Using MS-DIAL in metabolomics

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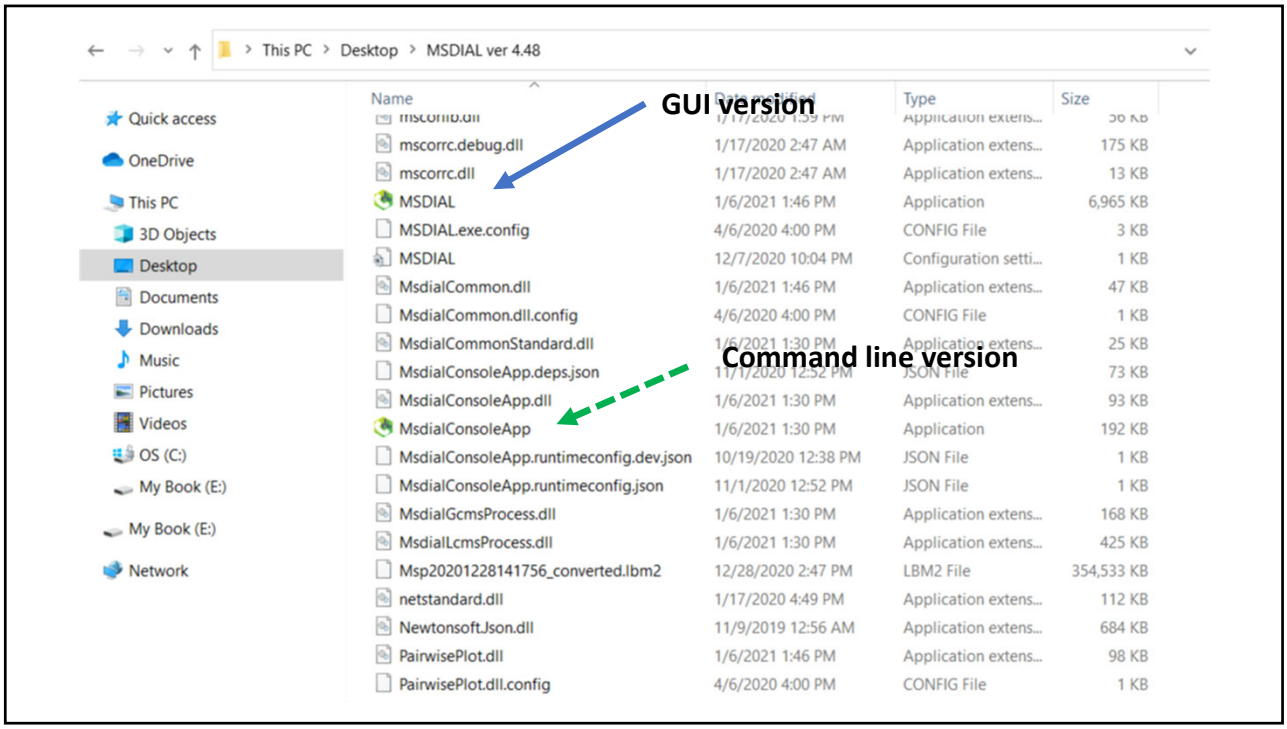
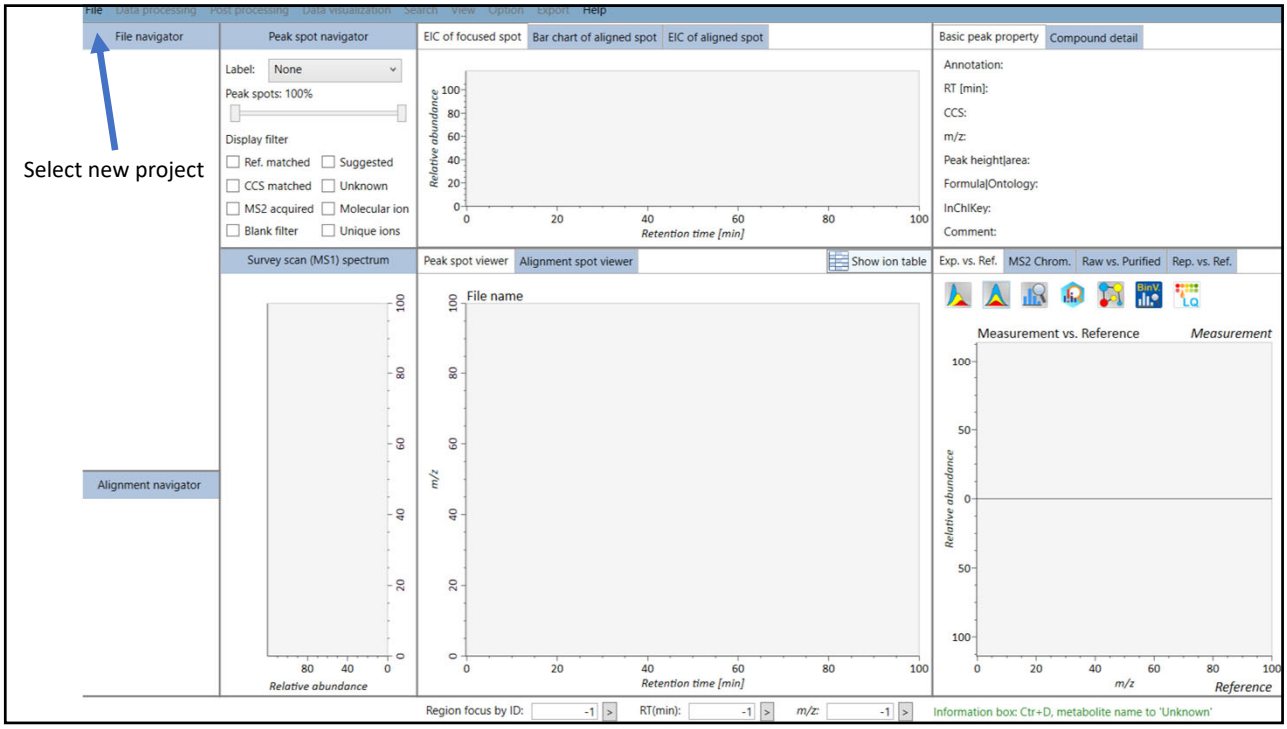
MS-DIAL

- **Download MS-DIAL from**
  - **http://prime.psc.riken.jp/compms/msdial/main.html**
- **Download ABF converter (scroll down – under "related" on right)**
  - **You will need to register its use**
- **Download the two public MSMS databases**
  - **http://prime.psc.riken.jp/compms/msdial/main.html#MSP**
- **Download MS-Finder**
  - **http://prime.psc.riken.jp/compms/msfinder/main.html**
- **Unzip MS-DIAL, ABF converter, MS-Finder and database zip files and place on desktop**
- **To use, locate the MS-DIAL folder (on your desktop)**
  - **Double click to open the folder**

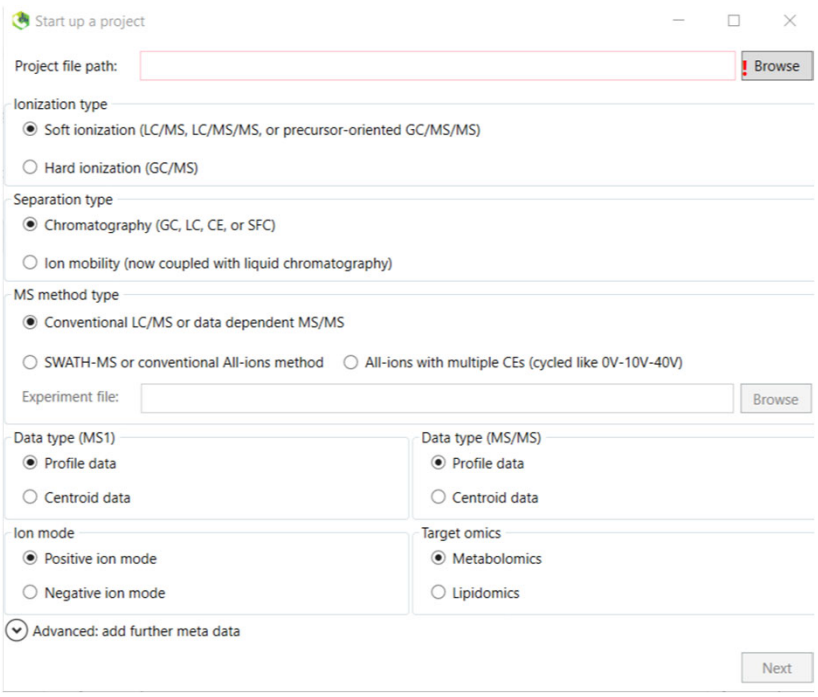
2


This PC > Desktop > MSDIAL ver 4.48
Quick access
OneDrive
This PC
3D Objects
Desktop
Documents
Downloads
Music
Pictures
Videos
OS (C:)
My Book (E:)
My Book (E:)
Network
Name
Type
Size
mscorrc.debug.dll 1/17/2020 2:47 AM Application extens... 175 KB
mscorrc.dll 1/17/2020 2:47 AM Application extens... 13 KB
MSDIAL 1/6/2021 1:46 PM Application 6,965 KB
MSDIAL.exe.config 4/6/2020 4:00 PM CONFIG File 3 KB
MSDIAL 12/7/2020 10:04 PM Configuration setti... 1 KB
MsdialCommon.dll 1/6/2021 1:46 PM Application extens... 47 KB
MsdialCommon.dll.config 4/6/2020 4:00 PM CONFIG File 1 KB
MsdialConsoleApp.dll 1/6/2021 1:30 PM Application extens... 93 KB
MsdialConsoleApp 1/6/2021 1:30 PM Application 192 KB
MsdialConsoleApp.runtimeconfig.dev.json 10/19/2020 12:38 PM JSON File 1 KB
MsdialConsoleApp.runtimeconfig.json 11/1/2020 12:52 PM JSON File 1 KB
MsdialGcmsProcess.dll 1/6/2021 1:30 PM Application extens... 168 KB
MsdialLcmsProcess.dll 1/6/2021 1:30 PM Application extens... 425 KB
Msp20201228141756_converted.lbm2 12/28/2020 2:47 PM LBM2 File 354,533 KB
netstandard.dll 1/17/2020 4:49 PM Application extens... 112 KB
NewtonsoftJson.dll 11/9/2019 12:56 AM Application extens... 684 KB
PairwisePlot.dll 1/6/2021 1:46 PM Application extens... 98 KB
PairwisePlot.dll.config 4/6/2020 4:00 PM CONFIG File 1 KB
GUI version
Command line version

