Published in final edited form as:

Mol Biosyst. 2015 March ; 11(3): 698–713. doi:10.1039/c4mb00586d.

Applications of Mass Spectrometry for Cellular Lipid Analysis

Chunyan Wang, Miao Wang, and Xianlin Han*

Diabetes and Obesity Research Center, Sanford-Burnham Medical Research Institute, Orlando, Florida 32827 USA

Abstract

Mass spectrometric analysis of cellular lipids is an enabling technology for lipidomics, which is a rapidly-developing research field. In this review, we briefly discuss the principles, advantages, and possible limitations of electrospray ionization (ESI) and matrix assisted laser desorption/ionization (MALDI) mass spectrometry-based methodologies for the analysis of lipid species. The applications of these methodologies to lipidomic research are also summarized.

Keywords

electrospray ionization mass spectrometry; lipidomics; matrix-assisted laser desorption/ionization mass spectrometry; quantification; shotgun lipidomics

1 Introduction

1.1 Lipids and lipidomics

Cellular lipids are the major constituents of animal and plant cells and main components of lipoproteins in serum. They represent a wide range of organic compounds in living organisms, and most of them are soluble in non-polar solvents, but not in water. The majority of cellular lipids are comprised of a polar hydrophilic part (usually called it as the head group) and a non-polar hydrophobic portion, which all belong to amphiphilic molecules (e.g., glycerophospholipids and sphingolipids). But some of the lipids (e.g., diacylglycerols (DAG), triacylglycerols (i.e., fats or oils) (TAG), wax, cholesterol, and cholesteryl esters) constitute predominantly with non-polar components. The polar head group defines the individual lipid class. In a few categories of lipids, individual class could diversify into different subclasses. For example, some of the glycerophospholipid classes are classified into three subclasses based on three linkages of the aliphatic chain to the hydroxyl group of glycerol at the *sn*-1 position (i.e., ester, ether, and vinyl ether, named as phosphatidyl-, plasmalyl-, and plasmenyl-, respectively). A group of sphingolipid species due to the absence of a *trans* double bond between C4 and C5 carbon atoms of the sphingoid base are usually called dihydrosphingolipids in the literature. A compendious lipid classification system has been recommended by the Lipid MAPS consortium, which classifies the cellular lipids into eight categories including fatty acyls, glycerolipids,

*To whom correspondence should be addressed: Xianlin Han, Ph.D., Diabetes and Obesity Research Center, Sanford-Burnham Medical Research Institute, 6400 Sanger Road, Orlando, Florida 32827, USA, Telephone: (407) 745-2139, Fax: (407) 745-2016, xhan@sanfordburnham.org.

glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides.¹

It has been well recognized that the majority of the cellular lipid molecular species are the combinations of a minimal number of building blocks. For example, all of the molecular species in the categories of glycerophospholipids and glycerolipids are centered with a glycerol molecule and all of these lipid species can be constructed with three building blocks of X, Y, and Z connected to the glycerol (Figure 1). Examples of these lipid classes include DAG, TAG, choline glycerophospholipid (PC), ethanolamine glycerophospholipid (PE), serine glycerophospholipid (PS), inositol glycerophospholipid (PI), glycerol glycerophospholipid (PG), and so forth, depending on the building blocks of Z. As an example, the molecular species of PS are multiple individual covalences of a glycerol backbone combined with all kinds of saturated or unsaturated (with variable degrees of unsaturation) aliphatic chains (usually containing 14 to 22 carbon atoms) at X or Y and with a polar head group of phosphoserine at Z. The different connections of the aliphatic chains at the *sn*-1 position of glycerol constitute the three subclasses of PS. There are more than 30 kinds of aliphatic chains in nature, due to the differences of carbon atom numbers, double-bond numbers, and double-bond locations. Therefore, the potential number of PS molecular species could be in the range of 2,700 (30 varieties of *sn*-1 aliphatic chains $\times$ 3 connections at the *sn*-1 position $\times$ 30 varieties of *sn*-2 aliphatic chains).

Similarly, all of the molecular species consisting of sphingoid-based backbones form the sphingolipidome.² Nomenclature and a comprehensive classification of the sphingolipidome can be found at www.sphingomap.com. Hundreds of thousands of sphingolipid molecular species of the sphingolipidome can be represented by a general molecular structure with a combination of three building blocks of X, Y, and Z (Figure 2).³

Lipids possess numerous essential functions in life including (1) the constituents of cellular membranes which are hydrophobic barriers to separate cellular compartments in biological organisms; (2) an optimal matrix to facilitate specific conformations, interactions, and dynamics for effective activities of trans-membrane proteins; (3) the energy reservoir (e.g., TAG) to store and supply energy for cellular needs; and (4) a storage depot of bioactive second messengers (e.g., eicosanoids, DAG, lysophospholipids, sphingosine, sphingosine-1-phosphate, etc.), which can be generated to propagate cellular signaling for cell growth, differentiation, death, and response to stimuli. Moreover, it is evidenced that a disorder of lipids is the risk factor for many human conditions and diseases, for example, cancer, psychiatric disorders, neurodegeneration, neurological disorders, infection, atherosclerosis, stroke, obesity, diabetes, etc.).⁴⁻¹¹

All of the lipids present in a cell type, a cellular organelle, an organ, a membrane microdomain, etc. constitute a cellular lipidome.¹² In other words, cellular lipidomes are variable and very complex at three levels of initial discrimination. First, the lipidome of each cell type is comprised of different compositions of specific lipid classes, subclasses, and molecular species. Second, cellular lipids are highly different among different cell types, species, cellular organelles, membranes, and membrane microdomains (e.g., caveola and/or rafts). Finally, the cellular lipidome is dynamically changing at any moment affected by

many factors, such as nutritional status, hormonal concentrations, health conditions, and exercise levels. Thus, hundreds of thousands of potential lipid molecular species are predictably present in the cellular lipidome, at the levels of attomole to nanomole of lipids per mg of protein.^{3, 13, 14}

The discipline that studies the entire cellular lipidome in a certain degree of high throughput is known as lipidomics.¹⁵⁻¹⁸ Specifically, lipidomics studies the pathways and networks of cellular lipids in biological systems (i.e., lipidomes) on a large scale and at intact molecule levels. This study involves the identification and quantitation of hundreds to thousands of cellular lipid molecular species, and attempts to understand their pathways and networks and to elucidate their interactions with other lipids, proteins, and other moieties *in vivo*. Investigators studying lipidomics examine the structures, functions, interactions, and dynamics of cellular lipids and the dynamic changes that occur during pathophysiologic perturbations. Many modern technologies, such as mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), fluorescence spectroscopy, and microfluidic devices, have been used in lipidomics for quantification of lipid species in biological systems.¹⁹ Among the development of lipidomics, MS techniques play an essential role in lipid characterization, identification, and quantitation (see^{15, 16, 20-25} for recent reviews).

1.2 Brief history in mass spectrometric analysis of lipids

Numerous methods have been developed and applied to determine the complexities inherent in cellular lipidomes. Historically, chromatographic techniques (TLC, GC, and HPLC) have been essential to identification and quantification of lipid molecular species.²⁶⁻³¹ Clearly, lipidomics is greatly facilitated by recent advances in two of the most popular and powerful technologies, electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted laser desorption/ionization (MALDI) MS, and their applications.^{15, 16, 20, 21, 32-39}

Electron ionization (EI) and chemical ionization (CI) have been applied to analyze lots of species of lipids since their introductions in the late 1940s⁴⁰ and late 1960s,⁴¹ respectively. In addition, fast atom bombardment (FAB) MS has also played an important role in the development of methods for lipid analyses.⁴²⁻⁴⁶ Atmospheric pressure chemical ionization (APCI) MS can ionize many classes of lipids and its spectra are relatively simple for analysis. For example, the protonated molecular ions (with dehydration in the case of sterols) of phospholipids, free fatty acids, sterols, and TAGs are the dominant base peaks in the spectra of APCI-MS.^{23, 47} However, the APCI is not soft enough as an ionization technique compared to ESI. The commercial ESI and MALDI mass spectrometers were available since late 1980s and early 1990s, and the advances in instrumentation revolutionize the analysis of most nonvolatile lipids in intact forms.⁴⁸⁻⁵² The majority of those studies on different lipid classes can be found in multiple, valuable review articles.^{20, 21, 32, 53, 54}

Other developments related to the ion sources in MS for analysis of lipids have also been made. For example, silver or gold ions as primary ions were employed in the approach of time-of-flight secondary ion mass spectrometry (TOF-SIMS) and it can be used to directly determine cellular lipids in tissue.⁵⁵⁻⁵⁸ MALDI-MS has been proven to be the most popular technique for imaging and can yield data with high mass accuracy and spatial resolution;⁵⁹

in addition, its application for analysis of lipid species *in vivo* has been well demonstrated.⁶⁰ Many lipid classes including neutral lipids and phospholipids have been analyzed by atmospheric pressure photoionization (APPI) MS. APPI-MS can also offer higher sensitivity and lower detection limitation in comparison to APCI-MS.⁶¹⁻⁶⁴ Mass spectrometers containing highly accurate mass analyzer (e.g., Fourier-transform ion cyclotron and Orbitrap) have also been important in lipidomics.^{52, 65-72} The great appreciation of MS applications for lipid analysis is largely due to the developments of novel techniques and the advanced instruments. In this article, we focus on ESI-MS and MALDI-MS analysis of lipids, briefly discuss the ionization features and methodologies for lipid analysis, and summarize the applications of these methodologies for biological research.

