MestReNova Guide: MS Plugin

1. Preface

This user guide is based on the modern interface settings of the MestReNova software and is divided into following parts:

A. Basic steps: Opening data files and generate chromatograms

B. Process Spectra and Chromatograms: Zoom in and out, create MS spectra with and without background subtraction, picking peaks in chromatograms and generate peak tables for masses and chromatographic peaks.

C. Deconvolution of Multiply Charged Ions: They are often observed in ESI-MS of large molecules. After deconvolution the spectrum of the related uncharged molecules is calculated.

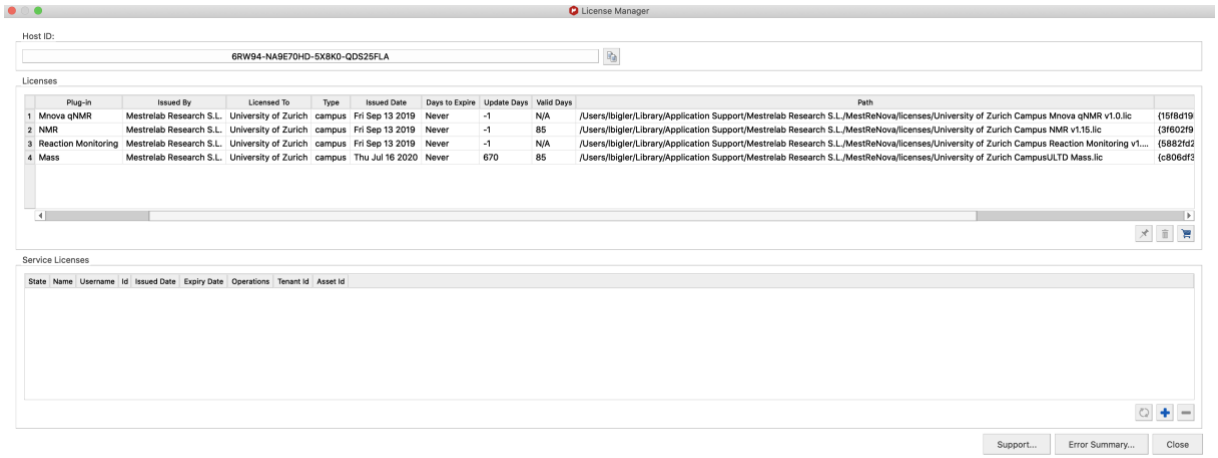
D. *M/z* value to Molecular Formulas: provide molecular formulas for measured masses including mass deviation in ppm compared to corresponding theoretical values.

E. Predict Molecular Masses and Isotopic Distributions: Simulation of a mass spectrum from its molecular formula.

F. Screening LC/GC-MS data for a compound of interest: Screening a GC- or LC-MS acquisition for a component with a definite chemical formula.

Furthermore, **UV chromatograms** can be generated from the UV data that was recorded during the LC-MS acquisition (see Chapter 3).

Before you start, check that the Mass Plugin has been installed:



The is a site license available at the chemistry department (for MS as for NMR data processing). Please ask our IT support if you need support for the installation.

For data access refer to the following:

LC-MS data can be accessed remotely in the UZH network (switch the VPN on if required). Access to your data with explorer (Win) or Mac (finder) using following link:

Windows: \ \CHEM-BIG-INT1.d.uzh.ch\Data_Synapt_OpenAccess
Mac: smb://CHEM-BIG-INT1.d.uzh.ch/Data_Synapt_OpenAccess

Login with your UZH shortname as well as your personal password. The shortname must be typed as followed.

uzh\"shortname"

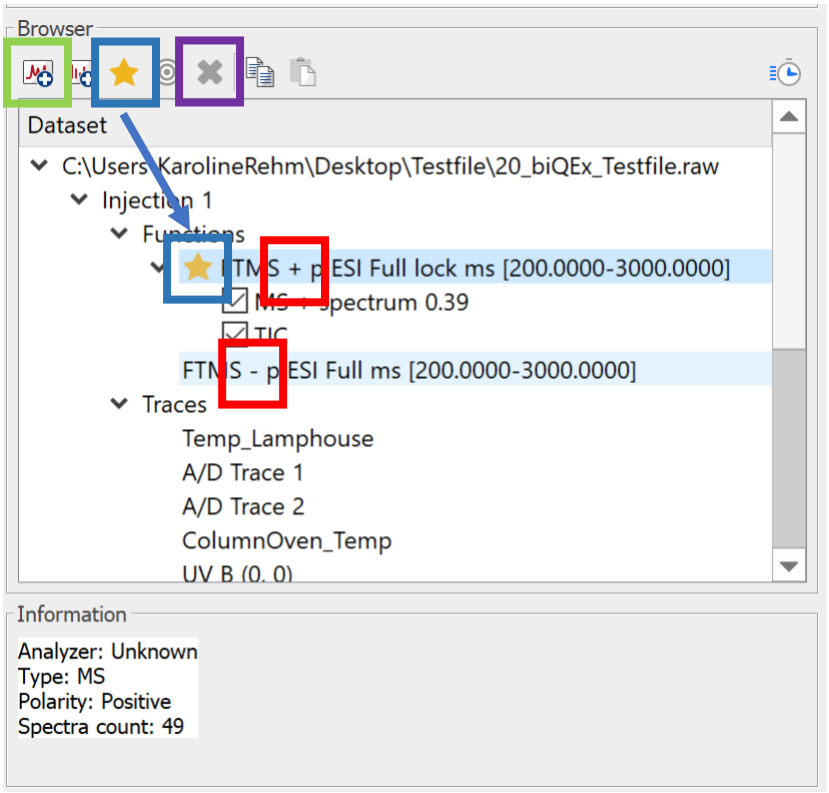
2. Processing LC-MS Data (locally, so download your data from the server first)

A. Basic Steps

➢ Open MestReNova.

➢ Go to **File/Page Setup/Orientation** and change the page orientation to portrait if you wish.

➢ Go to **Data Browser** to open any file in the folder containing raw data (Windows) or drag&drop (recommended for Mac users) the file from Explorer (Finder) into Mnova. Mnova will automatically convert your data and pick peaks. An MS browser should open at the beginning:



➢ Choose the ionization mode suitable for the compound that was measured (plus or minus (red box)). Set the desired ionization mode as active using the star symbol (blue box). For each ionization mode, you can generate chromatograms by clicking on “open new chromatogram” (green box). You can delete all unnecessary chromatograms and MS spectra by pressing “delete” (violet box).

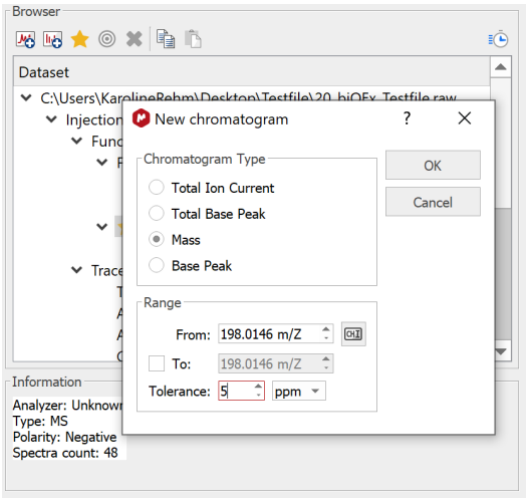
Available chromatogram options are:

- **Total ion current** (TIC): Gives you a chromatogram over all masses.

