Sample preparation

1. Non-volatile compounds, strong acid and bases are not compatible with Mass spectrometer. Avoid the following substances in your samples: DMSO, glycerol, salts, phosphate, borate and citrate buffers, inorganic acids, alkali metal bases, and surfactants.

If your samples contains above constituents, a preliminary cleaning step is necessary. Solid-Phase Extraction (SPE) is a prevalent approach used for the pre-cleansing. Below are two links to SPE cartridges. Follow the manufacturer' instructions for use.

https://www.phenomenex.com/Products/Strata-solid-phase-extraction-products#order

https://us.vwr.com/store/product/27986385/emporetm-solid-phase-extraction-cartridges-cds-analytical

Sample preparation

2. The detection limits of GC-MS system range from 0.05 ppm to 50 ppm (0.05-50 µg/ml) for individual compounds. If your sample has a high concentration of analytes, you need to dilute it to bring it within the range. Please ensure that the concentration **does not exceed 50 ppm (50 µg/ml, 0.1 mM).** Below is an example of chromatograms of limonene at 0.5 and 5 ppm.

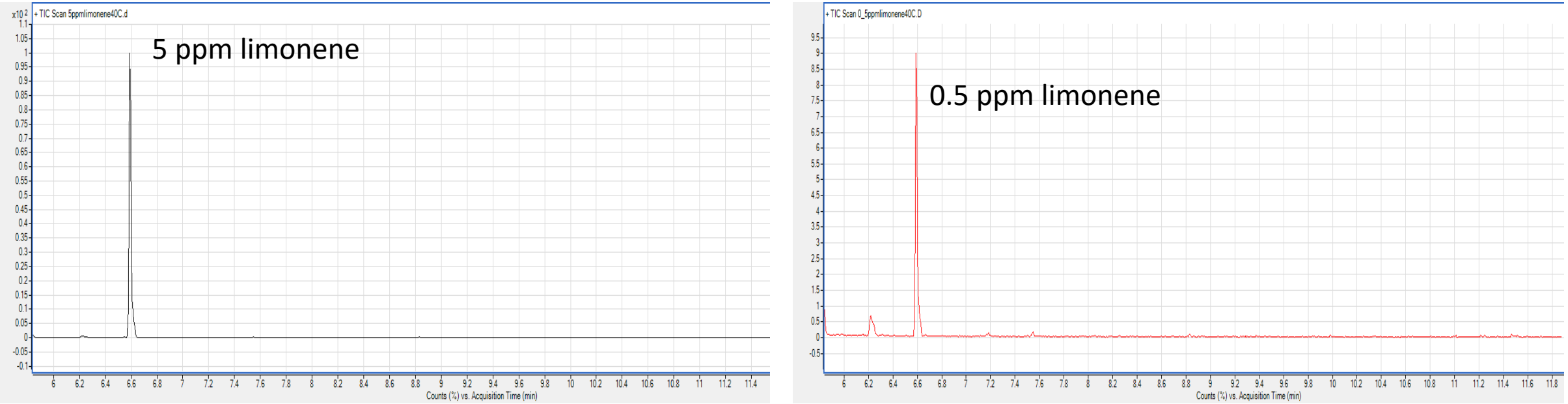


Fig. TIC Chromatogram of 5 ppm and 0.5 ppm limonene.

Sample preparation

If the sample's concentration is unknown, follow the procedure below:

1) Take 1 µl of the sample solution and add it to 1 ml of a suitable solvent to create sample A. Common solvents used in GCMS include hexane, dichloromethane, methanol, ethanol, acetone, and acetonitrile.
2) Take 1 µl of sample A and add it to 1 ml of the same solvent to generate sample B.
3) Analyze sample B and review the chromatogram and mass spectrum. If they are unsatisfactory, proceed to analyze sample A.

The concentration range mentioned previously is applicable to single-ingredient samples and simple mixtures.

Sample preparation

3. Ensure that the solution is clear. If it appears cloudy or contains visible particles, either filter it using a syringe filter or pipette filter tips, or centrifuge at 14,000 rpm for 10 minutes.

Links to syringe filters:

https://us.vwr.com/store/product?keyword=76479-010
Cat. No.76479-010

https://www.sigmaaldrich.com/US/en/products/filtration/laboratory-syringe-filters/millex-syringe-filters

4. Transfer 0.5-1.0 ml sample into a HPLC vial and cap it.

Links to vials and caps:

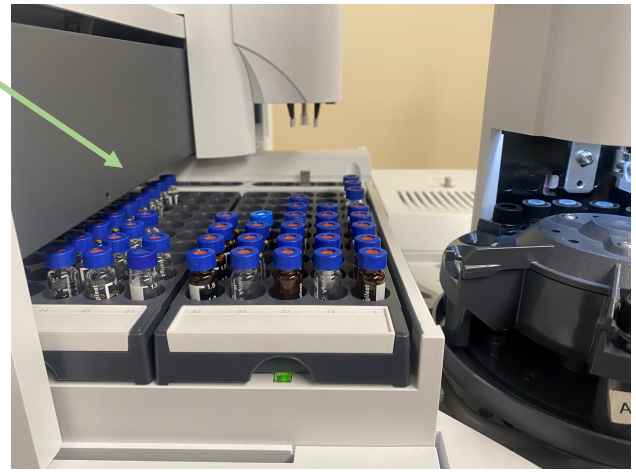
https://www.agilent.com/store/en_US/Prod-5182-0715/5182-0715

https://www.agilent.com/store/en_US/Prod-5182-0716/5182-0716

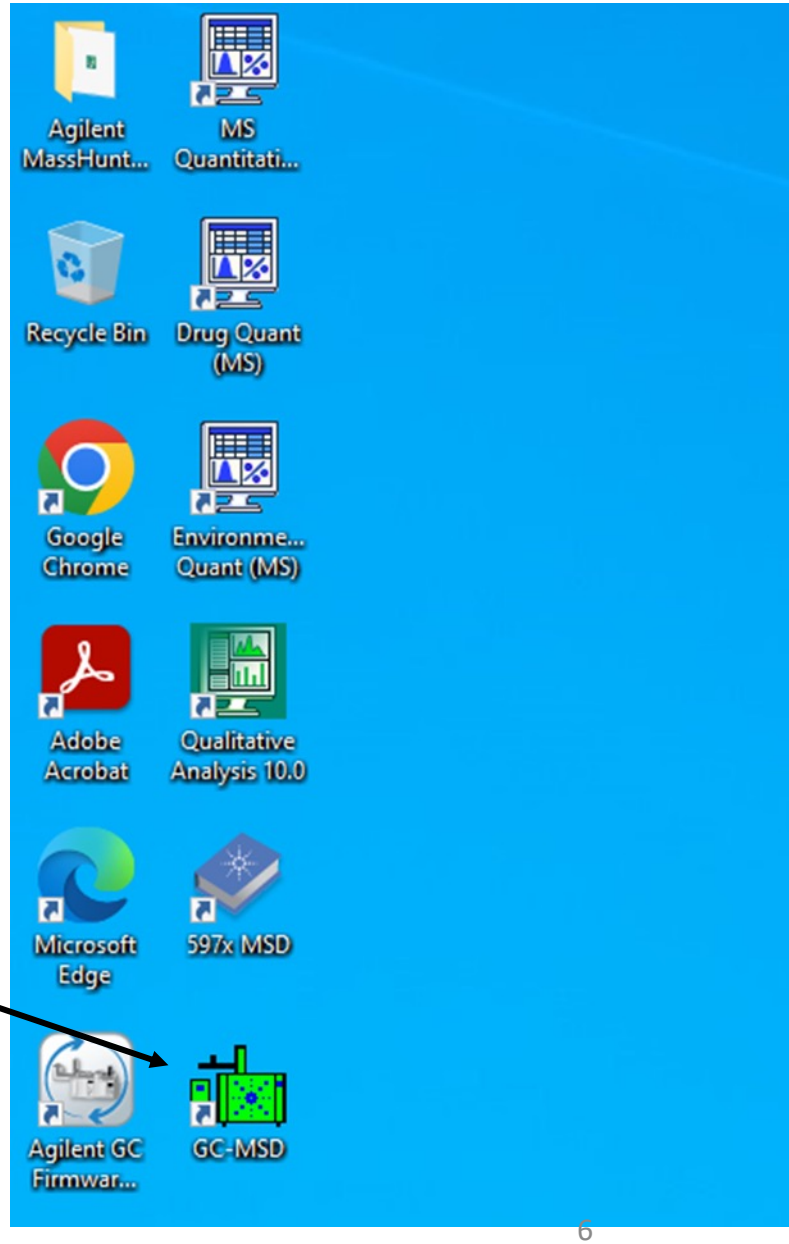
https://www.agilent.com/store/en_US/Prod-5182-0717/5182-0717

Data Acquisition

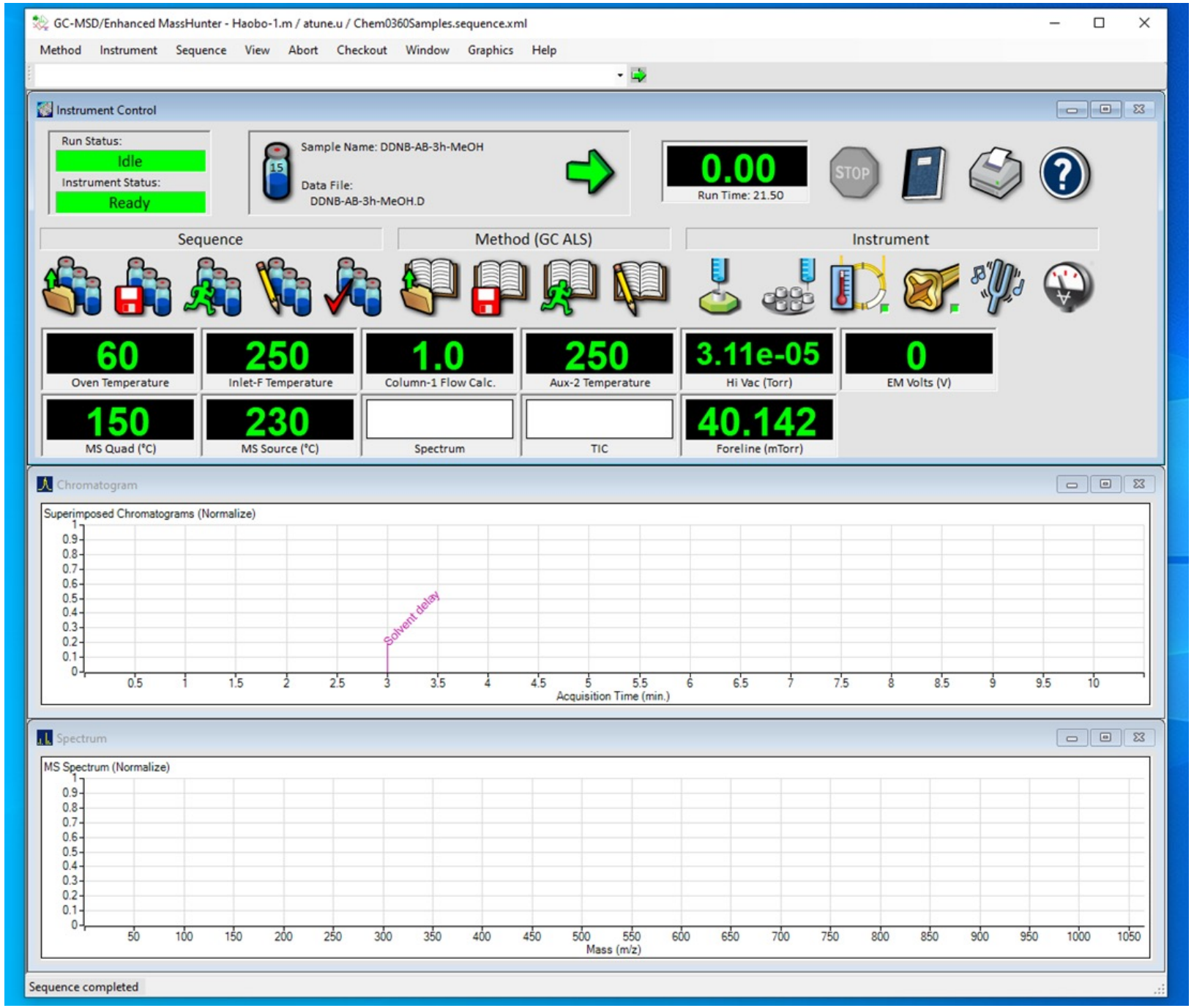
1. Place the sample vial in the autosampler.