2 ESI-MS of lipid analysis

2.1 Characterization of lipids by ESI-MS

ESI is one of the softest ionization techniques and the in-source CID can be neglected under proper instrument conditions. Therefore, only (quasi)molecular ions of lipids are displayed in the spectrum when ESI-MS is used for lipid analysis. Moreover, the ionization is very soft and some complexes dimers and solvent adducts can be detected during the ESI process.^{49, 73} ESI-MS offers multiple advantages.^{15, 16, 74} First, its ion source can act as a separation device to selectively ionize a certain category of lipid molecular species based on the charge property of lipid classes. Thus, it is feasible to analyze different lipid classes and individual molecular species with high efficiency without prior chromatographic separation.^{36, 75, 76} Second, the ionization efficiency of lipids in ESI-MS is incomparably higher than other traditional MS ion sources. Detection limitation at a concentration of amol/μl to low fmol/μl can be achieved⁷⁷ and will continue to improve as the instruments become more and more sensitive. Third, the instrument response factor of individual molecular species of a polar lipid class is essentially identical within experimental errors after ¹³C de-isotoping if the analysis is performed in a properly low concentration region of lipids.³ A low lipid concentration meant here is to avoid lipid aggregation, a process that depends on the physical properties of individual molecular species.⁷⁸ The minimal source fragmentation and selective ionization (see above) largely contribute to the identical response factor for the species of a polar lipid class. This feature makes the quantification of individual molecular species of a polar lipid class possible through direct comparison of ion peak intensities to that of a selected internal standard, or through the peak-area measurement in the case of LC-MS. Fourth, a nearly linear relationship between an ion peak intensity (or area) of a polar lipid molecular species and the concentration of the compound is present over a wide dynamic range in the proper concentration region of total lipids.^{79, 80} For the lipids without large dipoles, correction factors or calibration curves for each individual molecular species have to be pre-determined as previously demonstrated.⁸¹ Alternatively, modification of the charge properties of these less-ionizable lipid classes can be conducted through derivatization to enhance their ionization.^{82, 83} Finally, a reproducibility of > 95% from a prepared lipid sample in the presence of an internal standard can be readily achieved after direct infusion (currently this is generally known as shotgun lipidomics).^{16, 79, 84, 85} This reproducibility is essentially independent of laboratory, analyst, and instrument. This

high reproducibility guarantees the accuracy of analysis and reduces the required number of samples for replication. Accordingly, due these advantages, it is evident that ESI-MS-based lipid analysis has become an essential tool for measuring cellular lipidomes (see 15, 16, 20-22, 75, 86 for recent reviews).

2.1.1 ESI-MS analysis of lipids in the full-mass scan mode

2.1.1.1 Lipid analysis in the positive-ion mode: In general, lipids are apt to form small cation adducts in the positive-ion mode, due to the soft ionization process as aforementioned. The formation of cation adducts of lipid molecular species resulted from the affinity of the cations with the dipole that is present in the lipid species depends on the availability of the small cations in the matrix. For example, sodium adducts of lipids are often the abundant ions showed in the mass spectra of ESI-MS in the positive-ion mode when no modifiers are added into the sprayed solution, especially for PC (Figure 3) and sphingomyelin (SM).^{49, 77} This is because the sodium ion is the most common one in the natural source, and because it possesses high affinity with polar lipids and even non-polar lipids (e.g., TAG). However, lipids can also be adducted with other cations when modifier(s) are employed before the sample infused into ESI-MS.³⁶ For example, the majority of molecular species of lipid classes are presented as a protonated format in positive-ion ESI mass spectra when an organic acid is added as a modifier to the sprayed lipid solution. This is the common case for lipid analysis by LC-ESI-MS. Similarly, proton/ammonium adducts or lithium adducts of lipid molecular species could be the predominant peaks in positive-ion ESI mass spectra when an ammonium or lithium salt is added to a sprayed lipid solution.

2.1.1.2 Lipid analysis in the negative-ion mode: In negative-ion ESI-MS spectra, lipid species in the deprotonated form or with an anionic adduct are displayed depending on whether the lipid molecule species carry a net ionic bond. For example, PE, PI, PS, PA, PG, cardiolipin, sulfatide, non-esterified free fatty acid, lyso-cardiolipin, eicosanoids, acyl-CoA, phosphatidylinositol phosphate (PIP), etc. are all of acidic lipid classes (i.e., an ionic bond is present), and thus, are detected as deprotonated ions.^{16, 49, 87} On the other cases, cerebroside and many other glycolipids are the polar lipid classes without an ionizable bond or PC and SM are strong zwitterionic lipid classes, all of which can form as their anionic adducts with small anion(s) (e.g., Cl^- , CH_3COO^- , and HCOO^-) depending on the concentrations present in the matrix and their affinities with these lipid species.^{50, 77, 88}

2.1.2 ESI-MS analysis of lipids in the product-ion mode—As aforementioned, the (quasi)molecular ions of lipids are displayed in ESI full scan mass spectra. So structural determination of individual molecular species of lipid classes can be achieved by product-ion analysis with low-energy collision-induced dissociation (CID). Some review articles introduced the studies in this area in details.^{20, 21, 32} For example, the rules for fragmentation of different lipid classes are elucidated with much more details and a set of studies focusing on the structural characterization of each lipid class were conducted by Hsu and Turk.^{25, 89-91} Their work includes the characterization of triacylglycerols, the glycolipids that bear mycolic acids, and mono- and dihexoyl diacylglycerols.⁹¹⁻⁹⁴ Those who are interested in a detailed understanding of a specific lipid class on structural characterization by product-ion ESI-MS should consult these articles. Herein, only a brief

discussion which focuses on the features of phospholipids from their structural characterization is given as follows.

2.1.2.1 Product-ion analysis of lipids in the positive-ion mode: Product-ion mass spectra of protonated phospholipid species display little informative ions on structural characterization and usually they are very simple. For example, the protonated PC molecular species⁹⁵ produce a predominant phosphocholine fragment (m/z 184). However, richer fragment ions readily applicable for structural identification can be obtained by product-ion MS spectra of the phospholipid alkaline adducts in the forms of $[M+\text{alk}]^+$ and/or $[M+2\text{alk}-H]^+$ ions (alk = Li, Na, K, ...). The fragmentation patterns, as well as the proposed mechanisms leading to the fragments, have been described in detail.⁹¹ Moreover, *in silico* tandem MS-database searches can be used to identify the species, based on the product-ion mass spectra.⁹⁶ Additionally, *sn*-1 or *sn*-2 lysoPC and lysoPE can be also readily identified through alkaline adducts,^{95, 97} since the more labile loss of the fatty acyl substituent at *sn*-1 than that at the *sn*-2 position and the different role of charge-remote fragmentation process are present. Specifically, the peak intensity ratio of the fragment ions that correspond to sodiated five-membered ethylene phosphatidic acid and choline ions in product-ion spectra of sodiated lysoPC isomers can be used to determine the regiospecificity as described.^{95, 97} Similarly, the difference in the ratio of the relative intensity of the product-ion pair at $[M+Na-163]^+$ and $[M+Na-61]^+$ can distinguish the regioisomers of sodiated lysoPE.⁹⁷

2.1.2.2 Product-ion analysis of lipids in the negative-ion mode: Charge-driven fragmentation processes are very common for lipid classes with ESI-MS in the negative-ion mode. Moreover, determinant in the negative-ion mode is the loss of fatty acyl substituent, as an acid or as a ketene, that results from the gas-phase basicity of the precursor ions as previously described.⁹⁸ The gas phase basicity is the negative of the free energy change associated with the reaction of $M + H^+ \rightarrow MH^+$ as defined previously.⁹⁹ The fatty acyl substituents at *sn*-1 position can be distinct from the one at the *sn*-2 position, because the loss of the fatty acyl substituent from the *sn*-2 position and produced the $[M-H-R'_2CH=CO]^-$ or $[M-H-R_2CO_2H]^-$ (loss of a ketene or an acid) is more easily than the loss of fatty acyl substituent from the *sn*-1 position. Distinct product-ion mass spectra can be acquired from different lipid classes because these lipids possess the different head groups from which different fragment ions are yielded due to their different gas-phase basicities of the $[M-H]^-$ ions. Obviously, these distinct product-ion mass spectra can be used to distinguish these lipid classes. For example, the ions that reflect the (phospho)inositol head group are observed at m/z 315, 297, 259, 241, and 223. The m/z 297 ion ($[M-H-R_1CO_2H-R_2CO_2H]^-$) arises from consecutive losses of the fatty acyl substituents as acids. Moreover, the charge-driven fragmentation processes are also related with the degree of unsaturation in the fatty acyl substituents.⁷⁷

2.2 Applications of ESI-MS for lipid analysis

In this subsection, we briefly describe the principles of the methodologies related to the application of ESI-MS for lipid analysis rather than characterization of lipid molecular structures. Our intent is to describe the principles through the unique ESI ion source to separate and analyze different lipid classes. To this end, multidimensional MS (MDMS) was

developed to optimize the conditions for lipid analysis and the methods how we can use ESI-MS to perform identification and quantification of lipid species.³⁶

