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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's 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 $\mu\text{g}/\text{ml}$) 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 $\mu\text{g}/\text{ml}$, 0.1 mM)**. Below is an example of chromatograms of limonene at 0.5 and 5 ppm.

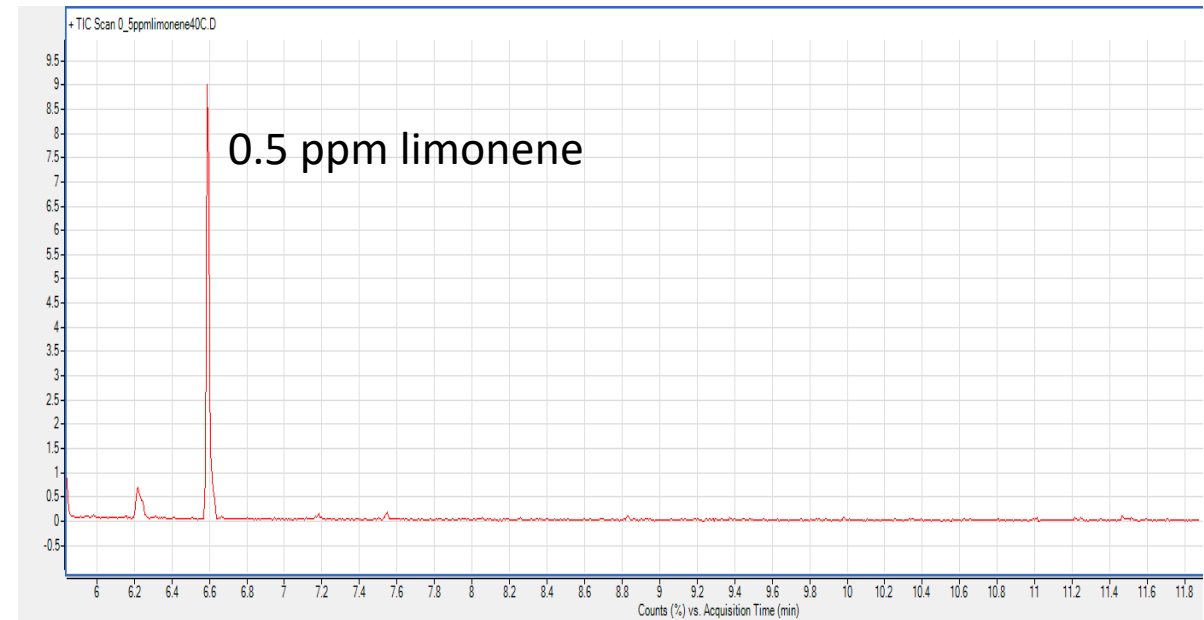
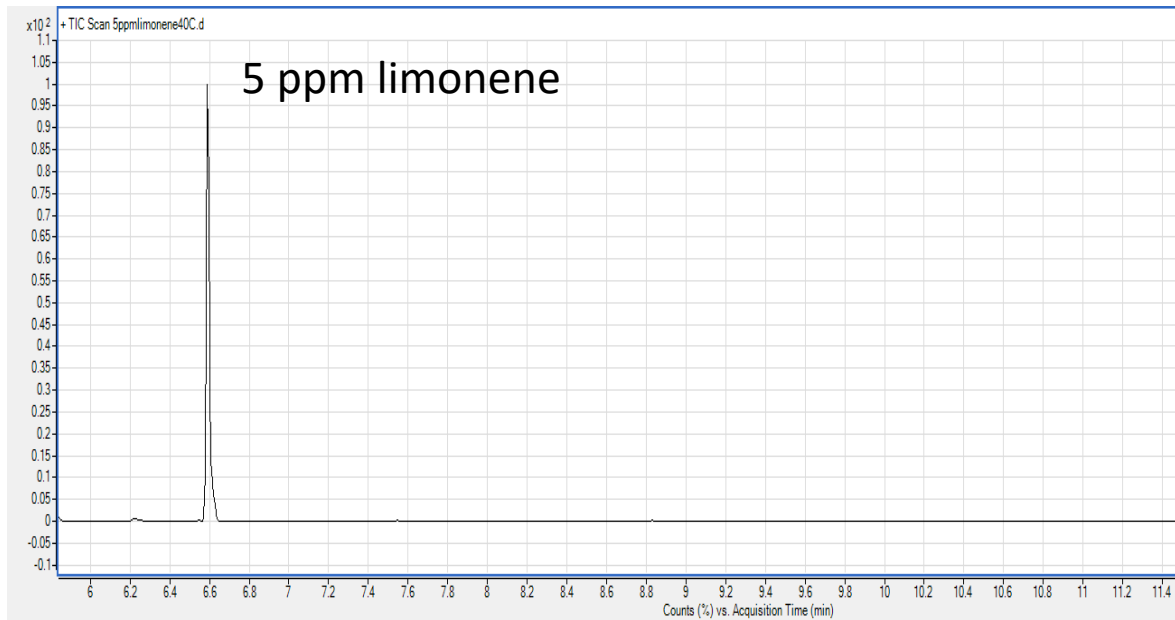


Fig. TIC Chromatogram of 5 ppm and 0.5 ppm limonene.

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.

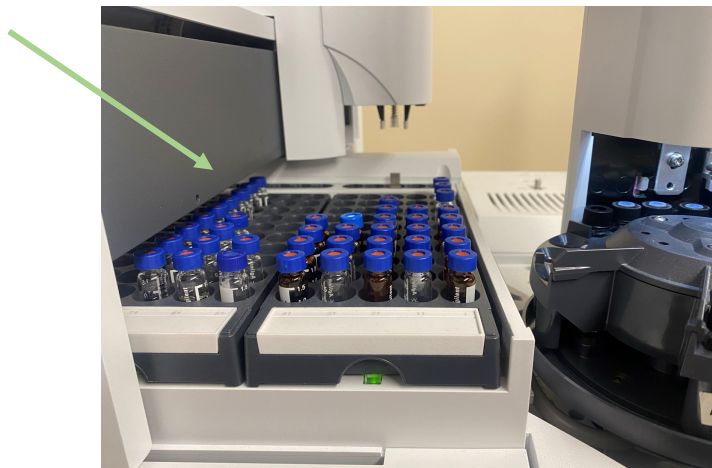
https://www.agilent.com/store/en_US/Prod-5182-0715/5182-0715

Links to vials and caps: https://www.agilent.com/store/en_US/Prod-5182-0716/5182-0716

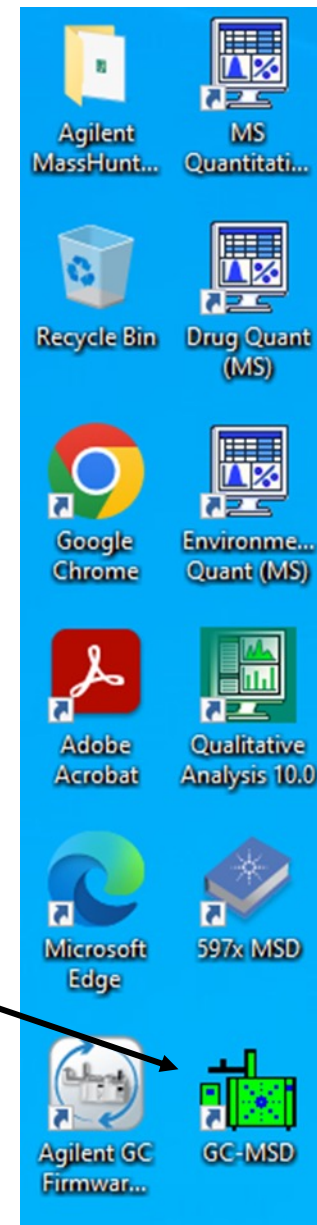
https://www.agilent.com/store/en_US/Prod-5182-0717/5182-0717

Data Acquisition

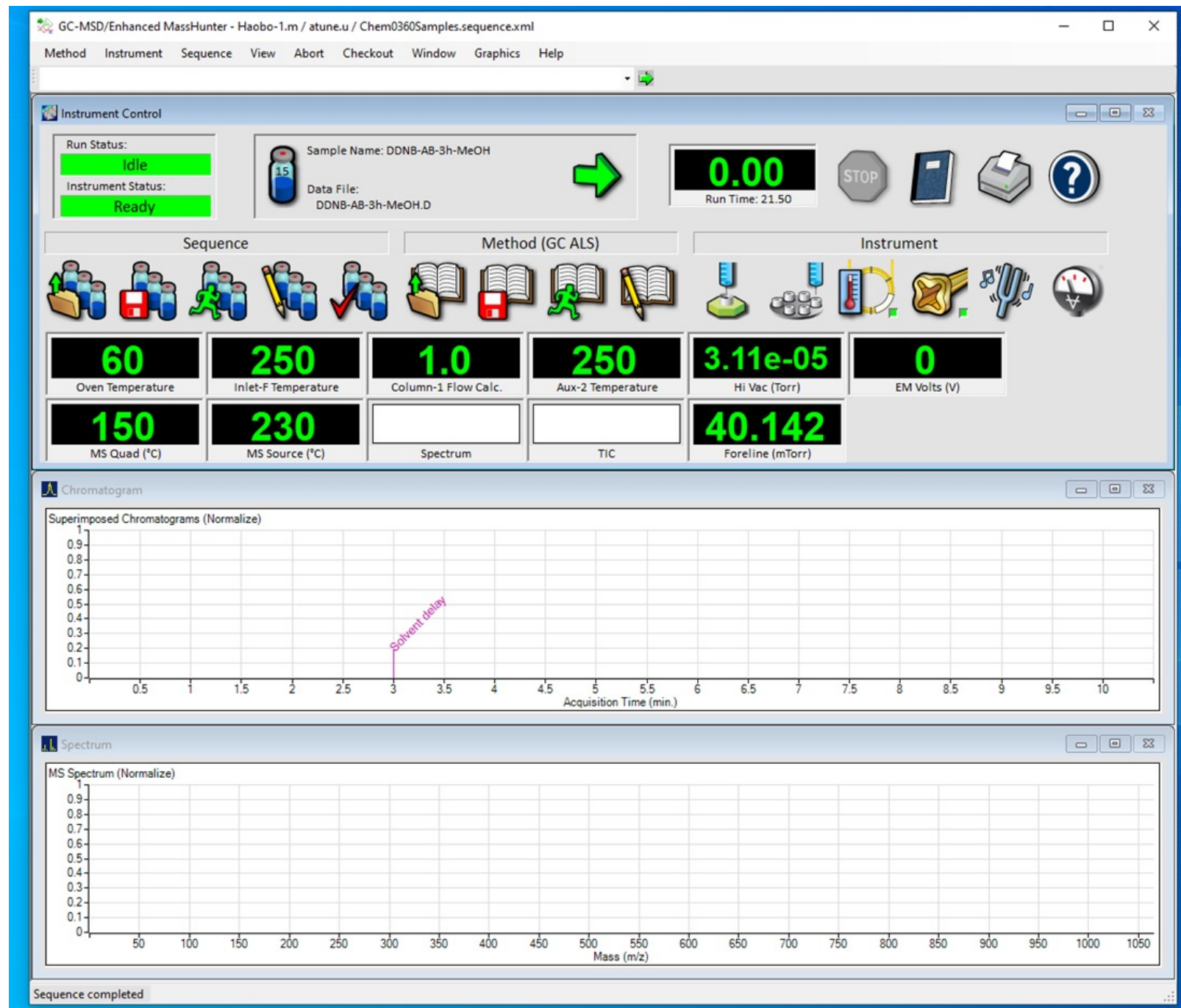
1. Place the sample vial in the autosampler.