2. Double click the GC-MSD icon to start data acquisition software.

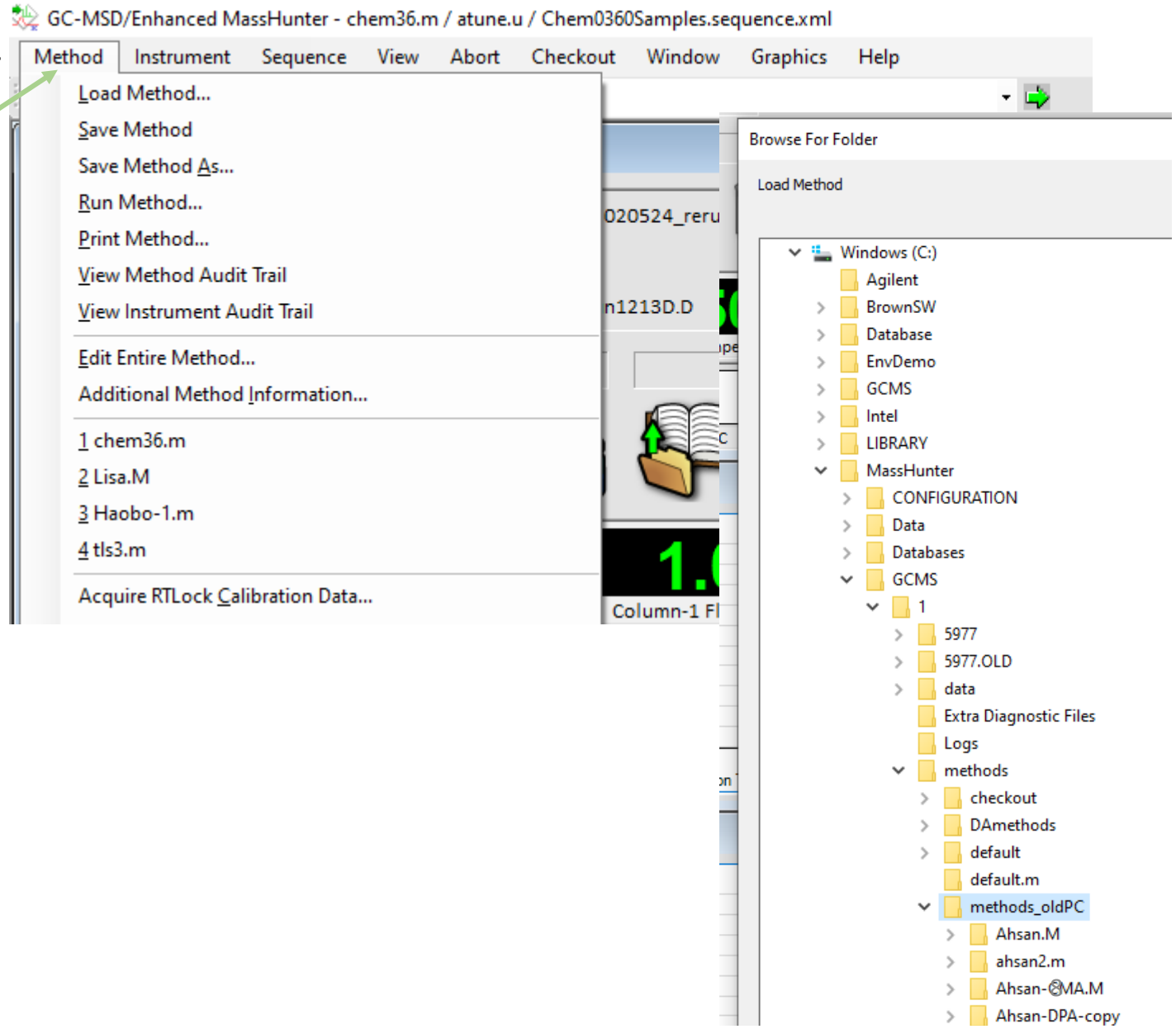


The user interface



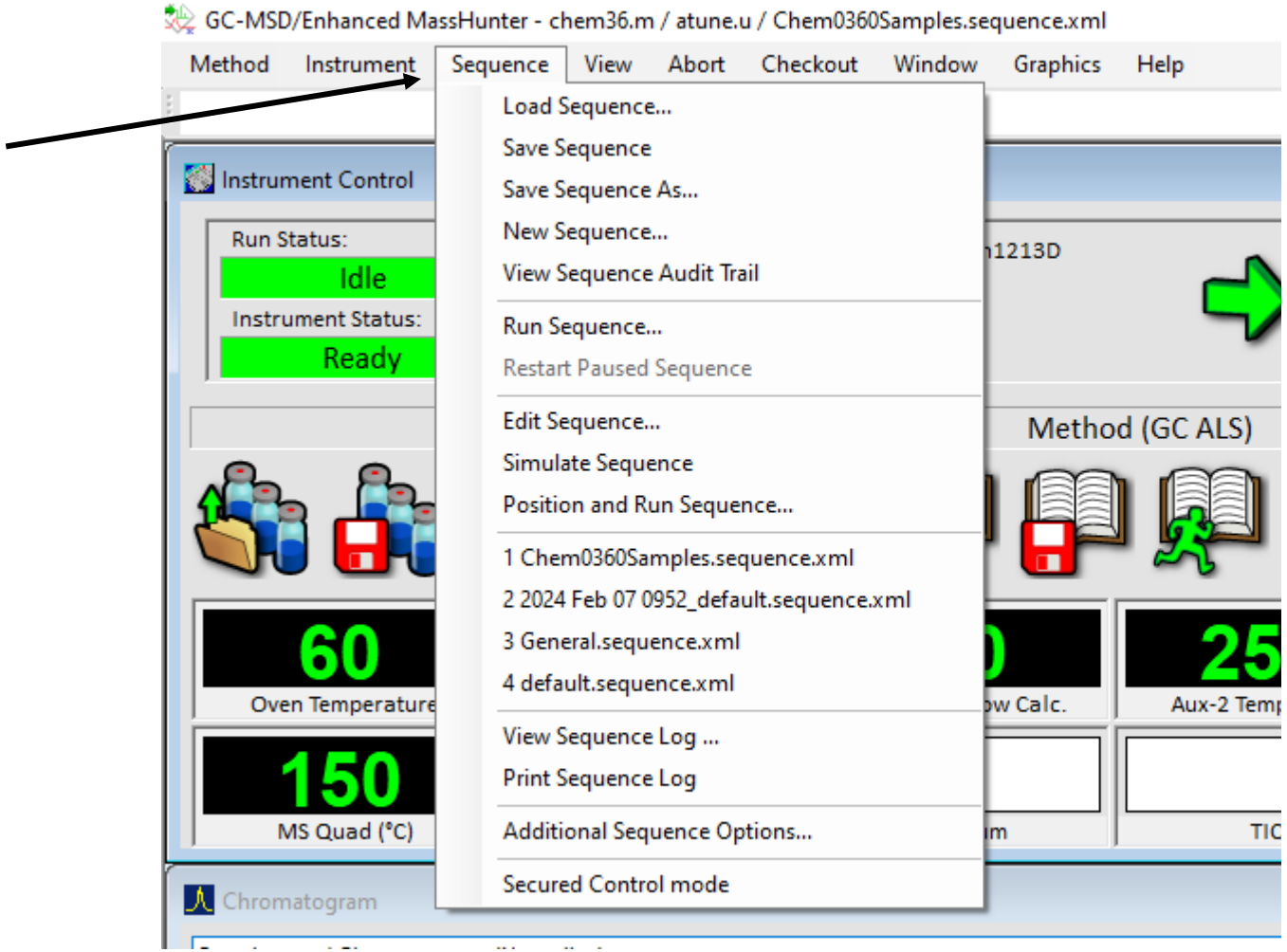
Data Acquisition

3. Click Method > Load Method > MassHunter > GCMS > 1> methods > methods_oldPC > tsl3.M. Click OK to load this method.



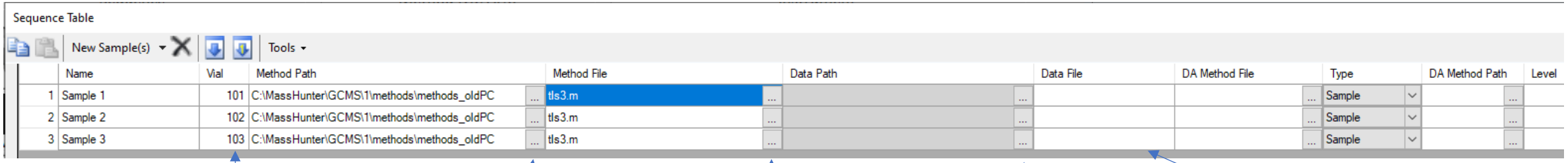
Data Acquisition

4. Click Sequence > New Sequence



Data Acquisition

6. In the sequence table opened, fill in the following information



Sample name

Vial position

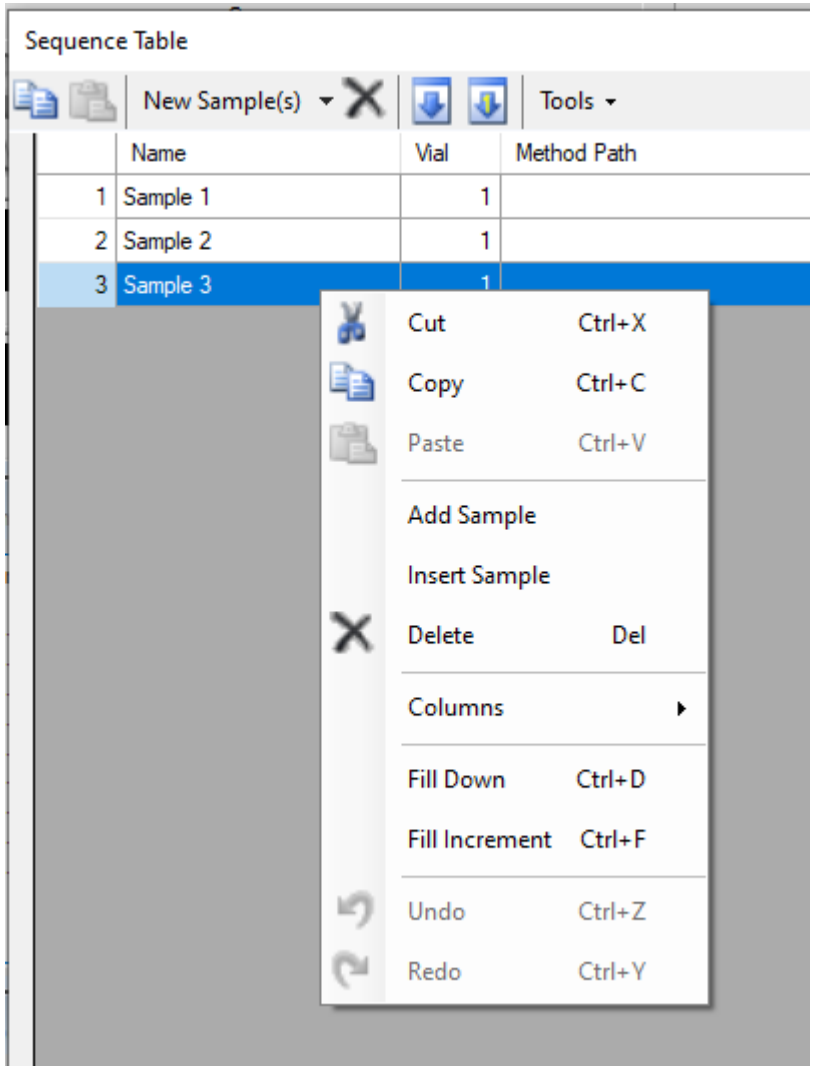
Click the … icon to choose Method path. Choose parent directory of the method file.

Click the … icon to choose Method

Click the … icon to choose file where the data will be saved. Please choose C:\MassHunter\GCMS\1\Users\group

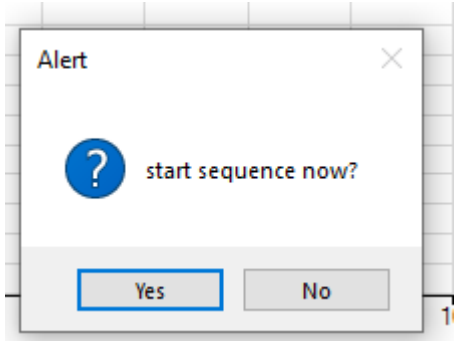
Input a name of your data

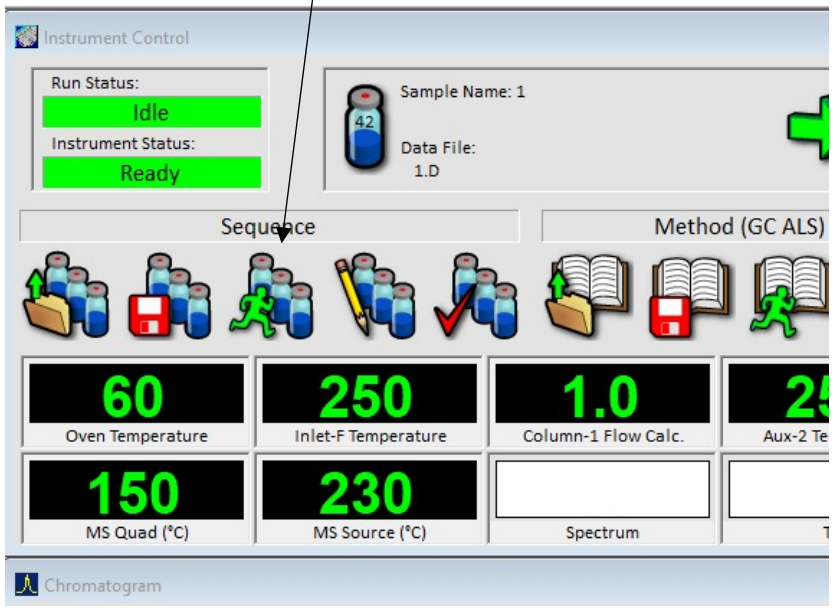
7. Right click the sequence table, delete or add rows



8. Click OK to finish the edit of sequence table.

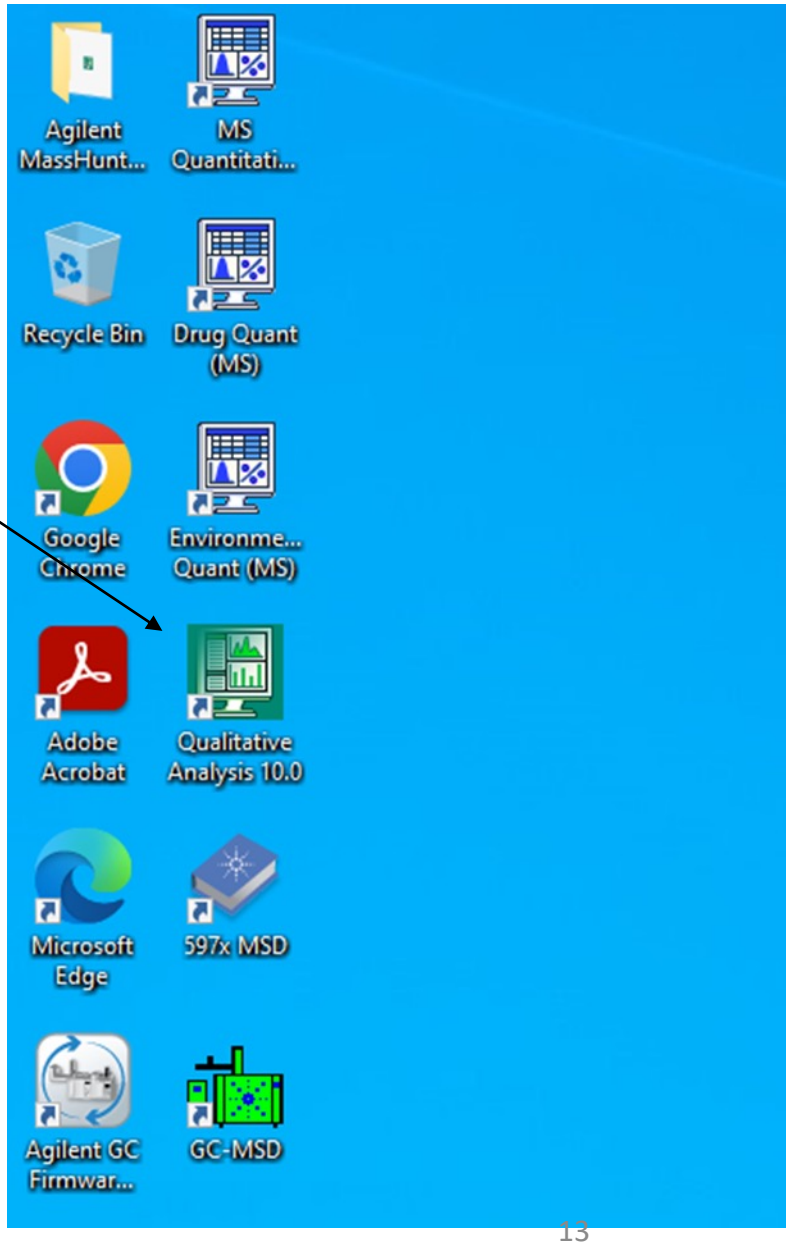
9. A dialogue box pops out. Click Yes to start data acquisition. Or click the Run Sequence icon > Run sequence to start.





