Automatic Characterization of Lipids from MALDI MS/MS Data Using SimLipid™ Software

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ABSTRACT

Within lipid research mass spectrometry is one of the most sophisticated technologies for identification and quantification of lipids from biological mixtures. MALDI TOF MS analysis has unique advantages for structural characterization of lipids and recently, Phosphatidylcholines or Glycerophosphocholines (PC) have been detected by direct tissue analysis using MALDI-TOF-MS.[1-4] However, the major challenge in mass spectrometric analysis of lipids by MS and MS/MS is the huge amount of data generated in the process. In addition, the structural analysis of lipids by mass spectrometry is not routine and often requires tedious, time consuming manual spectral interpretation. SimLipid™ software is a comprehensive informatics tool for characterizing lipids by MS and MS/MS data which streamlines this type of data analysis.

INTRODUCTION

Recently Phosphatidylcholines or Glycerophosphocholines (PC) have been detected by direct tissue analysis using MALDI-TOF-MS.[1,2,3,4] PCs are easily ionized in positive ion mode using MALDI mass spectrometry. Typical fragments observed in MALDI-MS spectra are corresponding to the loss of trimethylamine, the phosphocholine head group, and acyl groups and have been used for structural characterization of PCs.[1,2] A characteristic fragment, corresponding to the head group is readily detected at *m/z* 184 within MS^2 data acquired of PCs.[4] Additionally MALDI mass spectrometry has been applied to the direct analysis of Tissue (Tissue Imaging) of Lipids in lens tissue and rat brain tissue.[5, 6]
However, these studies did not allow for the structural characterization of PCs in tissue. As shown by the group of Woods, MALDI-TOF/TOF™ analysis of Lipids is extremely useful for the structure elucidation of Lipids. E.g. when analyzing PCs, the dominant neutral loss of trimethylamine was observed besides other fragments.[7] In a later study the same group published data corresponding to 32 Lipid species consisting of phosphatidylethanolamines, phosphatidylglycerol, phosphatidylinositols, phosphatidylserines, and sulfatides. As within their previous publication MALDI TOF/TOF™ analysis was applied to identify the structures of the species, highlighting again the usefulness of the fragmentation observed using this analyzer.[8]
However, the major challenge in mass spectrometric data analysis of Lipid MS and MS/MS data is the huge amount of data generated in the process. Some of the most important aspects of mass spectrometric lipidome data analysis are the high throughput quantitative analysis of crude lipid extracts and structural identification of lipids using precursor and product ion data. However, the structural analysis of Lipids by mass spectrometry is not routine and often requires tedious, time consuming manual spectral interpretation. SimLipid™ software is a comprehensive informatics tool for characterizing lipids using MS and product ion data.

MATERIALS AND METHODS

MALDI Sample Preparation: Lipid samples in different concentrations were prepared using DHB matrix (10 mg/mL) in Dichlormethane, Isopropanol, Acetonitril (2:1:1, v:v:v). Dried droplets were prepared with Lipid solution premixed in glass vials. Lipid standards of known structure were obtained from Avanti Lipids, Alabaster, Alabama, U.S.A. Lipids from commercial fats like olive oil, butter and margarine were analyzed as well, after lipid extraction using Dichlormethane, Isopropanol, Acetonitril (2:1:1, v:v:v).

Mass Spectrometry: Samples were analyzed using the AB SCIEX TOF/TOF™ 5800 in MS reflector mode and MS/MS mode. The instrument utilizes a Nd:YLF laser with λ = 354 nm wavelength and a repetition rate of up to 1000 Hz. Positive ion MS and MS/MS data were collected for all spectra. MS/MS data was acquired using 1 keV collision energy and air as collision gas. For MS/MS mode the operating pressure of the collision cell was set to 5 x 10^{-6} Torr. Up to 2000 laser shots were averaged for MSMS mode spectra, 1000 laser shots in MS mode.

MS data was statistically analyzed using principal component analysis (PCA) in MarkerView™ Software.

Key Features of SimLipid™ Software
- **Robust Lipid Structure Database**
- **Project Management**
- **High Throughput MS Lipid Search and MS/MS data analysis for Structural Elucidation**
- **High Throughput Isotopic Peak Correction of Lipid MS data and relative quantification using internal standards**
- **Mass Spectra Annotation with Identified Lipids and fragments**
- **Generate Reports**
- **Database Search**

