SimLipid[®]: Software platform for automating Shotgun, LC-MS and MALDI-MS based high-throughput lipidomics Ningombam Sanjib Meitei¹, Himani Gupta¹, Arun Apte²

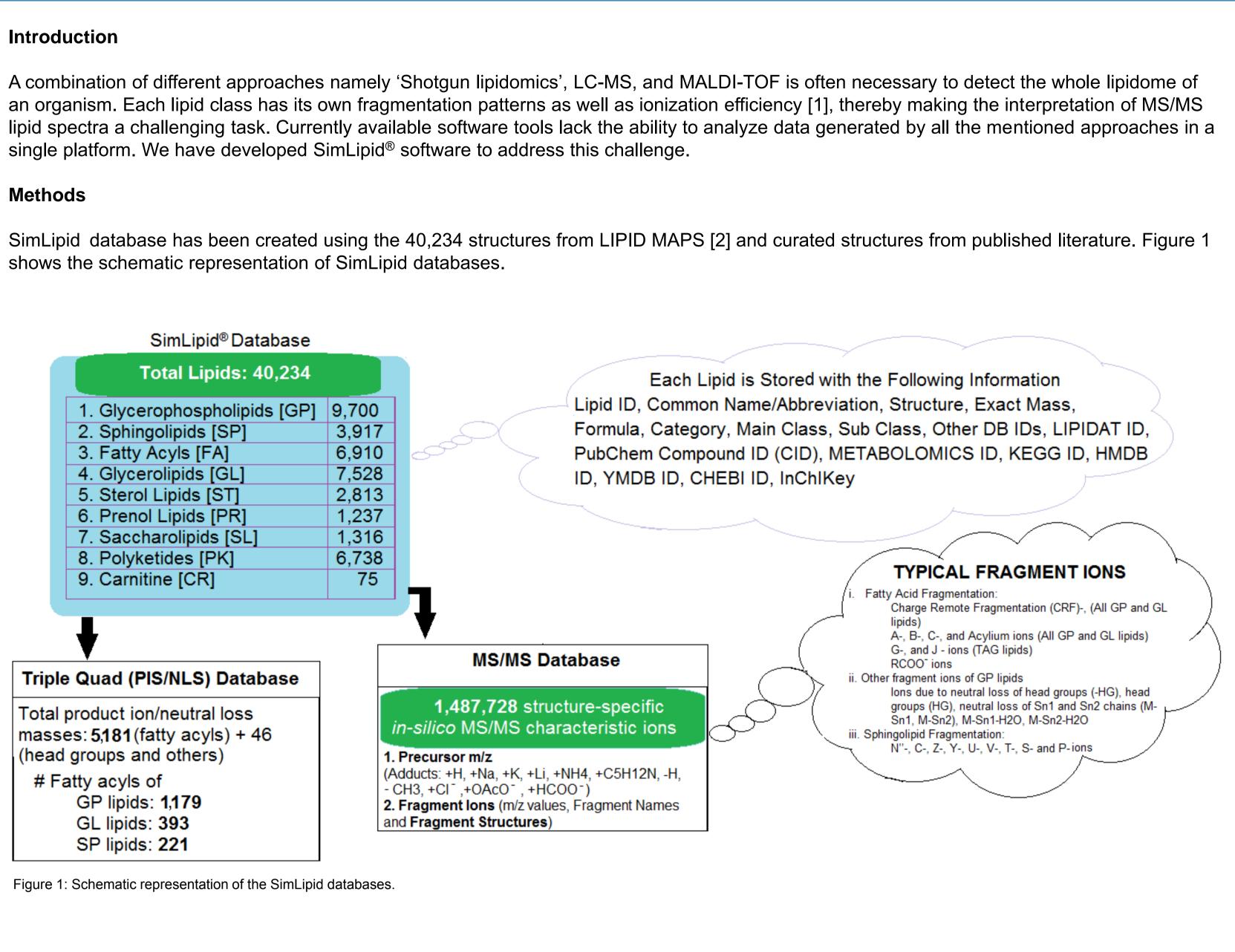
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Introduction

single platform. We have developed SimLipid[®] software to address this challenge.

Methods

shows the schematic representation of SimLipid databases.



MS/MS Database:

This contains 1,487,728 structure-specific *in-silico* MS/MS characteristic ions. The characteristic ions have been generated based on sub-class specific fragmentation rules reported in the literature [3, 5-8] as well as those observed in the 529 experimental MS/MS spectra of standard lipids listed in LIPID MAPS [4]. Typical fragment ions are charge remote fragmentation (CRF)-, A-, B-, C-, G-, J - ions [6-8] and acylium ions [8-9]. The CRF ions facilitate localization of double bonds and branching in fatty acid chains. Typical sphingolipid fragment ions are N"-, C-, Z-, Y-, U-, V-, T-, S- and P- ions that were observed in the 18 experimental MS/MS spectra of sphingolipid standards from LIPID MAPS. Similarly, fragmentation patterns for other categories were also developed.

Table 1 shows the typical fragment ions of TG(18:1(9Z)/ 18:1(9Z)/ 18:1(9Z)) with ion species [M+Na]¹⁺ by SimLipid software. Similarly, Table 2 shows the typical fragment ions of PC lipids.