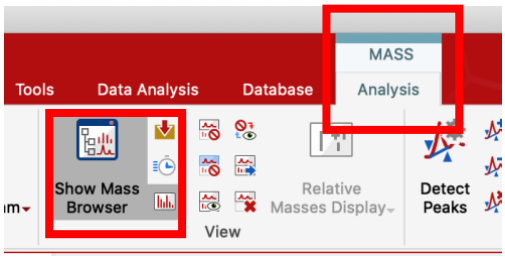
- **Total Base Peak** (BIC): Gives you a chromatogram similar to the TIC chromatogram but the background signals are reduced based on the most intense mass peaks in each scan event. Thus, a cleaner looking and (in most cases) more informative chromatogram is depicted. The BPC layout is recommended for LC-MS runs.

- **Mass**: Generate a chromatogram for a single mass (extracted ion chromatogram (EIC)). In the generated EIC, the mass tolerance should not be too large to ensure specificity but also not too small to avoid disjointed chromatographic peaks. A value of 5 ppm is a good starting point for a high-resolution EIC (chromatogram specific to a given chemical formula).

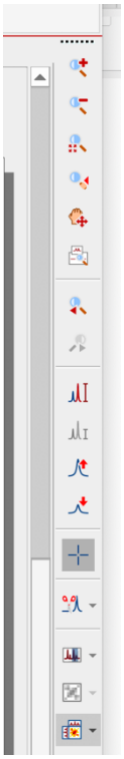
- **Base Peak**: Generate a base peak chromatogram over a limited mass range.



➢ Close the MS Browser when finished. Click 'Analysis' & 'Show Mass Browser' (see below), when you wish to reopen it and add or delete new chromatograms.



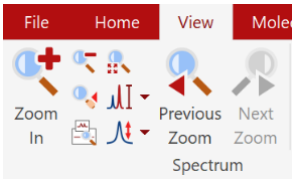
B. Process Spectra and Chromatograms

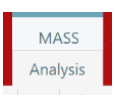


Preliminary remark: The most important commands that will be presented below are always displayed in a command bar on the right side of the window. If desired, this bar can be move to the top or the left side of the window.

➢ As an alternative, use **View** for selecting tools to zoom in and out of your spectrum. As a second alternative, you can use the following keyboard shortcuts:

"Z": Zoom in; "shift+Z": Zoom out; "Esc": End zoom mode

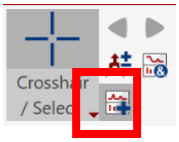


➢ MS processing tools are available under **Mass Analysis** 

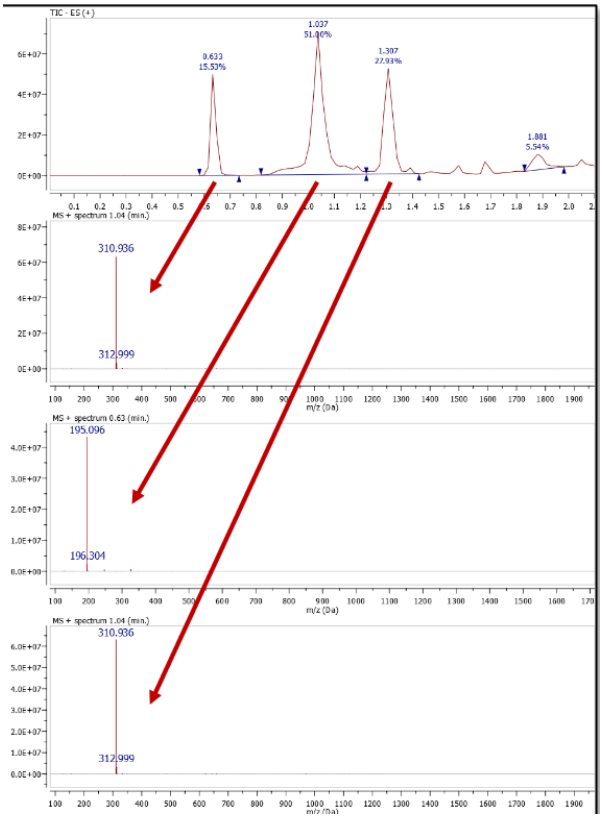
of the toolbar.

➢ Press the **Crosshair tool** 

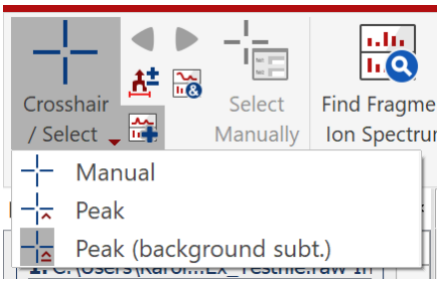
to switch to the crosshair cursor and click on the TIC to display the mass spectrum at a single retention time or click-and-drag to display co-added spectra (integration over a peak range).

➢ Press "**Append**" 
to change to appending mode if you want to display multiple mass spectra. Afterwards, click on the desired retention time (range) of the chromatogram to add a second mass spectrum.

➢ Additional tools appear by using **right-click button** of your mouse.



