MS-FINDER tutorial:

Universal program for metabolite annotation

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1. Prerequisite:

Software installation:

MS-DIAL last version**:** http://prime.psc.riken.jp/compms/msdial/main.html

MS-FINDER last version: http://prime.psc.riken.jp/compms/msfinder/main.html

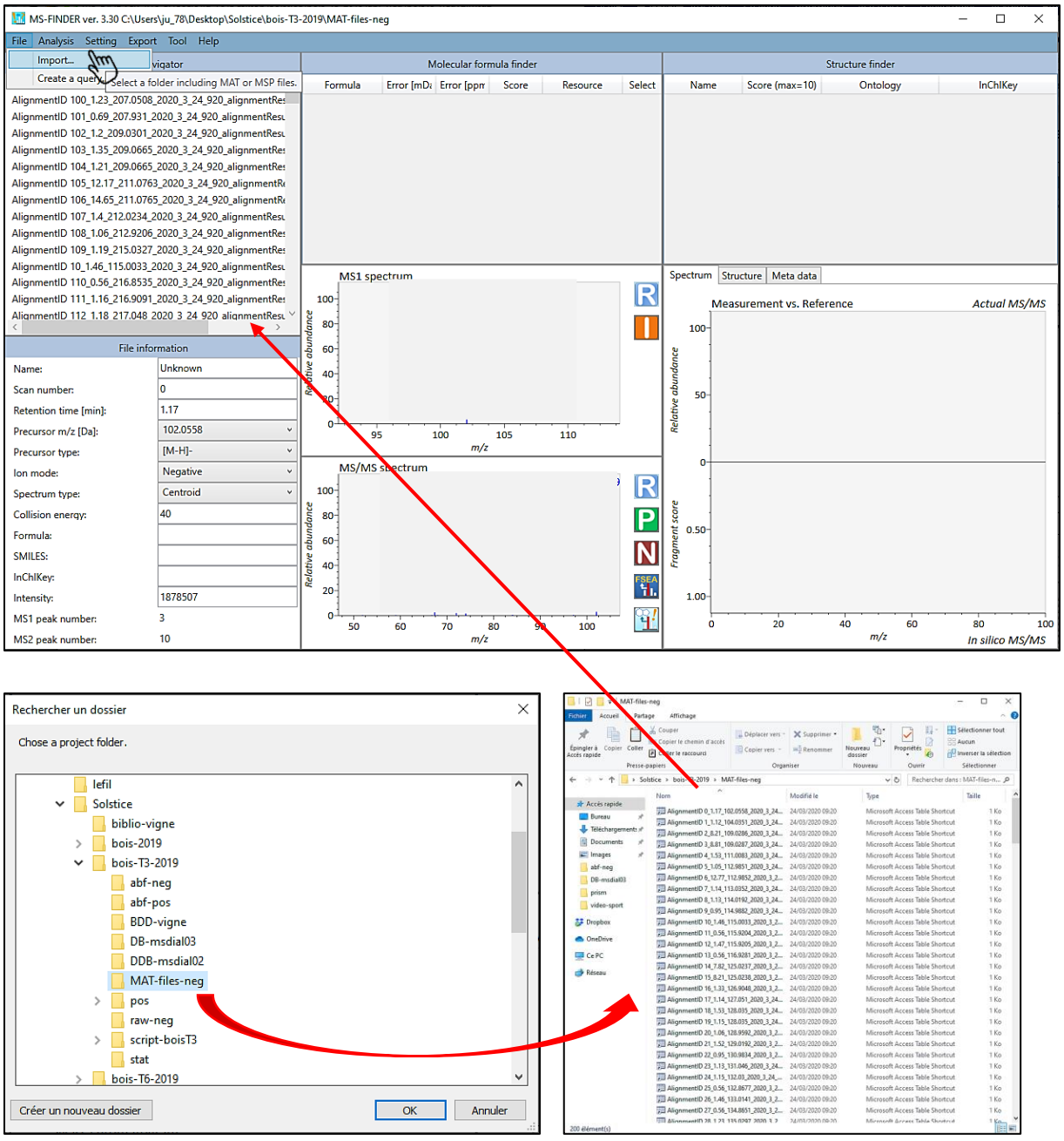
MSFileReader for Thermo data: see the following link which explains how to process http://fields.scripps.edu/rawconv/