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



The user interface

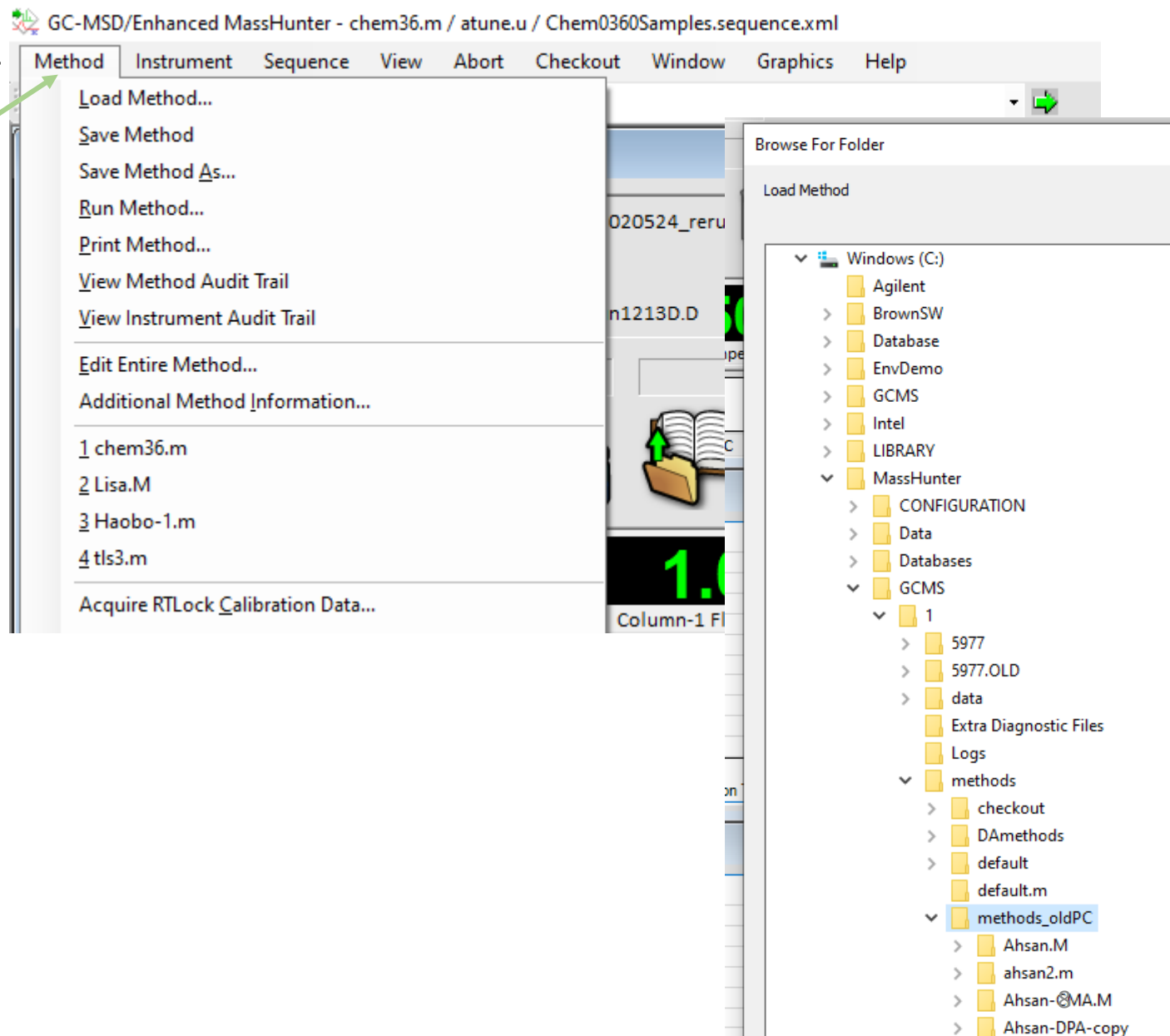


Data Acquisition

3. Click Method > Load Method > MassHunter

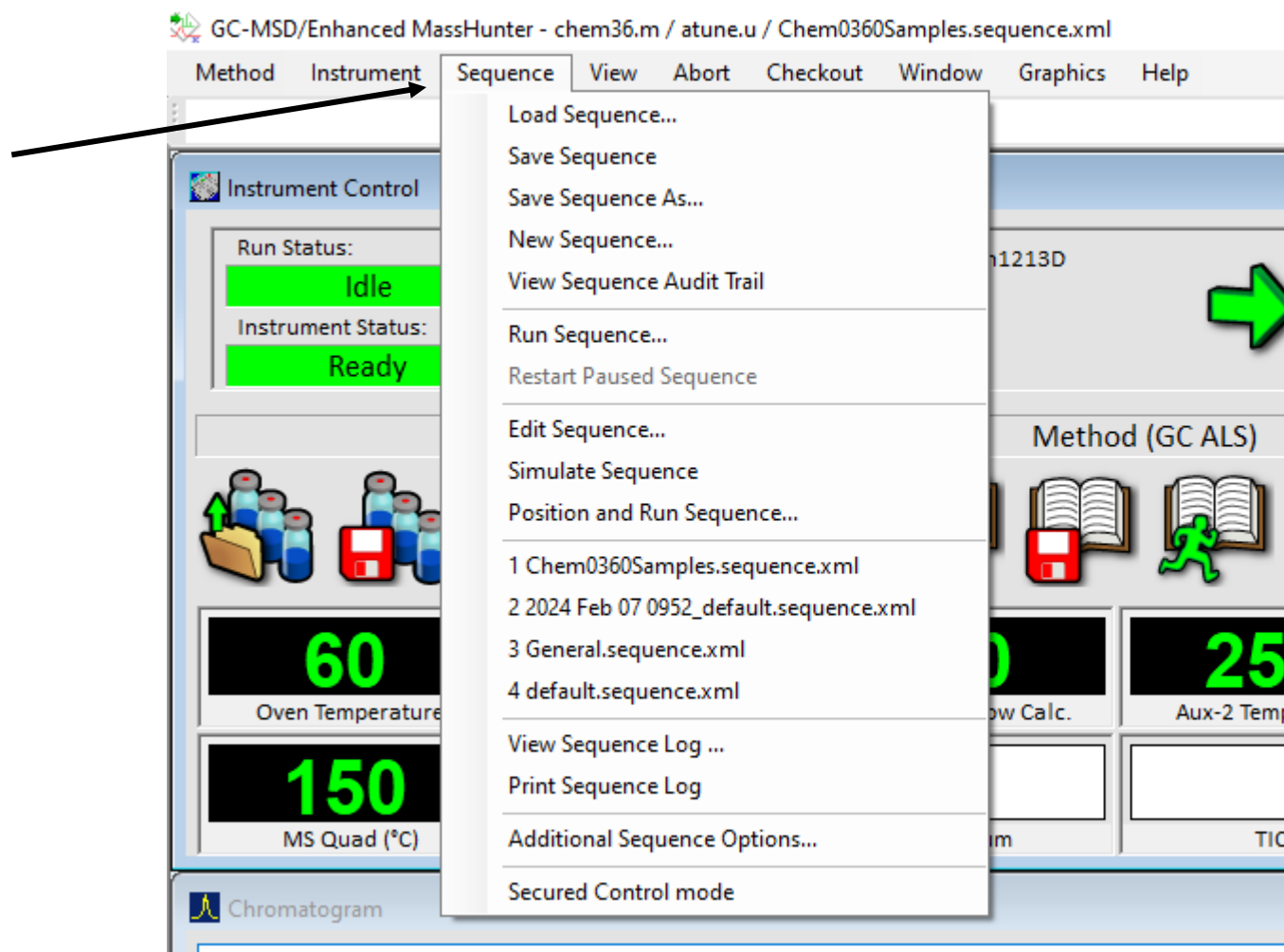
> GCMS > 1> methods > methods_oldPC

> 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

Sequence Table											
New Sample(s) X Tools											
	Name	Vial	Method Path	Method File	Data Path	Data File	DA Method File	Type	DA Method Path	Level	
1	Sample 1	101	C:\MassHunter\GCMS\1\methods\methods_oldPC	... tls3.m	Sample
2	Sample 2	102	C:\MassHunter\GCMS\1\methods\methods_oldPC	... tls3.m	Sample
3	Sample 3	103	C:\MassHunter\GCMS\1\methods\methods_oldPC	... tls3.m	Sample

Sample name

Vial position

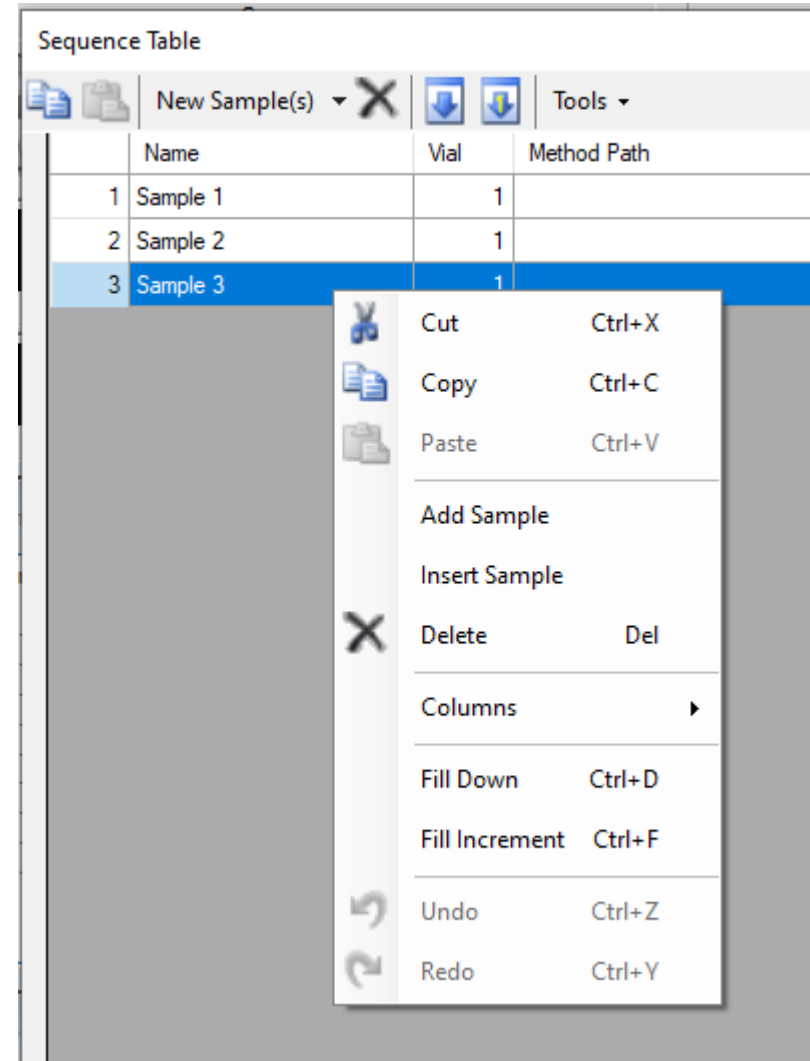
Click the ... icon to choose Method path. Choose parent directory of the method file.

