Analytica Chimica Acta 1024 (2018) 101–111

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Rapid profiling and quantification of phospholipid molecular species in human plasma based on chemical derivatization coupled with electrospray ionization tandem mass spectrometry

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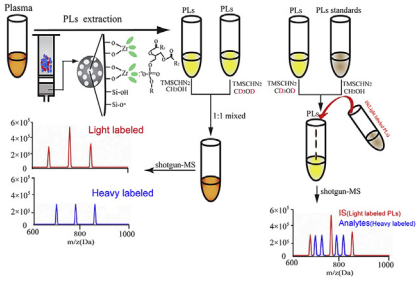
HIGHLIGHTS

- HybridSPE-PL allows rapid enrichment of PLs via Lewis acid-base interaction between zirconia and phosphate moiety of PLs.
- Derivatization with $TMSCHN_2$ leads to methylation of hydroxyl/amino in PLs and allows highly sensitive PL analysis by MS.
- An accuracy absolute quantification method for determination of PL molecular species in biological samples is developed.
- A novel strategy coupled SPE with MS based on $TMSCHN_2$ derivatization is explored for rapid analysis of PLs in human plasma.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 December 2017
Received in revised form
13 April 2018
Accepted 13 April 2018
Available online 18 April 2018

Keywords:
Phospholipids
Solid-phase extraction
Shotgun mass spectrometry
Derivatization
Quantification

ABSTRACT

In this study, we developed a novel strategy using solid-phase extraction (SPE) coupled with shotgun mass spectrometry (MS) based on trimethylsilyldiazomethane ($TMSCHN_2$) stable-isotope derivatization for rapid profiling and accurate quantification of phospholipids (PLs) in human plasma. HybridSPE-Phospholipid (HybridSPE-PL, zirconia coated silica stationary phase) was used for sample pretreatment via the Lewis acid-base interaction between zirconia and phosphate moiety of PLs. This step allows rapid enrichment and recovery of PLs from human plasma. Afterward, PLs were derivatized with $TMSCHN_2$, which leads to methylation of hydroxyl and amino groups in PLs and allows highly sensitive PL analysis by shotgun MS in positive ionization mode (limit of detection decreased up to 116.67 fold compared to underived PLs). We developed an accuracy quantification method for determination of PL molecular species in biological samples. Two or more PL standards were selected for each PL class and derivatized with $TMSCHN_2$ without stable-isotope coding. They were then used as the internal standards. PLs in biological samples were isotopic derivatized via acid-catalyzed H/D exchange and methanolysis of $TMSCHN_2$. For accurate quantification, a calibration curve for each class of PLs was typically constructed by using the internal standards to normalize the non-uniformity response caused by the differential fragmentation kinetics resulting from the distinct chemical constitution of individual PL species in the biological samples. This newly developed method was used to comprehensively analyze PL molecular

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https://doi.org/10.1016/j.aca.2018.04.012

species in human plasma samples. It is a promising methodology for rapid profiling and accurate quantification of complex lipid molecules in biological samples.

1. Introduction

Phospholipids (PLs) comprise an important kind of amphiphilic molecules, which have lipophilic acyl chains and ahydrophilic head. PLs consist of a glycerol backbone esterified with two fatty acids (FAs) at sn-1 and sn-2 positions. The backbone's sn-3 position is occupied by a phosphate group attached to a polar head of different nature. PL species can be classified into different classes based on their polar head groups, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylinositol (PI) and their lyso variants (lysoPLs). PLs are a major component of all cell membranes, and they are the source and reservoir of signaling molecules [1]. A cellular phospholipidome is a very complicated system, potentially consisting of hundreds of thousands of individual PL molecular species. Different molecular species of PLs play many diverse roles in cellular functions; any perturbation of a biological system can give rise to changes in the abundance or composition of the lipid pool [2]. Plasma is a highly complex and dynamic medium that contains multiple lipoprotein pools, each of which is made up of many lipid classes that contain hundreds or thousands of individual molecular lipid species [3]. The relationships between lipoprotein pools and metabolic diseases have long been recognized [4]. Determinations of acute or chronic changes in PL composition are likely to provide a wealth of information regarding multiple signaling events and pathological mechanisms.