Data Analysis

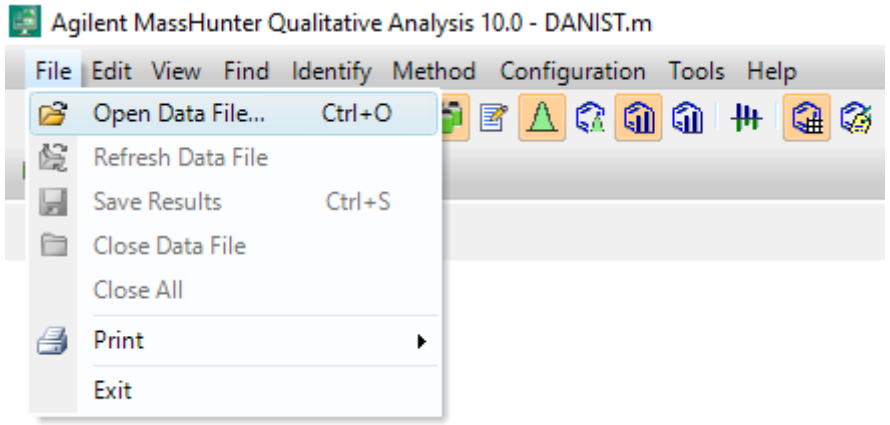
1. Double click the Qualitative Analysis icon to start qualitative data analysis software.



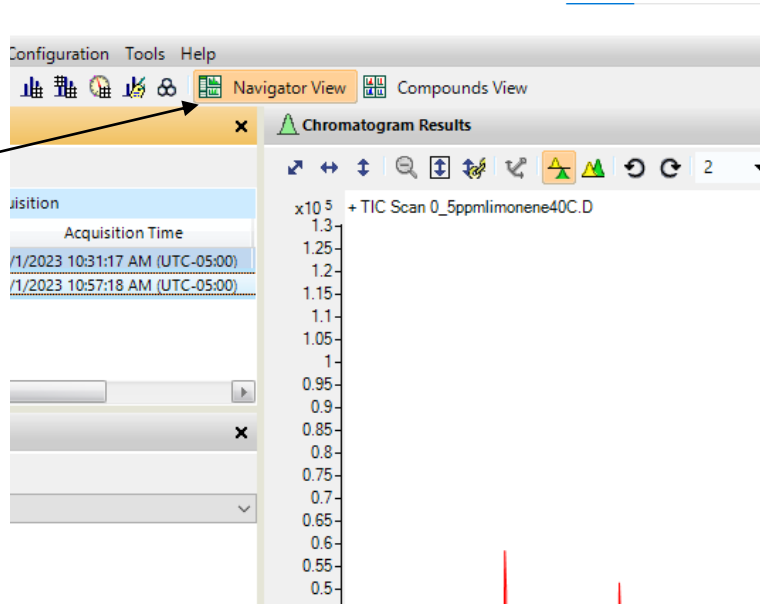
Data Analysis

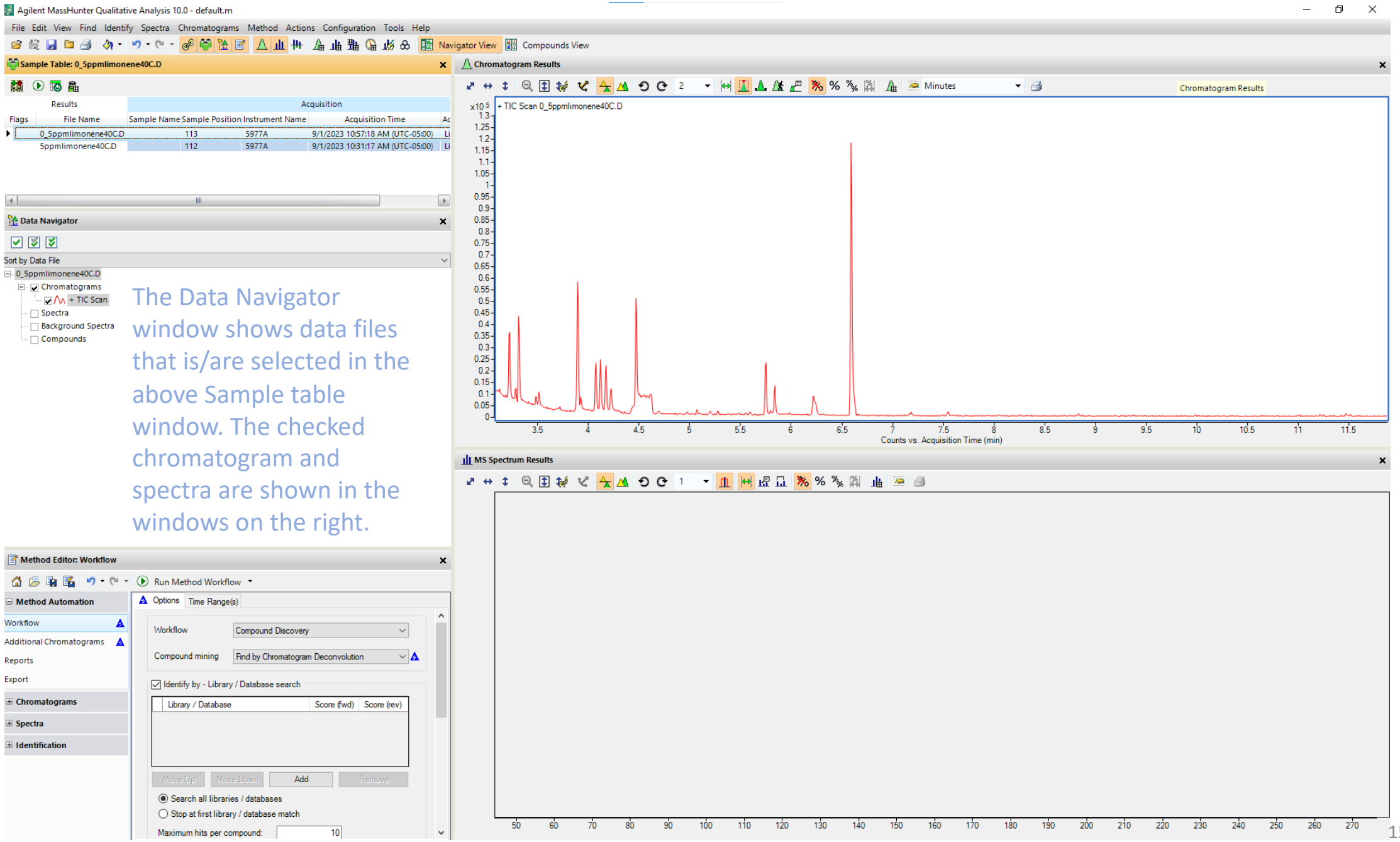
2. Click the File > Open Data File

load a data file



3. Make sure the Navigator View is selected

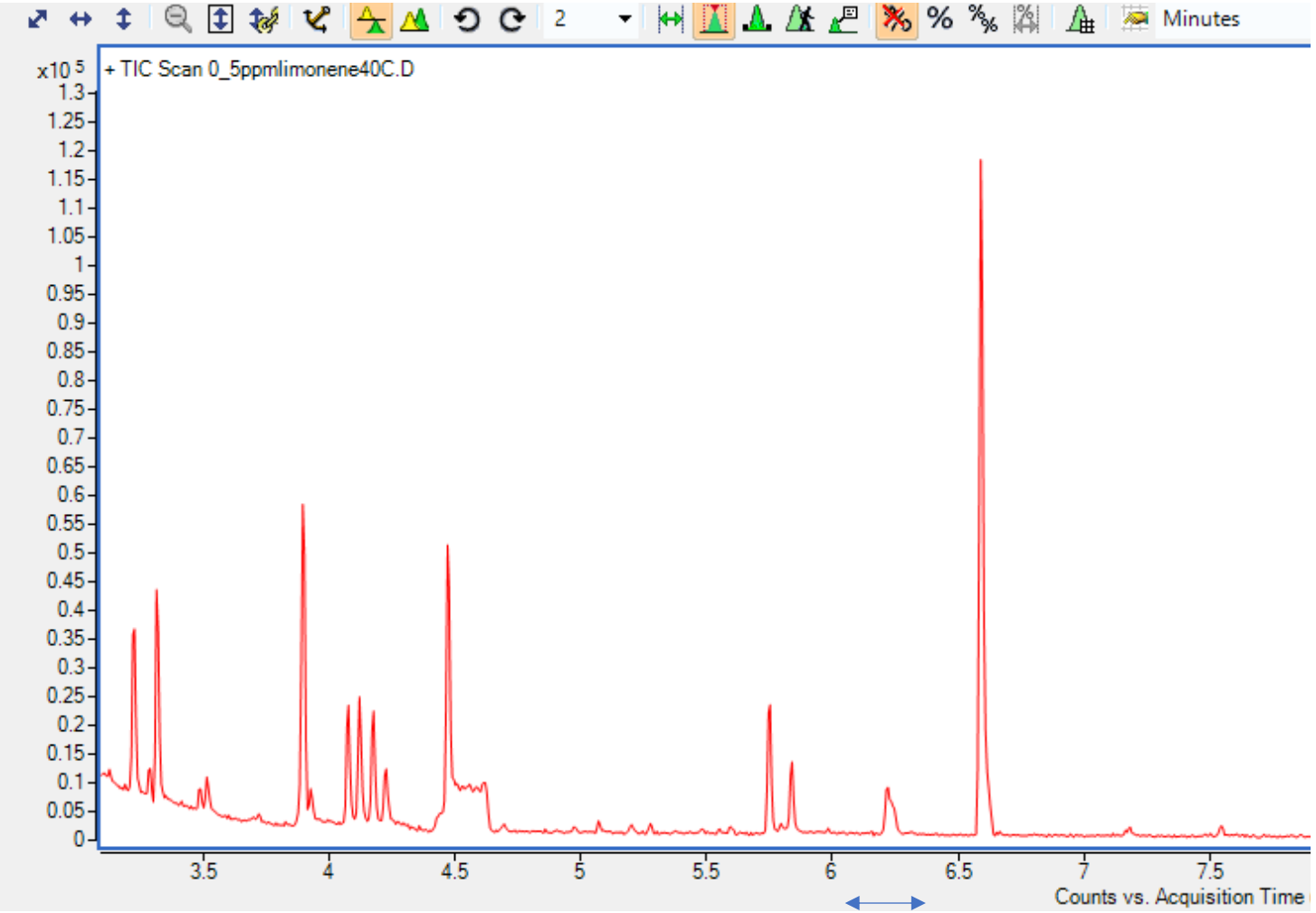




The Data Navigator window shows data files that is/are selected in the above Sample table window. The checked chromatogram and spectra are shown in the windows on the right.

Click Autoscale icon to zoom out

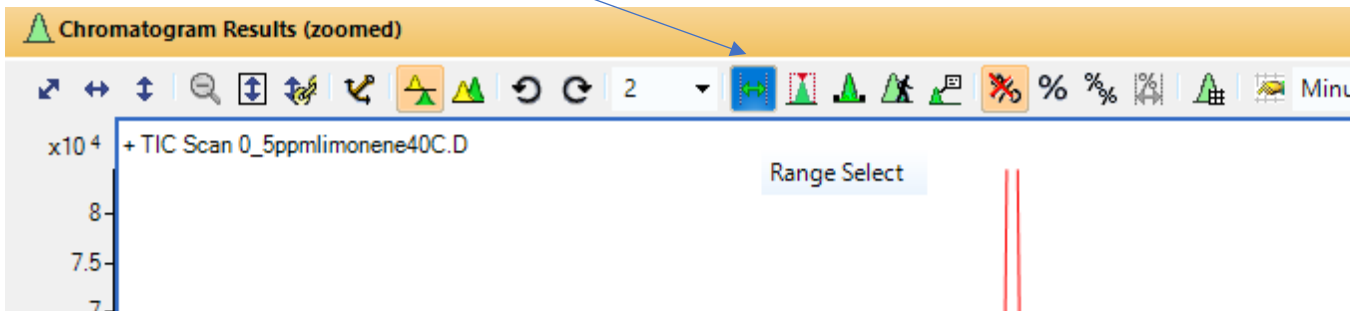
To zoom in on y-axis, move the cursor to y-axis, until a double vertical arrow ↕ appears, press the right mouse button and drag over an area.



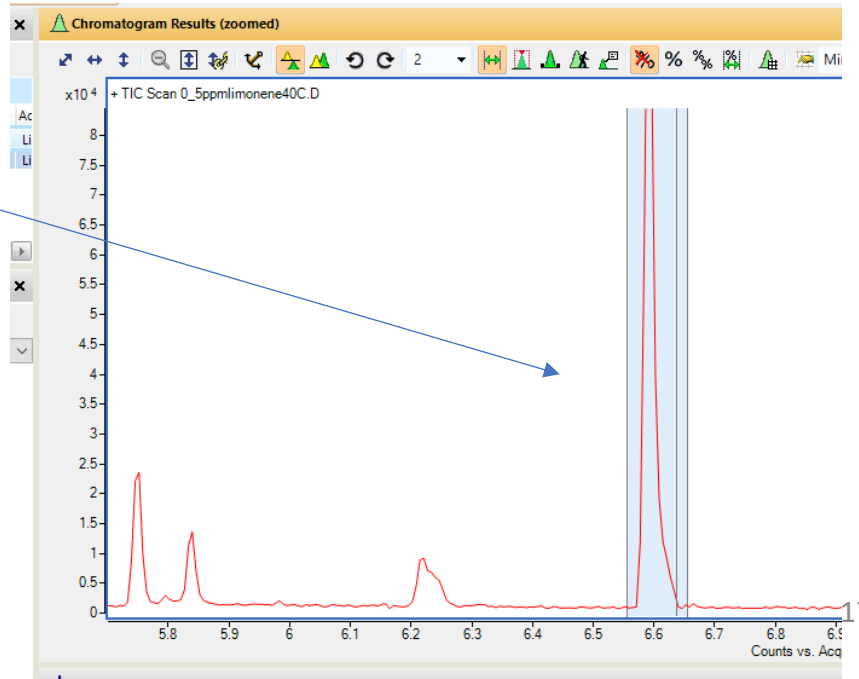
To zoom in on x-axis, move the cursor underneath x axe until a horizontal double arrow ↔ appears, then press the right mouse button and drag over an area.

