# *MS-CleanR tutorial:*
# *Peak list cleaning, data concatenation and peak annotation*
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## Prerequisite :
Software installation

## Downloading
**MS-DIAL** version up to 4.16:

http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL/index2.html

**MS-FINDER** version up to 3.30:

http://prime.psc.riken.jp/Metabolomics_Software/MS-FINDER/index2.html

**R version up to 3.6.1** : https://cran.r-project.org/

**R studio**: https://rstudio.com/products/rstudio/

## Installation
- **In R**, copy and paste the following command to update R version if necessary

```
if(!require(installr)) {
install.packages("installr"); require(installr)}
updateR()
```

- In **R studio**, update all your packages with the command

```
Setrepositories()
```

Select 1 and 2 for CRAN and BIOCONDUCTOR packages
Select the command Update on the right windows in the Package part

- Install MS-cleanR by copying and pasting the command :

```
devtools::install_github("eMetaboHUBMetatoul/mscleanr")
```

## MS-CleanR workflow

Within your project directory, create one subfolder for each ionization mode namely “pos” and “neg”. In each of this new directory, create another subfolder named “peaks”.

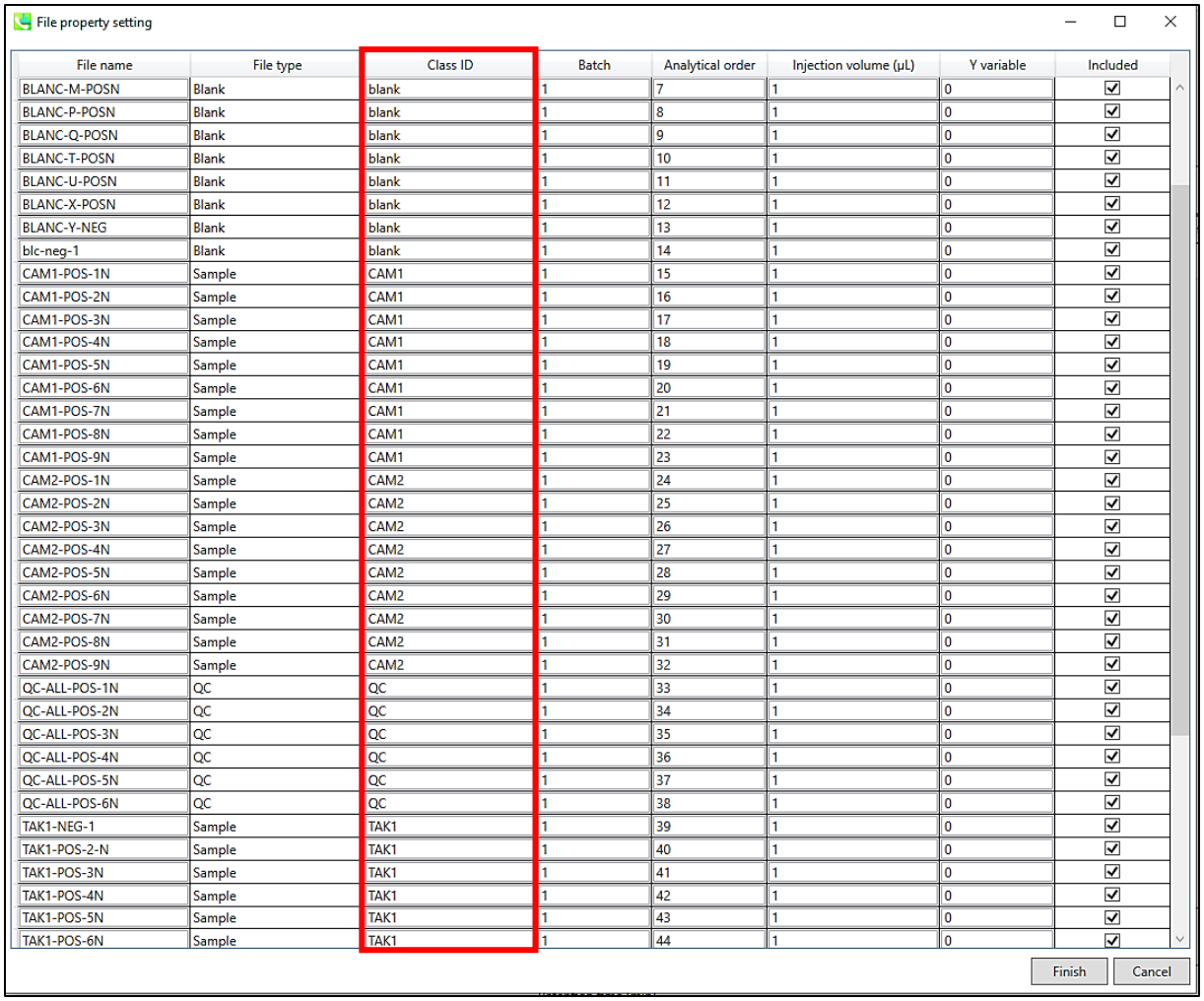
*Optional: Only one ionization mode can be treated by MS-CleanR*

## Process the data with MS-DIAL

Process data with MS-DIAL in either pos or neg mode or both according to the tutorial https://mtbinfo-team.github.io/mtbinfo.github.io/

## **Important notices:**

A) During data importation, it is important to note the type (Blank, QC or Sample) and class of every sample in **Class ID column and File Type** (blank, sample class, QC)
B) Be careful to have the **same number of samples** between pos and neg mode and in the **same order.**