RESULTS

MS and MSMS data was acquired in positive ion mode. Figure 1 shows an example of a Lipid standard showing five different Glycerolipids [GL] of the lipid class Triradylglycerols [GL03] and sub Class Triacylglycerols [GL0301]. Interestingly the Triacylglycerols are all detected as sodiated molecular species. As typical for MALDI analysis, all molecular ions are detected as singly charged ions. Several MALDI matrices have been tested for the analysis of Lipids, which include 2,5-dihydroxybenzoic acid (DHB), 2,4,6-trihydroxyacetophenone (THAP), 6-aza-2-thiothymine (ATT) and CMBT.[9,10] For the Lipids analyzed here the DHB matrix gave the best performance at the laser wavelength of λ = 354 nm as used here.
Each of the 5 Triacylglycerols (TAGs) was subjected to MS/MS analysis as well using 1 keV collision energy with air as collision gas. Figure 2 shows the result of an MS/MS spectrum obtained for the TAG at m/z 577.5, which corresponds to the 30:0 fatty acid chain distribution with 3 fatty acids of the same lengths.
As shown in figure 3, the dominant fragments observed in the MALDI MSMS spectrum are the result of the loss of a fatty acid and the loss of the fatty acid as sodium salt. In Figure 4 another example is shown for the TAG 48:0, exhibiting the same fragmentation behavior. Note the fragment ion signals of lower intensity as well, which are separated by mass difference of 14 Da, which indicate the loss of CH2 group, which are a result of the cleavages of C-C bonds within the fatty acid chains. Odd fragment masses indicate saturated fatty acids, while the double bonds can be identified using the losses of 12 Da, indicating the cleavage of a C=C bond. This analysis can be useful to identify the structure of the Lipid on top of the Lipid Species.

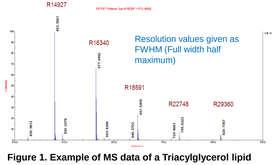

Figure 1. Example of MS data of a Triacylglycerol lipid standard acquired in positive ion mode using the AB SCIEX TOF/TOF™ 5800.

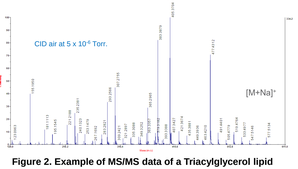

Figure 2. Example of MS/MS data of a Triacylglycerol lipid standard, precursor mass m/z 577.449, acquired in positive ion mode using the
AB SCIEX TOF/TOF™ 5800. The MSMS spectrum was acquired using 1kV lab frame collision energy.

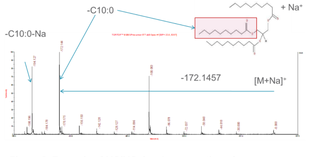

Figure 3. Example of MS/MS data Interpretation of a Triacylglycerol TAG 30:0 lipid standard, precursor mass m/z 577.449, acquired in positive ion mode using the AB SCIEX TOF/TOF™ 5800. The same spectrum as figure 2 is shown, but indicating here the dominant loss of either the C10:0 fatty acid or the corresponding salt [C10:0+Na] as neutral losses.

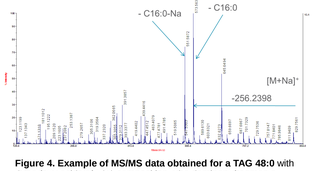

Figure 4. Example of MS/MS data obtained for a TAG 48:0 with three fatty acids of 16:0 composition. The dominant fragments corresponding to the neutral losses and the fatty acid 16:0 itself or the salt –[C16:0+Na] are indicated as well.

The MS/MS data was subjected to analysis in SimLipid™ Software. Figure 5 shows the typical identification result in SimLipid™ Software. The SimLipid™ user interface is organized such that important information is at the user's finger tips (Figure 5). The navigation window on the left makes it easy to move from one spectrum to the next within each project. Search results are displayed in two panes: The search results display pane and the annotation pane. Here the results for the MS/MS of the precursor m/z 577.449 (TAG 30:0) are shown. SimLipid™ ranks them on the basis of the peaks observed in the MS/MS data that correspond with diagnostic ions.
In Figure 6 the MS/MS Annotation tab of the matched m/z values in blue and unmatched m/z values for TAG 30:0 are shown. Additionally the Lipid Structure can be displayed as well (see Figure 6). SimLipid™ can either load MS or MS/MS data. As an examples the MS/MS search parameters, as required for data analysis of the MSMS of the precursor m/z 577.449 (TAG 30:0), are shown in Figure 7. The search results can be filtered using the charge state (in MALDI typical only singly charged molecules are detected), polarity, mass accuracies in MS and MS/MS mode and lipid category.

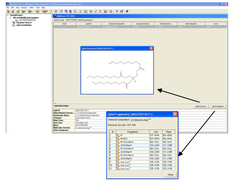

Figure 5. SimLipid™ Software Interface: Search results for TAG 30:0. The results are viewed in the Search Result tab. The MS/MS Annotation tab is shown in Figure 6.

