Knowledge that will change your world

The application of MZmine 2 to viewing metabolomics raw data

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The data sets come from this paper

PLOS ONE

RESEARCH ARTICLE

Impact of genistein on the gut microbiome of humanized mice and its role in breast tumor inhibition

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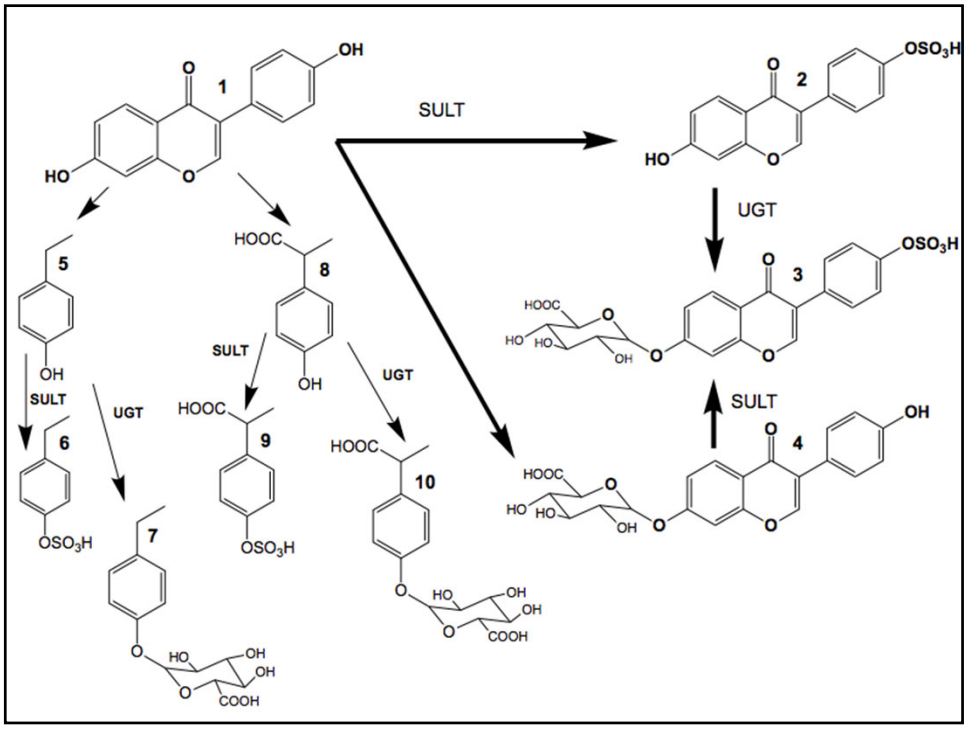
Check for updates

OPEN ACCESS



Answers to mass calculation question

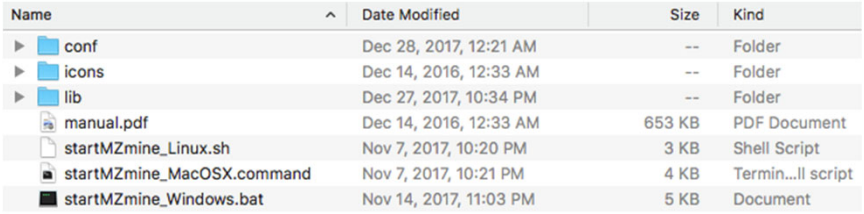
Reduce the problem - $C_{15}H_{10}O_5 + C_6H_{12}O_6 - H_2O = C_{21}H_{20}O_{10}$

- [M+H] Mass: 433.1129
- [M-H] Mass: 431.0983
- [M+H] Mass: 433.1035
- [M-H] Mass: 431.0889
- [M+H] Mass: 433.1129
- [M-H] Mass: 431.0984
- [M+H] Mass: 433.1129
- [M-H] Mass: 431.0984
- [M+H] Mass: 433.1129
- [M-H] Mass: 431.0984
- [M+H] Mass: 451.1235
- [M-H] Mass: 449.1089
- [M+H] Mass: 433.1129
- [M-H] Mass: 431.0984

Download MZmine 2.30

- Go to http://mzmine.github.io/
- Download
- Unzip the file and move the folder into Applications
  - There are three starting methods
    - Linux - startMZmine_Linux.sh
    - Mac - startMZmine_MacOSX.command
    - Windows - startMZmine_Windows.bat
  - Double click to start the program

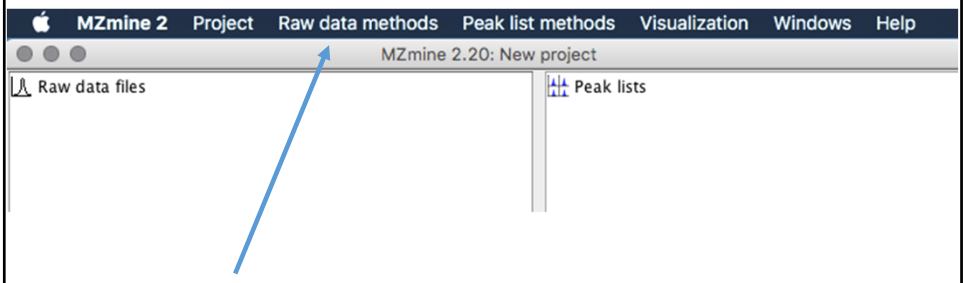
Starting point for MZmine



Name	Date Modified	Size	Kind
conf	Dec 28, 2017, 12:21 AM	--	Folder
icons	Dec 14, 2016, 12:33 AM	--	Folder
lib	Dec 27, 2017, 10:34 PM	--	Folder
manual.pdf	Dec 14, 2016, 12:33 AM	653 KB	PDF Document
startMZmine_Linux.sh	Nov 7, 2017, 10:20 PM	3 KB	Shell Script
startMZmine_MacOSX.command	Nov 7, 2017, 10:21 PM	4 KB	Termin...ll script
startMZmine_Windows.bat	Nov 14, 2017, 11:03 PM	5 KB	Document

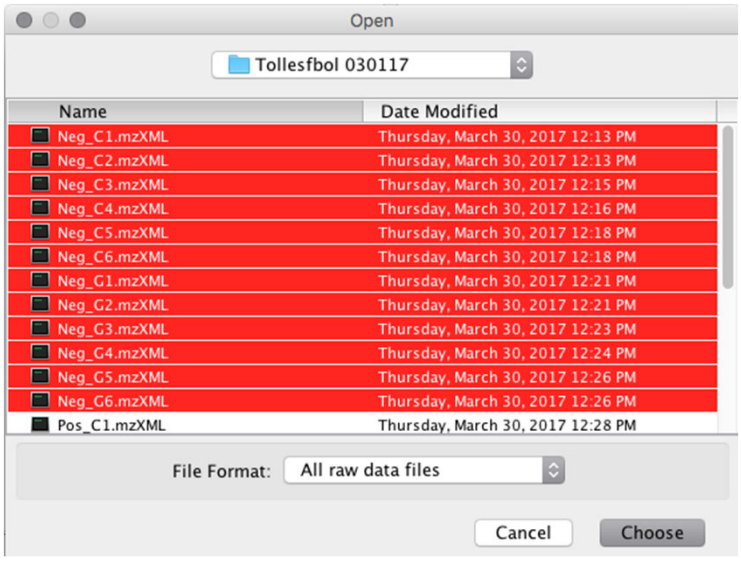
You will see Terminal open and the program load. Then Java will take over.

Starting off



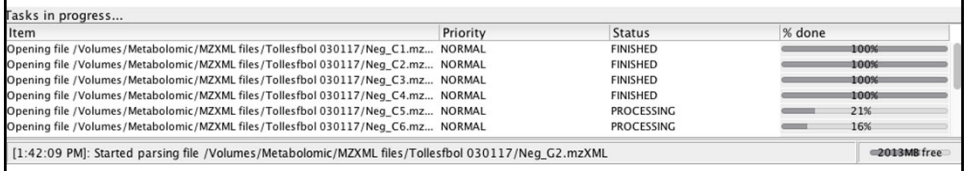
Click on Raw data methods and then select "raw data input" from the drop down box

Note the MZmine version number in 2019 is 2:37

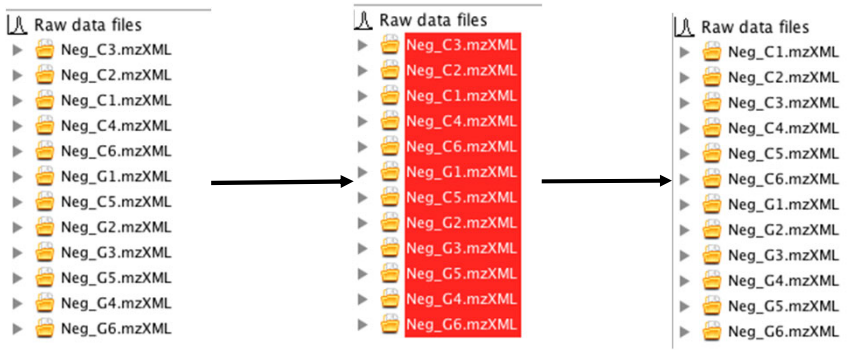


This is where the files are on my Mac. The highlighted ones are loaded by pressing <choose>. Note we just took two files (C1 and G1) this time.

