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Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry

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ABSTRACT

Liquid chromatography-mass spectrometry (LC-MS)-based lipidomics has undergone dramatic developments over the past decade. This review focuses on state of the art in LC-MS-based lipidomics, covering all the steps of global lipidomic profiling.

By reviewing 185 original papers and application notes, we can conclude that current advanced LC-MS-based lipidomics methods involve:

(1) lipid extraction schemes using chloroform/MeOH or methyl *tert*-butyl ether (MTBE)/MeOH, both with addition of internal standards covering each lipid class;
(2) LC separation of lipids using short microbore C18 or C8 columns with sub-2-μm or 2.6–2.8-μm (fused-core) particle size with analysis time <30 min;
(3) electrospray ionization in positive- and negative-ion modes with full spectra acquisition using high-resolution MS with capability to MS/MS.

Phospholipids (phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, phosphatidylserines, phosphatidylglycerols) followed by sphingomyelins, di- and tri-acylglycerols, and ceramides were the most frequently targeted lipid species.

Contents

Abbreviations: 2D-LC, Two-dimensional liquid chromatography; ACN, Acetonitrile; APCI, Atmospheric-pressure chemical ionization; BuOH, Butanol; CE, Cholesteryl ester; Cer, Ceramide; CID, Collision-induced dissociation; CL, Cardiolipin; DCM, dichloromethane; DDA, data-dependent acquisition; DG, Diacylglycerol; DGDG, Digalactosyldiacylglycerol; DIA, data-independent acquisition; ESI, Electrospray ionization; FA, Fatty acid; FWHM, Full width at half maximum; FT-ICR, Fourier transform ion cyclotron resonance; GM3, Monosialodihexosylganglioside; HCD, High-energy collisional dissociation; HexCer, Hexosylceramides; HILIC, Hydrophilic interaction chromatography; HPLC, High-performance liquid chromatography; HRMS, High-resolution mass spectrometry; IM-MS, Ion mobility-mass spectrometry; IT, Ion trap; LacCer, Lactosylceramide; LLE, Liquid-liquid extraction; (L)PA, (Lyso)phosphatidic acid; (L)PC, (Lyso)phosphatidylcholine; LPC(O), Lysoalkylphosphatidylcholine; (L)PE, (Lyso)phosphatidylethanolamine; (L)PG, (Lyso)phosphatidylglycerol; (L)PS, (Lyso)phosphatidylserine; MeOH, Methanol; MG, Monoacylglycerol; MGDG, Monogalactosyldiacylglycerol; MRM, Multiple reaction monitoring; MTBE, Methyl-*tert*-butyl ether; NARPLC, Non-aqueous reversed-phase LC; NMR, Nuclear magnetic resonance; NPLC, Normal-phase LC; PC(O), Alkylphosphatidylcholine; PC(P), Phosphatidylcholine plasmalogen; PI, Phosphatidylinositol; QLIT, Quadrupole/linear ion trap; QqQ, Triple quadrupole; QTOF, Quadrupole/time-of-flight; RPLC, Reversed-phase LC; SFC, Supercritical fluid chromatography; SIM, Selected ion monitoring; SM, Sphingomyelin; SPE, Solid-phase extraction; SQDG, Sulfoquinovosyl diacylglycerol; TG, Triacylglycerol; TOF, Time-of-flight; UHPLC, Ultrahigh-performance liquid chromatography.

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1. Introduction

Since its introduction in 2003 [1], lipidomics has emerged as one of the most promising research fields as a result of advances in mass spectrometry (MS). Direct infusion (shotgun) techniques were prevalent in the beginning of lipidomics research due to their relative simplicity of operation, fast analysis, and possibility to detect various lipid classes within a single run. In most cases, these methods used tandem MS in a class-specific or targeted way, so detection and subsequent identification of unknowns were impossible. This was followed by rapid progress in liquid-chromatography (LC) separation and computational methods [2–4]. The popularity of LC-MS-based methods can be explained by several advantages over direct infusion techniques, such as more reliable identification of individual lipid species, even at trace levels, separation of isomers and isobars, or reduced ion-suppression effects. In addition, current LC instruments permit more effective separation, and reduce analysis time and solvent consumption [5,6]. Currently, direct infusion-MS(/MS) and LC-MS(/MS) methods are reported in the scientific literature in almost equal ratio, while complementary techniques and their combinations, such as gas chromatography (GC), thin-layer chromatography (TLC) and nuclear magnetic resonance (NMR), are less frequently used in lipidomics (Fig. 1).

