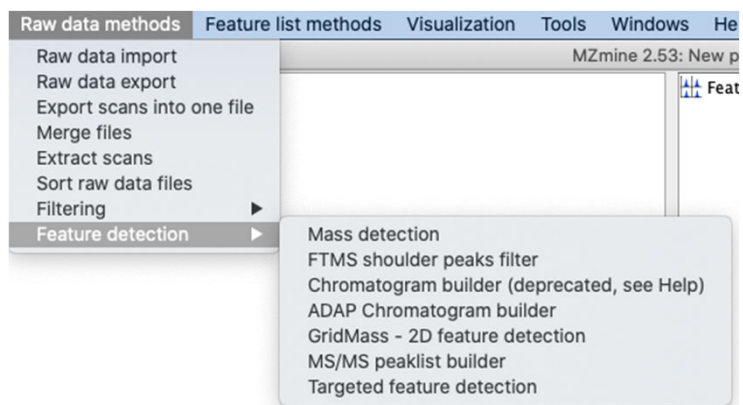


## Class on 02-01-21

1

## Detecting the masses



2

## Identifying the masses

Raw data files: 2 selected All raw data files

Scans: Retention time: 5.00 – 25.00 min. MS level: 1 Polarity: -

Mass detector: Wavelet transform

Mass list name: masses

CDF Filename (optional):