Showing uploading process

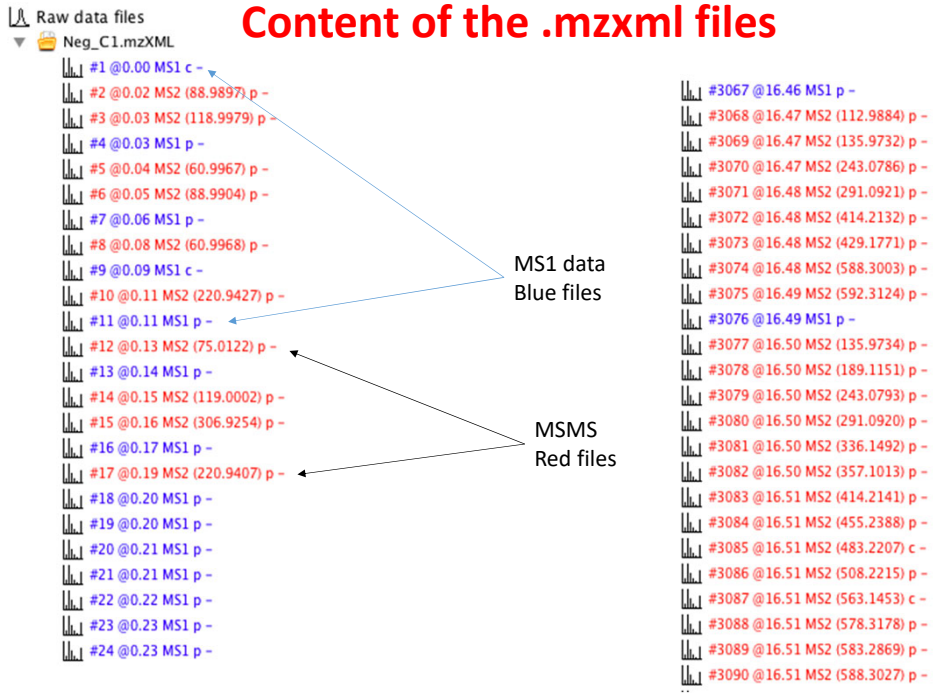


Files are now loaded

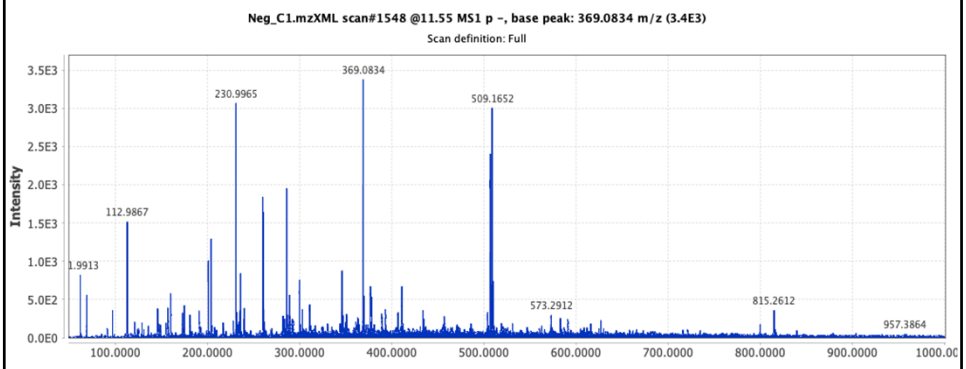


Highlight the files **Re-order the files**

Content of the .mzxml files

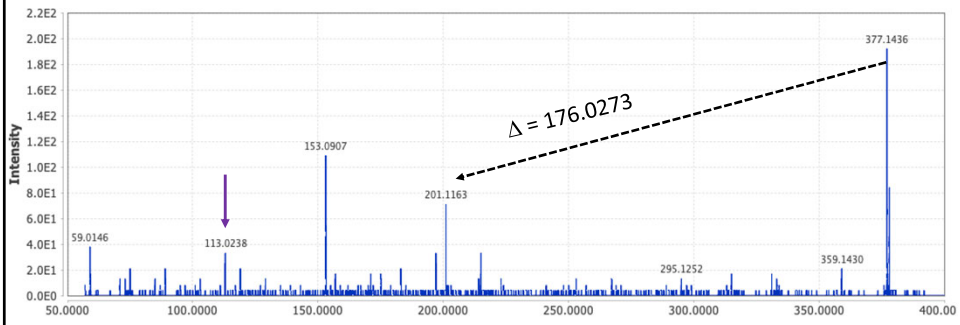


MS1 TOF spectrum of ions eluting at 11.55 min

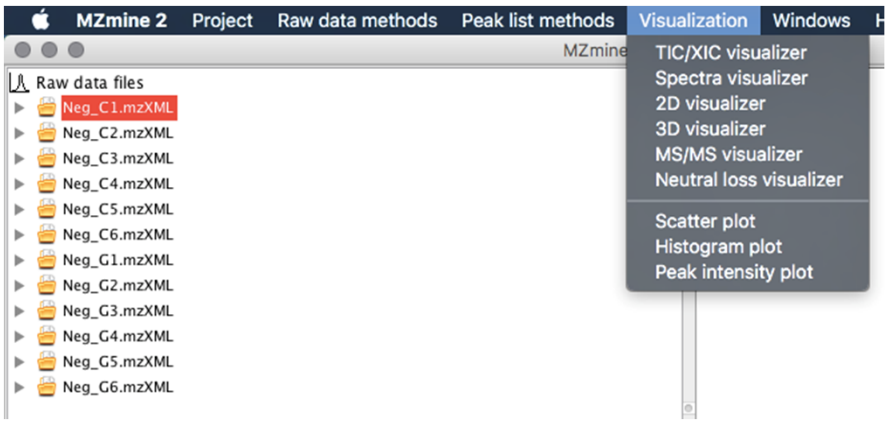


MSMS spectrum of ion eluting at 11.55 min

$C_6H_8O_6$ = 6 x 12 + 8 x 1.007825 + 6 x 15.99495
= 72 + 8.0626 + 95.9697
= 176.0323

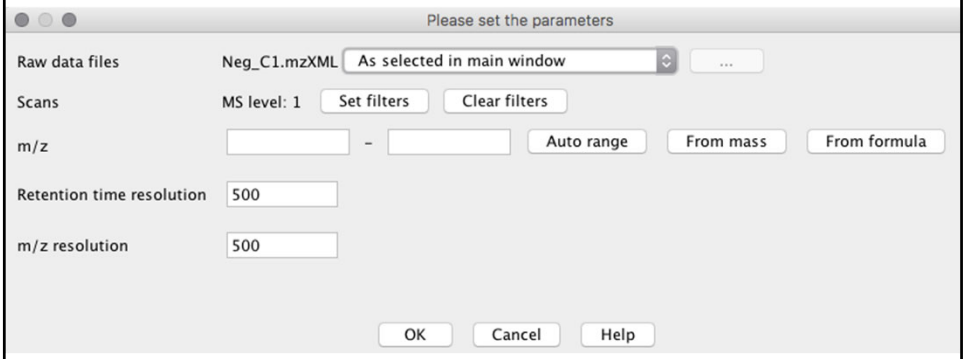


Visualization toolbar



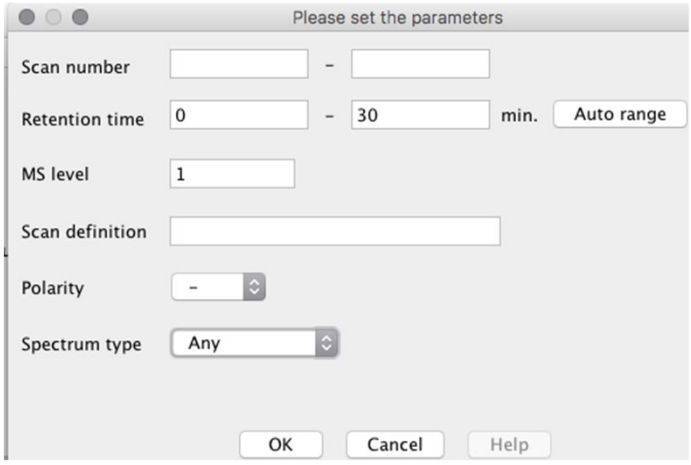
We'll start by selecting "3D visualizer" – this allows us to look at all the data

Selecting all the data

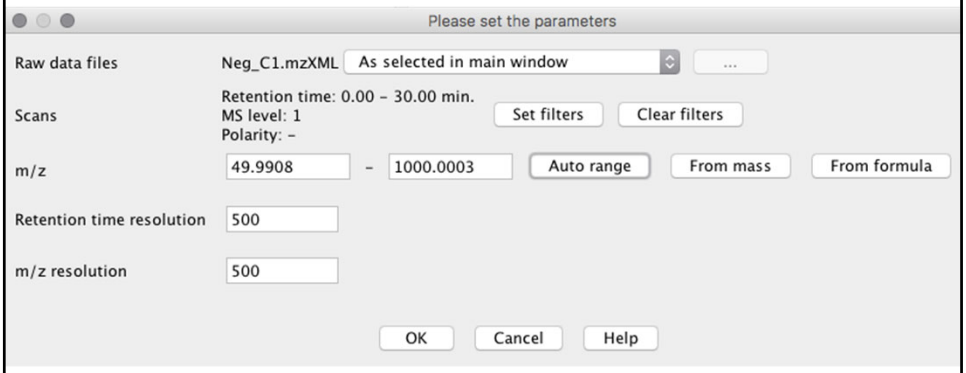


We'll select the file to open next. The 3D visualizer allows one at a time

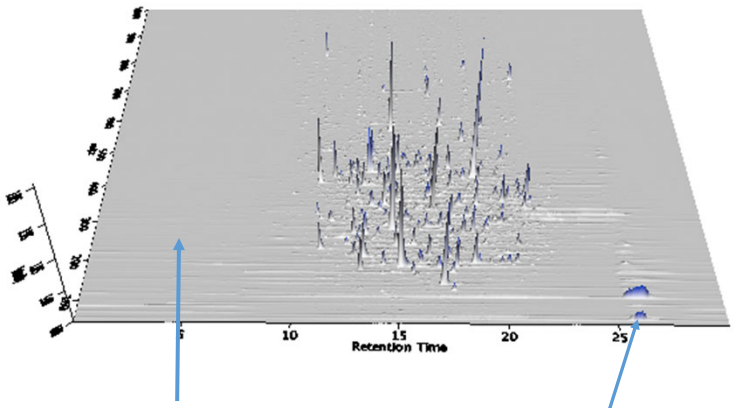
Setting 3D-parameters



Ready to view the 3D-plot



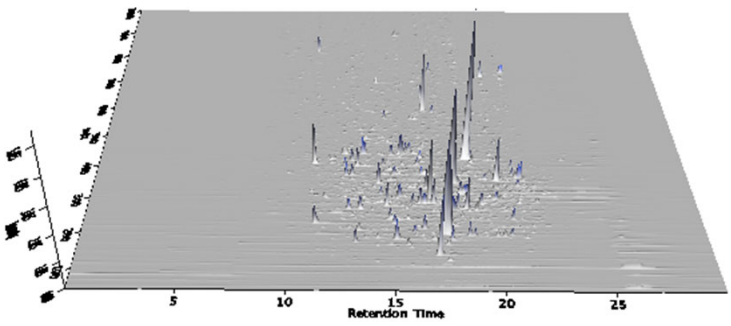
3D view of NegMode_C1



0-5 min – metabolites that didn't bind to the column

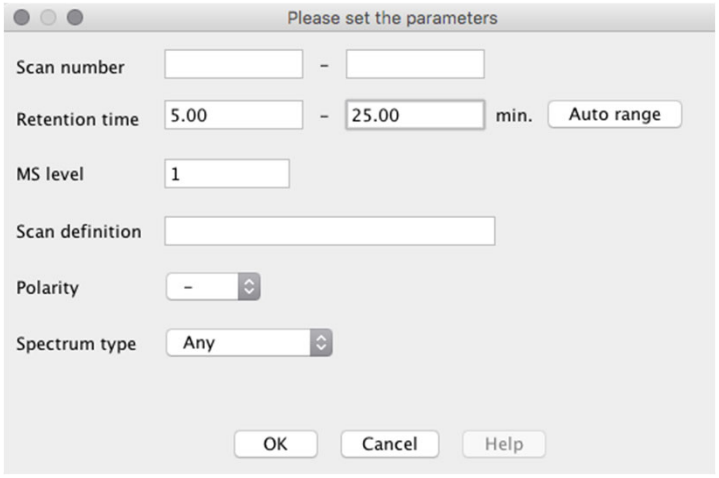
25-30 min – metabolites that stuck too hard to the column

