



A stoned cat

Data analysis in metabolomics

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Goals of the data analysis classes

- **To understand the nature of metabolomics data**
- **To become familiar with three programs**
 - MS-DIAL (current version 4.80)
 - Mzmine (current version 2.53)
 - Metaboanalyst 5.0 (online at <http://www.Metaboanalyst.ca>)
- **At this time, MS-DIAL is best run on Windows (10) as a GUI**
 - There are Linux and Mac versions
 - Can be run on a Windows box at the command line

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A data set on urines from colleagues at Harvard

- This is part of a study of urines collected from COVID-19 patients, some of whom developed acute kidney injury
- The study has been published in Kidney International Reports (Raines et al.)
- There were 14 patients in each group
- Additional samples run were 3 pooled samples, 3 extraction blanks and one reagent (methanol) blank
- Full scan MS1 data were collected at 4 Hz over a 0-8 min gradient
- The pooled sample was re-run, collecting MS1 data for 100 ms, followed by 8 computer selected, 50 ms MSMS spectra

3

Locate and launch MS-DIAL

4

Start a new project

Region focus by ID: RT (min): m/z: Information box: Ctrl+D, metabolite name to 'Unknown'

5

Define the path to the files

Start up a project

Project file path:

Ionization type

Soft ionization (LC/MS, LC/MS/MS, or precursor-oriented GC/MS/MS) Hard ionization (GC/MS)

Separation type

Chromatography (GC, LC, CE, or SFC) Ion mobility (now coupled with liquid chromatography)

MS method type

Conventional LC/MS or data dependent MS/MS SWATH-MS or conventional All-ions method All-ions with multiple CEs (cycled like 0V-10V-40V)

Experiment file:

Data type (MS1)

Profile data Centroid data

Data type (MS/MS)

Profile data Centroid data

Ion mode

Positive ion mode Negative ion mode

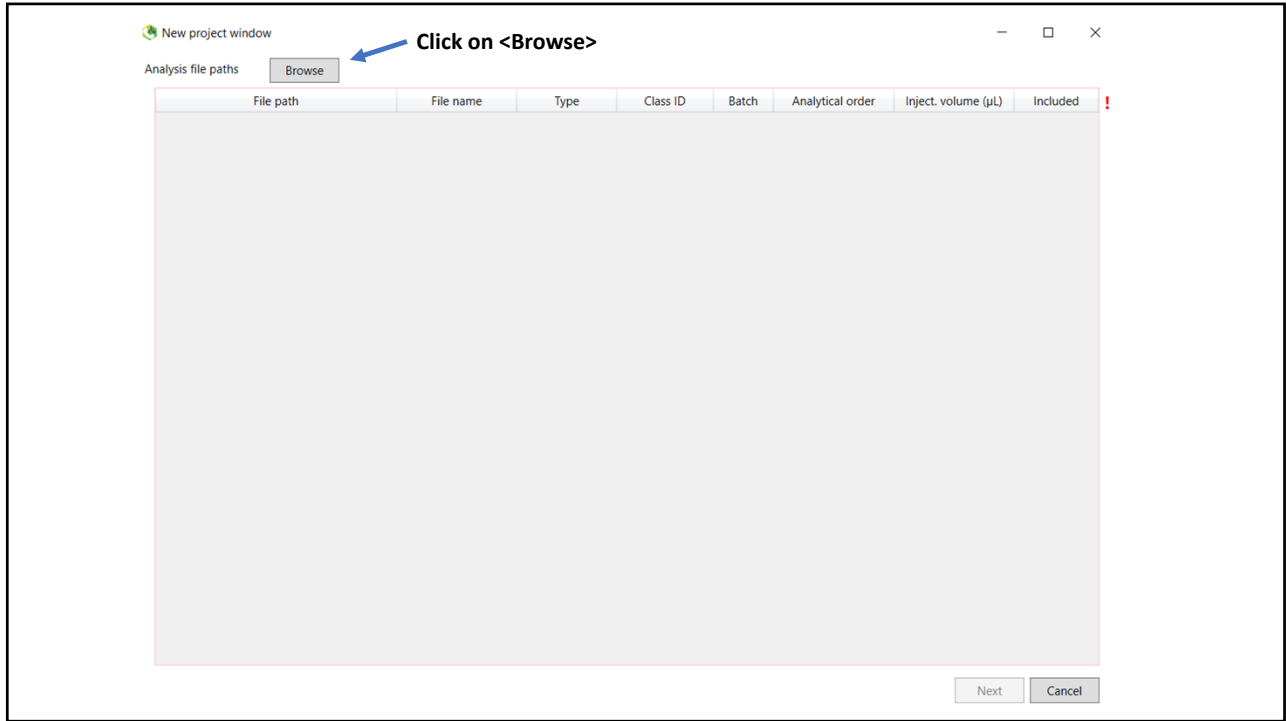
Target omics

Metabolomics Lipidomics

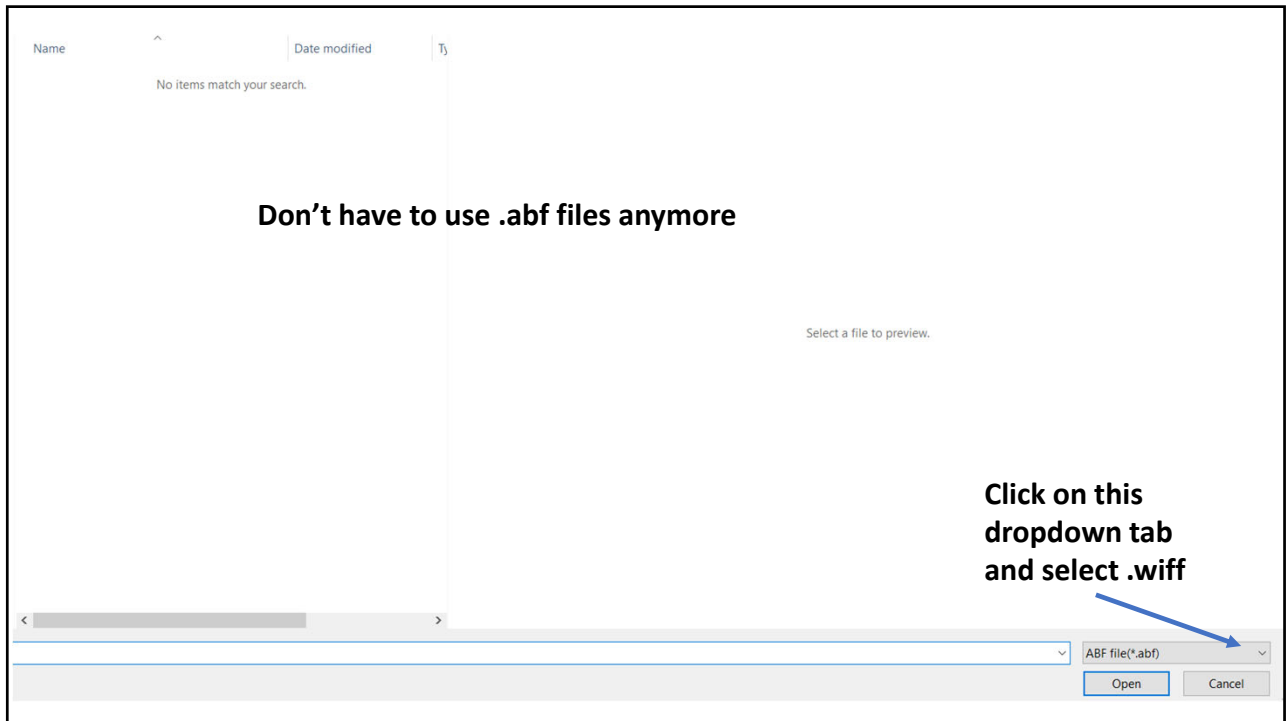
Advanced: add further meta data

This path should be changed to where the files are on your computer

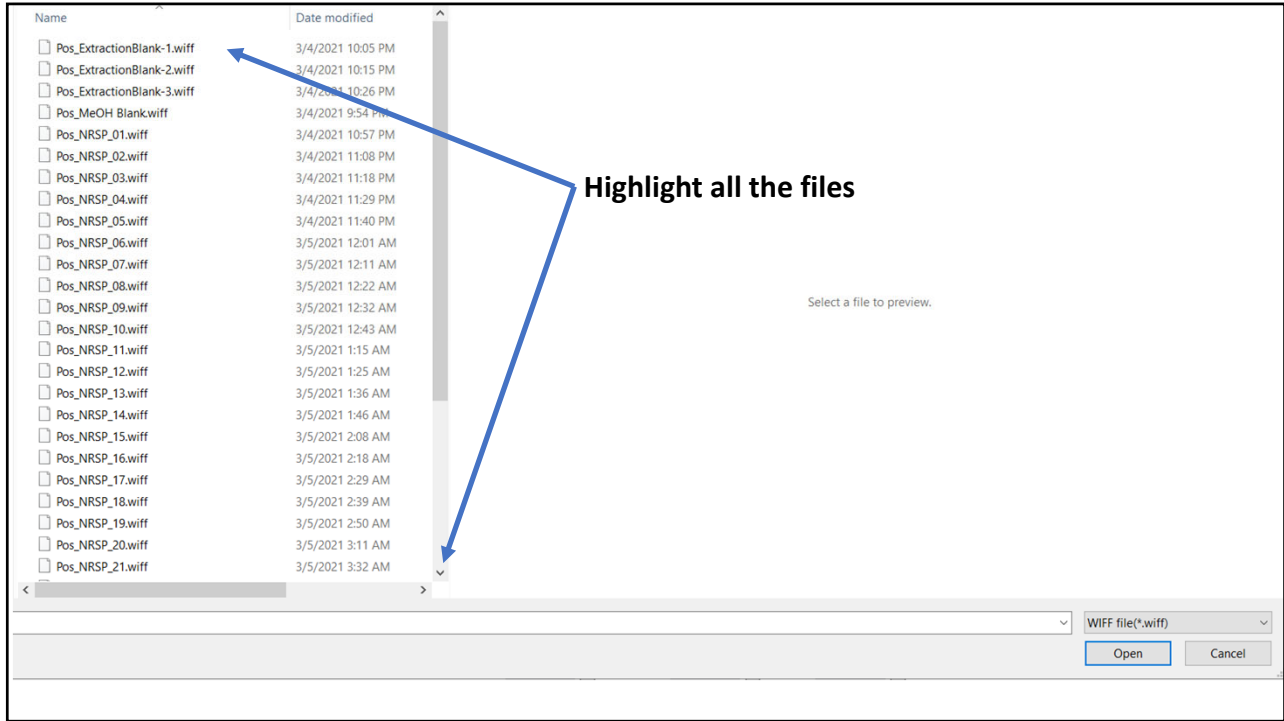
6



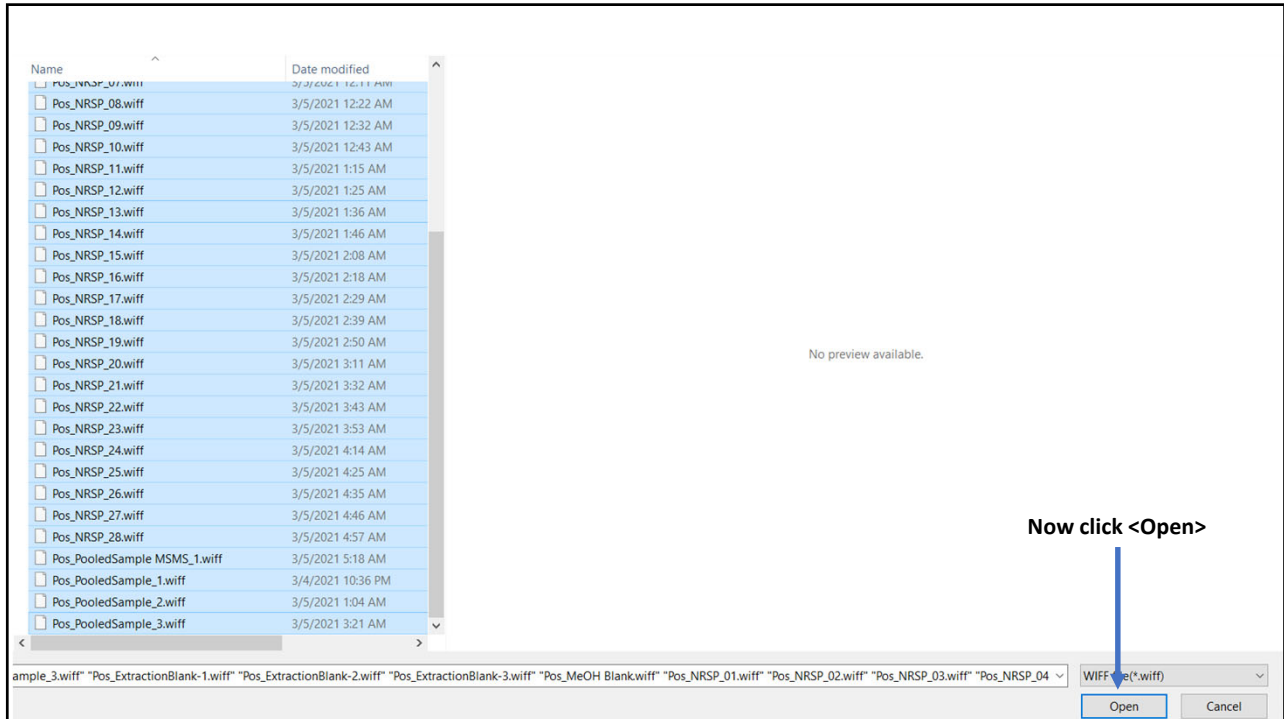
7



8



9



10

New project window

Analysis file paths

| File path | File name | Type | Class ID | Batch | Analytical order | Inject. volume (µL) | Included |
|--|-----------------------|--------|----------|-------|------------------|---------------------|-------------------------------------|
| E:\Harvard_class_pos\Pos_ExtractionBlank-1 | Pos_ExtractionBlank-1 | Sample | 1 | 1 | 1 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_ExtractionBlank-2 | Pos_ExtractionBlank-2 | Sample | 1 | 1 | 2 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_ExtractionBlank-3 | Pos_ExtractionBlank-3 | Sample | 1 | 1 | 3 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_MeOH Blank.wiff | Pos_MeOH Blank | Sample | 1 | 1 | 4 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_01.wiff | Pos_NRSP_01 | Sample | 1 | 1 | 5 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_02.wiff | Pos_NRSP_02 | Sample | 1 | 1 | 6 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_03.wiff | Pos_NRSP_03 | Sample | 1 | 1 | 7 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_04.wiff | Pos_NRSP_04 | Sample | 1 | 1 | 8 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_05.wiff | Pos_NRSP_05 | Sample | 1 | 1 | 9 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_06.wiff | Pos_NRSP_06 | Sample | 1 | 1 | 10 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_07.wiff | Pos_NRSP_07 | Sample | 1 | 1 | 11 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_08.wiff | Pos_NRSP_08 | Sample | 1 | 1 | 12 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_09.wiff | Pos_NRSP_09 | Sample | 1 | 1 | 13 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_10.wiff | Pos_NRSP_10 | Sample | 1 | 1 | 14 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_11.wiff | Pos_NRSP_11 | Sample | 1 | 1 | 15 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_12.wiff | Pos_NRSP_12 | Sample | 1 | 1 | 16 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_13.wiff | Pos_NRSP_13 | Sample | 1 | 1 | 17 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_14.wiff | Pos_NRSP_14 | Sample | 1 | 1 | 18 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_15.wiff | Pos_NRSP_15 | Sample | 1 | 1 | 19 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_16.wiff | Pos_NRSP_16 | Sample | 1 | 1 | 20 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_17.wiff | Pos_NRSP_17 | Sample | 1 | 1 | 21 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_18.wiff | Pos_NRSP_18 | Sample | 1 | 1 | 22 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_19.wiff | Pos_NRSP_19 | Sample | 1 | 1 | 23 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_20.wiff | Pos_NRSP_20 | Sample | 1 | 1 | 24 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_21.wiff | Pos_NRSP_21 | Sample | 1 | 1 | 25 | 1 | <input checked="" type="checkbox"/> |
| E:\Harvard_class_pos\Pos_NRSP_22.wiff | Pos_NRSP_22 | Sample | 1 | 1 | 26 | 1 | <input checked="" type="checkbox"/> |

Next

One can assign as class ID for each sample and use the stats packages in MS-DIAL. However, we'll defer this until later.