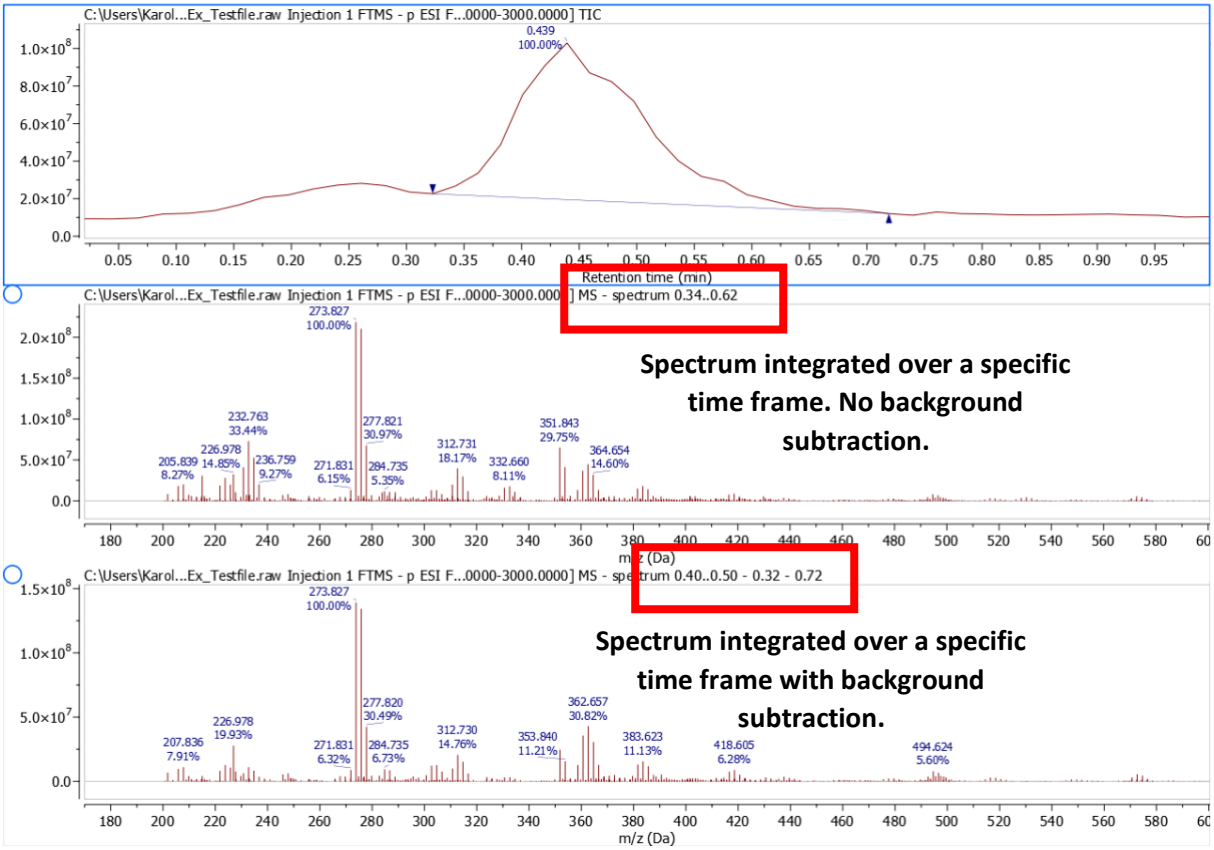
➢ Choose the Crosshair/Select drop-down menu to display mass spectra in different ways:



- **Manual mode**: Click to display a single MS, or click-and-drag to co-add multiple MS (default)

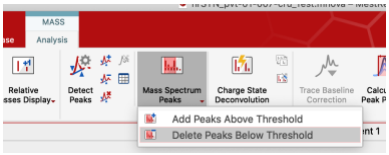
- **Peak mode**: Click on a peak to display the co-added MS within the peak range

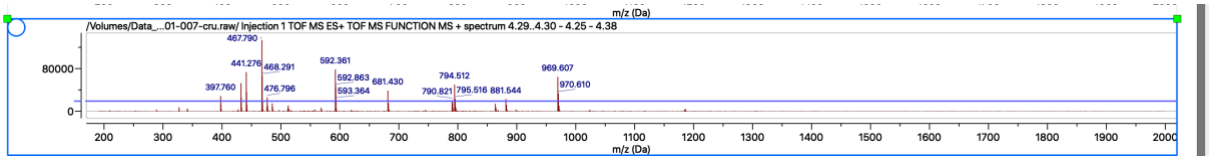
- **Peak (Background subtraction) mode:** Click on a peak to display spectra including background subtraction (first and last scan of the marked peak range (▼__▲). By this, all background signals from e.g. the solvent are removed. To see if a background was subtracted, see the MS description:



➢ In this tool bar you can add/subtract chromatographic peaks of the LC-run. By defining a peak, the generation of a mass spectrum becomes easier as no manual integration is necessary. Different modes are available: Manually add or delete peaks, clear all peaks and detect all peaks automatically by the software.

➢ You can generate a peak list for both LC chromatograms and MS spectra by choosing "**Show peaks table**"  while the desired spectrum is activated. In order to reduce the size of your list, define a threshold first by selecting 'Delete Peaks Below Threshold' over the entire mass range (blue line).

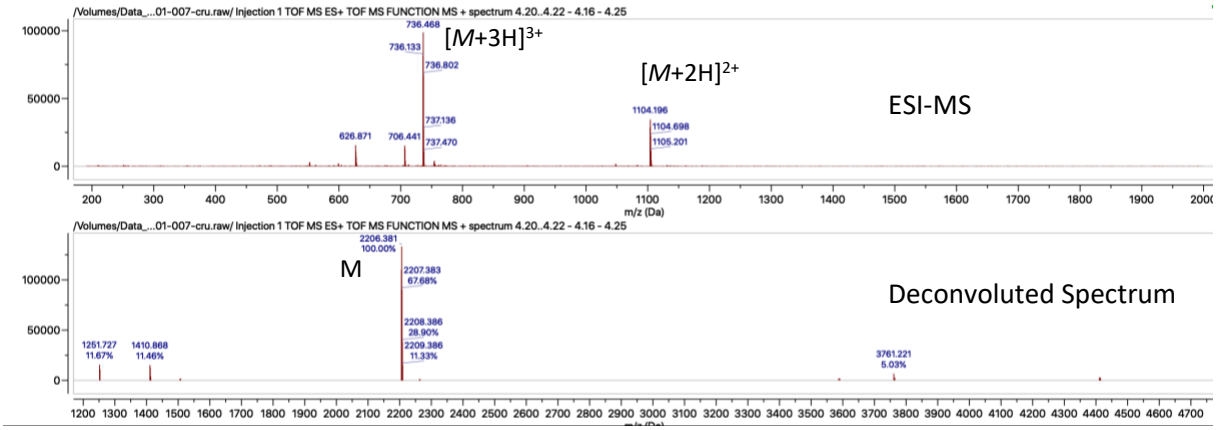




C. Deconvolution of Multiply Charged Ions

ESI-MS containing multiply charged ions can be deconvoluted in order to obtain the mass of the neutral molecules present in the sample. Selected the spectrum of interest and click on the 'Charge State Deconvolution' 

