Quantification of eicosanoids and their metabolites in biological matrices: a review

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The quantification of eicosanoids and their metabolites in biological samples remain an analytical challenge, even though a number of methodologies/techniques have been developed. The major difficulties encountered are related to the oxidation of eicosanoids and their low quantities in biological matrices. Among the known methodologies, liquid chromatography-mass spectrometry (LC-MS/MS) is the standard method for eicosanoid quantification in biological samples. Recently advances have improved the ability to identify and simultaneously quantitate eicosanoids in biological matrices. The present article reviews the quantitative analysis of eicosanoids in different biological matrices by LC and ultra performance liquid chromatography (UPLC)-MS/MS and discusses important aspects to be considered during the collection, sample preparation and the generation of calibration curves required for eicosanoid analysis.

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Eicosanoids are an important class of lipid mediators involved in inflammatory and neuropsychiatric processes. The bioprecursor to eicosanoids, arachidonic acid (AA), is an abundant cell membrane component [1]. Three different oxidative pathways comprising of cyclooxygenase (COX), lipoxygenase (LOX) and CYP450 enzymatically oxidize AA which is converted to different eicosanoids based on the pathway involved (Figure 1). These endogenous eicosanoids are found in biological fluids and tissues at low concentrations. Eicosanoids act as a regulator of important physiological processes (e.g., smooth muscle tone, platelet aggregation and vascular permeability) and pathophysiological conditions (inflammation, autoimmunity, allergy, HIV and cancer) [2,3]. Eicosanoid biosynthesis is a complex arrangement of interacting pathways producing a variety of metabolites and some eicosanoids can be produced by more than one enzyme [4,5]. The relation between eicosanoid concentration and disease progression has been extensively studied, to implicate their role in the pathogenesis of disease [6–8]. The relation between eicosanoid concentration and disease progression has driven the need for sensitive quantitation and specific identification of eicosanoids in biological matrices. However, the quantitation of eicosanoids has been problematic due to their similar structure, chemistry and physical properties. Moreover, the eicosanoids are derived from a single precursor (i.e., AA). To enhance our understanding of disease progression and eicosanoid concentration, measurement of the complete profile of produced eicosanoids is essential. A variety of methods to identify and quantitate eicosanoids in biological matrices have been reported; however each approach has its own potential limitation.

Methods for eicosanoid quantitation

The clinical interest of eicosanoids requires their targeted determination which in turn warrants the development of high-throughput analytical methodology. Bioassays were considered the standard for analysis before the development of specific immunoassays and analytical techniques for quantitation of eicosanoids. There are numerous examples in which bioassays were utilized to quantitate eicosanoids [3]. The main concern associated with the use of bioassay for eicosanoid quantitation is that additional eicosanoids may also be present, leading to biased quantitation [9]. Immunoassays including radio immune assays (RIA's) and ELISA's have also been accepted and used for eicosanoid

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Figure 1. Oxidative pathways of arachidonic acid.

quantitation [10,11] but the specific antibodies required to quantitate each eicosanoid makes it expensive and inefficient. The lack of the ability to determine multiple eicosanoids simultaneously also limits this approach. These drawbacks have largely been overcome by GC–MS methods, but this method also has limitations [12]. GC–MS analysis often requires chemical derivatization; however thermal stability and volatility may not be the same for all eicosanoids [13]. Several electrospray ionization mass spectrometry (ESI–MS) methods have been documented for eicosanoid quantitation [14–16]. The use of ESI–MS overcomes the need for a derivatization step and eicosanoids can be directly analyzed by MS from an aqueous sample but here the structure similarities of eicosanoids makes their separation complicated. In the last decade, one more analytical technique is rapidly growing and replacing other techniques, in the other word, LC–MS/MS for eicosanoids analysis in biological matrices [17,18] since it can utilize the improved resolving power of current LC systems with the detection specificity of MS/MS. Recently, there have been marked improvements in sample preparation techniques, methodology, calibration, sample volume, run time, sample pretreatment and instrumentation utilized for eicosanoids identification and simultaneous quantitation. The present article discusses eicosanoids quantitation in biological matrices, including human plasma, serum, urine, sputum and brain tissue by LC and UPLC–MS/MS.

Extraction approaches for biomatrix sample preparation

In order to reduce ion suppression resulting from the biological matrix, a variety of extraction approaches have been used for sample preparation including protein precipitation (PP), liquid–liquid extraction (LLE) and solid phase extraction (SPE). These techniques remove matrix interferences to a varying degree, and allow good analyte recovery, but there is potential for the generation of eicosanoids during the extraction process. Therefore, inhibitors of oxidation such as indomethacin or butylated hydroxyl toluene are added during the extraction process [5]. PP can be achieved by decreasing the dielectric constant via addition of an organic reagent (methanol or acetonitrile); salting out effect via addition of ammonium sulfate to increase the ionic strength; or changing the sample pH via addition of concentrated acids. In LLE, organic solvents are used wherein analytes have comparatively higher solubility than aqueous solubility, generally, hexane–ethyl acetate [19] chloroform–ethyl acetate [20], 2-propanol–hexane [21] or methanol–chloroform [22]. The upper organic layer is then removed, evaporated and reconstituted. SPE is widely used for sample preparation of eicosanoids due to the high extraction yields (above 90%), selectivity, precision, cost-effectiveness and minimal ion suppression [4]. In this technique, analytes are selectively retained to a particular adsorbent (sorbent) via hydrophobic, electrostatic or size exclusion interaction. This technique also can reduce the matrix effect, which is a common problem in bioanalytical sample preparation [23]. SPE is a popular and effective method for eicosanoid analysis since it is easy to perform, fast, and it removes the interfering matrix without the need to increase the temperature or to use external energy. Literature from last decade revealed that SPE

is a method of choice regardless of sample type (i.e., urine, plasma, saliva, etc.) [23–25]. SPE works based on the same principles as column chromatography, the majority of sample components are adsorbed to the stationary phase. These adsorbents are packed in different commercially available formats such as columns, cartridges or syringes or 96-well plates. An additional advantage of SPE is that it can be combined directly with HPLC, thus eliminating sample loss and also offer a fully automated system [26,27]. Ostermann *et al.* compared the SPE and LLE sample preparation techniques for the quantitative analysis of eicosanoids and other oxylipins in plasma and concluded SPE as the best approach in terms of recovery of added internal standards, extraction efficacy of oxylipins from plasma and reduction of ion-suppressing matrix [28].