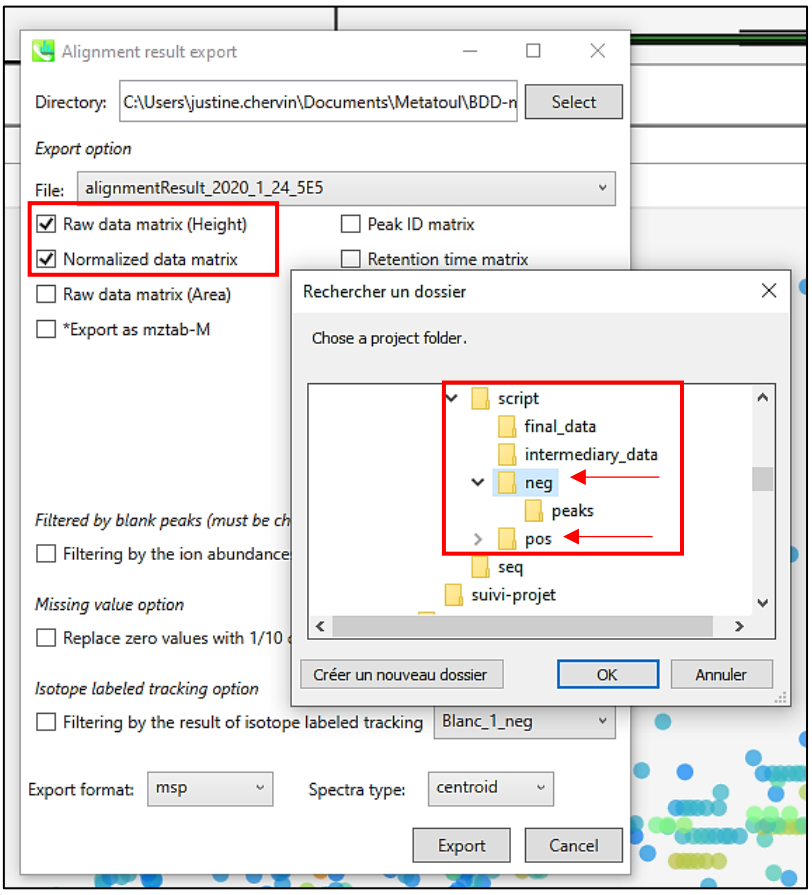

| File name | File type | Class ID | Batch | Analytical order | Injection volume (µL) | Y variable | Included |
|---|---|---|---|---|---|---|---|
| BLANC-M-POSN | Blank | blank | 1 | 7 | 1 | 0 | ☑ |
| BLANC-P-POSN | Blank | blank | 1 | 8 | 1 | 0 | ☑ |
| BLANC-Q-POSN | Blank | blank | 1 | 9 | 1 | 0 | ☑ |
| BLANC-T-POSN | Blank | blank | 1 | 10 | 1 | 0 | ☑ |
| BLANC-U-POSN | Blank | blank | 1 | 11 | 1 | 0 | ☑ |
| BLANC-X-POSN | Blank | blank | 1 | 12 | 1 | 0 | ☑ |
| BLANC-Y-NEG | Blank | blank | 1 | 13 | 1 | 0 | ☑ |
| blc-neg-1 | Blank | blank | 1 | 14 | 1 | 0 | ☑ |
| CAM1-POS-1N | Sample | CAM1 | 1 | 15 | 1 | 0 | ☑ |
| CAM1-POS-2N | Sample | CAM1 | 1 | 16 | 1 | 0 | ☑ |
| CAM1-POS-3N | Sample | CAM1 | 1 | 17 | 1 | 0 | ☑ |
| CAM1-POS-4N | Sample | CAM1 | 1 | 18 | 1 | 0 | ☑ |
| CAM1-POS-5N | Sample | CAM1 | 1 | 19 | 1 | 0 | ☑ |
| CAM1-POS-6N | Sample | CAM1 | 1 | 20 | 1 | 0 | ☑ |
| CAM1-POS-7N | Sample | CAM1 | 1 | 21 | 1 | 0 | ☑ |
| CAM1-POS-8N | Sample | CAM1 | 1 | 22 | 1 | 0 | ☑ |
| CAM1-POS-9N | Sample | CAM1 | 1 | 23 | 1 | 0 | ☑ |
| CAM2-POS-1N | Sample | CAM2 | 1 | 24 | 1 | 0 | ☑ |
| CAM2-POS-2N | Sample | CAM2 | 1 | 25 | 1 | 0 | ☑ |
| CAM2-POS-3N | Sample | CAM2 | 1 | 26 | 1 | 0 | ☑ |
| CAM2-POS-4N | Sample | CAM2 | 1 | 27 | 1 | 0 | ☑ |
| CAM2-POS-5N | Sample | CAM2 | 1 | 28 | 1 | 0 | ☑ |
| CAM2-POS-6N | Sample | CAM2 | 1 | 29 | 1 | 0 | ☑ |
| CAM2-POS-7N | Sample | CAM2 | 1 | 30 | 1 | 0 | ☑ |
| CAM2-POS-8N | Sample | CAM2 | 1 | 31 | 1 | 0 | ☑ |
| CAM2-POS-9N | Sample | CAM2 | 1 | 32 | 1 | 0 | ☑ |
| QC-ALL-POS-1N | QC | QC | 1 | 33 | 1 | 0 | ☑ |
| QC-ALL-POS-2N | QC | QC | 1 | 34 | 1 | 0 | ☑ |
| QC-ALL-POS-3N | QC | QC | 1 | 35 | 1 | 0 | ☑ |
| QC-ALL-POS-4N | QC | QC | 1 | 36 | 1 | 0 | ☑ |
| QC-ALL-POS-5N | QC | QC | 1 | 37 | 1 | 0 | ☑ |
| QC-ALL-POS-6N | QC | QC | 1 | 38 | 1 | 0 | ☑ |
| TAK1-NEG-1 | Sample | TAK1 | 1 | 39 | 1 | 0 | ☑ |
| TAK1-POS-2-N | Sample | TAK1 | 1 | 40 | 1 | 0 | ☑ |
| TAK1-POS-3N | Sample | TAK1 | 1 | 41 | 1 | 0 | ☑ |
| TAK1-POS-4N | Sample | TAK1 | 1 | 42 | 1 | 0 | ☑ |
| TAK1-POS-5N | Sample | TAK1 | 1 | 43 | 1 | 0 | ☑ |
| TAK1-POS-6N | Sample | TAK1 | 1 | 44 | 1 | 0 | ☑ |

## Export peak list

After alignment process:

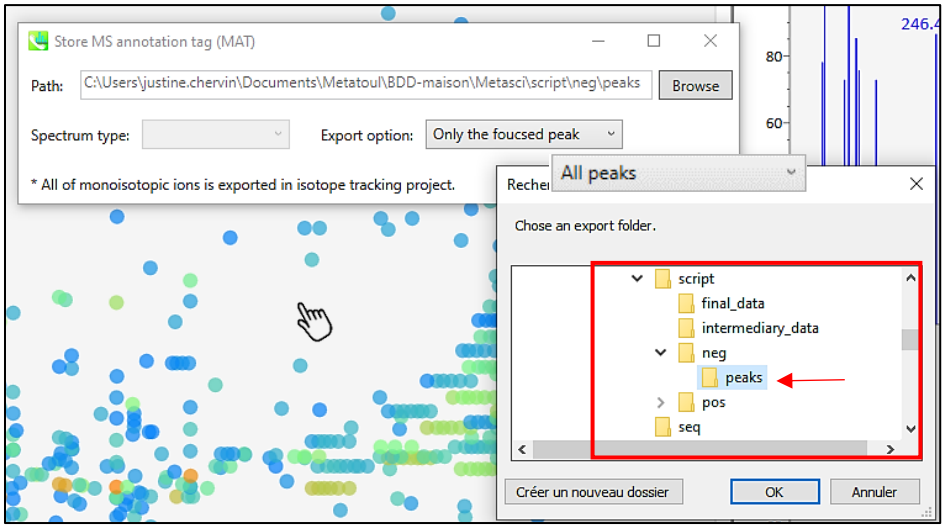
- **Normalized data** by Total ion chromatogram (TIC) or another normalization method

- Export alignment results: both **Raw data matrix (Height)** and **Normalized data matrix** respectively in previously created folders named “pos” and “neg”.




## Export all peaks

By clicking on one feature dot, export « **all peaks »** to the “peaks” directory respectively created in “pos” and “neg” folders.



