Sharing Data and Working with MassHunter

Data Processing may be performed in the computer lab using MassHunter Qual 10
Supporting Materials: Excel with mass calculator and template for polymer calculations

Video Tutorials

- Navigating MassHunter Qual 10
- Viewing data in MassHunter (scaling and zoom)

Data Conversion

After the analysis the data has to be converted by WIFF Translator located in programs under MassHunter workstation.

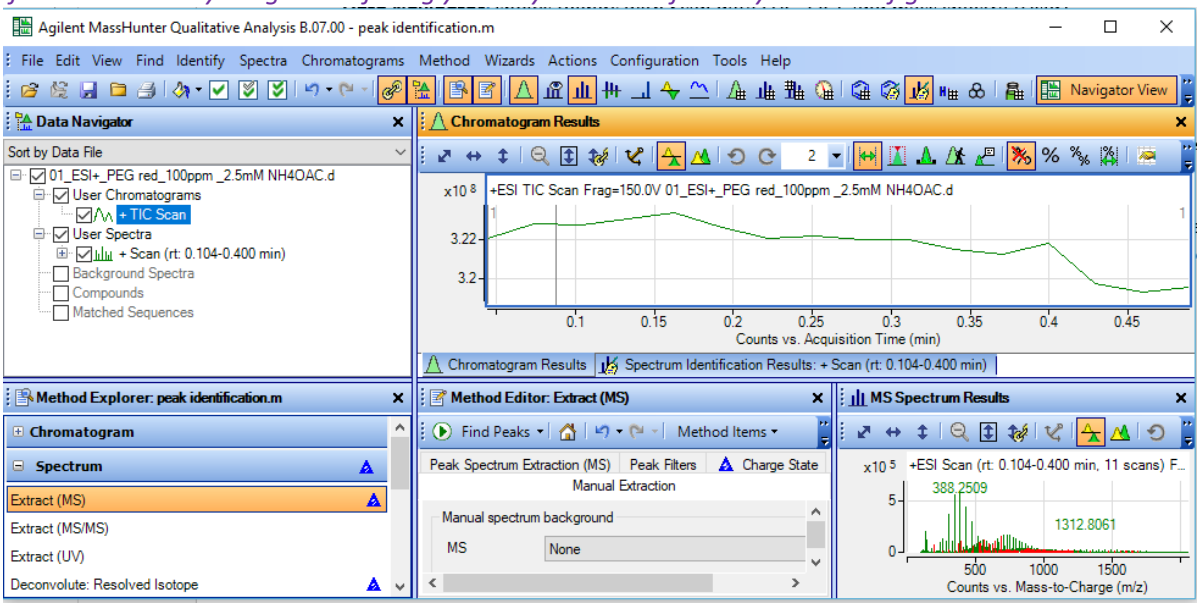
- In the right pane create folder in which the new files will be stored (should in the same project but new folder "data-MH."
- Uncheck "convert profile data to peak format" Make sure to select/enable profile spectra in main window of WIFF
- In the left pane right click on the directory with data to be converted (data folder) and select translate.

Viewing the data - setup of screen

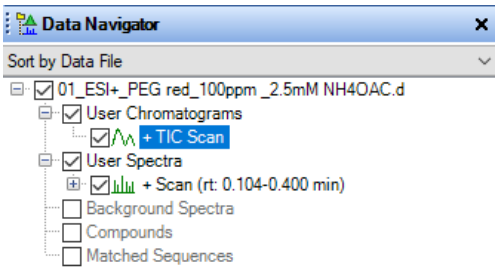
Open the MassHunter Qualitative software, currently the highest available version is B07.
Verify that correct windows for data processing are open, these include: **Data Navigator, Chromatogram Results, MS Spectrum Results, Method Explorer**. Additional windows you may need are **Method Editor** and **Mass Spectrum Peak List**. If any of those windows is not open click in the main menu, the **View tab** and select these windows (deselect the others).