Precursor Ion/Neutral Loss Scans Database: In order to facilitate lipid database search for precursor ion scan (PIS) or neutral loss scan (NLS) data, we stored the following lipid category specific target m/z and mass for GP: 1179, SP: 221, GL: 393 and Head groups: 49.

lon Type	Structure Displayed by SimLipid	Description	SimLipid Nomenclature
B - ion		Loss of one neutral fatty acid	M- <c:db>-H where <c:db> is the number of carbons and double bonds in the fatty acid E.g., M – 18:1-H</c:db></c:db>
C – ion		Loss of one sodium carboxylate residue	M- <c:db>-Na-H E.g., M – 18:1-Na H</c:db>
G - ion		Loss of 2 fatty acids; one from position 2 and the other from either position 1 or position 3. It contains all 3 carbons from glycerol backbone. SimLipid generates 3 G fragments by considering loss of any of the fatty acids.	fatty acid retained in the fragment
J - ion		Loss of 2 fatty acids at positions 1 and 3. It contains 2 carbons out of the 3 in glycerol backbone. SimLipid generates 3 J fragments by considering loss of any of the 2 fatty acids unconstrained to position 1 and 3.	Jk where k represents the fatty acid retained in the fragment structure. E.g., J3
A - ion	······································	Loss of a part of 1 fatty acid	Fj(Ri); i=1,2 & 3 and j = any no. between 2 to n where n = # carbons in the fatty acid chain – 2 - # double bonds in the fatty chain. E.g., F2(R1)
Acylium lon		Protonated acylium ion	RC=O ⁺ Where R represents the alkyl group that is attached to the CO group with a single bond. E.g., 17:1C=O+

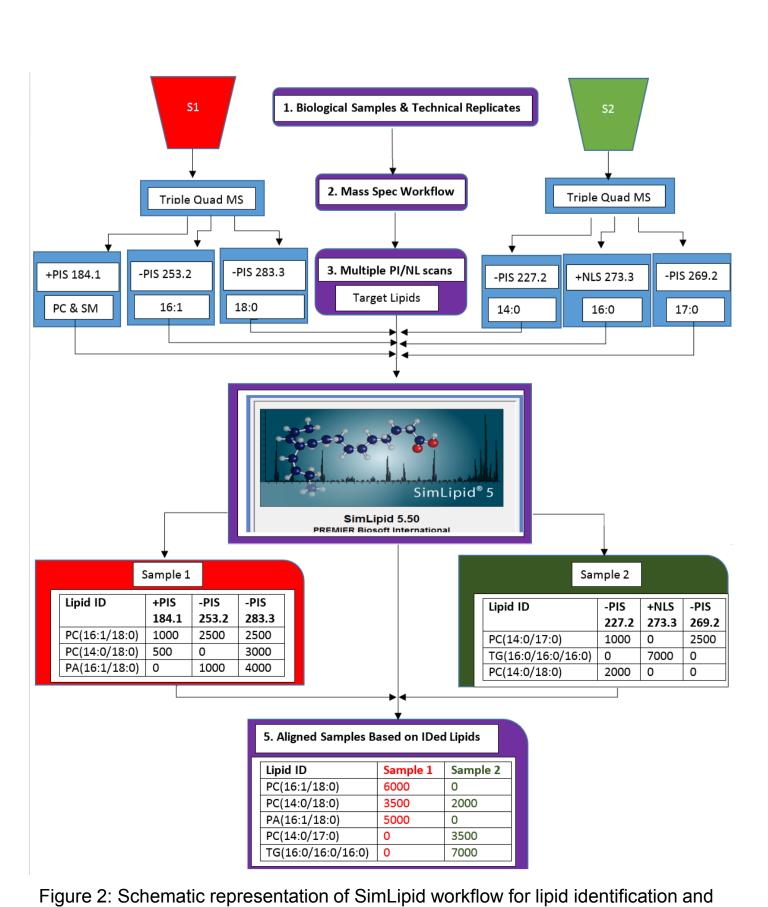
<u>SI.</u> #	Fragment Structure Displayed by SimLipid
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3	
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Га	ble 2: Typical fragment ions generated for PC lipids.

Table 1: Fragment ions generated for TG(18:1(9Z)/ 18:1(9Z)/ 18:1(9Z)) with ion species [M+Na]¹⁺ by SimLipid software.

Lipid Identification using MALDI-, ESI-, LC-ESI-, MS and MS/MS data: For MS1 data, SimLipid profiles lipids based on exact mass database search by assigning highest score to the candidate with minimum delta mass, that is the difference between the observed vs theoretical mass of a candidate lipid calculated in the units of Da (range: 0.0001-2), parts per million (ppm; range: 0.1-200), and milli-Daltons (mDa; range: 0.1-2000)). In case of LC-MS peaklist, the program performs the exact mass DB search for m/z at the M+0th peak of isotope clusters of each of the LC-compounds.

For MS/MS data, the program creates a list of candidate structures for each MS/MS spectrum based on precursor m/z value and other information. For each candidate, *in-silico* fragment ions are matched against the experimental MS/MS data. A scoring mechanism was developed in order to differentiate isobaric candidates.

Lipid Identification and Quantitation using PIS/NLS data: SimLipid performs exact mass database search by constraining candidate lipids based on the target fatty acid chain or lipid head group. Once the lipids are profiled, isotope correction of peaks are performed using algorithm described by Liebisch et al. (2004) [10]. Profiled Lipids can be aligned across multiple scans and biological samples based on (a) Short name (i.e., #C:#DB), (b) Similar fatty acyls (disregarding the position of Sn1, Sn2, Sn3 chains and position of double bonds), and (c) Common name/Abbreviation. Figure 2 shows the schematic representation of SimLipid workflow for lipid identification and quantitation using PIS/NLS data.



quantitation using PIS/NLS data.