2. Importing data into MS-FINDER

a. From a folder containing .MAT files

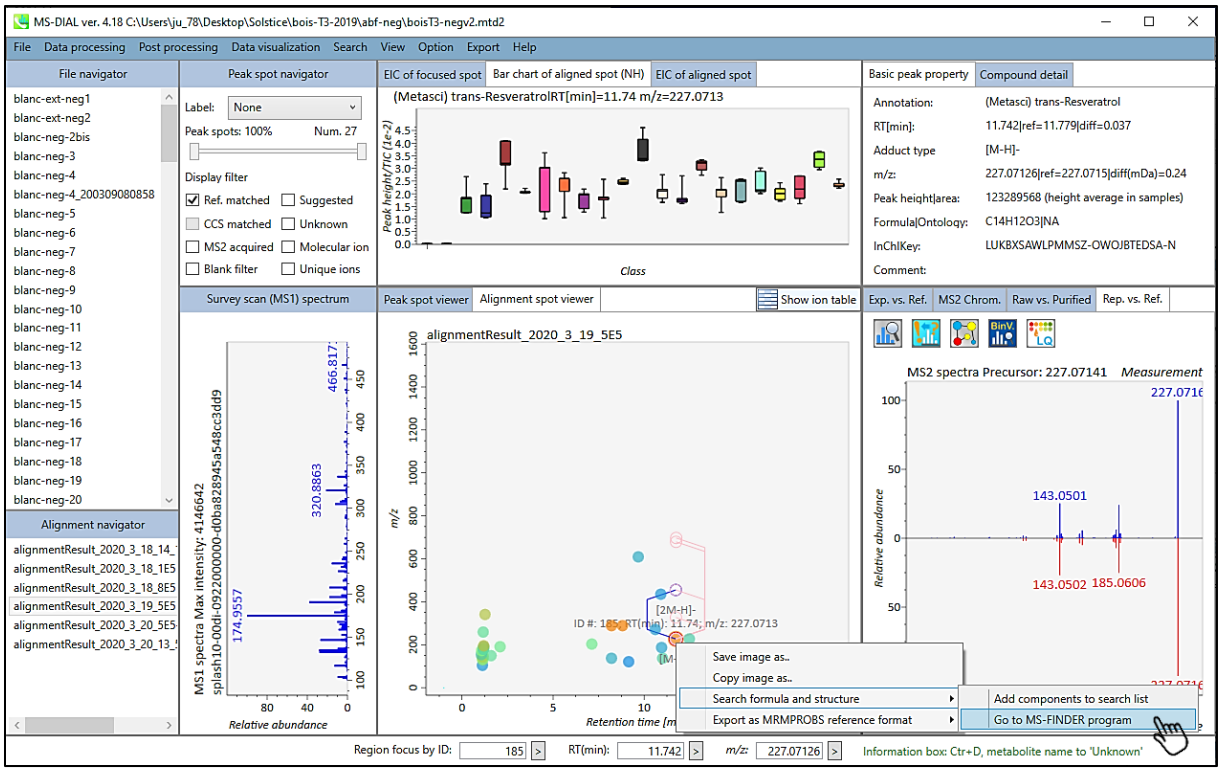
On the MS-DIAL interface, right-click on one point, then on the "**Search formula and structure**" menu select "**Add component to search list**" and export « **all peaks » as .MAT files** for further identification purpose with MS-FINDER.

Then on the MS-FINDER interface, in the “**File**” menu, click on “**Import**” and select the folder containing the .MAT files previously exported from MS-DIAL. The files imported are then visible in the top left-hand corner rectangle of the interface.

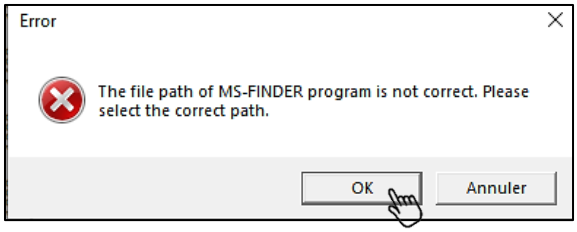


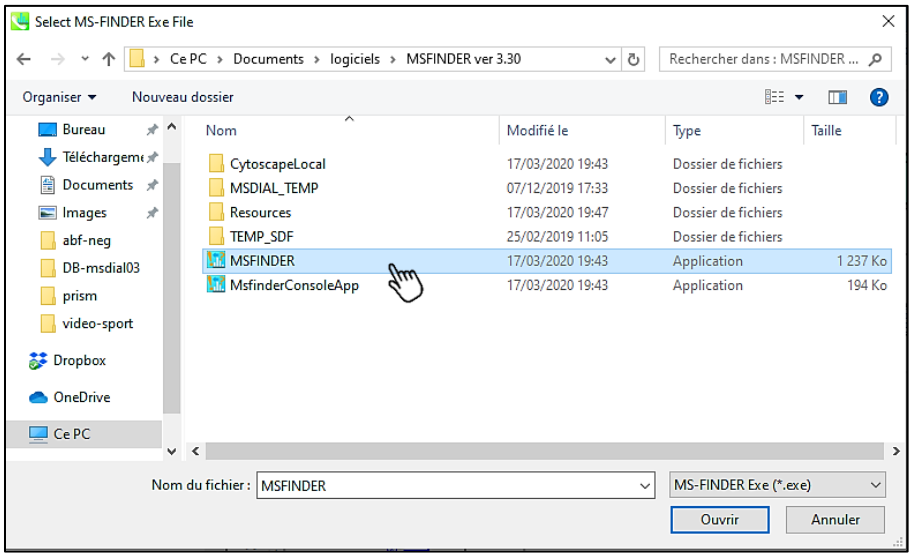
b. Directly into the MS-DIAL interface

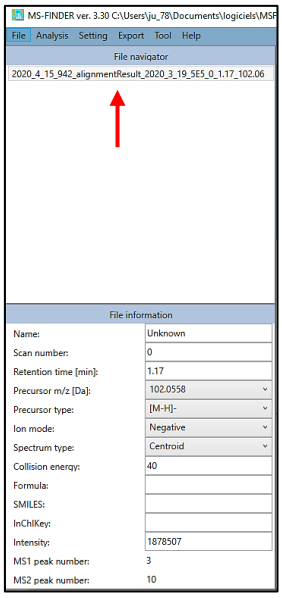
You can call MS-FINDER directly from Ms-DIAL. By clicking on one point, then on the “**Search formula and structure**” menu select “**Go to MS-FINDER program**” and export « **all peaks » as .MAT files** for further identification purpose with MS-FINDER.



