Supplementary Fig. 1. Examples of deconvolution and annotation result from hydrophilic interaction chromatography tandem mass spectrometry. Total 40 results including five standard compounds are described. NIST 12, MassBank, and ReSpect MS/MS libraries were used for peak annotations. Left panels (top and bottom) show the raw MS/MS chromatograms and the MS/MS spectrum of each metabolite. In middle-top panels, both raw- and deconvoluted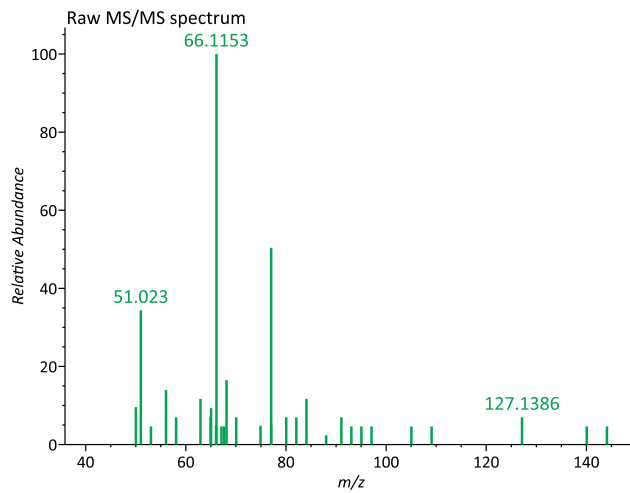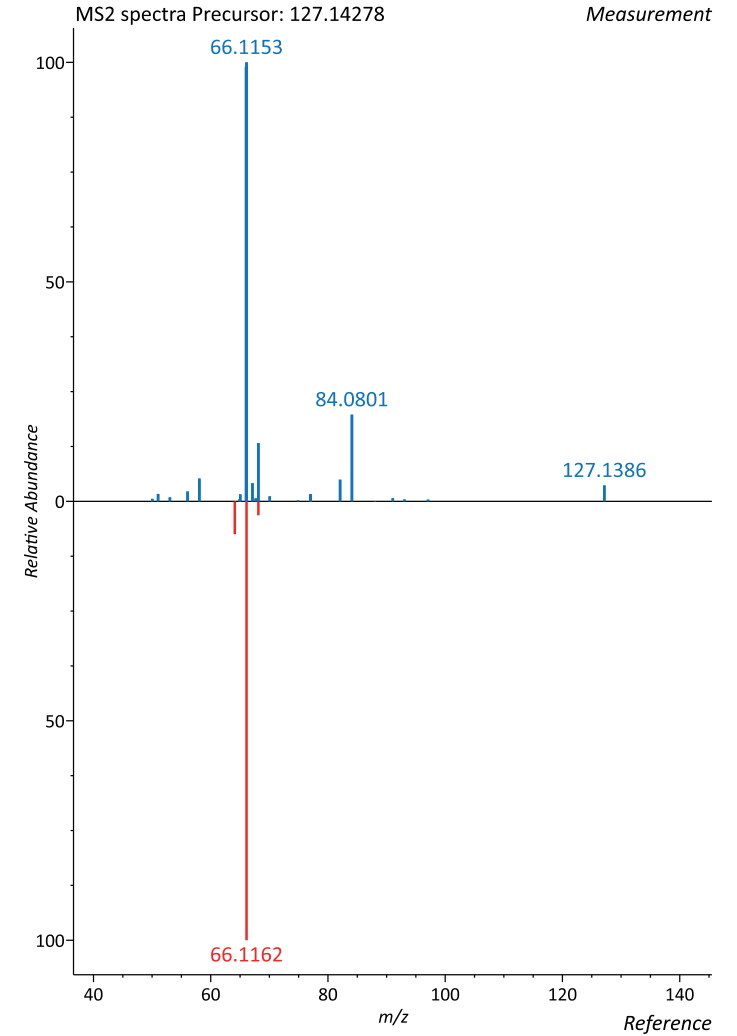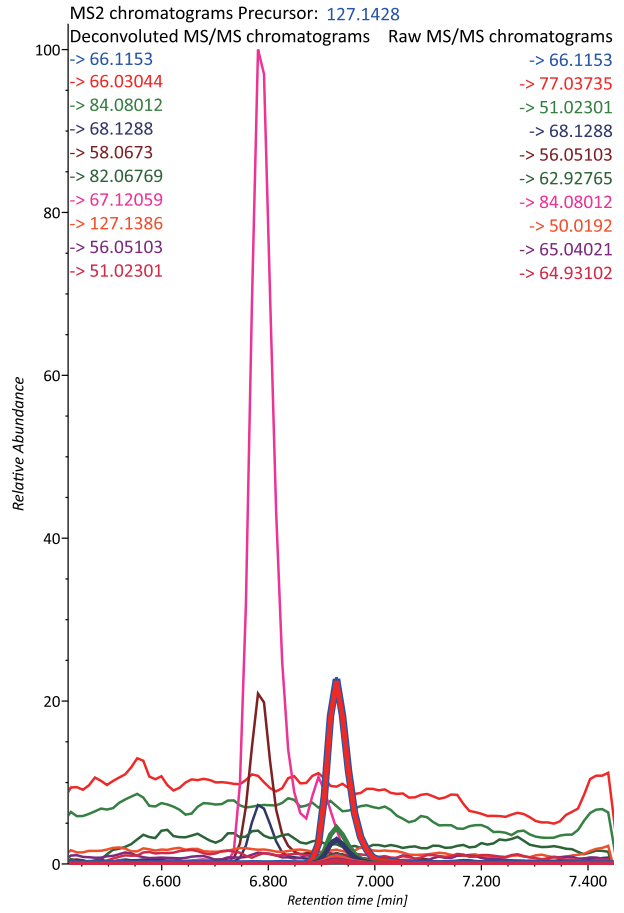 MS/MS chromatograms are described where regular and bold lines show raw- and deconvoluted chromatograms, respectively. The deconvoluted MS/MS spectrum is shown in middle-down panels. Right panels show the deconvoluted MS/MS spectrum in blue and the reference MS/MS spectrum in red.

Arginine-$^{15}N_2$

MS2 chromatograms Precursor: 177.1131

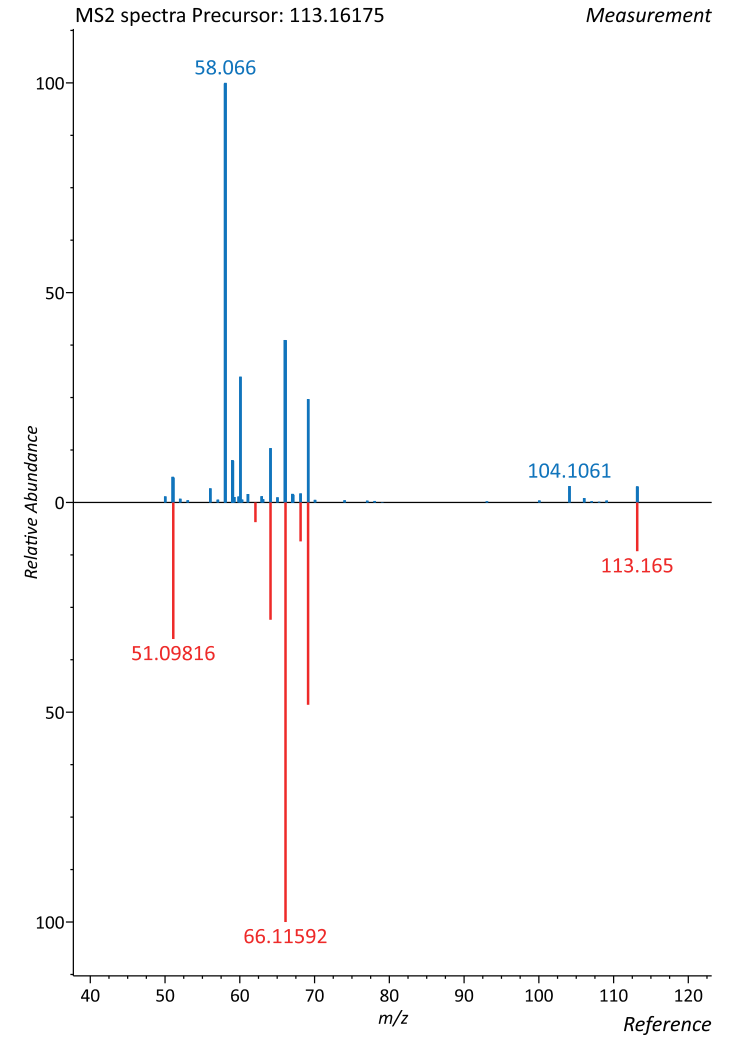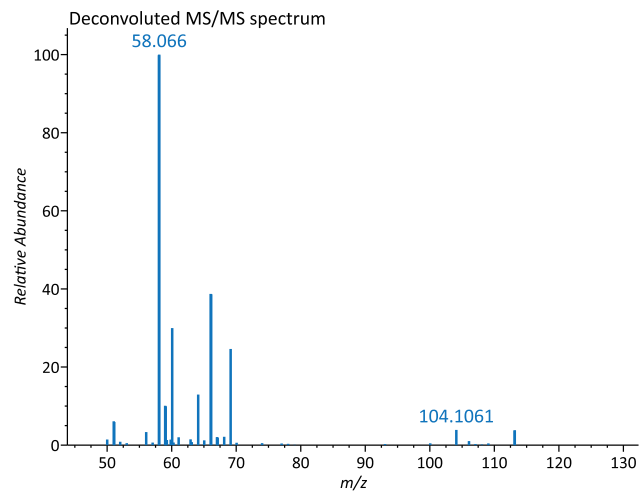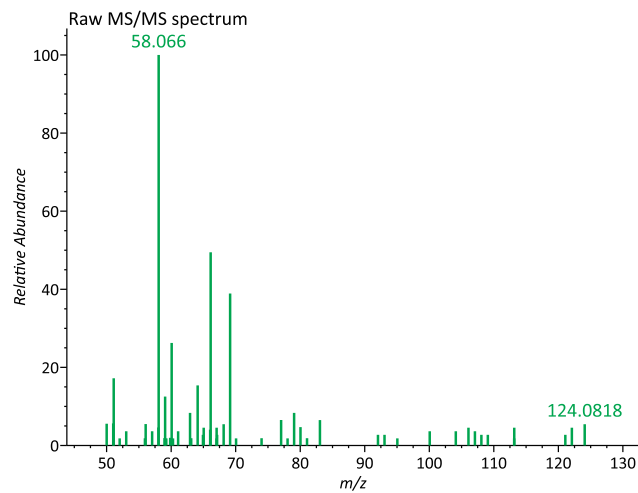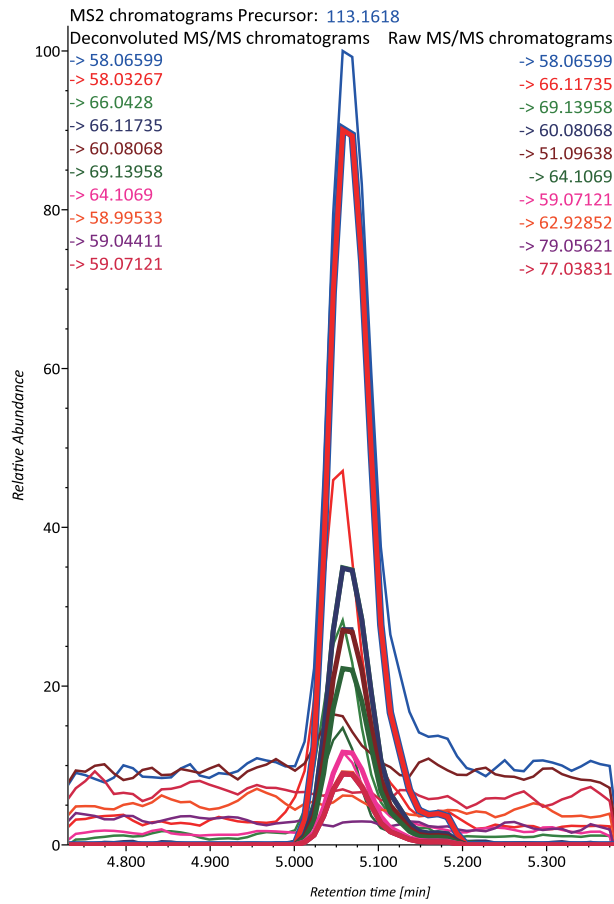Raw MS/MS chromatograms
-> 70.06609
-> 62.05319
-> 71.06755
-> 72.08331
-> 107.0505
-> 56.94174
-> 60.05679
-> 68.05304
-> 91.05882
-> 114.0905

Relative Abundance

Retention time [min]

MS2 chromatograms Precursor: 177.1131

Deconvoluted MS/MS chromatograms
-> 70.06609
-> 71.06755
-> 62.05319
-> 116.0473
-> 72.08331
-> 60.05679
-> 68.05304
-> 132.0529
-> 130.0598
-> 117.0557

Raw MS/MS chromatograms
-> 70.06609
-> 62.05319
-> 71.06755
-> 72.08331
-> 107.0505
-> 56.94174
-> 60.05679
-> 68.05304
-> 91.05882
-> 114.0905

Relative Abundance

Retention time [min]

MS2 spectra Precursor: 177.11307

Measurement

70.0661

116.0473

166.9826

132.0881

70.06557

Relative Abundance

m/z

Reference

Raw MS/MS spectrum

70.0661

107.0505

Relative Abundance

m/z

Deconvoluted MS/MS spectrum

70.0661

116.0473

166.9826

Relative Abundance

m/z

Betaine-D₃

MS2 chromatograms Precursor: 127.1428

Raw MS/MS chromatograms

-> 66.1153
-> 77.03735
-> 51.02301
-> 68.1288
-> 56.05103
-> 62.92765
-> 84.08012
-> 50.0192
-> 65.04021
-> 64.93102

MS2 chromatograms Precursor: 127.1428

Deconvoluted MS/MS chromatograms

-> 66.1153
-> 66.03044
-> 84.08012
-> 68.1288
-> 58.0673
-> 82.06769
-> 67.12059
-> 127.1386
-> 56.05103
-> 51.02301

Raw MS/MS chromatograms

-> 66.1153
-> 77.03735
-> 51.02301
-> 68.1288
-> 56.05103
-> 62.92765
-> 84.08012
-> 50.0192
-> 65.04021
-> 64.93102

Raw MS/MS spectrum

Deconvoluted MS/MS spectrum

MS2 spectra Precursor: 127.14278   *Measurement*

Reference

Choline-D$_9$

MS2 chromatograms Precursor: 113.1618

Raw MS/MS chromatograms
-> 58.06599
-> 66.11735
-> 69.13958
-> 60.08068
-> 51.09638
-> 64.1069
-> 59.07121
-> 62.92852
-> 79.05621
-> 77.03831

MS2 chromatograms Precursor: 113.1618

Deconvoluted MS/MS chromatograms
-> 58.06599
-> 58.03267
-> 66.0428
-> 66.11735
-> 60.08068
-> 69.13958
-> 64.1069
-> 58.99533
-> 59.04411
-> 59.07121

Raw MS/MS chromatograms
-> 58.06599
-> 66.11735
-> 69.13958
-> 60.08068
-> 51.09638
-> 64.1069
-> 59.07121
-> 62.92852
-> 79.05621
-> 77.03831

MS2 spectra Precursor: 113.16175   *Measurement*

58.066
104.1061
113.165
51.09816
66.11592

Reference

Raw MS/MS spectrum

58.066
124.0818

Deconvoluted MS/MS spectrum

58.066
104.1061

Glutamine-D$_5$

MS2 chromatograms Precursor: 152.106

Raw MS/MS chromatograms

-> 77.03976
-> 51.02399
-> 60.07542
-> 88.06944
-> 78.04484
-> 50.01519
-> 78.0386
-> 115.054
-> 55.05622
-> 59.07033

MS2 chromatograms Precursor: 152.106

Deconvoluted MS/MS chromatograms

-> 88.06944
-> 89.07058
-> 60.07542
-> 87.0687
-> 61.08237
-> 59.07033
-> 120.0821
-> 135.0792
-> 58.06404
-> 95.06139

Raw MS/MS chromatograms

-> 77.03976
-> 51.02399
-> 60.07542
-> 88.06944
-> 78.04484
-> 50.01519
-> 78.0386
-> 115.054
-> 55.05622
-> 59.07033

Raw MS/MS spectrum

Deconvoluted MS/MS spectrum

MS2 spectra Precursor: 152.10603

Measurement

Reference

Trimethylamine *N*-oxide-D$_9$ (TMAO-D$_9$)

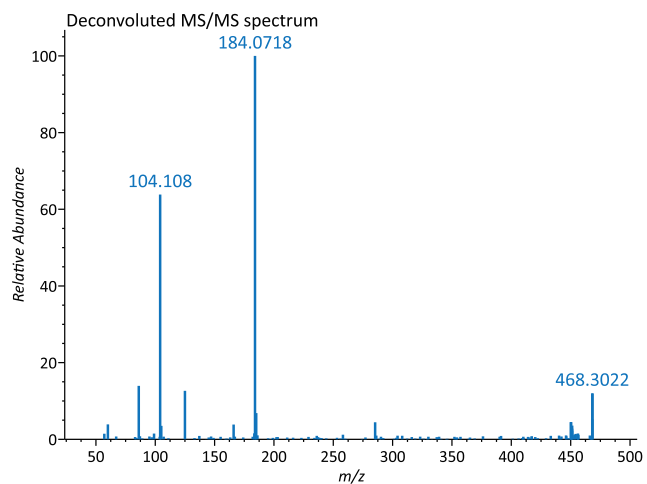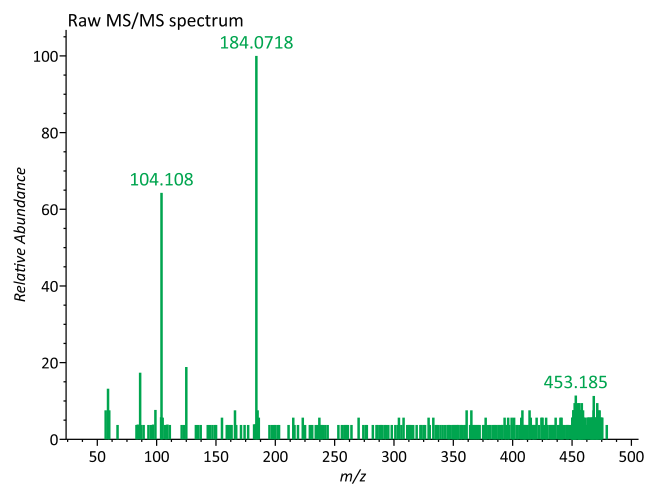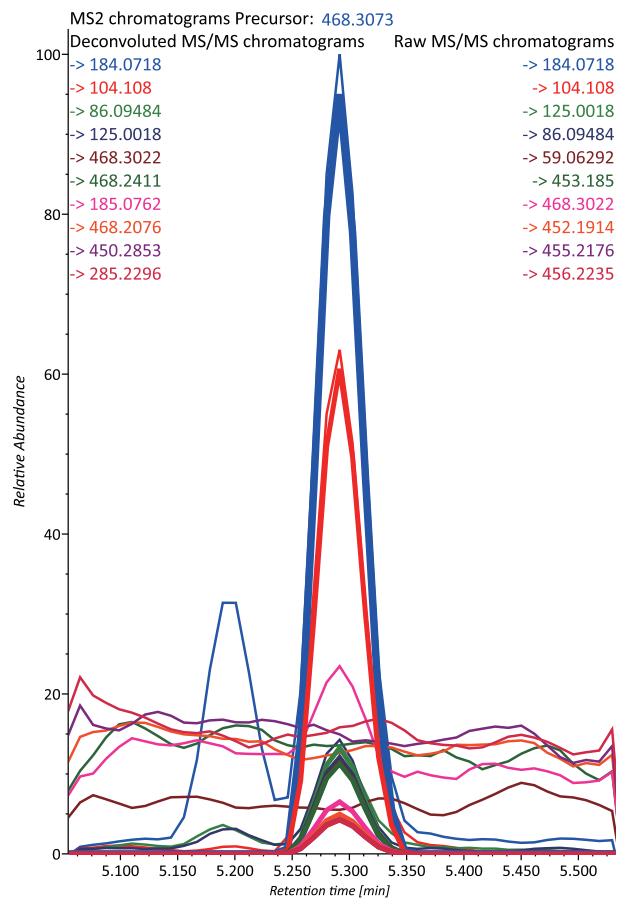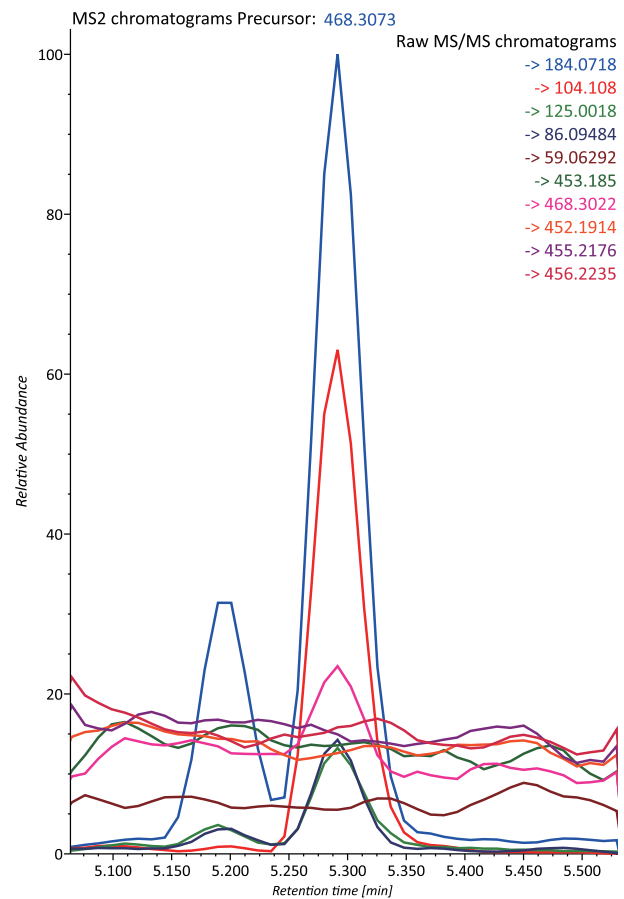# 1,2-Benzendicarboxylic acid

1-Methylhistamine

PC (14:0/0:0)

2-Amino-1-phenylethanol

3-Phenoxy-1-propanol

5-Aminovaleric acid

Acetylcarnitine

Arginine

Atenolol

MS2 chromatograms Precursor: 267.1706

Raw MS/MS chromatograms
-> 145.0646
-> 133.0617
-> 91.05206
-> 72.07965
-> 74.05866
-> 117.071
-> 56.05087
-> 190.0824
-> 107.0476
-> 178.0857

Relative Abundance

Retention time [min]

MS2 chromatograms Precursor: 267.1706

Deconvoluted MS/MS chromatograms
-> 145.102
-> 145.0646
-> 133.0617
-> 74.05866
-> 56.05087
-> 190.0824
-> 116.1067
-> 116.0504
-> 107.0476
-> 91.05206

Raw MS/MS chromatograms
-> 145.0646
-> 133.0617
-> 91.05206
-> 72.07965
-> 74.05866
-> 117.071
-> 56.05087
-> 190.0824
-> 107.0476
-> 178.0857

Relative Abundance

Retention time [min]

MS2 spectra Precursor: 267.17059

Measurement

145.102
74.0587
190.0824
267.1726
208.0967
267.1699
190.0862
74.06
145.0648

Relative Abundance

m/z

Reference

Raw MS/MS spectrum

145.0646
91.0521
190.0824
267.1726

Relative Abundance

m/z

Deconvoluted MS/MS spectrum

145.102
74.0587
190.0824
267.1726

Relative Abundance

m/z

Betaine

Caffeine

MS2 chromatograms Precursor: 195.0882

Raw MS/MS chromatograms
-> 138.0667
-> 91.05478
-> 94.06393
-> 77.03849
-> 117.0572
-> 59.04971
-> 55.05516
-> 104.0472
-> 110.0698
-> 115.0585

MS2 chromatograms Precursor: 195.0882

Deconvoluted MS/MS chromatograms
-> 138.0667
-> 110.0698
-> 123.0516
-> 123.086
-> 123.0735
-> 83.05854
-> 195.0746
-> 59.04971
-> 136.0746
-> 130.063

Raw MS/MS chromatograms
-> 138.0667
-> 91.05478
-> 94.06393
-> 77.03849
-> 117.0572
-> 59.04971
-> 55.05516
-> 104.0472
-> 110.0698
-> 115.0585

MS2 spectra Precursor: 195.08815

Measurement

138.0667
110.0698
59.0497
83.0585
195.0746
56.0493
69.0446
83.0603
123.0426
110.0712
138.066

Reference

Raw MS/MS spectrum
138.0667
91.0548
117.0572

Deconvoluted MS/MS spectrum
138.0667
110.0698
59.0497
83.0585
195.0746

Carnitine

1-Hexadecylpyridinium

13-Docosenamide

MS2 chromatograms Precursor: 338.3415

Raw MS/MS chromatograms
-> 69.07164
-> 57.07267
-> 97.09969
-> 83.08675
-> 55.054
-> 95.08604
-> 81.07107
-> 93.06898
-> 107.084
-> 100.075

Relative Abundance

Retention time [min]

MS2 chromatograms Precursor: 338.3415

Deconvoluted MS/MS chromatograms
-> 69.07164
-> 69.03529
-> 97.09969
-> 97.06771
-> 55.054
-> 57.07267
-> 83.05974
-> 83.08675
-> 95.04752
-> 95.08604

Raw MS/MS chromatograms
-> 69.07164
-> 57.07267
-> 97.09969
-> 83.08675
-> 55.054
-> 95.08604
-> 81.07107
-> 93.06898
-> 107.084
-> 100.075

Relative Abundance

Retention time [min]

MS2 spectra Precursor: 338.34152

Measurement

69.0716
227.2003
302.1056

42.9
149.2
338.2

70.9
111
56.8
83
97

Relative Abundance

m/z

Reference

Raw MS/MS spectrum

69.0716

227.2003

Relative Abundance

m/z

Deconvoluted MS/MS spectrum

69.0716

227.2003
302.1056

Relative Abundance

m/z

Benzyl tetradecyl dimethylammonium

Ecgonine methyl ester

MS2 chromatograms Precursor: 182.1162

Raw MS/MS chromatograms
- -> 82.06628
- -> 91.05342
- -> 65.03783
- -> 68.05069
- -> 94.06392
- -> 83.07137
- -> 77.0397
- -> 108.0821
- -> 67.05461
- -> 93.03366

MS2 chromatograms Precursor: 182.1162

Deconvoluted MS/MS chromatograms
- -> 82.06628
- -> 91.05342
- -> 68.05069
- -> 65.03783
- -> 94.06392
- -> 83.07137
- -> 67.0419
- -> 67.05461
- -> 93.05407
- -> 93.03366

Raw MS/MS chromatograms
- -> 82.06628
- -> 91.05342
- -> 65.03783
- -> 68.05069
- -> 94.06392
- -> 83.07137
- -> 77.0397
- -> 108.0821
- -> 67.05461
- -> 93.03366

MS2 spectra Precursor: 182.11621

Measurement

Reference

Raw MS/MS spectrum

Deconvoluted MS/MS spectrum

Midazolam

Tyrosine

Creatinine

MS2 chromatograms Precursor: 227.128

Raw MS/MS chromatograms
-> 77.03967
-> 114.0664
-> 140.9998
-> 158.0268
-> 78.03976
-> 145.0764
-> 196.1066
-> 171.0951
-> 91.05204
-> 183.0945

MS2 chromatograms Precursor: 227.128
Deconvoluted MS/MS chromatograms
-> 114.0664
-> 113.9865
-> 115.0464
-> 115.0676
-> 86.07226
-> 136.0467
-> 80.05045
-> 116.0519
-> 209.104
-> 72.04852

Raw MS/MS chromatograms
-> 77.03967
-> 114.0664
-> 140.9998
-> 158.0268
-> 78.03976
-> 145.0764
-> 196.1066
-> 171.0951
-> 91.05204
-> 183.0945