OK Cancel Help

Noise level: 1.0E2

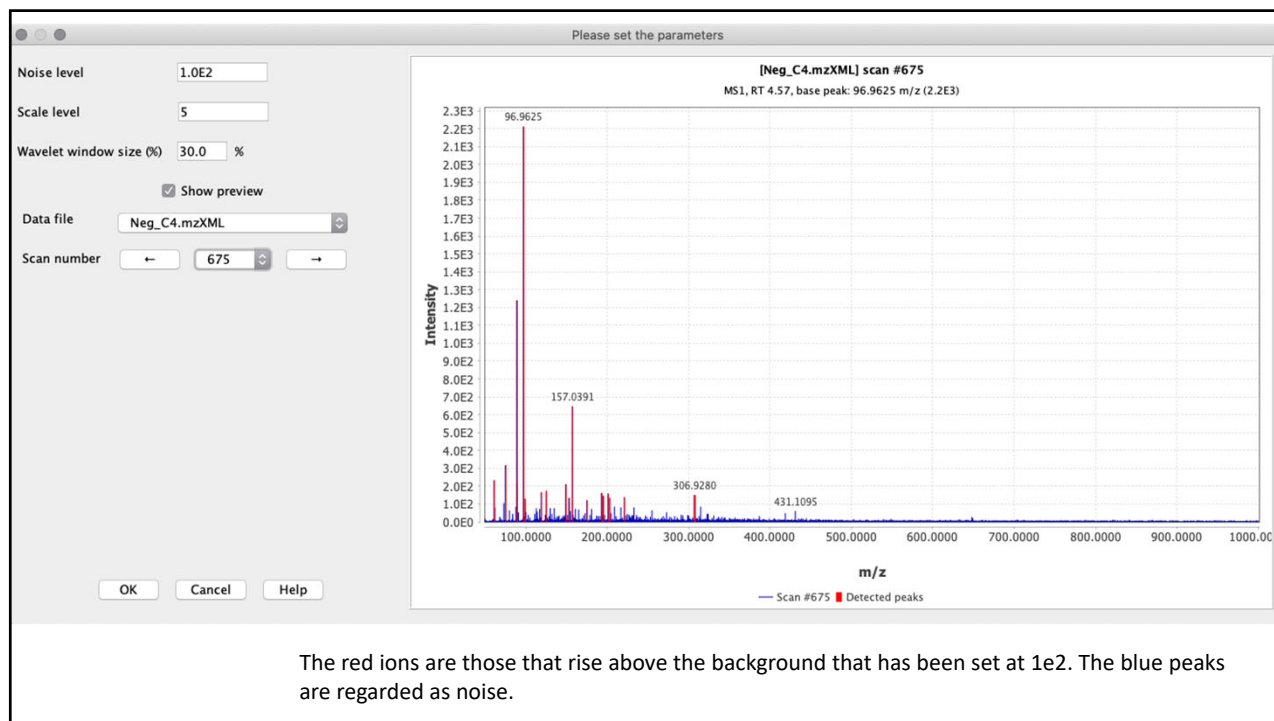
Scale level: 5

Wavelet window size (%): 30.0 %

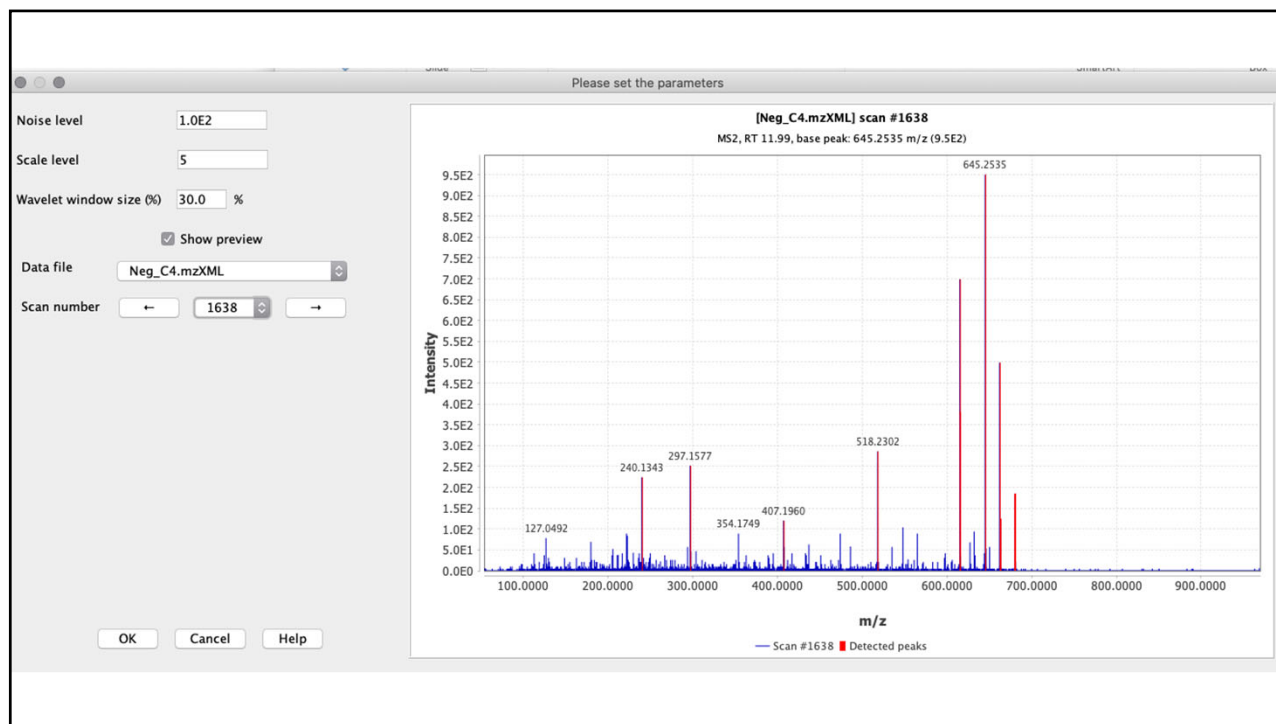
Show preview

OK Cancel Help

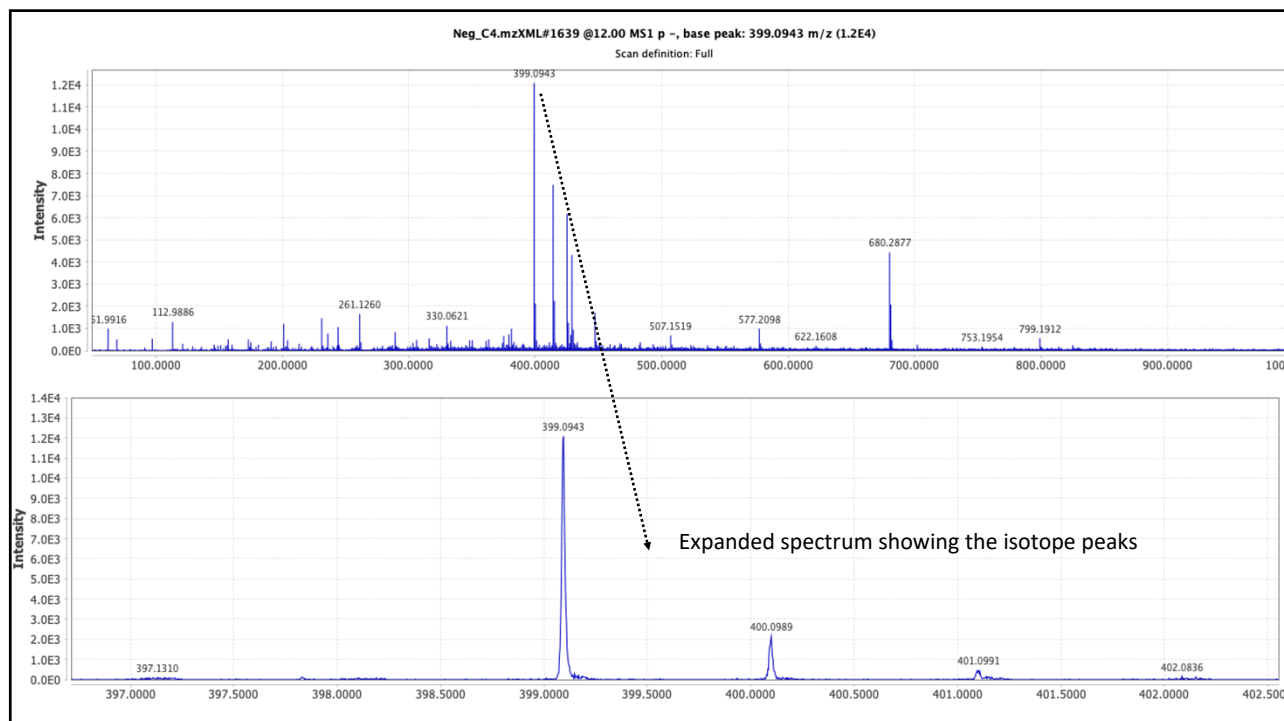
3



4



5



6

## Using the masses to create chromatograms

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

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

Scans MS level: 1 Set filters Clear filters

Mass list masses Choose...

