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Analytical Methods in Lipidomics and Their Applications

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CONTENTS

As a branch of metabolomics, lipidomics is aimed at full analysis of lipid species and their biological roles with respect to health and diseases and has attracted increasing attention of biological and analytical scientists. As lipidomics has been investigated for almost 10 years, there have been several reviews about it. In this review, we focus on the recent advances in analytical methods of lipidomics, especially in the past 2 years. Herein, an overview of mass spectrometry (MS)-based methods, chromatography-based methods, and spectroscopy methods are presented. The applications of these analytical methods in several lipidomics fields are also simply discussed based on research published in the last 2 years.

Lipids play multiple and critical roles in cellular functions, such as composing the membrane bilayer, providing an appropriate hydrophobic environment for membrane proteins and their interactions, and participating in cell growth, multiplication, and death. Simultaneously, some lipids are messengers in cell signaling transduction processes[1,2] and can be utilized as biomarkers of some diseases.[3−5]

Unlike other biomolecules, lipids are not characterized by a certain individual structure. The LIPID MAPS consortium defined lipids as hydrophobic or amphipathic small molecules that might originate entirely or in part by carbanion based condensations of ketoacyl thioesters and/or by carbocation based condensations of isoprene units.[6] On the basis of this definition, lipids have been divided into eight categories: fatty acyls (FA), glycerolipids (GL), glycerophospholipids (GP), sphingolipids (SP), sterol lipids (ST), prenol lipids (PR), saccharolipids (SL), and polyketides (PK).[6] Each category was further divided into classes, subclasses and, in the case of some subclasses of prenol lipids, fourth-level classes[7] (Table 1).

As for the lipid nomenclature, there are 11 key features adopted by the LIPID MAPS Consortium.[6,7] Among them, some very commonly used features are noted here, such as (1) the use of the stereospecific numbering (sn) to describe GL and GP, (2) the use of E/Z designations (as opposed to trans/cis) to define double-bond geometry, (3) the common term "*lyso*", denoting the position of lacking a radyl group, and (4) abbreviations such as TG(52:1) and DG(34:2), where the numbers within parentheses refer to the total number of carbons and double bonds of all the chains.

With the development of a lipid study, lipidomics was first put forward as a branch of metabolomics in 2003[8] and was defined as "the full characterization of lipid molecular species and of their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation".[9] Since then, lipidomics has attracted more and more attention from both academic and clinical communities and, now, has been considered as an essential tool for investigation of many diseases and physiological processes, such as atherosclerosis, Alzheimer's disease, and some cancers. Moreover, the application fields of lipidomics are constantly becoming wider and wider.

Because of the diversity of lipids, lipidomics analysis presents a challenge in the analytical chemistry area. Mass spectrometry (MS), nuclear magnetic resonance (NMR), and other spectroscopic approaches have become powerful approaches for lipid characterization. The direct infusion MS strategy does not need any previous separation, which makes it less time-

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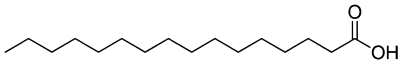
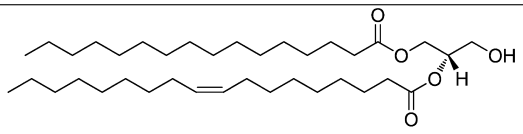
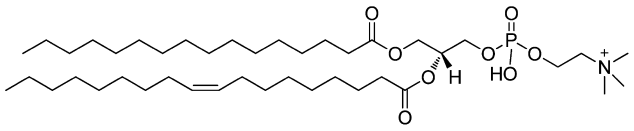
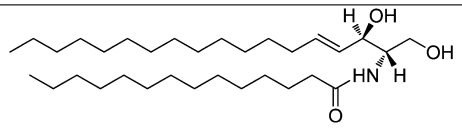
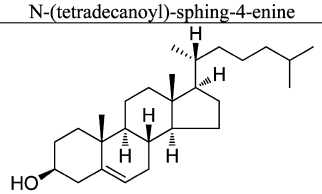
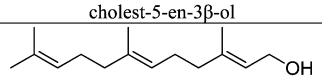
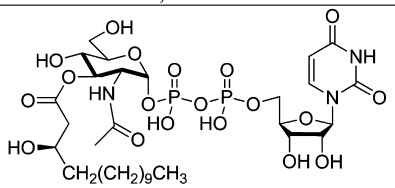
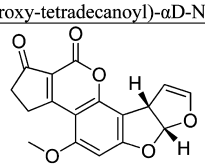
Table 1. Examples of Eight Categories of Lipids

Categories	Structures Examples	Typical Classes: Subclasses
Fatty acyls, FA	 hexadecanoic acid	Fatty acids: straight-chain fatty acids Eicosanoids Fatty alcohols Fatty esters Fatty amides
Glycerolipids, GL	 1-hexadecanoyl-2-(9Z-octadecenoyl)-*sn*-glycerol	Monoradylglycerols: monoacylglycerols Diradylglycerols: diacylglycerols Triradylglycerols: triacylglycerols
Glycerophospholipids, GP	 1-hexadecanoyl-2-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine	Glycerophosphocholines Glycerophosphoethanolamines Glycerophosphoserines Glycerophosphoglycerols Glycerophosphoglycerophosphates Glycerophosphoinositols Glycerophosphoglycerophosphoglycerols
Sphingolipids, SP	 N-(tetradecanoyl)-sphing-4-enine	Sphingoid bases Ceramides Phosphosphingolipids Neutral glycosphingolipids Acidic glycosphingolipids
Sterol lipids, ST	 cholest-5-en-3β-ol	Sterols Cholesterol and derivatives Steroids Bile acids and derivatives
Prenol lipids, PR	 2E,6E-farnesol	Isoprenoids Quinones and hydroquinones Polyprenols
Saccharolipids, SL	 UDP-3-O-(3R-hydroxy-tetradecanoyl)-αD-N-acetylglucosamine	Acylaminosugars Acylaminosugar glycans Acyltrehaloses Acyltrehalose glycans
Polyketides, PK	 aflatoxin B1	Macrolide polyketides Aromatic polyketides Nonribosomal peptide/polyketide hybrids

consuming. There is no doubt that it is the most widely used technology in lipidomics. On the other hand, various separation technologies, such as thin-layer chromatography (TLC), gas chromatography (GC), liquid chromatography (LC), supercritical fluid chromatography (SFC), and capillary electrophoresis (CE), are essential tools for comprehensive analysis of lipids in complex samples. When these are coupled to MS, different hyphenated techniques can provide a huge amount of information on lipidomics. Although there are some drawbacks in using spectroscopic approaches for lipidomics analysis, they should not be ignored for the ability of visualization analysis, which is quite useful in the study of dynamic biological processes. Up until now, thousands of publications on lipidomics analysis can be found, among which several comprehensive reviews have been published.[10−15]

■ ANALYTICAL METHODS OF LIPIDOMICS

Although there are several powerful methods widely used in lipidomics analysis, some novel methods have been developed in recent years. Nowadays, the development of novel methods primarily has three main aims: (1) to focus on the target molecules, the ideal method is specific for the target molecules with high speed and high sensitivity, (2) to explore comprehensive information, the ideal method could detect almost every lipid species simultaneously, (3) to investigate the dynamic biological processes, the ideal method may realize direct visualization for one single cell. Obviously, these three main aims cannot be realized simultaneously using only one method. Therefore, many techniques are used to develop a suitable method for different purposes in lipidomics analysis.

