



*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



RESEARCH ARTICLE

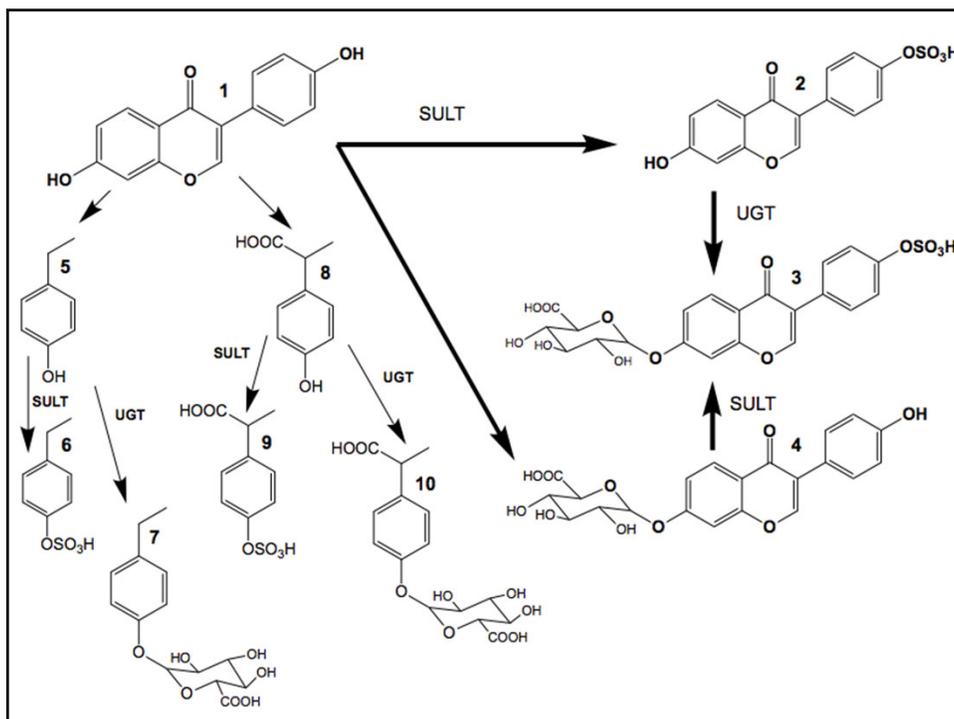
### Impact of genistein on the gut microbiome of humanized mice and its role in breast tumor inhibition

**Bidisha Paul<sup>1</sup>, Kendra J. Royston<sup>1,2</sup>, Yuanyuan Li<sup>1,2</sup>, Matthew L. Stoll<sup>3</sup>, Christine F. Skibola<sup>4</sup>, Landon S. Wilson<sup>5</sup>, Stephen Barnes<sup>2,5,6,7,8</sup>, Casey D. Morrow<sup>9</sup>, Trygve O. Tollefsbol<sup>1,2,7,8,10</sup>\***

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

## Download MZmine 2.30

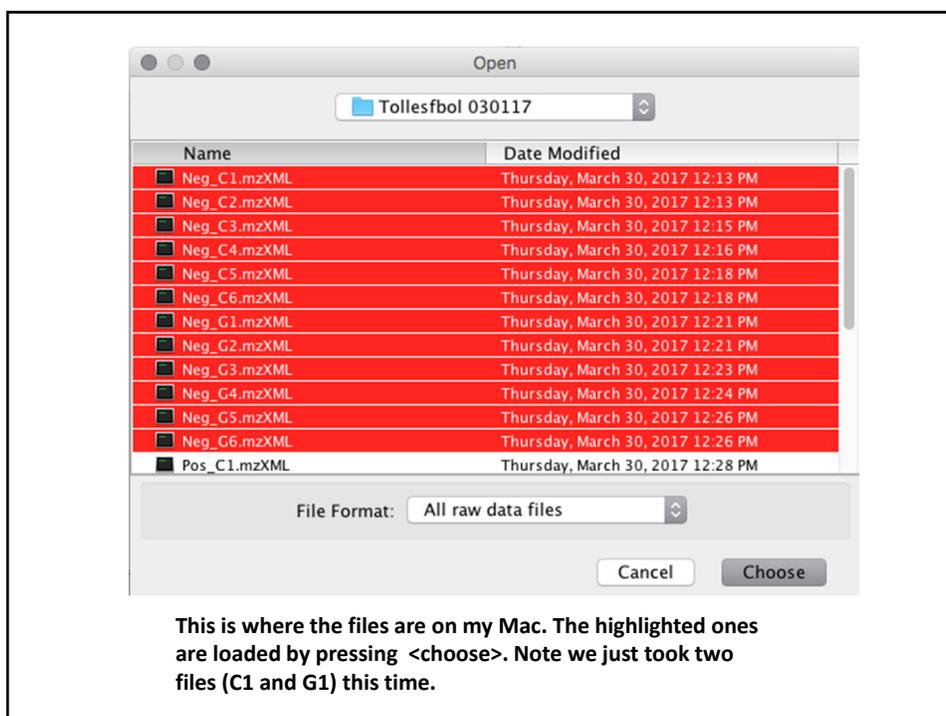
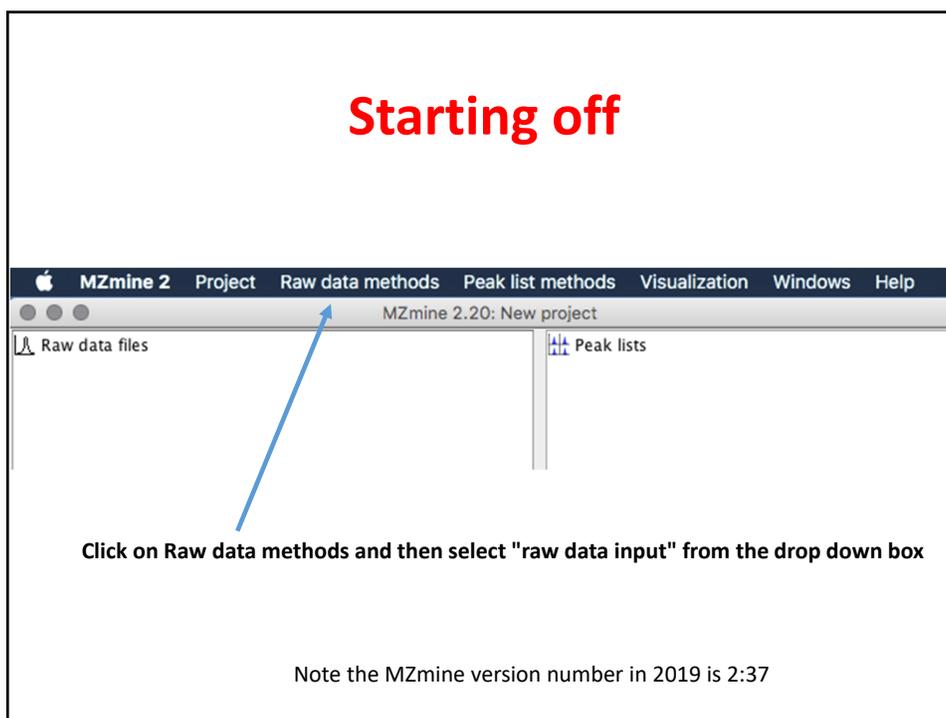
- Go to <http://mzmine.github.io/>
- Download
- Unzip the file and move the folder into Applications
  - There are three starting methods
    - Linux - startMZmine\_Linux.sh
    - Mac - startMZmine\_MacOSX.command
    - Windows - startMZmine\_Windows.bat
  - Double click to start the program

## 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



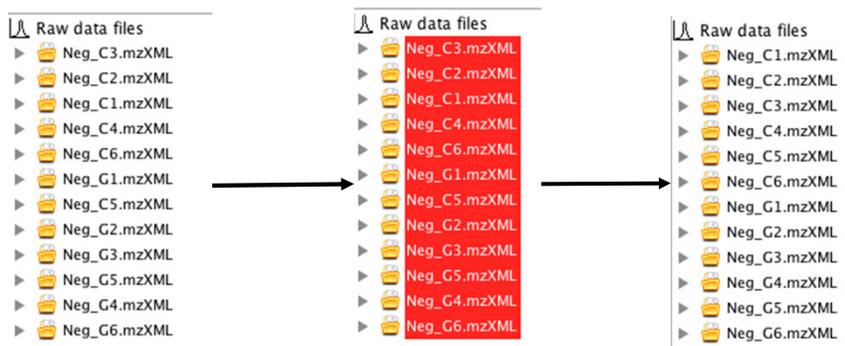
## Showing uploading process

Item	Priority	Status	% done
Opening file /Volumes/Metabolomic/MZXML files/Tollesbol 030117/Neg_C1.mz...	NORMAL	FINISHED	100%
Opening file /Volumes/Metabolomic/MZXML files/Tollesbol 030117/Neg_C2.mz...	NORMAL	FINISHED	100%
Opening file /Volumes/Metabolomic/MZXML files/Tollesbol 030117/Neg_C3.mz...	NORMAL	FINISHED	100%
Opening file /Volumes/Metabolomic/MZXML files/Tollesbol 030117/Neg_C4.mz...	NORMAL	FINISHED	100%
Opening file /Volumes/Metabolomic/MZXML files/Tollesbol 030117/Neg_C5.mz...	NORMAL	PROCESSING	21%
Opening file /Volumes/Metabolomic/MZXML files/Tollesbol 030117/Neg_C6.mz...	NORMAL	PROCESSING	16%