Min group size in # of scans 5

Group intensity threshold 1.0E2

Min highest intensity 5.0E2

m/z tolerance 0.01 m/z or 0.0 ppm

Suffix chromatograms

ADAP Module Disclaimer:  
 If you use the ADAP Chromatogram Builder Module, please cite the [MZmine2 paper](#) and the following article:  
[Myers OD, Sumner SJ, Li S, Barnes S, Du X: One Step Forward for Reducing False Positive and False Negative Compound Identifications from Mass Spectrometry Metabolomics Data: New Algorithms for Constructing Extracted Ion Chromatograms and Detecting Chromatographic Peaks. Anal Chem 2017. DOI: 10.1021/acs.analchem.7b00947](#)

OK Cancel Help

7

## When the chromatogram building ends

Raw data files

- ▶ Neg\_C4.mzXML
- ▶ Neg\_C5.mzXML
- ▶ Neg\_C6.mzXML
- ▶ Neg\_G4.mzXML
- ▶ Neg\_G5.mzXML
- ▶ Neg\_G6.mzXML

Feature lists

- ▶ Neg\_G6.mzXML chromatograms
- ▼ Neg\_C4.mzXML chromatograms
  - ▲ #1 61.9917 m/z @17.12
  - ▲ #2 62.0018 m/z @18.48
  - ▲ #3 62.0149 m/z @18.99
  - ▲ #4 68.9983 m/z @18.54
  - ▲ #5 69.0089 m/z @17.12
  - ▲ #6 75.0114 m/z @5.17
  - ▲ #7 79.9603 m/z @15.10
  - ▲ #8 88.9904 m/z @6.33
  - ▲ #9 96.9613 m/z @17.67
  - ▲ #10 107.0512 m/z @15.10
  - ▲ #11 107.0691 m/z @15.13
  - ▲ #12 112.9874 m/z @20.35
  - ▲ #13 112.9976 m/z @18.88
  - ▲ #14 113.0141 m/z @18.88
  - ▲ #15 113.0248 m/z @14.74
  - ▲ #16 113.9901 m/z @14.82

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## Genistein chromatograms

Raw data files

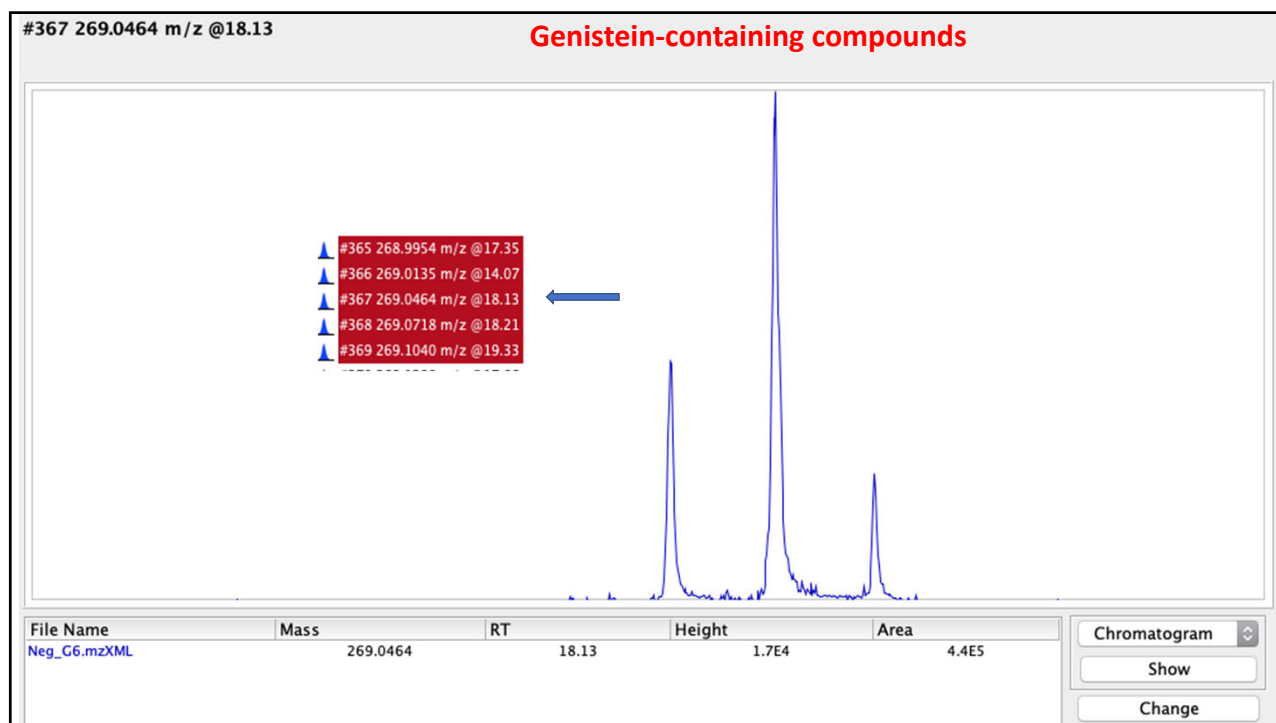
- Neg\_C4.mzXML
- Neg\_C5.mzXML
- Neg\_C6.mzXML
- Neg\_G4.mzXML
- Neg\_G5.mzXML
- Neg\_G6.mzXML