The following error window appears and you have to click on “OK” and go find the application MSFINDER.exe in your computer and open it.



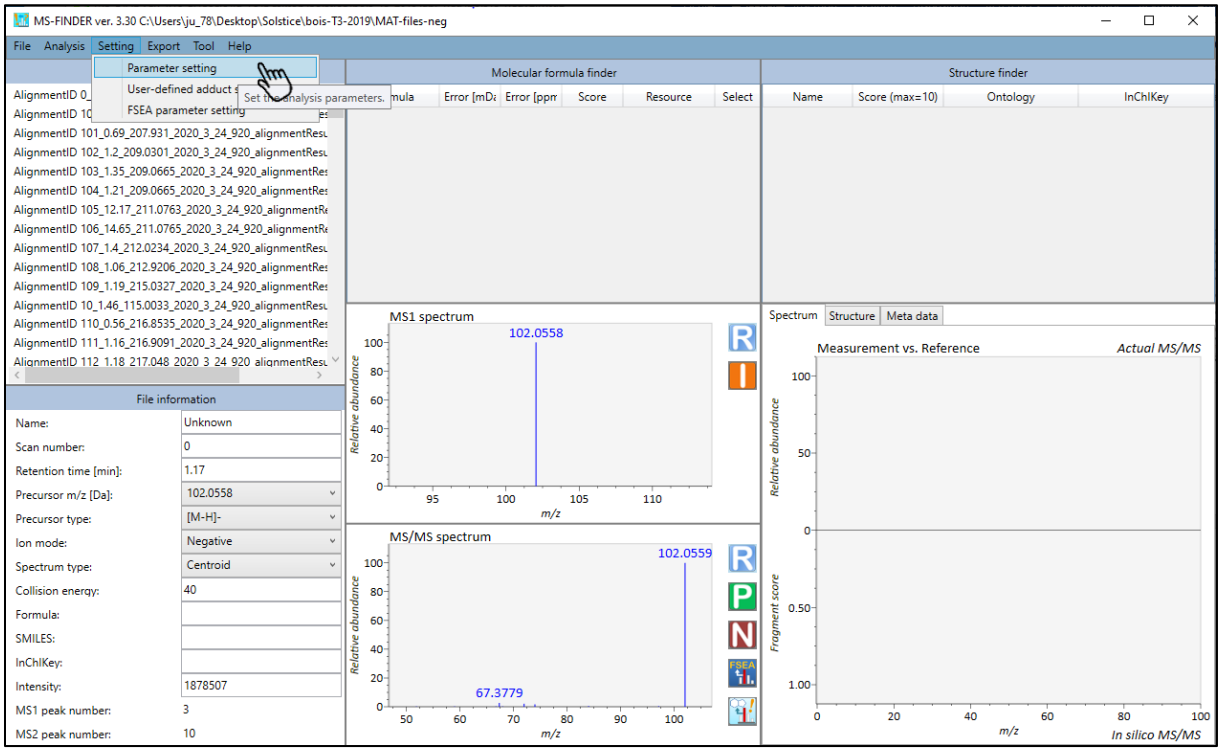




The MS-FINDER interface will then start with the importation of the peak you wanted to interrogate.

3. Define search parameters

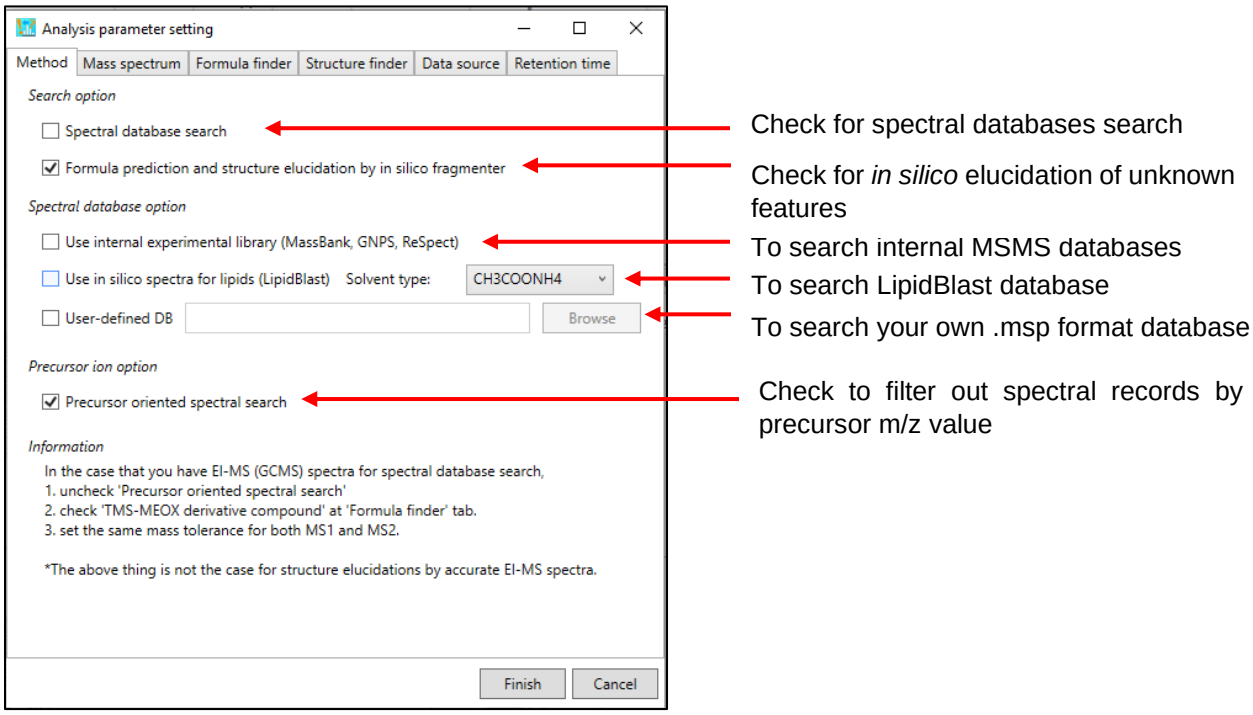
On the menu "**Setting**", select "**Parameter setting**" to define all the parameters of formula and structure search.



