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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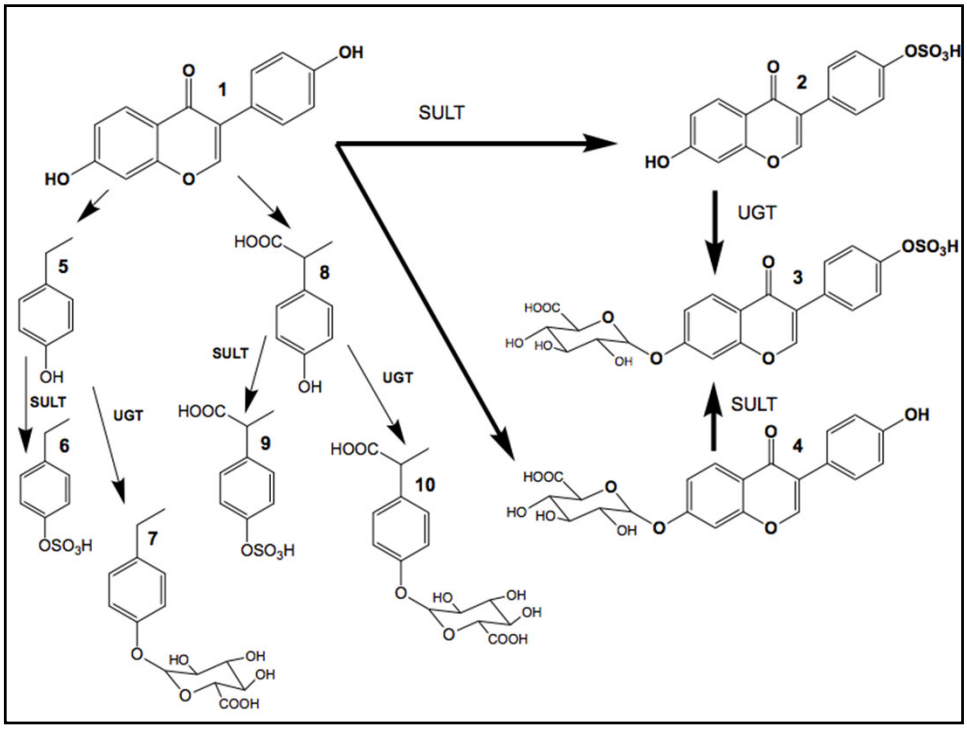
Check for updates

OPEN ACCESS

2



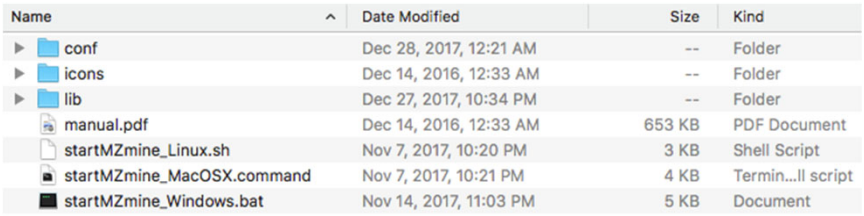
3

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

4

Starting point for MZmine

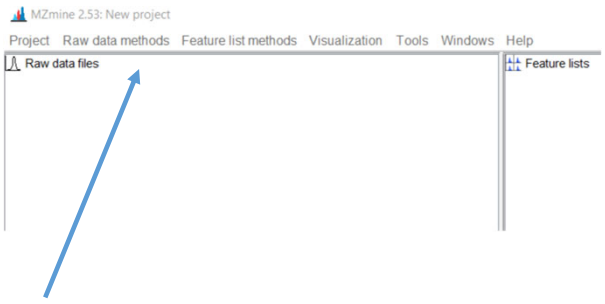


Name	Date Modified	Size	Kind
conf	Dec 28, 2017, 12:21 AM	--	Folder
icons	Dec 14, 2016, 12:33 AM	--	Folder
lib	Dec 27, 2017, 10:34 PM	--	Folder
manual.pdf	Dec 14, 2016, 12:33 AM	653 KB	PDF Document
startMZmine_Linux.sh	Nov 7, 2017, 10:20 PM	3 KB	Shell Script
startMZmine_MacOSX.command	Nov 7, 2017, 10:21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

5

Starting off

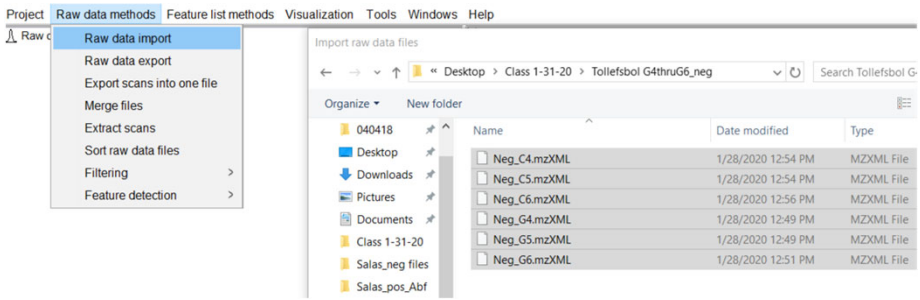


Click on Raw data methods and then select "raw data input" from the drop down box