2.2.1 Intrasource separation of lipid classes by ESI-MS—Charge separation can occur in the molecules that contain an ionic bond in a high electric field (e.g., the ESI ion source in which high electrical potentials (typically ~ 4 kV) are employed^{100, 101} or a nanomate device where regardless of a much lower voltage used, a short distance (i.e., a few microns) between the potential differences leads to a very high field). Specifically, in the positive-ion mode, the cations or the positively-charged moieties are selectively generated in the electrospray ion source, whereas the anionic parts or negatively-charged moieties after the oxidation process are selectively disposed as electrically neutral molecules to waste when the two types of charged moieties both present in the ESI sprayed solution.¹⁰² Similarly, in the negative-ion mode, the anions are eventually generated in the ion source and the cationic moieties are removed to waste after redox chemistry occurs. Therefore, the ESI source somewhat behaves as an electrophoretic device to selectively ionize a certain type of ions when it is conducting with a continuously equilibrating mobile phase.^{76, 100-102} In fact, ions can be generated in the gas-phase by ESI source from electrically neutral and not polar compounds. In this respect, the ESI source is more broadly suitable for separation of lipid classes than an electrophoretic cell. In such a high electric field, the compounds that are present in the sprayed solution do not carry separable charge(s) but rather possess intrinsic dipoles that can be induced to interact with whatever small cation(s) or anion(s) are available in the matrix (e.g., Na⁺, NH₄⁺, H⁺, Li⁺, K⁺, or OH⁻, Cl⁻, acetate, formate) to yield adduct ions in the positive- or negative-ion mode, respectively. The ionization efficiencies of these electrically neutral compounds depend on their inherent dipoles, the electrochemical properties of the resultant adducts, the concentration of the small matrix ions, and the affinity of the analytes with the small ions.¹⁶

Depending on the various charge properties of polar head group of each lipid classes, the different lipid classes can be separated and selectively ionized by intrasource separation through this physical property of the ESI ion source. Such a separation of each lipid classes in the ESI source is similar to use of an ion-exchange column to separate individual lipid classes.²⁷ However, the intrasource separation has many advantages compared to ion-exchange chromatography, such as rapidity, directness, and reproducibility, and can avoid the artifacts which are inherent in chromatography-based separation.¹⁰³ It should be emphasized that ion exchange chromatography also has advantages of its own in many applications.

Three main categories of all the lipid classes could be reclassified according to their charge properties.^{15, 16} Under the conditions of slightly acidic (i.e., near pH 5), a net negative charge is carried by lipid classes belonging to the first category. These classes of lipids are generally called anionic lipids (e.g., cardiolipin, PG, PI and its polyphosphate derivatives, PS, PA, sulfatide, acyl-CoA, and anionic lysophospholipids). In the second category, the lipid classes are lacking the net charge under weakly acidic conditions, but become negatively charged under alkaline conditions. Therefore, they are referred to as weakly anionic lipids. Lipid classes in this category include PE, lysoPE, non-esterified fatty acids and their modifiers, ceramide, bile acids, etc. The remaining lipid classes which are

electrically neutral, but polarizable belong to the third category. Lipid classes in this category include PC, lysoPC, SM, hexosylceramide, acylcarnitine, DAG, TAG, cholesterol and its esters, etc.

Therefore, a practical strategy for separation of these categories of lipids has been performed^{15, 16, 75, 104} and illustrated with a schematic diagram (Figure 4) based on the different charge properties mentioned above. Phospholipids in a lipid extract of mouse brain cortex can represent the three categories of lipids and the demonstration of such a strategy is shown in Figure 5. Anionic lipids are directly and selectively ionized from the diluted lipid solution by negative-ion ESI-MS. Weak anionic lipids are analyzed from the solution after being rendered to slightly basic condition by adding a suitable base solution (e.g., a LiOH solution) by ESI-MS in the negative-ion mode. The rest of the lipid classes belonging to the third category are analyzed by ESI in the positive-ion mode under the diluted and mildly alkalized lipid solution, because the anionic lipids in the first and second categories are un-ionizable under this condition. In summary, each category of lipids can be determined by ESI-MS with the inclusive sets of mass spectra under the different conditions of a sprayed solution for any biological samples.

2.2.2 Identification of lipids by shotgun lipidomics—In shotgun lipidomics, every single peak in the survey mass spectrum of the lipid extract of any biological sample could represent one or more molecular species, especially for the spectrum obtained from a unit resolution mass spectrometer. Hence, identification of individual molecular species underlying each ion peak could be performed with product-ion mass spectral analysis of the ion peaks, as described in the previous section. Alternatively, because biological lipid species are the combinations of a small number of building blocks, individual ion peaks in each of the mass spectra could also be identified through determination of the building blocks.⁷⁵ These building blocks can be determined by utilizing two other tandem MS techniques [i.e., precursor-ion scan (PIS) and neutral loss scan (NLS)]. Each PIS, which monitors a fragment ion as a building block, effectively determines all of the molecular ions that contain the building block. Similarly, each NLS that monitors a neutrally-lost fragment as a building block determines all of the molecular ions containing such a building block. All of these fragments (i.e., building blocks) can be derived from characterization of individual lipid molecular species of each lipid class as described above.

For example, if the interest is to identify the anionic phospholipid species present in a lipid extract of mouse brain cortex, we have to definitively identify the underneath lipid species of all ions displayed in the mass region between m/z 550 and 1000 of the mass spectrum acquired in the negative-ion mode and without addition of LiOH (similar to that of Figure 5A). From characterization of anionic phospholipids, we learned that the head group building blocks are the neutral loss of serine (87 Da) from PS, inositol phosphate (m/z 241) from PI, and the glycerophosphate (m/z 153) from all anionic phospholipids in addition to the fatty acyl carboxylates resulted from all fatty acyl chains. Detection of all of these building blocks constitutes a basic two-dimensional (2D) mass spectrum (Figure 6). One dimension (x-axis) is the mass to charge ratios of molecular ions and the other dimension (y-axis) is the building blocks. The crossing peaks of a given primary molecular ion in the first dimension with the second dimension show the building blocks (i.e., fragments) of this

given molecular ion. For example, the right broken line in Figure 6 indicates that the molecular ion at m/z 885.7 only crosses with the building blocks of PIS241 (inositol phosphate), PIS153 (glycerophosphate), PIS283 (18:0 FA), and PIS303 (20:4 FA) with a ratio greater than 1 of these two fatty acyl fragments. Hence, this set of information identifies the molecular ion at m/z 885.7 as 18:0-20:4 PI. It can be readily recognized that analysis of a handful building block is much more effective than product-ion analysis of individual ion in this mass region.

Employing various instrumental conditions such as changes in fragmentation conditions (e.g., collision gas pressure, collision energy, and collision gas) and in ionization conditions (e.g., source temperature and spray voltage), among others, can change the profiles and intensities of the basic 2D mass spectrum.^{36, 75} Therefore, additional dimensions of mass spectra to generate potentially information for lipid analyses can be obtained by changing a series of instrumental conditions.⁷⁵ A family of multi-dimensional mass spectrometry is constituted by all of these variables that has been previously described in detail.^{36, 75}

2.2.3 Quantitation of lipids by ESI-MS—ESI-MS is currently the most popular method for quantitative analysis of individual lipid molecular species, other than the qualitative elucidation of the structures of individual lipid species. The factors that affect accurate quantification have recently been discussed in detail.^{78, 105}

The LC-MS, tandem MS (MS/MS), and multidimensional MS are the three kinds of ESI-MS-based approaches for lipidomics practice. The advantages and some limitations of these approaches have been extensively discussed.¹⁶ Both of the shotgun lipidomics approaches (i.e., MS/MS and MDMS) are developed and potentially can be used as global analyses of individual lipid species by infusing a lipid extract of a biological sample without pre-chromatographic separation. The applications of LC-ESI-MS methods are well summarized in numerous recent reviews¹⁰⁶⁻¹⁰⁸ and so are not further discussed here. The advantages and limitations of shotgun lipidomics approaches are briefly discussed in the following section.

2.2.3.1 Shotgun lipidomics based on ESI-MS/MS: Many applications of ESI-MS/MS based shotgun lipidomics were performed to quantitate the lipids in biology samples and some principles of the methods are discussed as follows.^{22, 109, 110} First, at least two molecular species of a lipid class of interest should be added as internal standards into a lipid extract during extraction of a biological sample, largely in considering of the differential fragmentation patterns of individual lipid species of a lipid class after CID. The choice of the internal standards is important and should meet at least two criteria. One is that these compounds should represent the physical properties of the entire lipid class of interest as closely as possible (e.g., the similar structure of acyl chains). The other is that these standards should be absent or present in very minimal amounts in the lipid extracts from biological samples.

Next, a unique tandem MS analysis of a building block that is specific to the lipid class of interest, through either NLS or PIS, is performed. The concentration of each individual molecular species of the class of interest can be calculated from its ion-peak intensity from

the acquired tandem MS spectrum by comparing it to the ion-peak intensities of the internal standards. Many experimental factors that may introduce experimental errors are essentially eliminated by utilizing these internal standards. Since the tandem MS-based shotgun lipidomics is straightforward and have many advantages, this methodology has become very important to analyze many specific lipid classes.¹¹¹⁻¹¹³

But, there also exist some apparent limitations of this approach. For instance, the internal standards added before the extraction of lipids are not easy to be selected to meet the two criteria simultaneously; each molecular species of a lipid class which usually possesses differential fragmentation kinetics may lead to inaccurate quantitation results; the dynamic range of some lipid classes is limited if the tandem MS profile is not sensitive enough; and the structural identities and isobaric species are not determined; among others (see discussions in ref. ¹⁶).

