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Direct infusion electrospray ionization–ion mobility–mass spectrometry for comparative profiling of fatty acids based on stable isotope labeling

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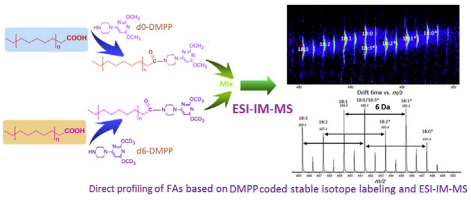
HIGHLIGHTS

- A novel method based on IM–MS and SIL was developed for FAs comparative profiling.
- Without LC separation, the method allowed direct infusion profiling of FAs in complex samples.
- Both of the efficiency and accuracy for FAs analyses were favorably enhanced by IM–MS and SIL.
- A significant increased content level of FFAs was confirmed in thyroid cancerous tissues.

GRAPHICAL ABSTRACT



Direct profiling of FAs based on DMPP coded stable isotope labeling and ESI-IM-MS

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ABSTRACT

A rapid method for fatty acids (FAs) comparative profiling based on carboxyl-specific stable isotope labeling (SIL) and direct infusion electrospray ionization–ion mobility–mass spectrometry (ESI–IM–MS) is established. The design of the method takes advantage of the three-dimensional characteristics of IM–MS including drift time, *m*/*z* and ion intensity, for comparison of *d*0-/*d*6-*2*,*4*-dimethoxy-6-piperazin-1-yl pyrimidine (DMPP)-labeled FAs. In particular, without chromatographic separation, the method allowed direct FAs profiling in complex samples due to the advantageous priority of DMPP in signal enhancement as well as the extra resolution that IM–MS offered. Additionally, the d0-/d6-DMPP-labeled FAs showed expected features, including very similar drift times, 6 Da mass deviations, specific reporter ions, similar MS responses, and adherence to the drift time rule regarding the influence of carbon chain length and unsaturation on relative drift times. Therefore, the introduction of isotope analogs minimized the matrix effect and variations in quantification and ensured accurate identification of non-targeted FAs by those typical features. Peak intensity ratios between d0-/d6-DMPP-labeled ions were subsequently used in relative quantification for the detected FAs. The established strategy has been applied

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successfully in the rapid profiling of trace free FAs between normal and cancerous human thyroid tissues. Sixteen free FAs were found with the increased level with a statistically significant difference ($p < 0.05$) compared to the normal tissue samples. The integrated SIL technique and ESI–IM–MS are expected to serve as an alternative tool for high-throughput analysis of FAs in complex samples.

1. Introduction

As an important component of lipids, fatty acids (FAs) are important for functions including membrane generation, protein modification and bioenergetic requirements [1,2]. Additionally, many cancer cells exhibit up-regulation of the synthase in de novo fatty acid synthesis [3,4]. Therefore, tracking the changes of FA levels in vivo provides a great opportunity to gain insights into the biological events relating to disease as well as the development of new drugs. The establishment of a rapid and reliable strategy for FA profiling offers a great research platform for monitoring disease progress.

Ion mobility–mass spectrometry (IM–MS) serves as a powerful tool for characterizing numerous biological compounds by providing three-dimensional (3D) data, including drift time, mass-to-charge ratio m/z and ion intensity [5–11]. The procedure separates ions on the basis of size-to-charge ratios and interactions with the bath gas under a certain drift voltage, therefore playing a role similar to chromatographic separation [12–15]. Due to the extra resolution, IM–MS allows more sensitive and more accurate detection of analytes compared to ordinary MS.

Recently, IM–MS was used in addition to GC–MS and high-resolution electrospray ionization–ion-mobility–mass spectrometry (ESI–MS) [16]. The samples were introduced into the IM–MS by direct infusion and the technique was used mainly to look for multiple structures and/or contaminants in the fatty acid samples from transgenic and non-transgenic plants that were being compared. In addition to accurate mass measurement, the study gave a comparison of the drift time of ions to achieve greater accuracy in FA identification. Besides, relative quantification was used by counting the peak intensity of target analytes among samples.