Calibration approaches for quantitation of eicosanoids by LC–MS/MS

The lack of a suitable blank matrix for eicosanoid quantitation remains a major challenge. Various approaches are available to deal with the blank matrices deficiency for the eicosanoids quantification by LC–MS/MS including, background subtraction [26,29,30], standard addition [31,32], neat solutions [33], artificial matrices of biological fluids [27,34], charcoal stripped matrices [13,23] and stable-isotope labeled analytes [35–41].

Method of background subtraction

In background subtraction, the calibration curve is constructed after subtracting the background eicosanoid analyte concentration in a pooled/representative matrix from the concentrations of the added standards. In this approach, the matrix used for the calibration curve preparation is identical to the samples to be analyzed so that there is no difference in the recovery and matrix effect between samples and calibration curves. The major drawback of this method is associated with its limit of quantitation (LOQ) which is limited by the endogenous background concentration in blank matrix not by the analytical sensitivity. Irreproducibility is another major drawback of this method which is resulted due to use of different batches of pooled biofluids as blank matrices requiring different background levels of endogenous analyte to subtract [42]. Teppner *et al.* utilized background subtraction for quantitative profiling of prostaglandins as oxidative stress biomarkers in rat plasma [26].

Method of standard addition

In the standard addition method, every study sample is divided into equal volume aliquots and all aliquots are spiked with analyte standards (known amounts) to construct a calibration curve. This standard calibration curve is used to determine the sample concentration as the negative x-intercept [43]. The major advantage of standard addition method is that exactly the same matrix is used of every study sample for the construction of its own calibration curve. The error chances are less in this method as direct quantitation of endogenous analytes is carried out without manual subtraction of background peak areas in contrast to background subtraction. The requirement of large sample volume makes this method time consuming and less suitable for pediatric profiling. Yang *et al.* profiled 39 oxylipin metabolome by LC–MS/MS involving standard addition method [32].

Surrogate matrices

It remains challenging to obtain an endogenous analyte free biological matrix for the preparation of calibration standards. Therefore, several other matrices are often used as surrogate matrices including mobile-phase solvents (i.e., neat) [33,44,45] or pure water [46–48]. Use of surrogate matrices enhances the method efficiency but it should be noted that if neat solutions are used as a surrogate matrix then extraction recovery and matrix effect should be comparable with the original matrix. Montuschi *et al.* quantified leukotriene B₄ in exhaled breath condensate (EBC) by using a neat solution as the surrogate matrix [49]. Other methods involving the use of a surrogate matrix include the use of artificial matrices and stripped matrices.

Artificial matrices

An artificial matrix can be prepared, if the matrix composition is known. This approach is generally utilized when utilizing cerebrospinal fluid (CSF), tears, vaginal fluid and sputum as the matrix of interest as they are difficult to obtain commercially. Simulated artificial matrices are authentic in terms of composition, salt content, analyte solubility, and have similar extraction recovery, and matrix effect to the original matrix. In general practice, artificial matrices to quantitate eicosanoids are prepared by adding bovine (BSA) or human serum albumin (HSA) to phosphate buffer saline (PBS) at a concentration of 20–80 g/l [50]. Zhang *et al.* quantified inflammatory biomarkers in human urine through LC–MS/MS detection method by using an artificial urine matrix [27]. In another study,

quantitation of polyunsaturated fatty acids and eicosanoids in human plasma was conducted by using isotonic saline solution as an artificial matrix [51].

Stripped matrices

Endogenous components can be stripped from particular biological matrices to generate analyte-free surrogate matrices for the calibration curve construction [52–54]. Activated-charcoal and heating are commonly utilized for this purpose. Special attention should be taken while using charcoal to remove all charcoal particles after stripping in order to avoid its binding with spiked analytes that can affect the result. The other main concern in using stripped matrices is that charcoal cannot remove all the endogenous analytes and inter batch variation may occur in stripping. Thakare *et al.* quantified eicosanoids and their metabolites in serum, sputum and bronchial alveolar lavage fluid (BALF) using charcoal stripping [55]. They reported similar extraction recoveries (90–115%) for these eicosanoids in diluted and undiluted serum and then the method was validated with two sets of calibration curves. In another study, Ogawa *et al.* determined salivary eicosapentaenoic acid (EPA) concentration to AA concentration ratio as a useful indicator to identify the cardiovascular disease risk, especially atherosclerosis by using charcoal stripping [13].

Surrogate analyte

The surrogate analyte method involves the use of stable-isotope-labeled standard as a surrogate analyte for the direct and sensitive quantification of analytes due to the lack of any endogenous background. The surrogate analytes are identical or have minimal differences in terms of extraction recovery, chromatographic retention and signal intensity due to similar physicochemical properties of the authentic and surrogate analytes. The response factor, in the other word, surrogate to analyte MS responses ratio should be close to unity and constant over the entire calibration range [56]. A limitation of this method is the requirement of costly-labeled standards. Care should be taken while storing labeled isotopes, as they may be prone to degradation during longer periods. In one study, Deems *et al.* performed eicosanoid quantitation by the stable isotope dilution method and for quantitation of each eicosanoid, a deuterated analog of the analyte was selected as an internal standard [57].

Eicosanoids quantitation in different biological matrices

Because of clinical interest of the quantitation of eicosanoids, there is a need for the development of a sensitive, high-throughput analytical method for the targeted determination of eicosanoids. There has been a recent increase in the literature available regarding quantitation of eicosanoids in different biological matrices via LC–MS/MS. We have summarized recent methods according to eicosanoid (including AA metabolites, oxidized lipids, and C18–C22 fatty acid derivatives) quantitation in different biomatrices in the following section.

Human serum, plasma, blood & CSF

A variety of research is available to describe the quantitation of eicosanoids in human serum, plasma, blood and CSF samples and the relation to their biological application. Although serum and plasma are usually considered to have similar compositions and properties, analytes have shown different concentrations in each matrix. Blood samples, especially plasma samples, are typically used for eicosanoids measurements since the intent is to determine levels as they are in blood just prior to collection. Serum is collected after a clotting reaction has occurred. This clotting reaction leads to a large increase in eicosanoids (mostly prostaglandins [PGs]) and allows for activity of prostaglandin dehydrogenase, epoxide hydrolases to act unhindered at room temperature for an indeterminate amount of time [33]. So, it becomes difficult to prove whether the detected eicosanoids were derived *in vivo* or *in vitro*. Table 1 provides the available research on eicosanoid quantitation utilizing human serum, plasma, blood and CSF matrix via the LC–MS/MS approach. A majority of literature is available for eicosanoid quantitation in plasma including quantification in human and monkey plasma utilizing a validated LC–MS/MS method [10]. Zhang *et al.* developed a LC–MS/MS method to investigate the eicosanoid metabolome in human and mouse plasma and mouse aorta after omega-3 PUFA supplementation. They simultaneously analyzed 32 eicosanoids by the developed LC–MS/MS method with limit of detection (LOD) between 0.0625 and 1 pg [58]. A combined extraction of 37 oxylipins and 14 endocannabinoid-related compounds from biological matrices by using LC–MS/MS was reported by Gouveia-Figueira *et al.* The developed method was successfully applied to different biological matrices such as tissues, cells, human plasma and milk [59]. A LC–MS/MS method has been developed to simultaneously analyze diverse AA metabolites, N-acyl ethanolamines and steroids in human plasma obtained from 32 healthy

Table 1. Summarizes representative examples of eicosanoids quantified in human serum, plasma and blood by LC-MS/MS or UPLC-MS/MS.