2.2.3.2 MDMS-based shotgun lipidomics: In the MDMS-based shotgun lipidomics approach, a two-step procedure has been developed to quantify lipid molecular species.^{75, 88, 114} First, ratiometric comparisons to a pre-selected internal standard of the class after ¹³C de-isotoping are applied for individual molecular species of the class, which are non-overlapping and abundant in the lipid extract. Next, the contents of other low-abundance or overlapping lipid molecular species are determined by using all of the already determined molecular species of the class including the pre-selected internal standard as standards *via* one or more tandem mass traces (each of which represents a specific building block of the class of interest). This second step is similar to the tandem MS-based shotgun lipidomics approach as described above. The difference is the use of standards to quantify lipid molecular species in the second step, which come endogenously and are not all added before the lipid extraction. The advantage is that the endogenous standards can represent much more comprehensive physical properties of structure similarity. The linear dynamic range of lipid quantitation can be extended by filtering the overlapping molecular species and by eliminating background noise through the quantitation process from this second step¹⁶. For example, for quantification of PS species, the full scan for analysis is acquired in the negative-ion mode. In the first-step quantitation, non-overlapping and high-abundance species were determined by ratiometric comparison with the selected internal standard (e.g., 14:0-14:0 PS, *m/z* 678) after baseline correction and de-isotoping. The second-step quantitation procedure is then performed to quantitate the rest of the overlapping and/or low-abundance species by using the abundant species already quantitated in the first step as the standards, in accordance with the data from the NLS87 scan that is quite specific to the species of PS class. A limitation of this methodology which should be recognized, however, is that the accuracy of the lipid molecular species in the second step is not good as those quantitated in the first step.

Therefore, it is essential to minimize any potential experimental error which can be propagated from the first step to the second step. Such as the experimental error resulted from baseline can be reduced by baseline correction. The propagation of experimental errors can also be minimized by using a proper amount of added internal standard to produce comparable ion peak intensities between the internal standard and the lipid species of a class.^{115, 116} It is noteworthy that different molecular species of non-polar lipid classes have

the different response factors, which is different from the polar lipid classes which essentially have identical response factor after ^{13}C de-isotoping in ESI-MS.^{75, 114, 117, 118} Therefore, the differential response factors for quantitation of individual lipid species of non-polar lipid classes need to be predetermined.⁸¹

3 MALDI-MS of lipid analysis

3.1 Behavior of lipid classes in MALDI-MS

In this section, we focus on illustrating the effects of lipid structures on the MALDI-MS responses, particularly for analysis of phospholipids. Sodium adducts and protonated molecular species of PC and SM are usually present simultaneously in the spectra of MALDI-MS in the positive-ion mode. The peak intensity ratio of the two kinds of ions depends on the availability of sodium ion in the matrix. The product-ion analysis of protonated molecular ion of PC and SM shows an exclusive fragment at m/z 184 in MALDI-MS through the post-source decay analysis and this fragment ion comes from the head group of these lipids.¹¹⁹ However, product-ion analysis of sodium adduct of these lipid species yields a more informative fragmentation pattern than that from protonated counterparts.¹¹⁹ An intense positive-ion signal can be detected from phosphocholine-containing lipid molecules, but the signal for ionization of these lipid molecular species in the negative-ion mode of MALDI-MS is relatively weak¹²⁰ although it was found that SM could be sensitively detected in the negative-ion mode with 9-aminoacridine (9-AA) as matrix.¹²¹ PE molecular species is identified by neutral loss of the head group resulted in a specific fragment ion in positive-ion mass spectra of MALDI-MS.^{119, 122} But, the higher sensitivity of PE species is present in the negative-ion mode than that in the positive-ion mode and the mass spectra of PE are usually less difficult to interpret in the negative-ion mode because no additional adducts overlap each other in the spectra.¹²³⁻¹²⁵

In addition to the presence of proton and sodium adducts of PS molecular species in positive-ion MALDI-MS mass spectra, molecular ion peaks corresponding to the adduct with two sodium ions can also be detected in the mass spectra. Similar as PE species, a fragment ion that corresponds to the neutral loss of head group serine is also exhibited in the positive-ion mass spectrum of PS, which indicates that the neutral loss of the head group from phospholipids is the most dominant fragment and can be used for characterization of phospholipids.¹¹⁹ A deprotonated base peak and singly-charged ion peak carrying a sodium adduct are shown in the negative-ion MALDI mass spectrum of PS.²⁴ Not only can the polar lipids be qualified through MALDI-MS,²⁴ but also characterization of non-polar lipid classes has been conducted by MALDI-MS.¹²⁶ For example, TAG is one of the non-polar lipid classes, the mass spectra of TAG as their sodium adducts are exclusively displayed in positive-ion MALDI. The MALDI mass spectrum of TAG is characterized by neutral loss of sodium fatty acyl carboxylate(s) resulted in a specific fragment ion in the positive-ion mode. As mentioned above, the technique of post-source decay has been applied to the characterization of PC and SM. But it is not the real tandem MS analysis of lipids. Along with commercially available for MALDI-TOF/TOF mass spectrometer, the true tandem MALDI-MS spectra of lipids are obtained and have been reported recently.¹²⁷⁻¹²⁹

3.2 Lipid analysis by MALDI-MS

3.2.1 Analysis of biological lipid extraction by MALDI-MS—Because the MALDI-MS has many advantages for lipid analysis, for example, the tremendous sensitivity and selectivity, the analysis can be performed in a short time, and the sample purified with less time consuming, so it is very convenient and useful for numerous number of lipid molecular species in the biological samples (see ^{24, 130-133} for reviews).

First of all, it is critical to choose the proper matrix in order to obtain the high quality and high intensity mass spectral data of the analytes. For example, 9-AA ¹³⁴ and 1,8-bis(dimethylamino)naphthalene (DMAN) ¹³⁵ are suitable for analysis of free fatty acids; 2,5-dihydroxylbenzoic acid (DHB) in acetone can provide lower limitation of detection for analysis of DAG and TAG ¹³⁶ and it is also a useful matrix for analyzing the molecular species of zwitterionic glycerophospholipids (PC and PE), ^{137, 138} and lyso-phosphatidic acid. ¹³⁹ Sphingosine-1-phosphate was detectable by using an inorganic zinc complex ([1,3-bis[bis(pyridine-2-ylmethyl)amino]propan-2-olato]dizinc(II)) as the matrix. ¹⁴⁰

Up to date, most of these studies by MALDI-MS have been focused on the analysis of PC class, mainly due to its higher sensitivity that results from the stable quaternary ammonium moiety in comparison to other cellular lipid classes. ¹²⁰ Direct analysis of individual species of other lipid classes present in a lipid extract is suppressed in the positive-ion mode due to the presence of PC molecular species. Unlike in ESI-MS, analysis of acidic phospholipids (e.g., PE) is also not sensitive, owing to the presence of severe source fragmentation with most matrices in the negative-ion mode of MALDI-MS in which fatty acyl carboxylates are predominant. Therefore, it seems necessary to conduct the pre-chromatographic separation of different lipid classes by HPLC or TLC, followed by analysis of these individual lipid classes in the positive- or negative-ion mode by using a acidity matrix such as *para*-nitro-aniline (PNA) as previously described. ¹⁴¹

Recently, it has been reported that very low-abundance sulfatide molecular species can be directly quantitated from crude lipid extracts by MALDI-TOF/MS in the negative-ion mode with 9-AA as matrix ¹⁴² (Figure 7). MALDI-Q-TOF/MS also proved to be a powerful hybrid spectrometer to obtain structural information on various lipids (e.g., PC, PE, PI, SM, TAG, and DAG), which allows rapid characterization of the different classes, including the fragmentation of sodium and lithium adducts of these lipids. ¹⁴³

Regardless MALDI-MS offers many advantages for analysis of lipid species such as speed, convenience, high sensitivity (pmol), repeatability (samples can often be re-analyzed at a later time), and high throughput, limitations are also obvious. First, the lipid analysis in the low mass-to-charge region is complicated with the general presence of severe matrix background. Second, the presence of multiple adducts and/or ion forms of each lipid molecular species as a common phenomenon not only complicates the analysis of individual molecular species of a lipid mixture for both identification and quantitation, but also reduces the sensitivity of the analysis. Third, the analytes are distributed in the sample spot heterogeneously with the majority of matrices, due to the presence of lipid-lipid interactions and lipid aggregation during crystallization. Fourth, although the post-source decay in MALDI-MS is useful for structural elucidation, it is problematic for quantitation due to the

differential fragmentational kinetics. Fifth, performing quantitative analysis of lipids by MALDI-MS is far less than ideal because of the other limitations. Therefore, MALDI-MS should be used more as a qualitative tool to rapidly screen the lipid profile of a biological sample than as a quantitative instrument suitable for lipidomics.