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

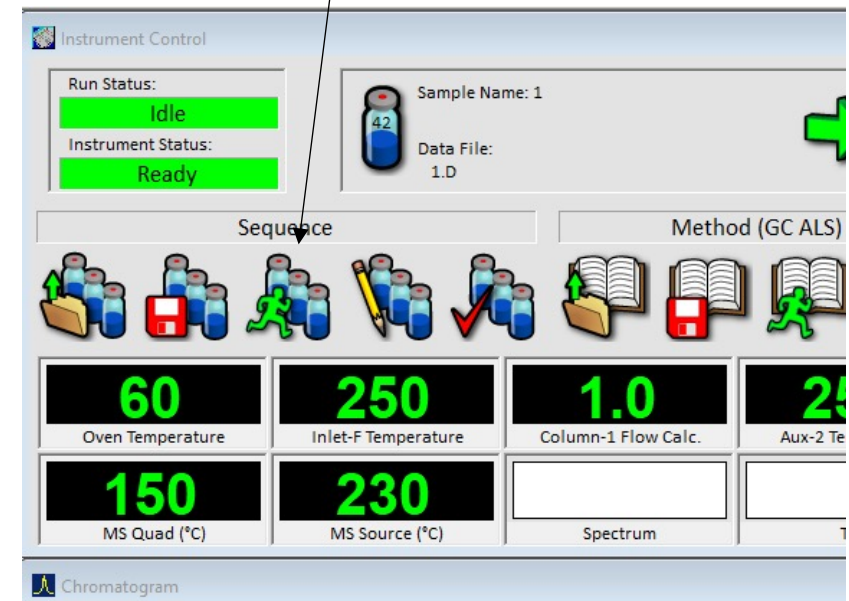
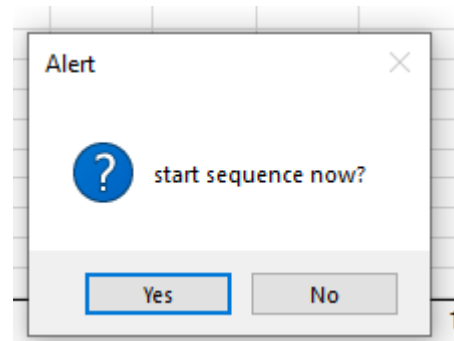
Input a name of your data

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



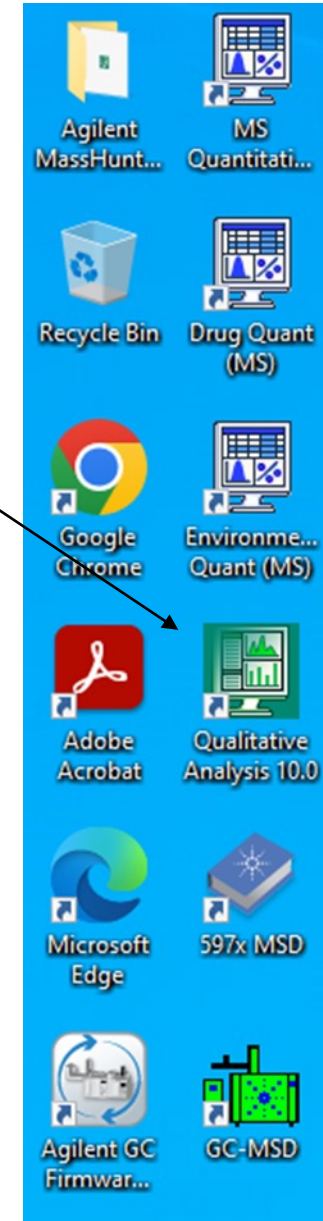
8. Click OK to finish the edit of sequence table.

