Automated Lipid Profiling of a Plasma Sample Using Ultra-High Resolution Qq-Time-Of-Flight Impact II™ Mass Spectrometer with SimLipid® Software

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Ningombam Sanjib Meitei[1,2], Himani Gupta[1], Arun Apte[2], Aiko Barsch[3]; Anjali Alving[4]; Juan J Aristizabal Henao[5]; Ken D Stark[5]
[1]PREMIER Biosoft, Indore, India; [2]PREMIER Biosoft, CA, US; [3]Bruker Daltonik GmbH, Bremen; [4]Bruker Daltonics, Billerica, MA; [5]University of Waterloo, Waterloo, Ontario
Corresponding author e-mail: sanjib@premierbiosoft.com

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INTRODUCTION

The field of lipidomics is fueled by analytical technology advances, particularly mass spectrometry and chromatography. The challenges with mass spectrometry based lipidomics lie in the complexity associated with identification of lipids that requires sophisticated software since automated interpretation of lipid MS/MS spectra is more challenging when compared to other biopolymers such as DNA, carbohydrates or peptides, since lipids show much less standardized fragment mass spectra. Each lipid class has its own fragmentation patterns as well as ionization efficiency [1]. We present a workflow for the global lipid profiling of a plasma sample using an LC system coupled to an Impact II, an Ultra-High Resolution Qq-Time-Of-Flight (UHR-QqTOF) mass spectrometer (Bruker Daltonik, Bremen, Germany) and SimLipid software.

METHODS

Sample Preparation: Mixed lipids were extracted from human whole blood using 2:1 chloroform-methanol (Folch) extraction.

HPLC Instrumentation: Agilent 1290 Infinity with Buffer A – 60:40 acetonitrile: water + 10 mM ammonium formate + 0.1% formic acid Buffer B – 90:10 isopropanol: acetonitrile + 10mM ammonium formate + 0.1% formic acid; Column – ACQUITY UPLC CSH C18 2.1 x 100 mm, 1.7 µm; Injection volume – 1 µL; and Column Temperature – 45°C.

MS Instrumentation: Data acquisition was performed with an Impact II QTOF (Bruker Daltonik, Bremen, Germany): Source – ESI positive mode; Calibration – Calibration with sodium formate (m/z 90-1500 Da) using valve switching; Capillary voltage – 4500V.

Data Analysis: SimLipid Software v. 6.00 (PREMIER Biosoft, USA) was used for LC-MS and MS/MS data analysis.

SIMLIPID DATABASE OVERVIEW

The SimLipid database contains 40,298 structures and 1,509,305 MS/MS ions structure-specific in-silico MS/MS characteristic ions. The ions were generated based on sub-class specific fragmentation rules reported in the literature [3-7], and the 529 experimental MS/MS spectra of standard lipids listed in LIPID MAPS [2].

RESULTS AND DISCUSSION

LC-MS data containing MS and data dependent MS2 spectra were processed for LC-peak detection, molecular feature finding, and association of MS/MS scans to detected LC-compounds using the parameters shown in Figure 1. The molecular feature finding algorithm could find 3186 LC-Compounds out of which 673 of them have MS/MS data.

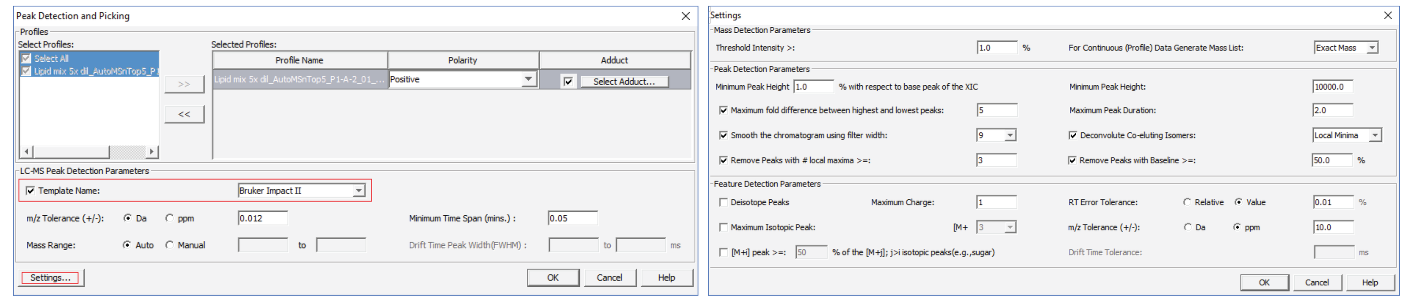


Figure 1: Typical SimLipid software graphical user interface (GUI) showing parameters used for the LC-MS and MS/MS data processing.