Data Analysis

4. Left click the Range Select icon

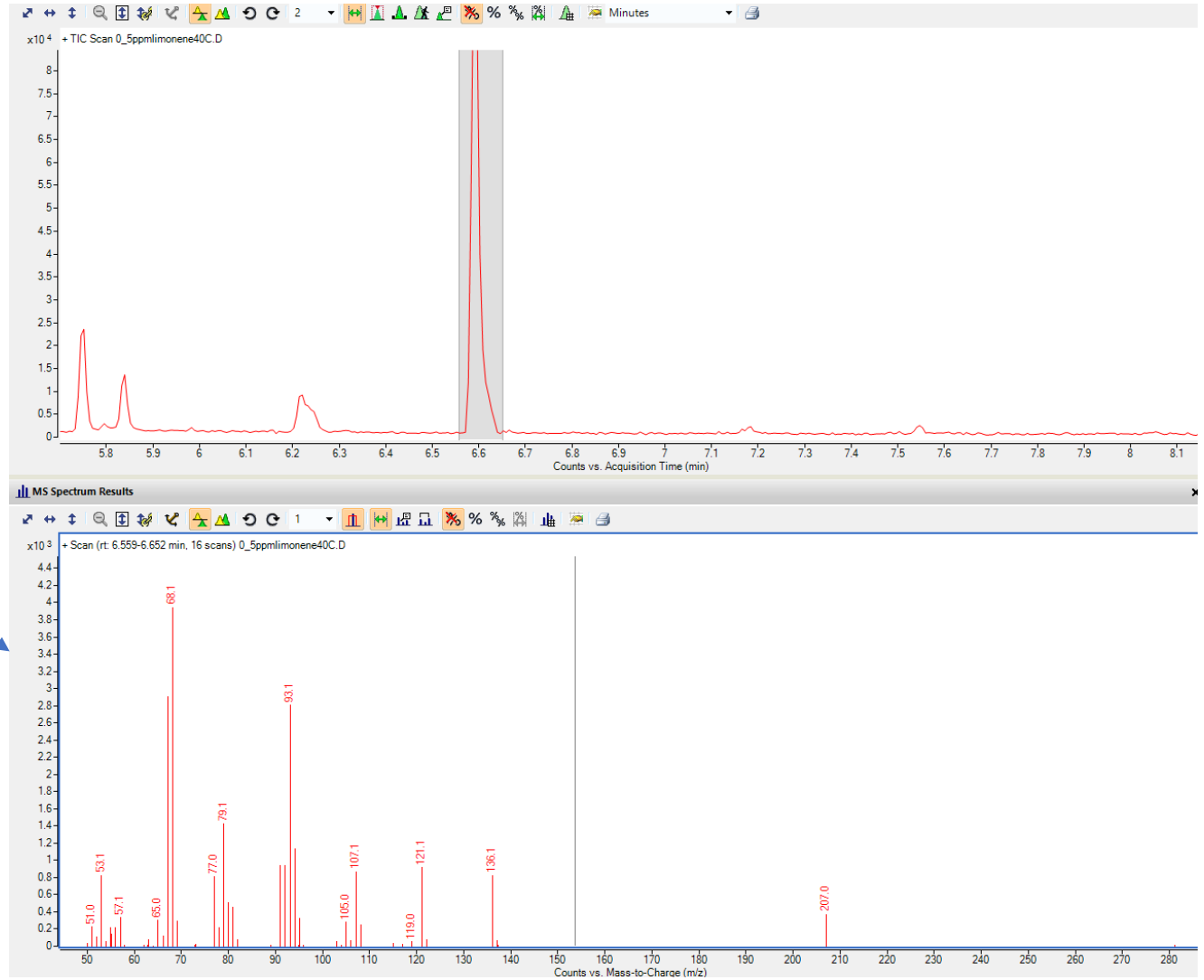


5. Press the left mouse button and drag over the peak of interest



Data Analysis

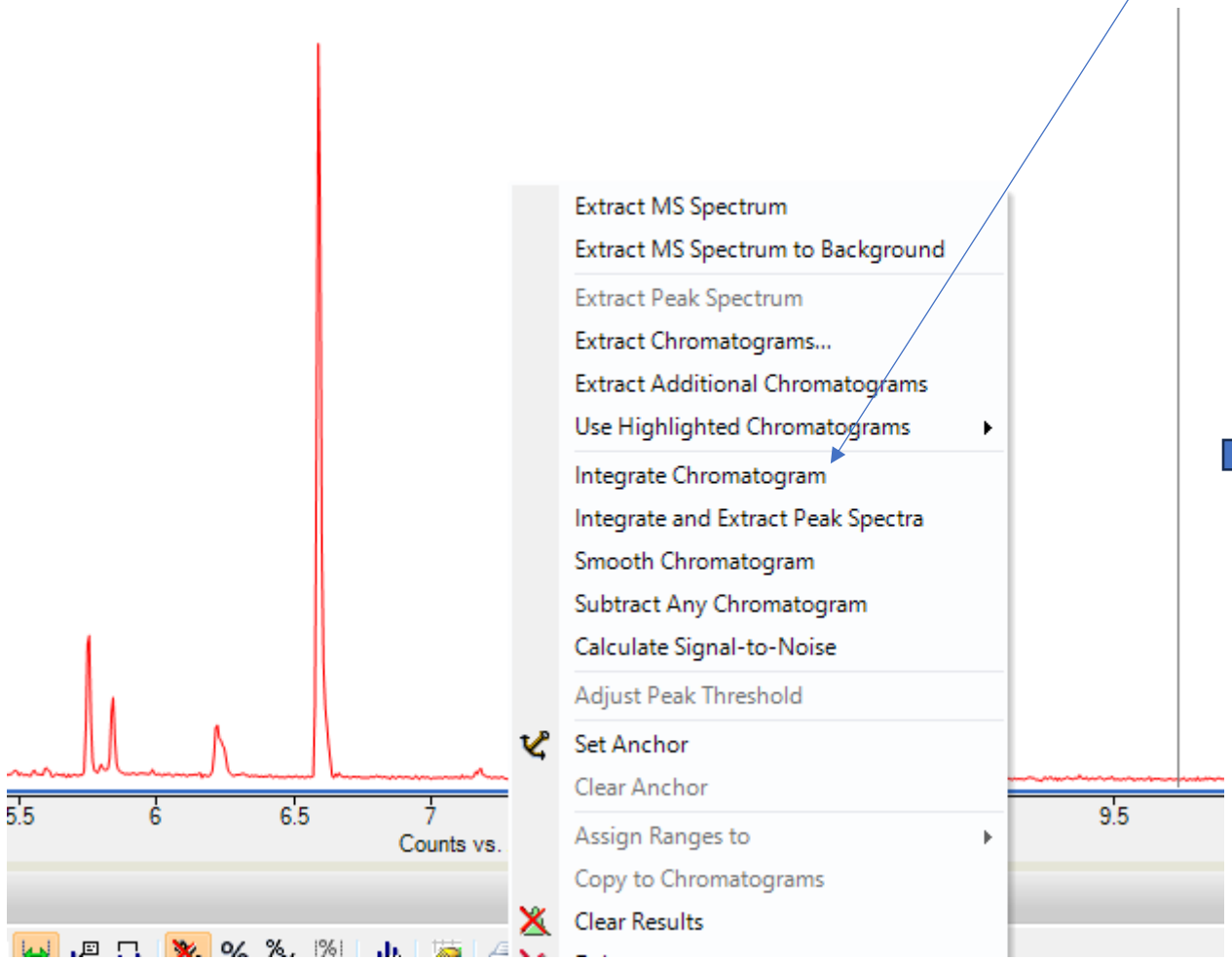
6. Double click the selected peak in the chromatogram. The peak spectrum is extracted and visible in the MS Spectrum Results window.

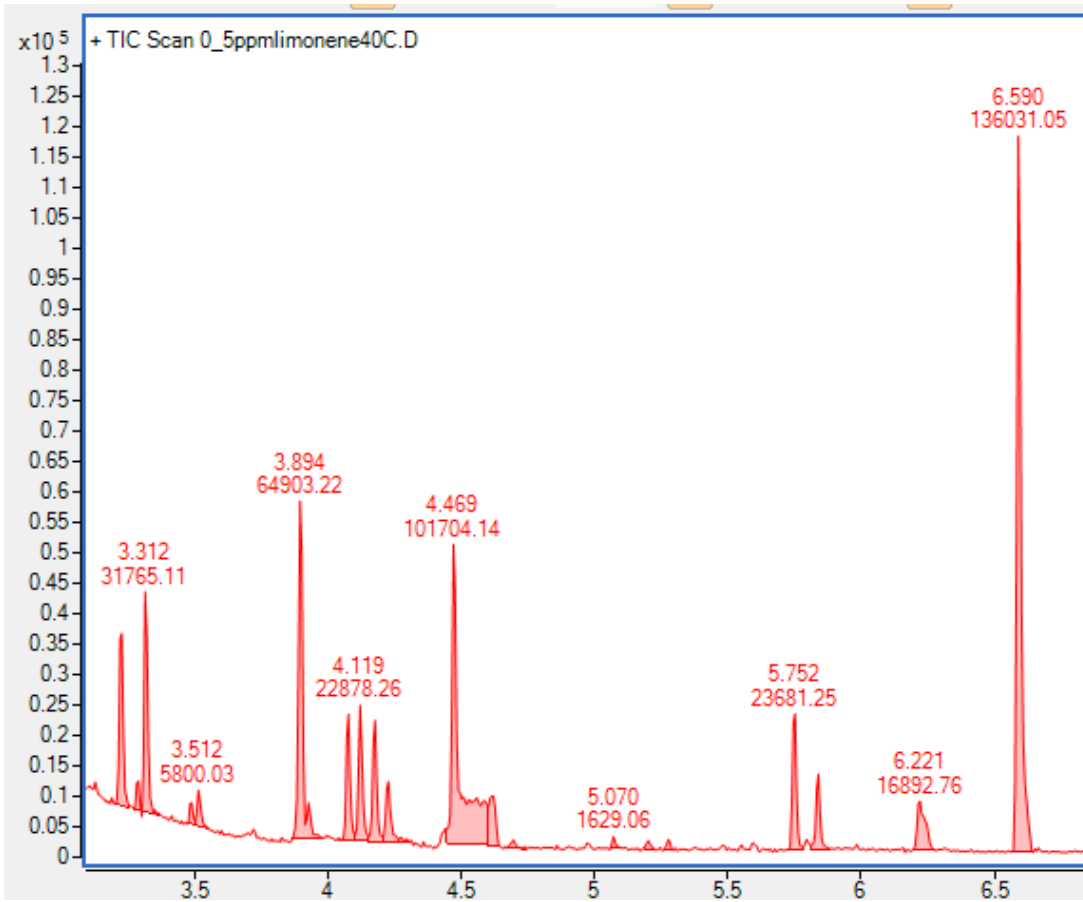


Data Analysis

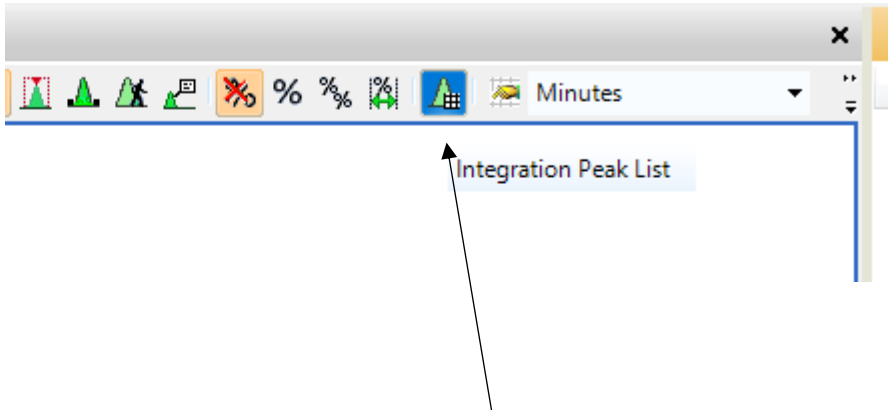
7. Right click in the chromatogram. You can integrate chromatogram or integrate and extract peak spectra as one action.

   Observe the retention time and peak area are displayed.

