Supplementary Information for

Detecting lysosomal storage disorders by glycomic profiling using liquid chromatography mass spectrometry

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Figure S1. Resolution of glucosylsphingosine from galactosylsphingosine.

ERNDIM Special Assays in Serum (SAS) samples were acquired using the targeted LC-MS/MS method. Representative extracted ion chromatograms show baseline resolution of glucosylsphingosine (1.88 min) from galactosylsphingosine-d5 (2.01 min). ERNDIM samples do not contain galactosylsphingosine. Resolution of unlabeled glucosylsphingosine and galactosylsphingosine is shown in Figure 1a.

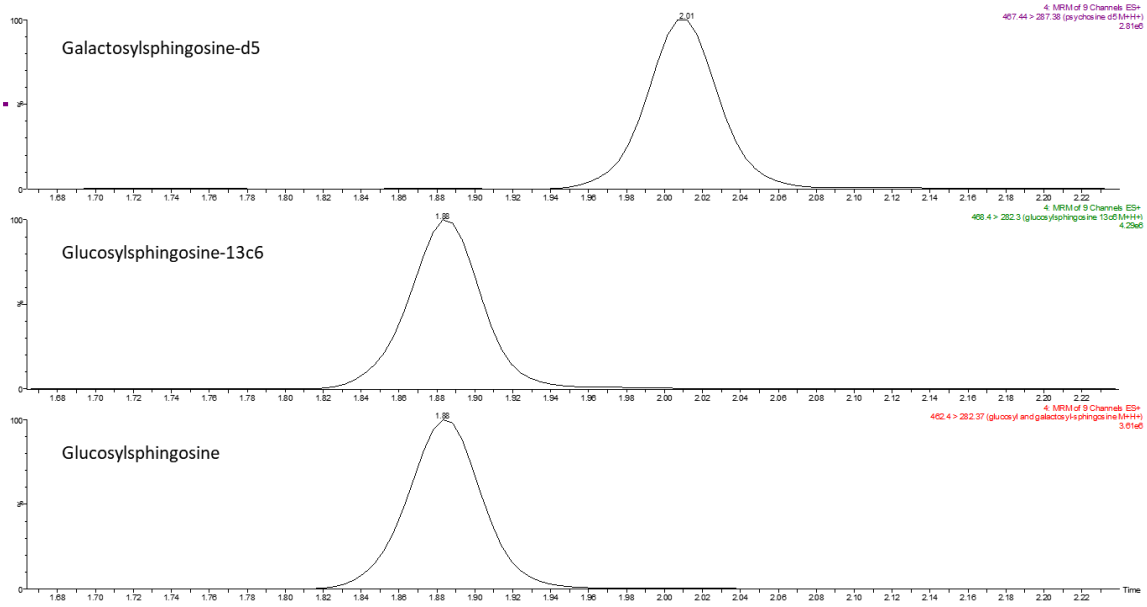


Figure S2. Chromatographic resolution of hex4 isomers that differentiate MOGS from Pompe disease.

Urines from patients with Mannosyl-oligosaccharide Glucosidase (MOGS) 1 deficiency and Pompe disease were acquired using the targeted LC-MS/MS method. Representative extracted ion chromatograms show baseline resolution of a compound annotated as glc3-man in MOGS (4.35 min) from glc4 in Pompe disease (4.6 min). Maltotetraose (authentic standard; 4.5 min) is also included to visualize chromatographic resolution.

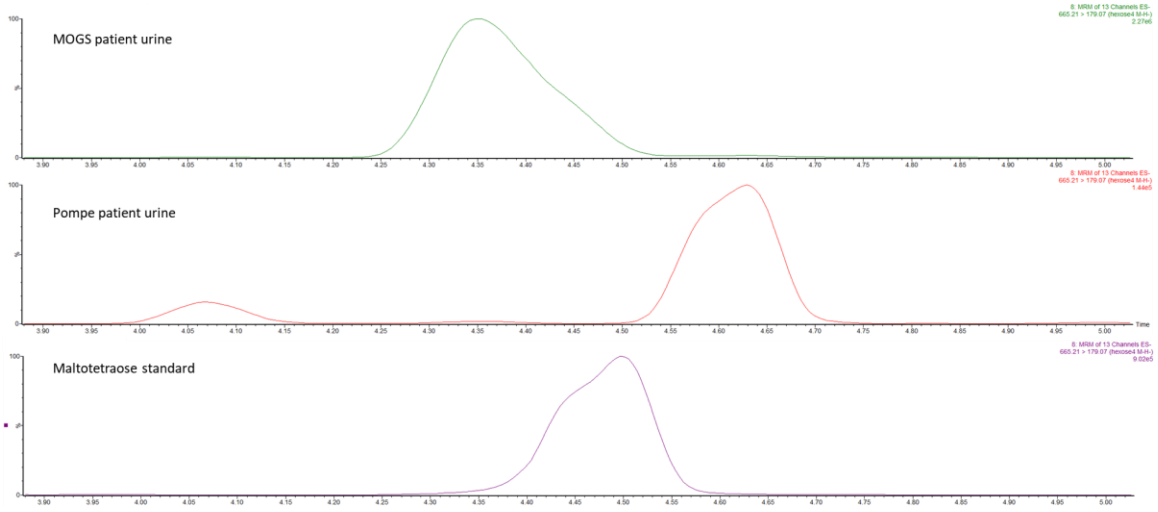
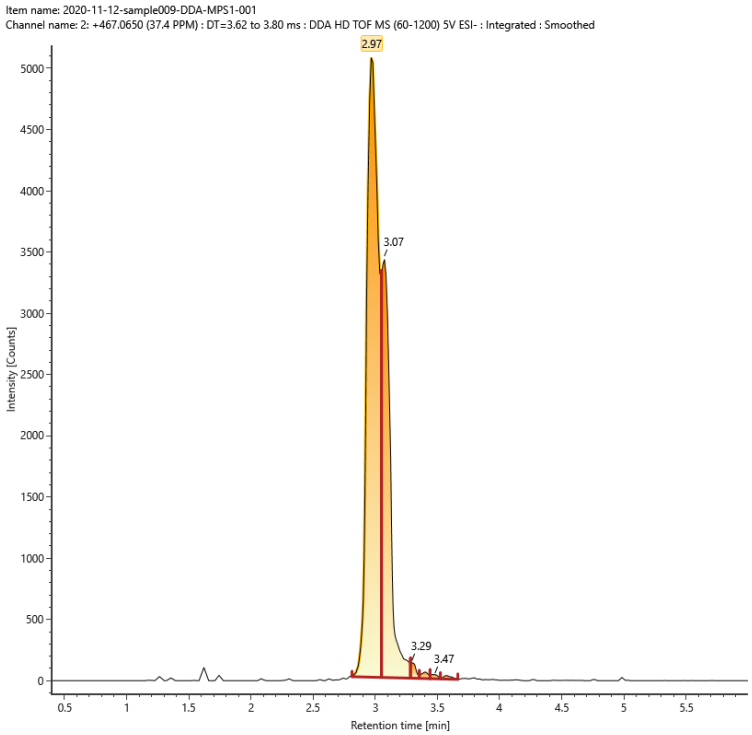


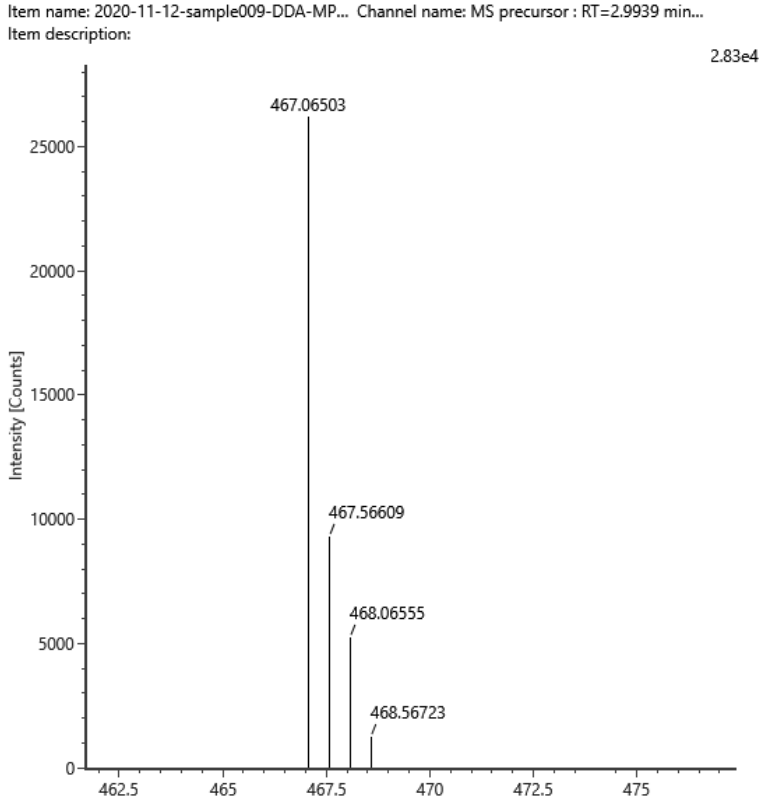
Figure S3. MPS biomarker spectra.

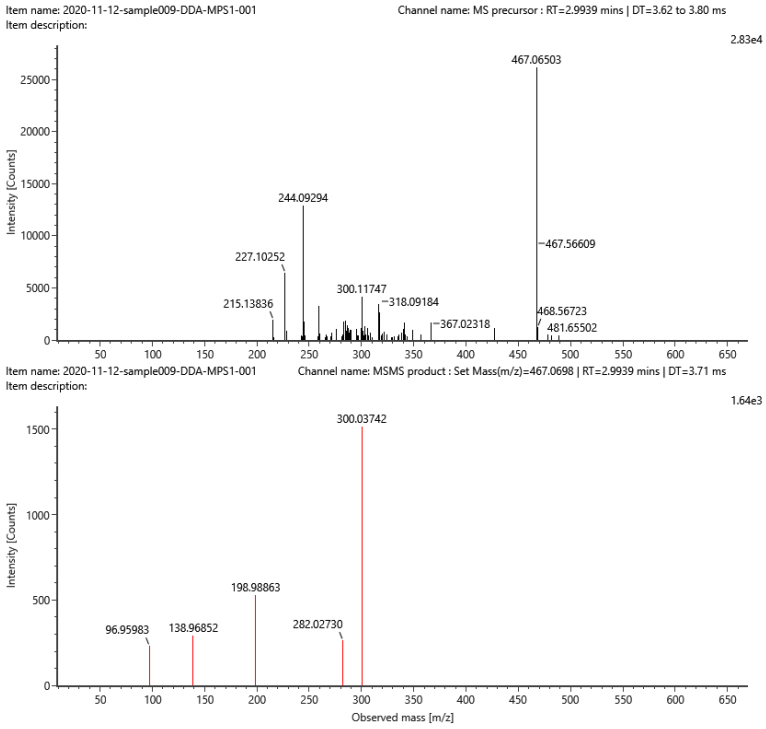
To validate spectra generated by Data Independent Acquisition (DIA) for each of the twenty MPS biomarkers, select urine samples were re-acquired on the Waters Vion using Data Dependent Acquisition (DDA) with ion-mobility enabled. For DDA, total MS cycle times ranged from 0.5-1 second, with each DDA scan set to 0.1-0.2 seconds. An MS/MS inclusion list containing each MPS biomarker's m/z and retention time was created and set to preferential or dedicated MS/MS acquisition. Various capillary voltages, cone voltages, and collision energy ramps were used to generate ideal DDA data. Representative DDA data is shown. DIA scans are included for comparison. Except where indicated, negative ESI was used. The Waters Unifi *in-silico* fragmentation algorithm was used to make structural assignments (indicated by blue dots or chemical structures above ions).

Biomarker number: 1

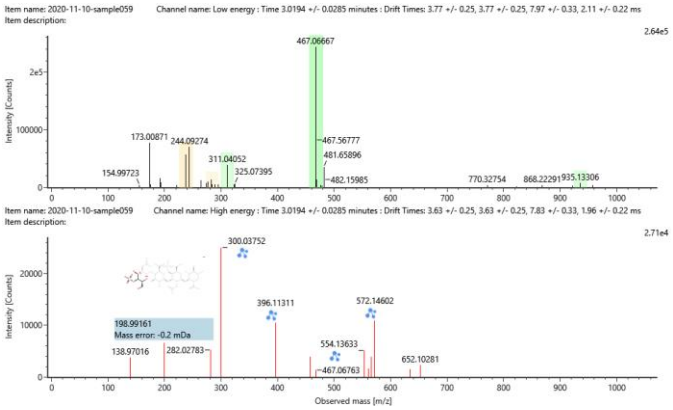
Annotation: hexNAc-s-UA-hexNAc-s-UA; Formula: C28H44N2O29S2







Left Shoulder-DIA



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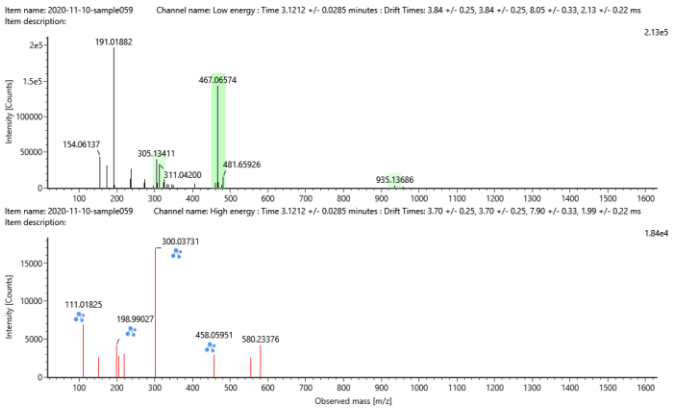
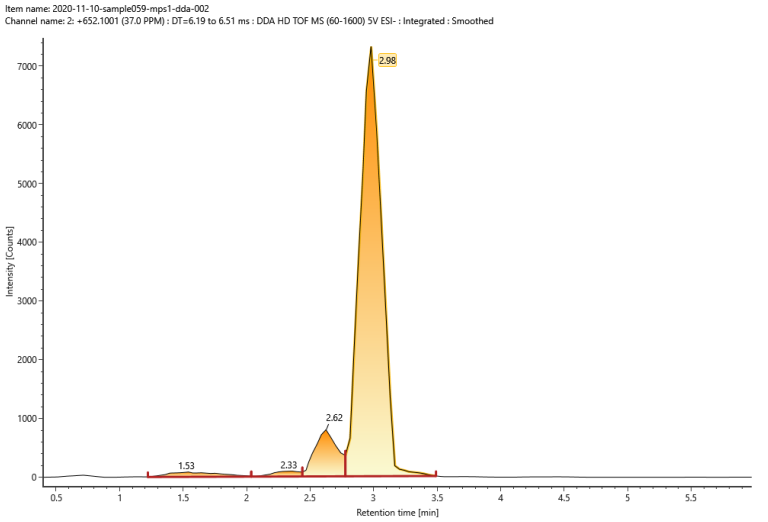
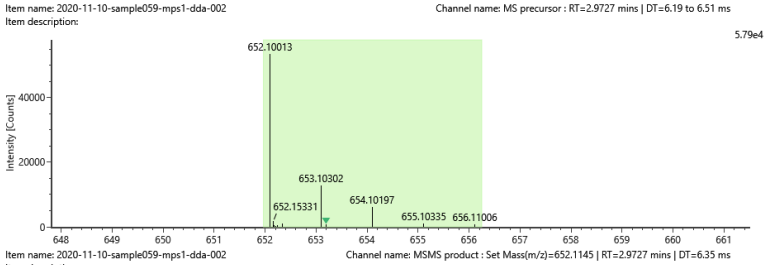
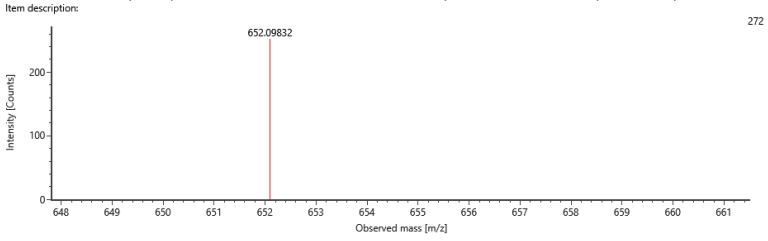


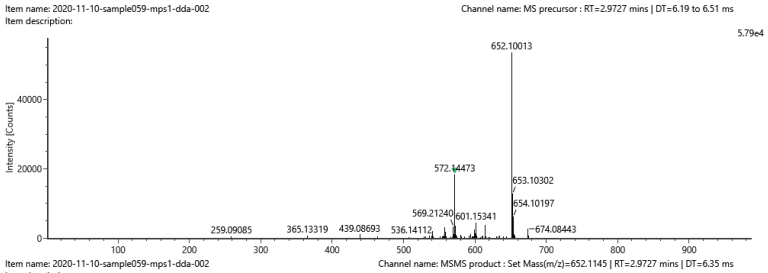
Biomarker number: 2

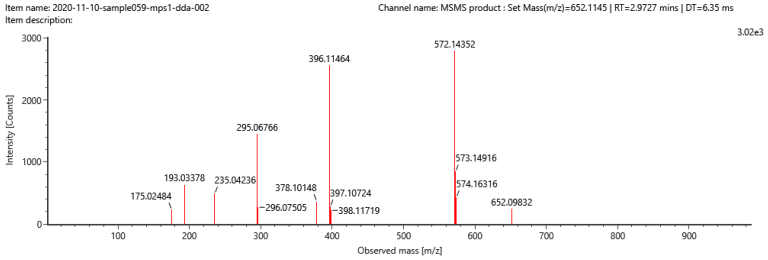
Annotation: UA-s-hexNAc-UA; Formula: C20H31NO21S