Direct-infusion MS, NMR, and other spectroscopic techniques have provided powerful approaches for lipids identification

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with high sensitivity and high throughput. However, without any separation procedure prior to detection, these methods showed some difficulties in distinguishing all different lipid molecules. As a result, various chromatographic technologies, such as TLC, GC, LC, and CE, usually coupled with MS as a detector, are well accepted in lipidomics research because of their high resolving power. The chromatography-MS hyphenated methods can obtain comprehensive information on almost all lipids in a sample, while chromatography separation may cost more time than direct-infusion MS. Spectroscopy and MS imaging techniques are suitable for investigation of dynamic biological processes, as they can reveal in vivo and real-time analysis.

In recent years, new advances in analytical technologies for lipidomics, such as two-dimensional (2D) NMR, new ionization techniques of MS, the improvement of MS imaging technology, and realization of two-dimensional liquid chromatography (2D LC) have significantly promoted the investigation of lipidomics.

Extraction Methods of Lipids. In order to obtain satisfactory results, extraction of lipids from complex biological matrixes, which aims at removing interfering agents in analysis of lipids, such as proteins, saccharides, or other small molecules, is usually indispensable before analysis. Generally, there are mainly two extraction methods, liquid−liquid extraction (LLE) and solid phase extraction (SPE) for sample preparation in lipidomics analysis.

The most widely used LLE method was developed by Folch and co-workers[16] in 1957 using chloroform/methanol (2:1, v/v) as the extraction solvent, which was improved later by Bligh and Dyer[17] through adding some water or other modifying agent, such as acetic acid, to increase the recovery and to prevent the degradation of lipids. This is a very efficient method for the extraction of both polar and nonpolar lipids, with their ability to penetrate the cell membrane system, higher polarity, and stronger interaction with hydrogen bonds.[18]

Another LLE method is based on a hexane/isopropanol system, with a typical ratio of hexane to isopropanol of 3:2.[19] Compared with the Folch method, the solvents in this method are much less toxic, but this method is not well accepted due to its relatively low extraction efficiency.

Recently, a novel chloroform-free LLE method for total lipid extraction has been developed by Löfgren and co-workers[20] based on a mixture of butanol and methanol (BUME) to extract total lipids from human plasma. The results showed high extraction efficiencies of CE, FC, TAG, DAG, PC, PE, PG, PA, and PS, which were comparable or better than the Folch method. Moreover, the BUME-based method was rapid and high throughput, which could automatically extract 96 samples in only 60 min.

In 2013, Chen and co-workers[21] developed a methyl tert-butyl ether (MTBE)-based LLE method to extract lipids and different classes of metabolites simultaneously. This novel approach realized a comprehensive analysis of metabolites including lipids in the same experiment after a single extraction, thus opening the way for a more complete characterization of lipids metabolism in health and diseases.

Besides the extraction of regular samples, such as blood, tissue, or cells, Gregory et al. in 2013 reported a full fecal lipidome LLE method.[22] This method utilized two separate, complementary extraction chemistries, one is dichloromethane (DCM) and another is a MTBE/hexafluoroisopropanol mixture, along with high pressure cycling. By following analytical LC-MS technique, 304 endogenous lipid species were identified in fecal material, which covered six of the eight categories defined by the LIPID MAPS as well as various related classes and subclasses. This method opened a door to the use of fecal lipid profiling for both scientific and clinical applications, but it still showed some limits, such as complexity of extraction procedures and low efficiencies for the low-abundance lipids.

As for SPE methods,[23,24] normal-phase stationary materials, such as bared or boned silica gel with −CN, $-NH_2$, or diol groups, are preferred in lipidomics analysis, with methanol, hexane, and chloroform as elution solvents. In comparison with LLE methods, SPE reduces the consumption of solvents and time. However, when dealing with a large volume of samples, the recovery will be significantly reduced due to the low peak capacity of SPE.

Whether LLE or SPE is more suitable for sample preparation in lipidomics analysis is a highly controversial topic. In 2012, C. Ferreiro-Vera and co-workers[25] tested both LLE and SPE methods to extract phospholipids from human serum. They compared the extraction efficiency of LLE using different mixtures of nonpolar−polar solvents at different ratios with that of SPE using three different eluents, showing that the highest selectivity and sensitivity was achieved by using SPE with MeOH as the optimum eluent for lipid extraction.

Generally speaking, the most appropriate extraction method depends on the analytical strategies. For nontargeted lipidomics, LLE methods may be preferred to extract more comprehensive lipids. For targeted lipidomics, SPE methods may perform better specificity and extraction efficiency.

Besides LLE and SPE methods, many new extraction methods have been utilized in lipidomics analysis in recent years, e.g., ultrasound-assisted extraction (UAE),[26] solid-phase microextraction (SPME),[27−29] pressurized fluid extraction (PFE),[30] and dispersive liquid−liquid microextraction (DLLME).[31] They all showed high efficiency, especially in targeted lipidomics.

Direct-Infusion MS-Based Lipidomics Analysis. Direct-infusion MS technologies, also known as direct-injection MS technologies, are analytical methods without any preseparation of lipids. They are accurate, reproducible, highly sensitive, and less time-consuming than other methods. Electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) are the most widely used ion sources in direct-infusion MS analysis, while emerged novel ionization techniques also promote the lipidomics investigation. Besides, MS imaging technology can provide visualization and distribution information, which is especially useful for the investigation of many biological processes.

Electrospray Ionization (ESI) MS. The introduction of lipidomics was based on the development of ESI-MS,[8] since then, ESI-MS has become the most frequently used MS method in lipids profiling from complex samples, such as blood,[32−34] fungi,[35−37] cells,[38,39] biofluids,[40,41] and biological tissues.[42−46] The recent progress in high-resolution MS techniques, such as LTQ Orbitrap MS[47−50] and Fourier transfer ion cyclotron resonance mass spectrometry (FTICR-MS),[51−54] has significantly influenced the research of lipidomics, which especially facilitated direct infusion ESI-MS for the simultaneous analysis of multiple lipid classes without the need for prior separation and even for extensive MS/MS analysis.[55] The ultrahigh resolution (≥100 000) provides the ability to separate lipid ions with the same nominal m/z values.