MS2 spectra Precursor: 227.12801

Measurement

114.0664

136.0467

209.104

72.05

86.07

114.07

Reference

Raw MS/MS spectrum

77.0397

114.0664

140.9998

Deconvoluted MS/MS spectrum

114.0664

209.104

Dioctyl phthalate

Glutamine

Guanidine

MS2 chromatograms Precursor: 114.0657

Raw MS/MS chromatograms
- -> 77.03719
- -> 79.05382
- -> 51.02491
- -> 62.93087
- -> 124.0866
- -> 114.0664
- -> 58.02956
- -> 53.04089
- -> 65.04007
- -> 83.05977

MS2 chromatograms Precursor: 114.0657

Deconvoluted MS/MS chromatograms
- -> 114.0664
- -> 72.04253
- -> 95.05442
- -> 96.06397
- -> 98.06274
- -> 65.04007
- -> 55.05404
- -> 51.02491
- -> 110.089
- -> 56.04774

Raw MS/MS chromatograms
- -> 77.03719
- -> 79.05382
- -> 51.02491
- -> 62.93087
- -> 124.0866
- -> 114.0664
- -> 58.02956
- -> 53.04089
- -> 65.04007
- -> 83.05977

MS2 spectra Precursor: 114.06569

Measurement

114.0664

72.0425

95.0544

65.0401

55.06   60.06   72.06   80.05   97.07

114.1

Reference

Raw MS/MS spectrum

77.0372

51.0249

62.9309

124.0866

Deconvoluted MS/MS spectrum

114.0664

72.0425

95.0544

Hypoxanthine

MS2 chromatograms Precursor: 137.0452

Raw MS/MS chromatograms
- -> 62.92868
- -> 77.04095
- -> 51.02394
- -> 64.92638
- -> 119.0331
- -> 50.01814
- -> 81.04574
- -> 92.04834
- -> 110.0313
- -> 67.03037

MS2 chromatograms Precursor: 137.0452

Deconvoluted MS/MS chromatograms
- -> 119.0331
- -> 55.03106
- -> 55.05618
- -> 137.0408
- -> 110.0313
- -> 137.0788
- -> 151.02394
- -> 94.04204
- -> 94.0612
- -> 120.0805

Raw MS/MS chromatograms
- -> 62.92868
- -> 77.04095
- -> 51.02394
- -> 64.92638
- -> 119.0331
- -> 50.01814
- -> 81.04574
- -> 92.04834
- -> 110.0313
- -> 67.03037

Raw MS/MS spectrum

Deconvoluted MS/MS spectrum

MS2 spectra Precursor: 137.04521

Measurement

Reference

Levamisole

Lysine

MS2 chromatograms Precursor: 147.1122

Raw MS/MS chromatograms

-> 77.03863
-> 51.02507
-> 84.07887
-> 57.93411
-> 62.92881
-> 55.93389
-> 59.93233
-> 56.04896
-> 56.94398
-> 65.03684

MS2 chromatograms Precursor: 147.1122

Deconvoluted MS/MS chromatograms

-> 84.07887
-> 56.04896
-> 76.95444
-> 77.03863
-> 67.05592
-> 85.08316
-> 85.94162
-> 54.95061
-> 55.93389
-> 70.07921

Raw MS/MS chromatograms

-> 77.03863
-> 51.02507
-> 84.07887
-> 57.93411
-> 62.92881
-> 55.93389
-> 59.93233
-> 56.04896
-> 56.94398
-> 65.03684

MS2 spectra Precursor: 147.11223 — *Measurement*

84.0789
56.049
76.9544
74.02
67.05
95.05
56.05
84.08

Reference

Raw MS/MS spectrum

77.0386
51.0251

Deconvoluted MS/MS spectrum

84.0789
56.049

PC (16:0/0:0)

PC (0:0/16:0)

Phenylalanine

MS2 chromatograms Precursor: 166.0865

Raw MS/MS chromatograms
-> 77.03964
-> 103.0552
-> 120.0805
-> 51.02589
-> 78.04347
-> 91.05472
-> 50.01607
-> 55.0561
-> 84.08382
-> 79.0563

MS2 chromatograms Precursor: 166.0865

Deconvoluted MS/MS chromatograms
-> 103.0552
-> 120.0805
-> 77.03964
-> 91.05472
-> 84.08382
-> 93.06899
-> 104.0544
-> 121.072
-> 121.0813
-> 56.05088

Raw MS/MS chromatograms
-> 77.03964
-> 103.0552
-> 120.0805
-> 51.02589
-> 78.04347
-> 91.05472
-> 50.01607
-> 55.0561
-> 84.08382
-> 79.0563

MS2 spectra Precursor: 166.08647

Measurement

103.0552
120.0805
77.0396
91.0547
56.0509
158.0747
51.02
65.04
77.04
91.05
120.08
103.05

Reference

Raw MS/MS spectrum
77.0396
103.0552
120.0805
51.0259

Deconvoluted MS/MS spectrum
103.0552
120.0805
77.0396
56.0509
158.0747

Phenylalanine-proline

Pipecolic acid

Proline

MS2 chromatograms Precursor: 116.0711

Raw MS/MS chromatograms
- -> 58.06524
- -> 51.02613
- -> 70.06742
- -> 58.03192
- -> 77.03864
- -> 59.07479
- -> 50.02131
- -> 91.05762
- -> 72.07987
- -> 69.84911

MS2 chromatograms Precursor: 116.0711

Deconvoluted MS/MS chromatograms
- -> 70.06742
- -> 51.02613
- -> 77.03864
- -> 50.02131
- -> 68.05087
- -> 69.84911
- -> 72.07987
- -> 65.04148
- -> 74.01761
- -> 61.18508

Raw MS/MS chromatograms
- -> 58.06524
- -> 51.02613
- -> 70.06742
- -> 58.03192
- -> 77.03864
- -> 59.07479
- -> 50.02131
- -> 91.05762
- -> 72.07987
- -> 69.84911

MS2 spectra Precursor: 116.07114

Measurement

70.0674
51.0261
116.0733
116.0711
70.0686

Reference

Raw MS/MS spectrum
58.0652
70.0674
91.0576
116.0733

Deconvoluted MS/MS spectrum
70.0674
51.0261
116.0733

Pyroglutamic acid

MS2 chromatograms Precursor: 130.0497

Raw MS/MS chromatograms
-> 84.04771
-> 56.05099
-> 77.03852
-> 51.025
-> 89.07458
-> 62.92873
-> 61.08016
-> 88.0668
-> 56.04677
-> 64.92984

MS2 chromatograms Precursor: 130.0497

Deconvoluted MS/MS chromatograms
-> 84.04771
-> 56.04677
-> 56.05099
-> 89.07458
-> 61.08016
-> 88.0668
-> 60.07432
-> 85.05051
-> 85.02579
-> 90.08408

Raw MS/MS chromatograms
-> 84.04771
-> 56.05099
-> 77.03852
-> 51.025
-> 89.07458
-> 62.92873
-> 61.08016
-> 88.0668
-> 56.04677
-> 64.92984

MS2 spectra Precursor: 130.04967

Measurement

Reference

Raw MS/MS spectrum

Deconvoluted MS/MS spectrum

Cinnamic acid

Trimethylamine *N*-oxide (TMAO)

Tryptophan

MS2 chromatograms Precursor: 188.072

Raw MS/MS chromatograms

-> 118.0652
-> 115.0554
-> 91.05338
-> 143.073
-> 117.0571
-> 142.0656
-> 116.061
-> 146.0568
-> 144.0805
-> 89.03985

Relative Abundance

Retention time [min]

MS2 chromatograms Precursor: 188.072

Deconvoluted MS/MS chromatograms

-> 118.0652
-> 115.0554
-> 91.05338
-> 143.073
-> 117.0571
-> 117.0403
-> 142.0656
-> 116.0473
-> 116.061
-> 144.0805

Raw MS/MS chromatograms

-> 118.0652
-> 115.0554
-> 91.05338
-> 143.073
-> 117.0571
-> 142.0656
-> 116.061
-> 146.0568
-> 144.0805
-> 89.03985

Relative Abundance

Retention time [min]

MS2 spectra Precursor: 188.07202

Measurement

Relative Abundance

118.0652
91.0534
143.073
170.0834
65.04 77.04
127.05
170.06
143.07
91.05
118.06

m/z

Reference

Raw MS/MS spectrum

118.0652
143.073
170.0576

Relative Abundance

m/z

Deconvoluted MS/MS spectrum

118.0652
91.0534
143.073
170.0834

Relative Abundance

m/z

Supplementary Fig. 2. Comparison of the SQDG MS/MS spectrum in the different precursor window and the accumulation time. Left panels show the MS/MS spectra of DDA at accumulation time 50 ms. Middle panels show the MS/MS spectra of SWATH acquisition with 21-Da isolation window at accumulation time 10 ms. Right panels show the MS/MS spectra of SWATH acquisition with 65-Da isolation window at accumulation time 30 ms. While m/z 225 and neutral loss fragment ions are not or hardly detected at 10 ms accumulation time, they were well-detected in 30 or 50 ms accumulation time conditions.

SQDG (14:0/16:0)

DDA
(Q1 window, 1Da; Accumulation time 50 ms)
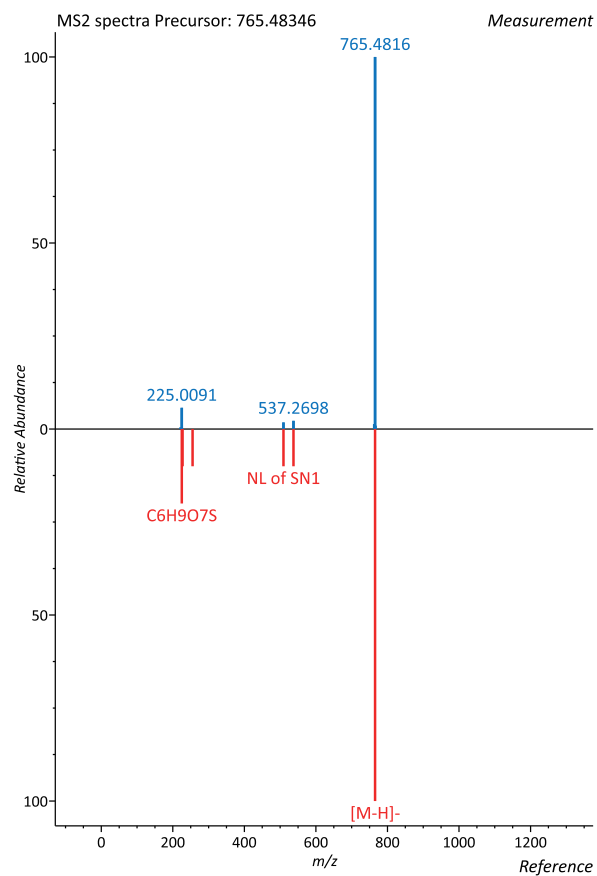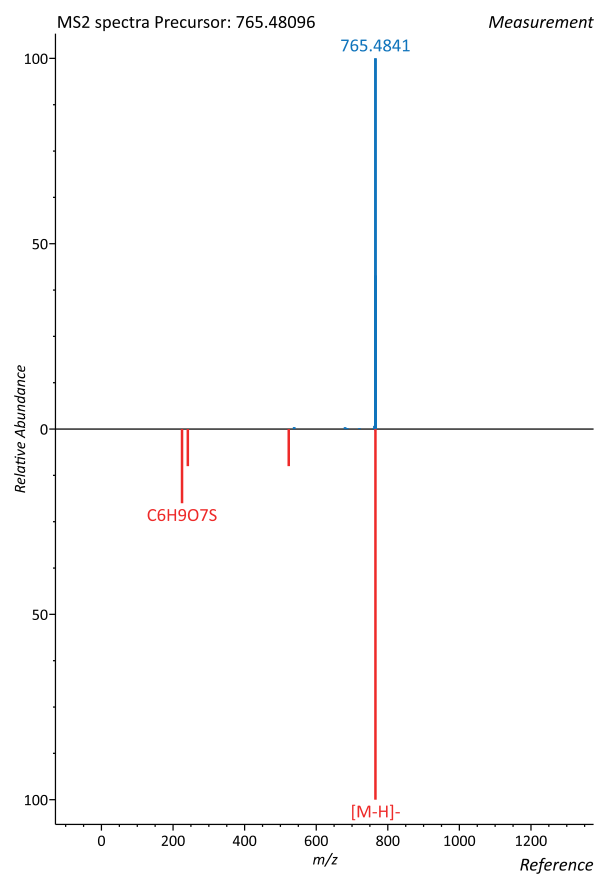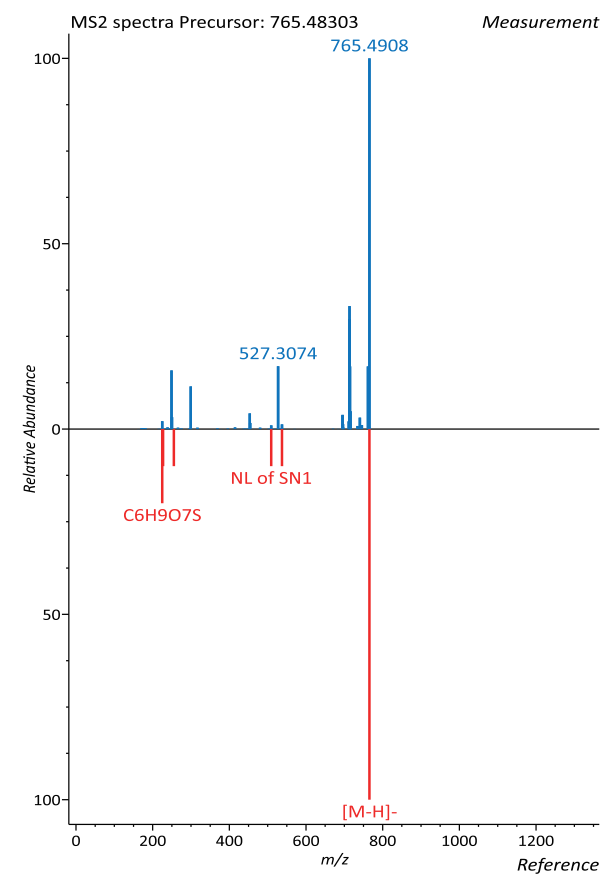
SWATH
(Q1 window, 21 Da; Accumulation time 10 ms)

SWATH
(Q1 window, 65 Da; Accumulation time 30 ms)

SQDG (16:0/16:0)
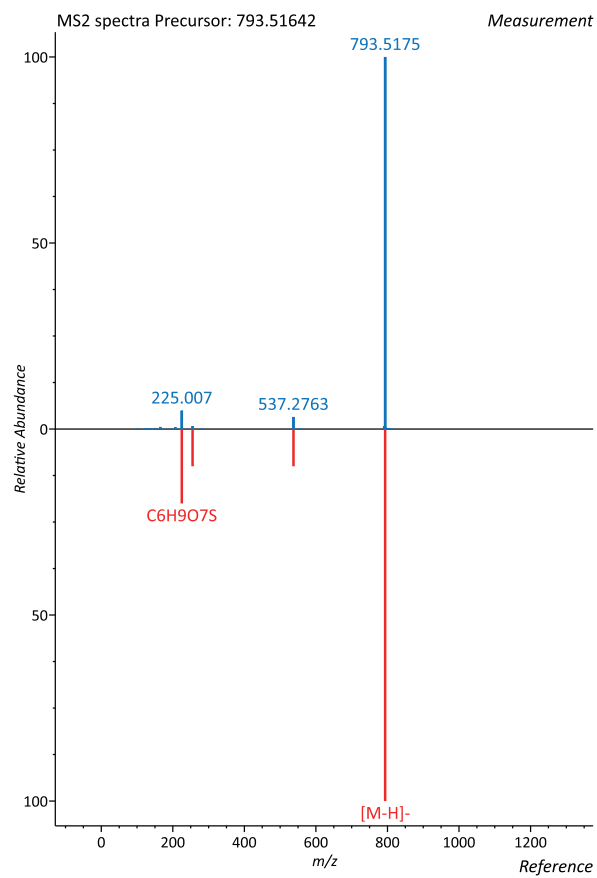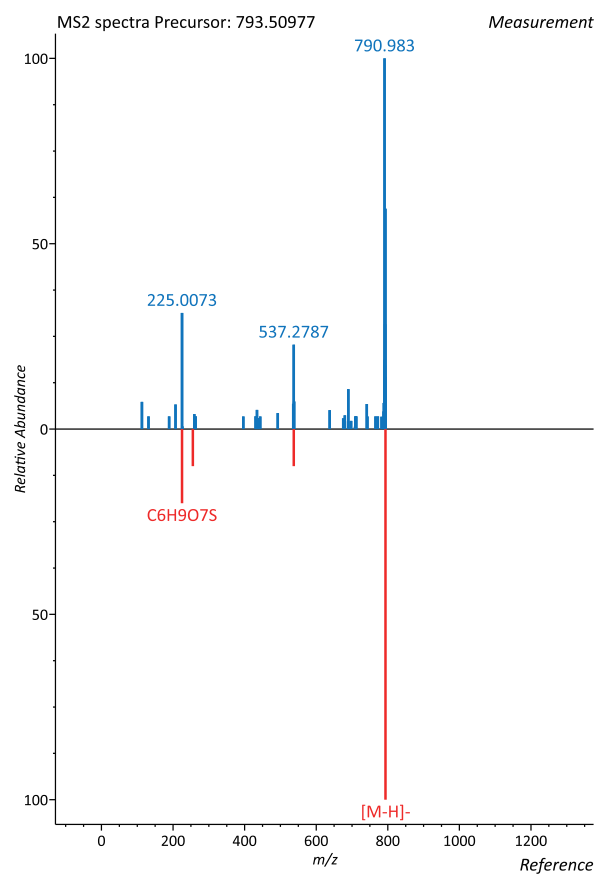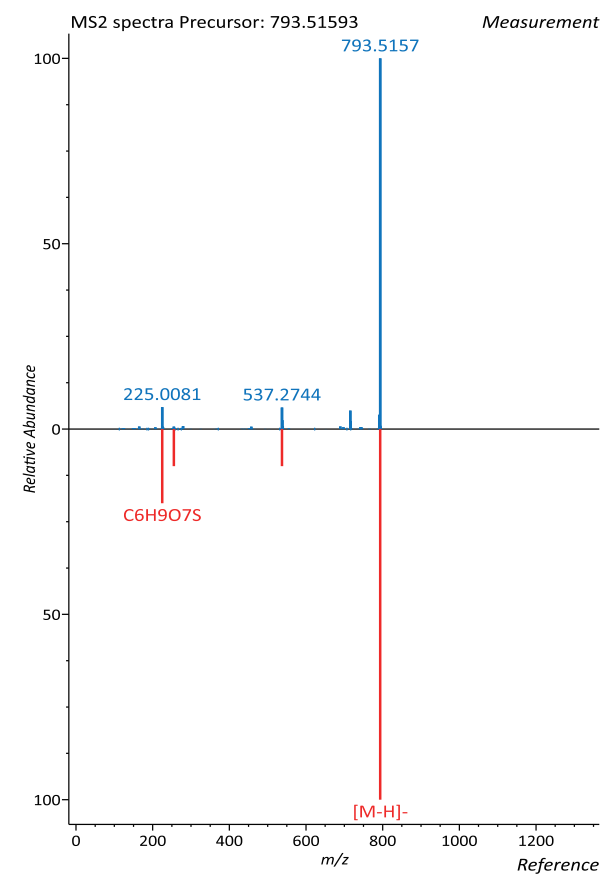
SQDG (16:0/18:1)

SQDG (16:0/18:2)

DDA

(Q1 window, 1Da; Accumulation time 50 ms)
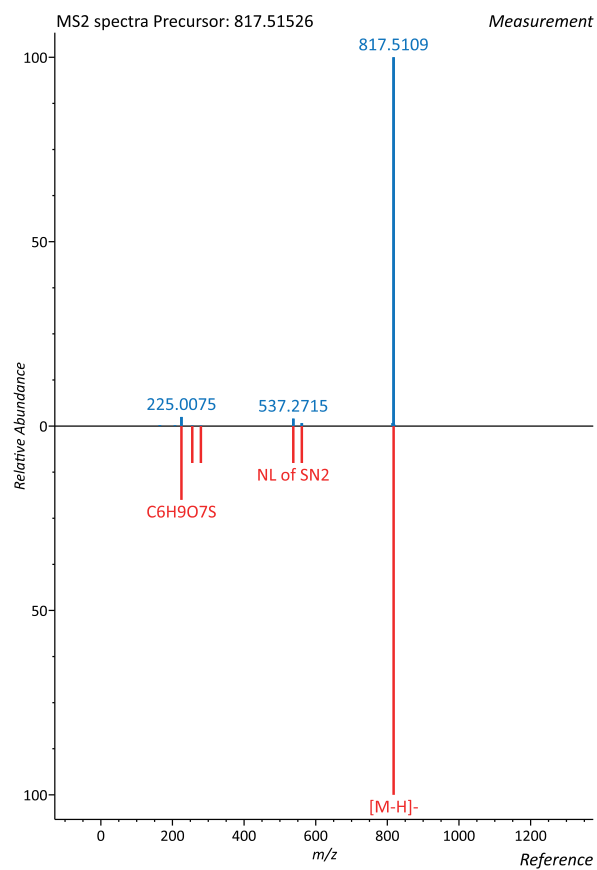
SWATH

(Q1 window, 21 Da; Accumulation time 10 ms)
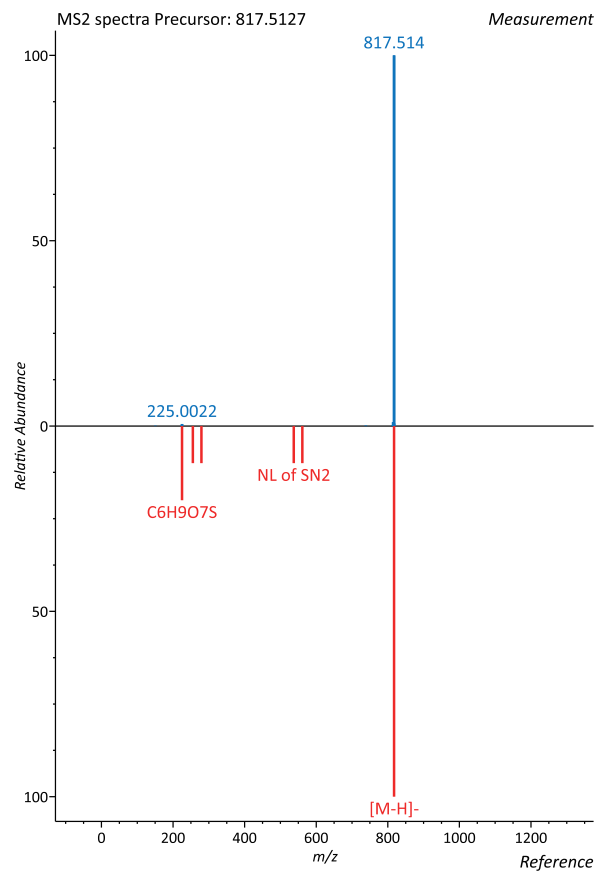
SWATH

(Q1 window, 65 Da; Accumulation time 30 ms)
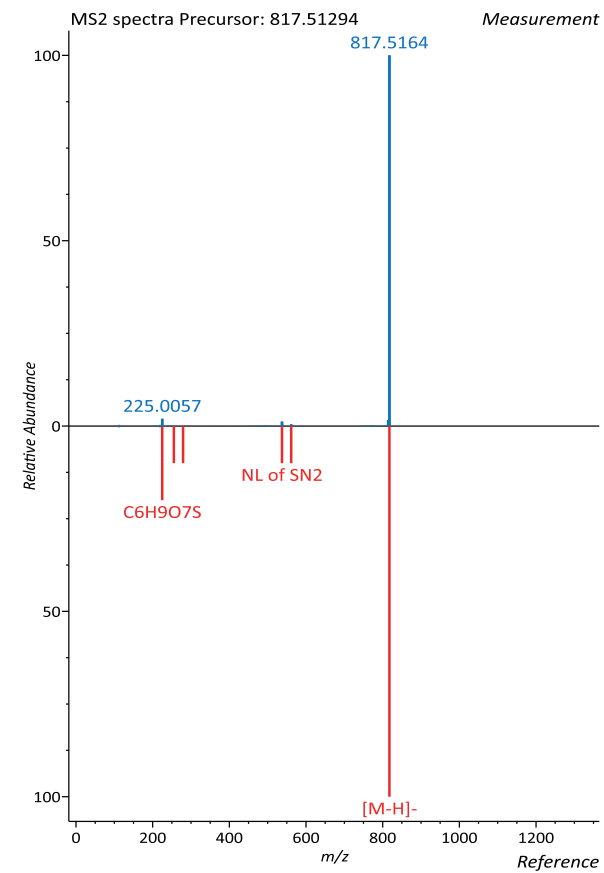
SQDG (16:0/18:3)