Note the MZmine version number in 2020 is 2:53

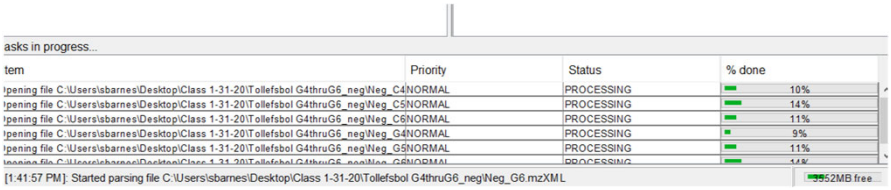
6

Select the files



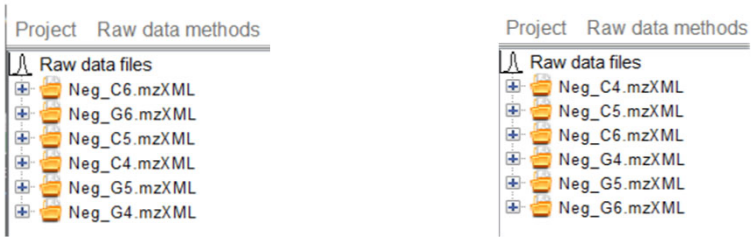
7

Files are loading



8

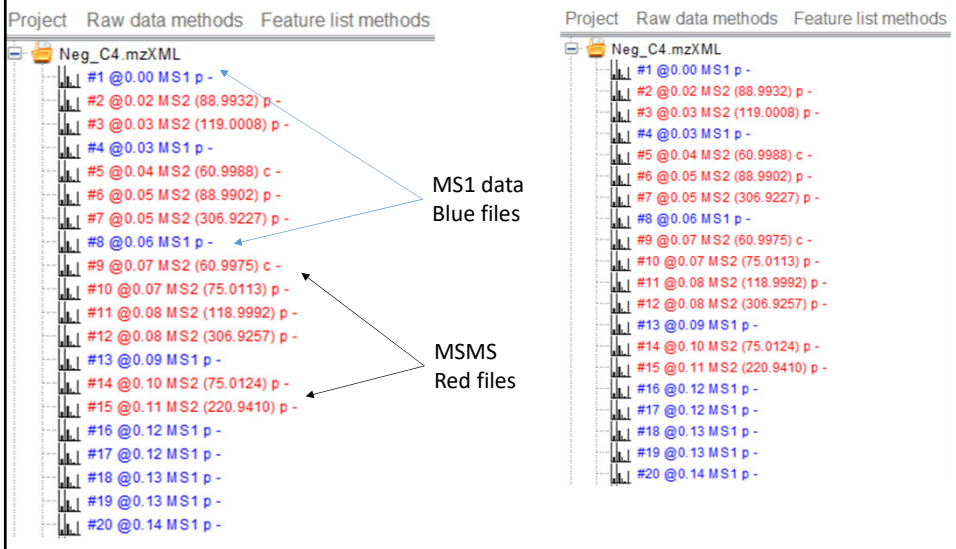
Sorting the files



Highlight files and sort under Raw data methods tab

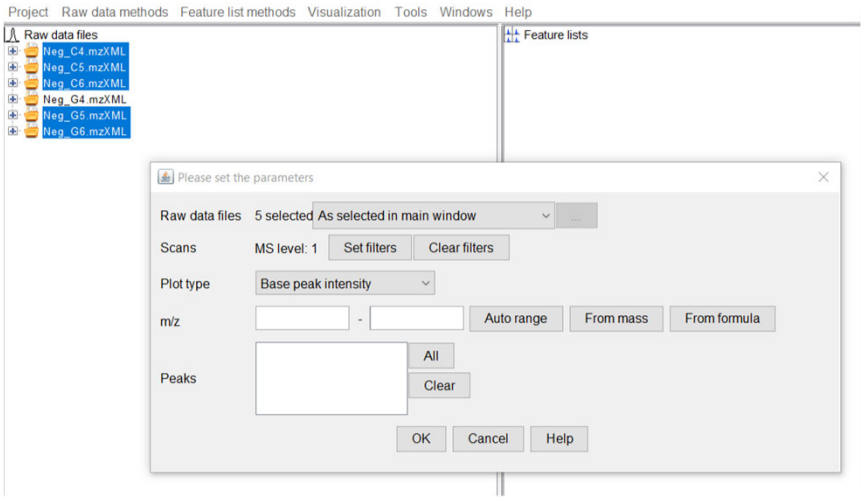
9

Content of the .mzxml files



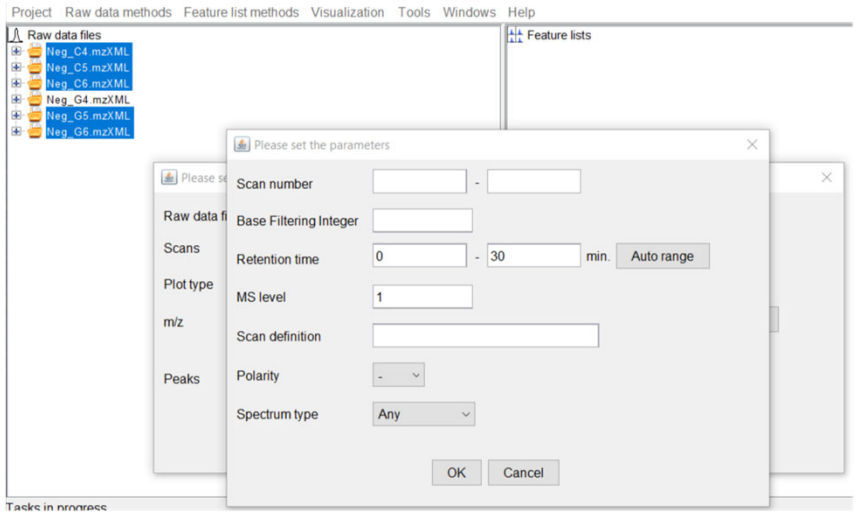
10

Comparing samples C4, C5, C6, G5 and G6

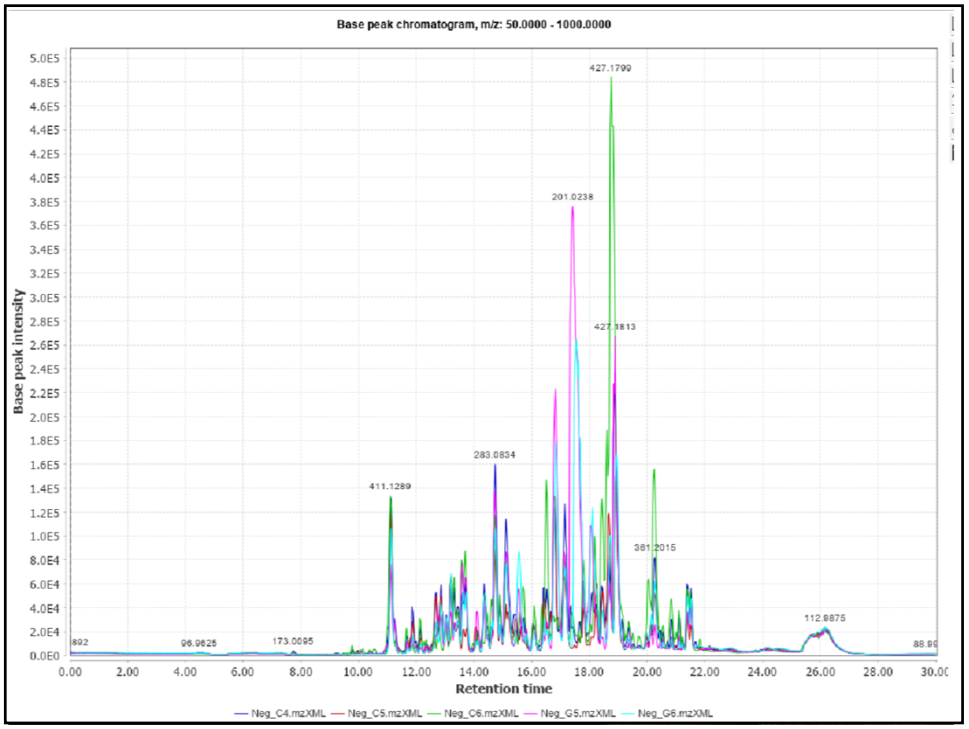


11

Setting the run time

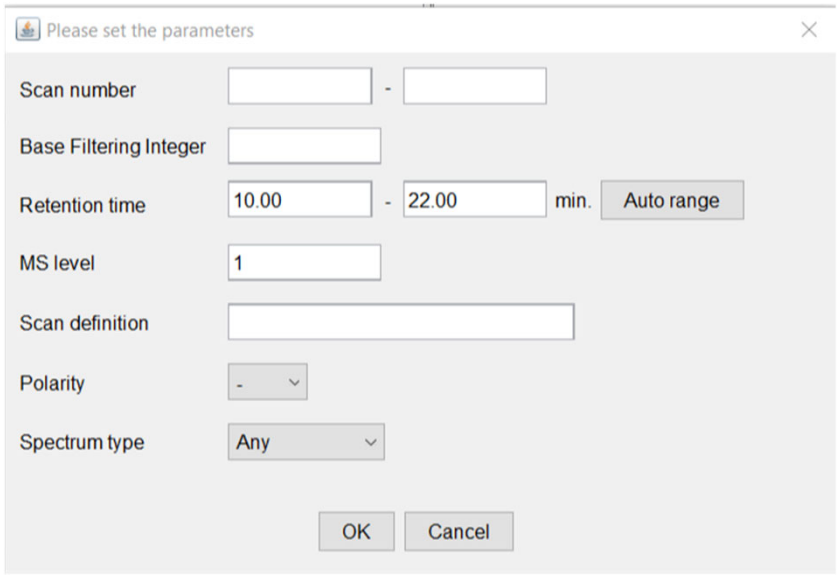


12



13

Resetting the run time



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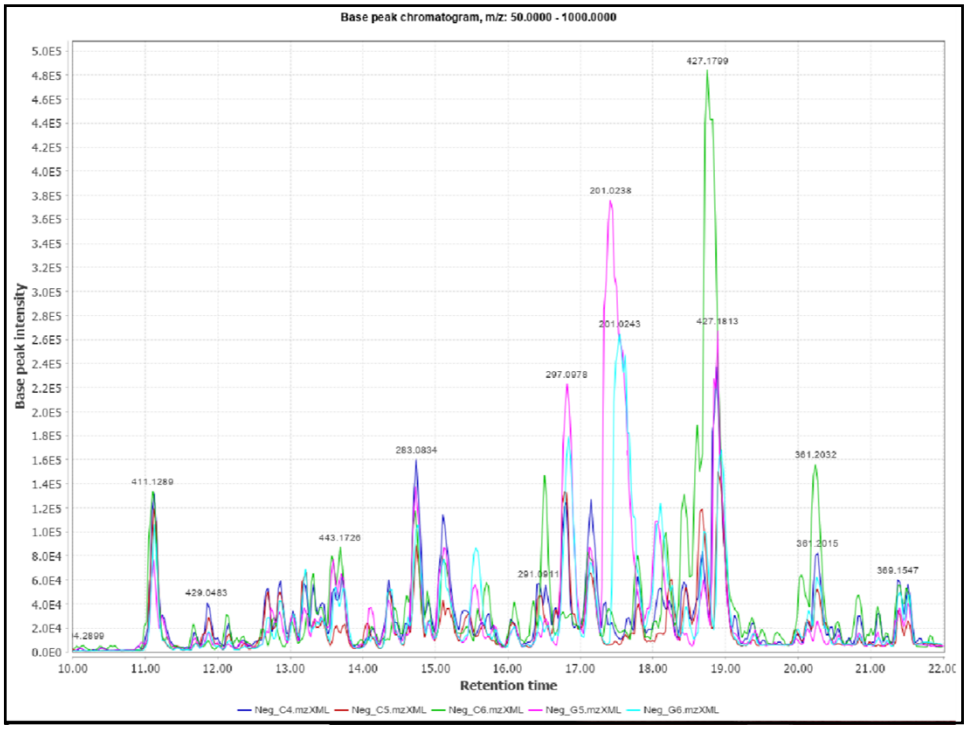
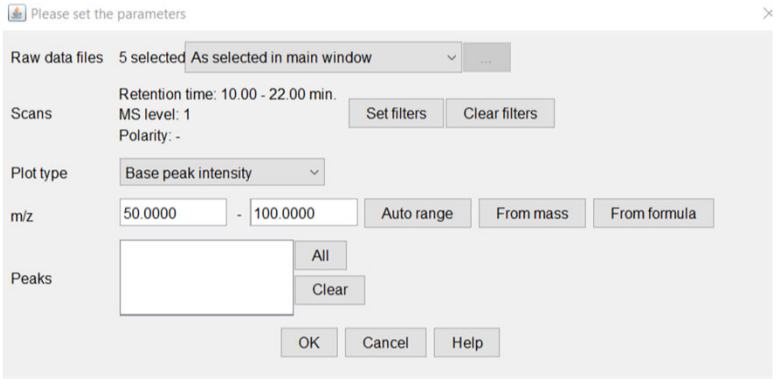
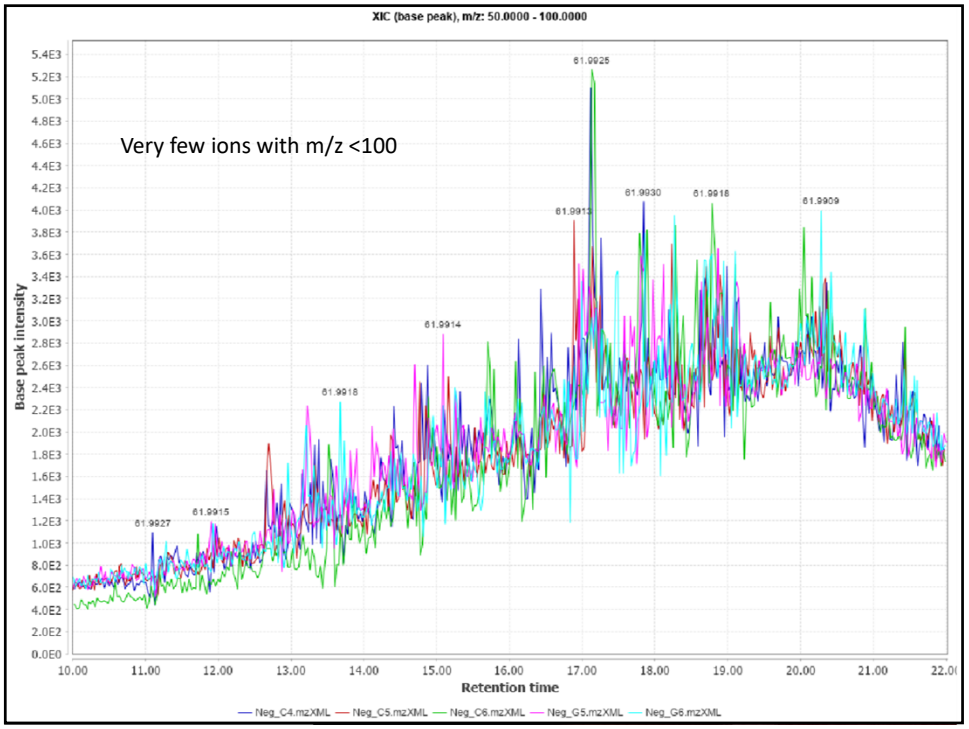


