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EVALUATION OF MS-DIAL AND MZMINE2 SOFTWARES FOR CLINICAL LIPIDOMICS ANALYSIS

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ABSTRACT. Lipidomics covers analysis of all lipid species in an organism. Lipid metabolism is one of the key factors to understand cellular processes at molecular level. Lipidomics has been used to find diagnostic and prognostic biomarkers in clinical sample (plasma, serum, urine, tissue). Today mass spectroscopy based approach dominates lipidomics and several computational platforms have been developed to process raw mass spectra data. However, there is no routine procedure for data processing in lipidomics. In present work, two different bioinformatics platforms, which are MS-DIAL and MZmine2, was compared for lipidomics analysis of plasma sample. Peak detection, identification and quantification parameters were investigated to understand advantages and disadvantages. In peak detection process, it was observed that MZmine2 detected more peak than MS-DIAL at same threshold level. In identification process, Lipidmaps database was used for identification. MZmine2 identifies more lipid than MS-DIAL. Semi-quantification is very important to find differentially expressed lipid species and biomarkers in clinical studies. MS-DIAL and MZmine2 calculated normalized peak intensities and results were compared to understand reproducibility. Average relative standard deviation of all peaks was calculated and results showed that MS-DIAL gives more reproducible results than MZmine2. In conclusion, MZmine2 and MS-DIAL could be used in clinical lipidomics studies.

1. INTRODUCTION

In recent years omics technologies have been emerged as essential tools to observe cellular processes at molecular level [1]

Genomics is the motherland of omics technologies. However, results of human genome project, which was ended in 2003, indicated that genome analysis is not

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sufficient to understand cellular process [2]. Because of this fact, post-genomic studies have drawn attention to complete genomics.

In post-genomic area, transcriptomics is the global analysis of mRNA, which is the product of transcription process [3]. Proteomics is another phoneme, which is the analysis of all gene-encoded proteins in cells and also post-translational modifications on protein structure [4]. Metabolomics is analysis of metabolites, which are side or end products of biological reactions (Figure 1) [5].

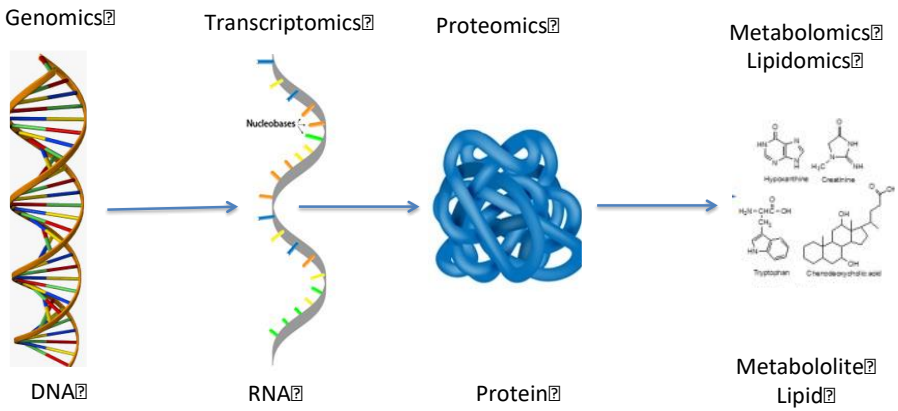

FIGURE 1. Omics technologies.

Lipidomics firstly described as a sub-discipline in metabolomics and covered analysis of all lipid spices in organism. Lipids play essential roles in cell structures, communication and trafficking [6]. In cell structure there are several thousand of lipid isoforms and in lipidomics each isoform structure should be evaluated in qualitative and quantitative analysis. In lipidomics workflow there are three steps. The first step is the extraction of lipids from biological specimen like cells, bacteria, tissue, plant, and body fluid. Currently various extraction methods are used for lipid isolation. Methanol/Chloroform and Methanol/terbutyl ether mixtures are most commonly used co-solvent systems in lipid extractions [7]. In second step, extracted lipid molecules are analyzed in analytical systems. Biological samples contained thousands of lipid molecules. Because of this biological complexity, there is a great demand for sensitive, reproducible analytical systems. Today liquid chromatography-mass spectroscopy system (LC/MS) is the most suitable combined

analytical system for lipidomics analysis [8-10]. Lipid species separated according to their polarity in LC system and analyzed in mass spectroscopy. Mass spectroscopy gives MS data for each lipid molecule. Commonly high sensitive LC/MS systems like LC/Q-TOF or LC/orbitrap system have been used in lipidomics analysis.

The last step in lipidomics is data process of raw MS data in computational platforms. In a single LC/MS analysis, thousand of MS data have been recorded. Analysis of this huge output is one of the biggest challenges in lipidomics analysis. Today various computational platforms have been developed for MS data processing [11-14]. Reliable lipid identification and semi quantification is the main purpose in computational lipidomics. Workflow of lipidomics software includes peak detection, filtering, deconvolution, intensity calculation, gap filling, identification and normalization.