MS DATABASE SEARCH

The 3186 LC-compounds were subjected into exact mass database search using 10 ppm as the m/z tolerance. The database search was restricted to thirteen main classes; DAG, TAG, PA, PC, PE, PG, PS, Acidic glycosphingolipids, Ceramides, Neutral glycosphingolipids, Phosphosphingolipids, Sphingoid bases, and Sterols. A total of 528 unique lipids were reported from the database search. For each LC-compound, the lipid with the minimum m/z delta mass – mass difference between the theoretical and the observed m/z values – of all the possible hits was reported as a potential lipid ID. Table 1 shows the summary of the database search result.

S. No.	Lipid Class	Frequency	S. No.	Lipid Class	Frequency
1	TAG	171	8	Ceramides	24
2	DAG	69	9	PE	17
3	PC	63	10	PA	16
4	Sterols	49	11	PS	11
5	Phosphosphingolipids	44	12	Sphingoid bases	4
6	Neutral glycosphingolipids	31	13	Acidic glycosphingolipids	2
7	PG	27		**Total**	**528**

Table 1: Class-wise frequency distribution of the lipids identified from exact mass SimLipid database search using 10 ppm mass tolerance.

MS/MS DATABASE SEARCH

The 673 LC-compounds that have MS/MS data were subjected into SimLipid database search for lipid identification using the parameters shown in Figure 2. The database search was performed with 10 ppm error tolerance for both the precursor and product ions. Figure 3 shows the total ion chromatogram annotated with LC time window for each lipid class.

UHR-QqTOF MS/MS SPECTRA: FRAGMENTATION PATTERNS OF PC, SM, PE, CERAMIDES, DAG, AND TAG LIPIDS

The MS/MS spectra of the identified lipids belonging to a specific class exhibited recognizable patterns.

PC LIPIDS

The UHR-QqTOF MS/MS spectra of PC lipids feature the following structure specific characteristic ions:
1. M: the parent ion
2. M-Fatty Acid chain + H, and M-Fatty Acid chain -H2O + H: the fragment ions formed due to loss of a fatty acid chain, and its daughter ion corresponding to neutral loss of water. SimLipid software represents a fatty acid chain by Number of Carbon: Number of double bond present in the chain. For example, the fragment M-18:2+H denotes the loss of fatty acid chain 18:2 from the parent ion.
3. M-NL+H: Product ion formed due to neutral loss of the phosphocholine headgroup
4. Acylium ion of the fatty acid chains. For example, 17:0 C = O + that confirms the identification of lipid with a fatty acid chain 18:0.

Parent ions of all the identified PC lipids were observed with [M+H]+ ion species. The characteristic ions corresponding to 1, 3, and at least one of the four ions of 2 were observed in the MS/MS spectra. The acylium ion were also observed for some of the PC lipids.

Figure 4 shows fragmentation patterns of the two identified lipids namely (A) PC(18:0/18:2), and (B) PC(16:0/18:1). The MS/MS spectra feature the characteristic ions 1- 3 for both the lipid species.. The observed fragmentation pattern exhibits the reported fragmentation patterns of lipids in ESI-MS workflows in the published literature [8]. Acylium ion is observed for some of the PC lipids.