[1:42:09 PM]: Started parsing file /Volumes/Metabolomic/MZXML files/Tollesbol 030117/Neg\_G2.mzXML

2019MB free

## Files are now loaded



Highlight the files

Re-order the files

### Content of the .mzxml files

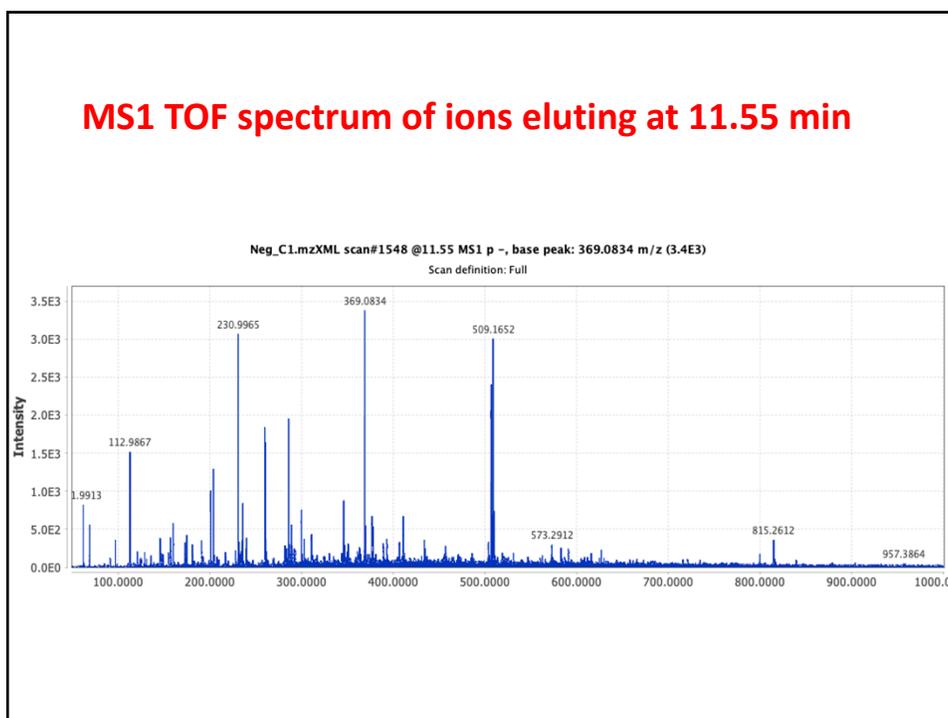
Raw data files

- Neg\_C1.mzXML
  - #1 @0.00 MS1 c -
  - #2 @0.02 MS2 (88.9897) p -
  - #3 @0.03 MS2 (118.9979) p -
  - #4 @0.03 MS1 p -
  - #5 @0.04 MS2 (60.9967) p -
  - #6 @0.05 MS2 (88.9904) p -
  - #7 @0.06 MS1 p -
  - #8 @0.08 MS2 (60.9968) p -
  - #9 @0.09 MS1 c -
  - #10 @0.11 MS2 (220.9427) p -
  - #11 @0.11 MS1 p -
  - #12 @0.13 MS2 (75.0122) p -
  - #13 @0.14 MS1 p -
  - #14 @0.15 MS2 (119.0002) p -
  - #15 @0.16 MS2 (306.9254) p -
  - #16 @0.17 MS1 p -
  - #17 @0.19 MS2 (220.9407) p -
  - #18 @0.20 MS1 p -
  - #19 @0.20 MS1 p -
  - #20 @0.21 MS1 p -
  - #21 @0.21 MS1 p -
  - #22 @0.22 MS1 p -
  - #23 @0.23 MS1 p -
  - #24 @0.23 MS1 p -

- #3067 @16.46 MS1 p -
- #3068 @16.47 MS2 (112.9884) p -
- #3069 @16.47 MS2 (135.9732) p -
- #3070 @16.47 MS2 (243.0786) p -
- #3071 @16.48 MS2 (291.0921) p -
- #3072 @16.48 MS2 (414.2132) p -
- #3073 @16.48 MS2 (429.1771) p -
- #3074 @16.48 MS2 (588.3003) p -
- #3075 @16.49 MS2 (592.3124) p -
- #3076 @16.49 MS1 p -
- #3077 @16.50 MS2 (135.9734) p -
- #3078 @16.50 MS2 (189.1151) p -
- #3079 @16.50 MS2 (243.0793) p -
- #3080 @16.50 MS2 (291.0920) p -
- #3081 @16.50 MS2 (336.1492) p -
- #3082 @16.50 MS2 (357.1013) p -
- #3083 @16.51 MS2 (414.2141) p -
- #3084 @16.51 MS2 (455.2388) p -
- #3085 @16.51 MS2 (483.2207) c -
- #3086 @16.51 MS2 (508.2215) p -
- #3087 @16.51 MS2 (563.1453) c -
- #3088 @16.51 MS2 (578.3178) p -
- #3089 @16.51 MS2 (583.2869) p -
- #3090 @16.51 MS2 (588.3027) p -

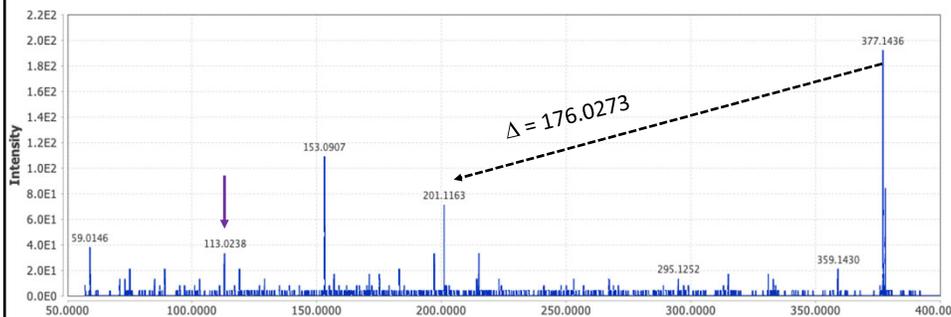
MS1 data  
Blue files

MSMS  
Red files

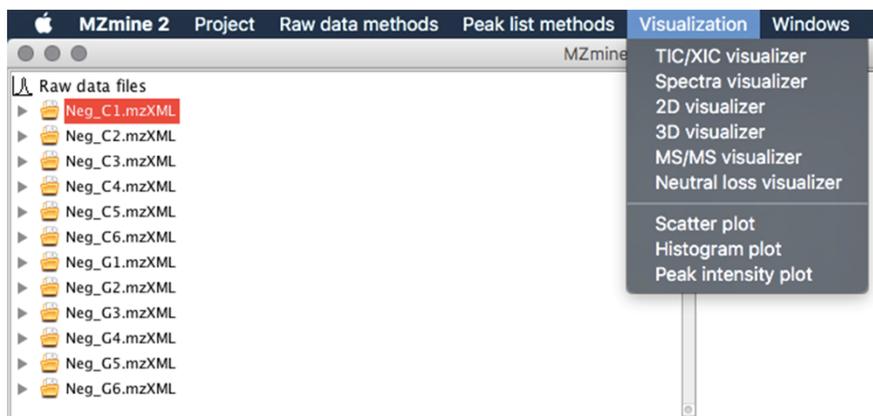


## MSMS spectrum of ion eluting at 11.55 min

$$\begin{aligned} \text{C}_6\text{H}_8\text{O}_6 &= 6 \times 12 + 8 \times 1.007825 + 6 \times 15.99495 \\ &= 72 + 8.0626 + 95.9697 \\ &= 176.0323 \end{aligned}$$



## Visualization toolbar



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

## Selecting all the data

Please set the parameters

Raw data files: Neg\_C1.mzXML (As selected in main window) ...

Scans: MS level: 1 (Set filters) (Clear filters)

m/z: [ ] - [ ] (Auto range) (From mass) (From formula)

Retention time resolution: 500

m/z resolution: 500

(OK) (Cancel) (Help)

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

## Setting 3D-parameters

Please set the parameters

Scan number: [ ] - [ ]

Retention time: 0 [ ] - 30 [ ] min. (Auto range)

MS level: 1

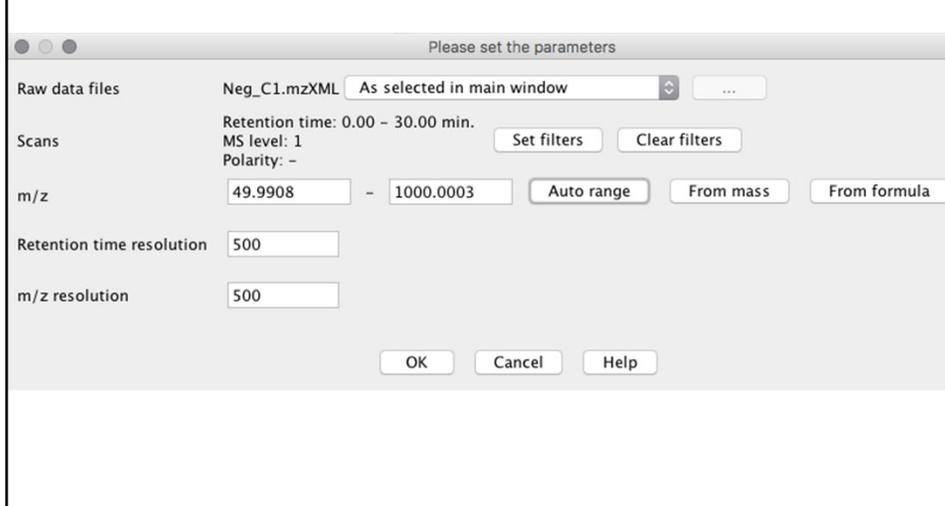
Scan definition: [ ]