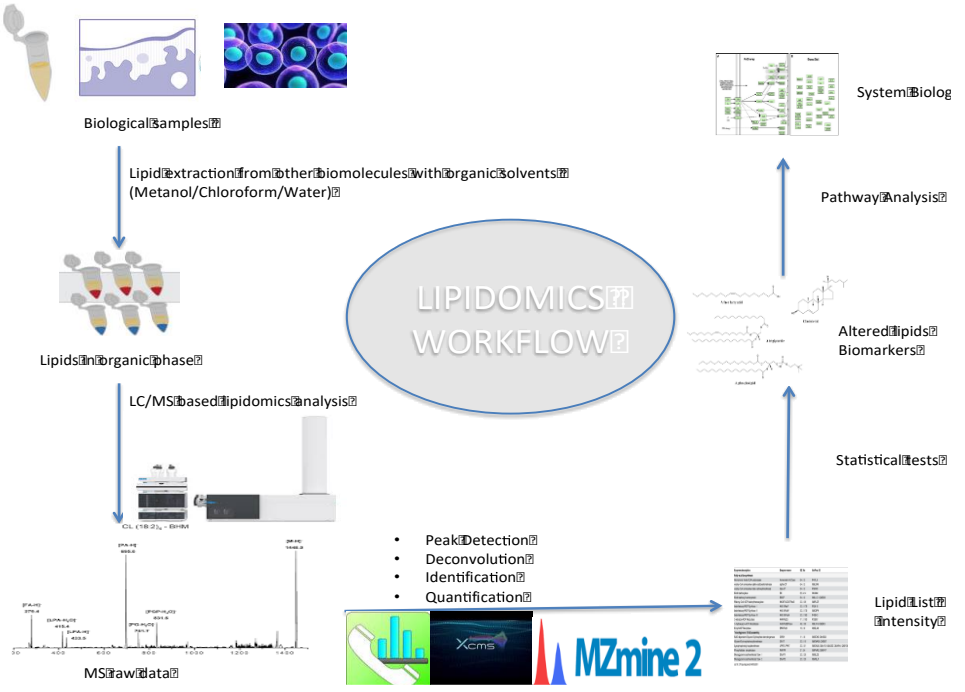

FIGURE 2. Classical LC/MS based lipidomics workflow.

MS-DIAL, which has been developed by Hiroshi Tsugova, has been used for untargeted metabolomics and lipidomics studies extensively [15-17]. In MS-DIAL

pipeline precursor ion peaks efficiently spotted by exploring two continuous data axes: retention times and accurate mass. Deconvolution mode is used to find individual peaks. Peak intensities are calculated according to peak height and area. Lipid molecules are identified with mass annotation (MS1 data) or MS2 data.
Another computational platform for metabolomics and lipidomics is Mzmine2 [18], which has similar workflow with MS-DIAL but it contains different peak detection, deconvolution and gap filling methods.

In present work, lipid profile of plasma was investigated by using Mzmine2 and MSDIAL. Peak detection, identification and quantification parameters were compared and discussed to understand advantages of software for clinical lipidomics.

2. EXPERIMENTAL

2.1. Sample Preparation

Lipidome structure of commercial plasma samples (100µL) (sigma) was analyzed in present work. Lipids in plasma were extracted by traditional methanol (300µL) /water (150µL)/chloroform (300µL) co solvent system. Lipids were extracted in chloroform phase. Chloroform was evaporated in vacuum centrifuge and lipids were solubilized in isopropyl alcohol and diluted mobile phase. We prepared three replicate for lipidomics analysis.
2.2. LC/MS Analysis

Lipids were separated in C18 column by performed Agilent 1290 HPLC system. In LC system, water (A) and ACN:Isopropyl alcohol (Sigma) (70:30 v/v) (B) were used a mobile phase. Aqueous and organic phase contained 1% 1M ammonium acetate - 0.1% acetic acid. Flow rate was adjusted as 0.20 mL/min. Elution gradient started with 55% B and it was risen up linearly 75% until 3^{th} minute, 89% until 8^{th} minute and 100% until 11^{th} minute. The ratio was kept constant till 15^{th} minute. The organic phase ratio will be decreased to 55% till 20th minute and 5-minute post run will be applied for further injections. Column temperature was set at 60^{o}C. After LC separation, lipids were analyzed in Agilent 6530 QTOF-MS system. Lipids were analyzed in positive mode. Scan range was 100-1700 m/z. Capillary voltage was 3500 V.

2.3. Data Processing

MS-DIAL workflow

The raw MS data, which was in MzmL format, were converted to abf. With abf converter (https://www.reifycs.com/AbfConverter/) format. MS-DIAL 3.30 was downloaded from http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL/. In first step, ionization mode (positive), data type (centroid) was selected. In peak detection process, threshold of peak intensity was selected as 10000 amplitude. Linear weighted moving average method was preferred for smoothing and peak width was adjusted as 5. In deconvolution process, sigma window value was selected as 0.5. Retention time and mass tolerance was selected 2 min. and 10 ppm for gap filling process. In peak alignment process, retention time tolerance and MS1 tolerance were set up at 0.05 min and 0.04 Da. Total peak areas were used for peak intensity calculation and total ion intensity was used for normalization (Figure 3).

MZmine2

In data process by performed MZmine2(http://mzmine.github.io/download.html), raw MS data in mzML format was imported. Mass detection and ADAP chromatogram builder was performed to detected peaks. Minimum intensity was selected as 10000 amplitudes as well MS-DIAL. In peak smoothing process, peak width was adjusted as 5 as well as MSDIAL. In deconvolution, wavelet (ADAP) algorithm was used to separate overlapped peaks. Retention time tolerance was adjusted as 2 min and mass tolerance 10 ppm as well as MS-DIAL for gap filling. In addition, Intensity tolerance, which is another option in Mzmine2, was selected default value (10%). Linear normalization algorithm was used to normalize peaks area for relative quantification. In normalization process, total ion intensity was used to avoid experimental errors (Figure 3).