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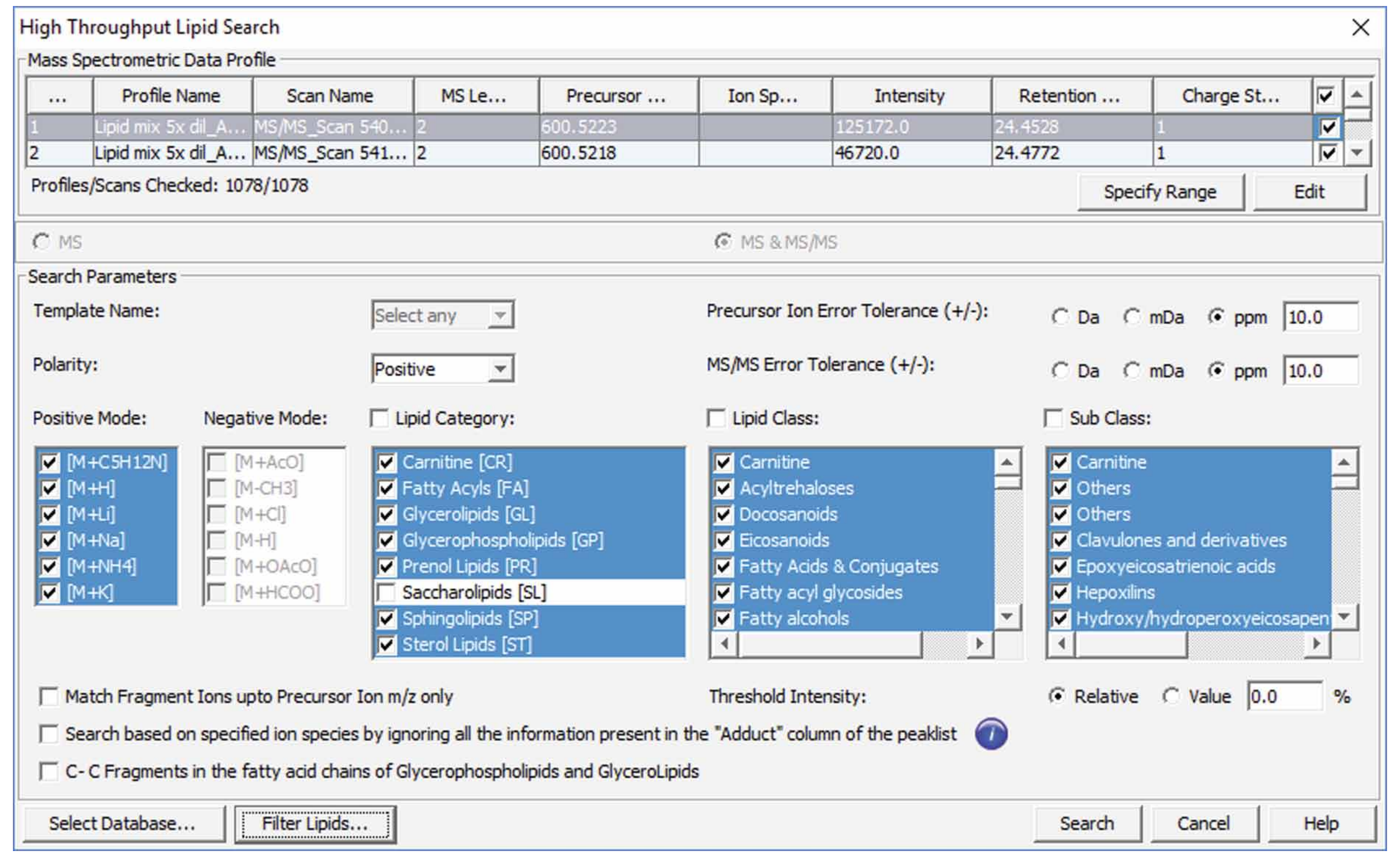


Figure 2: Typical SimLipid software GUI showing parameters for MS/MS database search.

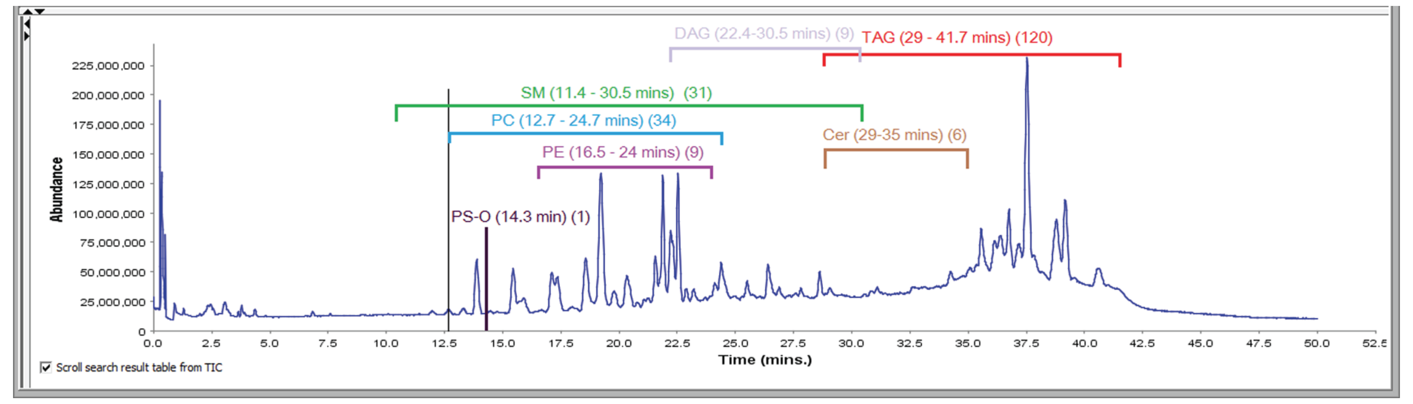

DAG (22.4-30.5 mins) (9)
TAG (29 - 41.7 mins) (120)
SM (11.4 - 30.5 mins) (31)
PC (12.7 - 24.7 mins) (34)
PE (16.5 - 24 mins) (9)
Cer (29-35 mins) (6)
PS-O (14.3 min) (1)
Abundance
Time (mins.)

Figure 3: Typical SimLipid software GUI showing total ion chromatogram (TIC). The TIC is manually annotated with Lipid Class (LC-time window of the lipid class) (No. of lipids belonging to the class).