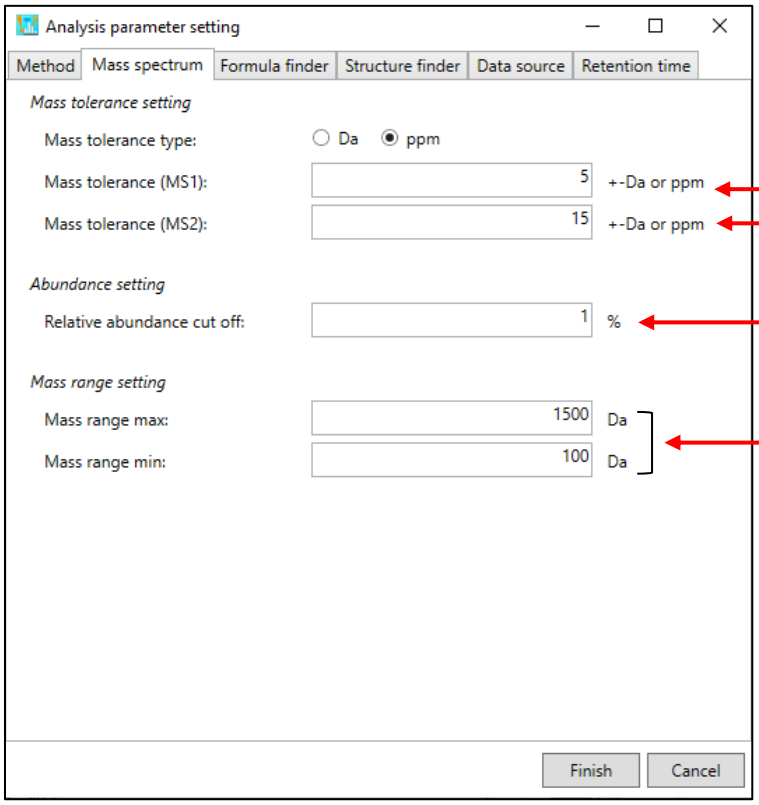
a. Method parameter

MS-FINDER proposes two options to annotate compounds:

- By spectral databases (*.msp)
- By formula and structure finder programs based on *in silico* fragmenter



b. Mass spectrum parameter



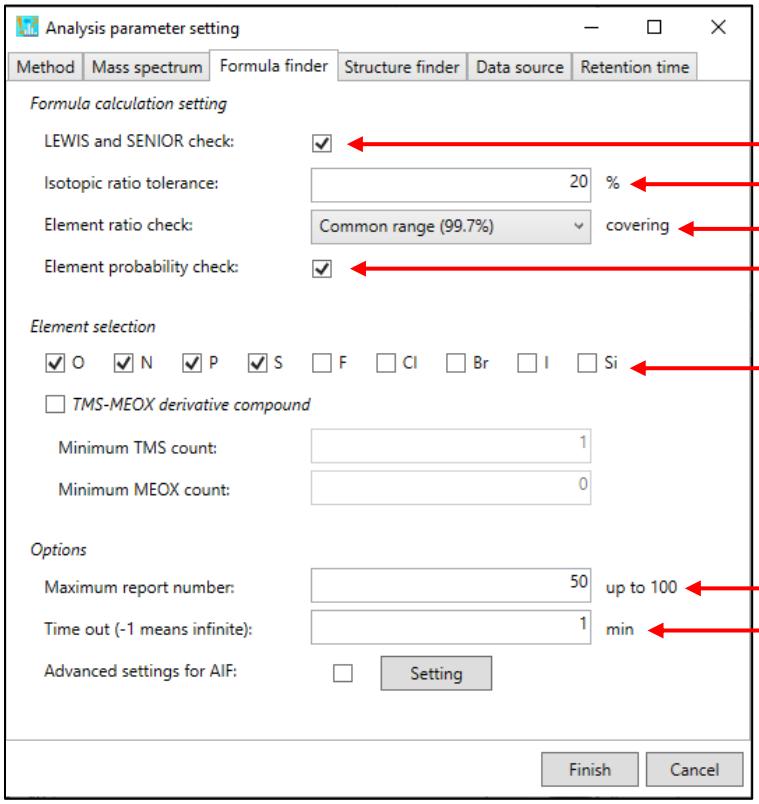
Set the mass tolerance for formula search