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Combined with higher energy collision dissociation (HCD) as a complementary fragmentation tool, high-resolution MS improves the confidence of molecular species assignment and accuracy of their quantification, especially for low-abundance lipid species.[56,57]

ESI-MS analysis for "shotgun" lipidomics was first presented by Han and Gross in 2003.[8] Since then, shotgun strategy is commonly used in lipidomics.[58−62] There are four main MS/MS modes that are particularly useful in shotgun lipidomics: product ion scan, precursor ion scan, neutral loss scan, and selected reaction monitoring.[63] The modes of product-ion scan, precursor-ion scan, and neutral-loss scan are inter-related. By using all of them to identify individual lipid molecular species, multidimensional mass spectrometry based shotgun lipidomics (MDMS-SL) was built up, creating a robust and highly informative analytical platform for MS analysis of individual lipid molecular species.[47,64] For targeted lipidomics analysis that focuses on one category or even one class of lipids, the shotgun strategy is much more appropriate to specific detection and sensitive quantification, especially after one-step derivatization.[65]

As it is easy to be implemented, nano-ESI is suitable for high-throughput analysis of trace volume samples.[66] In addition, microfluidics system based on ESI has been successfully accomplished,[67] improving the performance of shotgun lipidomics as an automated and high-throughput platform for global analysis of lipids. Because the microfluidics technique can integrate different functions on one single chip, such as lysis of cells, capture of lipids, and elution of captured lipids from a solid phase for microscale purification of lipids, it may present a highly efficient technique of comprehensive lipidomics research.[68]

However, discrimination of isomers of lipids is always a challenge for the direct infusion ESI-MS method. One solution to this problem is to compare the relative intensities of fragment ions. In 2011, Hou and co-workers[69] developed a novel methodology for the determination of stereoisomers of diacyl phospholipids, by observing the phenomenon that lyso-form fragment ions corresponding to the neutral loss of fatty acyl moieties attached to the sn2 position, as free fatty acids (FFA) and as ketenes, exhibited consistently higher intensity than their counterpart ions due to the neutral loss of fatty acyl moieties attached to the sn1 position. In comparison with the stereoisomers, the regio-isomers, with the difference in the double bonds' positions, are even more difficult to be distinguished. Yang et al. developed a novel approach for identification and quantitation of unsaturated FA regio-isomers.[70] They established the linearity by determining the relative or the normalized absolute intensity of the specific fragment ion at varied collision energies for each regio-isomer and the mixture. The slope of this linearity was exponential to the FA isomer composition in individual mixtures (i.e., a linear relationship of the ln(slope) vs the FA isomer composition).

Another solution to distinguish isomers is to bring in other chemicals to induce chemically selective dissociation of ions. Ozone-induced dissociation (OzID)[71] is a typical method to identify regio-isomers as the selective reaction between ozone and carbon−carbon double bonds may generate specific tandem mass spectrum. Radical-directed dissociation (RDD) has also been demonstrated to be able to discriminate lipid regio-isomers.[72,73] Although RDD has disadvantages in that it requires formation of a special adduct ion and has unpredictable complex fragmentations, compared with ozone induced dissociation (OzID), it is able to distinguish not only carbon−carbon double bond positions in regio-isomers but also chain-branching regio-isomers, as a consequence, the free radical driven processes can provide information on a wider array of structural variants.

Matrix-Assisted Laser Desorption Ionization (MALDI) MS. MALDI, like ESI, is one of the earliest ionization techniques used in lipidomics analysis. It is now still widely used in MS methods, especially for lipidomics related disease investigations.[74−78]

Developing and selecting suitable matrixes are very important for optimization of the MALDI-MS method, and new compositions and applications of matrix are continuously reported.[79] In 2012, Preianò et al.[80] described that, by using two different matrixes, 2,5-dihydroxybenzoic acid (DHB) and R-cyano-4-hydroxycinnamic acid (CHCA), proteome and lipidome profiles can be analyzed simultaneously from the same biological samples. This ability to rapidly reveal the overall pattern of changes in both lipidome and peptidome signatures could be of valuable interest for handling large numbers of samples for the purpose of finding new biomarkers.

The potential of simultaneous detection of different chemicals can be considered as an advantage of the MALDI-MS method, since the interest of correlation between different chemicals is increasing. For lipidomics, different MALDI-MS methods have been built up recently to study the interrelation between different lipids[81,82] or lipids with other chemicals, such as related proteins.[81] These simultaneous detection protocols have presented much more information about the interrelation, which may be related to some important biological activity procedures.

However, the quantitation capability and difficulty to hyphenate with other techniques are two main disadvantages of MALDI-MS. Nevertheless, adding internal standard is a good way to overcome this limit.[83] Niklas and co-workers utilized *N*-trifluoroacetyl-phosphatidylethanolamines as an internal standard to fulfill a high-throughput phospholipids quantitation.[84] The method was very sensitive, with the limit of detection for PC below 2 μg, and was easily applicable to any biological samples. On the other hand, Li et al. built up an offline liquid chromatography (LC)-MALDI-MS method in 2011,[85] in which the prior separation of phospholipids by HPLC is very valuable to remove the signal suppressing due to PC and to avoid the possible overlapping. This is a significant attempt to hyphenate separation techniques with MALDI-MS, which may significantly expand the applications of MALDI-MS in related areas.

Other Ionization Techniques Used in Lipidomics Analysis. Besides ESI and MALDI, many other ionization techniques, such as atmospheric pressure chemical ionization (APCI),[86] atmospheric pressure photoionization (APPI),[87] desorption electrospray ionization (DESI),[88] and especially some new "soft" ionization techniques, significantly promote the development of lipidomics investigations.

In 2012, Imbert and co-workers compared ESI, APCI, and APPI, which are all easily hyphenated to LC, for lipidomics analysis.[87] The results showed that ESI ranked first for the analysis of polar lipids, while APCI and APPI were more suitable for the analysis of nonpolar lipids. APCI could detect more lipids containing both polar and nonpolar classes than APPI, while APPI offered higher sensitivity for some low-polar and nonpolar lipids.

Desorption electrospray ionization (DESI) was first introduced by Cooks et al. in 2005[88] and can be used to directly

analyze biological samples, such as tissues or cells, without complex sample pretreatment.[89] DESI allows untargeted analysis and structural characterization of lipids ionized from the near-surface region of samples under ambient conditions. Therefore, DESI has become a high-throughput, powerful, and sensitive ionization technique for 2D and 3D imaging of lipids from unmodified complex biological samples.[90,91] Recently, nanospray DESI has been developed by Roach et al.[92] Since nanospray DESI realizes simultaneous detection of several lipid classes and other metabolites in a single biological tissue,[93,94] it may provide a promising tool for comprehensive lipidomics analysis.

Continuous flow extractive desorption electrospray ionization (CF-EDESI) is a novel ambient ionization technique that is derived from DESI, which was first reported in 2013.[95] This technique is properly applied to the analysis of analytes in "non-ESI friendly" solvents, such as hexane, chloroform, and ethyl acetate. Fatty acids were successfully analyzed by this ionization technique,[95] showing good potentials for lipidomics analysis. Compared to DESI, the CF-EDESI may be easier to be hyphenated to LC.

Surface acoustic wave nebulization (SAWN) was first reported by Yoon et al. in 2012[96] as a novel method to transfer nonvolatile analytes directly from the aqueous phase to the gas phase for MS analysis. The authors listed three advantages of the use of SAWN over conventional ionization methods: (1) ionization occurs from a planar device which facilitates higher throughput and ease of use, (2) SAWN produces ions of low internal energy, thereby preserving more of the labile precursor ions, and (3) no chemical matrix is required. Phospholipids and lipid A were successfully analyzed by detecting both positive and negative ions of them using this method, and fragmentation ions were also detected.