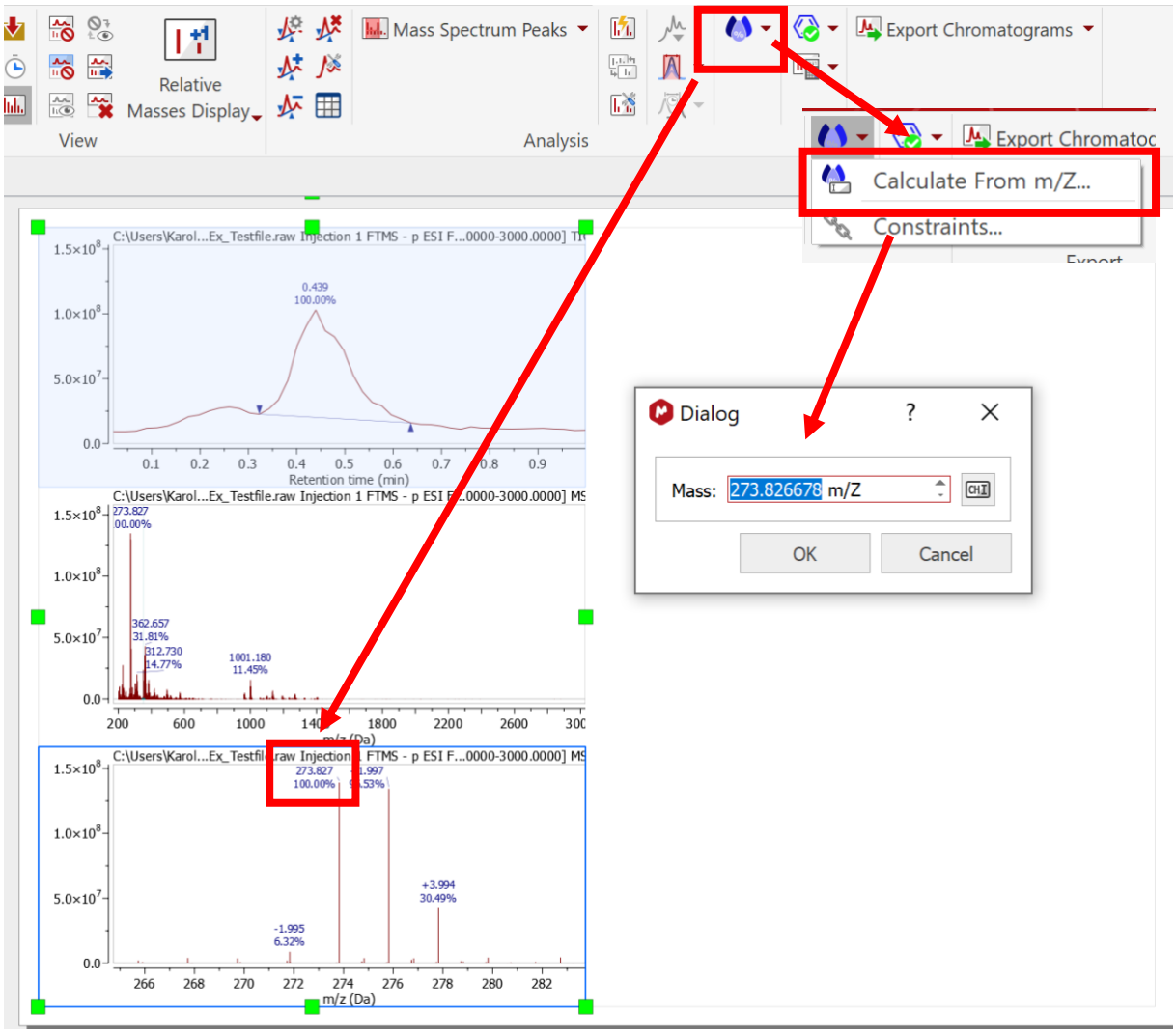
button. A new deconvoluted spectrum containing will be created.



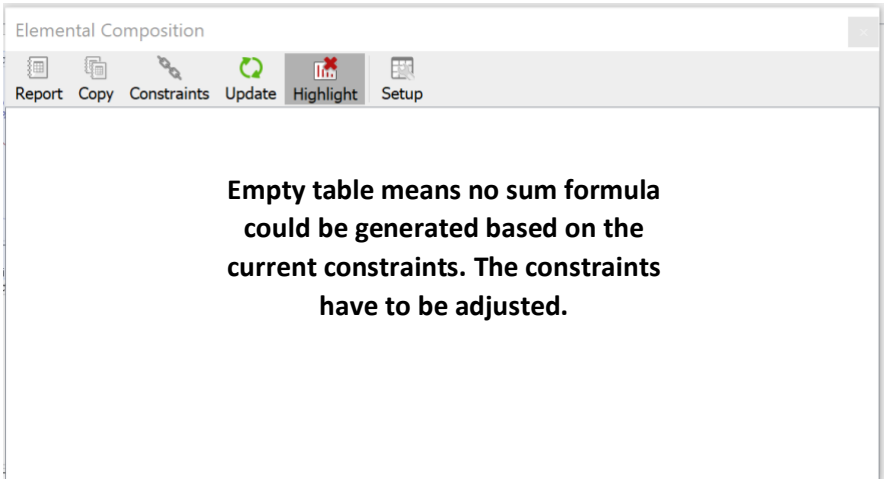
D. *M/z* value to Molecular Formulas (for HR-MS Data only!)

The acquisition of accurate masses (HR-MS) allows the generation of a list of possible chemical formulas for a specific *m/z* value. This procedure makes sense for ions of unknowns with a molecular mass ≤ 500 g/mol. For larger ions (≥ *m/z* 500), tight element constrains have to be defined and only the mass deviation (ppm or mDa) from a theoretical chemical formula will be obtained. See Chap. **F** for an alternative processing including simulation of isotopic distributions.

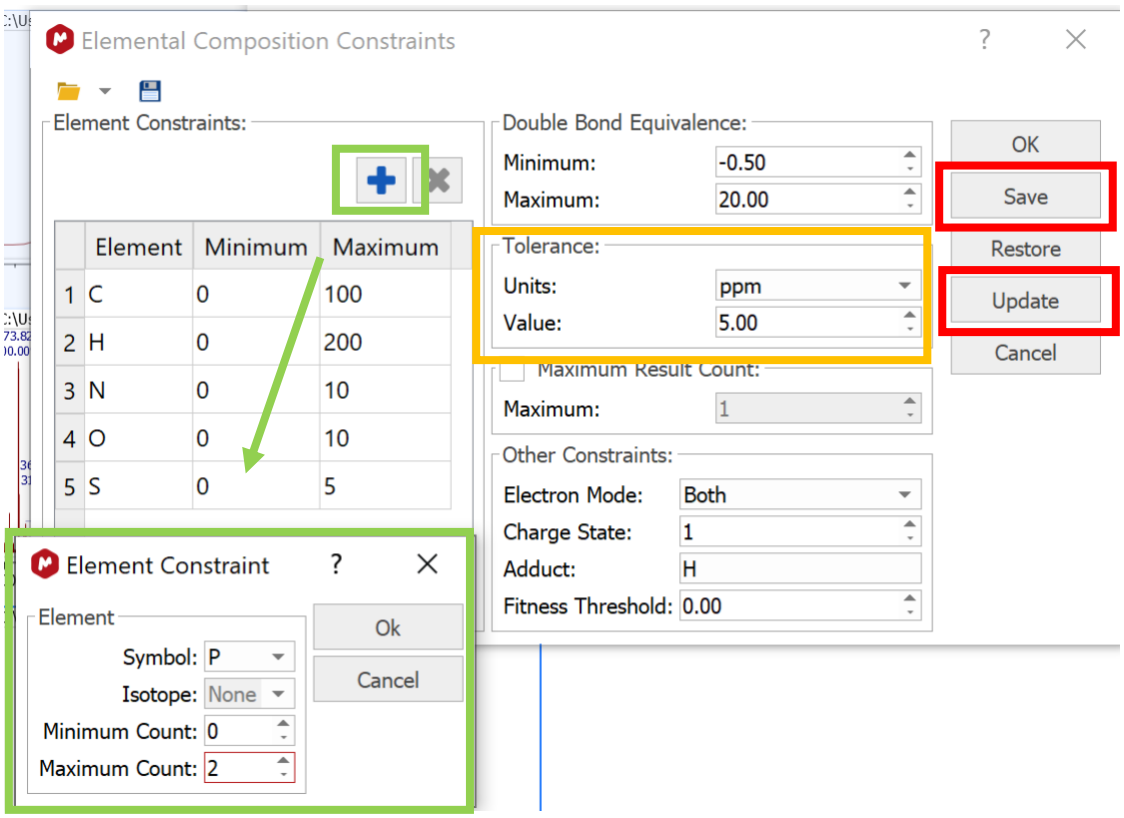
➢ In order to calculate the chemical formula of a molecular ion of interest, click on the **elemental composition** button  , select the mass of interest and press calculate from m/Z. Press OK in the Dialog window.