3

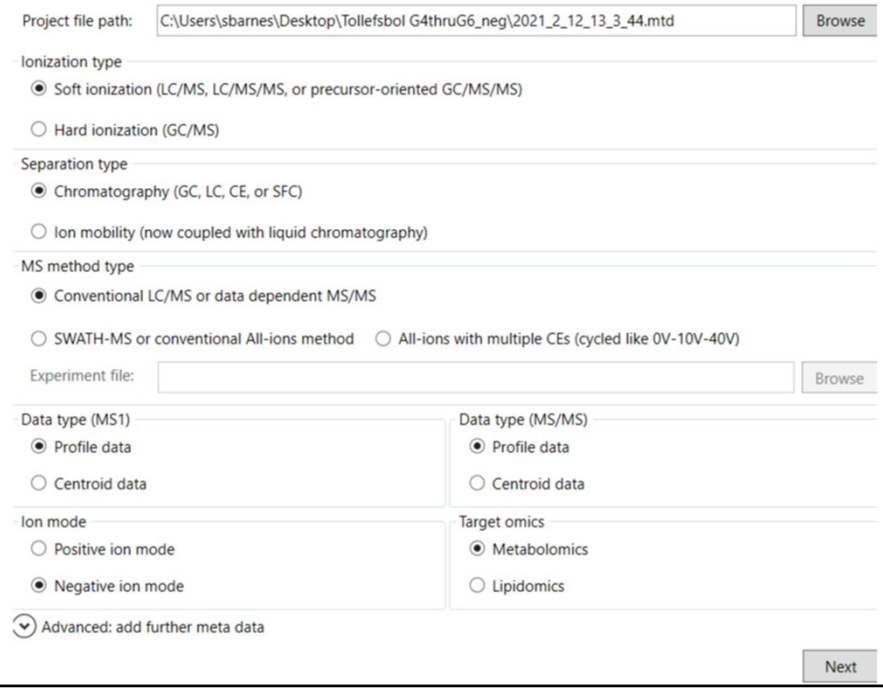

File navigator
Peak spot navigator
EIC of focused spot
Bar chart of aligned spot
EIC of aligned spot
Basic peak property
Compound detail
Label: None
Peak spots: 100%
Display filter
Ref. matched
Suggested
CCS matched
Unknown
MS2 acquired
Molecular ion
Blank filter
Unique ions
Annotation:
RT [min]:
CCS:
m/z:
Peak height|area:
Formula|Ontology:
InChIKey:
Comment:
Relative abundance
Retention time [min]
Survey scan (MS1) spectrum
Peak spot viewer
Alignment spot viewer
Show ion table
Exp. vs. Ref.
MS2 Chrom.
Raw vs. Purified
Rep. vs. Ref.
File name
m/z
Measurement vs. Reference
Measurement
Reference
Alignment navigator
Region focus by ID:
RT(min):
m/z:
Information box: Ctr+D, metabolite name to 'Unknown'
Select new project

4



Default opening page

5



Select the folder where the .abf data are located

Soft ionization

Chromatography

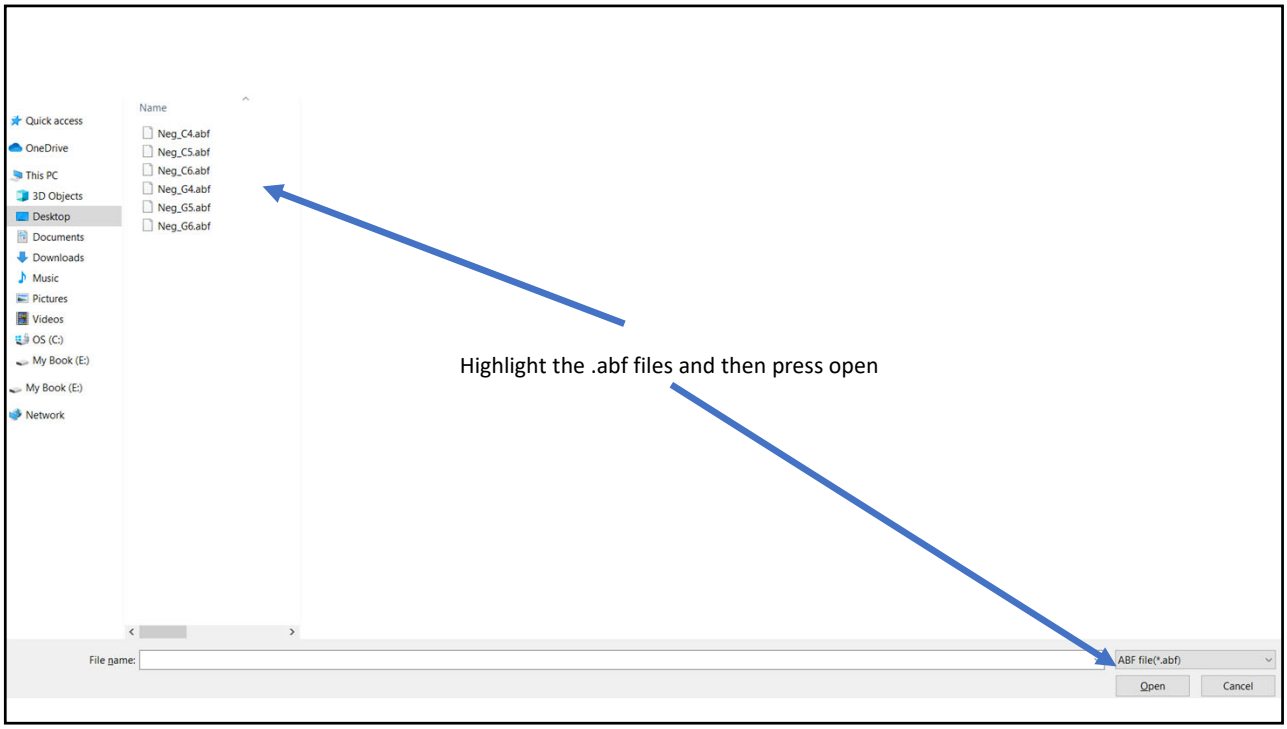
Conventional

Profile data for MS and MSMS

Ion mode = negative

Omics = metabolomics

6



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File path	File name	Type	Class ID	Batch	Analytical order	Inject. volume (µL)	Included
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_C4	Sample	1	1	1	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_C5	Sample	1	1	2	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_C6	Sample	1	1	3	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_G4	Sample	1	1	4	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_G5	Sample	1	1	5	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_G6	Sample	1	1	6	1	☑

Adjust the class ID to reflect the two groups

Analysis file paths Browse

File path	File name	Type	Class ID	Batch	Analytical order	Inject. volume (µL)	Included
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_C4	Sample	1	1	1	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_C5	Sample	1	1	2	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_C6	Sample	1	1	3	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_G4	Sample	2	1	4	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_G5	Sample	2	1	5	1	☑
C:\Users\sbarnes\Desktop\Tollefsbol G4thru	Neg_G6	Sample	2	1	6	1	☑

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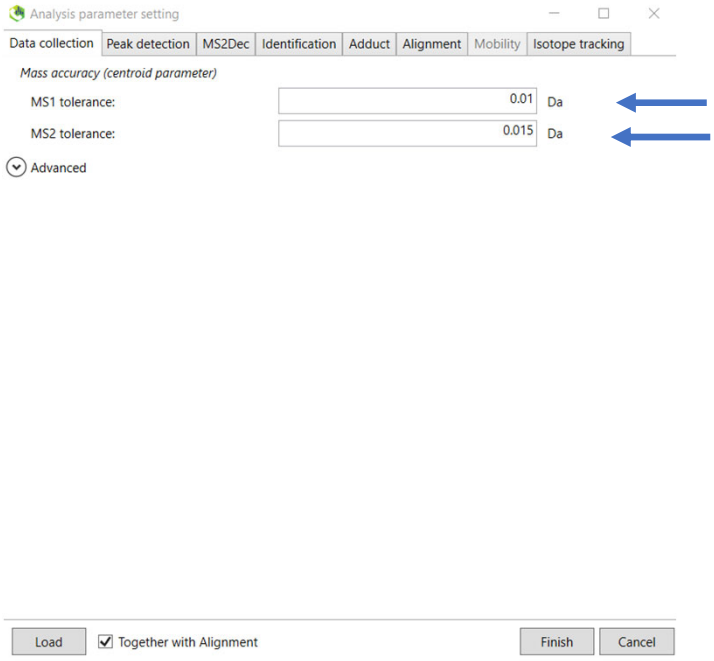
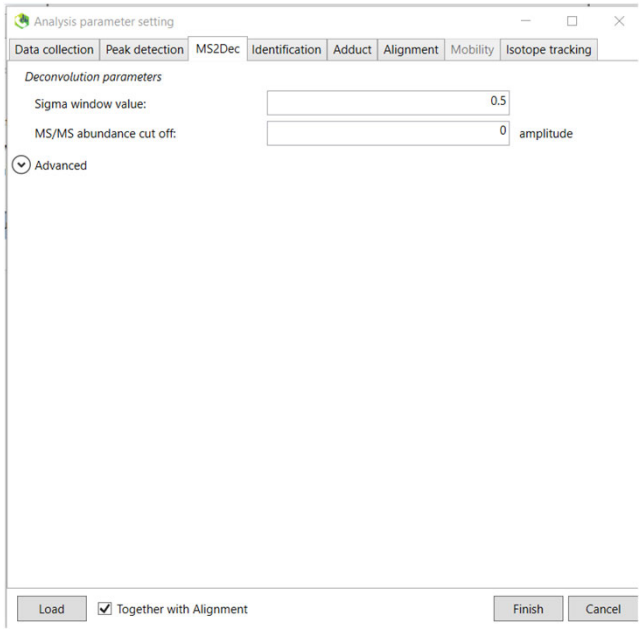


