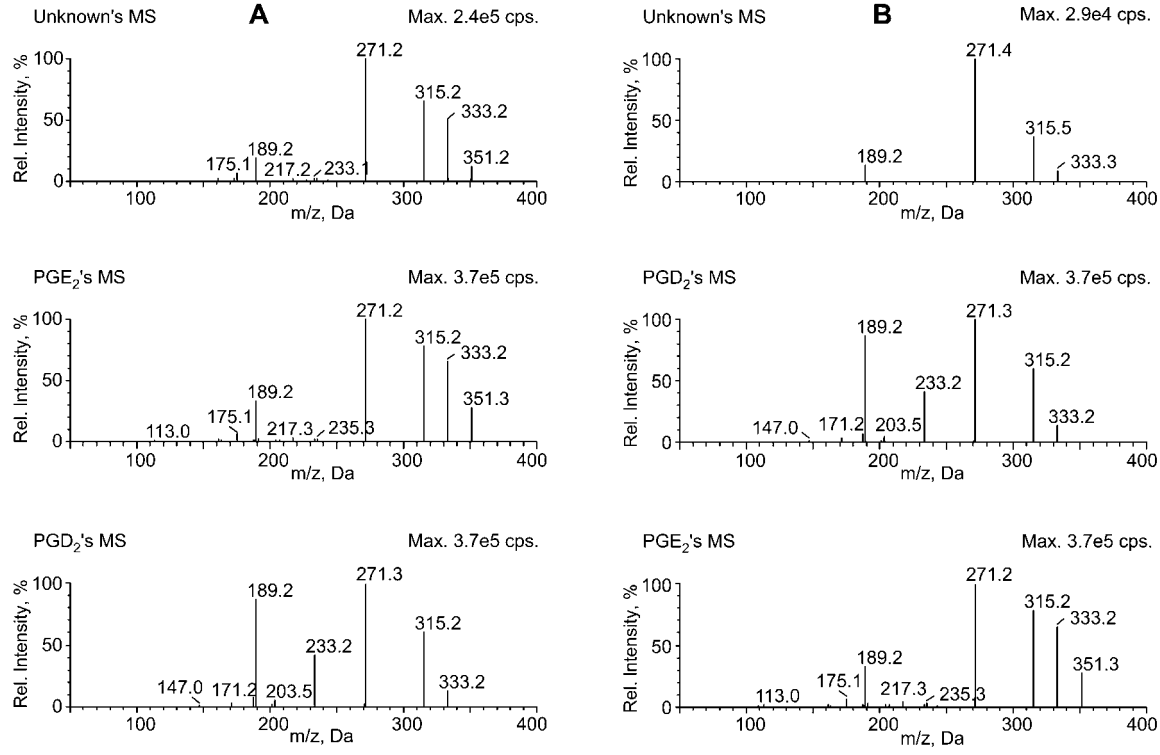


Figure 5 Peak purity fit for spiked human EDTA-plasma (c = 10 and 100 ng/mL).
An EPI library was generated using CE –30 eV/CES ±15 eV.

possible disturbing effects of background signals of the solvents. Spectra of three different CEs (–15, –30, and –45 eV) as well as one using the CES function (CE –30±15 eV) were obtained. The CES function allows the collection of data of three distinct CEs in one spectrum. Compared to three EPI scans with single CEs, CES reduces the duty cycle while providing information of low- and high-mass fragments in one spectrum. As shown in Figure 3 for the fragmentation of $PGF_{2\alpha}$, the CES results in the most significant fragment spectra. In the fragment spectrum at –15 eV (Figure 3A), the molecular ion and only a few fragments are detected. In contrast, the fragment spectrum at –45 eV results in a lot of unspecific low-molecular weight fragments. As the best compromise, CE –30 eV and CE –30±15 eV were chosen to generate two separate spectra libraries for the evaluation of library performance.

In Figure 4, the purity fit, a combination of the fit value (similarity of the signals in the reference spectrum with those in the unknown spectrum) and the reverse fit (RevFit) value (similarity of the signals in an unknown spectrum with those in a reference spectrum) of an aqueous eicosanoid standard mixture (100 ng/mL) is presented. Using CES for all analytes purity fit values above 70% were obtained, whereas a significant lower correlation was found for CE –30 eV. The fit and reverse fit values for all analytes are summarized in Tables 2 and 3.



	Name	Molecular weight	Fit	RevFit	Purity
1	PGE_2	352.2	95.06	91.76	89.55
2	PGD_2	352.2	75.03	68.96	63.65
3	LXA_4	352.2	30.00	30.94	28.16

	Name	Molecular weight	Fit	RevFit	Purity
1	PGD_2	352.2	51.12	65.57	37.86
2	PGE_2	352.2	48.45	63.25	35.88
3	LXA_4	352.2	4.85	39.30	3.59

Figure 6 Library search results for PGE_2 at two concentration levels of spiked EDTA-plasma (A) 100 ng/mL, (B) 10 ng/mL.

Library performance at high/low concentration levels

Finally, eicosanoid identification was carried out by a library search with the developed MS/MS library based on EPI spectra acquired with CES function (CE −30±15 eV). Spiked EDTA-plasma in two concentration levels (10 and 100 ng/mL) was used for testing the library performance. In Figure 5, the purity fit for the eicosanoids is plotted for the high and low spiked concentration. At 100 ng/mL purity fit, values above 80% were obtained for 12 eicosanoids. The identification of TxB_2 using the fragment spectra was not sufficient which might be caused by the broad peak shape in the chromatographic run. At low concentration, the purity fit was mostly in the range of 60–70%. As shown in Figure 6 with library search results for PGE_2 as an example, not all fragments of the library spectra could be detected in the plasma sample (Figure 6). Consequently, the library search was unable to differentiate between the regio-isomers PGD_2 and PGE_2 at low concentration levels (Figure 6B). Here, the retention time is necessary for a clear classification. However, at both concentration levels an appropriate pathway classification was possible. This could help in the future in the identification of unknown eicosanoid species. The library will now be enhanced to other enzymatically and non-enzymatically derived AA metabolites.

Conclusions

We developed a LC-QqLIT based spectra library for AA metabolite identification. The advantage of this newly developed method is its ability to detect and simultaneously identify metabolites in a single LC-MS/MS run. The use of the compound library enables the verification of eicosanoid metabolites in human plasma.

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