	Description	Positive Mode	Negative Mode	SimLipid Fragment Ion Nomenclature
	Protonated Acylium Ion	yes	no	RC=O* E.g., 16:0 C=O*
	Carboxylate Anion	no	yes	RCOO ⁻ where R represents the alkyl group that is attached to the COO group with a single bond. E.g., 16:0 COO ⁻
	Loss of Sn1 Chain	yes	yes	M - <c:db>+Adduct Where <c:db> is the number of carbons and double bonds in the fatty acid. E.g., M – 17:0 + Adduct</c:db></c:db>
	Loss of Sn2 Chain	yes	yes	M - <c:db> +Adduct where <c:db> is the number of carbons and double bonds in the fatty acid. E.g., M – 9:0 + Adduct</c:db></c:db>
	Loss of Sn1 and Sn2 Chains	yes	no	M- <c:db>-<c:db> Loss of Sn1 and Sn2 fatty acyl chains</c:db></c:db>
	Neutral loss of Phosphocholine Headgroup	yes	yes	M-NL+Adduct
	Phosphocholine Headgroup Fragment	yes	yes	HG+Adduct
~	Choline Headgroup Fragment	yes	yes	C5H13N
	Loss of Choline Headgroup	yes	yes	M-C5H13N

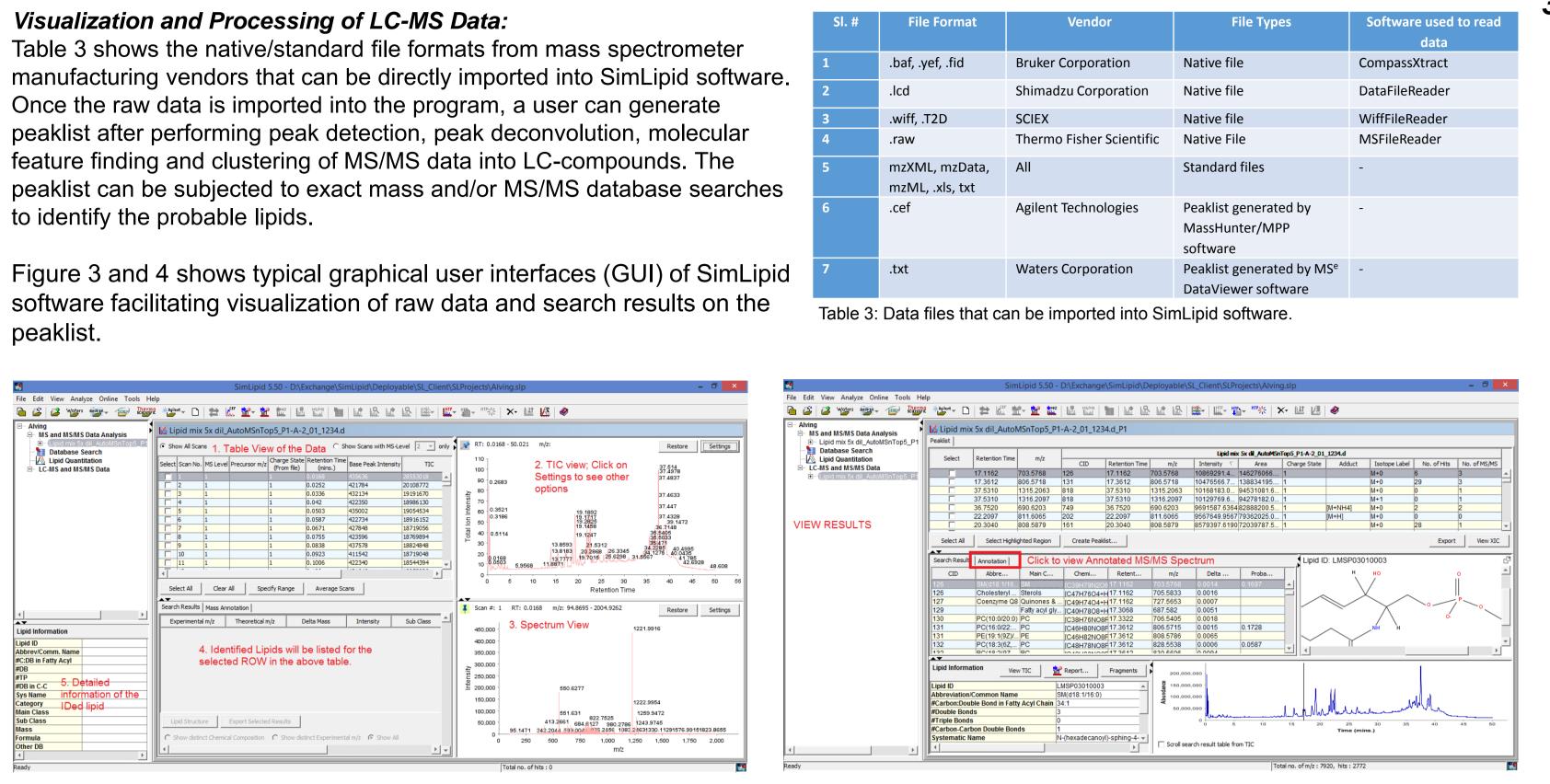


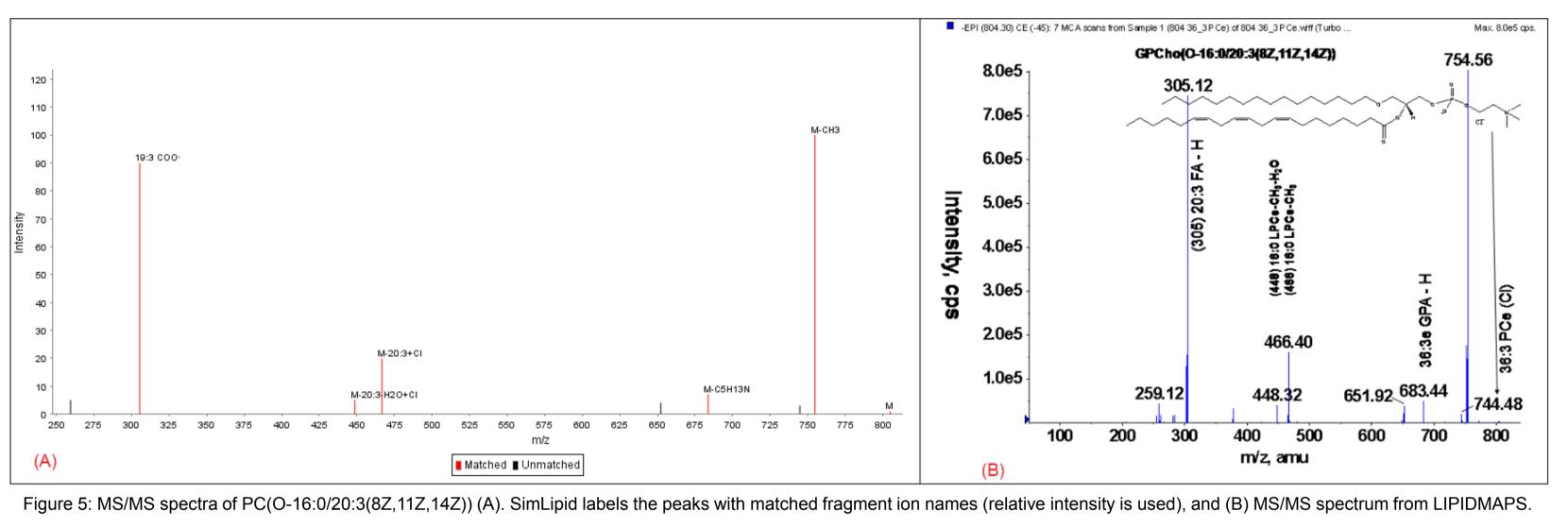
Figure 3: Typical GUI of SimLipid software showing the (1) tabular, (2) chromatogram, (3) spectrum views of LC-MS raw data. (4) Search results and (5) Lipid information is displayed after performing lipid database search.

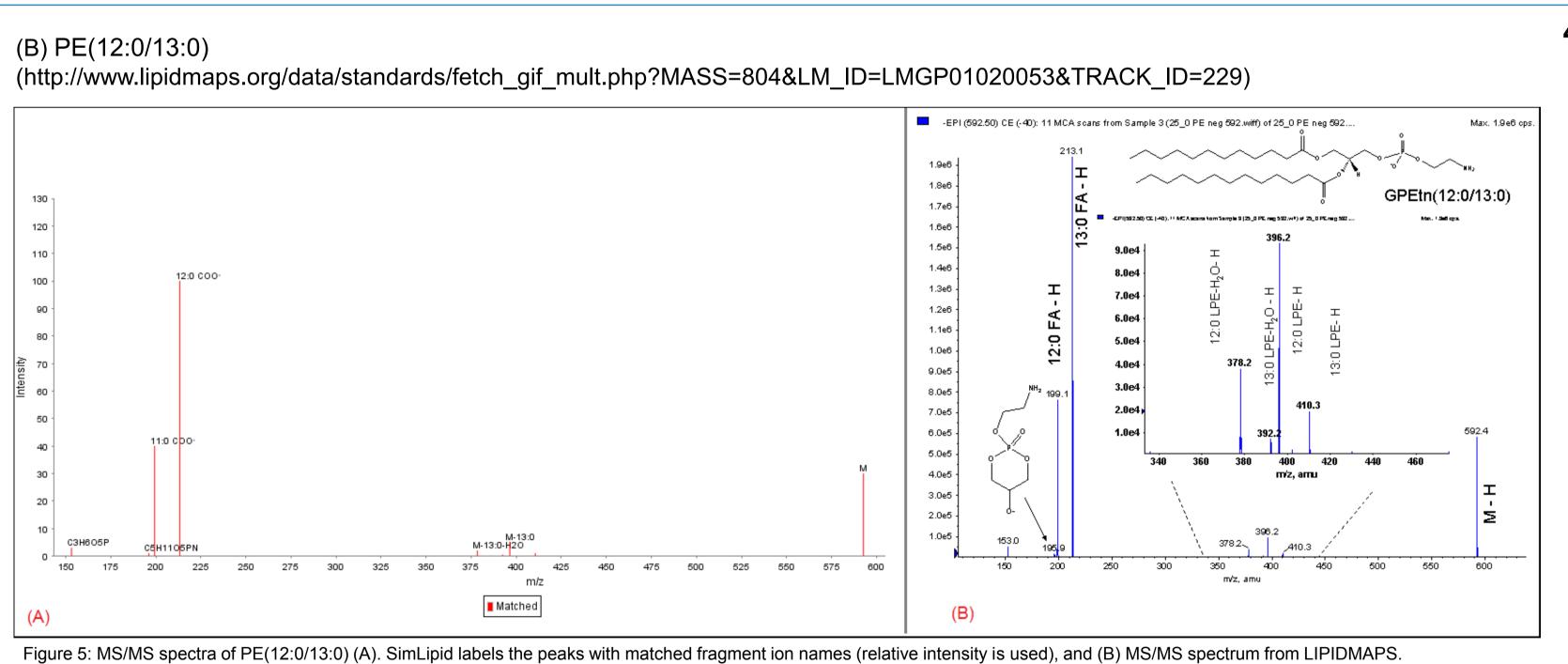
Result and Discussion

Validation of SimLipid results using standard MS/MS spectra from LIPIDMAPS

Experimental MS/MS spectra of various lipid standards from LIPIDMAPS (<u>http://www.lipidmaps.org/data/standards/search.html</u>) are subjected into SimLipid MS/MS database search. All the lipids are identified correctly. Comparison of the annotated MS/MS spectra reported by LIPIDMAPS and the ones generated by SimLipid are shown.