Instead click on <Next>

11

Analysis parameter setting

Data collection

Mass accuracy (centroid parameter)

MS1 tolerance: Da

MS2 tolerance: Da

Advanced

Set <>MS tolerance> to 0.015

Do not click on <Finish>

Together with Alignment

12

Analysis parameter setting

Data collection | Peak detection | MS2Dec | Identification | Adduct | Alignment | Mobility | Isotope tracking

Mass accuracy (centroid parameter)

MS1 tolerance: 0.01 Da

MS2 tolerance: 0.015 Da

Advanced

Data collection parameters

Retention time begin: 0 min

Retention time end: 100 min

MS1 mass range begin: 0 Da

MS1 mass range end: 2000 Da

MS/MS mass range begin: 0 Da

MS/MS mass range end: 2000 Da

Isotope recognition

Maximum charged number: 2

Consider Cl and Br elements:

Multithreading

Number of threads: 4

Execute retention time corrections:

Set MS1 begin to 50
Set MS1 end to 1000

Set <Number of threads> to 4

Do not click on <Finish>

Load Together with Alignment Finish Cancel

13

Analysis parameter setting

Data collection | Peak detection | MS2Dec | Identification | Adduct | Alignment | Mobility | Isotope tracking

Peak detection parameters

Minimum peak height: 1000 amplitude

Mass slice width: 0.1 Da

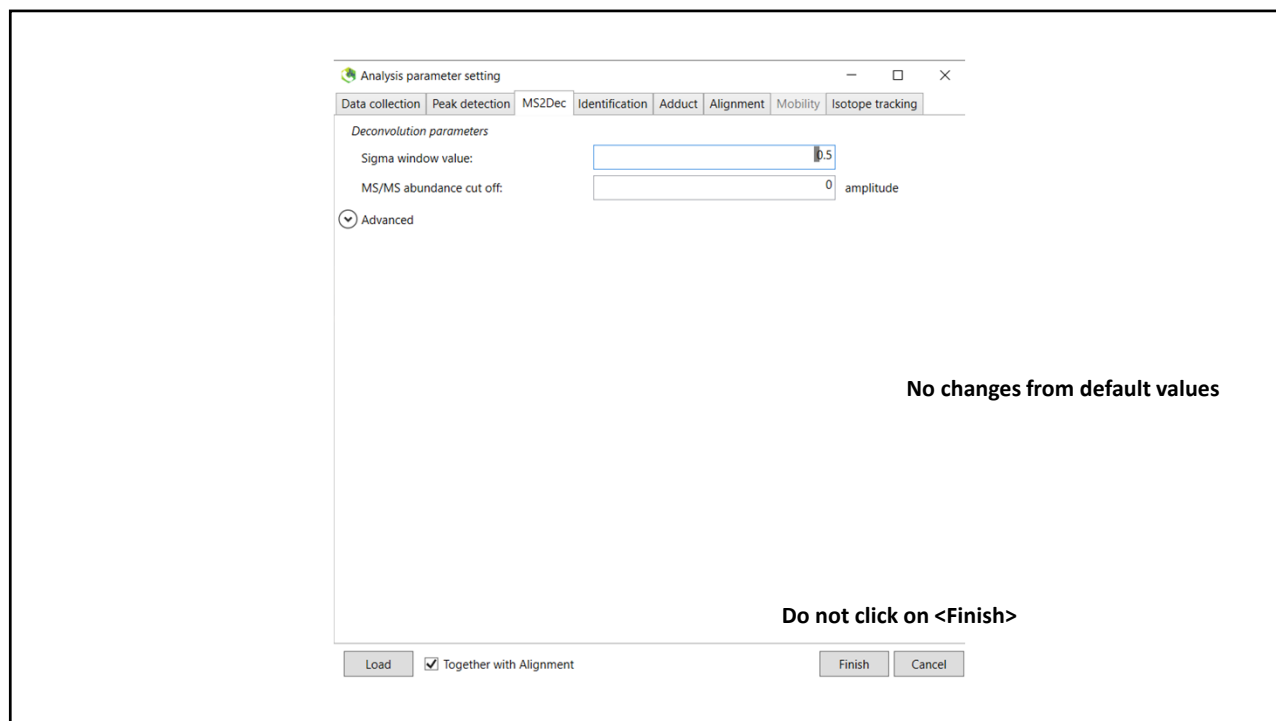
Advanced

No changes from default values

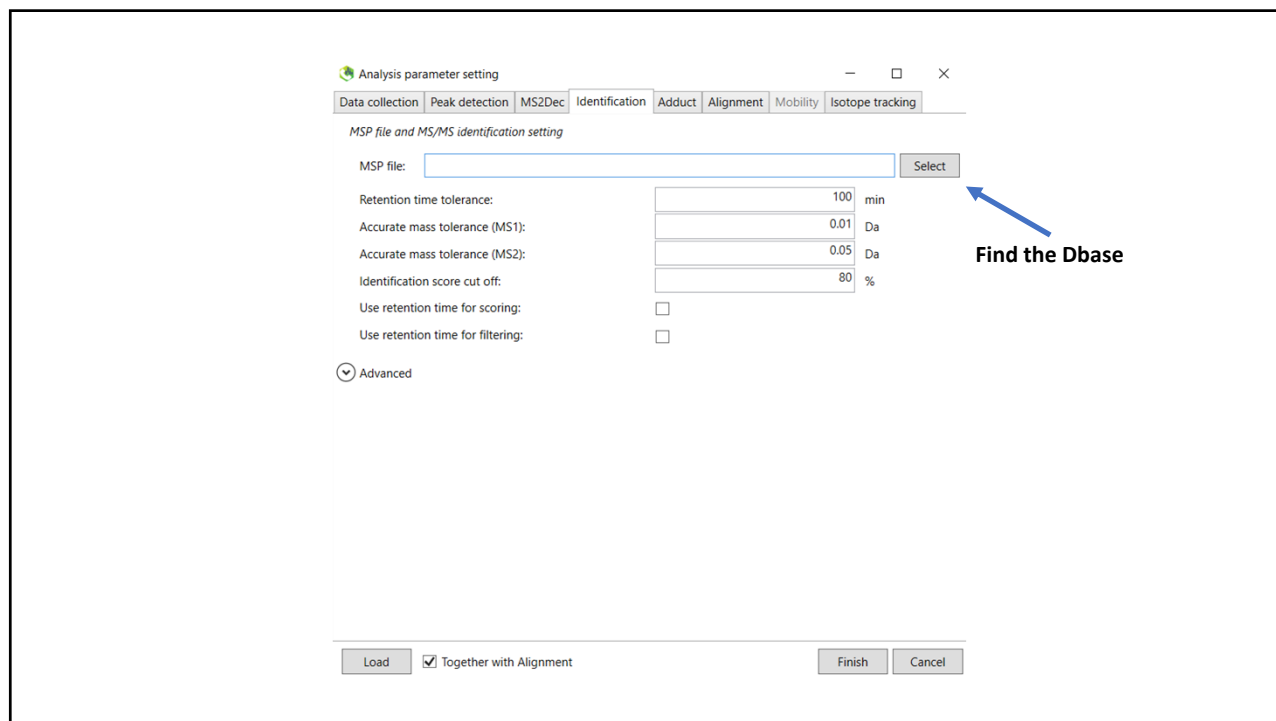
Do not click on <Finish>

Load Together with Alignment Finish Cancel

14



15



16

Analysis parameter setting


Data collection | Peak detection | MS2Dec | Identification | Adduct | Alignment | Mobility | Isotope tracking

MSP file and MS/MS identification setting

MSP file: C:\Users\sbarne\Desktop\Database\MSMS-Public-Pos-VS15.msp

Retention time tolerance: min

Accurate mass tolerance (MS1): Da

Accurate mass tolerance (MS2): Da 

Identification score cut off: %

Use retention time for scoring:

Use retention time for filtering:

Advanced

Do not click on <Finish>

Together with Alignment

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Analysis parameter setting

Data collection | Peak detection | MS2Dec | Identification | Adduct | Alignment | Mobility | Isotope tracking

Adduct ion setting

| Molecular species | Charge | Accurate mass [Da] | Included |
|-------------------------------|--------|--------------------|-------------------------------------|
| [M+H] ⁺ | 1 | 1.007276 | <input checked="" type="checkbox"/> |
| [M+NH4] ⁺ | 1 | 18.033823 | <input type="checkbox"/> |
| [M+Na] ⁺ | 1 | 22.989218 | <input checked="" type="checkbox"/> |
| [M+CH3OH+H] ⁺ | 1 | 33.033489 | <input type="checkbox"/> |
| [M+K] ⁺ | 1 | 38.963158 | <input type="checkbox"/> |
| [M+Li] ⁺ | 1 | 7.01600455 | <input type="checkbox"/> |
| [M+ACN+H] ⁺ | 1 | 42.033823 | <input type="checkbox"/> |
| [M+H-2H2O] ⁺ | 1 | -17.002191 | <input checked="" type="checkbox"/> |
| [M+H-2H2O] ⁺ | 1 | -30.012756 | <input checked="" type="checkbox"/> |
| [M+2Na-H] ⁺ | 1 | 44.97116 | <input checked="" type="checkbox"/> |
| [M+IsoProp+H] ⁺ | 1 | 61.06534 | <input type="checkbox"/> |
| [M+ACN+Na] ⁺ | 1 | 64.015765 | <input type="checkbox"/> |
| [M+2K-H] ⁺ | 1 | 76.91904 | <input type="checkbox"/> |
| [M+DMSO+H] ⁺ | 1 | 79.02122 | <input type="checkbox"/> |
| [M+2ACN+H] ⁺ | 1 | 83.06037 | <input type="checkbox"/> |
| [M+IsoProp+Na+H] ⁺ | 1 | 84.05511 | <input type="checkbox"/> |
| [M-C6H10O4+H] ⁺ | 1 | -145.050085 | <input checked="" type="checkbox"/> |
| [M-C6H10O5+H] ⁺ | 1 | -161.045 | <input checked="" type="checkbox"/> |
| [M-C6H8O6+H] ⁺ | 1 | -175.024265 | <input checked="" type="checkbox"/> |
| [2M+H] ⁺ | 1 | 1.007276 | <input checked="" type="checkbox"/> |
| [2M+NH4] ⁺ | 1 | 18.033823 | <input type="checkbox"/> |
| [2M+Na] ⁺ | 1 | 22.989218 | <input checked="" type="checkbox"/> |
| [2M+3H2O+2H] ⁺ | 1 | 28.02312 | <input type="checkbox"/> |
| [2M+K] ⁺ | 1 | 38.963158 | <input type="checkbox"/> |
| [2M+ACN+H] ⁺ | 1 | 42.033823 | <input type="checkbox"/> |

Select the likely adducts besides [M+H]⁺

Na⁺ adduct

Loss of water

2 Na⁺ adduct

Polar metabolites found in urine

Dimer

Do not click on <Finish>

Together with Alignment

18

Analysis parameter setting

Data collection | Peak detection | MS2Dec | Identification | Adduct | Alignment | Mobility | Isotope tracking

Adduct ion setting

| Molecular species | Charge | Accurate mass [Da] | Included |
|-------------------------------|--------|--------------------|-------------------------------------|
| [M+2ACN+H] ⁺ | 1 | 83.06037 | <input type="checkbox"/> |
| [M+IsoProp+Na+H] ⁺ | 1 | 84.05511 | <input type="checkbox"/> |
| [M-C6H10O4+H] ⁺ | 1 | -145.050085 | <input checked="" type="checkbox"/> |
| [M-C6H10O5+H] ⁺ | 1 | -161.045 | <input checked="" type="checkbox"/> |
| [M-C6H8O6+H] ⁺ | 1 | -175.024265 | <input checked="" type="checkbox"/> |
| [2M+H] ⁺ | 1 | 1.007276 | <input checked="" type="checkbox"/> |
| [2M+NH4] ⁺ | 1 | 18.033823 | <input type="checkbox"/> |
| [2M+Na] ⁺ | 1 | 22.989218 | <input checked="" type="checkbox"/> |
| [2M+3H2O+2H] ⁺ | 1 | 28.02312 | <input type="checkbox"/> |
| [2M+K] ⁺ | 1 | 38.963158 | <input type="checkbox"/> |
| [2M+ACN+H] ⁺ | 1 | 42.033823 | <input type="checkbox"/> |
| [2M+ACN+Na] ⁺ | 1 | 64.015765 | <input type="checkbox"/> |
| [M+2H] ²⁺ | 2 | 1.007276 | <input checked="" type="checkbox"/> |
| [M+H+NH4] ²⁺ | 2 | 9.52055 | <input type="checkbox"/> |
| [M+H+Na] ²⁺ | 2 | 11.998247 | <input type="checkbox"/> |
| [M+H+K] ²⁺ | 2 | 19.985217 | <input type="checkbox"/> |
| [M+ACN+2H] ²⁺ | 2 | 21.52055 | <input type="checkbox"/> |
| [M+2Na] ²⁺ | 2 | 22.989218 | <input checked="" type="checkbox"/> |
| [M+2ACN+2H] ²⁺ | 2 | 42.033823 | <input type="checkbox"/> |
| [M+3ACN+2H] ²⁺ | 2 | 62.547097 | <input type="checkbox"/> |
| [M+3H] ³⁺ | 3 | 1.007276 | <input checked="" type="checkbox"/> |
| [M+2H+Na] ³⁺ | 3 | 8.33459 | <input checked="" type="checkbox"/> |
| [M+H+2Na] ³⁺ | 3 | 15.76619 | <input checked="" type="checkbox"/> |
| [M+3Na] ³⁺ | 3 | 22.989218 | <input checked="" type="checkbox"/> |

Load Together with Alignment Finish Cancel

Do not click on <Finish>

Higher charge states

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Analysis parameter setting

Data collection | Peak detection | MS2Dec | Identification | Adduct | Alignment | Mobility | Isotope tracking