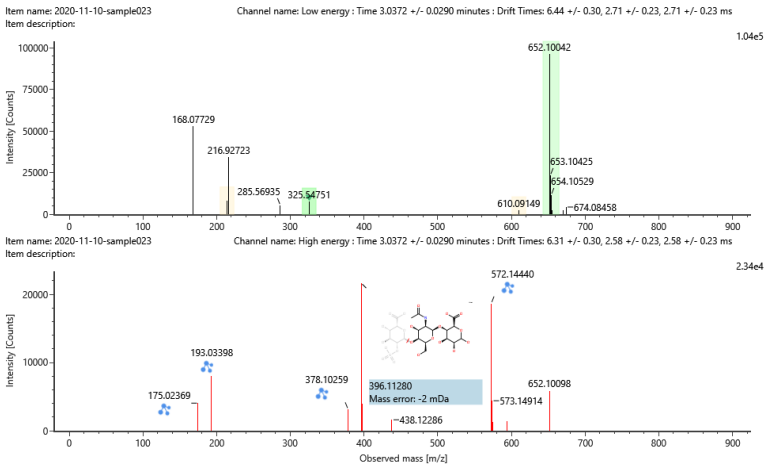








DIA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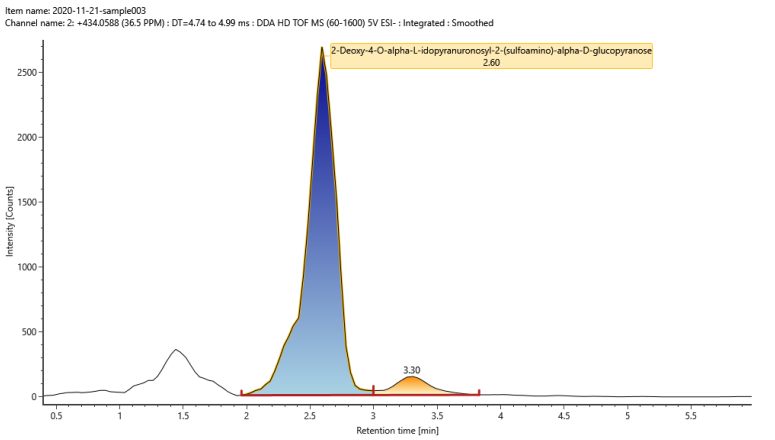
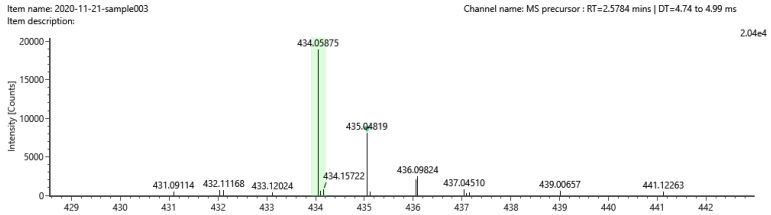
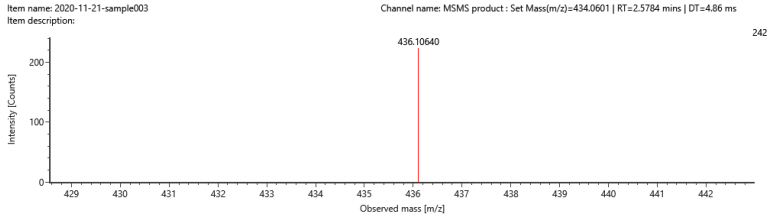
1.04e5
Intensity [Counts]
652.10042
168.07729
216.92723
653.10425
654.10529
285.56935
325.54751
610.09149
674.08458
Item name: 2020-11-10-sample023
Item description:
Channel name: High energy : Time 3.0372 +/- 0.0290 minutes : Drift Times: 6.31 +/- 0.30, 2.58 +/- 0.23, 2.58 +/- 0.23 ms
2.34e4
Intensity [Counts]
572.14440
193.03398
378.10259
396.11280
Mass error: -2 mDa
652.10098
175.02369
573.14914
438.12286
Observed mass [m/z]

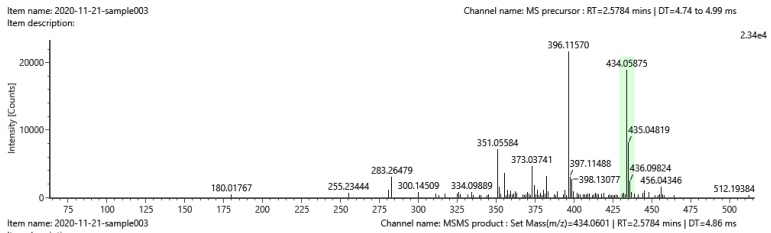
Biomarker number: 3

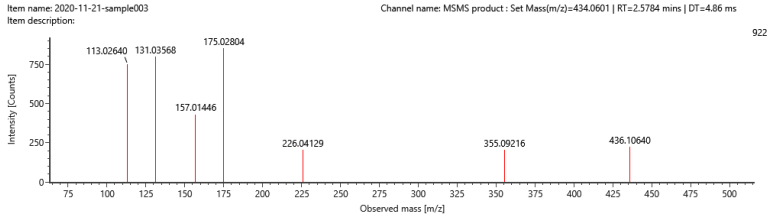
Annotation: hexN-s-UA; Formula: C12H21NO14S



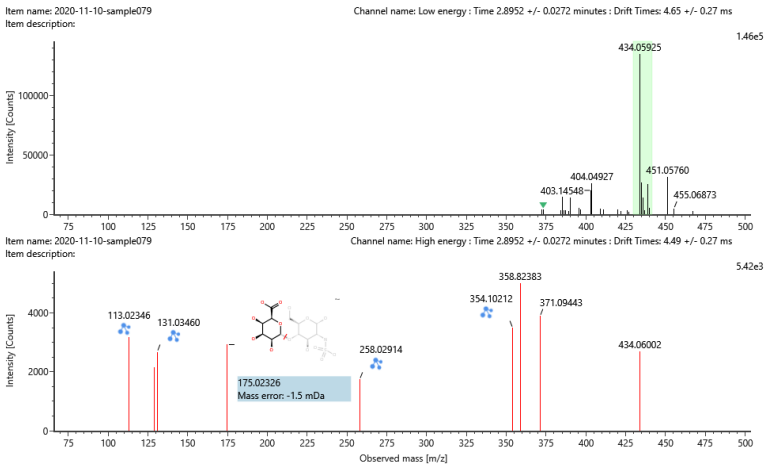






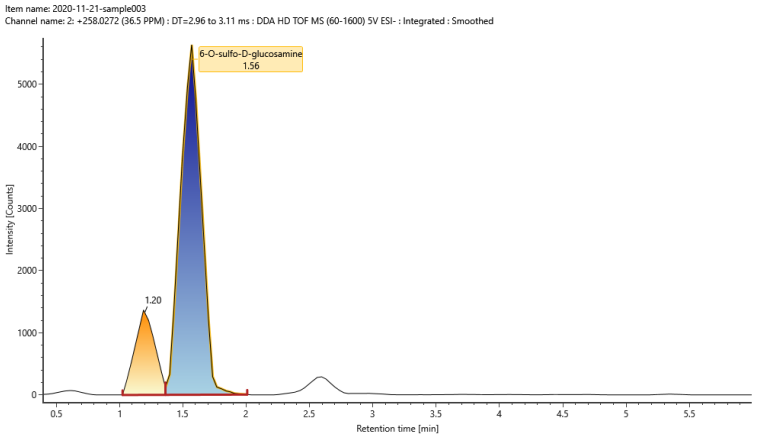


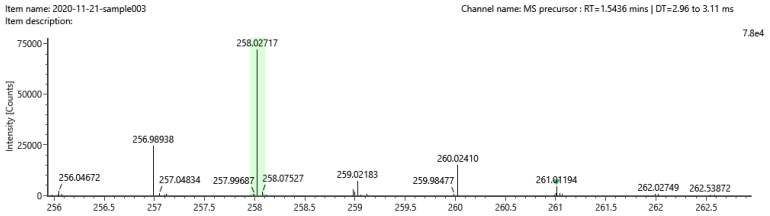
DIA

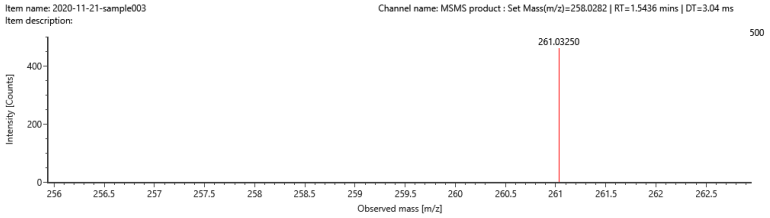

Item name: 2020-11-10-sample079
Item description:
Channel name: Low energy : Time 2.8952 +/- 0.0272 minutes : Drift Times: 4.65 +/- 0.27 ms
1.46e5
434.05925
404.04927
403.14548
451.05760
455.06873
Intensity [Counts]
Item name: 2020-11-10-sample079
Item description:
Channel name: High energy : Time 2.8952 +/- 0.0272 minutes : Drift Times: 4.49 +/- 0.27 ms
5.42e3
358.82383
354.10212
371.09443
113.02346
131.03460
258.02914
434.06002
175.02326
Mass error: -1.5 mDa
Intensity [Counts]
Observed mass [m/z]

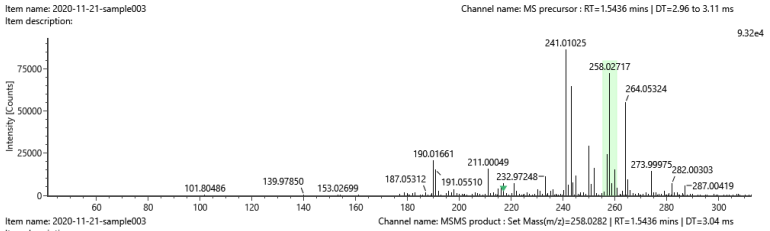
Biomarker number: 4

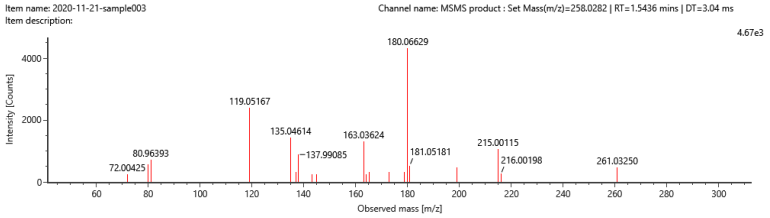
Annotation: hexN-s; Formula: C6H13NO8S







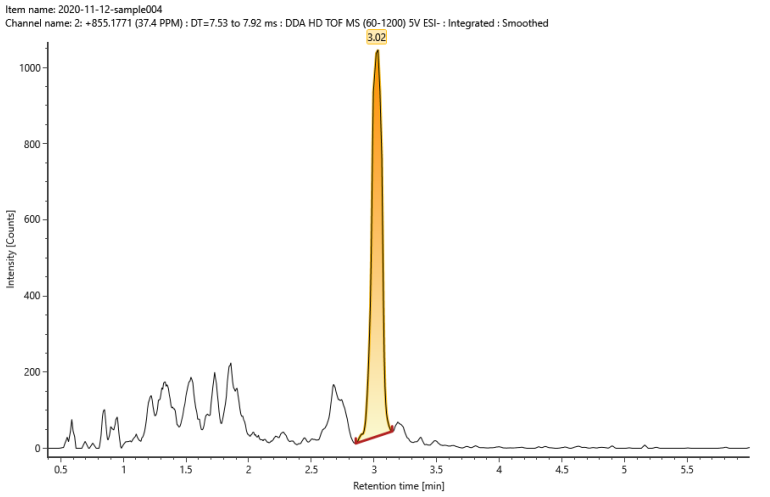


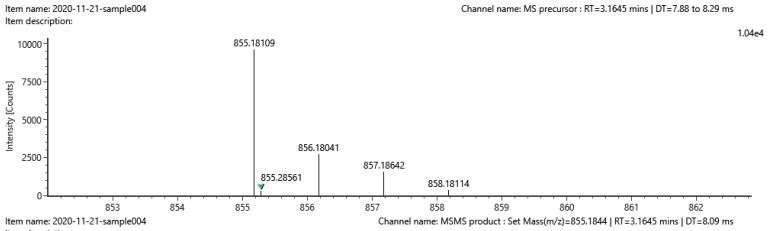


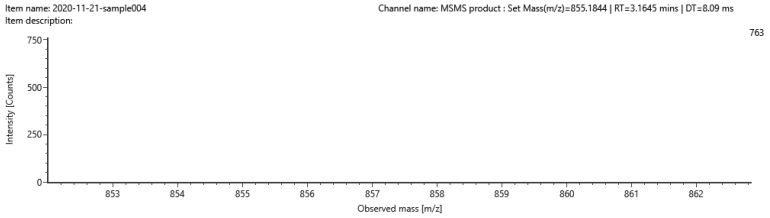
Clean DIA fragmentation spectra not available due to multiple co-eluting compounds with similar drift times.

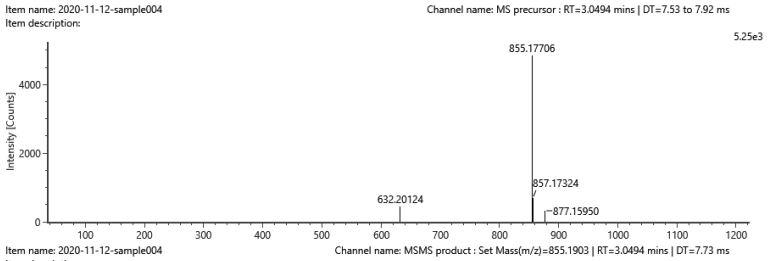
Biomarker number: 5

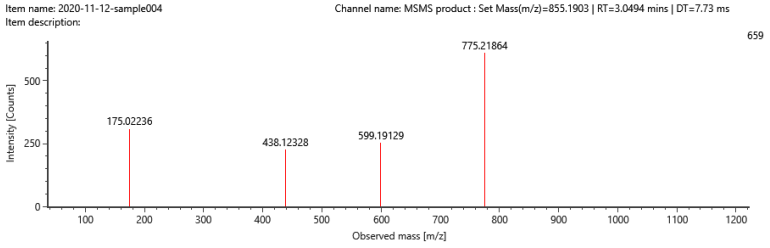
Annotation: hexNAc-s-UA-hexNAc-UA; Formula: C28H44O26N2S



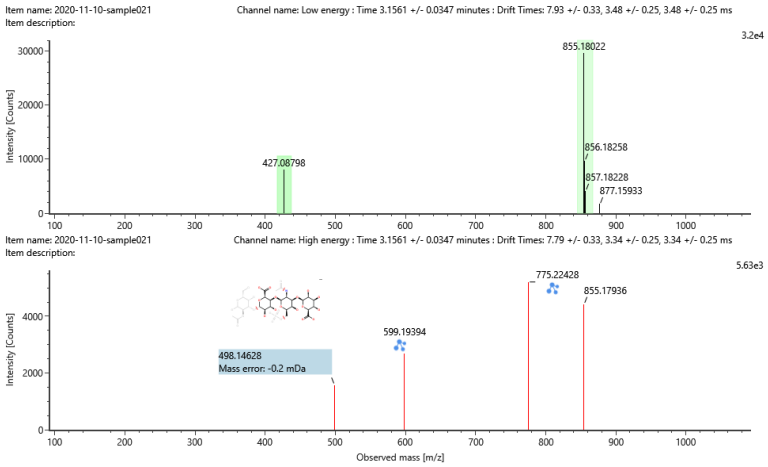








DIA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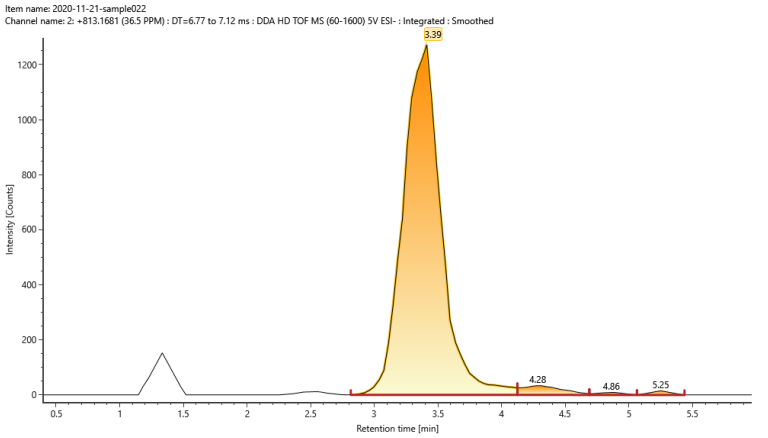
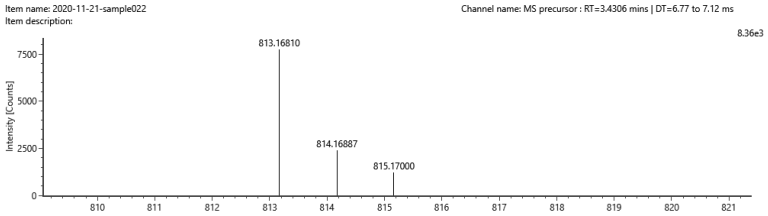
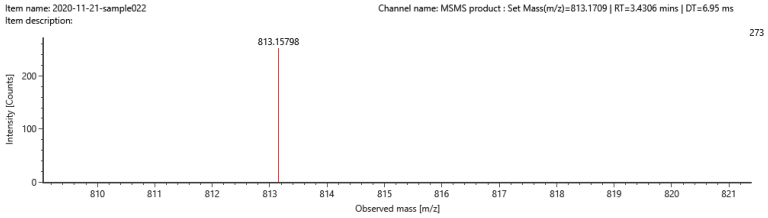
Item description:
Channel name: Low energy : Time 3.1561 +/- 0.0347 minutes : Drift Times: 7.93 +/- 0.33, 3.48 +/- 0.25, 3.48 +/- 0.25 ms
3.2e4
855.18022
856.18258
857.18228
877.15933
427.08798
Intensity [Counts]
Item name: 2020-11-10-sample021
Item description:
Channel name: High energy : Time 3.1561 +/- 0.0347 minutes : Drift Times: 7.79 +/- 0.33, 3.34 +/- 0.25, 3.34 +/- 0.25 ms
5.63e3
775.22428
855.17936
599.19394
498.14628
Mass error: -0.2 mDa
Intensity [Counts]
Observed mass [m/z]

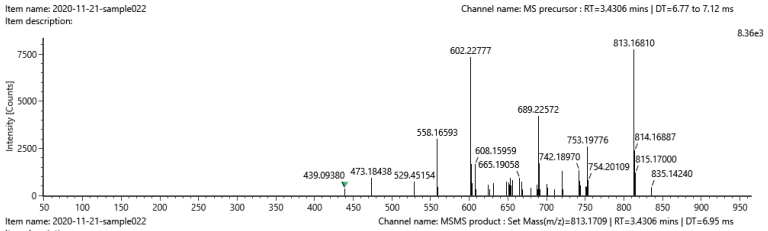
Biomarker number: 6

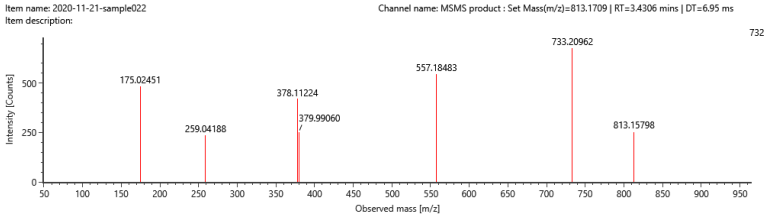
Annotation: no annotation



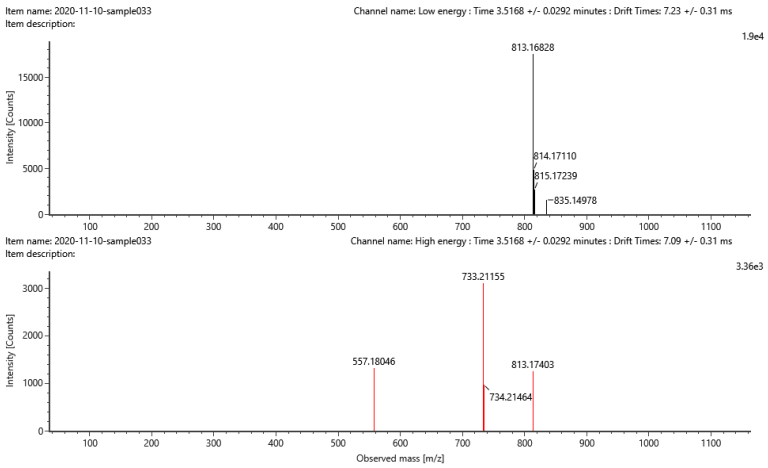






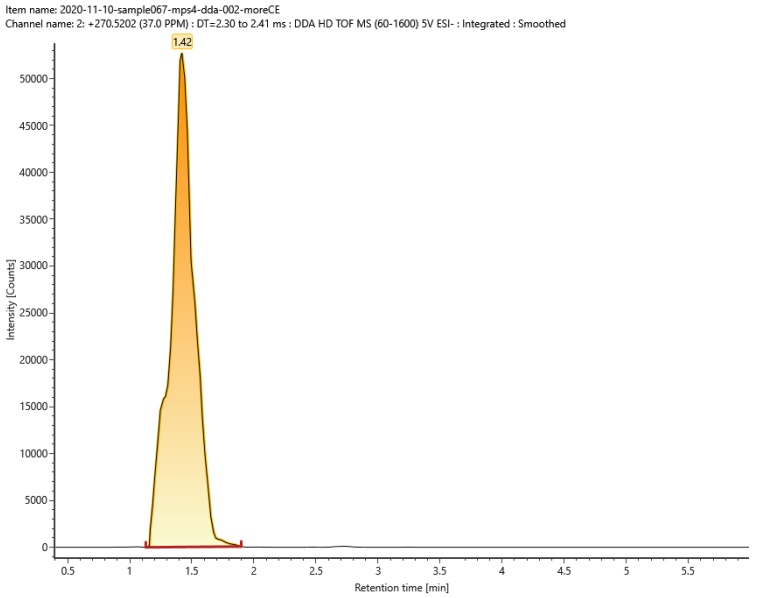


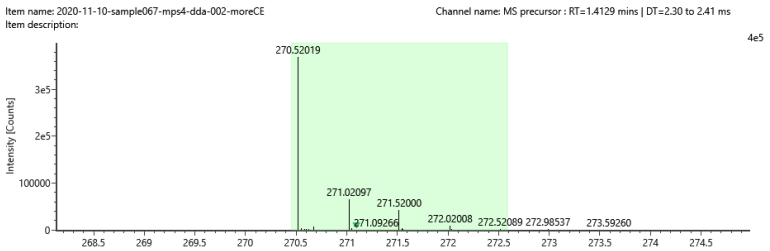
DIA

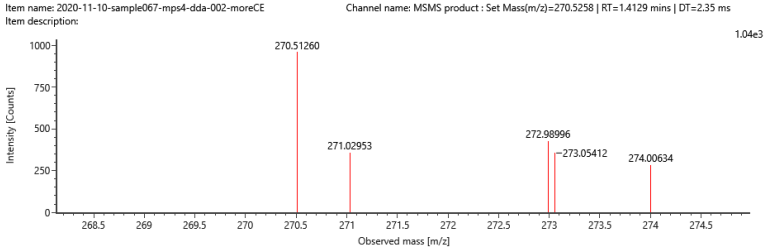

Item name: 2020-11-10-sample033
Item description:
Channel name: Low energy : Time 3.5168 +/- 0.0292 minutes : Drift Times: 7.23 +/- 0.31 ms
1.9e4
813.16828
814.17110
815.17239
835.14978
Intensity [Counts]
Item name: 2020-11-10-sample033
Item description:
Channel name: High energy : Time 3.5168 +/- 0.0292 minutes : Drift Times: 7.09 +/- 0.31 ms
3.36e3
733.21155
557.18046
813.17403
734.21464
Intensity [Counts]
Observed mass [m/z]