(A) PC(O-16:0/20:3(8Z,11Z,14Z)) (http://www.lipidmaps.org/data/standards/fetch_gif_mult.php?MASS=804&LM_ID=LMGP01020053&TRACK_ID=229)



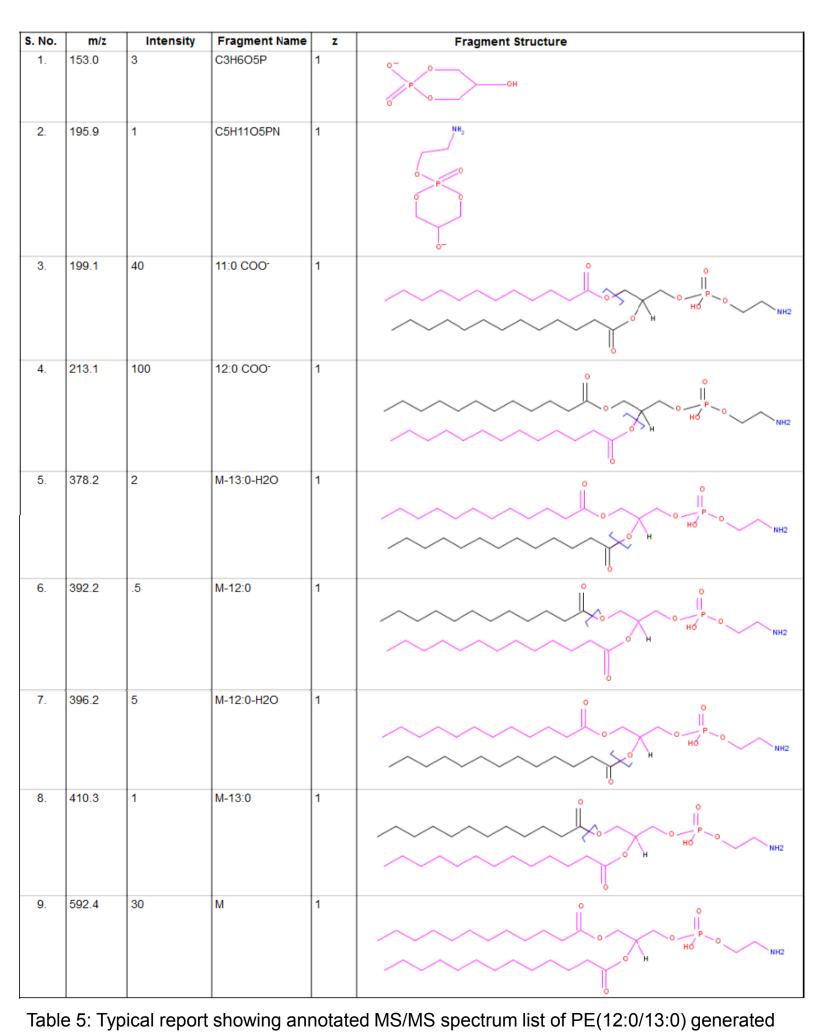


S. No.	m/z	Intensity	Fragment Name	z	Fragment Structure
1.	259.12	5		1	
2.	305.12		19:3 COO-	1	°
3.	448.32	5	M-20:3-H2O+CI	1	°
4.	466.40	20	M-20:3+Cl	1	۰۵ ٥
4.	400.40	20	IVI-20.3+CI	1	
5.	651.92			1	
6.	683.44	7	M-C5H13N	1	0
	744.40				ц,
7.	744.48			1	
8.	754.56	100	M-CH3	1	
9.	804.56	1	M	1	0

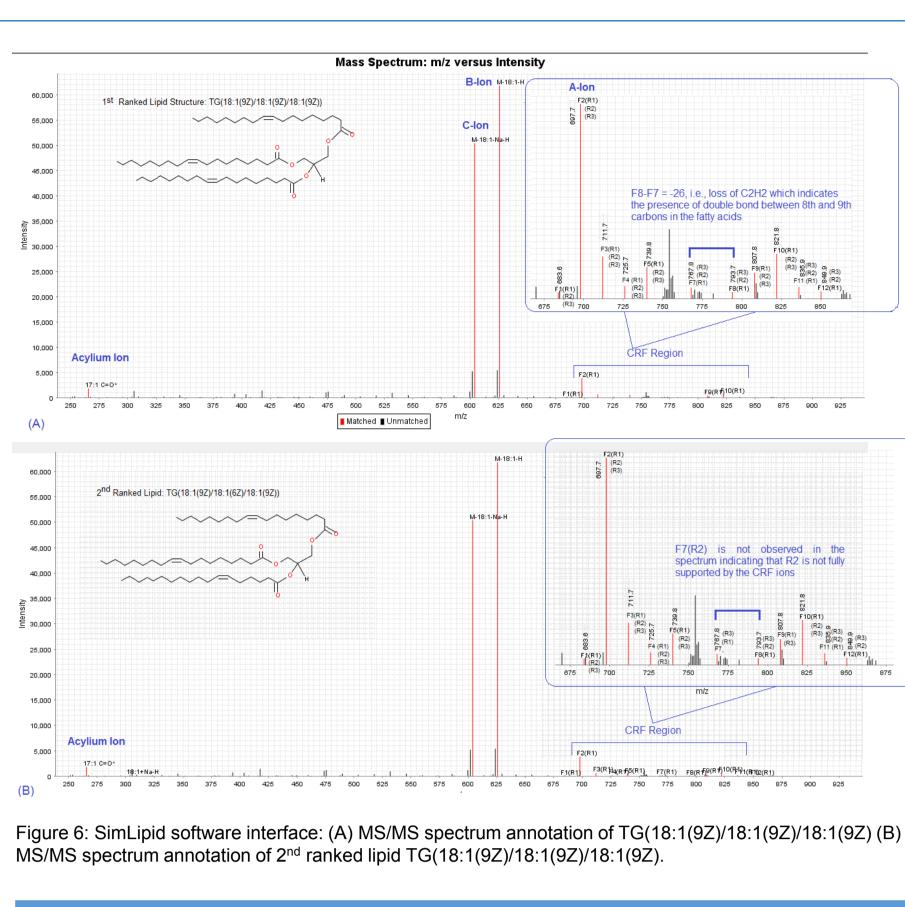
Table 4: Typical report snowing annotated spectrum list generated by SimLipid software. Fragment ions are displayed in red color.