Mass Spectrometry Imaging (MSI) in Lipidomics. MSI is a very popular and powerful tool for lipidomics analysis, as it creates images from individual mass spectra of a biological sample, particularly the complex tissues,[97] although the sensitivity of MSI is not as high as other existing imaging methods like fluorescence imaging. MSI can provide the visualization and distribution information of an individual molecule for the study of many biological processes involving the interaction and dynamic spatial distribution.

The first MALDI images of lipid distribution in tissues were obtained by Touboul et al. in 2004.[98] MALDI MSI is able to detect almost all classes of lipids,[99] which makes MALDI MSI the most widely used imaging method for lipidomics. In recent years, MALDI MSI has expanded its applications to focus on different health or diseases related tissues, such as mice brain,[100] Salamander retina,[101] cotton seed,[102] different parts of the human brain,[103] mice tongue,[104] normal and ischemic rat brain,[105,106] and four main layers of human skin.[107] As selection of the most appropriate matrix of a sample is greatly critical for MALDI MS analysis, new matrixes with improved characteristics for the detection of lipids appear constantly.[108] In 2011, Shanta et al.[109] demonstrated a binary matrix, combined by DHB and CHCA, as an appropriate matrix for phospholipids identification from a single brain tissue section. However, after comparing 12 different matrixes for the detection of lipids in both positive and negative ion modes, Thomas and co-workers[110] strongly recommended that 1,5-diaminonapthalene (DAN) was the most appropriate matrix for lipids imaging. The ability of DAN to offer rich lipid signatures in both positive and negative ion modes is particular useful to better cover and more deeply analyze the lipidome within tissue sections by high spatial resolution MSI, which is helpful to provide meaningful biological and clinical outputs.

DESI imaging has made significant contribution to lipidomics investigation since it was introduced.[111] Recently, the nanospray DESI imaging technology has been implemented,[112] which can significantly increase the spatial resolution and sensitivity of DESI imaging, especially in lipidomics analysis.[113] Combined with multivariate statistical analysis,[114] DESI imaging was applied to diagnose several kinds of cancers, such as renal cell cancer,[115] prostate cancer,[116] bladder cancer,[117] hepatocellular carcinoma,[118] and brain cancer[119] demonstrating that DESI-MS technology has the potential to identify the histology type of tumors. This can significantly help to define tumor margins by measuring the tumor cell concentration in a specimen. Therefore, DESI-MSI offers possibilities of realizing a near-real time tumor surgery guide by providing rapid diagnosis and tumor margin assessment.

As the initial development of the secondary ion MS (SIMS) technique was reported in 1971,[120] SIMS is an older method than ESI and MALDI. However, SIMS joined in the lipidomics field much later than ESI and MALDI.[121] Compared with ESI and MALDI, SIMS has the major advantage on ion imaging capability. SIMS can determine the molecular composition and individual compound localization on a tissue section with very high spatial resolution. Besides, the determination does not need any prior sample treatment, which makes the analyses easier and more straightforward and provides the closest possible to physiological conditions.[122] When combined with a microchannel plate (MCP)/Timepix detector, which was first built up by Kiss and co-workers in 2013,[123] SIMS imaging reached even higher spatial resolution and higher sensitivity due to the parallel detection of ions achieved with the pixelated detector.

As the spatial resolution of SIMS can reach to submicrometer grade, single cell detection by using SIMS becomes possible. It is a remarkable step for lipidomics study. Single cell analysis could give more details of some biological procedures. In 2011, Lanekoff and co-workers[124] used TOF-SIMS in combination with an in situ freeze fracture device enabled the acquisition of ion images from the plasma membrane in single PC12 cells. By incubating cells with different concentrations of deuterated phospholipids (PC and PE) and acquiring ion images at high mass resolution, phospholipids accumulation was shown in the membrane and the surrounding media. The relative amounts of phospholipids accumulation in the membrane depended on the concentration of PC or PE that were observed, which were sufficient to produce significant effects on important cellular processes, such as exocytosis in PC12 cells.

On the other hand, single cell analysis could give more information about intracellular correlation among different molecules. In 2013, Passarelli and co-workers mapped the localization of various intact lipid species across the surface of a single neuron using C60-SIMS.[125] With the high spatial resolution of C60-SIMS, visualization of the relative distribution of different molecules was successfully realized. A region of high colocalization between the vitamin E signal and compiled PC (16:0e/18:1) lipid signal could be seen on the top portion of the neurons soma, which might be caused by some unknown correlation between these two chemicals.

Chromatography-Based Lipidomics Analysis. Chromatography, with a unique high separation capability, is an

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essential tool for comprehensive analysis of complex samples. There are varieties of a commonly used chromatographic method, such as thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), and capillary electrophoresis (CE). They can satisfy different demands of lipidomics analysis. When hyphenated to MS, chromatography methods may provide a large amount of information on the lipidome.

TLC. In the field of lipidomics, TLC and its refined version, high-performance thin-layer chromatography (HPTLC), are indispensable tools, as they are simple, rapid, and inexpensive methods with acceptable resolution of different classes of lipids.[126] Because of the reduction of the particle size and the thickness of the layer, HPTLC[127] obviously increases the resolution compared to TLC. The implementation of 2-D TLC, by separating samples in two orthogonal directions, is also a commonly used technique to provide higher resolution and peak capacity.[128,129] As the realization of micro-TLC, combined with a multidimensional separation technique, a new planar chromatographic and electro-chromatographic chamber, as well as microfluidic paper-based device, the resolution of TLC is significantly increased, so that more than hundreds of spots consisting of low-molecular mass compounds can be separated.[130] This micro-TLC may become a very effective tool for lipidomics analysis.[131]

As well-known, the stationary phase has a great influence on the performance of TLC. The most popular stationary phases for lipid separations are silica gel, alumina, and kieselguhr, whereby silica gel is unequivocally the absolutely dominant phase.[132] Besides, Ag-TLC is also a great choice for lipidomics analysis.[133] In 2012, Dillon et al. first reported a silver-thiolate (AgTCM) chromatographic material as a stationary phase of TLC.[134] Compared to the traditional Ag-TLC, the AgTCM operates under the same principles, separating compounds by degree of unsaturation, but shows considerable advantages in terms of light stability and shelf lifetime. The mixture of fatty acid methyl esters (FAMEs), containing different number of double bonds and fatty acyl chains, can be successfully separated and detected by using this AgTCM TLC.

When combined with MS, such as MALDI-MS,[135−137] DESI-MS,[138] and ESI-MS,[139,140] TLC is important in the investigation and applications of the method in lipidomics. In addition to the direct hyphenation to MS, the TLC-blot-MALDI-MS is also useful for lipids analysis.[141,142] Also other detection methods can also be used to realize rapid visualization, or even quantitation of lipids,[143,144] making TLC a simple, rapid, and high-throughput method.