## Open the shiny interface of MS-CleanR

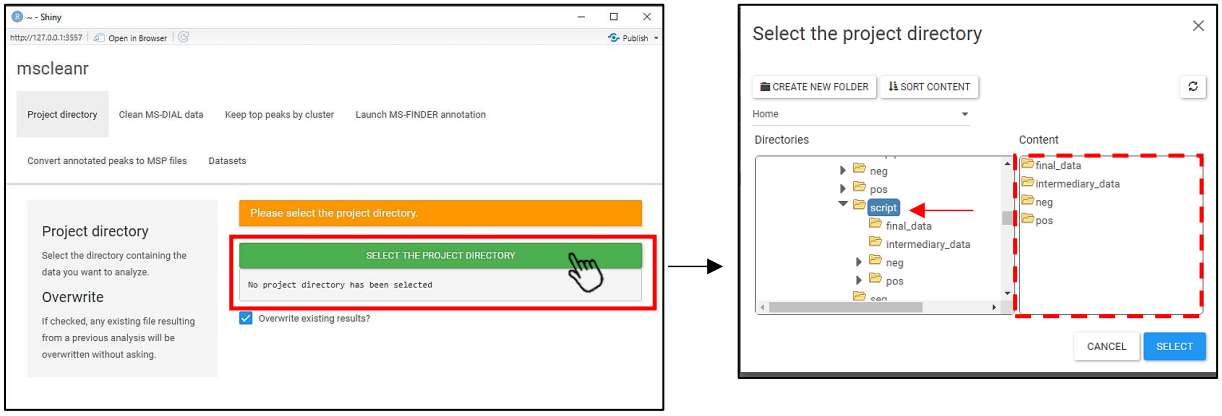
Select the MScleanR package in **Rstudio** and open the shiny interface using the following command:

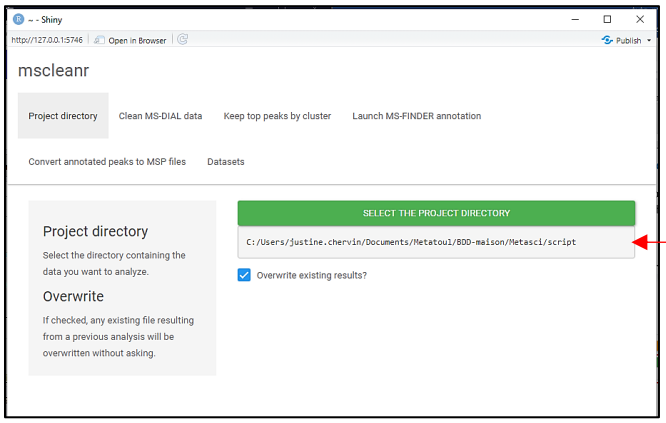
- *Note that if you encounter some issues, try to open the Shiny interface in internet browser.*
- *Sometimes Windows block file writing, close the shiny or R studio and run it again to solve the problem.*

```
runGUI()
```

### *Select the project directory*

First step is to define the project directory on the first tab called "**Project Directory**" by clicking on the green rectangle "*Select the project directory*" and by selecting the parent folder containing "pos" and "neg" folders.




When your project directory is selected, it is written in the grey rectangle.

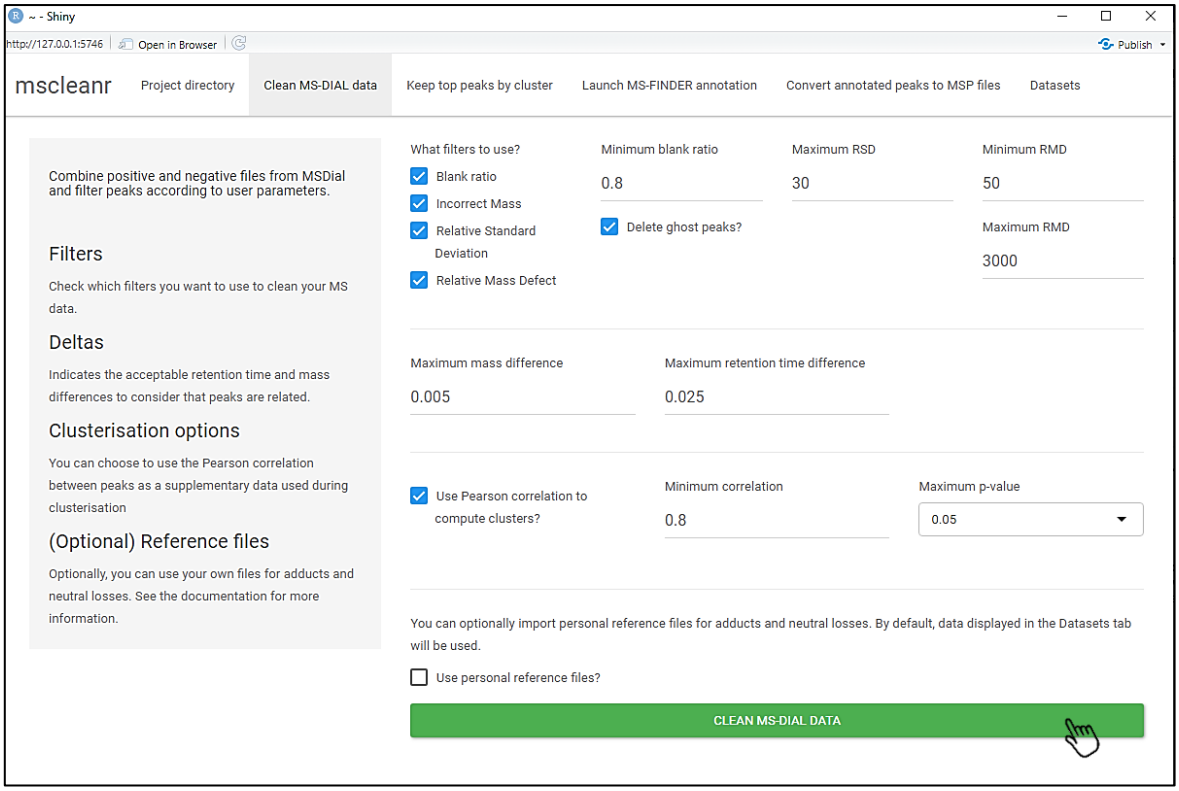
### *Define your parameter of filtration and Clean your data*

In the second tab called "**Clean MS-DIAL data**" various parameters can be personalized to filter your data. You can decide to select any filter according to your goal and experimental design.

| Command | Description |
|---|---|
| **Blank ratio** | Subtract blank peaks to samples based on the indicated "**Minimum blank ratio**" by default at 0.8.<br>This operation is done on the **Height files** between Blanks and QCs. |

| | |
|---|---|
| **Incorrect Mass** | Delete all peaks with a mass defect in X.8 and X.9 which appear to be artifacts. |
| **Relative standard Deviation (RSD)** | Filter based on the **Maximum RSD** value set at 30 by default.<br>The RSD is calculated on each defined class.<br>If RSD of one feature is under the defined value for all class, it is removed from the peak list. |
| **Relative Mass Defect (RMD)**[1] | RMD is calculated in ppm as ((mass defect/measured monoisotopic mass) × 10e6)<br>Analysis of natural products from the DNP shows that 95 % of RMD are comprised between 50 and 3000 (values by default). |
| **Delete ghost peaks** | Delete variables with *m/z* values corresponding to blank peaks but with a different RT in samples. |
| **Maximum mass difference** | m/z value tolerance set by default to 0.005 for Pearson correlation and pos/neg merging |
| **Maximum retention time difference** | RT value tolerance set by default to 0.025 (absolute value) for Pearson and pos/neg merging |
| **Use Pearson correlation to compute clusters?** | Extend MS-DIAL clusters with Pearson correlation.<br>**Minimum correlation** and **maximum p-value** are respectively set by default to 0.8 and 0.05 |

Once your parameters are fixed, click on the green rectangle named "*Clean MS-DIAL data*". A green window appears with the writing "*Cleaning data…*".