3.2.2 MALDI imaging mass spectrometry for lipid profiling of tissue samples—

MALDI imaging mass spectrometry (MALDI-IMS) has been successfully applied to imaging peptides, proteins, drugs, and drug metabolites to determine their distribution and relative concentration.¹⁴⁴⁻¹⁵³ The type of matrices used for MALDI-IMS is similar to that used for MALDI-MS as described above. For example, 9-AA was very suitable to analyze the phospholipids and sulfatides in rat brain tissue sections.¹⁵⁴ For analysis of PC and SM, it was demonstrated that a mixture of dihydroxyacetophenone (DHA), heptafluorobutyric acid (HFBA), and ammonium sulfate as matrix can provide better results for rat brain tissue.¹⁵⁵ Solvent-free matrix methods can be used for lipid analysis by MALDI-IMS.¹⁵⁶

Recently, MALDI-IMS technique has been extended to lipid profiling of single cells,¹⁵⁷ cells from lung,¹⁵⁸ lung tissues,¹⁵⁹ single zooplankton individuals,¹⁶⁰ muscle tissues,^{161, 162} brain tissues,^{127, 163, 164} and even entire bodies.¹⁶⁵ Ion peaks that correspond to PC molecular species are prominent in MALDI-MS analysis of lipid extracts of biological samples in the positive-ion mode, as discussed above, as well as in MALDI-IMS spectra of tissue analysis. Depending on the matrices and the other reagents used in MALDI-IMS, either sodium adducts¹⁶⁰ or protonated molecular species¹⁶³ of PC is displayed abundantly in these spectra of MALDI-IMS. Moreover, many lipid species (e.g., PI, PA, PG, PE, PS, sulfatides, and gangliosides) have been measured from adult mouse brain-tissue sections by MALDI-IMS in the negative-ion mode.¹⁶⁶ MALDI-IMS is becoming an established technique and can be another excellent choice for analysis of lipids.

4 Ion mobility mass spectrometry

Ion mobility mass spectrometry (IM-MS) is a relatively new method emerged in the mass spectrometry,¹⁶⁷ which combines an ion mobility apparatus with a mass spectrometer. In the method, samples are ionized by all kinds of ion source. For example, a radioactive ionization (RI) source and the secondary electrospray ionization (SESI) technique can be used for the vapor sample;^{168, 169} the ESI source can be used well for the liquid sample;^{170, 171} and an MALDI source is commonly used for the solid sample.¹⁷² Ionized molecules in a carrier buffer gas under the electric fields of a drift tube were separated based on the different ion mobilities (e.g., the differences between the charges, sizes, masses, and shapes) of the ions.¹⁷³ Therefore, IM-MS can provide an additional separation and characterization of lipids to the other existing methods for analysis of the complex lipid mixtures of biological samples. Moreover, any chromatographically co-eluting chemical noise can be resolved by IM-MS, leading to an enhanced signal to noise ratio. In this review, we summarized a few examples of IM-MS for analysis of lipids with ESI and MALDI as ion source.

Trimpin et al.¹⁷⁴ took the advantage of multidimensional IM-MS for profiling of phospholipids and other lipid structures in reducing the complexity of lipid mixtures. By

using this approach, they were able to analyze acidic, neutral, and basic lipid species. They also observed that the families of isomeric species could be readily discerned by multidimensional IM-MS.

Kim et al.¹⁷⁵ observed a good correlation between mass and mobility from saturated PC cations. In addition, greater separation and characterization of unsaturated PC cations can be achieved because a 5% reduction in drift time resulted from the double bond in the acyl chain and the drift time was reduced at a rate of ~1% for each additional double bond in the PC class.

The application of IM-MS for identification and quantification of glycerophospholipid isomers was demonstrated by analysis of complex biological extracts without any prior fractionation.¹⁷⁶ It was shown that IM-MS provided sufficient separation for the ions formed from the silver adduction of each phospholipid isomer. Similarly, separation and identification of isomeric and isobaric species of lipids were readily achieved by LC-IM-MS.^{170, 171}

MALDI-IM-MS has previously been used for analysis of phospholipids in complex mixtures in which the 2D separation of phospholipid species based upon plots of drift time *vs.* *m/z*.^{122, 177} It was shown that the head group, the length of fatty acyl chain, the degree of unsaturation, and the cationization of individual species of phospholipids were the important factors leading to the changes in the drift time of phospholipids. The advantages of imaging IM-MS have also been demonstrated from animal tissues in a fully automated and high throughput fashion. The 2D separations based on IM-MS can truly enhance the data dimensionality in the imaging process.^{172, 177}

5 Summary

Many diseases are accompanied with alteration of the lipid profile in the body fluids or tissues. Thus, establishment of the powerful methods for lipidome analysis is very important. Among the available methods in lipidomics, mass spectrometry-based techniques and especially the applications of ESI and MALDI ion source techniques for lipid analyses can provide more useful and reliable information of lipidomics. In this review, the methodologies based on ESI-MS and MALDI-MS for identification and quantitation of individual lipid molecular species are summarized. The potential limitations associated with each method for accurate quantitation of lipids are briefly discussed. In summary, ESI-MS possesses the impressive properties for global lipid analysis of biological lipid extracts and MALDI-MS can provide the fantastic large-scale lipid analyses as an indispensable tool for lipid imaging of biological samples from whole tissues. Research on lipidomics could be dramatically accelerated through combination of these techniques in the future.

ACKNOWLEDGEMENT

This work was supported by the National Institute of General Medical Sciences Grant R01 GM105724 and intramural institutional research funds.

References

1. Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH Jr. Murphy RC, Raetz CR, Russell DW, Seyama Y, Shaw W, Shimizu T, Spener F, van Meer G, VanNieuwenhze MS, White SH, Witztum JL, Dennis EA. *J. Lipid Res.* 2005; 46:839–861. [PubMed: 15722563]
2. Merrill AH Jr. Sullards MC, Allegood JC, Kelly S, Wang E. *Methods.* 2005; 36:207–224. [PubMed: 15894491]
3. Yang K, Cheng H, Gross RW, Han X. *Anal. Chem.* 2009; 81:4356–4368. [PubMed: 19408941]
4. Lecerf JM. *Nutr. Rev.* 2009; 67:273–283. [PubMed: 19386031]
5. Zamaria N. *Reprod Nutr Dev.* 2004; 44:273–282. [PubMed: 15460166]
6. Wijendran V, Hayes KC. *Annu. Rev. Nutr.* 2004; 24:597–615. [PubMed: 15189133]
7. Ginsberg HN, Elam MB, Lovato LC, Crouse JR 3rd, Leiter LA, Linz P, Friedewald WT, Buse JB, Gerstein HC, Probstfield J, Grimm RH, Ismail-Beigi F, Bigger JT, Goff DC Jr. Cushman WC, Simons-Morton DG, Byington RP. *N. Engl. J. Med.* 2010; 362:1563–1574. [PubMed: 20228404]
8. Amarenco P, Labreuche J. *Lancet Neurol.* 2009; 8:453–463. [PubMed: 19375663]
9. Hilvo M, Denkert C, Lehtinen L, Muller B, Brockmoller S, Seppanen-Laakso T, Budczies J, Bucher E, Yetukuri L, Castillo S, Berg E, Nygren H, Sysi-Aho M, Griffin JL, Fiehn O, Loibl S, Richter-Ehrenstein C, Radke C, Hyotylainen T, Kallioniemi O, Iljin K, Oresic M. *Cancer Res.* 2011; 71:3236–3245. [PubMed: 21415164]
10. Adibhatla RM, Hatcher JF. *Antioxid. Redox. Signal.* 2010; 12:125–169. [PubMed: 19624272]
11. Perera R, Riley C, Isaac G, Hopf-Jannasch AS, Moore RJ, Weitz KW, Pasa-Tolic L, Metz TO, Adamec J, Kuhn RJ. *PLoS Pathog.* 2012; 8:e1002584. [PubMed: 22457619]
12. Kishimoto K, Urade R, Ogawa T, Moriyama T. *Biochem. Biophys. Res. Commun.* 2001; 281:657–662. [PubMed: 11237708]
13. Wheelock CE, Goto S, Yetukuri L, D'Alessandri FL, Klukas C, Schreiber F, Oresic M. *Methods Mol. Biol.* 2009; 580:339–368. [PubMed: 19784609]
14. Han X, Jiang X. *Eur. J. Lipid Sci. Technol.* 2009; 111:39–52. [PubMed: 19690629]
15. Han X, Gross RW. *J. Lipid Res.* 2003; 44:1071–1079. [PubMed: 12671038]
16. Han X, Gross RW. *Mass Spectrom. Rev.* 2005; 24:367–412. [PubMed: 15389848]
17. Wenk MR. *Nat. Rev. Drug Discov.* 2005; 4:594–610. [PubMed: 16052242]
18. van Meer G. *EMBO J.* 2005; 24:3159–3165. [PubMed: 16138081]
19. Feng, L.; Prestwich, GD., editors. *Functional Lipidomics.* CRC Press, Taylor & Francis Group; Boca Raton, FL: 2006.
20. Pulfer M, Murphy RC. *Mass Spectrom. Rev.* 2003; 22:332–364. [PubMed: 12949918]
21. Griffiths WJ. *Mass Spectrom. Rev.* 2003; 22:81–152. [PubMed: 12820273]
22. Welti R, Wang X. *Curr. Opin. Plant Biol.* 2004; 7:337–344. [PubMed: 15134756]
23. Byrdwell WC. *Oily Press Lipid Library.* 2003; 16:171–253.
24. Schiller J, Suss R, Arnhold J, Fuchs B, Lessig J, Muller M, Petkovic M, Spalteholz H, Zschornig O, Arnold K. *Prog. Lipid Res.* 2004; 43:449–488. [PubMed: 15458815]
25. Hsu, F-F.; Turk, J. *Modern Methods for Lipid Analysis by Liquid Chromatography/Mass Spectrometry and Related Techniques.* Ill. Byrdwell, WC., editor. AOCS Press; Champaign: 2005. p. 61-178.
26. Cooper MJ, Anders MW. *J. Chromatogr. Sci.* 1975; 13:407–411. [PubMed: 1099110]
27. Gross RW, Sobel BE. *J. Chromatogr.* 1980; 197:79–85. [PubMed: 7430309]
28. Gross RW. *Biochemistry.* 1984; 23:158–165. [PubMed: 6419772]
29. Creer MH, Gross RW. *Lipids.* 1985; 20:922–928. [PubMed: 4094522]
30. McCluer RH, Ullman MD, Jungalwala FB. *Adv Chromatogr.* 1986; 25:309–353. [PubMed: 3518344]
31. Robins SJ, Patton GM. *J. Lipid Res.* 1986; 27:131–139. [PubMed: 3958616]
32. Murphy RC, Fiedler J, Hevko J. *Chem. Rev.* 2001; 101:479–526. [PubMed: 11712255]
33. Kilar A, Dornyei A, Kocsis B. *Mass Spectrom. Rev.* 2013; 32:90–117. [PubMed: 23165926]