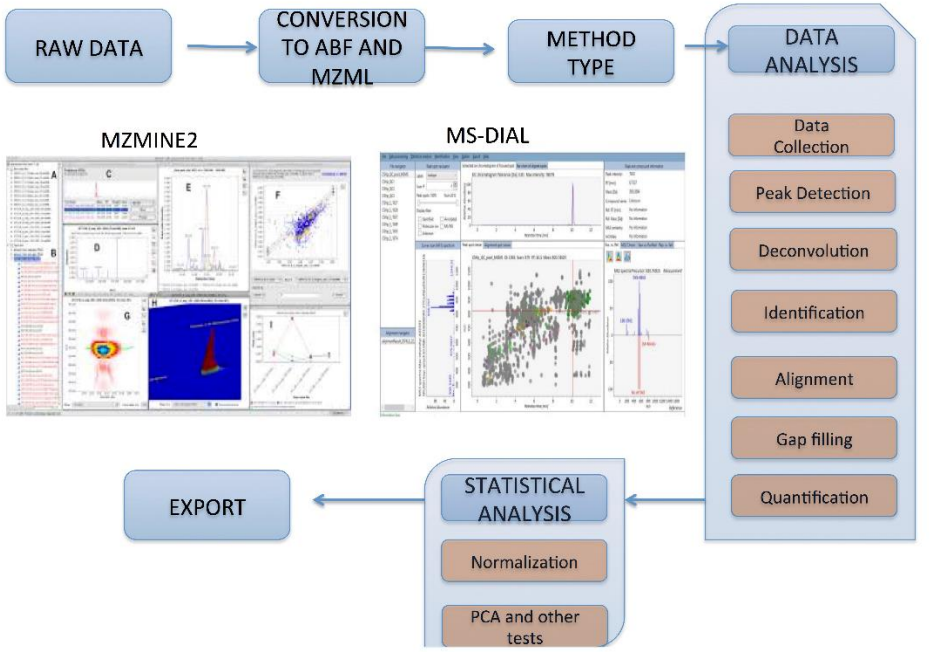

FIGURE 3. Workflow of MZmine2 and MS-DIAL

3. RESULTS AND DISCUSSION

Lipidomics, defined as the large-scale study of cellular lipids (i.e., the lipidome), has recently emerged as a rapidly expanding research field under the umbrella of systems biology. New analytical systems (NMR or Mass spectroscopy) have been developed in lipidomics to observe cellular process. However, there is a great demand to process raw outputs of analytical systems accurately. In present work, two promising platforms were compared for plasma lipidome profiling which is valuable specimen in clinical studies to find biomarkers. Peak detection, identification and quantification steps were evaluated for both platforms.

3.1. Peak Detection

The first step in lipidomics analysis is to detect peaks accurately. In peak detection, threshold of peak intensity is the most important parameter. High threshold causes

loss of many lipid species. Low threshold leads false negative identifications. In present work, threshold was selected as 10000 amplitudes for MS-DIAL and MZmine2. This level has been used in various lipidomics studies. In current stats, MS-DIAL detected 1157 and MZmine2 detected 1437 peaks
(Figure 4).

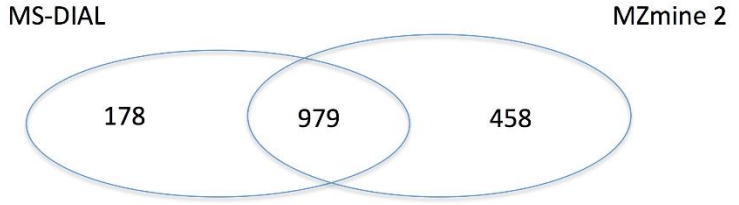

FIGURE 4. Number of detected peaks in MS-DIAL and MZmine2

Peak deconvolution is the second step after peak detection. The process of data deconvolution, sometimes called peak picking, is in itself a complex process caused by the complexity of the data and variation introduced during the process of data acquisition related to mass-to-charge ratio, retention time and chromatographic peak area. In deconvolution process, overlapped peaks separated to enhance analytical resolution.

The gap filling is another important parameter for lipidomics analysis. The gap filling process allowed us to connect identified metabolites and detection of metabolites that had been discarded during the data pre-processing or simply were not detected due to the LC/MS configuration. This is important to match identified and detected species between technical replicates. Mzmine2 software allows options to select gap filling ratio. MS-DIAL has automatic gap filling algorithm.

3.2. Peak Identification

In identification process MZmine2 offers two different routes. The first one is local MZmine2 lipid database and online lipidmaps database search option. MS-DIAL also offers two different identification routes. The first one lipidblast database module. The other way is transferring of MS-DIAL results to MS-FINDER, which include lipidmaps database, to match MS1 results with lipidmaps database. To evaluate identification yield of two softwares, lipidmaps database search option was used for both software. 189 lipid species were identified in MS-DIAL. In MZmine2, using lipidmaps database identified 155 lipid species. 121 lipid spices were

commonly identified with MZmine2 and MS-DIAL. 16.33% of detected peaks were identified in MS-DIAL and 10.78% of detected peaks were identified in MZmine 2. These lipids were listed in supplementary information.

3.3. Relative quantification of lipid species

In relative quantification process, peak intensities of lipid species in different experimental groups are compared to find statistically altered lipids in clinic samples. In lipidomics studies peak areas or peak heights are used for relative quantification between experimental groups. The peak of PG (16:0/0:0), which was detected in three replicates, was represented as an example in Figure 5.