3D view of NegMode_G1

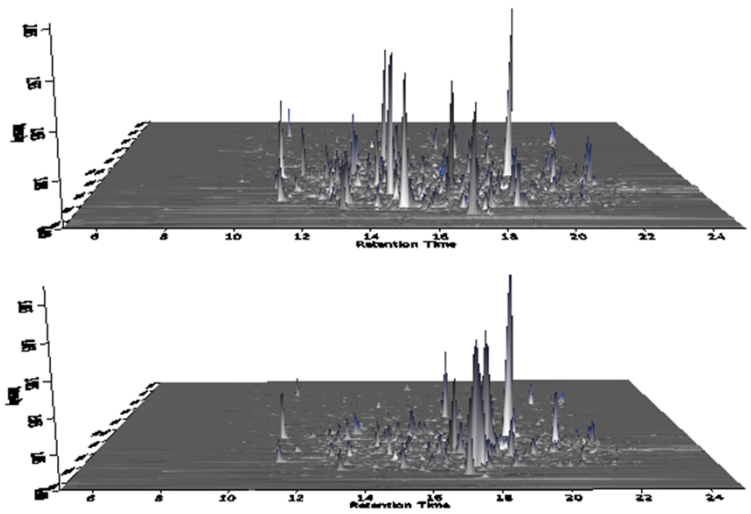


Note the large peaks – these are genistein metabolites

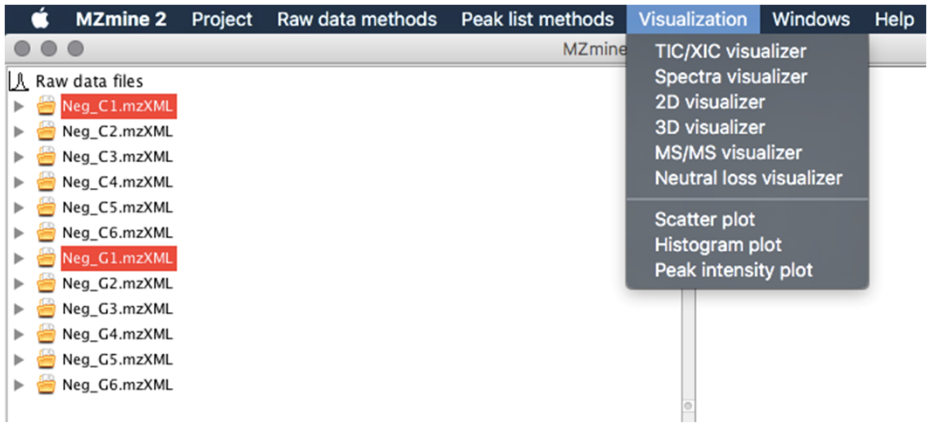
Resetting the parameters



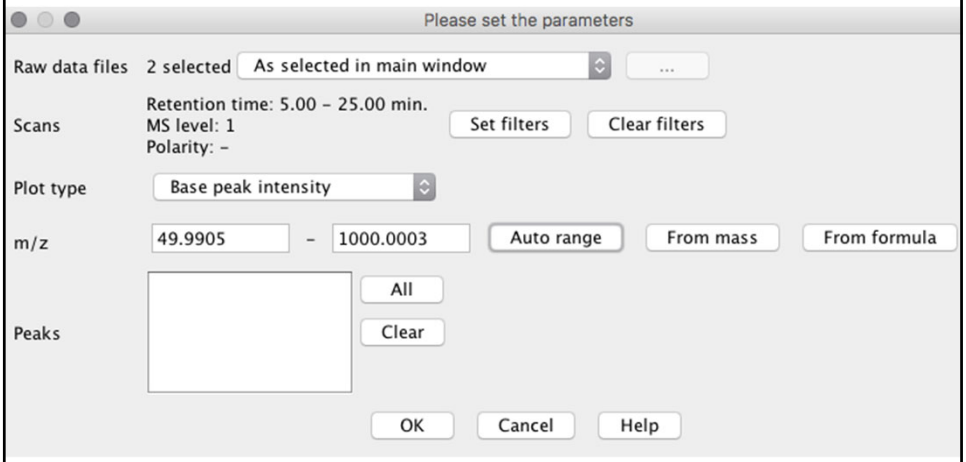
Data from 5-25 min



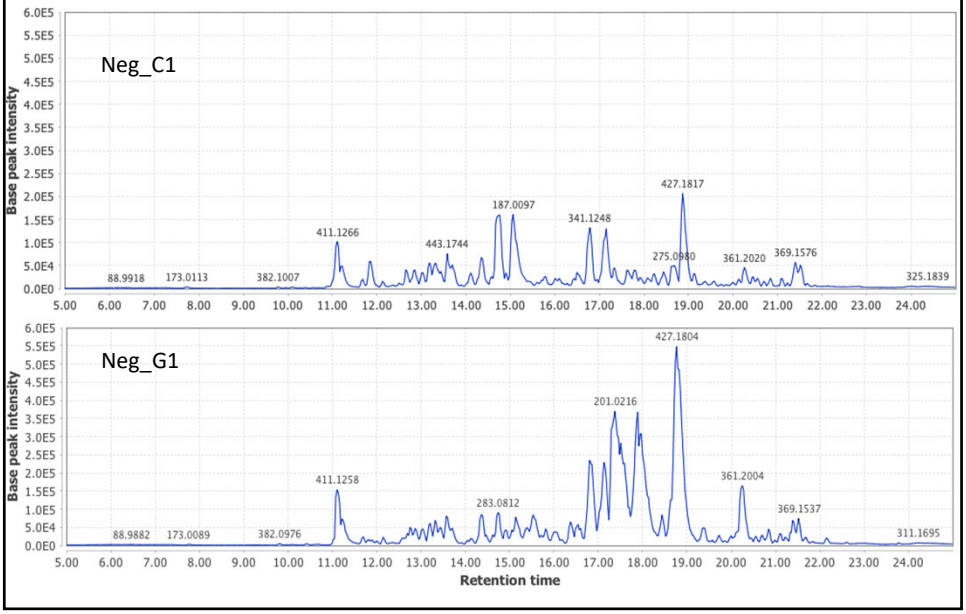
Total ion current



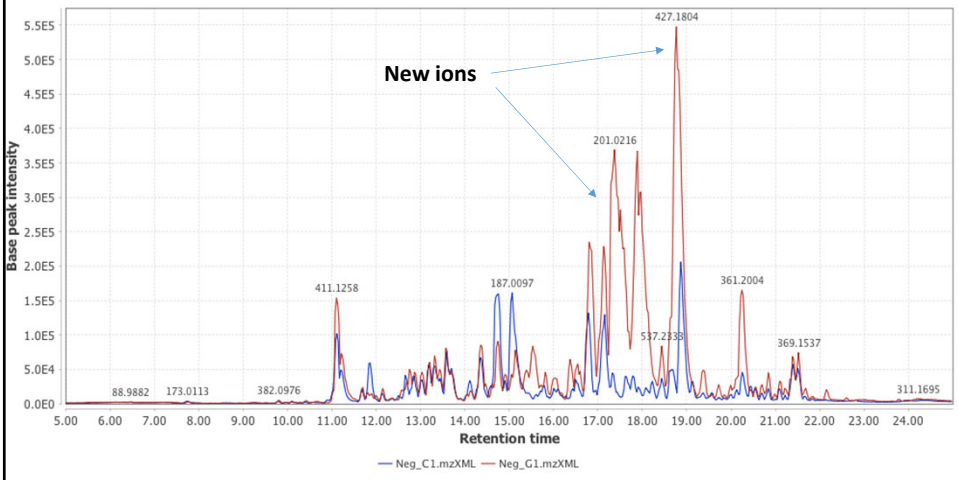
Setting the parameters



TIC of all ions from *m/z* 50-1000



TIC *m/z* 50-1000 C1/G1 comparison



To get this, highlight the files you want to compare, before invoking the XIC module

Let's calculate the mass of genistein $[M-H]^-$

- **The empirical formula of genistein is $C_{15}H_{10}O_5$**
- **If you open the mass calculator Excel file**

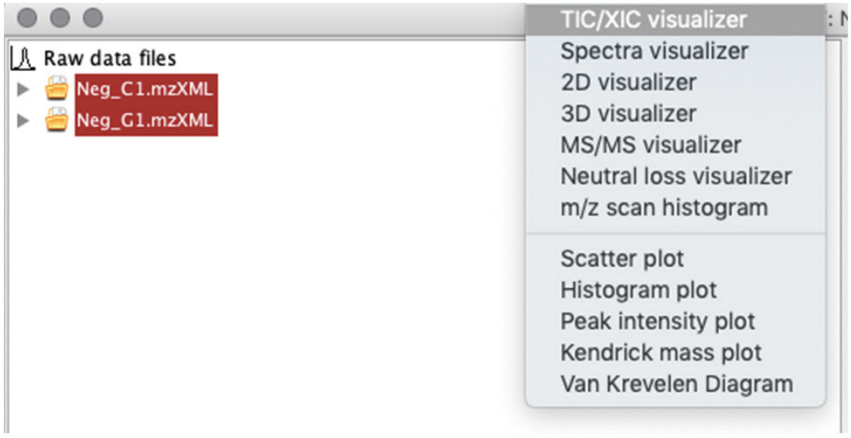
	A	B	C	D	E	F
			C	H	N	O
	Name	Empirical formula	12.000000000	1.007825032	14.003074004	15.9949146
	hexanol	C6H14O	6	14	0	1
	glucose	C6H12O6	6	12		6
	genistein	C15H10O5	15	10		5

MW	[M+H]+	[M-H]-
102.104469	103.111745	101.097193
180.063391	181.070668	179.056115
270.052826	271.060103	269.04555

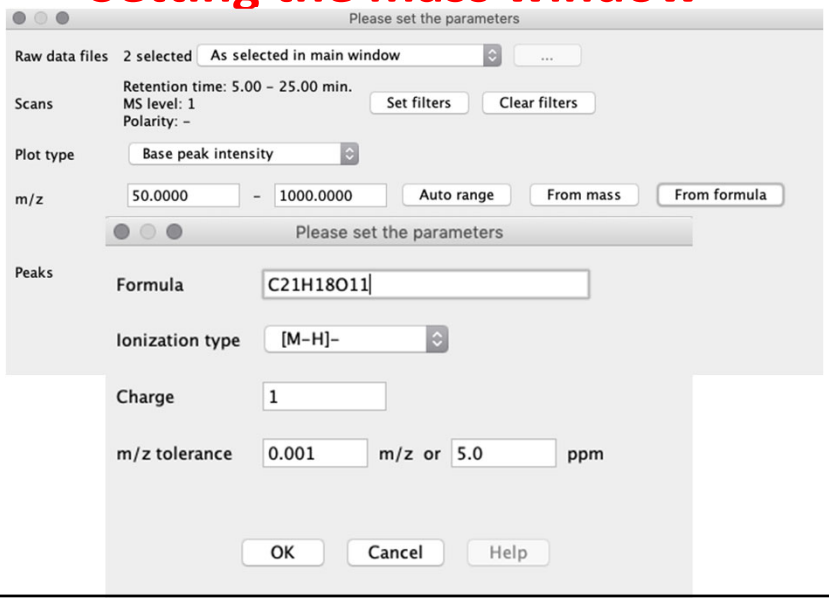
Ions of genistein and its conjugates

Name	Empirical formula	Mass (M)	[M-H]-
Genistein	$C_{15}H_{10}O_5$	270.05282	269.04557
Genistein sulfate	$C_{15}H_{10}O_8S$	350.00963	349.00238
Genistein β-glucuronide	$C_{21}H_{18}O_{11}$	446.08490	445.07765
Genistein β-glucuronide/sulfate	$C_{21}H_{18}O_{14}S$	526.04172	525.03444