Polarity: -

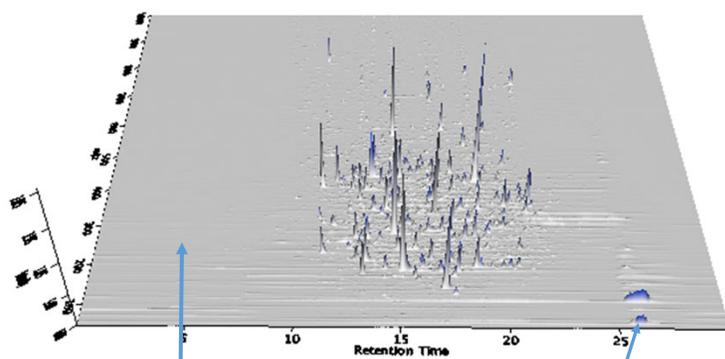
Spectrum type: Any

(OK) (Cancel) (Help)

## Ready to view the 3D-plot



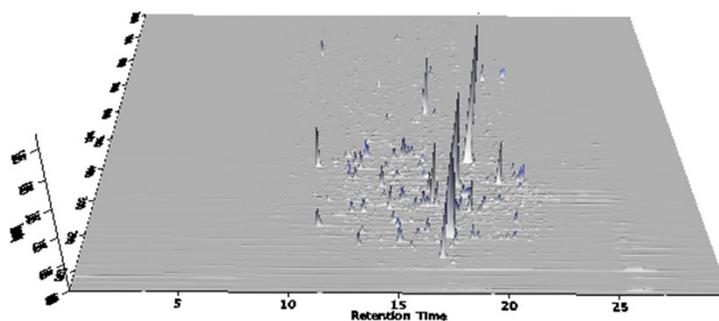
## 3D view of NegMode\_C1



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

Please set the parameters

Scan number  -

Retention time  -  min.

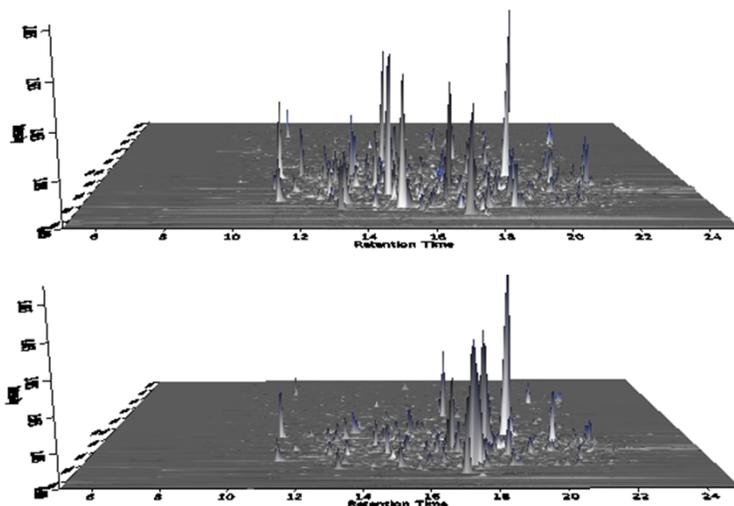
MS level

Scan definition

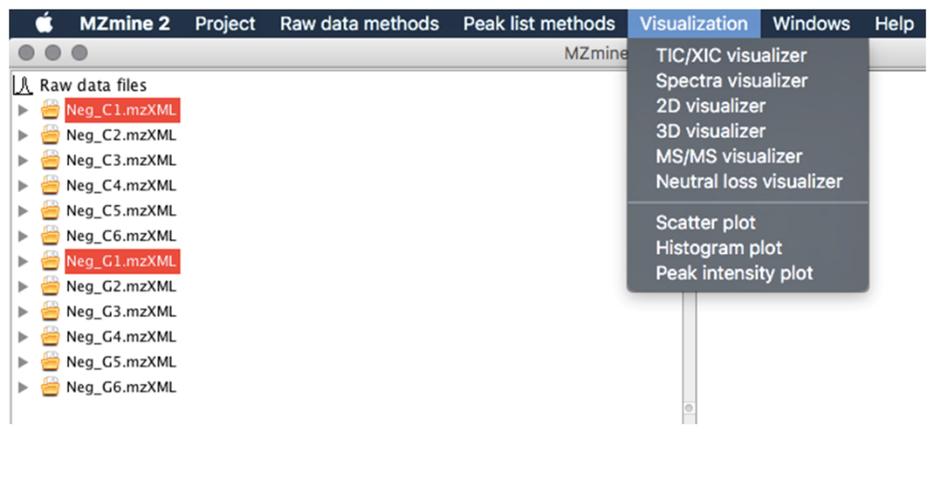
Polarity

Spectrum type

## Data from 5-25 min



## Total ion current



## Setting the parameters

Please set the parameters

Raw data files 2 selected As selected in main window ...

Scans Retention time: 5.00 - 25.00 min.  
MS level: 1 Set filters Clear filters  
Polarity: -

Plot type Base peak intensity

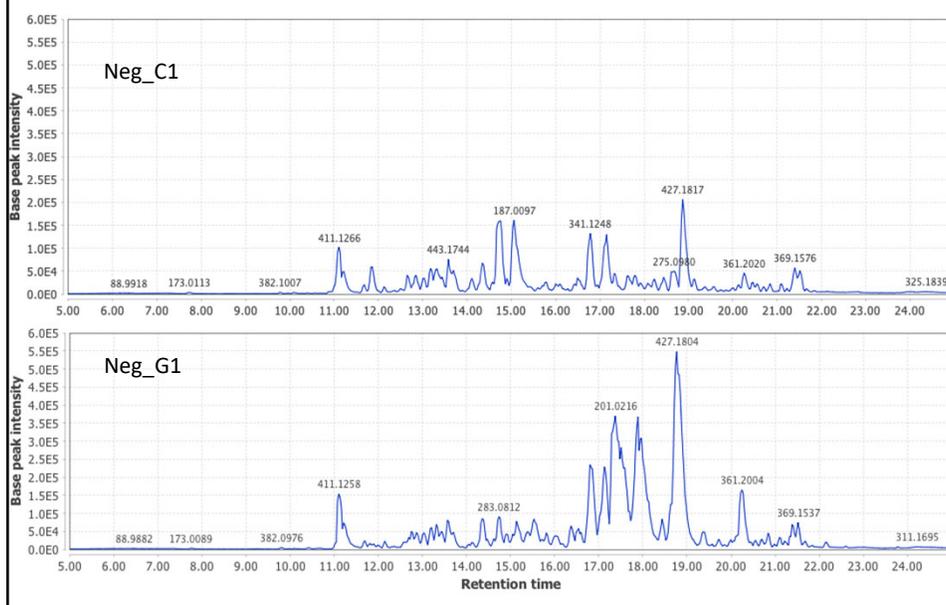
m/z 49.9905 - 1000.0003 Auto range From mass From formula

Peaks

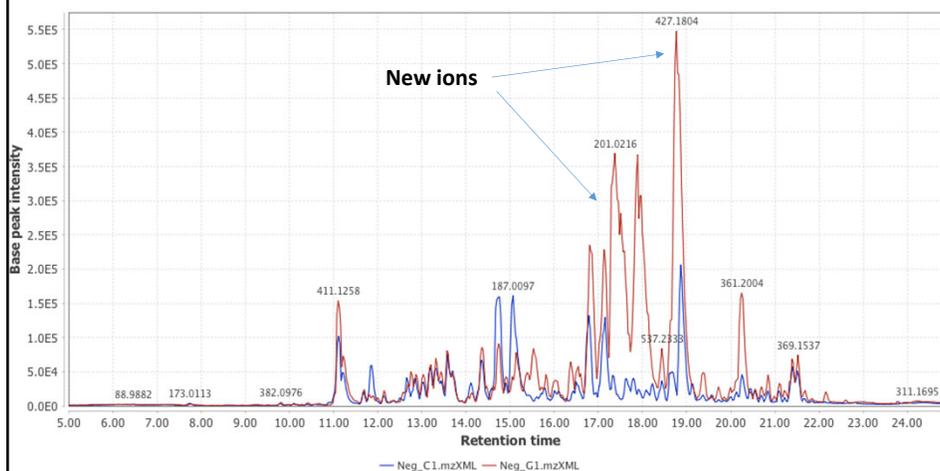
All  
Clear

OK Cancel Help

## 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

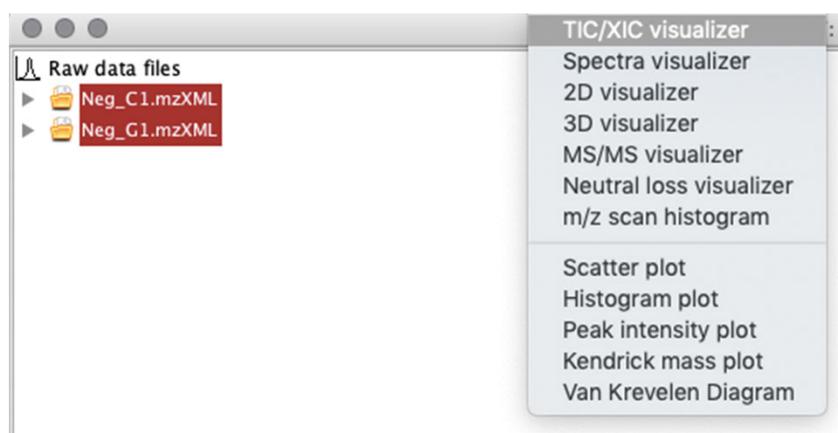
A	B	C	D	E	F
		C	H	N	O
Name	Empirical formula	12.000000000	1.007825032	14.003074004	15.9949146
hexanol	C6H14O	6	14	0	1
glucose	C6H12O6	6	12		6
genistein	C15H10O5	15	10		5