Setting the search parameters

Do not press the finish button

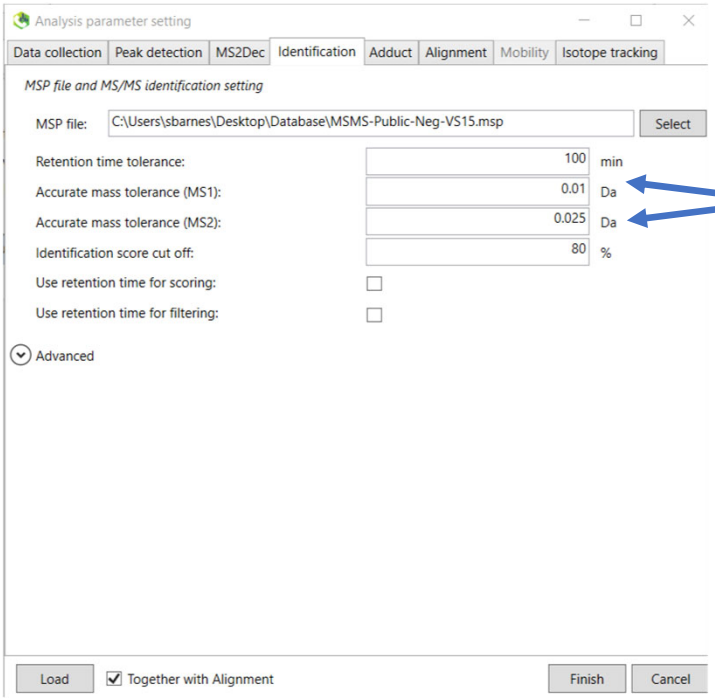
9

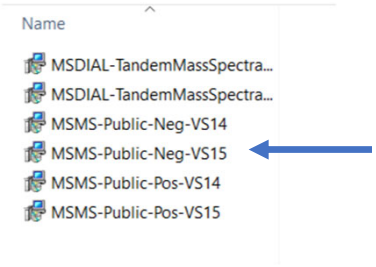


Making edits to the parameters using the strip

Do not press the finish button

10



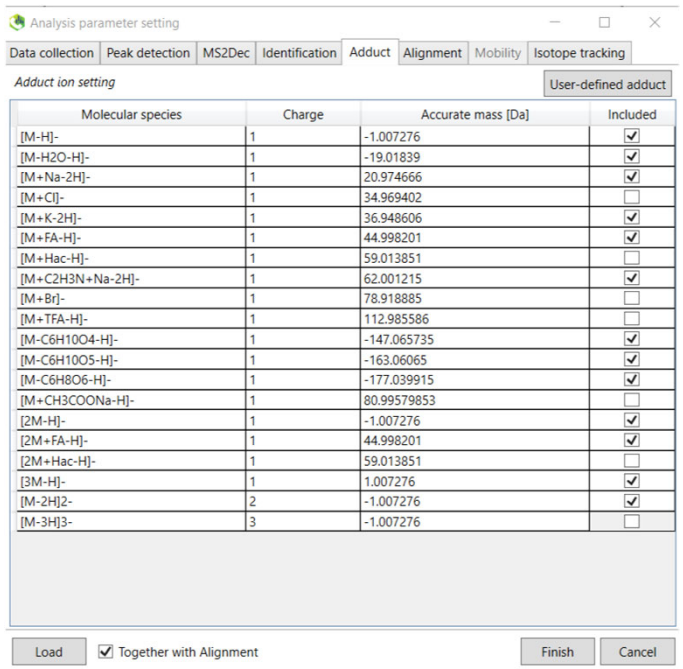


Select the MSMS database

Selecting the mass tolerance

Do not press the finish button

11



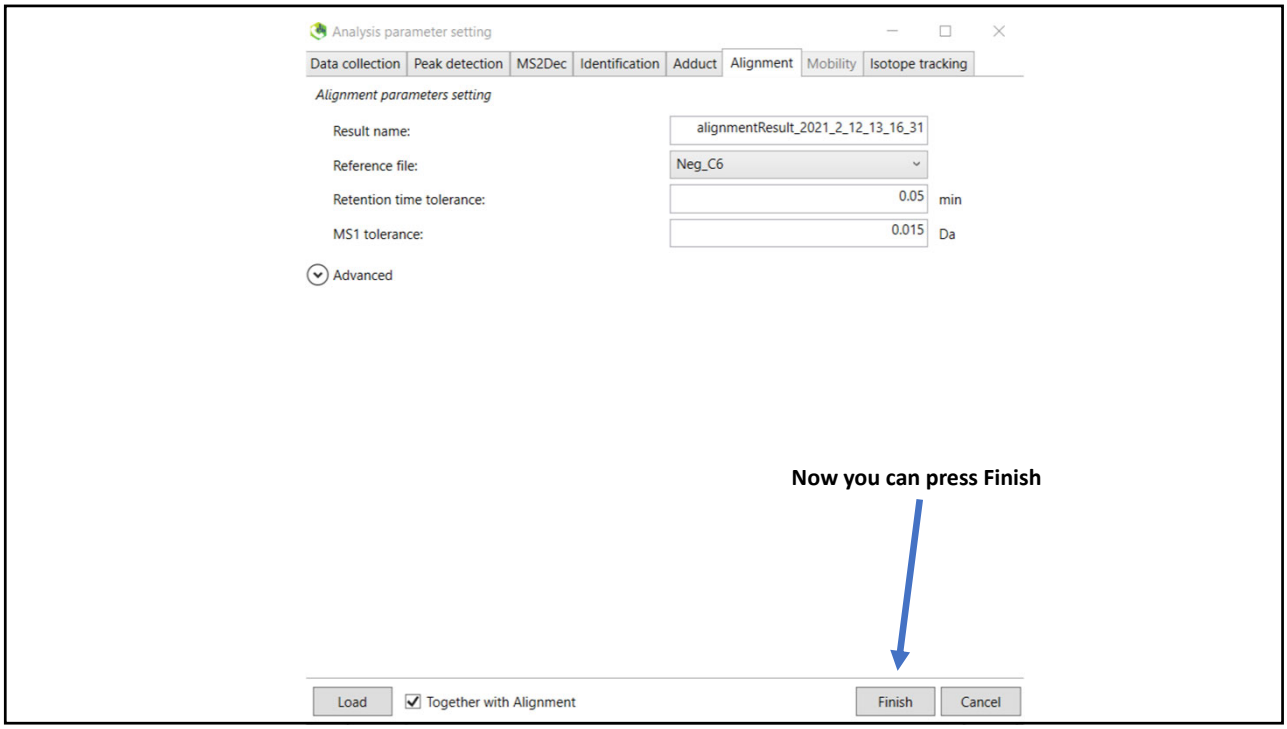
Molecular species	Charge	Accurate mass [Da]	Included
[M-H]-	1	-1.007276	☑
[M-H2O-H]-	1	-19.01839	☑
[M+Na-2H]-	1	20.974666	☑
[M+Cl]-	1	34.969402	☐
[M+K-2H]-	1	36.948606	☑
[M+FA-H]-	1	44.998201	☑
[M+Hac-H]-	1	59.013851	☐
[M+C2H3N+Na-2H]-	1	62.001215	☑
[M+Br]-	1	78.918885	☐
[M+TFA-H]-	1	112.985586	☐
[M-C6H10O4-H]-	1	-147.065735	☑
[M-C6H10O5-H]-	1	-163.06065	☑
[M-C6H8O6-H]-	1	-177.039915	☑
[M+CH3COONa-H]-	1	80.99579853	☐
[2M-H]-	1	-1.007276	☑
[2M+FA-H]-	1	44.998201	☑
[2M+Hac-H]-	1	59.013851	☐
[3M-H]-	1	1.007276	☑
[M-2H]2-	2	-1.007276	☑
[M-3H]3-	3	-1.007276	☐

Select the possible adducts – besides the molecular ion [M-H]-, we expect to see

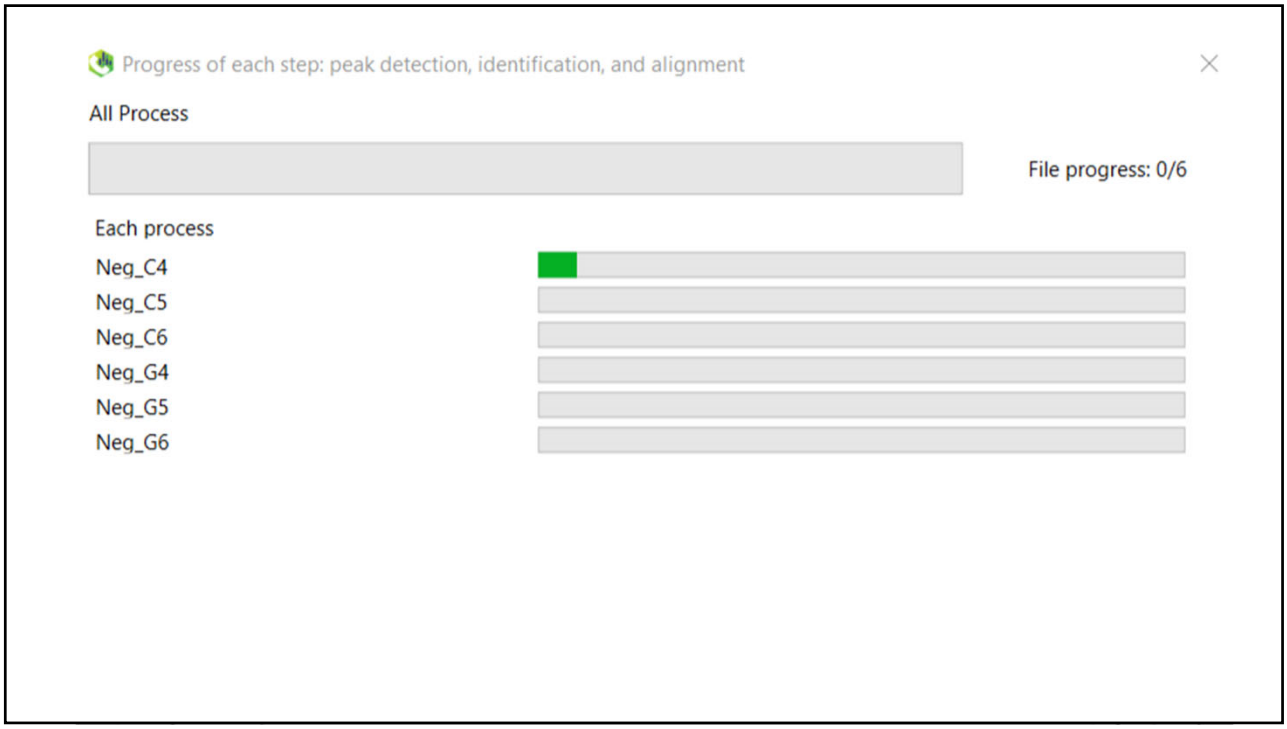
- **loss of water,**
- **Na and K adducts,**
- **formic acid adducts,**
- **ions formed in the interface, dimers and trimers,**
- **and multiply charged ions**

Do not press the finish button

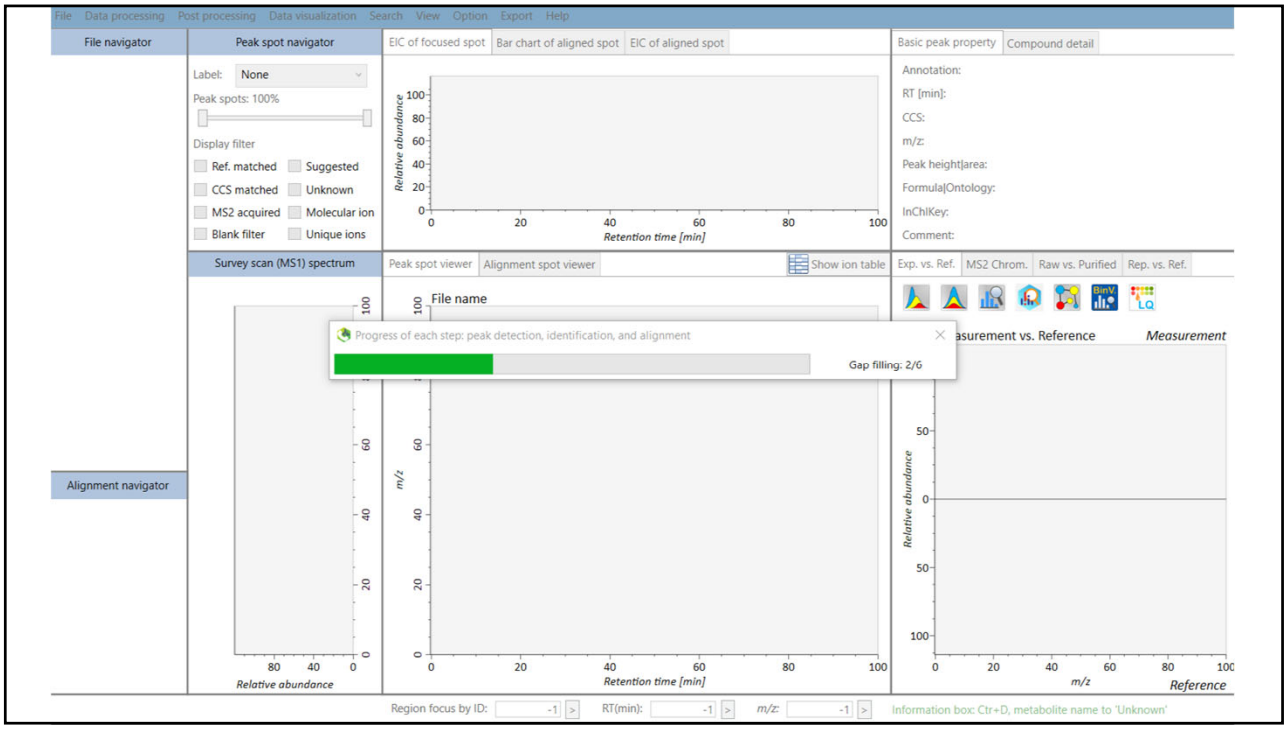
12


Analysis parameter setting
Data collection
Peak detection
MS2Dec
Identification
Adduct
Alignment
Mobility
Isotope tracking
Alignment parameters setting
Result name:
alignmentResult_2021_2_12_13_16_31
Reference file:
Neg_C6
Retention time tolerance:
0.05
min
MS1 tolerance:
0.015
Da
Advanced
Now you can press Finish
Load
Together with Alignment
Finish
Cancel