15

Setting the m/z range to 50-100

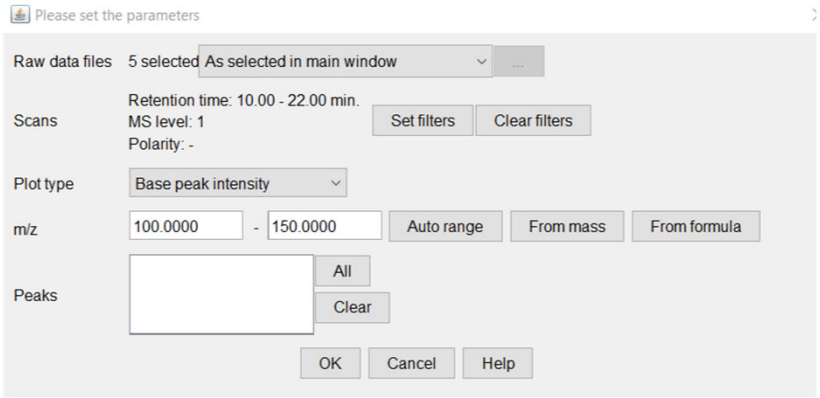


16

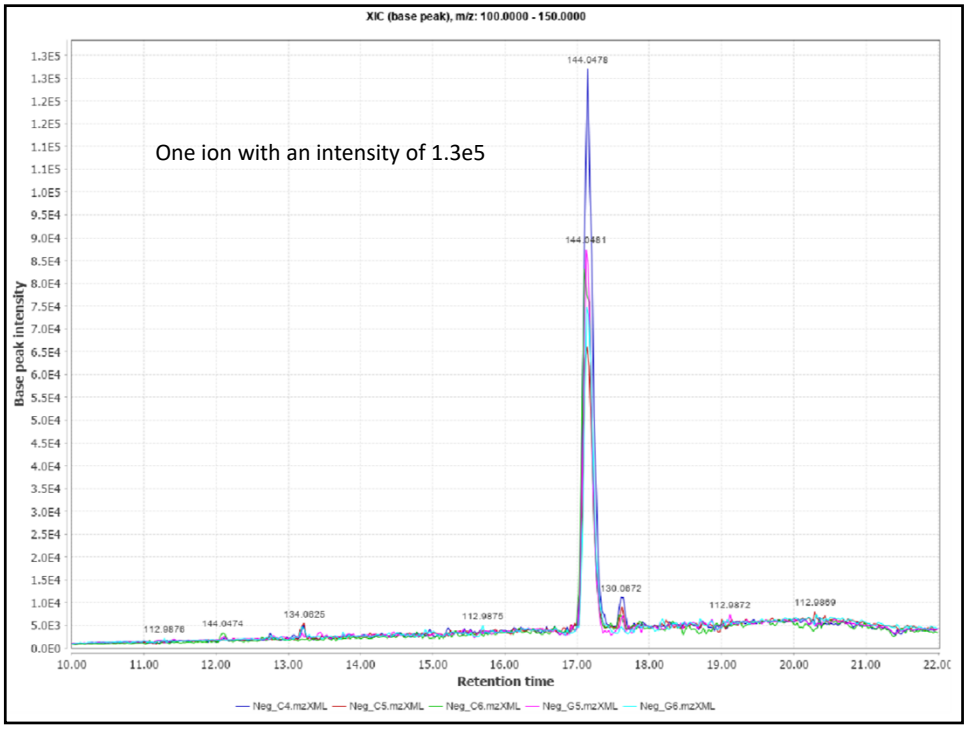


17

Setting the m/z range to 100-150



18


XIC (base peak), m/z: 100.0000 - 150.0000
One ion with an intensity of 1.3e5
Base peak intensity
Retention time
144.0478
144.0481
130.0672
112.9872
112.9869
112.9876
144.0474
134.0825
112.9875
Neg_C4.mzXML
Neg_C5.mzXML
Neg_C6.mzXML
Neg_G5.mzXML
Neg_G6.mzXML

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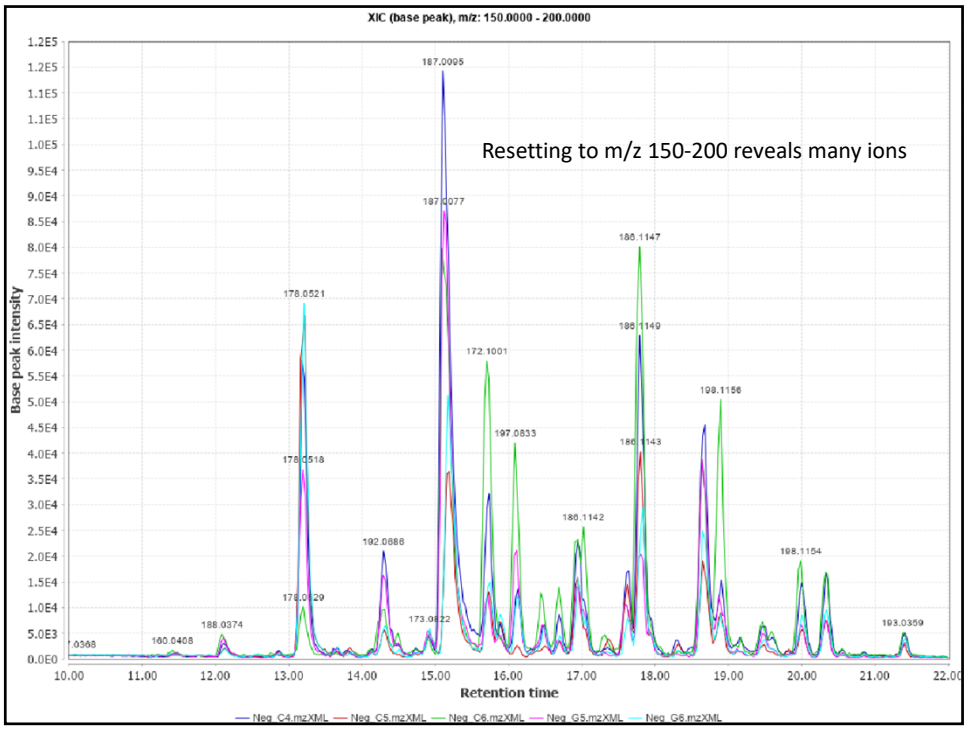
XIC (base peak), m/z: 150.0000 - 200.0000
Resetting to m/z 150-200 reveals many ions
Base peak intensity
Retention time
187.0095
187.0077
186.1147
186.1149
178.0521
178.0518
172.1001
198.1156
197.0833
186.1143
186.1142
192.0886
198.1154
178.0129
173.0822
188.0374
160.0408
193.0359
Neg_C4.mzXML
Neg_C5.mzXML
Neg_C6.mzXML
Neg_G5.mzXML
Neg_G6.mzXML