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



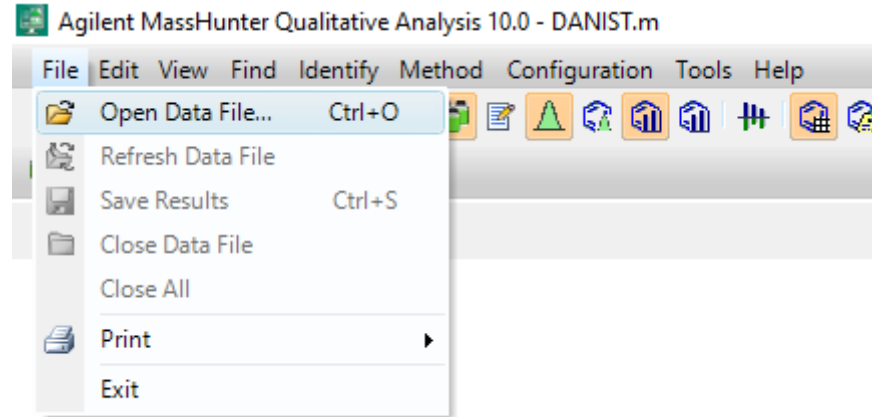
Data Analysis

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

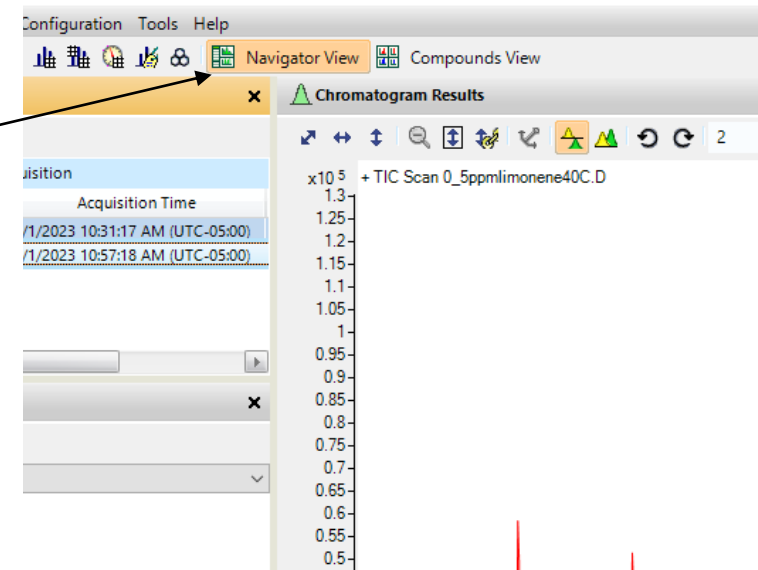


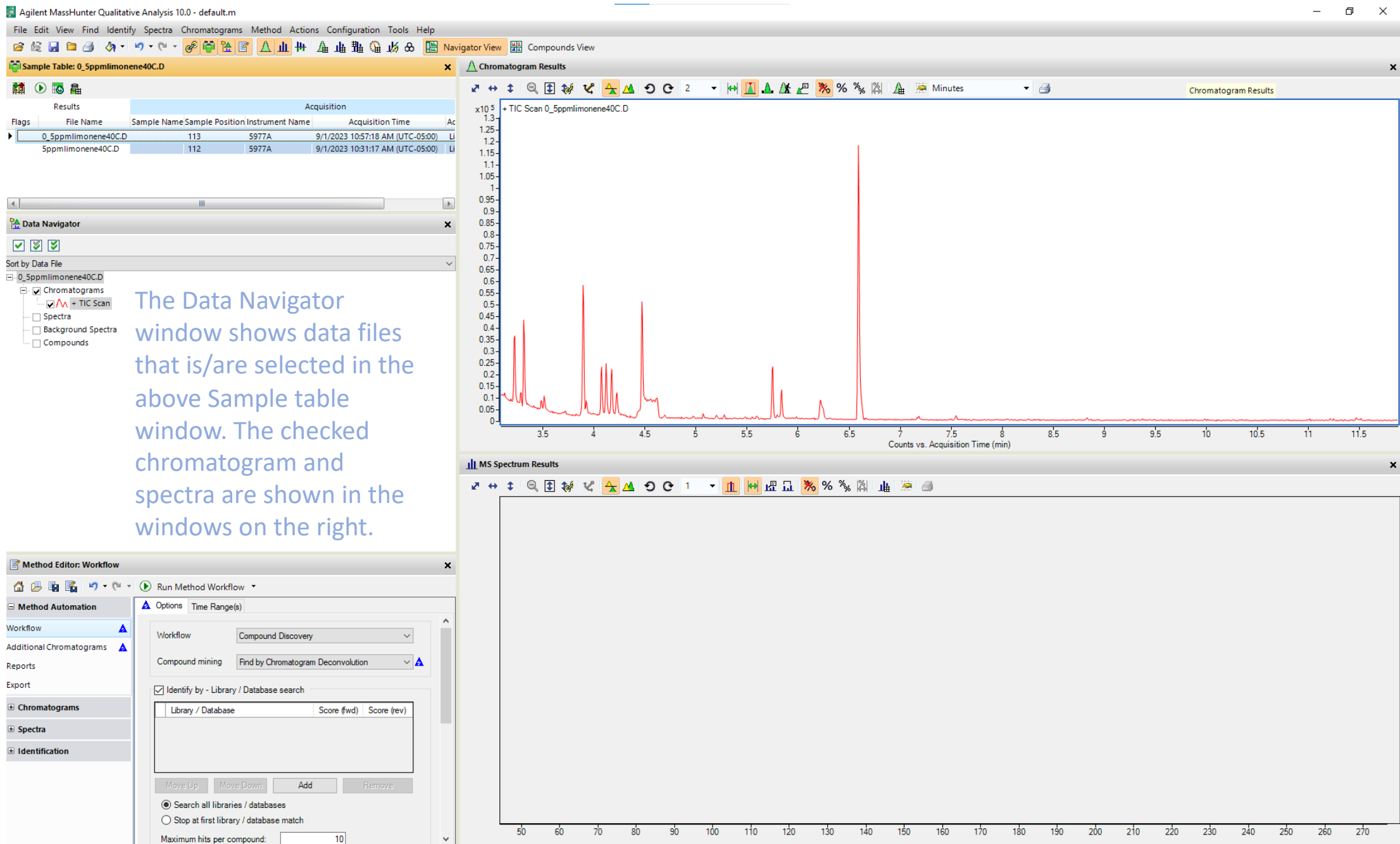
Data Analysis

2. Click the File > Open Data File
load a data file



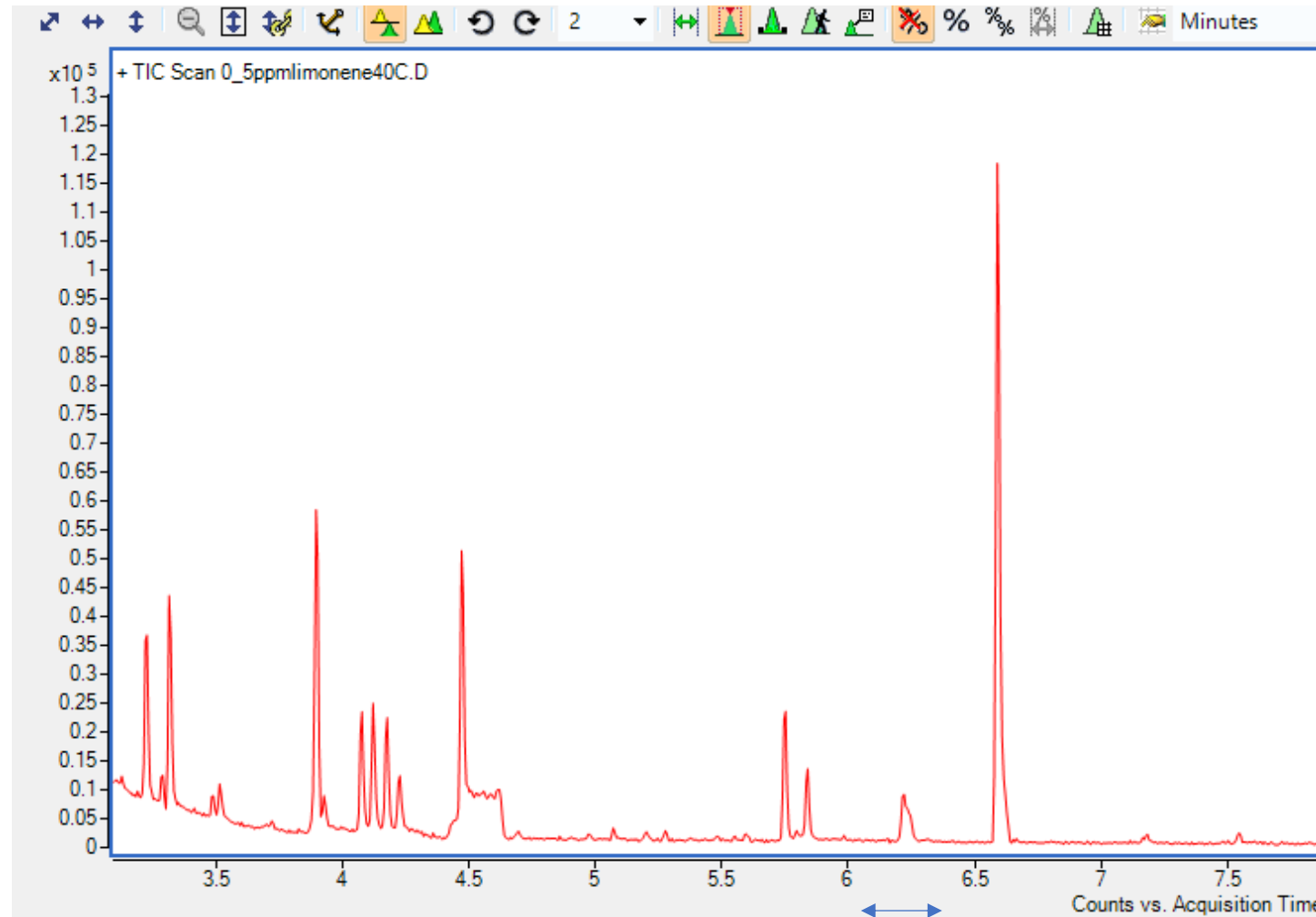
3. Make sure the Navigator View is selected





Click Autoscale icon to zoom out

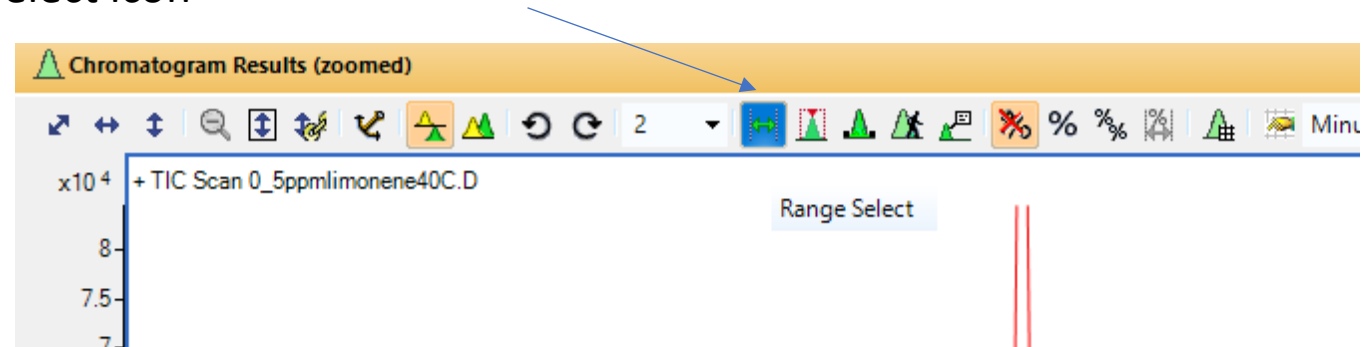
To zoom in on y-axis, move the cursor to y-axis, until a double vertical arrow \updownarrow appears, press the right mouse button and drag over an area.



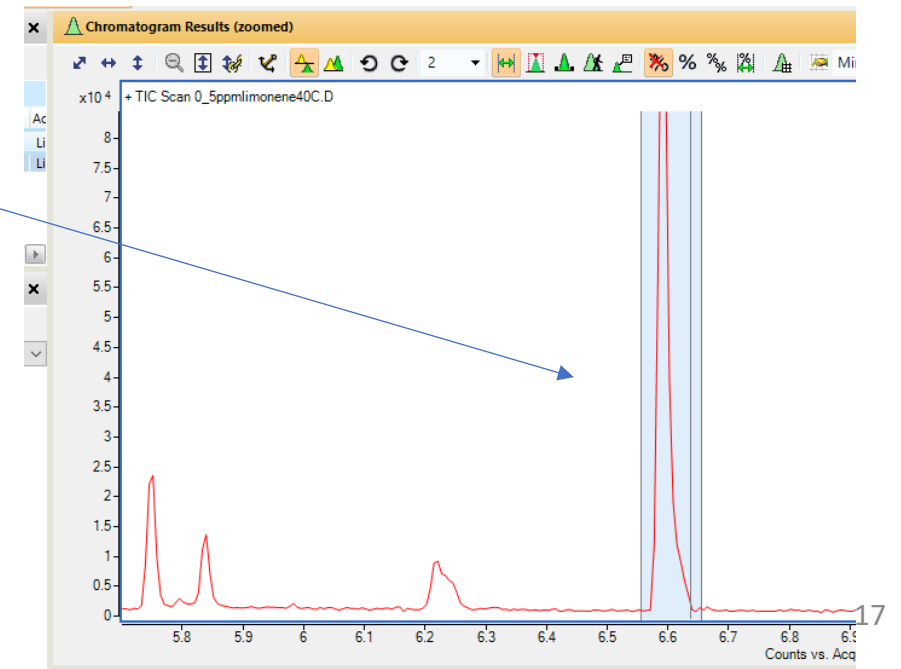
To zoom in on x-axis, move the cursor underneath x axis until a horizontal double arrow \leftrightarrow appears, then press the right mouse button and drag over an area.