LC-MS-based lipidomic analyses (Fig. 2) typically start with extraction of the lipids from the biological sample followed by LC separation, which can be performed based on lipid species [e.g., reversed-phase LC (RPLC)] or classes [e.g., normal-phase LC (NPLC)]. Once chromatographically separated, the molecules enter the ion source where they undergo ionization followed by detection of particular ions using a mass analyzer. This can be conducted in an untargeted (full spectra acquisition), class-specific (product-ion scanning, precursor-ion scanning or neutral-loss scanning) or targeted

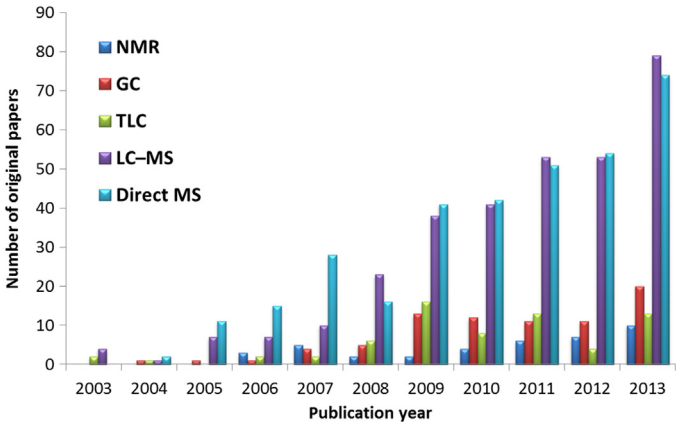

Fig. 1. Number of original papers published over 11 years dedicated to lipidomics and different instrumental platforms. Scopus (www.scopus.com) and Web of Knowledge (www.webofknowledge.com) databases used for citation analysis.

(multiple-reaction monitoring) way [7]. The data handling represents a post-acquisition phase, which is focused on identification and (semi)-quantification of detected lipids, followed by statistical analysis if the primary focus of the study is to distinguish groups of samples.

For this article, we reviewed 185 original LC-MS-based lipidomics papers and application notes published over the past decade (see references in Supplementary material, Tables S1 and S2). All the aspects, such as sample extraction, LC separation and MS detection are discussed in subsequent sections of this review. Since our primary focus was on the analysis of complex lipid mixtures in various biological systems, we omitted those papers dedicated to only a single lipid class (e.g., triacylglycerols or fatty acids).

2. Sample extraction

In general, lipidomics applications require sample-preparation methods that are fast, reproducible, and able to extract a wide range of analytes with different polarities, and that, at the same time, are compatible with the instrumental technique. Analytical strategies, which allow for increased coverage of metabolites determined in one sample, are therefore desirable [8,9]. In addition, samples may be available in only limited amounts, posing practical requirements to develop efficient, sample-saving experimental procedures. The reviewed studies were focused mainly on the analysis of lipids in plasma or serum, followed by animal tissues, cells, and plant tissues (Fig. 3A). Generally, 1–100 mg of tissue or 10–100 μL of biofluids (plasma/serum) per analysis were required in the reports reviewed here. Other matrices, which were less frequently studied, applied lipidomics to apicoplasts, microsomes, mitochondria, lipoprotein particles, milk, oxidized oils, *Drosophila*, microalgae, mesenteric lymph, cerebrospinal fluid, placental microvesicle, synovial fluid, tear samples, sebum, hetapocyte lipid droplets, urine, and solid fecal material (see references in Supplementary material, Table S1). Several sample-preparation methods were applied to biological samples with the goal of improving overall lipid coverage, including liquid-liquid extraction (LLE), organic solvent precipitation, and solid-phase extraction (SPE) [10].

Introduced more than 70 years ago by Folch et al. [11] and Bligh–Dyer [12] (Fig. 3B), most lipidomics studies still rely on these general extraction procedures, often in modified versions. The Folch method employs roughly a 20-fold excess of a mixture of chloroform/MeOH (2:1, *v*/*v*) for the extraction, while the Bligh–Dyer method is also based on a mixture of chloroform/MeOH (1:2, *v*/*v*), but uses a subsequent addition of 1 volume of chloroform and 1 volume of water. As a less toxic alternative, chloroform was replaced with dichloromethane (DCM) in some studies.

In 2008, Matyash et al. [13] introduced a novel sample-extraction procedure employing methyl *tert*-butyl ether (MTBE). The method involves addition of MeOH and MTBE (1.5:5, *v*/*v*) to the sample and phase separation is induced by adding water. The advantage of MTBE extraction over conventional two-phase chloroform-containing