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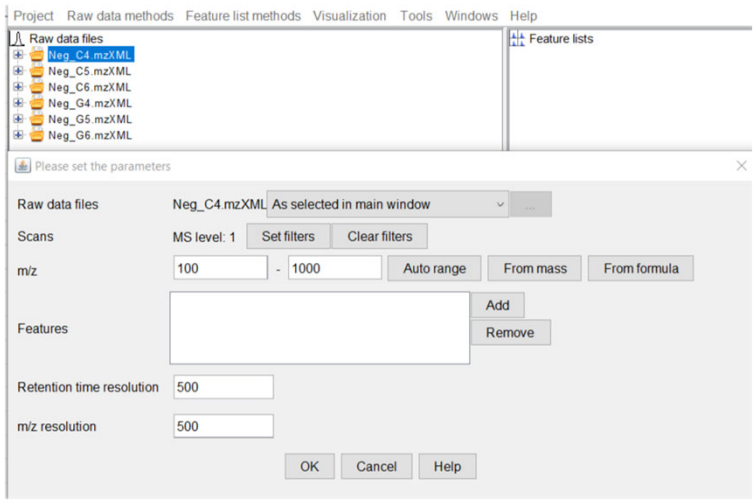
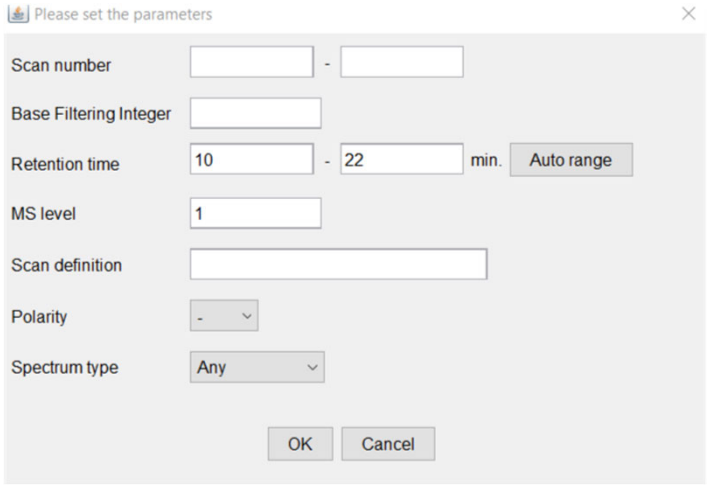
Using the 3D-visualizer (under the visualization tools tab)



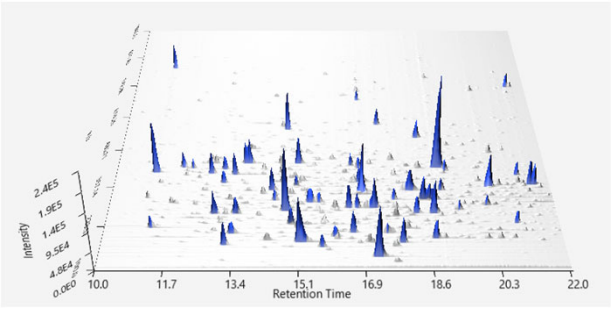
Note – set m/z to 100-1000 and time to 10-22 min

21

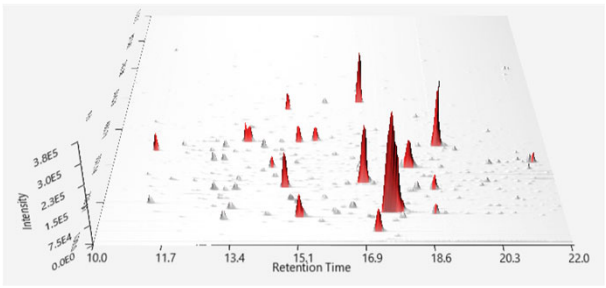


22

Control C4



Genistein G5



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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

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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

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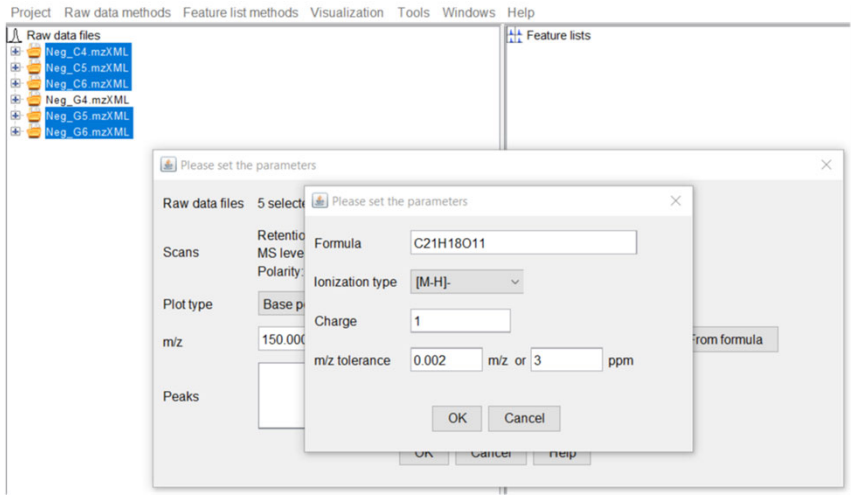
Setting the mass window for genistein β-glucuronide, C21H18O11



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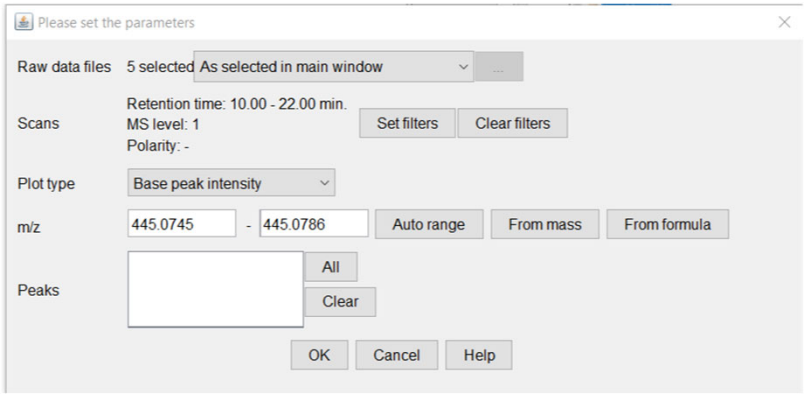
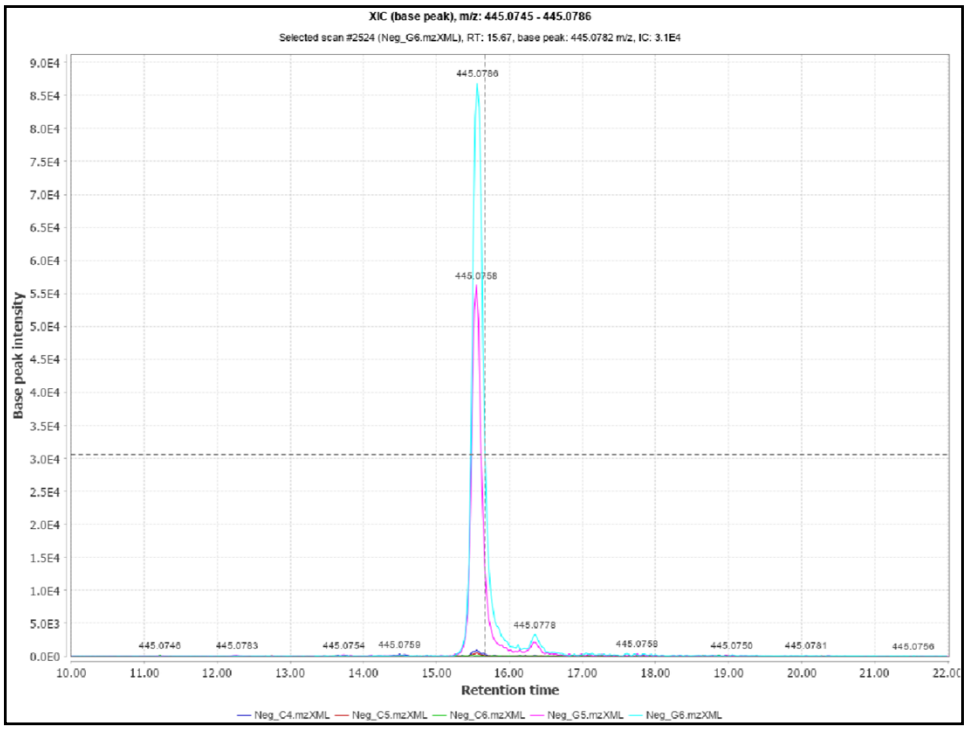
Mass window for Gen GlcA