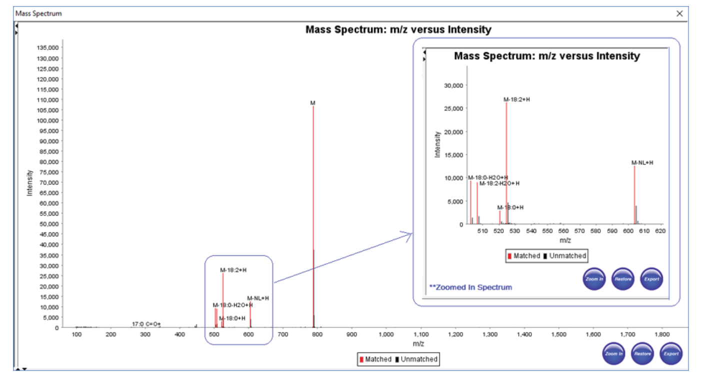


Figure 4(A): Typical SimLipid software GUI showing the MS/MS fragmentation of PC lipids (e.g., PC(18:0/18:2)). The zoomed in spectrum was manually inserted into the figure for clear visualization of the fragmentation pattern of the identified lipid.

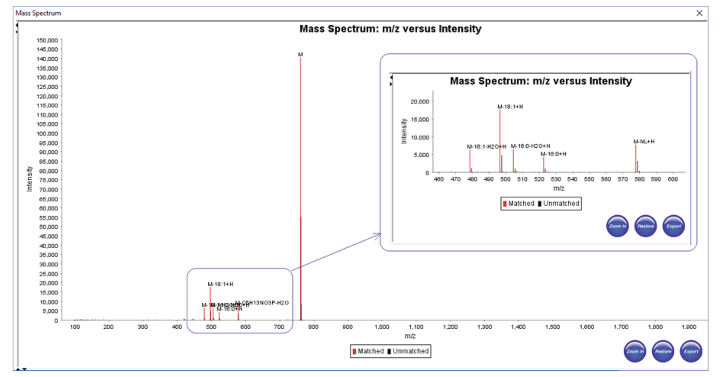


Figure 4(B): Typical SimLipid software GUI showing the MS/MS fragmentation of PC lipids (e.g., PC(16:0/18:1)).

SM LIPIDS

The MS/MS spectra of SM lipids feature the following structure specific characteristic ions:
1. M: the parent ion – all the observed parent ions of PC lipids have either +H, or +Na adducts
2. M-H2O: neutral loss of water from the parent ion
3. N": Product ions corresponding to the sphingosine – loss of the phophocholine headgroup, the fatty acid, and a water molecule as shown in Figure 5(A).
4. T": Product ions corresponding to the fatty acid chain – loss of the phophocholine headgroup, the sphingosine as shown in Figure 5(B).
5. M-Tetramethylamine: Product ion formed due to tetramethylamine from the parent ion as shown in Figure 5(C).
6. Product ion formed due to neutral loss of the phosphocholine headgroup, and its daughter ion formed due to further loss of H2O; we use the molecular formula of the fragment ion as shown in Figure 5(D).

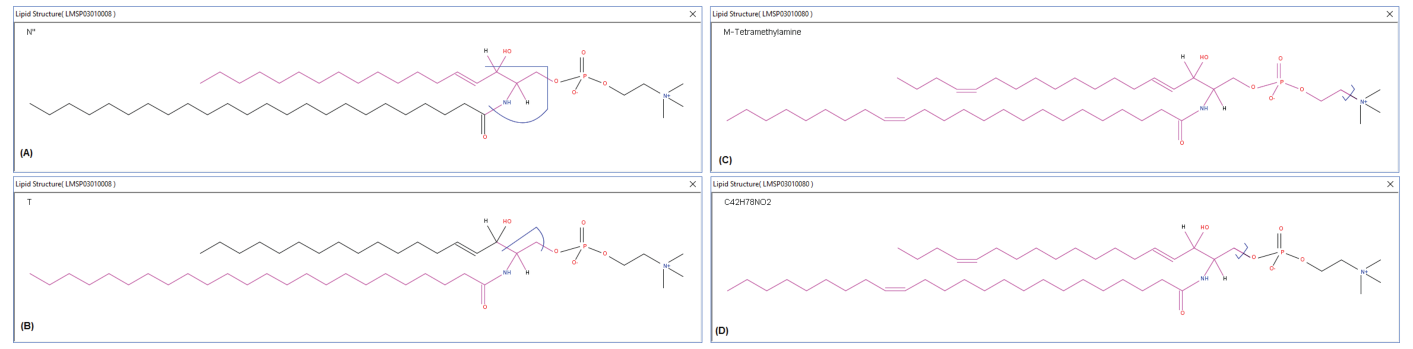


Figure 5: Typical SimLipid software GUI showing the structures of the fragment ions (A) N" and (B) T ions of SM(d18:1/24:0); (C) M-Tetramethylamine ion of SM(d18:2/24:1); and (D) loss of phospholine headgroup from the parent ion of SM(d18:2/24:1).

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The MS/MS spectra of SM lipids with [M+Na]+ parent ions have the fragmentation pattern – product ions 6, 5, and 1 in the descending order of intensity - displayed in Figures 6(A), and 6(B).