Lipid identification using MALDI TOF/TOF data: The MS/MS spectrum acquired using methods described in [11] was subjected to a search in SimLipid software which resulted in the identification of a TAG 54:3. The annotated MS/MS spectrum and the lipid structure are shown in Figure 6. In this case, CRF ions are the determinants for identifying the lipid structure as well as the position of double bonds in the fatty acyls.

Figure 4: Typical GUI of SimLipid software showing the peaklist, search results, structure of lipids, retention time of the IDed lipid in the chromatogram, and detailed lipid information.



by SimLipid software.



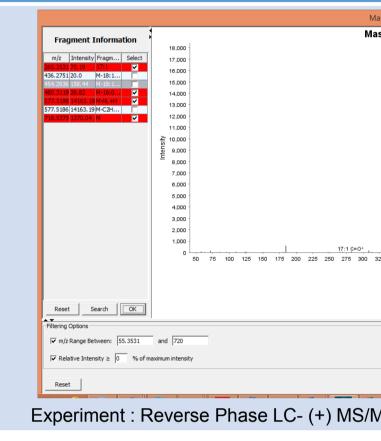


Table 6: MS/MS spectra from different experimental workflows wherein peaks are annotated with corresponding fragment ions from PE (16:0/18:1) lipid by SimLipid software. M-NL+H is the fragment ion generated due to the loss of head group i.e., loss of phosphoethanolamine from the lipid.

Table 6 shows the MS/MS spectra of PE(16:0/18:1) (Figure 7) from different experimental setups described in previously presented scientific poster [12]. Figure 8 shows the prototype results of lipids identified from LC- MS/MS data of both the ion modes exported into MS excel file. HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW ? 🗷 – 🗗 🗙

Multiplexed Precursor Ion/Neuti Data analysis:

Data from multiple precursor ion/n scans can be imported into SimLip using either native file formats or s formats. For an event e.g., PIS 184 all the scans can be summed up to the fluctuation in peak intensities a Users can model, experimental de the GUIs (Figure 9) by classifying according to biological/technical re

Identification of lipids based on target fatty acyls/head group fragment ions or their neutral losses is processed in batch mode. Isotope peak correction, if opted, is also done before loading the results into the software display windows (Figure 10). Lipids IDed across scans or samples are aligned (Figure 11) and corrected intensities of aligned lipids (across multiple precursor ion and neutral loss scans) are summed up.

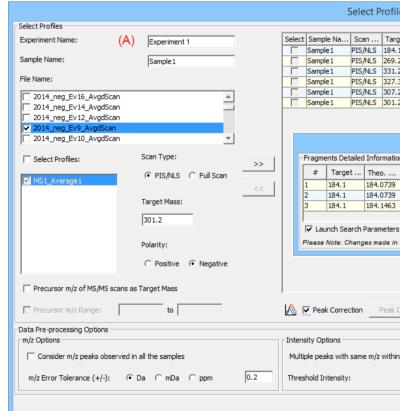


Figure 9: Typical SimLipid software GUIs for assigning target fatty acyls or lipid headgroups to product ions/neutral loss masses.

Conclusion

SimLipid[®] software provides the fo features:

- Comprehensive database cont lipids, 1,487,728 structure-spe MS/MS characteristic ions, and target masses of PIS/NLS.
- 2. A software tool capable of supp Shotgun, MALDI-, LC-, MS and workflows. Identify lipids for 10.000 MS/M
- batch 4. Isotope peak correction to facili quantitation of lipids.
- Portable reports in Microsoft ex CSV, JPG, PNG files.

References

- Murphy, R.C. (2002). Illuminati Press.
- 5. Ayumi et al. JASMS. (2013) 24:684Y68,
- Trimpin et al., JASMS. (2007) 18:1967.
- Cheng et al. Anal. Chem. (1998) 70:4417.
- McAnoy et al. J Am Soc Mass Spectrom. (2005) 16:1498–1509.



Lipid identification using MS/MS spectra in variable ion mode

In order to achieve structural information from tandem mass spectrometry data acquired in both the positive and negative mode ionizations, SimLipid software combined lipids identified by different experimental workflows and exported the fragment ions that are observed in different spectra, thereby facilitating easy review of the identified lipids.