Data Analysis

4. Left click the Range Select icon

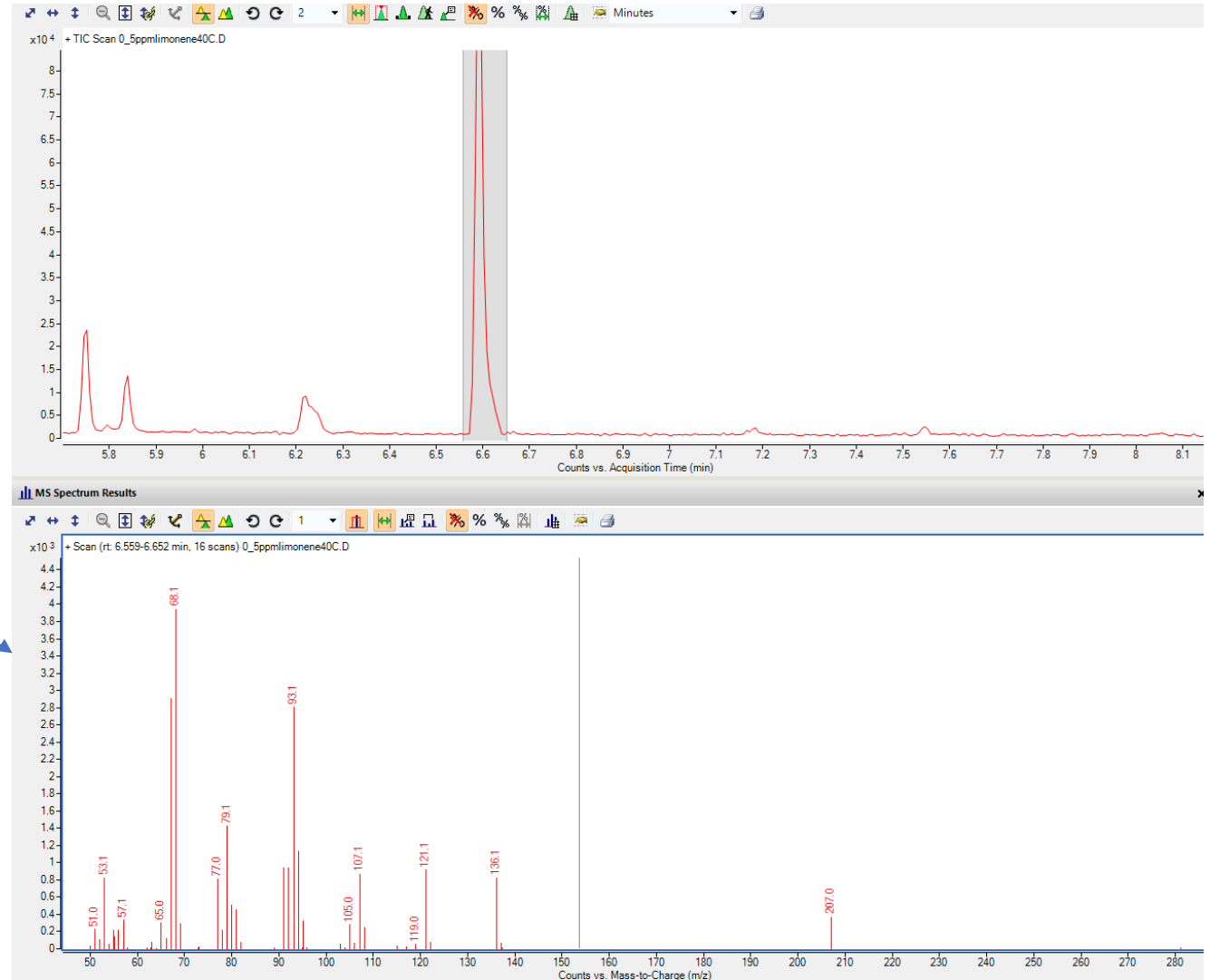


5. Press the left mouse button and drag over the peak of interest



Data Analysis

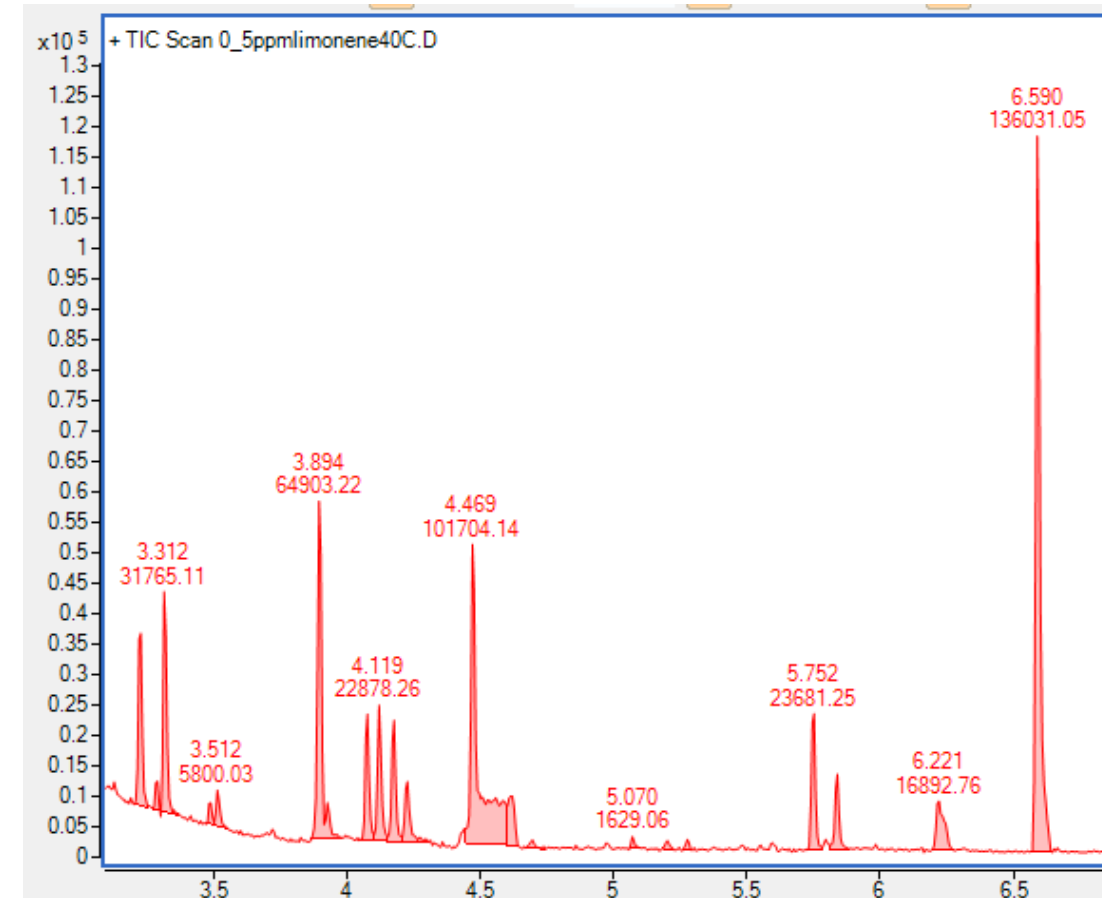
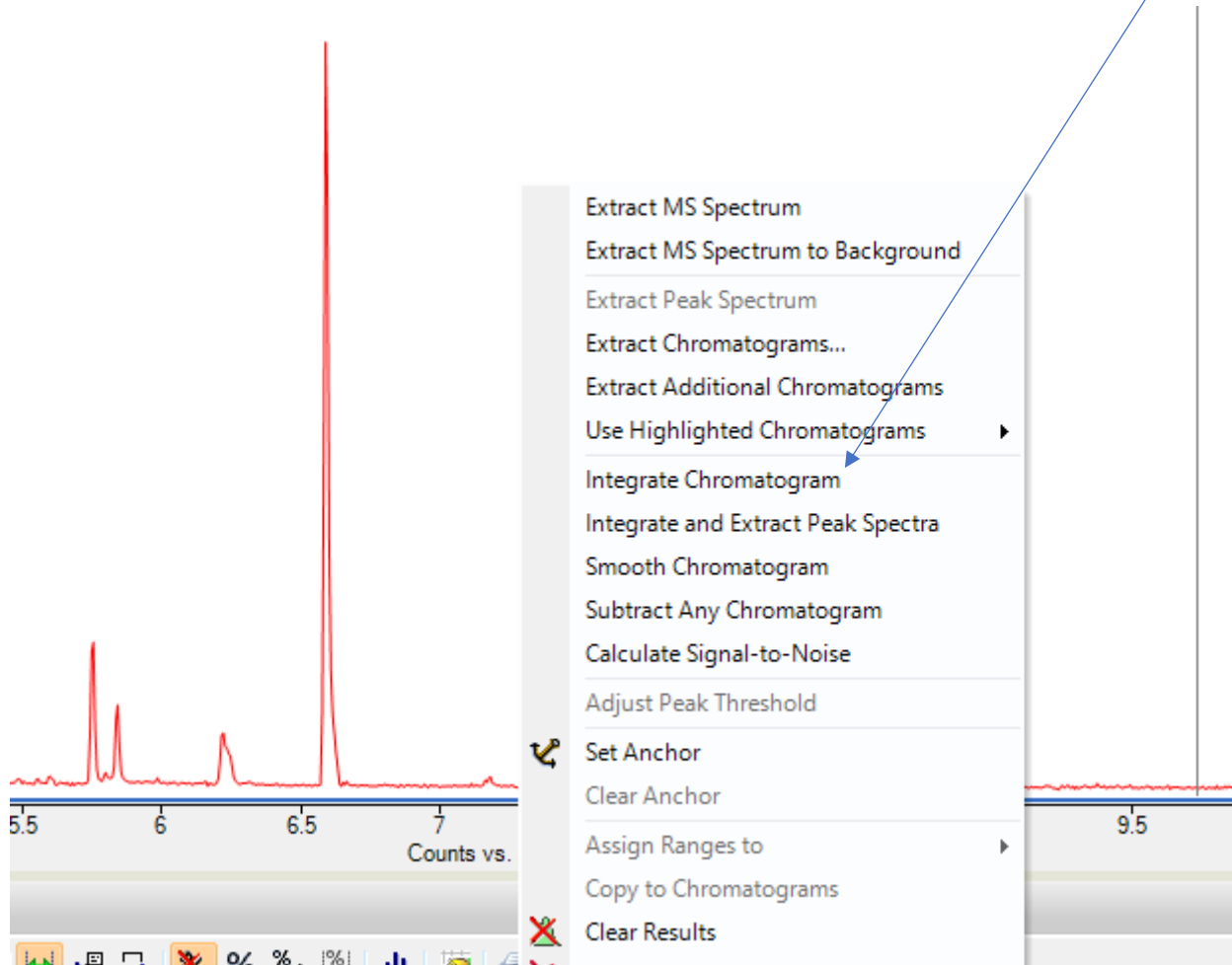
6. Double click the selected peak in the chromatogram. The peak spectrum is extracted and visible in the MS Spectrum Results window.