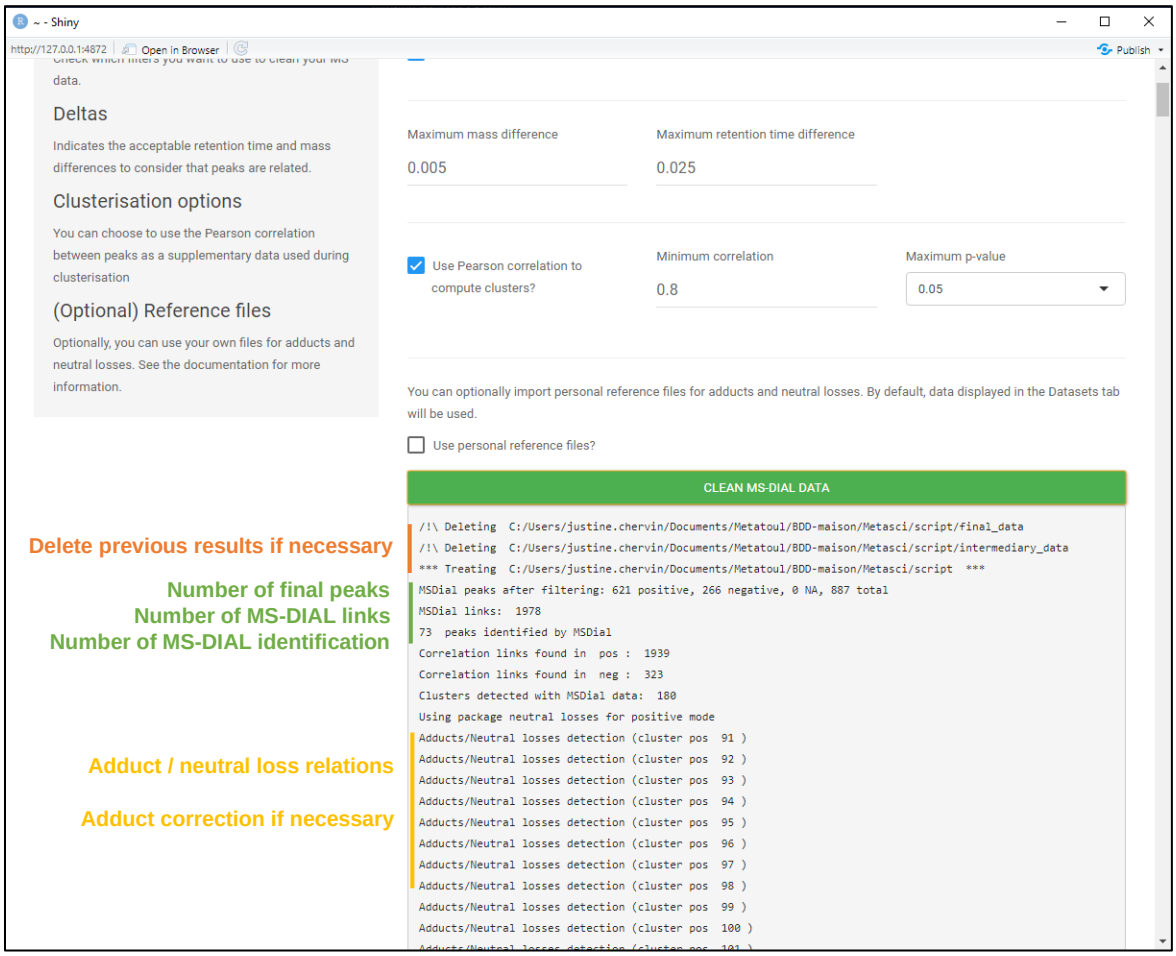


During the cleaning:

- Clusters are formed based on MS-DIAL "post curation column", Pearson correlation, links such as adducts, neutral losses, dimers, …;
- Adducts are corrected based on previous found links;
- Pos and Neg clusters are concatenated if relational links are found (adducts mass difference)

[1] Ekanayaka EA, Celiz MD, Jones AD. Relative mass defect filtering of mass spectra: a path to discovery of plant specialized metabolites. *Plant Physiol*. 2015;167(4):1221–1232. doi:10.1104/pp.114.251165

- Once the cleaning is done, one new folder is created named “intermediary_data”. Different information is obtained at the bottom of the index “Clean MS-DIAL data”.



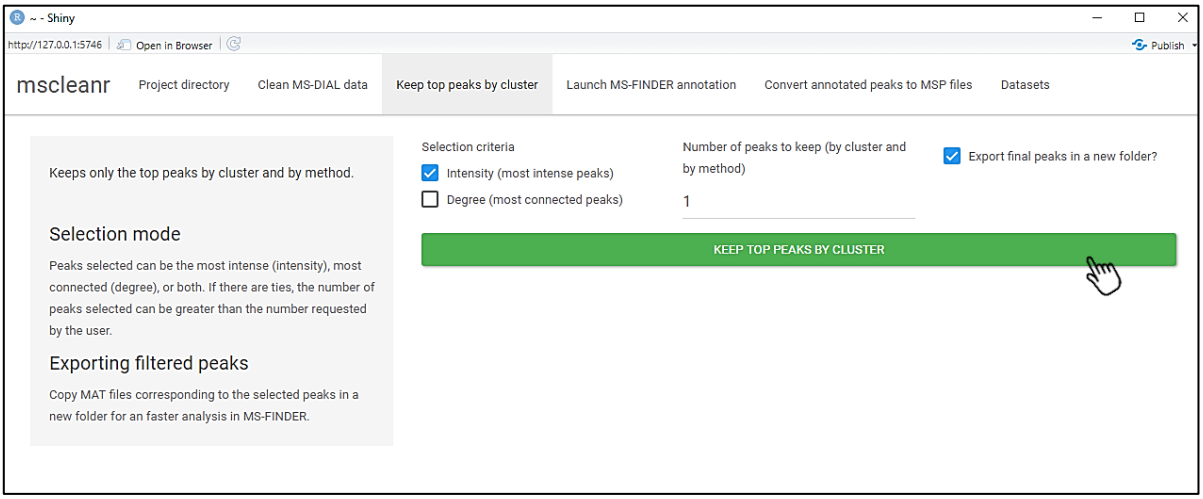

At this step, several files are created in the folder “intermediary_data”.

