Lipid Profiling of Grape Samples using Orbitrap Velos Pro Mass Spectrometer with SimLipid® Software

Vladimir Shulaev[1,2]; Giulia Chitarrini[1,3]; Himani Gupta[4]; Rajesh Pujari[4]; Urska Vrhovsek[4]; Fulvio Mattivi[3,5]; Ningombam Sanjib Meitei[4,6]

[1]Department of Biological Sciences, University of North Texas, Denton, TX; [2]Biodiscovery Institute, University of North Texas, Denton, TX; [3]Department of Food Quality and Nutrition, Research and Innovation Centre, Foundation Edmund Mach (FEM), San Michele all'Adige, Italy; [4]PREMIER Biosoft, Indore, India; [5]Center Agriculture Food Environment (CAFE), University of Trento, San Michele all'Adige, Italy; [6]PREMIER Biosoft, Palo Alto, CA

INTRODUCTION

Liquid chromatography—Mass spectrometry (LC-MS) provides one of the most popular platforms for lipidomics analysis. Recently, MS-based plant lipidomics research has been gaining popularity [1]. We developed LC-MS lipidomics method using Orbitrap Velos Pro Hybrid MS instrument to identify the lipid classes and lipid molecular species in grape samples. A challenge associated with this method was the analysis of large LC-MS data set. Besides the chemical complexity and large range of concentrations of thousands of lipid species that are present in the samples, there are co-eluting lipid species, especially from a class, in the LC domain and MS^2 spectra contain product ions of multiple lipid species. SimLipid has been re-developed in order to streamline such large scale data analysis generated by the method.

METHODS

Lipids were extracted from grape samples using Folch method. Two independent experiments using several grape genotypes were performed resulting in total of 89 biological samples.

LC-MS: LC-MS analysis was carried out on an Accela 1250 quaternary pump with Accela Open autosampler on-line coupled to an LTQ Orbitrap Velos Pro Hybrid MS (Thermo Fisher Scientific, USA). Lipid extracts were separated on an Accucore C18 2.1x150 mm 2.6 µm column using 30 min gradient [2].

Data Analysis: Lipid profiling: SimLipid v. 6.04 (PREMIER Biosoft, USA), and Statistical Analysis: SIMCA-P software.

OVERVIEW OF THE DATA ANALYSIS

The raw data from the 178 LC-MS runs in negative and positive modes were directly imported into the SimLipid software using Thermo's native file format (.raw). The negative mode data contains 247,125 MS/MS scans while the positive mode data contains 1,271,680 MS/MS scans, resulting in a total of 1,518,805 MS/MS scans. These scans were subjected to SimLipid MS/MS database search in multiple batches, each batches containing 100,000 MS/MS scans using 5 ppm tolerance for both the precursor ions, and the product ions; $[M+H]^{1+}$, $[M+Na]^{1+}$, and $[M+NH_4]^{1+}$ adduct ion species for positive mode, while $[M-H]^{1-}$, $[M-CH_3]^{1-}$, $[M+Formate]^{1-}$, $[M+Cl]^{1-}$, $[M+AcO]^{1-}$, and $[M+OAcO]^{1-}$ adduct ion species were selected for the negative mode MS/MS database search. Figure 1 (A) shows typical graphical user interfaces of SL software with optimized parameter settings for importing raw data generated by LTQ Orbitrap Velos Pro system. The MS/MS database search parameters shown in Figure 1(B) were used for lipid identification using SimLipid software.

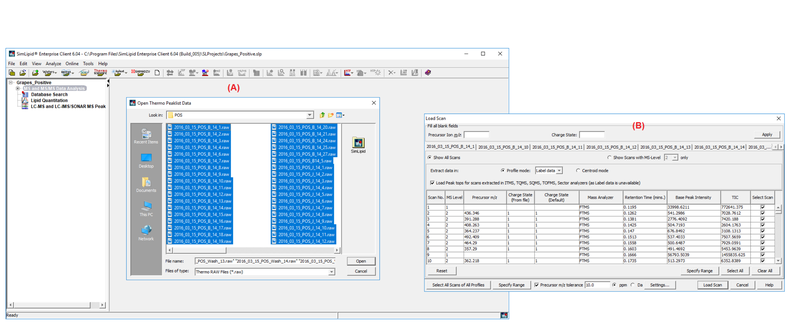

Figure 1: Typical SimLipid software GUI showing the raw data loading steps: (A) Select the .raw files, (B) Select the samples you want to read the data from.