DDA
(Q1 window, 1Da; Accumulation time 50 ms)
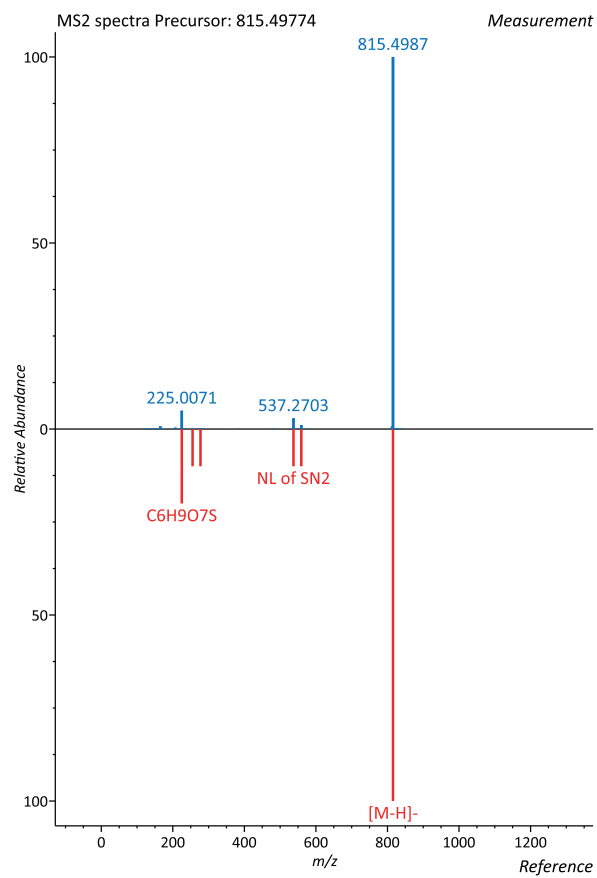
SWATH
(Q1 window, 21 Da; Accumulation time 10 ms)
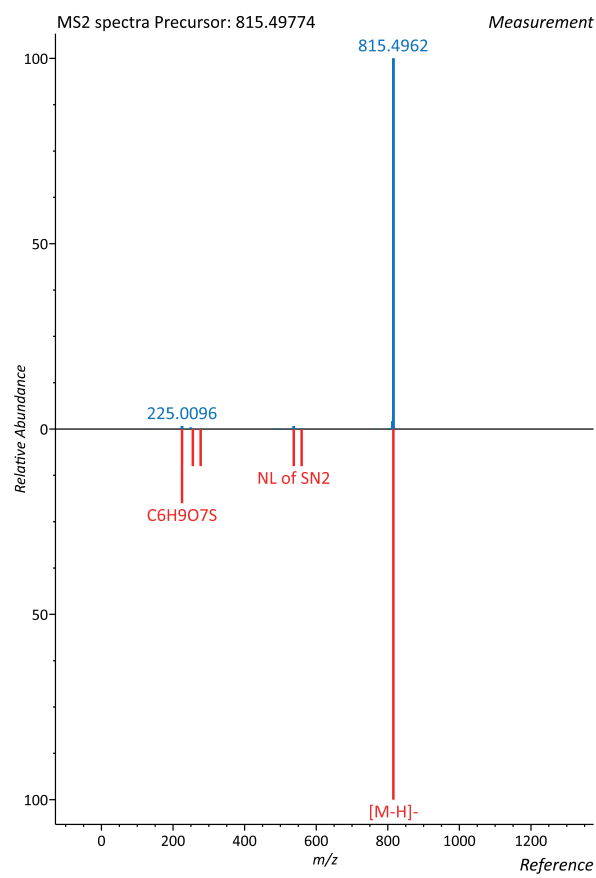
SWATH
(Q1 window, 65 Da; Accumulation time 30 ms)
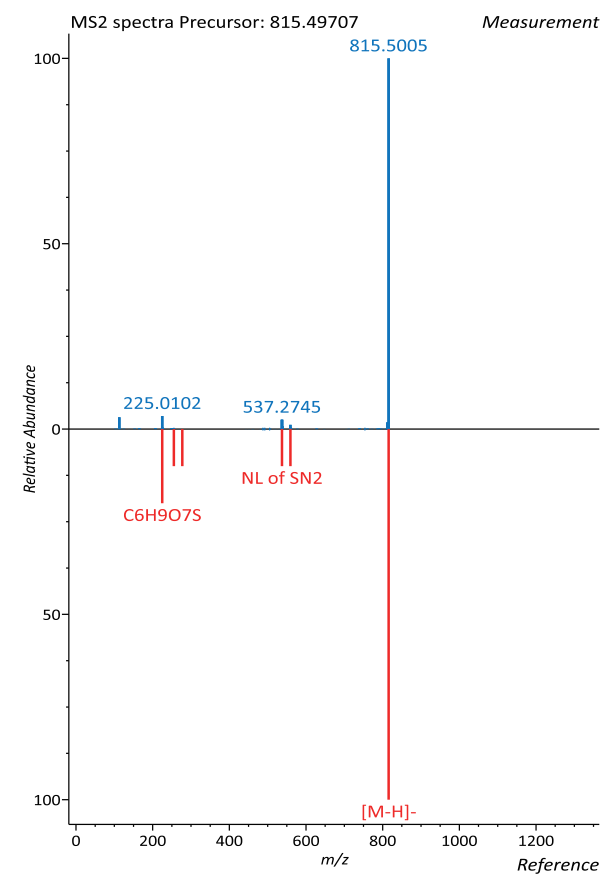
SQDG (16:2/18:2)
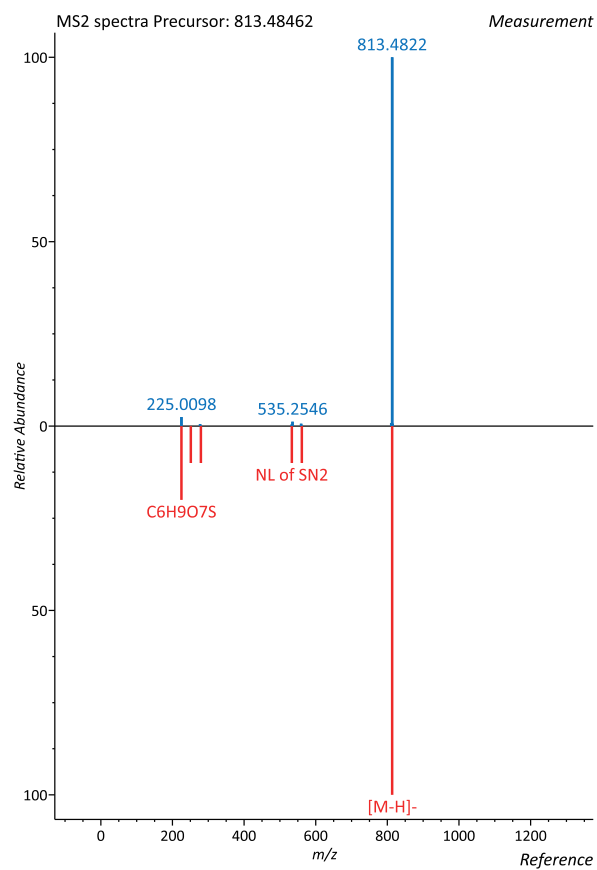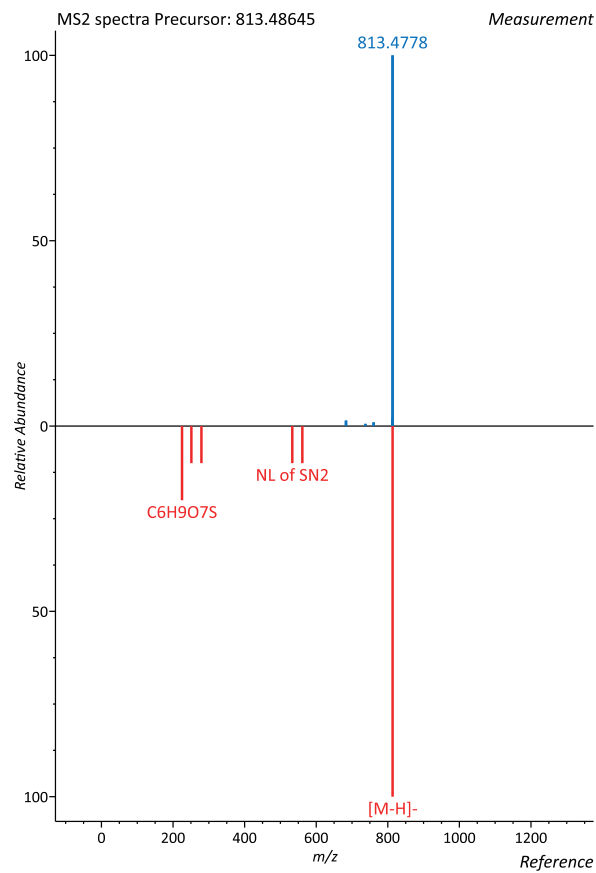
DDA	SWATH	SWATH
(Q1 window, 1Da; Accumulation time 50 ms)	(Q1 window, 21 Da; Accumulation time 10 ms)	(Q1 window, 65 Da; Accumulation time 30 ms)
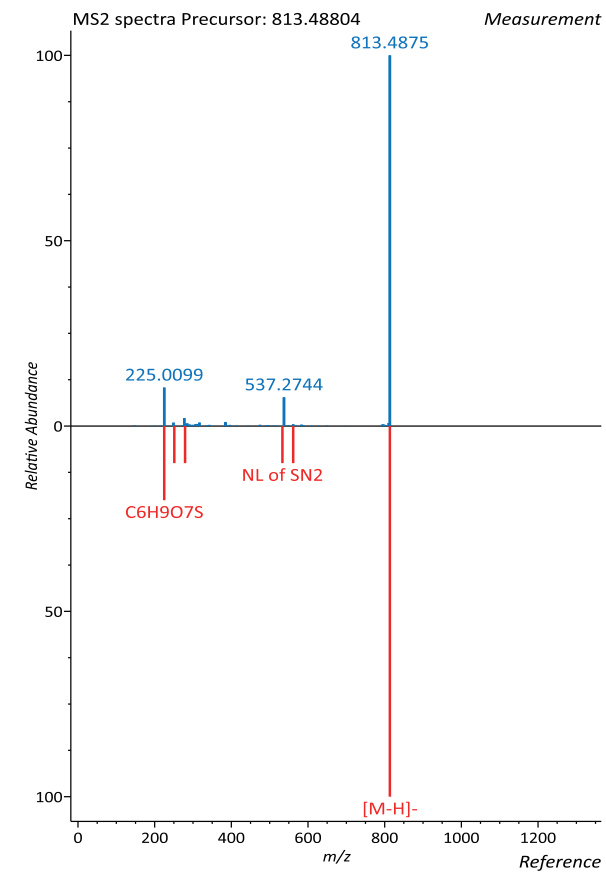
SQDG (16:2/18:3)

DDA

(Q1 window, 1Da; Accumulation time 50 ms)
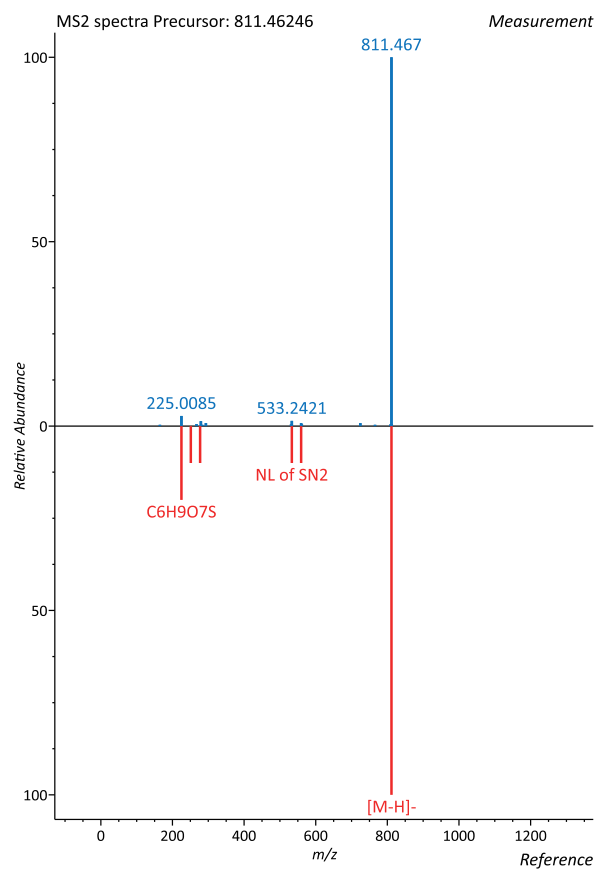
SWATH

(Q1 window, 21 Da; Accumulation time 10 ms)
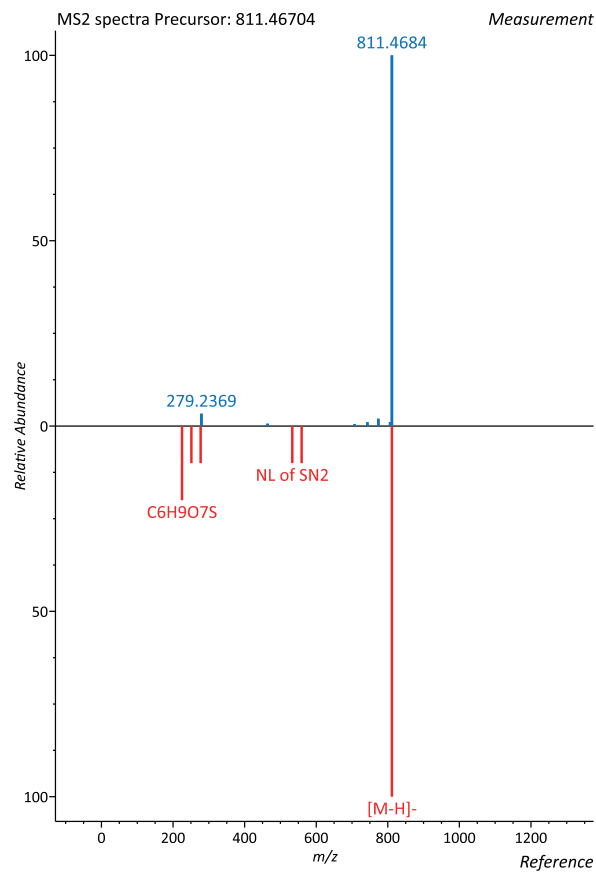
SWATH

(Q1 window, 65 Da; Accumulation time 30 ms)
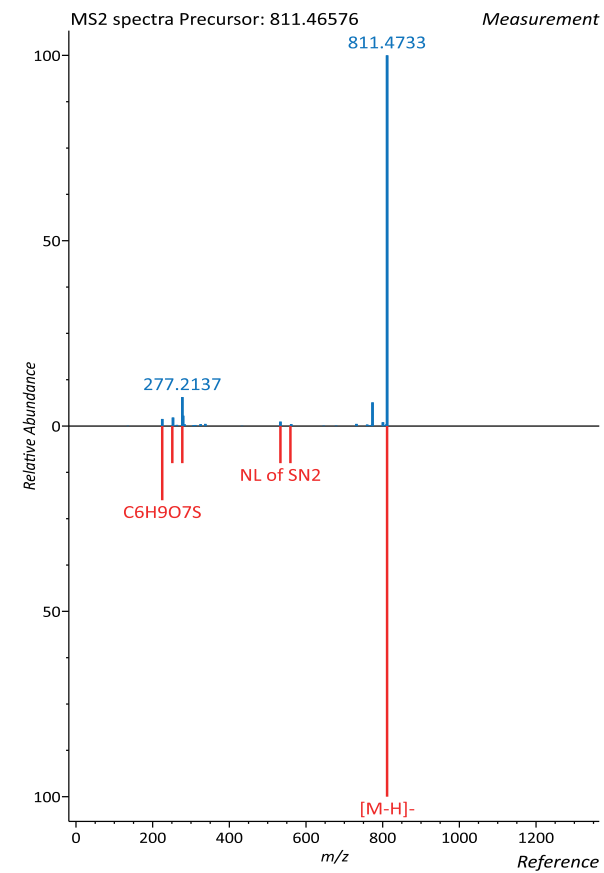
SQDG (16:3/18:3)
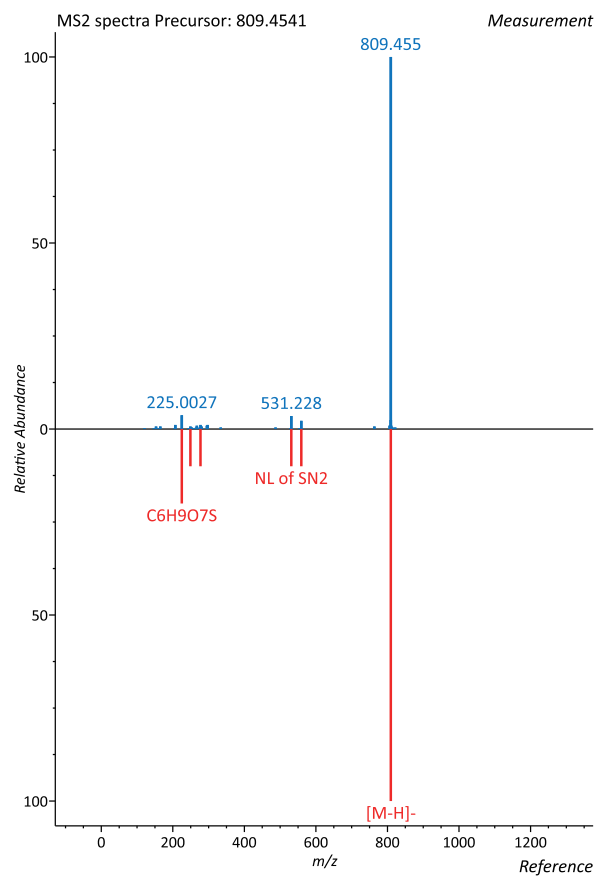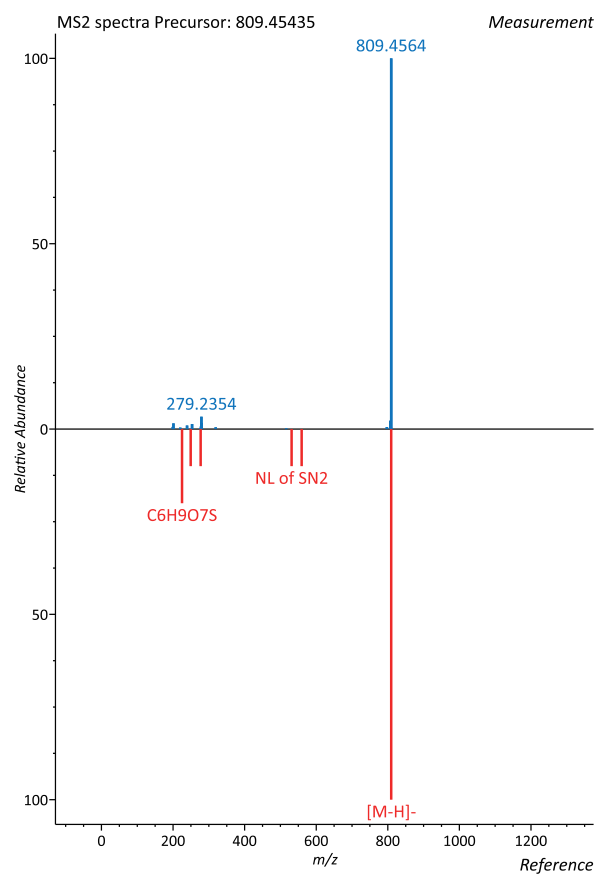
DDA	SWATH	SWATH
(Q1 window, 1Da; Accumulation time 50 ms)	(Q1 window, 21 Da; Accumulation time 10 ms)	(Q1 window, 65 Da; Accumulation time 30 ms)
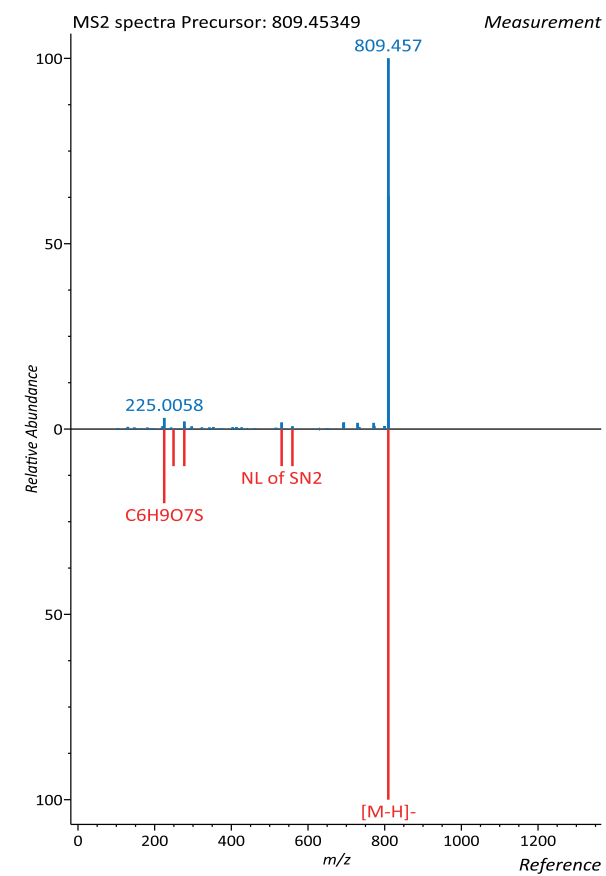
SQDG (16:4/18:3)

DDA
(Q1 window, 1Da; Accumulation time 50 ms)
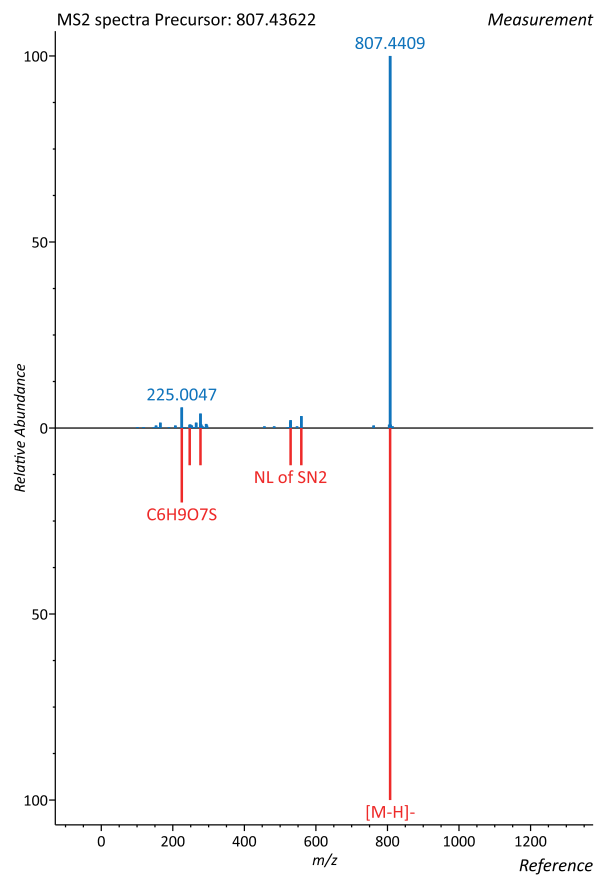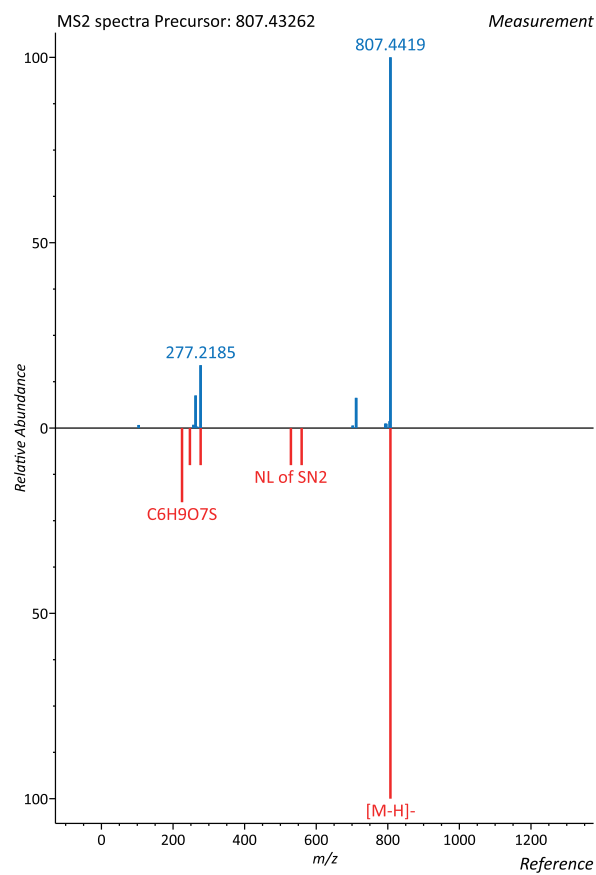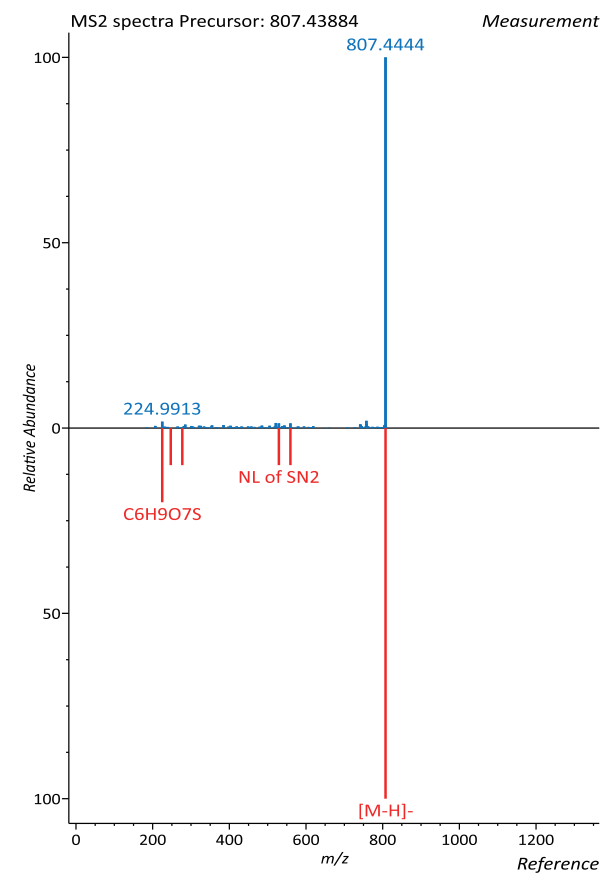
SWATH
(Q1 window, 21 Da; Accumulation time 10 ms)

SWATH
(Q1 window, 65 Da; Accumulation time 30 ms)

SQDG (18:3/18:3)
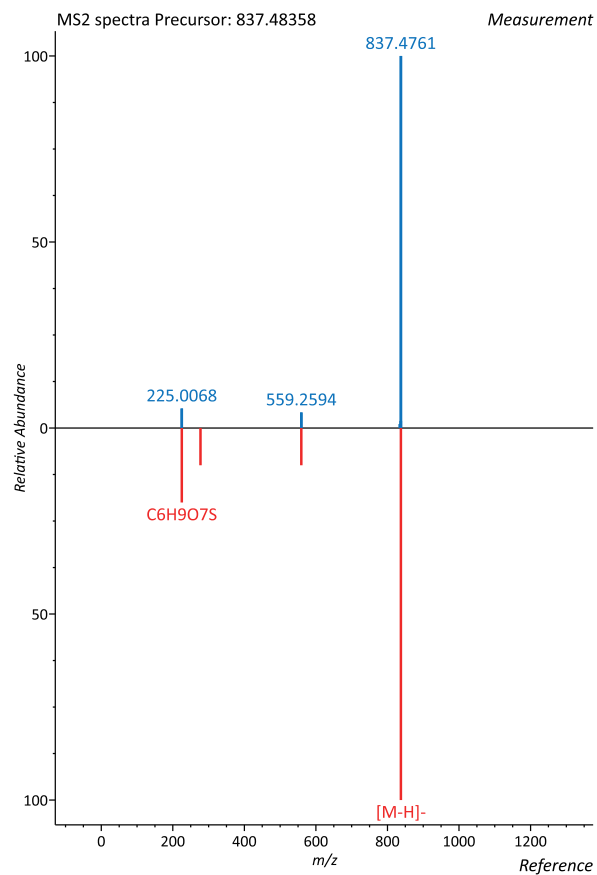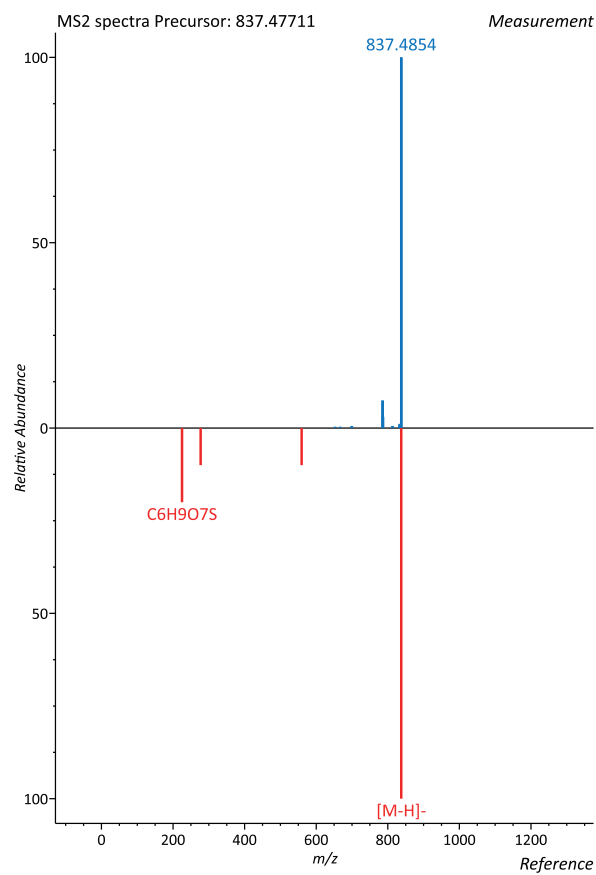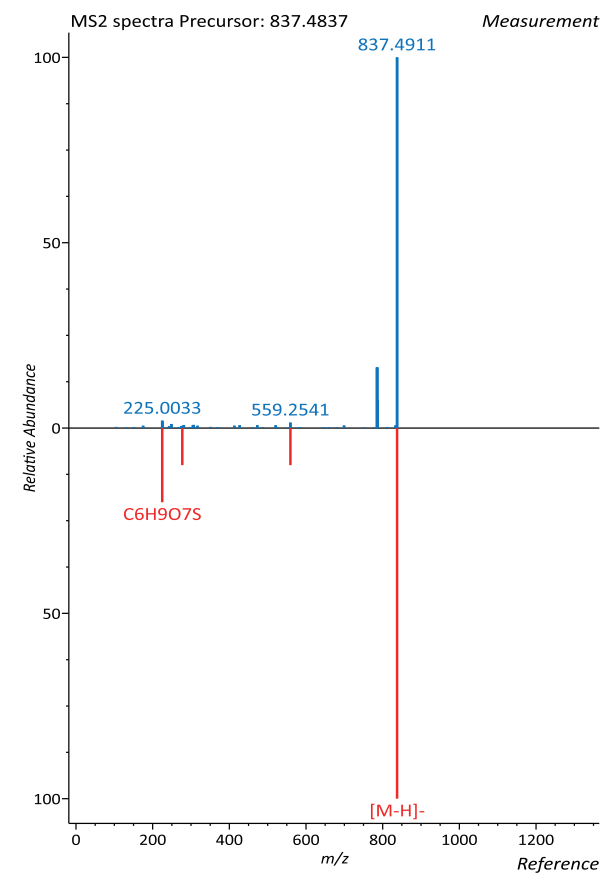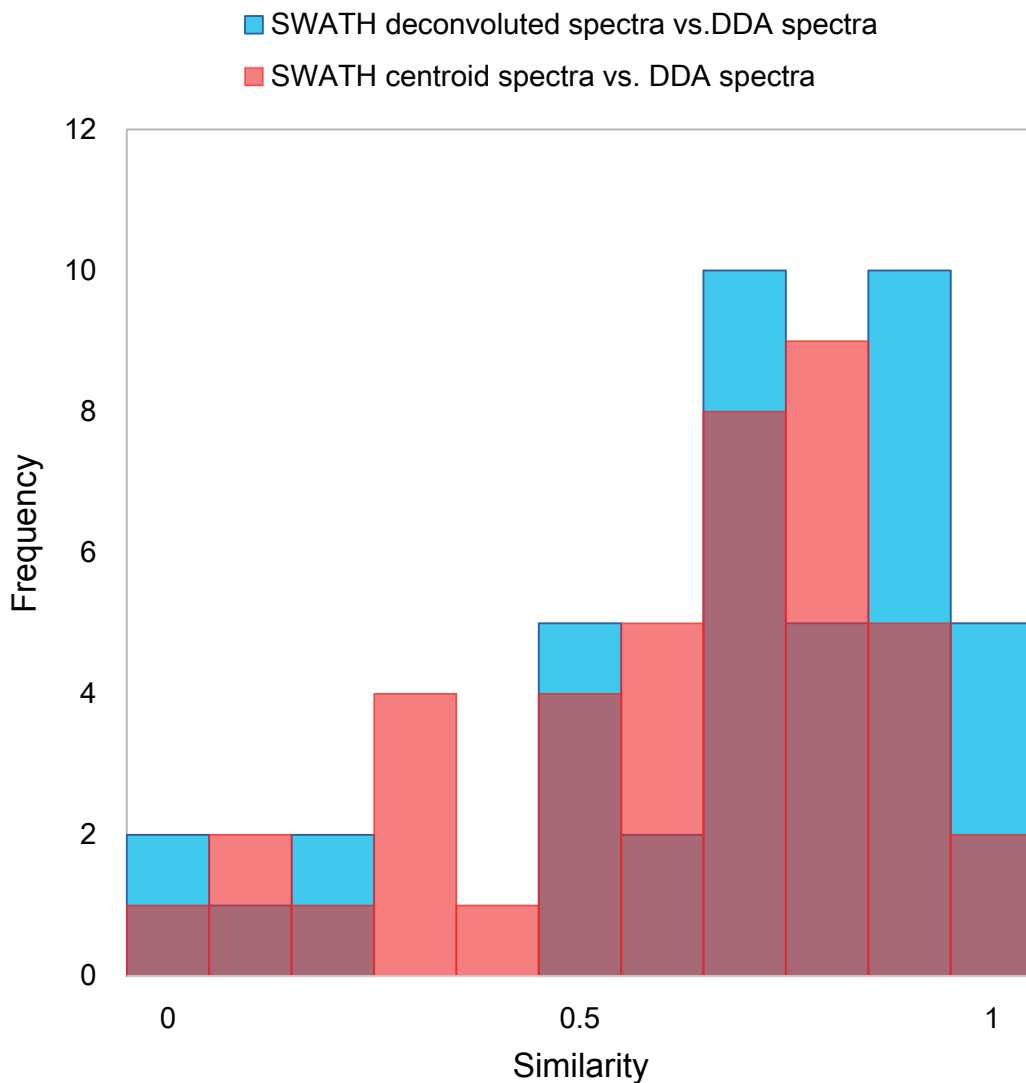
Supplementary Fig. 3. Comparison of mass spectra with respect to commonly identified lipids between SWATH and DDA methods. The 65-Da isolation window at 30 ms accumulation time was used for the SWATH acquisition. The blue histogram shows the spectra similarity between the deconvoluted spectra and DDA spectra. The red histogram shows the spectra similarity between the centroid spectra of non-deconvoluted spectra and DDA spectra. The dot product score is mostly greater than 0.7 even if the wider precursor window (65-Da) is used.