GC. As most lipids are not volatile and some lipids are easy to be degraded at high temperature, GC faces much more difficulties than TLC and HPLC when it is used for lipidomics analysis independently. In most cases, derivatization of lipids, which may significantly eliminate most structural information about lipid molecular species, is an essential step prior to GC separation. Nevertheless, because of the powerful abilities of isomers separation[133,145] and high sensitivity quantitation,[146] GC is also a powerful tool for lipidomics analysis. Usually, GC is used to analyze several classes of lipids and may present high resolution and sensitivity, especially when focusing on one class. Therefore, a very specific extraction, a preseparation by TLC or HPLC, and a derivatization procedure are important parts of GC-based lipidomic methods. It can sensitively detect most lipids, such as fatty acids,[147−151] phospholipids,[152−154] sphingolipids,[155,156] glycerol lipids,[157,158] cholesterol,[159] and steroids.[160−163]

High oven temperature of GC is essential for analysis of high molecular weight compounds.[164] Therefore, high temperature GC (HTGC) is a routine technique for the analysis of high boiling compounds which are eluted from the column with oven cycling up to >400 °C. Recently, a HTGC-TOF-MS system has been assembled by Sutton and co-workers.[165] This system using readily available equipments has been demonstrated to be viable for the online MS analysis (at up to 430 °C) of a wide range of high molecular weight compounds (m/z 1850), which is suitable for the analysis of very long chain lipids.[166]

Recently, the ionic liquid has been used as a novel stationary of GC column to separate lipids, especially the isomers of lipids. Delmonte et al. discussed the separation of cis and trans isomers of fatty acids,[167] showing successful separation of a series of geometric and positional isomers of monounsaturated fatty acids. By using this method, more than 10 individual peaks were obtained as different isomers of 18:1 FFA. After that, human hair and nail were analyzed as real samples by using this method.[168] It was proved that this ionic liquid GC capillary column could be recommended as the most suitable column for the analysis of total fatty acids or fatty acid methyl esters. It may offer more molecular species information for lipidomics analysis.

In recent years, the multidimensional GC system becomes more and more widely used to analyze complex samples. As two columns with different polarities are coupled together, the main advantage of comprehensive two-dimensional GC is improvement of the chromatographic resolution due to the increase of peak capacity. The two-dimensional GC has been applied to analyze fatty acids profiles in many different real samples, such as wastewater,[169] fish oils,[170] mouse heart,[171] human serum,[172] and various cells,[173,174] and becomes more and more popular in lipidomics analysis.

HPLC. HPLC is a commonly used separation technique in lipidomics with good reproducibility and high resolving power. Besides, HPLC systems are relatively isolated from the environment, a characteristic that can effectively reduce the contact between samples and air, thus avoiding the self-oxidation and degradation of lipids. Also almost all the lipid molecular species can be separated by HPLC. In recent years, the hyphenated technique of HPLC and ESI-MS has become the most widely used method in lipidomics analysis of various biological samples, such as blood,[175,176] cells,[177−179] and tissues.[180,181]

Normal-phase (NP) LC and reversed-phase (RP) LC have both been used for different purposes in lipidomics analysis: the NPLC method is regularly used to separate different classes of lipids based on the polar head groups and the RPLC method is often used to separate different molecular species in one class based on the different fatty-acyl chains. Recently, HILIC[182] and a mixed-mode column[183] have been introduced to lipids separation. The separation of lipids by using the HILIC column is similar to the NPLC column.[184] Although its resolution is a little lower than NPLC, HILIC uses the miscible mobile phases with RPLC, showing the potential of the two-dimensional LC (2D LC) method over NPLC. Compared to traditional RPLC, the mix-mode column shows similar separation principles with higher resolution.[183]

With the development of stationary phases, ultrahigh performance LC (UHPLC) columns and the core−shell

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columns have been used to bring the revolution of HPLC.[185] They both provide much higher resolution and are less time-consuming than traditional HPLC.[162] Although core−shell columns have less resolution than UHPLC, the lower column pressure makes less demands less of device,[77,186] but UHPLC columns are more widely used than core−shell columns in lipidomics. Nowadays, the UHPLC-MS technique has become a regular tool for lipids profiling of complex biological samples, including blood,[187−189] urine,[190] fungi,[191] plant,[192,193] cell,[194,195] and tissue.[196,197] Besides the revolution of stationary phase particles, the reduction of the inner diameter of the column, accompanied with the reduction of the flow rate of the mobile phase, leads to nano-LC[198] or capillary LC,[199] which could further increase detection sensitivity for trace amount of samples.[200] Recently, nano-LC has shown good performance for lipidomics analysis,[201] with the limit of detection close to femtomoles.[202]

The progress of LC-MS methods for lipidomics analysis is going primarily in two directions, targeted analysis and comprehensive analysis. To focus on target molecules, LC-MS methods are developed for highly sensitive detection of one class or even several species of lipids, such as fatty acids,[203] eicosanoids,[204] ceramides,[205] TAGs,[57] sterols,[162] and cardiolipins[206,207] in the recent 2 years. Two-dimensional LC (2D LC) is the best way to obtain comprehensive information on lipidomics, as the NPLC is powerful to discriminate different classes of lipids and the RPLC high resolution performance for different fatty acid chains in each class. However, as the mobile phase of NPLC is immiscible to RPLC, offline 2D LC could be established easily without much demand of the device.[208] The fractions are collected manually after a first dimensional separation and then are injected to a second dimensional column for further separation.[209]

In 2010, Nie et al.[210] first demonstrated a comprehensive online 2D LC system with a solvent evaporative interface for lipidomics analysis. The solvent evaporative interface can successfully resolve the incompatibility between the mobile phases from two dimensions. As a result, the ion suppression effects can be reduced and the sensitivity can be improved. In this work, we profiled the lipids from rat peritoneum, identified 721 lipid species from 12 lipid classes, and found 32 potential biomarkers of long-term peritoneum dialysis. On the basis of this system, Li et al.[211] improved this 2D LC system by updating a 10-port, 2 position valve as the interface. As two loops were used to trap and transfer the first dimensional elute to the second dimension separately, this new interface suppressed the sample band broadening in the first dimensional column, increased the recovery and repeatability of 2D LC interface, and offered the possibility for the realization of the non-stop-flow NP/RP 2D LC system for high-throughput separation.

