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Derivatization enhanced separation and sensitivity of long chain-free fatty acids: Application to asthma using targeted and non-targeted liquid chromatography-mass spectrometry approach

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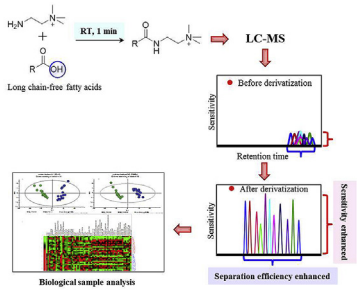
HIGHLIGHTS

- Cholamine-derivatization enhanced ionization and separation efficiency of LCFFAs.
- Sensitivity improved up to 2000-fold after derivatization.
- Developed a high efficient LC-MS method for derivatized LCFFAs.
- 35 LCFFAs were tentatively identified in asthma sera.
- This strategy was employed for identification of potential biomarkers in asthma.

GRAPHICAL ABSTRACT



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ABSTRACT

Long chain-free fatty acids (LCFFAs) play pivotal roles in various physiological functions, like inflammation, insulin resistance, hypertension, immune cell behavior and other biological activities. However, the detection is obstructed by the low contents, structural diversity, high structural similarity, and matrix interference. Herein, a fast cholamine-derivatization, within 1 min at room temperature, coupled with liquid chromatography-mass spectrometry (LC-MS) approach was developed to determine LCFFAs in complex samples. After derivatization, the ionization and separation efficiency were significantly improved, which resulted in up to 2000-fold increase of sensitivity compared with non-derivatization method, and the limits of detection were at low femtogram level. As well, this approach was applied successfully in the rapid profiling or quantification of targeted and non-targeted LCFFAs in the sera of healthy human and asthma patients. The targeted metabolomics method showed that the contents of 17 PUFAs were significantly changed in asthma patients, especially hydroxyeicosatetraenoic acids (HETEs), hydroperoxyeicosatetraenoic acid (HPETEs) and prostaglandins (PGs). The non-targeted method resulted in the tentatively identification of 35 LCFFAs including 31 saturated and mono-unsaturated LCFFAs, and 4 bile acids, except for 27 poly-unsaturated fatty acids (PUFAs), and the multivariate analysis indicated that eicosapentaenoic acid (EPA), ursodeoxycholic acid, deoxycholic acid, isodeoxycholic acid, palmitic acid, 2-lauroleic acid and lauric acid also have significant difference between healthy and asthma groups except for 17 PUFAs. To the best of our knowledge, this is the first report on the relationship of asthma

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with 5(S)-, 15(S)-HPETE, 8(S)-, 11(S)-HETE, 15(S)-HEPE, PGA2, PGB2, PGE1, PGF1α, PGJ2, and 13, 14-dehydro-15-keto PGF2α (DK-PGF2α).

Abbreviations

AA	Arachidonic acid
Cholamine	(2-aminoethyl)trimethylammonium chloride hydrochloride
COX	Cyclooxygenase
DK	13,14-dihydro-15-keto
EPOX	Epoxygenase
EPA	Eicosapentaenoic acid
LCFFAs	Long chain-free fatty acids
HETE	Hydroxyeicosatetraenoic acid
HEPE	Hydroxyeicosapentaenoic acid
HETrE	Hydroxyeicosatrienoic acid
HHTrE	Hydroxyheptadecatrienoic acid
HOTrE	Hydroxyoctadecatrienoic acid
HPODE	Hydroperoxyoctadecadienoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
HOBt	1-hydroxybenzotriazole hydrate
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
LOX	Lipoxygenase
PUFAs	Polyunsaturated fatty acids
PGs	Prostaglandins
TXB2	Thromboxane B2

1. Introduction

Long chain-free fatty acids (LCFFAs), which contain at least a carboxyl group and an aliphatic chain, represent a wide range family of lipids [1]. LCFFAs can be divided into saturated and unsaturated fatty acids, and the latter can further be divided into mono- and poly-unsaturated fatty acids (PUFAs) based on the numbers of carbon-carbon double bonds. ω-3 and ω-6 are two types of most investigated PUFAs. Prostaglandins (PGs), thromboxanes (TXs), hydroxyeicosatetraenoic acids (HETEs) and epoxyeicosatrienoic acids (EETs) belong to ω-6 PUFAs and derived from arachidonic acid (AA) through three main enzyme systems, cyclooxygenase (COX), lipoxygenase (LOX) and epoxygenase (EPOX) (Fig. 1) [2]. As well, hydroxyeicosapentaenoic acids (HEPEs) belong to ω-3 PUFAs [3]. LCFFAs play pivotal roles in many physiological functions, such as inflammation, immune cell behavior, membrane generation, apoptosis, tissue repair, and other biological activities [4–8]. Thus, the change of LCFFAs might be potential biomarkers of some diseases. For example, PGs were reported to be the main mediators of inflammation in asthma, a common respiratory disease associated with chronic airway inflammation [9,10]. HETEs are also indicated as important biological mediators of allergic airways inflammation [11,12]. Therefore, sensitive measurement of LCFFAs in biological samples is very important for the physiological and clinical implications.