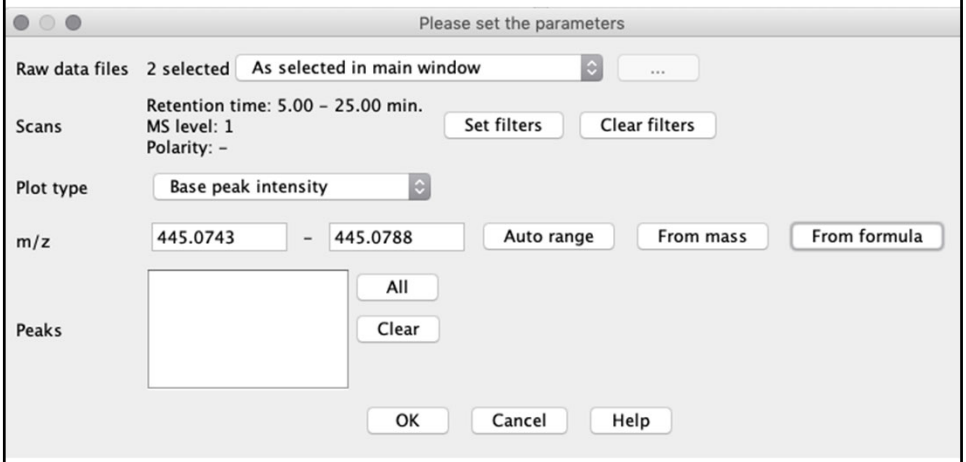
Setting the mass window



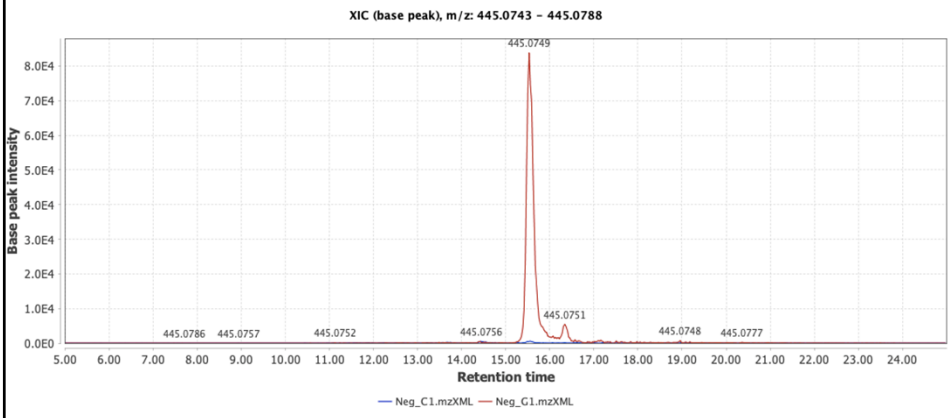
Setting the mass window



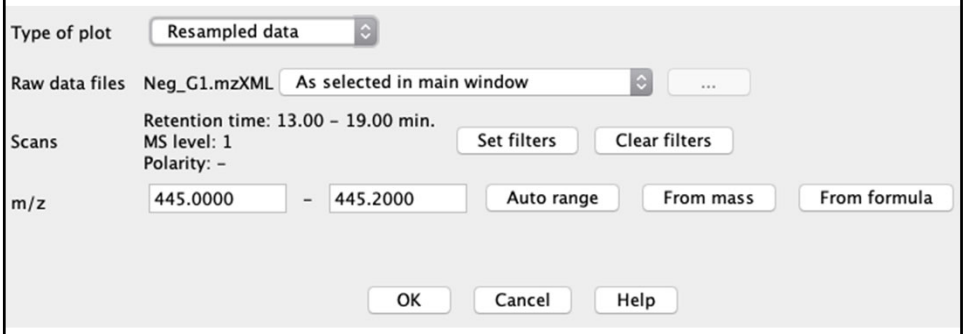
Finished the setup to find GenGlcA

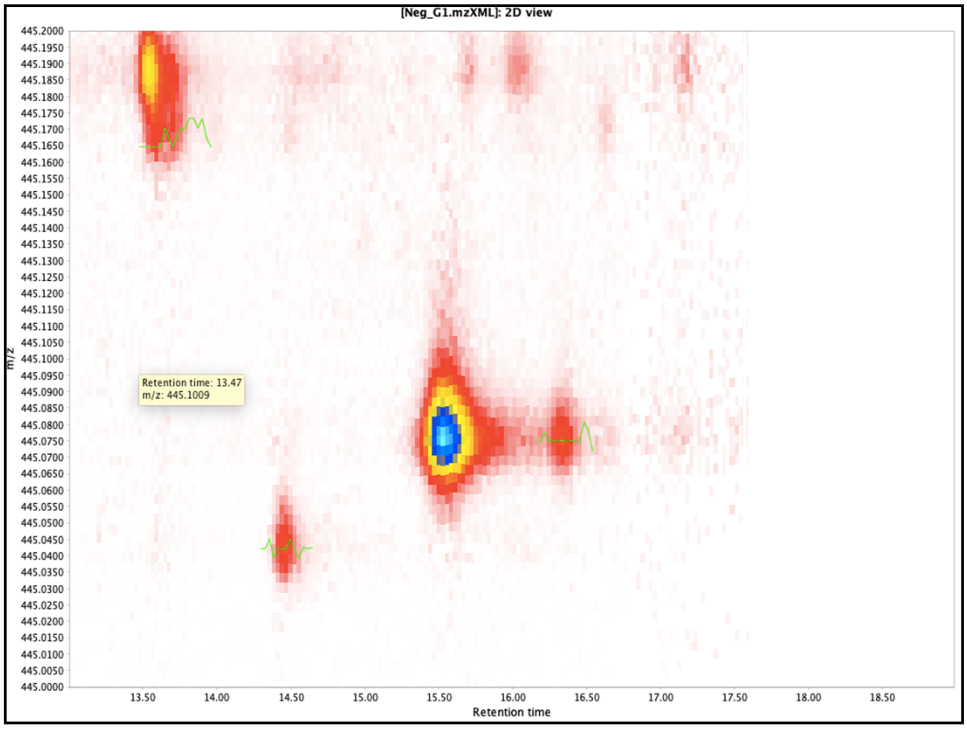


XIC of *m/z* 445.0788



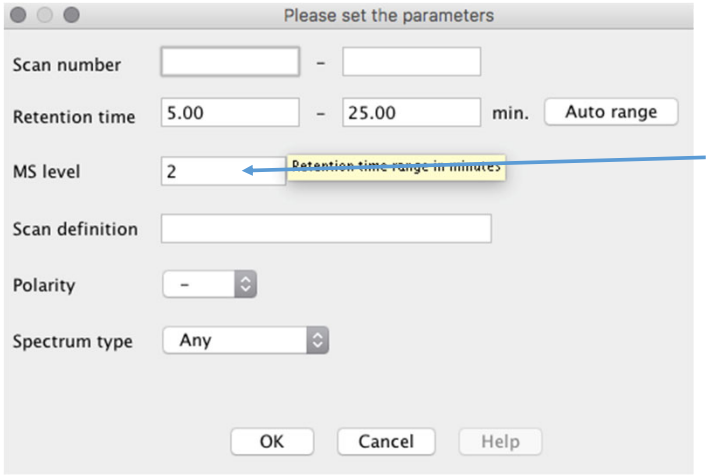
2D-plot for GENGlcA



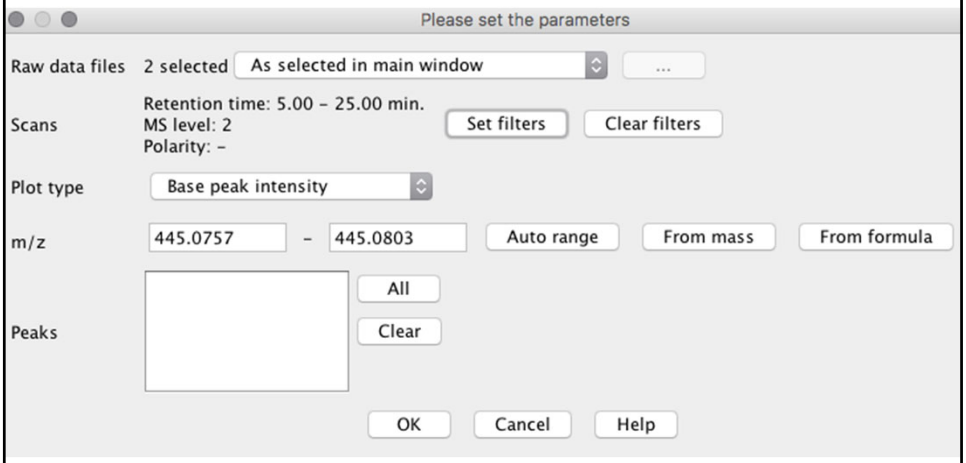


Getting MS/MS data

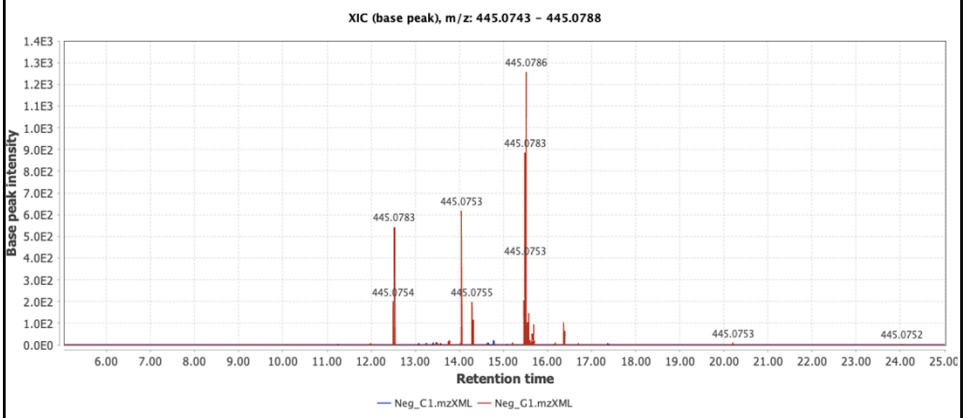
Select TIC/XIC and reset the parameters



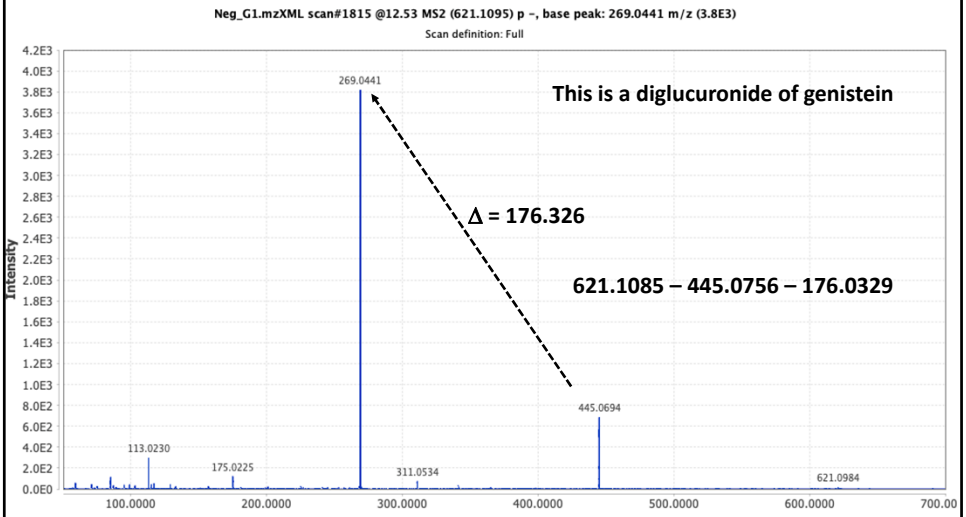
Ready to go



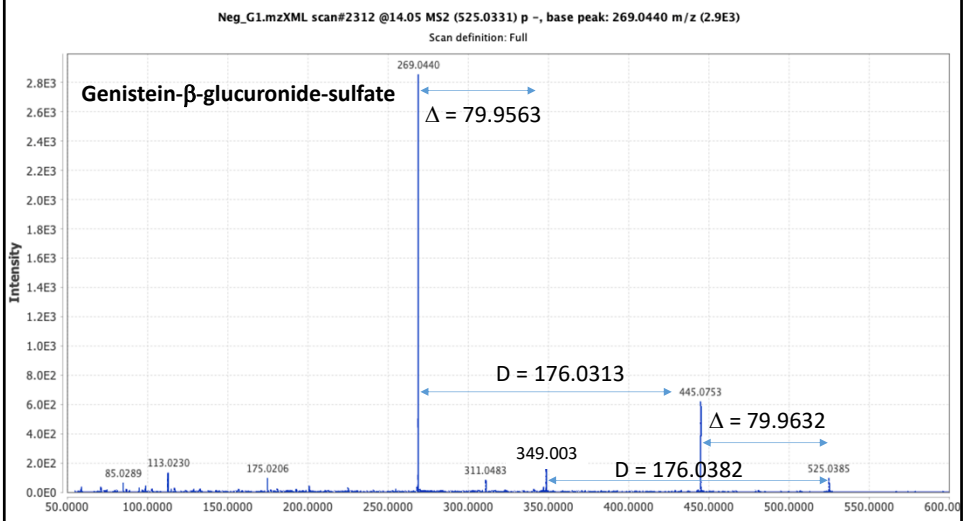