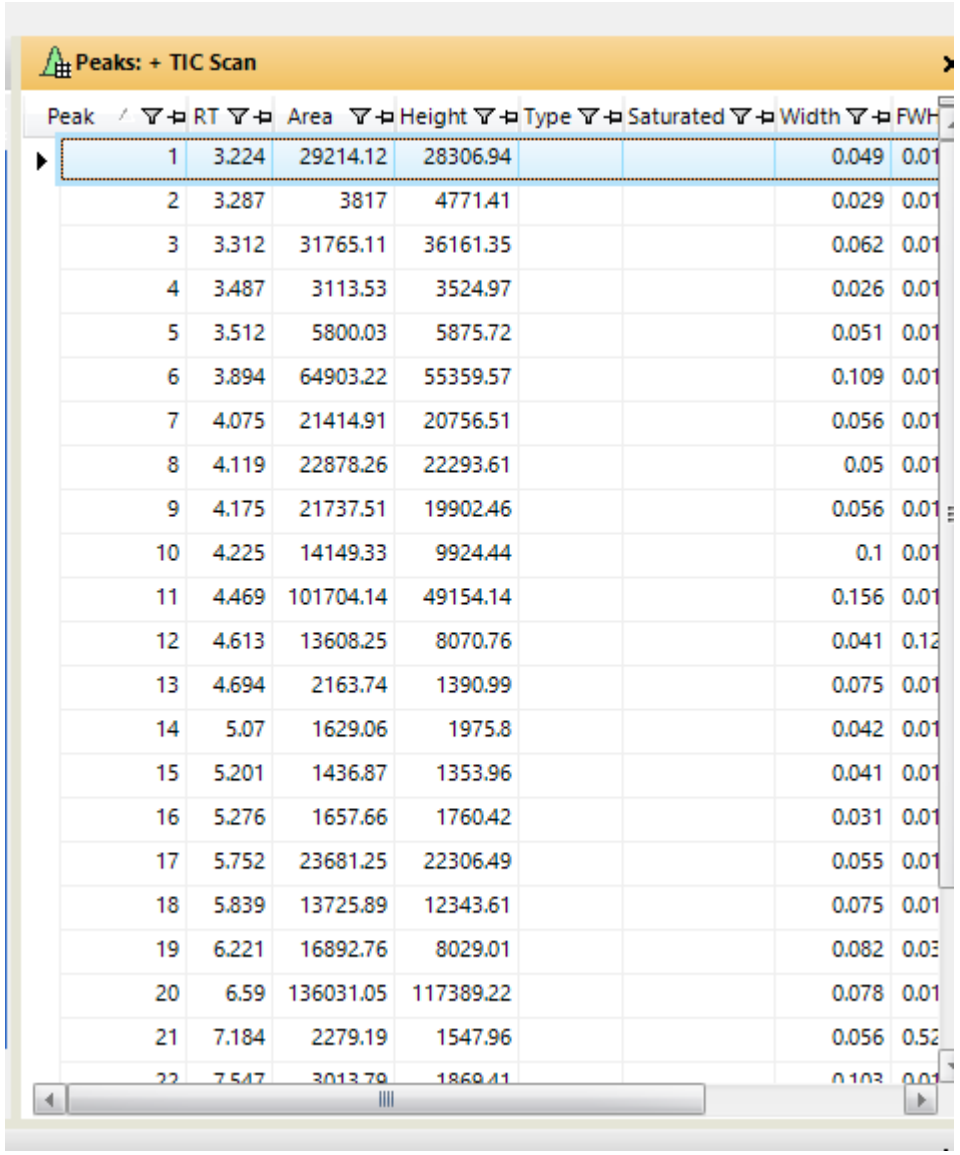




Data Analysis



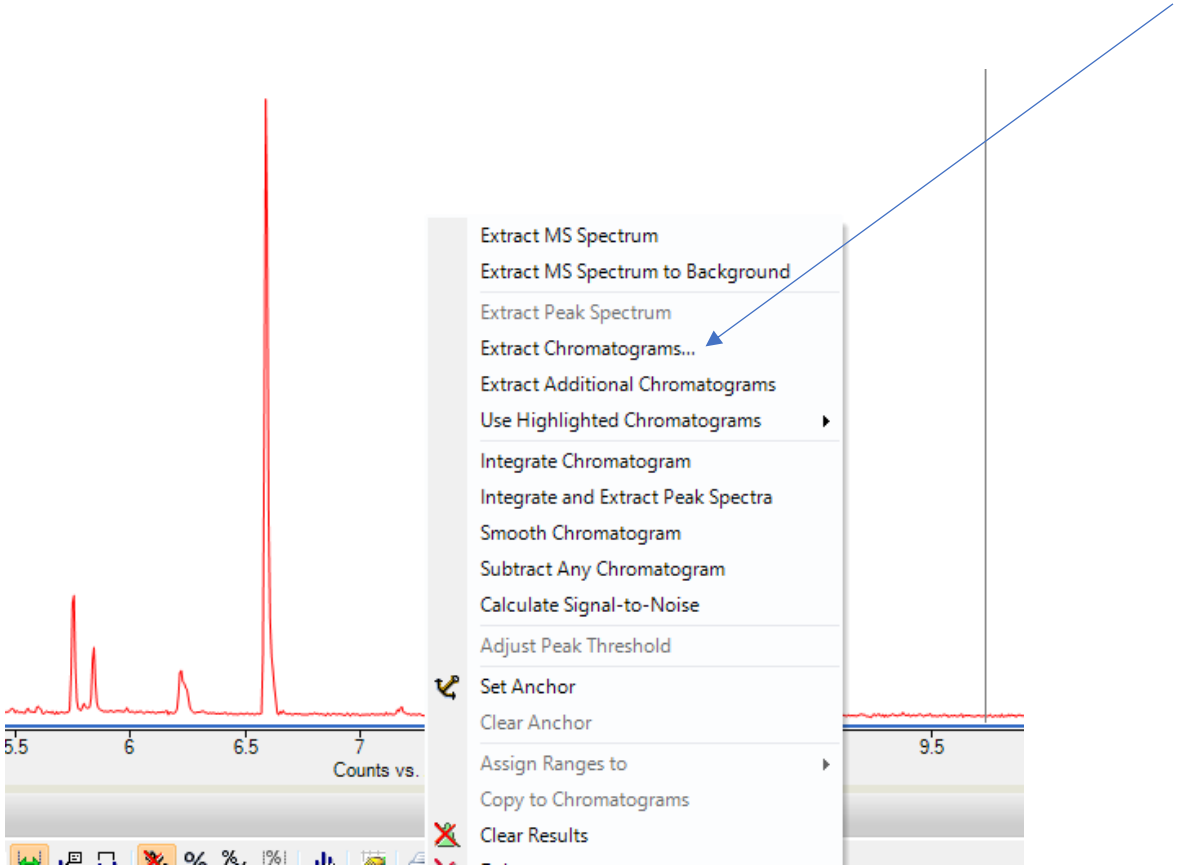
8. Click this icon to view peak list



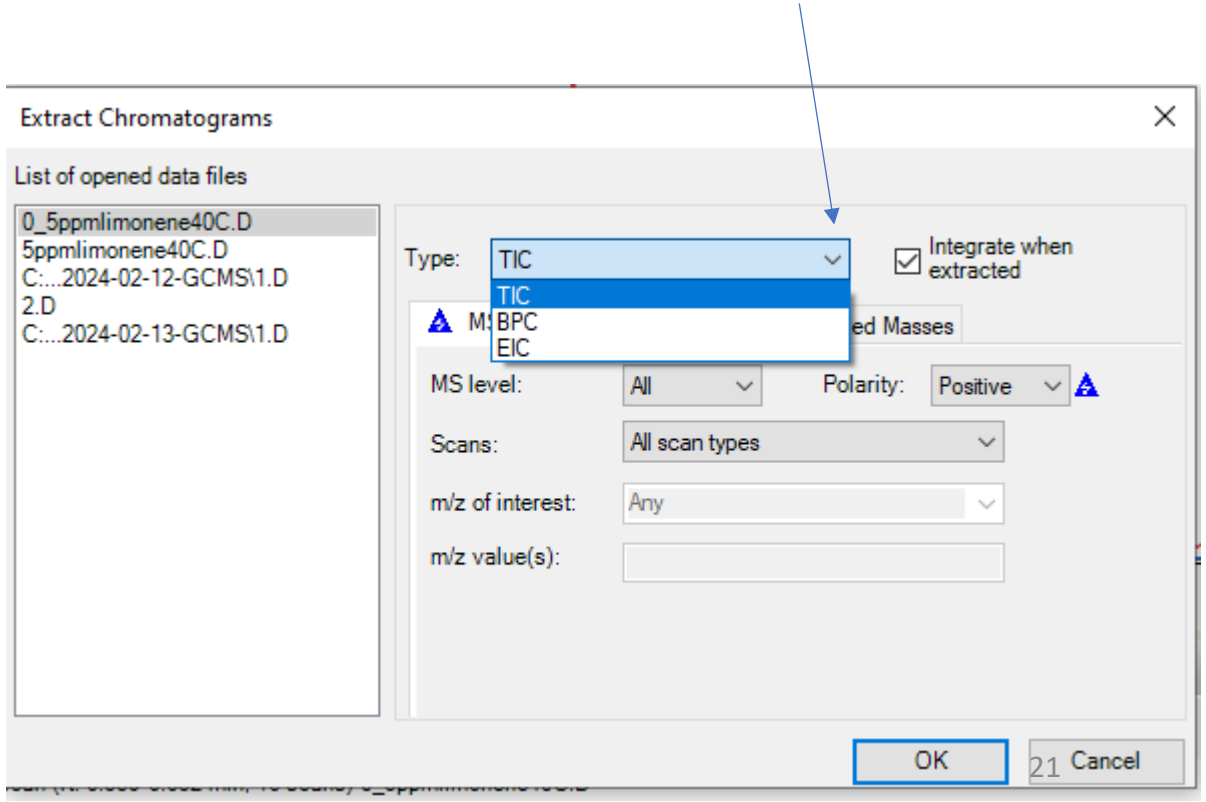
Peak	RT	Area	Height	Type	Saturated	Width	FWH
1	3.224	29214.12	28306.94			0.049	0.01
2	3.287	3817	4771.41			0.029	0.01
3	3.312	31765.11	36161.35			0.062	0.01
4	3.487	3113.53	3524.97			0.026	0.01
5	3.512	5800.03	5875.72			0.051	0.01
6	3.894	64903.22	55359.57			0.109	0.01
7	4.075	21414.91	20756.51			0.056	0.01
8	4.119	22878.26	22293.61			0.05	0.01
9	4.175	21737.51	19902.46			0.056	0.01
10	4.225	14149.33	9924.44			0.1	0.01
11	4.469	101704.14	49154.14			0.156	0.01
12	4.613	13608.25	8070.76			0.041	0.12
13	4.694	2163.74	1390.99			0.075	0.01
14	5.07	1629.06	1975.8			0.042	0.01
15	5.201	1436.87	1353.96			0.041	0.01
16	5.276	1657.66	1760.42			0.031	0.01
17	5.752	23681.25	22306.49			0.055	0.01
18	5.839	13725.89	12343.61			0.075	0.01
19	6.221	16892.76	8029.01			0.082	0.03
20	6.59	136031.05	117389.22			0.078	0.01
21	7.184	2279.19	1547.96			0.056	0.52
22	7.547	3013.79	1869.41			0.103	0.01

Data Analysis

9. Right click in the chromatogram. Click Extract Chromatograms.

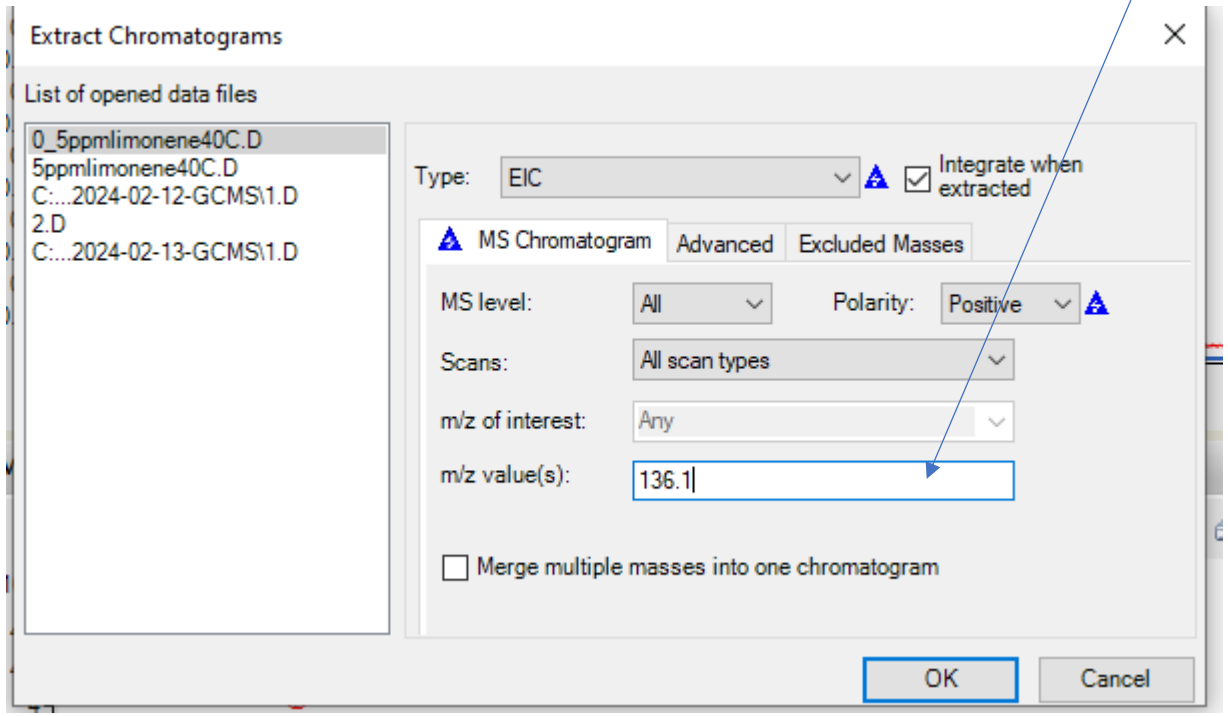


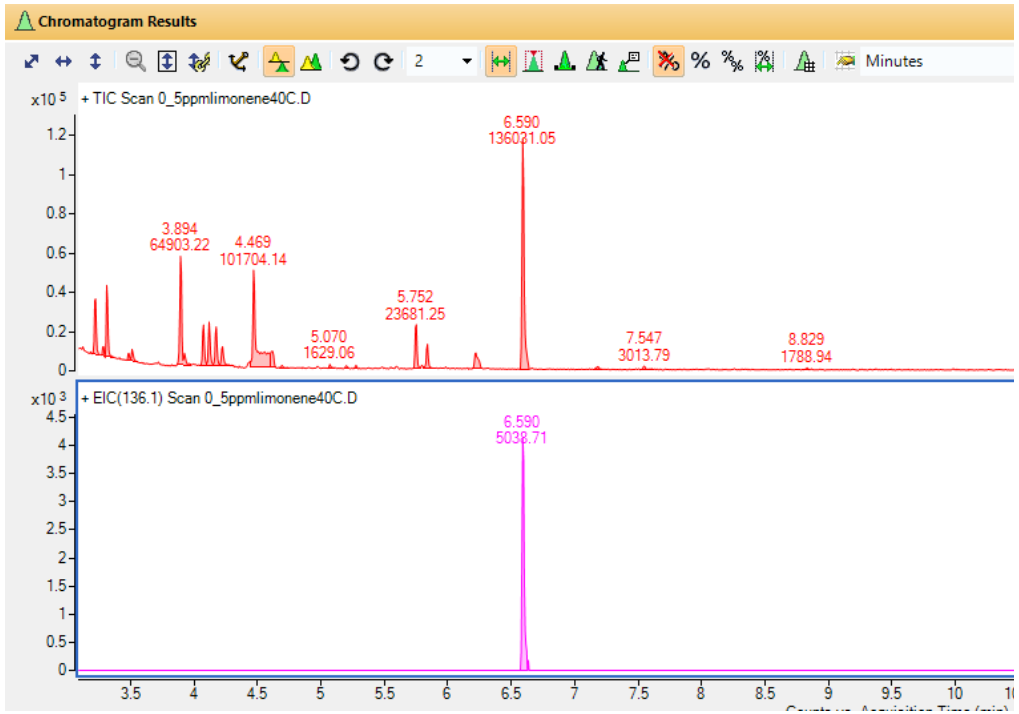
10. Click the down-arrow for type



Data Analysis

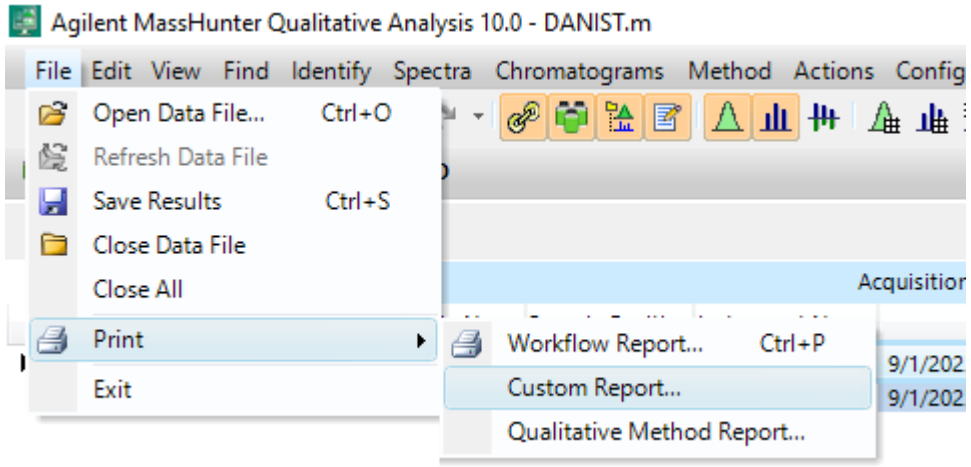
11. Click EIC. Input m/z value that you are interested in. Use commas to separate multiple values if needed. Click OK to extract an EIC (Extracted Ion Chromatogram). This could be used to search for a specific ion signal.