However, the MS/MS spectra of SM lipids with [M+H]+ parent ions have the fragmentation pattern – product ions 6, loss of phosphocholine headgroup along with additional loss of water; 2, neutral loss of water from the parent ion; 1, the protonated parent ion; and 6, loss of phosphocholine headgroup in the descending order of intensity - displayed in Figures 7(A), and 7(B).

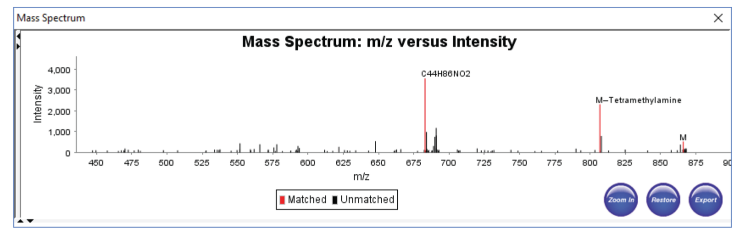


Figure 6(A): Typical SimLipid software GUI showing the MS/MS fragmentation of SM lipids with [M+Na]+ parent ions; annotated spectrum of SM(d18:1/26:0).

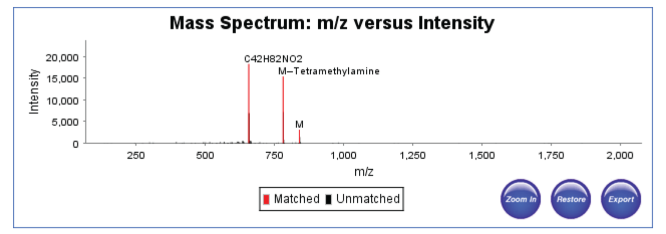


Figure 6(B): Typical SimLipid software GUI showing the MS/MS fragmentation of SM lipids with [M+Na]+ parent ions; annotated spectrum of SM(d18:1/24:0).

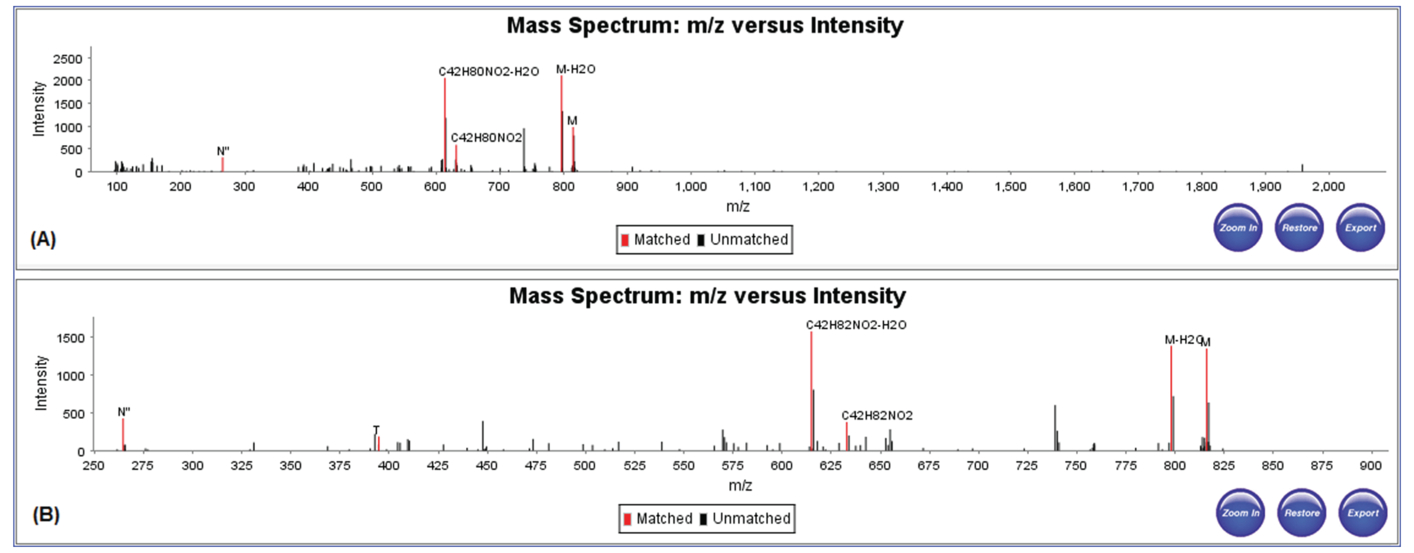


Figure 7: Typical SimLipid software GUI showing the MS/MS fragmentation of SM lipids with [M+H]+ parent ions; annotated spectrum of (A) SM(d18:1/24:1), and (B) annotated spectrum of SM(d18:1/24:0).