Number of eicosanoids quantified	Matrix	Matrix volume	MS	Analysis run time	Sample pretreatment	Application	Ref.
18	Human and monkey plasma	100 μ l	UPLC-MS/MS	6.0 min	SPE	To establish eicosanoids as biomarkers in atherosclerosis	[10]
32	Human plasma, mouse plasma and aorta	NA	LC-MS/MS	8 min	SPE	To study omega-3 PUFA supplement effect on human subject metabolism	[58]
51	Tissues, cell extracts, human plasma and milk	plasma (250 μ l)	LC-MS/MS	11 min	SPE	To facilitate discovery of tissue and species specificity of eicosanoid levels	[59]
10	Human plasma	0.5 ml	LC-MS/MS	11 min	SPE	To quantitate bioactive lipids in human plasma	[30]
184	Human plasma	20 μ l	UPLC-MS/MS	5 min	SPE	To establish eicosanoids as biomarker	[60]
05	Human plasma	200 μ l	LC-MS/MS	8 min	SPE	To develop omega-6, omega-3 and PGE2 levels as sickle cell disease biomarker	[61]
101	Human plasma	200 μ l	LC-MS/MS	13 min	SPE	To study plasma samples from healthy subjects.	[51]
06	Human plasma	400 μ l	LC-MS/MS	NA	LLE	To study elevated eicosanoids association and endothelial progenitor cells dysfunction	[62]
20	Human plasma and urine	200 μ l	LC-MS/MS	22 min	LLE	To analyze lipid peroxidation products in human plasma	[21]
02	Human plasma and urine	250 μ l	UPLC-MS/MS	7 min	LLE	To measure 2AG and AEA in human clinical plasma samples	[63]
32	Human plasma	1 ml	LC-MS/MS	18 min	SPE	To evaluate aspirin-derived changes in basal eicosanoid plasma levels	[64]
03	Human plasma and liver	200, 100 μ l	LC-MS/MS	47 min	LLE	To establish biomarkers in diseased conditions (liver dysfunction)	[20]
14	Human plasma, serum and tissue homogenate	20 μ l	LC-MS/MS	13 min	PPT	To analyze fatty acid metabolites in routine	[65]
104	Human plasma	250 μ l	LC-MS/MS	22 min	SPE	To determine increasing inflammatory oxylipins concentration after cardiac surgery	[29]
09	Human plasma	NA	LC-MS/MS	31 min	LLE	To profile 9-HETE and F2-isoprostanes as a biomarker in CAD	[66]
15	Human plasma	200 μ l	UPLC-MS/MS	8.0 min	LLE	To profile eicosanoids in for Alzheimer's medicine development	[67]
51	Human plasma	250 μ l	LC-MS/MS	25 min	SPE	To study air pollution adverse effects on human health	[68]
15d-PG12	Human plasma	500 μ l	LC-MS/MS	10 min	LLE	To study the role of 15d-PG12 biomarker in proinflammatory condition (diabetes)	[69]
EPA and DHA	Human plasma	500 μ l	LC-MS/MS	4.5 min	PPT	To assess the bioequivalence study of omega-fatty acid ethyl ester formulation	[70]
20	Human plasma	500 μ l	UPLC-MS/MS	12 min	SPE	To investigate the association between niacin administration and eicosanoid plasma level response	[71]
07	Human plasma	300 μ l	LC-MS/MS	7.5 min	PPE and LLE	To study the role of epoxyeicosatrienoic acid regioisomers in human physiology and diseases	[72]
34	Human serum	500 μ l	LC-MS/MS	25 min	SPE	To evaluate eicosanoids as biomarkers in human clinical trials	[55]
02 (TXB2 and 12(S)-HETE)	Human serum	50 μ l	LC-MS/MS	12 min	SPE	To screen COX and/or LOX inhibitors as potential new antiplatelet drugs	[33]
48	Serum, plasma, urine, and cell supernatant	100 μ l	UPLC-MS/MS	15 min	SPE	For large scale metabolomic studies	[73]
04	Human serum	100 μ l	LC-MS/MS	33 min	SPE	To study the influence of isotopic labeled internal standards on eicosanoids analysis in serum	[74]

2AG: 2-arachidonoyl glycerol; AEA: Arachidonoyl ethanolamide; CAD: Coronary artery disease; COX: Cyclooxygenase; CSF: Cerebrospinal fluid; DHA: Docosahexaenoic acid; HETE: Hydroxyeicosatetraenoic acids; LLE: Liquid-liquid extraction; LOX: Lipoxygenase; NA: Not available; PGE2: Prostaglandin E2; SAH: Subarachnoid hemorrhage; SPE: Solid phase extraction.

