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New Frontiers in Lipidomics Analyses using Structurally Selective Ion Mobility-Mass Spectrometry

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Abstract

The growth of lipidomics and the high isomeric complexity of the lipidome has revealed a need for analytical techniques capable of structurally characterizing lipids with a high degree of specificity. Lipids are morphologically diverse molecules that can exist as any one of a large number of isomeric species, and as such are often indistinguishable by mass spectrometry without a complementary separation method. Recent developments in the field of lipidomics aim to address these challenges by utilizing a combination of multiple analytical techniques which are selective to lipid primary structure. This review summarizes two emerging strategies for lipidomic analysis, namely, ion mobility-mass spectrometry and ion fragmentation via ozonolysis.

Keywords

Lipids; lipidomics; mass spectrometry; ion mobility-mass spectrometry; collision cross section; isomers; ozonolysis; molecular structure

1. Introduction

Lipids are an incredibly diverse class of molecules that regulate cellular activity, act as signaling molecules, and serve as the primary constituent of cell membranes, among other vital biological functions. As understanding of their complex roles in biological systems increases, lipids are gaining potential as therapeutic drug targets, especially given the intersection of lipid metabolic pathways with disease states such as cancer and diabetes [1–3]. The increasing relevance of lipids necessitates the development of analytical strategies for the discrimination and identification of these species in biological samples. Lipidomics, the broad scale analysis of the lipid content of a given system, poses unique analytical challenges due to the astounding structural diversity amongst lipids [3,4]. This issue is further complicated in that many lipid species are structural isomers, several examples of which are illustrated in Figure 1(a) for a common phosphatidylcholine species. Potentially

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hundreds of thousands of unique lipid molecules exist in biological samples, but only a small fraction of these have been putatively identified over recent decades. The source of this discrepancy is largely due to lipidomic workflows being generally developed for mass spectrometry (MS), a robust technology capable of identifying unknown species by mass [2–5]. Although MS allows for high-throughput analyses of complex samples, without a complementary separation method, MS measurements alone cannot distinguish isomeric components present in these samples. The necessity of the large degree of lipid isomerism produced in nature has yet to be determined; however, the specific, predictable lipid structural motifs have a distinctly biological basis. As an example, fatty acids (FAs) are used in the biosynthesis of six of the eight categories of lipids defined by the LipidMaps Consortium and therefore represent a useful model system for understanding broader lipid structural trends [6]. Biological synthesis of FAs is a widely conserved process across all eukaryotes and some prokaryotes, and although specific enzymes may differ between species, catalyzed condensation of acetyl-CoA units for chain elongation is generally involved [7,8]. For this reason, most endogenous FA molecules contain an even number of carbon atoms, and additional biological rules govern placement of double bonds via desaturation pathways. Attention to these biological guidelines provides researchers with a powerful tool to leverage in bioinformatics approaches for more sophisticated hypotheses for lipid analyses. As previously stated, one challenge in the field of lipidomics is the discrimination of isomeric species. The true number of naturally occurring isomeric lipid species is unknown, but it is estimated to be in the tens of thousands. The Human Metabolome Database (HMDB) released its most comprehensive update in 2018, along with a new category of metabolites termed “Expected” to represent species of known structures that have yet to be detected in the human body or for which the precise isomer has yet to be formally identified [9]. Over 90% of the 82,274 compounds reported in this category are lipids, indicating the potential scale of the isomer issue. A simple enumeration equation for FAs (Figure 1b) was developed by the authors to estimate the number of theoretical double bond positional isomers (DBPI) and *cis/trans* geometric isomers (GI) that can exist for unmodified (e.g., no hydroxylation, methylation, oxidation), straight chain fatty acids containing 18 carbon atoms, which are among the most abundant FA chain lengths encountered in nature. Established rules governing typical lipid structure were followed, which excludes double bonds positioned at the α - and ω -carbons and adjacent to additional double bonds. Calculation results for these equations are summarized in Table 1 and indicate that hundreds to tens of thousands of possible isomers exist for even the simplest FA lipids. A more rigorous enumeration of lipid isomers has been reported by Schuster et al. using generalized Fibonacci numbers, which in addition to DPBI and GI, considers the allowance of allenic and cumulenenic FAs as well as modifications to the carbon chain. In their analysis, a C18 FA can have over 1×10^9 possible forms [10]. Although only a fraction of these theoretical FA isomer species are expected to occur biologically, it is currently unknown to what extent. Therefore, chemical separation and analysis approaches capable of differentiating various isomeric forms are necessary in order to improve current lipidomic strategies and expand the breadth of analytical information that can be obtained. Moreover, detailed knowledge of experimentally-observed lipid structural trends present in multidimensional analytical data will become critically important in guiding the

development of predictive informatic tools and improve the confidence in lipid annotation and identification.