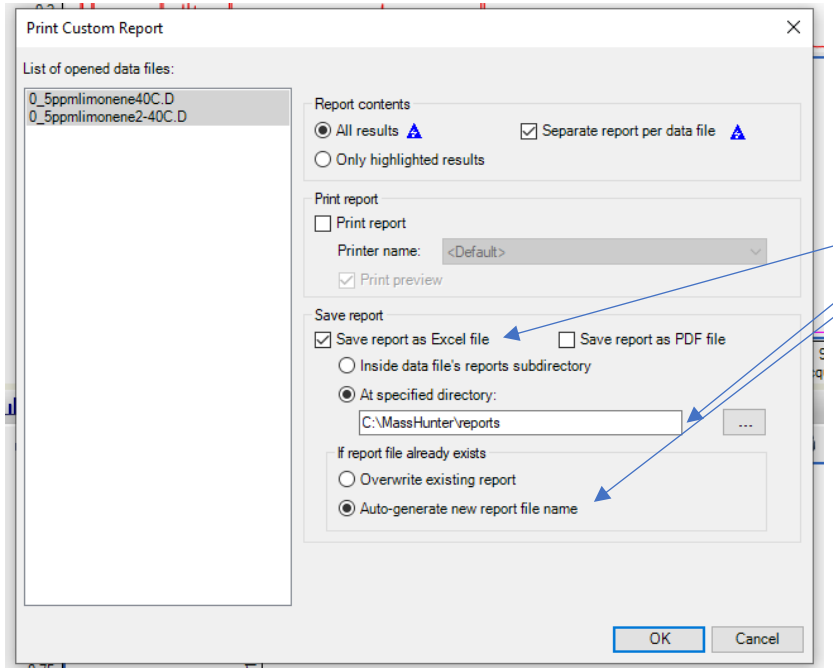




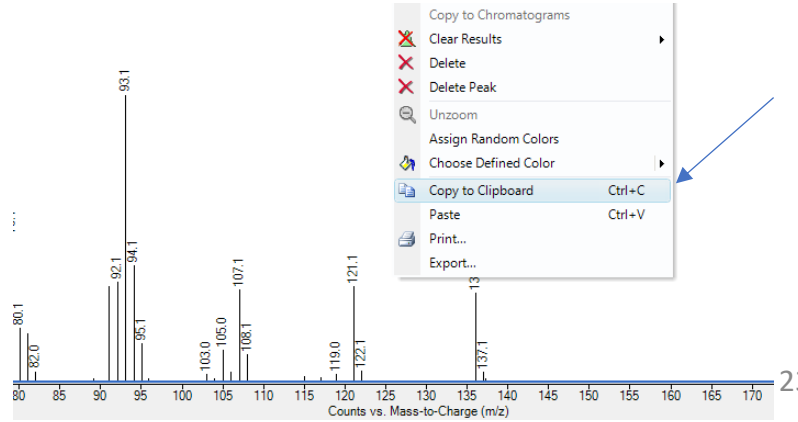
Data Analysis

12. To generate a report, left click File > Print > Custom Report



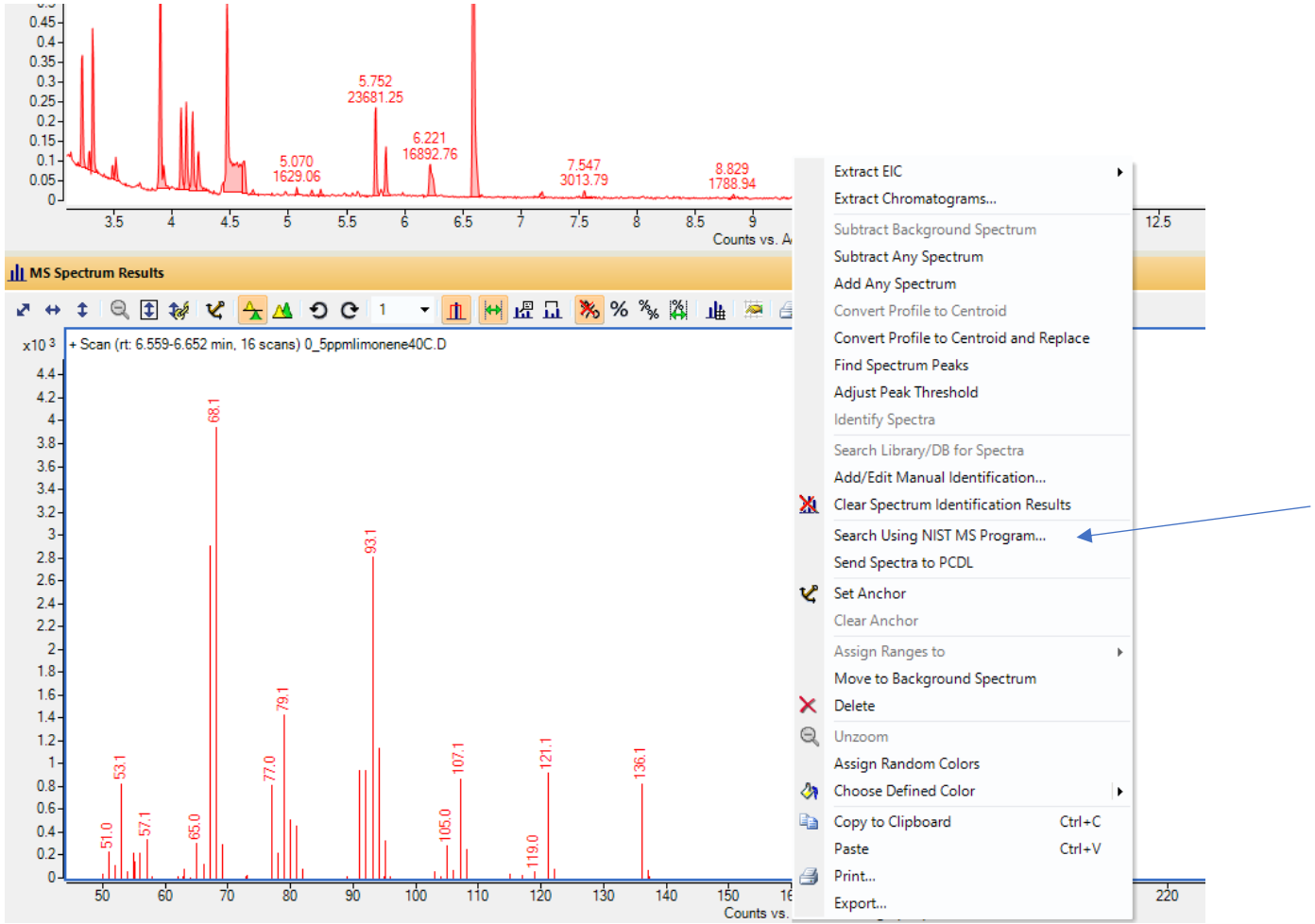


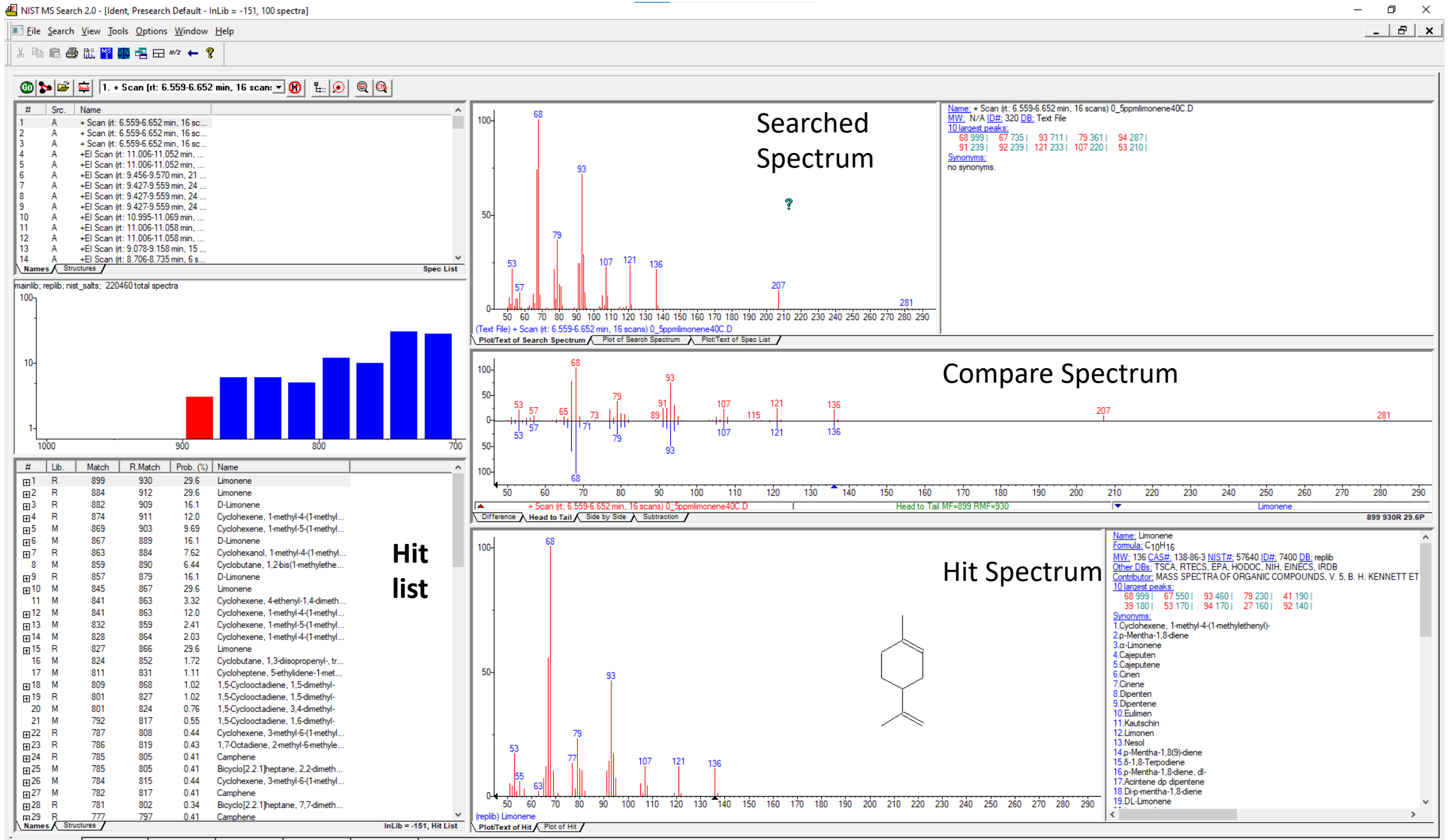
Set parameters in the dialog box that appears. Additionally, chromatograms and spectra can be copied and pasted into the report manually.



NIST library search

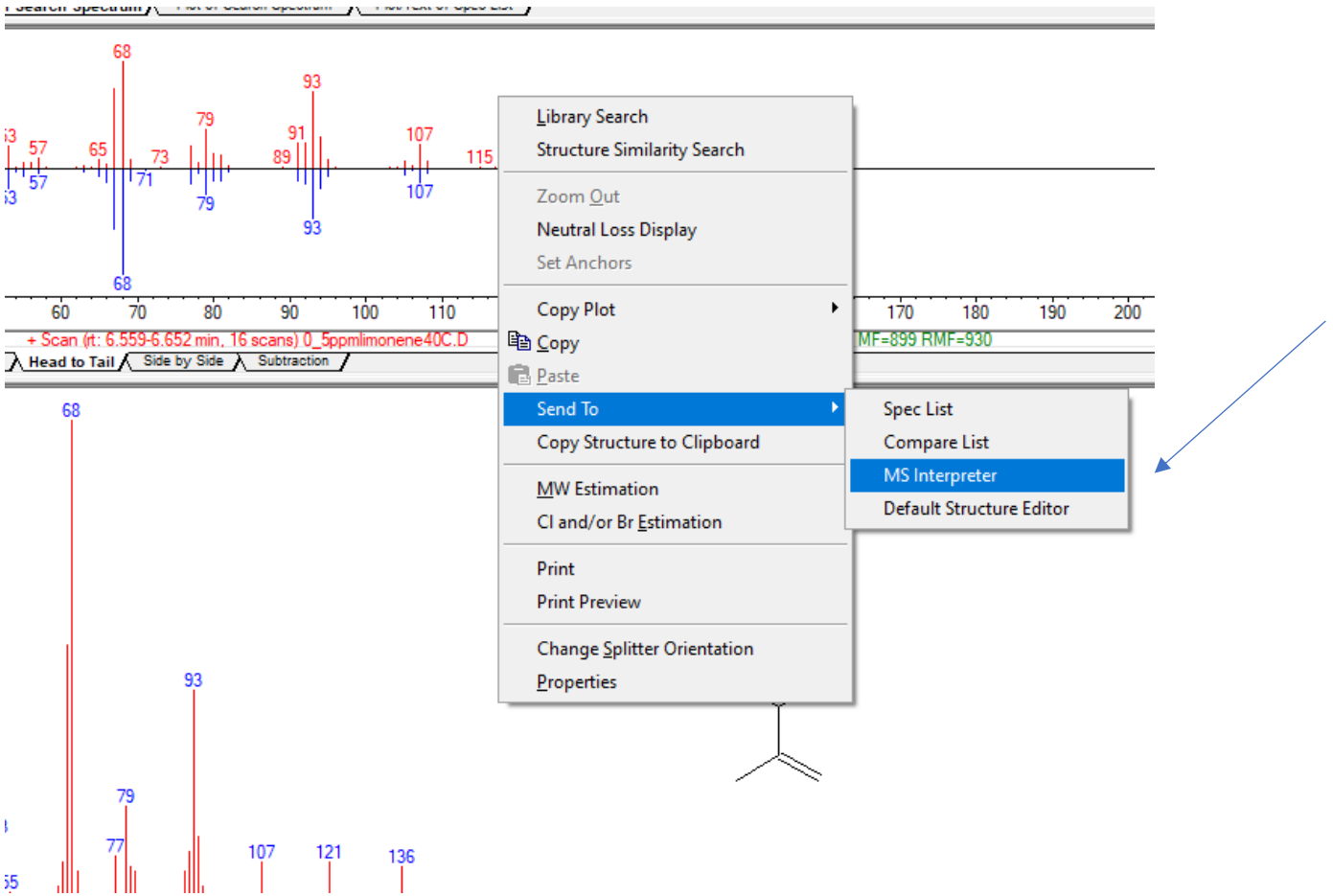
1. Right click a mass spectrum. Click Search Using NIST MS Program. This mass spectrum will be searched against the NIST library to find spectra of standards that are same or similar to that of your compound.