SFC. SFC is another high-resolution technique that can be used for the separation of various compounds including lipids. SFC-MS/MS methods can be utilized for comprehensive lipid profiling, biomarker discovery, and high-throughput screening of large numbers of samples. Although the use of SFC in lipidomics is not as common as HPLC, in recent years, as SFC-MS shows the maximum efficiency for the target analysis of lipids in a biological sample that includes many matrixes, this technique finds potential applications in lipidomics analysis.[212]

There are two main types of SFC: open tubular column SFC (OTSFC) and packed column SFC (PC-SFC).[213] OT-SFC can provide high-resolution separation when the sample is very complex and a large number of theoretical plates are mandatory, while PC-SFC provides high analysis speed and large sample capacity for the analysis of minor components and for preparative isolation, which is commonly used for lipidomic analysis now.[214]

Recently, SFC-MS/MS method has been developed for the separation of 12 different classes, from 4 different categories, of lipids. A total of 461 lipids, including phospholipids, ceramides, MAG, DAG, TAG, and cholesterol ester were detected from mouse plasma.[215] SFC-MS/MS also can implement separation of regio-isomers of oxidized PC.[216] Moreover, the sensitivity of SFC-MS/MS could reach to femtomoles when it is coupled online to an Orbitrap MS.[217]

CE. The majority of lipids is hydrophobic and has low UV absorbance; hence, CE with UV detection is rarely used for lipidomics analysis. To solve the problem, using an indirect UV detection can be an alternative. Gao et al.[218] reported a CE-UV method for analysis of six classes of phospholipids, including PE, PC, PI, PG, PS, and PA, which provided an acceptable reproducibility but relatively low sensitivity. Fluorescent labeling technique is also a solution to the low UV absorbance.[219] DAG, PI, PI4P, PI5P, PI(3,4)P2, PIP2, PI(3,5)P2, and PIP3 were separated and detected by CZE-LIF. The RSD of the migration time for all lipids was less than 1%, and the limit of detection could reach to 10−20 mol.

Because most lipids are difficult to be dissolved in water solutions used in common CE, nonaqueous CE (NACE) combined with MS was also used for the analysis of lipids.[220] In this work, phospholipids extracted from the rat peritoneal surface were successfully separated. The nonaqueous medium, because of its low surface tension and high volatility, can enhance the stability of electrospray, increase the efficiency of ionization, reduce the background intensity, and improve the sensitivity.

Open-tubular capillary electrochromatography (OT-CEC) was introduced to the lipidomics field[221] in 2011. The PLs from same class were separated based on different acyl chain length, while different classes of PLs with the same acyl chain length were separated by different polar head groups. Finally, 18 phospholipids from 5 classes were identified from human urinary by using OT-CEC-ESI-MS/MS. Besides, micellar electrokinetic capillary chromatography (MEKC) also made the first step of the lipidomics analysis.[222] PC, lysoPC, PE, PI, and PS were separated by MEKC. Although there were not very satisfactory results about sensitivity or quantitation from this first try, MEKC showed the potential for analysis of lipids from complex samples.

Spectroscopy. In recent years, spectroscopy technologies are surprisingly seldom used in lipidomics. There are several drawbacks of spectroscopy that limit its application, such as hard to discriminate information of individual molecules from a complex mixture, complicated spectrum which demands expert ability to get the information, relatively low sensitivity compared with MS, and so on. However, spectroscopy has the natural advantages of bringing visualization analysis, which is very important for investigation of biological processes. To achieve the aim of one single cell visualization detection, the development of spectroscopy based lipidomics should not be ignored.

Nuclear Magnetic Resonance (NMR). Although NMR spectroscopy is used predominantly in metabolomics for its low time-consumption and response to all organic molecules, it is not commonly used in lipidomics.[223] NMR has relatively low

sensitivity (particularly compared with MS), in the high micromolar to millimolar range, and has a small chemical-shift range, which produces crowded spectra when acquired as a 1D spectrum, meaning that discrimination of resonances from the various compounds in complex mixtures can be difficult.[224] Nevertheless, the distinction of different classes of phospholipids was accomplished by using ^{31}P NMR.[225] Recently, by combining ^{1}H NMR and ^{31}P NMR, a successful analysis of lipids from the plasma and liver of alcoholic liver disease rats has been obtained,[226] which can give a hand of pathogenesis of ethanol mediated liver injury. The 2D ^{31}P,^{1}H COSY NMR spectroscopy also proved to be valuable for the determination of the PL composition of cheese and fish.[227] Variations in the ^{31}P shifts indicated differences in the fatty acid chains attached to the individual PLs. The limit of detection can reach to 4 nmol. 2D NMR may bring new vitality for NMR in lipidomics analysis.

Raman Spectroscopy. Raman spectroscopy offers an attractive alternative for deriving direct, quantitative chemical information in a label-free, nondestructive, and real-time manner at the single-cell level without requiring any exogenous modification of samples. Recently, Raman spectroscopy has been used as an assistance tool to detect any variability of lipids in biological samples.[228,229] In 2011, a single-cell laser-trapping Raman spectroscopy method was used for direct, quantitative, in vivo lipid profiling of oil-producing microalgae.[230] As this approach was demonstrated in the quantitative determination of the degree of unsaturation and transition temperatures of constituent lipids within microalgae, Raman spectroscopy will find more applications in lipidomics.

APPLICATIONS OF LIPIDOMICS ANALYSIS IN LIFE SCIENCE

The pursuit of ideal analytical method is not the ultimate aim of lipidomics. As defined as "the full characterization of lipid molecular species and of their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation", lipidomics is a tool for investigation of life science, such as diseases, cellular biological processes, and so on. Some understanding of the applications is critical for the development of analytical methods. Herein, we simply discuss several applications of lipidomics analysis in life science. Table 2 summarizes the references on disease lipidomics in last 2 years.

Lipidomics of Cancers. The lipid profiles of body fluids reflect the general condition of the whole organism and can indicate the existence of certain diseases such as different cancers. Qualitative and quantitative assessment of lipids in blood and other body fluids could reveal novel biomarkers for early diagnosis of cancer; in addition, it may be useful in monitoring the efficacy and toxicity of anticancer treatment. A series of study on cancers-related lipidomics has been published in recent years, such as prostate cancer,[201,231] breast cancer,[38,224,232] hepatic carcinoma,[69,233] renal cell carcinoma,[234] thyroid papillary cancer,[235] and colorectal cancer.[51]

Since it is very indistinct of the initiation of cancers, the biomarkers of cancers cannot be identified easily. Although currently available data is promising, it is obtained from relatively small groups of donors (tens to hundreds of individuals), hence large-cohort multicenter clinical studies are definitively required. Further development in technology will surely increase resolution and sensitivity, which may allow extraction of all lipid information. In the near future, highly reliable lipidomic data may then be combined along with well-developed "of-the-moment" genomics and proteomics, delivering extended knowledge of lipids metabolism in the context of system biology approaches.

Lipidomics of Alzheimer's Disease (AD). There are many researches on AD-related lipidomics, but the progress is still very slow. Different conclusions could be obtained by using different analytical methods and ganglioside molecular species,[141] sphingomyelins, and ceramides,[59] or total PC and PUFAS, especially FA with 4−6 double bonds,[43] have been considered as potential biomarkers temporarily. With the development of analytical techniques, more comprehensive information was presented and showed so many molecular species changed in AD patients.[236] Therefore, exploration of the metabolic pathway is necessary to figure out real biomarkers. However, the pathway also needs evidence provided by analytical methods. Since each analysis is limited to the technique itself and the number of samples, the progress of disease lipidomics could not be remarkable and great efforts are needed in this field.