Data Navigator: shows folders with Scan data (TIC, EIC), and mass spectra (User)
Chromatogram Results: shows the record from chromatographic, or infusion runs typically in TIC, but also allows for extracted ions (EIC)
MS Spectrum Results: shows the mass spectra (do not use preview)
Method Explorer: allows for various settings of integration and data processing
Method Editor: shows specific setting for the processing method (e.g. formula generation, deconvolution etc.). Method editor changes depending, which method is selected in its menu **Method Items** the **Method Explorer**. (If Method editor opens as new window click on its top pane to embed as a new window)
Mass Spectrum Peak list: provides list of the peaks with m/z values and abundance in mass spectrum

If the windows layout gets confusing you may restore default layout in Configuration tab under Windows layout tab



Opening and Closing Data Files



In the **Data Navigator**, you can see all the files with chromatograms or mass spectra, which are open.

Check marks next to specific chart show visibility on screen. By unchecking the charts, panes will become invisible but will be still open. This way, you can have open the same file in various formats e.g., subtracted vs. unsubtracted, TIC or EICs open at the same time.

The files may be closed by going to File menu (or right click). When closing a file, all data processing may be saved (or discarded) by confirming/cancelling "**Do you want to save these results"**.

Note: Do not delete files, this will result in loss of all your data.

View Chromatogram/infusion (TIC)

Open data file using the **File menu.**

In the left pane in **Data Navigator** you will see the **User Chromatogram box checked** (even if data are from direct infusion only (No LC)) along with **TIC scan,** which is also checked.

View Mass Spectrum

In order to view the mass spectrum, left click-drag - then release the mouse over the area on the **Chromatogram Results** that you want to get the average mass spectrum for, The highlighted area will become a light blue. Double click in middle of the area to obtain the mass spectrum, which changes the highlighted area to a gray color

You can obtain several mass spectra from the same run or from different runs. In the left pane in **Data Navigator** you will see all open mass spectra under **User Spectra** folders.

These spectra can be viewed in **List mode** using the button on the MS Spectrum Results toolbar enabling comparison of separate panes. A number of panes is given by the number on the same toolbar. The mass spectra maybe also shown in **Overlaid mode** using the button.

Magnifying

The scales and magnifying is controlled by a mouse action in the charts or on the axes. ***Right click*** and drag in the spectrum/chromatogram allows for magnification of the particular area. ***Right click*** **and hold** **over the axis numbers**(if you drag to the right (horizontal) or to the top (vertical) you zoom in on the axis, if drag to the left (horizontal) or bottom (vertical), you zoom out on the axis). ***Left click***, **holding and moving over the axis numbers** scrolls the axis in the direction you are moving the mouse. To reset the scaling use the blue arrows in the toolbar.

Copy/Paste Graphics & Tables

To copy chromatograms or mass spectra, right click on the chart of interest and select **Copy to clipboard.** In the software you are copying to (e.g., MS Power Point/ MS Word) use command **Paste Special** and select **Enhanced Metafile**. In this format the file may be ungrouped and unwanted graphics (text, background grid) eliminated.

Note data itself should never be modified, these features are used only to improve quality of the final figure.

To Copy/Paste tables, right click on a header row of the Table and selecting **copy to clipboard** in the MS excel simply paste (the icon for copying should allow to import/conversion of the text as data. Optionally you also may **copy individual columns** click right on the header of the column and select copy column using new line separator to clipboard

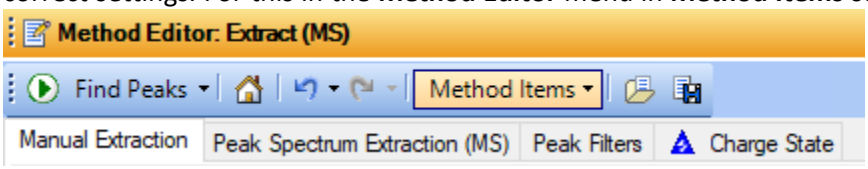
Mass Spectrum Interpretation

Subtract mass spectrum (A-B)

To subtract background mass spectrum (B) from the target compound mass spectrum (A). Go to the ***Data Navigator***. Right click on the mass spectrum (A) from which you want to subtract, select ***Subtract any spectrum***, and in the ***Data Navigator*** left click on the mass spectrum (B) you are subtracting. The subtracted mass spectrum will automatically appear under the original mass spectrum (A).