Set the mass tolerance matching experimental and reference fragments

Set an abundance cut off to remove noise

Indicate the range of MS1 mass

c. Formula finder parameter



To respect the valence rules of elements

To calculate the isotopic score

To respect satisfactory element ratios

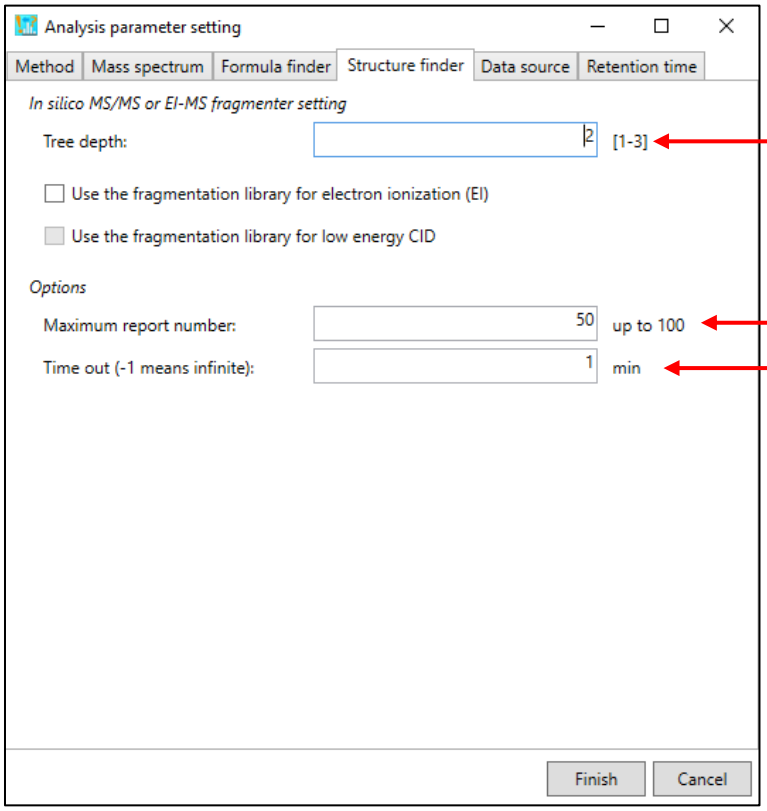
To respect heuristic rules (Seven Golden Rules)

Select the pertinent elements

Number of reported formula

Define a time-out to accelerate the search process

d. Structure finder parameter

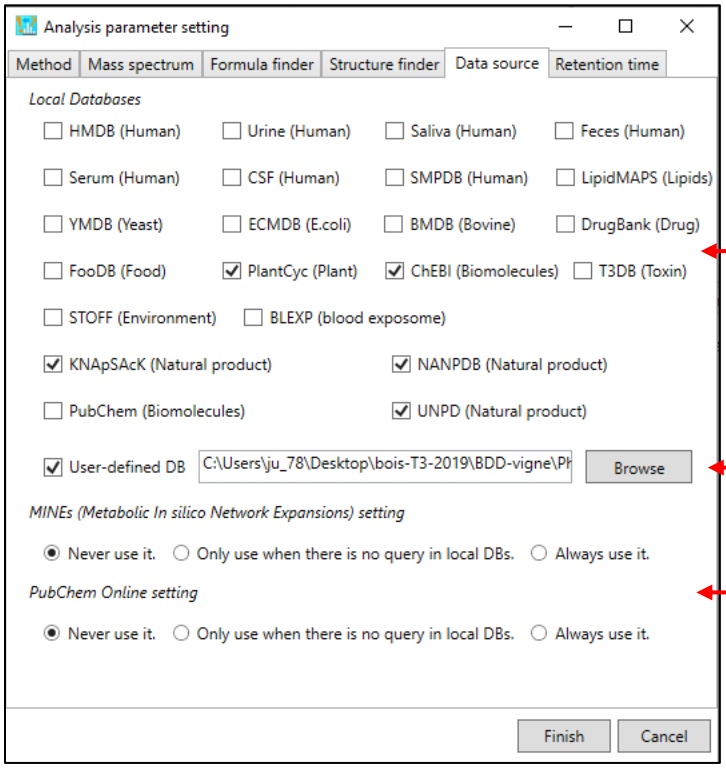


To restrict *in silico* cleavages. With 2, you generate fragments until product ions of a product ion