Supplementary Fig. 4. Lipid amounts of each lipid class in algal species. The X- and Y-axis show the lipid class names and the total peak heights of identified lipids. The results of SWATH-MS positive ion mode, SWATH-MS negative ion mode, DDA positive ion mode, and DDA negative ion mode are described in the respective pages.

SWATH-MS Positive ion mode

Figure showing nine bar charts of lipid class abundances (DG, DGDG, DGTS, lysoDGTS, lysoPC, lysoPE, MGDG, PC, PE, TG) for: *Chlamydomonas reinhardtii*, UTEX 2341, *Chlorella sorokiniana*, *Chlorella variabilis*, *Euglena gracilis*, *Nannochloropsis oculata*, *Dunaliella salina*, *Pleurochrysis carterae*, and *Pleurochrysis carterae*.

SWATH-MS Negative ion mode

Bar charts showing lipid class abundance (SWATH-MS negative ion mode) for nine microalgae species: *Chlamydomonas reinhardtii*, *UTEX 2341*, *Chlorella sorokiniana*, *Chlorella variabilis*, *Euglena gracilis*, *Nannochloropsis oculata*, *Dunaliella salina*, *Pleurochrysis carterae*, and *Pavlova lutheri*. The x-axis of each chart lists lipid classes: DGDG, FA, lysoPC, lysoPE, MGDG, PA, PC, PE, PG, PI, PS, SQDG.

DDA Positive ion mode

DDA Negative ion mode

Supplementary Fig. 5. Comparison of lipid properties in each algal species. The X- and Y-axis show the algae names and the total peak heights of identified lipids. The results of SWATH-MS positive ion mode, SWATH-MS negative ion mode, DDA positive ion mode, and DDA negative ion mode are described in the respective pages.

SWATH-MS Positive ion mode
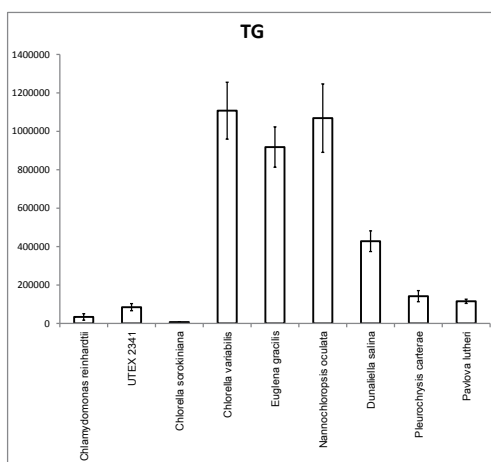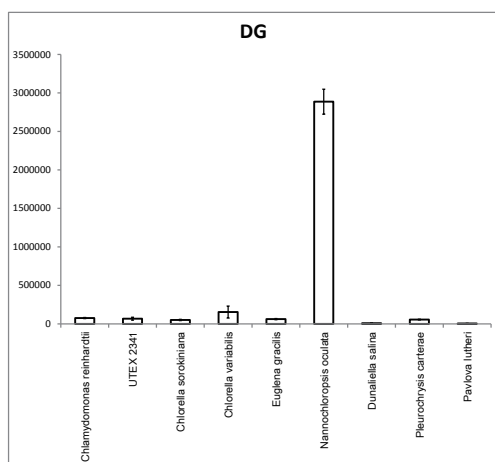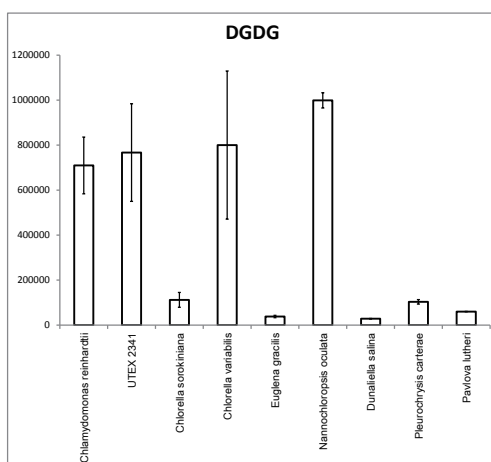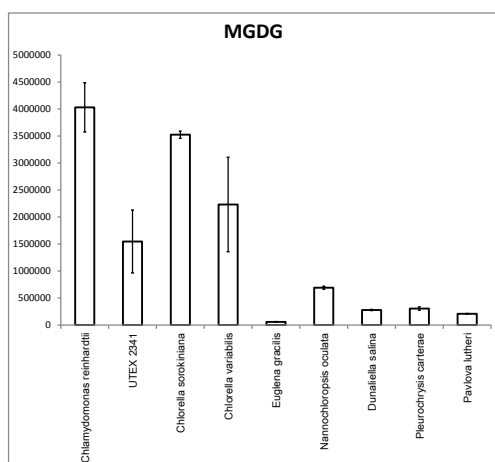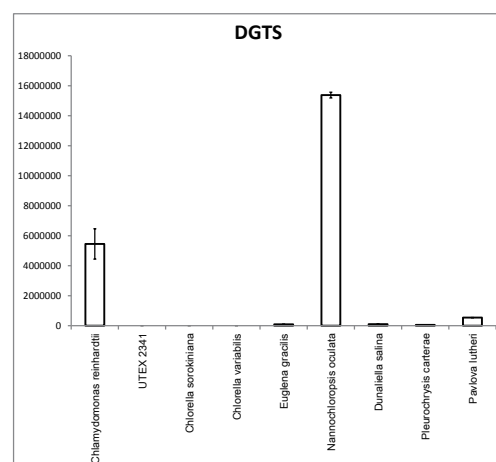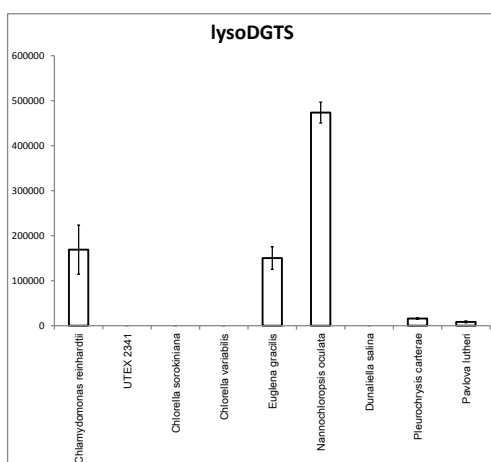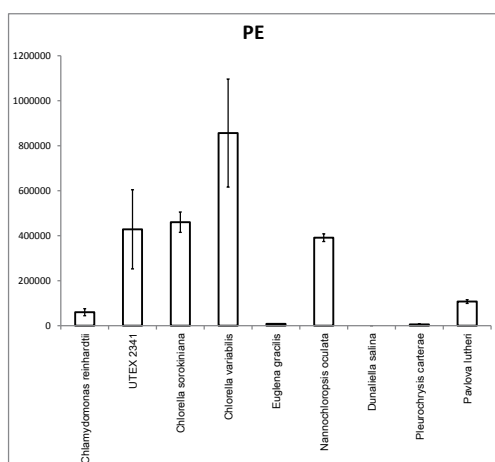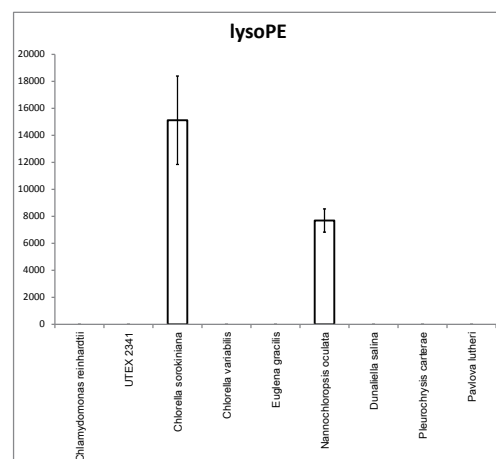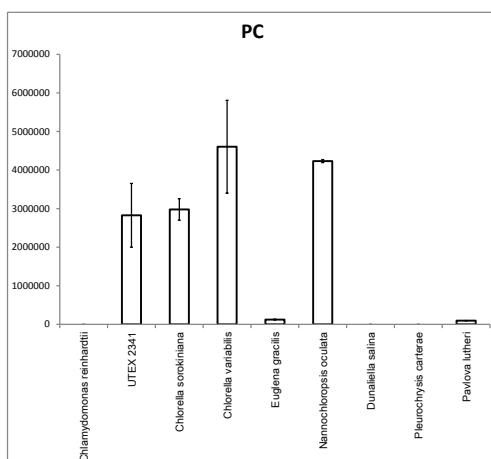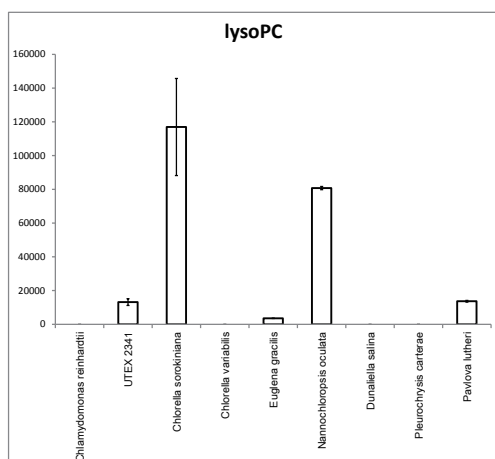
SWATH-MS Negative ion mode
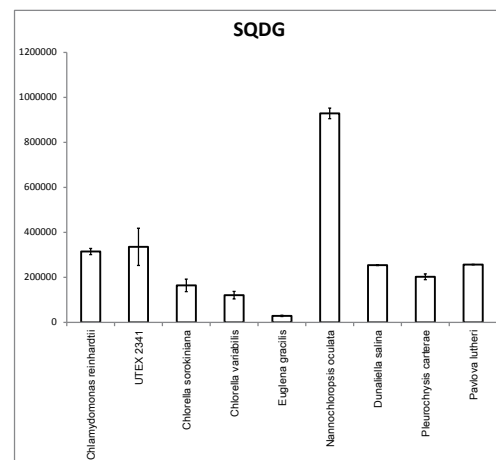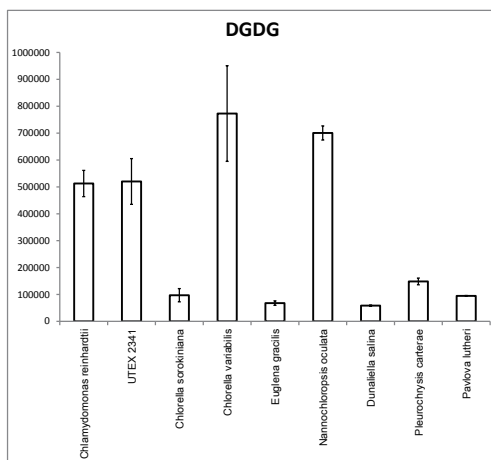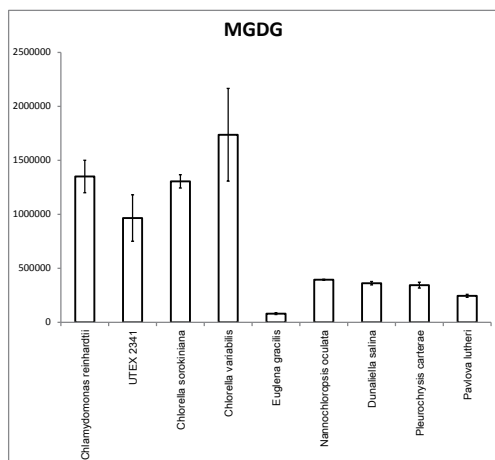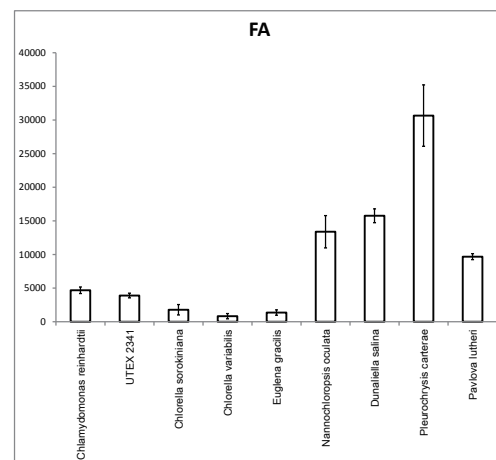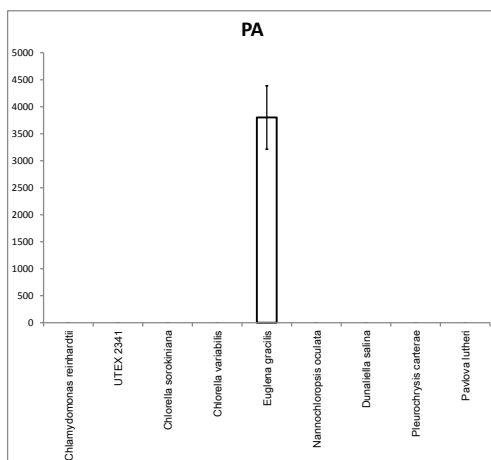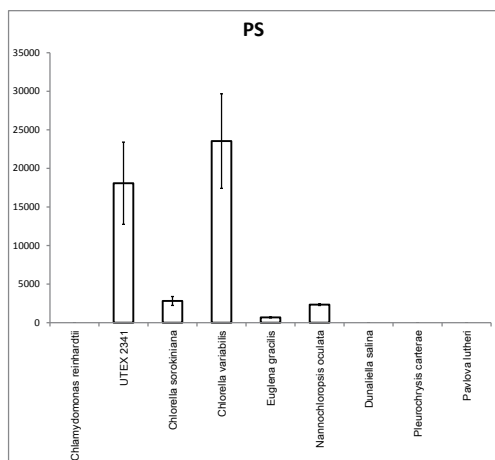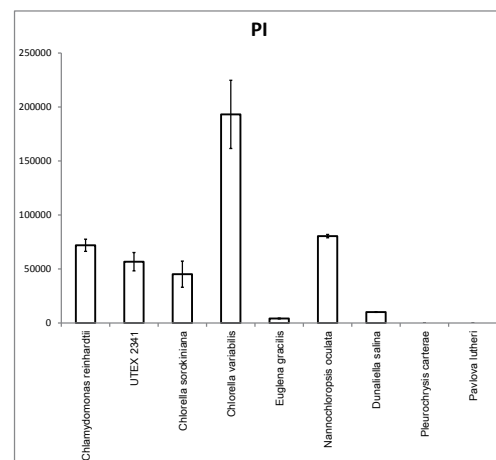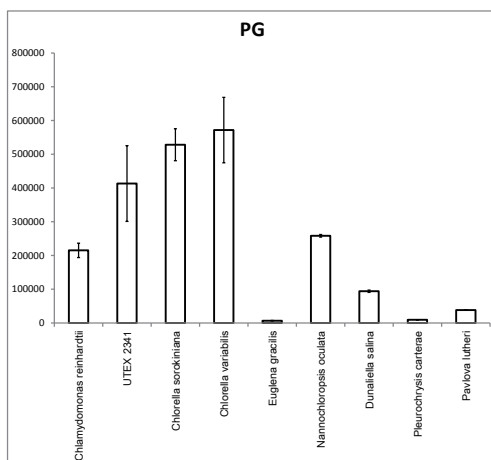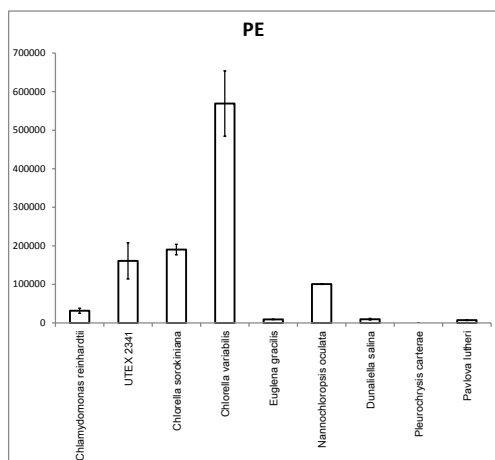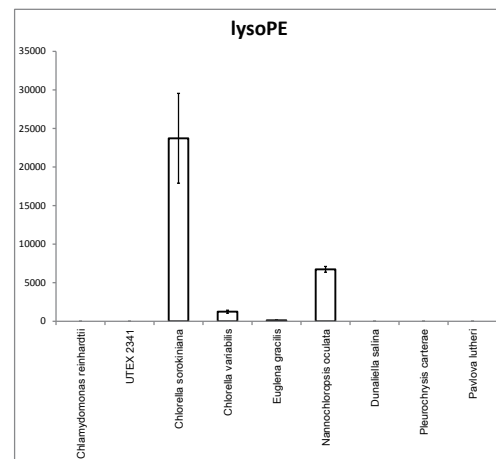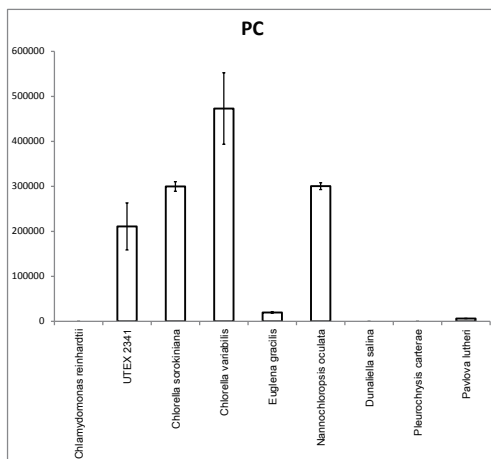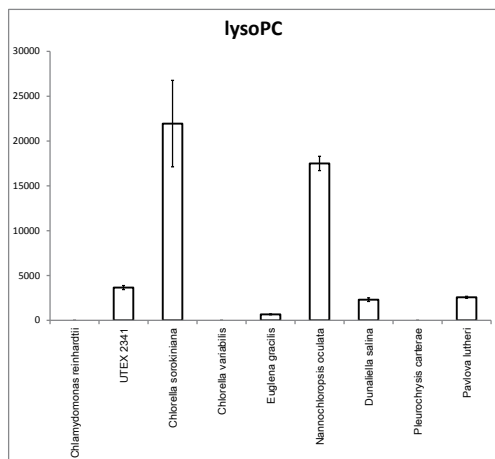
DDA Positive ion mode
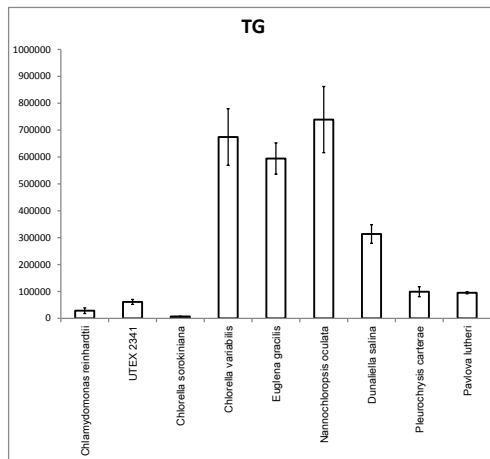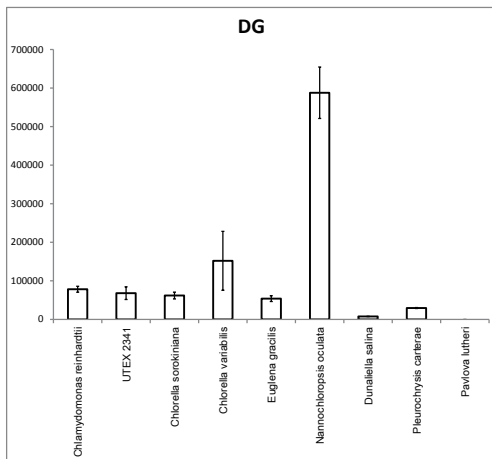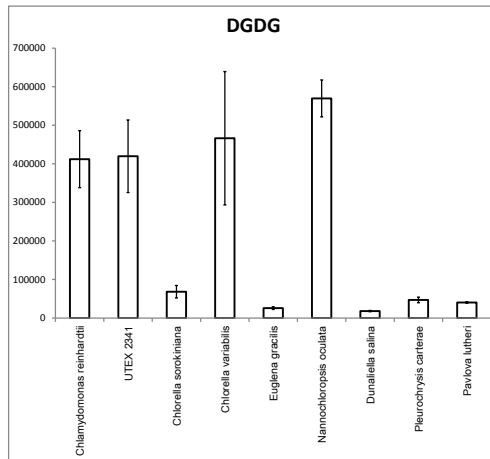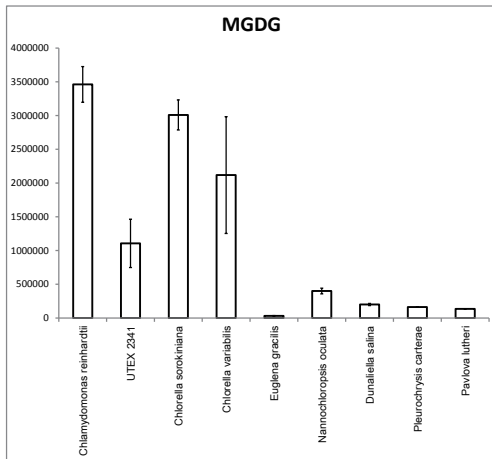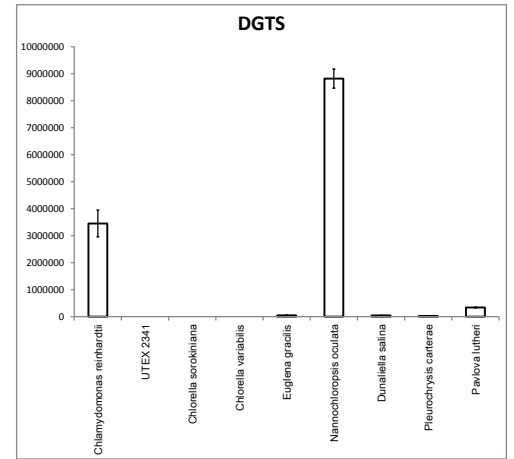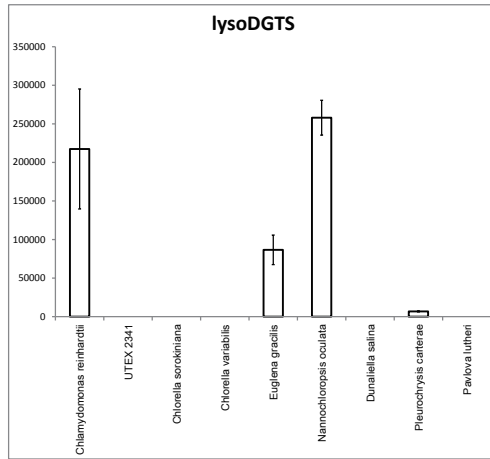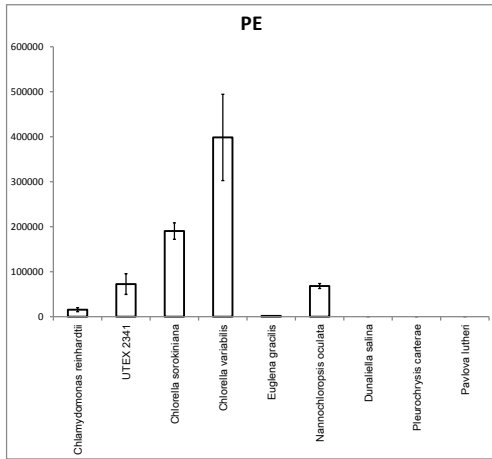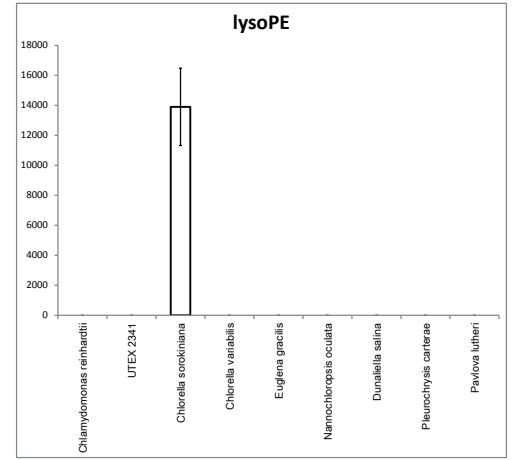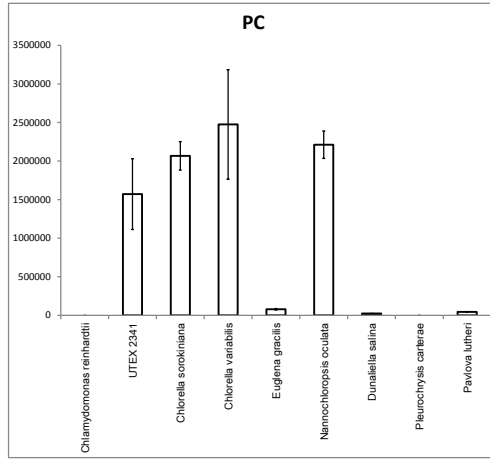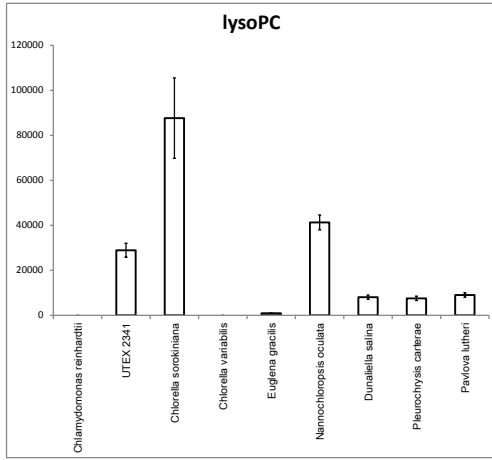
DDA Negative ion mode
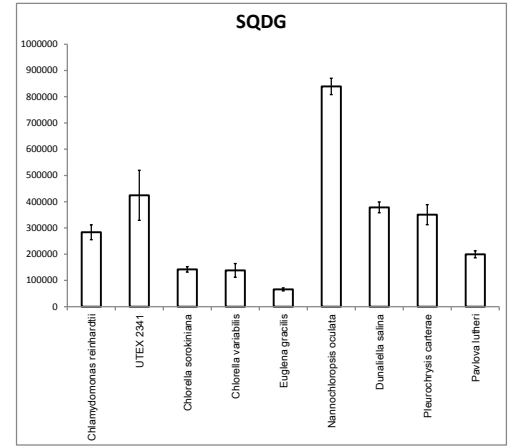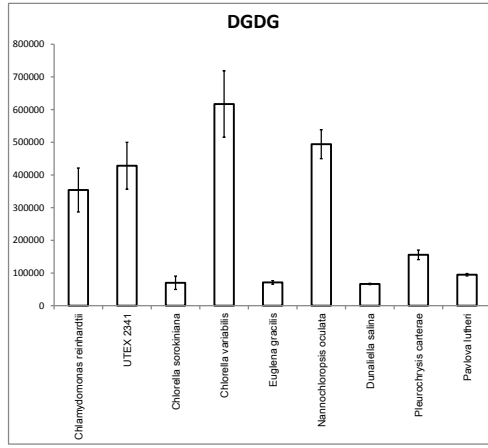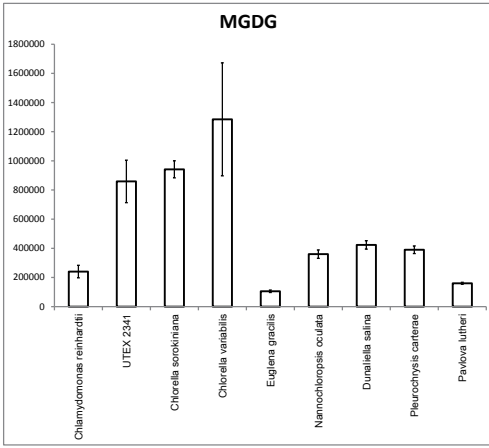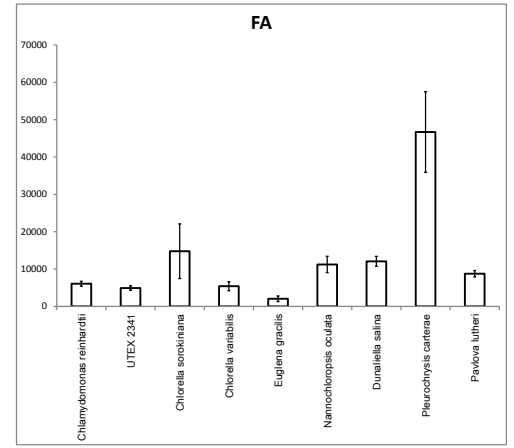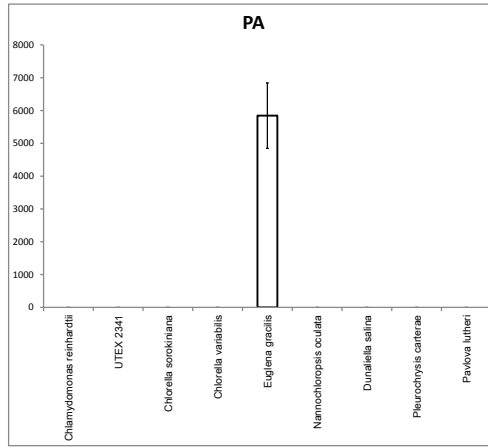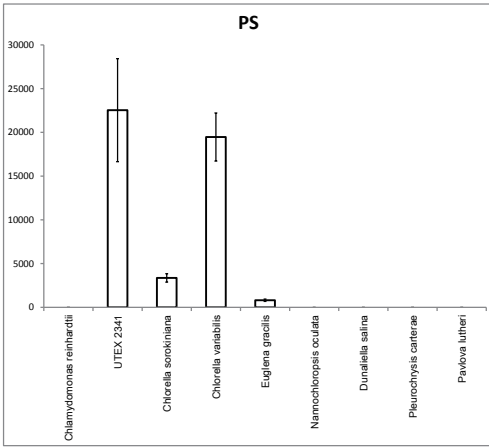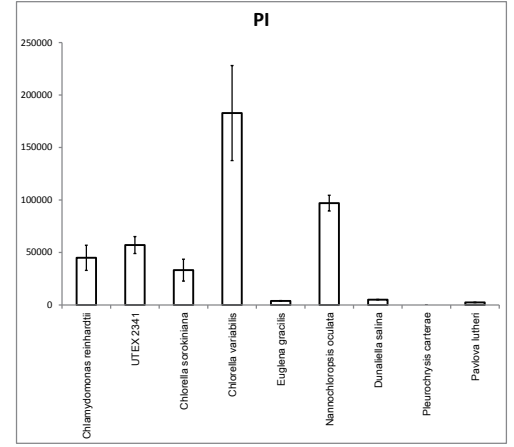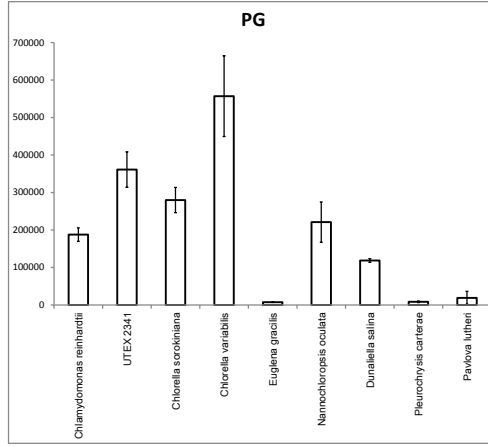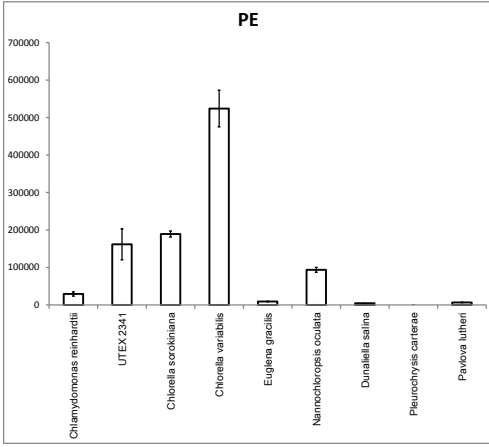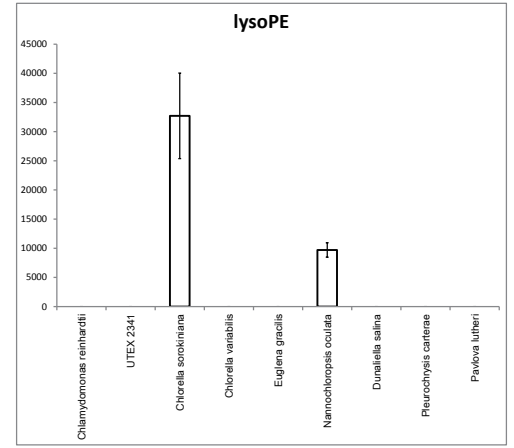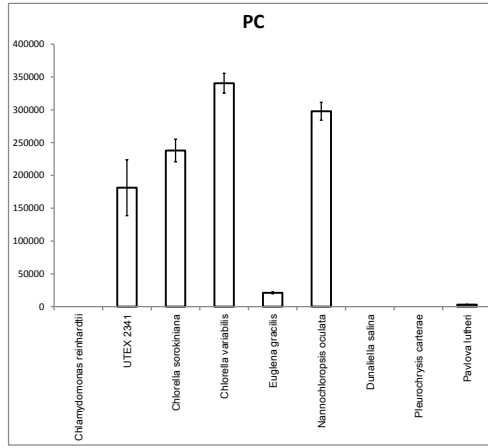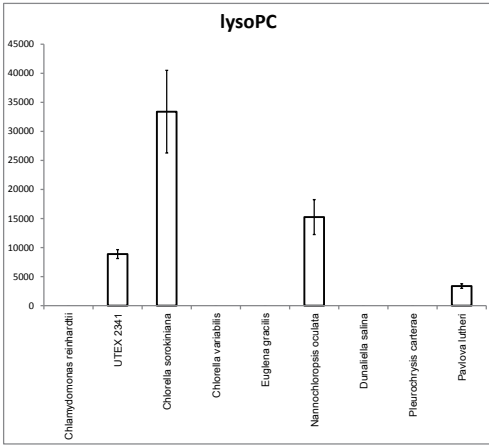
Supplementary Fig. 6. **Examples of lipid compounds that had little or not been reported previously.** Each panel shows the screenshot of MS-DIAL software. The retention time, mass accuracy, isotopic ratio, and MS/MS spectrum are confirmed for the lipid identifications.

