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Workflow development for targeted lipidomic quantification using parallel reaction monitoring on a quadrupole-time of flight mass spectrometry

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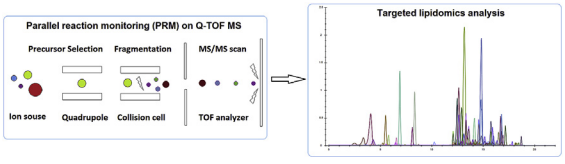
HIGHLIGHTS

- A PRM-based targeted lipidomics workflow was proposed on Q-TOF MS for the quantification of 222 lipids.
- This PRM-based quantification strategy provide high quality MS/MS spectra for both identification and quantification.
- A software solution was proposed to use multiple product ions for quantification, improving the reliability.
- The developed workflow was applied to discovery perturbed lipids in serum between different disease stages of SLE.

GRAPHICAL ABSTRACT



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ABSTRACT

Advances in high-resolution mass spectrometers with faster scanning capabilities and higher sensitivities have expanded these instruments' functionality beyond traditional data-dependent acquisition in targeted metabolomics. Apart from the traditional multiple reaction monitoring strategy, the parallel reaction monitoring (PRM) strategy is also used for targeted metabolomics quantification. The high resolution and mass accuracy of full-scan (MS1) and tandem mass spectrometry (MS/MS) scan result in sufficient selectivity by monitoring all MS/MS fragment ions for each target precursor and simultaneously providing flexibility in assay method construction and post-acquisition data analysis. In this study, using an orthogonal quadrupole-time of flight liquid chromatography-mass spectrometry system (QTOF LC-MS), we investigated the applicability of a large-scale targeted lipidomic assay using scheduled PRM. This method monitored 222 lipids belonging to 15 lipid species in serum. Robustness, reproducibility, and quantitative performance were assessed using chemical standards and serum samples. Finally, we demonstrated the application of this PRM-based targeted metabolomic workflow to systemic

Abbreviations: LC, Liquid chromatograph; MS, Mass Spectrometry; Q-TOF, Quadrupole-time of flight; PRM, Parallel reaction monitoring; MRM, Multiple reaction monitoring; DDA, Data-dependent acquisition; XIC, Extracted ion chromatogram; QC, Quality control; SLE, Systemic Lupus Erythematosus.

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lupus erythematosus, a severe autoimmunological disease. Results showed that 63 lipids belonging to 11 lipid species were significantly changed. In summary, at the first time, a robust targeted lipidomic workflow was established using PRM acquisition strategy on a Q-TOF platform, providing another powerful tool for targeted metabolomic analysis.

1. Introduction

Metabolomics aims at systematically studying the metabolites (less than 1000 Da) in biological samples, and the study has been widely applied in biological studies and disease research [1,2]. Untargeted metabolomic profiling and targeted metabolite quantification are the two major parts of modern metabolomics, and both of them have strengths and weaknesses. Untargeted profiling is often used for the discovery of candidate metabolites that are significantly changed between sample groups [3]. This method enables the comprehensive analysis of the metabolites and reliable identification of unknown metabolites, but it does not perform well in detecting low response metabolites, and does not yield accurate and reliable quantitative analysis compared to targeted quantification strategies [3]. On the other hand, the targeted metabolite quantification strategy can only analyze pre-selected metabolites, but it can provide reliable and accurate quantification [3,4]. With the development of mass spectrometry (MS) instrument and assay strategy, the targeted metabolomic strategy has become increasingly popular in metabolomic studies. This strategy expands the metabolite coverage of traditional targeted assay method and at the same time remains the quantification accuracy and analytical reproducibility, which have been proven to be powerful tools in biological studies [5,6] and clinical biomarker screening [7–9].