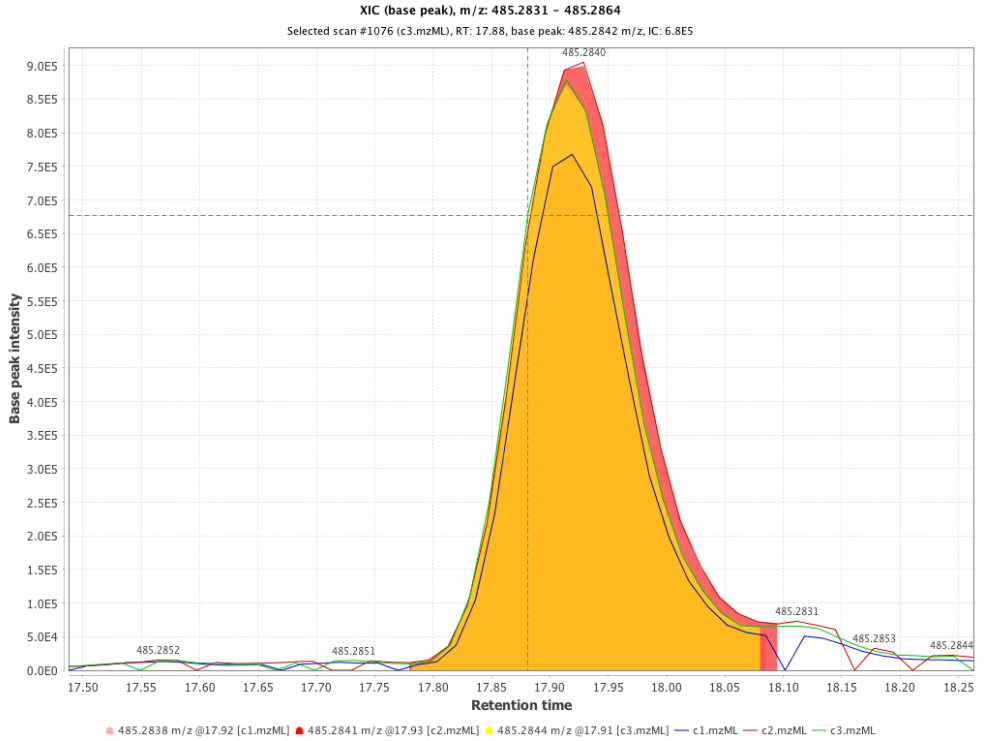

FIGURE 5. Peak of PG (16:0/0:0), wich was detected in three technical replicate. Peak area calculated by MS-DIAL and Mzmine2 automatically.

In MS-DIAL and MZmine2 workflow, peak heights or peak areas could be calculated. Normalization is an important step in relative quantification. In both platforms, internal standard could be used for normalization. Moreover, total ion

intensity could be also used for normalization process. In present work, total ion intensity of all identified lipids was used for normalization.

In present work, peak areas of lipid species were calculated by using MS-DIAL and MZmine2. Average relative standard deviation values were calculated for both software to understand relative reproducibility. Moreover, two sample t-test was used to observe if MSDIAL and MZmine2 give similar quantification results in plasma sample.

Average relative standard deviations of peak areas of identified lipids were calculated for both software. Average relative standard deviation was calculated as 10.2% for MS-DIAL and 16.3% for MZmine2. This result showed that MS-DIAL gives more reproducible results than MZmine2. We used two sample t-test to compare identified lipid intensities. Results showed there is no statistical difference for 98 lipid species. Intensities of 23 lipid species were differently calculated in MZmine2 and MS-DIAL. This result showed that MZmine2 and MS-DIAL give similar results for many lipid species in clinical samples.

4. Conclusion

In present work, MS-DIAL and MZmine2 software were compared by using LC/MS lipidomics data. In peak detection and identification process, MZmine2 provides to analyze more lipid species. However, MS-DIAL gives more reproducible results for quantification of lipid species. These two platforms have been used for lipidomics and metabolomics analysis. Present work will contribute to make more detailed and reliable lipidomics analysis.

Supplementary Information

No	Lipids
1	Xeniasterol-b;1alpha,25-dihydroxy-2beta-(3-hydroxypropoxy)vitamin D3 / 1alpha,25-dihydroxy-2beta-(3-hydroxypropoxy) cholecalciferol;
2	Wuhanic acid
3	Stoloniferone F;3alpha,7alpha,12alpha-Trihydroxy-24-methyl-5beta-cholest-23-en-26-oic acid
4	Spheroidene;Dihydroanhydrorhodovibrine/ Dihydromethoxylycopene
5	Rhodovibrin;3,4-Dihydrospheroidenone
6	PS(P-16:0/12:0)
7	PS(P-16:0/12:0)
8	PS(18:0/12:0);PS(17:0/13:0);PS(13:0/17:0);PS(12:0/18:0);PS(16:0/14:0);PS(15:0/15:0);PS(14:0/16:0)
9	PS(17:2(9Z,12Z)/0:0);Epothilone B