➢ An elemental composition table will open:

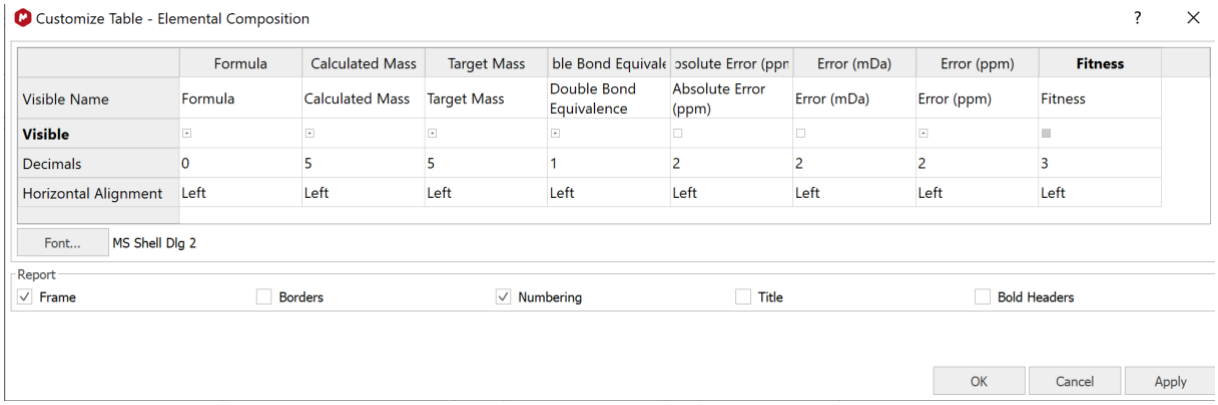


➢ Under **constraints** elements and other parameters can be defined. By default, only C, H, N, O and S atoms are considered. By clicking "**add**", a new window appears (green box) where additional elements that you assume to be in your compound can be added. You can additionally define the ppm tolerance (orange box). Mass errors under 5 ppm are accepted in publications. Click save and update to generate a new list of molecular formulas (red box).

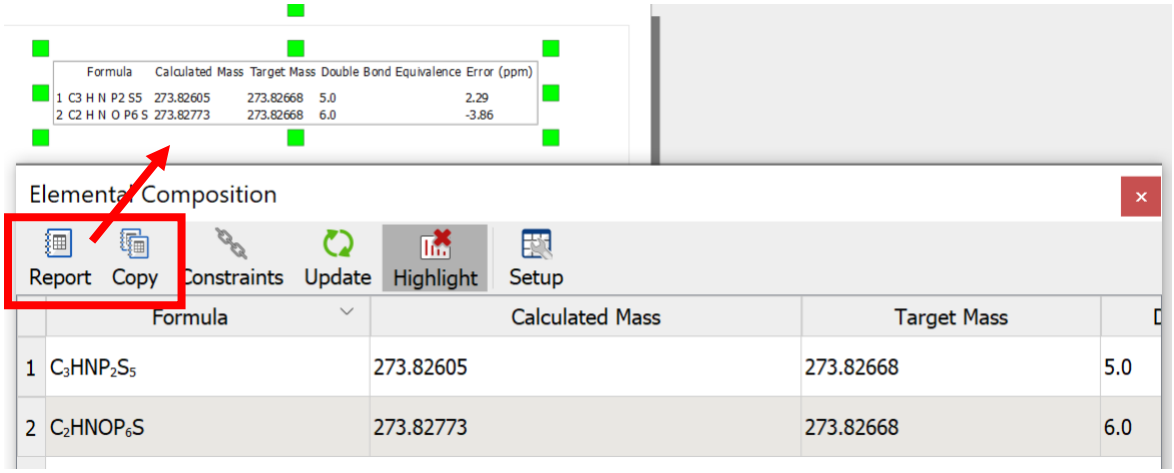


➢ Under **setup** 

, you can define the parameters displayed in your molecular formula list. There, you can choose the layout of your table and list, for example, absolute errors in mDa or ppm.



➢ Finally, you can press report to generate a Formula list directly into your Mnova document or copy to be able to paste it into excel etc. (red box).