Cellular Lipidomics. As mentioned above, lipids play a significant role in many cellular biological progresses, thus cellular lipidomics become an important branch of lipidomics. By investigating modeled cellular biological progresses, lipidomics provides evidence to the predesign or preguessed pathway, thus lipidomics analysis is targeted in this aspect. Different biological progresses have been studied during recent years, such as oxidative stress,[237] epithelial morphogenesis,[58] epithelial-to-mesenchymal transition,[58] cell incubation,[124] de novo sphingolipid biosynthesis,[238] cardiolipin remodeling,[239] fatty acid uptake from exogenous sources,[240] de novo fatty acid synthesis,[240] fatty acid elongation and degradation,[240] and acyl remodeling of cardiolipin.[83]

In addition to traditional detection methods, the imaging technique is very practical for progress investigation, as the direct visualization information is the more convincing evidence than any other detection results. Spectroscopy and MS imaging techniques may greatly promote the cellular lipidomics.

Plants. It is notable that not only human diseases or related cellular biological progresses are useful applications of lipidomics, the lipidome can also reveal important information in plant science. As the genome sequencing of *Arabidopsis thaliana* has been completed, there are many kinds of transgenic *Arabidopsis thaliana* to meet various demands of investigation. A series of *Arabidopsis thaliana* lipidome research was conducted in response to different temperatures and light,[241] demonstrating that the lipidome changed under different temperature and light. In addition, transgenic classes display a different lipidome from *Arabidopsis thaliana*,[242] especially in cold temperature. As using transgenic *Arabidopsis thaliana*, which reduced the sphingolipid D8 unsaturation, the glucosylceramides level was also decreased, and these mutants performed better freezing tolerance.[243] Further studies showed that, after 14 days of cold acclimation, the plants from most accessions had accumulated massive amounts of storage lipids, with most of the changes happening in long-chain unsaturated triacylglycerides, while the total amount of membrane lipids was only slightly changed. This is a sensible evidence to prove that the relative abundance of several lipid species is highly correlated with the freezing tolerance of the accessions, allowing the identification of possible marker lipids for plant freezing tolerance.[244]

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Table 2. Summary of Disease Lipidomics Researches in Latest Two Years

disease	sample	lipids	methods	literature number
	human brain	ganglioside	TLC-Blot/MALDI-TOF MS	245
	human brain tissue	MG, FAE, fatty acids; NAPE	RPLC-ESI-MS (ion trap XCT MS); RPLC-MS (ion trap XCT)	246
	human post mortem brain and mice brain	PC, LPC, pPC	FIA-MS/MS	43
Alzheimer's disease	human plasma	SM, Cer	triple-quadrupole MS	59
	human brain and mice forebrain	TAG, DAG, Cer, GluCER, GM3, LPC, LPCe, LPA, LPS, LPI, LPE, LPEp, PC, ePC, PA, PS, PI, PG, LBPA, PE, pPE, SM, GalCer, CE, Chol, Sulf	NPLC-QQQ MS	236
	plasma and cerebrospinal fluid	sterol	GC/MS and RPLC-APCI MS (LTQ Orbitrap)	161
Alzheimer's disease and Parkinson's disease	human brain tissue	GalCer, CSE, PE, PS, PC, SM, GM1, GD1a, GD1b, GT1b	TLC-Blot and MALDI-QIT-TOF MS	141
arthritis	rat serum	LPS	RPLC-QQQ MS	247
aspirin-intolerant asthma phenotype	exhaled breath condensate	eicosanoid lipids	RPLC-ESI-MS (QQQ) GC/MS	148
atherosclerotic plaques	human carotid plaque, plasma	LPS, PS, LPC, PC, PE, SM, ChoE	Chip-based nanospray + QQQ	248
bladder carcinomas	human bladder tissue	phospholipids (PS, FA, PI)	DESI-MS imaging (LTQ linear ion trap MS)	117
	human breast tissue	Cer, PC, PE, SM, TG, PI, GlcCer	URPLC-QTOF MS	232
breast cancer	EpH4, MC4-L5, and MC4-L2 Cell lines	LPC, SM, PC, PI, PS, PE	TLC and ESI-Qtrap and ESI-QQQ	38
coronary artery disease (CAD)	lipoproteins from human plasma	PC, CE, TAG, PG, PA, PI, LPG, LPA, LPI, LPC	online chip-type asymmetrical flow field-flow fractionation and ESI-MS/MS (LTQ Velos ion trap MS)	249
colorectal cancer	human serum	fatty acids, fatty amines, phospholipids	ESI-MS (Fourier transform ion cyclotron resonance mass spectrometry)	51
	human ileal fluid	bile acids	nano ESI-MS (LTQ-Orbitrap MS)	40
Crohn's disease	peripheral blood mononuclear cells	PC, PI, PS, PE, PG, PA Cer	nanoESI triple quadrupole MS and HPLC-MS	250
cystic fibrosis	human plasma	PC, LPC, TAG, FFA, Chol, CE, OEA, C18:3n-3	RPLC-MS (ion trap XCT MS)	185
depression and anxiety symptoms	human plasma	PC, LPC, PE, PLPE, SM, Cer	ESI-MS/MS (tandem quadrupole MS and ion trap MS)	34
essential hypertension	human plasma	PC, LPC, SM, TG, DG, ChoE	URPLC-MS (IT-TOF MS)	251
hypertension and type-2 diabetes mellitus	human plasma and whole blood	CE, TAG, DAG, LPC, LPE, PC, PCO, PE, PEO, PI, SM, Cer	apheresis by plasma lipidfiltration or whole blood dextran sulfate adsorption, chip-based nanoflow-MS (LTQ Orbitrap MS)	50
hyperlipidemia	human plasma and lipoproteins	PC, LPC, SM, OxPLs	RPLC-ESI-MS/MS (QTrap; triple quadrupole linear iontrap hybrid MS) MALDI-MS/MS (quadrupole iontrap-TOF tandem-MS)	77
inflammation	mice plasma, liver, ileum and adipose tissue	endocannabinoids, eicosanoids	URPLC-MS/MS (Xevo TQ-S MS; TSQ Quantum Discovery MS)	252
	serum and cerebrospinal fluid	PUFA-derived lipid mediators	RPLC-MS/MS (triple quadrupole linear ion trap MS)	253
multiple sclerosis	cerebrospinal fluid	products of lipid peroxidation	RPLC-Q-TOF MS	254
	human serum	LSM, PSM, GalCer, DMPE, DMPC, DMPG, PSM	CapRPLC-QTOF MS	255
neurodegenerative disease PHARC (polyneuropathy, hearing loss, ataxia, retinosis pigmentosa, cataract)	rat brain	MAG, LPS, LPC, LPE, LPG, LPI, LPA, BMP, DAG, PA, PC,PE, PI, PG, PS	RPLC-ESI-MS (TOF MS)	256
obesity	adipose tissue biopsies and 3T3-L1 adipocytes	PC, LPC, Cer, PE, TG; FA; Chol	URPLC-QTOF MS; GC-FID; GC/MS (MSD)	159
osseous arthritis	human plasma and synovial fluid	PC, LPC, SM	mesoporous aluminosilicate extraction, MALDI-TOF MS	80
Parkinson's disease	tissues of visual cortex, amygdala and anterior cingulate cortex	SM, Cer, GM3, SL, PA, PC, LPC, PE, LPE, PI, LPI, PS; DAG, TAG, CE; Chol	NPLC-MS (triple quadrupole/ion trap MS); RPLC-MS (Qtrap MS); RPLC-APCI-MS (Qtrap MS)	257
prostate cancer	human plasma	LPC, PE, ePE, SM, ePC, LPE, PC, PA, PE-Cer, CE, PI, ePS, PS	ESI-MS/MS (triple quadrupole MS)	231

