Knowledge that will change your world

The application of MZmine 2 to viewing metabolomics raw data

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Acknowledgements to

xiuxia.du@uncc.edu
Author of the ADAP routine in Mzmine
Listen to talk at UAB 2018 workshop
and hands-on session

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Author of MZmine

2

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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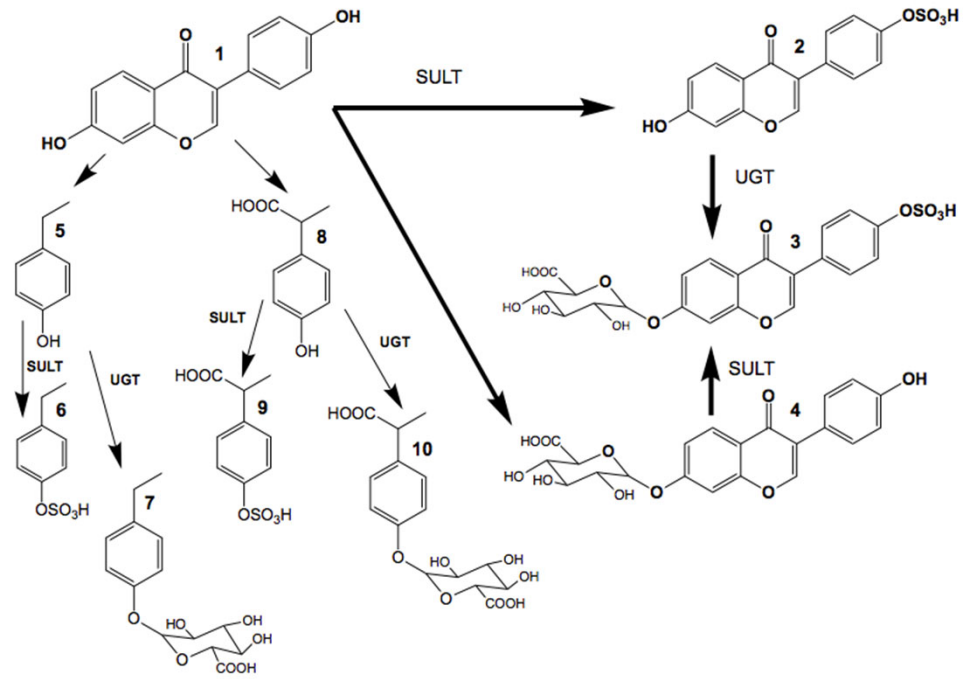

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Answers to mass calculation question

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

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Download MZmine 2.53

- 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

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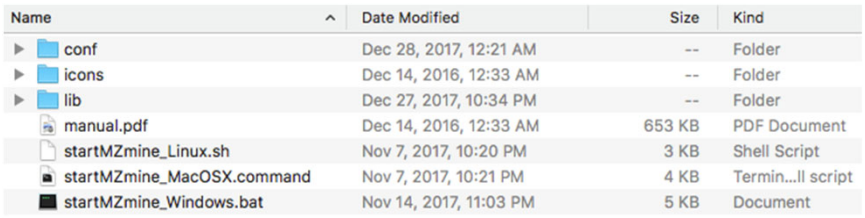
Starting point for MZmine



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.

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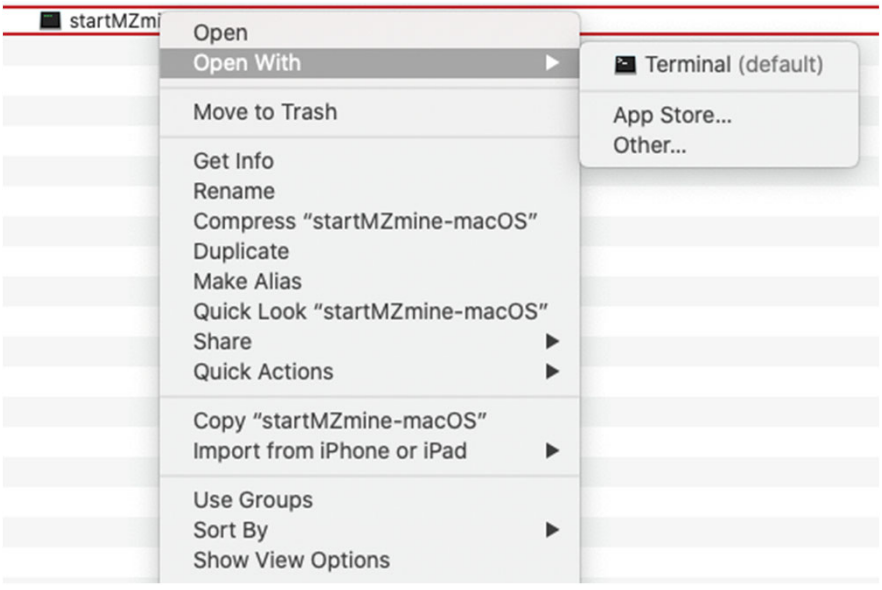
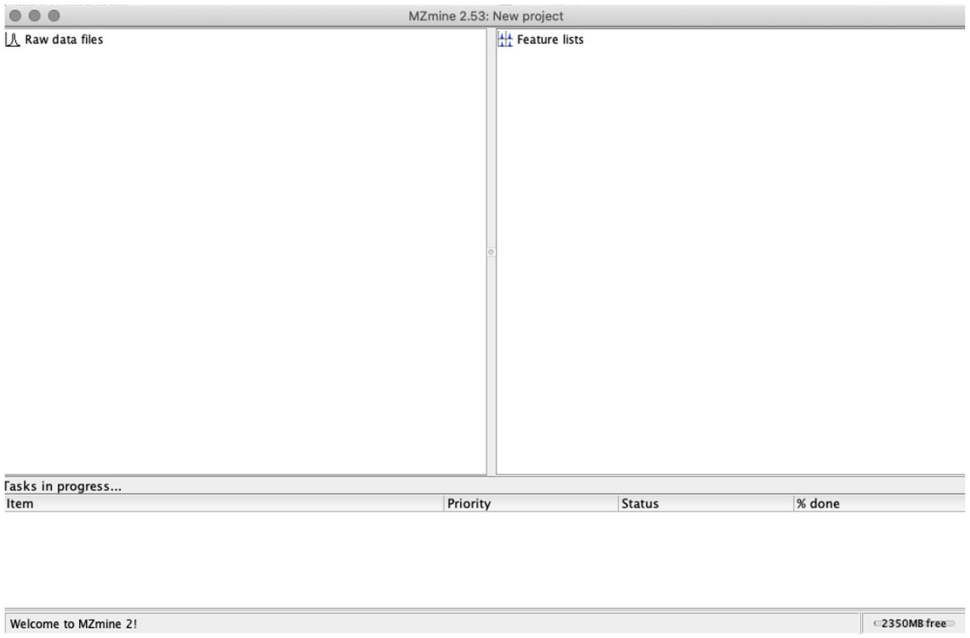


8



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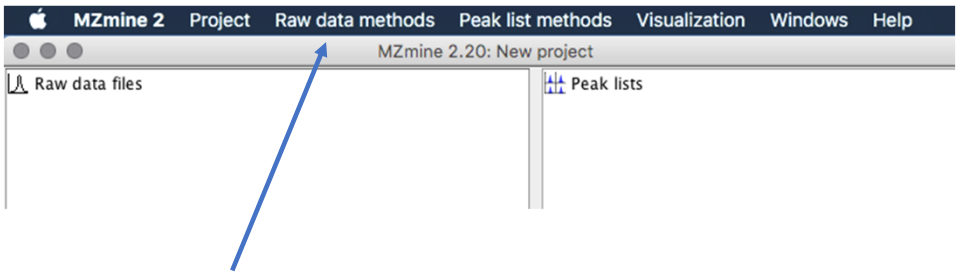
Starting off



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

Note the MZmine version number in 2021 is 2:53

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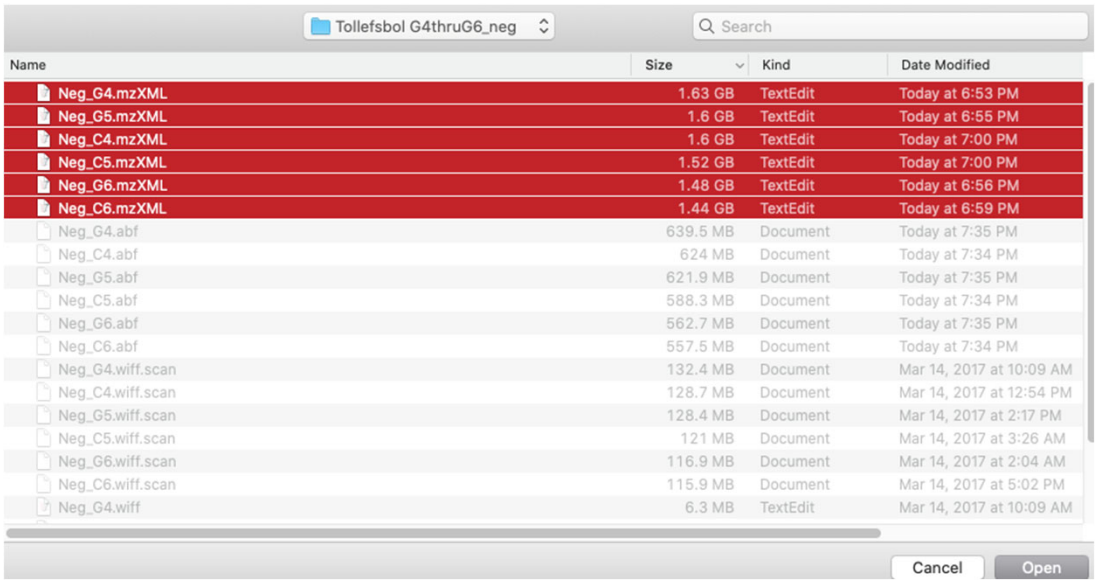

This is where the files are on my Mac. The highlighted ones are loaded by pressing <Open>.