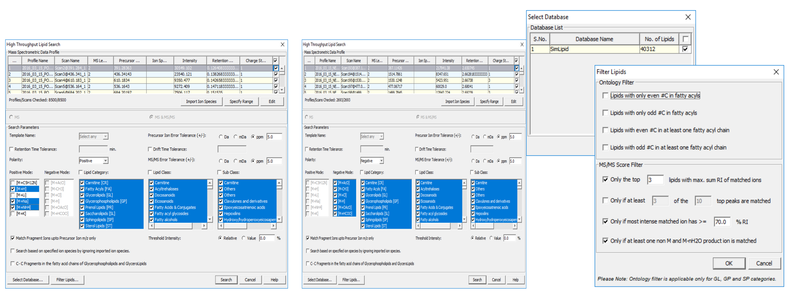

Figure 2: Typical GUIs of SimLipid software showing the MS/MS database search parameters for structural identification of lipids using product ions data.

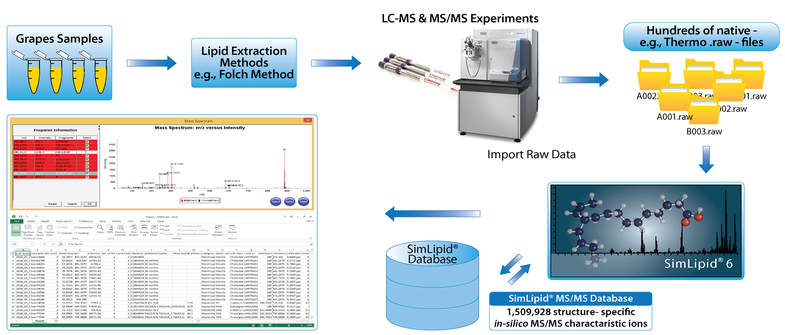

Figure 3: Schematic representation of LC-MS based large-scale lipidomics analysis using SimLipid software.

RESULTS AND DISCUSSION

A total of 1266 unique lipid species (Fatty Acyls [FA] (n=15), Glycerolipids [GL] (n=651), Glycerophospholipids [GP] (n=528), Prenol Lipids [PR](n=7), Saccharolipids [SL] (n=2), Sphingolipids [SP](n=42), Sterol Lipids [ST] (n=20)) were identified between the experimental runs without any filter criteria applied. However, a total of 1235 unique lipid species (Fatty Acyls [FA] (n=01), Glycerolipids [GL] (n=810), Glycerophospholipids [GP] (n=326), Prenol Lipids [PR](n=05), Sphingolipids [SP](n=22), Sterol Lipids [ST] (n=71)) were identified between the experimental runs with set filter criteria based on score (>=0.05) and Matched Sum RI (100).

	Sample "B"			
	Positive		Negative	
Sl. #	Main Class	Frequency	Main Class	Frequency
1	TAG	494	PC	37
2	PC	70	PA	32
3	DAG	40	PE	18
4	Sterols	38	TAG	5
5	PE	34	PG	4
6	PA	24	Neutral glycosphingolipids	3
7	PS	20	DAG	2
8	PI	8	Isoprenoids	2
9	Ceramides	7	PI	2
10	Oxidized Glycerophospholipids	7	PS	2
11	Glycosyldiradylglycerols	6	Acidic glycosphingolipids	1
12	PG	4	Glycosyldiradylglycerols	1
13	Neutral glycosphingolipids	2	Oxidized glycerophospholipids	1
14	Fatty esters	1	Total	110
15	Isoprenoids	1		
16	Secosteroids	1		
	Total	757		

Table 1: Class-wise frequency distribution of the lipids identified for Sample "B".