PE LIPIDS

The MS/MS spectra of PE lipids feature the following structure specific characteristic ions
1. M: the parent ion
2. M-NL+H: Product ion formed due to neutral loss of the phosphoethanolamine (PE) headgroup (Figure 8(A)).
3. M-Fatty Acid chain + H, and M-Fatty Acid chain -H2O+ H: the fragment ion formed due to loss of a fatty acid chain and its daughter ion corresponding to neutral loss of water (Figure 8(B)).
4. Fatty Acid chain PE: Product ion formed due to loss of both the PE headgroup, and the other fatty chain (Figure 8(C), and Figure 8(D)).

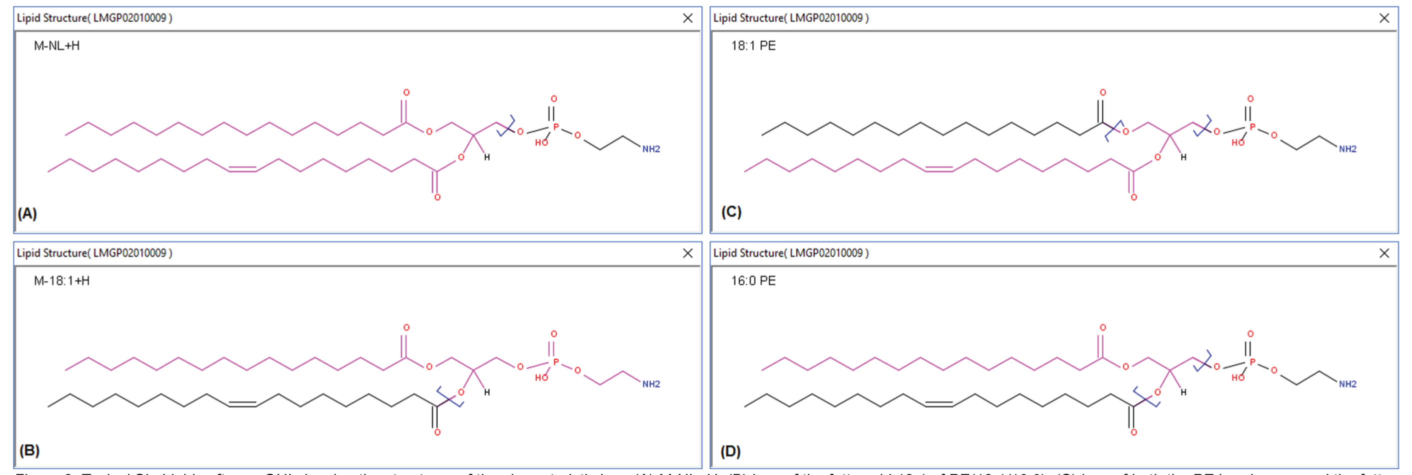


Figure 8: Typical SimLipid software GUI showing the structures of the characteristic ions (A) M-NL+H; (B) loss of the fatty acid 18:1 of PE(18:1/16:0); (C) loss of both the PE headgroup, and the fatty chain 16:0; and (D) loss of both the PE headgroup, and the fatty chain 18:1.

Parent ions of PE lipids were observed with either [M+H]+ or [M+Na]+ ion species. The characteristic ion 2 was observed as base peak in all the MS/MS spectra irrespective of the parent ion species.

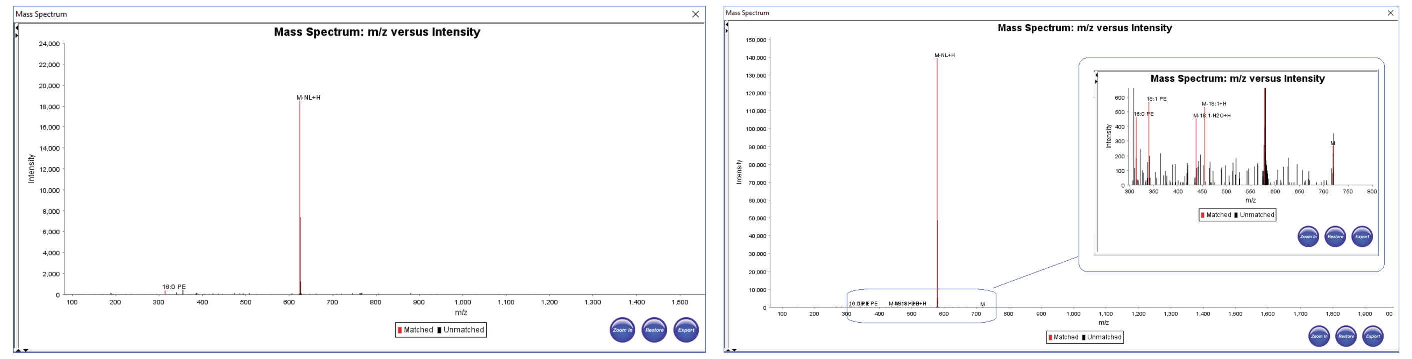


Figure 9: Typical SimLipid software GUI showing the MS/MS fragmentation of PE lipids; annotated spectrum of (A) PE(18:1/16:0), and (B) PE(16:0/22:6).