Odd chain lipid: *Chlamydomonas reinhardtii* DGTS (16:0/17:1)
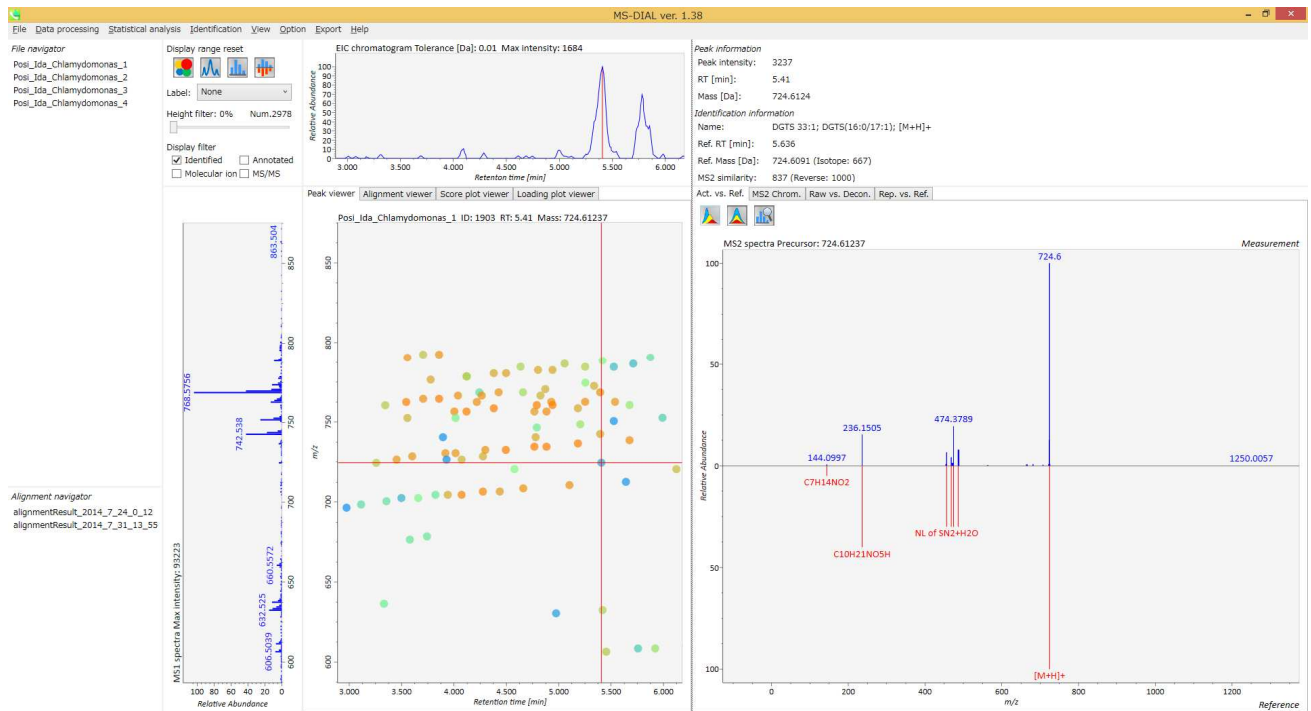
Odd chain lipid: *Chlamydomonas reinhardtii* DGTS (19:1/18:3)