	Sample "J"			
	Positive		Negative	
Sl. #	Main Class	Frequency	Main Class	Frequency
1	TAG	486	PC	41
2	PC	79	PA	23
3	Sterols	57	PE	17
4	DAG	56	TAG	4
5	PE	39	Neutral glycosphingolipids	3
6	PS	20	DAG	2
7	PA	13	Ceramides	1
8	Ceramides	11	Glycosyldiradylglycerols	1
9	Glycosyldiradylglycerols	10	Isoprenoids	1
10	Oxidized glycerophospholipids	6	Oxidized glycerophospholipids	1
11	PI	6	PG	1
12	PG	5	PI	1
13	Neutral glycosphingolipids	3	Total	96
14	Isoprenoids	2		
15	Fatty esters	1		
16	Other Glycerolipids	1		
	Total	795		

Table 2: Class-wise frequency distribution of the lipids identified for Sample "J".

	Sample "S"			
	Positive		Negative	
Sl. #	Main Class	Frequency	Main Class	Frequency
1	TAG	539	PC	42
2	PC	81	PE	18
3	DAG	50	PA	16
4	PE	41	DAG	4
5	Sterols	39	Neutral glycosphingolipids	3
6	PS	22	PG	3
7	PA	10	PI	3
8	Glycosyldiradylglycerols	7	TAG	2
9	PI	7	Cardiolipin	1
10	Ceramides	6	Glycosyldiradylglycerols	1
11	Oxidized glycerophospholipids	3	Isoprenoids	1
12	PG	3	Phosphosphingolipids	1
13	Neutral glycosphingolipids	2	PS	1
14	Phosphosphingolipids	2	Total	96
15	Fatty esters	1		
16	Secosteroids	1		
	Total	814		

Table 3: Class-wise frequency distribution of the lipids identified for Sample "S".

	Sample "WASH"			
	Positive		Negative	
Sl. #	Main Class	Frequency	Main Class	Frequency
1	TAG	297	PC	3
2	DAG	9	PE	3
3	Sterols	6	Oxidized glycerophospholipids	1
4	PE	4	Total	07
5	Oxidized glycerophospholipids	2		
6	PC	2		
7	PI	1		
	Total	321		

Table 4: Class-wise frequency distribution of the lipids identified for Sample "WASH".

Sl. #	Main Class	Frequency
1	TAG	712
2	PC	124
3	DAG	87
4	PE	74
5	Sterols	70
6	PA	61
7	PS	30
8	PI	17
9	Ceramides	12
10	PG	12
11	Glycosyldiradylglycerols	10

Sl. #	Main Class	Frequency
12	Oxidized glycerophospholipids	7
13	Neutral glycosphingolipids	6
14	Isoprenoids	5
15	Phosphosphingolipids	3
16	Acidic glycosphingolipids	1
17	Cardiolipin	1
18	Fatty esters	1
19	Other Glycerolipids	1
20	Secosteroids	1
	Total	1235

Table 5: Class-wise frequency distribution of the lipids identified from MS/MS SimLipid database search using 5 ppm mass tolerance under both positive and negative polarity.

Top 3 most abundant lipids from Sample "B" under positive ion mode

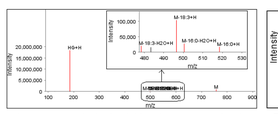

Figure 3a: Typical SimLipid software GUI showing the MS/MS fragmentation of PC lipids with [M+H]+ parent ions; annotated spectrum of PC(16:0_18:3)).

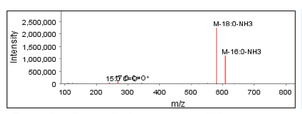

Figure 3b: Typical SimLipid software GUI showing the MS/MS fragmentation of TG lipids; annotated spectrum of TG(16:0_18:0_18:0) with [M+NH4]+ parent ion.

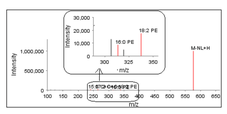

Figure 3c: Typical SimLipid software GUI showing the MS/MS fragmentation of PE lipids with [M+H]+ parent ions; annotated spectrum of PE(16:0_18:2)).