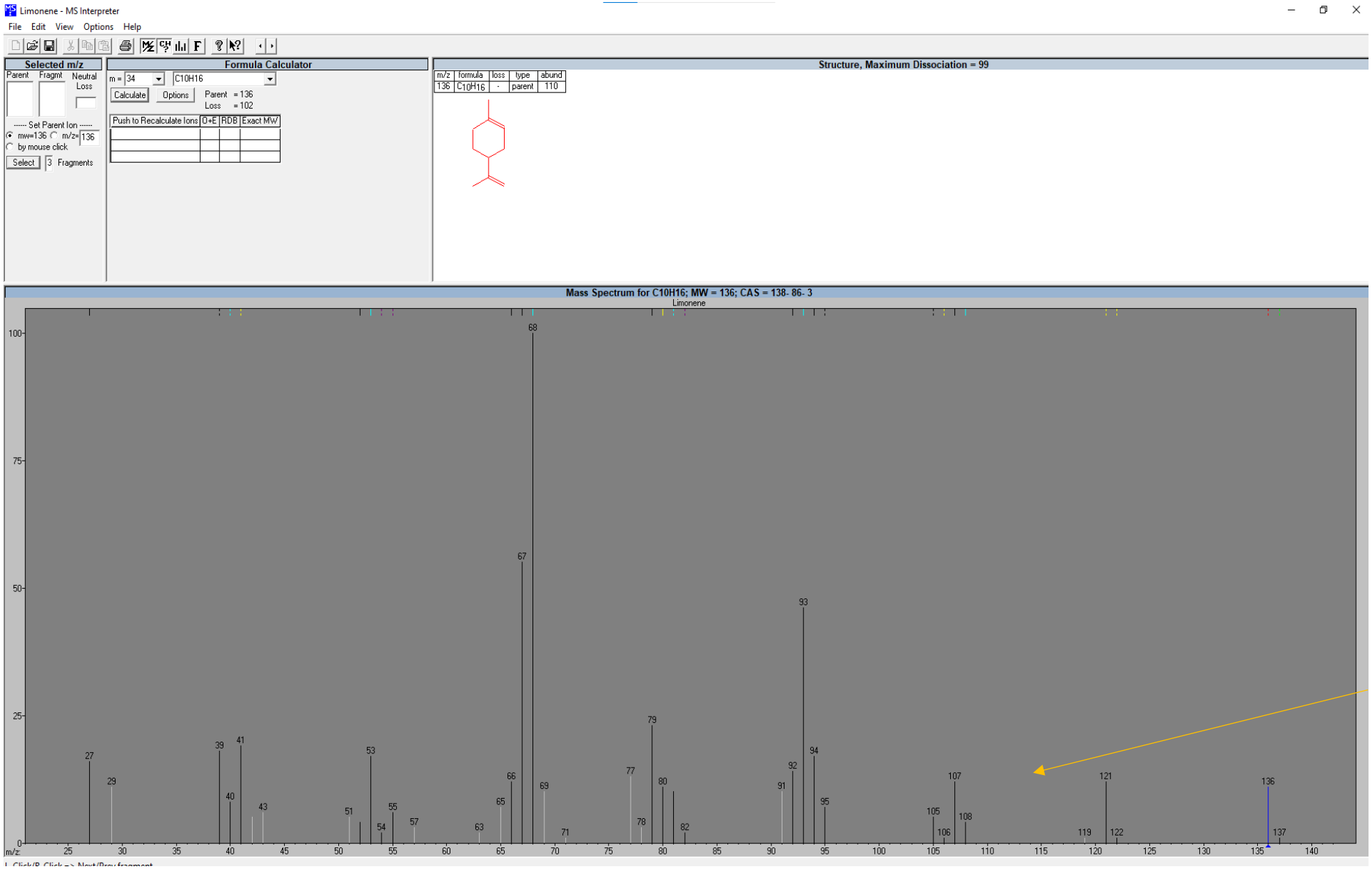



NIST MS Search 2.0 - [Ident, Presearch Default - InLib = -151, 100 spectra]
File Search View Tools Options Window Help
1. + Scan (rt: 6.559-6.652 min, 16 scan
Searched Spectrum
Compare Spectrum
Hit Spectrum
Hit list
mainlib; replib; nist_salts; 220460 total spectra
Lib. Search
Other Search
Names
Compare
Librarian
MSMS

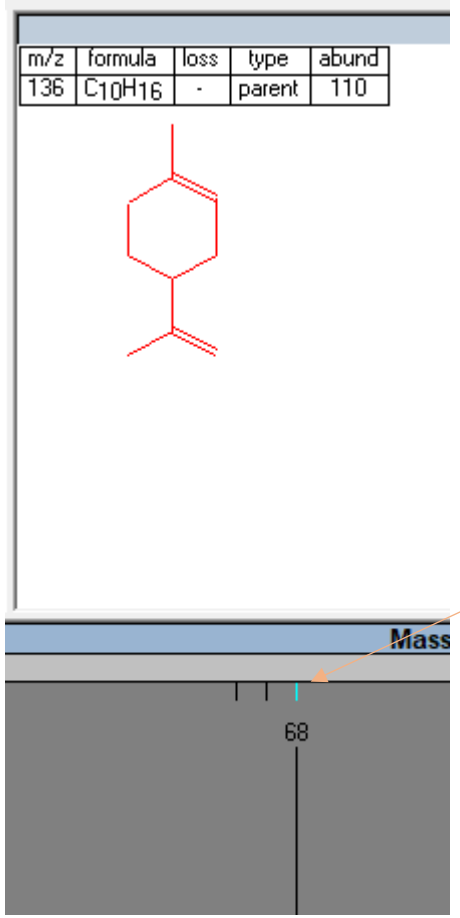
Utilize the NIST MS interpreter to assist in interpreting your spectrum.

2. In the interface of NIST, right click a hit spectrum > Send to > MS interpreter

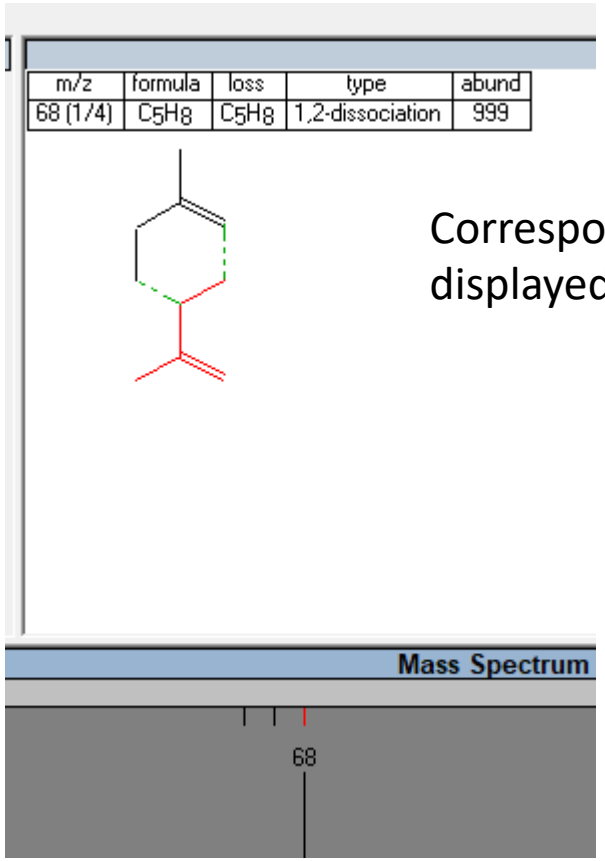




The black lines indicate the peaks in the mass spectrum that the MS Interpreter can explain. The white lines indicate the ones it cannot.



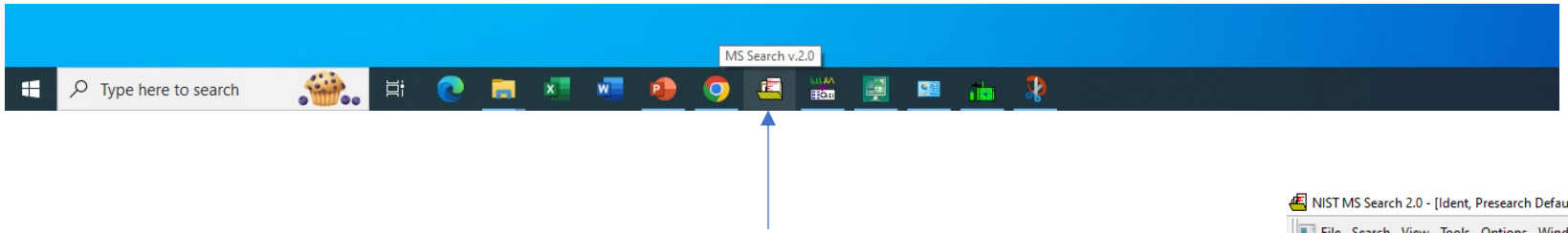
Left click the notch on top of the peak of interest, for example, the peak m/z 68



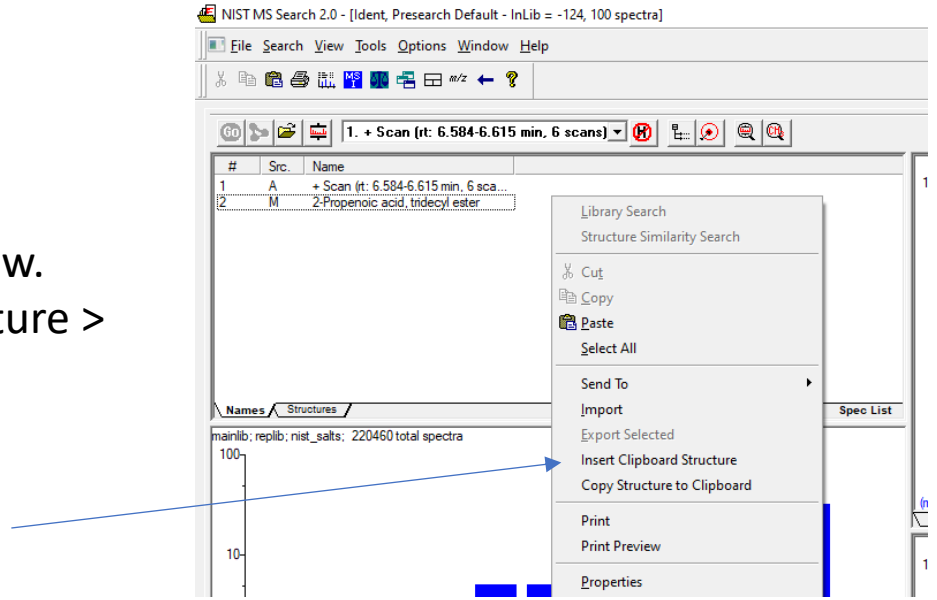
Corresponding fragments are displayed in red

Structure search allows the user to search the library for a structure.

1. Click the NIST icon in taskbar to open the NIST interface as shown on slide 25.

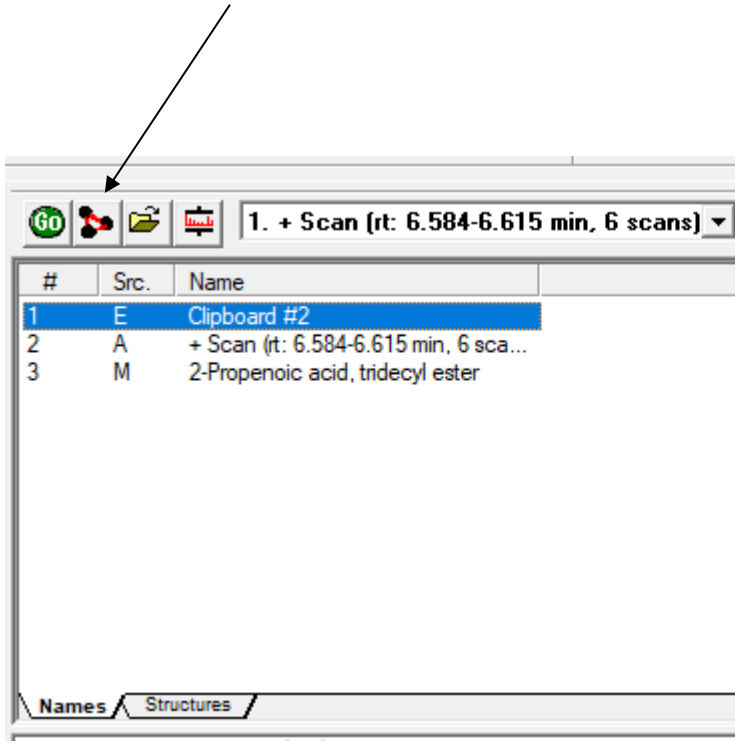


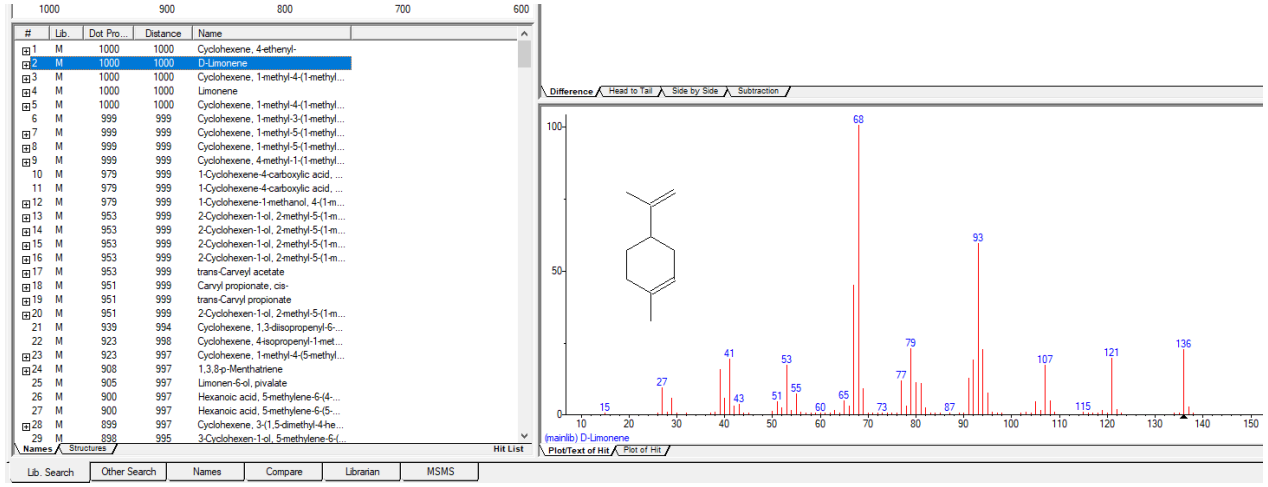
2. Copy a structure that you are interested in from ChemDraw.
Right Click Spec list window of NIST > Insert Clipboard Structure > OK