2. New Directions in Lipidomic Analyses

Recent advances in mass spectrometry and related analytical techniques have helped drive the field of lipidomics forward. In particular, ion activation/fragmentation techniques such as collision-induced dissociation (CID) and electron impact excitation of ions from organics (EIEIO) have enabled the discrimination of *sn*-regioisomers [11–13]. Additionally, techniques which promote carbon-carbon double bond cleavage have been developed in conjunction with MS to identify double bond position, in particular the Paternò-Büchi (PB) and ozonolysis (Oz) reactions [14–16]. However, at this time there is no single technique capable of resolving all types of isomers commonly encountered in lipid samples (Figure 1(a)) [5,17]. Therefore, integration of multiple approaches is necessary to expand lipidome coverage and to differentiate isomeric species.

In this review, we focus predominantly on the utilization of ion mobility-mass spectrometry (IM-MS) for lipidomic analyses and its combination with orthogonal analytical techniques. IM is particularly suited to tackling the specific structural issues endemic to lipidomics discussed previously. The IM technique separates gas-phase ions based on differences in their collision cross section (CCS), a parameter that correlates to the two-dimensional cross sectional area of the molecule in the gas phase [18]. Prior studies indicate that the multidimensional separations resulting from IM-MS analyses are capable of differentiating isomers and delineating molecules into respective biomolecular classes [19,20]. When applied to lipids, IM-MS analyses have previously revealed specific and reproducible mobility-mass correlations related to differences in headgroup, acyl chain length, and degree of unsaturation [21]. The relationship between lipid structure and gas-phase conformation via IM-MS analysis are explored in the following section, with subsequent expansion towards predictive approaches and complementary analytical techniques.

3. Mobility-Mass Trends and Prediction of CCS for Lipid Analyses

One of the more interesting aspects of IM-MS data is the existence of empirical correlations between the analyte ion size (mobility) and its mass, i.e., chemical class trendlines which correspond to the specific conformation that the analyte ion adopts within the anhydrous, gas-phase environment of the IM spectrometer. These mobility-mass correlations are related to the primary structure of the analyte and thus are highly-specific for each biomolecular class [20,22–27]. For lipids, it has been shown that gas-phase packing is inefficient, resulting in a relatively large size-to-mass ratio that allows lipids to be readily differentiated from other molecules within a 2-dimensional IM-MS spectrum. Recent developments in commercial IM-MS instrumentation have allowed for larger numbers of lipids to be measured with high precision and reproducibility, which has facilitated the development of IM-MS lipidomic databases incorporating both size (CCS) and mass measurement information [28,29]. This in turn, has allowed for numerous sub-classes of lipids to be analyzed for specific size-mass trends which can be quantitatively-mapped in support of utilizing IM information for identifying and characterizing unknowns [28–31].

Recent work from the authors' laboratory has focused on mapping IM-MS structural trends for broad classes of lipids (Figure 2a), yielding over 450 CCS measurements ($^{DT}CCS_{N_2}$) for 217 unique lipid species obtained from four classes of glycerophospholipids (PC, PE, PS, PA) and three classes of sphingolipids (GlcCer, Cer, SM) [28]. This dataset was sufficiently large to allow specific structural trends to be quantitatively mapped for each lipid sub-class as well as for different ion forms (e.g., H^+ , Na^+ , H^- , etc.) within each class, shown in Figure 2b for glucosylceramides. As with trends observed in smaller IM-MS studies using DTIMS [32–34] and TWIMS [35,36], the conformational orderings of lipids were found to be well-represented by linear functions [37] and reproducibly influenced by polar headgroup (lipid class), acyl chain length, degree of unsaturation, and ion form. Similar to previous findings [36], sphingolipids were found to adopt larger gas-phase conformations than phospholipids (quantitatively, by approximately 2–6% increase in CCS), with this observation being attributed to the limited degrees of unsaturation in sphingolipids due to the constraint imposed by the *sn1* sphingosine backbone [28]. Linear equations were fitted to groups of lipids differing only in their number of double bonds or acyl chain carbon atoms, and it was found that variations in degree of unsaturation were three to four times as influential on the CCS as alkyl chain length for similar masses. The influence of the ion form on the CCS was found to be correlated to the size of the charge-carrier adduct, i.e., $[M+2Na-H]^+ > [M+K]^+ > [M+Na]^+ > [M+H]^+ > [M-H]^-$.