Feature lists

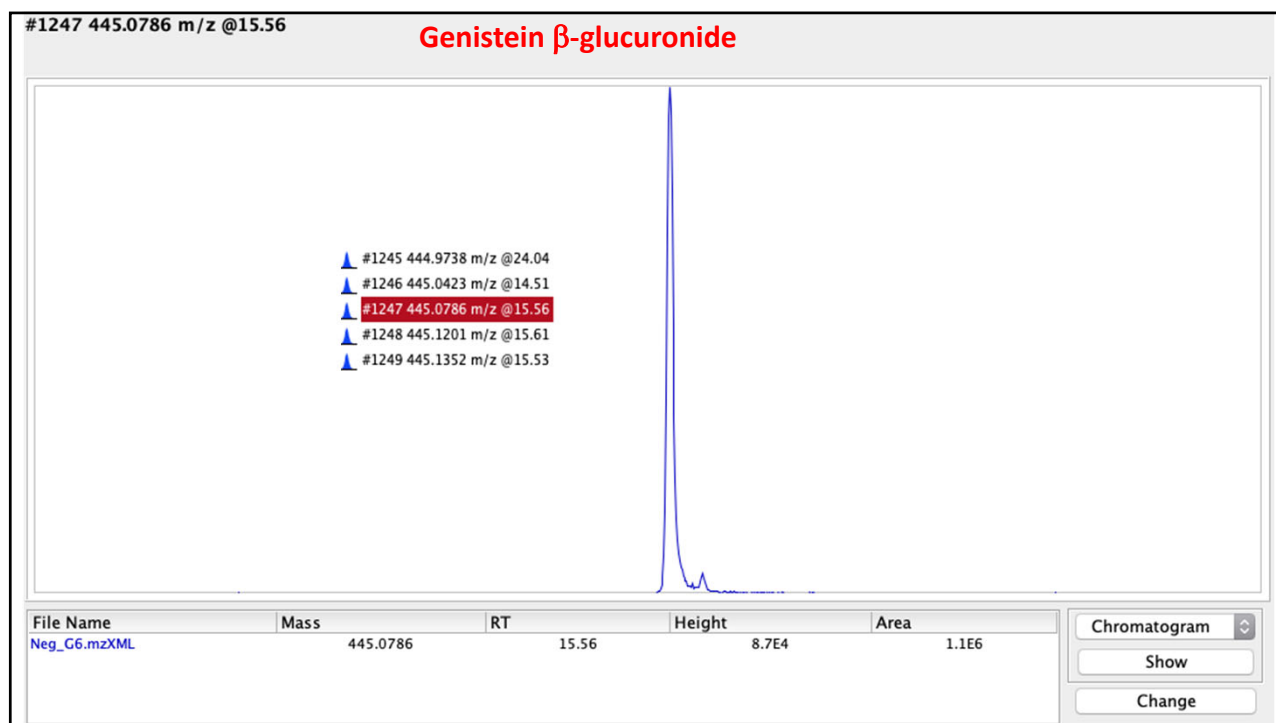
Neg\_G6.mzXML chromatograms

- #1 60.9965 m/z @6.38
- #2 61.9921 m/z @20.28
- #3 62.0035 m/z @17.57
- #4 62.0164 m/z @19.00
- #5 68.9987 m/z @18.48
- #6 69.0137 m/z @19.02
- #7 75.0118 m/z @5.16
- #8 79.9639 m/z @17.51
- #9 88.9906 m/z @6.37
- #10 92.0543 m/z @13.21
- #11 96.9620 m/z @17.75
- #12 107.0517 m/z @15.17
- #13 112.9875 m/z @20.31
- #14 112.9979 m/z @16.94
- #15 113.0116 m/z @18.97
- #16 113.0238 m/z @16.83

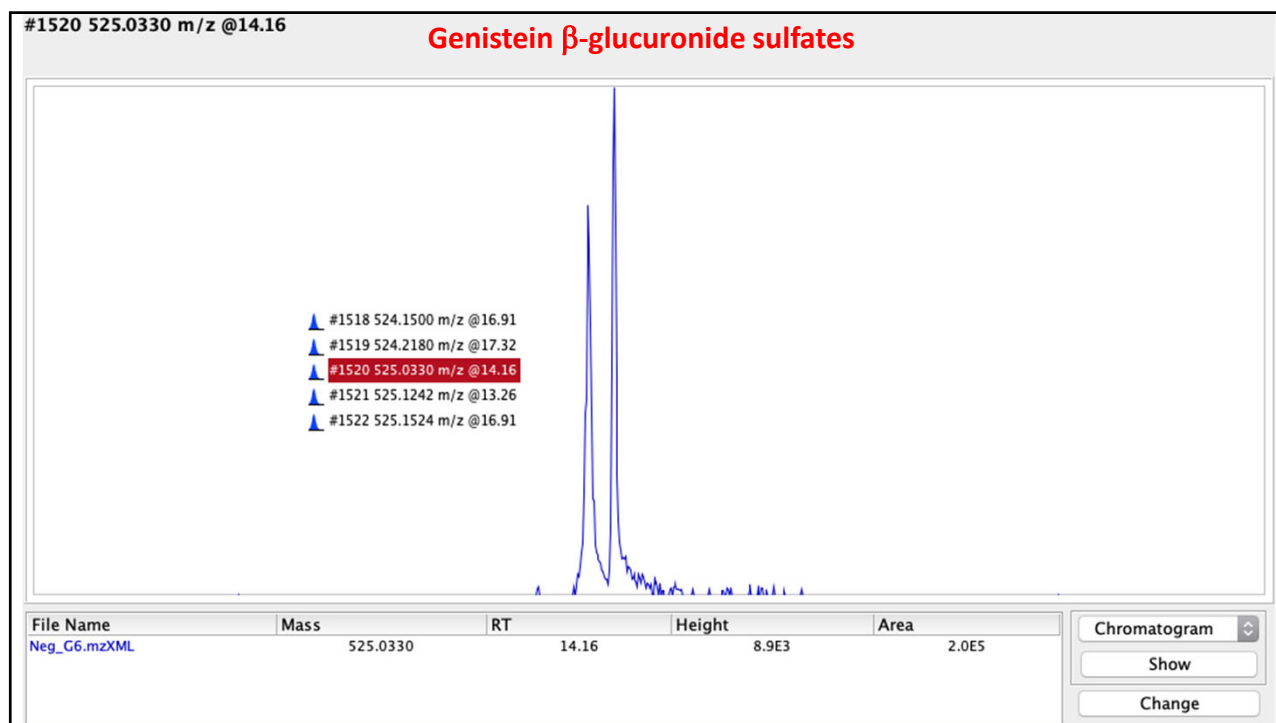
9



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## Chromatogram deconvolution

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

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

Suffix deconvoluted

Algorithm Wavelets (ADAP) ...

