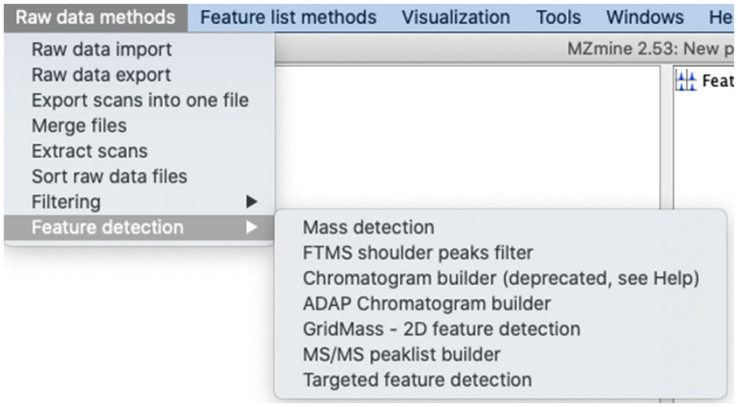
Class on 02-01-21

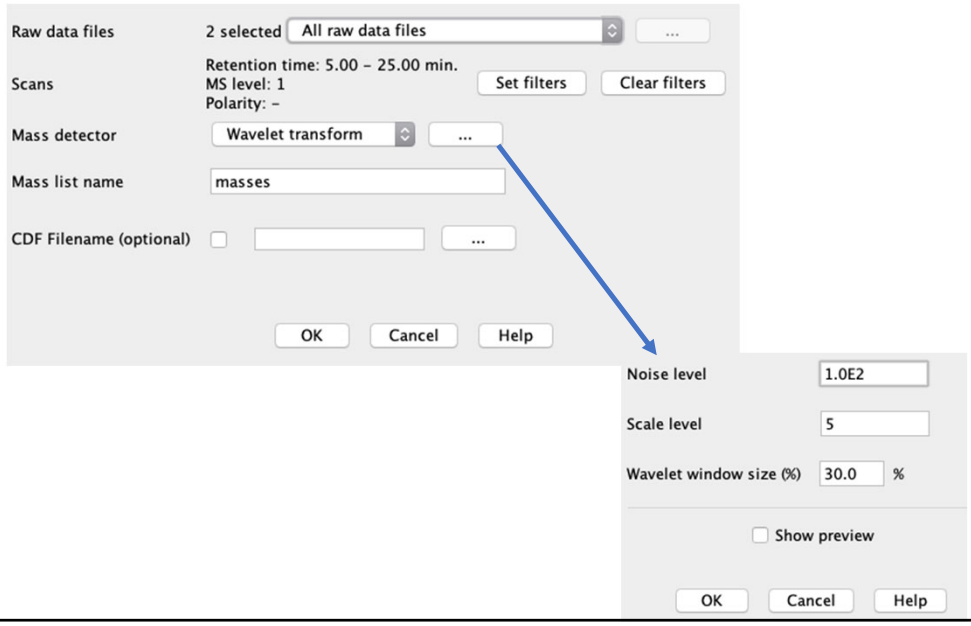
1

Detecting the masses

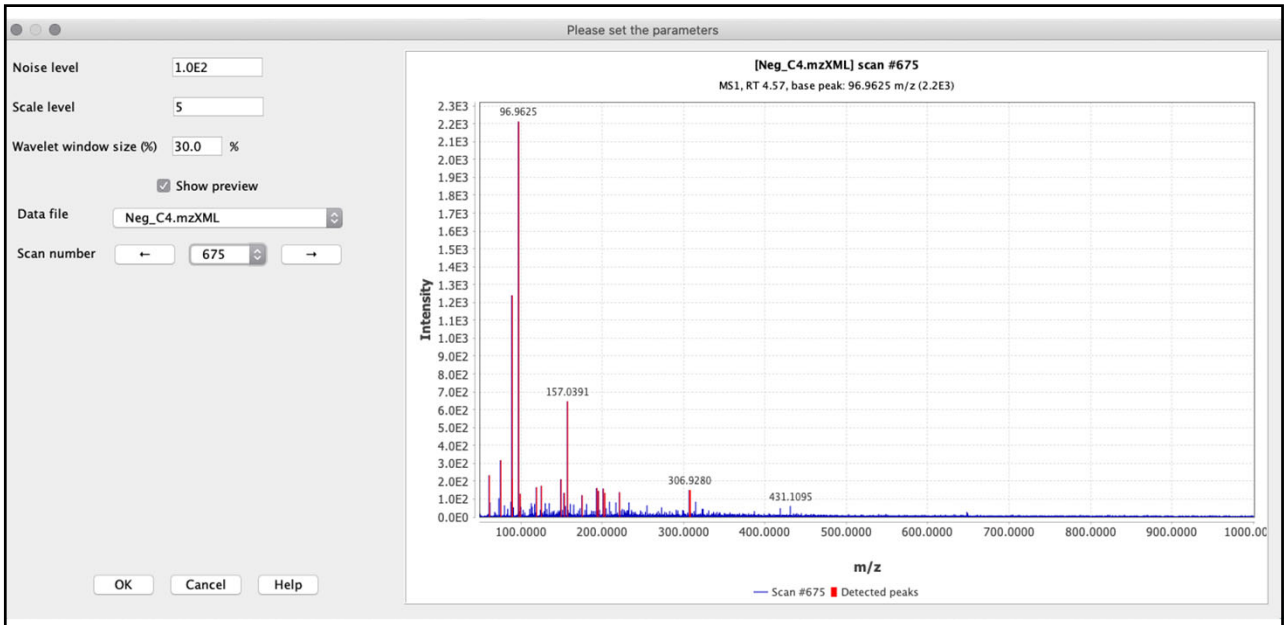


2

Identifying the masses

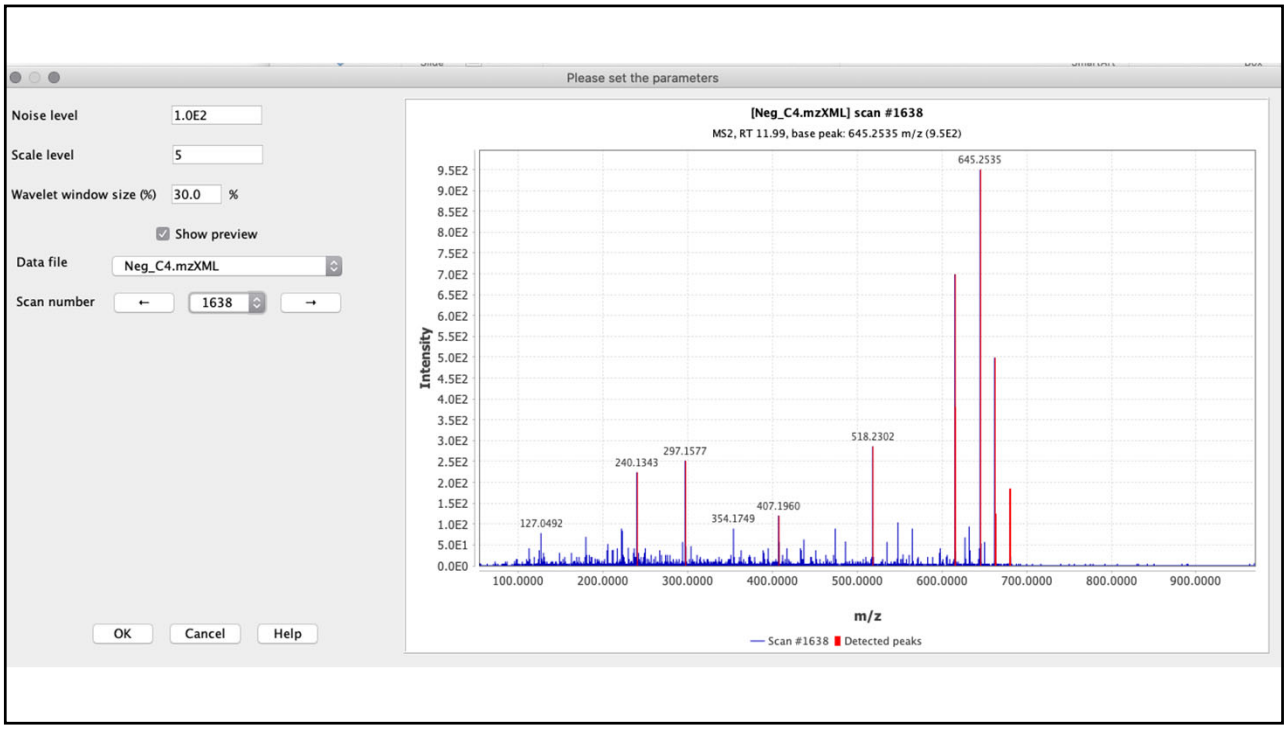


3

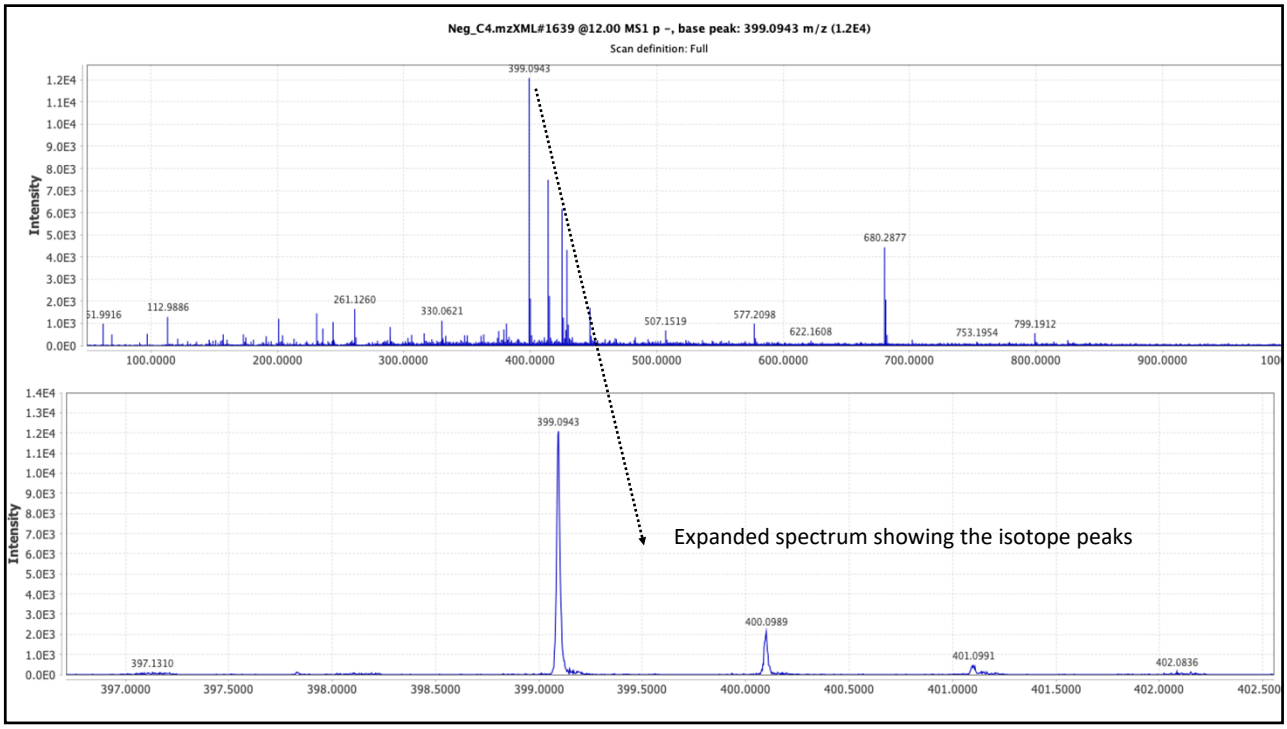


The red ions are those that rise above the background that has been set at 1e2. The blue peaks are regarded as noise.

4


Please set the parameters
Noise level
1.0E2
Scale level
5
Wavelet window size (%)
30.0
%
Show preview
Data file
Neg_C4.mzXML