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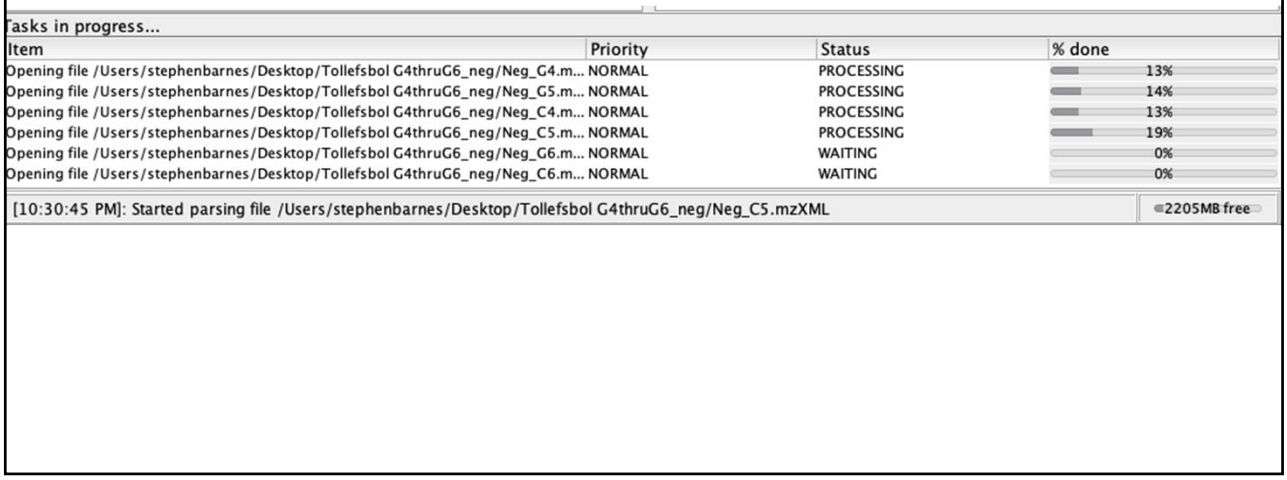
Showing uploading process



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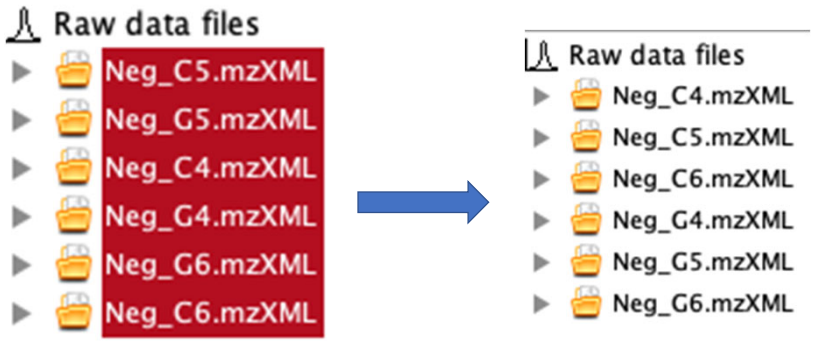
Files are now loaded



Highlight the files

Re-order the files

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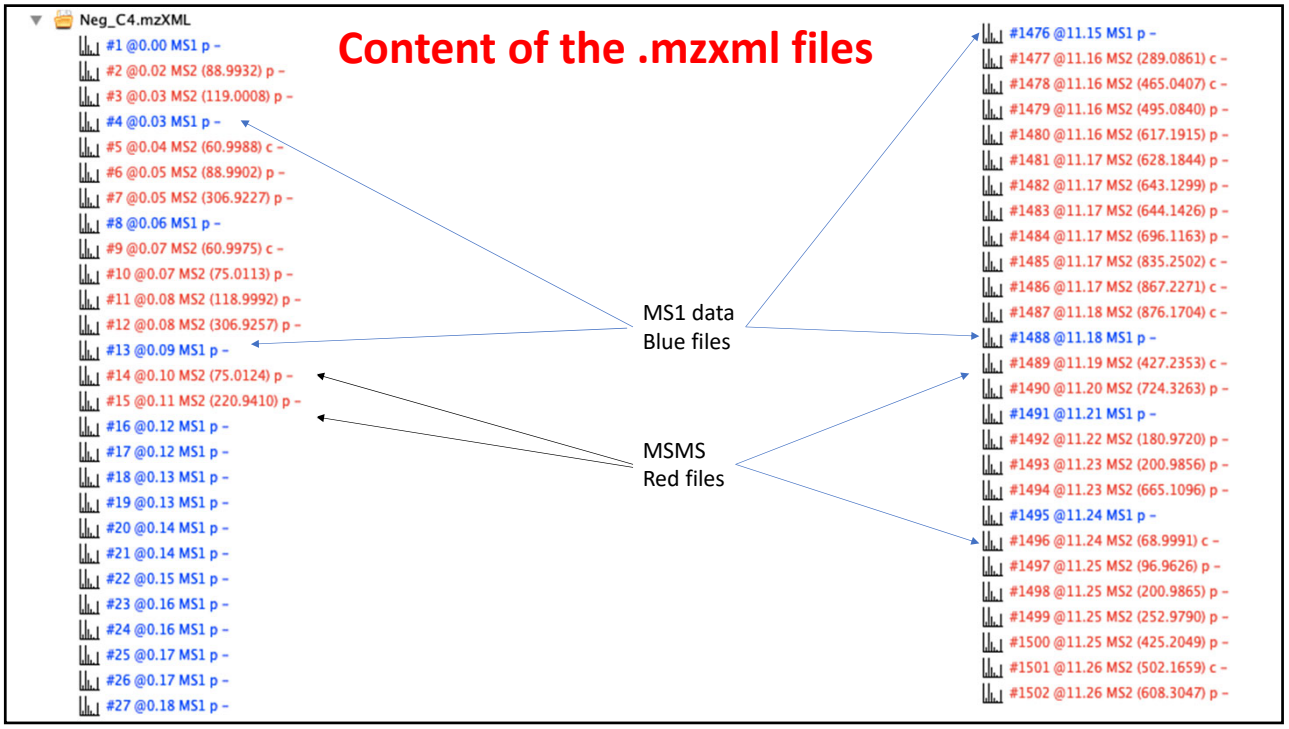
Content of the .mzxml files



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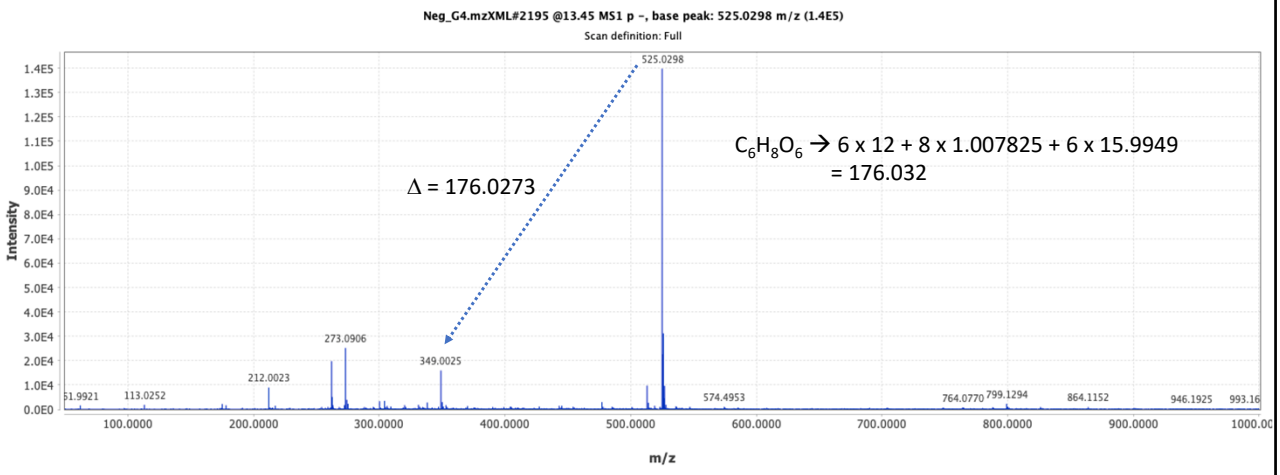
MS1 TOF spectrum of ions eluting at 13.45 min