MSMS spectra captured of *m/z* 445.077



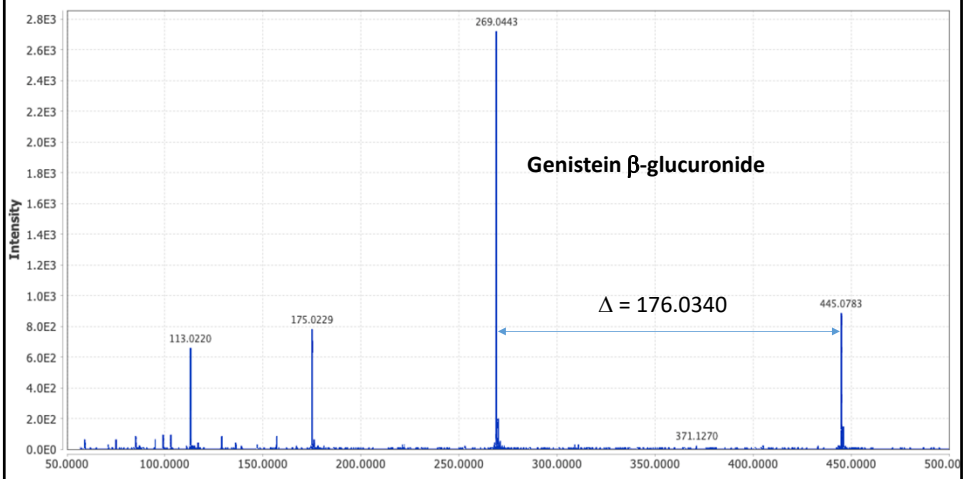
Where MSMS for *m/z* 445.079 were recorded (1)



Where MSMS for *m/z* 445.079 were recorded (2)

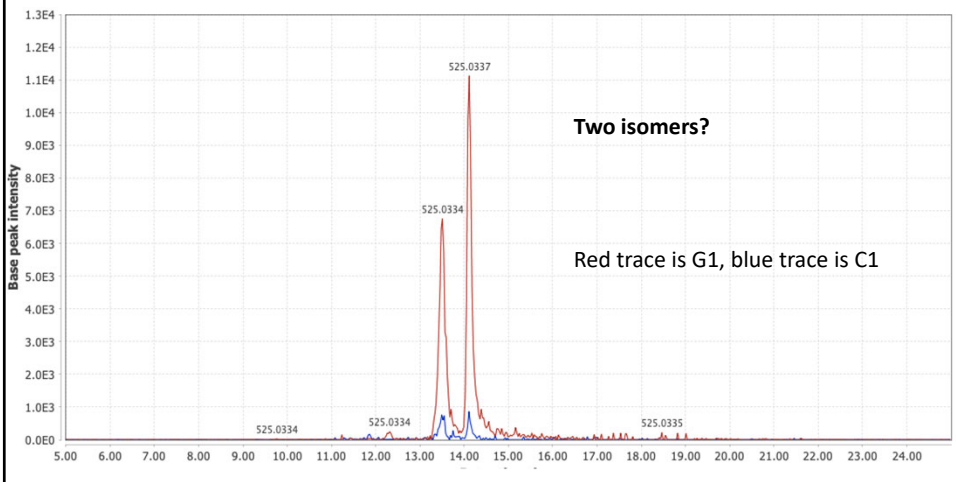


Where MSMS for *m/z* 445.079 were recorded (3)



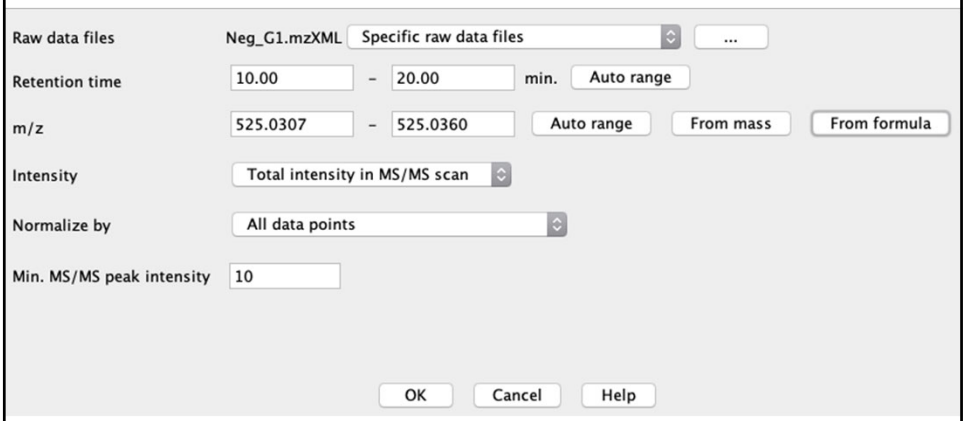
XIC of *m/z* 525.033

genistein β-glucuronide/sulfate

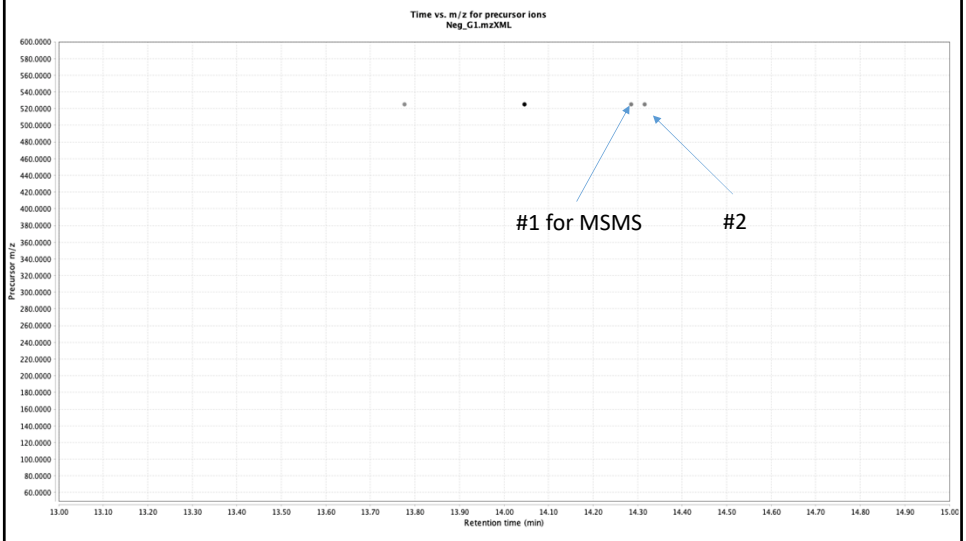


Another way to get MSMS spectra

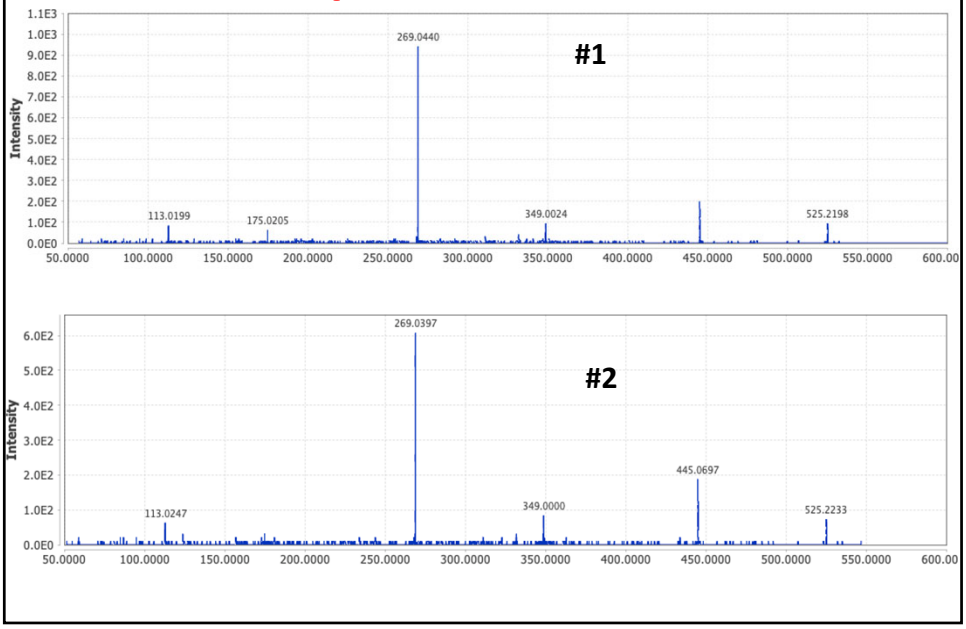
Select MSMS visualizer



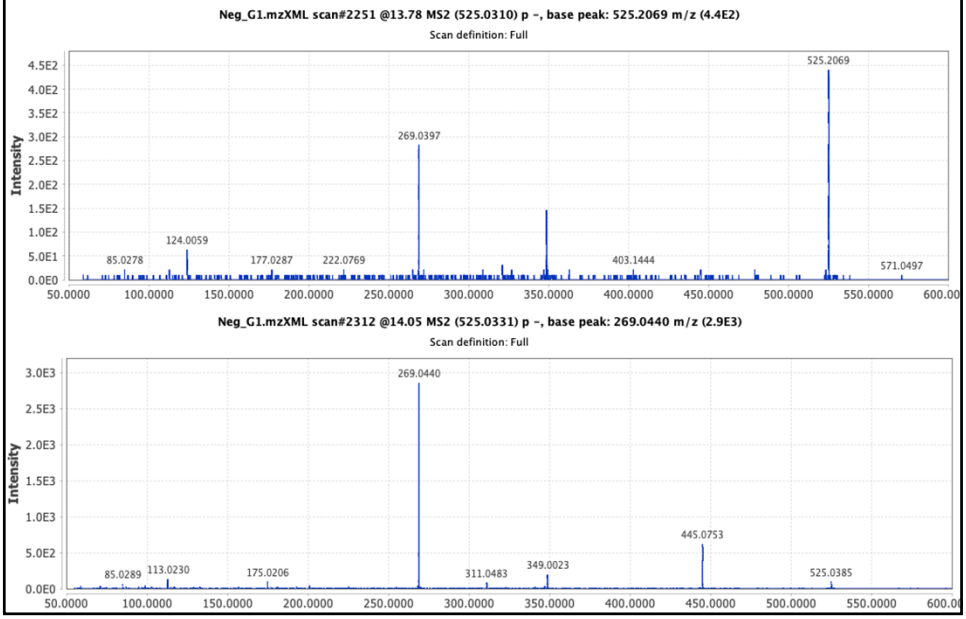
Four items detected by MSMS of *m/z* 523.3