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 $\beta$ -glucuronide	$C_{21}H_{18}O_{11}$	446.08490	445.07765
Genistein $\beta$ -glucuronide/sulfate	$C_{21}H_{18}O_{14}S$	526.04172	525.03444

## Setting the mass window



## Setting the mass window

The screenshot shows a dialog box titled "Please set the parameters" with the following settings:

- Raw data files: 2 selected, As selected in main window
- Scans: Retention time: 5.00 - 25.00 min., MS level: 1, Polarity: -
- Plot type: Base peak intensity
- m/z: 50.0000 - 1000.0000, with buttons for Auto range, From mass, and From formula
- Peaks: Formula: C<sub>21</sub>H<sub>18</sub>O<sub>11</sub>, Ionization type: [M-H]<sup>-</sup>, Charge: 1, m/z tolerance: 0.001 m/z or 5.0 ppm

Buttons at the bottom: OK, Cancel, Help

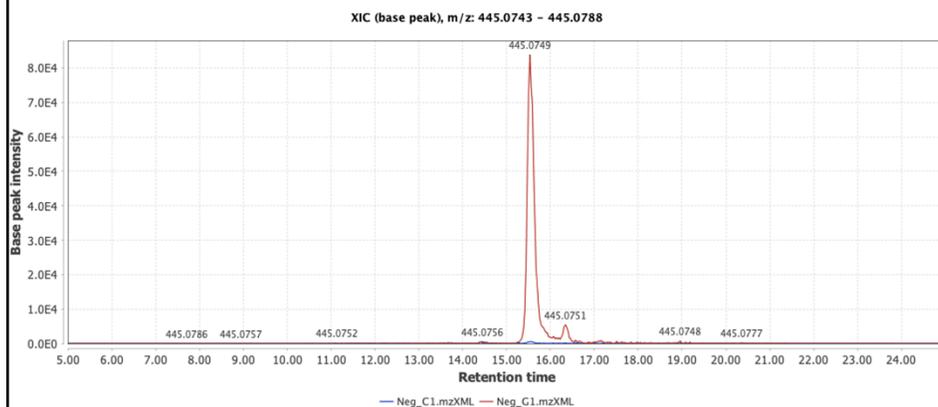
## Finished the setup to find GenGlcA

The screenshot shows the same dialog box with the following updated settings:

- Raw data files: 2 selected, As selected in main window
- Scans: Retention time: 5.00 - 25.00 min., MS level: 1, Polarity: -
- Plot type: Base peak intensity
- m/z: 445.0743 - 445.0788, with buttons for Auto range, From mass, and From formula
- Peaks: An empty list box with buttons for All and Clear

Buttons at the bottom: OK, Cancel, Help

## XIC of $m/z$ 445.0788



## 2D-plot for GENGLcA

Type of plot

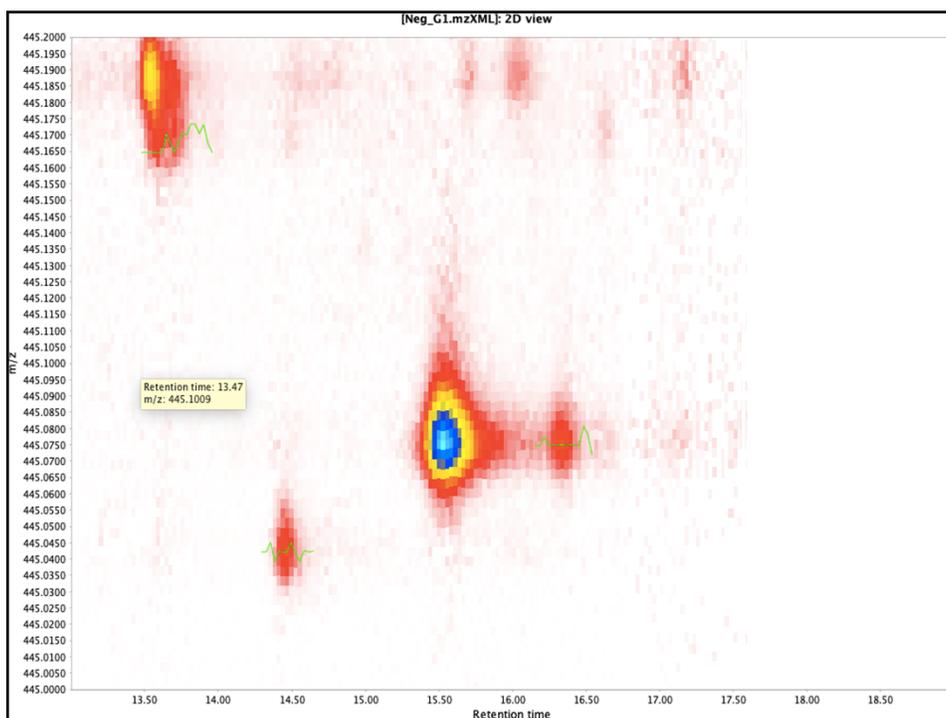
Raw data files

Scans Retention time: 13.00 - 19.00 min.

MS level: 1

Polarity: -

$m/z$   -



## Getting MS/MS data

Select TIC/XIC and reset the parameters

Please set the parameters

Scan number  -

Retention time  -  min.

MS level  Retention time range in minutes

Scan definition

Polarity  ▾

Spectrum type  ▾

# Ready to go

Please set the parameters

Raw data files 2 selected As selected in main window ...

Scans Retention time: 5.00 – 25.00 min.  
MS level: 2 Set filters Clear filters  
Polarity: -

Plot type Base peak intensity

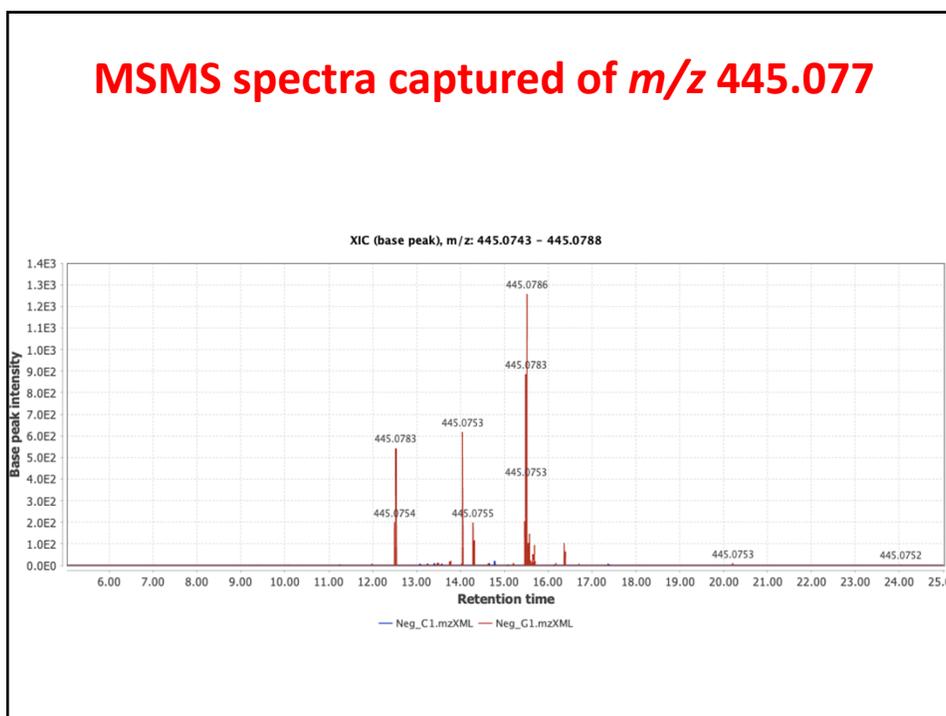
m/z 445.0757 - 445.0803 Auto range From mass From formula