Scan number
1638
OK
Cancel
Help
[Neg_C4.mzXML] scan #1638
MS2, RT 11.99, base peak: 645.2535 m/z (9.5E2)
Intensity
m/z
645.2535
518.2302
297.1577
240.1343
407.1960
354.1749
127.0492
Scan #1638
Detected peaks

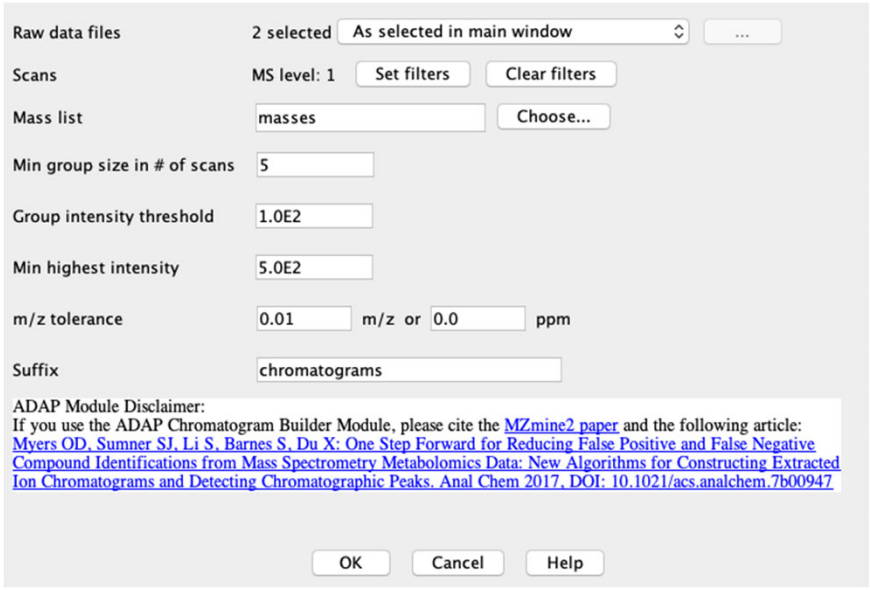
5


Neg_C4.mzXML#1639 @12.00 MS1 p −, base peak: 399.0943 m/z (1.2E4)
Scan definition: Full
Intensity
399.0943
680.2877
261.1260
112.9886
330.0621
507.1519
577.2098
622.1608
753.1954
799.1912
Expanded spectrum showing the isotope peaks
400.0989
397.1310
401.0991
402.0836