MSMS spectra of *m/z* 525.03

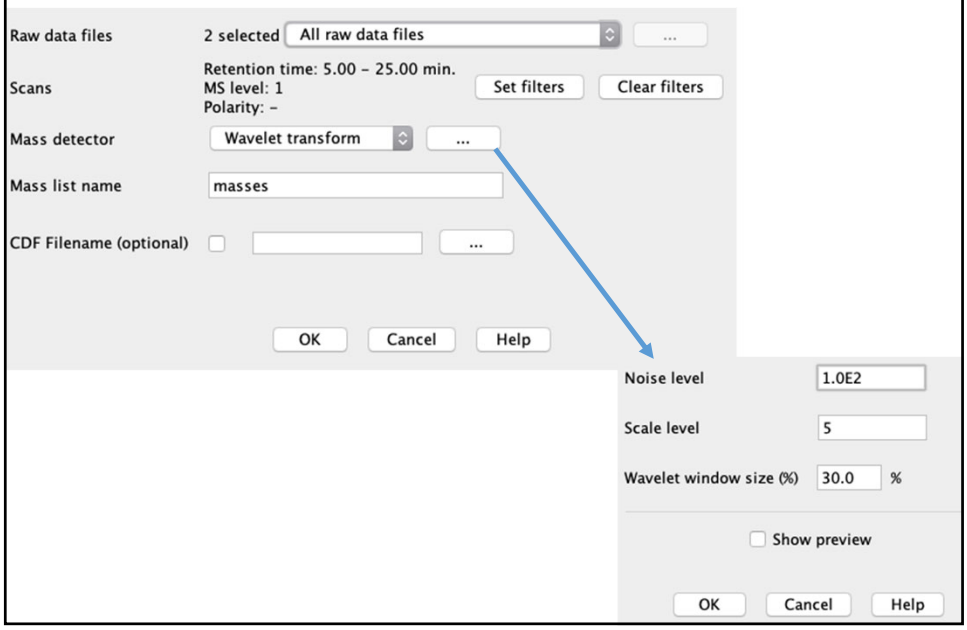


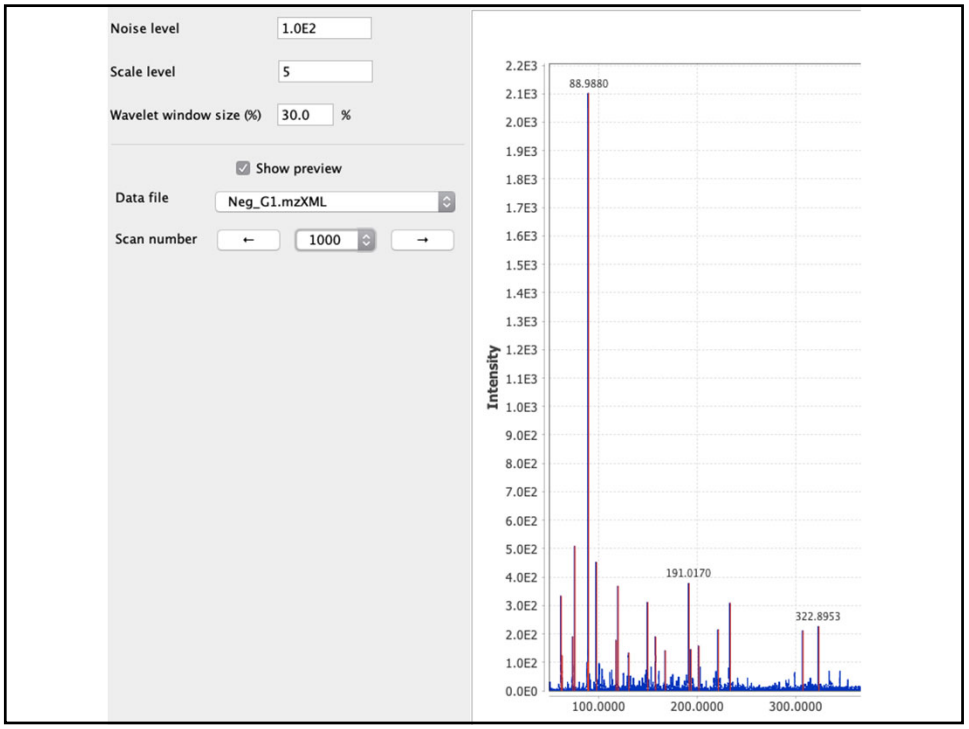
MSMS spectra of earlier *m/z* 525.03



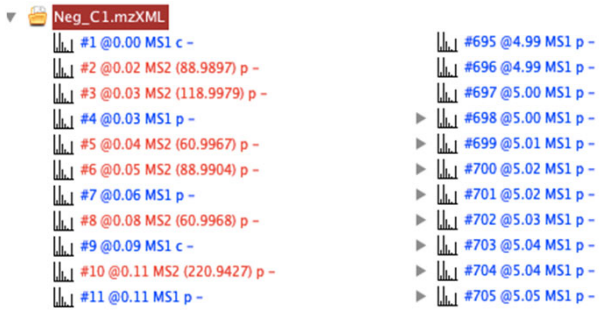
Class on 1-25-19

Identifying the masses





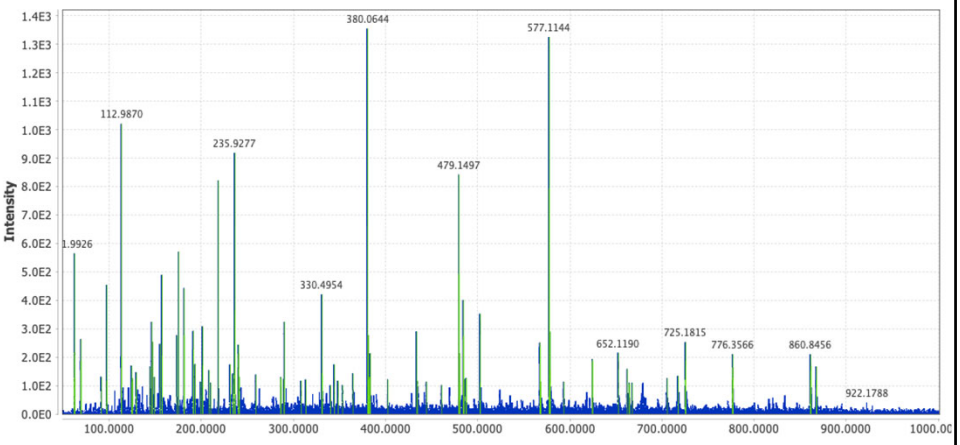
After identifying the masses



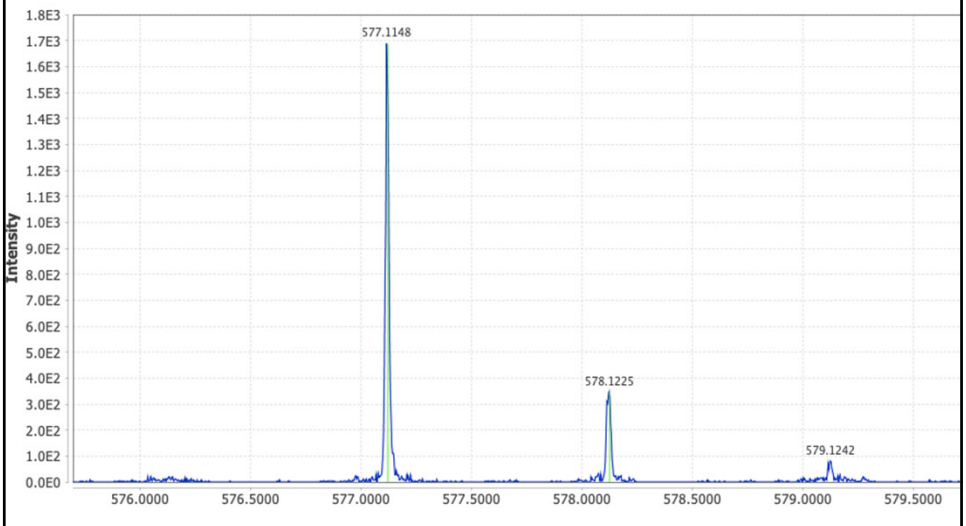
The mass identifications started at 5 min

Green masses identified

Blue are the background ions

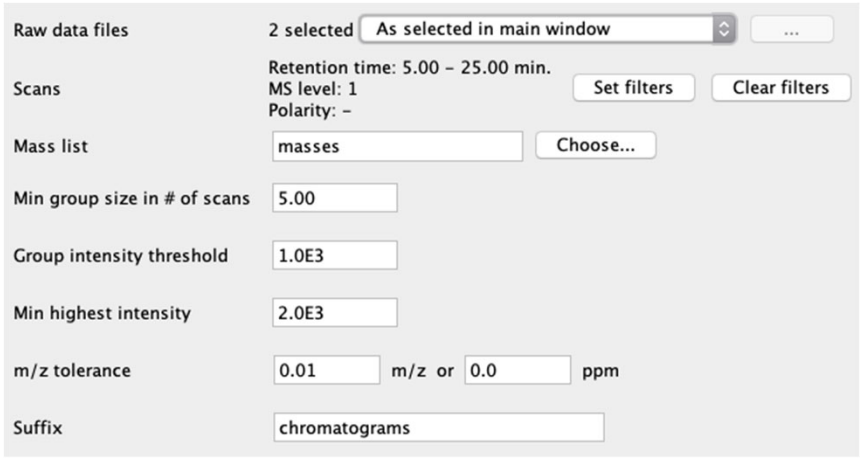


Isotope profile of *m/z* 577.115

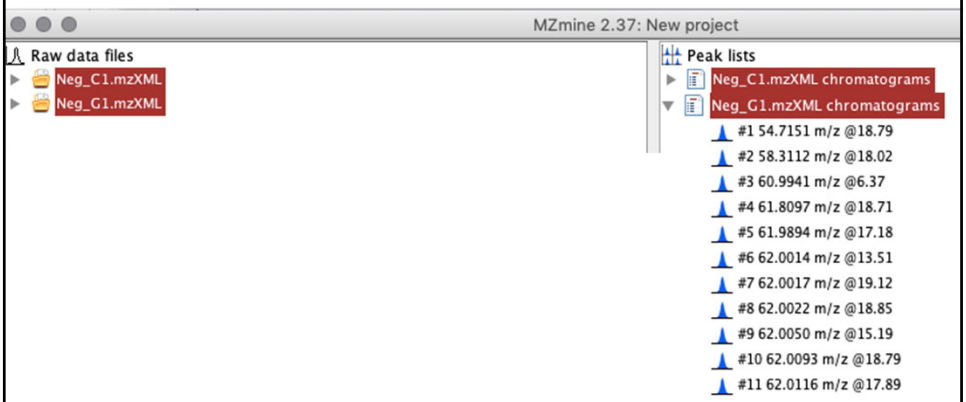


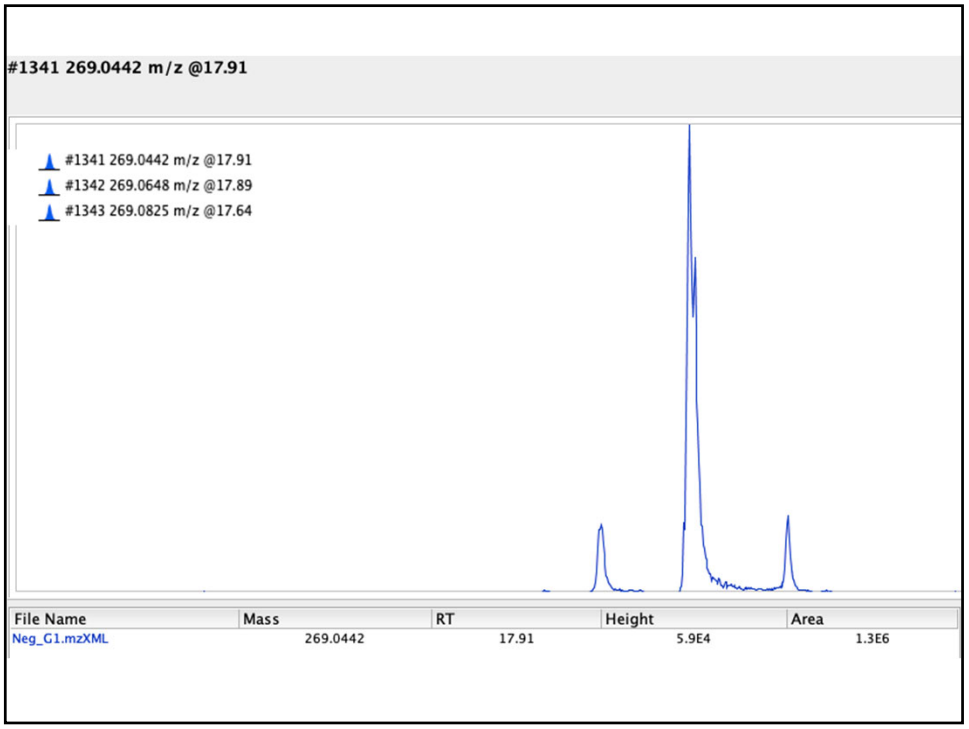
Using the masses to create chromatograms