27



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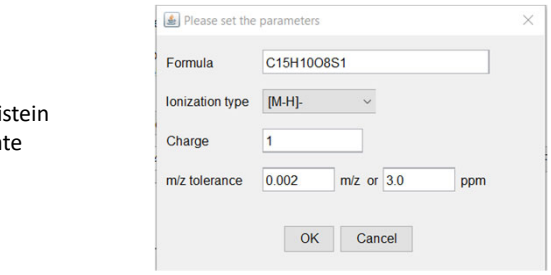
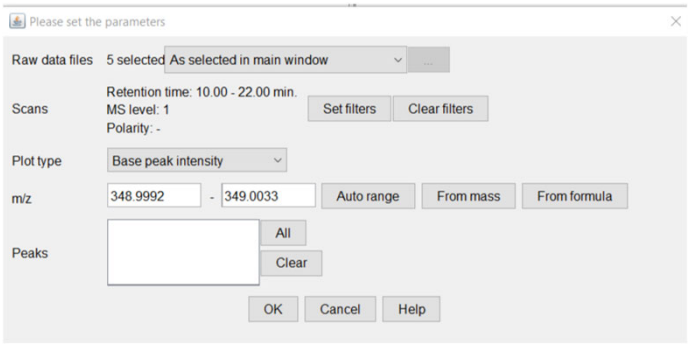
Genistein sulfate





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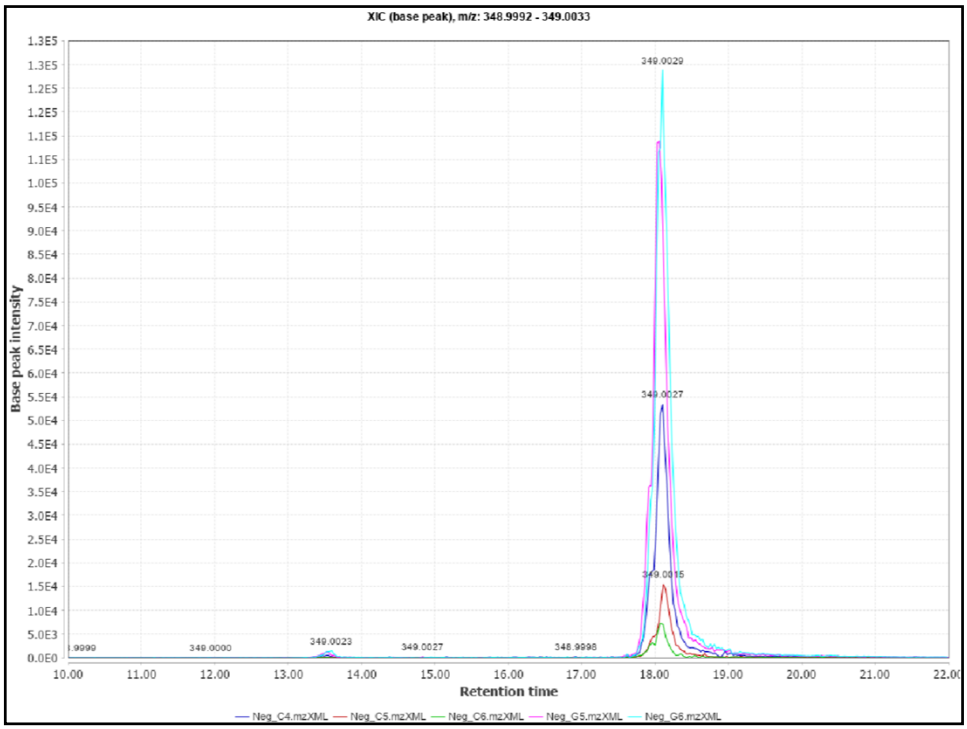
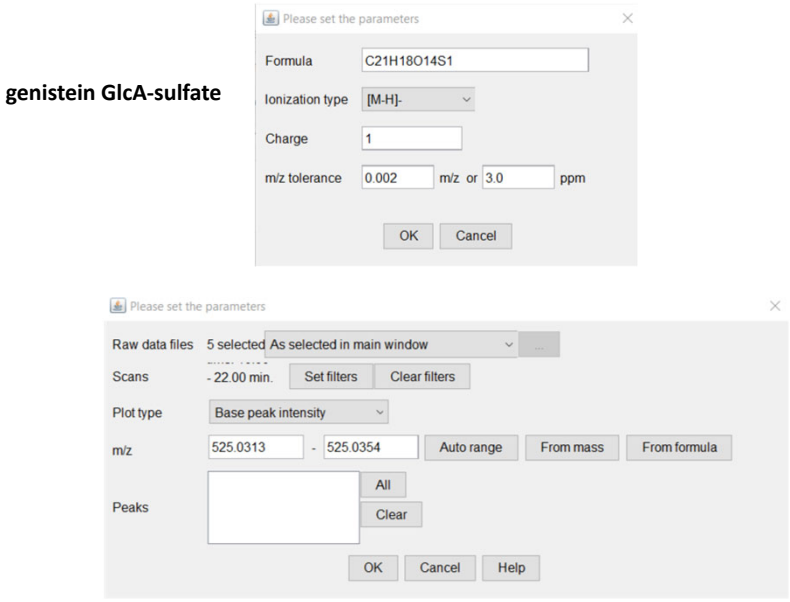
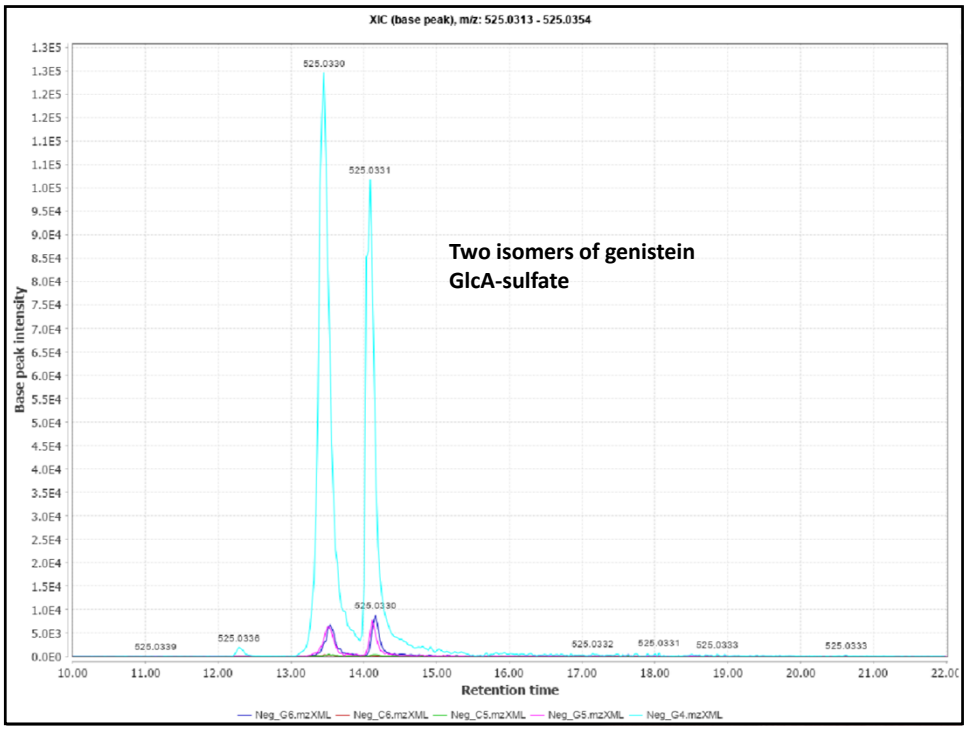


30

genistein GlcA-sulfate



31



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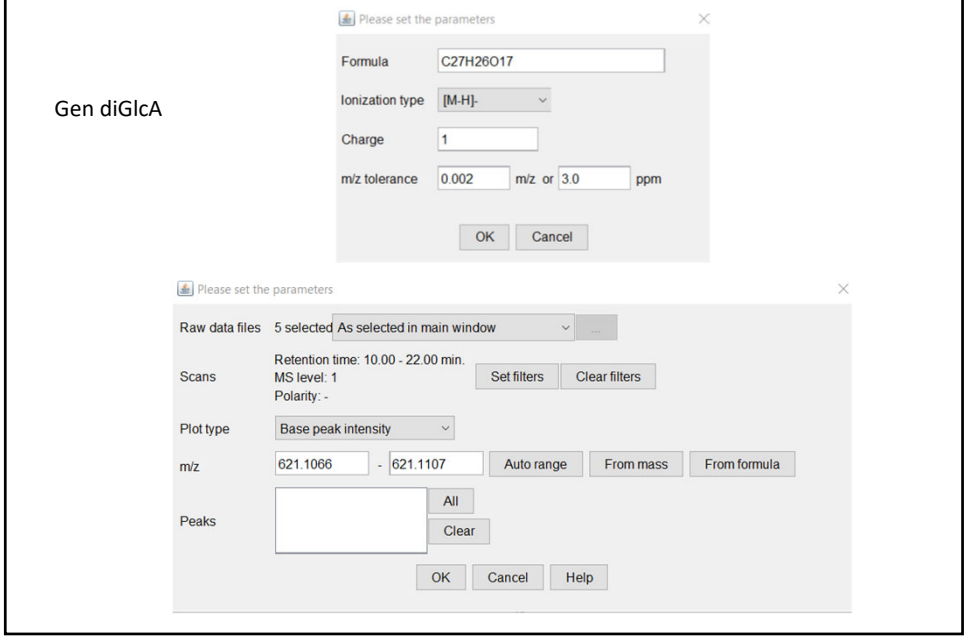
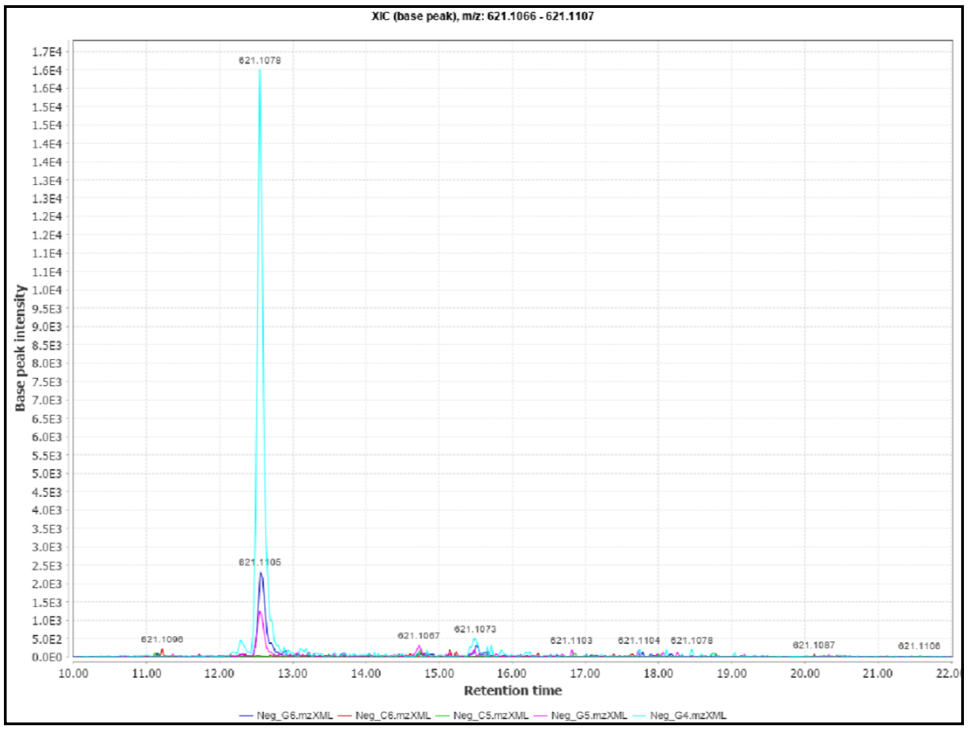
Gen diGlcA