Table 1. Summarizes representative examples of eicosanoids quantified in human serum, plasma and blood by LC–MS/MS or UPLC–MS/MS (cont.).							
Number of eicosanoids quantified	Matrix	Matrix volume	MS	Analysis run time	Sample pretreatment	Application	Ref.
4	Human serum	200 µl	LC–MS/MS	25 min	Automated SPE	To minimize human intervention, high precision and high throughput for routine epidemiological or clinical studies	[75]
9	Human serum and cell line	100 µl	LC–MS/MS	30 min	SPE	To quantify eicosanoid profile in human serum of obese persons	[76]
158	Human serum	200 µl	LC–MS/MS	30 min	SPE	To identify potential biomarkers of schizophrenia	[77]
36	Synovial fluid and whole blood	40 µl	LC–MS/MS	11 min	PPT	To analyze synovial fluid postmortem and whole blood samples in the presence of PUFA eicosapentaenoic acid	[78]
122	Human whole blood	200 µl	LC–MS/MS	6.5 min	SPE	To study whole blood samples for clinical and preclinical studies	[9]
29	Human whole blood	4.5 ml	LC–MS/MS	23 min	SPE	To evaluate lipid metabolite generation in physiological and pathophysiological conditions	[11]
20-HETE and 12-HETE	CSF	3 ml	LC–MS/MS	10 min	SPE	To establish 20-HETE and 12-HETE as biomarker SAH pathogenesis study	[79]
AA, DHA, 5- and 12-HETE)	CSF	1 ml	LC–MS/MS	38 min	SPE	To establish AA, DHA and 5- and 12-HETE as biomarkers of brain injury	[80]
2AG: 2-arachidonoyl glycerol; AEA: Arachidonoyl ethanolamide; CAD: Coronary artery disease; COX: Cyclooxygenase; CSF: Cerebrospinal fluid; DHA: Docosahexaenoic acid; HETE: Hydroxyeicosatetraenoic acids; LLE: Liquid–liquid extraction; LOX: Lipoxygenase; NA: Not available; PGE2: Prostaglandin E2; SAH: Subarachnoid hemorrhage; SPE: Solid phase extraction.							

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volunteers [30]. A total of 184 eicosanoids have been separated and quantitated in human plasma in a single 5 min UPLC run by Wang and co-workers [60].

A study was carried out to determine circulating omega-6, omega-3 polyunsaturated fatty acids and prostaglandin E2 (PGE2) levels in steady state sickle cell disease patients by UPLC–MS/MS and their levels confirms the proinflammatory state in sickle cell disease patients [61]. A rapid LC–MS/MS method was developed to profile PUFAs and 94 oxidized metabolites within 13 min analysis time. The method was successfully applied to quantify 20 eicosanoids in healthy human plasma samples [51]. In a clinical study, Issan *et al.* studied the effect of diabetes on serum levels of eicosanoids and reported the four- to fivefold increased level of 12-HETE and 12-HETE in plasma from diabetic patients with chronic myocardial ischemia [62]. A specific, sensitive, reproducible LC–MS/MS method has been developed for the analyses of lipid peroxidation products for the study of biologically relevant reactive oxygen species in human plasma and F2-isoprostanes in urine [21]. A UPLC–MS/MS method was developed and validated for simultaneous, accurate and precise measurement of 2-arachidonoyl glycerol (2AG) and arachidonoyl ethanolamide (AEA) in human plasma and urine [63]. 32 AA metabolites have been simultaneously analyzed in human plasma and the method was successfully applied to evaluate basal eicosanoid plasma levels and aspirin-derived changes in healthy volunteers. The plasma levels of TXB2 rapidly decreased (less than 10%), 2-h after aspirin administration and maintained up to 6 h [64]. 8-iso-prostaglandin F2 α (t8-iso-PGF2 α), total hydroxyoctadecadienoic acids (tHODEs), total hydroxyeicosatetraenoic acids (tHETEs) and total 7-hydroxycholesterol (7-OHCh) have been simultaneously measured in human plasma and liver sample. The developed LC–MS/MS method was applied to hepatitis C and hepatitis B virus-infected patients to determine liver dysfunction [20]. A rapid LC–MS assay was developed for routine pathophysiological sample analysis for the simultaneous determination of prostanoids, isoprostane and LOX-derived fatty acid metabolites in a small biological sample (20 μ l) [65]. A total number of 36 oxylipins including 14 eicosanoids have been quantified in human plasma samples and the developed LC–MS/MS method was successfully applied to determine increasing inflammatory oxylipins concentration after cardiac surgery. The hydroxyeicosatetraenoic acids, 12-HETE and 5-HETE metabolites were consistently increased after cardiac surgery [29]. Systemic levels of specific fatty acid oxidation products (hydroxyoctadecadienoic acids [HODEs], hydroxyeicosatetraenoic acids [HETEs] and F2-isoprostanes) were simultaneously measured by LC–MS/MS. 9-HETE and F2-isoprostanes concentrations were found significantly elevated in patients with angiographically defined coronary artery disease [66]. A total of 15 eicosanoids derived from AA have been quantified in human plasma via UPLC–MS/MS method with 8 min run time by Zhang *et al.* [67]. A study was conducted to detect circulating bioactive lipid metabolites alterations in response to biodiesel exhaust exposure using LC–MS/MS, with major emphasis on inflammation causing metabolites and effects on cardiovascular health. Among the 51 analyzed lipid metabolites, seven monohydroxy lipid metabolites displayed significant responsiveness after 24 h of biodiesel exhaust exposure [68]. Morgenstern *et al.* quantitated 15-deoxy- Δ 12,14-prostaglandin J2 (15d-PGJ2) in human plasma samples to understand the anti-inflammatory potential of 15-deoxy- Δ 12,14-prostaglandin J2 (15d-PGJ2) in severe long-term proinflammatory disorders like diabetes, cancer, or cardiovascular disease [69]. Ethyl esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been quantified in human plasma and the developed LC–MS/MS method has been implemented for pharmacokinetic study of omega-3 fatty acid ethyl esters drug formulations [70]. Miller *et al.* developed a sensitive UPLC–MS/MS method for 20 eicosanoids in human plasma and successfully applied the method to investigate the association between niacin administration and eicosanoid plasma level response [71]. A sensitive LC–MS/MS method was developed to simultaneously measure epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids (DHET) regioisomers to assess the endothelial function in human plasma [72]. Thakare *et al.* developed an LC–MS/MS method for simultaneous quantification of eicosanoids in human serum, BALF and sputum. The method was successfully applied for eicosanoid profiling in healthy human subjects [55]. Squellerio *et al.* simultaneously quantified both TXB2 (thromboxane) and 12(S)-HETE in human serum to detect the platelet enzymatic activity alterations. The developed assay may be applicable for the screening of novel antiplatelet drugs such as COX and LOX inhibitors [33]. In another study, Wolfer and group developed an UPLC–MS assay and quantified 48 oxylipins and PUFAs in human serum, plasma, urine, and cell culture samples. In future, oxylipin profiling may help in understanding the inflammation regulation in various medical conditions [73]. The influence of the stable isotopic labeled internal standard (SIL-IS) inclusion on the quantitative analysis of HETEs in human serum has been studied by Fernández-Peralbo *et al.* using LC–MS/MS [74]. A LC–MS/MS method was reported for targeted analysis of four epoxyeicosatrienoic acids regioisomers in human serum to minimize human intervention for routine analysis in epidemiological or clinical studies [75]. Nine eicosanoids have been simultaneously quantified via a developed LC–MS/MS approach and the