6

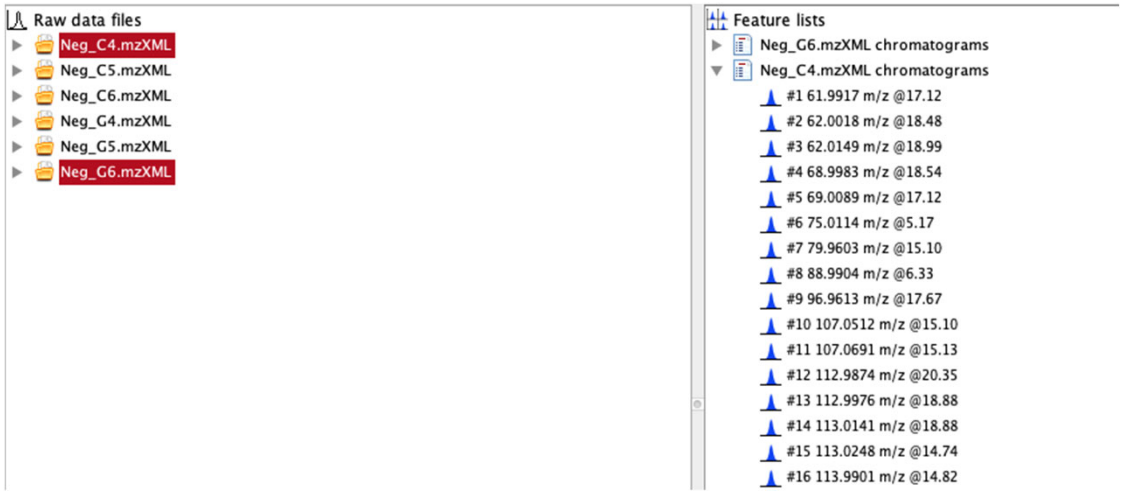
Using the masses to create chromatograms