Number of reported formula

Define a time-out to accelerate the search process

e. Data source parameter



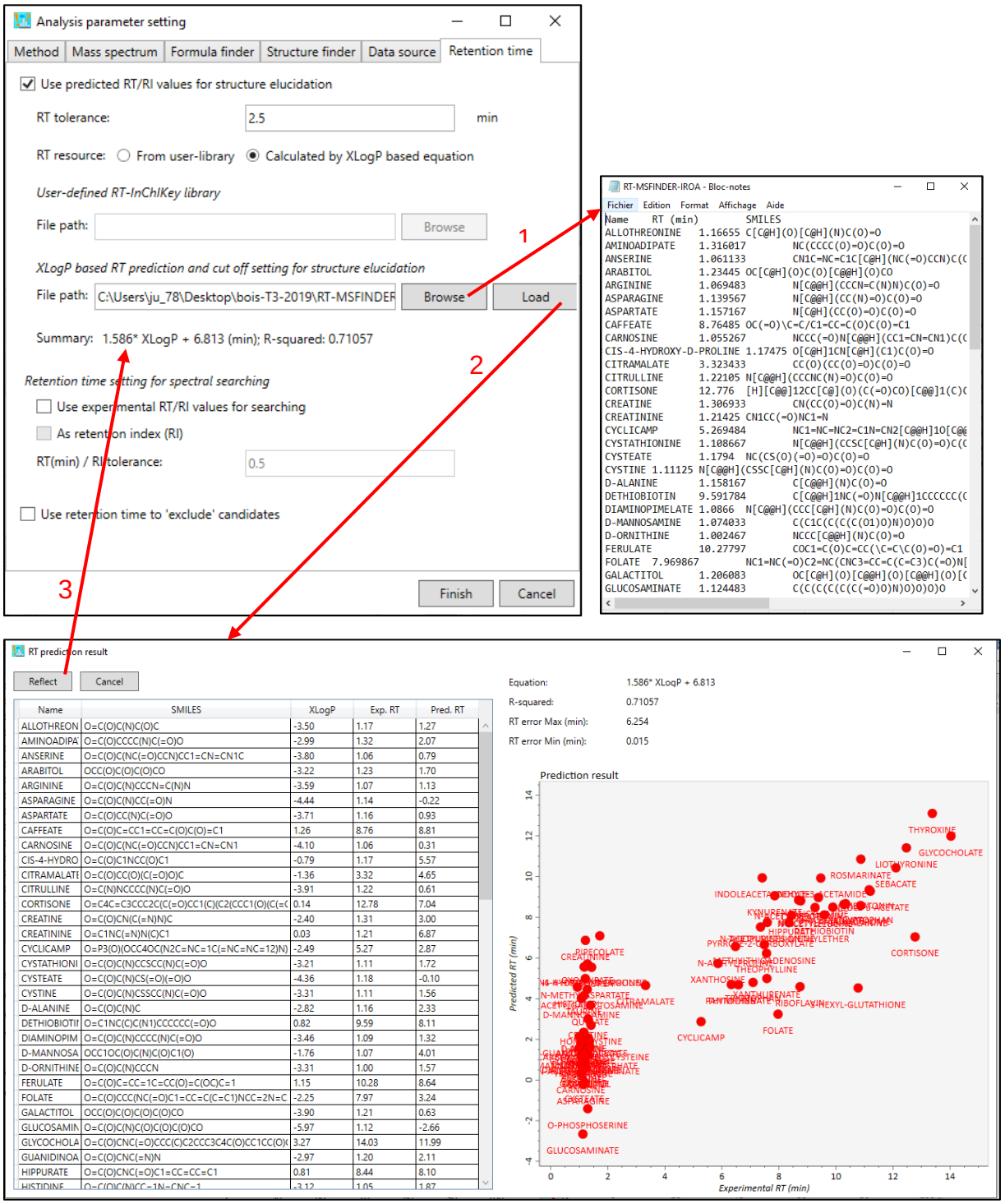
Select suitable databases among the 22 proposed local databases

Define our own text format database for *in silico* elucidation of unknown features

You can choose to widen your search by selecting MINEs and PubChem databases

f. Retention time parameter

You can add a tab-delimited text format file containing retention time of standards to bring a supplementary filter from structure elucidation. The three required columns should contain in the order the “metabolite name”, the “retention time” in min and the “smiles”. Check “**Use predicted RT/RI values**” and “**Calculated by XLogP based equation**”. Then load the text file and click on “**Reflect**” and the equation will appear.