method was successfully applied for quantification of eicosanoids in obese persons and found low level of PGE₂ in accordance to previous reports [76]. Wang *et al.* monitored 158 eicosanoids along with their metabolites in human serum sample of schizophrenia (SCZ) patients. They observed a significant increase in anandamide (AEA) and oleylethanolamine (OEA) concentrations and selected them as biomarkers in SCZ patients. After antipsychotic treatment the serum concentrations of AEA and OEA were both reduced in SCZ patients [77]. Jónasdóttir and co-workers developed a LC–MS/MS platform for the analysis of 36 specialized proresolving lipid mediators. The method was successfully applied in the analysis of synovial fluid post-mortem samples and whole blood treated with ionophore in the presence of PUFA eicosapentaenoic acid [78]. A total number of 122 eicosanoids have been quantified in human whole blood samples by a developed UPLC/MS method in order to determine responses of clinical subjects to experimental drugs. The method requires a small sample volume (2.5 µl) with a short LC run time (6.5 min) [9]. Gomolka *et al.* have successfully identified 29 eicosanoids including n-3 and n-6 PUFA-derived lipid metabolites in human and murine blood samples [11]. Eicosanoids levels has also been detected in CSF to establish them as biomarkers for brain injury. A study demonstrated the presence of 20-HETE and 12-HETE in the CSF of subarachnoid hemorrhage patients [79]. In an another study AA, DHA, 5- and 12- eicosatetraenoic acid (HETE) has been quantified in CSF which may act as biomarkers of brain injury [80].

Human urine

Urine is a noninvasive matrix sampling that can be collected longitudinally and temporally multiple times without affecting the patient reflects the metabolic status of the whole organism. Eicosanoids are produced in various physiological and pathophysiological processes and further metabolized and excreted in urine [81]. Urinary eicosanoids, though easy to measure, may have little to do with circulating levels, except to the extent that conditions in the kidney may mirror conditions elsewhere in the body. Recent studies suggest that they might largely only reflect production of eicosanoids in the kidney [82,83]. Table 2 summarizes the available research on eicosanoid quantitation utilizing urine matrix via the LC–MS/MS approach. F₂-isoprostanes and PGs were simultaneous quantified in urine of patients undergoing cardiac surgery by LC–MS/MS method to understand the role of oxidative stress plays in the pathophysiology of many human diseases [84]. Zhang *et al.* reported the first sensitive method for simultaneous estimation of tetranor PGDM and PGEM (tPGDM and tPGEM), PGD₂ and PGE₂ metabolites in urine utilizing on-line SPE in healthy human volunteers (smokers and nonsmokers) and chronic obstructive pulmonary disease (COPD) patients. Higher levels of tPGDM and tPGEM were observed in COPD patients than those of nonsmoking healthy volunteers [27]. Neale *et al.* developed a LC–MS/MS method to analyze tetranor PGE-M (PGE₂ metabolite) and study aspirin effect on urinary tetranor PGE-M levels in healthy male volunteers [85]. Urinary levels of tetranor-PGEM in infants with viral induced fever have been measured by Idborg *et al.* and found that tetranor-PGEM level increased as compared with controls. They further concluded urinary tetranor-PGEM as a potential noninvasive inflammatory biomarker for infants [86]. Sterz *et al.* described a novel UPLC–MS/MS method for robust and sensitive simultaneous profiling of PG tetranor PGE-M, 8-iso-, and 2,3-dinor-8-iso-PGF₂; the thromboxanes (TXs) 11-dehydro- and 2,3-dinor-TXB₂; leukotriene E₄; and 12-HETE in human urine samples. They performed the calibration by adding stable isotope-labeled eicosanoid species and the overall run time was 14 min [22]. A LC–MS/MS method has been developed for simultaneous determination of the oxidative stress biomarkers: 8-iso-prostaglandin F(2α), malondialdehyde and 4-hydroxynonenal. The method was successfully applied on samples obtained from asbestosis, pleural hyalinoses or silicosis patients [87]. Urinary free acids, 12- and 20-HETE has been quantified in urine samples of benign prostatic hypertrophy and prostate cancer patients. Results suggested that 12-HETE and 20-HETE levels increased in patients to indicate prostate gland abnormality [88]. Urinary isoprenoids were determined in 24 healthy male and female volunteers and results indicated the isoprenoids (IsoP) levels ranged from 685 to 3480 ng 24 h(-1) and from 864 to 7511 ng 24 h(-1) in urine from women and men, respectively [89].

A novel method has been developed to quantify lipid mediator metabolites in human urine of asthma patients. Results showed increase in metabolites of prostaglandin D₂, cysteinyl leukotrienes and isoprostanes in asthmatic patient urine [90]. Noble *et al.* have quantified 8-iso-prostaglandin F(2α) (8-iso-PGF[2α]) in human urine by UPLC–MS/MS method to be further utilized in pharmacodynamics study in human [91].

Dreisbach *et al.* profiled HETEs and diHETEs levels in urine and plasma samples of 262 African–American patients from the University of Mississippi Chronic Kidney Disease Clinic. They reported that the urinary excretion of 20-HETE, and diHETEs, other HETEs were correlated with estimated glomerular filtration rate in the patients with chronic kidney disease [92]. Dong and co-workers quantified urinary PGE-M concentrations to assess risk

Table 2. Summarizes representative examples of eicosanoids quantified in human urine by LC-MS/MS or UPLC-MS/MS.