Go to *Raw data methods, peak detection, ADAP chromatogram builder*



7

When the chromatogram building ends



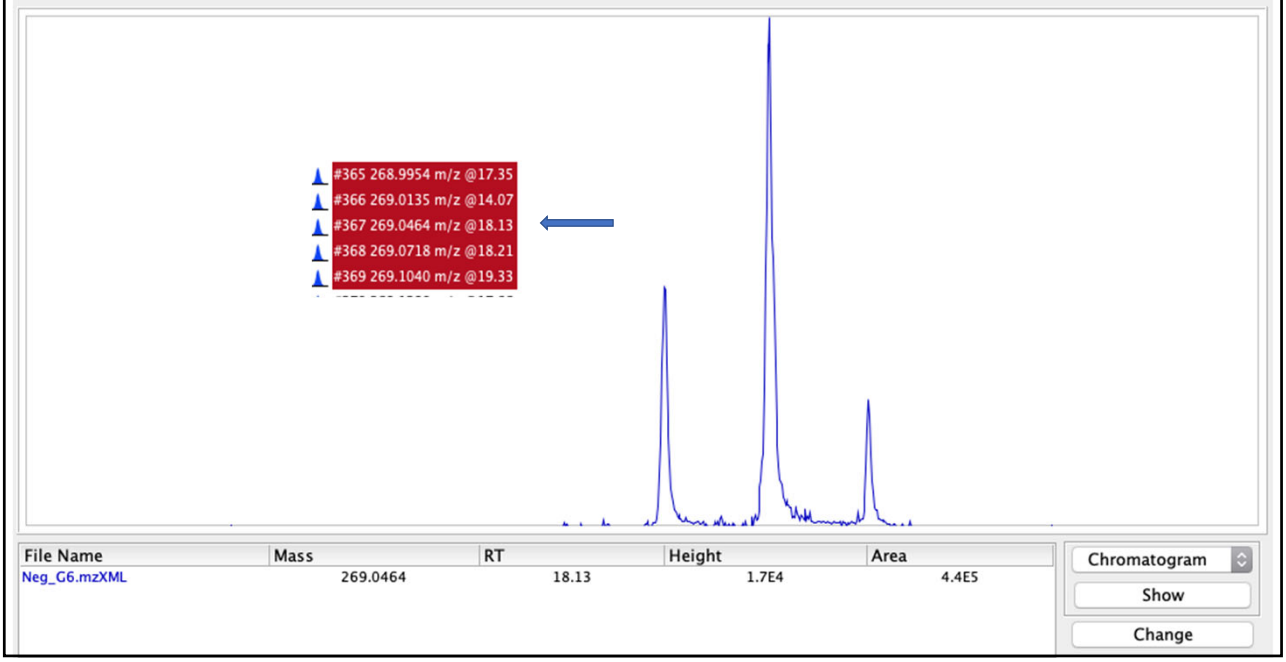
8

Genistein chromatograms

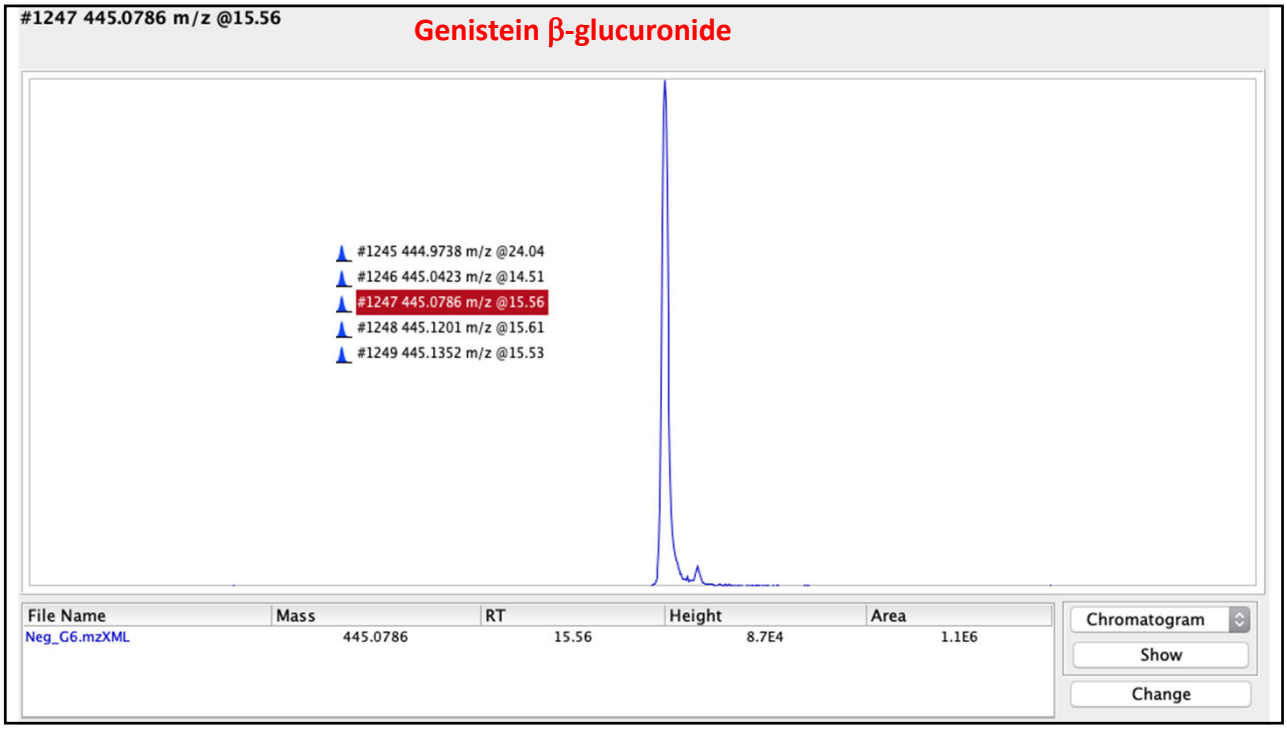
9

#367 269.0464 m/z @18.13

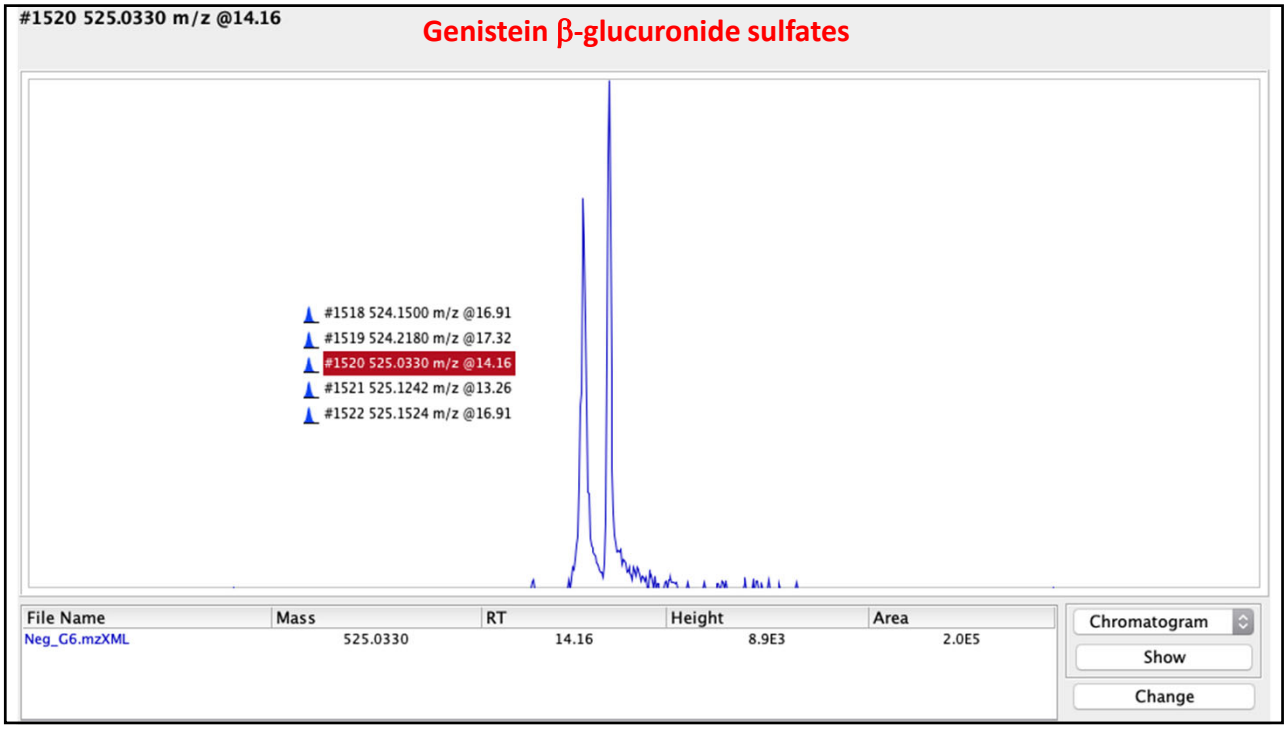
Genistein-containing compounds



10


#1247 445.0786 m/z @15.56
Genistein β-glucuronide
#1245 444.9738 m/z @24.04
#1246 445.0423 m/z @14.51
#1247 445.0786 m/z @15.56
#1248 445.1201 m/z @15.61
#1249 445.1352 m/z @15.53
File Name
Mass
RT
Height
Area
Neg_G6.mzXML
445.0786
15.56
8.7E4
1.1E6
Chromatogram
Show
Change

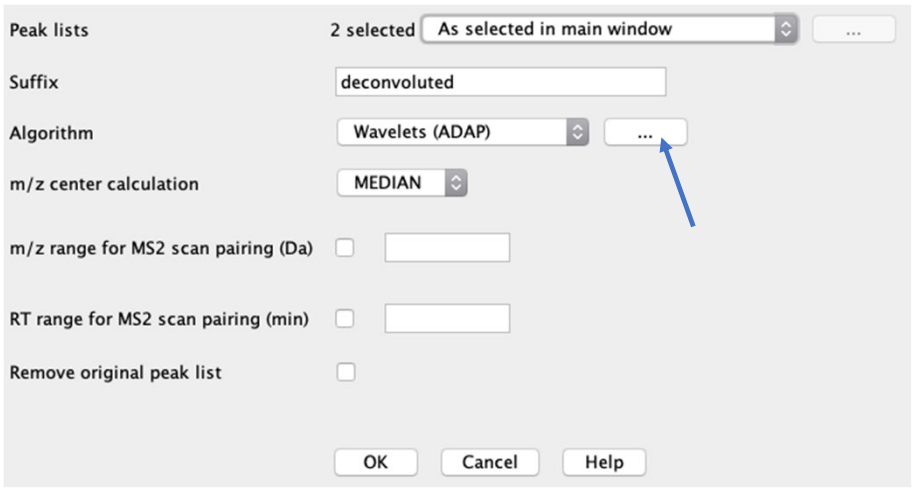
11


#1520 525.0330 m/z @14.16
Genistein β-glucuronide sulfates
#1518 524.1500 m/z @16.91
#1519 524.2180 m/z @17.32
#1520 525.0330 m/z @14.16
#1521 525.1242 m/z @13.26
#1522 525.1524 m/z @16.91
File Name
Mass
RT
Height
Area
Neg_G6.mzXML
525.0330
14.16
8.9E3
2.0E5
Chromatogram
Show
Change