Peaks

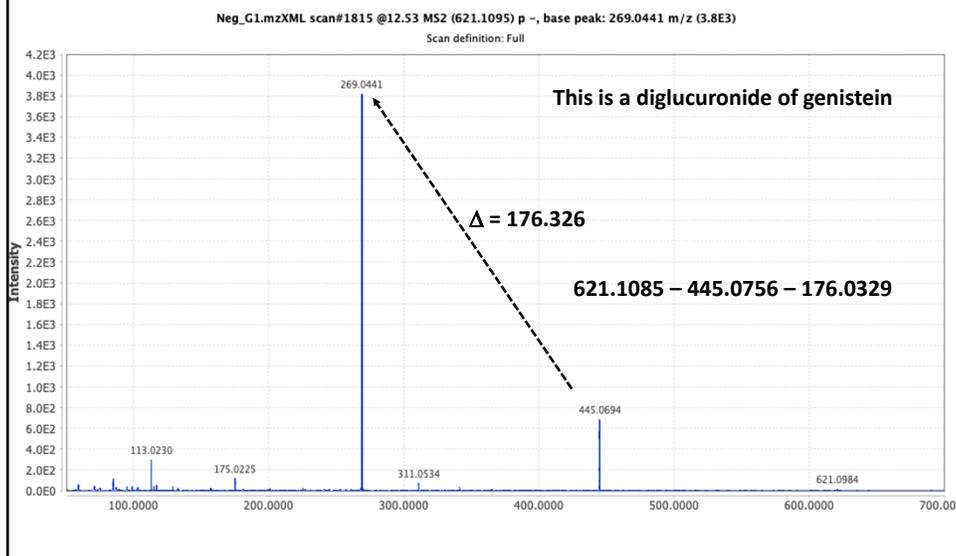
All  
Clear

OK Cancel Help

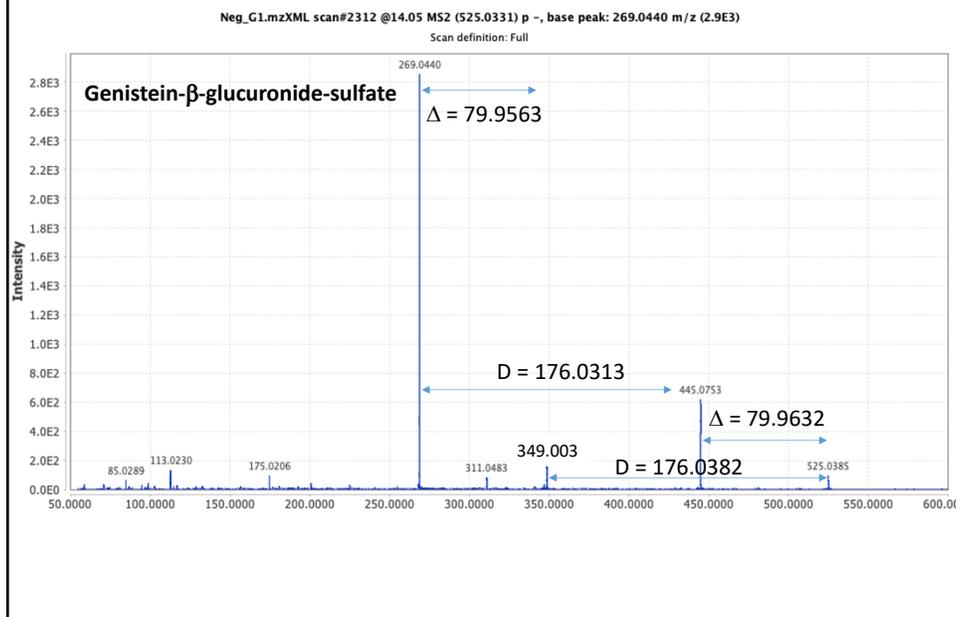
## MSMS spectra captured of $m/z$ 445.077

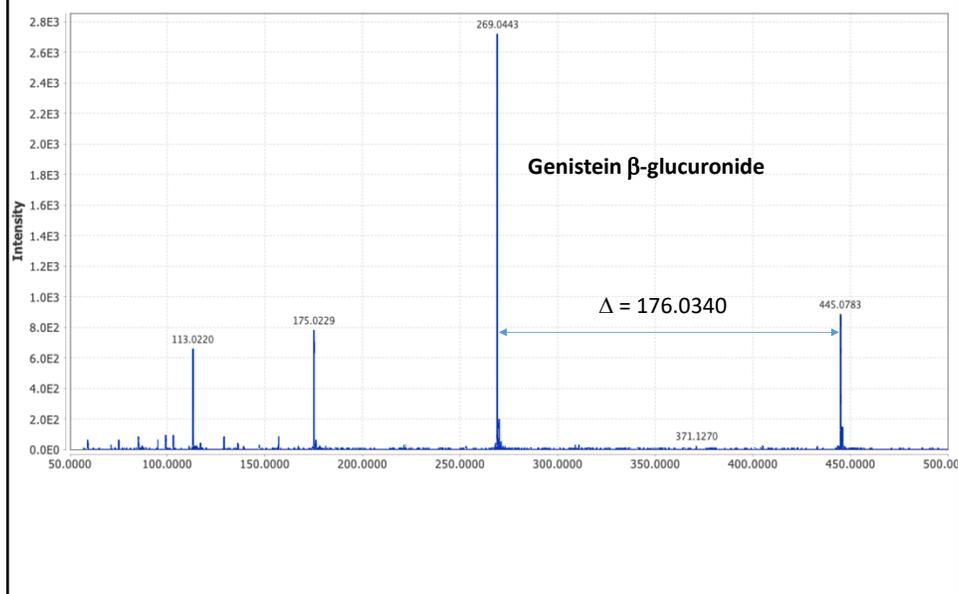
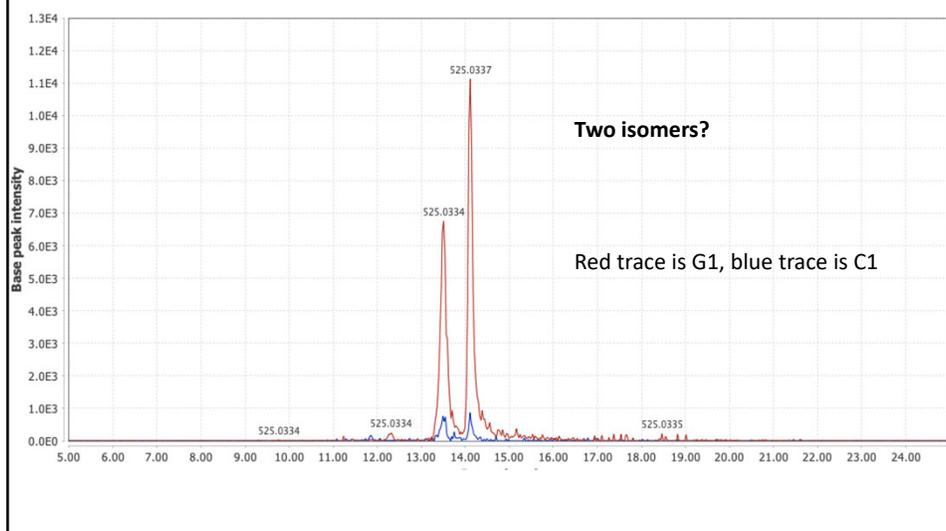


## Where MSMS for $m/z$ 445.079 were recorded (1)



## 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  $\beta$ -glucuronide/sulfate**

## Another way to get MSMS spectra Select MSMS visualizer

Raw data files: Neg\_G1.mzXML Specific raw data files

Retention time: 10.00 - 20.00 min. Auto range

m/z: 525.0307 - 525.0360 Auto range From mass From formula

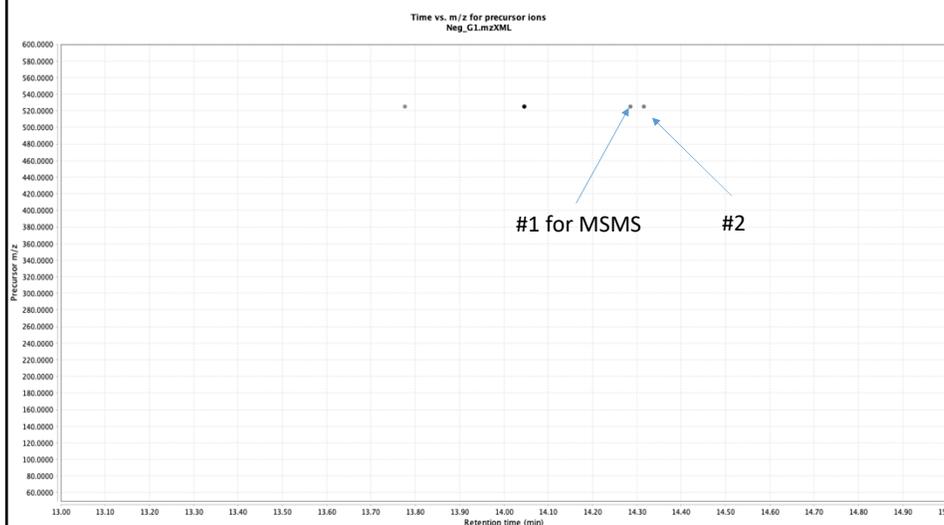
Intensity: Total intensity in MS/MS scan