Required settings for mass spectra operations

Before any work is started on the data processing of mass spectra, a **Mass Spectrum List of Peaks** must be generated using correct settings. For this in the **Method Editor** menu in **Method Items** select **Extract (MS)** and setup peak identification.



Next to green arrow should be "**Find peaks**" if not, click on black arrow (downward black triangle) and select it. Setting for tabs is as follows

Tab Manual Extraction: none;

Tab Peak Spectrum Extraction: average scans at 10% of height, exclude if above 10 % of saturation, never return empty, peak spectrum background: none

Tab Peak Filters: set absolute height =100 by ***checking*** box, relative height 0.1% of largest peak by ***checking*** and setting value; ***uncheck*** limit (by height) to the largest

Tab Charge State: Peaks spacing 0.0025 plus 7 ppm, isotope model unbiased, ***uncheck*** limit assigned charge states, ***check*** treat ions with unassigned charge as single charged

After ensuring the setting are correct, click on green arrow to **Find Peaks**

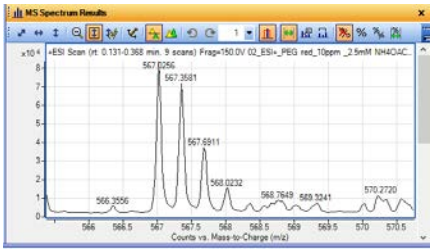
After this all peaks in mass spectra will have *m/z* value (upon magnification)

By Right clicking on the mass spectrum will show its **MS Spectrum Peak List 1** (if it is not visible)

Depending which spectrum is highlighted you will see different peak lists. (To highlight, left click on the spectrum)

Check yourself: Charge

Verify correct assignment of charges in the **MS Spectrum Peak List 1**
In the Peak list table find, the ion of higher abundance (>1000) with higher charge (3 and higher if possible). For example (shown), magnify the area of the spectrum with the cluster ions of a triple charge showing individual isotopes. Ensure you can explain charge assignment based on the isotopic pattern.

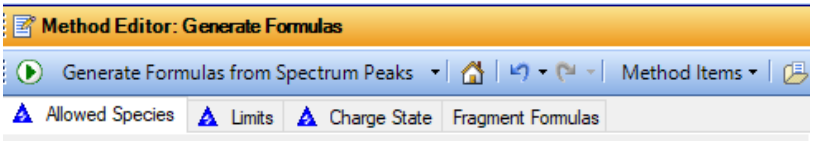


Verification of mass accuracy and isotopic distribution *Typically used for small molecules*

In the **Method Editor** menu in **Method Items** select ***Generate Formulas*** (scroll down)

*Another approach is to use the **Method Explorer**: select **Identify Compounds** and open new pane **Generate Formulas**.*

Ensure all tabs are setup correctly: in the **Allowed Species tab** the elements and their numbers of elements considered in isotopic calculation, in the **Limits tab** the mass and mass accuracy error, and in the **Charge State tab** peak spacing tolerance 0.025 + 10 ppm**,** using unbiased isotope model.