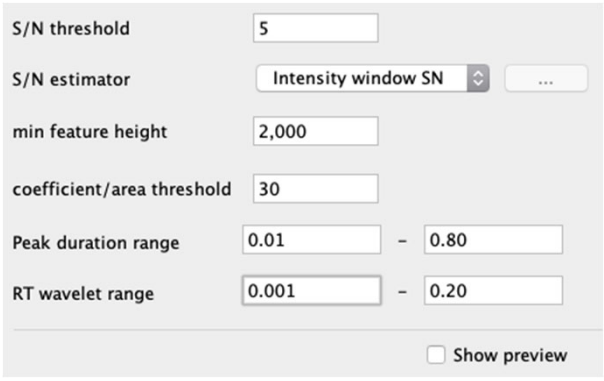
12

Chromatogram deconvolution

Go to *Feature list methods, feature detection, chromatogram deconvolution*

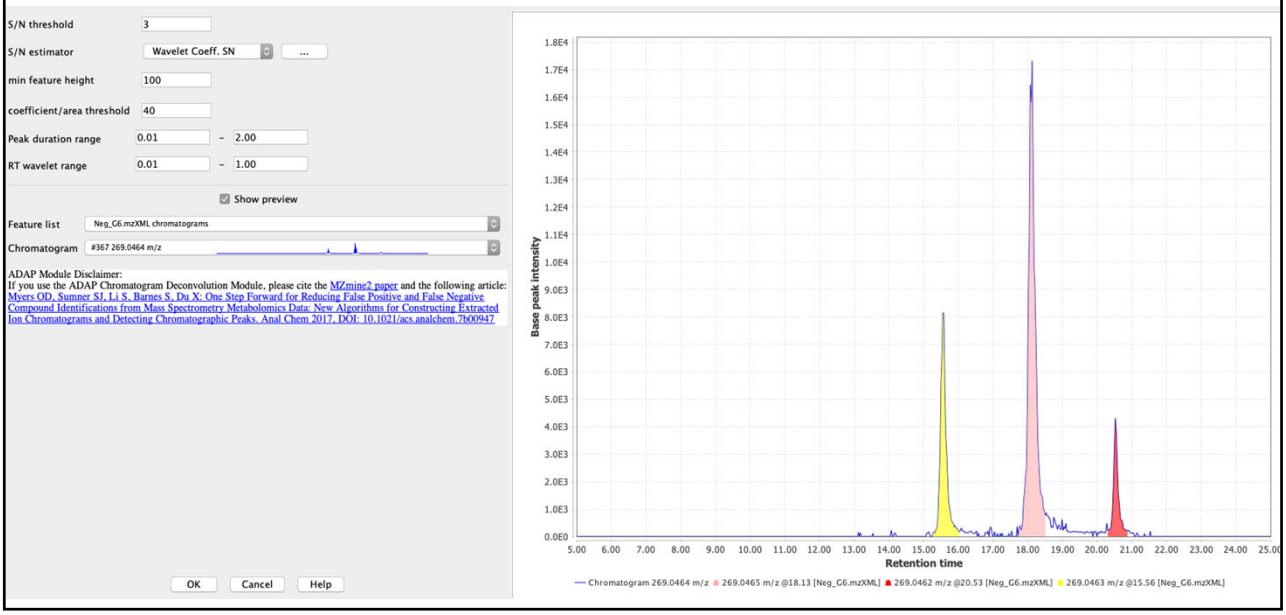


13



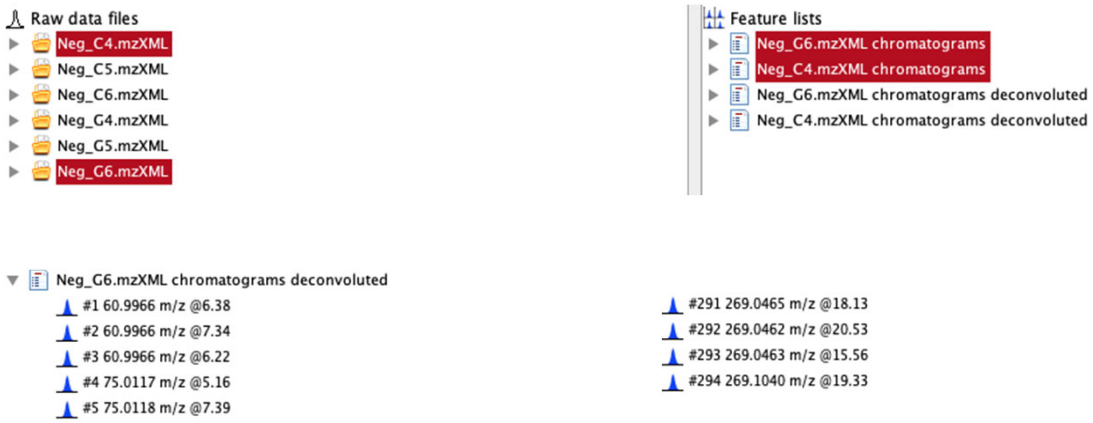
14

Preview of chromatographic deconvolution

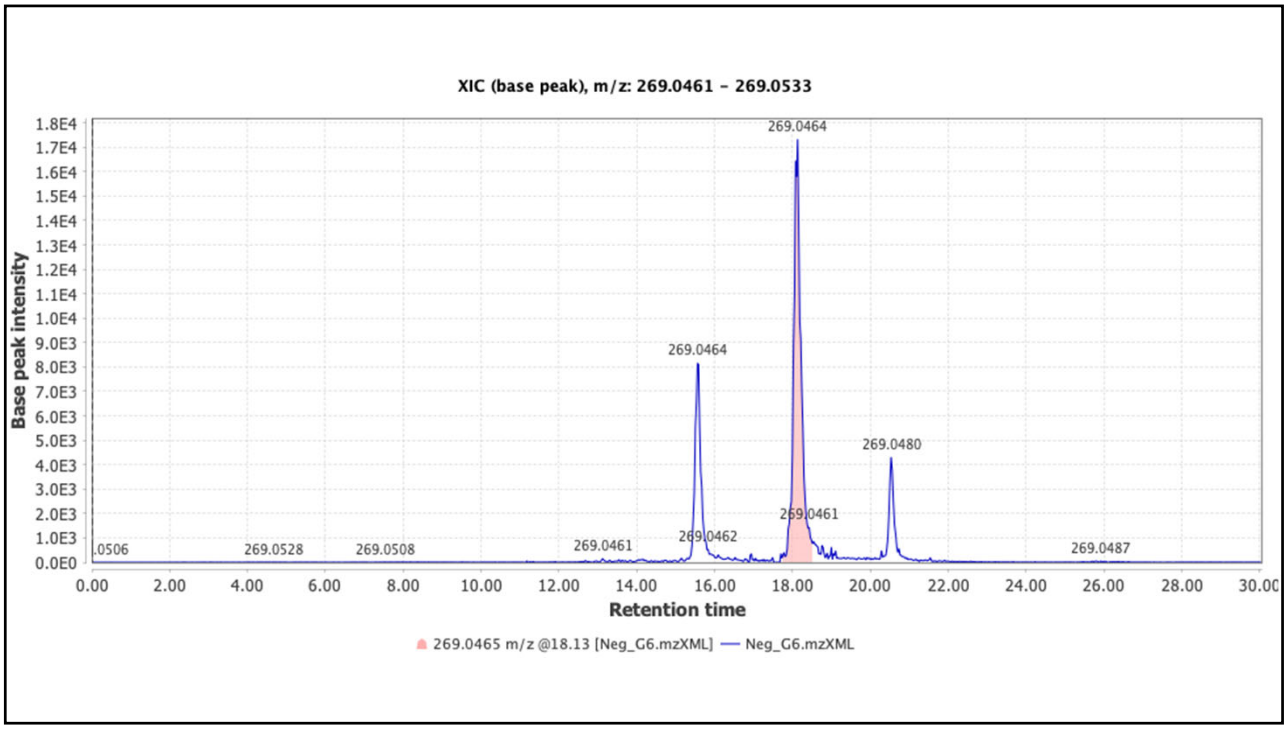


15

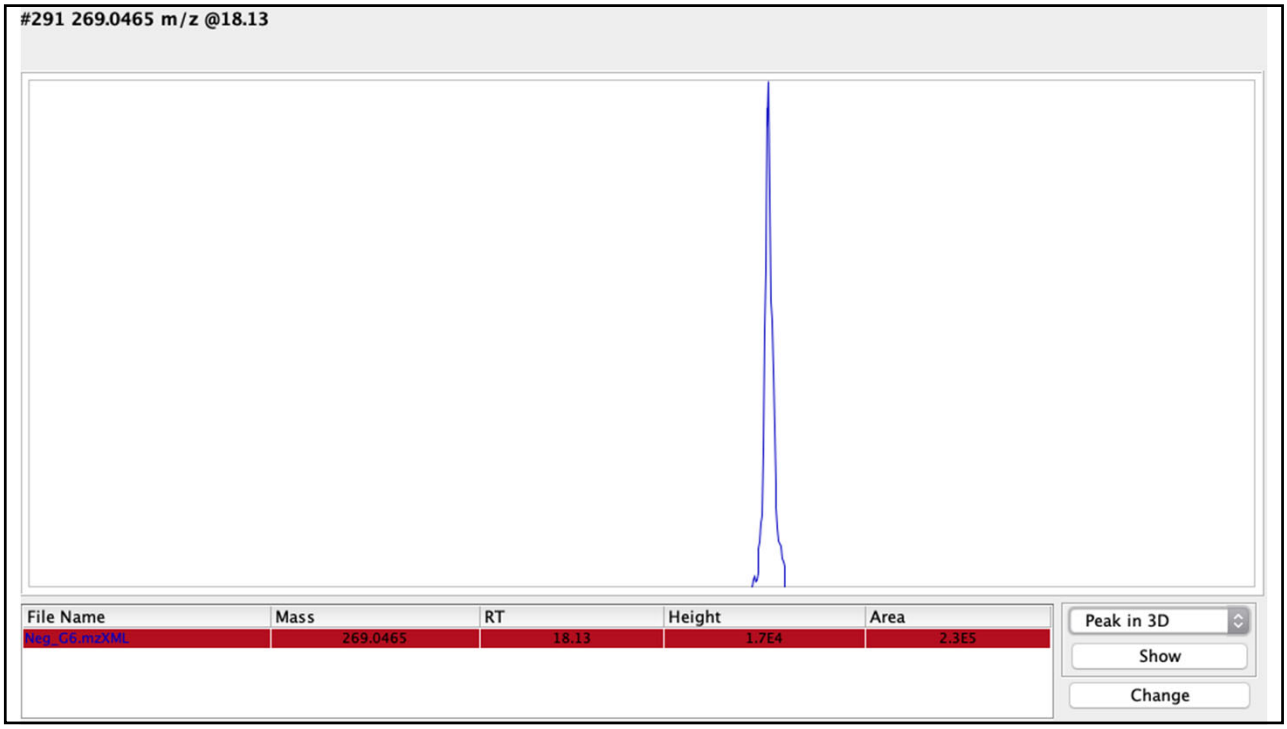
Chromatogram deconvolution output



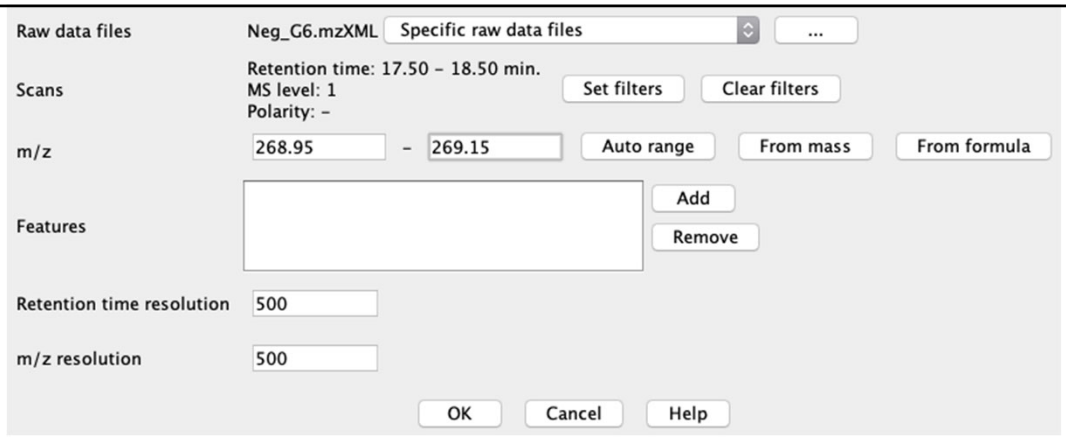
16


XIC (base peak), m/z: 269.0461 - 269.0533