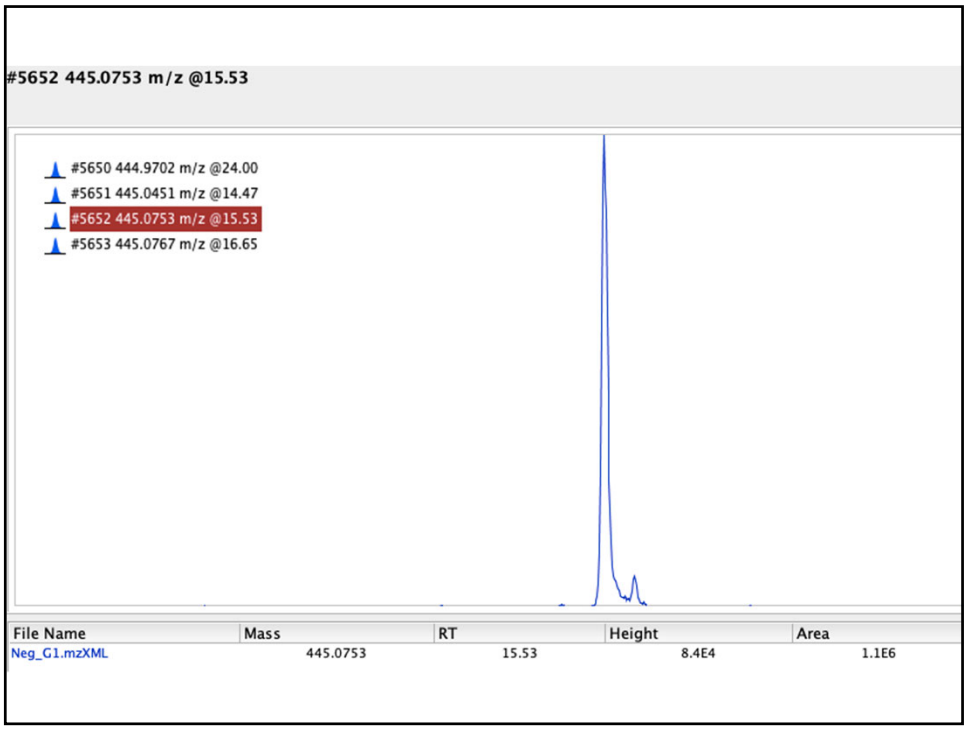
Go to *Raw data methods, peak detection, chromatogram builder*

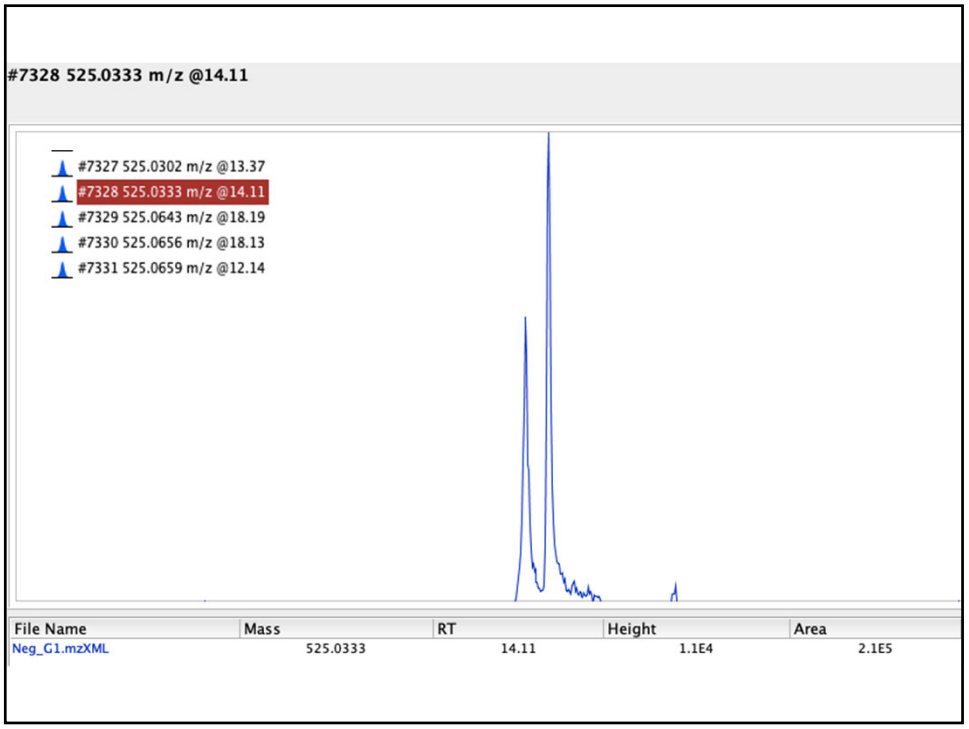


When the chromatogram building ends



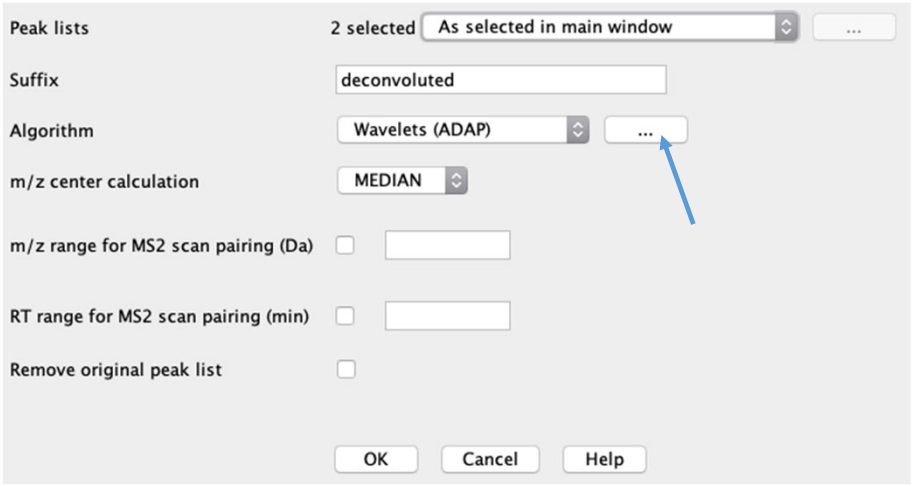

#1341 269.0442 m/z @17.91
#1341 269.0442 m/z @17.91
#1342 269.0648 m/z @17.89
#1343 269.0825 m/z @17.64
File Name
Mass
RT
Height
Area
Neg_G1.mzXML
269.0442
17.91
5.9E4
1.3E6

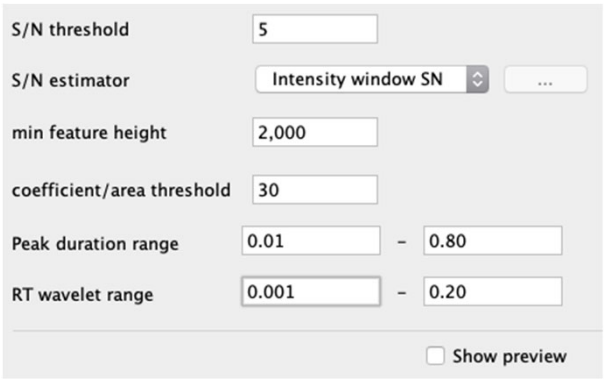

#5652 445.0753 m/z @15.53
#5650 444.9702 m/z @24.00
#5651 445.0451 m/z @14.47
#5652 445.0753 m/z @15.53
#5653 445.0767 m/z @16.65
File Name
Mass
RT
Height
Area
Neg_G1.mzXML
445.0753
15.53
8.4E4
1.1E6