Alignment parameters setting

Result name: alignmentResult_2021_10_1_14_15_26

Reference file: Pos_PooledSample_2

Retention time tolerance: 0.15 min

MS1 tolerance: 0.015 Da

Advanced

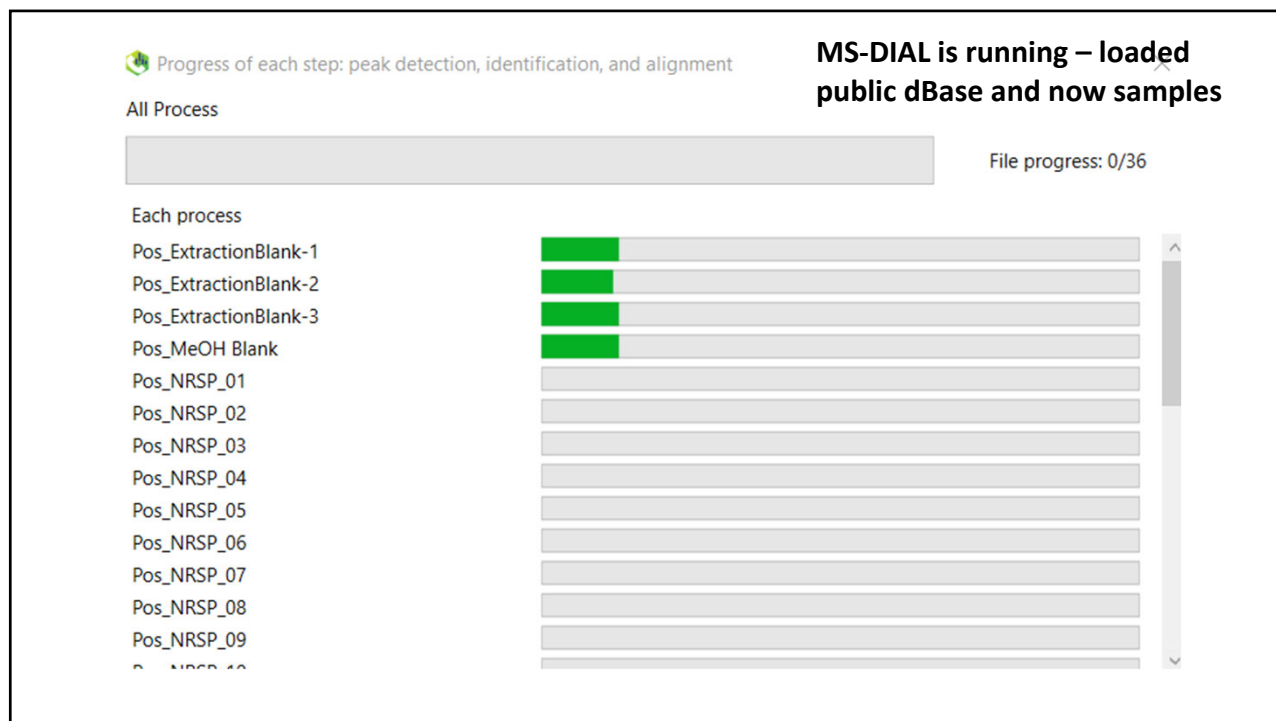
Load Together with Alignment Finish Cancel

Now you can click on <Finish>

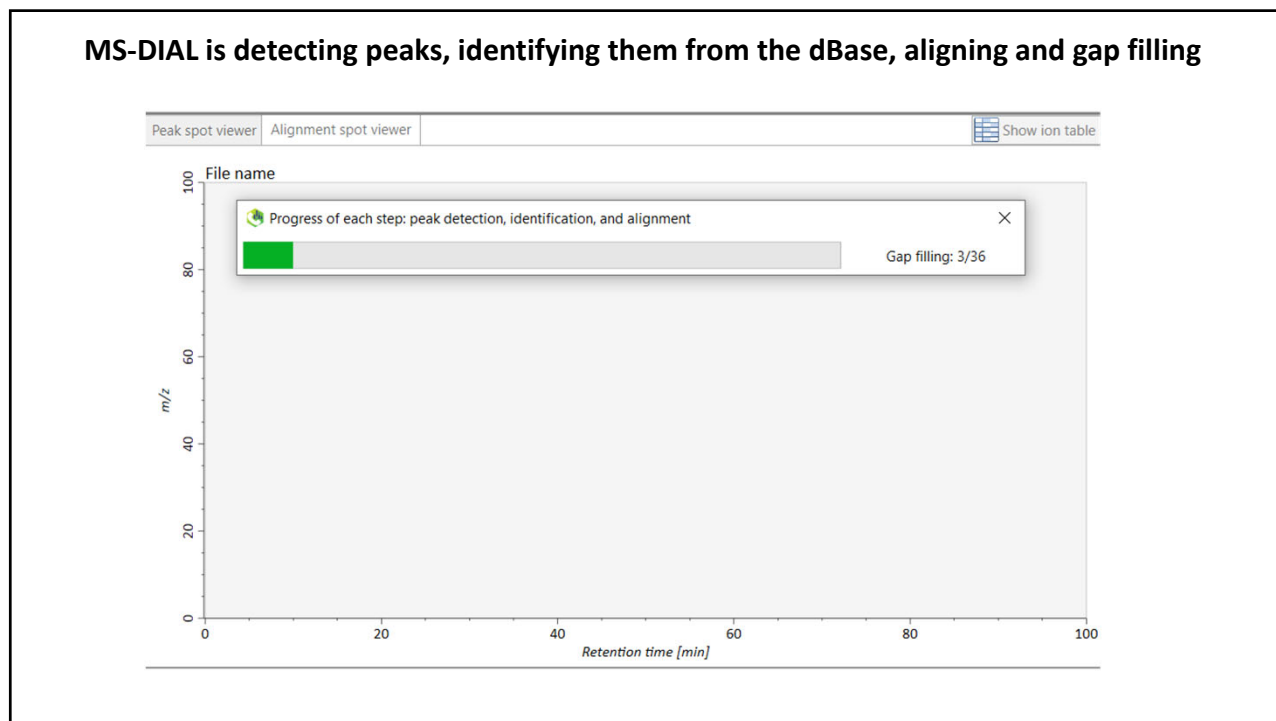
Set the reference sample to the middle sample or middle pooled sample

Set the retention time tolerance to 0.15 min

20



21



22

File Data processing Post processing Data visualization Search View Option Export Help

File navigator Peak spot navigator EIC of focused spot Bar chart of aligned spot (OH) EIC of aligned spot

Pos_ExtractionBlank-1
Pos_ExtractionBlank-2
Pos_ExtractionBlank-3
Pos_MeOH Blank
Pos_NRSP_01
Pos_NRSP_02
Pos_NRSP_03
Pos_NRSP_04
Pos_NRSP_05
Pos_NRSP_06
Pos_NRSP_07
Pos_NRSP_08
Pos_NRSP_09
Pos_NRSP_10
Pos_NRSP_11
Pos_NRSP_12
Pos_NRSP_13
Pos_NRSP_14
Pos_NRSP_15
Pos_NRSP_16
Pos_NRSP_17
Pos_NRSP_18

Label: None
Peak spots: 100% Num. 192

Display filter
 Ref. matched Suggested
 CCS matched Unknown
 MS2 acquired Molecular ion
 Blank filter Unique ions

Survey scan (MS1) spectrum
MS1 spectra Max intensity: 2899
splash!D:0100:790000000-b80c183284d9532471bf
60.3864 371.1018

Relative abundance

alignmentResult_2021_10_14_15_26
Retention time [min]

Region focus by ID: 0 RT(min): 4.168 m/z: 50.01407

Basic peak property Compound detail Structure

Annotation: Unknown
RT(min): 4.168
Adduct type [M+H]⁺
m/z: 50.01407
Peak height(are): 1068 (height average in samples)
Formula/Ontology: NAJNA
InChIKey: NA
Comment:

Exp. vs. Ref. MS2 Chrom. Raw vs. Purified Rep. vs. Ref.

Measurement vs. Reference Measurement
Relative abundance
Reference

Information box: Ctr+D, metabolite name to 'Unknown'

Double click this

23

Clicked Ref matched

File Data processing Post processing Data visualization Search View Option Export Help

File navigator Peak spot navigator EIC of focused spot Bar chart of aligned spot (OH) EIC of aligned spot

Pos_ExtractionBlank-1
Pos_ExtractionBlank-2
Pos_ExtractionBlank-3
Pos_MeOH Blank
Pos_NRSP_01
Pos_NRSP_02
Pos_NRSP_03
Pos_NRSP_04
Pos_NRSP_05
Pos_NRSP_06
Pos_NRSP_07
Pos_NRSP_08
Pos_NRSP_09
Pos_NRSP_10
Pos_NRSP_11
Pos_NRSP_12
Pos_NRSP_13
Pos_NRSP_14
Pos_NRSP_15
Pos_NRSP_16
Pos_NRSP_17
Pos_NRSP_18

Label: None
Peak spots: 100% Num. 129

Display filter
 Ref. matched Suggested
 CCS matched Unknown
 MS2 acquired Molecular ion
 Blank filter Unique ions

Survey scan (MS1) spectrum
MS1 spectra Max intensity: 2899
splash!D:0100:790000000-b80c183284d9532471bf
60.3864 371.1018

Relative abundance

alignmentResult_2021_10_14_15_26
Retention time [min]

Region focus by ID: 0 RT(min): 4.168 m/z: 50.01407

Basic peak property Compound detail Structure

Annotation: Unknown
RT(min): 4.168
Adduct type [M+H]⁺
m/z: 50.01407
Peak height(are): 1068 (height average in samples)
Formula/Ontology: NAJNA
InChIKey: NA
Comment:

Exp. vs. Ref. MS2 Chrom. Raw vs. Purified Rep. vs. Ref.

Measurement vs. Reference Measurement
Relative abundance
Reference

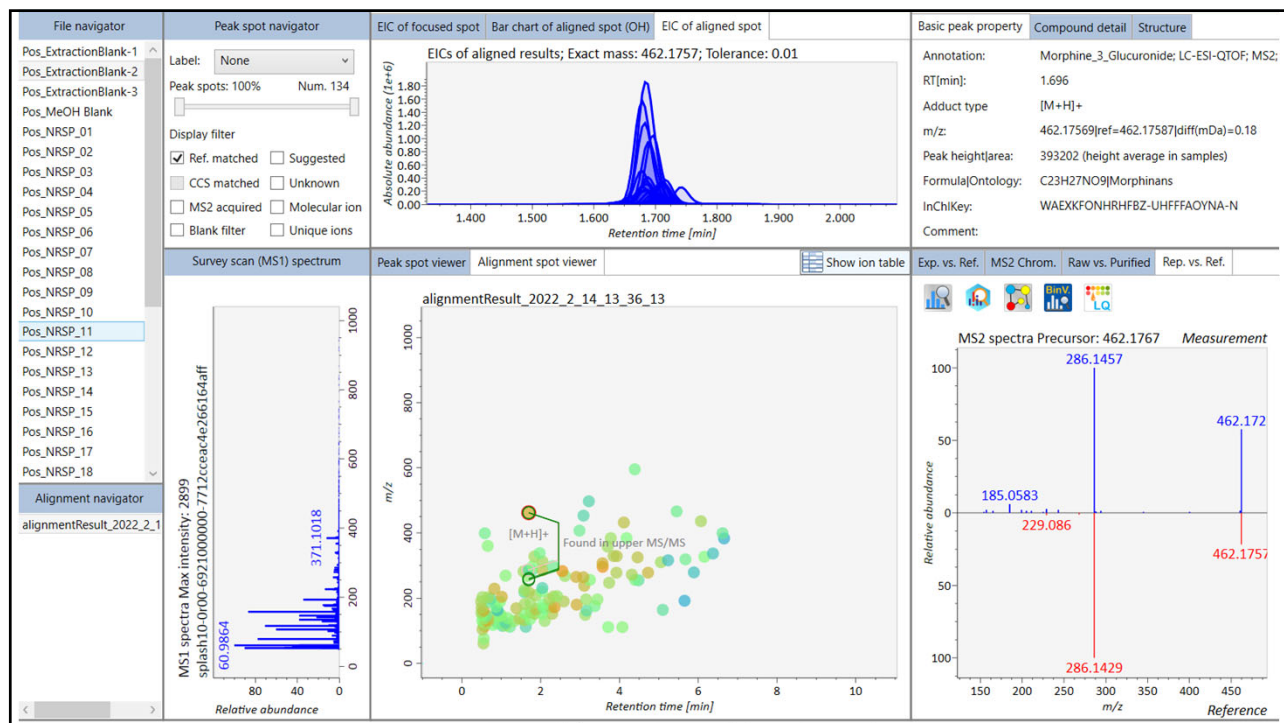
Information box: Ctr+D, metabolite name to 'Unknown'

24

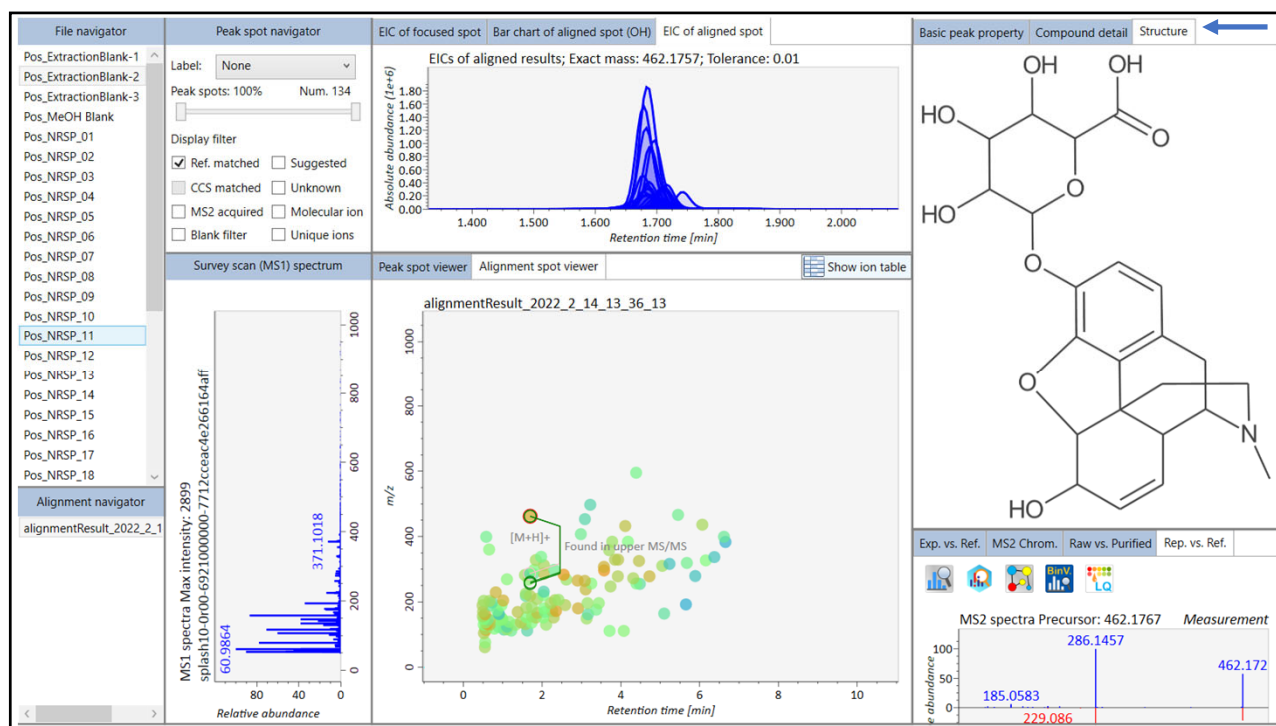
Click on individual spots to reveal identity and comparison with reference spectra