33



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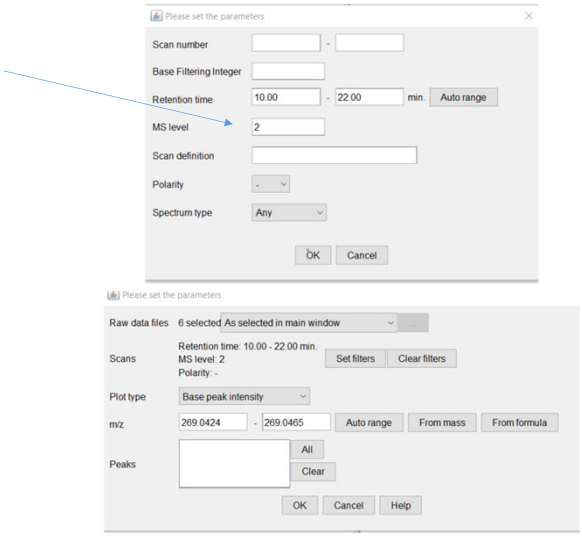
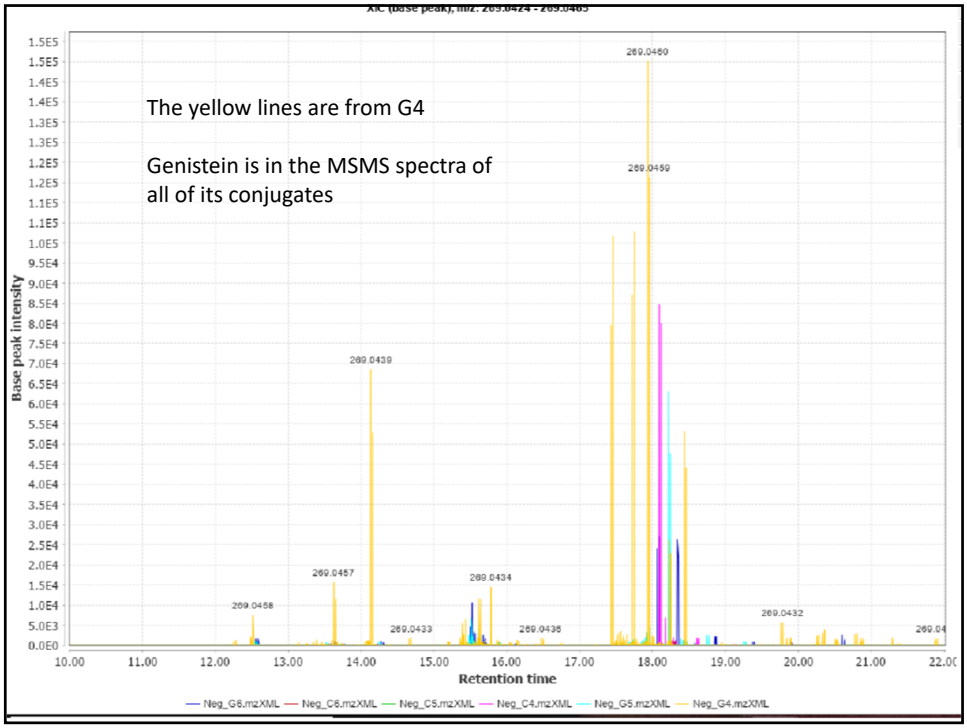
Getting MS/MS data

Select TIC/XIC and reset the parameters



35



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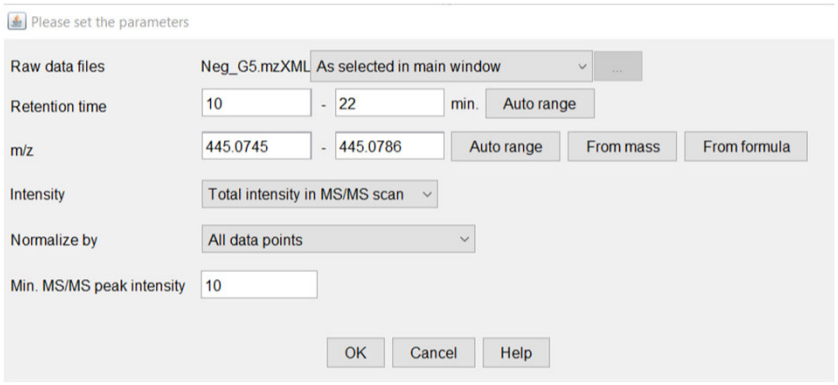
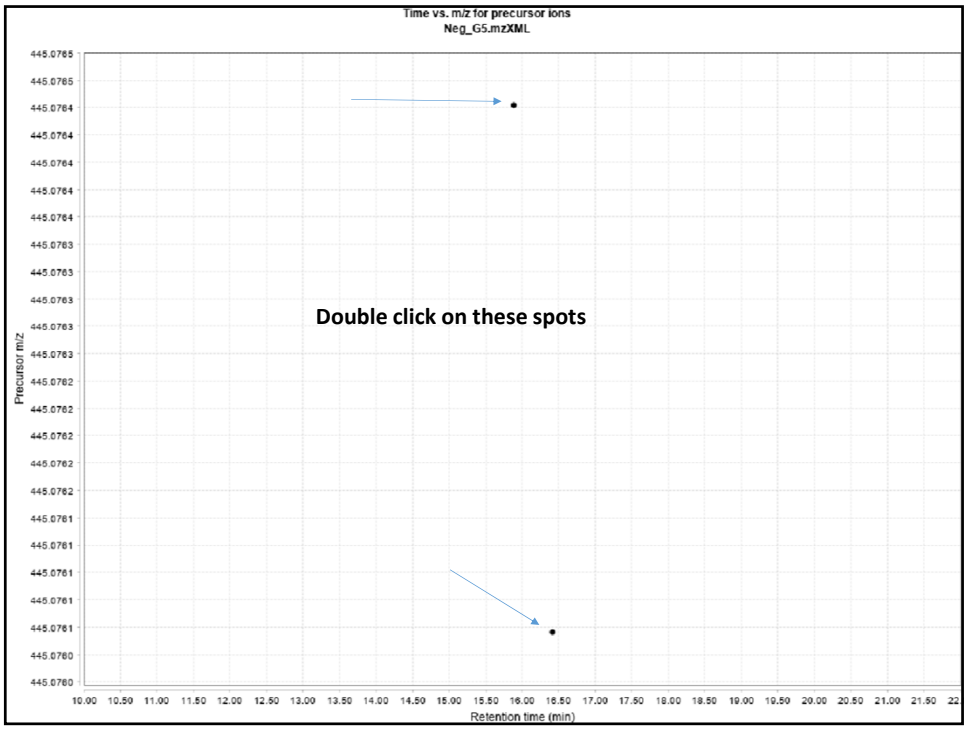
Another way to get MSMS spectra