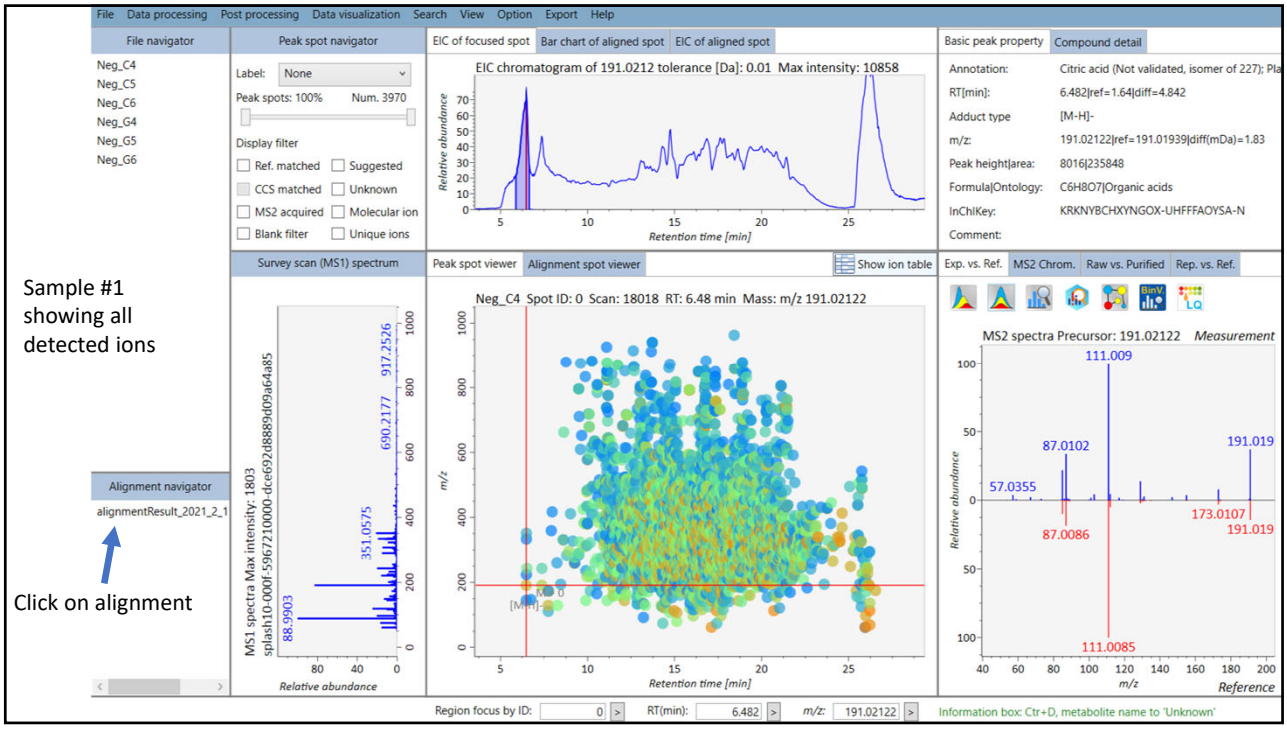
13


Progress of each step: peak detection, identification, and alignment
All Process
File progress: 0/6
Each process
Neg_C4
Neg_C5
Neg_C6
Neg_G4
Neg_G5
Neg_G6

14



15



Sample #1 showing all detected ions

Click on alignment

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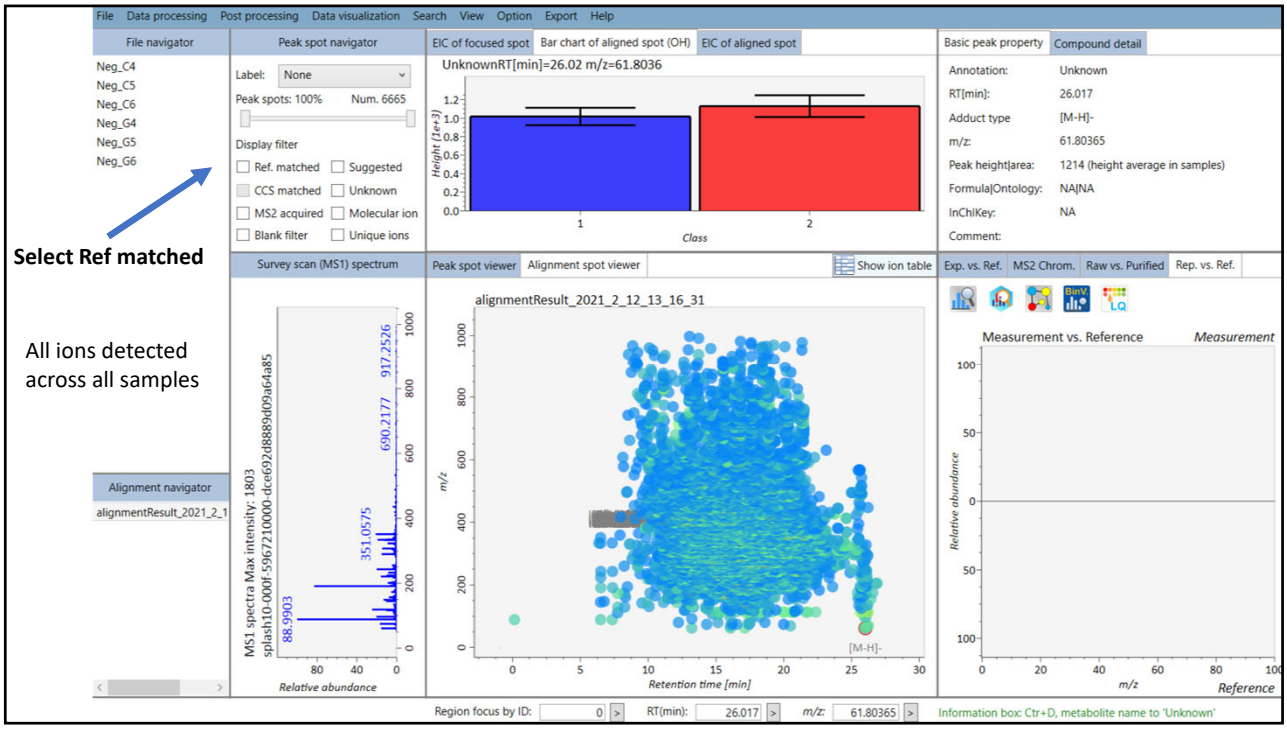
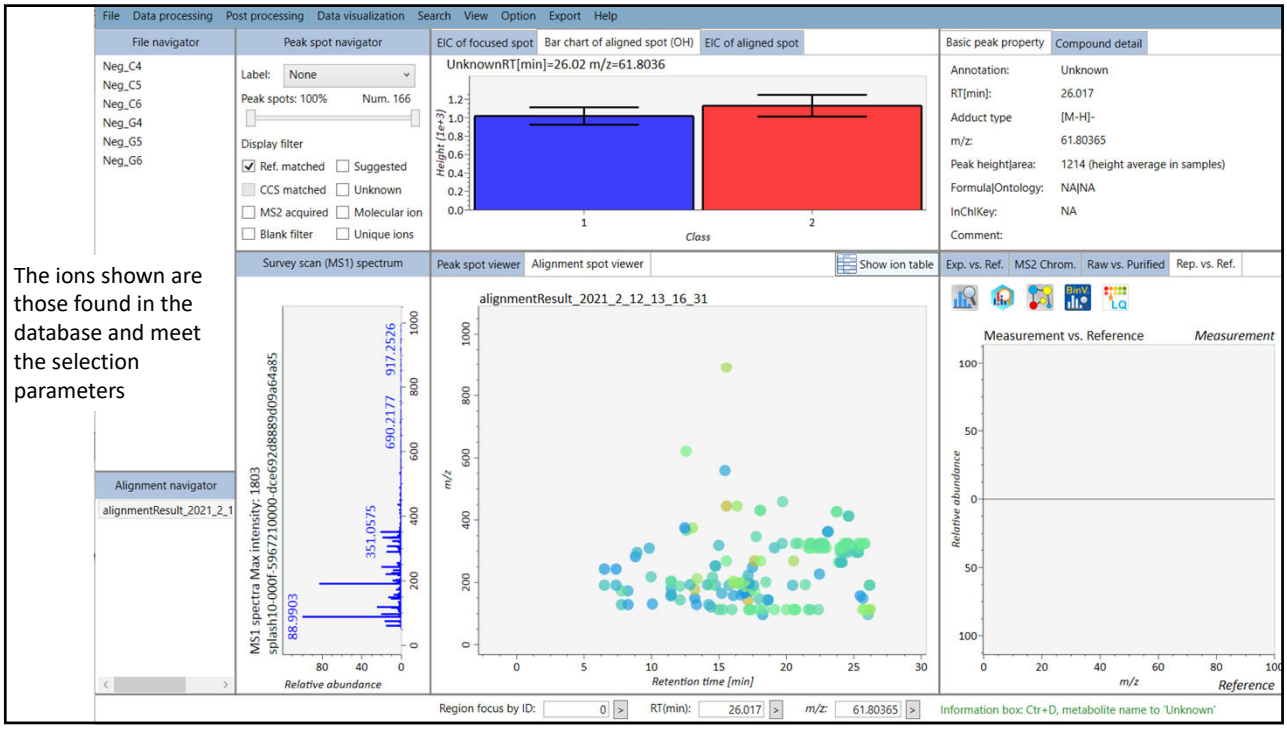
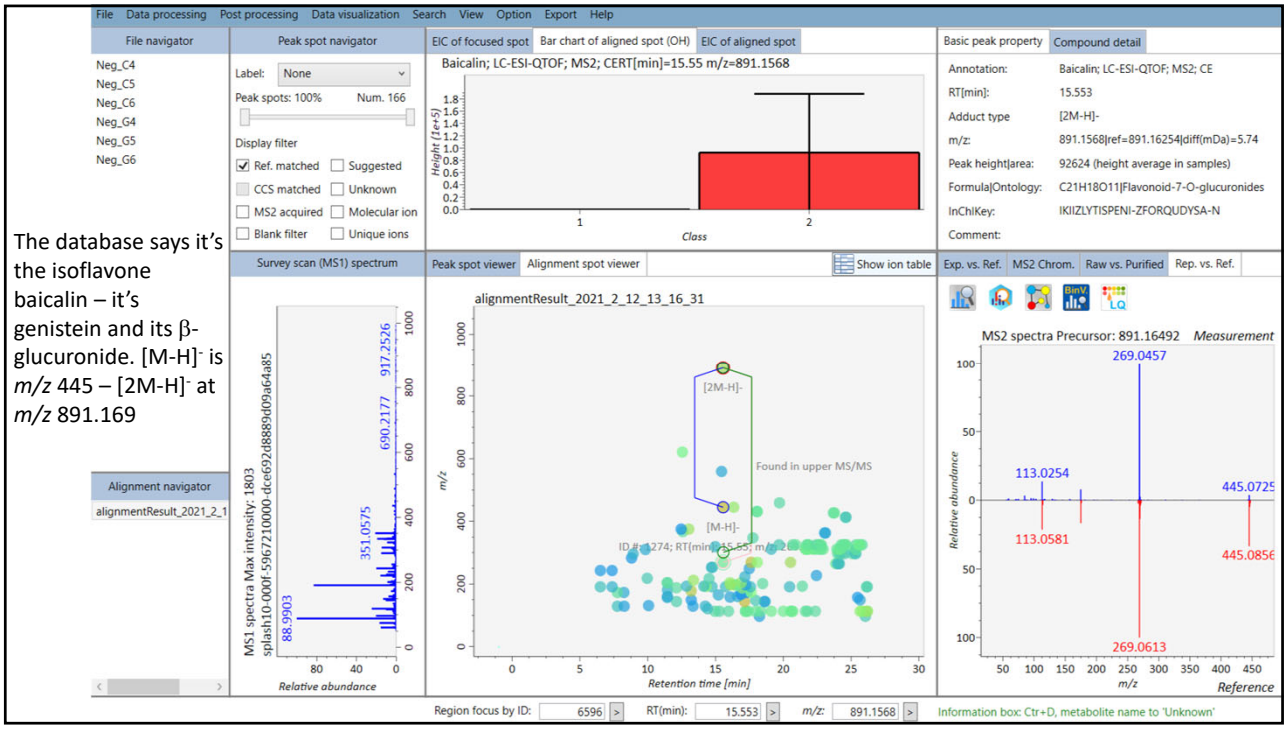