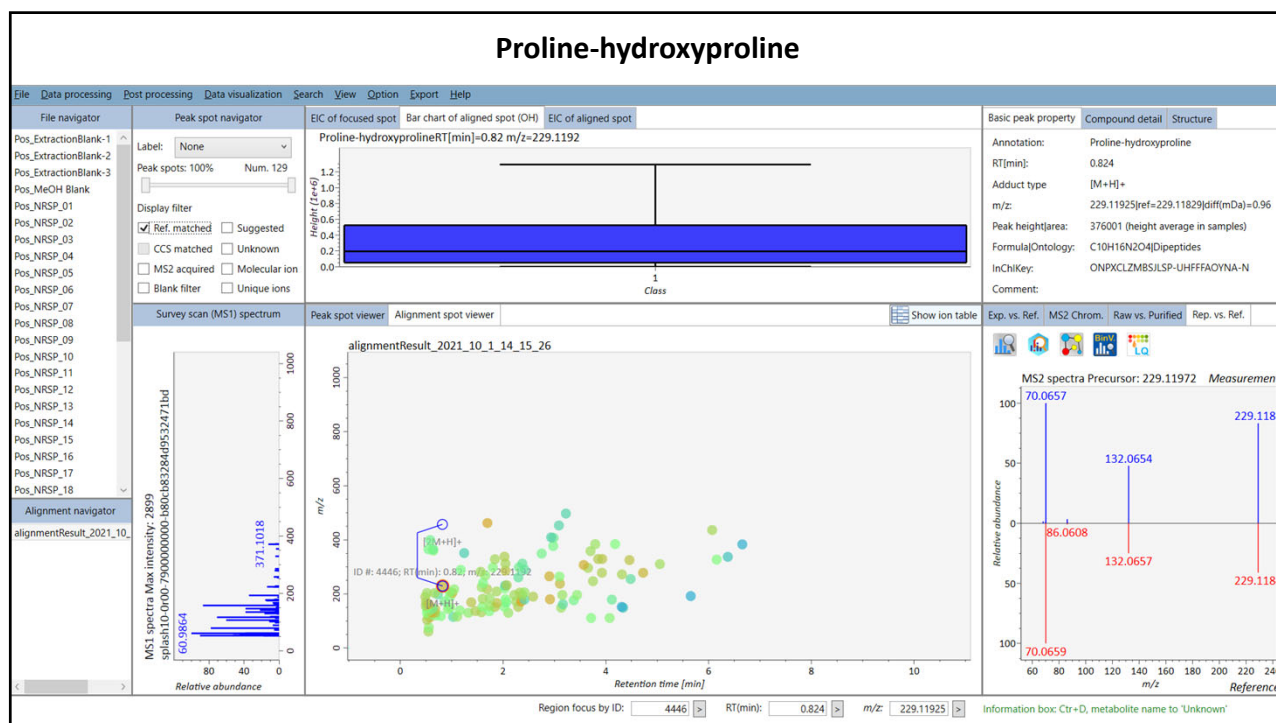
25



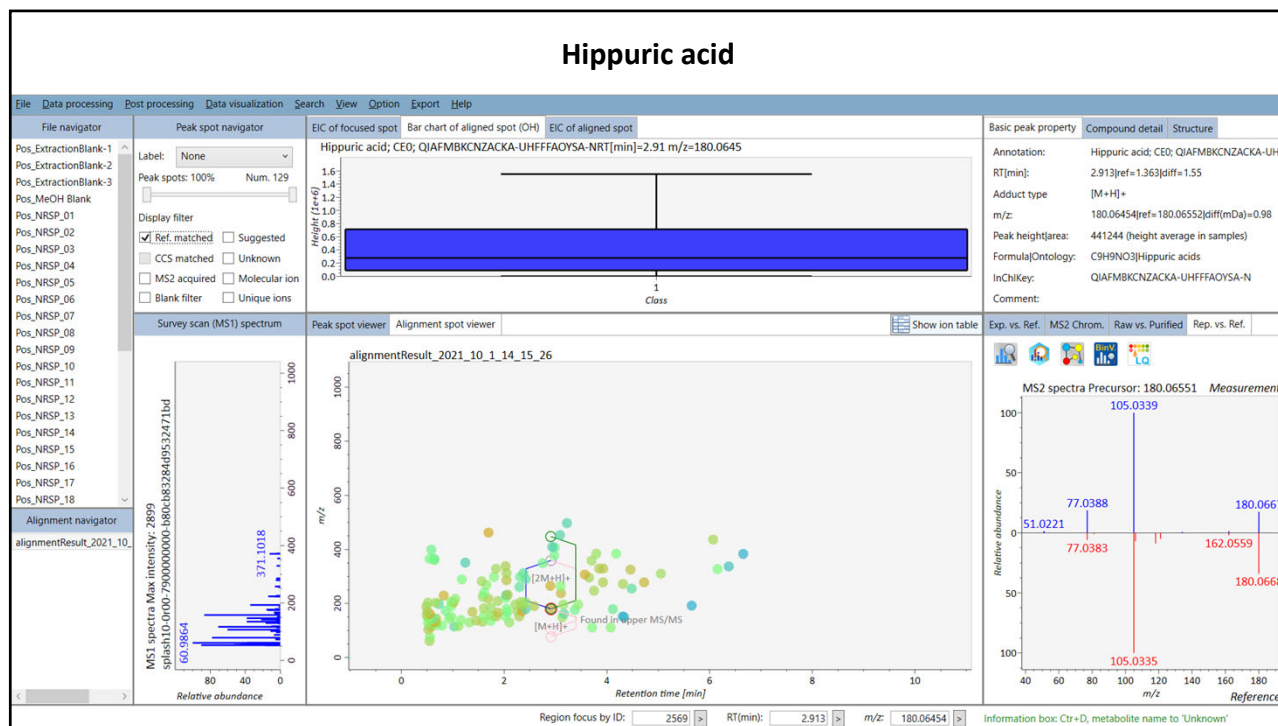
26



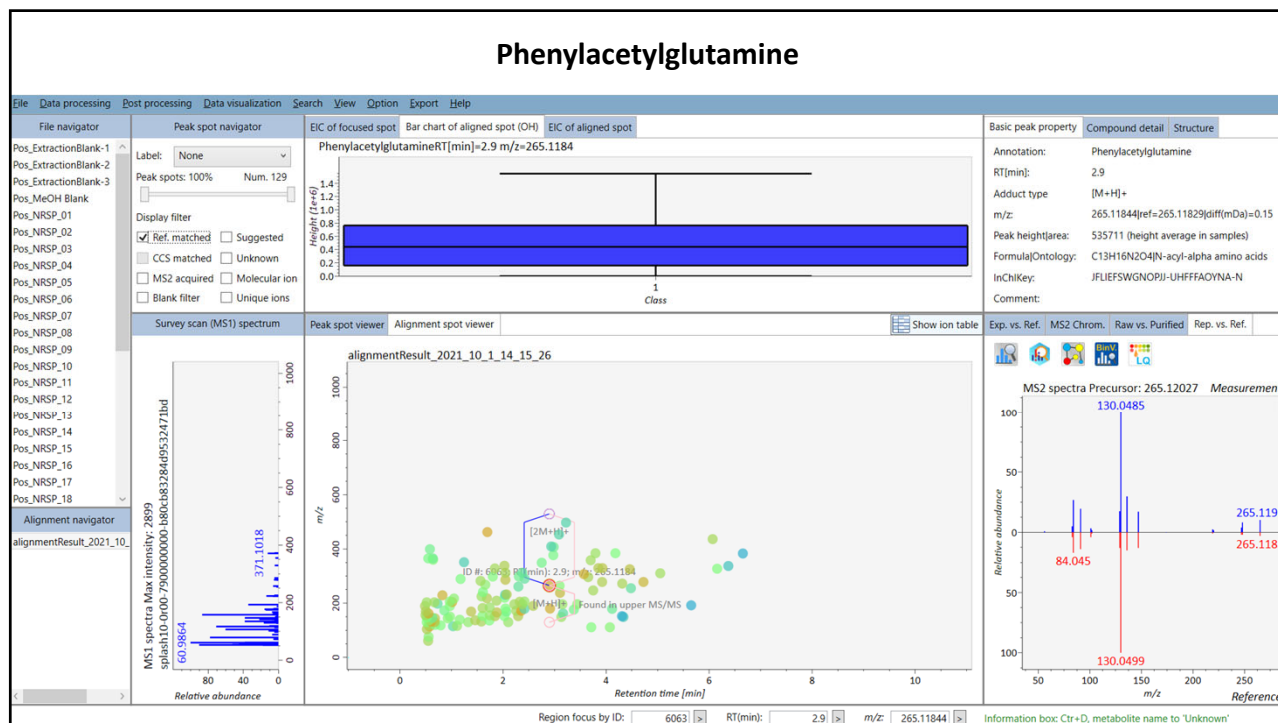
27



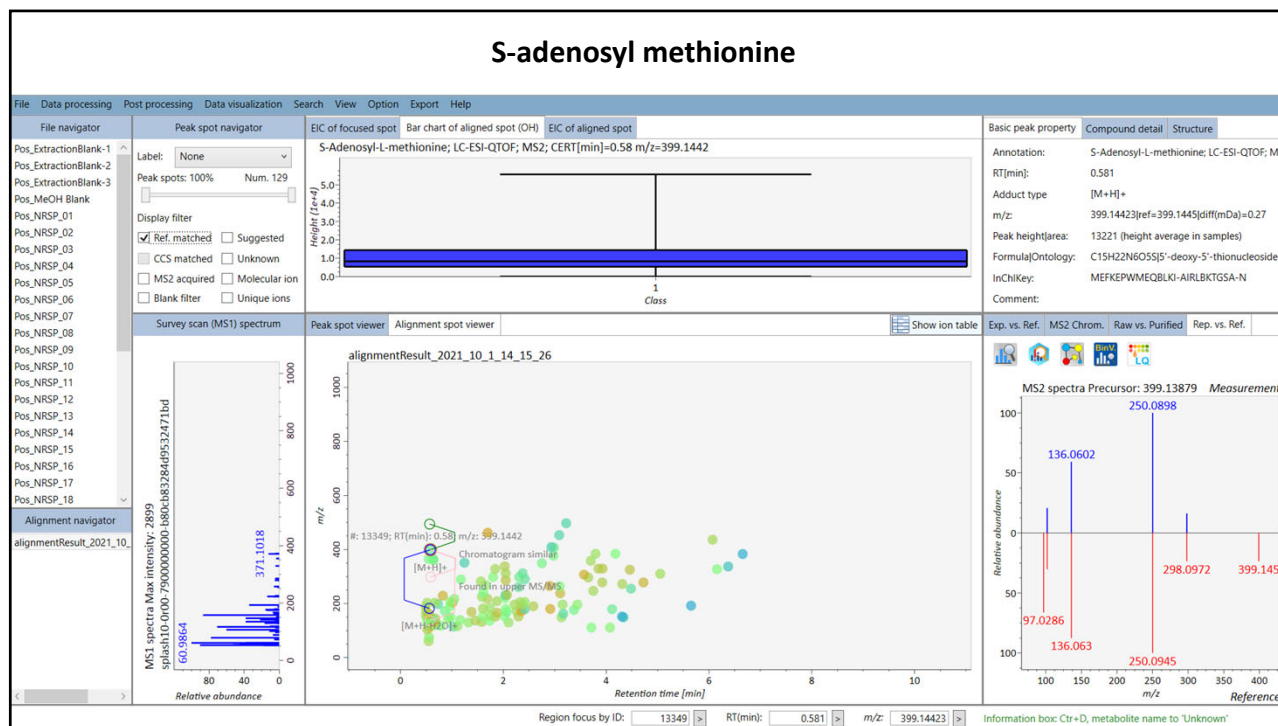
28



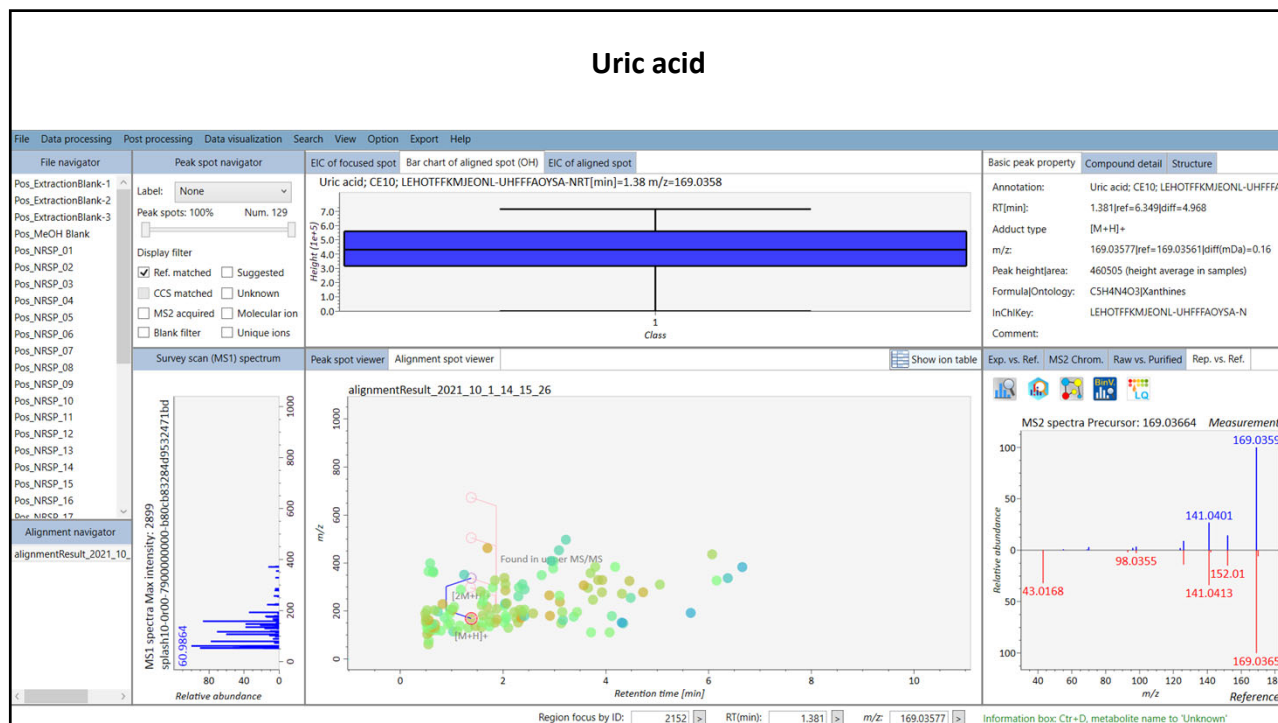
29



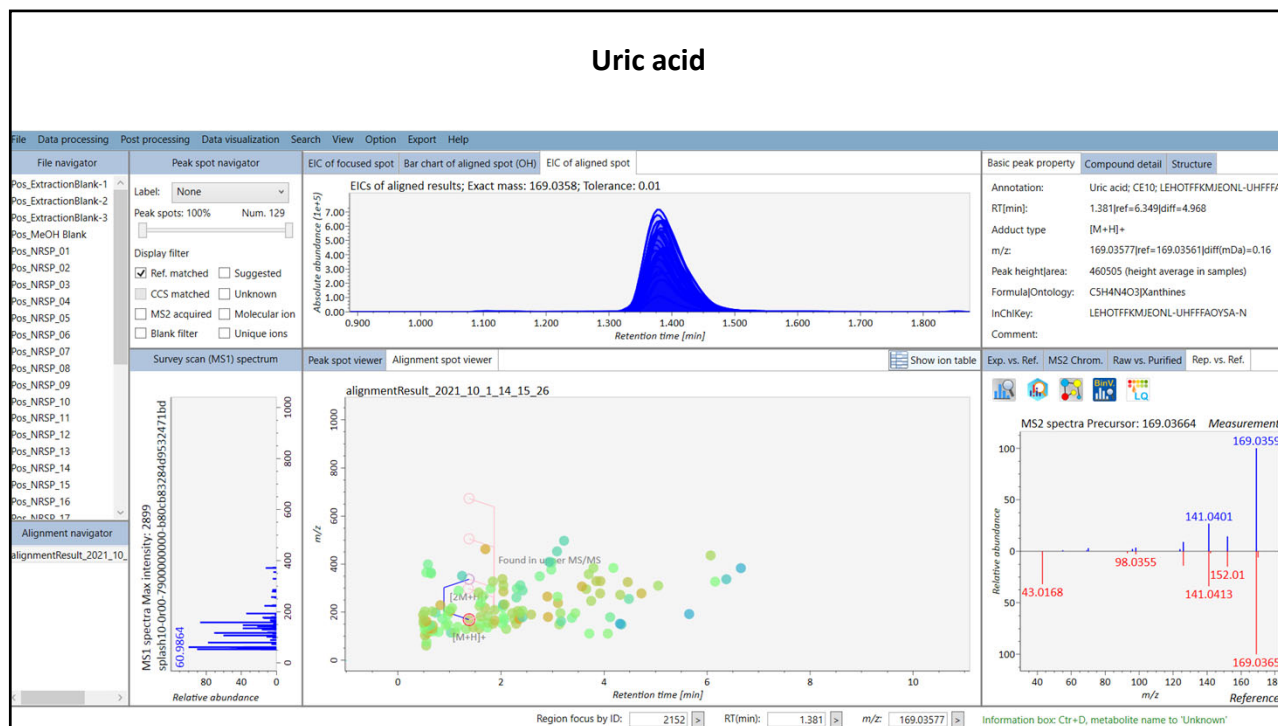
30



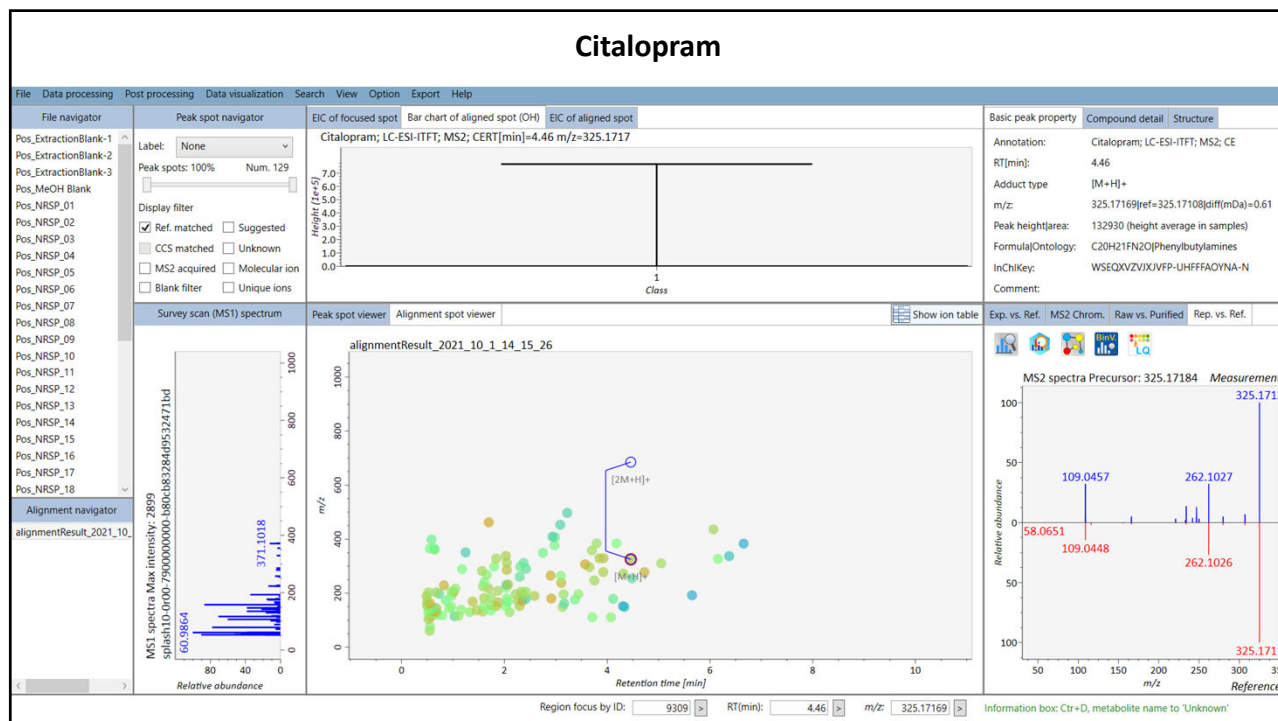
31



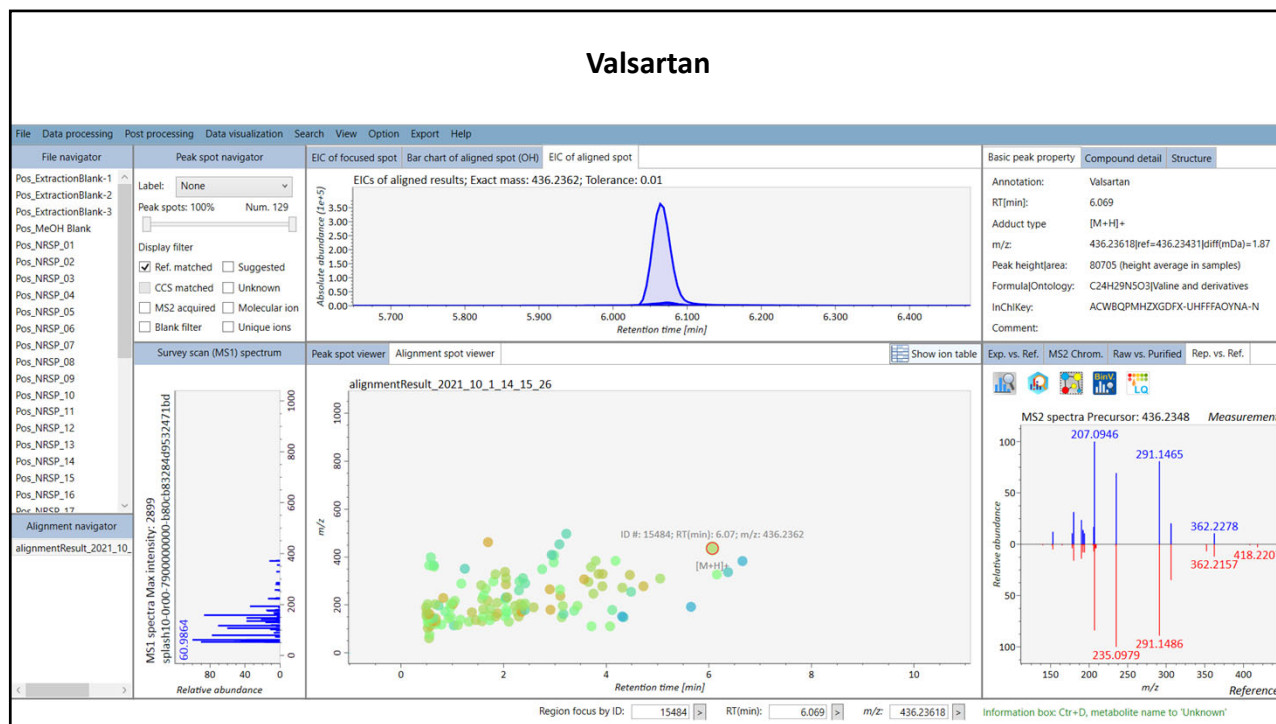
32



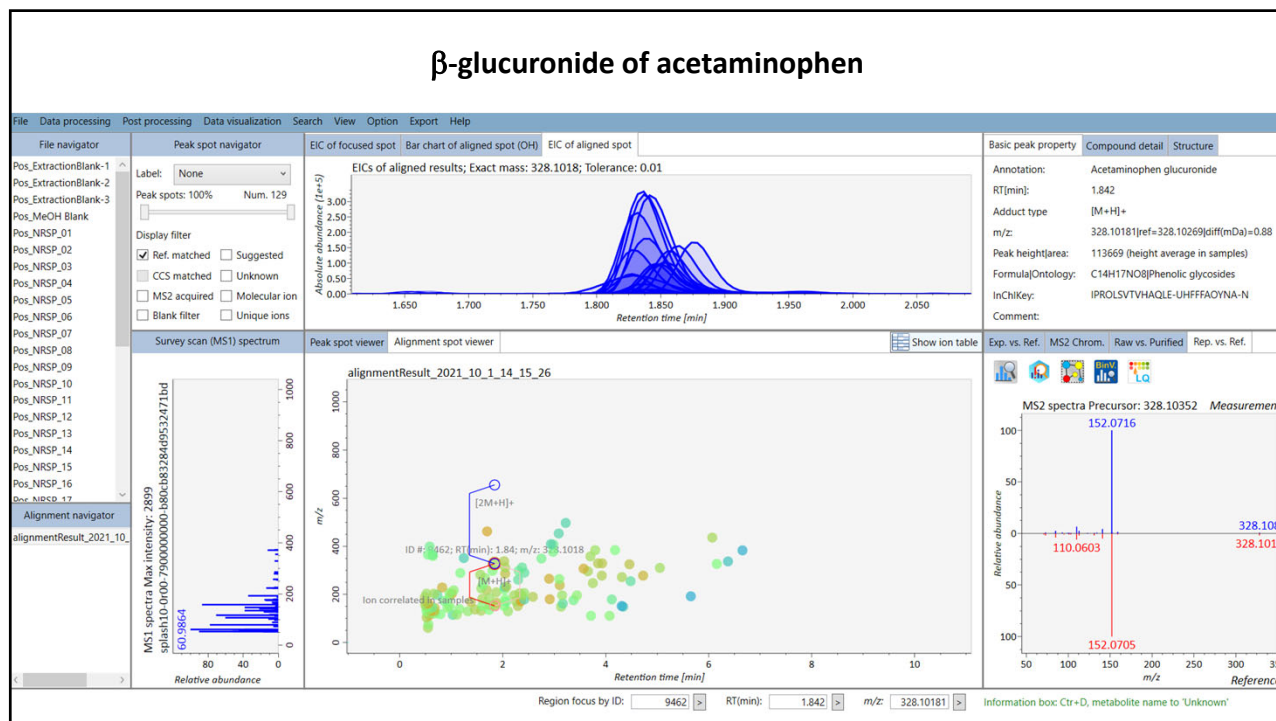
33



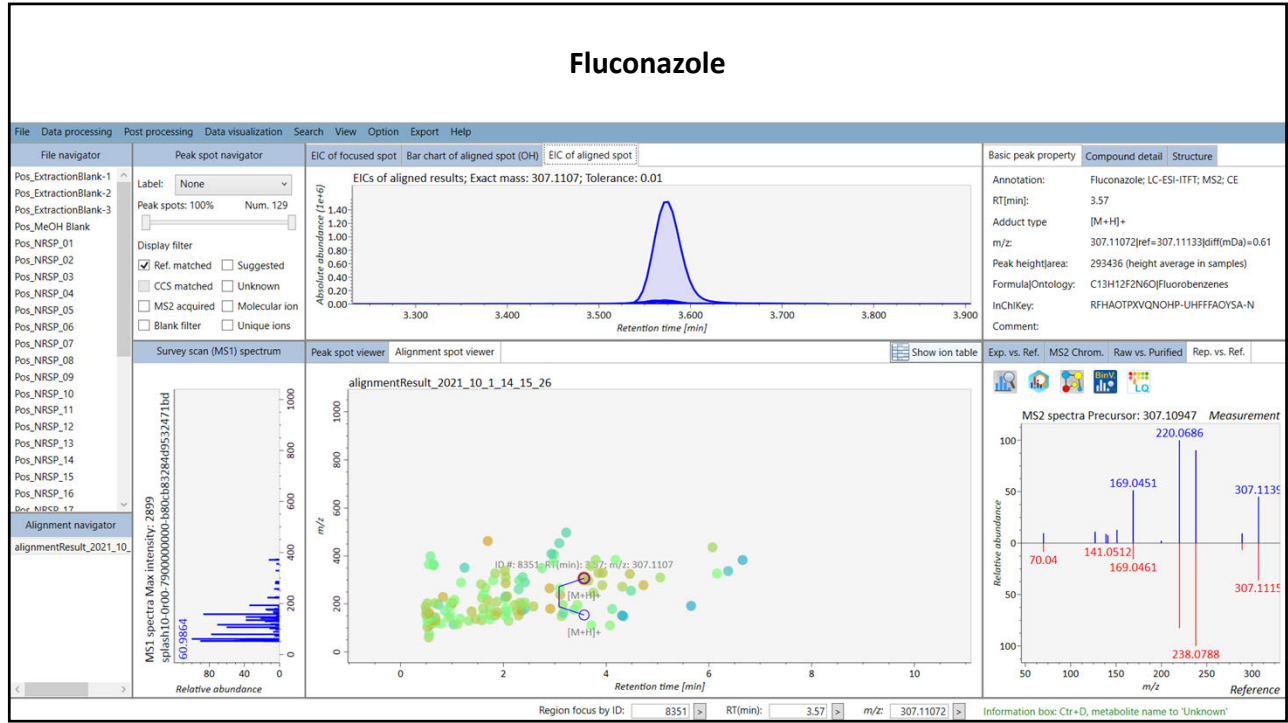
34



