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Liquid chromatography–tandem mass spectrometry for the analysis of eicosanoids and related lipids in human biological matrices: A review☆

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ABSTRACT

Today, there is an increasing number of liquid chromatography tandem-mass spectrometric (LC–MS/MS) methods for the analysis of eicosanoids and related lipids in biological matrices. An overview of currently applied LC–MS/MS methods is given with attention to sample preparation strategies, chromatographic separation including ultra high performance liquid chromatography (UHPLC) and chiral separation, as well as to mass spectrometric detection using multiple reacting monitoring (MRM). Further, the application in recent clinical research is reviewed with focus on preanalytical aspects prior to LC–MS/MS analysis as well as applications in major diseases of Western civilization including respiratory diseases, diabetes, cancer, liver diseases, atherosclerosis, and neurovascular diseases.

1. Introduction

Lipid mediators, derived from the metabolism of essential long chain polyunsaturated fatty acids (PUFAs) have numerous functions in the regulation of cell proliferation, tissue repair, coagulation, and immunity [1]. PUFA metabolites play an important role in the pathogenesis of various human diseases, including inflammation and cancer growth. Through enzymatic conversion with elongases and desaturases, linoleic acid (LA) can be converted into arachidonic acid (AA), and α-linolenic acid (ALA) into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as it is shown in Fig. 1. Eicosanoids are formed from the PUFAs through cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) pathways. Pro-inflammatory eicosanoids (prostanoids, hydroxytetraenoic acids, leukotrienes and epoxyeicosatrienoic acids) are mainly formed from the ω-6 fatty acid AA, which is part of the phospholipid domain of most cell membranes. Reactive oxygen species are also capable of converting AA into isoprostanes, which are in part also generated though enzymatic reactions [2]. Pro-inflammatory eicosanoids (*e.g.* the prostaglandins (PGs) PGE_2 and PGD_2) also signal the end of the inflammatory response by stimulating the local formation of metabolites derived from the ω-3 fatty acids EPA and DHA [3]. Anti-inflammatory metabolites of EPA and DHA are the protectin, resolvin and maresin families (see Fig. 1) [4–6].

The key for broadening our insight to the function of eicosanoids *in vivo* is the development of sensitive and specific methods for determination of eicosanoid concentrations in biological matrices. The analysis of PUFA-metabolites in cells, tissues and body fluids is of growing interest but challenging due to the low endogenous concentrations (ng/L-range), the multitude of isomeric and isobaric structures, and the risk of *in vitro* generation during sample pretreatment. In the past, eicosanoids were mainly analyzed by gas chromatography–mass spectrometry (GC–MS) [7–10], liquid chromatography [11,12] or immunoassay [13,14]. In this review we discuss LC–MS/MS based concepts and developments of the last 10 years for single analyte analysis or multi-target profiling in the field of PUFA-metabolism in human body fluids and tissue. Further, an overview of clinical studies applying LC–MS/MS for PUFA-metabolism analysis is given.

2. LC–MS/MS methods

Liquid chromatography (LC) especially ultra-high-performance liquid chromatography (UHPLC) combined with mass spectrometry (LC–MS) became more important for eicosanoid analysis during the last five years due to the simplification of sample pretreatment, and the potential for multi-analyte testing. An overview of currently

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Table 1
Overview of currently applied LC–MS/MS methods for analysis of eicosanoids, and related lipids.