Zhu and coworkers have recently developed a machine learning model to predict lipid CCS_{N_2} values [29]. Using a commercial DTIMS, the authors first empirically measured 458 lipid $^{DT}CCS_{N_2}$ values across ten classes of glycerophospholipids (PC, PE, PG, PS, PI, PA, LPC, LPE, LPI, LPS), two classes of glycerolipids (TG, DG), and three classes of sphingolipids (SM, Cer, GlcCer). Linear trends for acyl chain length and degree of unsaturation were observed, such as is shown for PE lipids in Figure 2(c). Using this large pool of CCS data, the authors then developed a prediction model called LipidCCS Predictor, which was capable of predicting lipid CCS values with an externally validated median relative error of ca. 1% (Figure 2(d)). The support vector regression-based prediction models were built using optimized molecular descriptors (45 for cations and 66 for anions) to generate a large-scale, theoretical database of lipid CCS values, containing 63,434 predicted lipid CCS values covering 22 common lipid classes [29]. Both the LipidCCS Predictor and LipidCCS database are freely-accessible. For CCS_{N_2} prediction, users can input SMILES structures into LipidCCS Predictor, which currently supports prediction for five commonly observed, singly charged adducts including $[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$, $[M+COOH]^-$, and $[M-H]^-$.

Fernandez and coworkers recently demonstrated another machine-learning approach for predicting lipid CCS values using only 2-dimensional molecular descriptors (numerical representatives of chemical structure) as inputs [30]. From a database of $^{TW}CCS_{N_2}$ measurements published by Astarita and coworkers [38], 195 values representing a wide variety of lipids were selected. An initial machine learning step prioritized 68 molecular descriptors of shape, symmetry, connectivity, chemical diversity, etc. upon which partial least squares (PLS) linear multivariate regression models were used to accurately predict CCS from 2D structures with a root mean square error for CCS prediction (RMSECP) of $1.72 \text{ \AA}^2$ (Figure 2(e)) [30]. These and other machine learning approaches to CCS prediction

are critically important for populating large databases of lipid CCS values where experimental measurements cannot be obtained, such as the case for the near 100,000 lipids which are only theoretically predicted to exist [9].

While the numbers of high-quality, experimentally-measured lipid CCS values have increased drastically within the last few years, there are relatively few initiatives to integrate this data into lipidomic informatic workflows. Recently, McLean and coworkers compiled over 3,800 experimental $^{DT}CCS_{N_2}$ values into an online, interactive resource called the Unified CCS Compendium. In addition to a large number of peptides, carbohydrates, and small molecule metabolites, this Compendium includes over 800 lipid CCS values, all of which have been scaled to reference values in order to make this resource self-consistent [31]. Regression models and predictive statistics were used in the Compendium to describe empirical size-mass correlations, and these mathematical relationships can serve as an identification filter in untargeted experimental workflows. As an example, the identification of an unknown biochemical species from a human serum sample was narrowed from 325 potential chemical formulas to 21 PC lipid isomers using the IM-MS information from the Compendium. This CCS filtering workflow results in higher confidence assignment of chemical class, and the predictive capabilities will support annotation of unknown chemical isomers which are commonly encountered in lipidomic and metabolomic studies. For lipid-specific analysis, Zhu and coworkers have recently developed a software tool known as LipidIMMS Analyzer to support the accurate identification of lipids using IM-MS [39]. The software incorporates a multi-dimensional database comprised of over 260,000 theoretical lipids annotated with predicted m/z , retention time, CCS, and fragmentation information and currently supports multiple data acquisition approaches and IM-MS instrumentation types. A test data set comprised of both lipid standards and biological samples yielded 500-600 lipid identifications through LipidIMMS Analyzer and indicated the increased identification confidence gained by utilizing a multidimensional approach [39]. While these and other IM-MS approaches provide important information for supporting identification of lipids from untargeted studies, comprehensive lipidomics requires more structural details than can yet be gleaned from trend and predictive analyses of CCS. Therefore, additional structurally-specific analytical techniques need to be employed, such as those addressed in the following section.