Odd chain lipid: *Chlorella minutissima* MGDG(17:2/18:3)
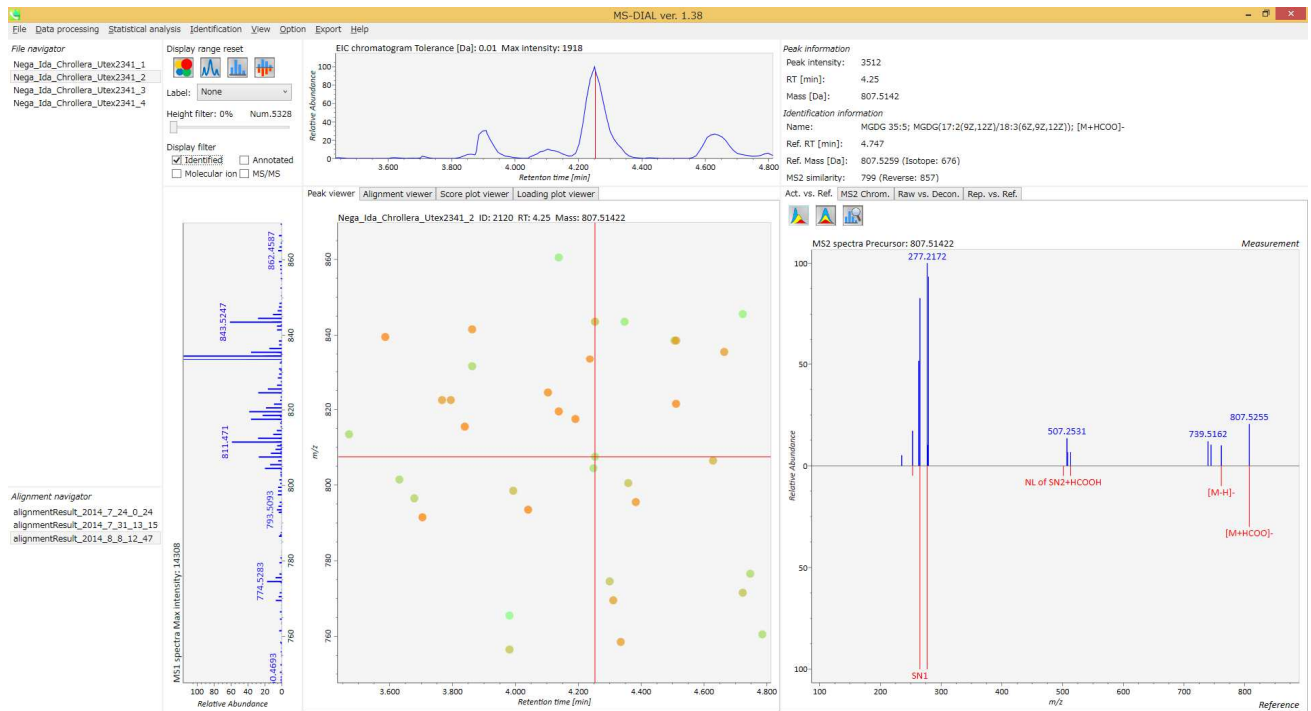
Odd chain lipid: *Chlorella minutissima* SQDG (15:0/18:3)
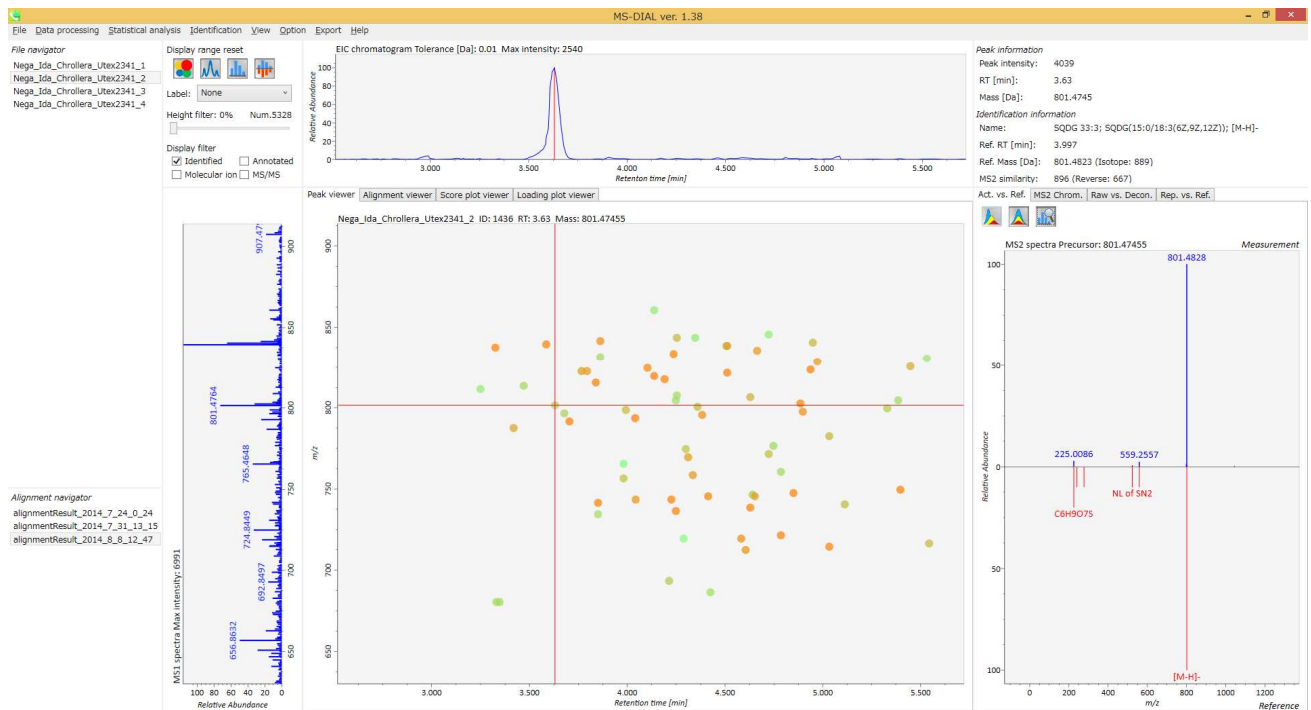
Odd chain lipid: *Chrollera sorokiniana* MGDG(16:2/17:1)
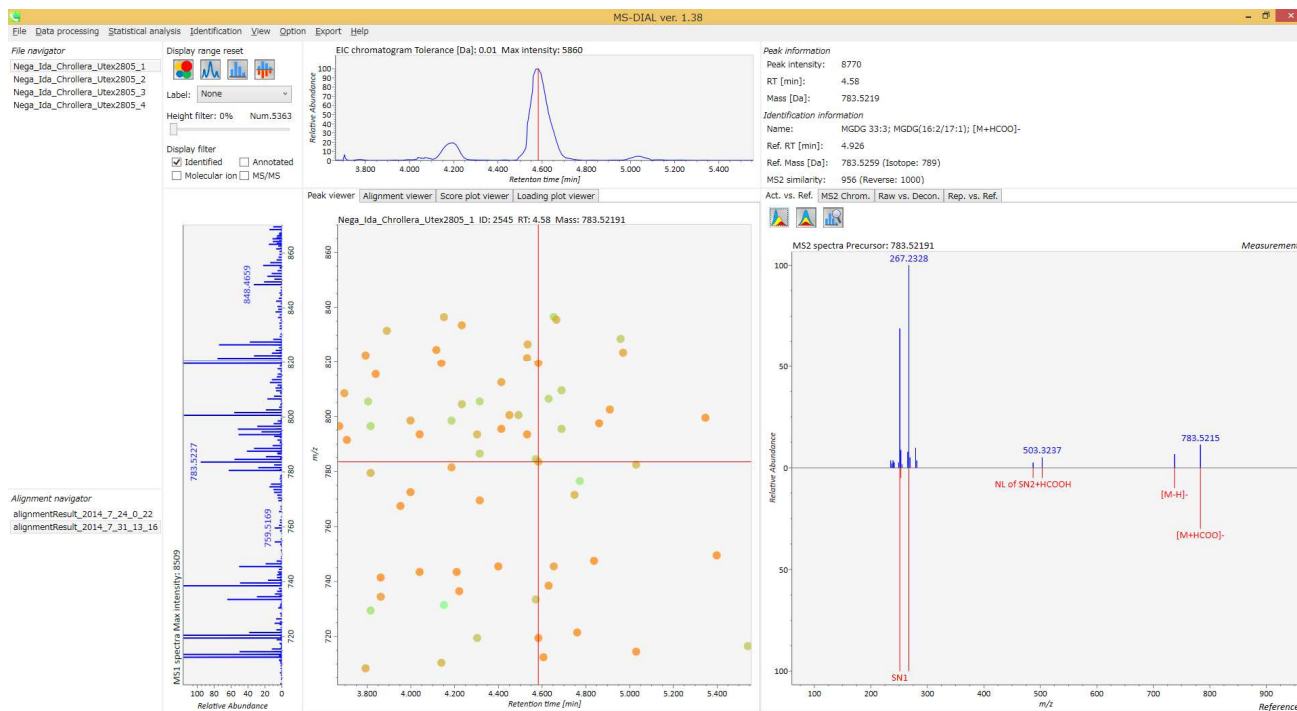
Odd chain lipid: *Chrollera sorokiniana* PC(16:3/17:3)
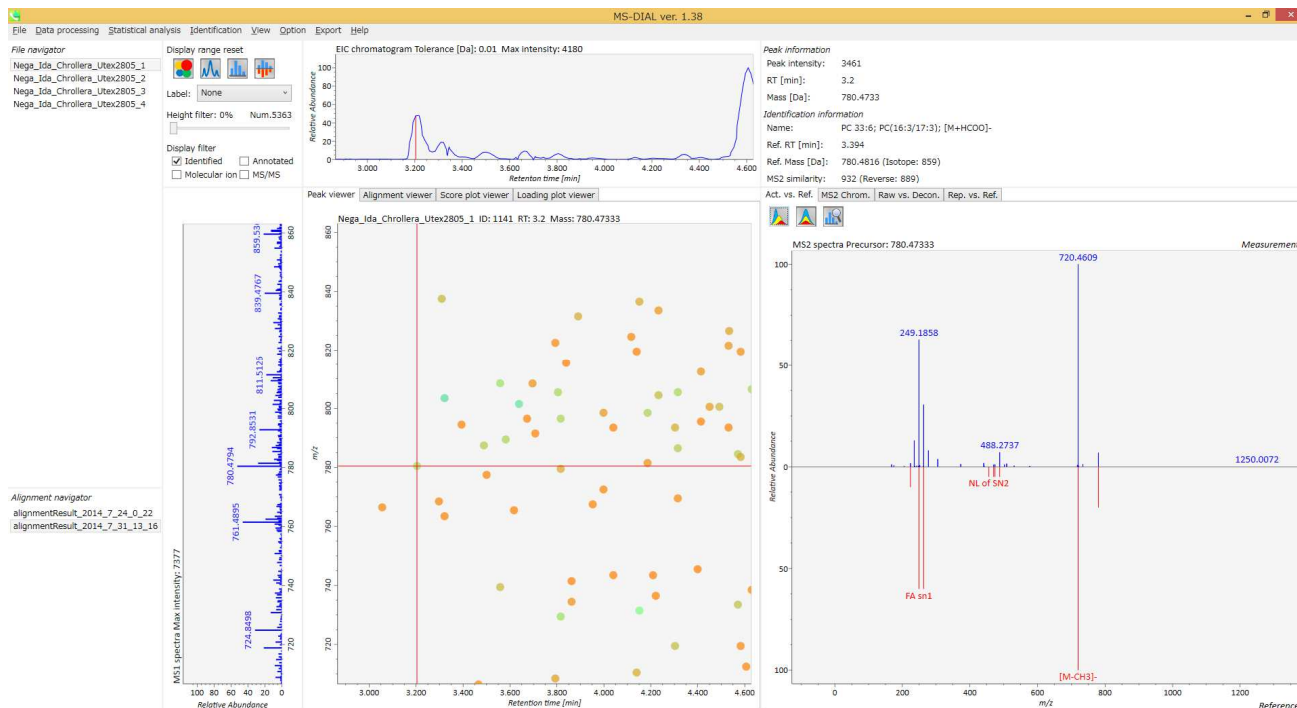
Odd chain lipid: *Chrollera variabilis* DGDG(16:3/17:3)
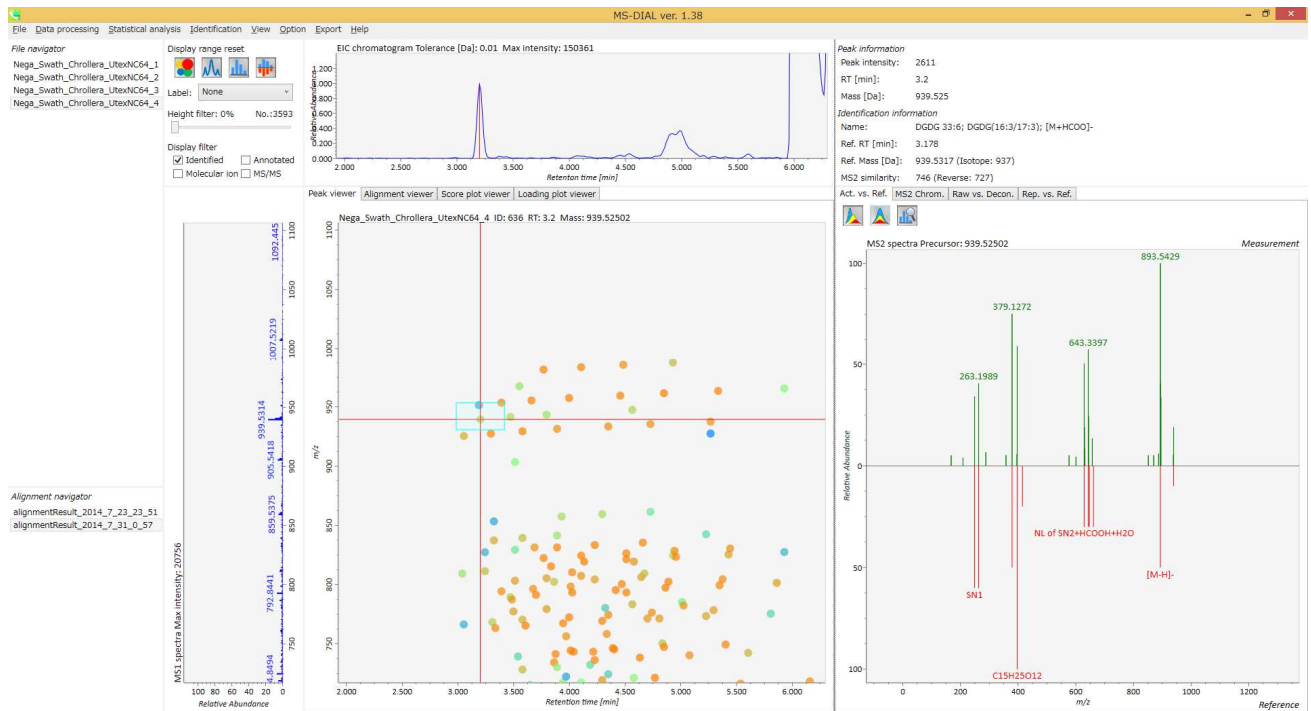
Odd chain lipid: *Euglena gracilis* DGDG(16:3/17:1)
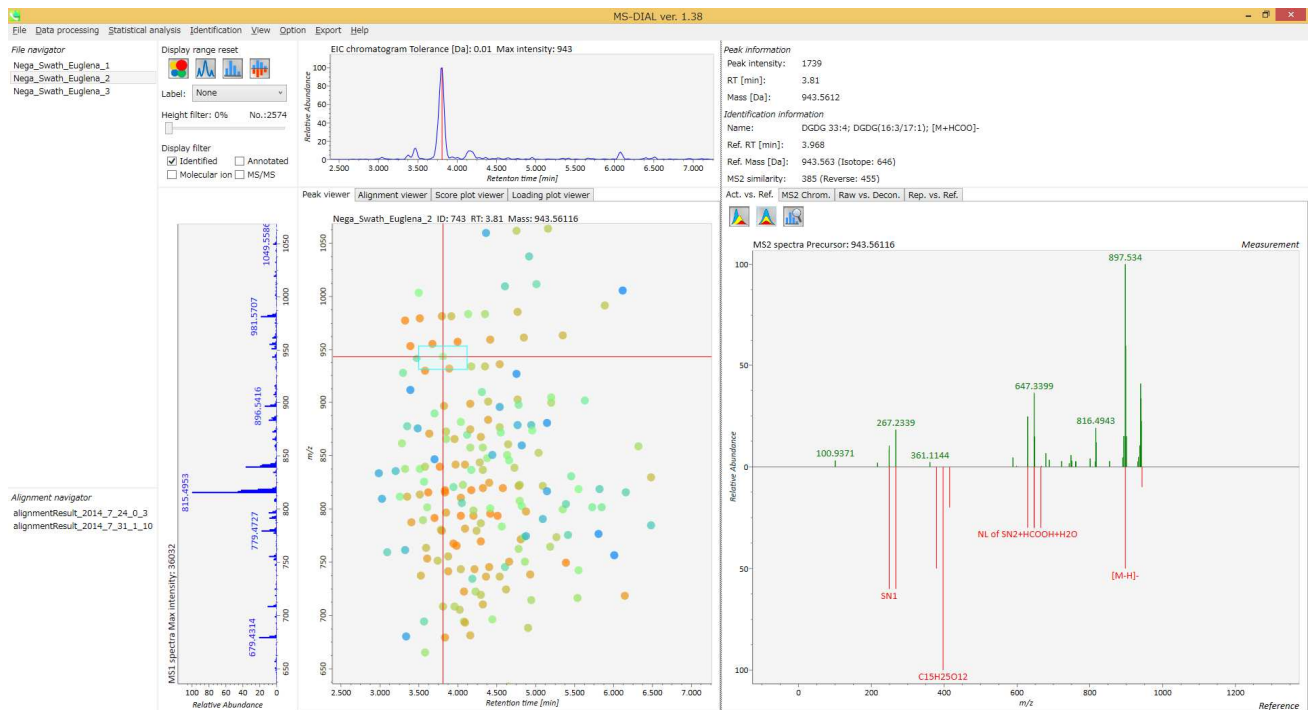
Odd chain lipid: *Nannochloropsis oculata* DGTS(17:1/20:5)
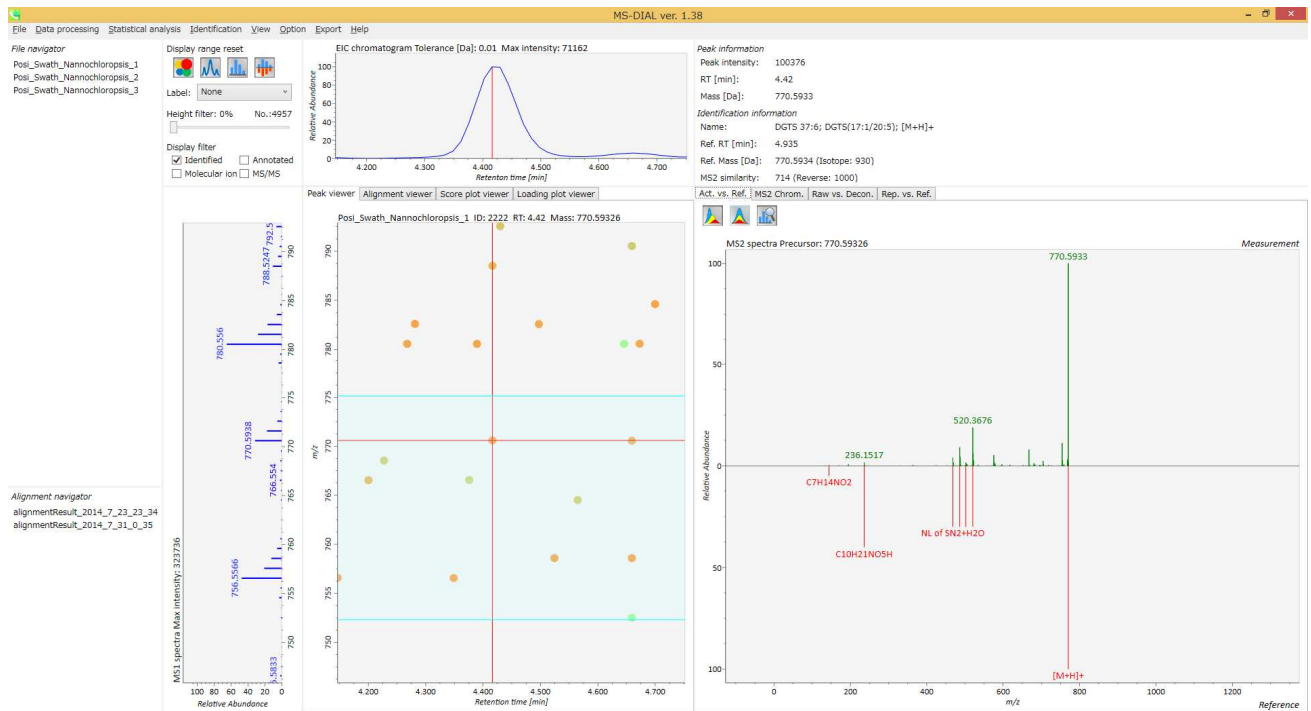
Odd chain lipid: *Dunaliella salina* DGTS(19:1/19:1)
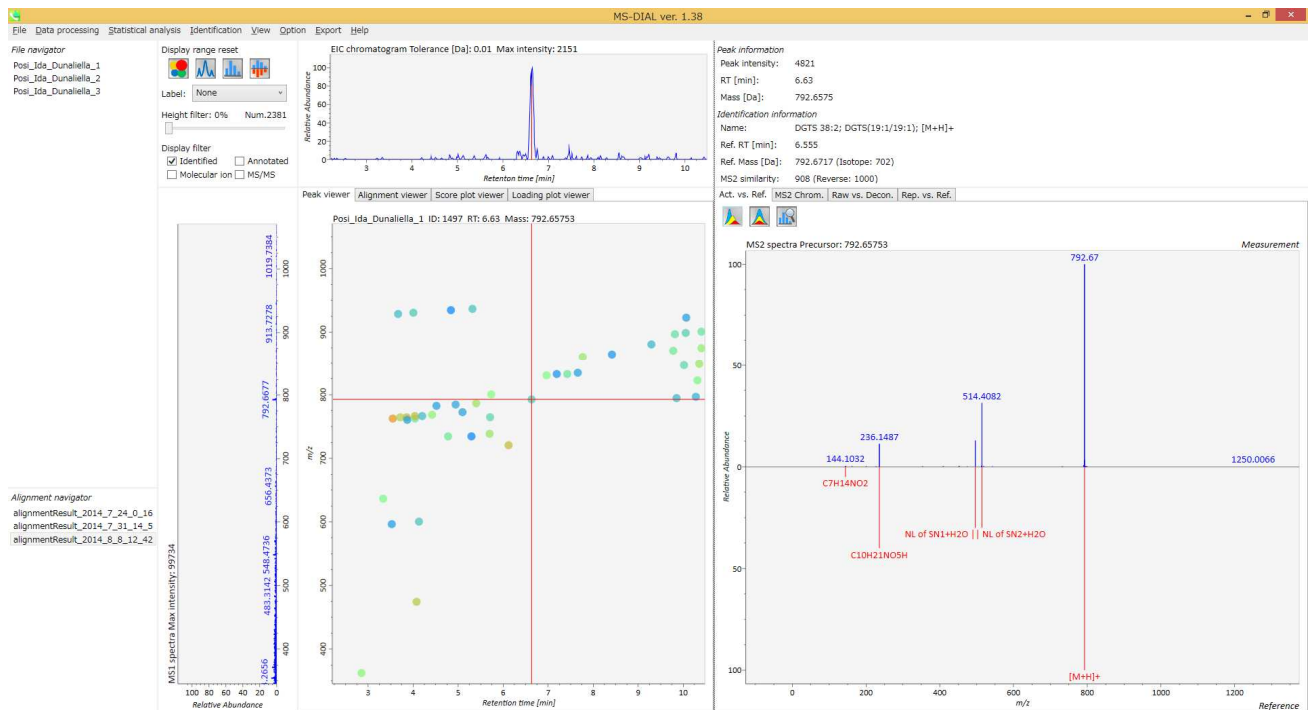
Odd chain lipid: *Pleurochrysis carterae* SQDG(16:0/17:1)
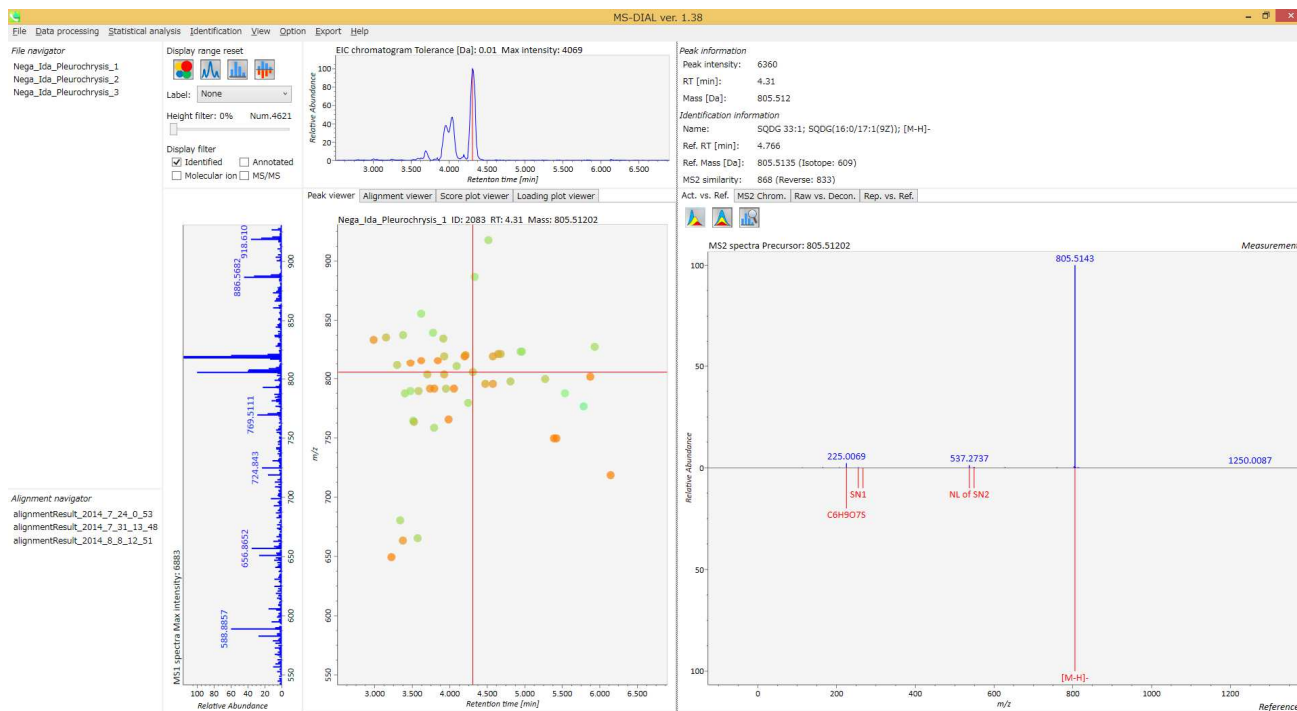
Odd chain lipid: *Pavlova lutheri* SQDG(14:0/15:0)
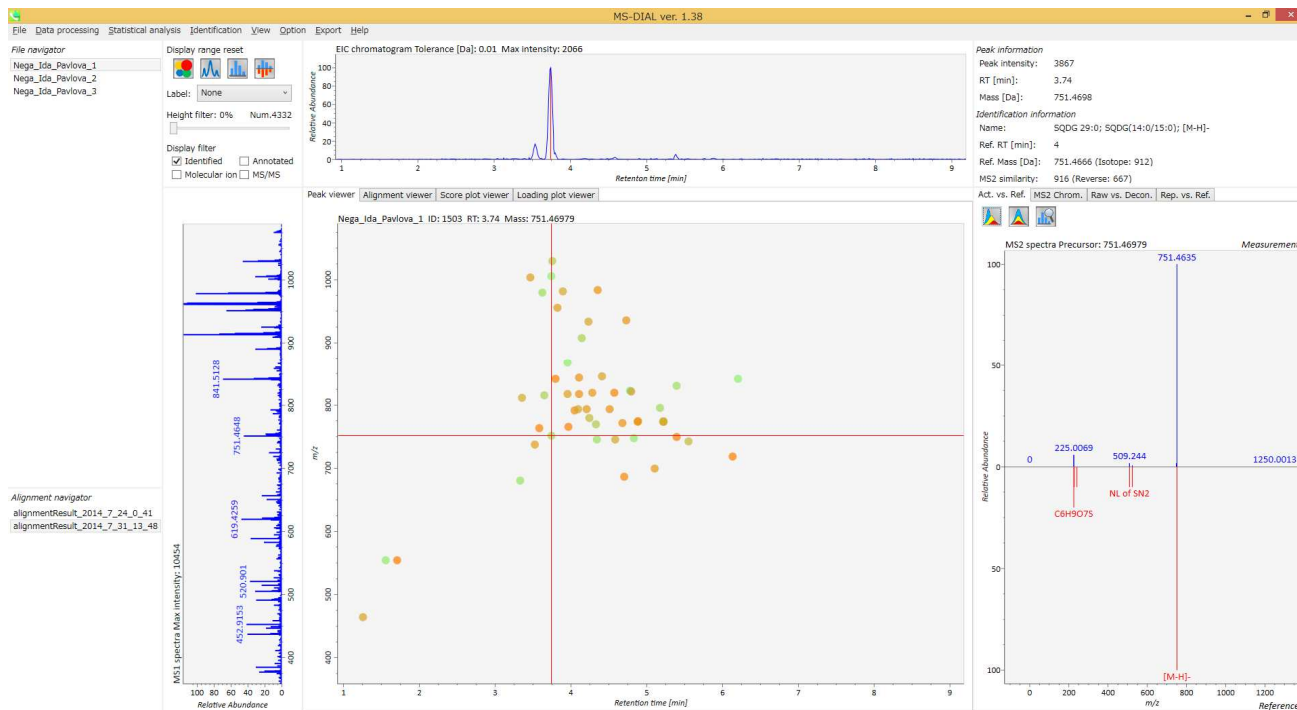
DGTS lipid: *Euglena gracilis* DGTS(16:1/18:1)
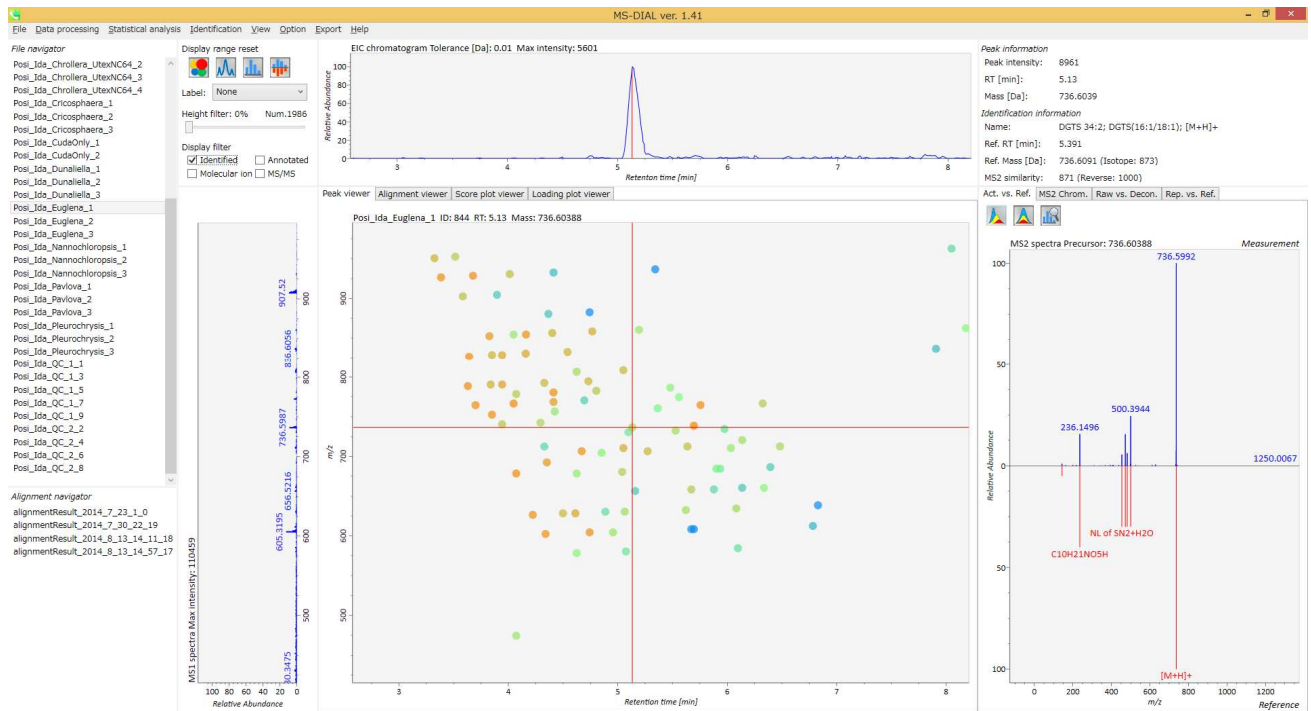
DGTS lipid: *Euglena gracilis* DGTS(16:0/18:1)
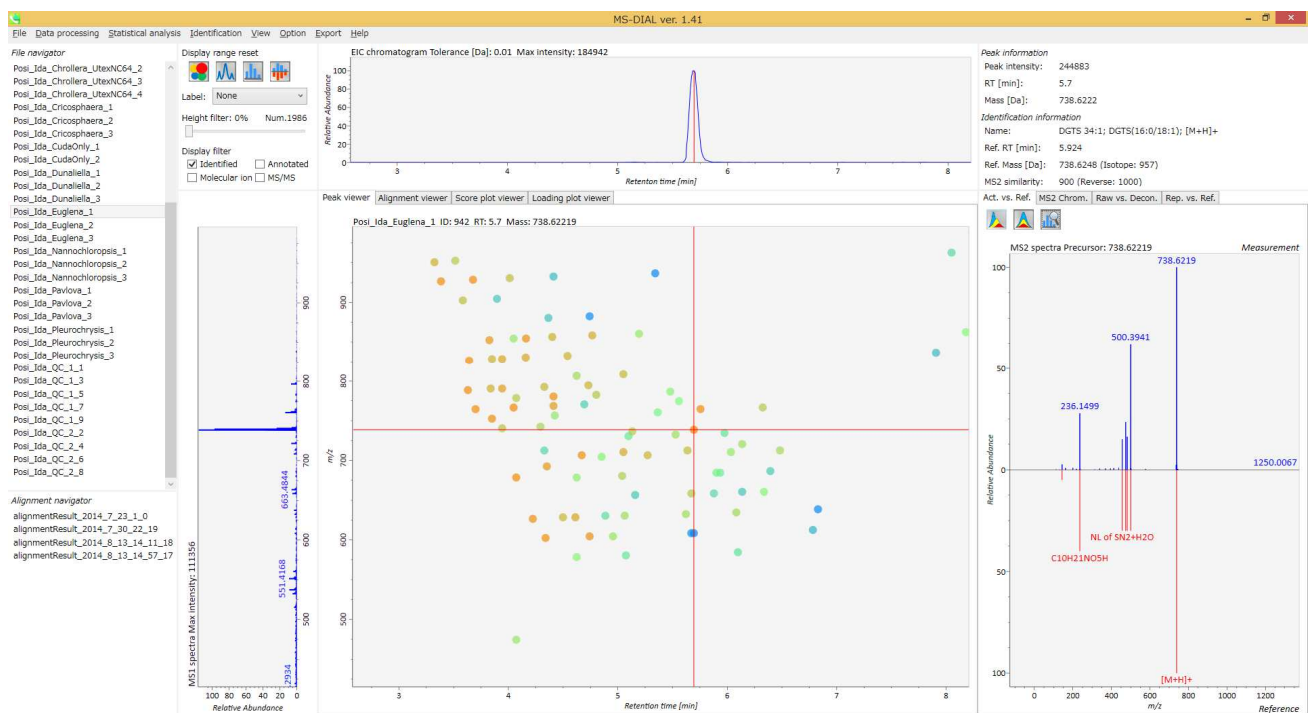
DGTS lipid: *Nannochloropsis oculata* DGTS(16:0/20:4)

DGTS lipid: *Nannochloropsis oculata* DGTS(17:0/20:5)