10	PS(17:0/22:0);PS(18:0/21:0);PS(19:0/20:0);PS(20:0/19:0);PS(22:0/17:0);PS(21:0/18:0)
11	PS(12:0/19:0);PS(13:0/18:0);PS(14:0/17:0);PS(17:0/14:0);PS(18:0/13:0);PS(19:0/12:0);PS(16:0/15:0);PS(15:0/16:0)
12	PKODiA-PA
13	PI(O-16:0/19:1(9Z));PI(O-18:0/17:1(9Z));PI(O-20:0/15:1(9Z));PI(P-16:0/19:0);PI(P-18:0/17:0);PI(P-20:0/15:0)
14	PI-Cer(d18:1/14:0)
15	PGF2alpha dimethyl amide;N-(3-oxo-octadecanoyl)-homoserine lactone;5,6-DiHETrE-EA;8,9-DiHETrE-EA;14,15-DiHETrE-EA;11,12-DiHETrE-EA
16	PG(P-20:0/19:1(9Z))
17	PG(P-16:0/20:5(5Z,8Z,11Z,14Z,17Z))
18	PG(P-16:0/15:1(9Z))
19	PG(O-16:0/20:2(11Z,14Z));PG(O-18:0/18:2(9Z,12Z));PG(P-16:0/20:1(11Z));PG(P-20:0/16:1(9Z));PG(P-18:0/18:1(9Z))
20	PG(O-16:0/18:2(9Z,12Z));PG(P-18:0/16:1(9Z));PG(P-20:0/14:1(9Z));PG(P-16:0/18:1(9Z))
21	PG(20:0/22:1(11Z));PG(20:1(11Z)/22:0);PG(22:1(11Z)/20:0);PG(22:0/20:1(11Z))
22	PG(17:0/22:1(11Z));PG(17:1(9Z)/22:0);PG(18:1(9Z)/21:0);PG(19:0/20:1(11Z));PG(19:1(9Z)/20:0);PG(20:0/19:1(9Z));PG(20:1(11Z)/19:0);PG(21:0/18:1(9Z));PG(22:0/17:1(9Z));PG(22:1(11Z)/17:0)
23	PG(16:0/0:0)
24	PG(16:0/0:0)
25	PG(13:0/22:1(11Z));PG(14:1(9Z)/21:0);PG(15:0/20:1(11Z));PG(15:1(9Z)/20:0);PG(16:0/19:1(9Z));PG(16:1(9Z)/19:0);PG(17:1(9Z)/18:0);PG(18:0/17:1(9Z));PG(18:1(9Z)/17:0);PG(19:0/16:1(9Z));PG(19:1(9Z)/16:0);PG(20:0/15:1(9Z));PG(20:1(11Z)/15:0);PG(21:0/14:1(9Z));PG(22:1(11Z)/13:0);PG(17:0/18:1(9Z));PG(16:0/18:0(11Cp))
26	Pectenolone;(3S,4R,3'R)-4-Hydroxyalloxanthin;(3S,4S,3'R)-4-Hydroxyalloxanthin;Phoenicoxanthin/ Adonirubin/ 3-Hydroxycanthaxanthin
27	PE(P-20:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))
28	PE(P-16:0/0:0)
29	PE(20:4(5Z,8Z,11Z,14Z)/0:0);PE(0:0/20:4(5Z,8Z,11Z,14Z));PE(0:0/20:4(8Z,11Z,14Z,17Z));PE(20:4(8Z,11Z,14Z,17Z)/0:0)
30	PE(20:3(8Z,11Z,14Z)/0:0);PE(0:0/20:3(11Z,14Z,17Z));PE(0:0/20:3(5Z,8Z,11Z));PE(0:0/20:3(8Z,11Z,14Z));PE(20:3(11Z,14Z,17Z)/0:0);PE(20:3(5Z,8Z,11Z)/0:0)
31	PE(12:0/16:1(9Z));PE(13:0/15:1(9Z));PE(14:0/14:1(9Z));PE(14:1(9Z)/14:0);PE(15:1(9Z)/13:0);PE(16:1(9Z)/12:0)
32	PE(12:0/15:1(9Z));PE(13:0/14:1(9Z));PE(14:1(9Z)/13:0);PE(15:1(9Z)/12:0)
33	PC(P-20:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))
34	PC(P-16:0/20:5(5Z,8Z,11Z,14Z,17Z))
35	PC(P-16:0/17:2(9Z,12Z));PE(O-18:0/18:3(6Z,9Z,12Z));PE(O-18:0/18:3(9Z,12Z,15Z));PE(O-16:0/20:3(8Z,11Z,14Z));PE(P-16:0/20:2(11Z,14Z));PE(P-18:0/18:2(9Z,12Z));GlcCer(d18:2(4E,8Z)/17:0(2OH[R]));GlcCer(d15:2(4E,6E)/20:0(2OH))
36	PC(O-18:2(9Z,12Z)/2:0);PC(20:2(11Z,14Z)/0:0)
37	PC(O-18:1(10E)/2:0);PC(O-18:1(9Z)/2:0);PC(P-18:0/2:0);PC(20:1(9Z)/0:0);PC(20:1(11Z)/0:0)
38	PC(O-16:1(11Z)/2:0);PC(P-16:0/2:0);PC(18:1(6Z)/0:0);PC(18:1(9E)/0:0);PC(18:1(9Z)/0:0);PC(0:0/18:1(6Z));PC(0:0/18:1(9E));PC(0:0/18:1(9Z));PC(18:1(9Z)/0:0)[rac];PC(18:1(11Z)/0:0)
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40	PC(O-16:0/0:0)
41	PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)

42	PC(22:5(4Z,7Z,10Z,13Z,16Z)/0:0);PC(22:5(7Z,10Z,13Z,16Z,19Z)/0:0)
43	PC(22:4(7Z,10Z,13Z,16Z)/0:0)
44	PC(22:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z));PC(22:2(13Z,16Z)/22:4(7Z,10Z,13Z,16Z));PC(22:4(7Z,10Z,13Z,16Z)/22:2(13Z,16Z));PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:0)
45	PC(21:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z));PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/21:0)
46	PC(20:5(5Z,8Z,11Z,14Z,17Z)/22:5(7Z,10Z,13Z,16Z,19Z));PC(20:4(5Z,8Z,11Z,14Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z));PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/20:4(5Z,8Z,11Z,14Z))
47	PC(20:4(5Z,8Z,11Z,14Z)/0:0);PC(0:0/20:4(5Z,8Z,11Z,14Z));PC(20:4(8Z,11Z,14Z,17Z)/0:0)
48	PC(20:3(8Z,11Z,14Z)/0:0);PC(20:3(5Z,8Z,11Z)/0:0)
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52	PC(15:0/0:0);PE(18:0/0:0);PE(0:0/18:0);1-(2-methoxy-6Z-heptadecenyl)-sn-glycero-3-phosphoethanolamine
53	PC(13:0/0:0);PE(16:0/0:0);PE(0:0/16:0);1-(2-methoxy-13-methyl-6Z-tetradecenyl)-sn-glycero-3-phosphoethanolamine;1-(2-methoxy-6Z-pentadecenyl)-sn-glycero-3-phosphoethanolamine
54	PA(P-16:0/14:1(9Z))
55	PA(O-20:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))
56	PA(O-18:0/17:2(9Z,12Z));PA(P-16:0/19:1(9Z));PA(P-18:0/17:1(9Z));PA(P-20:0/15:1(9Z));SM(d18:2/14:0);PE-Cer(d14:2(4E,6E)/21:0);PE-Cer(d15:2(4E,6E)/20:0);PE-Cer(d16:2(4E,6E)/19:0)
57	PA(O-16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z));PA(P-18:0/20:5(5Z,8Z,11Z,14Z,17Z))
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59	PA(O-16:0/17:2(9Z,12Z));PA(P-16:0/17:1(9Z));PA(P-18:0/15:1(9Z))
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61	PA(18:0/18:1(9Z));PA(14:0/22:1(11Z));PA(14:1(9Z)/22:0);PA(15:1(9Z)/21:0);PA(16:1(9Z)/20:0);PA(17:0/19:1(9Z));PA(17:1(9Z)/19:0);PA(19:0/17:1(9Z));PA(19:1(9Z)/17:0);PA(20:0/16:1(9Z));PA(20:1(11Z)/16:0);PA(21:0/15:1(9Z));PA(22:0/14:1(9Z));PA(22:1(11Z)/14:0);PA(18:1(9Z)/18:0);PA(16:0/20:1(11Z))
62	PA(17:0/22:1(11Z));PA(17:1(9Z)/22:0);PA(18:1(9Z)/21:0);PA(19:0/20:1(11Z));PA(19:1(9Z)/20:0);PA(20:0/19:1(9Z));PA(20:1(11Z)/19:0);PA(21:0/18:1(9Z));PA(22:0/17:1(9Z));PA(22:1(11Z)/17:0);PE-Cer(d15:2(4E,6E)/24:0(2OH))
63	PA(14:0/18:1(9Z));PA(12:0/20:1(11Z));PA(13:0/19:1(9Z));PA(14:1(9Z)/18:0);PA(15:0/17:1(9Z));PA(15:1(9Z)/17:0);PA(16:1(9Z)/16:0);PA(17:0/15:1(9Z));PA(17:1(9Z)/15:0);PA(18:0/14:1(9Z));PA(19:1(9Z)/13:0);PA(20:1(11Z)/12:0);PA(18:1(9Z)/14:0);PA(16:0/16:1(9Z))
64	OH-Spheroidenone
65	O-(17-carboxyheptadecanoyl)carnitine
66	N-palmitoyl tryptophan
67	N-oleoyl histidine
68	N-arachidonoyl D-serine;N-arachidonoyl L-serine;N-stearoyl taurine;15-HETE-Ala;12-HETE-Ala
69	N-(3E-hexadecenoyl)-deoxysphing-4-enine-1-sulfonate
70	Minabeolide-2
71	MG(16:0/0:0/0:0)[rac];MG(16:0/0:0/0:0);MG(0:0/16:0/0:0);1-O-(2R-methoxy-4Z-pentadecenyl)-sn-glycerol;1-O-(2R-hydroxy-4Z-hexadecenyl)-sn-glycerol
72	L-Isoleucic acid;DL-2-hydroxy caproic acid;DL-3-hydroxy caproic acid;DL-4-hydroxy caproic acid;5-hydroxy caproic acid;6-hydroxy caproic acid;5R-hydroxy-hexanoic acid;3R-hydroxy-hexanoic acid;2-hydroxy-3-methyl-pentanoic acid;Leucinic acid;2-ethyl-2-hydroxy-