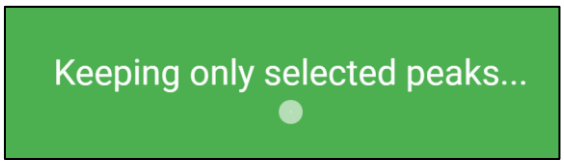
| Files | Description |
|---|---|
| Adducts_massdiff_filtered | Reference file for mass difference between regular adducts |
| Adducts_massdiff_total | Reference file for mass difference between all possible adducts |
| Adducts_detected_by_MSDIAL | Reference file for adduct ponderation of regular adducts found by MS-DIAL |
| Adducts_filtered.graphml | A graph to display feature clusters based on adducts links |
| Adducts_final_selection | Final adducts resulting from MSdial and modified after pos/neg concatenation |
| Adducts_initial.graphml | A graph to display feature clusters based on MSdial data |
| Annotated_MS-peaks-MSDial | List of annotated peaks based on the database (msp file) imported in MS-DIAL |
| Deleted_blank_ghosts | List of peaks deleted with “delete ghost peaks” |
| Deleted_blanks | List of peaks deleted with the filter “blank ratio” |
| Deleted_mz | List of peaks deleted with the filter “incorrect mass” |
| Deleted_rmd | List of peaks deleted with the filter “RMD” |
| Deleted_rsd | List of peaks deleted with the filter “RSD” |
| Links_clusters_final | List of correlation (adduct, neutral loss, msdial) between peaks in neg and pos |
| Links_post_selection | Feature links after adduct prioritization process |
| Links_pre_selection | Feature links with all adducts possibilities |
| MS_peaks-clusters.graphml | A graph of final clusters (MS-DIAL + Pearson) |
| MS_peaks-clusters_final | List of final clusters (MS-DIAL + Pearson) in both pos and neg ionization |
| MS_peaks-clusters_msdial | List of MS-DIAL clusters in both pos and neg ionization |
| parameters | List of parameter used for the cleaning |
| samples | List of samples with indication of sample name, class, file type, script class and column name |

### *Select number of retained peaks per cluster*

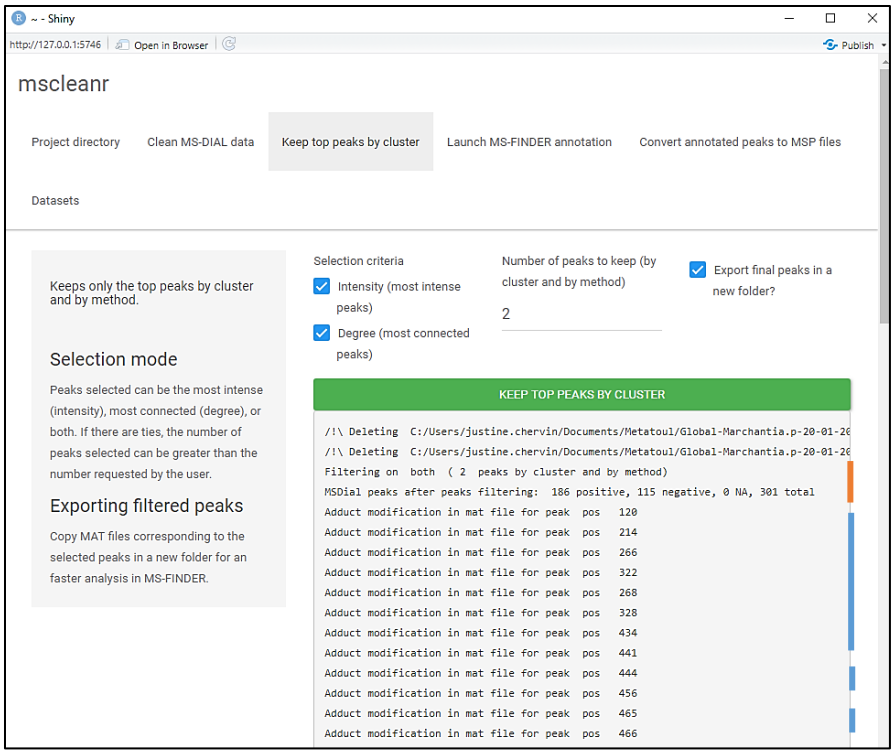
In the third tab "**Keep top peaks by cluster**" you can select the number of features you want to keep in each cluster.
This step is based on the hypothesis that in one cluster, only one unique metabolite is present. The other variables used to come from feature degeneration. Generally, this metabolite appears to be the **most intense** and/or **the most connected within the** graph (adducts, neutral loss, dimers…).

You can then choose to select as many peaks as you want and either the most intense(s) by clicking "**Intensity**", the most connected by clicking "**Degree**" or both.
We advise to select both criteria and keep 2 top peaks by cluster for further MS-finder request.




At this step, a new folder is created in both "pos" and "neg" folders named "**filtered peaks**". All .MAT files corresponding to kept peaks are copied from "peaks" folder and pasted in this new folder "filtered peaks".




**Number of kept peaks**

**Modification of adduct annotation directly in .MAT file for further MS-FINDER annotation**

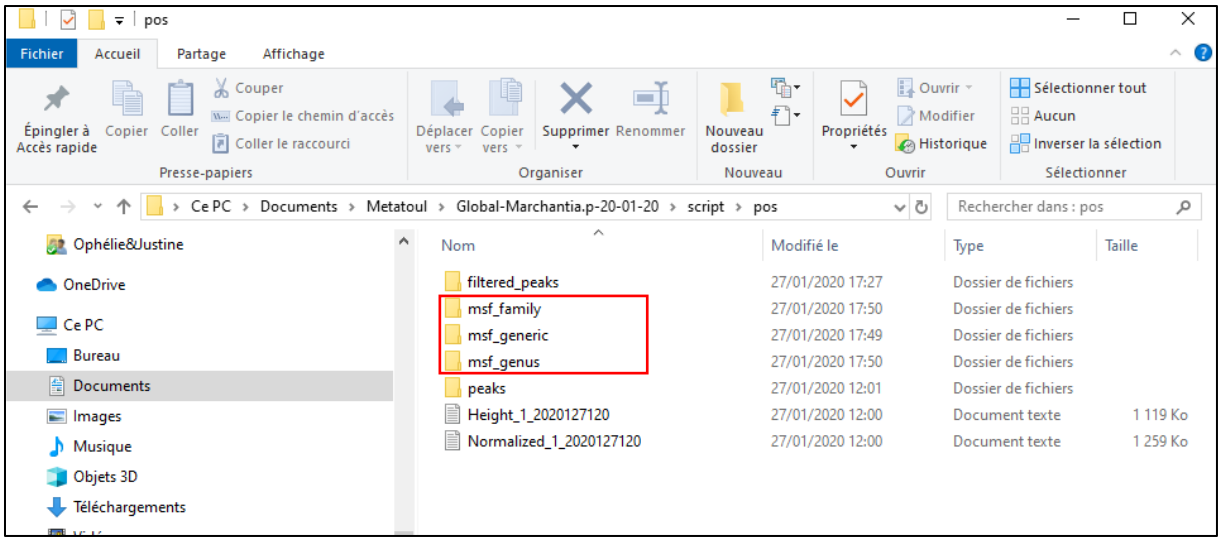
## *Interrogation of MS-FINDER*

From « **filtered peaks** » folder, interrogate MS-FINDER based on several databases of your choice (for example plant genus, plant family, generic databases from MS-FINDER, …).

*Optional: Add a "Compound_level" column within your in-house database for MS-FINDER. This level will be used for annotation ranking in the next step.*

The most **important thing** to do is to create respectively in "pos" and "neg" directories, new folders named "**msf_X**" (for example msf_genus) which correspond to the name of each database used for feature annotation. The msf_generic is mandatory and correspond to internal database in MS-Finder.