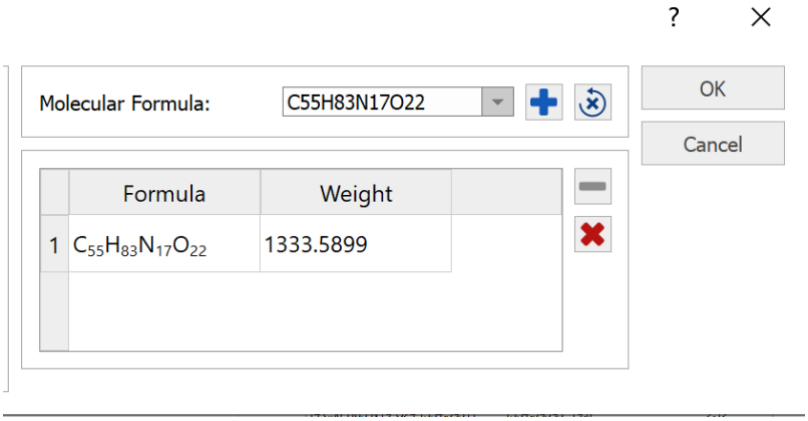


E. Predict molecular mass and isotopic pattern

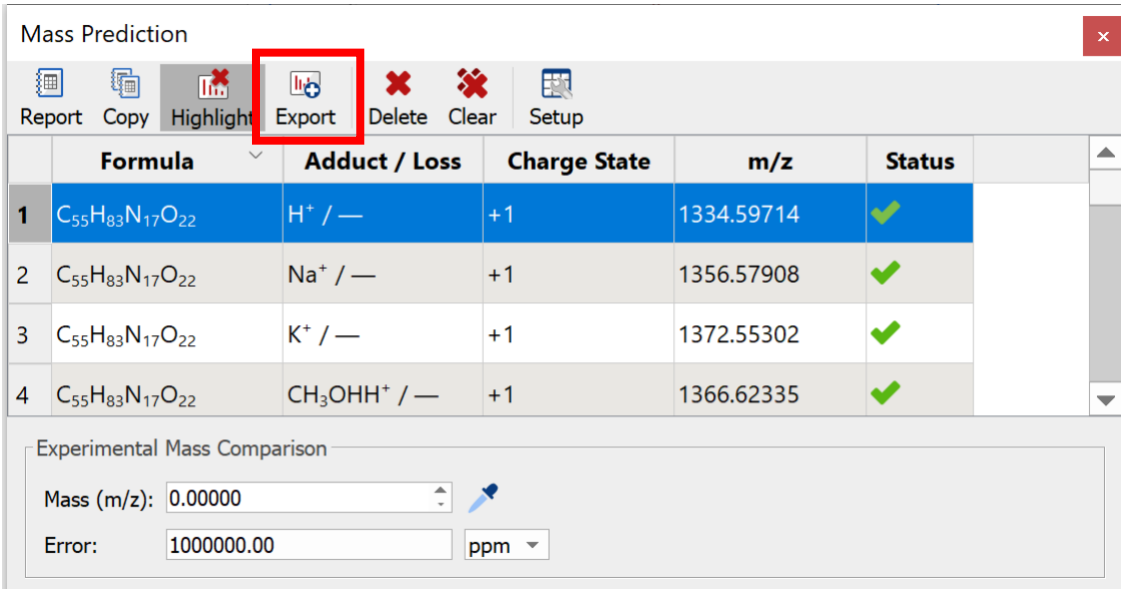
Isotopic pattern predictions are especially useful when a compound includes an element with an unusual isotope distribution, for example Pt, Fe, Re, Rh, Ga, Zn, B and so on. Thus, just by looking at the isotopic pattern one can confirm the presence (or absence) of a certain polyisotopic element.

➢ Click on the **predict** symbol 

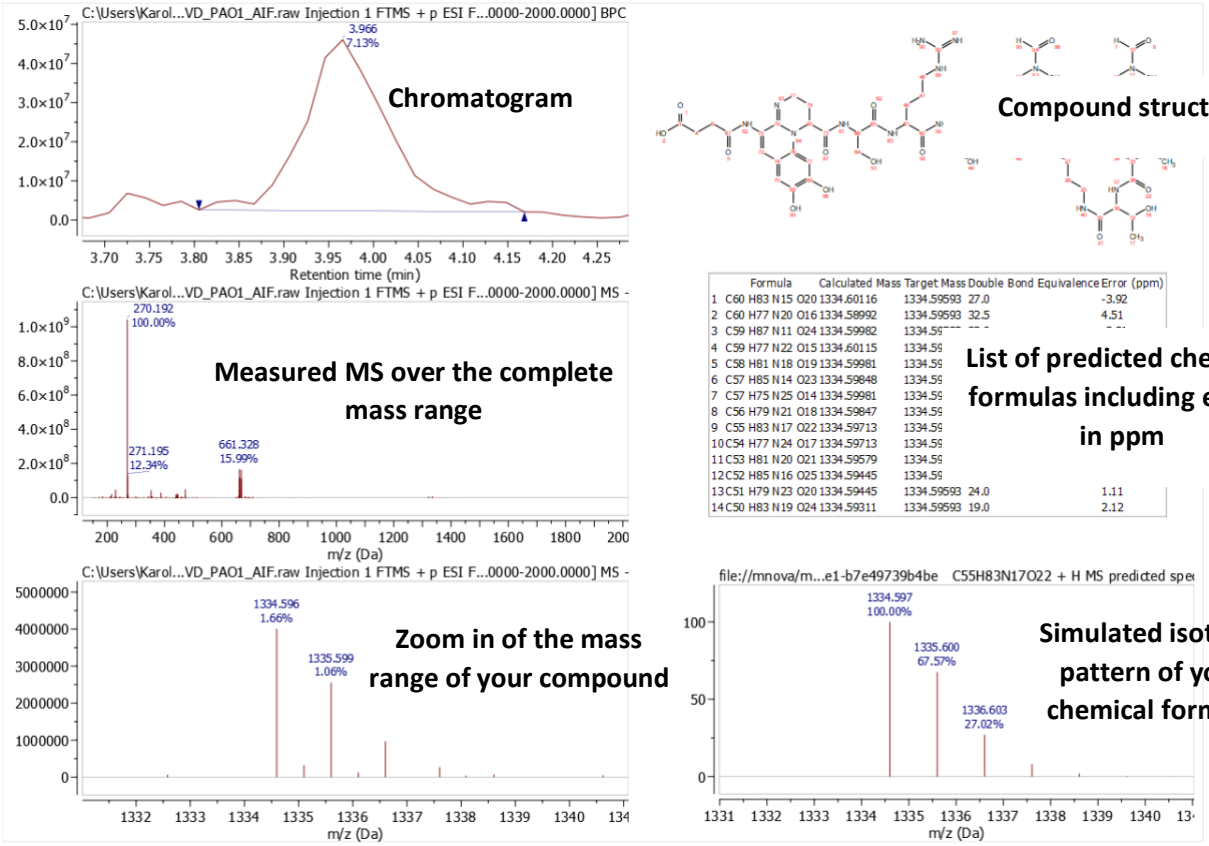
➢ Type in the molecular formula of the compound that you want to calculate the mass and isotopic pattern of. Click okay.



➢ Choose the desired MS adduct and export its predicted mass spectrum (red box).



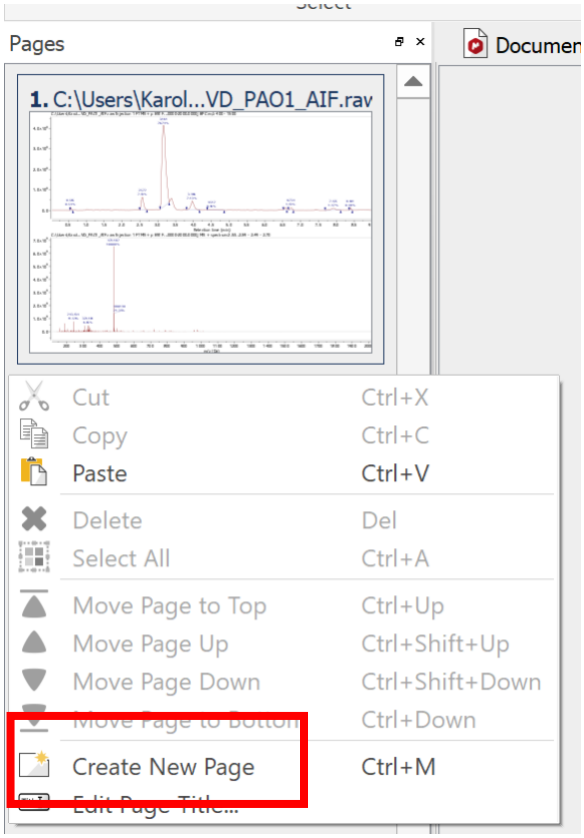
➢ Copy the simulated spectrum on your desired place. A complete report could look something like this:



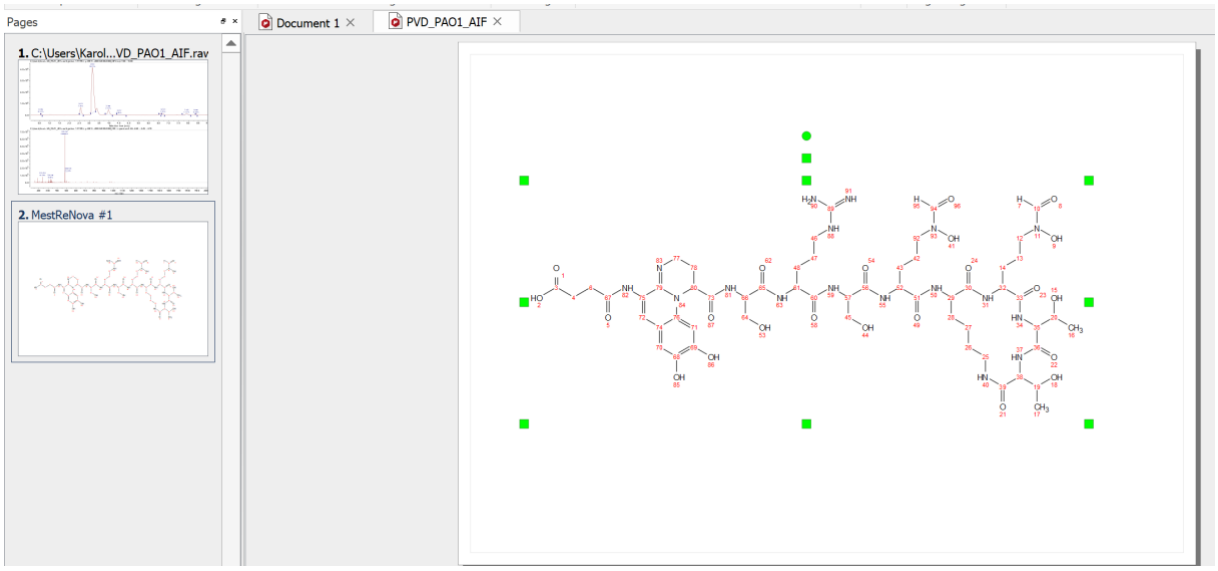
F. Screening LC/GC-MS data for a compound of interest

Molecule match analysis determines the possible presence of one or more given structures with definite molecular formulas within a mass spectral data set. The isotope cluster of each structure is computed and compared to each spectrum in the data set, and the mass spectrum with the best match that returns a score above the preset threshold is taken as a positive match. For this, GC/MS or LC/MS data sets and one or more structures are required. (Whole spectral libraries in *.sdf format can be also used).

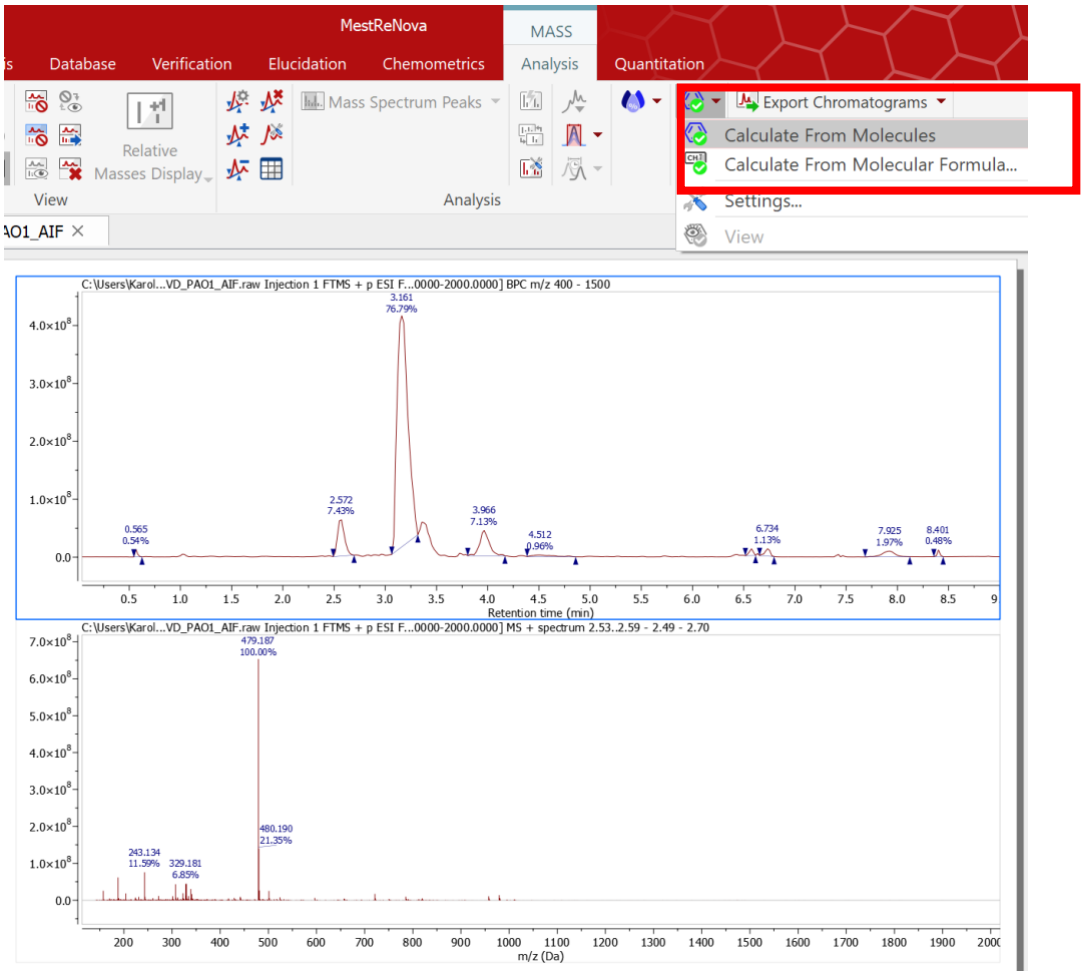
➢ Open your measurement file, right click under the pages settings and generate a **new page**.



➢ Copy paste the molecular structure of your compound(s) of interest into the new blank page directly from **Chemdraw**. Switch back to your measurement spectra.