Normalize by: All data points

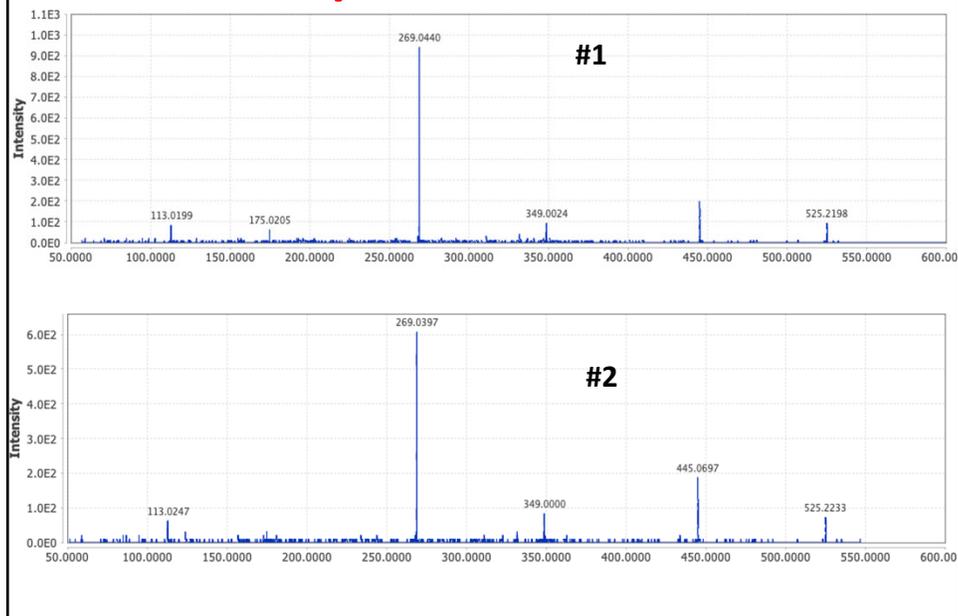
Min. MS/MS peak intensity: 10

OK Cancel Help

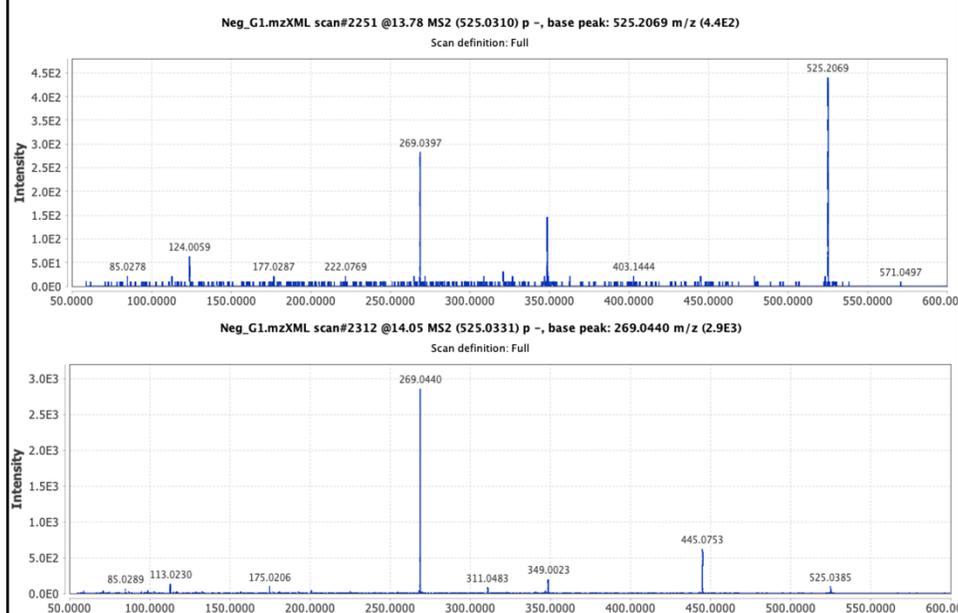
## Four items detected by MSMS of $m/z$ 523.3



## MSMS spectra of $m/z$ 525.03



## MSMS spectra of earlier $m/z$ 525.03



## Class on 1-25-19

## Identifying the masses

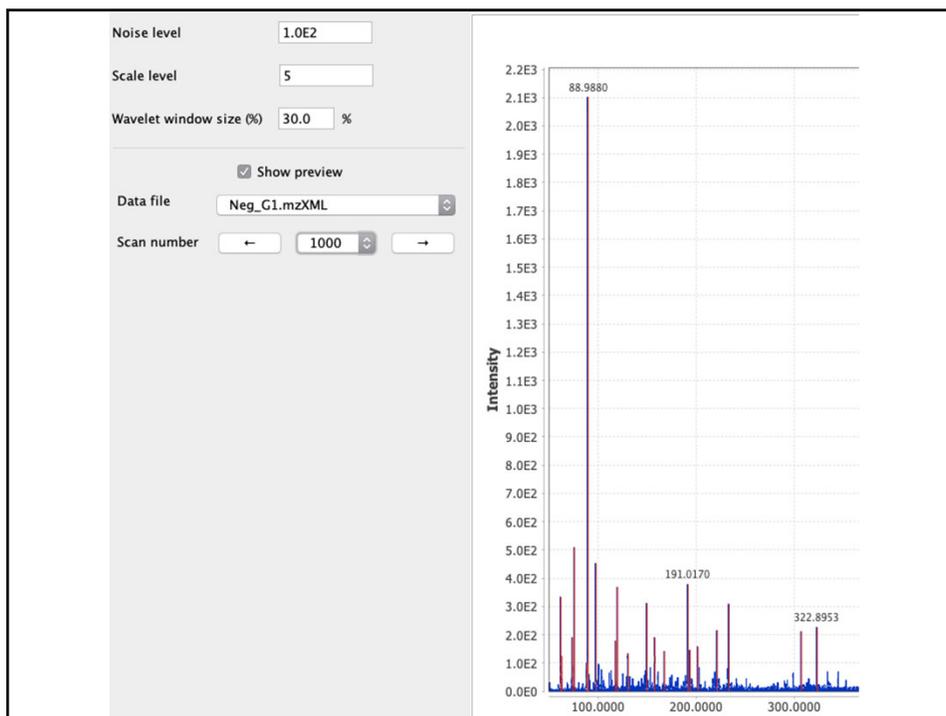
The image shows a software interface for identifying masses. It consists of a main dialog box and a secondary settings panel. The main dialog box has the following fields and controls:

- Raw data files: 2 selected, All raw data files (dropdown), ... (button)
- Scans: Retention time: 5.00 - 25.00 min., MS level: 1, Polarity: - (text), Set filters (button), Clear filters (button)
- Mass detector: Wavelet transform (dropdown), ... (button)
- Mass list name: masses (text)
- CDF Filename (optional):  (checkbox), (text), ... (button)
- Buttons: OK, Cancel, Help

The secondary settings panel, located to the right and below the main dialog, has the following fields and controls:

- Noise level: 1.0E2 (text)
- Scale level: 5 (text)
- Wavelet window size (%): 30.0 % (text)
- Show preview:  (checkbox)
- Buttons: OK, Cancel, Help

A blue arrow points from the '...' button next to the 'Wavelet transform' dropdown in the main dialog to the 'Noise level' field in the secondary settings panel.



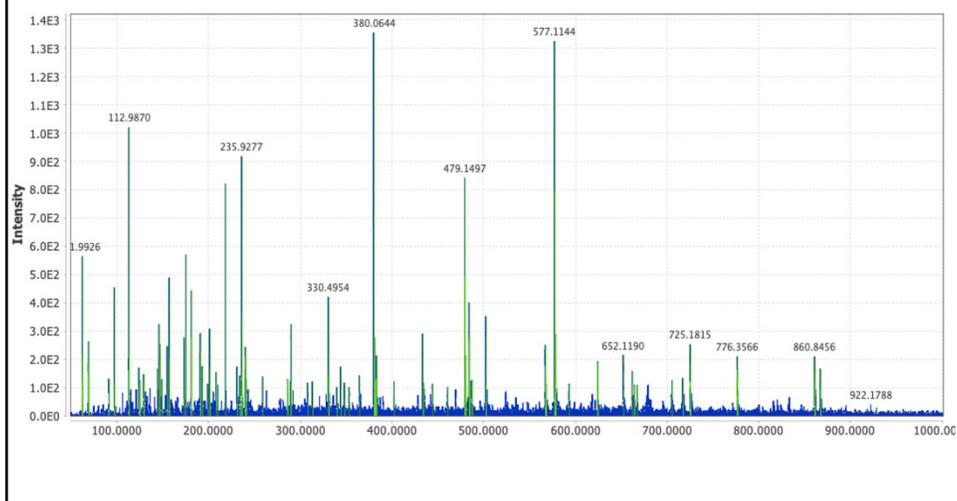
## After identifying the masses

- ▼ Neg\_C1.mzXML
  - #1 @0.00 MS1 c -
  - #2 @0.02 MS2 (88.9897) p -
  - #3 @0.03 MS2 (118.9979) p -
  - #4 @0.03 MS1 p -
  - #5 @0.04 MS2 (60.9967) p -
  - #6 @0.05 MS2 (88.9904) p -
  - #7 @0.06 MS1 p -
  - #8 @0.08 MS2 (60.9968) p -
  - #9 @0.09 MS1 c -
  - #10 @0.11 MS2 (220.9427) p -
  - #11 @0.11 MS1 p -
  - #695 @4.99 MS1 p -
  - #696 @4.99 MS1 p -
  - #697 @5.00 MS1 p -
  - #698 @5.00 MS1 p -
  - #699 @5.01 MS1 p -
  - #700 @5.02 MS1 p -
  - #701 @5.02 MS1 p -
  - #702 @5.03 MS1 p -
  - #703 @5.04 MS1 p -
  - #704 @5.04 MS1 p -
  - #705 @5.05 MS1 p -

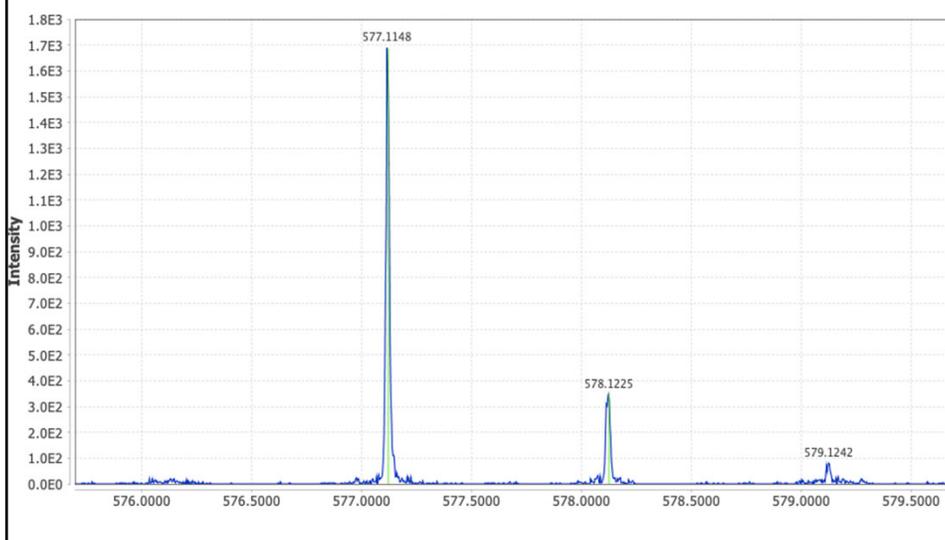
The mass identifications started at 5 min