Select MSMS visualizer

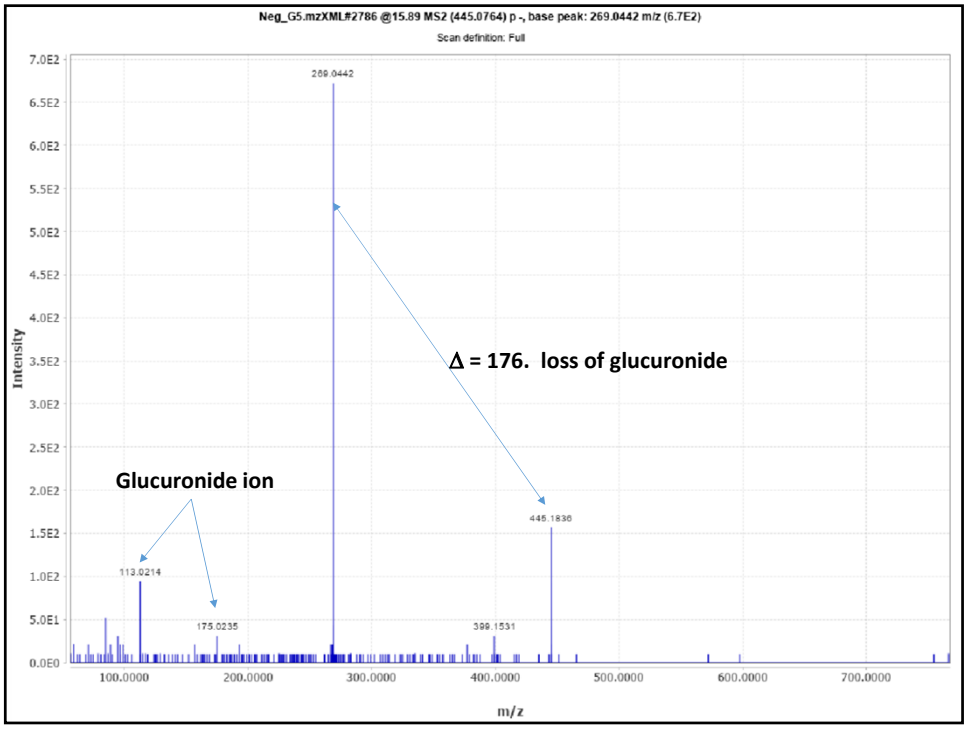
Genistein β-glucuronide (C21H18O11)



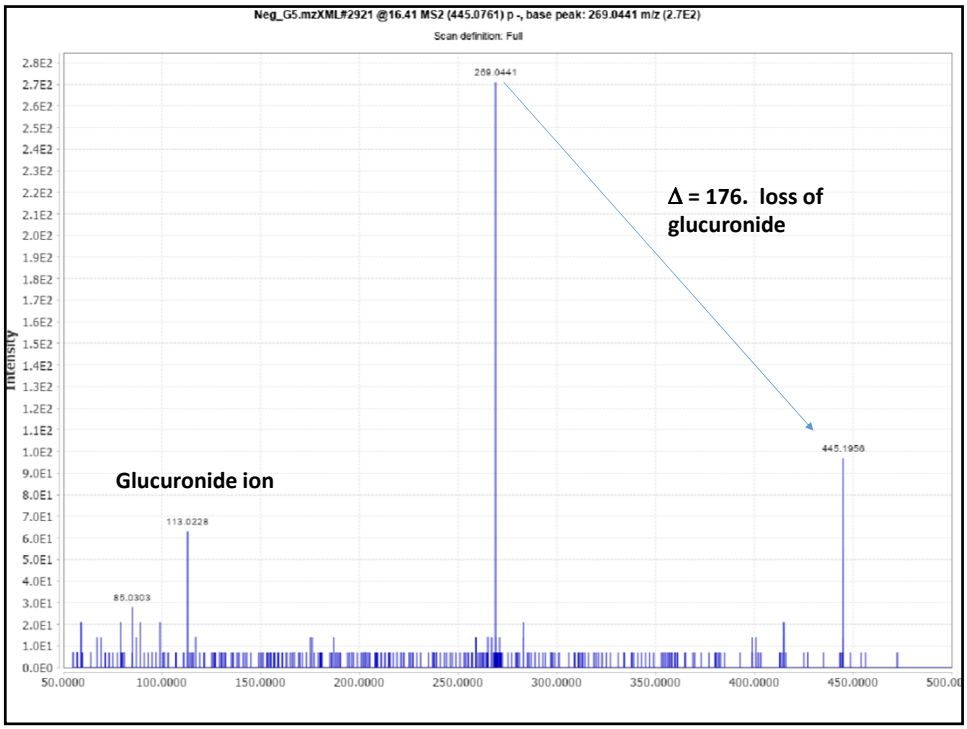
37



38

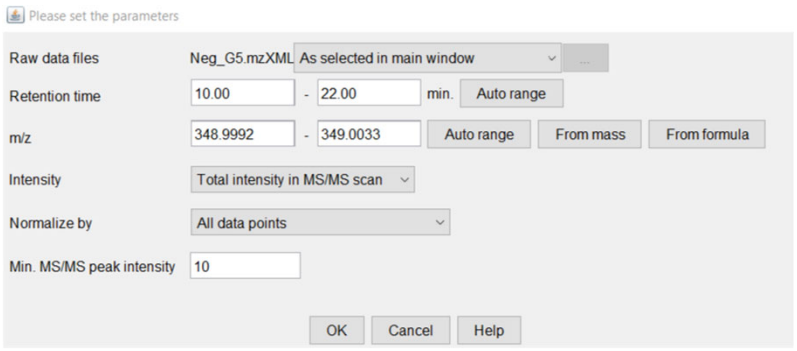

Neg_G5.mzXML#2786 @15.89 MS2 (445.0764) p -, base peak: 269.0442 m/z (6.7E2)
Scan definition: Full
269.0442
Δ = 176. loss of glucuronide
Glucuronide ion
445.1836
113.0214
175.0235
399.1531
Intensity
m/z

39

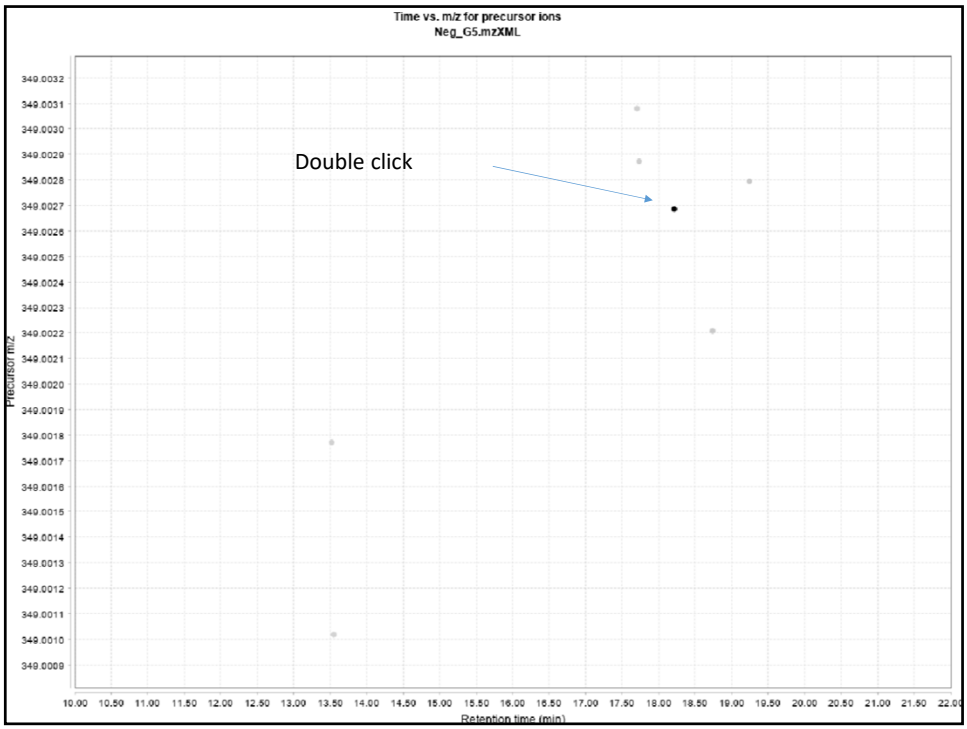

Neg_G5.mzXML#2921 @16.41 MS2 (445.0761) p -, base peak: 269.0441 m/z (2.7E2)
Scan definition: Full
269.0441
Δ = 176. loss of glucuronide
445.1956
Glucuronide ion
113.0228
85.0303
Intensity