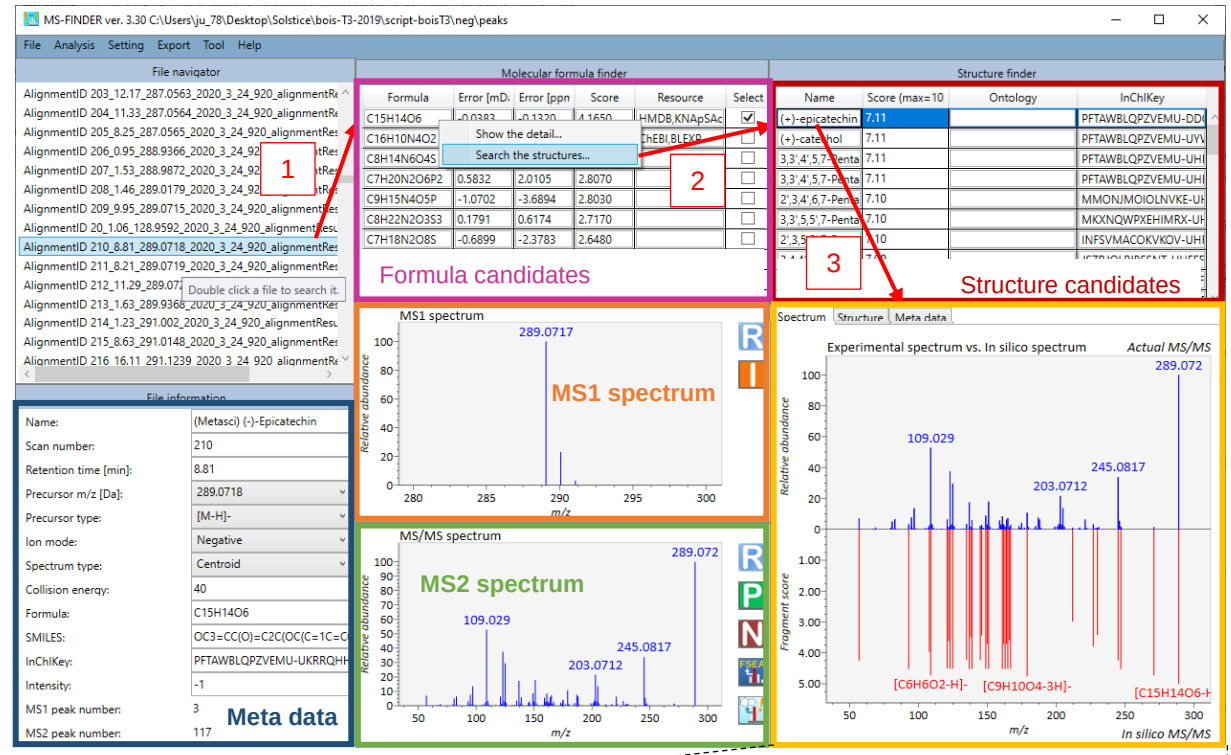
4. Annotation results

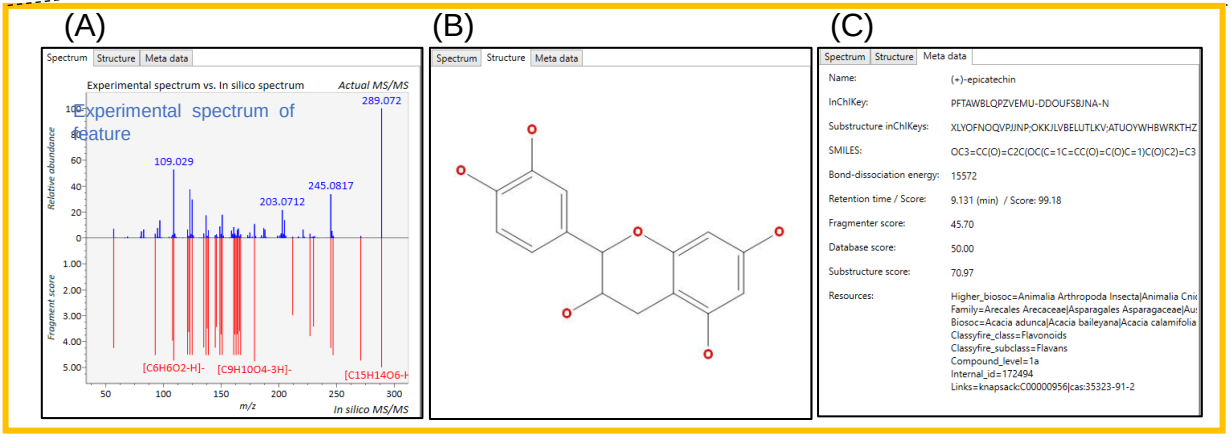
a. Single analysis

[1] For formula prediction, double-click on one file in the "**File navigator**". Check the metadata well first especially the "**Precursor type**".

[2] For structure prediction, select the formula you want to determine the structure, right-click on the formula result table and select "**Search the structures**"

[3] You obtain a ranking of structure candidates. Select one to see the experimental spectrum confronted to the *in silico* one of the reference (A), the structure (B) or some meta data (C).