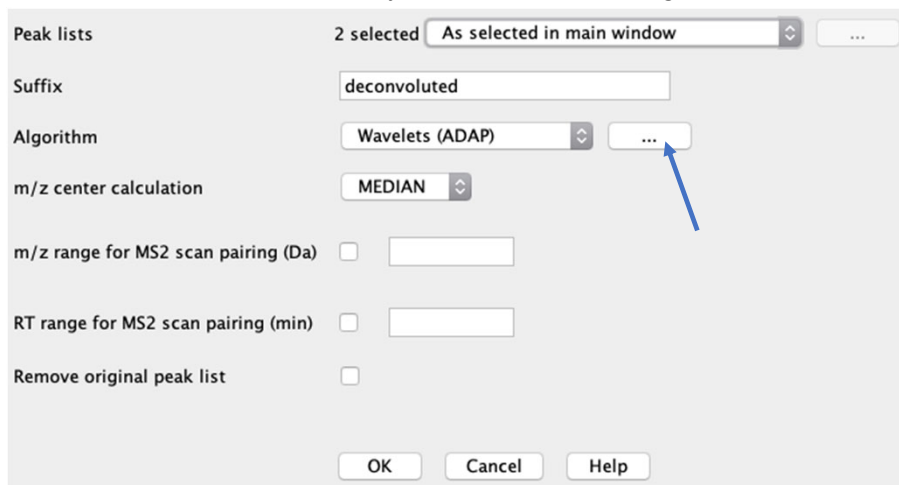
m/z center calculation MEDIAN

m/z range for MS2 scan pairing (Da)

RT range for MS2 scan pairing (min)

Remove original peak list

OK Cancel Help



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S/N threshold 5

S/N estimator Intensity window SN ...

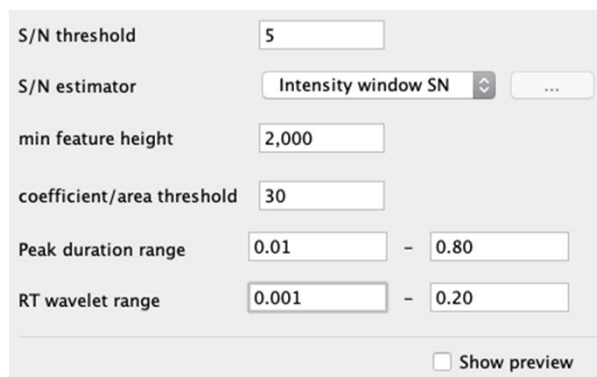
min feature height 2,000

coefficient/area threshold 30

Peak duration range 0.01 - 0.80

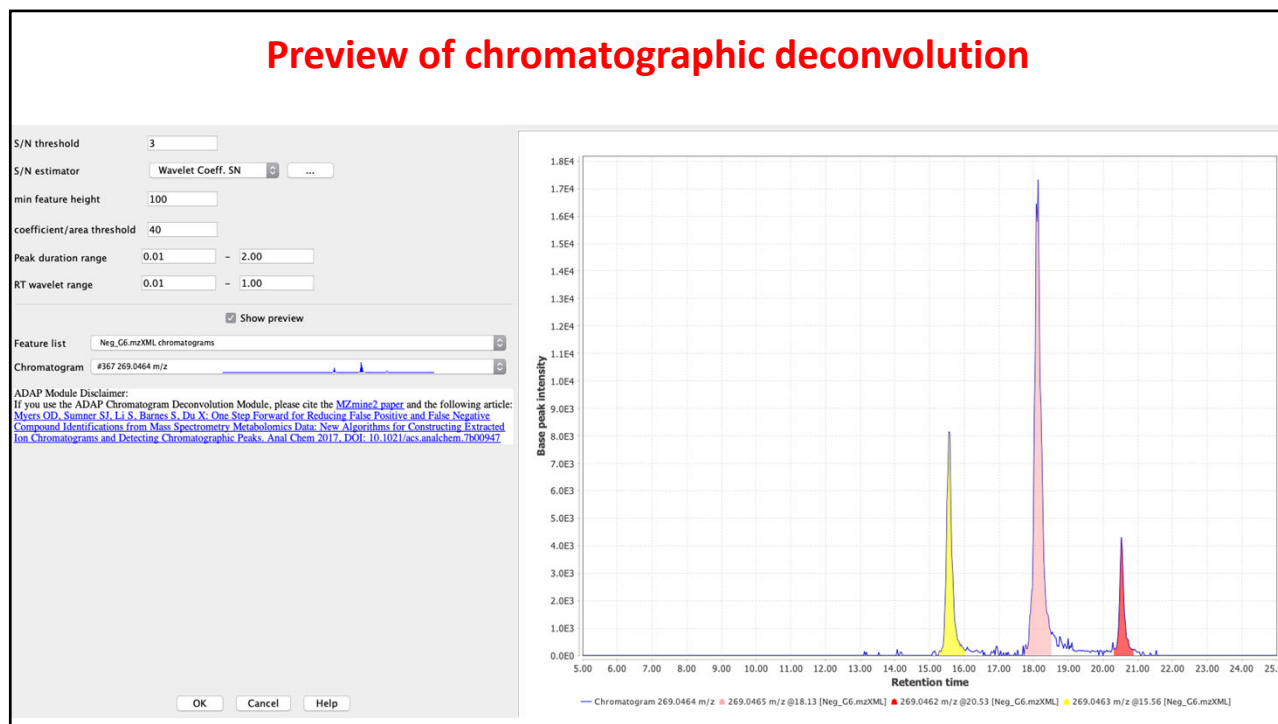
RT wavelet range 0.001 - 0.20

Show preview



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## Preview of chromatographic deconvolution