Select Ref matched
All ions detected across all samples
Alignment navigator
alignmentResult_2021_2_1

17

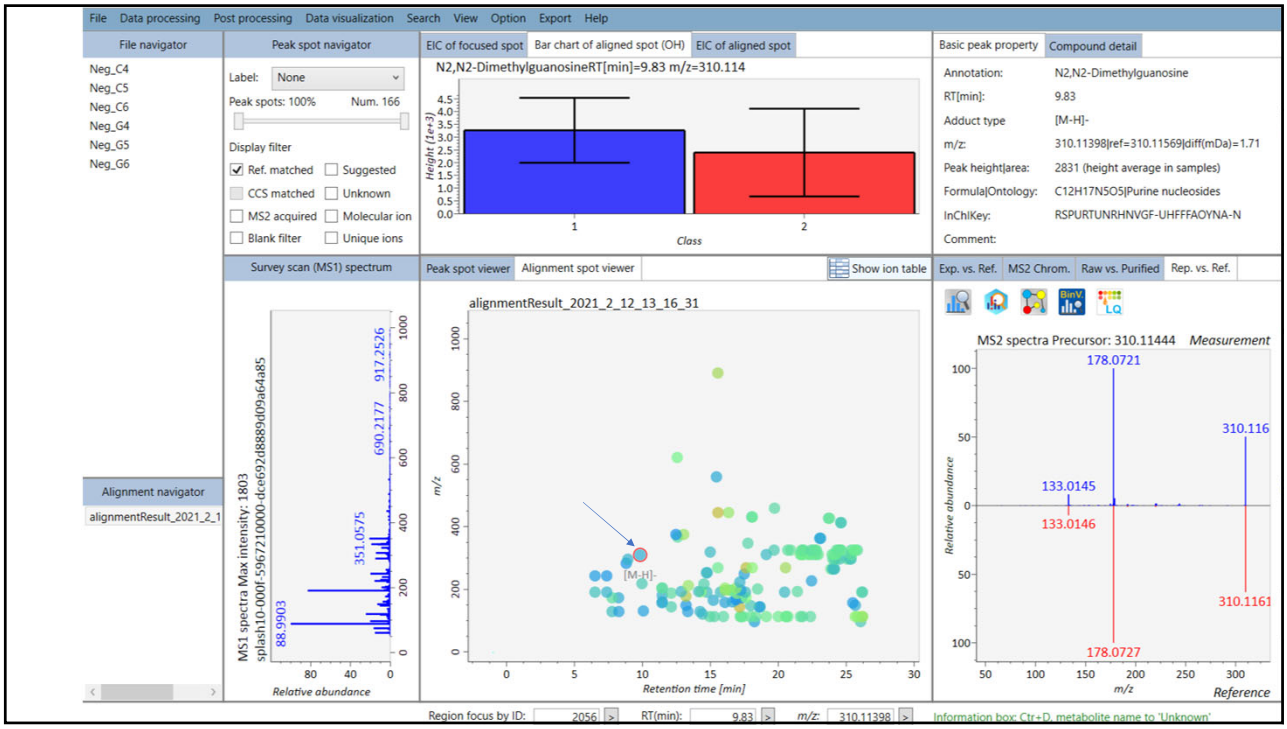

The ions shown are those found in the database and meet the selection parameters
Alignment navigator
alignmentResult_2021_2_1

18

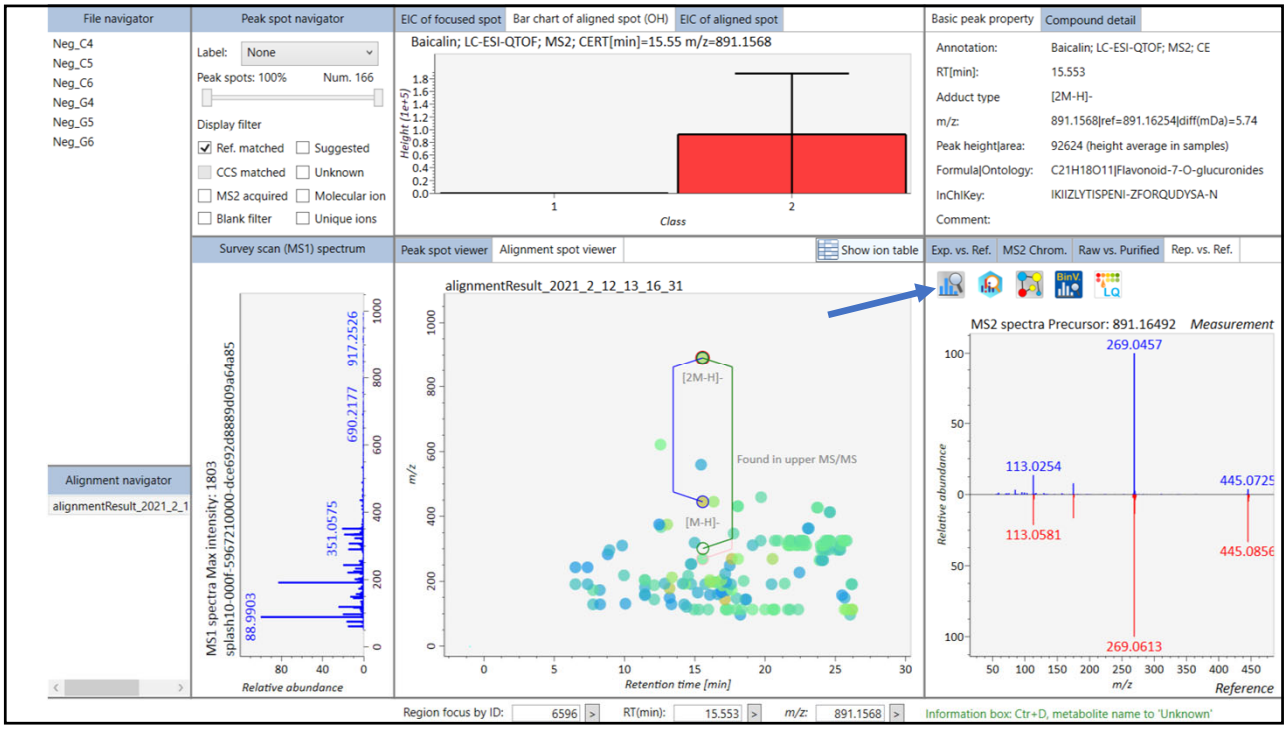
The database says it's the isoflavone baicalin – it's genistein and its β-glucuronide. $[M-H]^-$ is *m/z* 445 – $[2M-H]^-$ at *m/z* 891.169