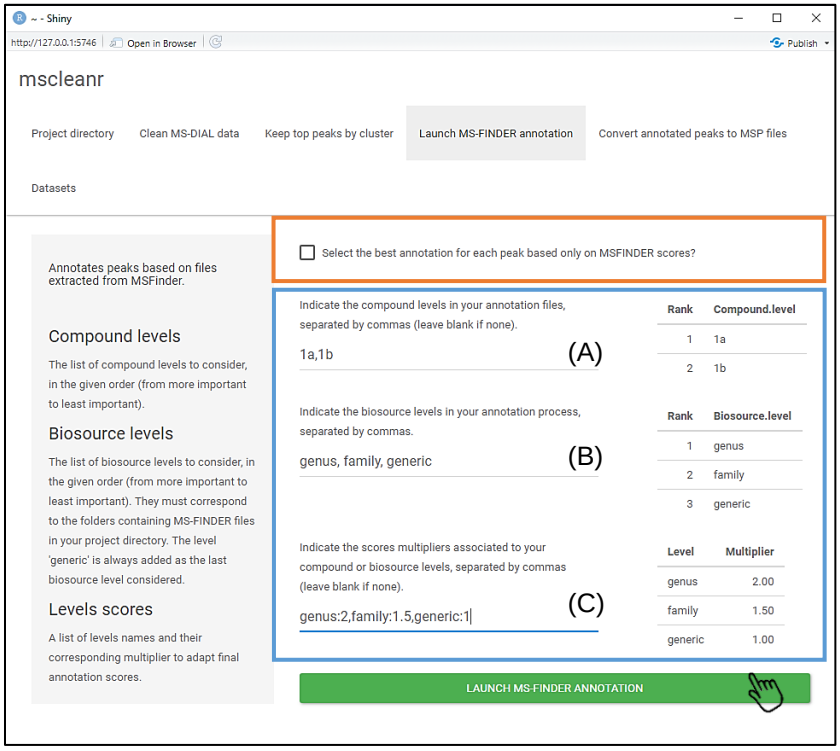
For each database used, export "structure" and "formula" as a single file in the corresponding folder.




## *Launch MS-FINDER annotation*

Once all your MS-FINDER interrogations are done and your folder “msf_X” filled with “**structure**” and “**formula**” files, go to the fourth tab called “**Launch MS-FINDER annotation”**.

This step will merge feature annotation to the dataset based either only on the score of MS-FINDER or on the prioritization of the different databases, used to indicate the more pertinent annotation.



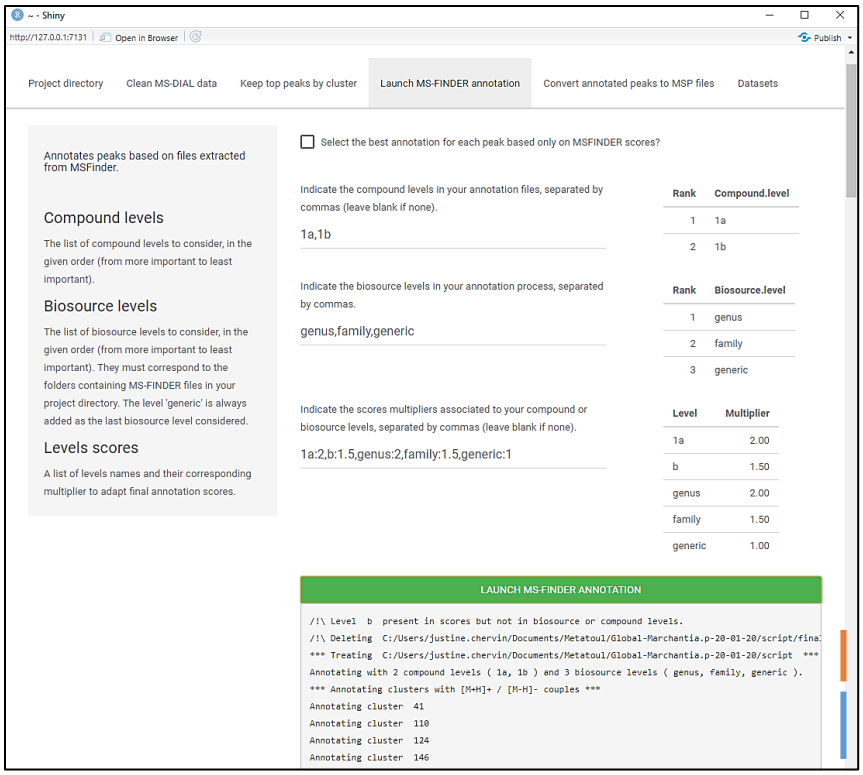

**This option is used to report the identification with the best MS-FINDER score**

**This option is used when you want to prioritize some databases.**

In (A) you have to indicate the compound level within your database

In (B) you have to order your database

In (C) you can dedicate to your database levels a multiplier to calculate new scores from MS-FINDER ones.




**Summary of compounds and biosource levels used**

**Paste of annotation in the final peak list**

Two files are created in the "**final-data**" folder:

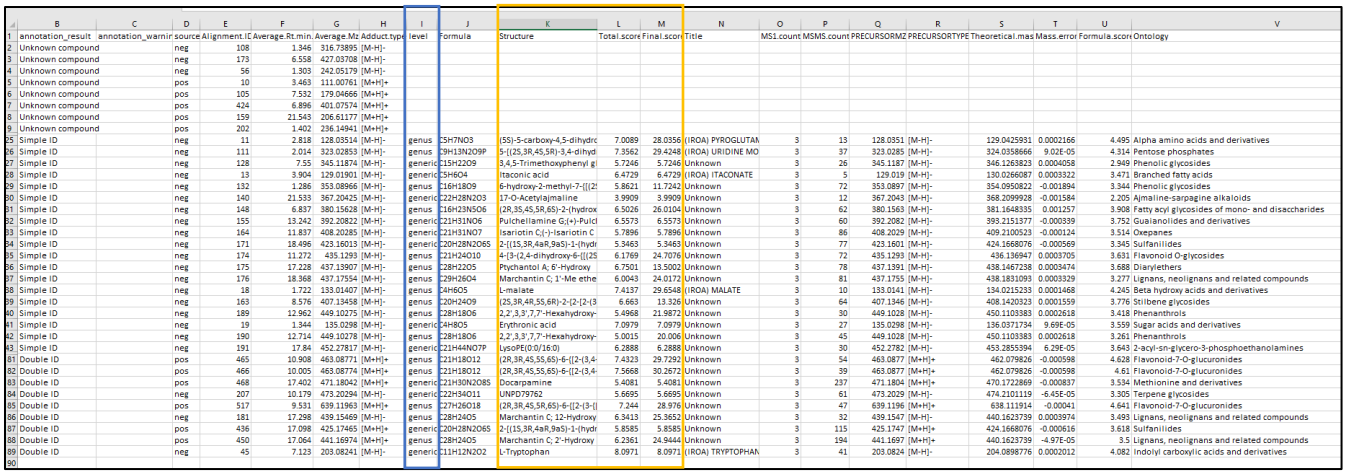
- **Annotated MS peaks cleaned** = the final peak list with annotation from MS-FINDER
- **Annotated MS peaks normalized** = the final peak list renormalized based on total peak area

The final peak list looks like as follow. Different information are available such as:

- The average m/z value;
- The average RT value;
- The annotation based on MS-FINDER interrogation on the "**Structure**" column with the associated **Total score** of MS-FINDER and **Final score** calculated from the indicated multipliers.
- The source of the annotation in the "level" column;
- The ontology of the compound; ...