	butyric acid;D-Leucic acid;hydroxy-isocaproic acid;(S)-3-hydroxyhexanoic acid;(2R,3S)-2-hydroxy-3-methylpentanoic acid;(2S,3R)-3-hydroxy-2-methylpentanoic acid
73	GM4(d18:1/20:0)
74	GlcCer(d18:2(4E,8Z)/16:0(2OH[R]));GlcCer(d18:2(4E,8E)/16:0(2OH[R]));GlcCer(d14:2(4E,6E)/20:0(2OH));GlcCer(d16:2(4E,6E)/18:0(2OH))
75	GlcCer(d15:2(4E,6E)/20:0)
76	GlcCer(d14:1/18:1);GlcCer(d14:2(4E,6E)/18:0)
77	ent-9-L1-PhytoP;16-B1-PhytoP;9-L1-PhytoP;ent-16-B1-PhytoP;9-B1-PhytoP;ent-9-B1-PhytoP;16-A1-PhytoP;16-epi-16-A1-PhytoP;9-A1-PhytoP;9-epi-9-A1-PhytoP;ent-16-A1-PhytoP;ent-16-epi-16-A1-PhytoP;ent-9-A1-PhytoP;ent-9-epi-9-A1-PhytoP;16-J1-PhytoP;16-epi-16-J1-PhytoP;9-J1-PhytoP;9-epi-9-J1-PhytoP;ent-16-J1-PhytoP;ent-16-epi-16-J1-PhytoP;ent-9-J1-PhytoP;ent-9-epi-9-J1-PhytoP;14,14,14-Trifluoro-11E-tetradecenyl acetate;14,14,14-Trifluoro-11Z-tetradecenyl acetate
78	dolichyl-4-D-xylosyl phosphate
79	Diketospirilloxanthin/ 2,2'-Diketospirilloxanthin
80	DGCC(16:0/20:5);DGCC(20:5/16:0)
81	DG(22:5(7Z,10Z,13Z,16Z,19Z)/22:5(7Z,10Z,13Z,16Z,19Z)/0:0);DG(22:4(7Z,10Z,13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)[iso2];PA(15:0/22:1(11Z));PA(15:1(9Z)/22:0);PA(16:1(9Z)/21:0);PA(17:0/20:1(11Z));PA(17:1(9Z)/20:0);PA(18:0/19:1(9Z));PA(18:1(9Z)/19:0);PA(19:0/18:1(9Z));PA(19:1(9Z)/18:0);PA(20:0/17:1(9Z));PA(20:1(11Z)/17:0);PA(21:0/16:1(9Z));PA(22:0/15:1(9Z));PA(22:1(11Z)/15:0)
82	DG(12:0/17:1(9Z)/0:0)[iso2];DG(13:0/16:1(9Z)/0:0)[iso2]
83	delta2-THA;5beta-Chola-3,8(14),11-trien-24-oic Acid
84	Cervonyl carnitine
85	bromosuccinic acid
86	Bombykol;10-propyl-5,9-tridecadien-1-ol;7,11-hexadecadien-1-ol;6E,11Z-hexadecadien-1-ol;6Z,11Z-hexadecadien-1-ol;10E,12E-Hexadecadien-1-ol;11E,13E-Hexadecadien-1-ol;11E,13Z-Hexadecadien-1-ol;4E,6Z-Hexadecadien-1-ol;11Z,13E-Hexadecadien-1-ol;7Z,11E-Hexadecadien-1-ol;11Z,13Z-Hexadecadien-1-ol;7Z,11Z-Hexadecadien-1-ol;1,3-Hexadecadien-1-ol;2-hexadecenal;7-hexadecenal;9-hexadecenal;11-hexadecenal;2Z-hexadecenal;10E-Hexadecenal;10Z-Hexadecenal;11Z-Hexadecenal;12Z-Hexadecenal;7Z-Hexadecenal;9Z-Hexadecenal;cis-11-Hexadecenal;(Z)-hexadec-13-enal;1,15-Hexadecadien-3-one;1-Hexadecen-3-one
87	Behenic acid;Isobehenic acid;19-methyl-heneicosanoic acid;3-methyl-heneicosanoic acid;14,19-dimethyl-eicosanoic-acid;Eicosyl acetate;octadecyl butyrate;hexadecyl hexanoate;tetradecyl octanoate;dodecyl decanoate;hexyl hexadecanoate;6,10,13-Trimethyltetradecyl 3-methylbutanoate;16-Methylheptadecyl isobutyrate;Octadecyl isobutyrate;Butyl octadecanoate
88	bayogenin;Acacic acid;Arjunolic acid;11-acetoxy-3beta,6alpha-dihydroxy-24-methylene-9,11-seco-5alpha-cholesta-7,22E-dien-9-one. ;11-acetoxy-3beta,6alpha-dihydroxy-24-methyl-9,11-seco-5alpha-cholesta-7,22Z-dien-9-one. ;cimigenol
89	Axillarenic acid;Nebraskanic acid;Tetracosanedioic acid
90	Angelic acid;Tiglic acid;beta,beta-dimethyl acrylic acid;Isopropenylacetic acid;2-ethyl acrylic acid;beta-ethyl acrylic acid;beta-penteic acid;Allyl acetic acid;cis-pent-2-enoic acid;cis-pent-3-enoic acid;ethyl 2E-propenoate;gamma-valerolactone;Pentane-2,4-dione
91	9-Keto heptadecylic acid;16-oxo-heptadecanoic acid;2-oxo-heptadecanoic acid;3-oxo-heptadecanoic acid;2-methoxy-5Z-hexadecenoic acid;2-methoxy-6Z-hexadecenoic acid;Avocadyne;Muricatacin
92	6,8-dihydroxy-octanoic acid
93	5-(L-alanin-3-yl)-2-hydroxy-cis,cis-muconate 6-semialdehyde
94	4R-aminopentanoic acid;4S-aminopentanoic acid;4-amino-pentanoic acid;5-amino-pentanoic acid;2S-amino-pentanoic acid;2-Amino-3-methylbutanoic acid
95	4,8,12-Trimethyl-1,3E,7E,11-tridecatetraene;3,6,9-Hexadecatriene
96	4,4'-Diapo-zeta-carotene