Figure 7: PE(16:0/18:1) lipid was identified in all the four MS/MS runs by SimLipid

Lipid Structure(LMGP02010009

MS/MS spectra annot	tated by SimLipid
Mass Spectrum	Mass Spectrum
Mass Spectrum: m/z versus Intensity	Fragment Information Mass Spectrum: m/z versus Intensity m/z Intensity Fragm Select 196.0395 59.4 G411 1 196.0 1 1 196.0 1 1 196.0 1 1 196.0 1 1 196.0 1 1 196.0 1 1 196.0 1 1 196.0 1
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S/MS, Precursor m/z: 718.5378, and Adduct: [M+H] ⁺	Experiment : Reverse Phase (-) MS/MS, Precursor m/z: 716.5243, and Adduct: [M-H
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	Ret	ention ne	Lipid ID	Mass	#Carbon	#Double Bond	e Chemical Composition	Experimental m/z		tank	Score	Matched Fragments	Retention Time	the second strength of	Delta Mass(ppm)	Rank	Score	Matched Fragments
		2.635	LMGP0401086	834.635	40		0 (C46H91O10P+H) 1+	835.6417	0.6454	1	0.034	M-H2O:817.6291:36.66	2.643	833.6283	1.3609	1	0.035	19:0 COO-:311 2949:7(19:0 COO-:311 2949:7(M:833 6297 2649 32
m	6	2.987	LMSP0501AB0:	805.5551	30	,	1 [C42H79NO13+Na] 1	828.5442	0.8692	1	0.098	M-C30H58N02:365.1015:2.29, M-C6H1105:666.4862:27.57, M:828.5433:16421.83	2.988	804.5485	1.499	1		M-C6H11O5:642.4945: M:804.5482:1570.86
2	5		LMGP0401033		40	,	5 [C46H81O10P+Na] 1			1		HG+Na:195.0027:38.18	4.394	823.55	1.3815	1		HG-H2O:152.9941:63.7 C3H8O5P:152.9941:63. C3H6O5P:152.9941:63. M:823.5491:1207.44
		4 485	LMGP0401003	776 5567	36		1 [C42H81O10P+Na] 1	- 799.545	1.9022		0.05	HG+Na:195.0038:454.22, M-NL+Na:627.5349:1078.45, M-C3H805P- H20:627.5349:1078.45, M.799.5453:1659.2	4 489	775.5493	0.4439	1	0.059	17:1 COO-:281.2478:39 M-18:1:511.3059:116.3 M:775.5494:2769.89

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PISNLS_184.1_Pos	893.6	893.6742	0.0742	1592.9	1592.9	2704.7495	[C51H90NO8P+NH4		PC(21:0/22:6(4Z	43:6	PC(21:0/22:6)	PC(43:6)	LMGP010	~	
PISNLS_331.2_Neg	644.5	644.4524	0.0476	1522	1522	2132.8722	[C29H52NO7P+C5H	1	PC(21:4(6Z,9Z,1	21:4	PC(21:4/0:0)	PC(21:4)	LMGP010	~	
	894.7	894.6952	0.0048 <	1827.8889	1451.7815	2485.6082	[C52H96NO8P+H]		PC(22:0/22:4(7Z	44:4	PC(22:0/22:4)	PC(44:4)	LMGP010	~	
PISNLS_307.2_Neg	898.9	898.7265	0.1735	1765.8718	1765.8718	3024.8601	[C52H100NO8P+H]		PC(22:1(13E)/22	44:2	PC(22:1/22:1)	PC(44:2)	LMGP010	~	
	892.7	892.6795	0.0205	2577.0833	2577.0833	4411.157	[C52H94NO8P+H]	PC	PC(22:1(11Z)/22	44:5	PC(22:1/22:4)	PC(44:5)	LMGP010	~	
Experiment2	888.6	888.6089	0.0089	1863.2372	1863.2372	26853.8073	[C52H84NO8P+Li]	PC	PC(22:4(7Z,10Z,	44:10	PC(22:4/22:6)	PC(44:10)	LMGP010	~	
LC-WS and WS/WS Data	606.3	606.2956	0.0044	2358.5625	2283.162	3249.5157	[C30H50NO7P+K]		PC(22:6(4Z,7Z,1	22:6	PC(22:6/0:0)	PC(22:6)	LMGP010	~	
	895.6	895.596	0.004	3230.5192	3005.4752	5152.9154	[C52H80NO8P+NH4		PC(22:6(4Z,7Z,1	44:12	PC(22:6/22:6)	PC(44:12)	LMGP010	~	
	882.7	882.6922	0.0078	929.0833	929.0833	1539.5907	[C49H98NO8P+Na]		PC(23:0/18:0)	41:0	PC(23:0/18:0)	PC(41:0)	LMGP010	~	4
	646.4	646.4208	0.0208	1768.0833	1768.0833	2576.2015	[C32H66NO7P+K]		PC(24:0/0:0)	24:0	PC(24:0/0:0)	PC(24:0)	LMGP010	-	_
	861.5	861.5279	0.0279	884.5	884.5	1462.02	[C49H78NO8P+Na]		PE(22:4(7Z,10Z,	44:10	PE(22:4/22:6)	PE(44:10)	LMGP010	~	
	605.5	605.4653	0.0347	1105.9167	1105.9167	1513.229	[C32H65N2O6P+H]		PE-Cer(d14:1(4E	30:1	PE-Cer(d14:1/1	. PE-Cer(30:1)	LMSP030	~	
	649.3	649.4915	0.1915 <	451.3333	305.0219	27.7984	[C34H69N2O7P+H]		PE-Cer(d14:1(4E	32:1	PE-Cer(d14:1/1	. PE-Cer(32:1)	LMSP030	~	
	633.2	633.4966	0.2966	1061.5	1061.5	1485.5397	[C34H69N2O6P+H]		PE-Cer(d14:1(4E	32:1	PE-Cer(d14:1/1	. PE-Cer(32:1)	LMSP030	, ⊽	- 1
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gure 10: Typical SimLipid software GUI displaying targeted lipids IDed of PIS 184.1 Phosphocholine) scan. Encircled columns shows the observed peak intensity and isotope corrected peak intensities