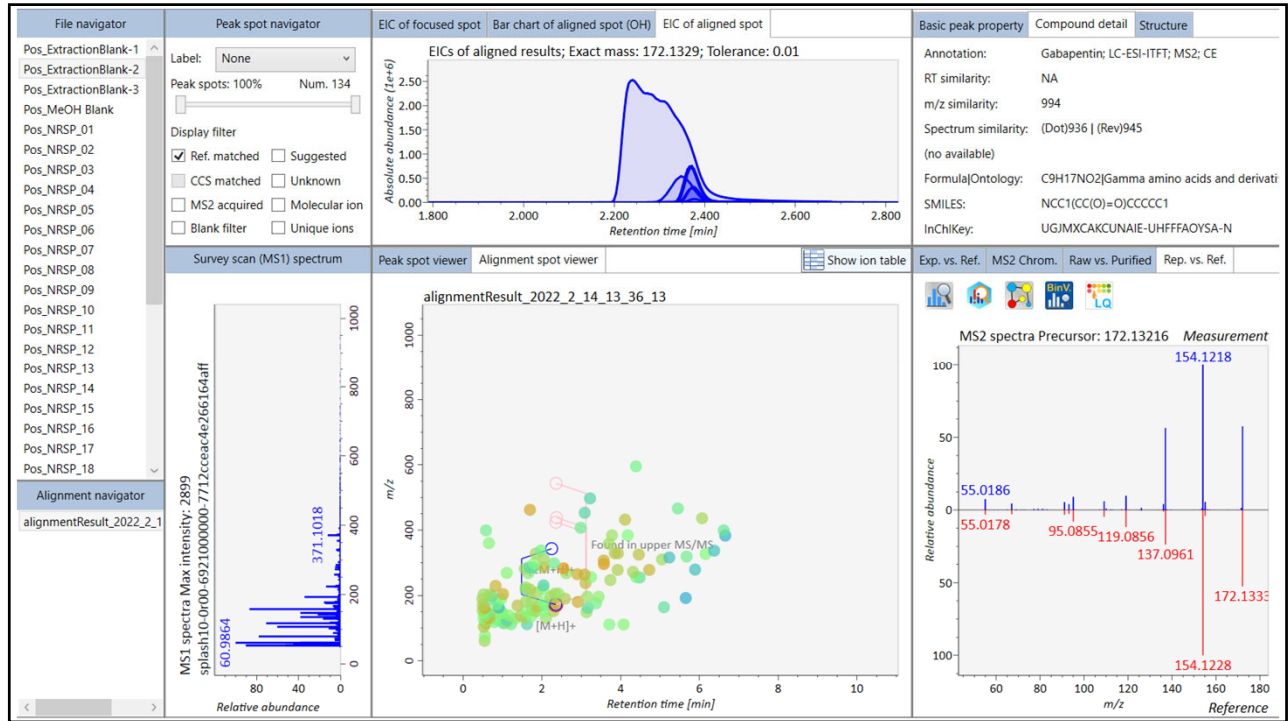
35



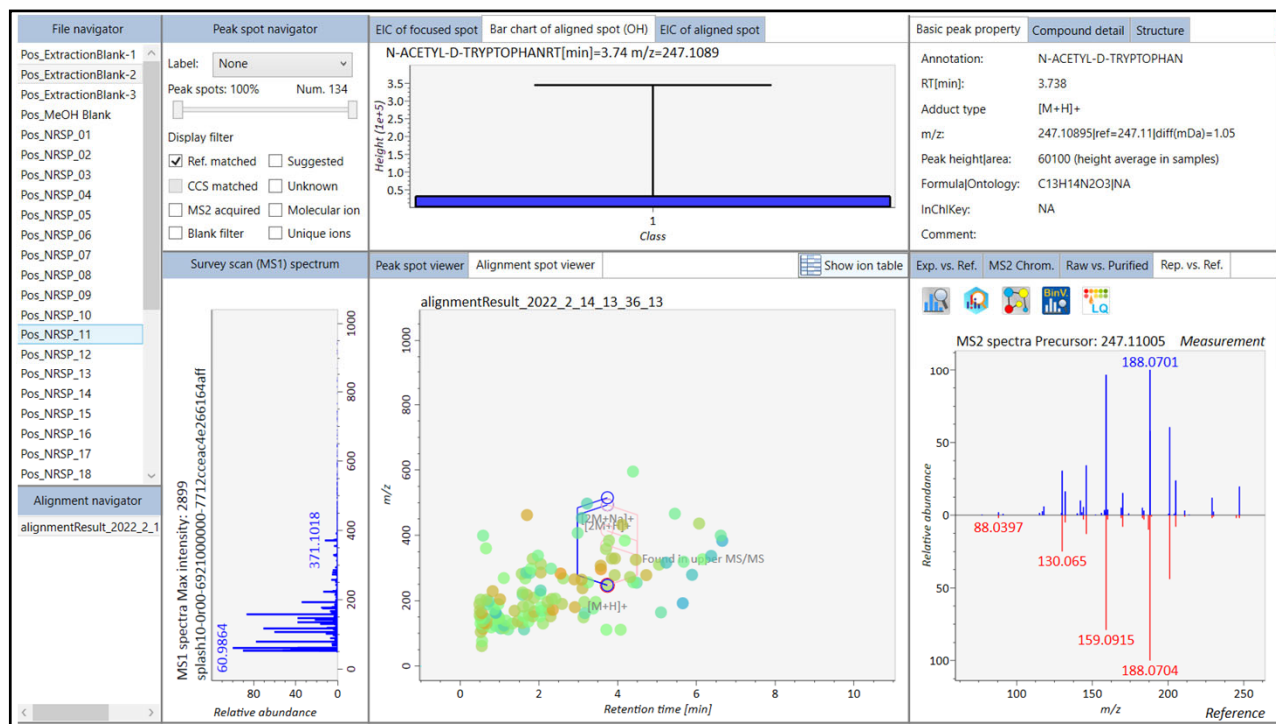
36



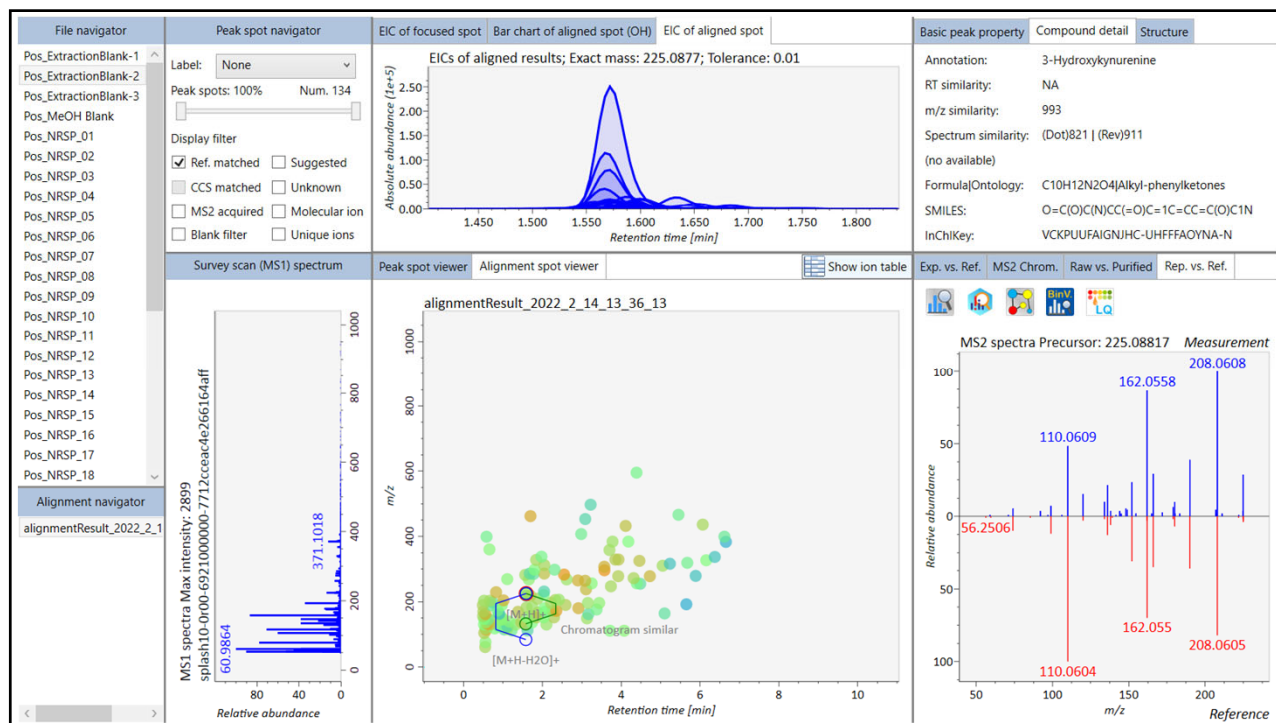
37



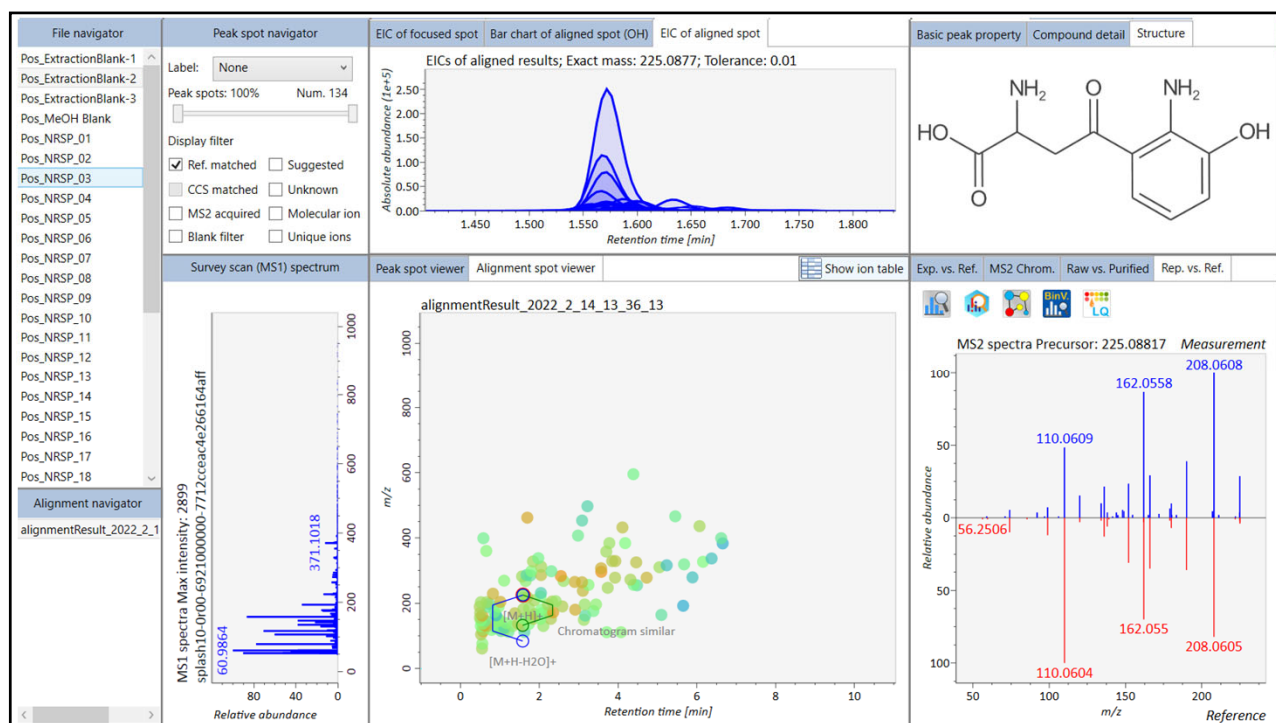
38



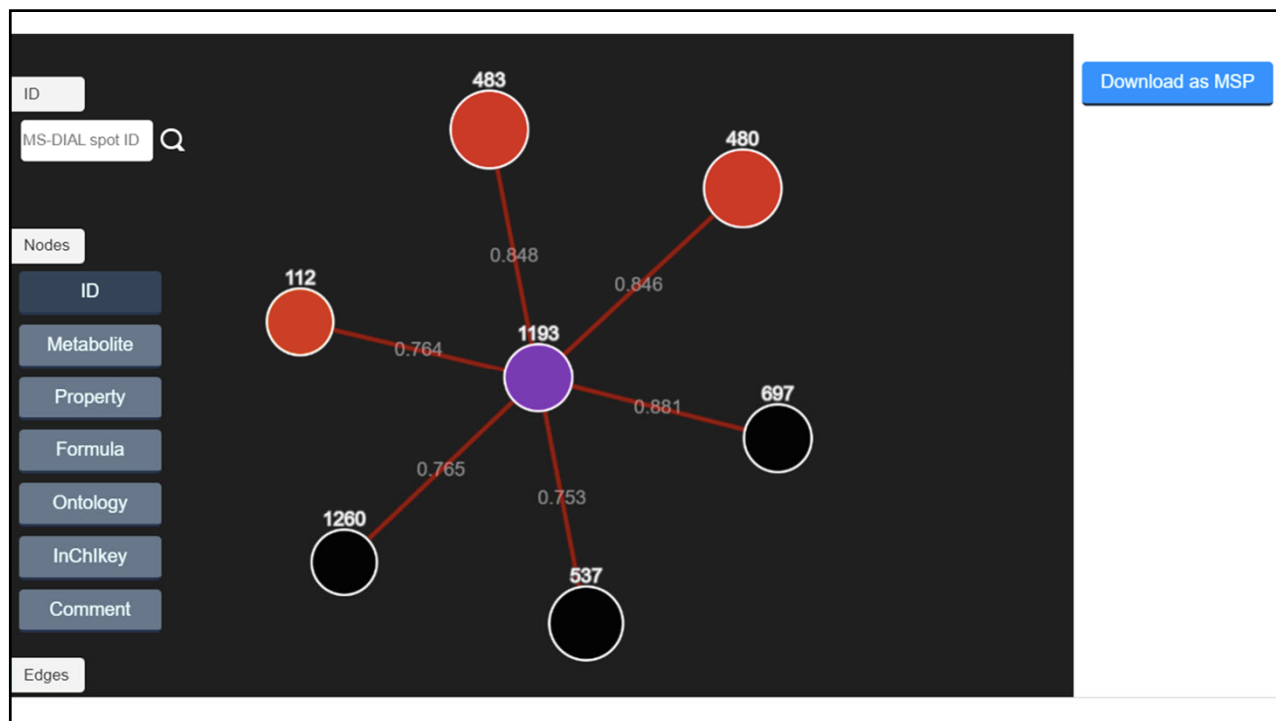
39



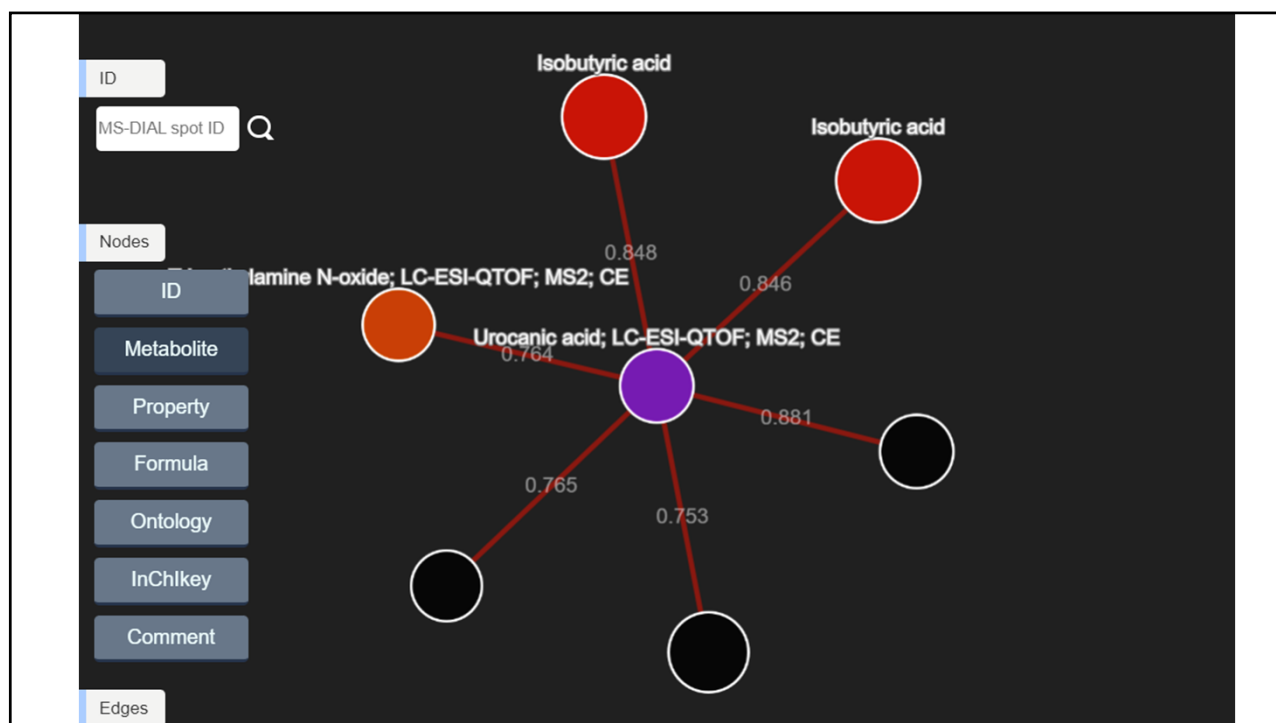
40



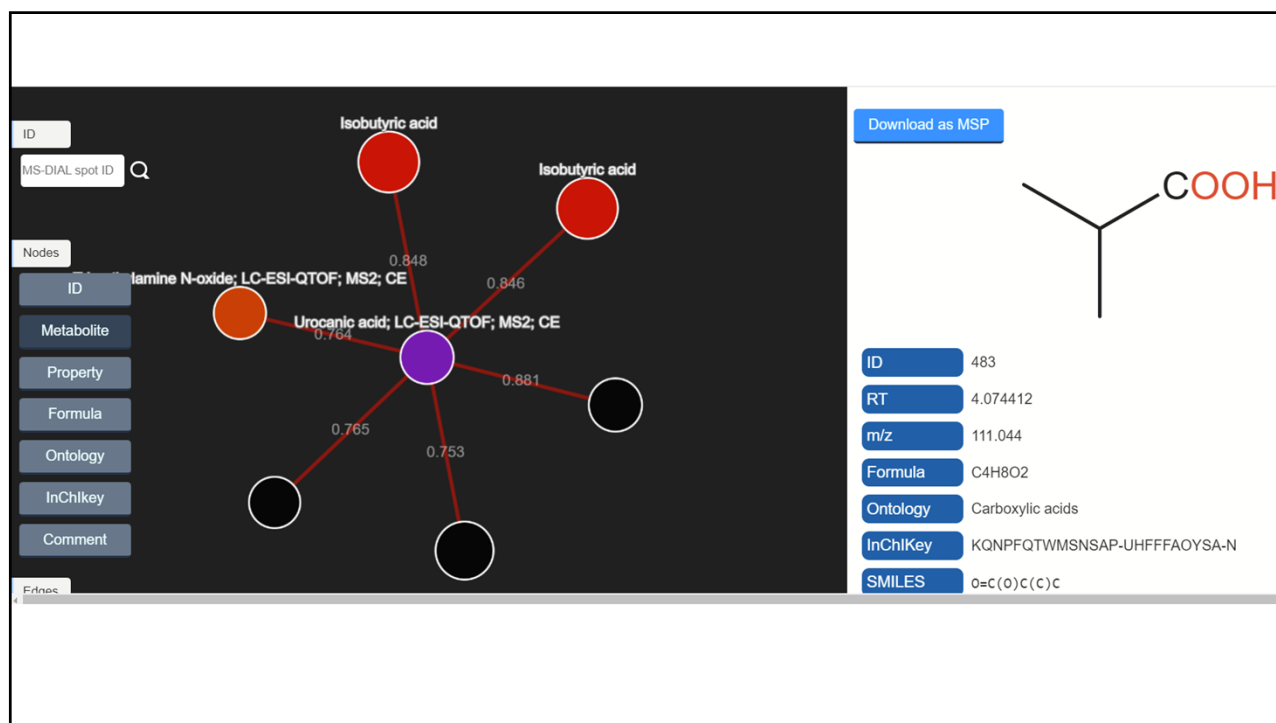
41



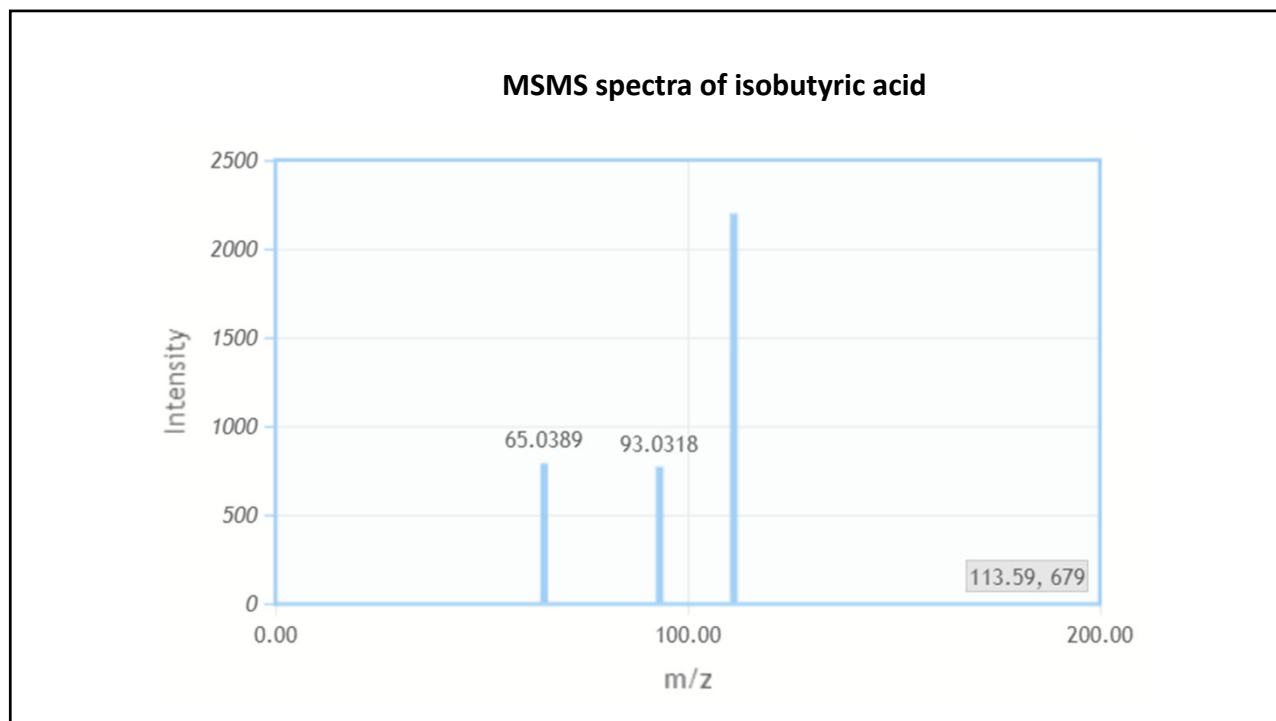
42



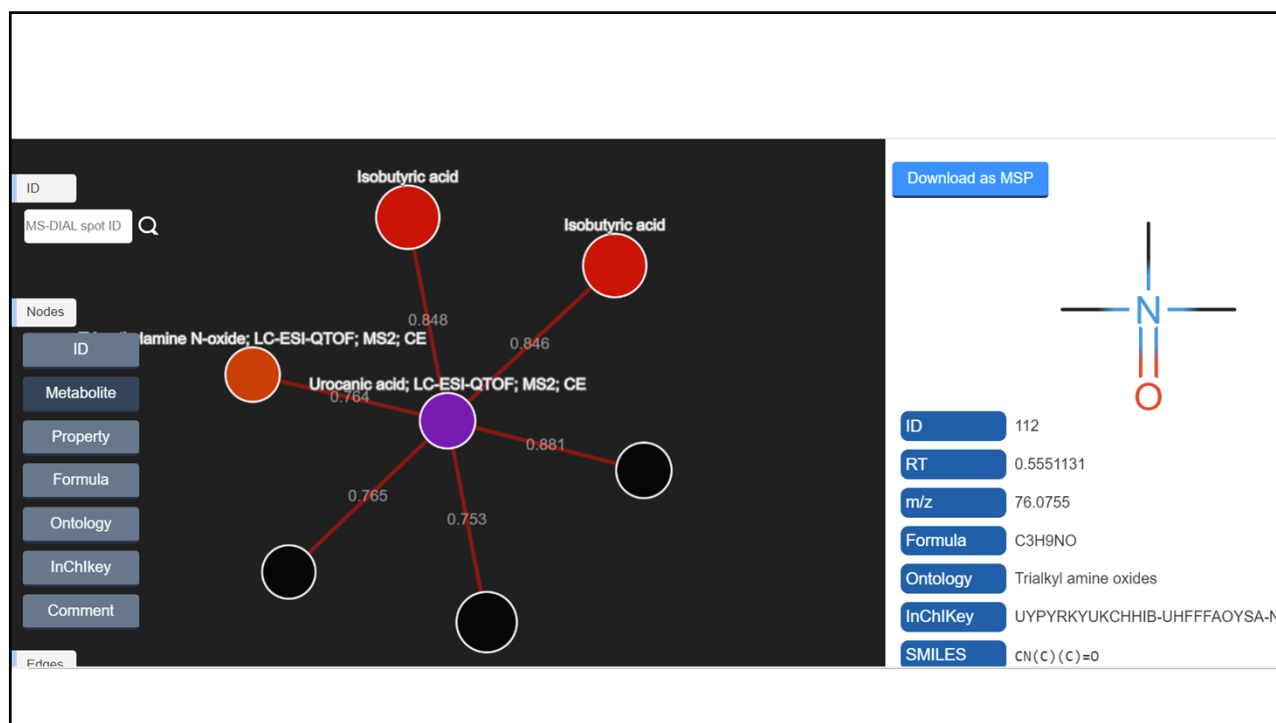
43



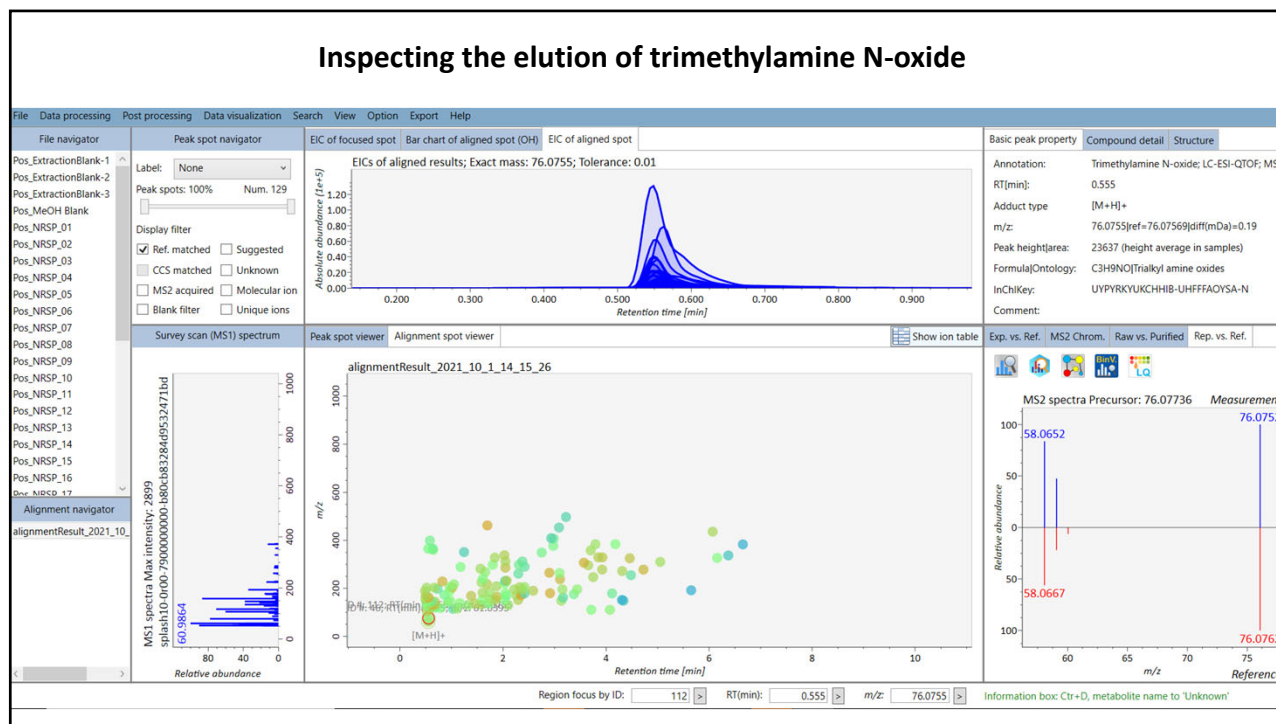
44