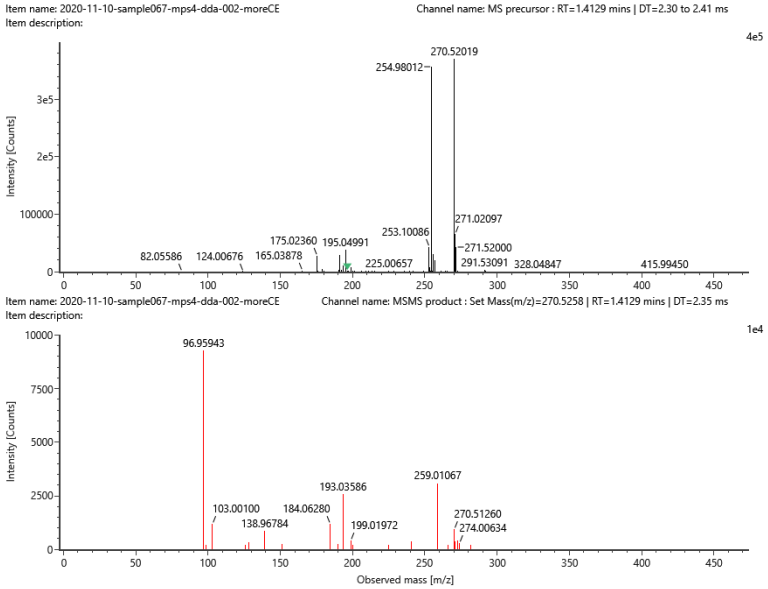
Biomarker number: 7

Annotation: hex-s-hexNAc-s; Formula: C14H25NO17S2

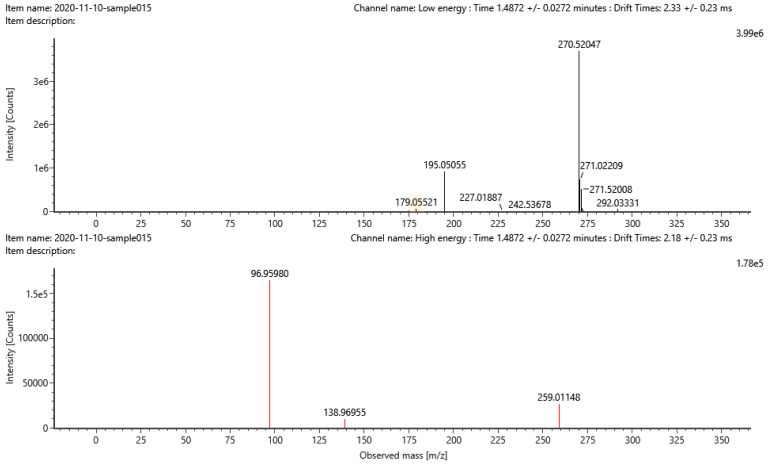






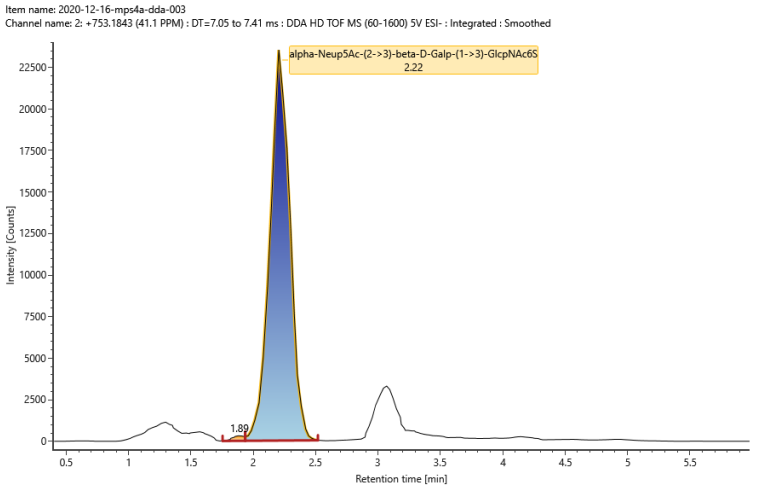

Item name: 2020-11-10-sample067-mps4-dda-002-moreCE
Item description:
Channel name: MS precursor : RT=1.4129 mins | DT=2.30 to 2.41 ms
4e5
Intensity [Counts]
270.52019
254.98012
271.02097
253.10086
271.52000
175.02360
195.04991
165.03878
82.05586
124.00676
225.00657
291.53091
328.04847
415.99450
Item name: 2020-11-10-sample067-mps4-dda-002-moreCE
Item description:
Channel name: MSMS product : Set Mass(m/z)=270.5258 | RT=1.4129 mins | DT=2.35 ms
1e4
96.95943
259.01067
193.03586
103.00100
184.06280
138.96784
199.01972
270.51260
274.00634
Observed mass [m/z]

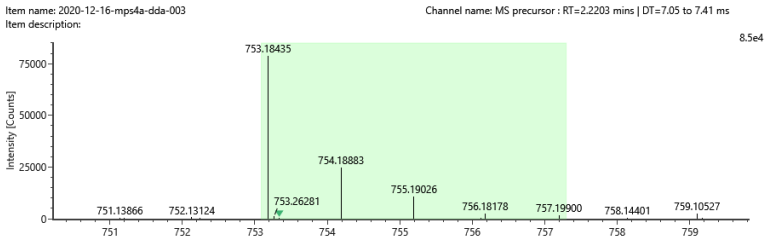
DIA

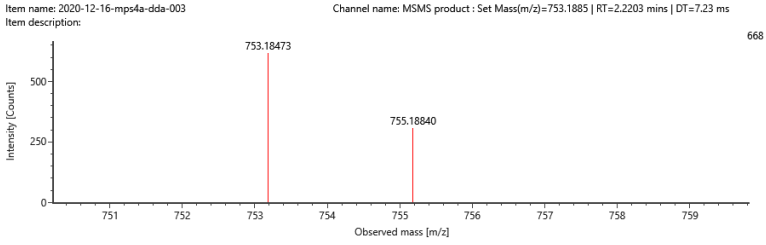

Item name: 2020-11-10-sample015
Item description:
Channel name: Low energy : Time 1.4872 +/- 0.0272 minutes : Drift Times: 2.33 +/- 0.23 ms
3.99e6
Intensity [Counts]
270.52047
195.05055
271.02209
271.52008
179.05521
227.01887
242.53678
292.03331
Item name: 2020-11-10-sample015
Item description:
Channel name: High energy : Time 1.4872 +/- 0.0272 minutes : Drift Times: 2.18 +/- 0.23 ms
1.78e5
96.95980
259.01148
138.96955
Observed mass [m/z]

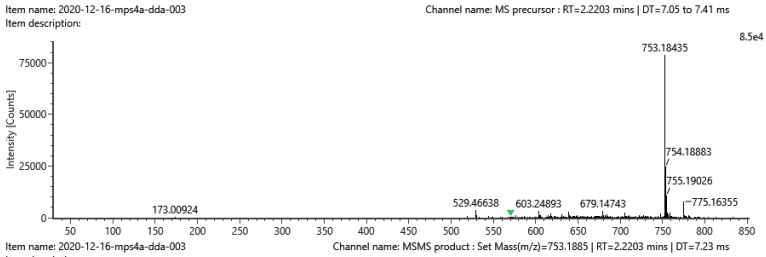
Biomarker number: 8

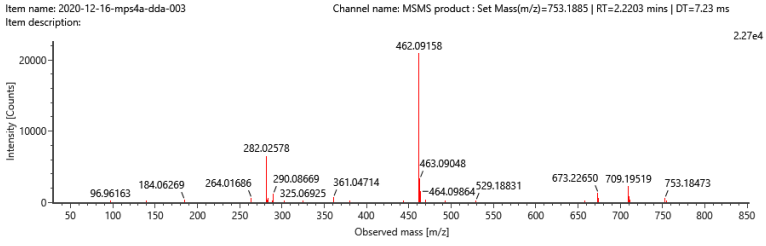
Annotation: neuAC-hex-hexNAc-s; **Formula**: C25H42N2O22S



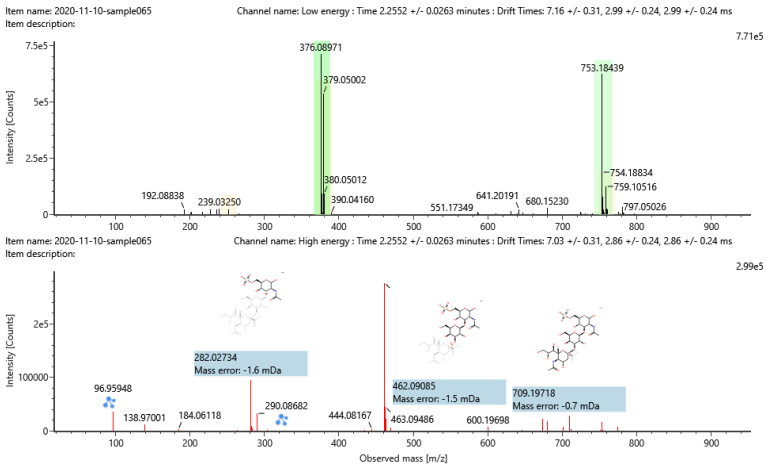






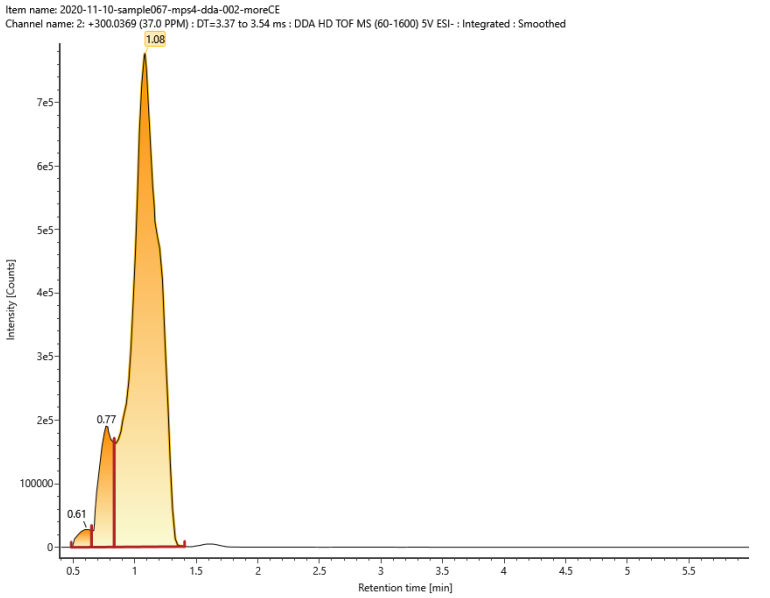


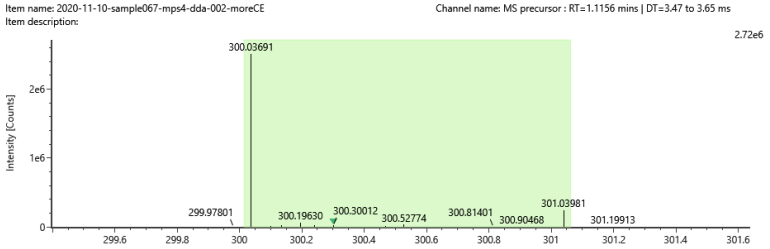
DIA

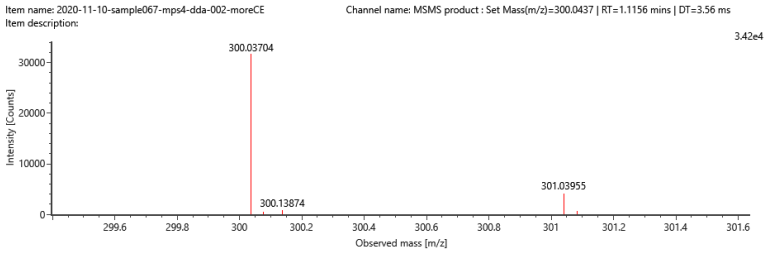

Item name: 2020-11-10-sample065
Item description:
Channel name: Low energy : Time 2.2552 +/- 0.0263 minutes : Drift Times: 7.16 +/- 0.31, 2.99 +/- 0.24, 2.99 +/- 0.24 ms
7.71e5
Intensity [Counts]
376.08971
379.05002
753.18439
380.05012
390.04160
754.18834
759.10516
192.08838
239.03250
551.17349
641.20191
680.15230
797.05026
Item name: 2020-11-10-sample065
Item description:
Channel name: High energy : Time 2.2552 +/- 0.0263 minutes : Drift Times: 7.03 +/- 0.31, 2.86 +/- 0.24, 2.86 +/- 0.24 ms
2.99e5
282.02734
Mass error: -1.6 mDa
462.09085
Mass error: -1.5 mDa
709.19718
Mass error: -0.7 mDa
96.95948
138.97001
184.06118
290.08682
444.08167
463.09486
600.19698
Observed mass [m/z]

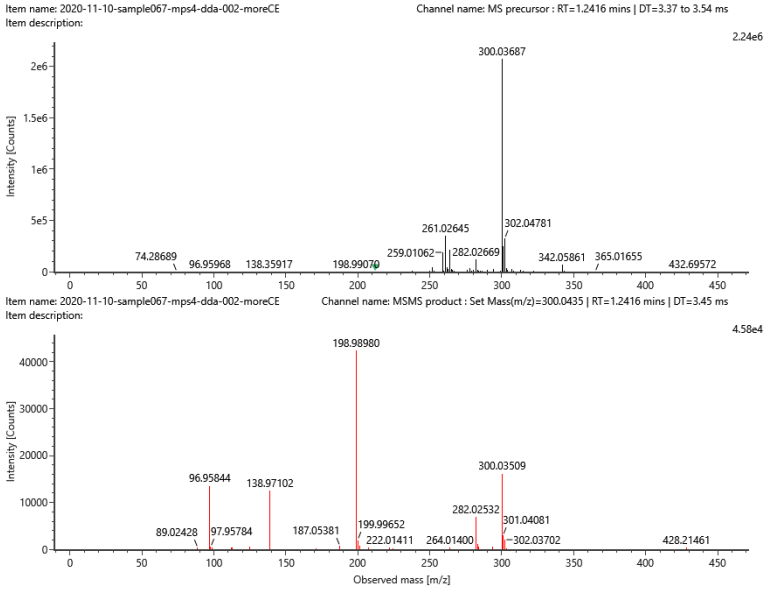
Biomarker number: 9

Annotation: hexNAc-s; **Formula**: C8H15NO9S

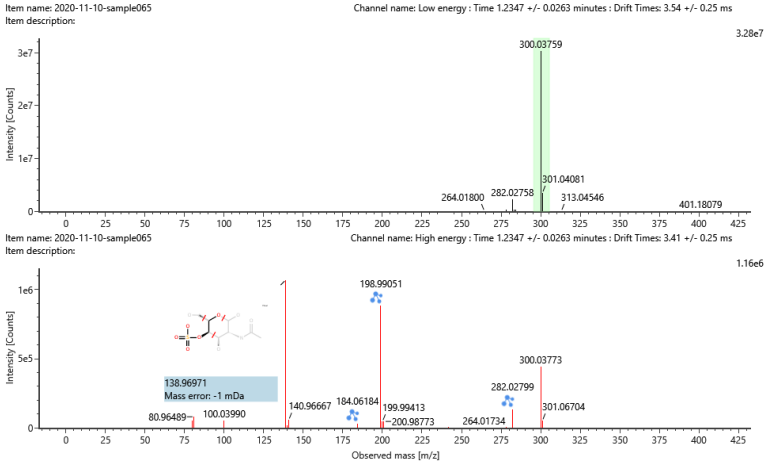







Item name: 2020-11-10-sample067-mps4-dda-002-moreCE
Item description:
Channel name: MS precursor : RT=1.2416 mins | DT=3.37 to 3.54 ms
2.24e6
Intensity [Counts]
300.03687
261.02645
302.04781
259.01062
282.02669
74.28689
96.95968
138.35917
198.99079
342.05861
365.01655
432.69572
Item name: 2020-11-10-sample067-mps4-dda-002-moreCE
Item description:
Channel name: MSMS product : Set Mass(m/z)=300.0435 | RT=1.2416 mins | DT=3.45 ms
4.58e4
Intensity [Counts]
198.98980
96.95844
138.97102
300.03509
282.02532
301.04081
89.02428
97.95784
187.05381
199.99652
222.01411
264.01400
302.03702
428.21461
Observed mass [m/z]