Base peak intensity
1.8E4
1.7E4
1.6E4
1.5E4
1.4E4
1.3E4
1.2E4
1.1E4
1.0E4
9.0E3
8.0E3
7.0E3
6.0E3
5.0E3
4.0E3
3.0E3
2.0E3
1.0E3
0.0E0
269.0464
269.0464
269.0480
269.0461
269.0462
269.0461
.0506
269.0528
269.0508
269.0487
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Retention time
269.0465 m/z @18.13 [Neg_G6.mzXML]
Neg_G6.mzXML

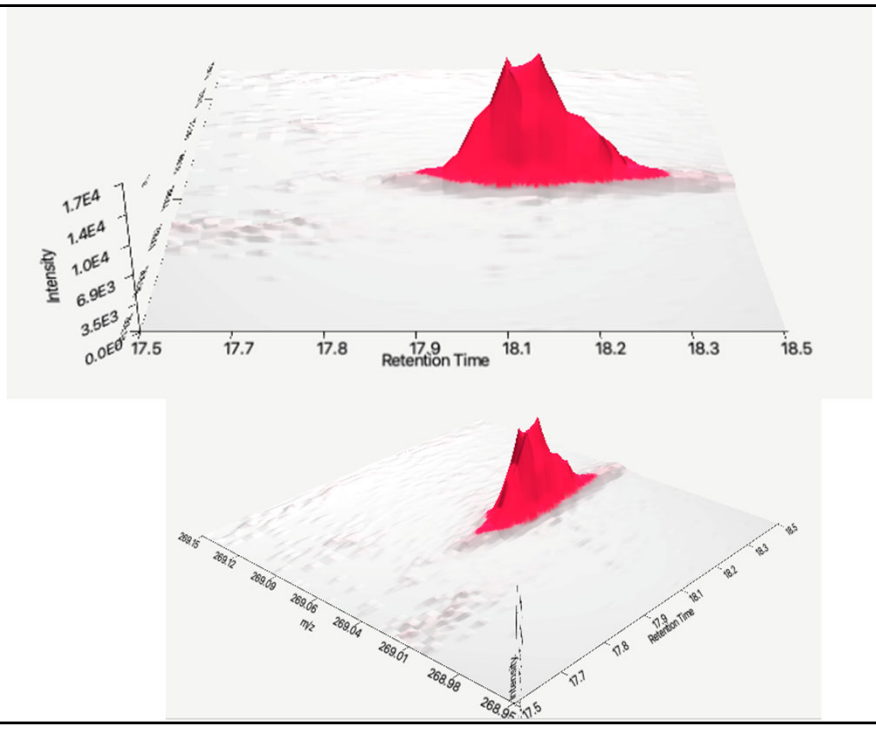
17


#291 269.0465 m/z @18.13
File Name
Mass
RT
Height
Area
Neg_G6.mzXML
269.0465
18.13
1.7E4
2.3E5
Peak in 3D
Show
Change