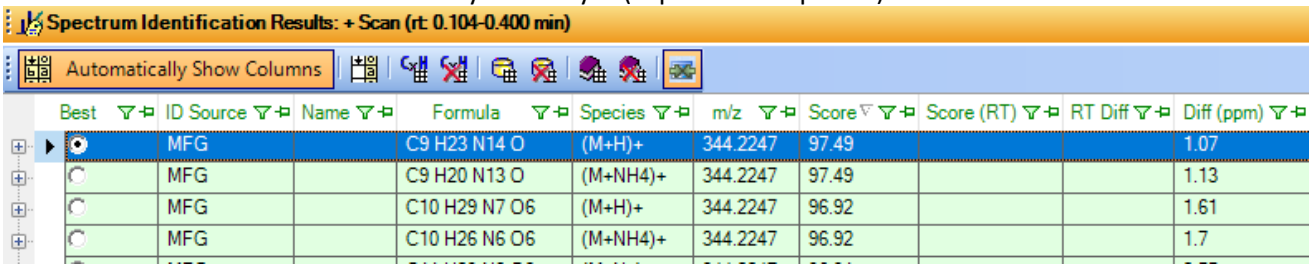


In the mass spectrum, magnify the ion of interest and highlight the isotopic peak you want to evaluate.

Ensure that next to the green arrow **Generate Formulas from <u>the spectrum peaks</u>** is selected.

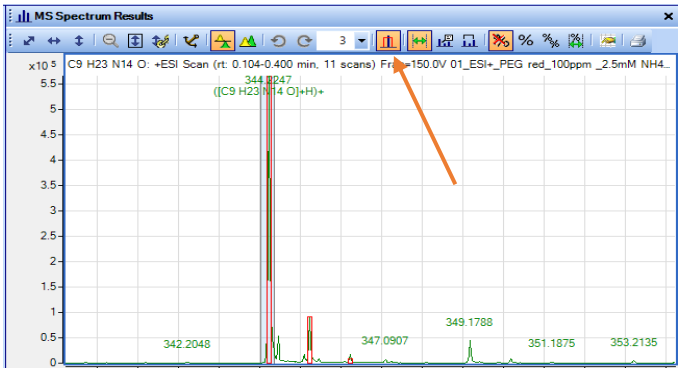
Click on green arrow or ***right click*** **on the selected peak** and select ***Generate Formulas from the spectrum peaks***, this will generate a table of possible compounds including mass accuracy error in ppm for each of the compounds proposed

To verify isotopic pattern, in the list of proposed compound in the table click and check under "the best" column on the in front of the row with the formula of your analyte (expected compound).



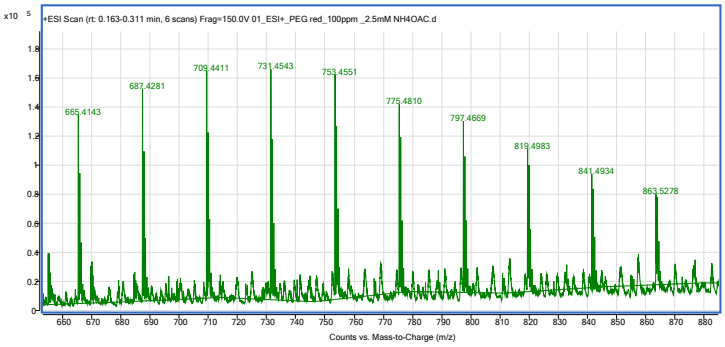
Best	ID Source	Name	Formula	Species	m/z	Score	Score (RT)	RT Diff	Diff (ppm)
◉	MFG		C9 H23 N14 O	(M+H)+	344.2247	97.49			1.07
○	MFG		C9 H20 N13 O	(M+NH4)+	344.2247	97.49			1.13
○	MFG		C10 H29 N7 O6	(M+H)+	344.2247	96.92			1.61
○	MFG		C10 H26 N6 O6	(M+NH4)+	344.2247	96.92			1.7

On the mass spectrum menu select the icon ***Show predicted isotope distribution*** (note this will change as you change the target analyte).



Check yourself: Mass Accuracy

The mass accuracy error should be verified independently either using **Mass Calculator in MS Excel.** Another option is to use function **Show Formula Calculator** in the menu **Tools**, then fill in the measured mass, ensure the ranges for elements are reasonable and click green arrow at the top of the window.