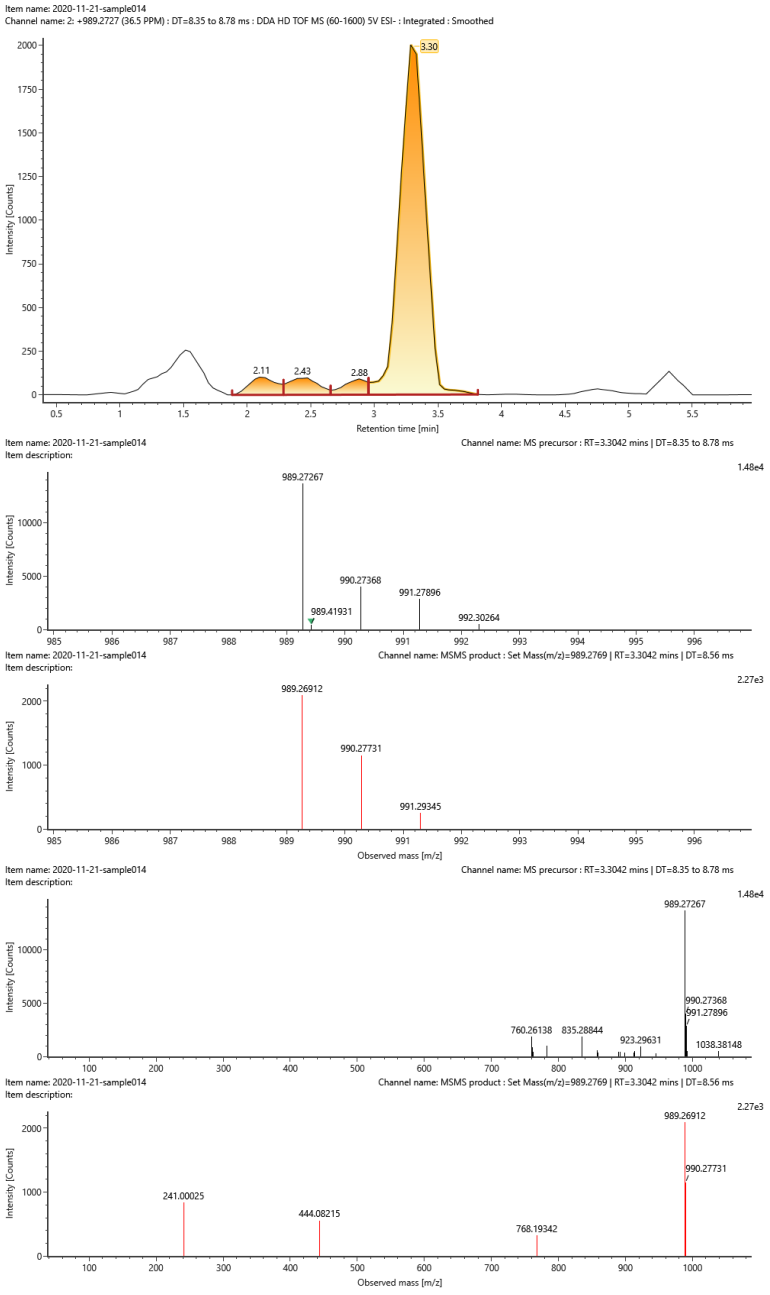
DIA


Item name: 2020-11-10-sample065
Item description:
Channel name: Low energy : Time 1.2347 +/- 0.0263 minutes : Drift Times: 3.54 +/- 0.25 ms
3.28e7
Intensity [Counts]
300.03759
301.04081
264.01800
282.02758
313.04546
401.18079
Item name: 2020-11-10-sample065
Item description:
Channel name: High energy : Time 1.2347 +/- 0.0263 minutes : Drift Times: 3.41 +/- 0.25 ms
1.16e6
Intensity [Counts]
198.99051
138.96971
Mass error: -1 mDa
300.03773
282.02799
140.96667
184.06184
199.99413
301.06704
80.96489
100.03990
200.98773
264.01734
Observed mass [m/z]

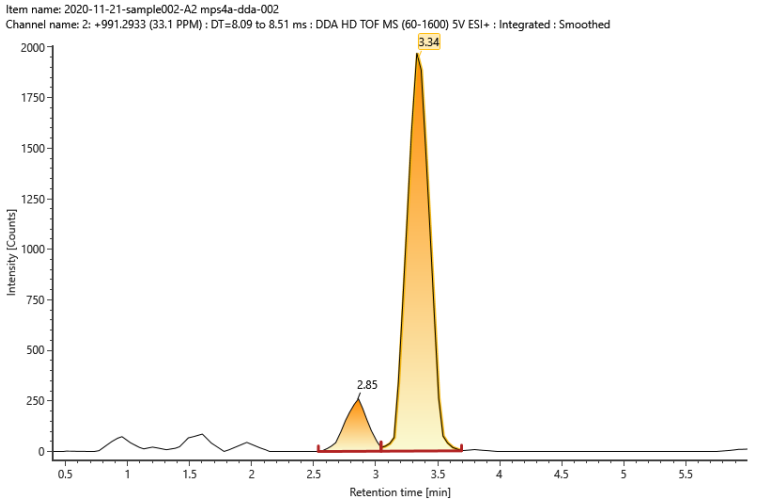
Biomarker number: 10

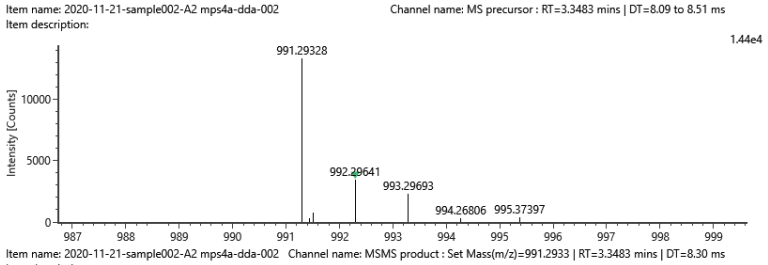
Annotation: hex3-hexNAc2-s; **Formula**: C34H58N2O29S

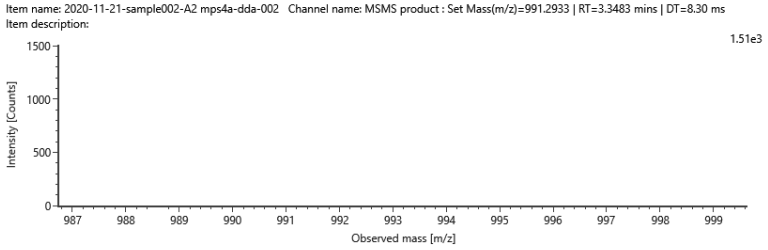
Neg ESI

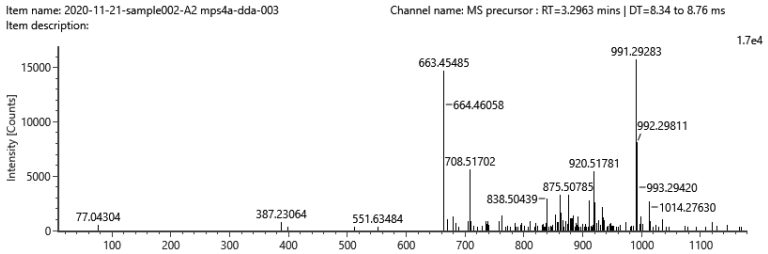


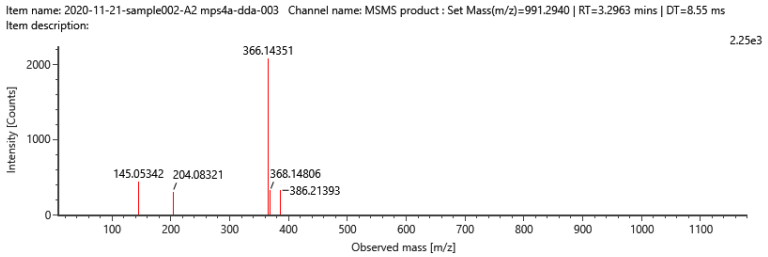
Pos ESI









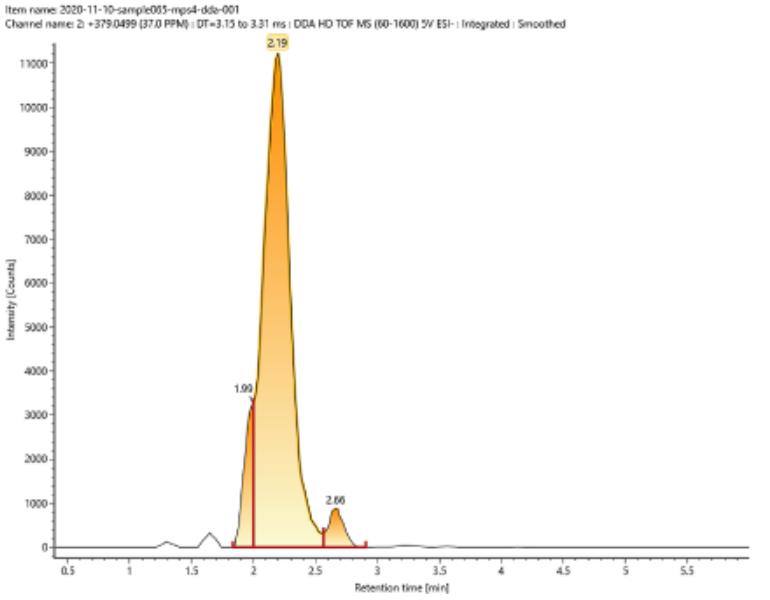


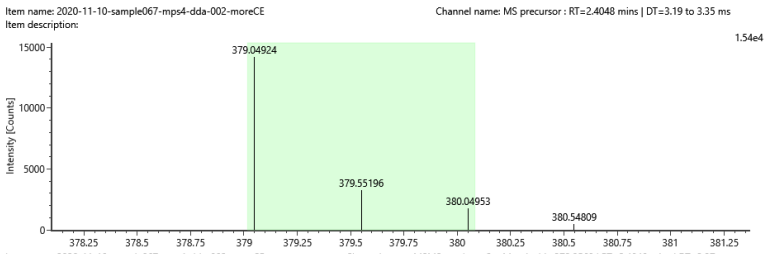
Uninformative DIA fragmentation spectra due to insufficient collision energy. Higher collision energies were used for the DDA acquisition.

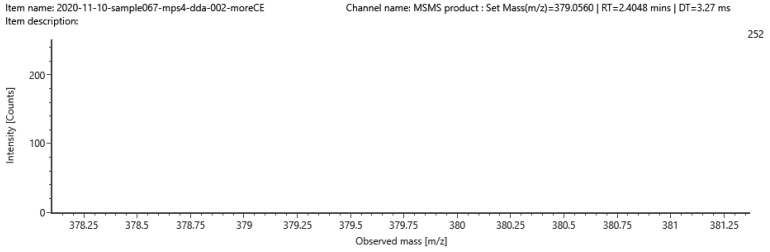
Biomarker number: 11

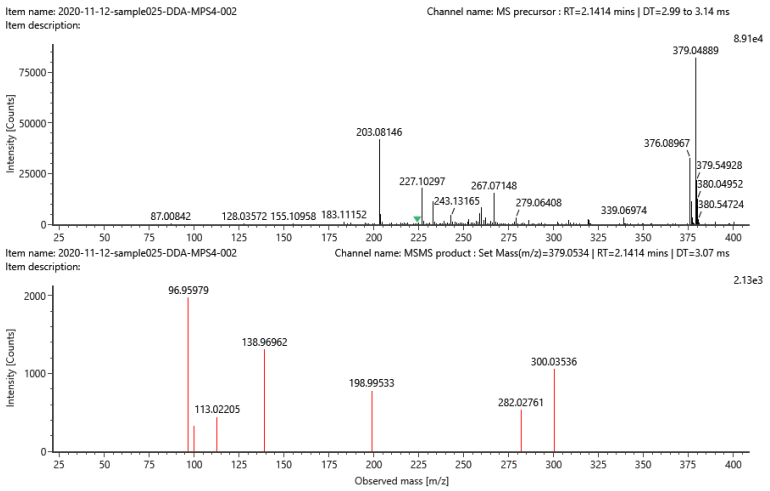
Annotation: hexNAc-s-UA-hexNAc-s; Formula: C22H36N2O23S2

MPS 4 sample

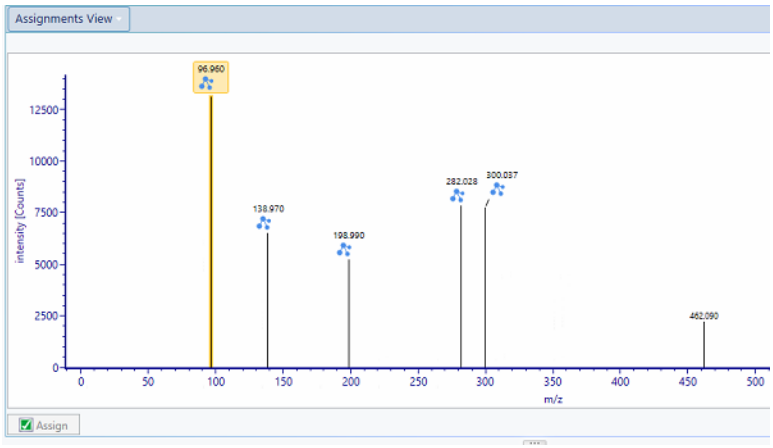




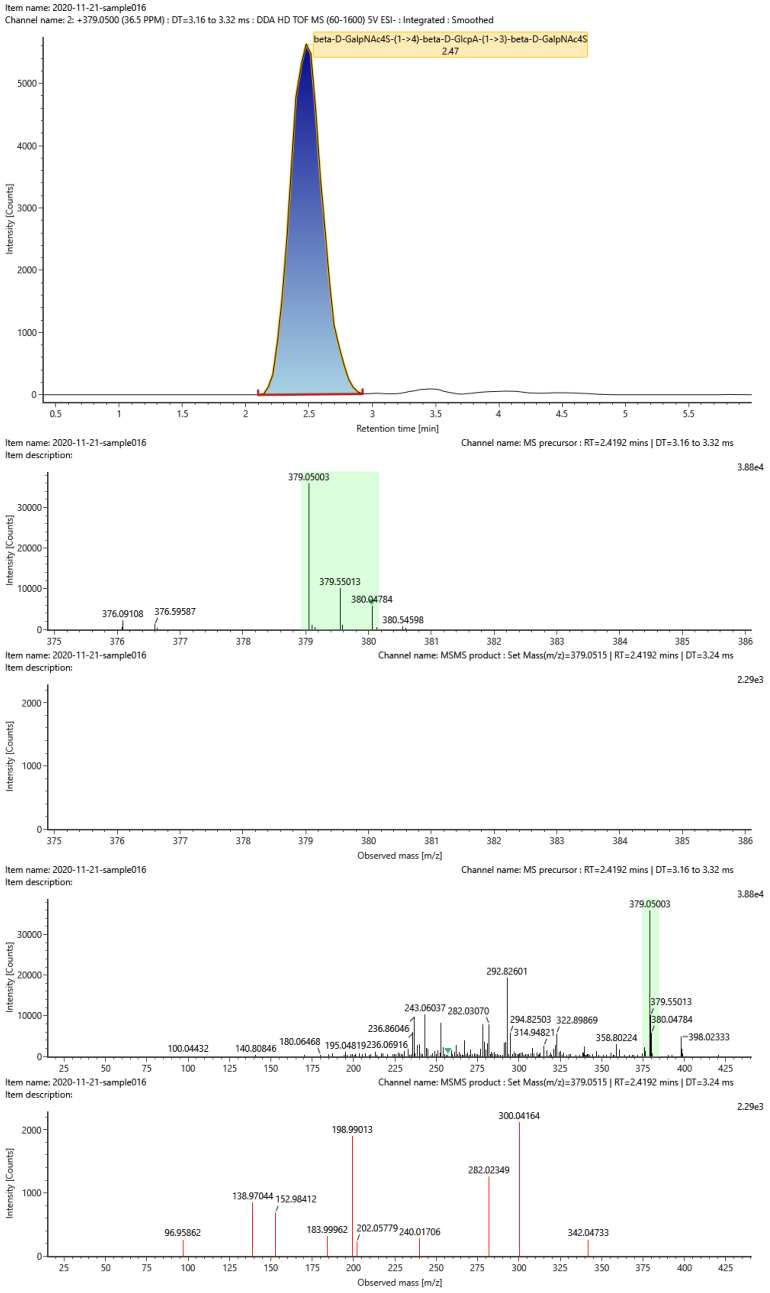



Item name: 2020-11-12-sample025-DDA-MPS4-002
Item description:
Channel name: MS precursor : RT=2.1414 mins | DT=2.99 to 3.14 ms
8.91e4
379.04889
203.08146
376.08967
379.54928
227.10297
267.07148
380.04952
243.13165
279.06408
380.54724
87.00842
128.03572
155.10958
183.11152
339.06974
Intensity [Counts]
Item name: 2020-11-12-sample025-DDA-MPS4-002
Item description:
Channel name: MSMS product : Set Mass(m/z)=379.0534 | RT=2.1414 mins | DT=3.07 ms
2.13e3
96.95979
138.96962
300.03536
198.99533
113.02205
282.02761
Intensity [Counts]
Observed mass [m/z]

DIA


Assignments View
96.960
138.970
198.990
282.028
300.037
462.090
intensity [Counts]
m/z
Assign

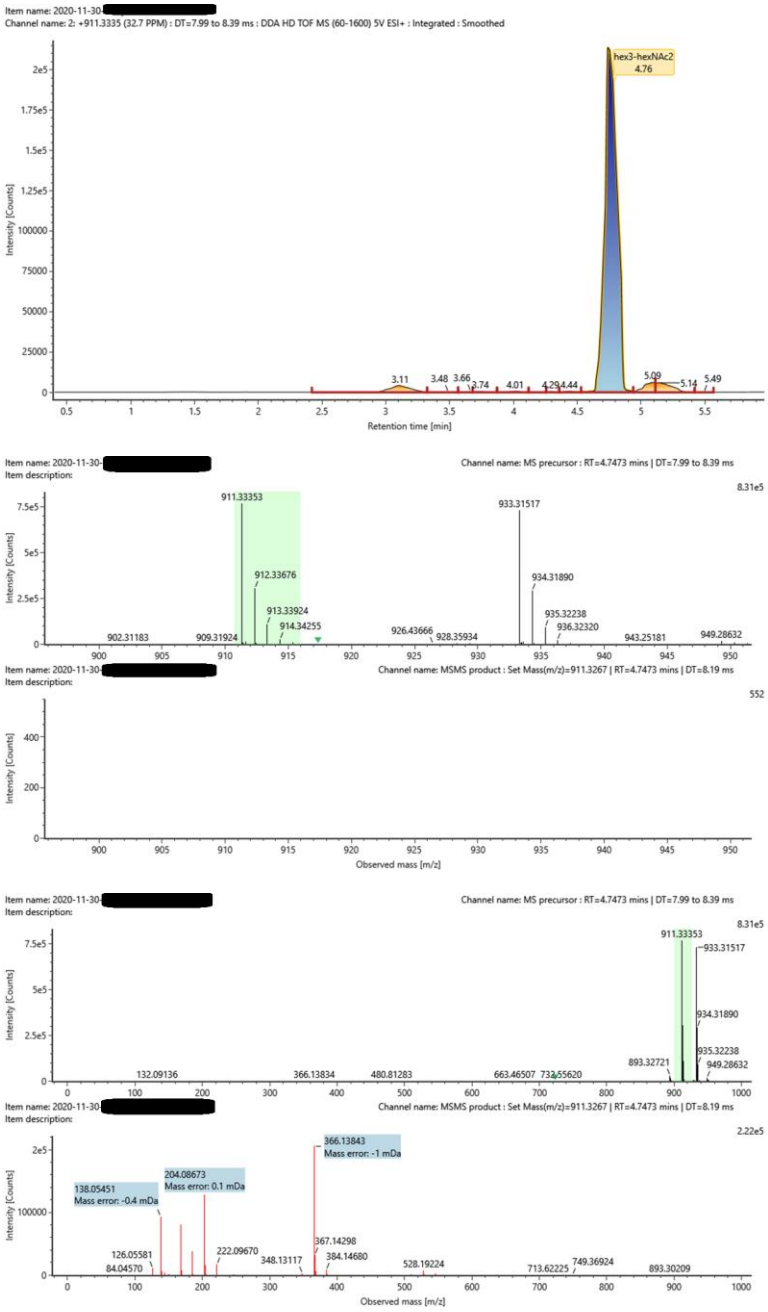
MPS 6 sample


Item name: 2020-11-21-sample016
Channel name: 2: +379.0500 (36.5 PPM) : DT=3.16 to 3.32 ms : DDA HD TOF MS (60-1600) 5V ESI- : Integrated : Smoothed
beta-D-GalpNAc4S-(1->4)-beta-D-GlcpA-(1->3)-beta-D-GalpNAc4S
2.47
Intensity [Counts]
Retention time [min]
Item name: 2020-11-21-sample016
Item description:
Channel name: MS precursor : RT=2.4192 mins | DT=3.16 to 3.32 ms
3.88e4
379.05003
379.55013
380.04784
376.09108
376.59587
380.54598
Item name: 2020-11-21-sample016
Item description:
Channel name: MSMS product : Set Mass(m/z)=379.0515 | RT=2.4192 mins | DT=3.24 ms
2.29e3
Observed mass [m/z]
Item name: 2020-11-21-sample016
Item description:
Channel name: MS precursor : RT=2.4192 mins | DT=3.16 to 3.32 ms
3.88e4
379.05003
292.82601
379.55013
243.06037
282.03070
294.82503
322.89869
236.86046
314.94821
380.04784
358.80224
398.02333
100.04432
140.80846
180.06468
195.04819
236.06916
Item name: 2020-11-21-sample016
Item description:
Channel name: MSMS product : Set Mass(m/z)=379.0515 | RT=2.4192 mins | DT=3.24 ms
2.29e3
300.04164
198.99013
282.02349
138.97044
152.98412
96.95862
183.99962
202.05779
240.01706
342.04733
Observed mass [m/z]