Top 3 most abundant lipids from Sample "J" under positive ion mode

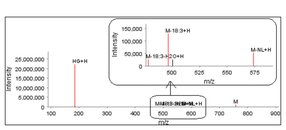

Figure 4a: Typical SimLipid software GUI showing the MS/MS fragmentation of PC lipids with [M+H]+ parent ions; annotated spectrum of PC(16:0_18:3)).

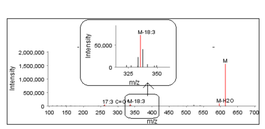

Figure 4b: Typical SimLipid software GUI showing the MS/MS fragmentation of DG lipids; annotated spectrum of DG(18:3_18:3_0:0) with [M+H]+ parent ion.

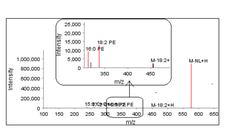

Figure 4c: Typical SimLipid software GUI showing the MS/MS fragmentation of PE lipids with [M+H]+ parent ions; annotated spectrum of PE(16:0_18:2)).

Top 3 most abundant lipids from Sample "S" under positive ion mode

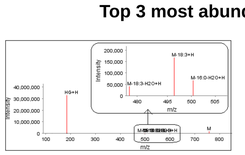

Figure 5a: Typical SimLipid software GUI showing the MS/MS fragmentation of PC lipids with [M+H]+ parent ions; annotated spectrum of PC(16:0_18:3)).

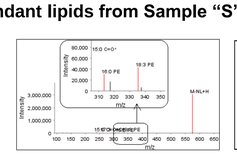

Figure 5b: Typical SimLipid software GUI showing the MS/MS fragmentation of PE lipids with [M+H]+ parent ions; annotated spectrum of PE(16:0_18:3)).

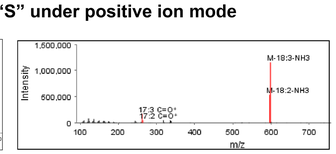

Figure 5c: Typical SimLipid software GUI showing the MS/MS fragmentation of TG lipids; annotated spectrum of TG(12:2_18:3_18:3) with [M+NH4]+ parent ion.

Top 3 most abundant lipids from Sample "WASH" under positive ion mode

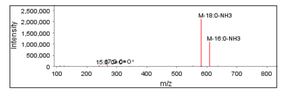

Figure 6a: Typical SimLipid software GUI showing the MS/MS fragmentation of TG lipids; annotated spectrum of TG(16:0_18:0_18:0) with [M+NH4]+ parent ion.

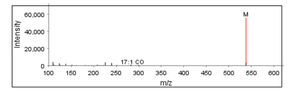

Figure 6b: Typical SimLipid software GUI showing the MS/MS fragmentation of FAHFA lipids; annotated spectrum of FAHFA(18:1_16:0) with [M+H]+ parent ion.

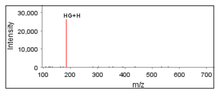

Figure 6c: Typical SimLipid software GUI showing the MS/MS fragmentation of PC lipids with [M+H]+ parent ions; annotated spectrum of PC(16:0_18:3)).

Top 3 most abundant lipids from Sample "B" under negative ion mode

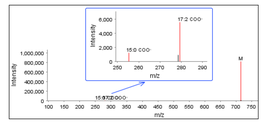

Figure 7a: Typical SimLipid software GUI showing the MS/MS fragmentation of PC lipids with [M+HCOO]- parent ions; annotated spectrum of PC(16:0_18:2).

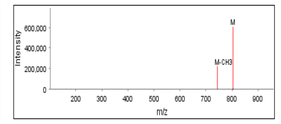

Figure 7b: Typical SimLipid software GUI showing the MS/MS fragmentation of PE lipids with [M-H]- parent ions; annotated spectrum of PE(16:0_18:2).

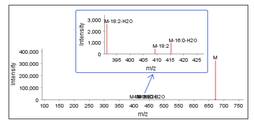

Figure 7c: Typical SimLipid software GUI showing the MS/MS fragmentation of PA lipids with [M-H]- parent ions; annotated spectrum of PA(16:0_18:2).

Top 3 most abundant lipids from Sample "J" under negative ion mode

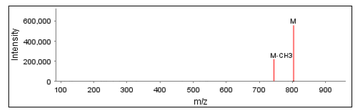

Figure 8a: Typical SimLipid software GUI showing the MS/MS fragmentation of PC lipids with [M+ HCOO]- parent ions; annotated spectrum of PC(16:0_18:2).