## 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*

Raw data files	2 selected	As selected in main window	...	
Scans	Retention time: 5.00 – 25.00 min.	MS level: 1	Set filters	Clear filters
	Polarity: -			
Mass list	masses	Choose...		
Min group size in # of scans	5.00			
Group intensity threshold	1.0E3			
Min highest intensity	2.0E3			
m/z tolerance	0.01	m/z or	0.0	ppm
Suffix	chromatograms			

## When the chromatogram building ends

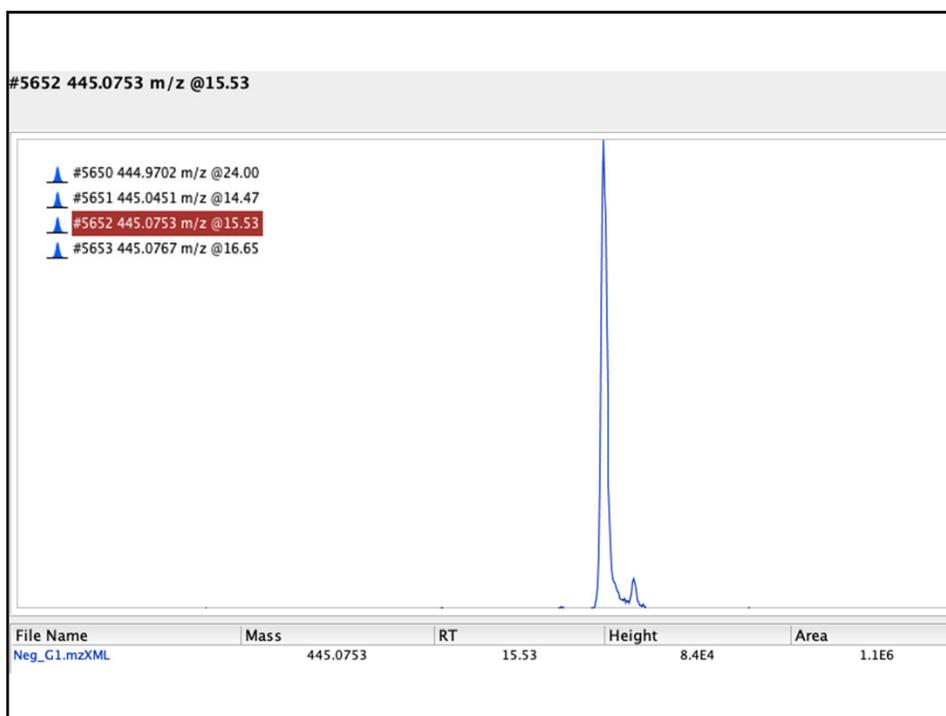
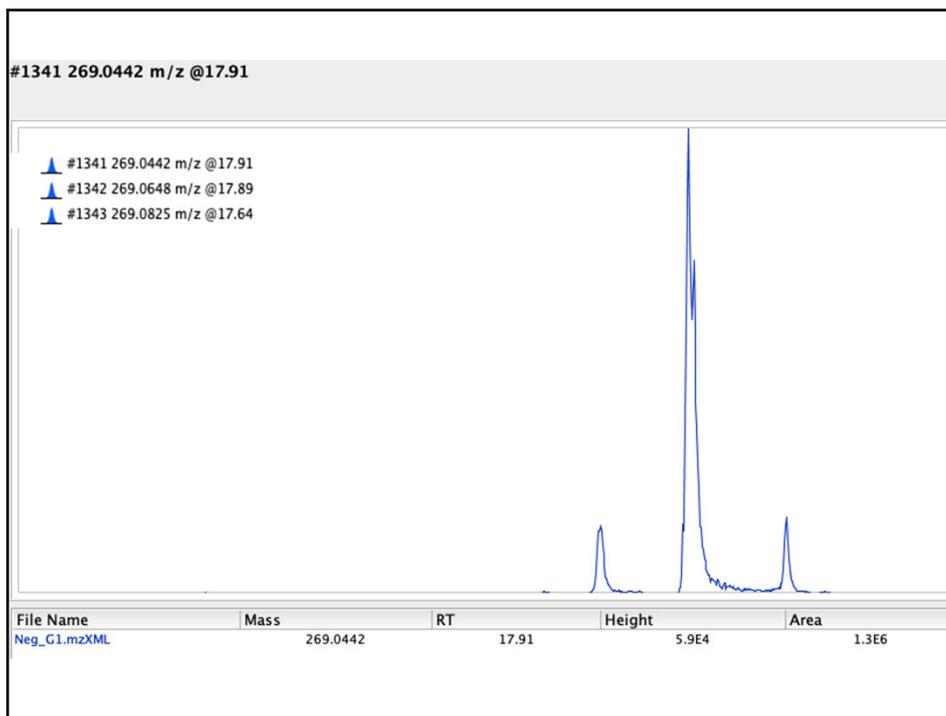
MZmine 2.37: New project

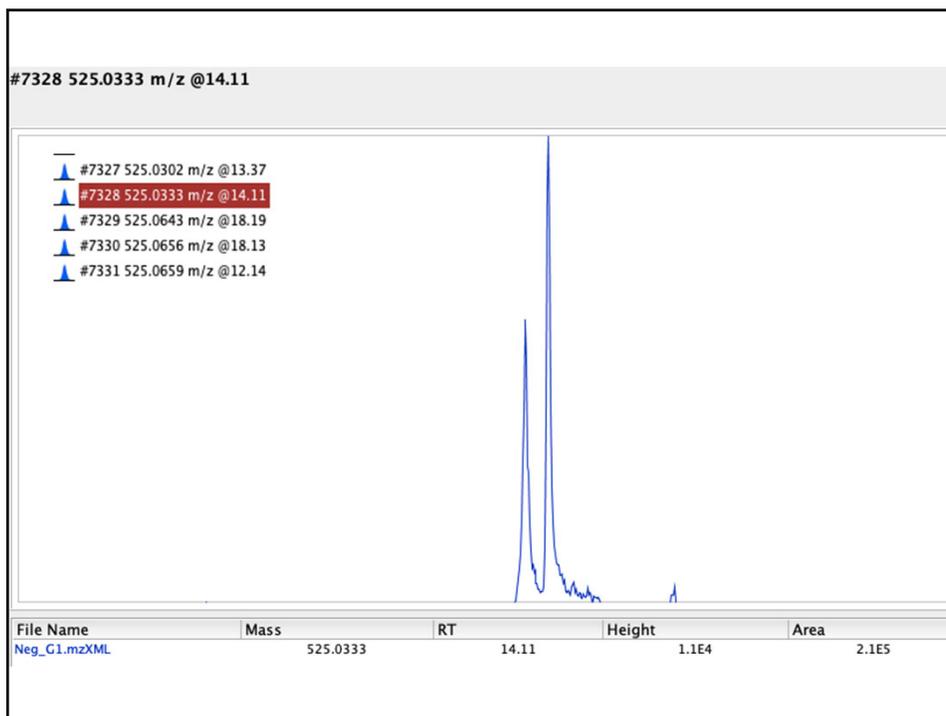
Raw data files

- Neg\_C1.mzXML
- Neg\_G1.mzXML

Peak lists

- Neg\_C1.mzXML chromatograms
- Neg\_G1.mzXML chromatograms
  - #1 54.7151 m/z @18.79
  - #2 58.3112 m/z @18.02
  - #3 60.9941 m/z @6.37
  - #4 61.8097 m/z @18.71
  - #5 61.9894 m/z @17.18
  - #6 62.0014 m/z @13.51
  - #7 62.0017 m/z @19.12
  - #8 62.0022 m/z @18.85
  - #9 62.0050 m/z @15.19
  - #10 62.0093 m/z @18.79
  - #11 62.0116 m/z @17.89





## Chromatogram deconvolution

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

Peak lists 2 selected As selected in main window ...