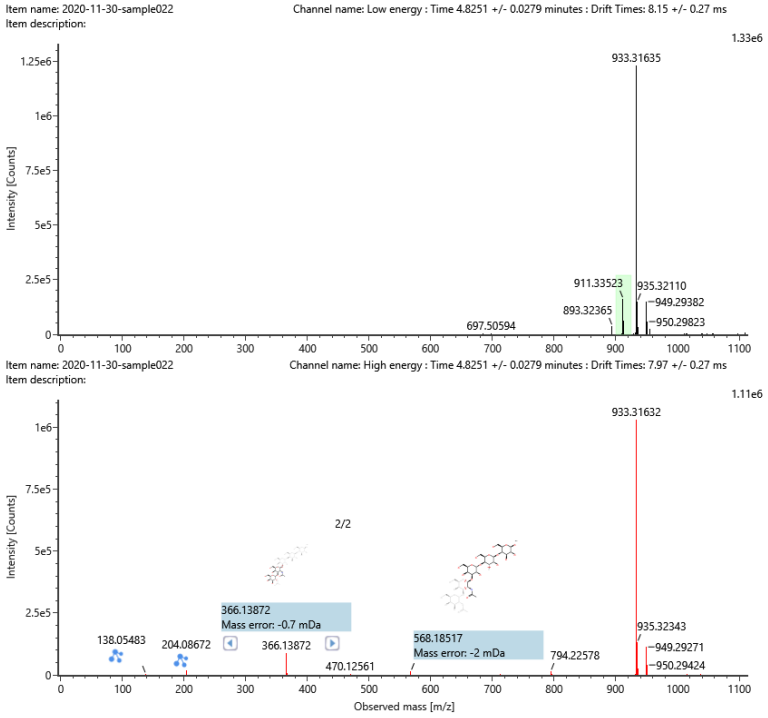
Biomarker number: 12

Annotation: hex3-hexNAc2; Formula: C34H58N2O26

Pos ESI



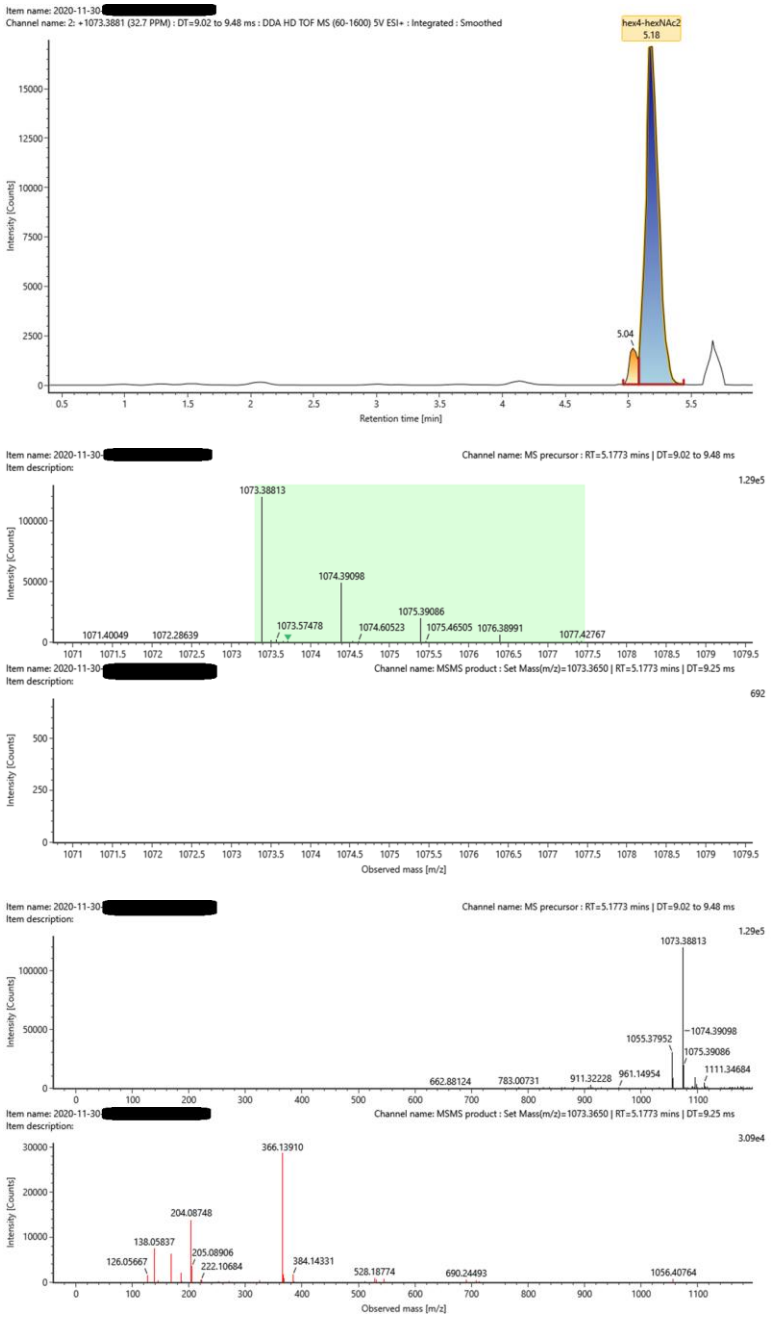
DIA


Item name: 2020-11-30-sample022
Item description:
Channel name: Low energy : Time 4.8251 +/- 0.0279 minutes : Drift Times: 8.15 +/- 0.27 ms
1.33e6
933.31635
911.33523
935.32110
949.29382
893.32365
950.29823
697.50594
Intensity [Counts]
Item name: 2020-11-30-sample022
Item description:
Channel name: High energy : Time 4.8251 +/- 0.0279 minutes : Drift Times: 7.97 +/- 0.27 ms
1.11e6
933.31632
2/2
366.13872
Mass error: -0.7 mDa
568.18517
Mass error: -2 mDa
138.05483
204.08672
366.13872
470.12561
794.22578
935.32343
949.29271
950.29424
Intensity [Counts]
Observed mass [m/z]

Biomarker number: 13

Annotation: hex4-hexNAc2; Formula: C40H68N2O31

Pos ESI

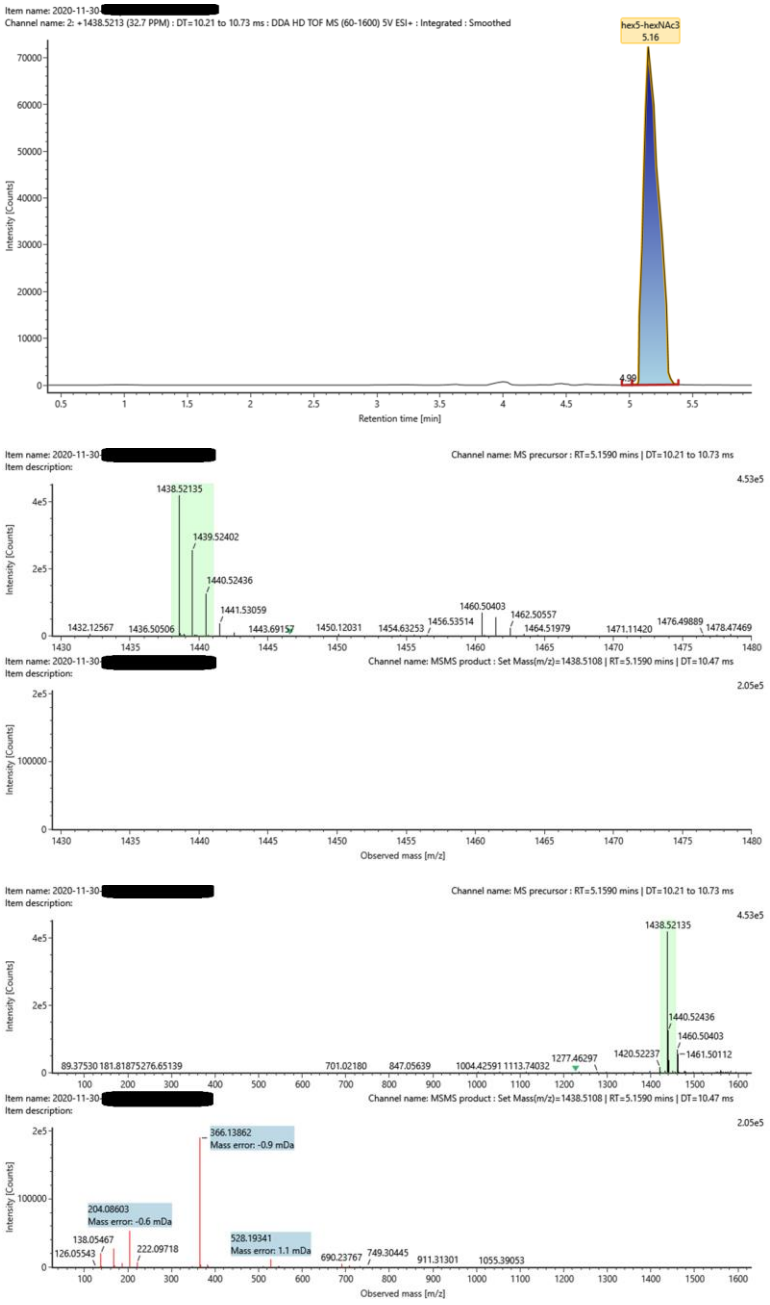


Uninformative DIA fragmentation spectra due to insufficient collision energy. Higher collision energies were used for the DDA acquisition.

Biomarker number: 14

Annotation: hex5-hexNAc3; Formula: C54H91N3O41

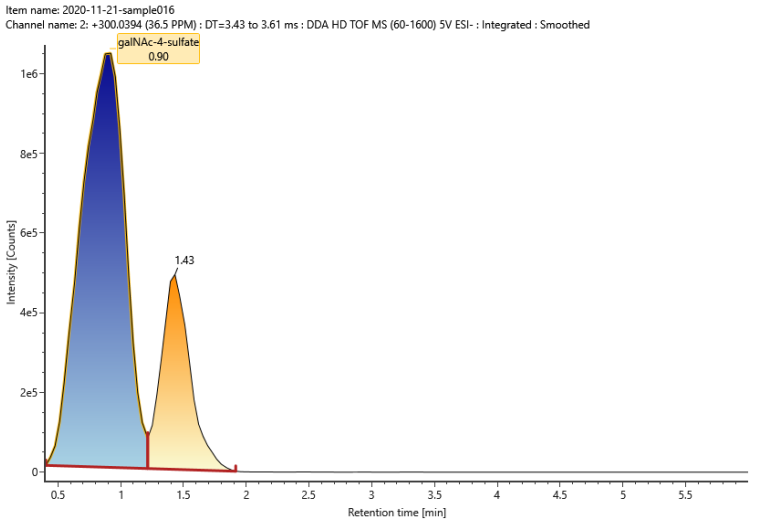
Pos ESI

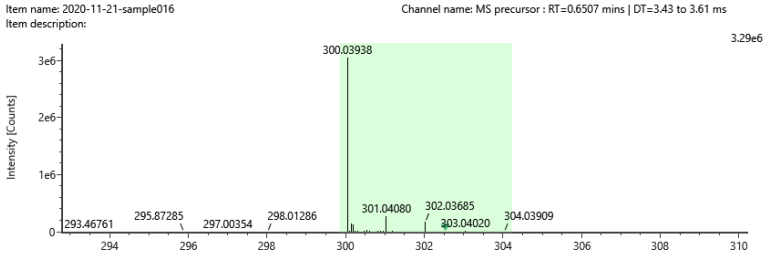


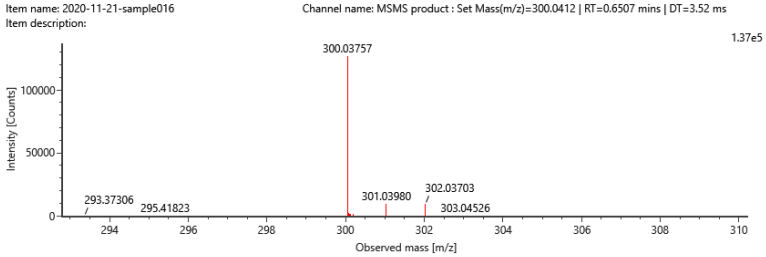
Uninformative DIA fragmentation spectra due to insufficient collision energy. Higher collision energies were used for the DDA acquisition.

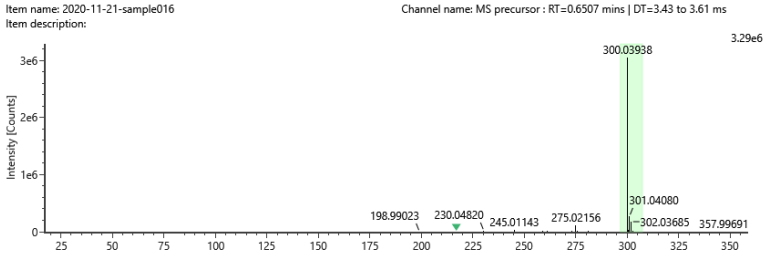
Biomarker number: 15

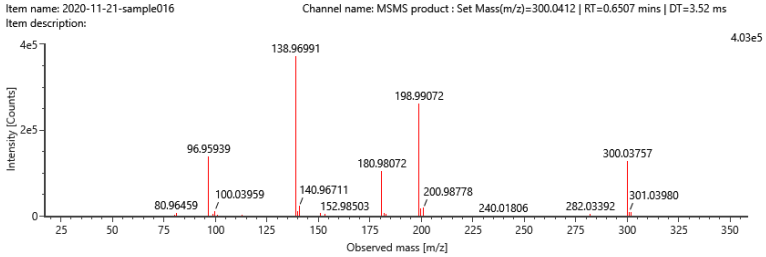
Annotation: hexNAc-s; **Formula**: C8H15NO9S



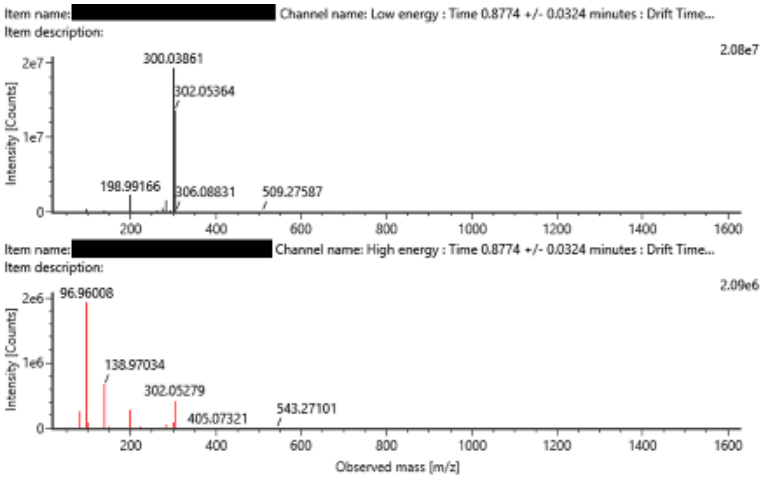






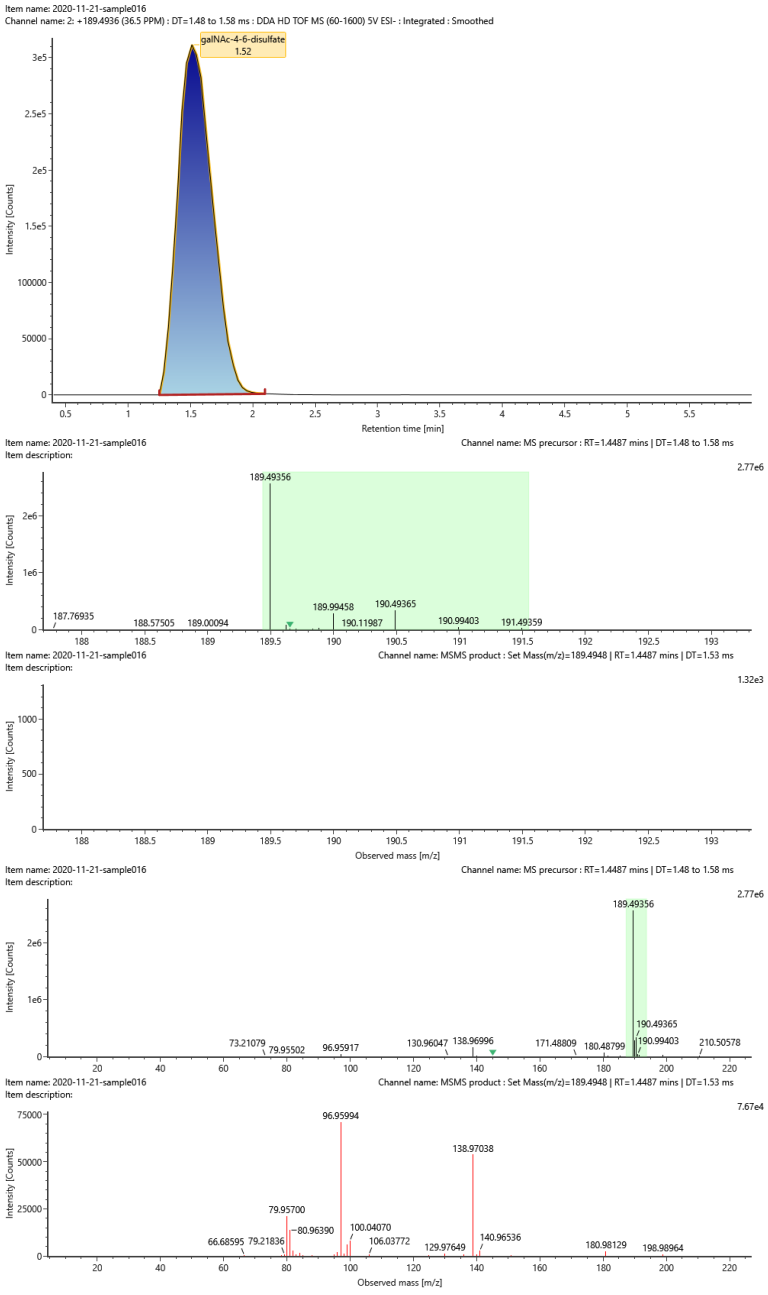


DIA

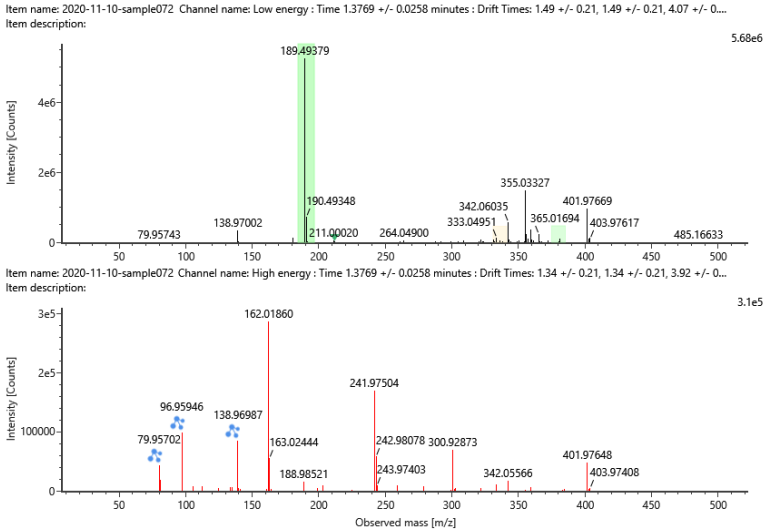

Item name: [redacted] Channel name: Low energy : Time 0.8774 +/- 0.0324 minutes : Drift Time...
Item description:
2.08e7
300.03861
302.05364
198.99166
306.08831
509.27587
Intensity [Counts]
2e7
1e7
0
200
400
600
800
1000
1200
1400
1600
Item name: [redacted] Channel name: High energy : Time 0.8774 +/- 0.0324 minutes : Drift Time...
Item description:
2.09e6
96.96008
138.97034
302.05279
405.07321
543.27101
2e6
1e6
Observed mass [m/z]