Number of eicosanoids quantified	Matrix	Matrix volume	MS	Analysis run time	Sample pretreatment	Application	Ref.
04	Human urine	200 µl	LC-MS/MS	20 min	SPE	To evaluate isoprostanes and prostaglandins as inflammatory biomarkers	[84]
02	Human urine	500 µl	LC-MS/MS	7 min	SPE	To provide disease stage insight	[27]
01	Human urine	1 ml	LC-MS/MS	10 min	SPE	To observe the effect of aspirin on urinary tetranor PGE-M levels	[85]
01	Human urine	1.5 ml	LC-MS/MS	30 min	SPE	To establish urinary tetranor-PGEM as a potential biomarker of inflammation in infants	[86]
07	Human urine	3 ml	UPLC-SRM/MS	14 min	LLE	To profile eicosanoid biomarkers in clinical/toxicological studies	[22]
03	EBC, plasma and Human urine	0.2 ml	LC-MS/MS	70 min	SPE	To study oxidative stress biomarkers in patients with asbestos or silica-induced lung diseases	[87]
02	Human urine	2 ml	LC-MS/MS	33 min	SPE	To detect prostate abnormality via detecting increased 12-HETE and 20-HETE levels	[88]
13	Human urine	400 µl	LC-MS/MS	13.5 min	SPE	To establish urinary IsoPs as oxidative stress biomarkers	[89]
13	Human urine	2 ml	LC-MS/MS	20 min	SPE	To provide insight into the inflammatory and oxidative stress status of the individual	[90]
01	Human urine	1 ml	LC-MS/MS	10 min	SPE	To support pharmacodynamic study in humans	[91]
09	Human urine	2 ml	LC-MS/MS	36 min	SPE	To establish correlations in eGFR and urinary 20-HETE and 19-HETE levels	[92]
01	Human urine	0.75 ml	LC-MS/MS	15 min	SPE	To establish urinary PGE-M levels as biomarker of gastric cancer among women	[93]
06	Human urine	0.6 ml	LC-MS/MS	32 min	SPE	To establish urinary F(2)-IsoP concentrations as a oxidative stress biomarker	[94]
07	Human urine	2 ml	LC-MS/MS	20 min	SPE	To investigate the pathophysiological role of lipid peroxidation associated diseases	[95]
03	Human urine	1 ml	UFLC-MS/MS	3.5 min	SPE	To study urinary prostanoids levels correlation between exercise and oxidative stress	[96]
01	Human urine	1 ml	LC-MS/MS	15 min	SPE	To study the correlation between PGE-M levels and PGE ₂ formation in pathophysiological processes	[97]
16	Human urine	2 ml	UPLC-MS/MS	20.5 min	SPE	To develop omega-6 and omega-3 PUFA metabolites as efficient biomarkers	[98]
11-dehydro-thromboxane B2	Human urine	1–2 ml	LC-MS/MS	13.5 min	LLE	To establish TXA2 as biomarker of CVS diseases	[14]
15	Human urine	0.5 ml	LC-MS/MS	75 min	LLE	To establish PGE-M level as biomarker of breast cancer risk independent of estrogen level	[15]
8-iso-prostaglandin F _{2α} (8-iso-PGF _{2α})	Human urine	1 ml	LC-MS/MS	15 min	SPE	To establish urinary 8-iso-PGF _{2α} as oxidative stress biomarker mainly in children	[17]
PGE-M	Human urine	0.75 ml	LC-MS/MS	15 min	SPE	To establish urinary PGE-M as a cancer biomarker to predict pancreatic cancer risk	[99]
PGE-M	Human urine	0.75 ml	LC-MS/MS	15 min	SPE	To profile urinary PGE-M, together with helicobacter pylori as promising model to assess risk of gastric cancer	[100]
04	Human urine	1 ml	LC-MS/MS	26 min	SPE	To profile 8-oxo-dG, R-cdA, S-cdA and 8-iso-PGF _{2α} as early disease markers (diabetes)	[101]

CVS: Cardiovascular system; EBC: Exhaled breath condensate; HETE: Hydroxyeicosatetraenoic acids; IsoP: Isoprenoids; LLE: liquid-liquid extraction; SPE: Solid phase extraction; UFLC: Ultra flow liquid chromatography.

of development of gastric cancer in the Shanghai Women's Health Study. They reported higher levels of urinary PGE-M as marker of inflammation associated with risk of gastric cancer [93]. A novel LC/MS–MS method has been developed to quantify oxidative stress biomarkers such as F2-Isoprostanes (F2-IsoPs), regio- and stereoisomers of prostaglandin F2 α (PGF2 α) and urinary F2-IsoP metabolites including 2,3-dinor-5,6-dihydro-8-iso-PGF2 α (2,3-dinor-8-iso-PGF1 α [2,3-dinor-F1]) and 2,3 dinor-8-iso-PGF2 α (2,3-dinor-F2). The method was clinically applied in smokers and nonsmoker population resulting into higher F2-IsoPs level in smokers [94]. Yan and co-workers quantified isoprostane isomers in human urine from smokers and nonsmokers for investigation of the pathophysiological role of lipid peroxidation in associated human diseases [95]. A high-throughput UPLC–MS/MS method has been developed by Blatnik *et al.* for the analysis of urinary prostanoid biomarkers of proinflammatory responses, tetranor PGE_m, 6-keto PGF(1 α) and 2,3-dinor-6-keto PFG(1 α) to study the association of exercise and oxidative stress. Tetranor PGE_m levels increased from 1.5- to 6-fold postexercise and 6-keto PGF1 α levels increased from 2- to 55-fold postexercise and the prostanoid 2,3-dinor-6-keto PGF1 α remained unchanged postexercise [96]. In a study by Murphey *et al.*, levels of PGE-M (PGE2 metabolites) have been quantified in human urine with developed LC/MS–MS method and were found increased in non-small-cell lung cancer, and this increase is reduced by administration of the COX-2 inhibitor celecoxib [97]. Sasaki and co-workers determined omega-6 and omega-3 PUFA metabolites in human urine samples using UPLC/MS/MS [98]. Urinary 11-dehydro-thromboxane (TX)B2 has been described as a potential predictive biomarker in patients with acute myocardial infarction [14]. In another studies, urinary 11 α -hydroxy-9,15-dioxo-2,3,4,5-tetranorprostaglandin-1,20-dioic acid (PGE-M) level has been identified as biomarker for breast, pancreatic, gastric cancer [15,99,100] and urinary 8-iso-PGF2 α level for the assessment of oxidative stress mainly in children and potential oxidative stress biomarkers in the patients with prediabetes [17,101].

Sputum & bronchial alveolar lavage fluid

Sputum analysis for cellular and soluble mediators is rapidly increasing as a noninvasive method to assess airway inflammation [12,102]. Table 3 sights representative examples of eicosanoids quantified in human sputum and BALF. Leukotriene B4 (LTB4) has been accepted as a key target for the treatment of asthma. Jian *et al.* developed a highly selective and sensitive UPLC–MS/MS assay for quantitation of LTB4 in human sputum as a biomarker for LTB4 biosynthesis inhibition to indicate the effectiveness of the drug dithiothreitol (DTT) [50]. LTB4 was also quantified in BALF by Montuschi *et al.* [49]. A total number of 34 eicosanoids have been simultaneously identified in human sputum and BALF to define them a biomarker for various pathophysiological conditions [23]. Chappell *et al.* developed a specific, sensitive and accurate UPLC/MS–MS assay method for the determination of cysteinyl leukotrienes and LTB4 in human sputum samples for pathway study of these leukotrienes [103]. In a study correlation between oxylipin profiles and lung function has been determined via detecting proinflammatory and anti-inflammatory lipid mediators in freshly obtained sputum from cystic fibrosis patients [104]. Exhaled breath condensate (EBC) of healthy subjects has been quantitated for eicosanoid concentration using LC/MS–MS. The developed method was applied for eicosanoid quantitation in smokers and nonsmokers, with smokers found to have higher concentration of 5-HETE and 8-iso-PGF2 α [105]. 39 oxylipins has been simultaneously detected in serum and BALF samples to enhance the understanding of a disease process and to treat and prevent inflammatory diseases [32]. A novel LC/MS–MS method has been developed to determine the EPA to AA concentration ratio. The salivary EPA to AA EPA/AA ratio was determined to act as noninvasive alternative to serum or plasma quantitation and to be useful as an indicator to identify the risk of cardiovascular disease [13].