97	3alpha-Hydroxy-6-oxo-5beta-cholan-24-oic Acid;3beta-Hydroxy-6-oxo-5beta-cholan-24-oic Acid;3alpha-Hydroxy-6-oxo-5alpha-cholan-24-oic Acid;3beta-Hydroxy-6-oxo-5alpha-cholan-24-oic Acid;7-oxolithocholic acid;3beta-Hydroxy-7-oxo-5beta-cholan-24-oic Acid;3beta-Hydroxy-7-oxo-5alpha-cholan-24-oic Acid;3alpha-Hydroxy-11-oxo-5beta-cholan-24-oic Acid;3beta-Hydroxy-11-oxo-5beta-cholan-24-oic Acid;3alpha-Hydroxy-12-oxo-5beta-cholan-24-oic Acid;3beta-Hydroxy-12-oxo-5beta-cholan-24-oic Acid;12beta-Hydroxy-3-oxo-5beta-cholan-24-oic Acid;6alpha-Hydroxy-3-oxo-5beta-cholan-24-oic Acid;6beta-Hydroxy-3-oxo-5beta-cholan-24-oic Acid;6alpha-Hydroxy-3-oxo-5alpha-cholan-24-oic Acid;7alpha-Hydroxy-3-oxo-5beta-cholan-24-oic Acid;7beta-Hydroxy-3-oxo-5beta-cholan-24-oic Acid;7alpha-Hydroxy-3-oxo-5alpha-cholan-24-oic Acid;7alpha-Hydroxy-12-oxo-5beta-cholan-24-oic Acid;7beta-Hydroxy-12-oxo-5beta-cholan-24-oic Acid;7alpha-Hydroxy-12-oxo-5alpha-cholan-24-oic Acid;7beta-Hydroxy-12-oxo-5alpha-cholan-24-oic Acid;12alpha-Hydroxy-3-oxo-5beta-cholan-24-oic Acid;12alpha-Hydroxy-3-oxo-5alpha-cholan-24-oic Acid;12alpha-Hydroxy-7-oxo-5alpha-cholan-24-oic Acid;3beta,6beta-Dihydroxychol-4-en-24-oic Acid;3beta,7alpha-Dihydroxychol-4-en-24-oic Acid;3beta,7alpha-Dihydroxychol-5-en-24-oic Acid;3beta,7beta-Dihydroxychol-5-en-24-oic Acid;3beta,12alpha-Dihydroxychol-5-en-24-oic Acid;3alpha,12alpha-Dihydroxy-5beta-chol-6-en-24-oic Acid;3alpha,12beta-Dihydroxy-5beta-chol-6-en-24-oic Acid;3alpha,12alpha-Dihydroxy-5beta-chol-7-en-24-oic Acid;3alpha,12alpha-Dihydroxy-5beta-chol-8-en-24-oic Acid;3alpha,12alpha-Dihydroxy-5beta-chol-8(14)-en-24-oic Acid;3alpha,12alpha-Dihydroxy-5beta-chol-9(11)-en-24-oic Acid;7alpha,12alpha-Dihydroxy-5beta-chol-3-en-24-oic Acid;(22E)-3alpha,7alpha-Dihydroxy-5beta-chol-22-en-24-oic Acid;(22E)-3alpha,7beta-Dihydroxy-5beta-chol-22-en-24-oic Acid;(22E)-3alpha,12alpha-Dihydroxy-5beta-chol-22-en-24-oic Acid;1alpha-Hydroxy-3-oxo-5beta-cholan-24-oic Acid;3alpha,7alpha-Dihydroxychol-5-en-24-oic Acid;3alpha,7beta-Dihydroxychol-5-en-24-oic Acid;3alpha,7alpha-Dihydroxy-5beta-chol-11-en-24-oic Acid;3alpha,7beta-Dihydroxy-5beta-chol-11-en-24-oic Acid;3alpha,12alpha-Dihydroxy-5beta-chol-14-en-24-oic Acid;3alpha,12beta-Dihydroxy-5beta-chol-9(11)-en-24-oic Acid;3alpha-Hydroxy-12-oxo-5alpha-cholan-24-oic Acid;7alpha,12alpha-Dihydroxy-5beta-chol-2-en-24-oic Acid;12alpha-Hydroxy-7-oxo-5beta-cholan-24-oic Acid;3beta,19-Dihydroxychol-5-en-24-oic Acid;3alpha-Hydroxy-7-oxo-5alpha-cholan-24-oic Acid;3alpha,7alpha-Dihydroxy-5beta-chol-16-en-24-oic Acid
98	3,4,3',4'-Tetrahydrospirilloxanthin
99	3,4-dihydroxy-4-methylhexadecanoic acid;1-O-(2R-hydroxy-4Z-tetradecenyl)-sn-glycerol
100	3-Methylsubericacid;Azelaic acid;cis- and trans-Ethyl 2,4-dimethyl-1,3-dioxolane-2-acetate
101	3-hydroxypalmitoleoylcarnitine;N-stearoyl glutamic acid
102	2,5-Diaminopentanoic acid
103	2,4-diamino-butyric acid
104	2-deoxyecdysone;25-deoxyecdysone;Porrigenin A;Digitogenin;Agigenin;Paniculogenin;Neoagigenin;Tokorogenin;Epimetagenin;Metagenin;Convallagenin A;Neotokorogenin;Hispigenin;Solaspigenin;Neosolaspigenin;(24R)-6,19-epidioxy-1alpha,24-dihydroxy-6,19-dihydrovitamin D3 / (24R)-6,19-epidioxy-1alpha,24-dihydroxy-6,19-dihydrocholecalciferol;(6R)-6,19-epidioxy-1alpha,25-dihydroxy-6,19-dihydrovitamin D3 / (6R)-6,19-epidioxy-1alpha,25-dihydroxy-6,19-dihydrocholecalciferol;(23S,25R)-1alpha,23,25,26-tetrahydroxyvitamin D3 / (23S,25R)-1alpha,23,25,26-tetrahydroxycholecalciferol;1alpha,23R,25S,26-Tetrahydroxyvitamin D3;7beta,12alpha-Dihydroxy-3-oxo-5beta-cholestan-26-oic acid;7alpha,12alpha-Dihydroxy-3-oxo-5beta-cholestan-26-oic acid;7alpha,12alpha-Dihydroxy-3-oxo-5alpha-cholestan-26-oic acid;3alpha,12alpha-Dihydroxy-7-oxo-5beta-cholestan-26-oic acid;3alpha,7alpha,12alpha-Trihydroxy-5beta-24E-cholesten-26-oic acid;3alpha,7alpha,12alpha-Trihydroxy-5beta-cholest-23-en-26-oic acid;3alpha,7alpha-Dihydroxy-12-oxo-5alpha-cholestan-26-oic acid;3alpha,7alpha,12alpha-trihydroxy-5alpha-23E-cholesten-26-oic acid;3alpha,7alpha,12alpha-Trihydroxy-5beta-24Z-cholesten-26-oic acid
105	17-phenyl-trinor-PGF2alpha amide;N-linoleoyl taurine
106	17-oxo-20Z-hexacosenoic acid;Ficulinic acid A;22-keto-26-Hexacosanolide
107	12beta-hydroxy-24-norcholesta-1,4,22E-trien-3-one
108	10-oxo-nonadecanoic acid;18-oxo-nonadecanoic acid;2-oxo-nonadecanoic acid;3-oxo-nonadecanoic acid