4. Diagnostic Double Bond Cleavage Coupled to IM-MS

Given the strong influence of molecular structure on biological function, the discrimination of lipid double bond positional isomers (Figure 1(a)) is an analytical challenge of great interest to the lipidomic community. Analyses combining MS and X-ray crystallography have shown that the position and degree of unsaturation of a lipid's acyl chain(s) affect the ability of that species to fit in the binding pocket of an interacting protein partner [40,41]. Given the potential existence of thousands of double bond positional isomers in biological samples, the identification and separation of these species is a laborious yet crucial task. Therefore, it is beneficial to combine multiple stages of analytical techniques to enhance lipid isomer separation and identification [17].

While IM has been successfully applied to the separation of *cis/trans* lipid isomers, the differences in CCS of double bond positional isomers is generally too small to be resolved with current IM instrumentation [25,42]. Thus, to distinguish between double bond positional isomers, alternative strategies are necessary. While multiple analytical strategies have been developed for this purpose, including radical-derived processes such as ultraviolet photodissociation (UVPD) [43], electron impact excitation from organics (EIEIO) [13], and radical-directed dissociation (RDD) [44], here we will focus primarily on ion-molecule reactions that specifically target the C=C double bond.

Ozonolysis (Oz) [15,16,45–49] and Paternò-Büchi (PB) reactions [14,50–53] have both been utilized to target sites of unsaturation in lipids. A detailed comparison of these two reactions can be found in a recent review article, to which this work is complementary [5]. When interfaced with MS, Oz and/or PB reactions induce fragmentation at the C=C double bond, resulting in diagnostic fragment ions whose mass pinpoints the double bond location in the lipid precursor ion. The two approaches have been utilized in both shotgun [16,46,48,54] and HPLC-coupled workflows [55–58] and are capable of readily identifying a multitude of lipid C=C isomers in complex samples. In order to facilitate data analysis, custom software have been developed by both the Xia and Mitchell groups to automate the annotation of reaction products originating from Oz and PB reactions [57,59].

More recently, ozonolysis has been combined with IM-MS as an orthogonal dimension of separation [58,60–62]. Differential mobility spectrometry (DMS) as a front-end separation for ozonolysis has been used for structural characterization of unsaturated PCs, as well as 1-deoxysphingosine and its isomers [60–62]. Conversely, ozonolysis can also be performed prior to IM as part of an LC-Oz-IM-MS workflow (Figure 3(a)). In this case, IM separates the diagnostic ozonolysis fragment ions produced by the reaction, allowing further structural discrimination and improving sensitivity for low abundance fragments. For this workflow, the ozonolysis reaction can be performed either in solution or in the gas phase, with the latter implementation termed OzID [56,58]. The solution-phase method utilizes a low-pressure mercury lamp mounted parallel with the fluidics to convert dissolved oxygen gas into ozone that reacts with lipids before ionization in the ESI source. Alternatively, the gas phase approach requires modification of the IM-MS instrument such that ozone is introduced into the trapping ion funnel, allowing controlled reactions of ozone with lipid ions on timescales of 1-90 ms prior to injection into the IM drift tube. Because the rate of reaction is sufficiently fast, ozonolysis and OzID product ions can be correlated to precursors based on common retention times when LC separation is included.

An example of how ozonolysis can be used to elucidate double bond location is depicted in Figure 3(b), where the IM-MS spectrum of a mixture of PC 18:1(9Z)/18:1(9Z) and PC 18:1(6Z)/18:1(6Z) double bond positional isomers is shown. The primary spectral peaks at m/z 786.6 and m/z 808.6 represent the $[M+H]^+$ and $[M+Na]^+$ cations, respectively. The isomer with double bonds at the 9Z positions reacted with ozone to form products at m/z 676.5 $[M+H]^+$ and m/z 698.4 $[M+Na]^+$, while the isomer with double bonds at the 6Z positions gave products at m/z 634.4 $[M+H]^+$ and m/z 656.4 $[M+Na]^+$. Each of these species possesses a unique drift time, which corresponds to the time taken for that particular ion to traverse through the drift tube. This drift time is proportional to the size and shape of the

molecule in the gas phase, and it has previously been observed that changing the position and orientation of a lipid species' double bond changes the molecule's resulting drift time [25].