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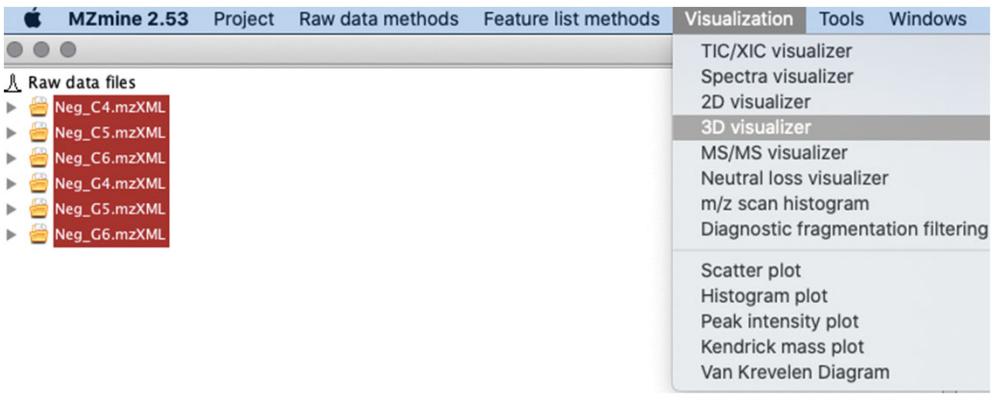
Visualization toolbar



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

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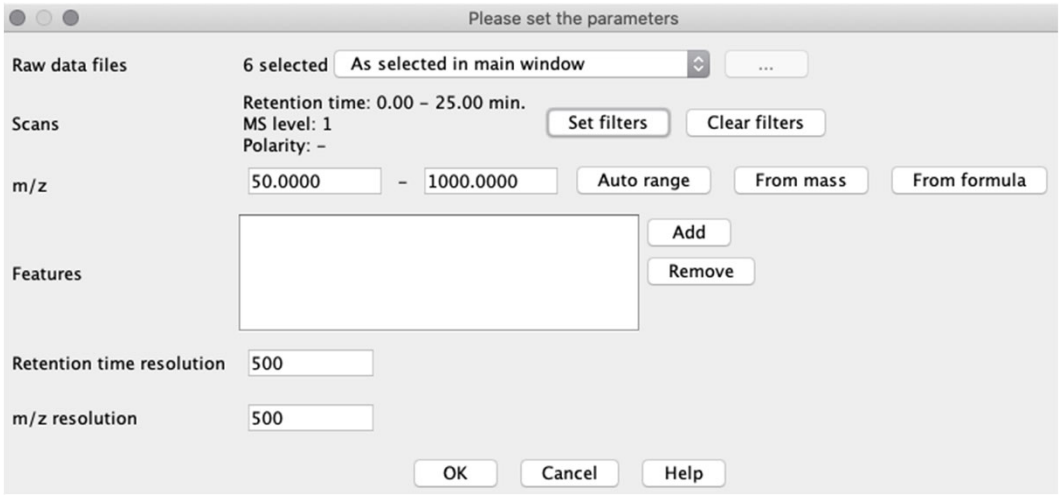
Selecting all the data



We'll select the file to open next. The 3D visualizer allows all to be examined.

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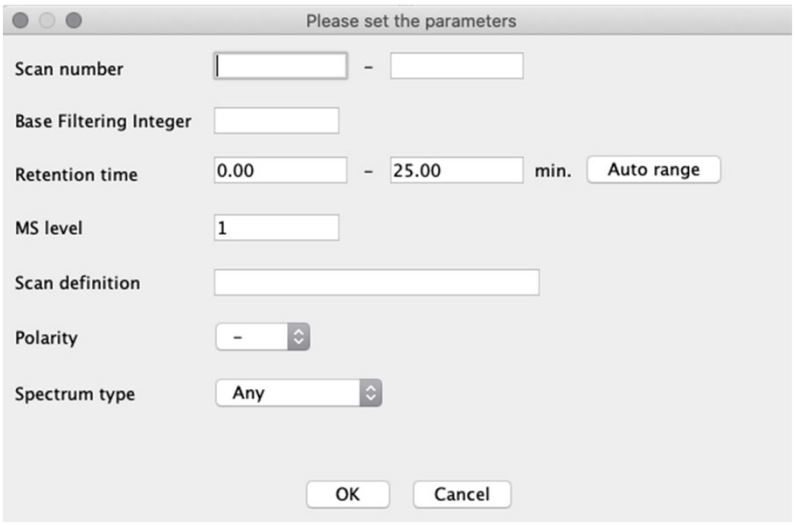
Setting 3D-parameters



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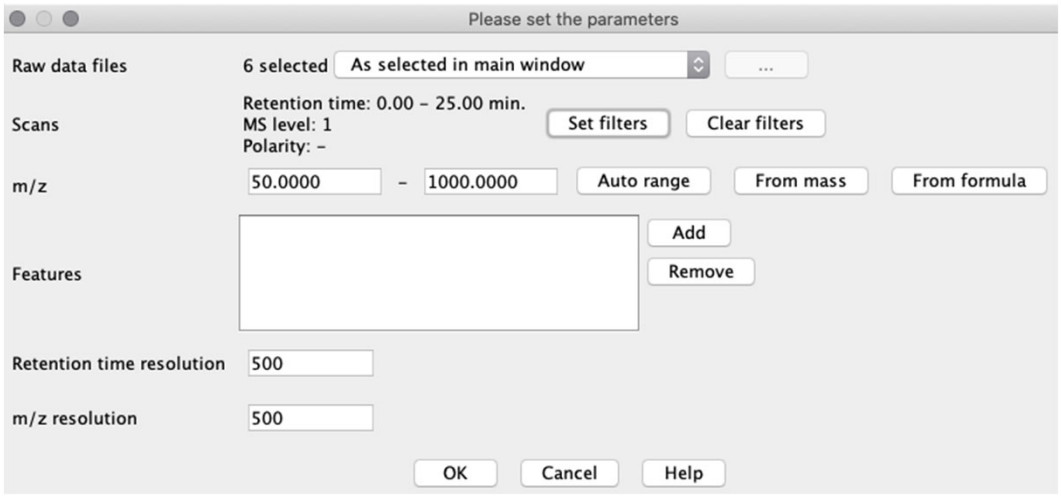
Ready to view the 3D-plot



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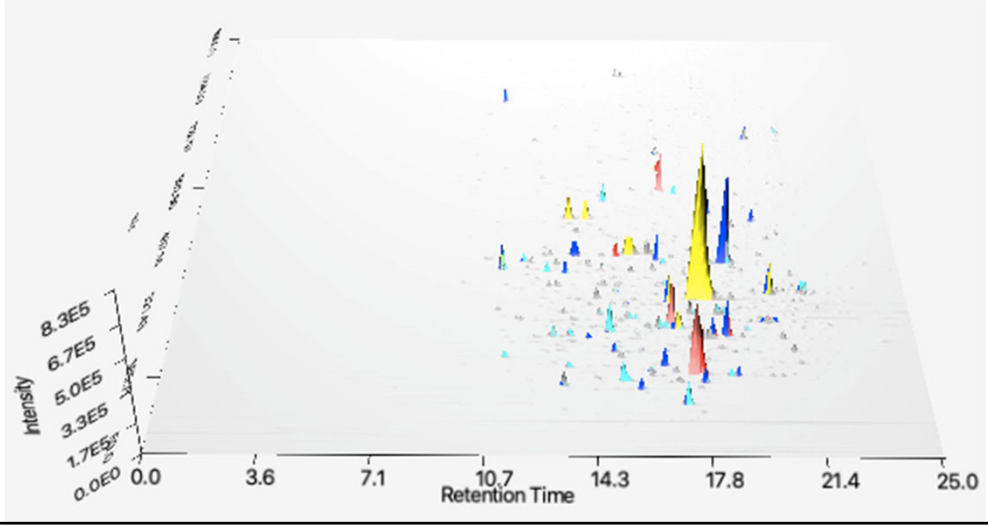
Genistein – red, orange,yellow