Check yourself: Repeating unit in Polymers

For polymers, the Gaussian distribution of ion clusters apart by monomeric unit should be observed. To determine occurrence of the unit and confirm its mass accuracy the **Mass Calculator in MS Excel tab with repeating unit can be used.** To do this correctly, one must ensure a correct charge assignment to the ions. (see MS Excel for the example)

Mass Spectra Deconvolution

In the **Method Editor** menu in **Method Items** select **Deconvolute: resolved isotope** and setup as follows

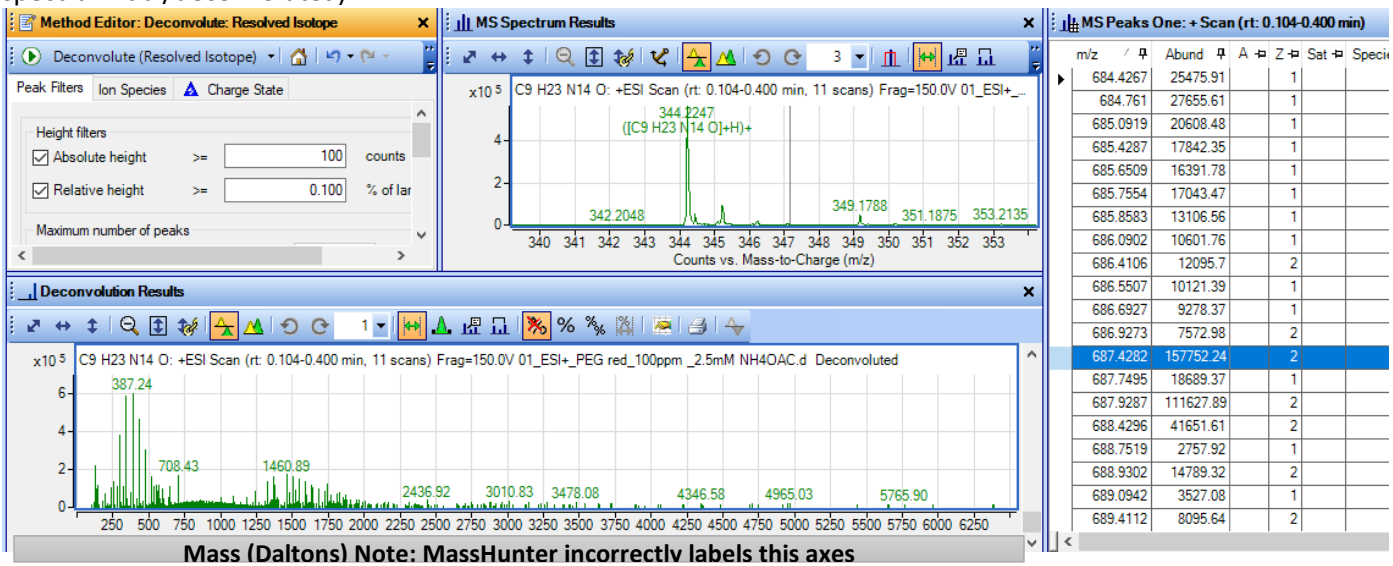
Tab Peak Filters: set absolute height =100, relative height 0.1% of largest peak, ***uncheck*** Limit (by height) to the largest

Tab Ion Species: charge carrier typically +H

Tab Charge State: Peaks spacing 0.0025 plus 7 ppm, isotope model unbiased, ***uncheck*** limit assigned charge states, optionally check treat ions with unassigned charge as singly charged.

Click on the mass spectrum you want to deconvolute and then on a green arrow in the method editor

The new deconvolution spectrum will appear along with the peak table (the peak table will change depending in which spectra initial/deconvoluted).



Check yourself: M_n, M_w, M_z

MS Excel template with formulas is provided to determine polymers and molecular weights. Copy and paste columns with mass of deconvoluted ions and their abundances (double check you are copying deconvoluted data).