Blanksby and coworkers have demonstrated that differences in unique drift times for *cis/trans* isomer precursors allows for correlation to and identification of their product ions in combined OzID-IM experiments [56]. When running a mixture of PE *cis/trans* isomers, namely PE 18:1(9Z)/18:1(9Z) and PE 18:1(9E)/18:1(9E), it was observed that the *cis* isomer exhibited a lower drift time than the *trans* isomer (Figure 3(c)). This relationship was retained post-OzID in the fragment ions, and although the two resulting products had the same mass, they could be identified based upon their IM elution order.

Ion mobility separations and ozonolysis reactions exist as orthogonal techniques, and when combined serve as a powerful analytical tool for lipid isomer discrimination and identification. The work described here illustrates that combined LC-Oz/OzID-IM-MS workflows allow for the differentiation of both *cis/trans* isomers and double bond positional isomers, two of the four isomer types described in Figure 1(a). The ongoing work to integrate these techniques into a standard LC-MS lipidomics workflow will rely on the development of bioinformatics software for the automated assignment of spectral peaks that, once completed, will enable thousands of lipid identifications from a single experiment, bringing the field closer to the capability of cataloguing the entirety of the lipidome.

5. Conclusions

Lipidomics as a field has experienced enormous growth in past decades, but still faces critical challenges due to the structural complexity of lipid species and the high degree of isomerism hindering putative identifications. New analytical developments leverage the highly-conserved rules of lipid biosynthesis on expected lipid structure to more fully characterize lipid samples. In particular, IM-MS analysis of lipids reveals quantitative structural trends based on headgroup, acyl chain length, and degree of unsaturation. Combining IM with targeted ion-molecule reactions such as Oz and PB provide orthogonal dimensions of separation for further discrimination of isomeric species. Future directions to further interrogate lipid structural trends include bioinformatics approaches, such as predicting CCS, and the integration of IM and novel ion activation techniques into existing lipidomic workflows, both of which will promote improved identifications and expansion of the current lipidomic landscape.

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Abbreviations

HMDB	The Human Metabolome Database
MS	Mass spectrometry
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
HPLC	High pressure liquid chromatography
ESI	Electrospray ionization
CID	Collision-induced dissociation
EIEIO	Electron impact excitation of ions from organics
PB	Paternò-Büchi
Oz	Ozonolysis
OzID	Ozone-induced dissociation
UVPD	Ultraviolet photodissociation
RDD	Radical-directed dissociation
IM	Ion mobility spectrometry
IM-MS	Ion mobility-mass spectrometry
CCS	Collision cross section
^{DT}CCS_{N2}	Drift tube CCS measured in nitrogen drift gas
TWIMS	Traveling wave ion mobility spectrometry
DMS	Differential mobility spectrometry
FA	Fatty acid
PA	Phosphatidic acid
PE	Phosphatidylethanolamine
PC	Phosphatidylcholine
PS	Phosphatidylserine
GlcCer	Glucosylceramide
Cer	Ceramide
SM	Sphingomyelin
PI	Phosphatidylinositol

PG	Phosphatidylglycerol
LPC	Lyso-phosphatidylcholine
LPE	Lyso-phosphatidylethanolamine
LPI	Lyso-phosphatidylinositol
LPS	Lyso-phosphatidylserine
TG	Triacylglycerides
DG	Diacylglycerides
GP	Glycerophospholipids
SP	Sphingolipids
GL	Glycerolipids
GI	Geometric isomers
DBPI	Double bond positional isomers

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Highlights:

- Discusses the biological basis for lipid structure and using empirical structural patterns to guide predictive tools
- Reviews lipid mobility-mass trends in light of headgroup, acyl chain length, and degree of unsaturation
- Explores new directions in lipidomic analyses including CCS predictions and double-bond targeted reactions
- Emphasizes combination of multiple analytical approaches for full lipidome coverage

(b) **Enumeration Equations for C18:N Fatty Acid (FA) Isomers** (C = Carbons; N = Double Bonds)

$$DBPI = \sum_{i=1}^{C-2N} \frac{i(i+1)(i+2)}{6} (C-2N-i+1) \qquad GI = 2^N \cdot DBPI - 2 \left[\sum_{i=1}^{C-2N} \frac{1}{2} i(i+1)(C-2N-i+1) \right]$$

Double Bond Positional Isomers (DBPI) *Geometric (cis / trans) Isomers (GI)*

Fig. 1.