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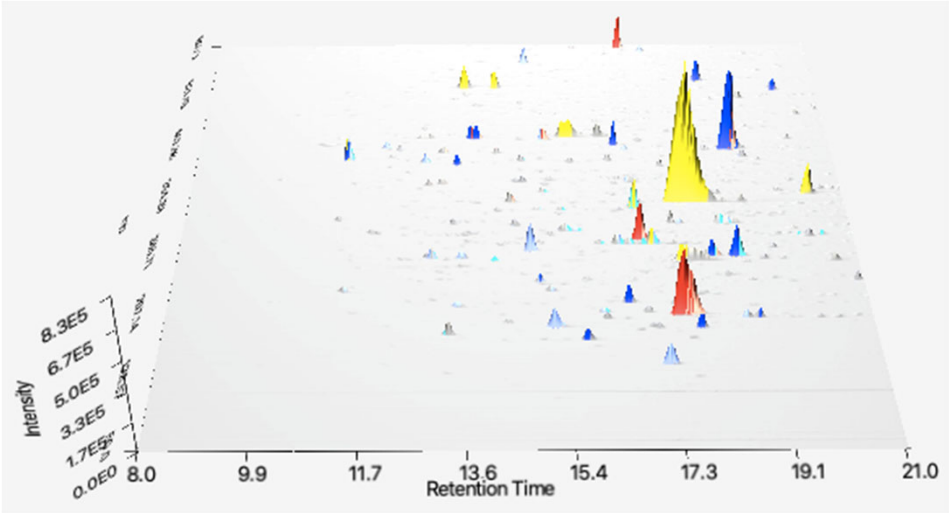
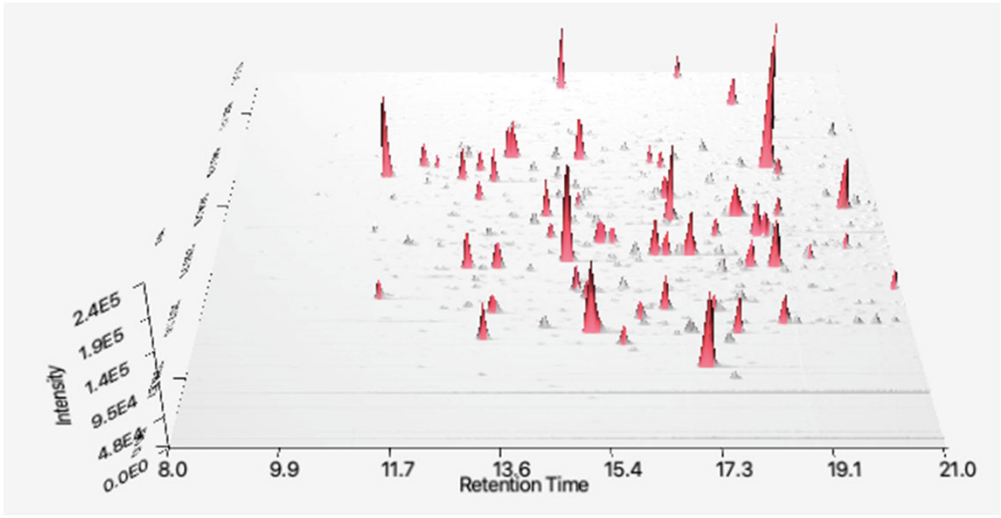
Expanded 3D-view



22

Control sample 3D-look



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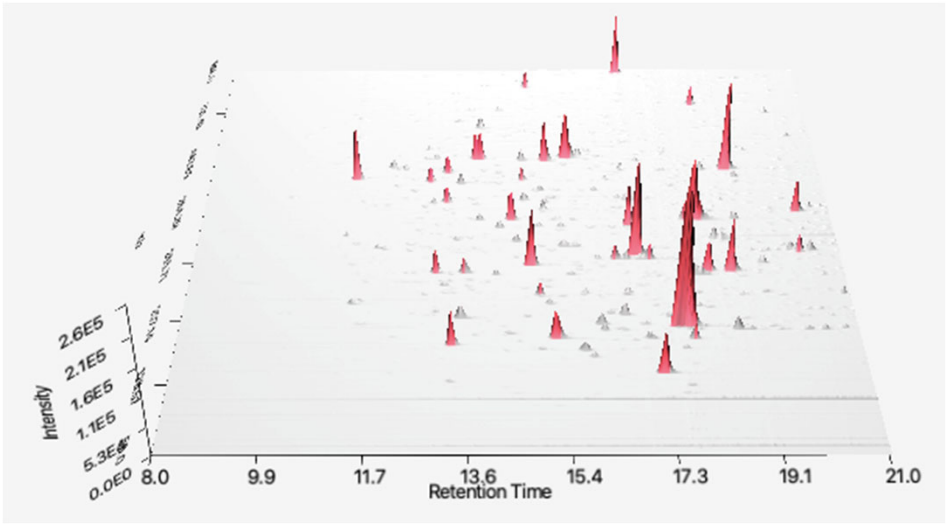
Genistein sample 3D-look



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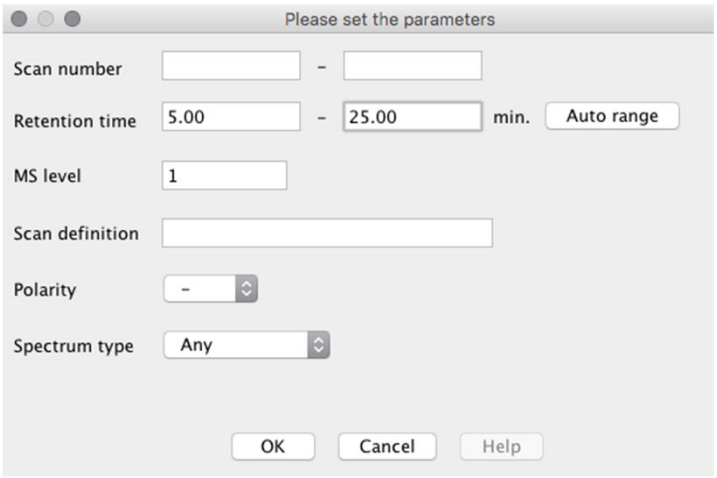
Resetting the parameters



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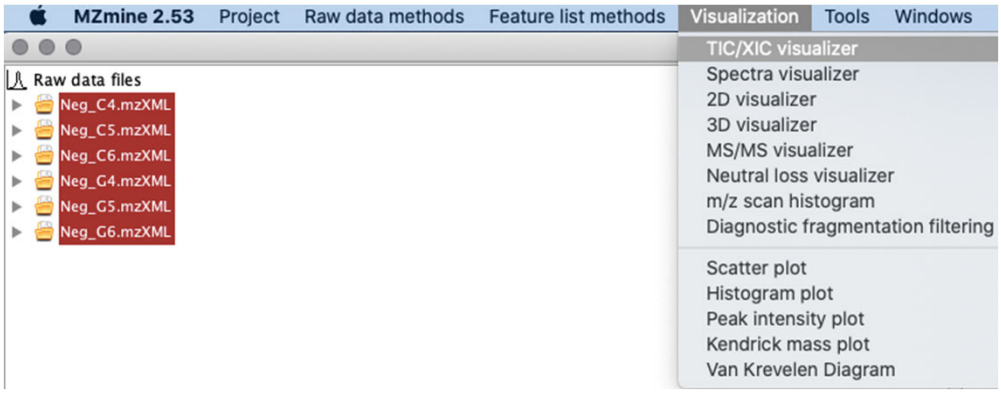
Total ion current



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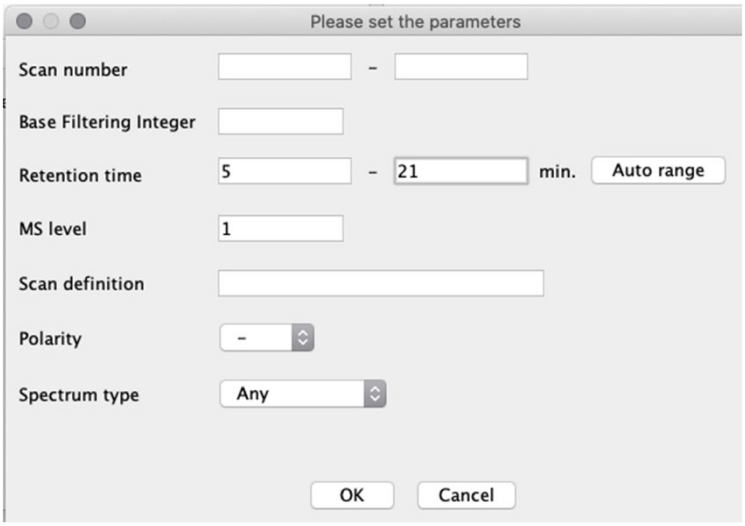
Setting the parameters



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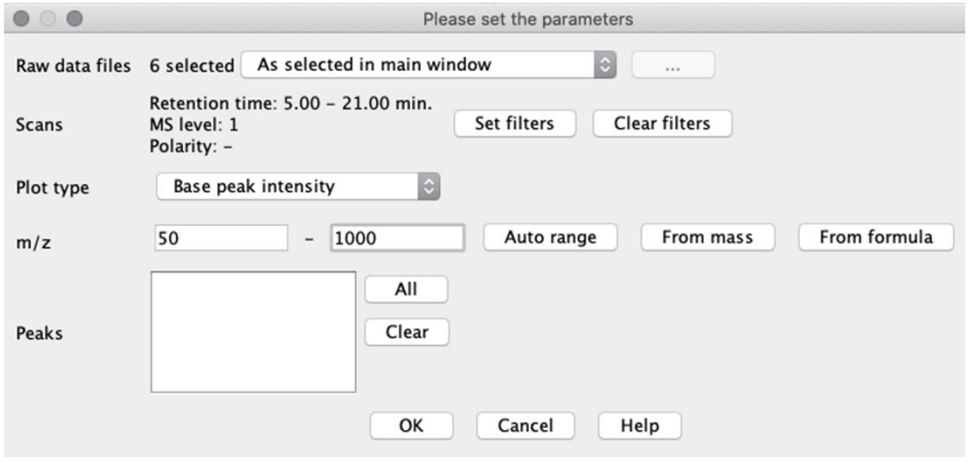
Ready to view the total ion current