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## Chromatogram deconvolution output

### Raw data files

- ▶ Neg\_C4.mzXML
- ▶ Neg\_C5.mzXML
- ▶ Neg\_C6.mzXML
- ▶ Neg\_G4.mzXML
- ▶ Neg\_G5.mzXML
- ▶ Neg\_G6.mzXML

### Feature lists

- ▶ Neg\_G6.mzXML chromatograms
- ▶ Neg\_C4.mzXML chromatograms
- ▶ Neg\_G6.mzXML chromatograms deconvoluted
- ▶ Neg\_C4.mzXML chromatograms deconvoluted

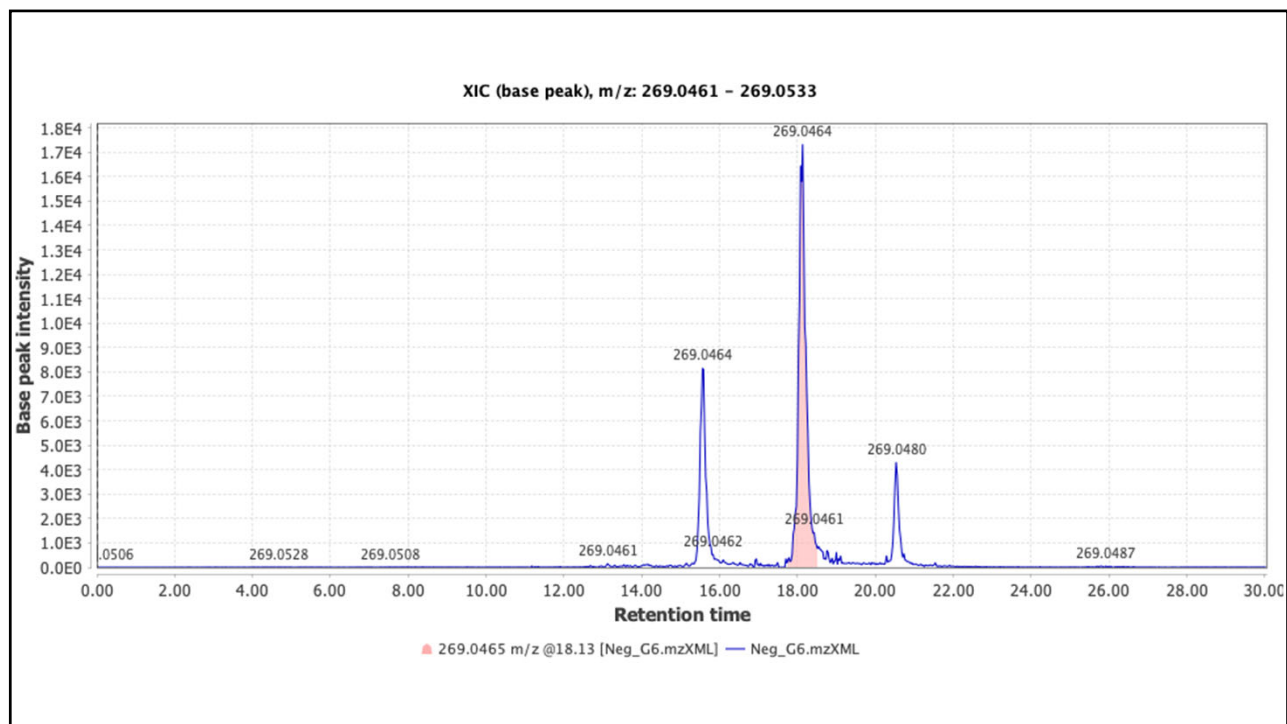
### ▼ Neg\_G6.mzXML chromatograms deconvoluted

- ▲ #1 60.9966 m/z @6.38
- ▲ #2 60.9966 m/z @7.34
- ▲ #3 60.9966 m/z @6.22
- ▲ #4 75.0117 m/z @5.16
- ▲ #5 75.0118 m/z @7.39