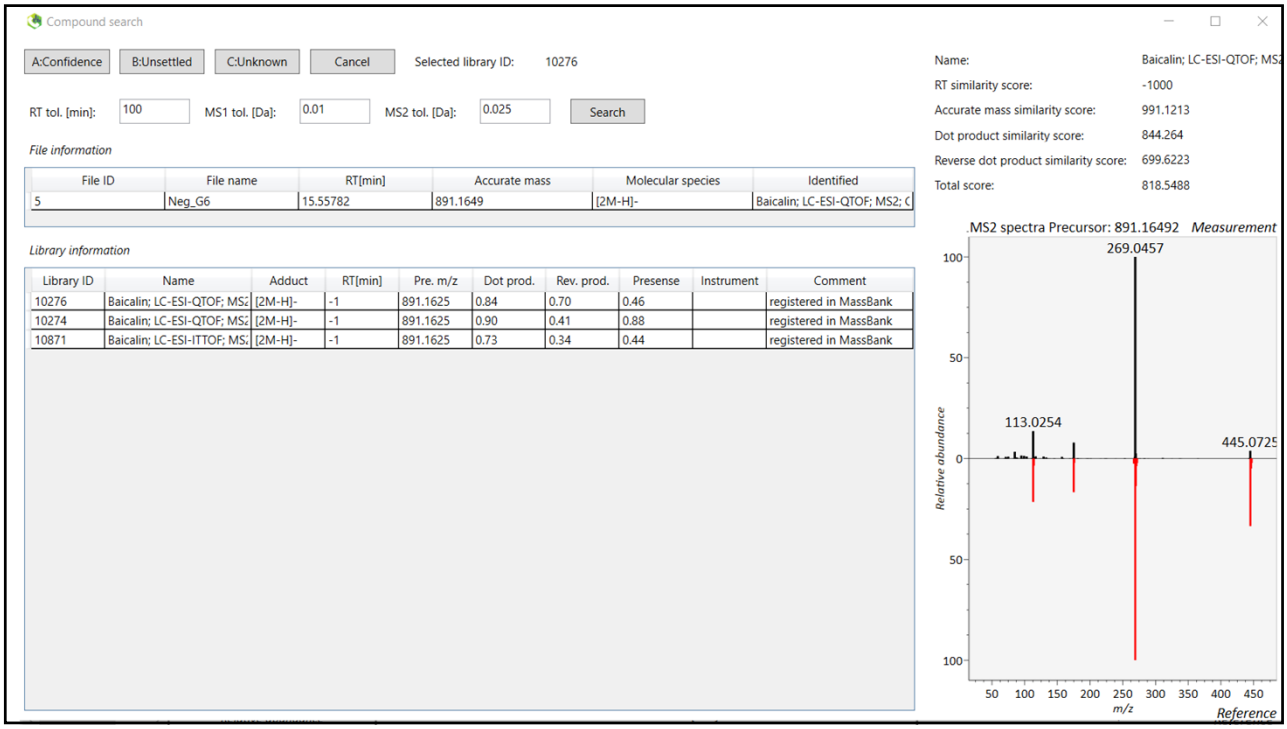
19



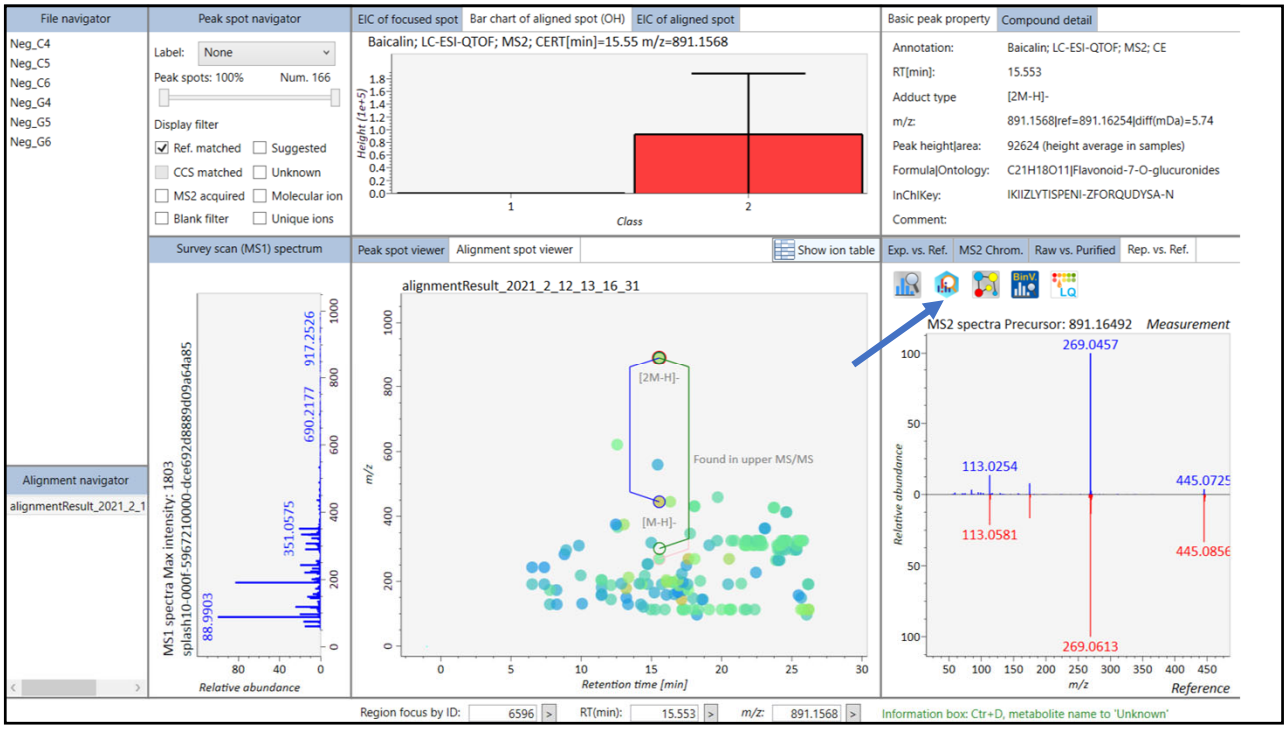
20


File navigator
Neg_C4
Neg_C5
Neg_C6
Neg_G4
Neg_G5
Neg_G6
Alignment navigator
alignmentResult_2021_2_1
Peak spot navigator
Label: None
Peak spots: 100% Num. 166
Display filter
Ref. matched
Suggested
CCS matched
Unknown
MS2 acquired
Molecular ion
Blank filter
Unique ions
EIC of focused spot
Bar chart of aligned spot (OH)
EIC of aligned spot
Baicalin; LC-ESI-QTOF; MS2; CERT[min]=15.55 m/z=891.1568
Height (1e+5)
Class
Basic peak property
Compound detail
Annotation: Baicalin; LC-ESI-QTOF; MS2; CE
RT[min]: 15.553
Adduct type [2M-H]-
m/z: 891.1568|ref=891.16254|diff(mDa)=5.74
Peak height|area: 92624 (height average in samples)
Formula|Ontology: C21H18O11|Flavonoid-7-O-glucuronides
InChIKey: IKIIZLYTISPENI-ZFORQUDYSA-N
Comment:
Survey scan (MS1) spectrum
MS1 spectra Max intensity: 1803
splash10-000f-5967210000-dce692d88889d09a64a85
88.9903
351.0575
690.2177
917.2526
Relative abundance
Peak spot viewer
Alignment spot viewer
Show ion table
alignmentResult_2021_2_12_13_16_31
[2M-H]-
[M-H]-
Found in upper MS/MS
m/z
Retention time [min]
Exp. vs. Ref.
MS2 Chrom.
Raw vs. Purified
Rep. vs. Ref.
MS2 spectra Precursor: 891.16492 Measurement
269.0457
113.0254
113.0581
445.0725
445.0856
269.0613
Relative abundance
m/z
Reference
Region focus by ID: 6596
RT(min): 15.553
m/z: 891.1568
Information box: Ctr+D, metabolite name to 'Unknown'

21


Compound search
A:Confidence
B:Unsettled
C:Unknown
Cancel
Selected library ID: 10276
RT tol. [min]: 100
MS1 tol. [Da]: 0.01
MS2 tol. [Da]: 0.025
Search
File information
File ID | File name | RT[min] | Accurate mass | Molecular species | Identified
5 | Neg_G6 | 15.55782 | 891.1649 | [2M-H]- | Baicalin; LC-ESI-QTOF; MS2; C
Library information
Library ID | Name | Adduct | RT[min] | Pre. m/z | Dot prod. | Rev. prod. | Presense | Instrument | Comment
10276 | Baicalin; LC-ESI-QTOF; MS2 | [2M-H]- | -1 | 891.1625 | 0.84 | 0.70 | 0.46 | | registered in MassBank
10274 | Baicalin; LC-ESI-QTOF; MS2 | [2M-H]- | -1 | 891.1625 | 0.90 | 0.41 | 0.88 | | registered in MassBank
10871 | Baicalin; LC-ESI-ITTOF; MS2 | [2M-H]- | -1 | 891.1625 | 0.73 | 0.34 | 0.44 | | registered in MassBank
Name: Baicalin; LC-ESI-QTOF; MS2
RT similarity score: -1000
Accurate mass similarity score: 991.1213
Dot product similarity score: 844.264
Reverse dot product similarity score: 699.6223
Total score: 818.5488
MS2 spectra Precursor: 891.16492 Measurement
269.0457
113.0254
445.0725
Relative abundance
m/z
Reference

22


File navigator
Neg_C4
Neg_C5
Neg_C6
Neg_G4
Neg_G5
Neg_G6
Alignment navigator
alignmentResult_2021_2_1
Peak spot navigator
Label: None
Peak spots: 100% Num. 166
Display filter
Ref. matched
Suggested
CCS matched
Unknown
MS2 acquired
Molecular ion
Blank filter
Unique ions
Survey scan (MS1) spectrum
MS1 spectra Max intensity: 1803
splash10-000f-5967210000-dce692d8889d09a64a85
Relative abundance
EIC of focused spot
Bar chart of aligned spot (OH)
EIC of aligned spot
Baicalin; LC-ESI-QTOF; MS2; CERT[min]=15.55 m/z=891.1568
Height (1e+5)
Class
Peak spot viewer
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Show ion table
alignmentResult_2021_2_12_13_16_31
[2M-H]-
[M-H]-
Found in upper MS/MS
m/z
Retention time [min]
Basic peak property
Compound detail
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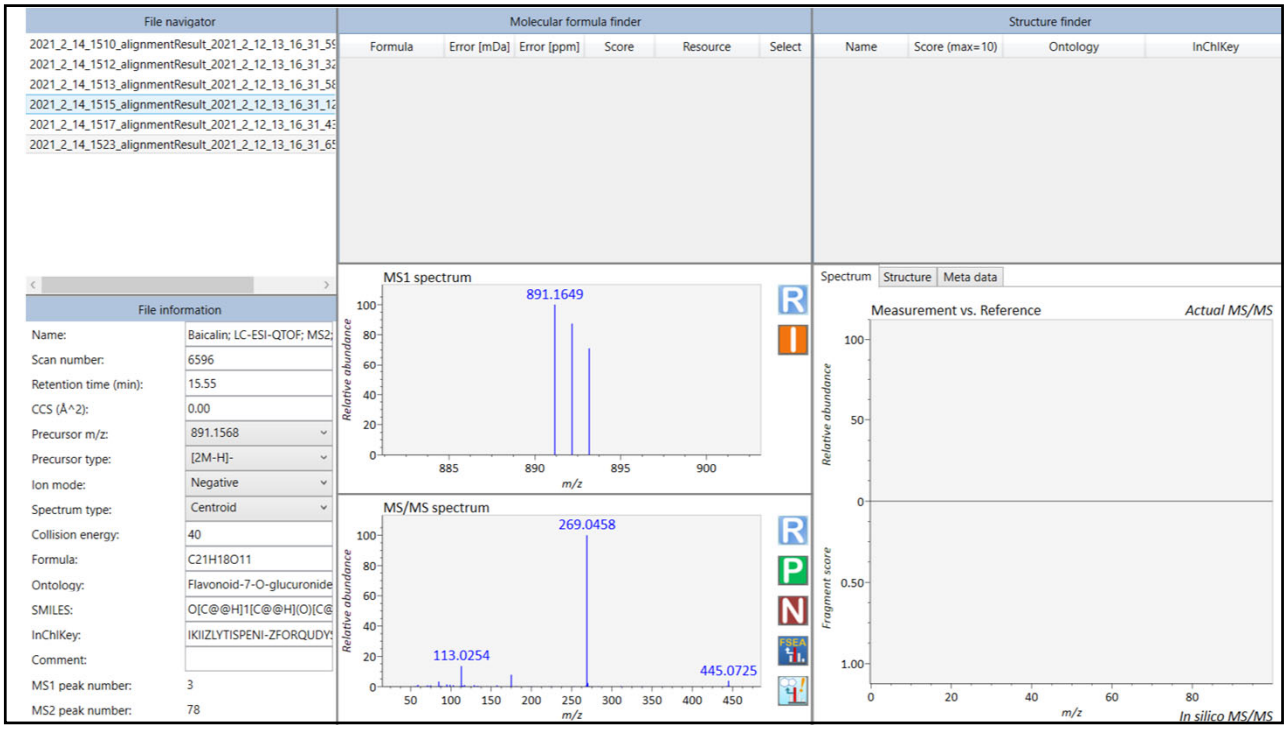
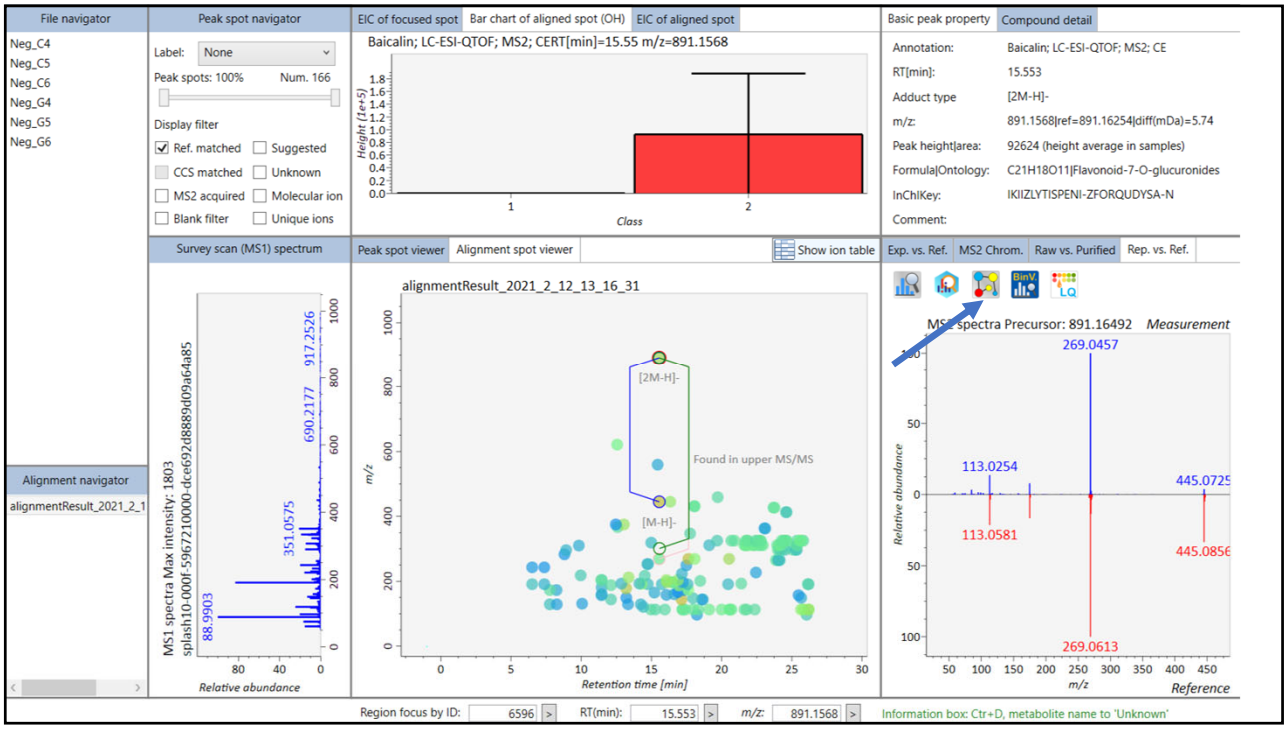
Comment:
Exp. vs. Ref.
MS2 Chrom.
Raw vs. Purified
Rep. vs. Ref.
MS2 spectra Precursor: 891.16492 Measurement
Reference
Region focus by ID: 6596
RT(min): 15.553
m/z: 891.1568
Information box: Ctr+D, metabolite name to 'Unknown'