Biomarker number: 16

Annotation: hexNAc-disulfate; **Formula**: C8H15NO12S2



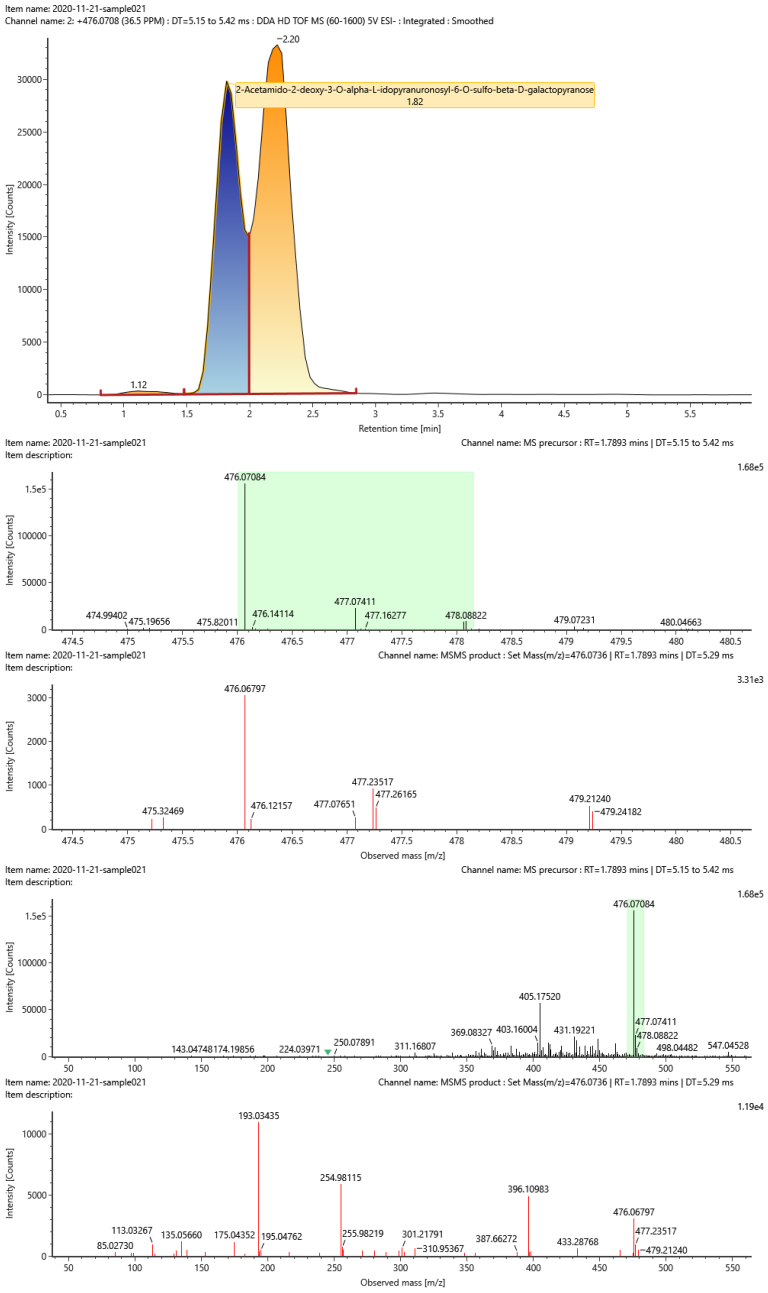
DIA


Item name: 2020-11-10-sample072 Channel name: Low energy : Time 1.3769 +/- 0.0258 minutes : Drift Times: 1.49 +/- 0.21, 1.49 +/- 0.21, 4.07 +/- 0....
Item description:
5.68e6
Intensity [Counts]
189.49379
190.49348
355.03327
342.06035
401.97669
365.01694
333.04951
403.97617
138.97002
79.95743
211.00020
264.04900
485.16633
Item name: 2020-11-10-sample072 Channel name: High energy : Time 1.3769 +/- 0.0258 minutes : Drift Times: 1.34 +/- 0.21, 1.34 +/- 0.21, 3.92 +/- 0...
Item description:
3.1e5
Intensity [Counts]
162.01860
241.97504
96.95946
138.96987
79.95702
163.02444
242.98078
300.92873
243.97403
401.97648
188.98521
342.05566
403.97408
Observed mass [m/z]

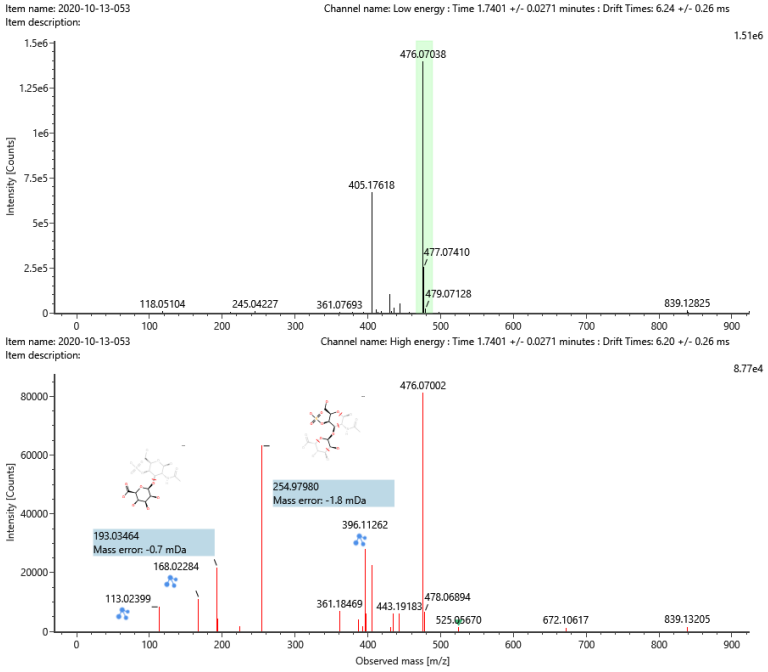
Biomarker number: 17

Annotation: hexNAc-s-UA; Formula: C14H23NO15S

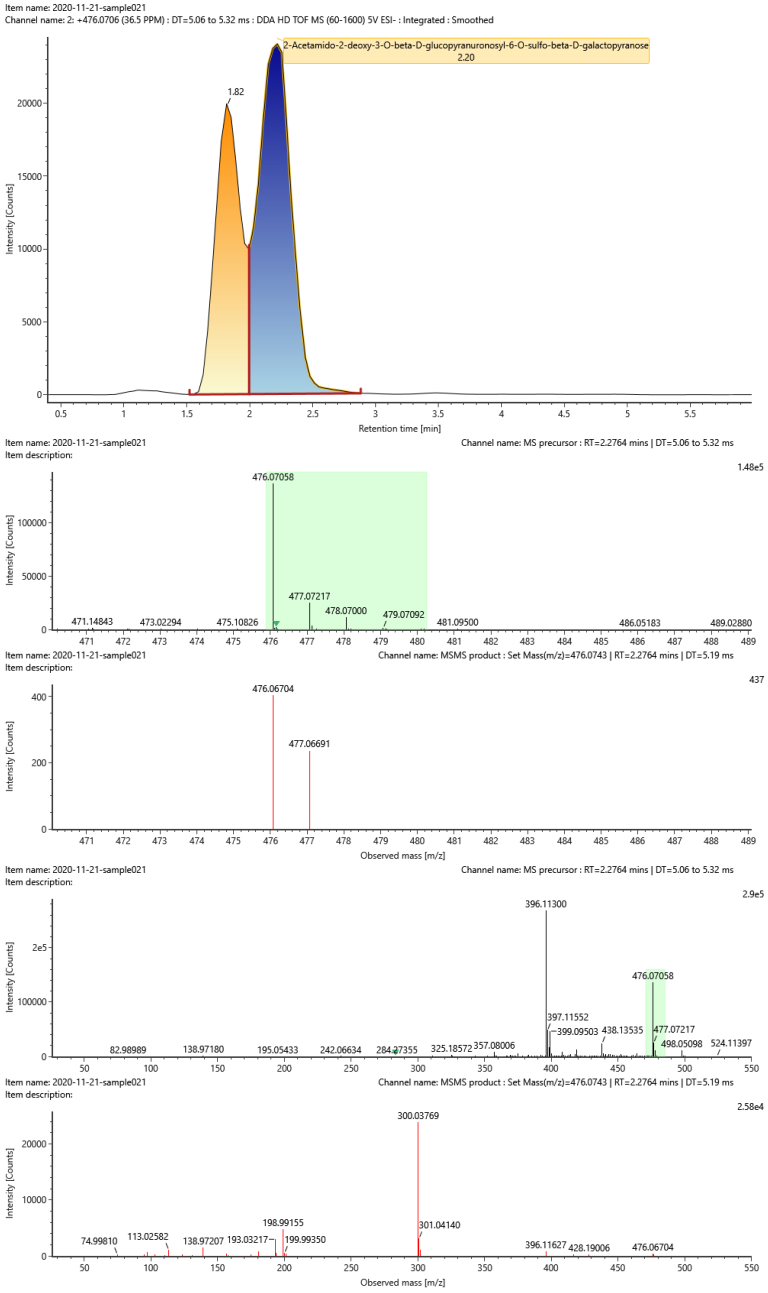
Left peak



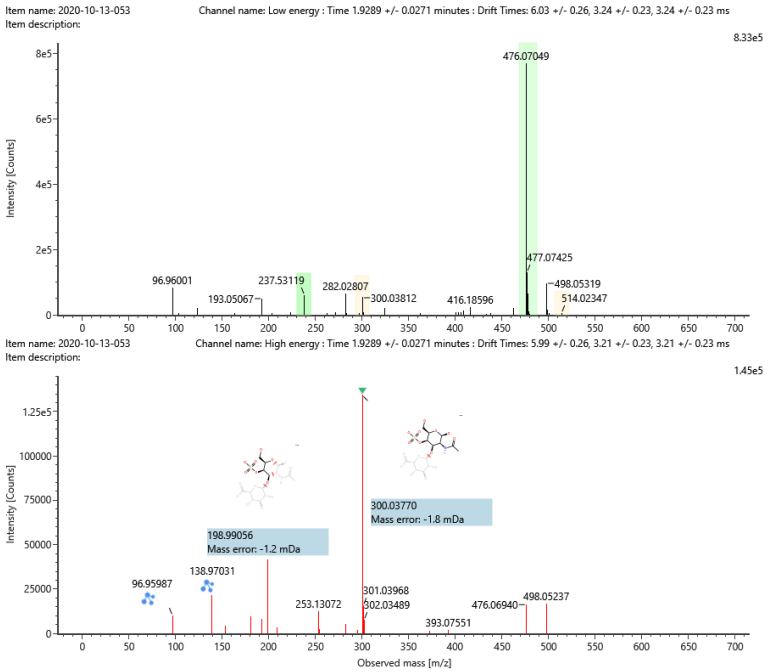
DIA


Item name: 2020-10-13-053
Item description:
Channel name: Low energy : Time 1.7401 +/- 0.0271 minutes : Drift Times: 6.24 +/- 0.26 ms
1.51e6
476.07038
405.17618
477.07410
479.07128
118.05104
245.04227
361.07693
839.12825
Intensity [Counts]
Item name: 2020-10-13-053
Item description:
Channel name: High energy : Time 1.7401 +/- 0.0271 minutes : Drift Times: 6.20 +/- 0.26 ms
8.77e4
476.07002
254.97980
Mass error: -1.8 mDa
193.03464
Mass error: -0.7 mDa
396.11262
168.02284
113.02399
361.18469
443.19183
478.06894
525.05670
672.10617
839.13205
Intensity [Counts]
Observed mass [m/z]

Right peak (note the different fragmentation pattern)

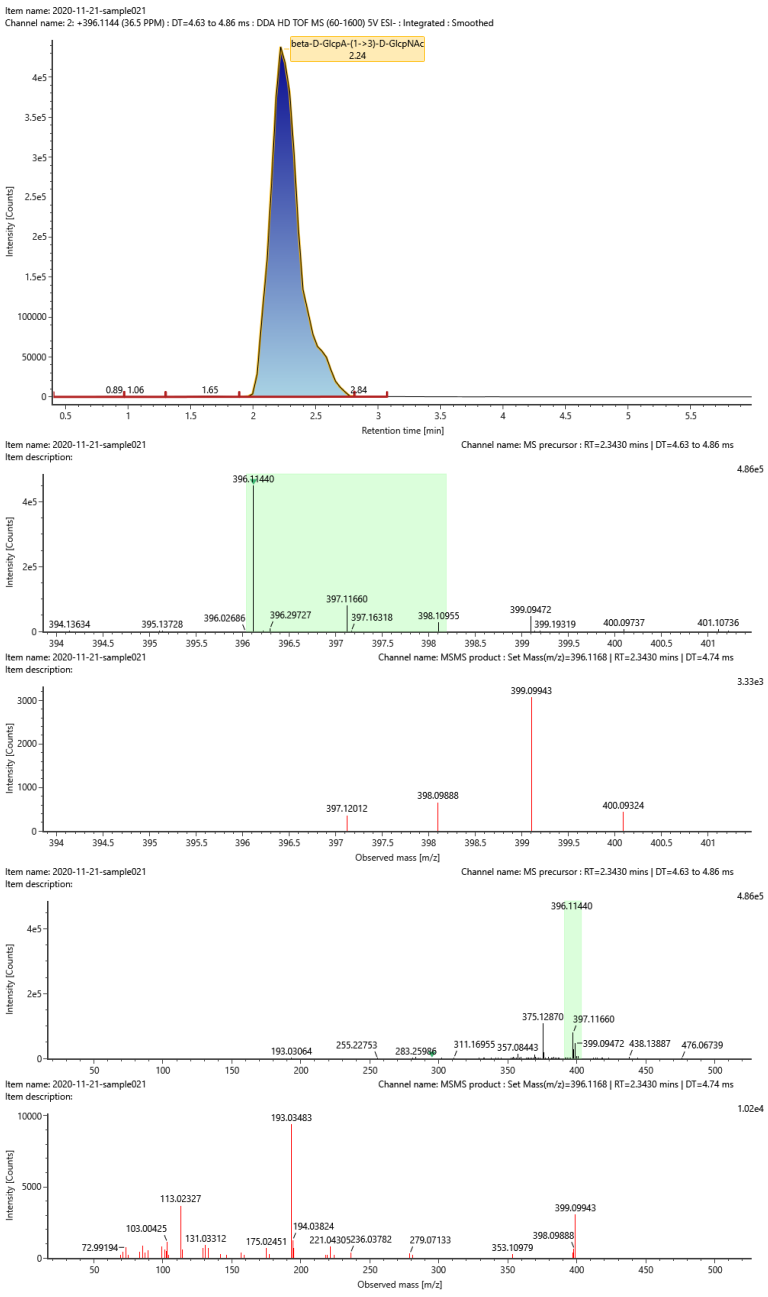


DIA

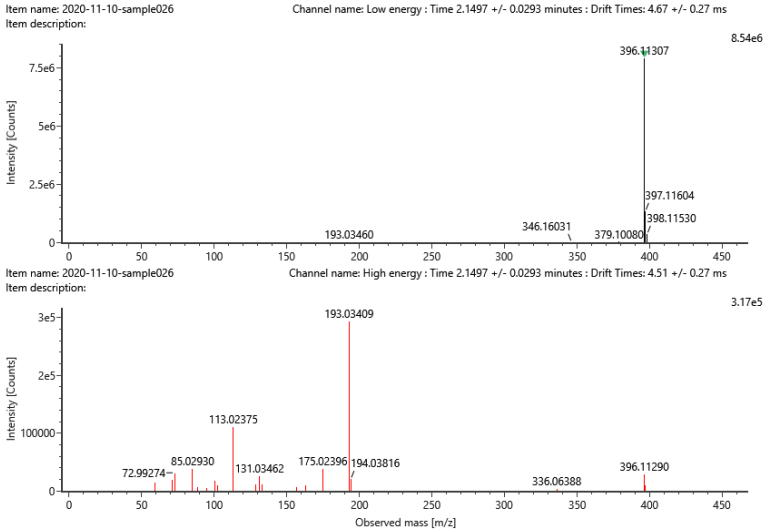

Item name: 2020-10-13-053
Item description:
Channel name: Low energy : Time 1.9289 +/- 0.0271 minutes : Drift Times: 6.03 +/- 0.26, 3.24 +/- 0.23, 3.24 +/- 0.23 ms
8.33e5
476.07049
477.07425
96.96001
237.53119
282.02807
193.05067
300.03812
416.18596
498.05319
514.02347
Intensity [Counts]
Item name: 2020-10-13-053
Item description:
Channel name: High energy : Time 1.9289 +/- 0.0271 minutes : Drift Times: 5.99 +/- 0.26, 3.21 +/- 0.23, 3.21 +/- 0.23 ms
1.45e5
300.03770
Mass error: -1.8 mDa
198.99056
Mass error: -1.2 mDa
138.97031
96.95987
253.13072
301.03968
302.03489
476.06940
498.05237
393.07551
Intensity [Counts]
Observed mass [m/z]