Rat/mouse plasma & tissues

A variety of work has been carried out related to method development and eicosanoid quantitation in rat/mouse plasma and tissues. Table 4 summarizes those representative examples. Zhang *et al.* developed an LC–MS/MS-based method to simultaneous quantify 32 arachidonic acid (AA) metabolites and 37 omega-3 PUFA-derived products in mouse plasma and mouse aorta samples [58]. A large number of fatty acid-related metabolites (137) with different ionization polarity have been quantified in mouse tissues via a LC–MS/MS method with high speed continuous ionization polarity switching approach [106]. Wong *et al.* profiled bioactive oxylipins including both pro-inflammatory and anti-inflammatory to provide a useful analytical tool for biological investigation. They carried out the tissue distribution study in rat to analyze role of different oxylipins in biological processes. They further analyzed tissue samples from rat model of osteoarthritis (OA) pain and showed that concentrations of 12-HETE were significantly increased in the OA rat knee joint compared with control [107]. Shaik *et al.* simultaneously

Table 3. Summarizes representative examples of eicosanoids quantified in human sputum and bronchial alveolar lavage fluid by LC-MS/MS or UPLC-MS/MS.

Number of eicosanoids quantified	Matrix	Matrix volume	MS	Analysis run time	Sample pretreatment	Application	Ref.
2	Saliva	1 ml	LC-MS/MS	10 min	SPE	To identify the risk of cardiovascular disease, especially atherosclerosis	[13]
34	Sputum and BALF	500 µl	LC-MS/MS	25 min	SPE	To assess eicosanoid biomarkers in human clinical trials	[23]
1	EBC (BALF)	1 ml	LC-MS/MS	15 min	Direct concentrate via evaporation under nitrogen	To measure LTB4 as a noninvasive assessment of airway inflammation	[49]
01	Human Sputum	100 µl	LC-MS/MS	4.5 min	Automated LLE	To quantitate LTB4 in human sputum as a biomarker	[50]
04	Human Sputum	500 µl	LC-MS/MS	10 min	SPE	To assess pharmacodynamics changes in sputum as biomarker of airway inflammation	[103]
22	Human Sputum	1.5 g	LC-MS/MS	22 min	SPE and LLE	To detect proinflammatory and anti-inflammatory lipid mediators for clinical progression of lung disease	[104]
20	EBC	1 ml	LC-MS/MS	12.5 min	LLE	To investigate clinical progression of inflammatory lung diseases	[105]
39	Serum, BALF	250 µl	LC-MS/MS	21 min	SPE	To evaluate complex regulatory oxylipin responses in different studies	[32]

BALF: Bronchial alveolar lavage fluid; EBC: Exhaled breath condensate; LLE: Liquid-liquid extraction; LTB4: Leukotriene B4; SPE: Solid phase extraction.

Table 4. Summarizes representative examples of eicosanoids quantified in rat/mouse plasma and tissues by LC-MS/MS or UPLC-MS/MS.						
Number of eicosanoids quantified	Matrix	Matrix volume	MS	Analysis run time	Sample pretreatment	Application
28	Rat brain, liver	500 mg	LC-MS/MS	30 min	SPE	To understand the role of lipid mediators in health and disease
23	Rat kidney tissue	100 mg	LC-MS/MS	60 min	SPE	To provide further insight into the inflammatory mechanisms
32	Human plasma and mouse aorta	NA	LC-MS/MS	8 min	SPE	To be used for comprehensive eicosanoid study
137	Mouse tissues	Whole organ tissues	LC-MS/MS	20.1 min	SPE	To establish a standard and sensitive approach for mediator lipidomics analysis
40	Rat organ tissues	Whole isolated tissue	LC-MS/MS	30 min	SPE	To understand the disease progress
11	Rat brain	Whole brain	LC-MS-MS	12 min	SPE	To understand the role of prostanooids in various neuroinflammatory and cerebrovascular disorders
24	Mouse colonic tissue	Whole colon	LC-MS/MS	8.5 min	SPE	To investigate PUFA metabolite profile during inflammation and resolution
2	Rat spinal cord microdialysates	75 µl	LC-MS/MS	6 min	LLE	To elucidate role of PGE2 and PGD2 in inflammation
10	Colon samples from CB56/bl6 mice	50 µl	UPLC-MS/MS	19 min	SPE	To establish role of eicosanoids in cell signaling and colon inflammation
25	Serum and hepatic tissue	Whole liver tissue	LC-MS/MS	5.0 min	LLE	The findings strengthen the current knowledge about eicosanoids involvement in cancer progression
48	C57BL/6 mouse serum and tissue samples	150 µl serum and 5 mg tissue	UPLC-MS/MS	8 min	SPE	To determine the disease-linked association between inflammation and oxidative stress in disease
54	ArFachidonate and oxidation products	Mouse and human brain tissue	LC-MS/MS	34.5 min	LLE	To profile oxidation products of arachidonate for AD pathophysiology study
19	Rat brain tissue	20–40 mg	LC-MS/MS	35 min	SPE	To elucidate the mechanism of eicosanoid metabolites in TBI as well as other disease
PGE2 and PGD2	Mice brain tissue	30 mg	UPLC-MS/MS	4.5 min	SPE	This method increases the recovery of PGs
18	Rat plasma	200 µl	UPLC-MS/MS	12 min	Protein precipitation	To establish eicosanoid biomarkers as diagnostics of rheumatoid arthritis
18	Rat brain	1.5–2 gm	UPLC-MS/MS	27 min	SPE	DHA metabolite profiling can be further used as a reference for further neuropathological disorder research
AD: Alzheimer's disease; DHA: Docosahexaenoic acid; LLE: Liquid-liquid extraction; NA: Not available; SPE: Solid phase extraction.						