Suffix deconvoluted

Algorithm Wavelets (ADAP) ...

m/z center calculation MEDIAN

m/z range for MS2 scan pairing (Da)

RT range for MS2 scan pairing (min)

Remove original peak list

OK Cancel Help

S/N threshold

S/N estimator  ...

min feature height

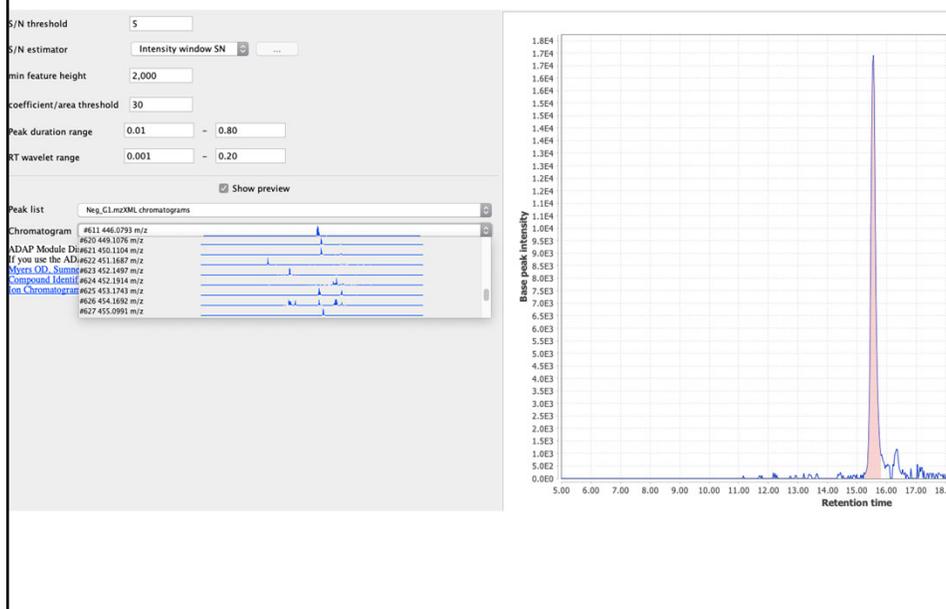
coefficient/area threshold

Peak duration range  -

RT wavelet range  -

Show preview

## Preview of chromatographic deconvolution



## Chromatogram deconvolution output

Raw data files

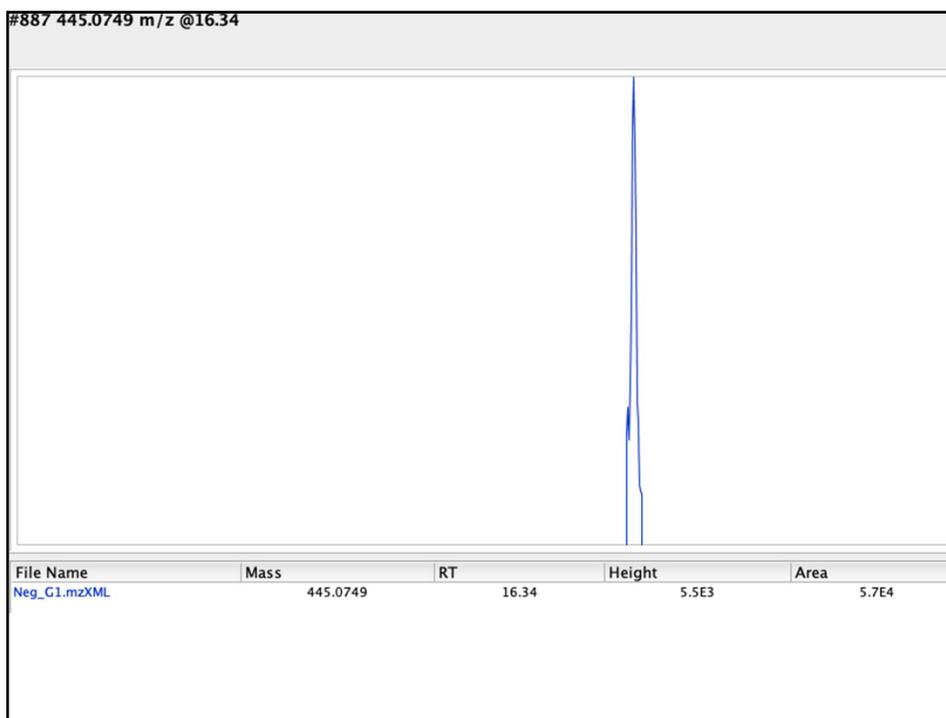
- NegMode\_IR1.mzXML
- NegMode\_NR1.mzXML

Neg\_C1.mzXML chromatograms deconvoluted

- Neg\_C1.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 lists

- NegMode\_NR1.mzXML chromatograms
- NegMode\_IR1.mzXML chromatograms
- NegMode\_NR1.mzXML chromatograms deconvoluted
- NegMode\_IR1.mzXML chromatograms deconvoluted



## Peak and chromatogram alignment

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

A dialog box for peak and chromatogram alignment. It features several input fields and checkboxes. The 'Peak lists' field shows '2 selected' and a dropdown menu set to 'As selected in main window'. The 'Peak list name' field contains 'Aligned peak list'. The 'm/z tolerance' field is set to '0.015' with a unit of 'm/z or 5.0 ppm'. The 'Weight for m/z' field is '0.5'. The 'Retention time tolerance' field is '0.5' with a unit of 'absolute (min)'. The 'Weight for RT' field is '0.5'. There are three checkboxes: 'Require same charge state' (unchecked), 'Require same ID' (unchecked), and 'Compare isotope pattern' (checked). A 'Setup..' button is next to the checked checkbox. At the bottom are 'OK', 'Cancel', and 'Help' buttons.

Peak lists	2 selected	As selected in main window
Peak list name	Aligned peak list	
m/z tolerance	0.015	m/z or 5.0 ppm
Weight for m/z	0.5	
Retention time tolerance	0.5	absolute (min)
Weight for RT	0.5	
Require same charge state	<input type="checkbox"/>	
Require same ID	<input type="checkbox"/>	
Compare isotope pattern	<input checked="" type="checkbox"/>	Setup..

OK Cancel Help

## Isotope grouping

A dialog box for isotope grouping. It features several input fields and checkboxes. The 'Peak lists' field shows '2 selected' and a dropdown menu set to 'As selected in main window'. The 'Name suffix' field contains 'deisotoped'. The 'm/z tolerance' field is set to '0.01' with a unit of 'm/z or 0.0 ppm'. The 'Retention time tolerance' field is '0.2' with a unit of 'absolute (min)'. There are three checkboxes: 'Monotonic shape' (unchecked), 'Remove original peaklist' (unchecked), and 'Maximum charge' (set to '2'). The 'Representative isotope' dropdown menu is set to 'Most intense'. At the bottom are 'OK', 'Cancel', and 'Help' buttons.

Peak lists	2 selected	As selected in main window	...
Name suffix	deisotoped		
m/z tolerance	0.01	m/z or 0.0	ppm
Retention time tolerance	0.2	absolute (min)	
Monotonic shape	<input type="checkbox"/>		
Maximum charge	2		
Representative isotope	Most intense		
Remove original peaklist	<input type="checkbox"/>		

OK Cancel Help

## Peak and chromatogram alignment

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

Peak lists 2 selected As selected in main window

Peak list name Aligned peak list

m/z tolerance 0.02 m/z or 0.0 ppm

RT tolerance 0.2 absolute (min)

RT tolerance after correction 0.1 absolute (min)

RANSAC iterations 5000

Minimum number of points 50.0 %

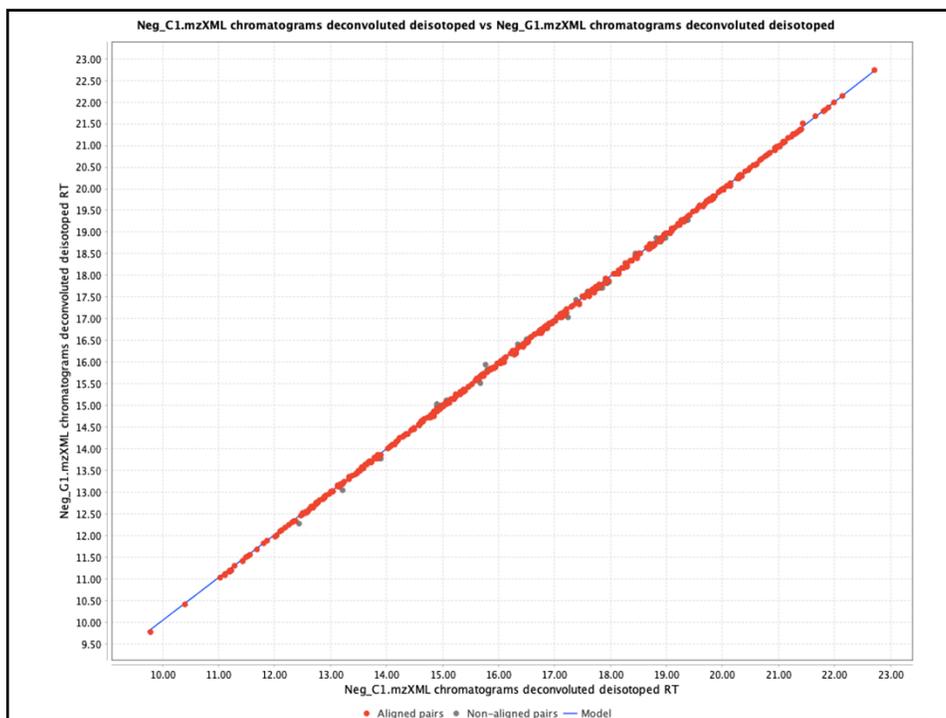
Threshold value 0.067

Linear model

Require same charge state

Show preview of RANSAC alignment

OK Cancel Help



## Aligned data

- ▼  **Aligned peak list**
-  #1 427.1787 m/z @18.86
  -  #2 187.0068 m/z @15.10
  -  #3 283.0823 m/z @14.74
  -  #4 567.1720 m/z @14.73
  -  #5 341.1242 m/z @16.76
  -  #6 144.0467 m/z @17.14
  -  #7 411.1262 m/z @11.10
  -  #8 443.1727 m/z @13.57
  -  #9 349.0926 m/z @14.35
  -  #10 441.1571 m/z @15.05

## 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

- Export row ID
- Export row m/z
- Export row retention time
- Export row identity (main ID)
- Export row identity (all IDs)
- Export row identity (main ID + details)
- Export row comment

- Peak status
- Peak m/z
- Peak RT
- Peak RT start
- Peak RT end
- Peak duration time
- Peak height

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- Peak area
- Peak charge
- Peak # data points
- Peak FWHM
- Peak tailing factor
- Peak asymmetry factor