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Table 2. continued

disease	sample	lipids	methods	literature number
	urine	PC, PE, PS, PI, PA, PG	nanoRPLC-ESI-MS/MS (ion trap MS)	201
renal cell carcinoma	urine	MAG, DAG, TAG, PA, PC, PE, PG, Pn, PIP, GL,LPA, LPG, LPE, LPS, LPI	CapRPLC-QTOF MS	234
rheumatoid arthritis	human synovial fluid	PC, LPC, CE, SM,PE,LPE,PI, LPI,TG,FA,Chol; Maresin 1, lipoxin A4, resolving D5, 5S,12S-diHETE	RPLC-MS/MS(IT-MS); RPLC-MS/MS (Qtrap MS)	200
	human plasma	plasmalogens, fatty acids	TLC, GC-FID	153
schizophrenia	human serum and brain tissue	LPC, TG, SM	URPLC-QToF MS	258
schizophrenia and other psychotic disorders	human serum	PC,PE, PG, Cer, PS, PA, MG, DG, TG	URPLC-QToF MS	33
streptozotocin-induced diabetes	renal cortex of rats	SM, HexCer, Cer	MALDI TOF/TOF MS	74
thyroid papillary cancer	human tissue section	PC, SM	MALDI-TOF/TOF-IMS	235
type 1 diabetes	mice and rats plasma, liver, and heart	FA; ceramide, SM, cerebroside	GC-FID; RPLC-ESI-MS/MS (Q Trap MS)	259
	human peripheral plasma	CE, PE, PI, LPC	ESI-MS/MS (triple quadrupole MS)	260
	rat plasma	TG, HDL-c, VLDL-c	LC-MS (IT-TOF)	261
type 2 diabetes mellitus	human plasma	TG, FFA, DAG, PC, LPC, Chol, Cer, CE, NEFA	RPLC-MS (Qtrap MS)	262
	human ApoB-containing lipoproteins	CE, TAG, DAG, PC, LPC, SM, Cer, LPC	HPLC-MS	263
type 2 diabetes mellitus and diabetic nephropathy	human plasma	PE, PG, PI, PS, PC, SM, LPC	NPLC-MS (ion trap MS)	264

CONCLUSIONS

After a decade of development, lipidomics has been considered an essential tool for investigation of many diseases and physiological processes, such as cancers and Alzheimer's disease. In order to separate and determine various lipids with the diversity of structures, many methods have been developed for lipidomics analysis. LLE is one of most often used procedures for sample preparation, and SPE has also shown great potential in this field. MS, Raman, and NMR are powerful approaches for lipid characterization, while direct infusion MS strategies are accurate, reproducible, highly sensitive, and less time-consuming. ESI-MS and MALDI-MS are the most widely used techniques for both comprehensive and targeted analysis, and MS imaging could provide the information on interaction and dynamic spatial distribution of biological samples. Various chromatographic technologies are used for the separation of lipids in complex sample matrixes, especially for comprehensive analysis. TLC is a simple, rapid, and inexpensive technology that can achieve the separation of different lipid classes; GC has the advantage of isomer separation and highly sensitive quantitation, although derivatization of lipids is usually needed. LC is definitely an essential tool for lipid separation, and 2D LC has the highest resolution and shows great applicability when it is coupled online to MS or MS/MS. SFC is suitable for nontargeted lipid profiling due to high resolution, while CE, usually coupled with MS, is gradually accepted in lipidomics research. NMR and Raman possess the advantage of providing visualization analysis even in a single cell, which is very important for the study of biological processes. In recent years, new advances in analytical technologies for lipidomics, such as 2D NMR, new ionization modes of MS, the improvement of MS imaging technology, and the realization of 2D LC have significantly promoted the investigation of lipid profiling and other aspects in lipidomics research.

Nevertheless, with the aims to realize target molecule analysis and to obtain comprehensive information or monitor dynamic biological processes, the development of novel methods is still urgently needed, especially these methods with higher sensitivity for low abundance lipid. In addition, for the purpose of full characterization of lipid molecular species and of their biological roles and functions, great effort is desired in the research on the applications of lipids analytical methods. Moreover, the progress of new techniques may also significantly affect the development of lipidomics analysis methods. To sum up, the exploration of new methods of lipidomics analysis and their applications is still challenging for both analytical chemists and other scientists working in life science areas.

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Notes

The authors declare no competing financial interest.

Biographies

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(NP/RP) two-dimensional (2D) LC-based method. She has twice won first prize in the Guanghua Scholarship of Peking University in 2011 and 2013, respectively, for her research on lipid profiling by using novel NP/RP 2D LC−MS.

Yang Li received her Bachelor's Degree from Wuhan University, China, in 2012 as an Outstanding Student. She won many scholarships, such as Freshman Scholarship and the National Encouragement Scholarship. She is currently a Ph.D. student of Analytical Chemistry at Peking University, China. Her current research activities include developing novel analytical methods for lipidomics analysis and the applications of these methods in clinical study.

Yu Bai graduated from Jilin University, China, with a Bachelor's Degree in chemistry in 1998 and obtained her Ph.D. in chemistry from the Changchun Institute of Applied Chemistry, Chinese Academy of Sciences in 2004. During 2002−2004, she studied at the University of Konstanz, and then she worked at the University of Toronto with a Healthy Research (CIHR) Postdoc Fellowship from 2007 to 2008. After that, she joined the College of Chemistry and Molecular Engineering, Peking University, as faculty member, and now she is an Associate Professor. Her major research interests focus on the applications of nanomaterials in the separation and analysis of biological samples, disease-related metabolomic research, and development of sensitive detection methods for bioactive molecules analysis and screening based on mass spectrometry.

Huwei Liu graduated from Beijing Institute of Technology, China, with a Bachelor's Degree in polymer material in 1982, and received a Ph.D. in applied chemistry in 1990. Then he joined the Department of Chemistry of Peking University and now is a full professor. His research interest focuses on bioseparation and detection, including chromatographic and electromigration techniques as well as their online coupling to mass spectrometry used in pharmaceutical and clinical analysis. He also serves as an associate editor for *Journal of Separation Science* (2010−) and *Analytical Instruments* (in Chinese) (2009−), is a member of the editorial advisory board for *Analytical Chemistry* (2013−) and *Analytical and Bioanalytical Chemistry* (2008−), and a member of the editorial advisory board for more than 10 Chinese scientific journals.

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