Mass spectrometry (MS)-based shotgun lipidomics, an emerging powerful technique in lipidomics, holds much promise for the characterization of PLs [5]. Through effective intrasource separation of predetermined groups of lipid classes according to their intrinsic electrical propensities, lipid extracts can be analyzed directly without pre-separation of the lipids. A unique feature of tandem-quadrupole mass spectrometers is the ability to detect specific compounds using either neutral-loss scanning (NLS) or precursor-ion scanning (PIS). These scan modes are relevant to the detection of PL classes as well as PLs with a particular cassette of fatty-acyl substituents on the glycerol backbone by direct infusion “shotgun” MS equipped with an electrospray ionization (ESI) source. However, not all PLs are suitable for the shotgun-ESI-MS method, since the ionization efficiencies of several PLs are extremely low and such compounds cannot be sensitively detected. Furthermore, matrix effects in biological samples are unavoidable and may cause a negative impact like ion suppression on the analysis, which leads to decrease of MS signals of lipid species, in case of minor lipid species the signal may completely disappear. Therefore, eliminating endogenous interference and improving the ionization efficiency of PLs would be particularly valuable.

Shotgun-MS-based methods permit high-throughput qualitative analysis of PLs with high selectivity. However, using tandem MS to accurately quantify complex PL mixtures poses significant problems because of the non-uniformity response caused by the differential fragmentation kinetics of individual PL species. The differential fragmentation kinetics results from the distinct chemical constitution (including acyl chain lengths and unsaturation) of individual species and can lead to species-dependent mass spectra after collision-induced dissociation (CID) [6,7]. The use of isotope internal standards of each analyte ensures high accuracy of quantitative measurement during MS analysis. However, commercially available isotope internal standards of PLs are extremely limited and expensive. Alternatively, two or more internal standards are selected for each class of PLs, and a calibration curve is typically constructed using the internal standards to quantify of the species of the entire class. The selected internal standards for each class of PLs should not be present in the biological samples but should represent the chemical structures of the entire class, because of the numerous PLs in biological samples and the limited range of commercially available standards; they may require synthesis in the laboratory.

Chemical derivatization of the analyte is often used to enhance the ionization efficiency and detection sensitivity. Derivatization enhances the ESI response of an analyte, either by making it more easily charged or by increasing its hydrophobicity [8–10]. The role of the methylation strategy in MS has become increasingly prominent. Diazomethane has been used to react with phosphate moieties and primary amines. This reaction concomitantly produces a fixed positive charge on the lipid by forming of a quaternary ammonium ion and neutralizes the negative charge of the phosphate group [11]. The MS detection sensitivity was significantly improved particularly in tandem mass spectrometry (MS/MS) experiments because the ion fragmentation was consolidated to only one or two channels. A methodology based on phosphate methylation has been introduced to analysis of phosphoinositides and polyphosphoinositides [12,13].

The pattern change of the lipid species in a lipidome has been widely used in relative quantification measures by integrating derivatization with MS analysis. In this method, two samples, one from a control condition and the other from an experimental condition, are metabolically or chemically labeled. A given heavy nucleus (e.g., ^{2}H, ^{13}C, or ^{15}N) is incorporated into one of the samples for comparative quantification. The light and heavy populations of molecules have high chemical equivalence, but they remain distinguishable by MS. An integrated method that combines direct-infusion MS and isotopic labeling through phosphate methylation for relative quantitation of phosphoinositides from biological samples was developed [14]. This method was further expanded to simultaneous profiling and relative quantification PLs from PC3 prostate cancer cell lines [7].

In contrast to relative quantification method, absolute quantification determines the mass levels of individual lipid molecular species, which is critical for the elucidation of biochemical mechanism responsible for the changes and for pathway/network analysis. A novel method based on derivatization and differential stable-isotope labeling was proposed in this study for the absolute quantification of PLs. In this method, two or more PL standards are selected for each class of PLs and light labeled, which were then used as the internal standards; the PLs in a biological sample are heavy labeled. To accurately quantify the species of the entire PL class, a calibration curve is typically constructed by using the internal standards to normalize the non-uniformity response of individual PL species in the biological samples. The innovation reduces isotope internal standard dependence for absolute quantification and enlarges the choice range of PL internal standards, for it obviates selecting PL standards absent in the biological samples, which make it easy to choose many PL standards representing the