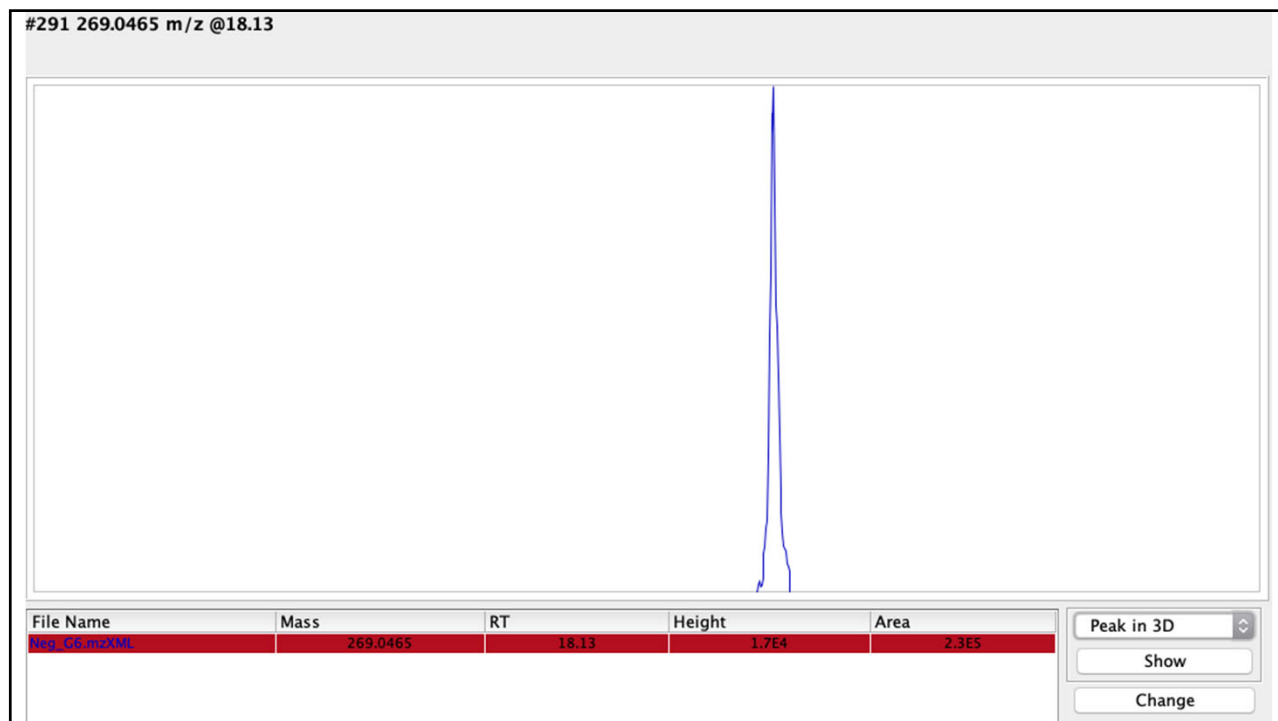
- ▲ #291 269.0465 m/z @18.13
- ▲ #292 269.0462 m/z @20.53
- ▲ #293 269.0463 m/z @15.56
- ▲ #294 269.1040 m/z @19.33

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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

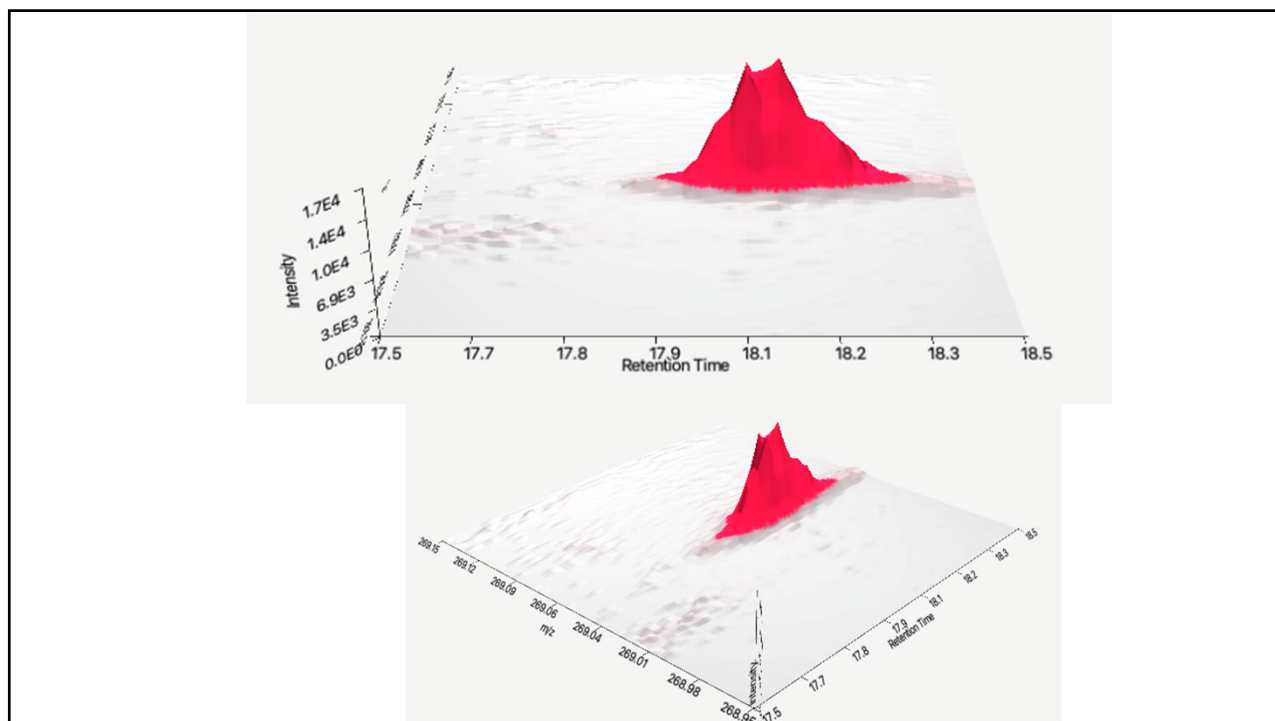
Features: Add Remove

Retention time resolution: 500

m/z resolution: 500

OK Cancel Help

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## Peak and chromatogram alignment

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

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

Require same ID

Compare isotope pattern  Setup..

OK Cancel Help

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

Feature list name Aligned feature list

m/z tolerance 0.015 m/z or 10.0 ppm

Weight for m/z 0.5

Retention time tolerance 0.5 absolute (min)

Weight for RT 0.5

Require same charge state

Require same ID

Compare isotope pattern  Setup..

Compare spectra similarity  Setup..