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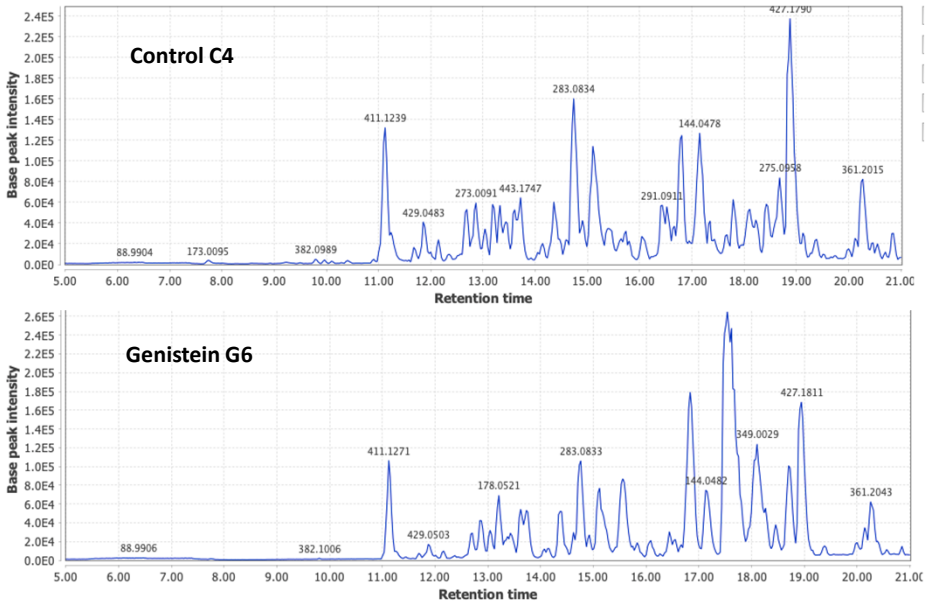
TIC of all ions from *m/z* 50-1000



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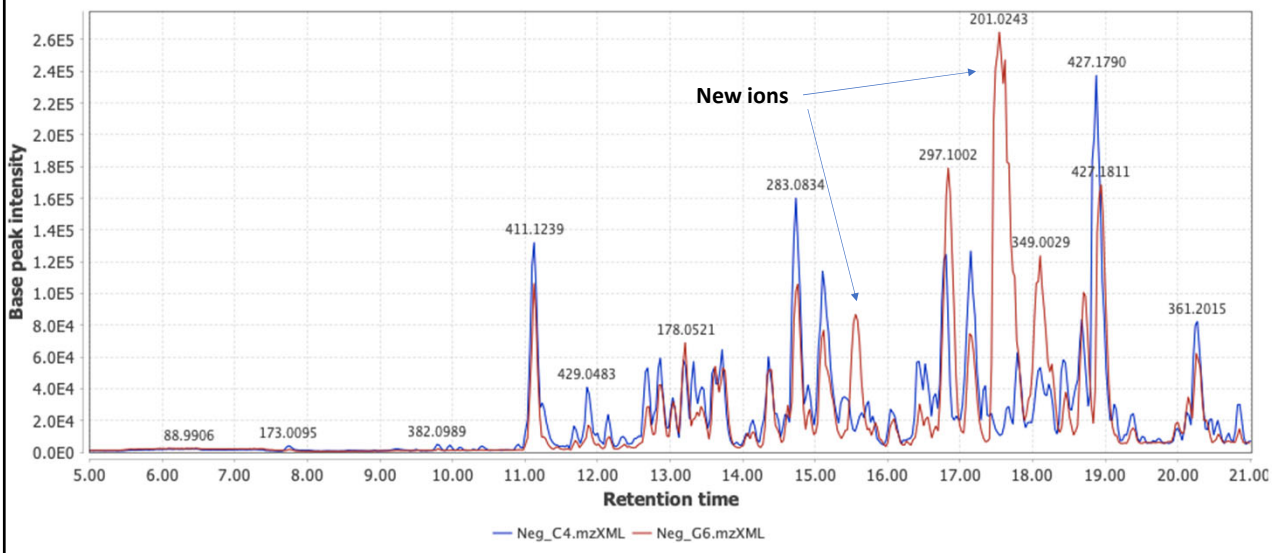
TIC *m/z* 50-1000 C4/G6 comparison



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

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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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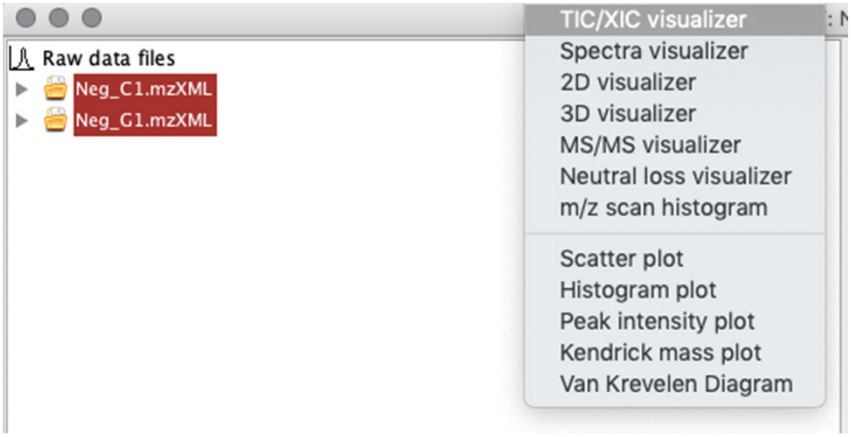
Setting the mass window



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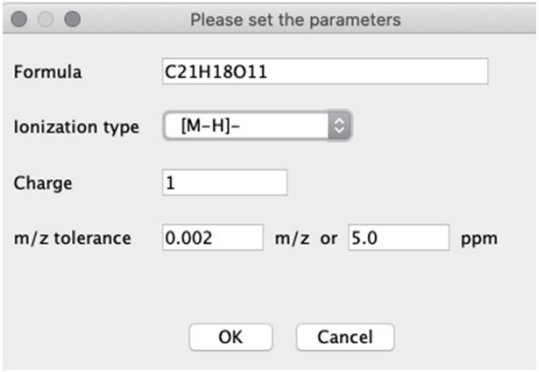
Setting the mass window



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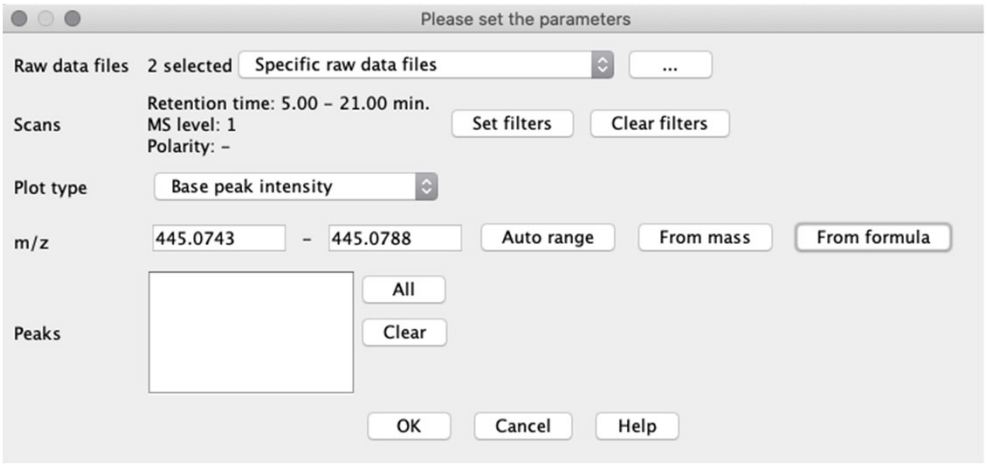
Finished the setup to find GenGlcA