23


File navigator
Molecular formula finder
Formula
Error [mDa]
Error [ppm]
Score
Resource
Select
Structure finder
Name
Score (max=10)
Ontology
InChIKey
File information
Name: Baicalin; LC-ESI-QTOF; MS2
Scan number: 6596
Retention time (min): 15.55
CCS (Å^2): 0.00
Precursor m/z: 891.1568
Precursor type: [2M-H]-
Ion mode: Negative
Spectrum type: Centroid
Collision energy: 40
Formula: C21H18O11
Ontology: Flavonoid-7-O-glucuronide
SMILES: O[C@@H]1[C@@H](O)[C@
InChIKey: IKIIZLYTISPENI-ZFORQUDY
Comment:
MS1 peak number: 3
MS2 peak number: 78
MS1 spectrum
MS/MS spectrum
Relative abundance
m/z
Spectrum
Structure
Meta data
Measurement vs. Reference
Actual MS/MS
Fragment score
In silico MS/MS

24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Neg_C4
Neg_C5
Neg_C6
Neg_G4
Neg_G5
Neg_G6
Alignment navigator
alignmentResult_2021_2_1
Peak spot navigator
Label: None
Peak spots: 100% Num. 166
Display filter
Ref. matched
Suggested
CCS matched
Unknown
MS2 acquired
Molecular ion
Blank filter
Unique ions
EIC of focused spot
Bar chart of aligned spot (OH)
EIC of aligned spot
Baicalin; LC-ESI-QTOF; MS2; CERT[min]=15.55 m/z=891.1568
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m/z
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Reference
Region focus by ID: 6596
RT(min): 15.553
m/z: 891.1568
Information box: Ctr+D, metabolite name to 'Unknown'

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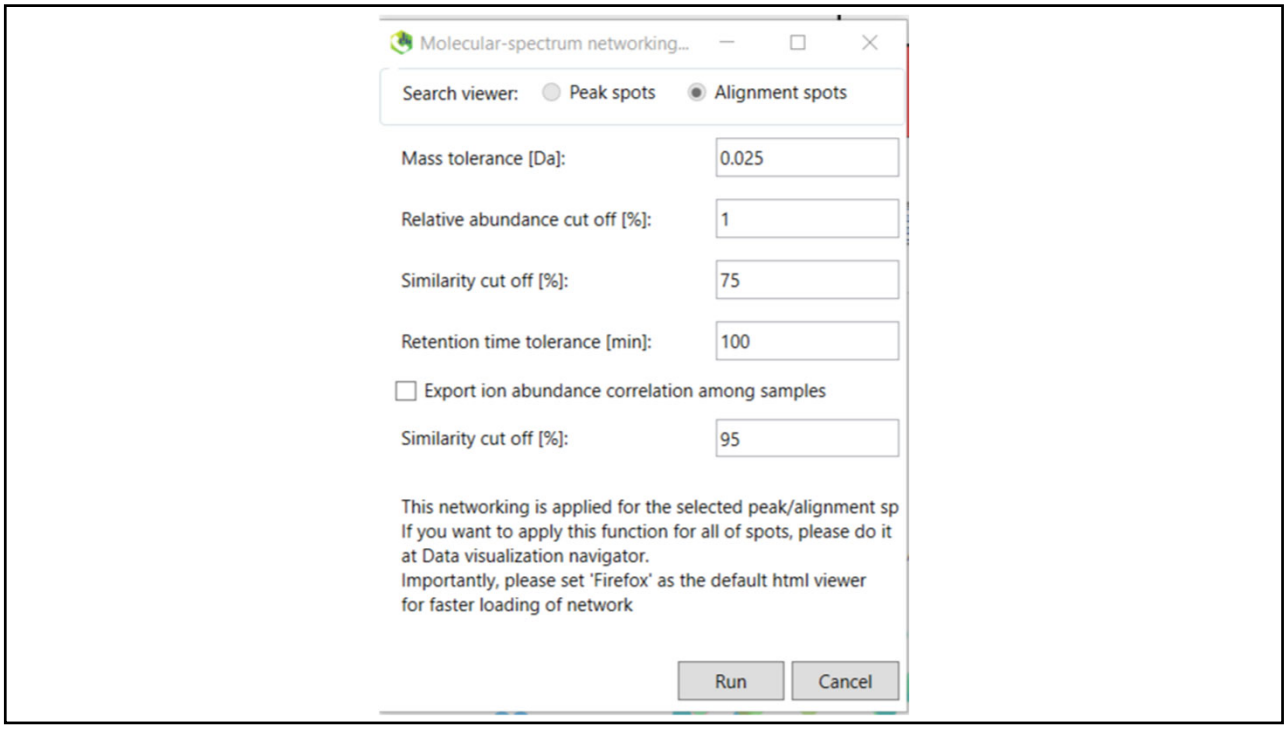
Molecular-spectrum networking...
Search viewer: Peak spots Alignment spots
Mass tolerance [Da]: 0.025
Relative abundance cut off [%]: 1
Similarity cut off [%]: 75
Retention time tolerance [min]: 100
Export ion abundance correlation among samples
Similarity cut off [%]: 95
This networking is applied for the selected peak/alignment sp
If you want to apply this function for all of spots, please do it
at Data visualization navigator.
Importantly, please set 'Firefox' as the default html viewer
for faster loading of network
Run
Cancel

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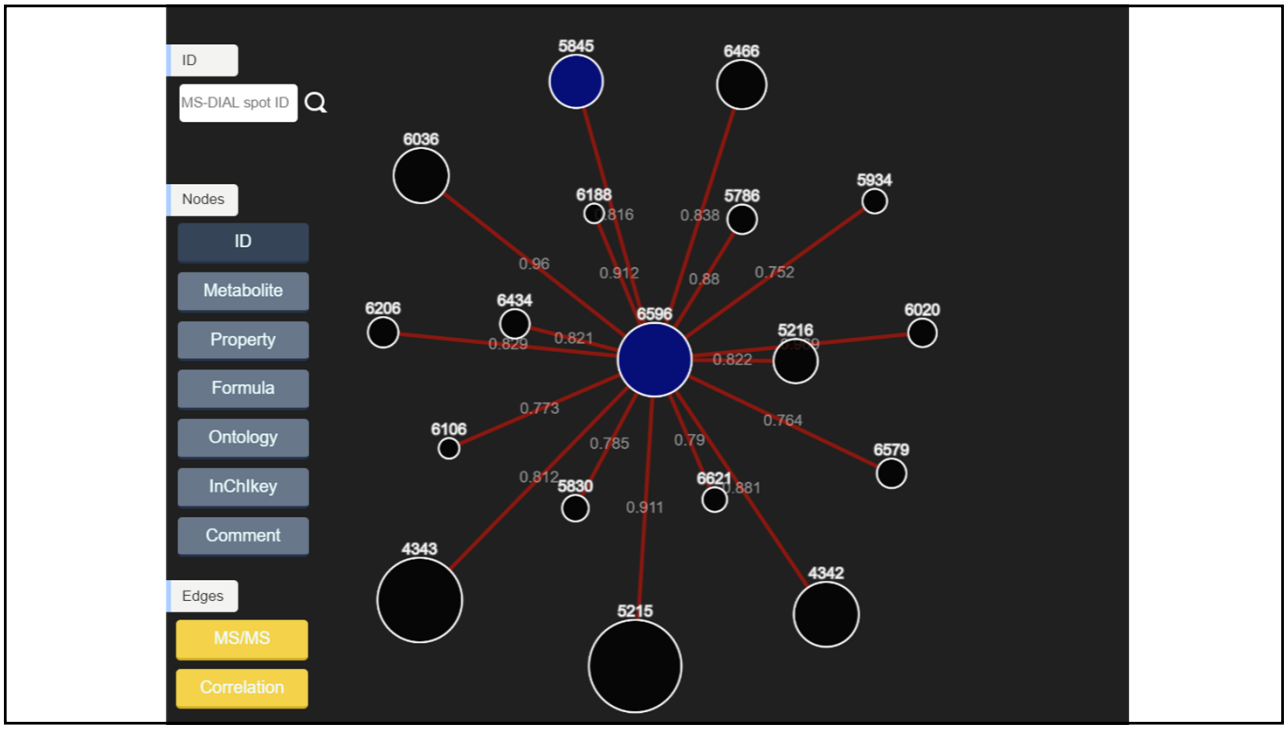
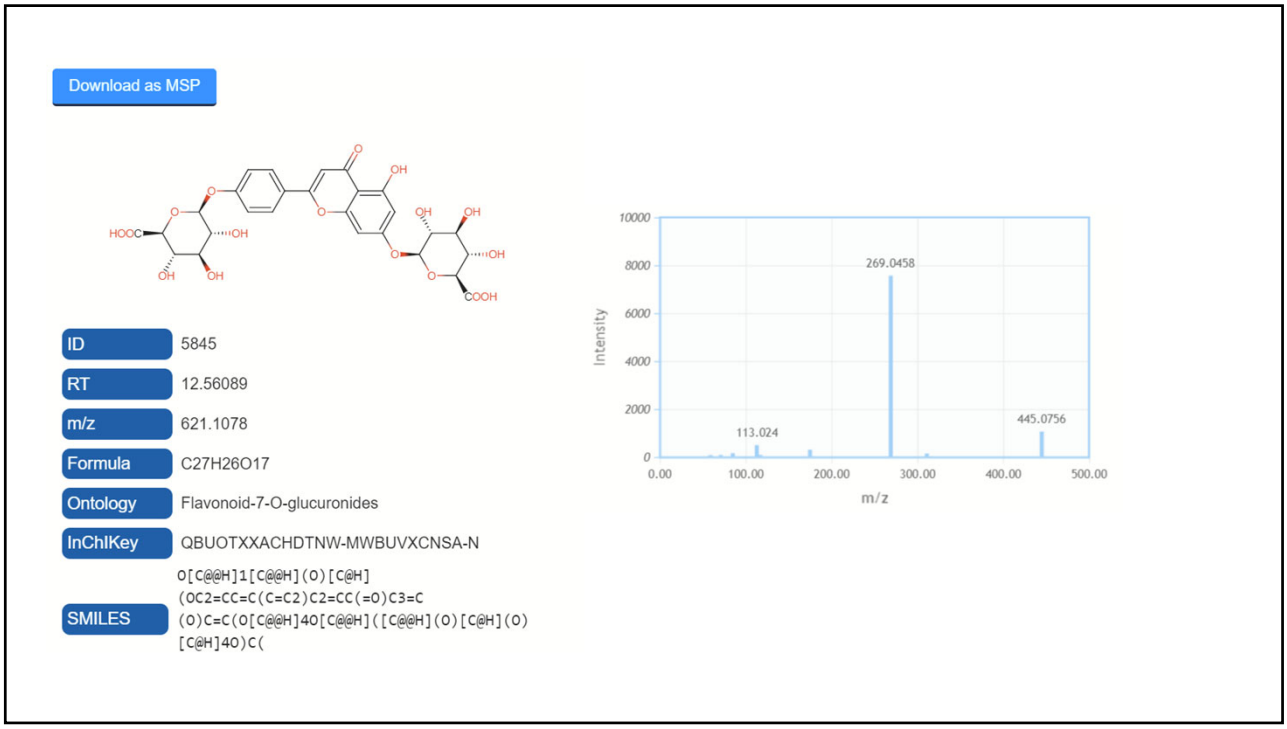