Biomarker number: 18

Annotation: hexNAc-UA; **Formula**: C14H23NO12

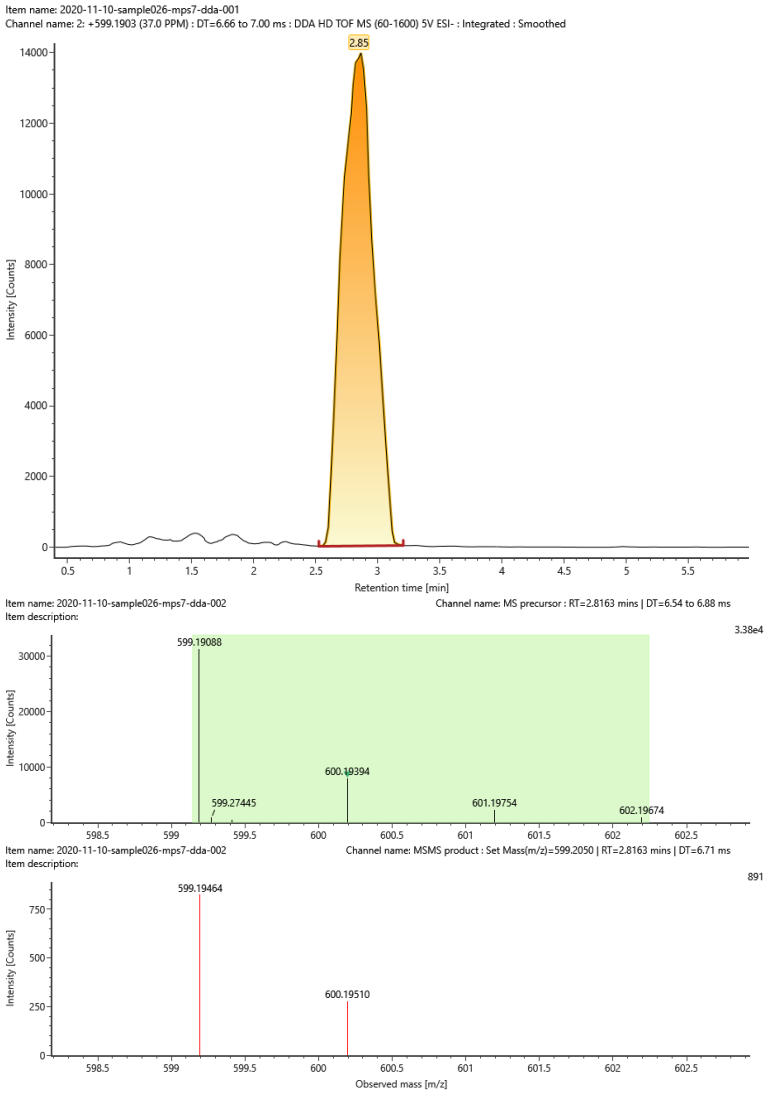


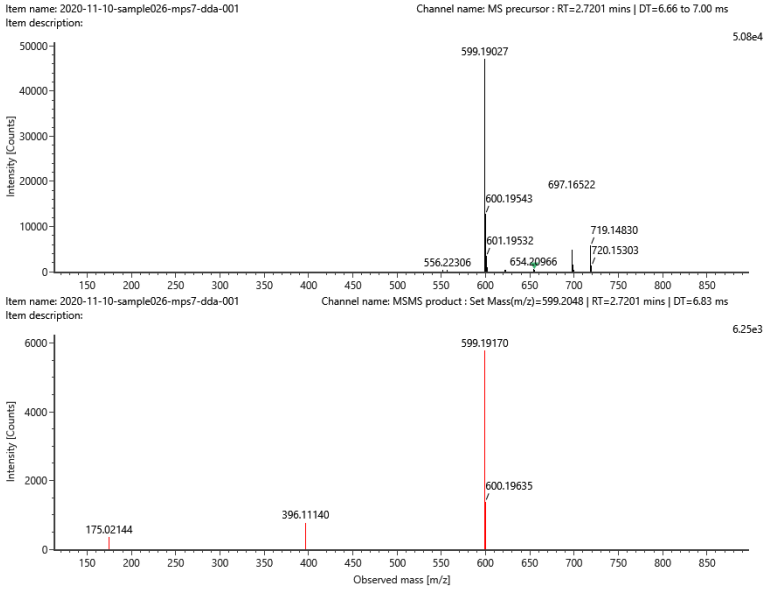
DIA


Item name: 2020-11-10-sample026
Item description:
Channel name: Low energy : Time 2.1497 +/- 0.0293 minutes : Drift Times: 4.67 +/- 0.27 ms
8.54e6
396.11307
397.11604
398.11530
193.03460
346.16031
379.10080
Intensity [Counts]
Item name: 2020-11-10-sample026
Item description:
Channel name: High energy : Time 2.1497 +/- 0.0293 minutes : Drift Times: 4.51 +/- 0.27 ms
3.17e5
193.03409
113.02375
85.02930
72.99274
131.03462
175.02396
194.03816
336.06388
396.11290
Intensity [Counts]
Observed mass [m/z]

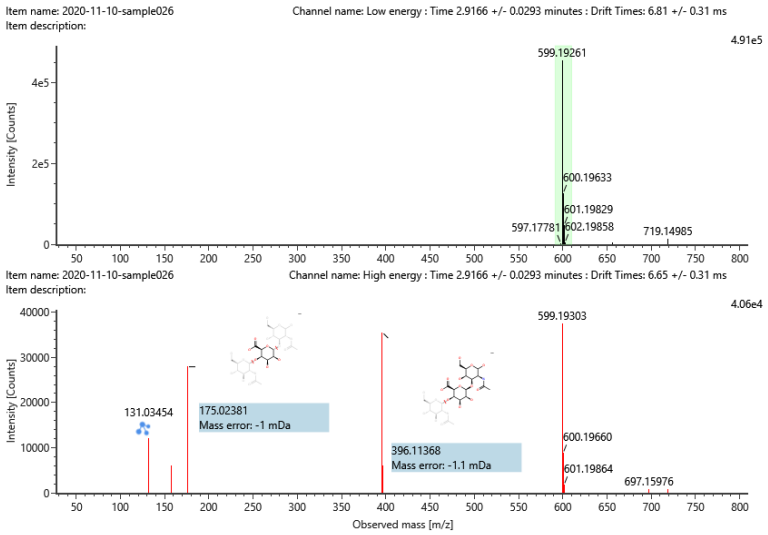
Biomarker number: 19

Annotation: hexNAc-UA-hexNAc; **Formula**: C22H36N2O17




Item name: 2020-11-10-sample026-mps7-dda-001
Item description:
Channel name: MS precursor : RT=2.7201 mins | DT=6.66 to 7.00 ms
5.08e4
599.19027
697.16522
600.19543
719.14830
601.19532
720.15303
556.22306
654.20966
Intensity [Counts]
Item name: 2020-11-10-sample026-mps7-dda-001
Item description:
Channel name: MSMS product : Set Mass(m/z)=599.2048 | RT=2.7201 mins | DT=6.83 ms
6.25e3
599.19170
600.19635
396.11140
175.02144
Intensity [Counts]
Observed mass [m/z]

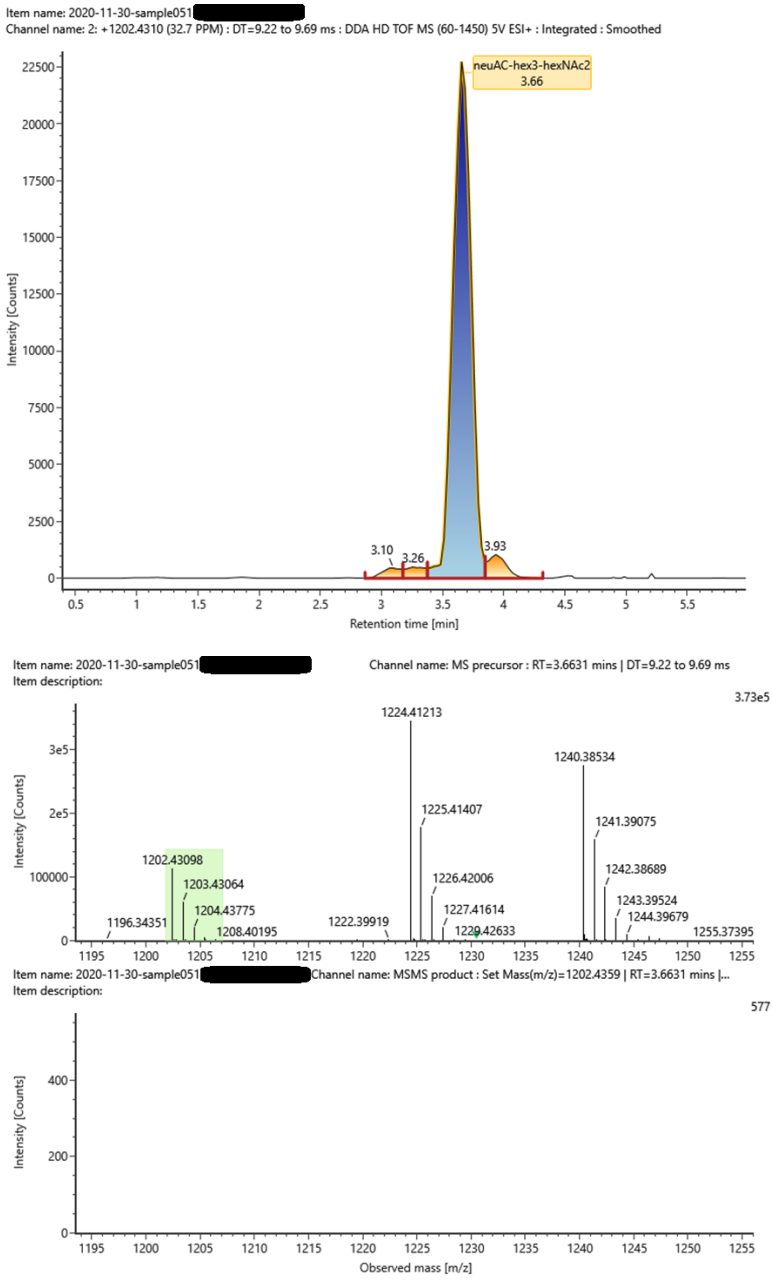
DIA


Item name: 2020-11-10-sample026
Item description:
Channel name: Low energy : Time 2.9166 +/- 0.0293 minutes : Drift Times: 6.81 +/- 0.31 ms
4.91e5
599.19261
600.19633
601.19829
597.17781
602.19858
719.14985
Intensity [Counts]
Item name: 2020-11-10-sample026
Item description:
Channel name: High energy : Time 2.9166 +/- 0.0293 minutes : Drift Times: 6.65 +/- 0.31 ms
4.06e4
599.19303
131.03454
175.02381
Mass error: -1 mDa
396.11368
Mass error: -1.1 mDa
600.19660
601.19864
697.15976
Intensity [Counts]
Observed mass [m/z]

Biomarker number: 20

Annotation: neuAC-hex3-hexNAc2; Formula: C45H75N3O34

Pos ESI



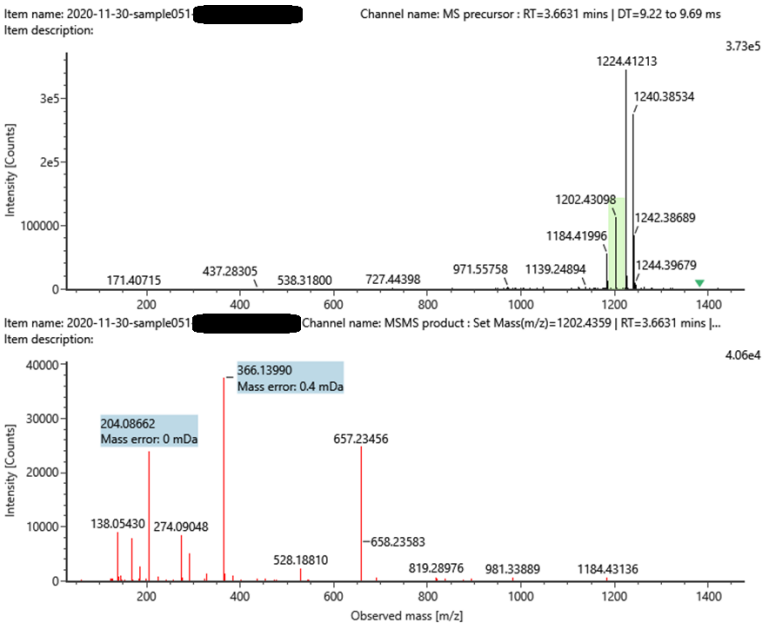
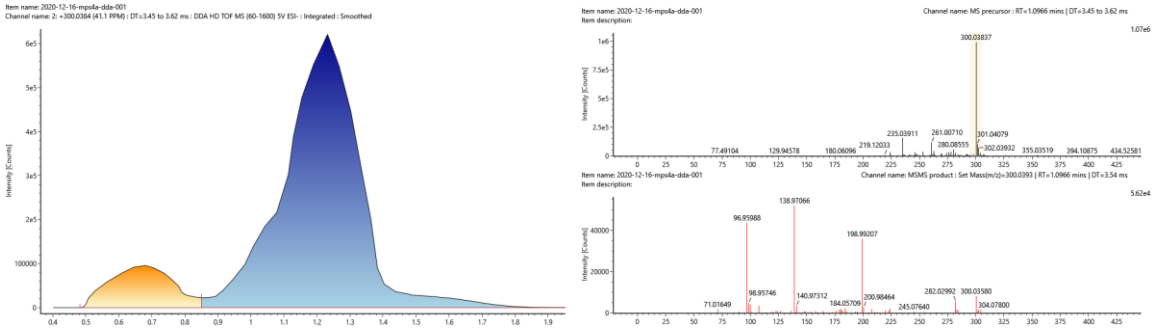

Item name: 2020-11-30-sample051[redacted]
Channel name: MS precursor : RT=3.6631 mins | DT=9.22 to 9.69 ms
Item description:
3.73e5
1224.41213
1240.38534
1202.43098
1242.38689
1184.41996
1244.39679
171.40715
437.28305
538.31800
727.44398
971.55758
1139.24894
Intensity [Counts]
Item name: 2020-11-30-sample051[redacted]
Channel name: MSMS product : Set Mass(m/z)=1202.4359 | RT=3.6631 mins |...
Item description:
4.06e4
366.13990
Mass error: 0.4 mDa
204.08662
Mass error: 0 mDa
657.23456
138.05430
274.09048
658.23583
528.18810
819.28976
981.33889
1184.43136
Intensity [Counts]
Observed mass [m/z]

Figure S4. Chromatographic resolution of two ion features both annotated as hexNAc-s that can differentiate MPS 4a from MPS 6.

Representative extracted ion chromatograms showing retention time differences between two ion features annotated as hexNAc-s (300.0384 m/z; ESI-) for A) MPS 4a and B) MPS 6. Urine samples were analyzed on the Vion using DDA with ion-mobility enabled. Corresponding spectra are included. There were no appreciable differences in drift time. To control for retention time shifts due to mobile phase or batch effects, the samples were acquired consecutively without system changes.

A) MPS 4a urine

Drift Time = 3.45-3.62 ms



B) MPS 6 urine

Drift Time = 3.51-3.69 ms

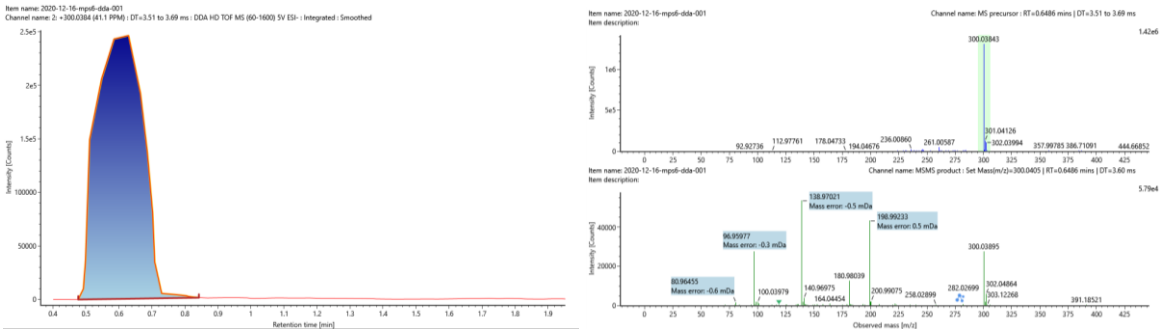


Figure S5. Detailed Z-scores for each sample.

Z-scores are overlayed onto the heatmap given in Figure 3.