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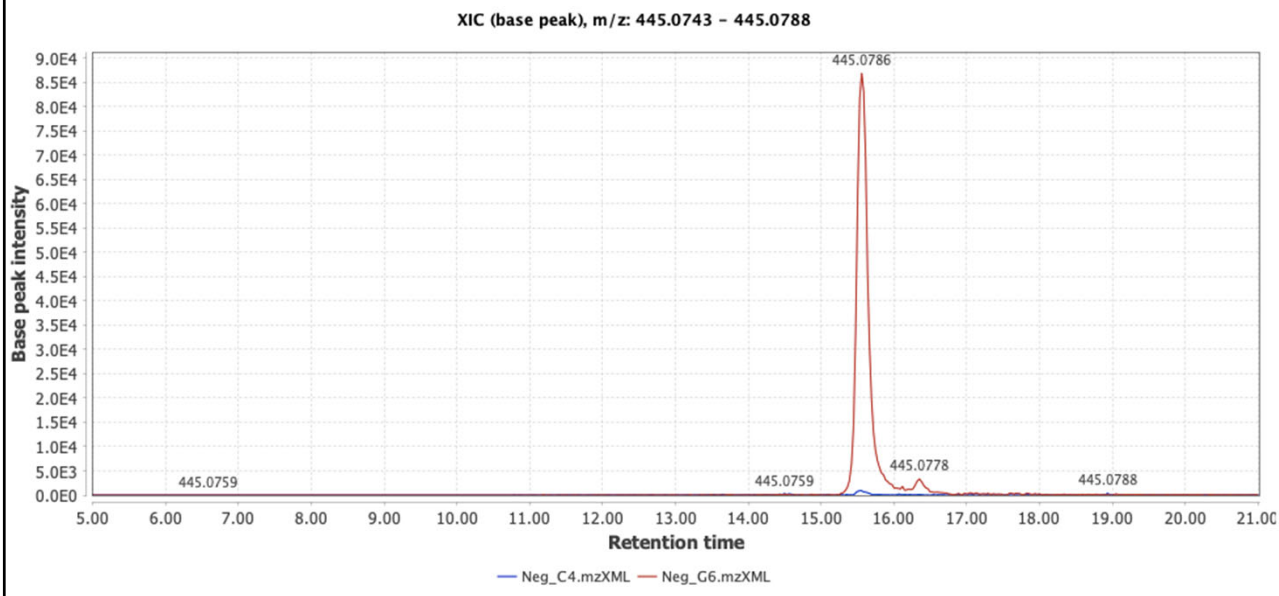
XIC of *m/z* 445.0788



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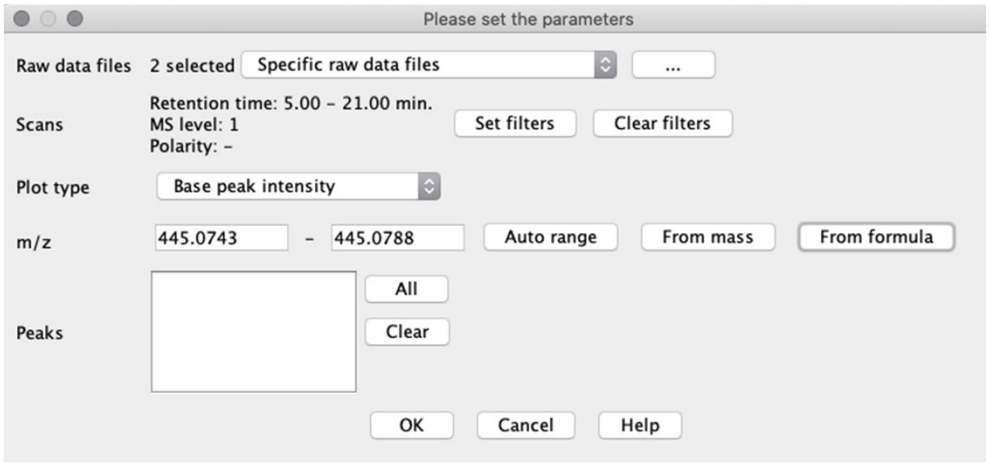
2D-plot for GENGlcA



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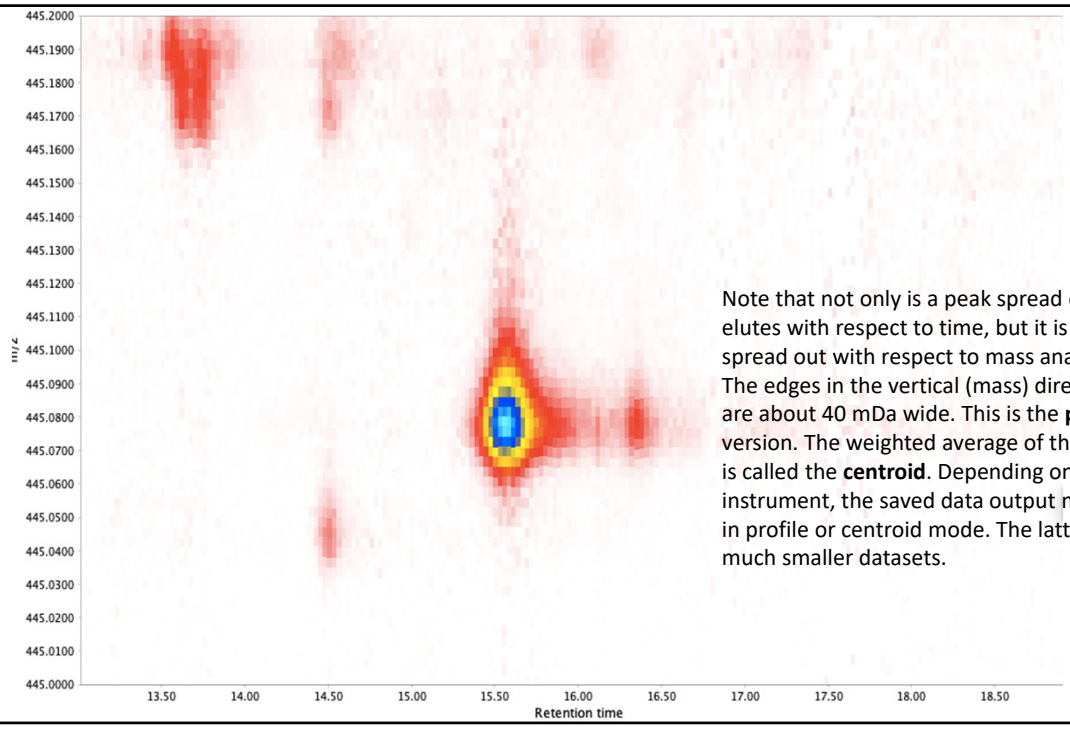

Note that not only is a peak spread out as it elutes with respect to time, but it is also spread out with respect to mass analysis. The edges in the vertical (mass) direction are about 40 mDa wide. This is the **profile** version. The weighted average of the peak is called the **centroid**. Depending on the instrument, the saved data output may be in profile or centroid mode. The latter are much smaller datasets.

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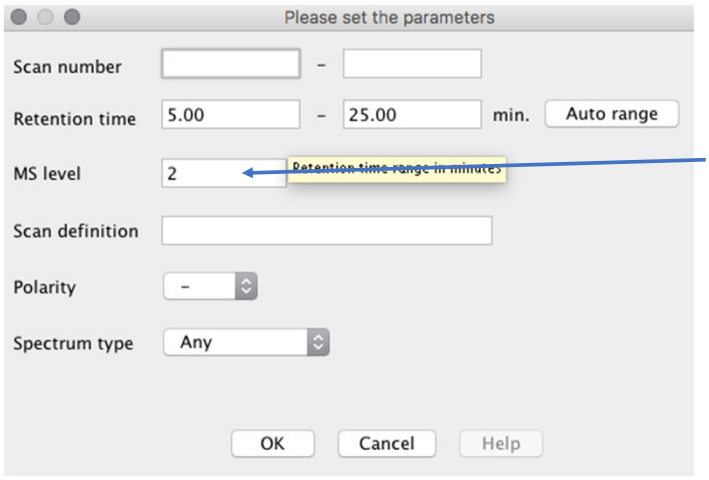
Getting MS/MS data

Select TIC/XIC and reset the parameters



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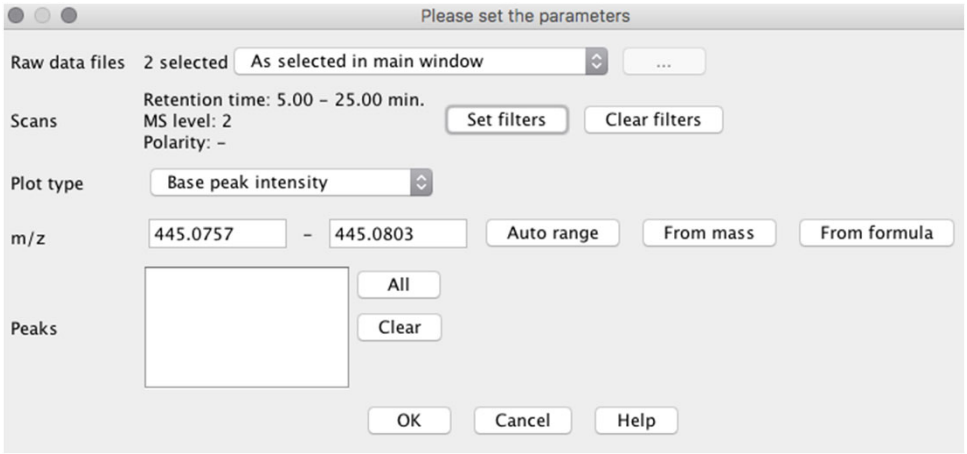
Ready to go