ID
MS-DIAL spot ID
Nodes
ID
Metabolite
Property
Formula
Ontology
InChIkey
Comment
Edges
MS/MS
Correlation
5845
6466
6036
6188
5786
5934
6206
6434
6596
6020
5216
6106
6579
5830
6621
4343
4342
5215
0.816
0.838
0.96
0.912
0.88
0.752
0.829
0.821
0.822
0.773
0.764
0.785
0.79
0.812
0.881
0.911

27


Download as MSP
ID 5845
RT 12.56089
m/z 621.1078
Formula C27H26O17
Ontology Flavonoid-7-O-glucuronides
InChIKey QBUOTXXACHDTNW-MWBUVXCNSA-N
SMILES O[C@@H]1[C@@H](O)[C@H](OC2=CC=C(C=C2)C2=CC(=O)C3=C(O)C=C(O[C@@H]4O[C@@H]([C@@H](O)[C@H](O)[C@H]4O)C(
Intensity
m/z
269.0458
445.0756
113.024

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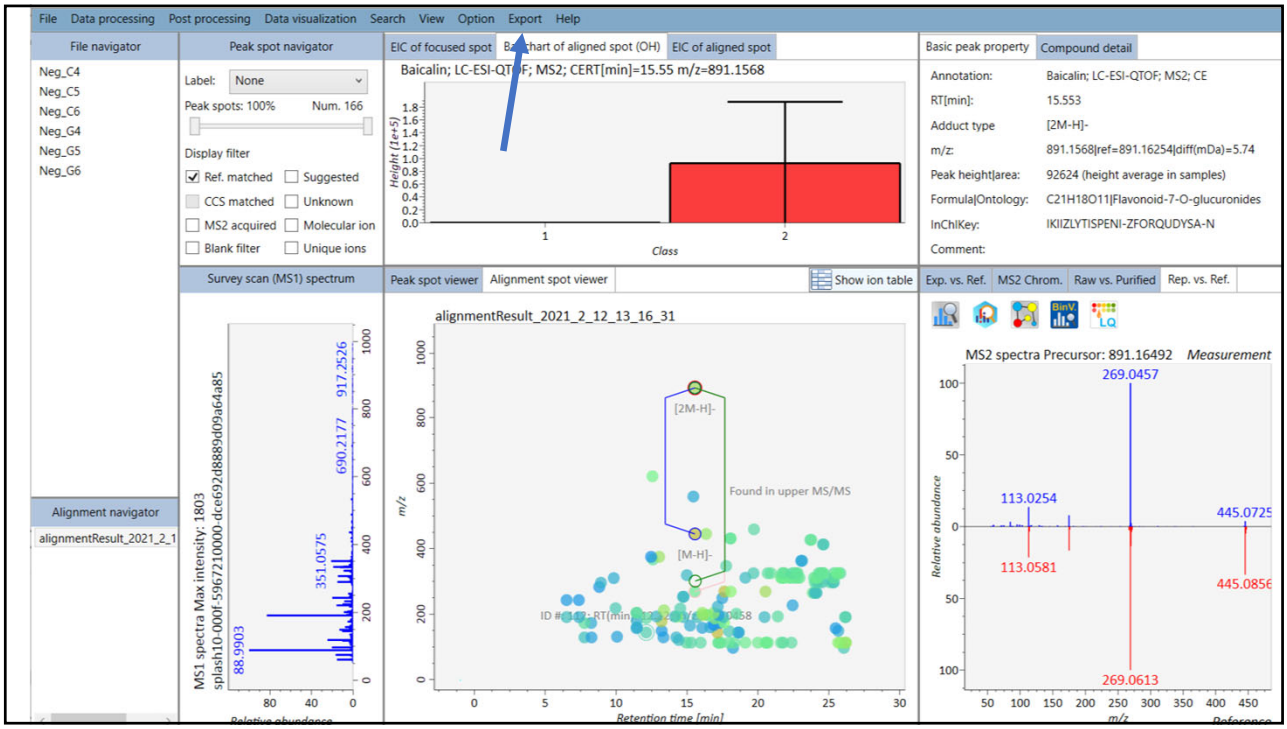
File Data processing Post processing Data visualization Search View Option Export Help
File navigator
Neg_C4
Neg_C5
Neg_C6
Neg_G4
Neg_G5
Neg_G6
Peak spot navigator
Label: None
Peak spots: 100% Num. 166
Display filter
Ref. matched Suggested
CCS matched Unknown
MS2 acquired Molecular ion
Blank filter Unique ions
EIC of focused spot
Baicalin; LC-ESI-QTOF; MS2; CERT[min]=15.55 m/z=891.1568
Height (1e+5)
Class
Basic peak property
Compound detail
Annotation: Baicalin; LC-ESI-QTOF; MS2; CE
RT[min]: 15.553
Adduct type [2M-H]-
m/z: 891.1568|ref=891.16254|diff(mDa)=5.74
Peak height|area: 92624 (height average in samples)
Formula|Ontology: C21H18O11|Flavonoid-7-O-glucuronides
InChIKey: IKIIZLYTISPENI-ZFORQUDYSA-N
Comment:
Survey scan (MS1) spectrum
MS1 spectra Max intensity: 1803
splash10-000f-596721000-dce692d8889d09a64a85
88.9903
351.0575
690.2177
917.2526
Peak spot viewer
Alignment spot viewer
Show ion table
alignmentResult_2021_2_12_13_16_31
[2M-H]-
[M-H]-
Found in upper MS/MS
m/z
Retention time [min]
Exp. vs. Ref.
MS2 Chrom.
Raw vs. Purified
Rep. vs. Ref.
MS2 spectra Precursor: 891.16492 Measurement
269.0457
113.0254
113.0581
445.0725
445.0856
269.0613
Relative abundance
Alignment navigator
alignmentResult_2021_2_1

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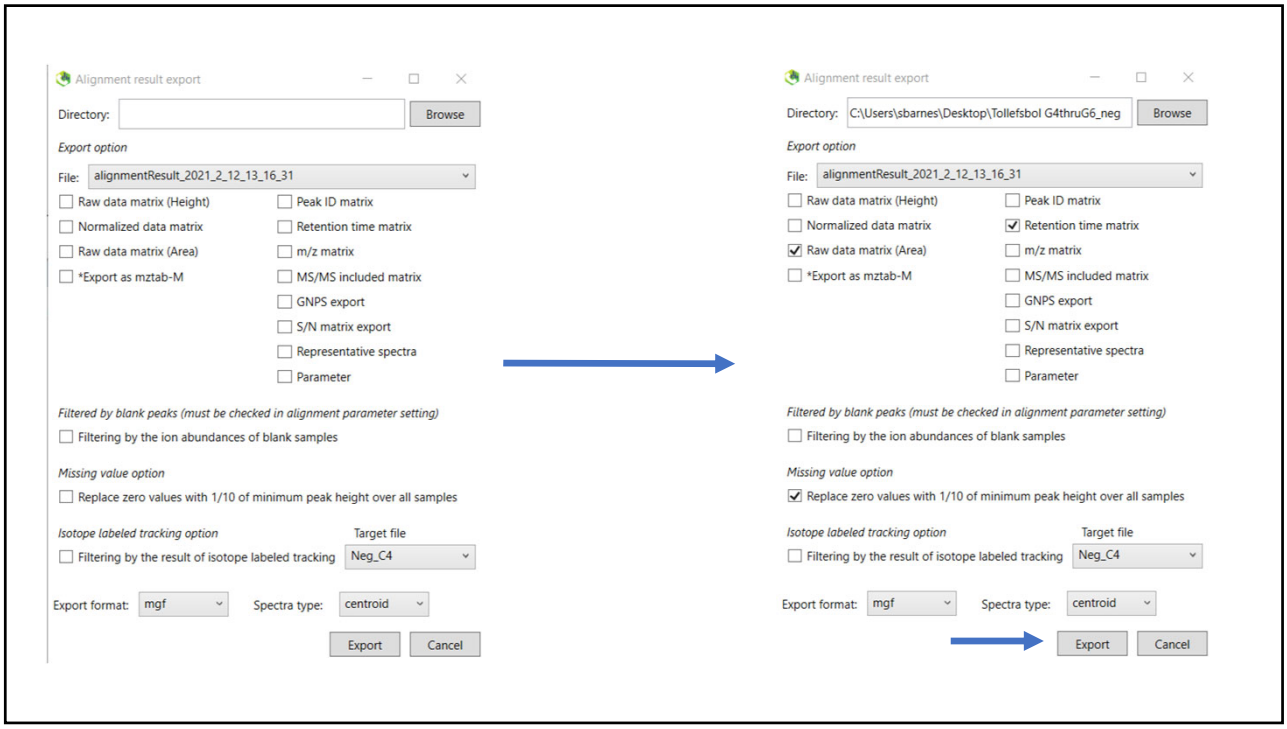
Alignment result export
Directory:
Browse
Export option
File: alignmentResult_2021_2_12_13_16_31
Raw data matrix (Height)
Normalized data matrix
Raw data matrix (Area)
*Export as mztab-M
Peak ID matrix
Retention time matrix
m/z matrix
MS/MS included matrix
GNPS export
S/N matrix export
Representative spectra
Parameter
Filtered by blank peaks (must be checked in alignment parameter setting)
Filtering by the ion abundances of blank samples
Missing value option
Replace zero values with 1/10 of minimum peak height over all samples
Isotope labeled tracking option
Filtering by the result of isotope labeled tracking
Target file
Neg_C4
Export format: mgf
Spectra type: centroid
Export
Cancel
Alignment result export
Directory: C:\Users\sbarnes\Desktop\Tollefsbol G4thruG6_neg
Browse
Export option
File: alignmentResult_2021_2_12_13_16_31
Raw data matrix (Height)
Normalized data matrix
Raw data matrix (Area)
*Export as mztab-M
Peak ID matrix
Retention time matrix
m/z matrix
MS/MS included matrix
GNPS export
S/N matrix export
Representative spectra
Parameter
Filtered by blank peaks (must be checked in alignment parameter setting)
Filtering by the ion abundances of blank samples
Missing value option
Replace zero values with 1/10 of minimum peak height over all samples
Isotope labeled tracking option
Filtering by the result of isotope labeled tracking
Target file
Neg_C4
Export format: mgf
Spectra type: centroid
Export
Cancel

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