For the targeted metabolomic workflow, the MRM strategy on triple-quadrupole MS is the most commonly used [10–12]. Precursor-product ion pairs representing the given metabolites are monitored methodically in a typical MRM assay, and its advantages includes fast scan speed, high selectivity, and reliable quantitative performance [13]. PRM strategy can also be used for targeted compound quantification. This strategy is performed on high-resolution MS platforms, in which the entire tandem mass spectrometry (MS/MS) results for the given metabolites are recorded at high resolution [14]. As PRM is utilized more in targeted proteomic analysis [15], reported applications of PRM on targeted metabolomic analysis are lacking. Recently, Zhou et al. constructed a PRM assay method on a quadrupole-Orbitrap platform, targeting 237 hydrophilic metabolites in biological samples [16]. Their results ensured the applicability of PRM strategy on targeted metabolomic analysis and drew several advantages of PRM compared with the traditional MRM strategy. The advantages include higher selectivity and reproducibility and less dependence on chemical standards [16]. But to the best of our knowledge, no application of PRM strategy on targeted lipidomic analysis as well as on Q-TOF platform has been reported. In the present study, we further extend the PRM acquisition strategy to another popular high-resolution MS platform, the quadrupole-time of flight (QTOF). After constructing a targeted lipidomic assay method, which targets 222 lipids belonging to 15 lipid species, we systematically evaluated the analytical performance and feasibility of this assay method on the TripleTOF 4600 LC-MS platform. Reproducibility, analytical reliability, and dynamic range were evaluated using chemical standards and serum samples. The PRM data processing procedure was also improved to enable the use of multiple product ions for peak extraction and metabolite quantification.

The constructed PRM-based workflow was applied to biomarker screening of different SLE disease stages. SLE is a devastating systemic autoimmune disease that predominately affects young women [17]. The disease is more common in African-Americans and Orientals than in Caucasians and results in poor quality of life and considerable morbidity and mortality [18]. The disease activity is divided into two stages based on the SLE disease activity index (SLEDAI) [19], i.e., the inactive stage (SLEDAI < 3) and active stage (SLEDAI ≥ 3). Studies on the disease's pathology and biomarkers have mainly focused on the immune cells [20] and auto-antibodies [21]. A few serum metabolites, such as fatty acids (FAs) and amino acids, have been found to change between the different SLE stages [22]. However, to the best of our knowledge, no detailed targeted lipidomic study has been performed yet on serum samples from different SLE disease stages. Our biomarker screening for SLE patient serum can provide valuable information for disease diagnosis as well as pathogenesis study.

2. Material and method

2.1. Chemical materials

Ammonium formate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid, HPLC grade isopropanol, acetonitrile and methanol were purchased from Fisher Scientific. Deionized water was produced by a Milli-Q system. The chemical standards were of analytical grade with typical purity of >99%. Fatty acids (12:0, 14:0, 16:0, 18:0, 18:1, 18:2, 20:0, 20:2, 20:5 and 22:0) were purchased from Sigma. Ceramides (16:0, 18:0, 18:1, 20:0, 22:0, 24:0 and 24:1) and LPC (16:0, 18:0, 18:1, 20:0, 22:0, 24:0) were purchased from Avanti polar lipids (USA).

2.2. Samples preparation and metabolite extraction

Primary serum specimens from 66 SLE patients or healthy people were obtained from Beijing Third Hospital. The study was approved by the local ethics committee for clinical studies. The samples were obtained only from patients or healthy people who agreed to undergo the exam for the purpose of laboratory research. Analyzed serum samples were obtained from three groups: 24 active SLE patients, 22 inactive SLE patients, and 20 healthy controls. The three groups were matched with age and gender. Lipids were extracted from the serum samples using isopropanol precipitation [23] as follows: 100 μL serum samples were precipitated by addition of 3 vol of IPA precooled to −20 °C. Samples were vortex mixed for 1 min. After 10 min of incubation at room temperature, samples were stored overnight at −20 °C to improve protein precipitation and then centrifuged at 14 000 g for 20 min. The supernatant was collected and evaporated in vacuum, then stored at −80 °C awaiting MS analysis.

2.3. Lipid list generation for large-scale targeted metabolomics analysis

Combining the identification results generated from multiple