40

MSMS of genistein sulfate



41



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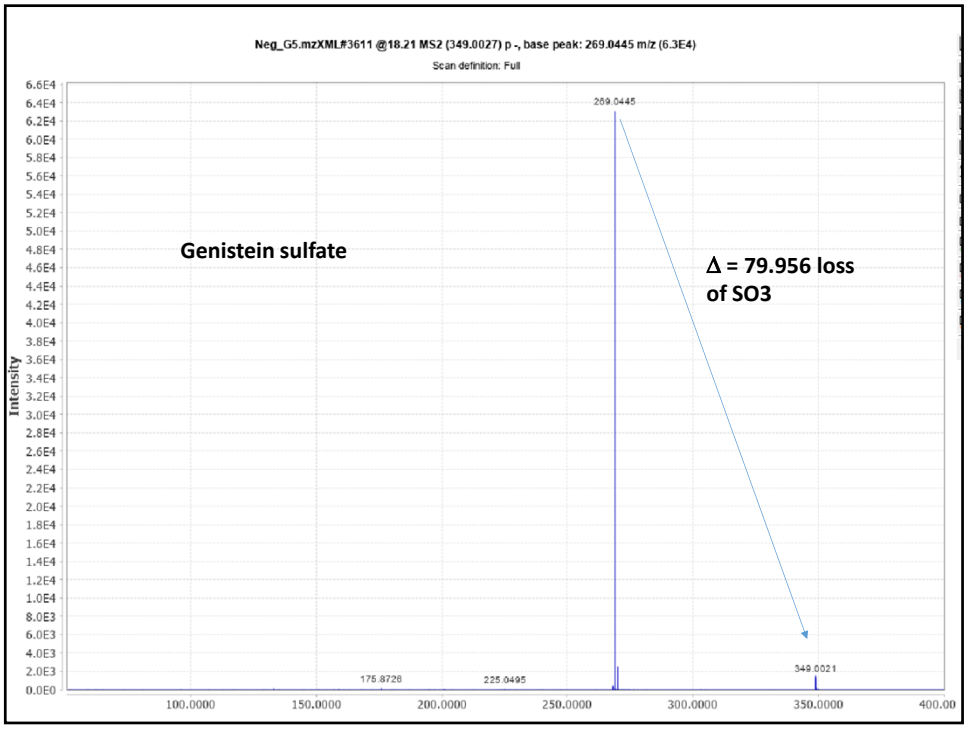

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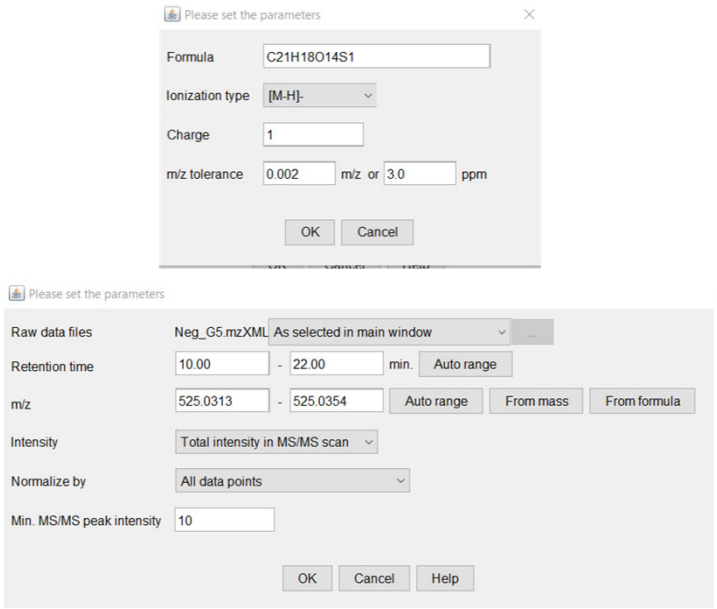
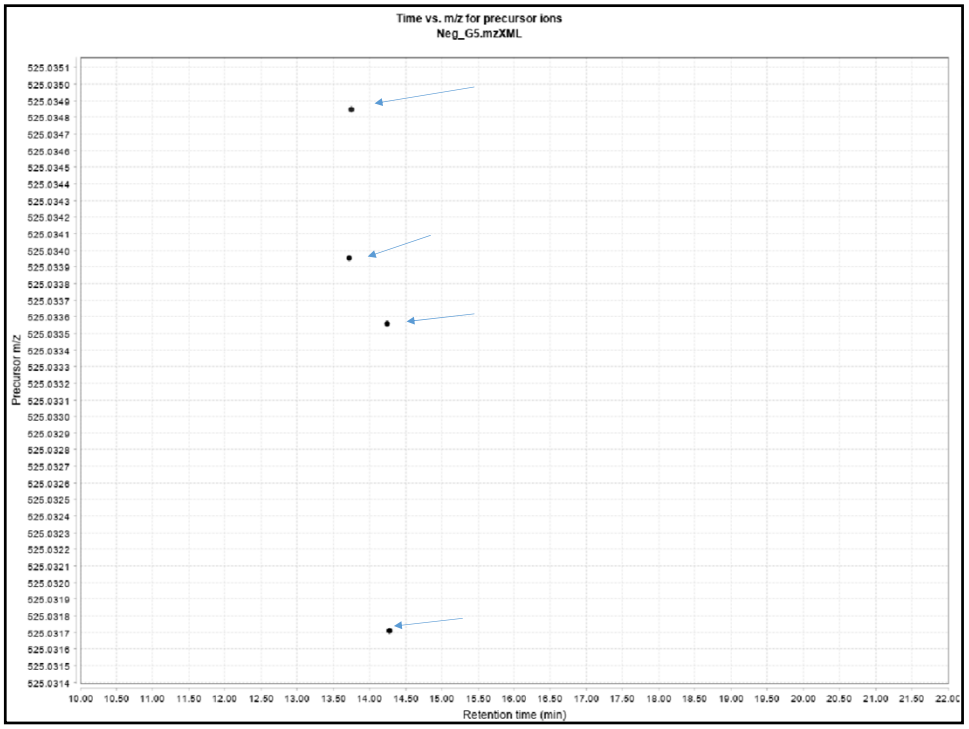
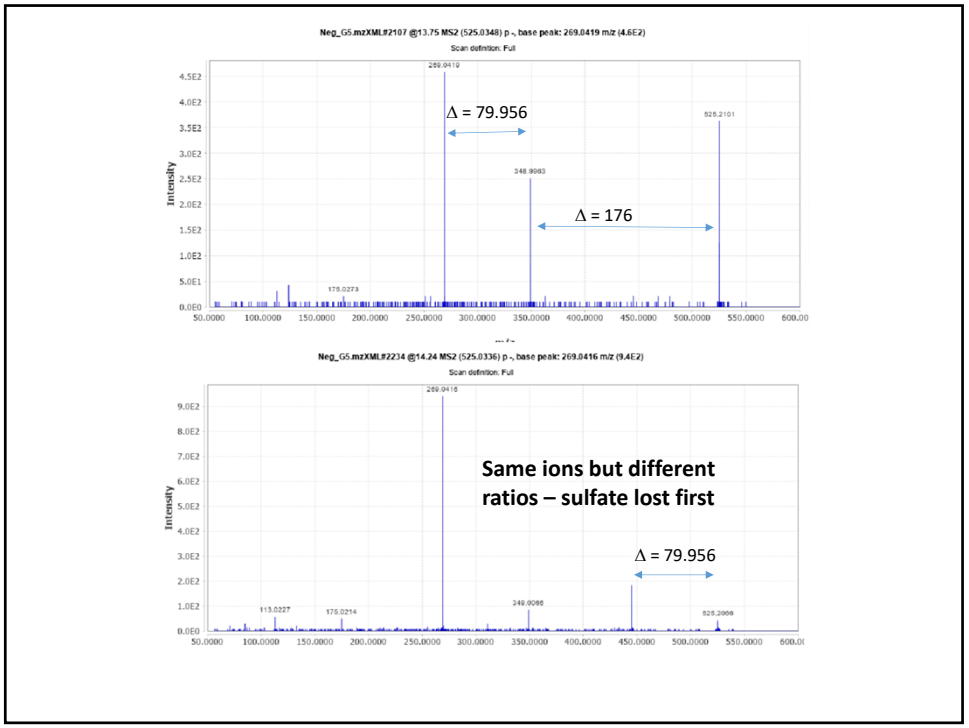
MSMS of genistein β-glucuronide sulfate



44


Time vs. m/z for precursor ions
Neg_G5.mzXML
Precursor m/z
Retention time (min)

45


Neg_G5.mzXML#2107 @13.75 MS2 (525.0348) p -, base peak: 269.0419 m/z (4.6E2)
Scan definition: Full
Δ = 79.956
Δ = 176
Intensity
Neg_G5.mzXML#2234 @14.24 MS2 (525.0336) p -, base peak: 269.0416 m/z (9.4E2)
Scan definition: Full
Same ions but different ratios – sulfate lost first
Δ = 79.956
Intensity

46

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.

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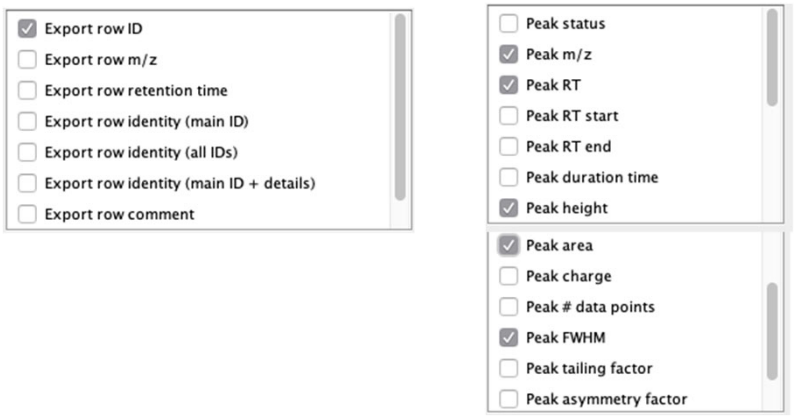
Export to .CSV file



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