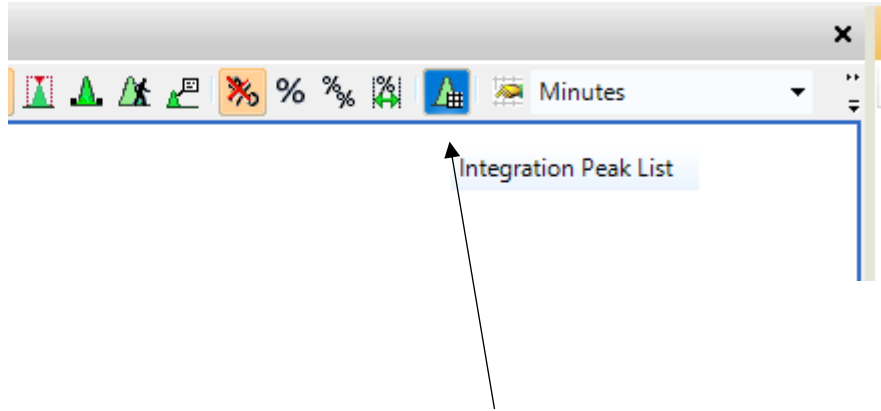
Data Analysis

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

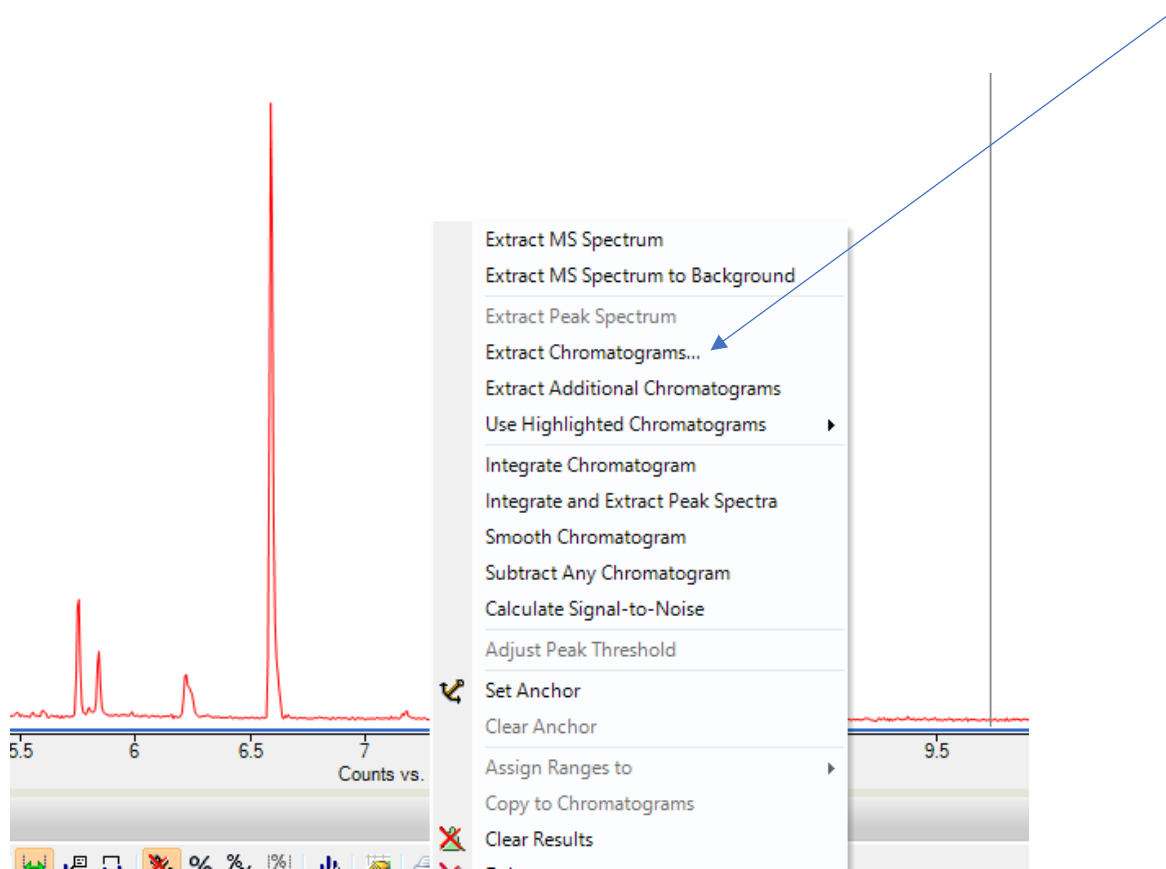


8. Click this icon to view peak list

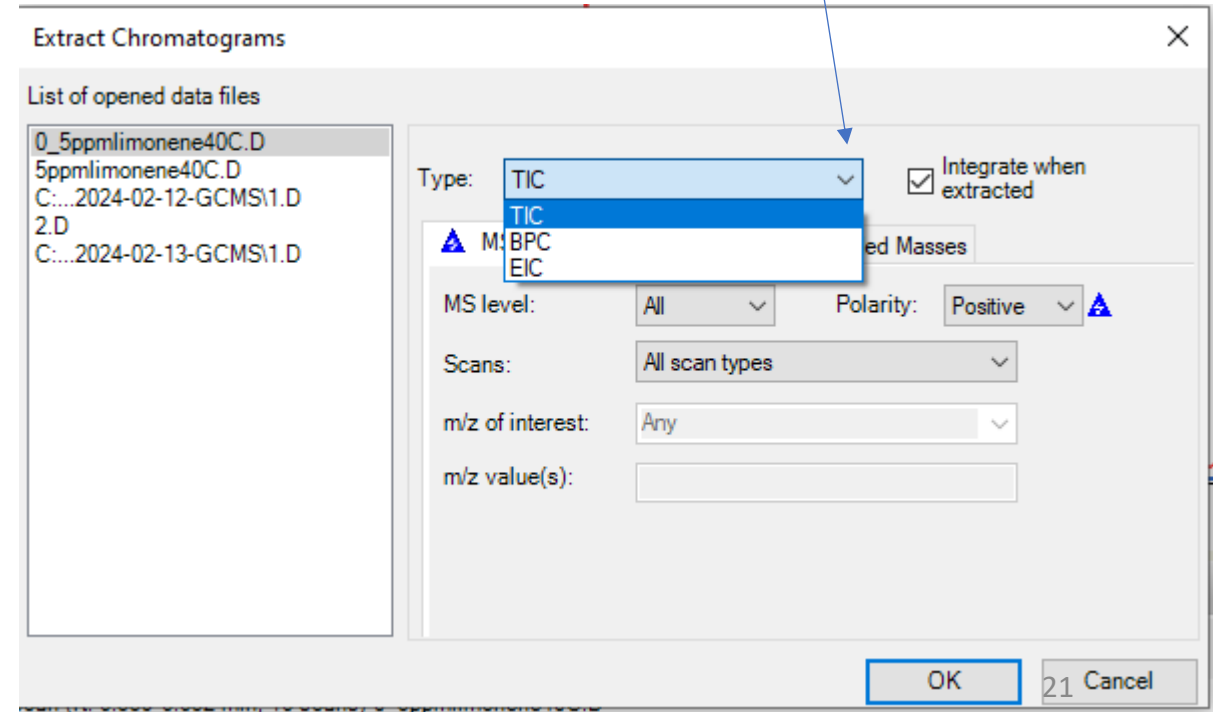
Peaks: + TIC Scan									
Peak	RT	Area	Height	Type	Saturated	Width	FWHM		
1	3.224	29214.12	28306.94			0.049	0.01		
2	3.287	3817	4771.41			0.029	0.01		
3	3.312	31765.11	36161.35			0.062	0.01		
4	3.487	3113.53	3524.97			0.026	0.01		
5	3.512	5800.03	5875.72			0.051	0.01		
6	3.894	64903.22	55359.57			0.109	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.

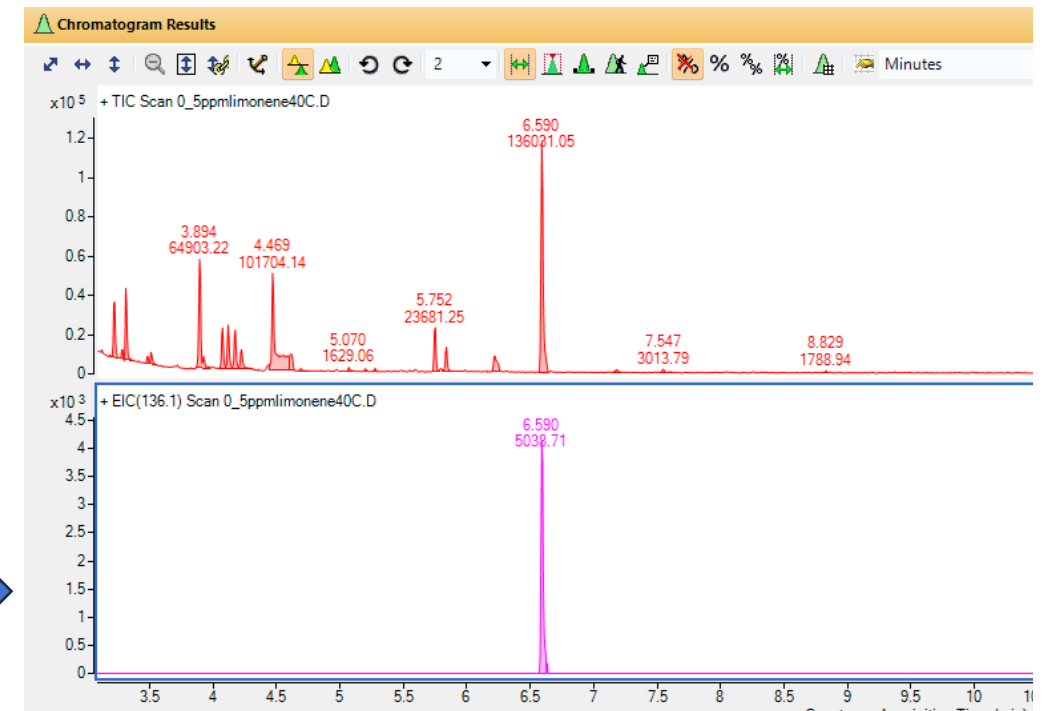
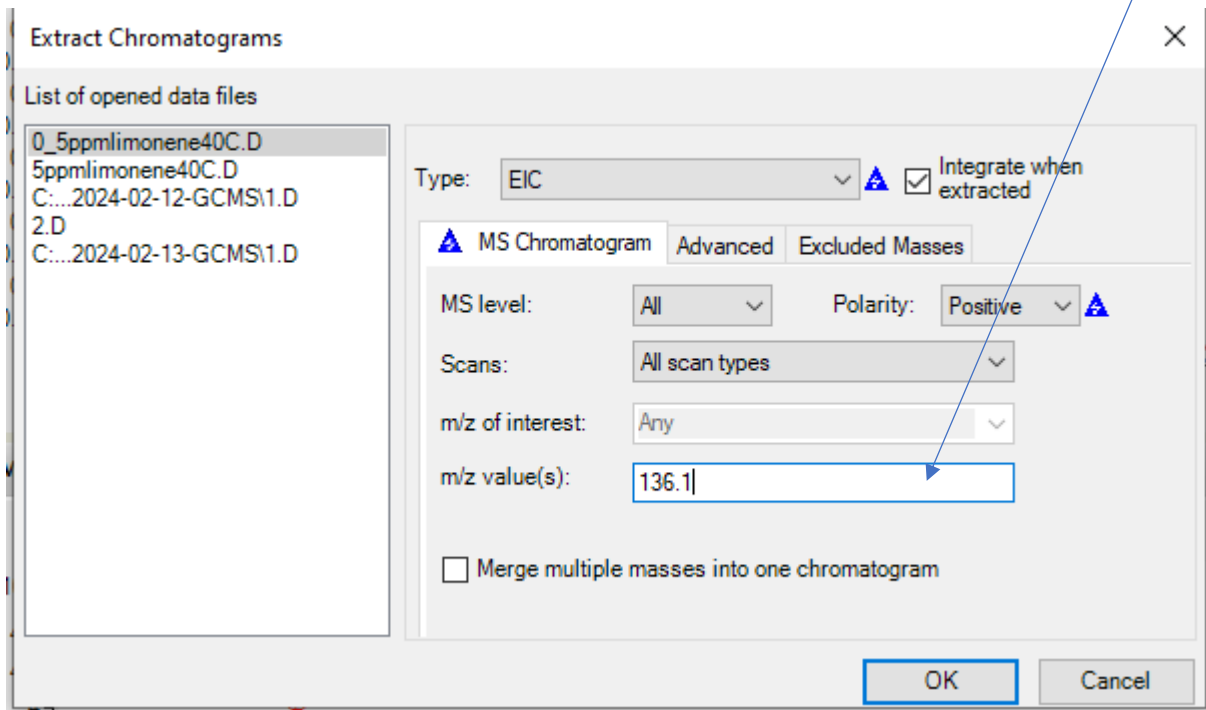


10. Click the down-arrow for type



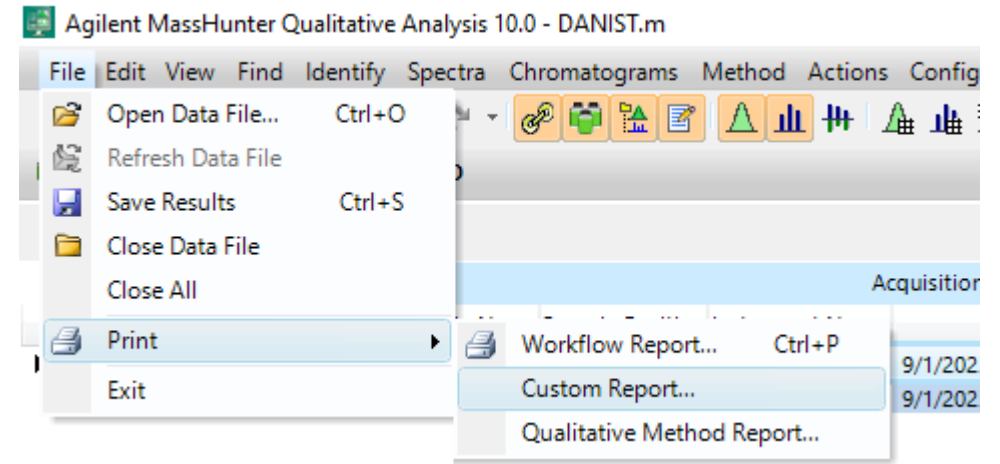
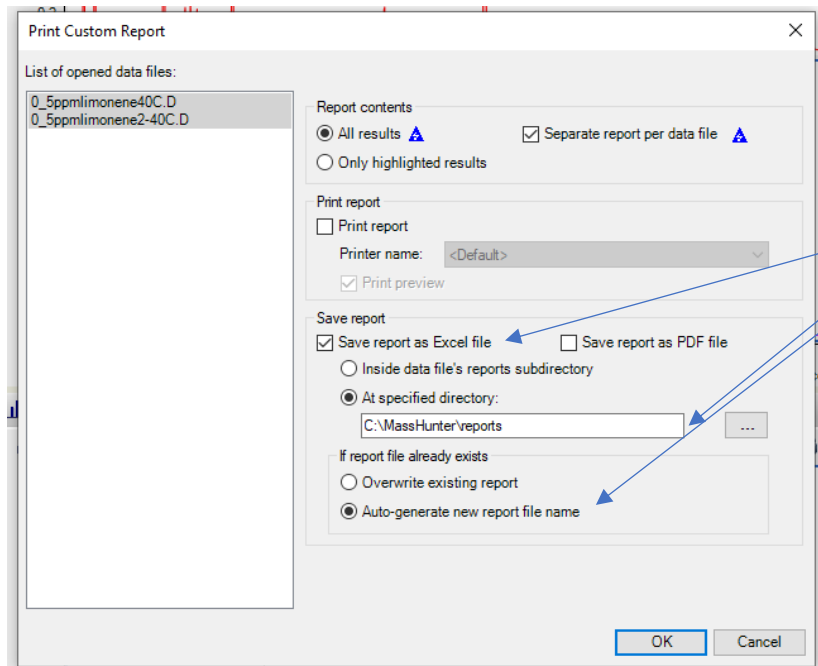
Data Analysis

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.