DGTS lipid: *Dunaliella salina* DGTS(16:0/18:3)
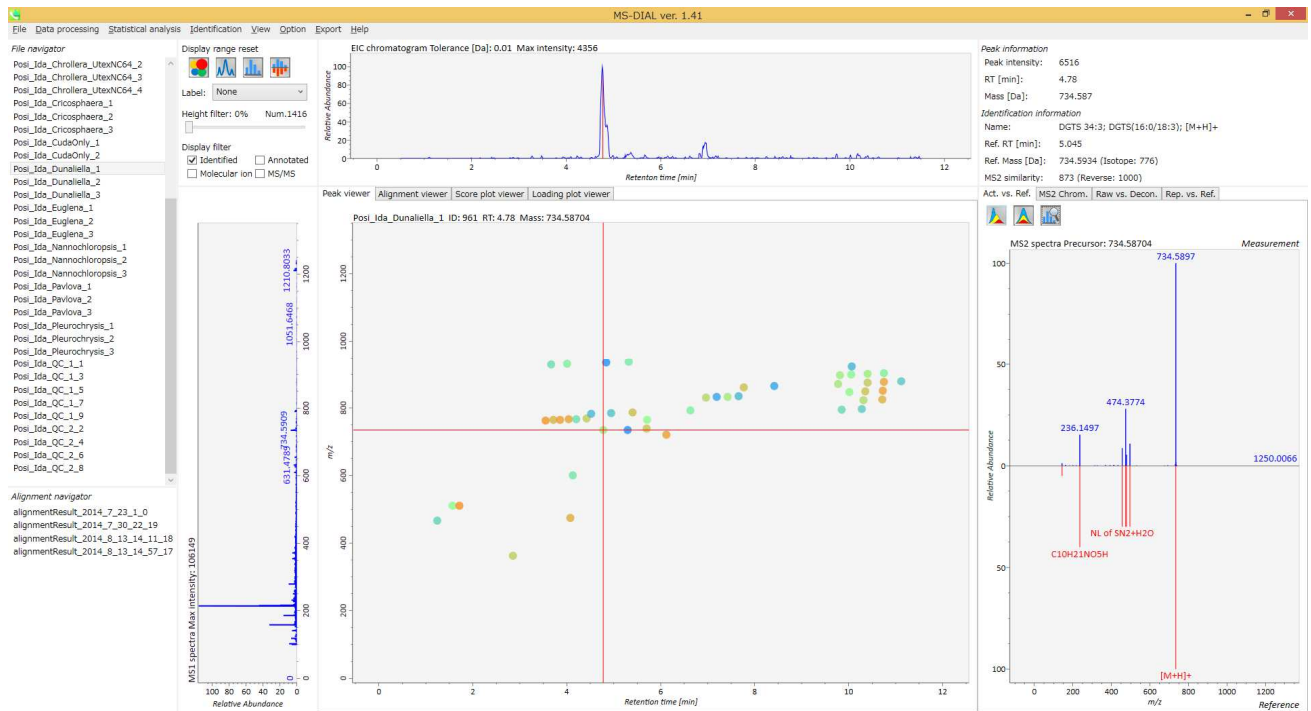
DGTS lipid: *Dunaliella salina* DGTS(16:0/18:1)
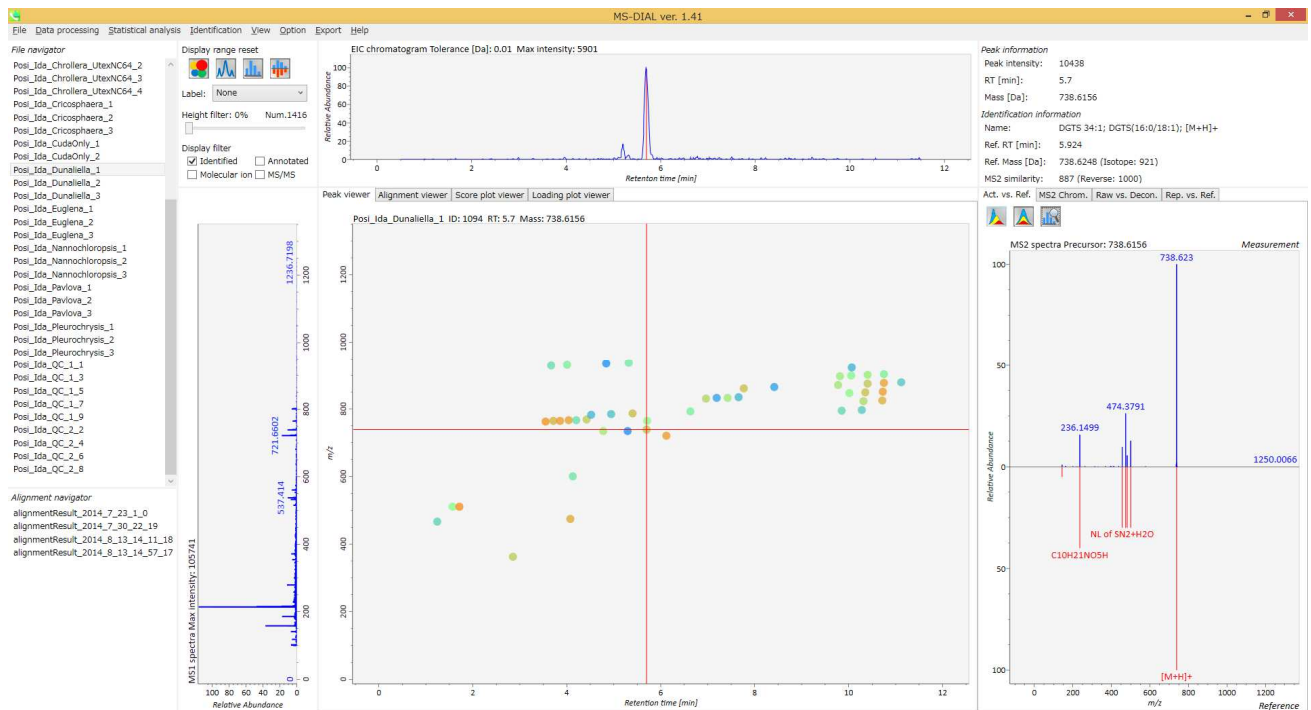
DGTS lipid: *Pleurochrysis carterae* DGTS(16:0/22:6)
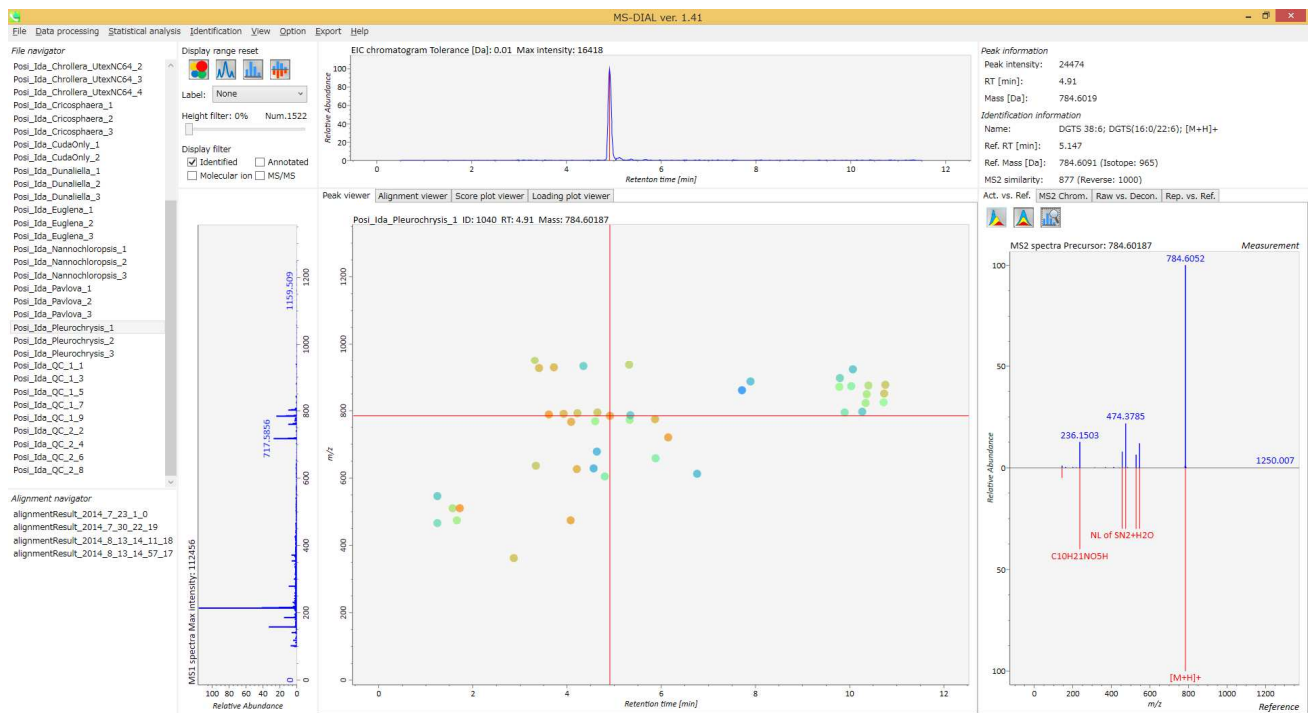
DGTS lipid: *Pleurochrysis carterae* DGTS(16:0/22:5)
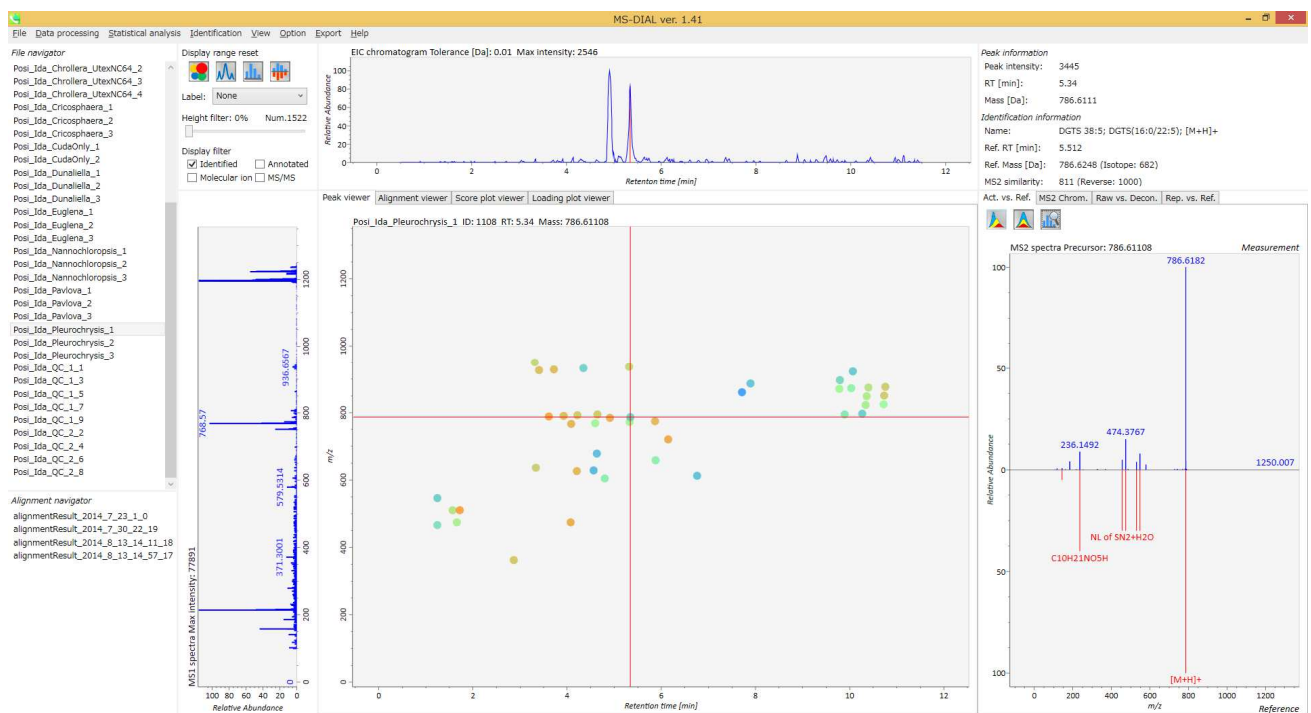
DGTS lipid: *Pavlova lutheri* DGTS(14:0/16:1)
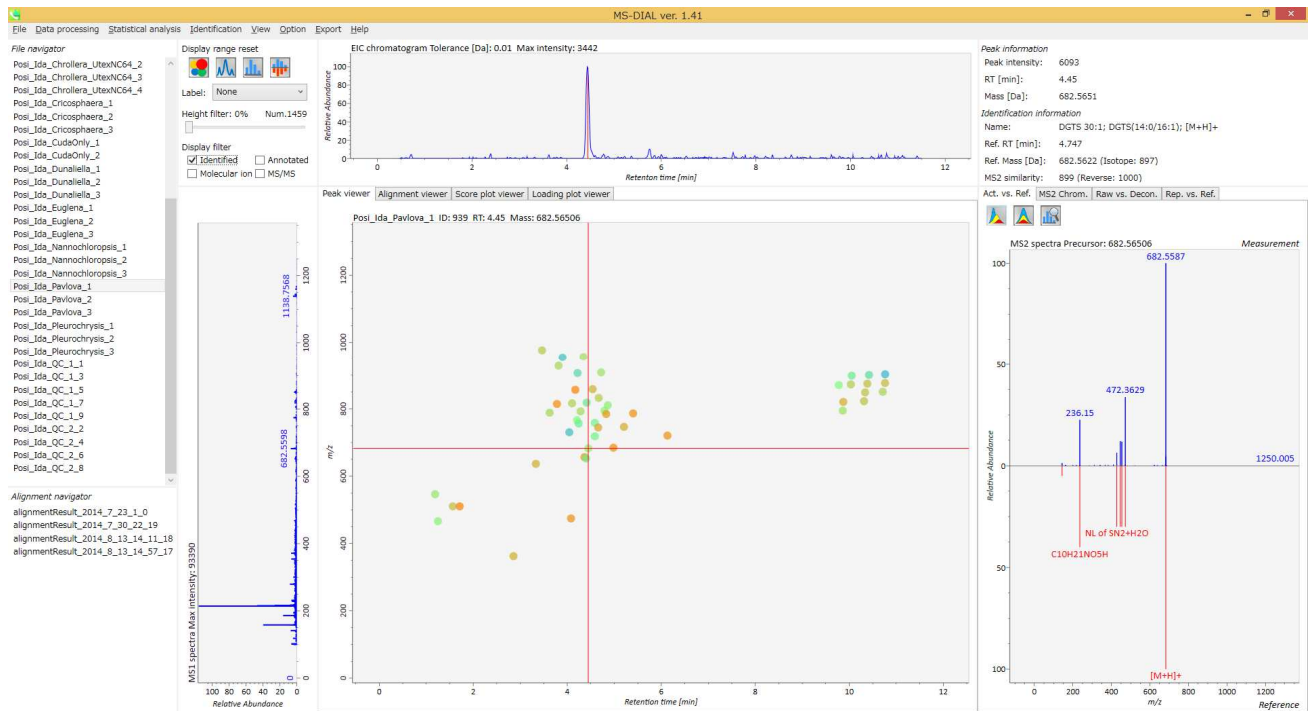
DGTS lipid: *Pavlova lutheri* DGTS(16:0/22:6)
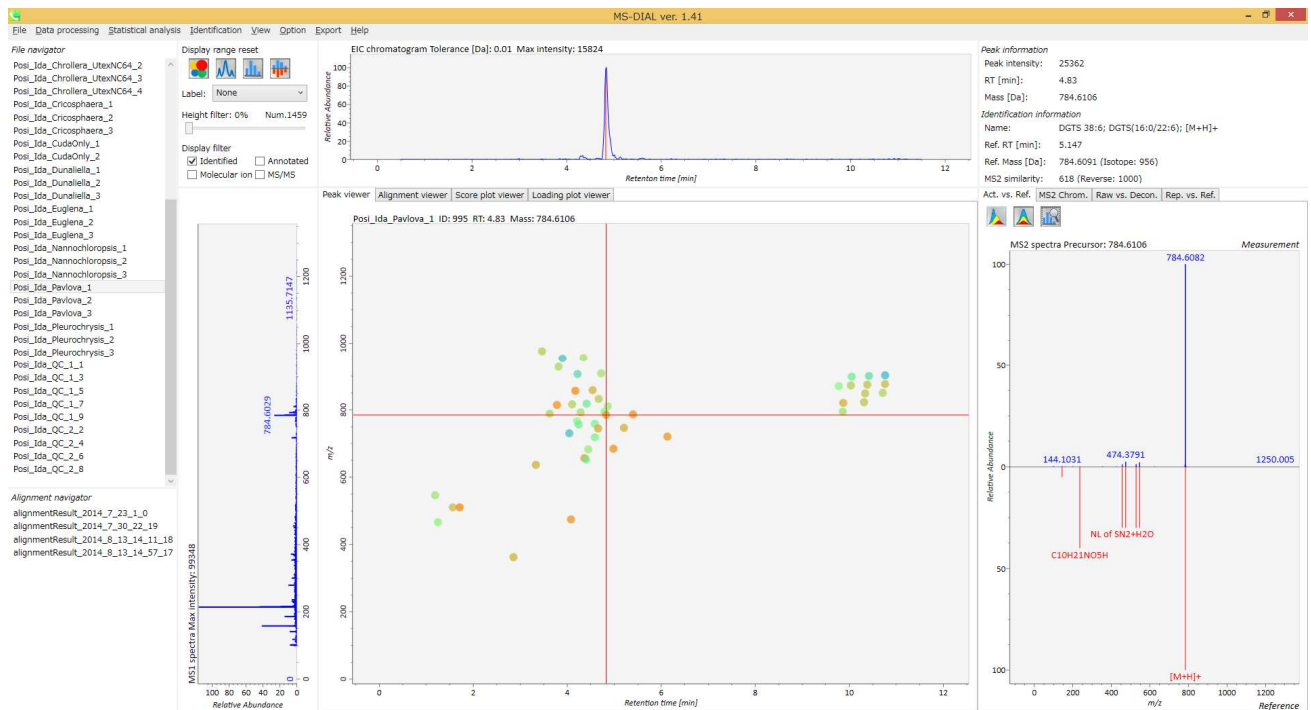
18:5 PUFA: *Chlamydomonas reinhardtii* DGTS(18:5/18:5)

18:5 PUFA: *Nannochloropsis oculata* DGTS(18:5/20:5)

18:5 PUFA: *Pleurochrysis carterae* DGDG(16:2/18:5)
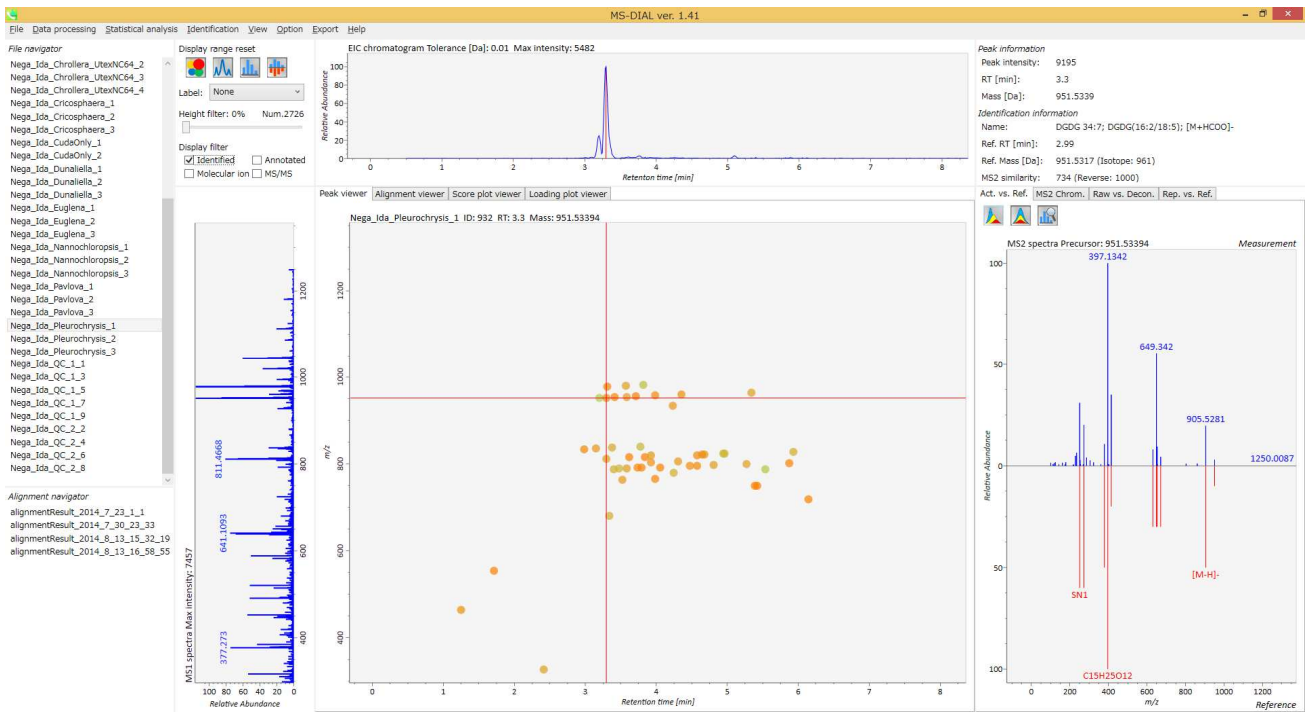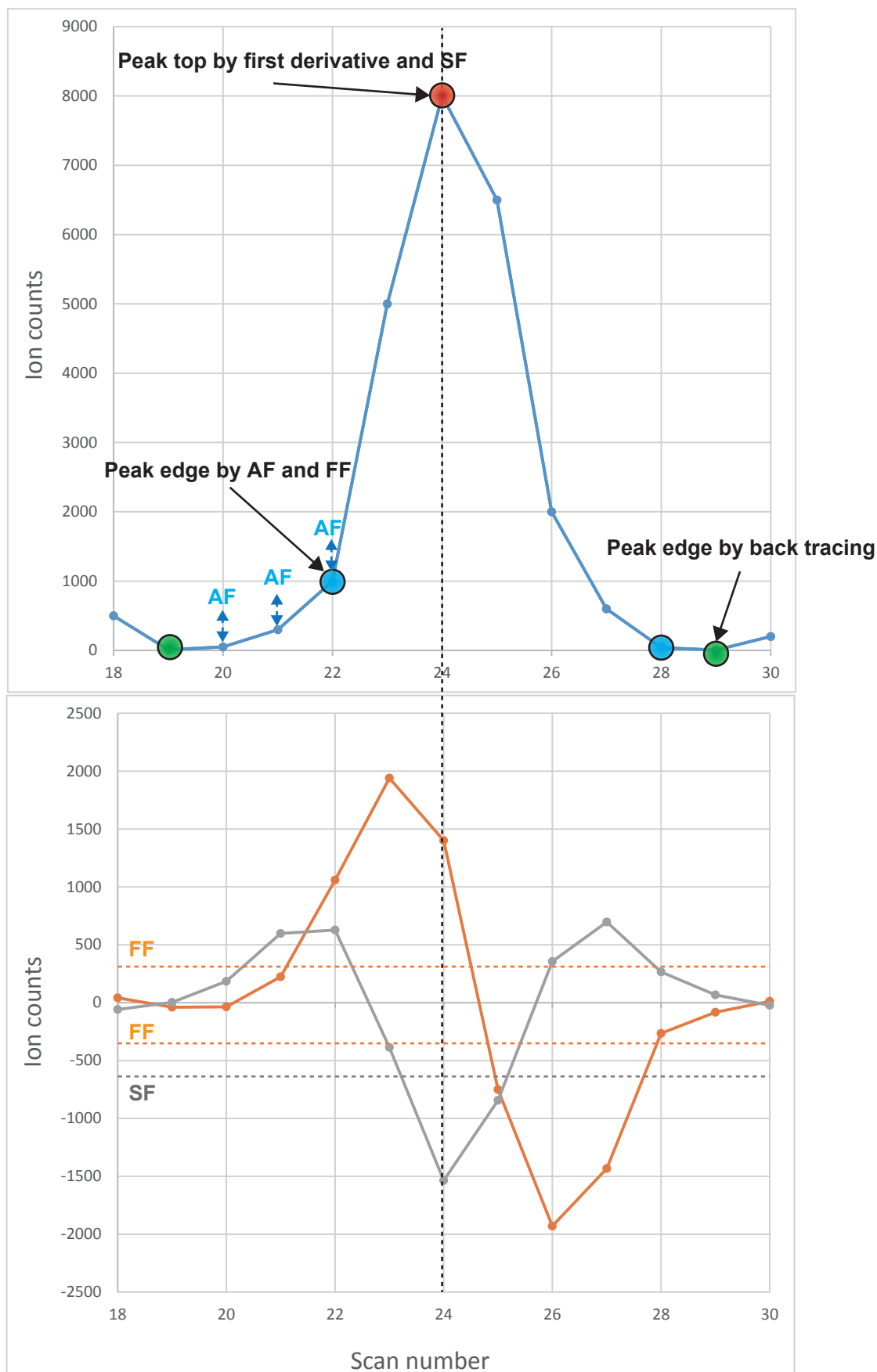
Supplementary Fig. 7. Peak detection by three threshold values and back-tracing. Blue, orange, and gray lines show the line plots of raw data points, first derivatives, and second derivatives, respectively. Blue dash with arrow, orange dash, and gray dash show the threshold values of amplitude filter (AF), first derivative filter (FF), and second derivative filter (SF), respectively. Blue circle shows the peak left- and right edge decided by the AF and FF thresholds. Green circle shows the final peak left- and right edge by the back-tracing method. The peak top described by the red circle is decided by the sign (positive/negative) change of the FF value and the SF threshold.

a
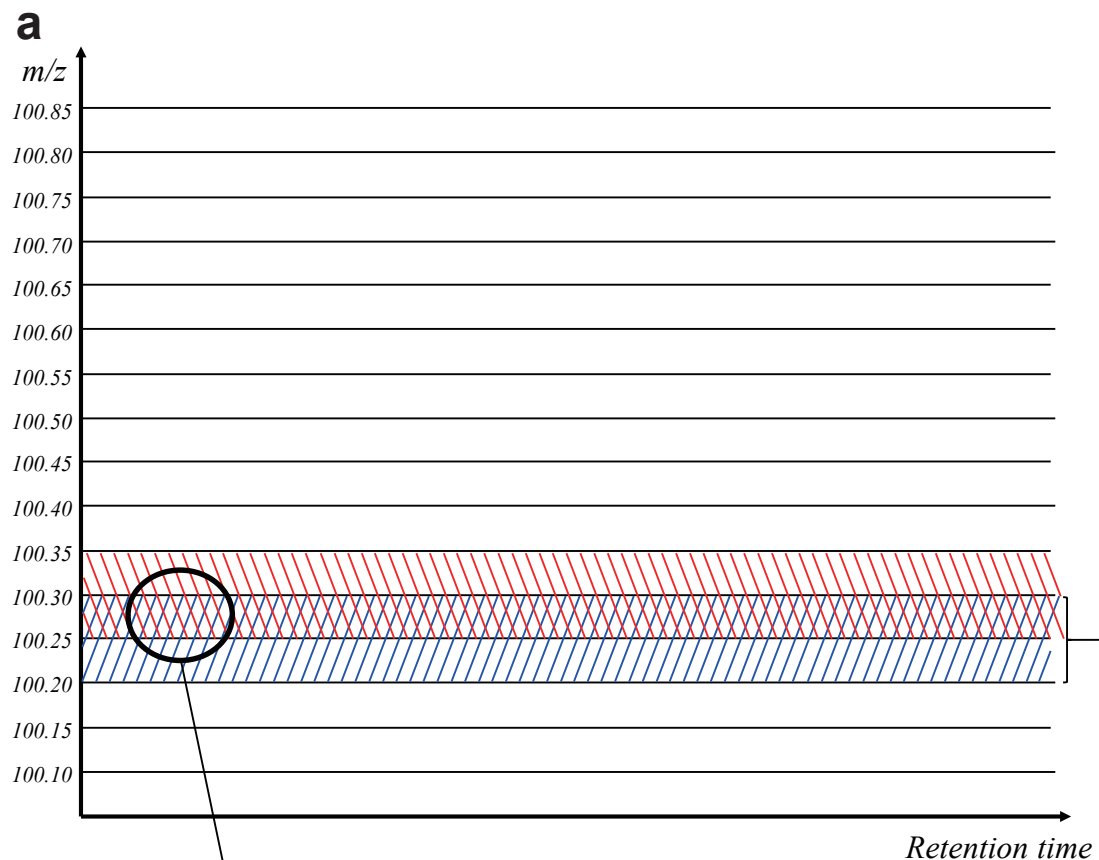
Scan number	Retention time [min]	Base peak *m/z*	Base peak intensity
1	0.1	100.2054	1
2	0.12	100.2053	10
3	0.14	100.2053	5
4	0.16	100.2052	50
5	0.18	100.2051	200
6	0.2	100.2054	1500
7	0.22	100.2054	3000
8	0.24	100.2054	1700
9	0.26	100.2053	180
10	0.28	100.205	60

Base peak chromatogram within 0.1 Da

b

Higher is kept, i.e. either is removed.
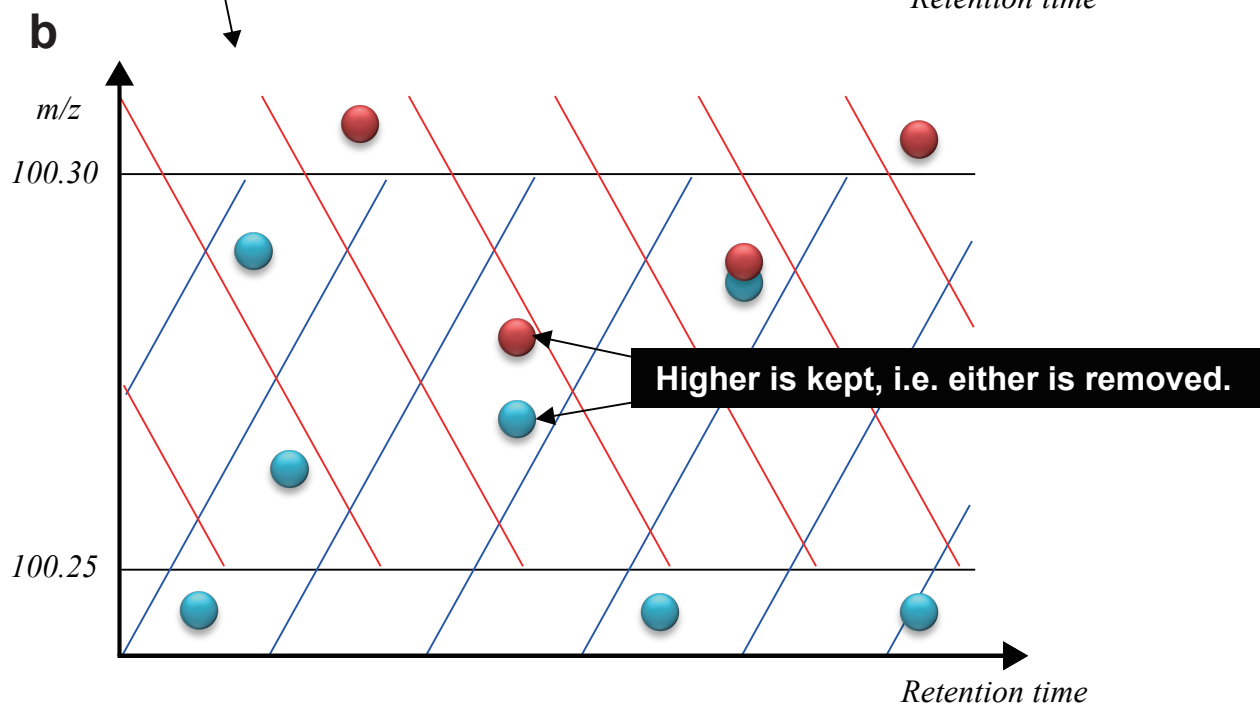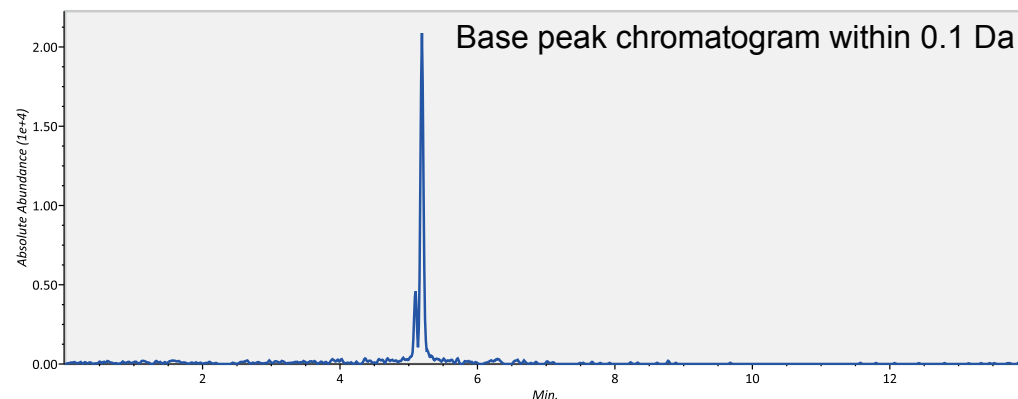
Supplementary Fig. 8. Summary of the peak spotting method.
(**a**) The base peak chromatogram is extracted for each mass slice of 0.1 Da (default). The retention time, base peak m/z, and peak intensity are shown in the top right table and the red color text indicates a spot.
(**b**) The blue and red spots show the detected peaks in m/z 100.20-100.30 and 100.25-100.35 bins, respectively. The spots of the same retention time and close m/z detected in adjacent bins are simply merged, i.e. the one with the higher intensity is kept.
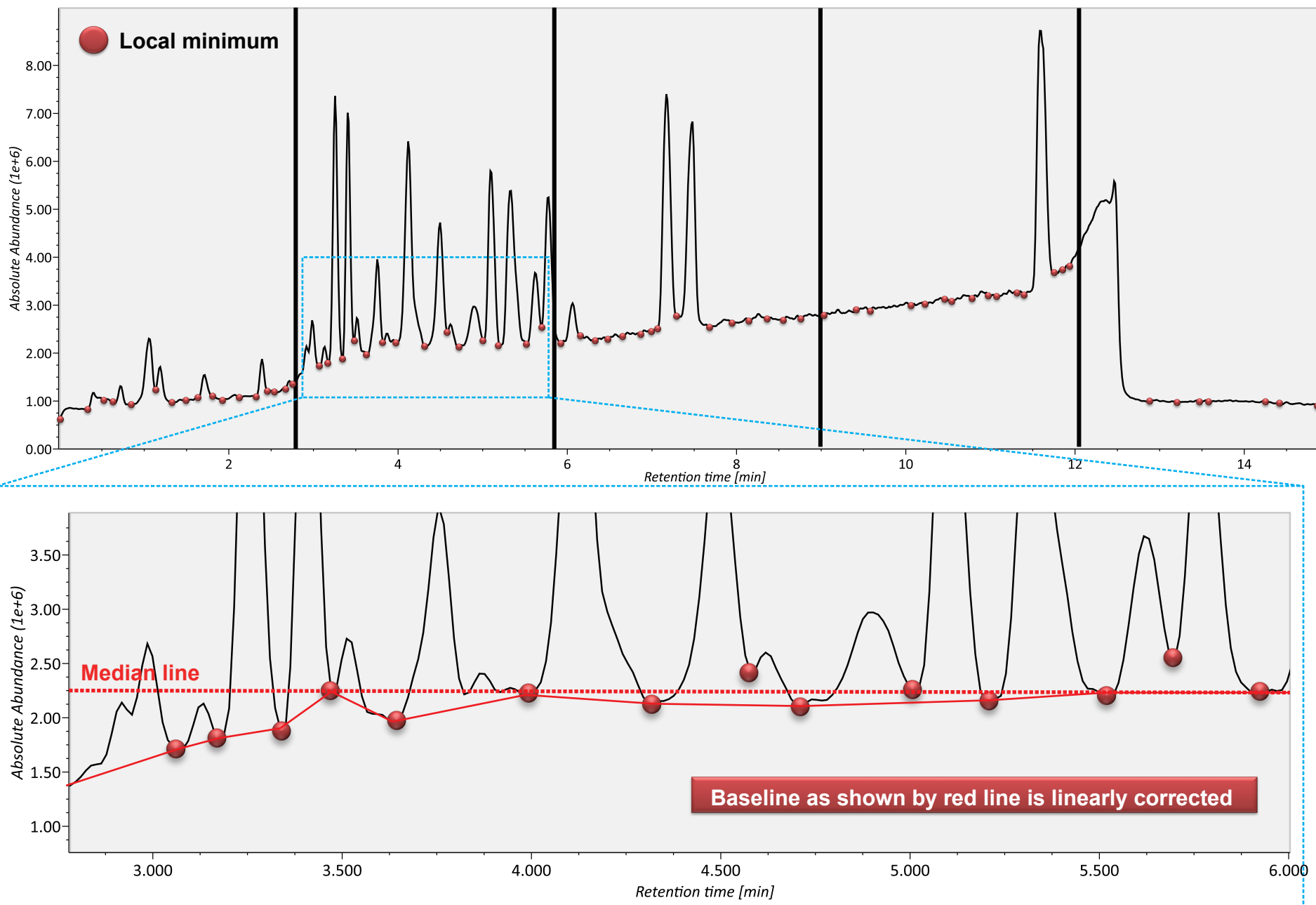
Supplementary Fig. 9. Baseline correction of MS-DIAL program. The local minima denoted by red circles are determined in each segment separated by black lines. The median value of the local minima is calculated for each segment. The local minima less than the median value are connected, from which all data points are subtracted.