The variable are also identified as:

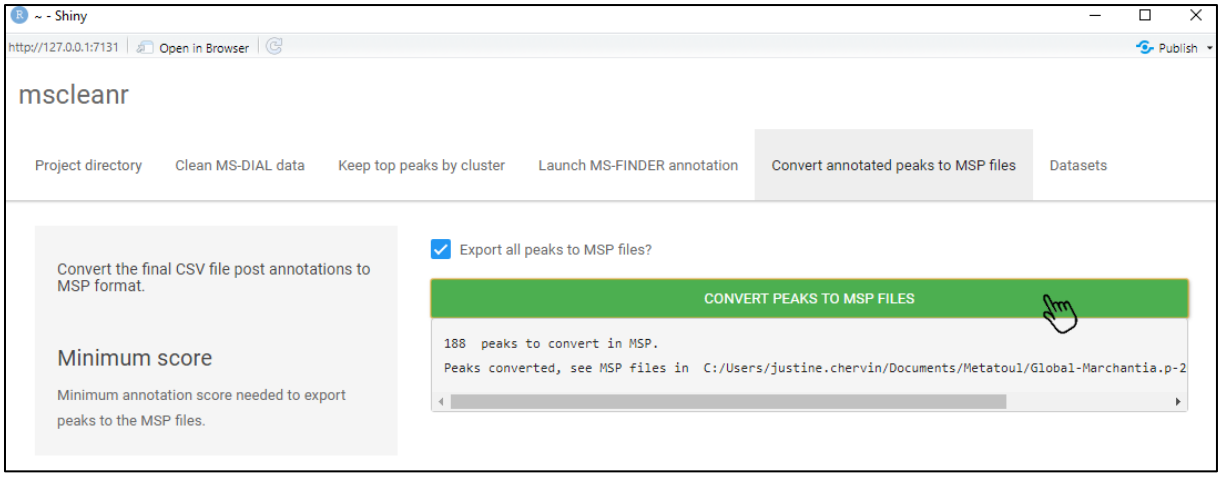
- Unknown compound = variable with no annotation
- Simple ID = based on a single feature in pos or neg mode
- Double ID =based on same annotation retrieve in pos and neg mode



| | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | annotation_result | annotation_warnir | source | Alignment.ID | Average.Rt.min. | Average.Mz | Adduct.type | level | Formula | Structure | Total.score | Final.score | Title | MS1.count | MSMS.count | PRECURSORMZ | PRECURSORTYPE | Theoretical.mas | Mass.error | Formula.score | Ontology |
| 2 | Unknown compound | | neg | 108 | 1.346 | 316.73895 | [M-H]- | | | | | | | | | | | | | | |
| 3 | Unknown compound | | neg | 173 | 6.558 | 427.03708 | [M-H]- | | | | | | | | | | | | | | |
| 4 | Unknown compound | | neg | 56 | 1.303 | 242.05179 | [M-H]- | | | | | | | | | | | | | | |
| 5 | Unknown compound | | pos | 10 | 3.463 | 111.00761 | [M+H]+ | | | | | | | | | | | | | | |
| 6 | Unknown compound | | pos | 105 | 7.532 | 179.04666 | [M+H]+ | | | | | | | | | | | | | | |
| 7 | Unknown compound | | pos | 424 | 6.896 | 401.07574 | [M+H]+ | | | | | | | | | | | | | | |
| 8 | Unknown compound | | pos | 159 | 21.543 | 206.61177 | [M+H]+ | | | | | | | | | | | | | | |
| 9 | Unknown compound | | pos | 202 | 1.402 | 236.14941 | [M+H]+ | | | | | | | | | | | | | | |
| 25 | Simple ID | | neg | 11 | 2.818 | 128.03514 | [M-H]- | genus | C5H7NO3 | (5S)-5-carboxy-4,5-dihydrc | 7.0089 | 28.0356 | (IROA) PYROGLUTAN | 3 | 13 | 128.0351 | [M-H]- | 129.0425931 | 0.0002166 | 4.495 | Alpha amino acids and derivatives |
| 26 | Simple ID | | neg | 111 | 2.014 | 323.02853 | [M-H]- | genus | C9H13N2O9P | 5-[(2S,3R,4S,5R)-3,4-dihyd | 7.3562 | 29.4248 | (IROA) URIDINE MO | 3 | 37 | 323.0285 | [M-H]- | 324.0358666 | 9.02E-05 | 4.314 | Pentose phosphates |
| 27 | Simple ID | | neg | 128 | 7.55 | 345.11874 | [M-H]- | genus | C15H22O9 | 3,4,5-Trimethoxyphenyl gl | 5.7246 | 5.7246 | Unknown | 3 | 26 | 345.1187 | [M-H]- | 346.1263823 | 0.0004058 | 2.949 | Phenolic glycosides |
| 28 | Simple ID | | neg | 13 | 3.904 | 129.01901 | [M-H]- | genus | C5H6O4 | Itaconic acid | 6.4729 | 6.4729 | (IROA) ITACONATE | 3 | 5 | 129.019 | [M-H]- | 130.0266087 | 0.0003322 | 3.471 | Branched fatty acids |
| 29 | Simple ID | | neg | 132 | 1.286 | 353.08966 | [M-H]- | genus | C16H18O9 | 6-hydroxy-2-methyl-7-{[(2S | 5.8621 | 11.7242 | Unknown | 3 | 72 | 353.0897 | [M-H]- | 354.0950822 | -0.001894 | 3.344 | Phenolic glycosides |
| 30 | Simple ID | | neg | 140 | 21.533 | 367.20425 | [M-H]- | genus | C22H28N2O3 | 17-O-Acetylajmaline | 3.9909 | 3.9909 | Unknown | 3 | 12 | 367.2043 | [M-H]- | 368.2099928 | -0.001584 | 2.205 | Ajmaline-sarpagine alkaloids |
| 31 | Simple ID | | neg | 148 | 6.837 | 380.15628 | [M-H]- | genus | C16H23NSO6 | (2R,3S,4S,5R,6S)-2-(hydrox | 6.5026 | 26.0104 | Unknown | 3 | 62 | 380.1563 | [M-H]- | 381.1648335 | 0.001257 | 3.908 | Fatty acyl glycosides of mono- and disaccharides |
| 32 | Simple ID | | neg | 155 | 13.242 | 392.20822 | [M-H]- | genus | C21H31NO6 | Puchellamine G;(+)-Pulcl | 6.5573 | 6.5573 | Unknown | 3 | 60 | 392.2082 | [M-H]- | 393.2151377 | -0.000339 | 3.752 | Guaianolides and derivatives |
| 33 | Simple ID | | neg | 164 | 11.837 | 408.20285 | [M-H]- | genus | C21H31NO7 | Isariotin C;(-)-Isariotin C | 5.7896 | 5.7896 | Unknown | 3 | 86 | 408.2029 | [M-H]- | 409.2100523 | -0.000124 | 3.514 | Oxepanes |
| 34 | Simple ID | | neg | 171 | 18.496 | 423.16013 | [M-H]- | genus | C20H28N2O6S | 2-[(1S,3R,4aR,9aS)-1-(hydr | 5.3463 | 5.3463 | Unknown | 3 | 77 | 423.1601 | [M-H]- | 424.1668076 | -0.000569 | 3.345 | Sulfanilides |
| 35 | Simple ID | | neg | 174 | 11.272 | 435.1293 | [M-H]- | genus | C21H24O10 | 4-[3-(2,4-dihydroxy-6-[[(2S | 6.1769 | 24.7076 | Unknown | 3 | 72 | 435.1293 | [M-H]- | 436.136947 | 0.0003705 | 3.631 | Flavonoid O-glycosides |
| 36 | Simple ID | | neg | 175 | 17.228 | 437.13907 | [M-H]- | genus | C28H22O5 | Ptychantol A; 6'-Hydroxy | 6.7501 | 13.5002 | Unknown | 3 | 78 | 437.1391 | [M-H]- | 438.1467238 | 0.0003474 | 3.688 | Diarylethers |
| 37 | Simple ID | | neg | 176 | 18.368 | 437.17554 | [M-H]- | genus | C29H26O4 | Marchantin C; 1'-Me ethe | 6.0043 | 24.0172 | Unknown | 3 | 81 | 437.1755 | [M-H]- | 438.1831093 | 0.0003329 | 3.277 | Lignans, neolignans and related compounds |
| 38 | Simple ID | | neg | 18 | 1.722 | 133.01407 | [M-H]- | genus | C4H6O5 | L-malate | 7.4137 | 29.6548 | (IROA) MALATE | 3 | 10 | 133.0141 | [M-H]- | 134.0215233 | 0.0001468 | 4.245 | Beta hydroxy acids and derivatives |
| 39 | Simple ID | | neg | 163 | 8.576 | 407.13458 | [M-H]- | genus | C20H24O9 | (2S,3R,4R,5S,6R)-2-[2-[2-(3 | 6.663 | 13.326 | Unknown | 3 | 64 | 407.1346 | [M-H]- | 408.1420323 | 0.0001559 | 3.776 | Stilbene glycosides |
| 40 | Simple ID | | neg | 189 | 12.962 | 449.10275 | [M-H]- | genus | C28H18O6 | 2,2',3,3',7,7'-Hexahydroxy- | 5.4968 | 21.9872 | Unknown | 3 | 30 | 449.1028 | [M-H]- | 450.1103383 | 0.0002618 | 3.418 | Phenanthrols |
| 41 | Simple ID | | neg | 19 | 1.344 | 135.0298 | [M-H]- | genus | C4H8O5 | Erythronic acid | 7.0979 | 7.0979 | Unknown | 3 | 27 | 135.0298 | [M-H]- | 136.0371734 | 9.69E-05 | 3.559 | Sugar acids and derivatives |
| 42 | Simple ID | | neg | 190 | 12.714 | 449.10278 | [M-H]- | genus | C28H18O6 | 2,2',3,3',7,7'-Hexahydroxy- | 5.0015 | 20.006 | Unknown | 3 | 45 | 449.1028 | [M-H]- | 450.1103383 | 0.0002618 | 3.261 | Phenanthrols |
| 43 | Simple ID | | neg | 191 | 17.84 | 452.27817 | [M-H]- | genus | C21H44NO7P | LysoPE(0:0/16:0) | 6.2888 | 6.2888 | Unknown | 3 | 30 | 452.2782 | [M-H]- | 453.2855394 | 6.29E-05 | 3.643 | 2-acyl-sn-glycero-3-phosphoethanolamines |
| 81 | Double ID | | pos | 465 | 10.908 | 463.08771 | [M+H]+ | genus | C21H18O12 | (2R,3R,4S,5S,6S)-6-[[2-(3,4- | 7.4323 | 29.7292 | Unknown | 3 | 54 | 463.0877 | [M+H]+ | 462.079826 | -0.000598 | 4.628 | Flavonoid-7-O-glucuronides |
| 82 | Double ID | | pos | 466 | 10.005 | 463.08774 | [M+H]+ | genus | C21H18O12 | (2R,3R,4S,5S,6S)-6-[[2-(3,4- | 7.5668 | 30.2672 | Unknown | 3 | 39 | 463.0877 | [M+H]+ | 462.079826 | -0.000598 | 4.61 | Flavonoid-7-O-glucuronides |
| 83 | Double ID | | pos | 468 | 17.402 | 471.18042 | [M+H]+ | genus | C21H30N2O8S | Docarpamine | 5.4081 | 5.4081 | Unknown | 3 | 237 | 471.1804 | [M+H]+ | 470.1722869 | -0.000837 | 3.534 | Methionine and derivatives |
| 84 | Double ID | | pos | 207 | 10.179 | 473.20294 | [M-H]- | genus | C22H34O11 | UNPD79762 | 5.6695 | 5.6695 | Unknown | 3 | 61 | 473.2029 | [M-H]- | 474.2101119 | -6.45E-05 | 3.305 | Terpene glycosides |
| 85 | Double ID | | pos | 517 | 9.531 | 639.11963 | [M+H]+ | genus | C27H26O18 | (2R,3R,4S,5R,6S)-6-[[2-[3-(] | 7.244 | 28.976 | Unknown | 3 | 47 | 639.1196 | [M+H]+ | 638.111914 | -0.00041 | 4.641 | Flavonoid-7-O-glucuronides |
| 86 | Double ID | | neg | 181 | 17.298 | 439.15469 | [M-H]- | genus | C28H24O5 | Marchantin C; 12-Hydroxy | 6.3415 | 25.3652 | Unknown | 3 | 32 | 439.1547 | [M-H]- | 440.1623739 | 0.0003974 | 3.493 | Lignans, neolignans and related compounds |
| 87 | Double ID | | pos | 436 | 17.098 | 425.17465 | [M+H]+ | genus | C20H28N2O6S | 2-[(1S,3R,4aR,9aS)-1-(hydr | 5.8585 | 5.8585 | Unknown | 3 | 115 | 425.1747 | [M+H]+ | 424.1668076 | -0.000616 | 3.618 | Sulfanilides |
| 88 | Double ID | | pos | 450 | 17.064 | 441.16974 | [M+H]+ | genus | C28H24O5 | Marchantin C; 2'-Hydroxy | 6.2361 | 24.9444 | Unknown | 3 | 194 | 441.1697 | [M+H]+ | 440.1623739 | -4.97E-05 | 3.5 | Lignans, neolignans and related compounds |
| 89 | Double ID | | neg | 45 | 7.123 | 203.08241 | [M-H]- | genus | C11H12N2O2 | L-Tryptophan | 8.0971 | 8.0971 | (IROA) TRYPTOPHAN | 3 | 41 | 203.0824 | [M-H]- | 204.0898776 | 0.0002012 | 4.082 | Indolyl carboxylic acids and derivatives |
| 90 | | | | | | | | | | | | | | | | | | | | | |

## *Export peaks as .msp files*

In the fifth tab "**Convert annotated peaks to MSP files**", you will be able to create two .msp files named "peaks-neg.msp" and "peaks-pos.msp" in the folder "final_data". All peaks can be converted, or user can choose a scoring threshold based on multiplied MSfinder score. One metadata file per ionization mode is also created containing annotation results and average peak area of each class.




These two files could then be imported in MetGem software or GNPS facility to create mass spectral similarity networks.