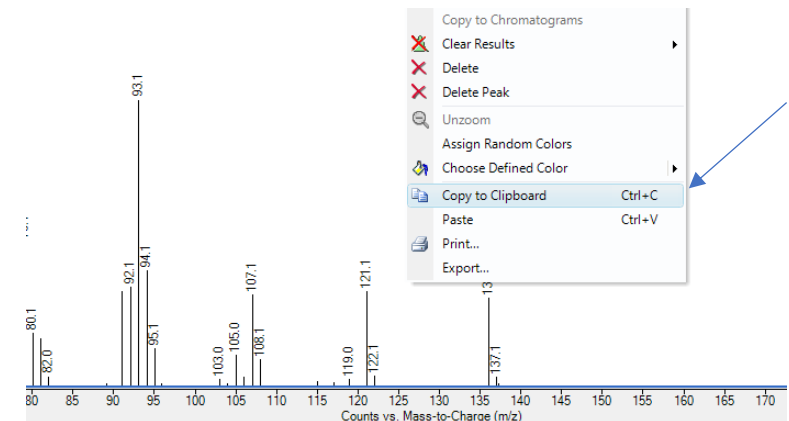


Data Analysis

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

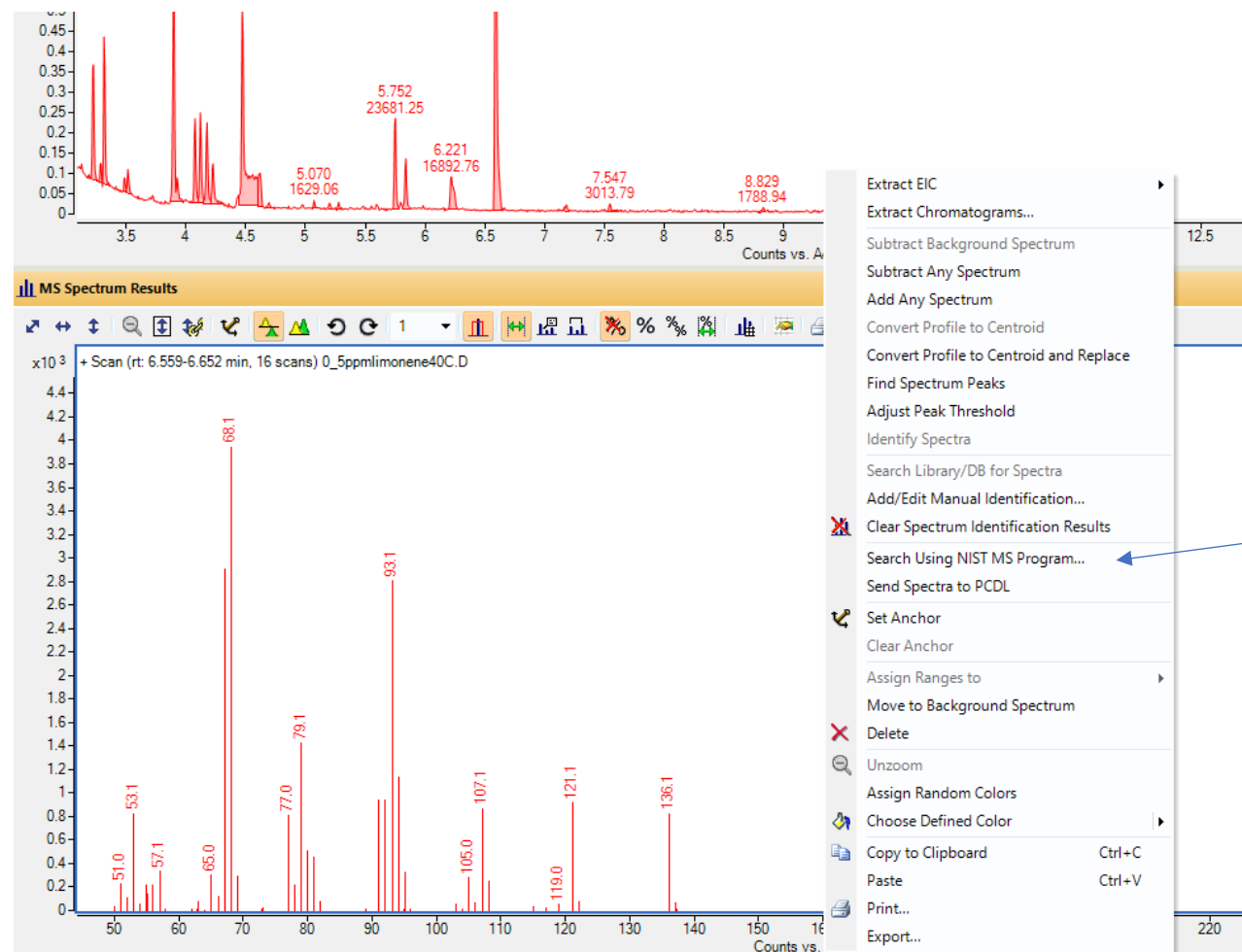


Set parameters in the dialog box that appears. Additionally, chromatograms and spectra can be copied and pasted into the report manually.



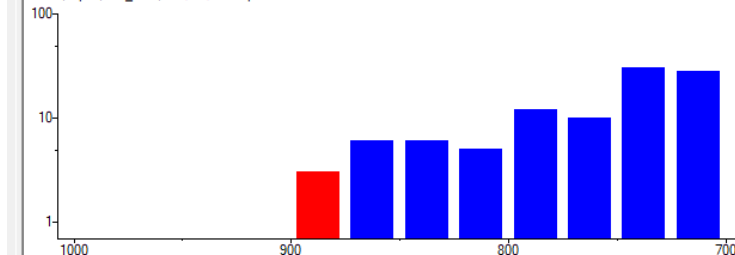
NIST library search

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.



#	Src.	Name
1	A	+ Scan (rt: 6.559-6.652 min, 16 sc...
2	A	+ Scan (rt: 6.559-6.652 min, 16 sc...
3	A	+ Scan (rt: 6.559-6.652 min, 16 sc...
4	A	+EI Scan (rt: 11.006-11.052 min, ...
5	A	+EI Scan (rt: 11.006-11.052 min, ...
6	A	+EI Scan (rt: 9.456-9.570 min, 21 ...
7	A	+EI Scan (rt: 9.427-9.559 min, 24 ...
8	A	+EI Scan (rt: 9.427-9.559 min, 24 ...
9	A	+EI Scan (rt: 9.427-9.559 min, 24 ...
10	A	+EI Scan (rt: 10.995-11.069 min, ...
11	A	+EI Scan (rt: 11.006-11.058 min, ...
12	A	+EI Scan (rt: 11.006-11.058 min, ...
13	A	+EI Scan (rt: 9.079-9.158 min, 15 ...
14	A	+EI Scan (rt: 8.706-8.735 min, 6 s...

mainlib; replib; nist_salts; 220460 total spectra

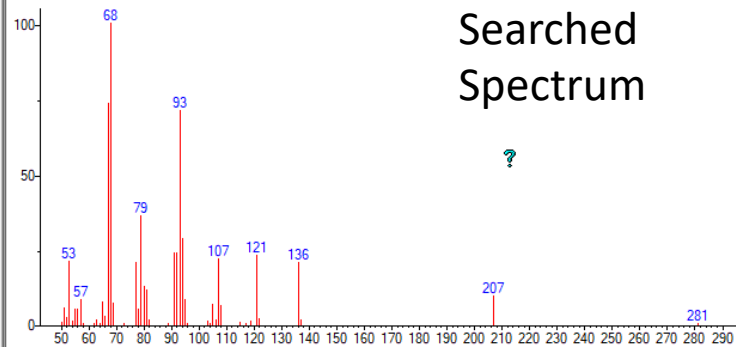


#	Lib.	Match	R.Match	Prob. (%)	Name
1	R	899	930	29.6	Limonene
2	R	884	912	29.6	Limonene
3	R	882	909	16.1	D-Limonene
4	R	874	911	12.0	Cyclohexene, 1-methyl-4-(1-methyl...
5	M	869	903	9.69	Cyclohexene, 1-methyl-5-(1-methyl...
6	M	867	889	16.1	D-Limonene
7	R	863	884	7.62	Cyclohexanol, 1-methyl-4-(1-methyl...
8	M	859	890	6.44	Cyclobutane, 1,2-bis(1-methylethe...
9	R	857	879	16.1	D-Limonene
10	M	845	867	29.6	Limonene
11	M	841	863	3.32	Cyclohexene, 4-ethenyl-1,4-dimeth...
12	M	841	863	12.0	Cyclohexene, 1-methyl-4-(1-methyl...
13	M	832	859	2.41	Cyclohexene, 1-methyl-5-(1-methyl...
14	M	828	864	2.03	Cyclohexene, 1-methyl-4-(1-methyl...
15	R	827	866	29.6	Limonene
16	M	824	852	1.72	Cyclobutane, 1,3-diisopropenyl-, tr...
17	M	811	831	1.11	Cycloheptene, 5-ethylidene-1-met...
18	M	809	868	1.02	1,5-Cyclooctadiene, 1,5-dimethyl-
19	R	801	827	1.02	1,5-Cyclooctadiene, 1,5-dimethyl-
20	M	801	824	0.76	1,5-Cyclooctadiene, 3,4-dimethyl-
21	M	792	817	0.55	1,5-Cyclooctadiene, 1,6-dimethyl-
22	R	787	808	0.44	Cyclohexene, 3-methyl-6-(1-methyl...
23	R	786	819	0.43	1,7-Octadiene, 2-methyl-6-methyle...
24	R	785	805	0.41	Camphene
25	M	785	805	0.41	Bicyclo[2.2.1]heptane, 2,2-dimeth...
26	M	784	815	0.44	Cyclohexene, 3-methyl-6-(1-methyl...
27	M	782	817	0.41	Camphene
28	R	781	802	0.34	Bicyclo[2.2.1]heptane, 7,7-dimeth...
29	R	777	797	0.41	Camphene

Hit list

InLib = -151, Hit List

Searched Spectrum



(Text File) + Scan (rt: 6.559-6.652 min, 16 scans) 0_5ppmlimonene40C.D

Plot/Text of Search Spectrum Plot of Search Spectrum Plot/Text of Spec List

Name: + Scan (rt: 6.559-6.652 min, 16 scans) 0_5ppmlimonene40C.D

MW: N/A ID#: 320 DB: Text File

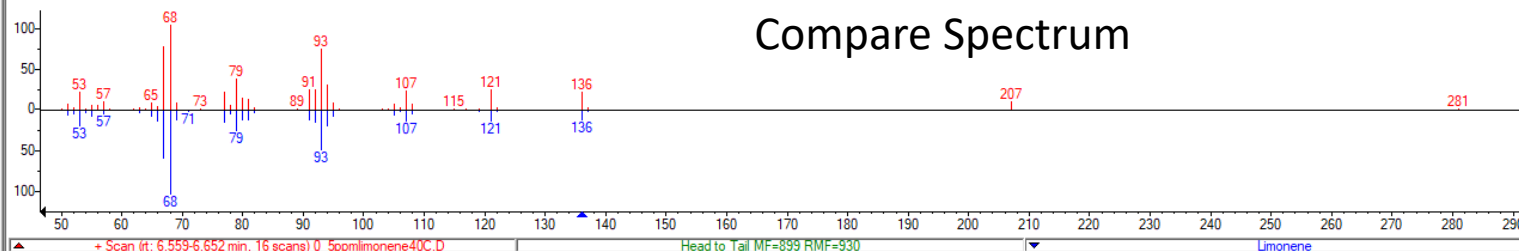
10 largest peaks:

68 999 | 67 735 | 93 711 | 79 361 | 94 287 |
91 239 | 92 239 | 121 233 | 107 220 | 53 210 |

Synonyms:

no synonyms.