a. Making a reference peak table

Reference file

Peak no.	RT [min]	m/z
1	0.73	434.5876
2	1.26	842.1221
3	1.82	254.1078
4	2.11	332.0043
5	2.29	111.0078
k-1	7.09	982.9814
k	7.11	512.3321
k+1	7.12	687.8406
n	12.88	1230.412

Sample A

Peak no.	RT [min]	m/z
p	7.11	721.9012

Try to find Equation 8

Find or not → Yes → *Next peak*

No → *Add to reference*

Repeat for all samples

Peak no.	RT [min]	m/z
1	0.73	434.5876
2	0.88	541.9048
3	1.26	842.1221
4	1.82	254.1078
5	1.99	771.3782
6	2.11	332.0043
7	2.13	659.6631
8	2.29	111.0078
n-2	11.35	1050.772
n-1	12.85	1001.289
n	12.88	1230.412

b. Fitting each sample peak table to reference peak table

Reference peak table

Peak no.	RT [min]	m/z
1	0.73	434.5876
2	0.88	541.9048
3	1.26	842.1221
4	1.82	254.1078
5	1.99	771.3782
6	2.11	332.0043
7	2.13	659.6631
8	2.29	111.0078
n-2	11.35	1050.772
n-1	12.85	1001.289
n	12.88	1230.412

Sample A

Peak no.	RT [min]	m/z
k	1.99	771.3761

Joint to matched peak Equation 9

Repeat for all samples

Aligned peak table

Alignment ID	RT Ave	m/z Ave.	Sample A	Sample B	Sample C
1	0.72	434.5878	2500	1800	4000
2	0.88	541.9050	N.D.	1500	2000
3	1.25	842.1220	53000	62000	40000
4	1.81	254.1079	100	50	730
5	1.99	771.3765	N.D.	N.D.	N.D.
6	2.12	332.0049	14500	7800	25000
7	2.13	659.6631	90000	150000	120000
8	2.28	111.0082	8500	N.D.	N.D.
n-2	11.36	1050.775	5000	4500	N.D.
n-1	12.85	1001.282	58000	20000	25000
n	12.87	1230.412	10000	12000	9000

c. Filtering aligned peaks

Condition
1. Peak count filter: 80%
2. QC 'at least' filter: ON

Aligned peak table

Alignment ID	RT Ave.	m/z Ave.	Sample A	Sample B	Sample C	QC 1	QC 2	QC 3	
1	0.72	434.5878	2500	1800	4000	1000	N.D.	2000	← *Excluded (Step 3)*
2	0.88	541.9050	N.D.	1500	2000	1400	2000	1500	
3	1.25	842.1220	53000	62000	40000	45000	30000	35000	
4	1.81	254.1079	100	50	730	100	50	730	
5	1.99	771.3765	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	← *Excluded (Step 1)*
6	2.12	332.0049	14500	7800	25000	14500	7800	25000	
7	2.13	659.6631	90000	150000	120000	75000	70000	72000	
8	2.28	111.0082	8500	N.D.	N.D.	8500	8800	9000	← *Excluded (Step 2)*
n	12.87	1230.412	10000	12000	9000	15000	12000	13000	

d. Interpolating missing values

The maximum of raw data point within the blue range (equation 10) is interpolated.

Alignment ID	RT Ave.	m/z Ave.	Sample A	Sample B	Sample C	QC 1	QC 2	QC 3
1'	0.88	541.9050	N.D.	1500	2000	1400	2000	1500
2'	1.25	842.1220	53000	62000	40000	45000	30000	35000
3'	1.81	254.1079	100	50	730	100	50	730
4'	2.11	332.0049	14500	7800	25000	14500	7800	25000
5'	2.13	659.6631	90000	150000	120000	75000	70000	72000
n'	12.87	1230.412	10000	12000	9000	15000	12000	13000

Sample A raw data
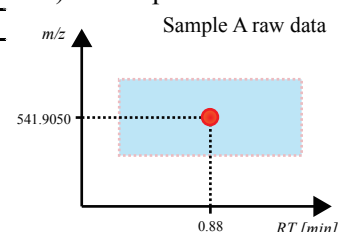
Supplementary Fig. 10. Flow chart and summary of peak alignment. The algorithm consists of four parts: (a) making a reference peak table, (b) fitting each sample peak table to reference peak table, (c) filtering aligned peaks, and (d) interpolating missing values.

Supplementary Table 1. LipidBlast MS/MS information. The focused 13 lipid classes, fatty acid length, precursor ion, and MS/MS spectrum are described. (**a**) LipidBlast MS/MS spectrum in positive ion mode. (**b**) LipidBlast MS/MS spectrum in negative ion mode. The 'Fatty acid' and 'Record' columns show the coverage of chain length and the total lipid number included in the LipidBlast library, respectively. The bracket shows the relative ion abundance standardized to Max 999 which is utilized for dot-product search. The word NL means neutral loss. In this study, the presence of fragment ions, which represent the lipid class or fatty acids, was manually confirmed after the automatic peak identification was finished in MS-DIAL software. The fragment ions described in 'supplemental fragment' column were also utilized to complement the identification result.

(a) LipidBlast MS/MS spectrum in positive ion mode

Lipid class	Short	Fatty acid	Record	Target precursor	Class fragment	Fatty acid fragment	Supplemental fragment
Diacylglyceryltrimethylhomoserines	DGTS	10-26	8280	[M+H]+ (999)	C10H21NO5H (400)	NL of SN1 (300); NL of SN2 (300)	C7H14NO2 (50); NL of SN1+H2O (300); NL of SN1+H2O (300)
Lysodiacylglyceryltrimethylhomoserines	lysoDGTS	10-26	91	[M+H]+ (400)	C10H21NO5H (999)	NL of SN1|SN2+H2O (50)	[M+H-H2O]+ (200); C7H14NO2 (100)
Phosphatidylcholines	PC	10-26	4720	[M+H]+ (400)	C5H15NO4P (999)	NL of SN1 (50); NL of SN2 (50)	NL of SN1+H2O (50); NL of SN1+H2O (50)
Lysophosphatidylcholines	lysoPC	10-26	76	[M+H]+ (400)	C5H15NO4P (999)	NL of SN1|SN2 (50)	[M+H-H2O]+ (150); NL of SN1|SN2+H2O (50)
Phosphatidylethanolamines	PE	10-26	4720	[M+H]+ (50)	NL of C2H8NO4P (999)	SN1-O (50); SN2-O (50)	N/A
Lysophosphatidylethanolamines	lysoPE	10-26	76	[M+H]+ (10)	NL of C2H8NO4P (999)	N/A	N/A
Monoacylglycerols	MAG	10-26	70	[M+NH4]+ (100)	N/A	N/A	[M+H]+ (50); [M+H-H2O]+ (999)
Diacylglycerols	DAG	10-26	1964	[M+NH4]+ (100)	N/A	NL of SN1+NH3 (999); NL of SN2+NH3 (999)	[M+H]+ (50); [M+H-H2O]+ (400)
Triacylglycerols	TAG	10-26	6170	[M+NH4]+ (100)	N/A	NL of SN1+NH3 (999); NL of SN2+NH3 (999); NL of SN3+NH3 (999)	[M+H]+ (50)
Monogalactosyldiacylglycerols	MGDG	10-26	4720	[M+NH4]+ (100)	NL of C6H11O6 (600)	NL of SN1+C6H11O6 (999); NL of SN2+C6H11O6 (999)	NL of C6H11O6+H2O (600)
Digalactosyldiacylglycerols	DGDG	10-26	4720	[M+NH4]+ (100)	NL of C12H21O11 (999)	NL of SN1+C12H21O11 (999); NL of SN2+C12H21O11 (999)	NL of C12H21O11+H2O (999)

(b) LipidBlast MS/MS spectrum in negative ion mode

Lipid class	Short	Fatty acid	Record	Target precursor	Class fragment	Fatty acid fragment	Supplemental fragment
Phosphatidylcholines	PC	10-26	4720	[M+HCOO]- (200)	[M-CH3]- (999)	SN1 (600); SN2 (600)	NL of SN1+CH3 (50); NL of SN2+CH3 (50); NL of SN1+CH3+H2O (50); NL of SN2+CH3+H2O (50); C7H15NO5P (100)
Lysophosphatidylcholines	lysoPC	10-26	76	[M+HCOO]- (10)	[M-CH3]- (400)	SN1|SN2 (999)	C7H15NO5P (100)
Phosphatidylethanolamines	PE	10-26	4720	[M-H]- (700)	C5H11NO5P (100)	SN1 (999); SN2 (999)	NL of SN1 (50); NL of SN2 (50); NL of SN1+H2O (50); NL of SN2+H2O (50)
Lysophosphatidylethanolamines	lysoPE	10-26	76	[M-H]- (200)	C5H11NO5P (100)	SN1 |SN2 (999)	NL of SN1|SN2 (100); NL of SN1|SN2+H2O (100)
Phosphatidylserines	PS	10-26	4720	[M-H]- (500)	NL of C3H5NO2 (600)	SN1 (999); SN2 (999)	NL of C3H5NO2+SN1 (200); NL of C3H5NO2+SN2 (200); NL of C3H5NO2+SN1+H2O (200); NL of C3H5NO2+SN2+H2O (200)
Phosphatidic acids	PA	10-26	4720	[M-H]- (400)	C3H6O5P (300)	SN1 (999); SN2 (999)	NL of SN1 (100); NL of SN2 (100); NL of SN1+H2O (100); NL of SN2+H2O (100)
Phosphatidylinositols	PI	10-26	4720	[M-H]- (999)	C9H14O9P (200)	SN1 (200); SN2 (200)	NL of SN1 (100); NL of SN2 (100); NL of SN1+H2O (100); NL of SN2+H2O (100); C6H10O8P (200)
Phosphatidylglycerols	PG	10-26	4720	[M-H]- (500)	N/A	SN1 (999); SN2 (999)	NL of SN1 (100); NL of SN2 (100); NL of SN1+H2O (100); NL of SN2+H2O (100)
Free fatty acid	FA	10-26	43	[M-H]- (999)	N/A	N/A	N/A
Monogalactosyldiacylglycerols	MGDG	10-26	4720	[M+HCOO]- (300)	C9H17O8 (50)	SN1 (999); SN2 (999)	[M-H]- (100); NL of SN1+HCOOH (50); NL of SN2+HCOOH (50)
Digalactosyldiacylglycerols	DGDG	10-26	4720	[M+HCOO]- (100)	C15H25O12 (999)	SN1 (999); SN2 (999)	[M-H]- (500); NL of SN1+HCOOH (300); NL of SN2+HCOOH (300); NL of SN1+HCOOH+H2O (300); NL of SN2+HCOOH+H2O (300); C15H27O13 (200); C15H23O11 (500)
Sulfoquinovosyldiacylglycerols	SQDG	10-26	4720	[M-H]- (999)	C6H9O7S (200)	SN1 (100); SN2 (100)	NL of SN1 (100); NL of SN2 (100)

Supplementary Table 2. Identification result of major lipid classes of 9 algal species.

	Chlamydomonas reinhardtii	UTEX2341	Chlorella sorokiniana	Chlorella variabilis	Euglena gracilis	Nannochloropsis oculata	Dunaliella salina	Pleurochrysis carterae	Pavlova lutheri	Total
Positive										
DAG	10 / 10	6 / 6	8 / 12	12 / 13	31 / 28	17 / 14	1 / 1	7 / 3	1 / 0	93 / 87
TAG	11 / 9	15 / 10	4 / 4	31 / 26	141 / 112	53 / 42	26 / 20	23 / 16	16 / 13	320 / 252
MGDG	13 / 13	9 / 9	15 / 12	15 / 14	13 / 9	11 / 6	7 / 5	13 / 8	12 / 10	108 / 86
DGDG	11 / 10	12 / 12	10 / 7	14 / 14	11 / 9	21 / 15	4 / 3	11 / 6	8 / 7	102 / 83
lysoDGTS	14 / 11	0 / 0	0 / 0	0 / 0	5 / 6	17 / 16	0 / 0	2 / 1	1 / 0	39 / 34
DGTS	64 / 49	0 / 0	0 / 0	0 / 0	8 / 5	78 / 57	6 / 4	3 / 2	5 / 10	164 / 127
lysoPC	0 / 0	3 / 4	9 / 8	0 / 0	4 / 1	5 / 6	0 / 1	0 / 1	1 / 1	22 / 22
PC	0 / 0	14 / 13	25 / 18	29 / 16	23 / 28	49 / 33	0 / 2	0 / 0	4 / 2	144 / 112
lysoPE	0 / 0	0 / 0	3 / 3	0 / 0	0 / 0	2 / 0	0 / 0	0 / 0	0 / 0	5 / 3
PE	3 / 1	7 / 6	16 / 12	17 / 13	8 / 2	14 / 5	0 / 0	1 / 0	3 / 0	69 / 39
Total	126 / 103	66 / 60	90 / 76	118 / 96	244 / 200	267 / 194	44 / 36	60 / 37	51 / 43	1066 / 845
Negative										
MGDG	16 / 12	14 / 14	25 / 19	24 / 21	23 / 18	13 / 14	7 / 6	20 / 18	15 / 12	157 / 134
DGDG	16 / 13	12 / 12	15 / 10	24 / 18	29 / 17	27 / 22	8 / 6	19 / 15	12 / 10	162 / 123
SQDG	7 / 12	6 / 12	10 / 6	4 / 8	8 / 22	12 / 20	2 / 6	5 / 15	4 / 11	58 / 112
lysoPC	0 / 0	3 / 3	8 / 6	0 / 0	4 / 0	8 / 5	1 / 0	0 / 0	1 / 1	25 / 15
PC	0 / 0	14 / 13	19 / 16	18 / 15	23 / 17	26 / 27	0 / 0	0 / 0	2 / 1	102 / 89
lysoPE	0 / 0	0 / 0	6 / 4	2 / 0	1 / 0	3 / 3	0 / 0	0 / 0	0 / 0	12 / 7
PE	6 / 4	11 / 8	19 / 13	17 / 15	16 / 11	10 / 9	3 / 1	0 / 0	2 / 1	84 / 62
PG	8 / 8	11 / 9	18 / 16	11 / 10	6 / 5	12 / 10	7 / 7	1 / 1	5 / 3	79 / 69
PI	3 / 2	3 / 3	9 / 5	4 / 7	3 / 4	6 / 9	2 / 1	0 / 0	0 / 1	30 / 32
PS	0 / 0	3 / 3	1 / 1	3 / 2	1 / 1	1 / 0	0 / 0	0 / 0	0 / 0	9 / 7
PA	0 / 0	0 / 0	0 / 0	0 / 0	4 / 5	0 / 0	0 / 0	0 / 0	0 / 0	4 / 5
FA	2 / 2	1 / 1	1 / 3	1 / 3	2 / 2	3 / 2	3 / 1	5 / 6	2 / 1	20 / 21
Total	58 / 53	78 / 78	131 / 99	108 / 99	120 / 102	121 / 121	33 / 28	50 / 55	43 / 41	742 / 676

The numbers to the left and right of the slash indicate the number of identified lipids in SWATH and DDA methods, respectively. The row and column include lipid class names and algal names targeted in this study.

Supplementary Table 3. Identification result of Chlamydomonas reinhardtii under data-dependent acquisition (DDA accumulation time 50 ms) and SWATH (isolation window, 65 Da; accumulation time, 30 ms).

	SWATH	DDA
MGDG	17	12
DGDG	23	13
SQDG	16	12
lysoPC	0	0
PC	0	0
lysoPE	0	0
PE	7	4
PG	15	8
PI	4	2
PS	0	0
PA	0	0
FA	3	2
Total	86	53

Supplementary Table 4. Data processing parameters of MS-DIAL software used in this study. (**a**) SWATH positive ion mode in the hydrophilic metabolite analysis, (**b**) SWATH/DDA positive ion mode in the lipid analysis, and (**c**) SWATH/DDA negative ion mode in the lipid analysis.

(a) SWATH positive ion mode in the hydrophilic metabolite analysis

Retention time begin	0 min
Retention time end	14 min
Mass range begin	50 Da
Mass range end	500 Da
MS^1 tolerance for centroid	0.01 Da
MS^2 tolerance for centroid	0.1 Da
Peak detection-based	Checked
Smoothing method	Linear weighted moving average
Smoothing level	2 scan
Minimum peak width	5 scan
Minimum peak height	1000 amplitude
Mass slice width	0.1 Da
Band width	5 scan
Segment number	1
Peak consideration	Both
Sigma window value	0.001 min
Exclude after precursor	Checked
Retention time tolerance for peak identification	0.5 min
Accurate mass tolerance (MS^1) for peak identification	0.025
Accurate mass tolerance (MS^2) for peak identification	0.25
Identification score cut off	80%
Exclusion mass list (tolerance: 0.005 Da)	59.061, 77.0379, 100.0731, 118.0863, 122.0966, 124.0398, 124.0874, 140.999, 141.9576, 158.0261, 159.0302, 160.0226, 213.4248, 213.4351, 214.089, 214.105, 215.0933, 216.086, 216.1044, 279.0919, 354.286, and 371.3115

(b) SWATH/DDA positive ion mode in the lipid analysis

Retention time begin	0.5 min
Retention time end	11.5 min
Mass range begin	100 Da
Mass range end	1250 Da
MS^1 tolerance for centroid	0.01 Da
MS^2 tolerance for centroid	0.1 Da
Peak detection-based	Unchecked
Smoothing method	Linear weighted moving average
Smoothing level	2 scan
Minimum peak width	5 scan
Minimum peak height	2000 amplitude
Mass slice width	0.1 Da
Band width	5 scan
Segment number	1
Peak consideration	Both
Sigma window value	0.001 min
Exclude after precursor	Checked
Retention time tolerance for peak identification	0.5 min
Accurate mass tolerance (MS^1) for peak identification	0.025
Accurate mass tolerance (MS^2) for peak identification	0.25
Identification score cut off	70%
Retention time tolerance for peak alignment	0.1 min
MS^1 tolerance for peak alignment	0.025 Da
Retention time factor	0.5
MS^1 factor	0.5
Peak count filter	0%
QC at least filter	Checked
Gap filling option	Checked
Exclusion mass list (tolerance: 0.005 Da)	100.9993, 101.1073, 102.0901, 118.0854, 140.9984, 158.0264, 184.1788, 186.0576, 214.0881, 215.0947, 216.0849, and 279.0926

(c) SWATH/DDA negative ion mode in the lipid analysis

Retention time begin	0.5 min
Retention time end	10 min
Mass range begin	100 Da
Mass range end	1250 Da
MS^1 tolerance for centroid	0.01 Da
MS^2 tolerance for centroid	0.1 Da
Peak detection-based	Unchecked
Smoothing method	Linear weighted moving average
Smoothing level	2 scan
Minimum peak width	4 scan
Minimum peak height	500 amplitude
Mass slice width	0.1 Da
Band width	5 scan
Segment number	1
Peak consideration	Both
Sigma window value	0.001 min
Exclude after precursor	Checked
Retention time tolerance for peak identification	0.5 min
Accurate mass tolerance (MS^1) for peak identification	0.025
Accurate mass tolerance (MS^2) for peak identification	0.25
Identification score cut off	70%
Retention time tolerance for peak alignment	0.1 min
MS^1 tolerance for peak alignment	0.025 Da
Retention time factor	0.5
MS^1 factor	0.5
Peak count filter	0%
QC at least filter	Checked
Gap filling option	Checked
Exclusion mass list (tolerance: 0.005 Da)	100.9342, 112.9862, 115.9207, 130.9668, 132.9243, 139.0088, 158.9769, 182.9895, 186.9327, 212.0751, 213.0784, 248.9672, 316.9489, 384.9353, 452.9176, 520.9081, 588.8986, 656.8814, and 724.8645

Supplementary Note: This not describes the mathematical equations implemented in the MS-DIAL

program, cited in the **Online Methods**.

Equation 1 is the function of linearly weighted smoothing average.

$$\text{Equation 1: } f(x)_{new} = \frac{\sum_{i=-n}^{n}(n-i+1) \times f(x+i)}{n^2}$$

n is the number of data points integrated by the smoother.

Equation 2 and **Equation 3** are the mathematical functions to calculate first derivative and second

derivative values, respectively.

$$\text{Equation 2: First derivative: } f^{'}(x) = \frac{-2x_{-2} - x_{-1} + x_{+1} + 2x_{+2}}{10}$$

$$\text{Equation 3: Second derivative: } f^{''}(x) = \frac{2x_{-2} - x_{-1} - 2x_0 - x_{+1} + 2x_{+2}}{7}$$

x_0 is the focused data point to calculate its derivatives that are calculated by five-point approximations.

Equation 4 is the function of spectral centroiding.

$$\text{Equation 4: Centroid} = \frac{\sum_n \text{ion counts}(n) \times m/z(n)}{\sum_n \text{ion counts}(n)} \; (n \in centroid\ tolerance)$$

n is the spans the user-defined region between the peak's left and right edges for each detected peak in

the mass chromatogram.

Equation 5, **6**, and **7** are the functions to calculate the ideal slope value of detected peaks.

$$\text{Equation 5: } ideal\ slope_{left} = \sum_{k=left\ edge}^{peak\ top} f^{'}(k) \quad with\ f^{'}(k) \geq 0$$

$$\text{Equation 6: } ideal\ slope_{right} = \sum_{k=right\ edge}^{peak\ top} \left| f'(k) \right| \quad with\ f'(k) \leq 0$$

$$\text{Equation 7: } ideal\ slope = \frac{ideal\ slope_{left} + ideal\ slope_{right}}{\sum_{k=left\ edge}^{right\ edge} \left| f'(k) \right|}$$

$f'(k)$ is calculated by **Equation 2** (**Online Methods**) and the value evaluates the smoothness, i.e., whether the peak contains any spike noise within its left- and right edges.

Equation 8, **9**, and **10** are the functions to calculate the sharpness value of detected peaks.

$$\text{Equation 8: } sharpness_{left} = \max_{n} \frac{A_{max} - A(n)}{n\sqrt{A_{max}}}$$

$$\text{Equation 9: } sharpness_{right} = \max_{n} \frac{A_{max} - A(n)}{n\sqrt{A_{max}}}$$

$$\text{Equation 10: } sharpness = \frac{sharpness_{left} + sharpness_{right}}{2}$$

Here, A_{max} and $A(n)$ denote the height of the peak top, and the height of data point n-step away from the peak top, respectively. The sharpness value evaluates peak symmetry in combination with the absolute intensity.

Equation 11 is the second Gaussian derivative filter fitted to the array of sharpness values (default delta = 0.001 min).

$$\text{Equation 11: Matched filter: } \left\{ 1 - \left(\frac{x}{\delta} \right)^2 \right\} \times \exp\left\{ -\frac{1}{2} \left(\frac{x}{\delta} \right)^2 \right\} \text{ (eq. 11)}$$

Equation 12 is the function of reverse dot product (also see **Equation 6** of **Online Methods**).

$$\text{Equation 12: } dot\ product_{reverse} = \frac{\left(\sum wA_{act.} wA_{lib.} \right)^2}{\sum wA_{act.}^2 \sum wA_{lib.}^2} \quad (against\ spectrum\ included\ in\ library)$$

Equation 13, **14**, and **15** are the functions to calculate total scores. If retention time information is not available for compound identification, the total score is calculated as **Equation 13**. If the formula information is not available, the total score is calculated as **Equation 14**. If both retention time and formula information are not available, the total score is calculated as **Equation 15**.

$$\text{Equation 13:} \; Total \; score \; = \; \frac{\text{MS/MS similarity} \; + \; \text{MS1 similarity} \; + \; 0.5 \; \times \text{isotope ratio similarity}}{2.5} \times 100$$

$$\text{Equation 14:} \; Total \; score \; = \; \frac{\text{MS/MS similarity} \; + \; \text{MS1 similarity} \; + \; \text{RT similarity}}{3} \times 100$$

$$\text{Equation 15:} \; Total \; score \; = \; \frac{\text{MS/MS similarity} \; + \; \text{MS1 similarity}}{2} \times 100$$