Analytes	No. of analytes	Specimen	Volume	Column	MS	Ionization	Analysis time	Sample Pretreatment	Ref.
PGs, Tx	6	Cell culture media	500 μL	BEH C18 50 × 2.1 mm i.d., 1.7 μm	QqQ	Neg. ESI	0.5 min	LLE	[31]
F_2-IsoPs, PG	4	Urine	200 μL	Hydro-RP 250 × 2.0 mm i.d.	QqQ	Neg. ESI	20 min	Off-line SPE	[26]
Tetranor-PGs	2	Urine	500 μL	Kinetex C18 50 × 3 mm i.d. 2.6 μm	QqQ	Neg. ESI	7 min	Derivatisation with methoxyamine on-line SPE	[37]
PGs, TxB_2, HETEs, HEPE, LTs, EETs, HdoHE, Rvs, PD	24	Tissue culture media	n.s.	Luna C18 150 × 2 mm i.d. 3 μm	QqQ	Neg. ESI	20 min	Off-line SPE	[20]
F_2-IsoPs, Total HODE, Total HETE	3	Plasma, liver extracts	200 μL, 100 μL	Hypersil Gold 100 ×2.1 mm i.d. 1.9 μm	QqQ	Neg. ESI	47 min	LLE	[33]
PGs, Tx, isoPs, HODEs, HETEs, LTs, PUFAs	14	Plasma, serum, tissue homogenate	20 μL	ZORBAX Eclipse XDB C18 100 × 3.0 mm i.d. 3.5 μm	QqQ	Neg. ESI	13 min	PP, Filtration	[35]
Tetranor-PG	1	Urine	1 mL	Synergi Hydro-RP 75 × 2.0 mm i.d. 4 μm	QqQ	Neg. ESI	10 min	Off-line SPE	[24]
PUFAs, DiHETEs, DHETs, EETs, HETEs, HHT, HpETEs, LTs, LXs, oxoETEs, PGs, Txs	79	Cell culture media	2 mL	Grace-Vydac C18 250 × 2.1 mm i.d. 5 μm Chiralpak AD-H 250 × 4.6 mm i.d.	QqQ QqLIT	Neg. ESI	19 min	Off-line SPE	[25]
PGs, DHETs, HETEs, EETs	16	Brain tissue	20 mg	Symmetry C18 250 × 4.6 mm i.d. 5 μm	QqQ	Pos. ESI	100 min	Off-line SPE	[11]
PUFAs, DiHETEs, DHETs, EETs, HETEs, HHT, HpETEs, LTs, LXs, oxoETEs, PGs, Tx, Rvs, PD, others	122	Human whole blood	n.s.	Kinetex C18 150 × 2.1 mm i.d. 1.7 μm	QqQ	Neg. ESI	6.5 min	Off-line SPE	[21]
PGs, Tx	20	Cutaneous blister fluid, plasma, tissue cell culture medium	<200 μL to 5 mL liquid sample 45–200 mg tissue 10–5 mL cell culture	Luna C18 150 × 2.0 mm i.d. 5 μm Kinetex C18 100 × 2.1 mm i.d. 2.6 μm	QqQ	Neg. ESI	30 min 35 min	Off-line SPE	[19]
HODEs, HEPEs, HETEs, HDHAs, LTs, PD, Rvs, Lx, oxoETE, EETs, DHET, HETrE	36			LUX Cellulose-1 150 × 2.0 mm i.d. 3 μm			70 min		
Lx, Rvs, HEPEs, HDHAs	12								
PUFAs, HETEs, EETs, oxoETEs, HODEs, PGs, oxoODE	20	Plasma, urine	200 μL	XPERTEX C18 250 × 2.1 mm i.d. 5 μm	QqQ	Neg. ESI	22 min	LLE	[34]
PUFAs, DiHETE, DHETs, EETs, HETEs, HHT, HpETEs, LTs, LXs, oxoETEs, PGs, Tx, Rv, others	101	Plasma	200 μL	Kinetex C18 100 × 2.1 mm i.d. 2.6 μm	QqQ QqLIT	Neg. ESI	13 min	PP on-line SPE	[15]
LT	1	Sputum	100 μL	Acquity BEH C18 50 × 2.1 mm i.d. 1.7 μm	QqQ	Neg. ESI	4.5 min	Automated LLE	[32]
EETs	4	Serum	200 μL	MediterraneaSeaC18 150 × 4.6 mm id. 3 μm	QqQ QqTOF	Neg. ESI	25 min	Automated off-line SPE	[22]