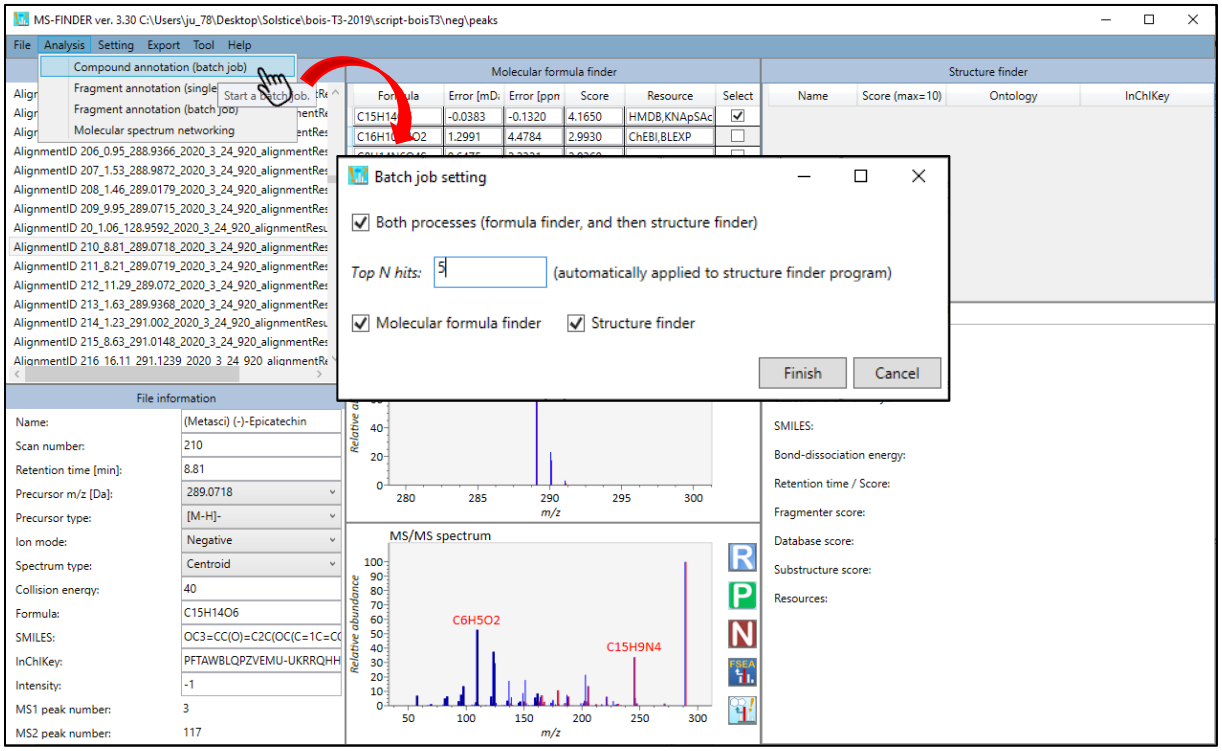


Show raw data spectrum
Show the isotopic ions

Show product ion
Show neutral loss ion
Show the result of fragment set enrichment analysis
Show assigned substructures

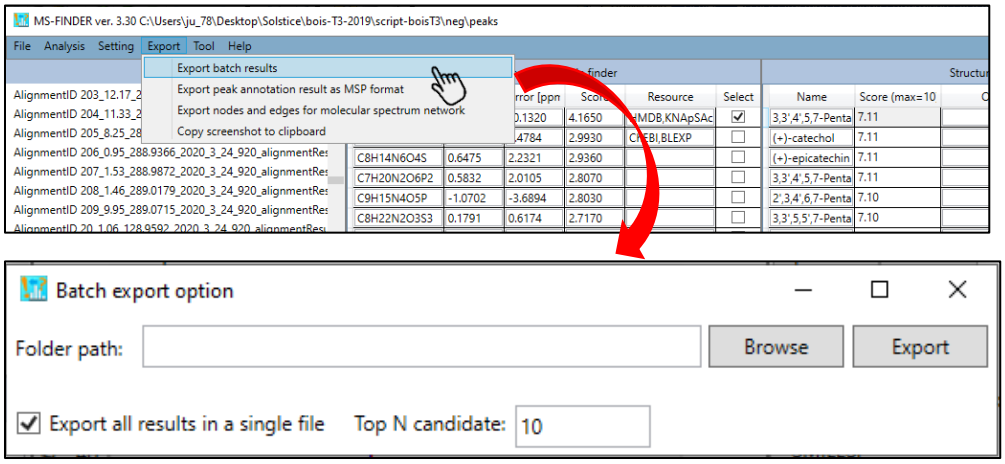
b. Batch analysis

You can also perform a batch analysis for both formula and structure searches. On the "**Analysis**" menu, select "**Compound annotation (Batch job)**". You can process both formula and structure finders by selecting "Both processes" or choose to do them separately. Fix the number of Top formula hits to be processed by structure finder. *More than 2 hits greatly increase processing time.*

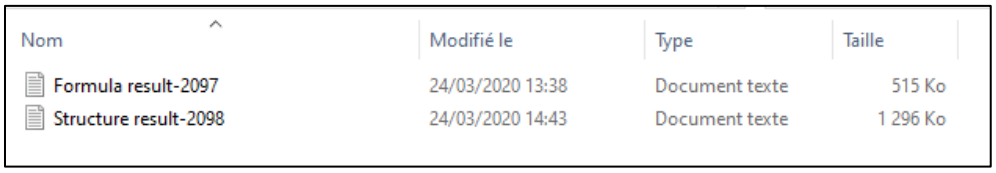


5. Export results

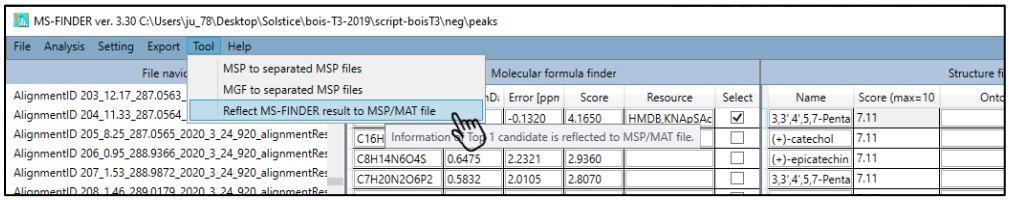
You can export Formula and Structure results on the menu "**Export**" by selecting "**Export batch results**". Define the folder of arrival and fix the number of Top candidates you want to export.



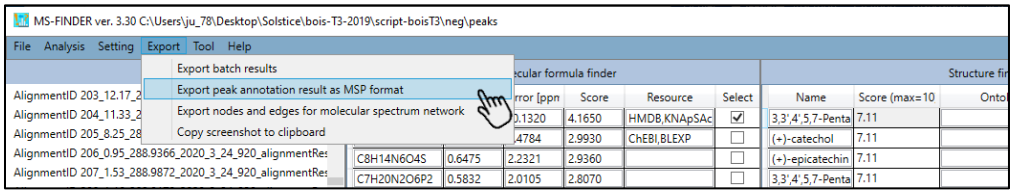
Two text format files will then be created:



You can also choose to export results as .msp file. On the "**Tool**" menu select "**Reflect MS-FINDER results to MSP/MAT file**".



Then on the "**Export**" menu, select "**Export peak annotation result as MSP format**". Choose the folder of arrival and name your file.



Follow the MS-CleanR tutorial to merge results from MS-DIAL and MS-FINDER.