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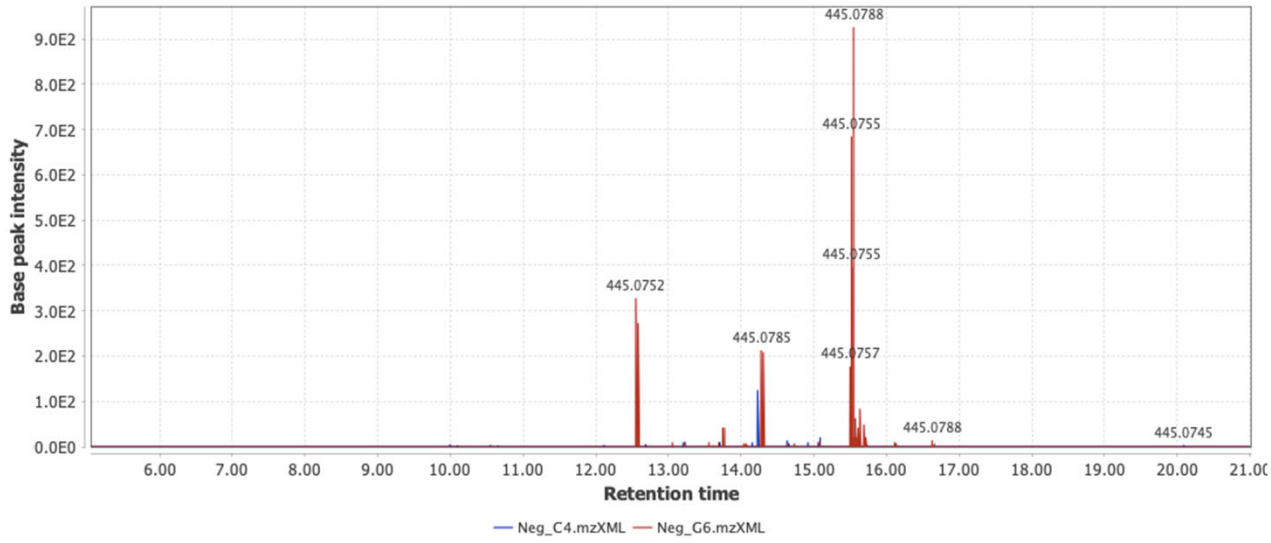
MSMS spectra captured of *m/z* 445.077



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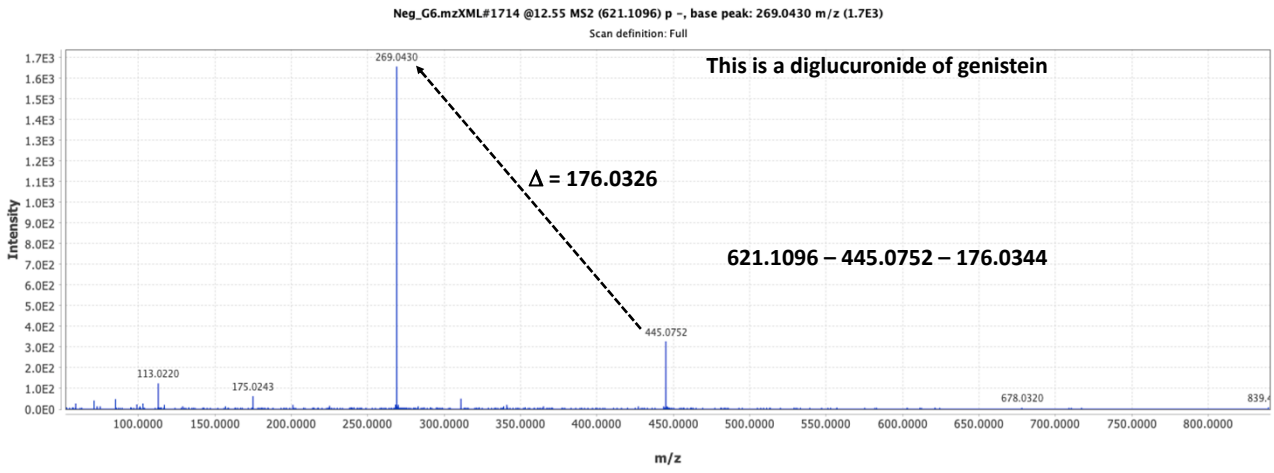
Where MSMS for *m/z* 445.079 were recorded (1)



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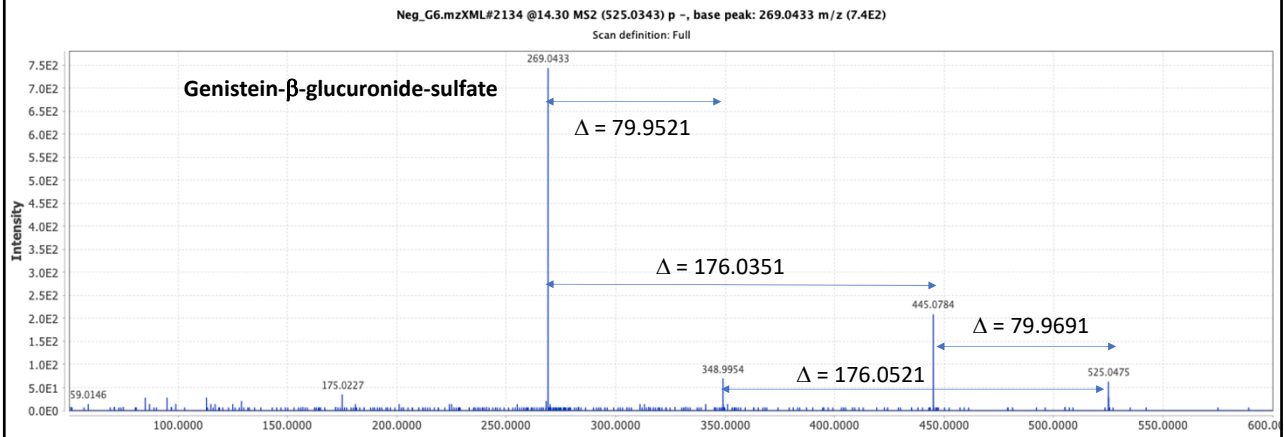
Where MSMS for *m/z* 445.079 were recorded (2)



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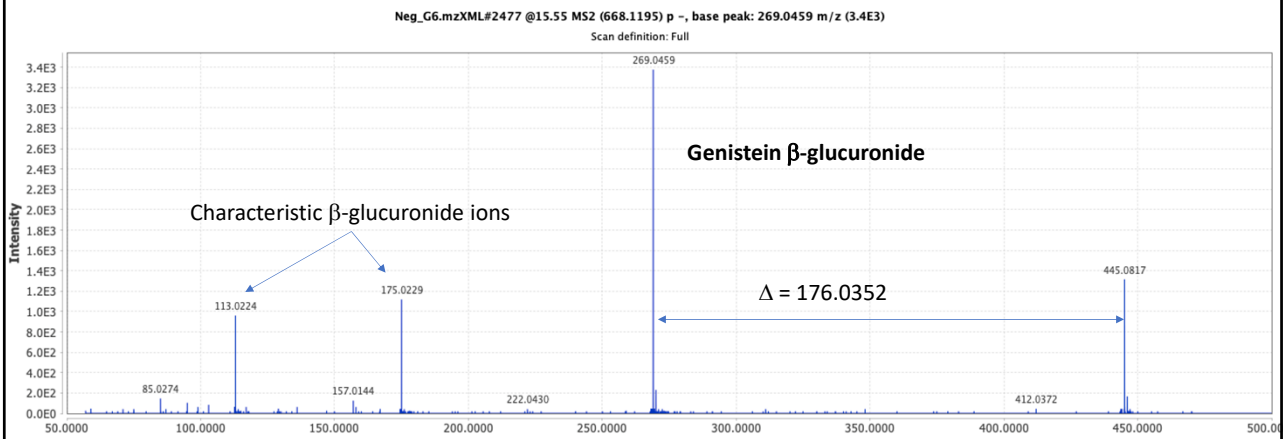
Where MSMS for *m/z* 445.079 were recorded (3)



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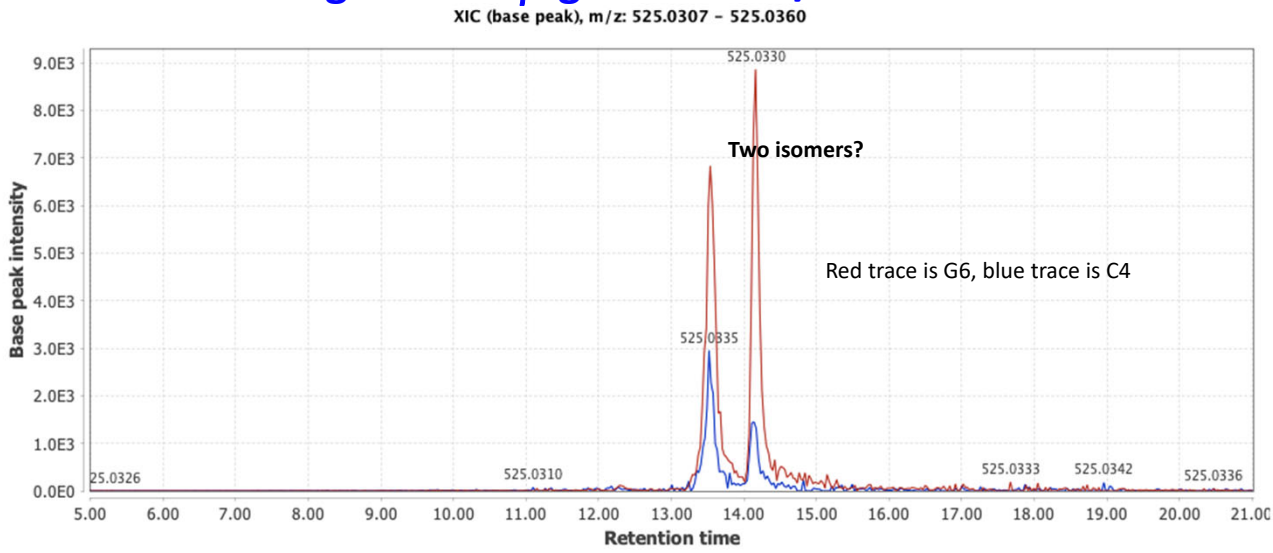
XIC of *m/z* 525.033