CERAMIDES LIPIDS

The MS/MS spectra of Ceramides lipids feature the following structure specific characteristic ions:
1. M-H2O: the parent ion with neutral loss of water
2. N": Product ions corresponding to the sphingosine – loss of the fatty acid, and two water molecules as shown in Figure 10(A), and 10(B).

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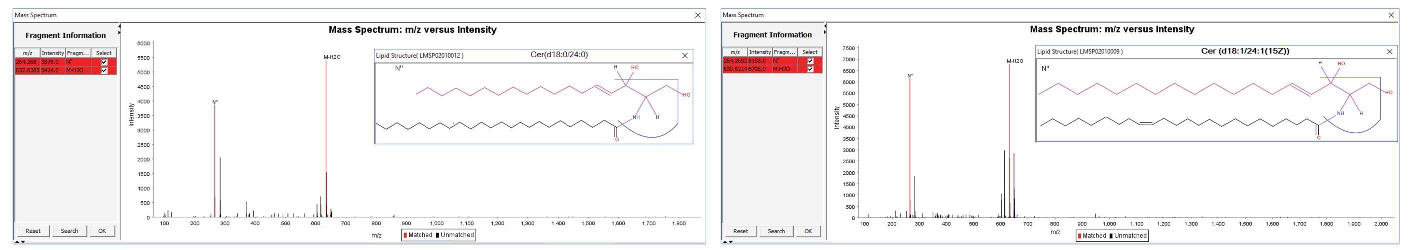


Figure 10: Typical SimLipid software GUI showing the MS/MS fragmentation of Ceramides lipids; annotated spectrum of (A) Cer(d18:0/24:0), and (B) Cer(d18:1/24:1). The structure of N" ion was manually inserted into the annotated MS/MS spectra exported from SimLipid software.

DAG LIPIDS

The MS/MS spectra of DAG lipids feature the following structure specific characteristic ions:
1. M-H2O: the parent ion with neutral loss of water
2. M-Fatty Acid chain + H: the fragment ion formed due to loss of a fatty acid chain
3. M-Fatty Acid chain + Adduct + H: the fragment ion formed due to loss of a fatty acid chain and an adduct - metal adduct e.g., Na or an ammonia (NH3) - based on the parent ion species.

Parent ions with [M+H]+, [M+NH4]+, and [M+Na]+ ion species were observed for DAG lipids. The characteristic ions 1 was observed as the base peak, and 2 or 3 were observed in all the MS/MS spectra depending on the parent ion species.

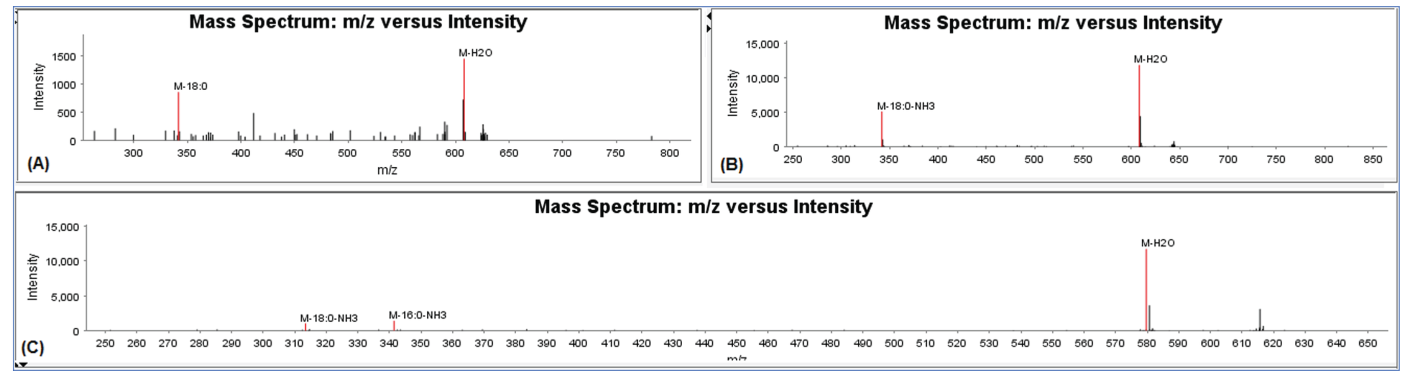


Figure 11: Typical SimLipid software GUI showing the MS/MS fragmentation of DAG lipids; annotated spectrum of (A) DAG(18:0/18:0/0:0) with [M+H]+ parent ion, (B) DAG(18:0/18:0/0:0) with [M+NH4]+ parent ion, and (C) DAG(18:0/16:0/0:0) with [M+NH4]+ parent ion.