34. Tam VC, Quehenberger O, Oshansky CM, Suen R, Armando AM, Treuting PM, Thomas PG, Dennis EA, Aderem A. *Cell*. 2013; 154:213–227. [PubMed: 23827684]
35. Wenk MR. *Cell*. 2010; 143:888–895. [PubMed: 21145456]
36. Han X, Yang K, Gross RW. *Mass Spectrom. Rev.* 2012; 31:134–178. [PubMed: 21755525]
37. Blanksby SJ, Mitchell TW. *Annu. Rev. Anal. Chem.* 2010; 3:433–465.
38. Ivanova PT, Milne SB, Myers DS, Brown HA. *Curr. Opin. Chem. Biol.* 2009; 13:526–531. [PubMed: 19744877]
39. Divito EB, Davic AP, Johnson ME, Cascio M. *Anal. Chem.* 2012; 84:2388–2394. [PubMed: 22283789]
40. Nier AO. *Rev. Sci. Instr.* 1947; 18:398–411.
41. Munson MSB, Field FH. *J. Am. Chem. Soc.* 1966; 88:2621–2630.
42. Fenwick GR, Eagles J, Self R. *Biomed. Mass Spectrom.* 1983; 10:382–386.
43. Ayanoglu E, Wegmann A, Pilet O, Marbury GD, Hass JR, Djerassi C. *J. Am. Chem. Soc.* 1984; 106:5246–5251.
44. Jensen NJ, Tomer KB, Gross ML. *Lipids*. 1986; 21:580–588. [PubMed: 3762331]
45. Jensen NJ, Tomer KB, Gross ML. *Lipids*. 1987; 22:480–489. [PubMed: 3626775]
46. Costello CE, Vath JE. *Methods Enzymol.* 1990; 193:738–768. [PubMed: 2074845]
47. Byrdwell WC. *Lipids*. 2001; 36:327–346. [PubMed: 11383683]
48. Weintraub ST, Pinckard RN, Hail M. *Rapid Commun. Mass Spectrom.* 1991; 5:309–311. [PubMed: 1841648]
49. Han X, Gross RW. *Proc. Natl. Acad. Sci. U.S.A.* 1994; 91:10635–10639. [PubMed: 7938005]
50. Kerwin JL, Tuininga AR, Ericsson LH. *J. Lipid Res.* 1994; 35:1102–1114. [PubMed: 8077849]
51. Kim HY, Wang TC, Ma YC. *Anal. Chem.* 1994; 66:3977–3982. [PubMed: 7810900]
52. Marto JA, White FM, Seldomridge S, Marshall AG. *Anal. Chem.* 1995; 67:3979–3984. [PubMed: 8633761]
53. Bresler K, Pyttel S, Paasch U, Schiller J. *Chem. Phys. Lipids*. 2011; 164:696–702. [PubMed: 21827741]
54. Wei Y, Li S, Wang J, Shu C, Liu J, Xiong S, Song J, Zhang J, Zhao Z. *Anal. Chem.* 2013; 85:4729–4734. [PubMed: 23574540]
55. Sjoval P, Lausmaa J, Nygren H, Carlsson L, Malmberg P. *Anal. Chem.* 2003; 75:3429–3434. [PubMed: 14570193]
56. Sjoval P, Lausmaa J, Johansson B. *Anal. Chem.* 2004; 76:4271–4278. [PubMed: 15283560]
57. Altelaar AF, Klinkert I, Jalink K, de Lange RP, Adan RA, Heeren RM, Piersma SR. *Anal. Chem.* 2006; 78:734–742. [PubMed: 16448046]
58. Dufresne M, Thomas A, Breault-Turcot J, Masson JF, Chaurand P. *Anal. Chem.* 2013; 85:3318–3324. [PubMed: 23425078]
59. Shariatgorji M, Svenningsson P, Andren PE. *Neuropsychopharmacology*. 2014; 39:34–49. [PubMed: 23966069]
60. Gode D, Volmer DA. *Analyst*. 2013; 138:1289–1315. [PubMed: 23314100]
61. Raffaelli A, Saba A. *Mass Spectrom. Rev.* 2003; 22:318–331. [PubMed: 12949917]
62. Cai SS, Syage JA. *Anal. Chem.* 2006; 78:1191–1199. [PubMed: 16478111]
63. Kostianinen, R.; Kauppila, TJ. *Modern Methods for Lipid Analysis by Liquid Chromatography/ Mass Spectrometry and Related Techniques*. Ill. Byrdwell, WC., editor. AOCS Press; Champaign: 2005. p. 472–487.
64. Delobel A, Touboul D, Laprevote O. *Eur. J. Mass Spectrom.* 2005; 11:409–417.
65. Fridriksson EK, Shipkova PA, Sheets ED, Holowka D, Baird B, McLafferty FW. *Biochemistry*. 1999; 38:8056–8063. [PubMed: 10387050]
66. Ivanova PT, Cerda BA, Horn DM, Cohen JS, McLafferty FW, Brown HA. *Proc. Natl. Acad. Sci. U.S.A.* 2001; 98:7152–7157. [PubMed: 11416200]
67. Jones JJ, Batoy SM, Wilkins CL. *Comput. Biol. Chem.* 2005; 29:294–302. [PubMed: 16039161]

68. Ishida M, Yamazaki T, Houjou T, Imagawa M, Harada A, Inoue K, Taguchi R. *Rapid Commun. Mass Spectrom.* 2004; 18:2486–2494. [PubMed: 15384179]
69. Hardman M, Makarov AA. *Anal. Chem.* 2003; 75:1699–1705. [PubMed: 12705605]
70. Yang HJ, Park KH, Lim DW, Kim HS, Kim J. *Rapid Commun. Mass Spectrom.* 2012; 26:621–630. [PubMed: 22328215]
71. Wang M, Huang Y, Han X. *Rapid Commun. Mass Spectrom.* 2014; 28:2201–2210. [PubMed: 25178724]
72. Fauland A, Kofeler H, Trotzmuller M, Knopf A, Hartler J, Eberl A, Chitraju C, Lankmayr E, Spener F. J. *Lipid Res.* 2011; 52:2314–2322. [PubMed: 21960706]
73. Cheng H, Jiang X, Han X. *J. Neurochem.* 2007; 101:57–76. [PubMed: 17241120]
74. Zehethofer N, Pinto DM. *Anal. Chim. Acta.* 2008; 627:62–70. [PubMed: 18790128]
75. Han X, Gross RW. *Expert Rev. Proteomics.* 2005; 2:253–264. [PubMed: 15892569]
76. Han X, Yang K, Yang J, Fikes KN, Cheng H, Gross RW. *J. Am. Soc. Mass Spectrom.* 2006; 17:264–274. [PubMed: 16413201]
77. Han X, Gross RW. *J. Am. Soc. Mass Spectrom.* 1995; 6:1202–1210. [PubMed: 24214071]
78. Yang K, Han X. *Metabolites.* 2011; 1:21–40. [PubMed: 22905337]
79. Han X, Yang K, Gross RW. *Rapid Commun. Mass Spectrom.* 2008; 22:2115–2124. [PubMed: 18523984]
80. Stahlman M, Ejsing CS, Tarasov K, Perman J, Boren J, Ekroos K. *J. Chromatogr. B.* 2009; 877:2664–2672.
81. Han X, Gross RW. *Anal. Biochem.* 2001; 295:88–100. [PubMed: 11476549]
82. Wang M, Han RH, Han X. *Anal. Chem.* 2013; 85:9312–9320. [PubMed: 23971716]
83. Wang M, Hayakawa J, Yang K, Han X. *Anal. Chem.* 2014; 86:2146–2155. [PubMed: 24432906]
84. Crane AM, Hua HU, Coggin AD, Gugiu BG, Lam BL, Bhattacharya SK. *Invest Ophthalmol Vis Sci.* 2012; 53:7122–7130. [PubMed: 22956606]
85. Eilertsen HC, Huseby S, Degerlund M, Eriksen GK, Ingebrigtsen RA, Hansen E. *Molecules.* 2014; 19:16373–16380. [PubMed: 25314600]
86. Ivanova PT, Milne SB, Forrester JS, Brown HA. *Mol. Interv.* 2004; 4:86–96. [PubMed: 15087482]
87. Jiang X, Han X. *J. Lipid Res.* 2006; 47:1865–1873. [PubMed: 16682747]
88. Han X, Cheng H. *J. Lipid Res.* 2005; 46:163–175. [PubMed: 15489545]
89. Hsu, FF.; Turk, J. *The encyclopedia of mass spectrometry.* Caprioli, RM., editor. Vol. 3. Elsevier; New York: 2005. p. 430-447.
90. Hsu, FF.; Turk, J. *The encyclopedia of mass spectrometry.* Caprioli, RM., editor. Vol. 3. Elsevier; New York: 2005. p. 473-492.
91. Hsu FF, Turk J. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2009; 877:2673–2695.
92. Tatituri RV, Brenner MB, Turk J, Hsu FF. *J. Mass Spectrom.* 2012; 47:115–123. [PubMed: 22282097]
93. Hsu FF, Turk J. *J. Am. Soc. Mass Spectrom.* 2010; 21:657–669. [PubMed: 20171120]
94. Hsu FF, Wohlmann J, Turk J, Haas A. *J. Am. Soc. Mass Spectrom.* 2011; 22:2160–2170. [PubMed: 21972013]
95. Hsu F-F, Turk J, Thukkani AK, Messner MC, Wildsmith KR, Ford DA. *J. Mass Spectrom.* 2003; 38:752–763. [PubMed: 12898655]
96. Kind T, Liu KH, Lee do Y, Defelice B, Meissen JK, Fiehn O. *Nat. Methods.* 2013; 10:755–758. [PubMed: 23817071]
97. Han X, Gross RW. *J. Am. Chem. Soc.* 1996; 118:451–457.
98. Cheng C, Gross ML. *Mass Spectrom. Rev.* 2000; 19:398–420. [PubMed: 11199379]
99. Lias SG, Liebman JF, Levin RD. *J. Phys. Chem. Ref. Data.* 1984; 13:695–808.
100. Ikonomou MG, Blades AT, Kebarle P. *Anal. Chem.* 1991; 63:1989–1998.
101. Tang L, Kebarle P. *Anal. Chem.* 1991; 63:2709–2715.
102. Gaskell SJ. *J. Mass Spectrom.* 1997; 32:677–688.