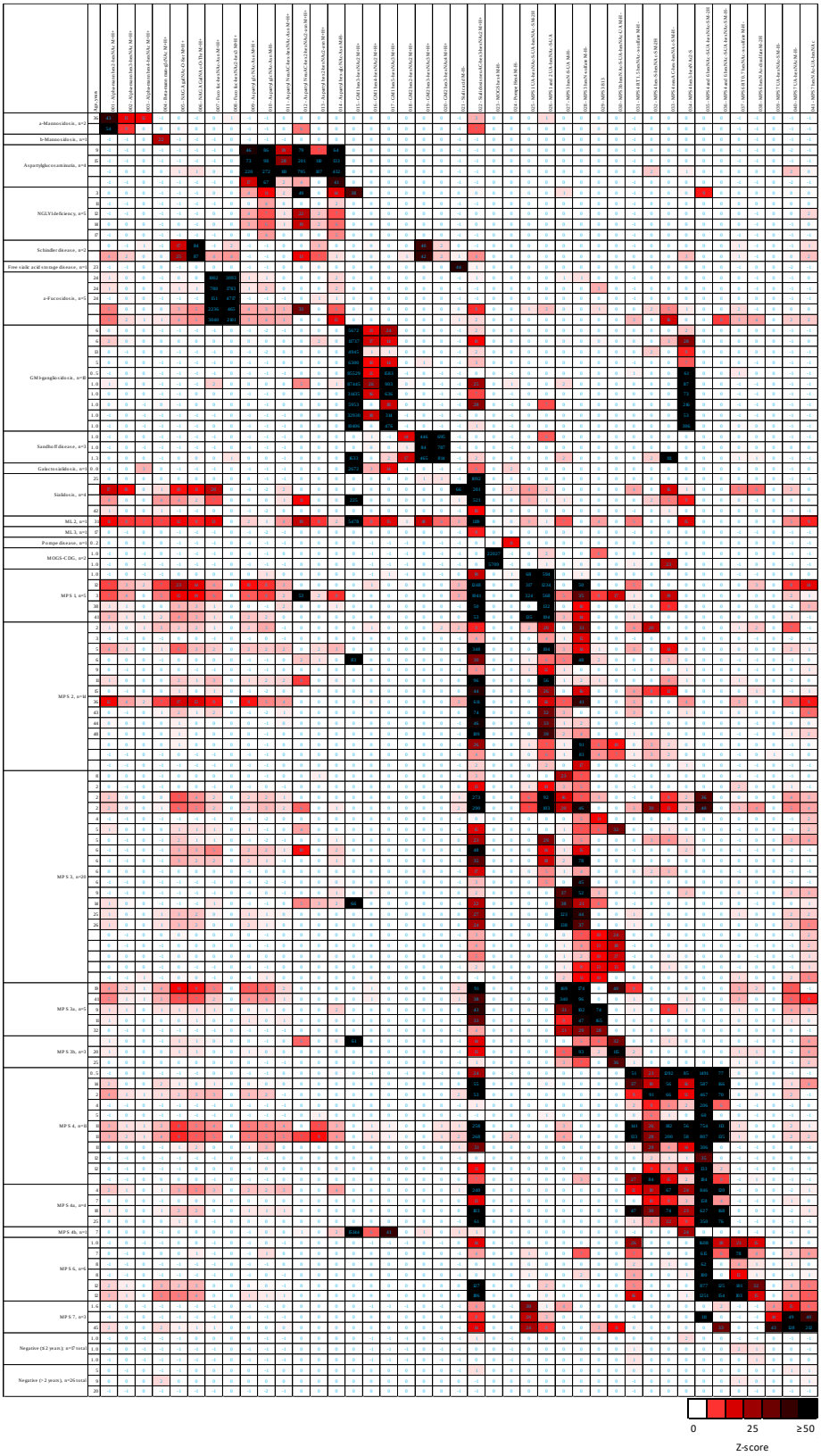
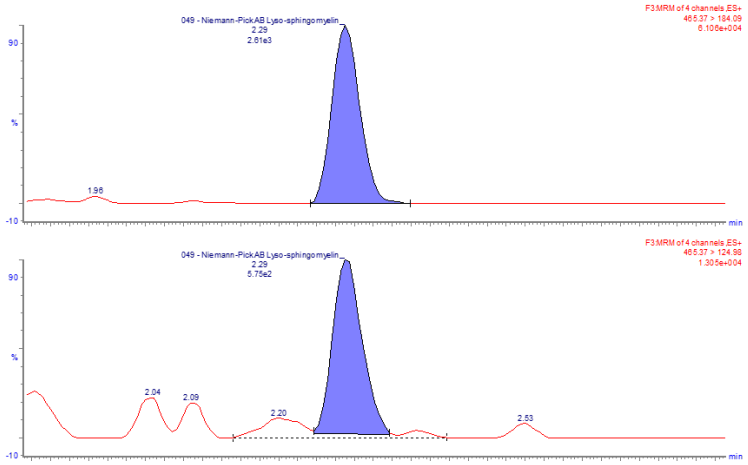


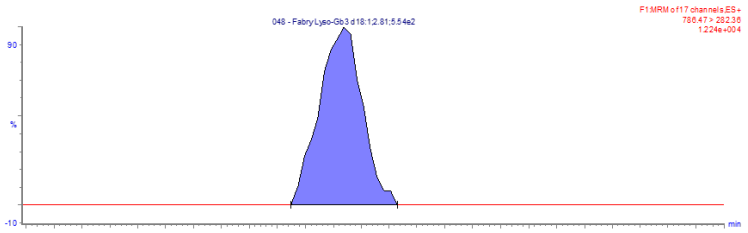
Figure S6. Limits of detection for glycolipids.

The limits of detection for glycolipids were assessed using ERNDIM SAS samples. Extracted ion chromatograms of lyso-SM, lyso-Gb3, and glucosylsphingosine are shown. For lyso-SM and glucosylsphingosine, the lowest concentrations in the survey year were detected. For lyso-Gb3, the second lowest concentration was detected. Both the quantifier ion (top trace) and the qualifier ion (bottom trace) were detected for lyso-SM and glucosylsphingosine. Only the quantifier ion was detected for lyso-Gb3.

A) Lyso-SM (3.75 nmol/L); peak height = 61,060 counts



B) Lyso-Gb3 (12.5 nmol/L); peak height = 12,240 counts



C) Glucosylsphingosine (0.6 nmol/L); peak height = 9,091 counts

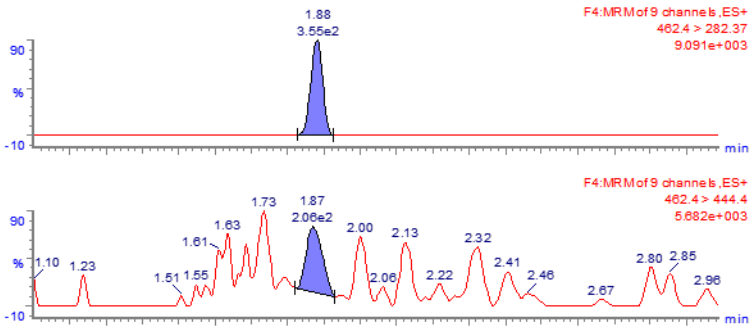


Table S1. Detailed methods for the LC-MS analyses.

Detailed methods for A) the Vion ion-mobility quadrupole time of flight MS (IMS-qTOF MS) and B) the TQ-S Micro tandem quadrupole MS.

A) Vion: Untargeted analyses were performed with a Waters Acquity H-Class UPLC system coupled to a Waters Vion ion-mobility quadrupole time of flight MS (IMS-qTOF MS). Positive and negative electrospray ionization (ESI) was used in sensitivity mode. Profile-mode data was acquired from 60-1600 m/z over a scan time of 0.2 seconds with Data Independent Acquisition (DIA) and ion mobility (High Definition MS^e). Soft transmission and a nitrogen-filled collision cell were used. The capillary voltage was 2.5 kV, the cone voltage was 35 V, the source temperature was 120°C, the desolvation gas temperature was 500°C, the cone gas flow was 50 L/h, and the nebulizer gas flow was 1000 L/h, and the collision energy ramp was set to 25-65 V. Leucine-enkephalin was used for lock-mass correction. Before every batch, CCS calibration was performed according to manufacturer directions. Waters Unifi 1.9.4 was used to control the system and analyze data.

B) TQ-S Micro: Targeted analyses were performed on a Waters Acquity H-Class UPLC system coupled to a Waters TQ-S Micro tandem quadrupole MS. Positive and negative ESI was used with rapid polarity switching. Multiple reaction monitoring (MRM) was used at unit resolution. The capillary voltage was 2.5 kV, the source temperature was 150°C, the desolvation gas temperature was 425°C, the cone gas flow was 50 L/h, and the nebulizer gas flow was 1000 L/h. Waters MassLynx 4.2 was used to control the system and TargetLynx XS was used for data analysis. The following MRM table was used for the acquisition method:

	Compound	Polarity	Q1	Q3	Cone (V)	Collision (eV)	RT start, min	RT end, min
1	NAGA galNAc-O-Ser M+H+	Pos	309.13	126.05	10	15	2.3	5
2	NAGA galNAc-O-Ser M+H+	Pos	309.13	186.07	10	15	2.3	5
3	NAGA GalNAc-O-Thr M+H+	Pos	323.14	126.05	10	15	2.3	5
4	NAGA GalNAc-O-Thr M+H+	Pos	323.14	186.08	10	15	2.3	5
5	Alpha-mann hex2-hexNAc M+H+	Pos	546.2	138.06	40	40	2.3	5
6	Alpha-mann hex2-hexNAc M+H+	Pos	546.2	204.09	40	20	2.3	5
7	GM2 hex2-hexNAc2 M+H+	Pos	749.28	204.09	30	30	2.3	5
8	GM2 hex2-hexNAc2 M+H+	Pos	749.28	366.3	30	20	2.3	5
9	Fabry Lyso-Gb3 d18:1 M+H+	Pos	786.47	264.33	31	32	2.3	5
10	Fabry Lyso-Gb3 d18:1 M+H+	Pos	786.47	282.36	31	34	2.3	5
11	Aspartyl NeuAC-hex-hexNAc-Asn M+H+	Pos	789.29	274.09	50	30	2.3	5
12	Aspartyl NeuAC-hex-hexNAc-Asn M+H+	Pos	789.29	336.14	50	15	2.3	5
13	Aspartyl NeuAC-hex2-hexNAc2-Asn M+H+	Pos	1154.42	204.09	50	50	2.3	5
14	Aspartyl NeuAC-hex2-hexNAc2-Asn M+H+	Pos	1154.42	366.14	50	30	2.3	5
15	Aspartyl NeuAC-hex2-hexNAc2-Asn M+H+	Pos	1154.42	657.23	50	20	2.3	5
16	Sialidosis neuAC-hex3-hexNAc2 M+H+	Pos	1202.42	204.08	20	50	2.3	5
17	Sialidosis neuAC-hex3-hexNAc2 M+H+	Pos	1202.42	366.14	20	40	2.3	5

	Compound	Polarity	Q1	Q3	Cone (V)	Collision (eV)	RT start, min	RT end, min
18	Aspartyl glcNAc-Asn M+H+	Pos	336.14	126.1	30	20	2.9	5.8
19	Aspartyl glcNAc-Asn M+H+	Pos	336.14	133.1	30	10	2.9	5.8
20	Fuco fuc-hexNAc-Asn M+H+	Pos	482.2	126.05	25	35	2.9	5.8
21	Fuco fuc-hexNAc-Asn M+H+	Pos	482.2	133.06	20	20	2.9	5.8
22	beta-mannosidosis man-glcNAc M+H+	Pos	384.15	186.08	20	15	1.8	3.8
23	beta-mannosidosis man-glcNAc M+H+	Pos	384.15	204.09	20	15	1.8	3.8
24	Niemann-Pick AB Lyso-sphingomyelin M+H+	Pos	465.37	124.98	35	52	1.8	3.8
25	Niemann-Pick AB Lyso-sphingomyelin M+H+	Pos	465.37	184.09	35	20	1.8	3.8
26	glucosyl and galactosyl-sphingosine M+H+	Pos	462.4	282.37	38	22	1.1	3
27	glucosyl and galactosyl-sphingosine M+H+	Pos	462.4	444.4	24	12	1.1	3
28	psychosine d5 M+H+	Pos	467.44	287.38	38	22	1.1	3
29	psychosine d5 M+H+	Pos	467.44	449.44	38	14	1.1	3
30	glucosylsphingosine 13c6 M+H+	Pos	468.4	282.3	30	18	1.1	3
31	glucosylsphingosine 13c6 M+H+	Pos	468.4	450.37	30	14	1.1	3
32	Fabry Ceramide Trihexoside d18:1/16:0 M+H+	Pos	1024.67	520.5	30	30	1.1	3
33	Fabry Ceramide Trihexoside d18:1/16:0 M+H+	Pos	1024.67	844.62	30	30	1.1	3
34	Fabry Ceramide Trihexoside d18:1/22:0 M+H+	Pos	1108.77	604.6	30	30	1.1	3
35	Alpha-mann hex3-hexNAc M+H+	Pos	708.26	204.09	40	30	3.8	6
36	Alpha-mann hex3-hexNAc M+H+	Pos	708.26	222.1	40	20	3.8	6
37	Aspartyl hex2-hexNAc2-asn M+H+	Pos	863.33	204.09	40	20	3.8	6
38	Aspartyl hex2-hexNAc2-asn M+H+	Pos	863.33	366.14	40	30	3.8	6
39	Alpha-mann hex4-hexNAc M+H+	Pos	870.31	204.09	40	30	3.8	6
40	Alpha-mann hex4-hexNAc M+H+	Pos	870.31	222.1	40	20	3.8	6
41	GM1 hex3-hexNAc2 M+H+	Pos	911.33	138.1	44	76	3.8	6
42	GM1 hex3-hexNAc2 M+H+	Pos	911.33	366.14	44	35	3.8	6
43	GM1 hex3-hexNAc2 M+Na+	Pos	933.31	388.12	45	60	3.8	6
44	Fuco fuc-hexNAc2-hex3 M+H+	Pos	1057.39	366.17	25	35	3.8	6
45	Fuco fuc-hexNAc2-hex3 M+H+	Pos	1057.39	512.59	25	15	3.8	6
46	GM1 hex4-hexNAc2 M+H+	Pos	1073.38	204.08	50	45	3.8	6
47	GM1 hex4-hexNAc2 M+H+	Pos	1073.38	366.14	50	35	3.8	6
48	GM1 hex4-hexNAc2 M+Na+	Pos	1095.36	388.12	50	80	3.8	6
49	GM1 hex4-hexNAc2 M+Na+	Pos	1095.36	550.17	50	80	3.8	6
50	GM2 hex3-hexNAc3 M+H+	Pos	1114.42	204.09	80	45	3.8	6
51	GM2 hex3-hexNAc3 M+H+	Pos	1114.42	911.34	30	15	3.8	6
52	GM2 hex3-hexNAc4 M+H+	Pos	1317.49	204.09	80	50	3.8	6
53	GM2 hex3-hexNAc4 M+H+	Pos	1317.49	1114.42	30	20	3.8	6

	Compound	Polarity	Q1	Q3	Cone (V)	Collision (eV)	RT start, min	RT end, min
54	GM1 hex5-hexNAc3 M+H+	Pos	1438.52	204.08	100	50	3.8	6
55	GM1 hex5-hexNAc3 M+H+	Pos	1438.52	366.14	100	35	3.8	6
56	Hexose M-H	Neg	179	58.9	32	16	0.4	2.5
57	Hexose M-H	Neg	179	88.93	32	6	0.4	2.5
58	Galactitol M-H	Neg	181.05	58.97	5	20	0.4	2.5
59	Galactitol M-H	Neg	181.05	100.91	5	14	0.4	2.5
60	MPS 6 hexNAc-4-6-disulfate M-2H	Neg	189.49	96.96	10	10	0.4	2.5
61	MPS 6 hexNAc-4-6-disulfate M-2H	Neg	189.49	138.97	10	10	0.4	2.5
62	MPS 3 hexN-sulfate M-H	Neg	258.02	79.95	30	30	0.4	2.5
63	MPS 3 hexN-sulfate M-H	Neg	258.02	119.05	30	30	0.4	2.5
64	MPS 3 hexN-sulfate M-H	Neg	258.02	137.98	30	25	0.4	2.5
65	MPS 4 hex-S-hexNAc-S M-2H	Neg	270.51	96.96	40	20	0.4	2.5
66	MPS 4 hex-S-hexNAc-S M-2H	Neg	270.51	259.01	40	10	0.4	2.5
67	hexNAc-x-sulfate M-H	Neg	300.03	96.96	10	20	0.4	2.5
68	hexNAc-x-sulfate M-H	Neg	300.03	138.98	10	20	0.4	2.5
69	MPS 6 hexNAc-4-6-disulfate M-H	Neg	379.99	300.03	10	10	0.4	2.5
70	Sialic acid M-H	Neg	308.1	87.01	30	16	0.8	3.6
71	Sialic acid M-H	Neg	308.1	170.05	30	12	0.8	3.6
72	MPS 7 UA-hexNAc-S M-H	Neg	476.07	96.96	40	40	0.8	3.6
73	MPS 7 UA-hexNAc-S M-H	Neg	476.07	193.03	40	20	0.8	3.6
74	MPS 7 UA-hexNAc-S M-H	Neg	476.07	254.98	40	20	0.8	3.6
75	MPS 7 UA-hexNAc-S M-H	Neg	476.07	300.03	40	20	0.8	3.6
76	Aspartyl glcNAc-Asn M-H	Neg	334.13	113.04	40	15	2.2	6
77	Aspartyl glcNAc-Asn M-H	Neg	334.13	196.07	40	15	2.2	6
78	Aspartyl hex-glcNAc-Asn M-H	Neg	496.18	196.07	25	30	2.2	6
79	Aspartyl hex-glcNAc-Asn M-H	Neg	496.18	214.08	25	20	2.2	6
80	hexose3 M-H	Neg	503.16	88.98	35	48	2.2	6
81	hexose3 M-H	Neg	503.16	161.04	35	20	2.2	6
82	hexose3 M-H	Neg	503.16	179.07	35	30	2.2	6
83	MOGS Pompe hexose4 M-H	Neg	665.21	88.98	44	48	2.2	6
84	MOGS Pompe hexose4 M-H	Neg	665.21	161.04	44	20	2.2	6
85	MOGS Pompe hexose4 M-H	Neg	665.21	179.07	44	30	2.2	6
86	hexose5 M-H	Neg	827.27	88.98	45	48	2.2	6
87	hexose5 M-H	Neg	827.27	161.04	45	20	2.2	6
88	hexose5 M-H	Neg	827.27	179.07	45	30	2.2	6
89	MPS 4 and 6 hexNAc-S-UA-hexNAc-S M-2H	Neg	379.04	96.96	40	30	1.2	3
90	MPS 4 and 6 hexNAc-S-UA-hexNAc-S M-2H	Neg	379.04	198.99	40	20	1.2	3
91	MPS 4 and 6 hexNAc-S-UA-hexNAc-S M-2H	Neg	379.04	300.03	40	10	1.2	3

	Compound	Polarity	Q1	Q3	Cone (V)	Collision (eV)	RT start, min	RT end, min
92	MPS 7 UA-hexNAc M-H	Neg	396.11	113.02	50	10	1.2	3
93	MPS 7 UA-hexNAc M-H	Neg	396.11	193.03	50	10	1.2	3
94	MPS 3 hexN-S-UA M-H	Neg	434.06	113	40	25	1.2	3
95	MPS 3 hexN-S-UA M-H	Neg	434.06	131.03	40	25	1.2	3
96	MPS 3 hexN-S-UA M-H	Neg	434.06	157	40	20	1.2	3
97	MPS 3 hexN-S-UA M-H	Neg	434.06	175.02	40	20	1.2	3
98	MPS 4 neuAC-hex-hexNAc-S M-H	Neg	753.18	96.96	20	60	1.2	3
99	MPS 4 neuAC-hex-hexNAc-S M-H	Neg	753.18	282.02	20	40	1.2	3
100	MPS 4 neuAC-hex-hexNAc-S M-H	Neg	753.18	462.09	20	20	1.2	3
101	MPS 4 and 6 hexNAc-S-UA-hexNAc-S M-H	Neg	759.1	175.02	40	50	1.2	3
102	MPS 4 and 6 hexNAc-S-UA-hexNAc-S M-H	Neg	759.1	396.11	40	40	1.2	3
103	MPS 4 and 6 hexNAc-S-UA-hexNAc-S M-H	Neg	759.1	599.2	40	30	1.2	3
104	MPS 1 UA-hexNAc-S-UA-hexNAc-S M-2H	Neg	467.06	198.99	25	30	1.7	4.4
105	MPS 1 UA-hexNAc-S-UA-hexNAc-S M-2H	Neg	467.06	300.03	55	15	1.7	4.4
106	MPS 7 hexNAc-UA-hexNAc M-H	Neg	599.19	175.02	60	20	1.7	4.4
107	MPS 7 hexNAc-UA-hexNAc M-H	Neg	599.19	396.11	60	20	1.7	4.4
108	MPS 1 and 2 UA-hexNAc-S-UA M-H	Neg	652.1	175.02	55	40	1.7	4.4
109	MPS 1 and 2 UA-hexNAc-S-UA M-H	Neg	652.1	193.03	55	40	1.7	4.4
110	MPS 1 and 2 UA-hexNAc-S-UA M-H	Neg	652.1	396.11	55	30	1.7	4.4
111	MPS 1 and 2 UA-hexNAc-S-UA M-H	Neg	652.1	572.14	55	10	1.7	4.4
112	MPS 3 813	Neg	813.16	175.02	40	40	1.7	4.4
113	MPS 3 813	Neg	813.16	557.18	40	30	1.7	4.4
114	MPS 3b hexNAc-S-UA-hexNAc-UA M-H	Neg	855.18	175.02	15	45	1.7	4.4
115	MPS 3b hexNAc-S-UA-hexNAc-UA M-H	Neg	855.18	775.2	20	15	1.7	4.4
116	MPS 4 hex3-hexNAc2-S M-H	Neg	989.26	96.96	80	70	1.7	4.4
117	MPS 4 hex3-hexNAc2-S M-H	Neg	989.26	989.27	80	12	1.7	4.4