As a rapid and reliable strategy for profiling FAs, direct sample infusion coupled with IM–MS analysis is an attractive approach. One potential drawback is that detection sensitivity might be compromised under direct sample infusion conditions without chromatographic separation due to ion suppression effects. Besides, without appropriate internal standards, the accuracy of quantification relied on the intensity of ions, which is easily affected by many factors, including matrix effect and instrument status. The problem is more serious when encountering FAs of low abundance in complex samples.

To address the challenges described above, the introduction of stable isotope labeling (SIL) by derivatization with specific labeling reagents might solve this problem [17–19]. For the commercially available targeted FAs, their isotope analogs were obtained simply by attaching stable isotopic tags onto the parent FAs [20–23]. The SIL strategy has been shown to be a powerful tool for the analysis of FA samples that circumvent the problems of matrix effects and variations in sample detection. Additionally, the detection sensitivity of analytes was favorably enhanced by introducing a chemical group with high MS response. Therefore, the SIL technique coupled with direct sample infusion IM–MS might provide great potential in FAs analysis with high levels of efficiency and accuracy. Due to the nature of SIL, the strategy was often used in relative quantification. To avoid error in absolute quantification, one major solution was to add the isotope-labeled analytes to the test sample before the treatment procedure.

Earlier, we reported 2,4-dimethoxy-6-piperazin-1-yl pyrimidine (DMPP)-based carboxyl-specific stable isotope labeling technique for the liquid chromatography tandem mass spectroscopy (LC-MS/MS) analysis of diversified FAs with high levels of sensitivity and accuracy [24]. Here, combining the strengths of the DMPP-based SIL technique, a rapid FAs comparative profiling strategy was established using direct infusion ESI–IM–MS. The design of the method has been built on the IM–MS characteristics of interested isotope ions including drift time, m/z and peak intensity. It allowed the non-targeted FAs to be recognized easily by comparison of a series of typical features between light and heavy forms of labeled ions. Peak intensity ratios between d0-and d6-DMPP-FA ions were used for relative quantification of FAs in mixed samples. The entire workflow was shown to be an efficient, rapid and reliable method for the metabolic analysis of FAs in complex tissue samples.

2. Experimental

2.1. Chemicals and reagents

HPLC grade methanol, acetonitrile (ACN) ethanol and chloroform were obtained from Merck (Darmstadt, Germany). Fatty acid standards (purity >98%) were purchased from Shanghai Aladdin Reagent Co., Ltd (Shanghai, China). Deionized water was produced by a Direct-Q water purification system (Millipore, El Paso, TX, USA). DMPP was synthesized in our laboratory with purity >99.5% and 732-strong-acid cation exchange resin (gel type) was obtained from Shanghai Resin Factory Co., Ltd (Shanghai, China). All other chemicals were of analytical grade and used without further purification. A procedure for the extraction of free fatty acids from thyroid tissue and related sample information are shown in the Supplementary materials 1.1 and 1.2.

2.2. Labeling procedure

Solutions of d0-/d6-DMPP (50 nmol μL^{-1}) in ACN (30 μL) and 1-hydroxy-7-azabenzotriazole (HOAt; 15 nmol μL^{-1}) in ACN (10 μL) and 200 μL methanol were added sequentially to the dried samples. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC; 15 nmol μL^{-1}) in ACN (20 μL) was then added to the solution to initiate the reaction. Immediately after mixing for 15 s at room temperature the solvent was removed by evaporation with nitrogen gas to terminate the derivatization. The residue was dissolved in 50% (v/v) ACN (50 μL) in water containing 0.1% (v/v) trifluoroacetic acid. After that, the d0-DMPP labeled and d6-DMPP labeled samples were mixed further. An equal volume of resin (100 μL) was added to the solution, with slight agitation for 10 s, to adsorb specifically any excess labeling reagent as well as other chemical interference. The standard pretreatment of the resin is described in the Supplementary material 1.3. The resulting resin was washed with ethanol (300 μL), together with ultrasound (100 W, 40 kHz) for 5 min at room temperature. The washing solution was assayed by IM–MS analysis directly.