103. DeLong CJ, Baker PRS, Samuel M, Cui Z, Thomas MJ. *J. Lipid Res.* 2001; 42:1959–1968. [PubMed: 11734568]
104. Han X, Yang J, Cheng H, Ye H, Gross RW. *Anal. Biochem.* 2004; 330:317–331. [PubMed: 15203339]
105. Christie, WW.; Han, X. *Lipid Analysis: Isolation, Separation, Identification and Lipidomic Analysis*. Fourth. The Oily Press; Bridgwater, England: 2010.
106. Brouwers JF. *Biochim. Biophys. Acta.* 2011; 1811:763–775. [PubMed: 21851861]
107. Myers DS, Ivanova PT, Milne SB, Brown HA. *Biochim. Biophys. Acta.* 2011; 1811:748–757. [PubMed: 21683157]
108. Zoerner AA, Gutzki FM, Batkai S, May M, Rakers C, Engeli S, Jordan J, Tsikas D. *Biochim. Biophys. Acta.* 2011; 1811:706–723. [PubMed: 21875688]
109. Brugger B, Erben G, Sandhoff R, Wieland FT, Lehmann WD. *Proc. Natl. Acad. Sci. U.S.A.* 1997; 94:2339–2344. [PubMed: 9122196]
110. Ekroos K, Shevchenko A. *Rapid Commun. Mass Spectrom.* 2002; 16:1254–1255. [PubMed: 12112281]
111. Liebisch G, Scherer M. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2012:883–884. 141-146.
112. Brugger B. *Annu. Rev. Biochem.* 2014; 83:79–98. [PubMed: 24606142]
113. Shiva S, Vu HS, Roth MR, Zhou Z, Marepally SR, Nune DS, Lushington GH, Visvanathan M, Welti R. *Methods Mol. Biol.* 2013; 1009:79–91. [PubMed: 23681526]
114. Han X, Yang K, Yang J, Cheng H, Gross RW. *J. Lipid Res.* 2006; 47:864–879. [PubMed: 16449763]
115. Han X. *Front. Biosci.* 2007; 12:2601–2615. [PubMed: 17127266]
116. Yang K, Fang X, Gross RW, Han X. *J. Am. Soc. Mass Spectrom.* 2011; 22:2090–2099. [PubMed: 21952772]
117. Han X, Gubitosi-Klug RA, Collins BJ, Gross RW. *Biochemistry.* 1996; 35:5822–5832. [PubMed: 8639543]
118. Koivusalo M, Haimi P, Heikinheimo L, Kostianinen R, Somerharju P. *J. Lipid Res.* 2001; 42:663–672. [PubMed: 11290839]
119. Al-Saad KA, Zabrouskov V, Siems WF, Knowles NR, Hannan RM, Hill HH Jr. *Rapid Commun. Mass Spectrom.* 2003; 17:87–96. [PubMed: 12478559]
120. Petkovic M, Schiller J, Muller M, Benard S, Reichl S, Arnold K, Arnhold J. *Anal. Biochem.* 2001; 289:202–216. [PubMed: 11161314]
121. Eibisch M, Schiller J. *Rapid Commun. Mass Spectrom.* 2011; 25:1100–1106. [PubMed: 21452388]
122. Jackson SN, Ugarov M, Post JD, Egan T, Langlais D, Schultz JA, Woods AS. *J. Am. Soc. Mass Spectrom.* 2008; 19:1655–1662. [PubMed: 18703352]
123. Zhou Z, Ribeiro AA, Lin S, Cotter RJ, Miller SI, Raetz CR. *J. Biol. Chem.* 2001; 276:43111–43121. [PubMed: 11535603]
124. Estrada R, Yappert MC. *J. Mass Spectrom.* 2004; 39:412–422. [PubMed: 15103655]
125. Fuchs B, Schiller J, Suss R, Schurenberg M, Suckau D. *Anal. Bioanal. Chem.* 2007; 389:827–834. [PubMed: 17673987]
126. Lee ES, Shon HK, Lee TG, Kim SH, Moon DW. *Atherosclerosis.* 2013; 226:378–384. [PubMed: 23245508]
127. Jackson SN, Wang HY, Woods AS. *J. Am. Soc. Mass Spectrom.* 2005; 16:2052–2056. [PubMed: 16253515]
128. Dong W, Shen Q, Baibado JT, Liang YM, Wang P, Huang YQ, Zhang ZF, Wang YX, Cheung HY. *Int. J. Mass Spectrom.* 2013; 343:15–22.
129. Kubo A, Satoh T, Itoh Y, Hashimoto M, Tamura J, Cody RB. *J. Am. Soc. Mass Spectrom.* 2013; 24:684–689. [PubMed: 23247968]
130. Schiller J, Suss R, Fuchs B, Muller M, Zschornig O, Arnold K. *Front. Biosci.* 2007; 12:2568–2579. [PubMed: 17127263]

131. Cozzolino R, De Giulio B. *Eur. J. Lipid Sci. Technol.* 2011; 113:160–167.
132. Fuchs B, Schiller J. *Eur. J. Lipid Sci. Technol.* 2009; 111:83–89.
133. Fuchs B, Suss R, Schiller J. *Prog. Lipid Res.* 2010; 49:450–475. [PubMed: 20643161]
134. Shroff R, Muck A, Svatos A. *Rapid Commun. Mass Spectrom.* 2007; 21:3295–3300. [PubMed: 17880046]
135. Shroff R, Svatos A. *Rapid Commun. Mass Spectrom.* 2009; 23:2380–2382. [PubMed: 19575397]
136. Schiller J, Suss R, Fuchs B, Muller M, Petkovic M, Zschornig O, Waschipky H. *Eur. Biophys. J.* 2007; 36:517–527. [PubMed: 17047951]
137. Wallace WE, Arnould MA, Knochemuss R. *Int. J. Mass Spectrom.* 2005; 242:13–22.
138. Gellermann GP, Appel TR, Davies P, Diekmann S. *Biol. Chem.* 2006; 387:1267–1274. [PubMed: 16972796]
139. Tanaka T, Tsutsui H, Hirano K, Koike T, Tokumura A, Satouchi K. *J. Lipid Res.* 2004; 45:2145–2150. [PubMed: 15314093]
140. Morishige J, Urikura M, Takagi H, Hirano K, Koike T, Tanaka T, Satouchi K. *Rapid Commun. Mass Spectrom.* 2010; 24:1075–1084. [PubMed: 20213695]
141. Estrada R, Yappert MC. *J. Mass Spectrom.* 2004; 39:1531–1540. [PubMed: 15578747]
142. Cheng H, Sun G, Yang K, Gross RW, Han X. *J. Lipid Res.* 2010; 51:1599–1609. [PubMed: 20124011]
143. Calvano CD, De Ceglie C, Aresta A, Facchini LA, Zamboni CG. *Anal. Bioanal. Chem.* 2013; 405:1641–1649. [PubMed: 23232957]
144. Stauber J, MacAleese L, Franck J, Claude E, Snel M, Kaletas BK, Wiel IM, Wisztorski M, Fournier I, Heeren RM. *J. Am. Soc. Mass Spectrom.* 2010; 21:338–347. [PubMed: 19926301]
145. Grey AC, Chaurand P, Caprioli RM, Schey KL. *J. Proteome Res.* 2009; 8:3278–3283. [PubMed: 19326924]
146. Andersson M, Groseclose MR, Deutch AY, Caprioli RM. *Nat. Methods.* 2008; 5:101–108. [PubMed: 18165806]
147. Rowell F, Hudson K, Seviour J. *Analyst.* 2009; 134:701–707. [PubMed: 19305918]
148. Cornett DS, Frappier SL, Caprioli RM. *Anal. Chem.* 2008; 80:5648–5653. [PubMed: 18564854]
149. Hazarika P, Jickells SM, Wolff K, Russell DA. *Angew. Chem. Int. Ed. Engl.* 2008; 47:10167–10170. [PubMed: 19039814]
150. Wiseman JM, Ifa DR, Zhu Y, Kissinger CB, Manicke NE, Kissinger PT, Cooks RG. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:18120–18125. [PubMed: 18697929]
151. Nemes P, Woods AS, Vertes A. *Anal. Chem.* 2010; 82:982–988. [PubMed: 20050678]
152. Manicke NE, Dill AL, Ifa DR, Cooks RG. *J. Mass Spectrom.* 2010; 45:223–226. [PubMed: 20049747]
153. Miura D, Fujimura Y, Yamato M, Hyodo F, Utsumi H, Tachibana H, Wariishi H. *Anal. Chem.* 2010; 82:9789–9796. [PubMed: 21043438]
154. Cerruti CD, Benabdellah F, Laprevote O, Touboul D, Brunelle A. *Anal. Chem.* 2012; 84:2164–2171. [PubMed: 22300189]
155. Delvolve AM, Colsch B, Woods AS. *Anal. Methods.* 2011; 3:1729–1736. [PubMed: 21961026]
156. Hankin JA, Barkley RM, Murphy RC. *J. Am. Soc. Mass Spectrom.* 2007; 18:1646–1652. [PubMed: 17659880]
157. Li L, Garden RW, Sweedler JV. *Trends Biotechnol.* 2000; 18:151–160. [PubMed: 10740261]
158. Fernandez R, Lage S, Abad-Garcia B, Barcelo-Coblijn G, Teres S, Lopez DH, Guardiola-Serrano F, Martin ML, Escriba PV, Fernandez JA. *J. Am. Soc. Mass Spectrom.* 2014; 25:1237–1246. [PubMed: 24760294]
159. Berry KA, Li B, Reynolds SD, Barkley RM, Gijon MA, Hankin JA, Henson PM, Murphy RC. *J. Lipid Res.* 2011; 52:1551–1560. [PubMed: 21508254]
160. Ishida Y, Nakanishi O, Hirao S, Tsuge S, Urabe J, Sekino T, Nakanishi M, Kimoto T, Ohtani H. *Anal. Chem.* 2003; 75:4514–4518. [PubMed: 14632058]
161. Touboul D, Piednoel H, Voisin V, De La Porte S, Brunelle A, Halgand F, Laprevote O. *Eur. J. Mass Spectrom.* 2004; 10:657–664.