109	1-tetradecanyl-2-(8-[3]-ladderane-octanyl)-sn-glycerophosphoethanolamine;GlcCer(d15:2(4E,6E)/18:0)
110	1-tetradecanyl-2-(8-[3]-ladderane-octanyl)-sn-glycerophosphoethanolamine
111	1-O-(2R-hydroxy-hexadecyl)-sn-glycerol
112	1-chloro-3-(5'-(penta-1,3-diyn-1-yl)-[2,2'-bithiophen]-5-yl)prop-2-yn-1-ol
113	1-(6-[3]-ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-sn-glycerophosphocholine
114	1-(2-methoxy-eicosanyl)-sn-glycero-3-phosphoethanolamine
115	(3'-sulfo)Galbeta-Cer(d18:1/22:0)
116	(2S,3R,4E,8E)-3-Hydroxy-2-[methyl(stearoyl)amino]-4,8-octadecadien-1-yl hydrogen sulfate
117	(2E,5Z,7E)-decatrienoylcarnitine
118	(25S)-5alpha-cholestan-3beta,6alpha,7beta,8beta,15alpha,16beta,26-heptol;(25S)-5alpha-cholestan-3beta,4beta,6alpha,8beta,15alpha,16beta,26-heptol
119	(22E)-26,26,26,27,27,27-hexafluoro-25-hydroxy-22,23-didehydrovitamin D3 / (22E)-26,26,26,27,27,27-hexafluoro-25-hydroxy-22,23-didehydrocholecalciferol
120	(11Z)-eicoseneoylcarnitine
121	(11Z,14Z)-eicosadienoylcarnitine

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