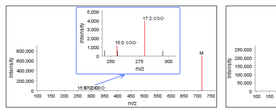

Figure 8b: Typical SimLipid software GUI showing the MS/MS fragmentation of PE lipids with [M-H]- parent ions; annotated spectrum of PE(16:0_18:2).

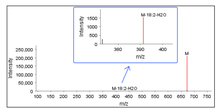

Figure 8c: Typical SimLipid software GUI showing the MS/MS fragmentation of PA lipids with [M-H]- parent ions; annotated spectrum of PA(16:0_18:2).

Top 3 most abundant lipids from Sample "S" under negative ion mode

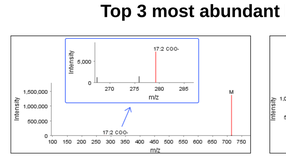

Figure 9a: Typical SimLipid software GUI showing the MS/MS fragmentation of PC lipids with [M-H]- parent ions; annotated spectrum of PC(14:0_18:2).

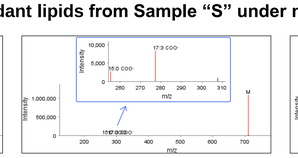

Figure 9b: Typical SimLipid software GUI showing the MS/MS fragmentation of PE lipids with [M-H]- parent ions; annotated spectrum of PE(16:0_18:3).

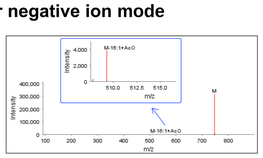

Figure 9c: Typical SimLipid software GUI showing the MS/MS fragmentation of PA lipids with [M+AcO]- parent ions; annotated spectrum of PA(16:1_19:1).

Sl. #	Functionality	Data Type	File Format	Instrument Name	File Size (in Gigabytes)		Number of Scans		Time (in minutes)	
					Previous version	Current Version	Previous version	Current Version	Previous version	Current Version
1.	Loading Raw Data	Orbitrap Data	.raw	Thermo Scientific LTQ Orbitrap XL system	2.94	1.04			50	40
2.	MS/MS Database Search in Batch Mode	Orbitrap Data	.raw	Thermo Scientific LTQ Orbitrap XL system	--	--	20,000	100,000		
3.	Import Raw Data or Peaklists	Orbitrap Data	.raw	Thermo Scientific LTQ Orbitrap XL system	--	--	5,00,000	2,000,000	--	--
4.	Export search results	Orbitrap Data	.raw	Thermo Scientific LTQ Orbitrap XL system	--	--	50,000	100,000		
5.	HTP Dialog Launch	Orbitrap Data	.raw	Thermo Scientific LTQ Orbitrap XL system	--	--	--	--	45.1962	16.9422
6.	LC-MS Dialog Launch	Orbitrap Data	.raw	Thermo Scientific LTQ Orbitrap XL system	--	--	--	--	10.50	6.75

Table 6: Large scale lipidomics analysis performance matrix of SimLipid Software.

* The performance of the SimLipid was assessed by removing the limitations pertaining to the number of data files, MS scans, or MS/MS scans that can be processed in batch mode.

CONCLUSION

SimLipid alleviates the challenges posed by large scale MS based lipidomic data analysis facilitating lipid profiling of complex biological samples very quickly. The program has been re-developed to support large scale lipidomics data sets generated by high-resolution mass spectrometry (HRMS). Key features of SimLipid supporting MS based large scale lipidomic data analysis are:

- Import raw data from 2 million MS or MS/MS scans at a click of a button
- Database search for 100,000 MS or MS/MS scans in batch mode
- Filter results based on Score and Matched RI for high confidence results.
- Faster MS and MS/MS database Search.

NOVEL ASPECT

Global lipid profiling of grapes samples using LC-MS method.

REFERENCES

[1] Shulaev et al., (2017), *Biochim. Biophys. Acta* 1862 (8): 786-791.
[2] Hu, C., et al., (2008), *Proteome Res.* 7: 4982–4991.