Mass spectrometry (MS) serves as a powerful technique in characterizing LCFFAs. Gas chromatography-MS (GC-MS) and liquid chromatography-MS (LC-MS) methods have been widely used for LCFFAs analysis [13–17]. However, the detection is obstructed by the structure diversity, hydrophobicity, high structural similarity, trace amounts, and complicated matrix. To solve these problems, several derivatization approaches have been described, e.g. N-(4-aminomethylphenyl)pyridinium (AMPP) derivatization [18] and trimethylaminoethyl ester (TMAE) derivatization [19]. Although the sensitivity increased, long reaction time, high reaction temperature, or poor chromatography separation efficiency restricted their application for high-throughput analysis or complex biological samples. Hence, it is urgent to develop a simple, quick and high efficient approach for the determination of LCFFAs.

It was reported that using (2-aminoethyl)trimethylammonium chloride hydrochloride (cholamine) as the derivatization reagent, 10 LCFFAs in eggs were successfully quantified [20], however the reaction time was too long and large amounts of reagents were consumed. In this research, the derivatization reaction was first optimized and applied to 34 LCFFA standards within 1 min at room temperature. Then, an ultra-high performance liquid chromatography-quadruple-time of flight/MS (UHPLC-Q-TOF/MS) approach was developed to quantify targeted LCFFAs in biological samples. In addition, based on the MS/MS characteristics of cholamine derivatives, non-targeted LCFFAs were also identified. Finally, using the developed method, the potential biomarkers in the sera of asthma patients were determined.

2. Experimental section

2.1. Chemicals and reagents

All fatty acid standards mixtures were purchased from Cayman Chemical (Ann Arbor, MI). They are (S)-hydroxyeicosatetraenoic acid (HETE) HPLC mixture containing 5(S)-, 8(S)-, 11(S)-, 12(S)-, and 15(S)-HETE; hydroperoxy HPLC mixture containing 5(S)-, 12(S)-, and 15(S)-hydroperoxyeicosatetraenoic acid (HPETE), 9(S)- and 13(S)-hydroperoxyoctadecadienoic acid (HPODE); ω-3 hydroxy acid HPLC mixture containing 5(S)-, 12(S)-, and 15(S)-hydroxyeicosapentaenoic acid (HEPE), 13(S)-hydroxyoctadecatrienoic acid (HOTrE), and 15(S)-Hydroxyeicosatrienoic acid (HETrE); cyclopentenone prostaglandin (PG) HPLC mixture containing PGA2, PGB2, PGD2, PGE2, PGJ2 and 15-deoxy-$\Delta^{12,14}$-PGJ2; prostaglandin HPLC mixture containing PGE1, PGE2, PGF1α, 6-keto PGF1α and PGF2α; vasoactive eicosanoid HPLC mixture containing thromboxane B2 (TXB2), 11-dehydro TXB2, 6-keto PGF1α, 2,3-dinor-6-keto PGF1α and 12(S)-hydroxyheptadecatrienoic acid (HHTrE); prostaglandin metabolite HPLC mixture containing 13,14-dihydro-15-keto PGD2 (DK-PGD2), 13,14-dihydro-15-keto PGE2 (DK-PGE2), 11β-PGF2α, 13,14-dihydro-15-keto PGF2α (DK-PGF2α) and PGF2α. The isotope labeled eicosanoids, 12(S)-HETE-d_8, PGD2-d_4, PGE2-d_9, TXB2-d_4 and 6-keto PGF1α-d_4, were used as internal standards (ISs).

1-Hydroxybenzotriazole hydrate (HOBt), 1-[bis(dimethylamino) methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) and triethylamine (TEA) were purchased from Sigma-Aldrich Laboratories, Inc. (St. Louis, MO). Cholamine was obtained from Santa Cruz (Indian Gulch, CA). Acetonitrile (MS grade), dimethyl sulfoxide (DMSO) (MS grade) and ethyl acetate (ACS grade) were provided by J.T. Baker (Danville, PA). Deionized