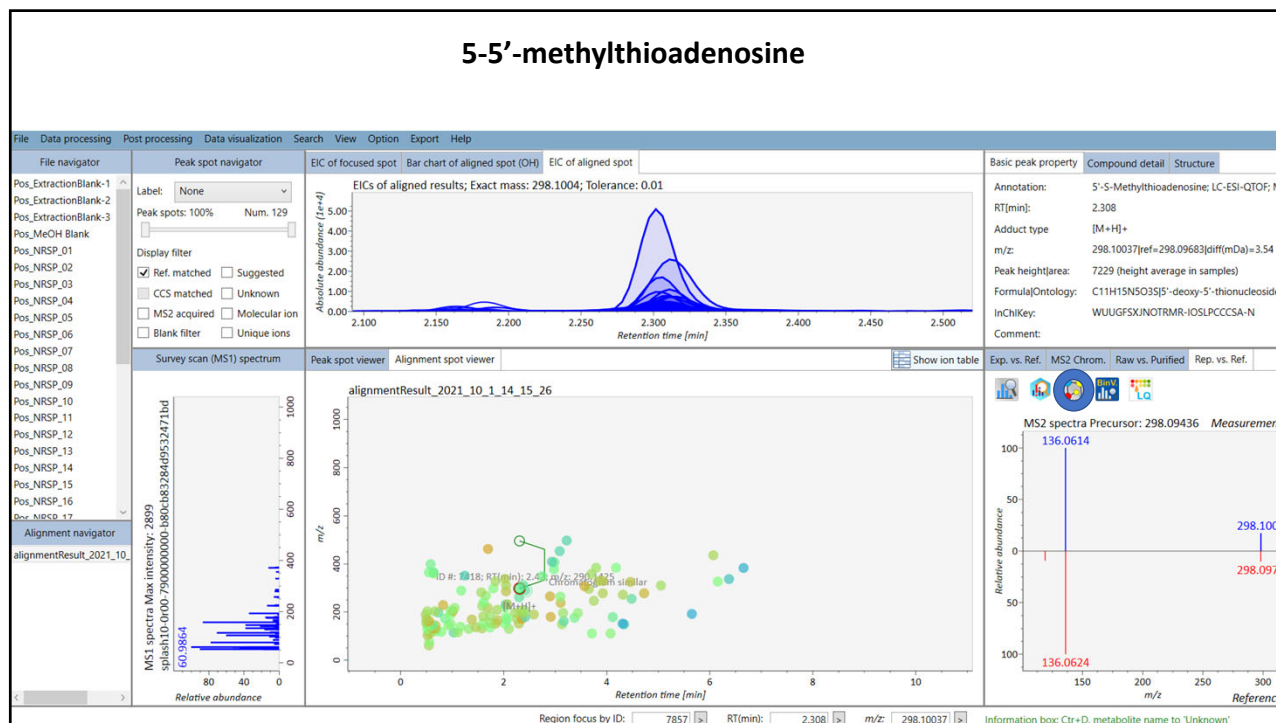
45



46



47



48

Nodes

Download as MSP

7857
 2.30782
 298.1004
 C11H15N5O3S
 5'-deoxy-5'-thionucleosides
 WUUGFSXJNOTRMR-IOSLPCCSA-N
 CSC[C@H]1O[C@H]([C@H](O)

49

Nodes

Download as MSP

6201
 1.655442
 268.1055
 C10H13N5O4

 OIRDYQYFTABQOQ-KQYNXXCUSA-N
 OCC1OC(N2C=NC=3C(=NC=NC32)N)C(O)C1O

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Changing pane to show structure

File Data processing Post processing Data visualization Search View Option Export Help

File navigator Peak spot navigator EIC of focused spot Bar chart of aligned spot (OH) EIC of aligned spot Basic peak property **Compound detail** Structure

Pos_ExtractionBlank-1
Pos_ExtractionBlank-2
Pos_ExtractionBlank-3
Pos_MeOH Blank
Pos_NRSP_01
Pos_NRSP_02
Pos_NRSP_03
Pos_NRSP_04
Pos_NRSP_05
Pos_NRSP_06
Pos_NRSP_07
Pos_NRSP_08
Pos_NRSP_09
Pos_NRSP_10
Pos_NRSP_11
Pos_NRSP_12
Pos_NRSP_13
Pos_NRSP_14
Pos_NRSP_15
Pos_NRSP_16
Pos_NRSP_17