(a) Visualization of potential isomeric forms of PC 34:1 including *sn*-regioisomers, double bond positional isomers, geometric *cis/trans* isomers, and chiral stereoisomers. (b) Enumeration equations for the determination of double bond positional isomers and geometric isomers for simple, straight-chain fatty acids.

Fig. 2.

(a) Experimentally measured $^{DT}CCS_{N2}$ vs mass measurements and linear regressions plotted for the two lipid categories, sphingolipids and phospholipids. (b) Quantitative correlations observed within GlcCer cation data corresponding to variation in either acyl chain length (*i.e.* XX:00) or degree of unsaturation (*i.e.* 42:YY). (c) Linear trends for three common PE lipid modifications (colored markers) to investigate the effect of acyl chain length and degree of unsaturation in $[M+H]^+$ ions. (d) Intra-lab validation for correlation of predicted and experimental $^{DT}CCS_{N2}$ values for cations of three lipid classes. (e) Correlation of cross

validation (CV)-predicted and experimental $^{TW}CCS_{N_2}$ with root mean square error of calibration (RMSEC), cross validation (RMSECV), and test set prediction (RMSECP), as well as bias and fitness. Figure 2a adapted from K.L. Leaptrot, J.C. May, J.N. Dodds, J.A. McLean, Ion Mobility Conformational Lipid Atlas for High Confidence Lipidomics, *Nat. Commun.* 10 (2018) 1–9. Figures 2c, 2d adapted with permission from Z. Zhou, J. Tu, X. Xiong, X. Shen, Z.-J. Zhu, LipidCCS: Prediction of Collision Cross-Section Values for Lipids with High Precision To Support Ion Mobility–Mass Spectrometry-Based Lipidomics, *Anal. Chem.* 89 (2017) 9559–9566. Figure 2e adapted with permission from M.T. Soper-Hopper, A.S. Petrov, J.N. Howard, S.S. Yu, J.G. Forsythe, M.A. Grover, F.M. Fernández, Collision Cross Section Predictions Using 2-Dimensional Molecular Descriptors, *Chem. Commun.* 53 (2017) 7624–7627.

Fig. 3.

(a) Example workflows combining IM and ozonolysis techniques. (b) A mixture of PC double bond positional isomers is converted to diagnostic products using LC-Oz-IM-MS. (c) IM elution order of *cis/trans* PE isomers is retained post-ozonolysis, allowing for identification of precursors. Figure 2b is adapted using data from R.A. Harris, J.C. May, C.A. Stinson, Y. Xia, J.A. McLean, Determining Double Bond Position in Lipids Using Online Ozonolysis Coupled to Liquid Chromatography and Ion Mobility-Mass Spectrometry, *Anal. Chem.* 90 (2018) 1915–1924. Figure 3c is adapted with permission

from B.L.J. Poad, X. Zheng, T.W. Mitchell, R.D. Smith, E.S. Baker, S.J. Blanksby, Online Ozonolysis Combined with Ion Mobility-Mass Spectrometry Provides a New Platform for Lipid Isomer Analyses, *Anal. Chem.* 90 (2018) 1292–1300. Copyright 2018 American Chemical Society.

Table 1

Theoretical number of double bond positional isomers and *cis/trans* isomers for unmodified, straight chain fatty acids containing 18 carbon atoms and 0-5 double bonds. Isomer numbers are calculated from the equations presented in Fig. 1b.

Fatty Acid (FA)	Molecular Formula	Exact Mass (Da) ([M-H] ⁻)	Number of Carbons	Number of Double Bonds	Number of Possible Double Bond Isomers	Number of Possible <i>cis</i> / <i>trans</i> Isomers
FA 18:0	C ₁₈ H ₃₆ O	283.2637	18	0	0	0
FA 18:1	C ₁₈ H ₃₄ O	281.2480	18	1	16	30
FA 18:2	C ₁₈ H ₃₂ O	279.2324	18	2	105	392
FA 18:3	C ₁₈ H ₃₀ O	277.2167	18	3	364	2756
FA 18:4	C ₁₈ H ₂₈ O	275.2011	18	4	715	11000
FA 18:5	C ₁₈ H ₂₆ O	273.1854	18	5	792	25104