18


Raw data files
Neg_G6.mzXML
Specific raw data files
...
Scans
Retention time: 17.50 – 18.50 min.
MS level: 1
Polarity: –
Set filters
Clear filters
m/z
268.95
–
269.15
Auto range
From mass
From formula
Features
Add
Remove
Retention time resolution
500
m/z resolution
500
OK
Cancel
Help

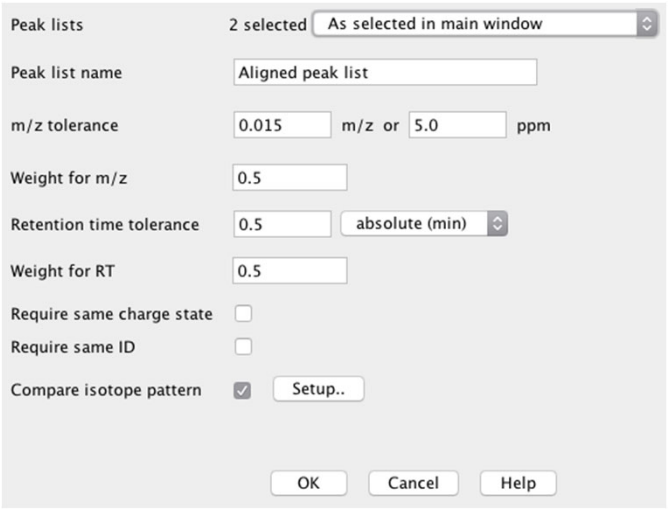
19


Intensity
1.7E4
1.4E4
1.0E4
6.9E3
3.5E3
0.0E0
17.5
17.7
17.8
17.9
18.1
18.2
18.3
18.5
Retention Time
m/z
269.15
269.12
269.09
269.06
269.04
269.01
268.98
268.95

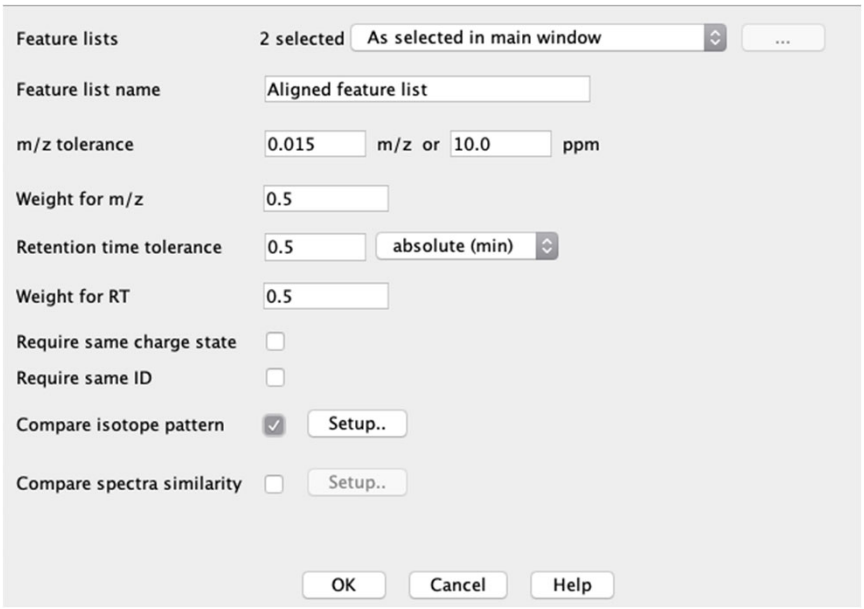
20

Peak and chromatogram alignment

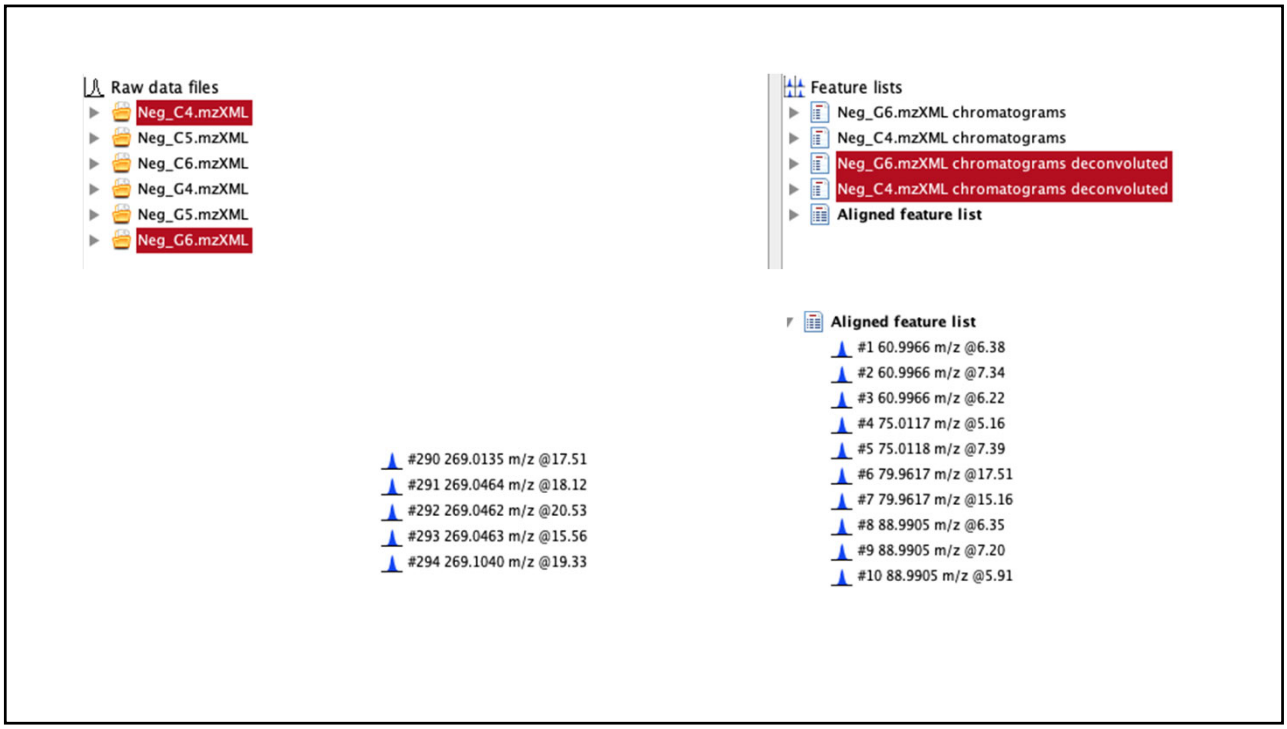
Go to *Feature list method, alignment, join aligner*