Table 5. Summarizes representative examples of eicosanoids quantified in Cell line or cell culture media by LC-MS/MS or UPLC-MS/MS.						
Number of eicosanoids quantified	Matrix	MS	Analysis time	Sample pretreatment	Application	Ref.
07	Rat and human hepatocytes	LC-MS/MS	13.5 min	Online SPE	To establish isoprostanes as biomarker of oxidative stress conditions	[26]
14	RAW264.7 cells	LC-MS/MS	6 min	SPE	To quantitate other eicosanoids and their metabolites	[119]
05	Murine mast cells	LC-MS/MS	16 min	SPE	To understand the multiple roles of prostanoids in physiological and pathophysiological processes	[120]
04	Atlantic salmon head kidney cells	LC-MS/MS	20 min	SPE	To understand the signaling mechanism of eicosanoids, their roles in inflammatory pathologies in mammals and fish	[121]
26	Colon cancer cell lines	LC-MS/MS	6.5 min	Online SPE	To study the role of hydroxylated polyunsaturated fatty acids in inflammation	[122]
05	Epithelial cell line	LC-MS/MS	37 min	SPE	To establish 11-oxoeicosatetraenoic acid as endogenous antiproliferative eicosanoid	[123]
14	Caco-2 cells	LC-MS/MS	20 min	SPE	To support the role of arachidonic acid metabolites in intestinal epithelial cell proliferation and colorectal tumorigenesis	[23]
04	Prostate cancer cell lines (PC-3)	LC-MS/MS	20 min	SPE	To determine the production of prostaglandin from human prostate cancer cells with different degree of invasiveness	[34]
06	Cell culture media bone marrow-derived macrophage	UPLC-MS/MS	0.5 min	LLE	To simultaneously quantitate eicosanoids and their metabolites at a faster rate	[19]
60	DMEM cell culture media	LC-MS/MS	16 min	Off line SPE	To simultaneously quantitate a large number of eicosanoids and determine their role in physiological and pathophysiological conditions	[57]
02	A549 and RAW 264.7 cell culture	LC-MS/MS	10 min	SPE	To understand the role of PGD2 and PGE2 IN pathogenesis of COPD, bronchiectasis, asthma and lung cancer	[124]
14	Murine 3T6 fibroblasts cell culture	LC-MS/MS	20 min	SPE	To study eicosanoids role in physiological and pathophysiological conditions	[125]
COPD: Chronic obstructive pulmonary disease; LLE: Liquid-liquid extraction; PGE2: Prostaglandin E2;SPE: Solid phase extraction.						

measured 11 prostanoids including PGs and cyclopentenone metabolites in the rat brain cortical tissue via UPLC–MS/MS method [108]. One method developed by Faouder and co-workers in mouse colonic tissue can be used to quantify pro-inflammatory and pro-resolving PUFA metabolites mediators in different cultured cells, in fluids and in colonic tissues [109]. Blewett *et al.* simultaneously quantified 23 eicosanoids and Masoodi *et al.* quantified 28 eicosanoids via a developed LC–MS/MS method [16,18]. Schimdt *et al.* analyzed prostaglandin E2 and D2 in microdialysis samples of rats and the method has further applicability to elucidate role of PGE2 and PGD2 in inflammation [110]. 18 eicosanoids have been simultaneous profiled in rat plasma and brain to identify them as a potential biomarkers for rheumatoid arthritis [111,112]. A total number of 25 eicosanoids quantified in hepatic tissue of mouse to establish their role in cancer progression [31]. Furman and group quantified 54 Arachidonate and oxidation products to study their role in Alzheimer's disease (AD) progression [113].

Cell line or cell culture media

Cell line or cell culture media is extensively and progressively used as a substitute for animal experimentation to facilitate the study of the mechanisms involved in various physiological and pathophysiological processes [118]. Table 5 consists of research work carried out for sensitive and robust LC–MS/MS method development and validation for simultaneous quantitation of a variety of eicosanoids in different cell lines, rat and human hepatocytes [26], RAW264.7 cells [119], murine mast cells [120], Atlantic salmon head kidney cells cell line [121], colon cancer cell lines HCA-7 and SW-480 [122], Human colorectal adenocarcinoma (LoVo) epithelial cell line [123], caco-2 cells [23] and Bovine coronary artery endothelial cells (ECs) and human prostate cancer cells (PC-3) [34] or cell culture media viz. bone marrow-derived macrophage [19], Dulbecco's Modification of Eagle's Medium [57], A549 cell culture [124] and Murine 3T6 fibroblasts (ATCC CCL96) cell culture [125].

Conclusion & future perspective

Eicosanoids comprise of a large family of potent bioactive lipid mediators with numerous physiological and pathophysiological roles. For that reason, eicosanoids are actively quantified to establish their roles in various diseases and applications. The demand of analytical techniques for accurate quantification of eicosanoids and their diverse metabolites at lower level (picogram) is intensifying. The development of UPLC and LC–MS/MS methods has renovated the approach to analyze eicosanoids, as they do not require derivatization and reduce the time and cost. In this article, we reviewed the LC–MS/MS and UPLC–MS/MS methodology to identify and quantify a wide range of eicosanoids in different available biomatrices such as human plasma, serum, urine, sputum and brain tissue using different calibration approaches. Our assessment of reported studies in the area of eicosanoid quantitation indicates that LC–MS/MS is a versatile and reliable analytical tool for the sensitive, rapid and simultaneous analysis of multiple eicosanoids and their metabolites in order to study the physiology and pathophysiology of eicosanoids in different biomatrices under various conditions. Finally, UPLC further increased the resolution, speed and sensitivity for eicosanoid analysis. Regardless of these significant advances and efforts, major challenges associated with the dynamic range of measurements are quantitation accuracy, analysis throughput and the robustness of present instrumentation must be addressed for efficient clinical applications. Future instrument advancement will permit simpler higher throughput methods to improve selectivity, sensitivity and lower down the detection limits.

Executive summary

Background

- Important aspects during sample preparation, calibration curve generation and bioanalysis required for eicosanoid quantitation in different biological matrices are discussed.

Methods & results

- Research articles on eicosanoids analysis in biomatrices from the past 20 years are reviewed and identifies the application of eicosanoid quantitation in various physiological and pathophysiological conditions.
- The accurate quantitation requires continuous improvements in analytical methodologies (including sample preparation techniques, methodology, calibration, sample volume, run time, sample pretreatment and instrumentation).

Conclusion

- Eicosanoid quantitation with UPLC and LC–MS/MS provides a versatile and reliable analytical tool for the sensitive, rapid and simultaneous analysis of multiple eicosanoids and their metabolites in different biomatrices.

Financial & competing interests disclosure

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