genistein β-glucuronide/sulfate



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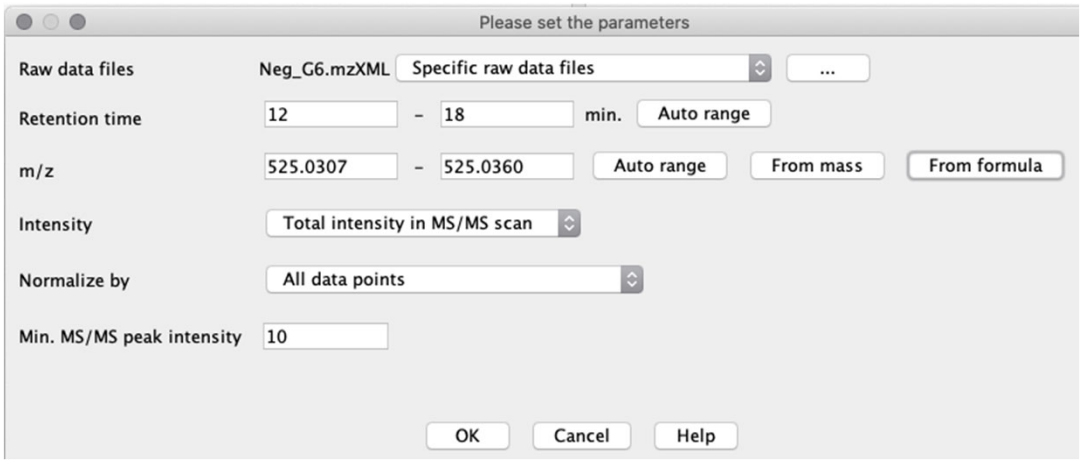
Another way to get MSMS spectra

Select MSMS visualizer



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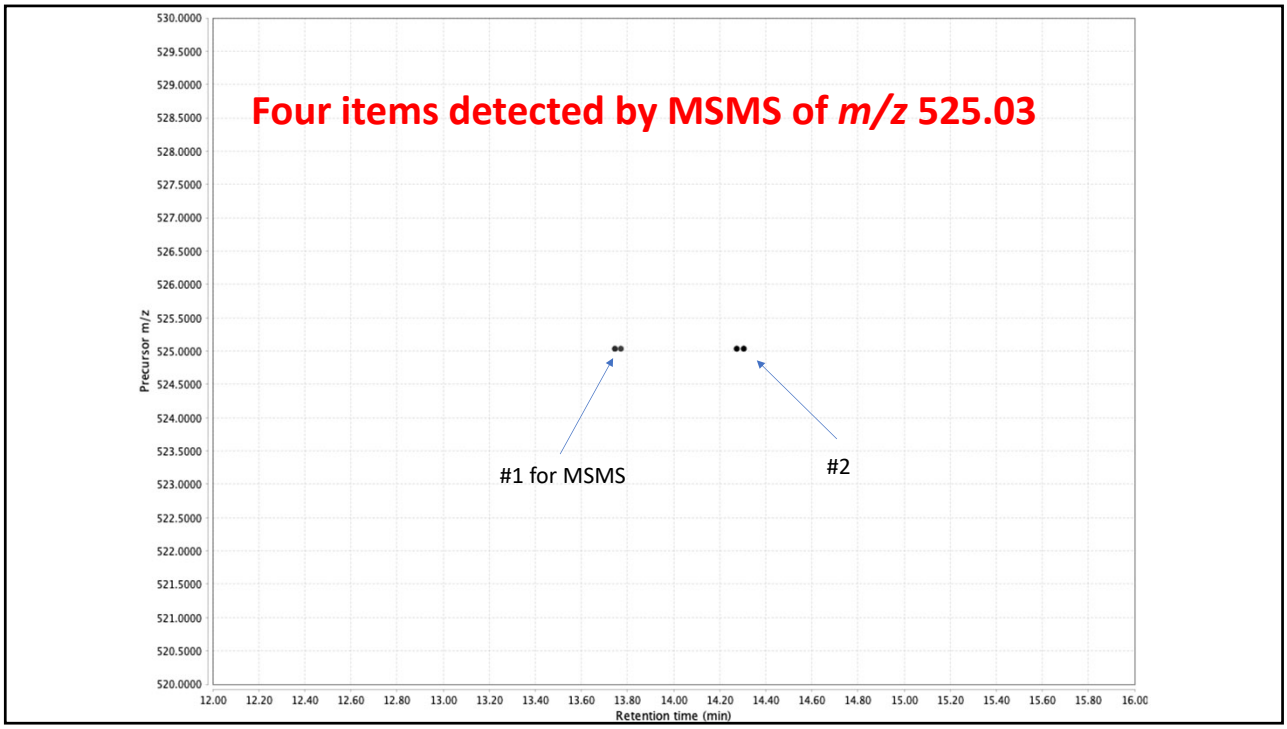
Four items detected by MSMS of *m/z* 525.03
Precursor m/z
Retention time (min)
#1 for MSMS
#2

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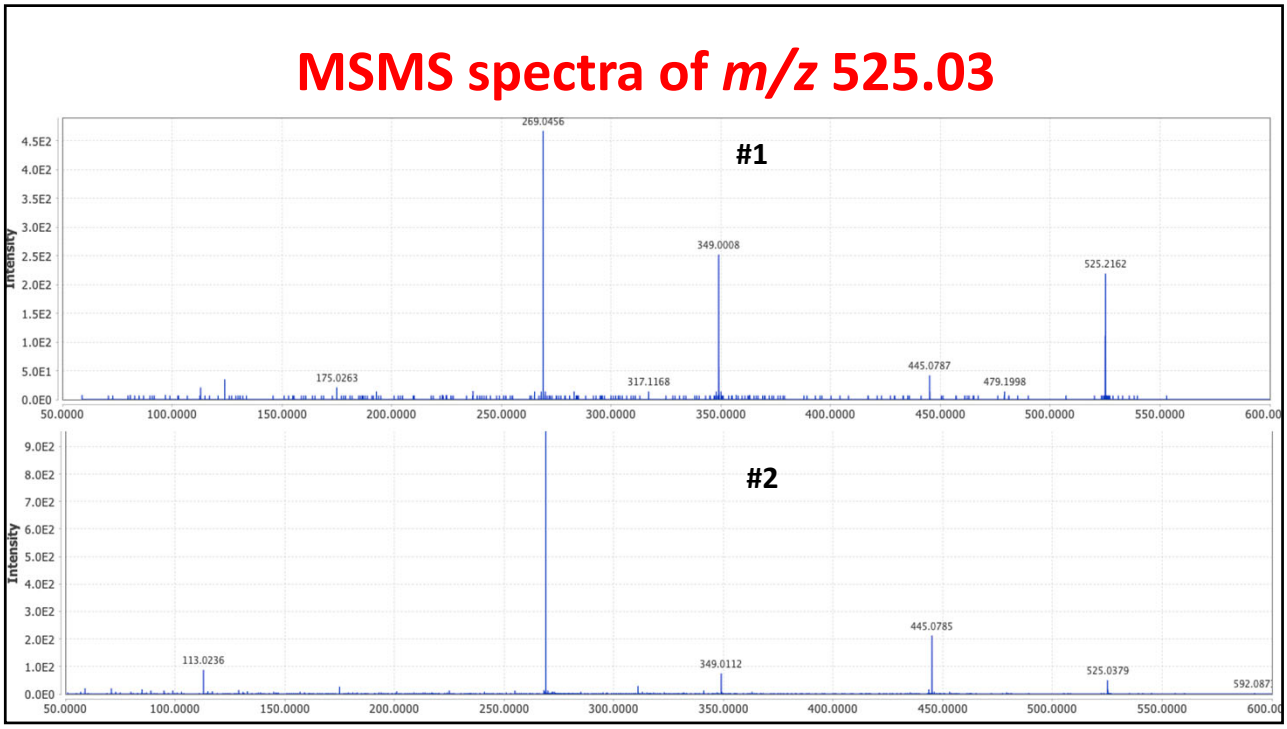
MSMS spectra of *m/z* 525.03
#1
269.0456
349.0008
525.2162
175.0263
317.1168
445.0787
479.1998
#2
113.0236
349.0112
445.0785
525.0379
592.087
Intensity

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