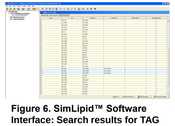

Figure 6. SimLipid™ Software Interface: Search results for TAG 30:0, The MS/MS Annotation tab displays matched m/z values in blue and unmatched m/z values

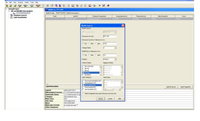

Figure 7. SimLipid™ Search Parameters. The MS/MS Search window displays the required parameters for a lipid search. An example of data input for a MALDI MS/MS spectrum containing sodium adducts is shown. Again the data from MS/MS of the precursor m/z 577.449 (TAG 30:0) was used.

To show the features of SimLipid™ Software, we applied the technology on the analysis of lipids from different origins such as like olive oil brands, margarine and butter. Figure 8 indicates different MS mode spectra obtained for these fats showing distinct differences in their lipid profile. A proper assessment of the differences between the different lipid fractions requires a statistical analysis of the MS data. MS data was compared using MarkerView™ Software, which provides principal component analysis (PCA) among other statistical techniques.
Figure 9 shows the PCA results for the comparison of lipid fractions from different origins. The loadings plot (to the right) indicates the relevant m/z values. In this case the lipid with precursor m/z 907.742 was found to have elevated ion intensities for margarine and olive oils. Figure 10 shows a plot of the intensity profile for this precursor. The MS/MS spectrum was subjected to a search in SimLipid™ Software and resulted in the identification of a TAG 54:3. See the annotation report and the lipid structure in Figure 12. The structure of a TAG 54:3 is show, and additionally some fragment ions annotated by the software, which helped to identify the lipid.

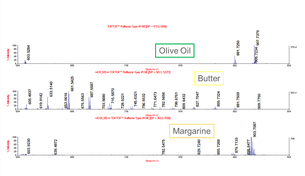

Figure 8. MS profiles for lipid fractions of different origin acquired in reflector mode using the AB SCIEX TOF/TOF™ 5800.

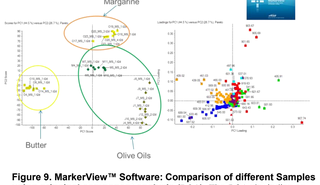

Figure 9. MarkerView™ Software: Comparison of different Samples using principal component analysis (PCA). The PCA plot indicates the separation of the lipid fractions from different origin, in this case from different olive oil brands, margarine and butter. The loadings plot (to the right) indicates the m/z values causing differentiation of the samples.

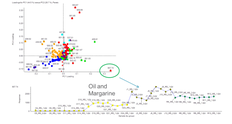

Figure 10 MarkerView™ Software: Intensity profile of m/z 907.742 for olive oil brands and margarine and butter, which indicates higher signal response for this ion in margarine and even higher signal response in olive oils. To identify the molecular species of m/z 907.742 the ion was selected as precursor for an MS/MS experiment. Figure 11 and 12 shows the resulting MS/MS spectrum.

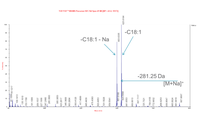

Figure 11. MS/MS of *m/z* 907.742 from olive oil. Some fragment ions are indicated as well.

Figure 12. Identification and annotation of the product ion spectrum of the unknown lipid from olive oil at *m/z* 907.742 using SimLipid™ Software. The structure of a TAG 54:3 was identified.

Additional lipids could be identified within this workflow as well, which include e.g. *m/z* 689.75 from butter which were identified as Diradylglycerols and Diacylglycerophosphogycerols and from Margarine, ID of *m/z* 901.65, which lead to an unsaturated TAG as well (data not shown)

CONCLUSIONS

Because of its large mass range, high sensitivity, and soft desorption and ionization conditions, MALDI time of-flight (MALDI-TOF) and MALDI TOF/TOF™ mass spectrometry are particularly useful in the analysis of Lipids. MALDI MS/MS under high energy-CID conditions supplies highly detailed fragmentation information for the structure elucidation of lipids: Metastable or unimolecular decay spectra of sodiated ions from lipids are dominated by fragment ion signals of losses of the fatty acids and the head group, whereas the high-energy CID MS/MS data additionally exhibits fragments which are the result of a cleavage of carbon-carbon bonds of the fatty acids. The information of the MS/MS data can be used to determine the lipid class or even the lipid species structure using a novel software called SimLipid™, which utilizes a database of lipids for identification. SimLipid™ is a powerful program for the analysis of Lipids. For every precursor m/z, all the possible lipid structures are ranked based on SimLipid's proprietary search and scoring algorithm. Lipids randomly matching the precursor m/z are removed from the list of candidates by filtering out those lipids that do not have any of its characteristic fragments match the product ions observed on the MS/MS spectra. In combination with statistical analysis routines such as PCA that allow for unbiased differentiation of lipid preparations, SimLipid™ Software provides a rapid means of identifying relevant lipid species at high confidence. This was demonstrated identifying unknown lipids from fats, e.g. m/z 907.742, which was identified as TAG 54:3 within olive oil.

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