TAG LIPIDS

The MS/MS spectra of TAG lipids feature the following structure specific characteristic ions:
1. M: Parent ion
2. M-H2O: the parent ion with neutral loss of water
3. M-Fatty Acid chain + H: the fragment ion formed due to loss of a fatty acid chain
4. M-Fatty Acid chain + Adduct+ H: the fragment ion formed due to loss of a fatty acid chain and an adduct - metal adduct e.g., Na, K, C5H12N or an ammonia (NH3) - based on the parent ion species.
5. Acylium ion of the fatty acid chains.

Parent ions with [M+NH4]+, [M+Na]+, [M+K]+, and [M+C5H12N]+ ion species were observed for TAG lipids. The characteristic ions 3 or 4 corresponding to the fatty acid chains were observed in all the MS/MS spectra depending on the parent ion species. The observed fragmentation patterns are similar to the reported fragmentation patterns of lipids in ESI-MS workflows [8].

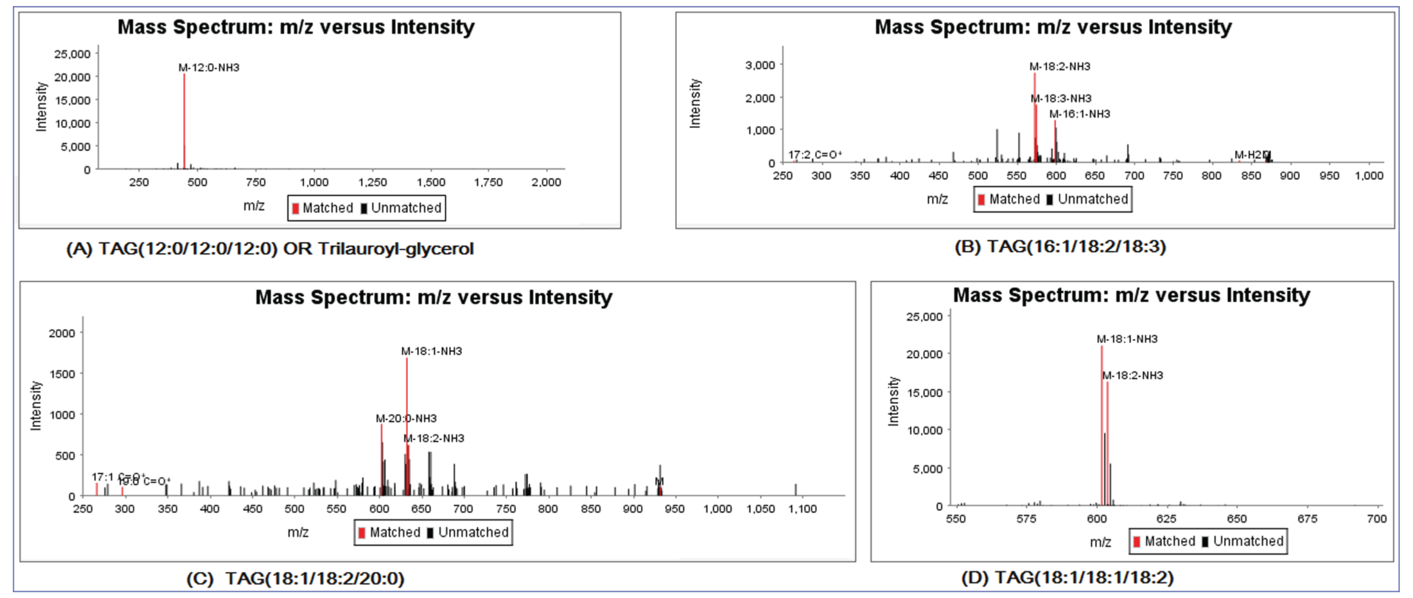

(A) TAG(12:0/12:0/12:0) OR Trilauroyl-glycerol
(B) TAG(16:1/18:2/18:3)
(C) TAG(18:1/18:2/20:0)
(D) TAG(18:1/18:1/18:2)

Figure 12: Typical SimLipid software GUI showing the MS/MS fragmentation of TAG lipids; annotated spectrum of (A) TAG Trilauroyl-glycerol, (B) TAG(16:1/18:2/18:3), (C) TAG(18:1/18:2/20:0).

CONCLUSION

Improvements in analytical methods, especially liquid chromatography and mass spectrometry, have driven the recent advances in the development of lipid profiling methods. We have presented a robust workflow for the global lipid profiling of a plasma sample using an LC system coupled to an Impact II™ Ultra-High Resolution Qq-Time-Of-Flight (UHR-QqTOF) mass spectrometer (Bruker Daltonik, Bremen, Germany) with > 50,000 Full-Sensitivity Resolution and SimLipid® informatics software (PREMIER Biosoft, USA).

SimLipid software supports automated data analysis for lipid identification using MS/MS data. The program enables clear understanding of fragmentation patterns of lipids belonging to different classes observed in the UHR-QqTOF-MS/MS spectra. Besides, the program allows its users to view the raw data; review the results; automatically annotate thousands of MS/MS spectra; and export the results into MS excel files.

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