Structure search

3. Highlight the structure in Spec List by click it. Left click the Structure Search icon to start the search.

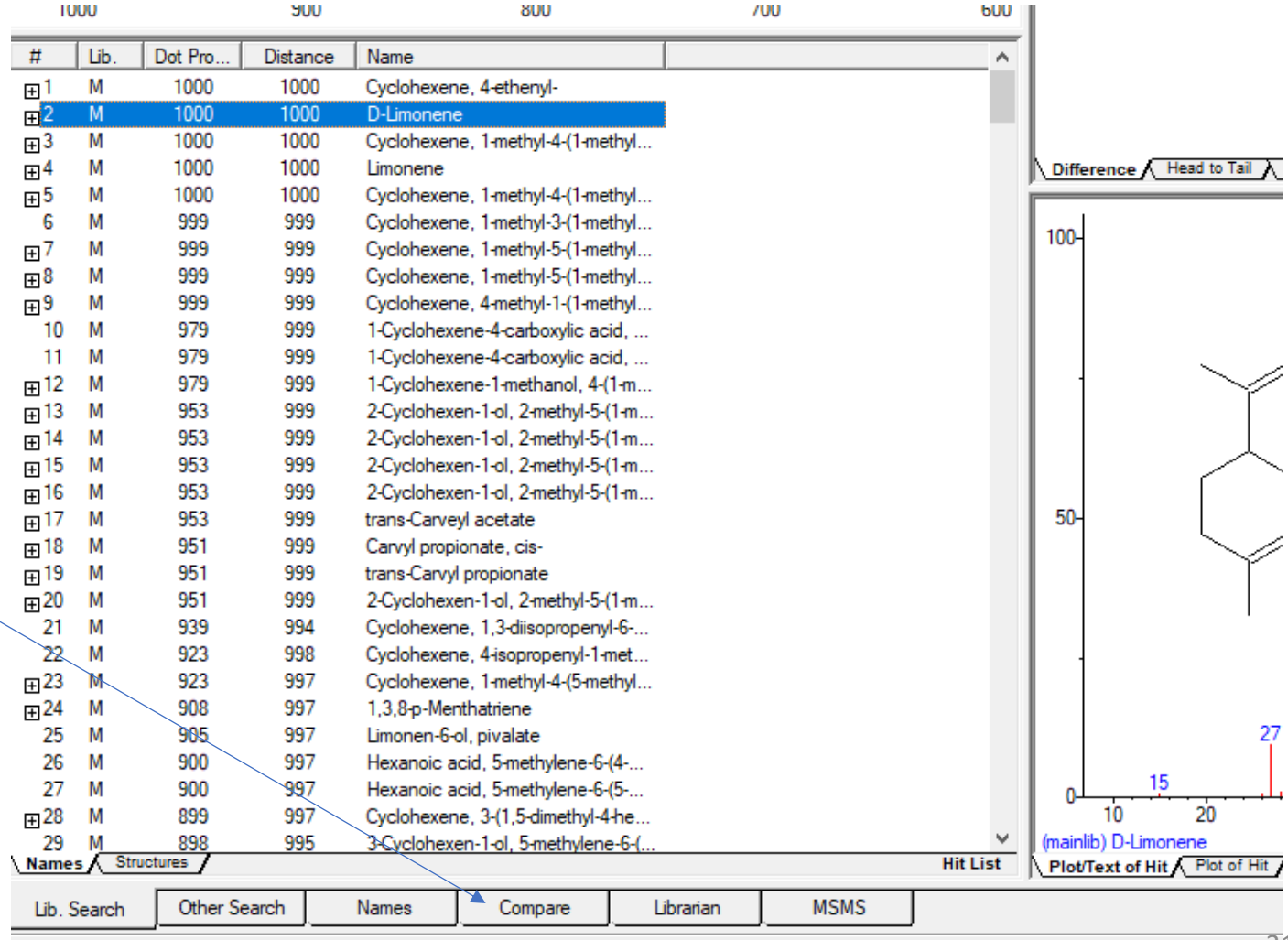




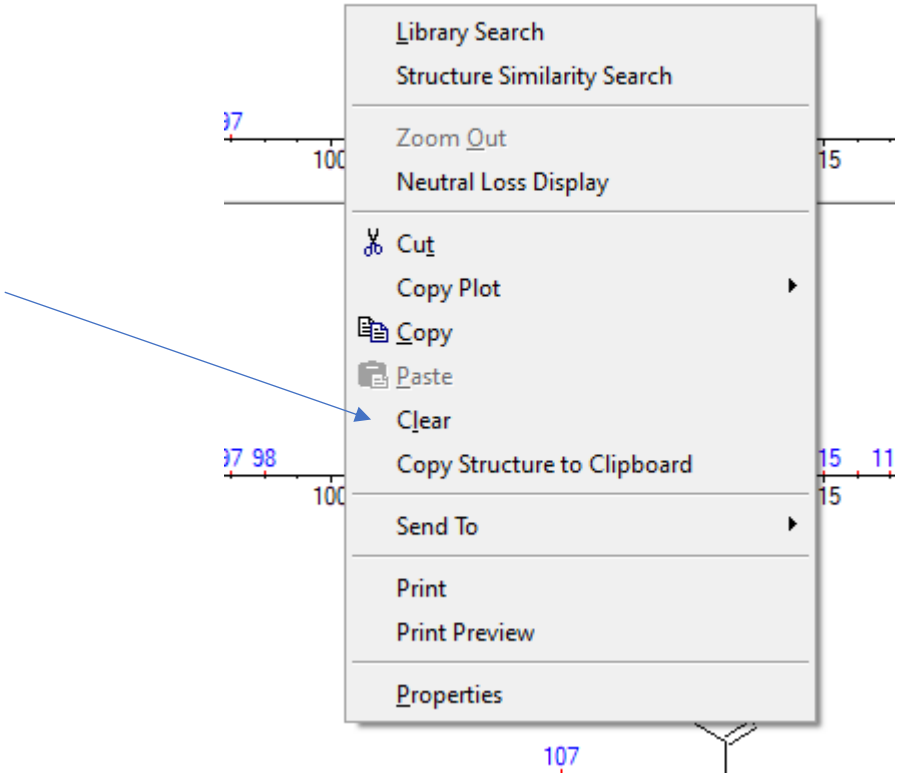
Structure search

You could further use the found hits to compare the spectra of isomers.

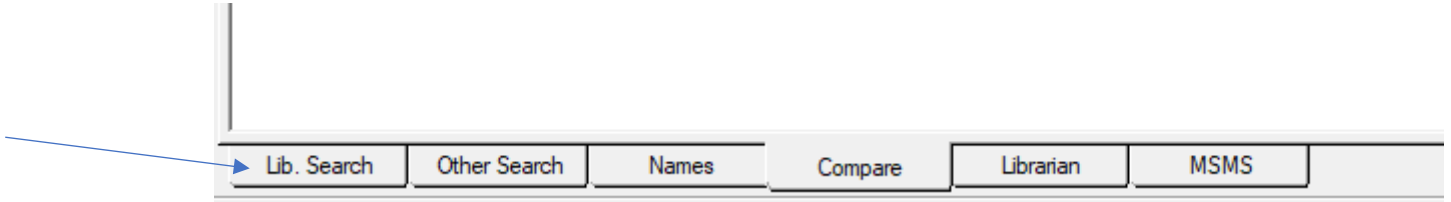
4. Click Compare



5. Right Click the spectra > Clear

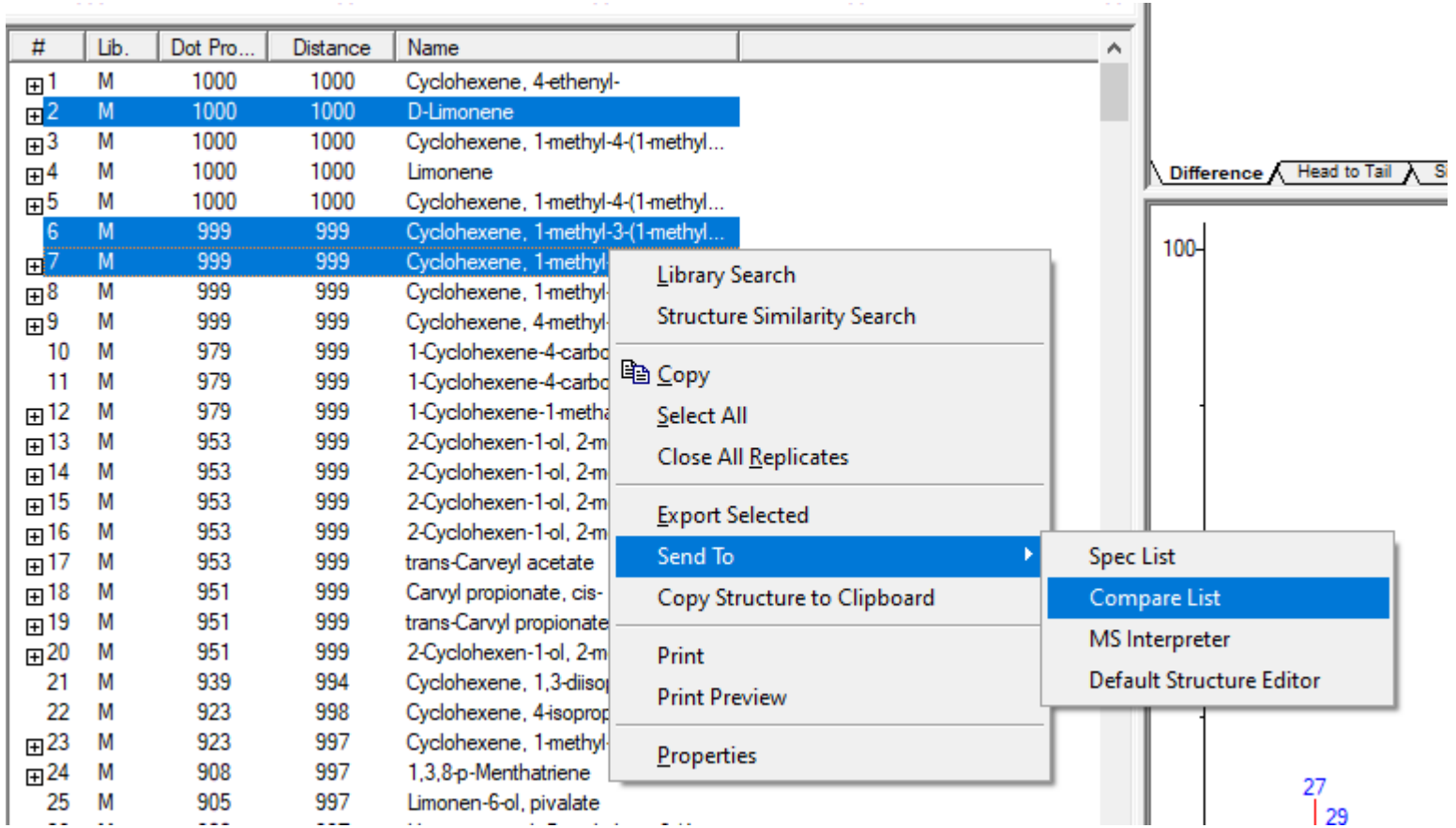


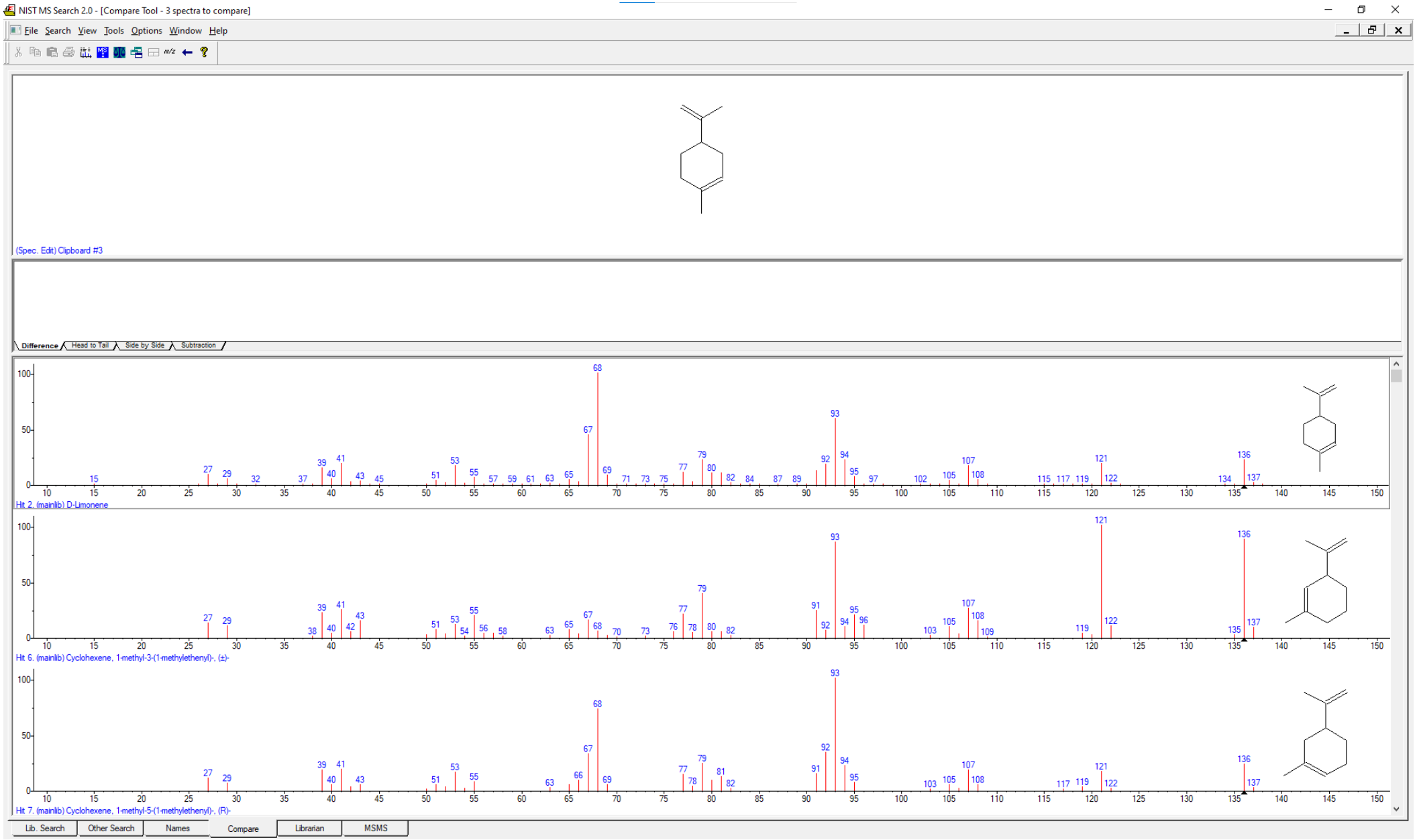
6. Left Click Lib. Search to go back the search interface



7. Choose the isomers of interest in hit list window. Right Click > Send to > Compare List.

The result is as shown in next slide.




NIST MS Search 2.0 - [Compare Tool - 3 spectra to compare]
File Search View Tools Options Window Help
(Spec. Edit) Clipboard #3
Difference
Head to Tail
Side by Side
Subtraction
Hit 2. (mainlib) D-Limonene
Hit 6. (mainlib) Cyclohexene, 1-methyl-3-(1-methylethenyl)-, (±)-
Hit 7. (mainlib) Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)-
Lib. Search
Other Search
Names
Compare
Librarian
MSMS

To conduct quantitative analysis and omics studies, additional software is required. Please contact me for training