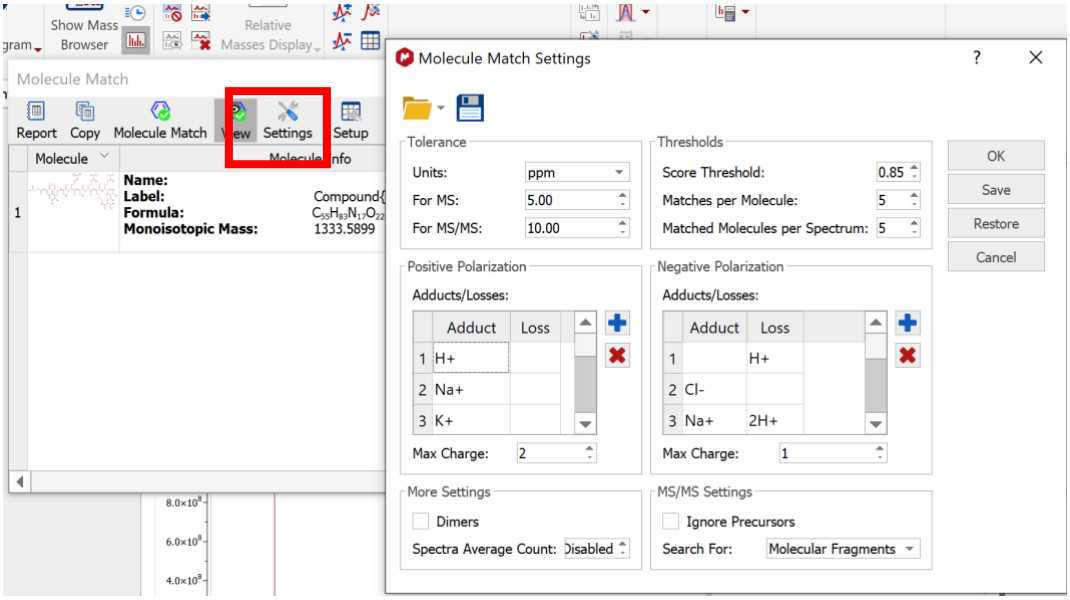


➢ By clicking on the **Molecule Match** (red box) feature you can now screen your measurement for your compound that was copied in ChemDraw.

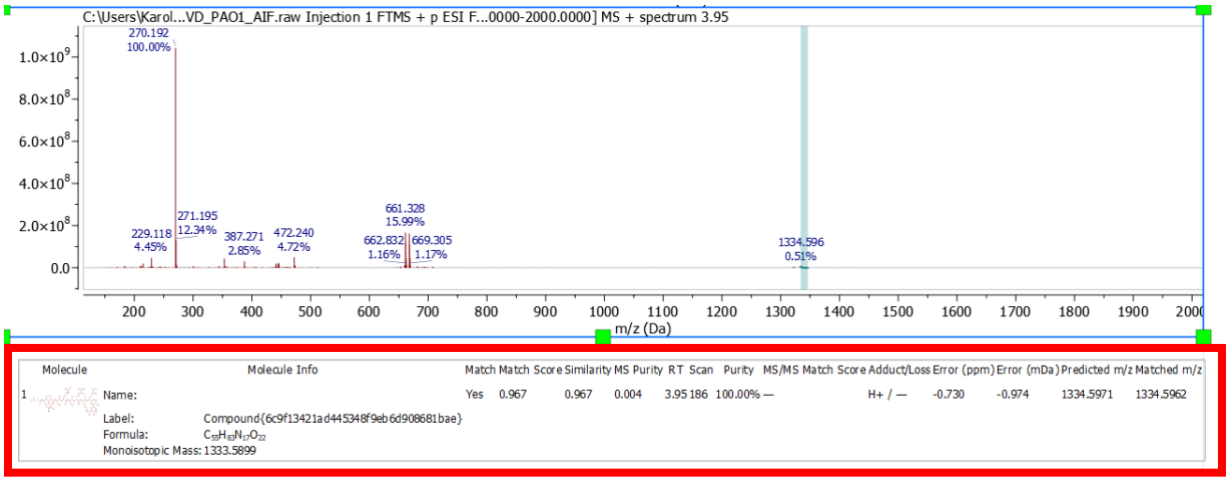


➢ Under **Settings** 

, you can adjust constraints such as type of possible adducts as well as ppm error limits. If you change these settings, you have to manually press molecule match to recalculate the results.

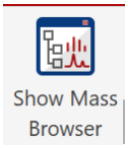


➢ Finally, you can press report to generate a **match list** (red box) directly into your Msnova document or press copy to be able to paste it into excel etc.

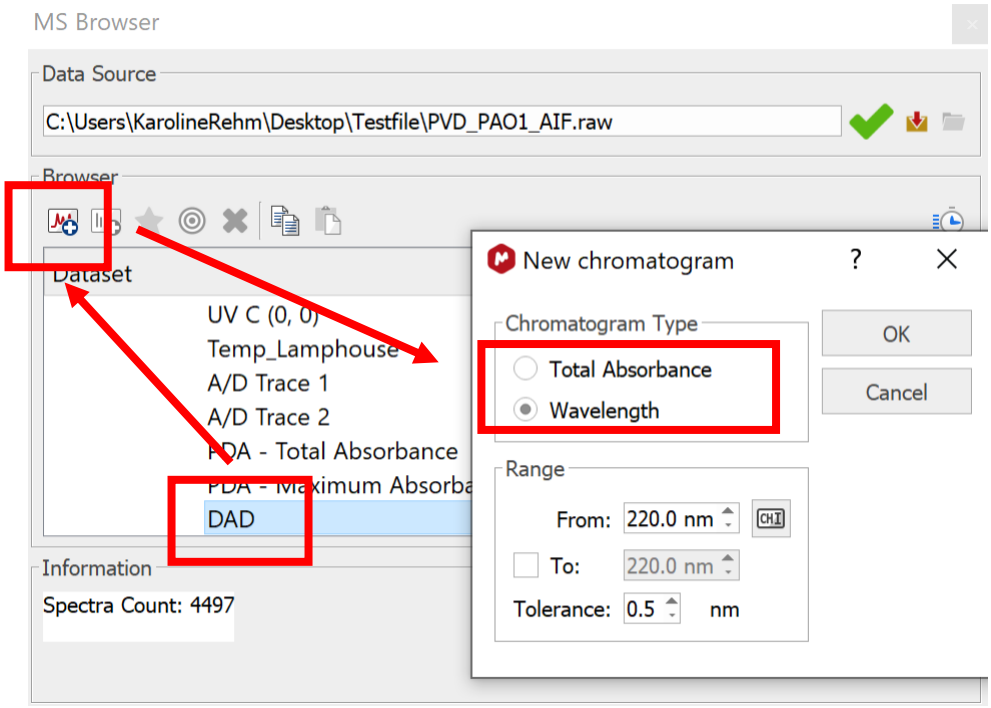


3. Processing of UV chromatograms

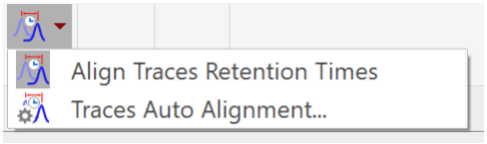
Before you start to process, make sure that UV data has been acquired (selection of the appropriate instrument method and DAD detector switched on).

➢ Click 

to open the MS browser, choose DAD in the list and create a “Total Absorbance” or “Wavelengths” item.



➢ Align a DAD, or another trace, to a TIC using the auto-alignment settings.



4. Additional Resources

Additional information on the software can be found on the Mestrelab website:

https://mestrelab.com/learn_support/mnova/ms/

https://resources.mestrelab.com/

https://mestrelab.com/downloads/mnova/manuals/MestReNova-14.1.0_Manual.pdf