21



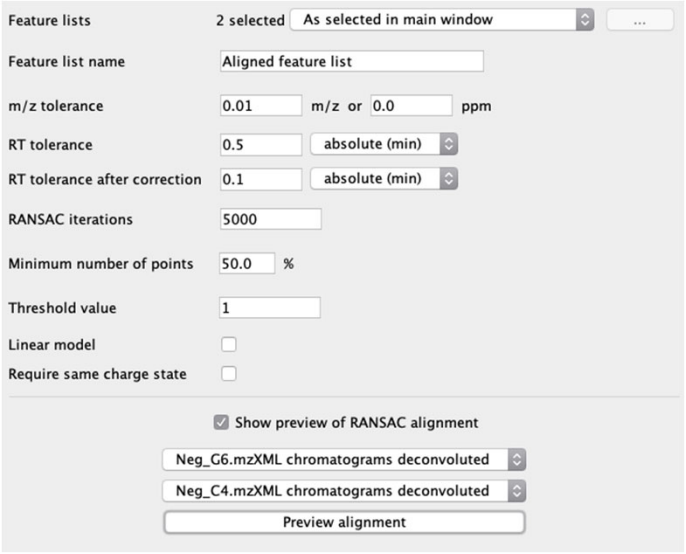
22



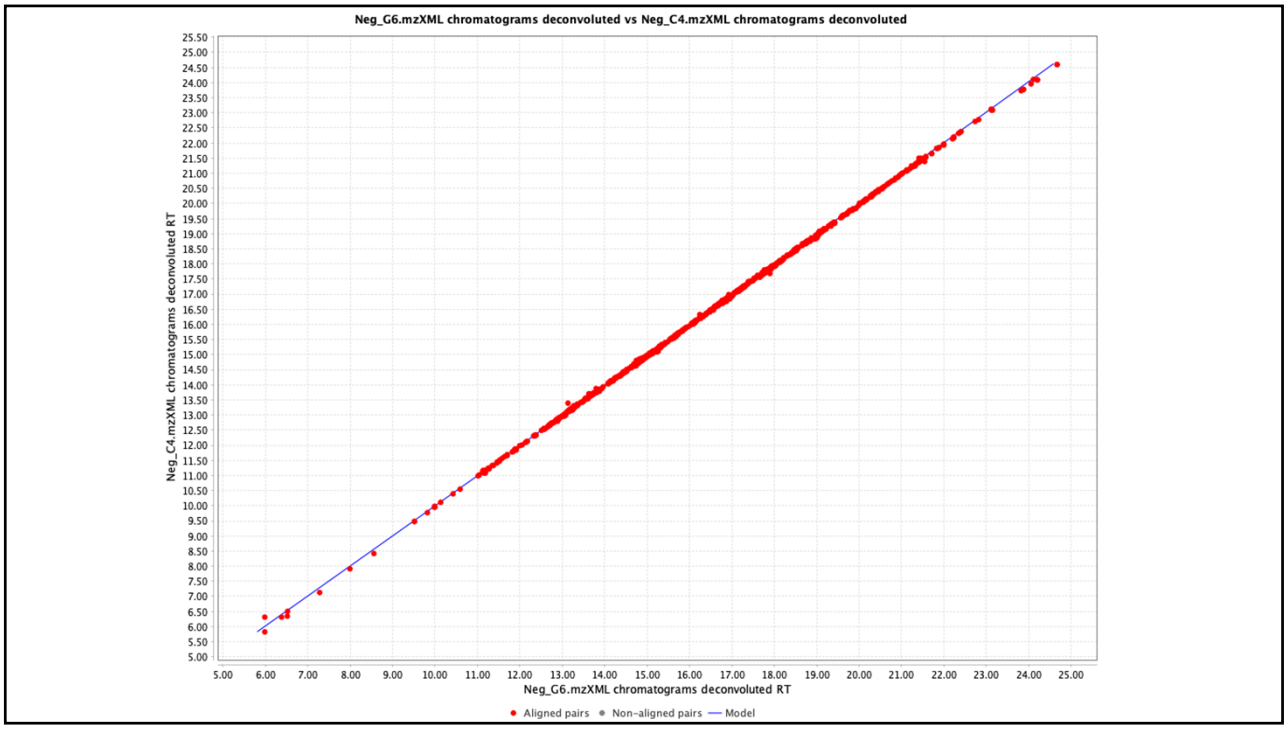
23

Peak and chromatogram alignment

Go to *Feature list method, alignment, RANSAC aligner*



24



25

Double click on Aligned peak list

ID: m/z: RT: Identity:

ID	Average m/z	Average RT	Peak shape	Neg_G6.mzXML Stat...	Neg_G6.mzXML Height	Neg_G6.mzXML Area	Neg_C4.mzXML Status	Neg_C4.mzXML Height	Neg_C4.mzXML Area
1259	778.3463	18.95			7.2E3	3.8E4		1.3E4	7.1E4
1260	793.2615	15.61			8.2E2	6.1E3			
1261	794.8021	9.50			8.1E2	5.1E3		1.7E3	1.1E4
1262	795.3037	9.50			5.6E2	3.7E3		1.3E3	7.9E3
1263	799.2599	10.57			1.2E3	7.6E3		1.2E3	9.8E3
1264	813.2678	17.65			8.1E2	5.1E3			
1265	823.2578	11.12			2.2E4	1.0E5		6.5E4	3.1E5
1266	879.2910	15.53			1.1E3	6.6E3			
1267	891.1604	15.59			1.5E4	1.1E5			
1268	892.1640	15.56			7.2E3	5.3E4			
1269	893.1763	15.56			5.7E3	3.8E4			
1270	894.1765	15.56			2.2E3	1.4E4			

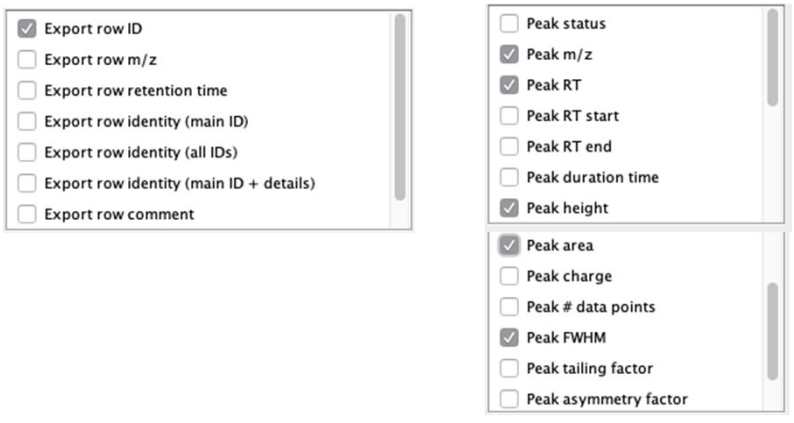
26

Class exercise

- Load the C4-C6 and G4-G6 .mzxml files
- Locate the ions that have the ion (in negative) for p-ethylphenol glucuronide ($C_{14}H_{18}O_7$) and p-ethylphenol sulfate ($C_8H_{10}O_4S$) - what are their *m/z* values?
  - Get MSMS spectra of each one
- Identify all the *masses* in each file -– from these generate chromatograms, and then deconvolute the chromatograms
- Output the data into a .csv file (choose row ID, m/z, retention time, peak height, peak area and FWHM)
- Sort the file by retention time – identify ions that are co-eluting and are isotopes.

27

Export to .CSV file



28