162. Goto-Inoue N, Manabe Y, Miyatake S, Ogino S, Morishita A, Hayasaka T, Masaki N, Setou M, Fujii NL. *Anal. Bioanal. Chem.* 2012; 403:1863–1871. [PubMed: 22349342]
163. Jackson SN, Wang HY, Woods AS. *Anal. Chem.* 2005; 77:4523–4527. [PubMed: 16013869]
164. Carter CL, McLeod CW, Bunch J. J. *Am. Soc. Mass Spectrom.* 2011; 22:1991–1998. [PubMed: 21952770]
165. Shahidi-Latham SK, Dutta SM, Prieto Conaway MC, Rudewicz PJ. *Anal. Chem.* 2012; 84:7158–7165. [PubMed: 22827834]
166. Angel PM, Spraggins JM, Baldwin HS, Caprioli R. *Anal. Chem.* 2012; 84:1557–1564. [PubMed: 22243218]
167. Stach J, Baumbach JI. *Int. J. Ion Mobility Spectrom.* 2002; 5:1–21.
168. Steiner WE, Klopsch SJ, English WA, Clowers BH, Hill HH. *Anal. Chem.* 2005; 77:4792–4799. [PubMed: 16053290]
169. Wu C, Siems WF, Hill HH Jr. *Anal. Chem.* 2000; 72:396–403. [PubMed: 10658336]
170. Damen CW, Isaac G, Langridge J, Hankemeier T, Vreeken RJ. *J. Lipid Res.* 2014; 55:1772–1783. [PubMed: 24891331]
171. Baker PR, Armando AM, Campbell JL, Quehenberger O, Dennis EA. *J. Lipid Res.* 2014; 55:2432–2442. [PubMed: 25225680]
172. McLean JA, Ridenour WB, Caprioli RM. *J. Mass Spectrom.* 2007; 42:1099–1105. [PubMed: 17621390]
173. Mclean, JA.; Schultz, JA.; Woods, AS. *Electrospray and MALDI Mass Spectrometry: Fundamentals, Instrumentation, Practicalities, and Biological Applications.* Cole, RB., editor. John Wiley & Sons, Inc.; Hoboken, NJ: 2010. p. 411-439.
174. Trimpin S, Tan B, Bohrer BC, O'Dell DK, Merenbloom SI, Pazos MX, Clemmer DE, Walker JM. *Int. J. Mass Spectrom.* 2009; 287:58–69.
175. Kim HI, Kim H, Pang ES, Ryu EK, Beegle LW, Loo JA, Goddard WA, Kanik I. *Anal. Chem.* 2009; 81:8289–8297. [PubMed: 19764704]
176. Maccarone AT, Duldig J, Mitchell TW, Blanksby SJ, Duchoslav E, Campbell JL. *J. Lipid Res.* 2014; 55:1668–1677. [PubMed: 24939921]
177. Jackson SN, Woods AS. *J. Chromatogr. B.* 2009; 877:2822–2829.
178. Han X, Cheng H, Mancuso DJ, Gross RW. *Biochemistry.* 2004; 43:15584–15594. [PubMed: 15581371]
179. Wang C, Wang M, Zhou Y, Dupree JL, Han X. *Mol. Neurobiol.* 2014; 50:88–96. [PubMed: 24395133]

Figure 1. General structure of glycerol-based lipids. Three building blocks are linked to the hydroxy groups of a glycerol backbone.

Figure 2.

General structure of sphingoid-based lipids. The building block X represents a different polar moiety (linked to the oxygen at the C1 position of sphingoid base). The building block Y represents fatty acyl chains (acylated to the primary amine at the C2 position of sphingoid base) with or without the presence of a hydroxyl group which is usually located at the alpha or omega position. The building block Z represents the aliphatic chains in all of possible sphingoid bases, which are carbon-carbon linked to the C3 position of sphingoid bases and vary with the aliphatic chain length, degree of unsaturation, the presence of branch, and the presence of an additional hydroxyl group.

Figure 3.

The spectra of lipid extracts from the hippocampus, heart, muscle, and brain cortex of mice in the positive-ion mode. The lipid extracts were prepared by a modified Bligh and Dyer method.¹⁰⁵ Positive-ion ESI mass spectra were directly acquired from the diluted lipid extracts by using a TSQ Vantage ESI mass spectrometer (Thermo Fisher Scientific, San Jose, CA) with an automated nanospray device (TriVersa NanoMate, Advion Bioscience Ltd., Ithaca, NY).⁷⁹ PC and TAG denote phosphatidylcholine and triacylglycerol, respectively.

Figure 4.
Schematic diagram of shotgun lipidomics based on intrasource separation directly from a crude extract of a biological sample.

Figure 5.

An example of electrospray ionization mass spectra of lipid classes resolved by intrasource separation from a crude lipid extract of a mouse brain cortex homogenate. Mouse brain cortex lipid extracts were prepared as previously described. 178 ESI mass spectra were acquired in the negative-ion mode after the extracts were diluted to a total lipid concentration of ~ 10 pmol/ μ l with 1:1 chloroform/methanol (v/v) (panel A), acquired in the negative-ion mode from the diluted lipid solution after addition of 50 nmol LiOH/mg of protein (panel B), and acquired in the positive-ion mode from the diluted lipid solution after addition of 50 nmol LiOH/mg of protein (panel C). IS denotes internal standard; PS, PG, PI, PE, PC, FA, and DAG stand for phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidylcholine, fatty acid, and diacylglycerol, respectively. All mass spectral traces are displayed after normalization to the base peak in each individual spectrum.

Figure 6.

An example of a two-dimensional electrospray ionization mass spectrum of a mouse brain cortex chloroform extract in the negative-ion mode. A conventional ESI mass spectrum was acquired in the negative-ion mode directly from a diluted cortex lipid extract prior to analysis of lipid building blocks in the second dimension by precursor-ion (PIS) and neutral loss scanning (NLS) as indicated. Each mass spectral scan was acquired as described previously.¹⁰⁴ IS denotes internal standard; m:n indicates an acyl chain containing m carbon atoms and n double bonds. All mass spectral traces were displayed after normalization to the base peak in each individual spectrum.

Figure 7.

Negative-ion MALDI-TOF/MS analysis of lipid solutions of spinal cord from cerebroside sulfotransferase (CST) null, CST heterozygous, and their wild type littermates. Spinal cord lipid extracts of the wild type (WT) littermate controls (Panel A), CST heterozygous (Het.) (Panel B), and CST null (Panel C) mice at 48 days of age were prepared by a modified Bligh and Dyer procedure. MALDI mass spectrometric analysis of the lipid extracts was performed with 9-aminoacridine as matrix as described previously.¹⁴² Mass spectra are displayed after normalization to the internal standard (IS) peaks (i.e., the peaks corresponding to IS are equally intense in each spectrum) for direct comparisons. PS and PI stand for phosphatidylserine and phosphatidylinositol, respectively. (Reprinted from the ref.¹⁷⁹ with permission).