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		PISNLS_184.1_Pos		PC(18:0/22:3(10Z,13Z,16Z				PC(40:3)	[C48H90NO8P+		5729.5331	9397.7016		174.8577	
ining 40,234		PISNLS_331.2_Neg		PC(20:0/20:2(11Z,14Z))	-//			PC(40:2)	[C48H92N08P+		3153.7472	6010.3038			
11111y 40,234		PISNLS_327.3_Neg	V	PC(18:4(6Z,9Z,12Z,15Z)/2	2:6(4Z,7Z,1.	40:10	PC(18:4/22:6)	PC(40:10)	[C48H76N08P+		4688.1067	8911.7658			
fin in allian		PISNLS_307.2_Neg		PC(16:0/24:1(15Z))		40:1		PC(40:1)	[C48H94N08P+		1259.6667	2071.0868		1259.6667	
fic <i>in-silico</i>		PISNLS_301.2_Neg		PC(14:0/26:0)		40:0	PC(14:0/26:0)	PC(40:0)	[C48H96NO8P+	-LPC	9248.746	40341.0096		1986.3571	
	Experim ₩ LC MS	and MS/MS Data		PC(17:2(9Z,12Z)/22:6(4Z,				PC(39:8)	[C47H78N08P+	-kPC	1199.3314	2056.5265		1199.3314	
over 5,200	LC-M30	anu mo/mo Data		PC(17:1(9Z)/22:6(4Z,7Z,10				PC(39:7)	C47H80NO8P+		2371.6966	4067.795		2371.6966	
				PC(19:1(9Z)/20:5(5Z,8Z,1				PC(39:6)	[C47H82NO8P+		3730.2	6263.1806			
				PC(19:0/20:5(5Z,8Z,11Z,1		39:5		PC(39:5)	[C47H84NO8P+		5426.2727	8995.7869			
				PC(17:0/22:4(7Z,10Z,13Z,				PC(39:4)	[C47H86NO8P+		5014.7291	8319.2642	3942.3094	0000 40	_
<i></i>				PC(17:1(9Z)/22:2(13Z,16Z PC(17:0/22:2(13Z,16Z))	.))		PC(17:1/22:2) PC(17:0/22:2)		[C47H88NO8P+		2023.19 17577.1571	3275.9644 30373.5557		2023.19 2039.8793	
orting				PC(17:0/22:2(132,102)) PC(17:0/22:1(11Z))			PC(17:0/22:2) PC(17:0/22:1)		[C47H90N08P+		4685.6003	7792.3684	4685.6003	2039.0793	_
•				PC(17:0/22:0)			PC(17:0/22:0)		[C47H92NO8P+ [C47H94NO8P-		8442.5018	13678.8897	8442.5018		_
MS/MS				PC(18:4(6Z,9Z,12Z,15Z)/2	0.5(57.87.1		PC(18:4/20:5)		[C46H74N08P+		9105.5778	16676.8462		3925.6778	_
				PC(18:3(6Z,9Z,12Z)/20:5(PC(38:8)	[C46H76N08P+		3205.7738	5438.0141		3205.7738	
	Linid Information			PC(16:1(9Z)/22:6(4Z,7Z,10				PC(38:7)	[C46H78N08P-		11189.3115	17909.4922			
	Lipid Information			PC(18:4(6Z,9Z,12Z,15Z)/2				PC(38:6)	[C46H80NO8P+		10203.2715	18332.1597			
	Lipid ID	LMGP01010821		PC(16:1(9Z)/22:4(7Z,10Z,	13Z,16Z))	38:5	PC(16:1/22:4)	PC(38:5)	[C46H82NO8P+		11414.8782	18527.3945			3117
scans in a		me PC(18:0/22:6(4Z,7		PC(16:0/22:4(7Z,10Z,13Z,	16Z))	38:4	PC(16:0/22:4)	PC(38:4)	[C46H84NO8P+	++PC	58161.2547	93383.9853		52257.535	
ocario in a	#C:DB in Fatty Acyl	40:6		PC(18:1(9Z)/20:2(11Z,14Z	.))	38:3	PC(18:1/20:2)	PC(38:3)	[C46H86NO8P-	HPC	4371.147	6998.0816		1	
	#DB #TP	0													►
	#DB in C-C	6		how distinct Chemical Formula	Show Set	lected O	Show All		Update Se	lection Cle	ar All Lipid St	tructure Mark	Unmark Internal Stand	lard Qua	ntitation
	Sys Name	1-octadecanoyl-2-		now distinct enemican ormala	se onow oc								onnark Internarotana		Tatadon
ate accurate	Category	Glycerophospholi		Scan Name	-	Observed m/	7	Theoretical r	m/z	Adduct		Charge State		Delta Mass	
	Main Class	PC	PISNI	LS_184.1_Pos	840.6	obberveaniy		.6089	[M+I		1	enarge etat	0.0089	Denamado	
	Sub Class	Diacylglycerophos		LS_331.2_Neg	833.8		833		[M-F		1		0.206		
	Mass	833.59345598		LS_327.3_Neg	833.6		833		[M-H		1		0.006		
cel, HTML,	Formula	C48H84NO8P		LS_307.2_Neg	892.7			6073	[M+/		1		0.0927		
	Other DB	PubChem Substa		LS_301.2_Neg	833.7		833		[M-H		1		0.106		
	◀	•													

Figure 11: Typical SimLipid software GUI showing aligned lipids across multiple PIS/NLS data.

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