OK Cancel Help

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**Raw data files**

- Neg\_C4.mzXML
- Neg\_C5.mzXML
- Neg\_C6.mzXML
- Neg\_G4.mzXML
- Neg\_G5.mzXML
- Neg\_G6.mzXML

**Feature lists**

- Neg\_G6.mzXML chromatograms
- Neg\_C4.mzXML chromatograms
- Neg\_G6.mzXML chromatograms deconvoluted
- Neg\_C4.mzXML chromatograms deconvoluted
- Aligned feature list

**Aligned feature list**

- #1 60.9966 m/z @6.38
- #2 60.9966 m/z @7.34
- #3 60.9966 m/z @6.22
- #4 75.0117 m/z @5.16
- #5 75.0118 m/z @7.39
- #6 79.9617 m/z @17.51
- #7 79.9617 m/z @15.16
- #8 88.9905 m/z @6.35
- #9 88.9905 m/z @7.20
- #10 88.9905 m/z @5.91

#290 269.0135 m/z @17.51  
 #291 269.0464 m/z @18.12  
 #292 269.0462 m/z @20.53  
 #293 269.0463 m/z @15.56  
 #294 269.1040 m/z @19.33

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## Peak and chromatogram alignment

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

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

Feature list name Aligned feature list

m/z tolerance 0.01 m/z or 0.0 ppm

RT tolerance 0.5 absolute (min)

RT tolerance after correction 0.1 absolute (min)

RANSAC iterations 5000

Minimum number of points 50.0 %

Threshold value 1

Linear model

Require same charge state

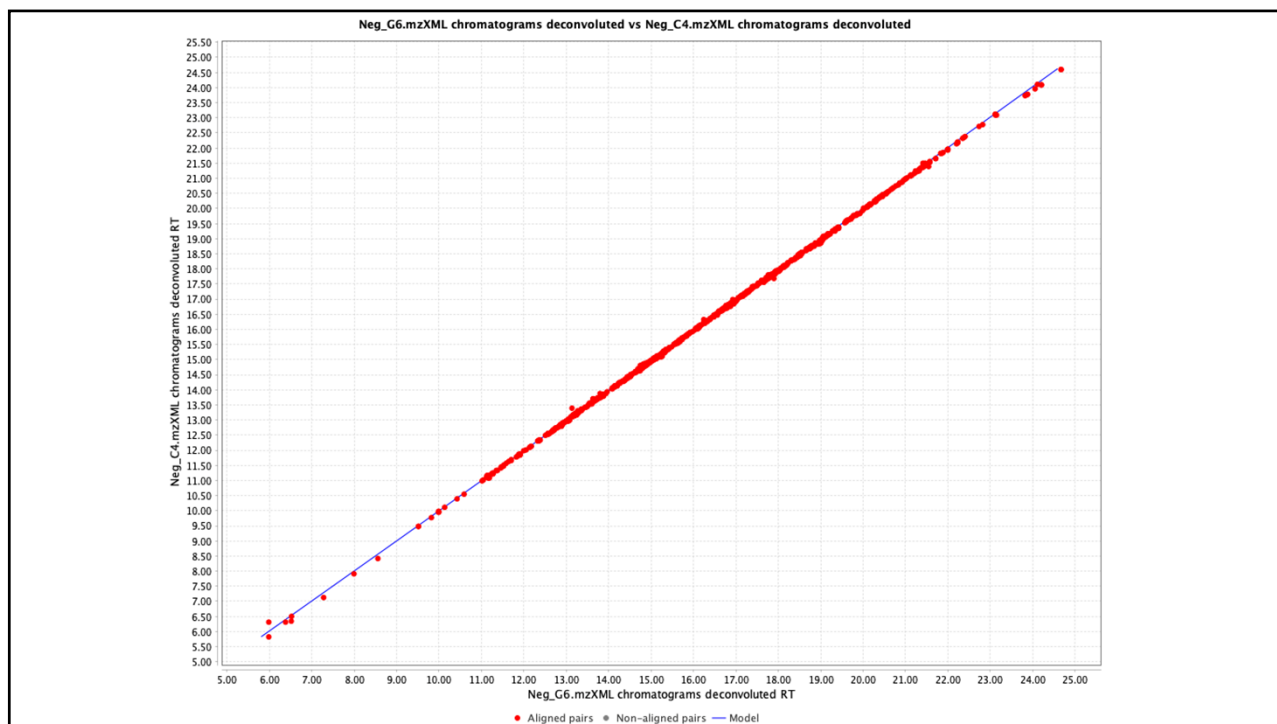
Show preview of RANSAC alignment

Neg\_G6.mzXML chromatograms deconvoluted

Neg\_C4.mzXML chromatograms deconvoluted

Preview alignment

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### Double click on Aligned peak list

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

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## 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

<input checked="" type="checkbox"/> Export row ID	<input type="checkbox"/> Peak status
<input type="checkbox"/> Export row $m/z$	<input checked="" type="checkbox"/> Peak $m/z$
<input type="checkbox"/> Export row retention time	<input checked="" type="checkbox"/> Peak RT
<input type="checkbox"/> Export row identity (main ID)	<input type="checkbox"/> Peak RT start
<input type="checkbox"/> Export row identity (all IDs)	<input type="checkbox"/> Peak RT end
<input type="checkbox"/> Export row identity (main ID + details)	<input type="checkbox"/> Peak duration time
<input type="checkbox"/> Export row comment	<input checked="" type="checkbox"/> Peak height
	<input checked="" type="checkbox"/> Peak area
	<input type="checkbox"/> Peak charge
	<input type="checkbox"/> Peak # data points
	<input checked="" type="checkbox"/> Peak FWHM
	<input type="checkbox"/> Peak tailing factor
	<input type="checkbox"/> Peak asymmetry factor

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