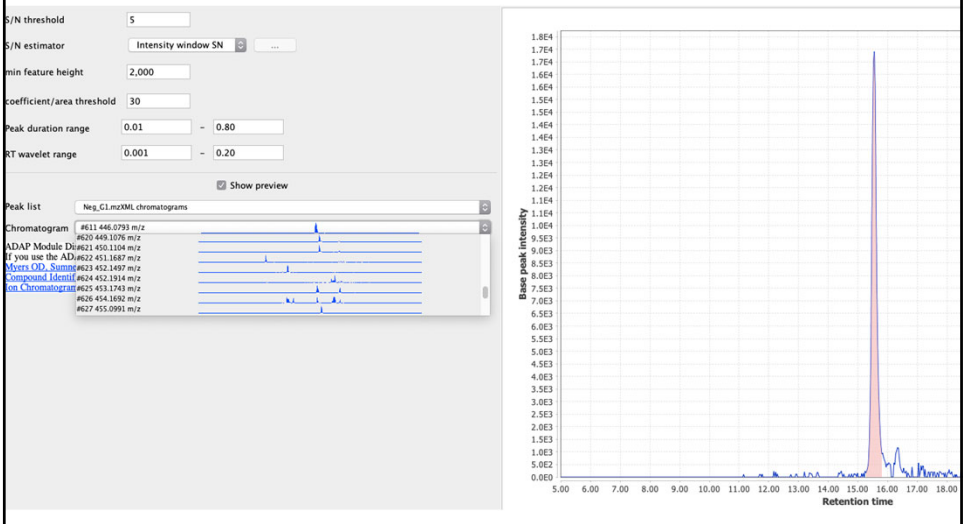
Chromatogram deconvolution

Go to *Peak list methods, peak detection, chromatogram deconvolution*





Preview of chromatographic deconvolution

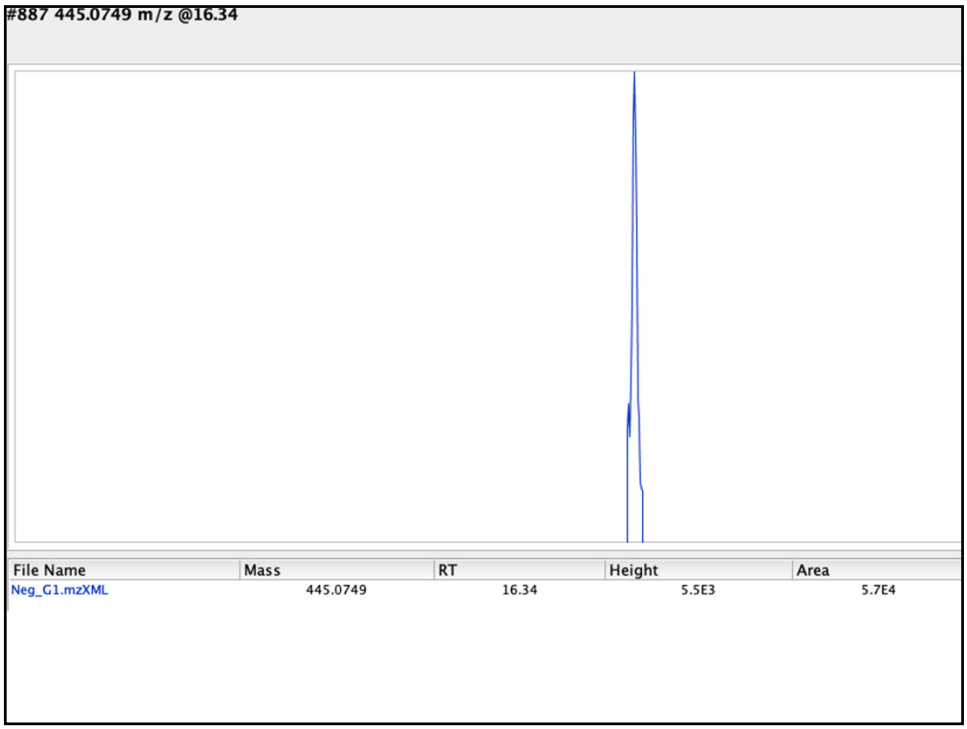


Chromatogram deconvolution output

Raw data files
- NegMode_IR1.mzXML
- NegMode_NR1.mzXML

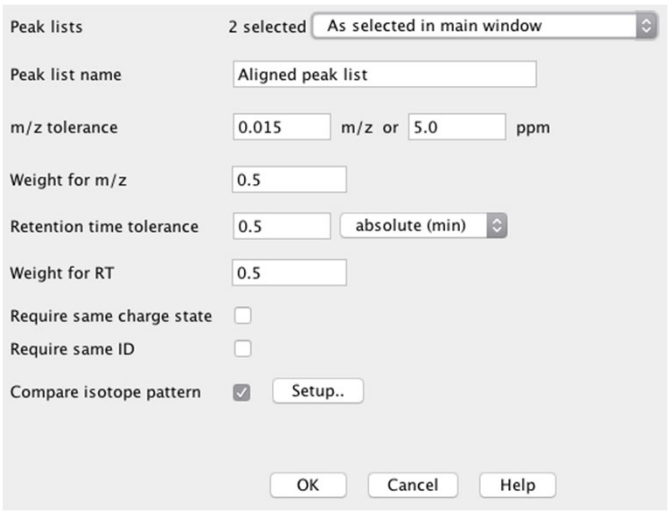
Peak lists
- NegMode_NR1.mzXML chromatograms
- NegMode_IR1.mzXML chromatograms
- NegMode_NR1.mzXML chromatograms deconvoluted
- NegMode_IR1.mzXML chromatograms deconvoluted

- Neg_C1.mzXML chromatograms deconvoluted
- Neg_G1.mzXML chromatograms deconvoluted
  - #1 79.9583 m/z @17.40
  - #2 112.9849 m/z @20.33
  - #3 112.9848 m/z @16.40
  - #4 121.0654 m/z @17.40
  - #5 129.0541 m/z @14.23
  - #6 130.0651 m/z @17.64
  - #7 134.0604 m/z @13.20
  - #8 143.5970 m/z @17.15

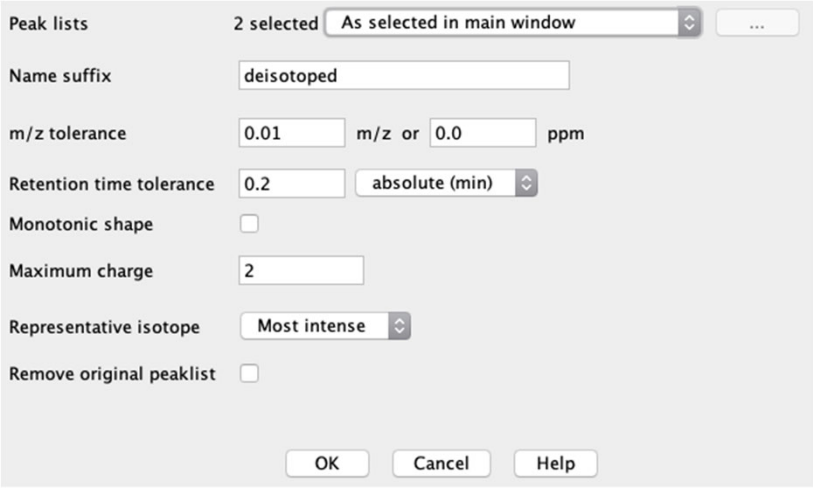


Peak and chromatogram alignment

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

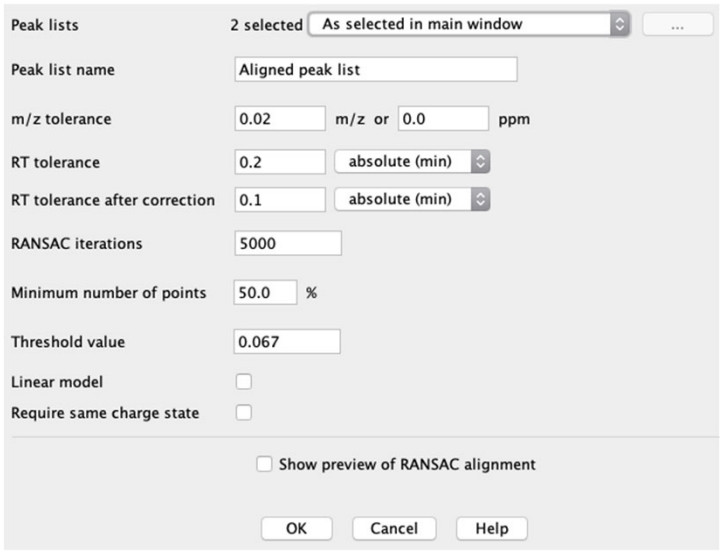


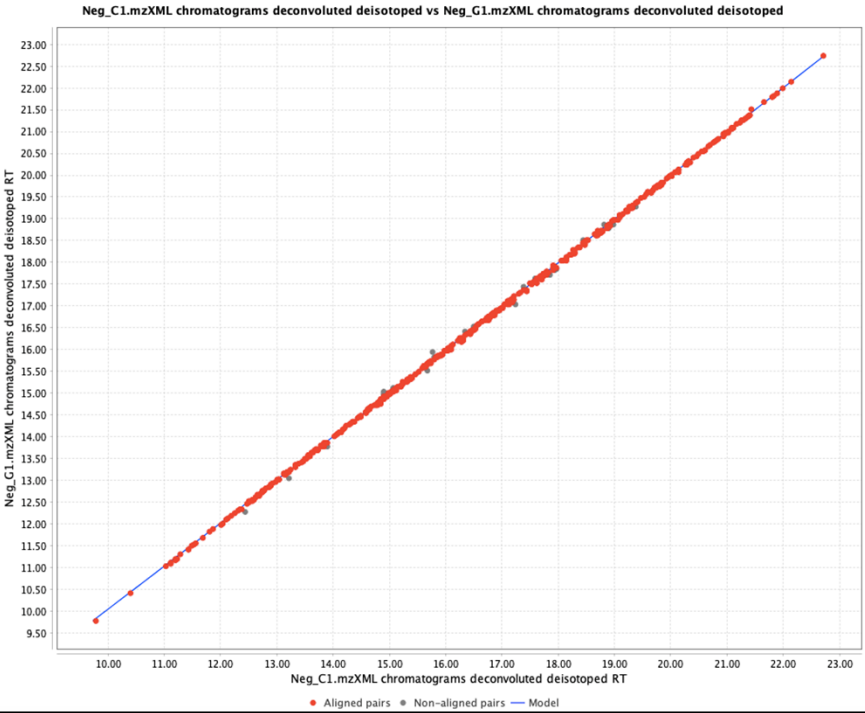
Isotope grouping



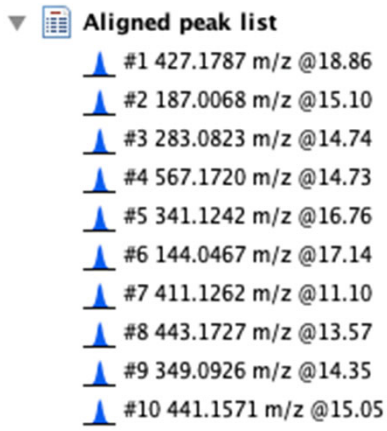
Peak and chromatogram alignment

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





Aligned data



Double click on Aligned peak list

ID	Average		Identity	Comment	Peak shape	Neg_C1.mzXML			Neg_G1.mzXML		
	m/z	RT				Status	Height	Area	Status	Height	Area
1	427.1787	18.86					2.1E5	1.8E6			
2	187.0068	15.10					1.6E5	1.9E6		7.8E4	7.3E5
3	283.0823	14.74					1.6E5	1.7E6		9.1E4	7.0E5
4	567.1720	14.73					1.4E5	9.3E5		2.9E4	1.5E5
5	341.1242	16.76					1.3E5	1.1E6		8.5E4	5.9E5
6	144.0467	17.14					1.3E5	1.2E6		2.3E5	2.1E6
7	411.1262	11.10					1.0E5	7.0E5		1.5E5	1.2E6
8	443.1727	13.57					7.6E4	7.7E5		8.1E4	8.2E5
9	349.0926	14.35					6.7E4	5.1E5		8.6E4	6.7E5
10	441.1571	15.05					6.7E4	5.5E5		4.3E4	3.0E5
11	429.0501	11.88					5.9E4	4.6E5		1.5E4	1.0E5
12	369.1571	21.40					5.8E4	7.4E5			
13	178.0508	13.19					5.6E4	4.3E5		6.0E4	4.5E5
14	212.0027	13.35					5.5E4	6.1E5		2.6E4	2.8E5
15	275.0968	18.66					5.0E4	4.4E5		1.3E5	1.1E6
16	230.9958	11.20					4.9E4	4.3E5		7.3E4	7.1E5
17	198.1139	18.66					4.8E4	5.7E5		5.7E4	6.2E5
18	361.2012	20.25					4.6E4	3.7E5		1.7E5	1.6E6
19	587.3065	17.32					4.5E4	3.1E5		3.1E4	2.3E5
20	495.1188	13.71					4.2E4	3.5E5		1.0E4	8.8E4

Organizing by retention time

ID	Average		...	...	Peak shape	NegMode_NR1.mzXML			NegMode_IR1.mzXML		
	m/z	RT ▲				Status	Height	Area	Status	Height	Area
748	270.0457	14.14	P...							3.4E3	1.5E4
853	477.1528	14.14	P...							7.6E3	2.0E4
921	647.1545	14.14	P...							2.6E3	1.1E4
471	490.1299	14.14	P...				4.1E3	1.9E4			
375	432.0996	14.16	P...				6.0E3	3.9E4		4.9E3	4.0E4
462	478.1060	14.16	P...				2.3E4	2.1E5		2.7E4	1.9E5
463	479.1084	14.16	P...				6.4E3	4.3E4		9.0E3	4.9E4
852	477.1022	14.17	P...							9.5E4	8.7E5
854	477.1674	14.17	P...							2.3E3	1.2E4
138	269.0447	14.17	P...				1.4E4	1.4E5		1.6E4	1.5E5
367	431.0963	14.18	P...				2.2E4	8.6E4		2.0E4	1.8E5
636	670.1571	14.20	P...				3.9E3	2.0E4			
156	283.0604	14.22	P...				1.3E3	5.4E3			

Class exercise

- Load the C1-C3 and G1-G3 .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.

Export to .CSV file