Label: None
Peak spots: 100% Num. 129
Display filter
 Ref. matched Suggested
 CCS matched Unknown
 MS2 acquired Molecular ion
 Blank filter Unique ions

Survey scan (MS1) spectrum
MS1 spectra Max intensity: 2899
splash10-0000-7900000000-1880c183284d9532471bd
60.9864

EICs of aligned results; Exact mass: 298.1004; Tolerance: 0.01

Peak spot viewer Alignment spot viewer Show ion table

alignmentResult_2021_10_1_14_15_26

MS2 spectra Precursor: 298.09436 Measurement
136.0614
298.1006
298.0974
136.0624

Region focus by ID: 7857 RT(min): 2.308 m/z: 298.10037

Information box: Ctr+D, metabolite name to 'Unknown'

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File Data processing Post processing Data visualization Search View Option Export Help

File navigator Peak spot navigator EIC of focused spot Bar chart of aligned spot (OH) EIC of aligned spot Basic peak property Compound detail **Structure**

Pos_ExtractionBlank-1
Pos_ExtractionBlank-2
Pos_ExtractionBlank-3
Pos_MeOH Blank
Pos_NRSP_01
Pos_NRSP_02
Pos_NRSP_03
Pos_NRSP_04
Pos_NRSP_05
Pos_NRSP_06
Pos_NRSP_07
Pos_NRSP_08
Pos_NRSP_09
Pos_NRSP_10
Pos_NRSP_11
Pos_NRSP_12
Pos_NRSP_13
Pos_NRSP_14
Pos_NRSP_15
Pos_NRSP_16
Pos_NRSP_17

Label: None
Peak spots: 100% Num. 129
Display filter
 Ref. matched Suggested
 CCS matched Unknown
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Survey scan (MS1) spectrum
MS1 spectra Max intensity: 2899
splash10-0000-7900000000-1880c183284d9532471bd
60.9864

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alignmentResult_2021_10_1_14_15_26

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136.0614
298.1006
298.0974
136.0624

Region focus by ID: 7857 RT(min): 2.308 m/z: 298.10037

Information box: Ctr+D, metabolite name to 'Unknown'

NC1=NC=NC2=C1N=CN2[C@@H]3O[C@H](CS)[C@@H](O)[C@H]3O

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Other candidates from the Dbase

Selected library ID: 252956

RT tol. [min]:
 MS1 tol. [Da]:
 MS2 tol. [Da]:

Name: 5'-S-Methylthioadenosine
 RT similarity score: -1000
 Accurate mass similarity score: 969.91
 Dot product similarity score: 682.2743
 Reverse dot product similarity score: 815.5943
 Total score: 806.0453

File information

| File ID | File name | RT[min] | Accurate mass | Molecular species | Identified |
|---------|----------------------|----------|---------------|--------------------|------------------------------|
| 32 | Pos_PooledSample.MSN | 2.294167 | 298.0944 | [M+H] ⁺ | 5'-S-Methylthioadenosine; LC |

Library information

| Library ID | Name | Adduct | RT[min] | Pre. m/z | Dot prod. | Rev. prod. | Presense | Instrument | Comment |
|------------|---------------------------|--------------------|---------|----------|-----------|------------|----------|------------|---------------------------|
| 252956 | 5'-S-Methylthioadenosine; | [M+H] ⁺ | -1 | 298.0968 | 0.68 | 0.82 | 0.67 | | registered in MassBank |
| 262048 | 5'-Deoxy-5'-Methylthioad | [M+H] ⁺ | -1 | 298.0968 | 0.68 | 0.82 | 0.67 | | registered in Respect |
| 421 | Adenosine + C2H5S; PlaSN | [M+H] ⁺ | 2.7 | 298.097 | 0.69 | 0.70 | 0.10 | | Annotation level-2; PlaSM |
| 731073 | 5'-Methylthioadenosine | [M+H] ⁺ | -1 | 298.0968 | 0.57 | 0.66 | 0.25 | | DB#=FiehnHILIC000114; o |
| 231915 | 5'-Methylthioadenosine | [M+H] ⁺ | -1 | 298.0968 | 0.54 | 0.54 | 0.18 | | DB#=FiehnHILIC001694; o |
| 241714 | NCGC00263645-02_C11H1 | [M+H] ⁺ | -1 | 298.097 | 0.43 | 0.66 | 0.20 | | DB#=CCMSLIB000008539f |
| 237771 | 5'-METHYLTHIOADENOSIN | [M+H] ⁺ | -1 | 298.1 | 0.43 | 0.69 | 0.17 | | DB#=CCMSLIB000005778f |
| 235438 | Erythraline | [M+H] ⁺ | -1 | 298.1 | 0.19 | 0.13 | 0.25 | | DB#=CCMSLIB00000812z |
| 235441 | Erythratidinone | [M+H] ⁺ | -1 | 298.1 | 0.22 | 0.01 | 0.03 | | DB#=CCMSLIB00000812z |
| 261053 | 5'-Deoxy-5'-Methylthioad | [M+H] ⁺ | -1 | 298.0968 | 0.00 | 0.00 | 0.00 | | registered in Respect |
| 261055 | 5'-Deoxy-5'-Methylthioad | [M+H] ⁺ | -1 | 298.0968 | 0.00 | 0.00 | 0.00 | | registered in Respect |
| 261120 | 5'-Deoxy-5'-Methylthioad | [M+H] ⁺ | -1 | 298.0968 | 0.00 | 0.00 | 0.00 | | registered in Respect |
| 261121 | 5'-Deoxy-5'-Methylthioad | [M+H] ⁺ | -1 | 298.0968 | 0.00 | 0.00 | 0.00 | | registered in Respect |
| 261127 | 5'-Deoxy-5'-Methylthioad | [M+H] ⁺ | -1 | 298.0968 | 0.00 | 0.00 | 0.00 | | registered in Respect |
| 261138 | 5'-Deoxy-5'-Methylthioad | [M+H] ⁺ | -1 | 298.0968 | 0.00 | 0.00 | 0.00 | | registered in Respect |

MS2 spectra Precursor: 298.09436 Measurement

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Indole-3-carboxylic acid – tryptophan metabolite

File Data processing Post processing Data visualization Search View Option Export Help

File navigator
 Pos_ExtinctionBlank-1
 Pos_ExtinctionBlank-2
 Pos_ExtinctionBlank-3
 Pos_MeOH Blank
 Pos_NRSP_01
 Pos_NRSP_02
 Pos_NRSP_03
 Pos_NRSP_04
 Pos_NRSP_05
 Pos_NRSP_06
 Pos_NRSP_07
 Pos_NRSP_08
 Pos_NRSP_09
 Pos_NRSP_10
 Pos_NRSP_11
 Pos_NRSP_12
 Pos_NRSP_13
 Pos_NRSP_14
 Pos_NRSP_15
 Pos_NRSP_16
 Pos_NRSP_17
Alignment navigator
 alignmentResult_2021_10_

Peak spot navigator
 Label: None
 Peak spots: 100% Num. 129
 Display filter:
 Ref. matched Suggested
 CCS matched Unknown
 MS2 acquired Molecular ion
 Blank filter Unique ions

EIC of aligned results; Exact mass: 162.0543; Tolerance: 0.01

Survey scan (MS1) spectrum

Peak spot viewer Alignment spot viewer
 alignmentResult_2021_10_14_15_26

Basic peak property
 Annotation: 1H-indole-3-carboxylic acid; LC-ESI-QTOF; M
 RT[min]: 3.165
 Adduct type: [M+H]⁺
 m/z: 162.05429[ref=162.05496]diff(mDa)=0.67
 Peak height[area]: 10560 (height average in samples)
 Formula[Ontology]: C9H7NO2[Indolecarboxylic acids and derivat
 InChIKey: KMAKOBLOCCGIP-UHFFFAOYSA-N
 Comment:

MS2 spectra Precursor: 162.05305 Measurement

Region focus by ID: RT(min): m/z:

Information box: Ctr+D, metabolite name to 'Unknown'

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Compound search

A:Confidence B:Unsettled C:Unknown Cancel Selected library ID: 251286

Records corresponding to the mass

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 |
|---------|----------------------|----------|---------------|--------------------|------------------------------|
| 32 | Pos_PooledSample MSN | 3.157433 | 162.0531 | [M+H] ⁺ | 1H-indole-3-carboxylic acid; |

Library information

| Library ID | Name | Adduct | RT[min] | Pre. m/z | Dot prod. | Rev. prod. | Presence | Instrument | Comment |
|------------|-----------------------------|---------------------|----------|----------|-----------|------------|----------|------------|---------------------------|
| 251286 | 1H-indole-3-carboxylic ac | [M+H] ⁺ | -1 | 162.055 | 0.89 | 0.89 | 1.00 | | registered in MassBank |
| 251440 | Indole-3-carboxylic acid; L | [M+H] ⁺ | -1 | 162.055 | 0.98 | 0.91 | 0.57 | | registered in MassBank |
| 251441 | Indole-3-carboxylic acid; L | [M+H] ⁺ | -1 | 162.055 | 0.95 | 0.83 | 0.50 | | registered in MassBank |
| 262109 | 1H-indole-3-carboxylic ac | [M+H] ⁺ | -1 | 162.055 | 0.93 | 0.78 | 0.57 | | registered in Respect |
| 253153 | Indole-3-carboxylic acid; L | [M+H] ⁺ | -1 | 162.055 | 0.93 | 0.78 | 0.57 | | registered in MassBank |
| 237458 | Indole-3-carboxylic acid; 1 | [M+H] ⁺ | -1 | 162.055 | 0.97 | 0.79 | 0.36 | | DB# = CCMSLIB000004784 |
| 231385 | Indole-3-carboxylic acid; L | [M+H] ⁺ | -1 | 162.055 | 0.94 | 0.83 | 0.33 | | DB# = FiehnHILIC000439; c |
| 251870 | Indole-3-carboxylic acid; l | [M+H] ⁺ | -1 | 162.055 | 0.66 | 0.71 | 1.00 | | registered in MassBank |
| 2472 | Indole-3-carboxylic acid; C | [M+H] ⁺ | 1.455419 | 162.0549 | 0.85 | 0.73 | 0.36 | | MS2 deconvoluted using l |
| 251871 | Indole-3-carboxylic acid; l | [M+H] ⁺ | -1 | 162.055 | 0.65 | 0.70 | 1.00 | | registered in MassBank |
| 251287 | 1H-indole-3-carboxylic ac | [M+H] ⁺ | -1 | 162.055 | 0.79 | 0.67 | 0.57 | | registered in MassBank |
| 251442 | Indole-3-carboxylic acid; L | [M+H] ⁺ | -1 | 162.055 | 0.81 | 0.62 | 0.57 | | registered in MassBank |
| 251872 | Indole-3-carboxylic acid; l | [M+H] ⁺ | -1 | 162.055 | 0.61 | 0.65 | 1.00 | | registered in MassBank |
| 231845 | 2,8-Quinolinediol | [M+H] ⁺ | -1 | 162.055 | 0.63 | 0.87 | 0.38 | | DB# = FiehnHILIC001623; c |
| 251873 | Indole-3-carboxylic acid; l | [M+H] ⁺ | -1 | 162.055 | 0.58 | 0.62 | 1.00 | | registered in MassBank |
| 1132 | Unknown (carbon number | [M+H] ⁺ | 3.27 | 162.0566 | 0.66 | 0.68 | 0.60 | | Annotation level-4; PlaSM |
| 2471 | Indole-3-carboxylic acid; C | [M+H] ⁺ | 1.455419 | 162.0549 | 0.71 | 0.62 | 0.40 | | MS2 deconvoluted using l |
| 232231 | Indole-3-carboxylic acid | [M+H] ⁺ | -1 | 162.055 | 0.62 | 0.72 | 0.33 | | DB# = FiehnHILIC002011; c |
| 2469 | Indole-3-carboxylic acid; C | [M+H] ⁺ | 1.455419 | 162.0549 | 0.87 | 0.24 | 0.50 | | MS2 deconvoluted using l |
| 2468 | Indole-3-carboxylic acid; C | [M+H] ⁺ | 1.455419 | 162.0549 | 0.68 | 0.23 | 0.50 | | MS2 deconvoluted using l |
| 230984 | 2,8-Quinolinediol | [M+H] ⁺ | -1 | 162.055 | 0.29 | 0.46 | 1.00 | | DB# = FiehnHILIC000035; c |
| 96514 | 3-hydroxy-1,2-dimethylpy | [M+Na] ⁺ | 3.22 | 162.05 | 0.24 | 0.73 | 0.50 | Orbitrap | spec_id=103220; origin=B |

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Exporting the aligned data

File Data processing Post processing Data visualization Search View Option Export Help

File navigator Peak spot navigator EIC of focused spot Peak list result

Pos_ExtractionBlank-1
Pos_ExtractionBlank-2
Pos_ExtractionBlank-3
Pos_MeOH Blank
Pos_NRSP_01
Pos_NRSP_02
Pos_NRSP_03
Pos_NRSP_04
Pos_NRSP_05
Pos_NRSP_06
Pos_NRSP_07
Pos_NRSP_08
Pos_NRSP_09
Pos_NRSP_10
Pos_NRSP_11
Pos_NRSP_12
Pos_NRSP_13
Pos_NRSP_14
Pos_NRSP_15
Pos_NRSP_16
Pos_NRSP_17
Alignment navigator
alignmentResult_2021_10_14_15_26

Label: None
Peak spots: 100% Num. 129
Display filter
 Ref. matched Suggested
 CCS matched Unknown
 MS2 acquired Molecular ion
 Blank filter Unique ions

Basic peak property Compound detail Structure

Annotation: 1H-indole-3-carboxylic acid; LC-ESI-QTOF; M
RT[min]: 3.165
Adduct type [M+H]⁺
m/z: 162.05429(ref=162.05496)(diff(mDa)=0.67)
Peak height[area]: 10560 (height average in samples)
Formula[Ontology]: C9H7NO2[Indolecarboxylic acids and deriva
InChIKey: KMAKOBLOCCQJIP-UHFFFAOYSA-N
Comment:

Exp. vs. Ref. MS2 Chrom. Raw vs. Purified Rep. vs. Ref.

MS1 spectra Max intensity: 2899
splash1!0-0100-7900000000-b80c4b832864f9532471hd
60.3864

MS2 spectra Precursor: 162.05305 Measurement
118.0639 144.0449 162.0538
118.068 144.045 162.06

Region focus by ID: 1917 RT(min): 3.165 m/z: 162.05429 Information box: Ctrl+D, metabolite name to 'Unknown'

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Alignment result export

Directory: C:\Users\sbarne\Desktop\Data analysis class

Export option

File: alignmentResult_2022_2_14_13_36_13

Raw data matrix (Height) Peak ID matrix
 Normalized data matrix Retention time matrix
 Raw data matrix (Area) m/z matrix
 *Export as mztab-M 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: Pos_ExtractionBlank

Export format: msp

Before exiting MS-DIAL, go to Export (top bar) and select <alignment result>. Then select where you are going to save the export file.

De-select raw data matrix (height) and select raw data matrix (area) and retention time matrix.

Then <Export>. The resulting file is in .txt format and can be read into Excel on any platform and then saved in a .xlsx format – we'll do that on Friday.

Also in MS-DIAL, make sure to save the MS-DIAL file – click on <file> (top left corner) and <save>.