Compare Spectrum

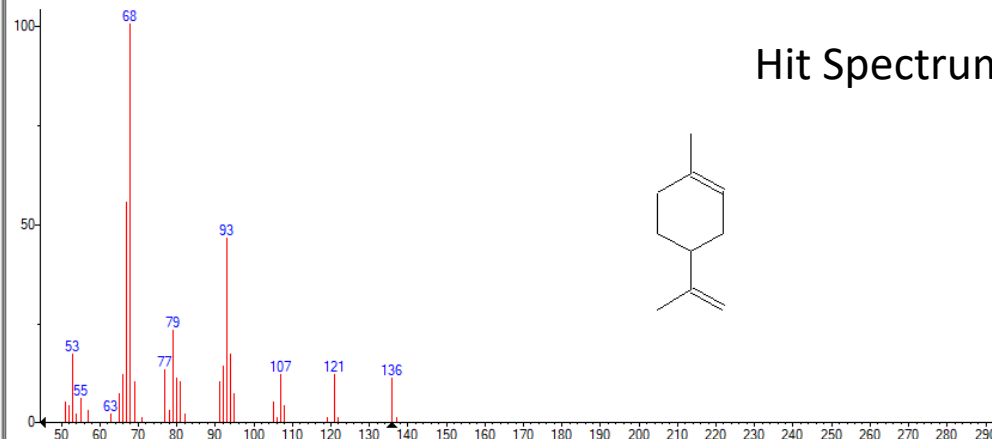


+ Scan (rt: 6.559-6.652 min, 16 scans) 0_5ppmlimonene40C.D Head to Tail MF=899 RMF=930

Limonene

899 930R 29.6P

Hit Spectrum



(replib) Limonene

Plot/Text of Hit Plot of Hit

Name: Limonene

Formula: C10H16

MW: 136 CAS#: 138-86-3 NIST#: 57640 ID#: 7400 DB: replib

Other DBs: TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB

Contributor: MASS SPECTRA OF ORGANIC COMPOUNDS, V. 5. B. H. KENNETT ET

10 largest peaks:

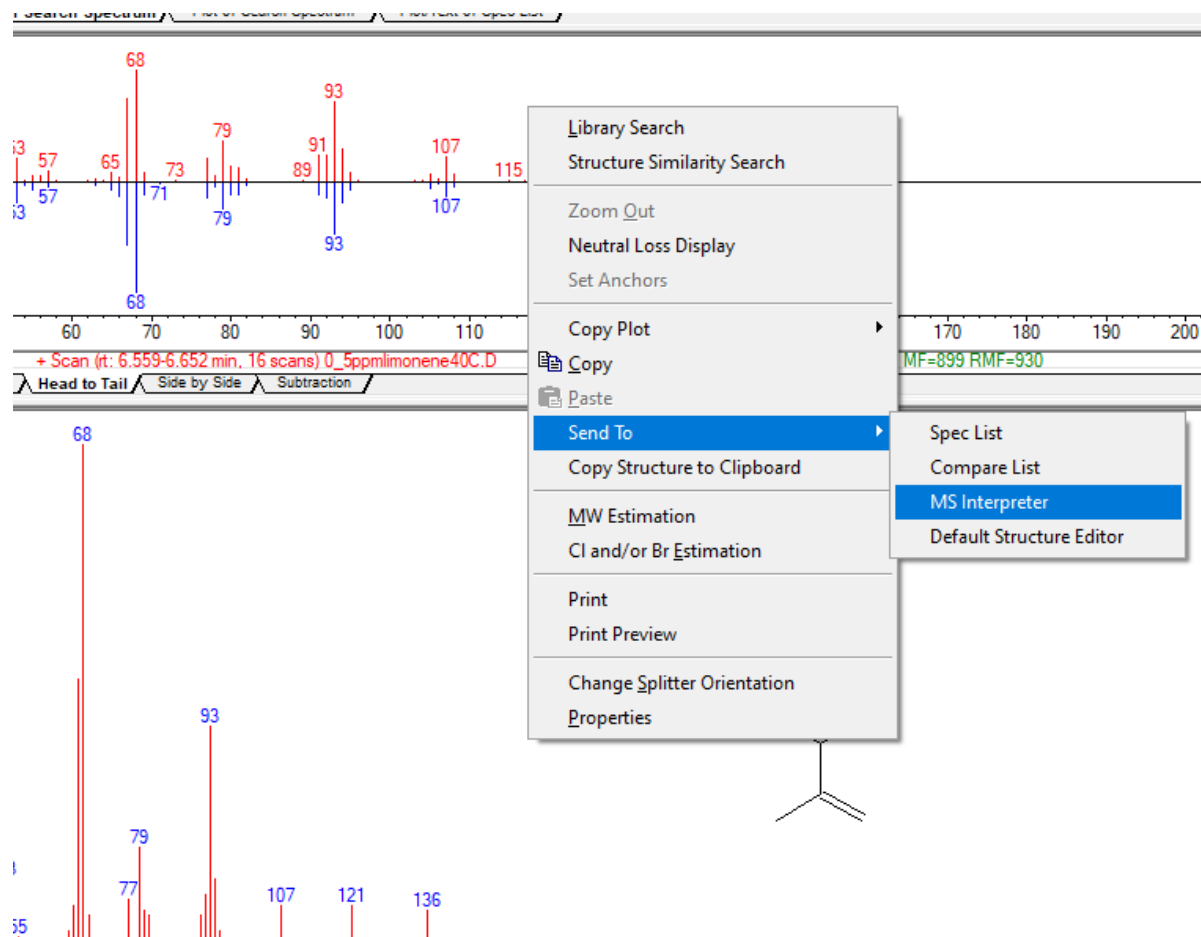
68 999 | 67 550 | 93 460 | 79 230 | 41 190 |
39 180 | 53 170 | 94 170 | 27 160 | 92 140 |

Synonyms:

1. Cyclohexene, 1-methyl-4-(1-methylethenyl)-
2. p-Mentha-1,8-diene
3. α-Limonene
4. Cajeputen
5. Cajeputene
6. Cinen
7. Cinene
8. Dipenten
9. Dipentene
10. Eulimen
11. Kautschin
12. Limonen
13. Nesol
14. p-Mentha-1,8(9)-diene
15. δ-1,8-Terpodiene
16. p-Mentha-1,8-diene, di-
17. Acintene dp dipentene
18. Di-p-mentha-1,8-diene
19. DL-Limonene

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



Limone - MS Interpreter

File Edit View Options Help

Selected m/z

Parent Fragment Neutral Loss

Formula Calculator

m = 34 C10H16

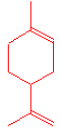
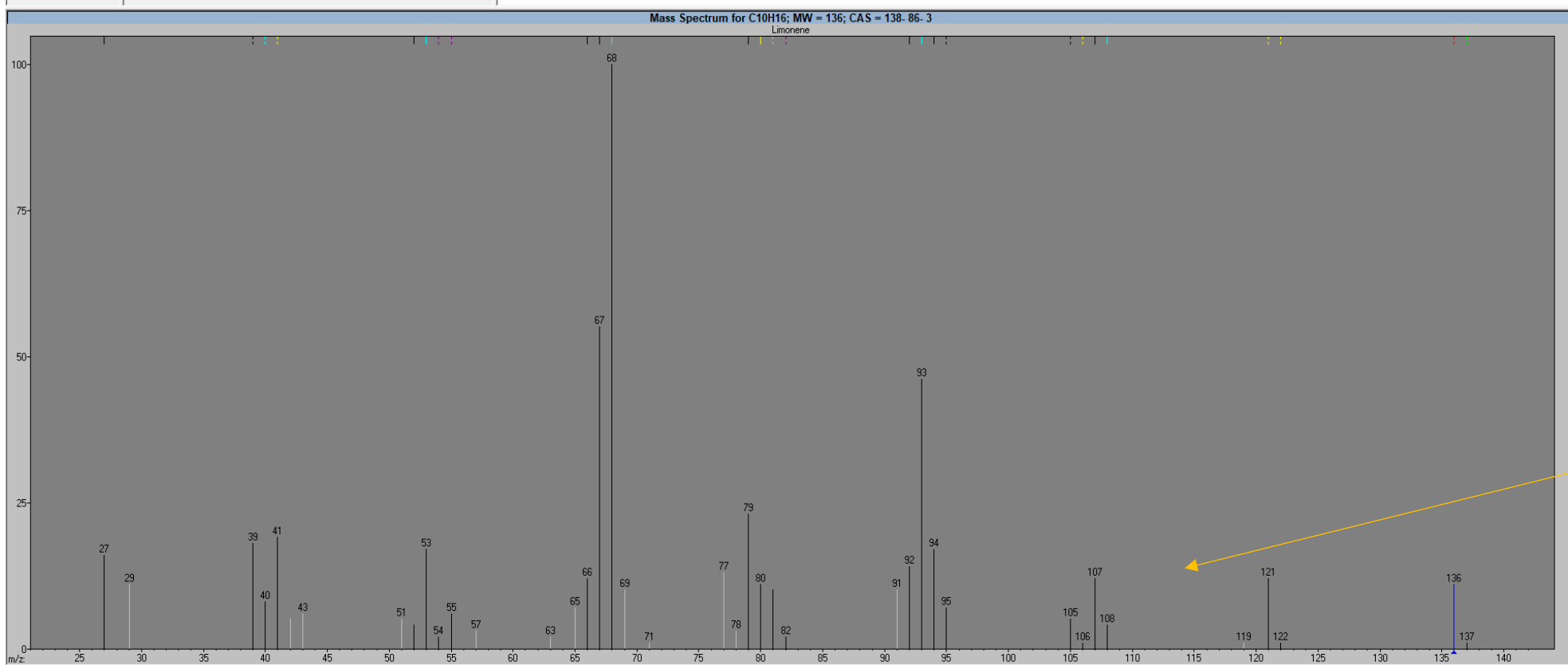
Calculate Options Parent = 136 Loss = 102

Push to Recalculate Ions D+E RDB Exact MW

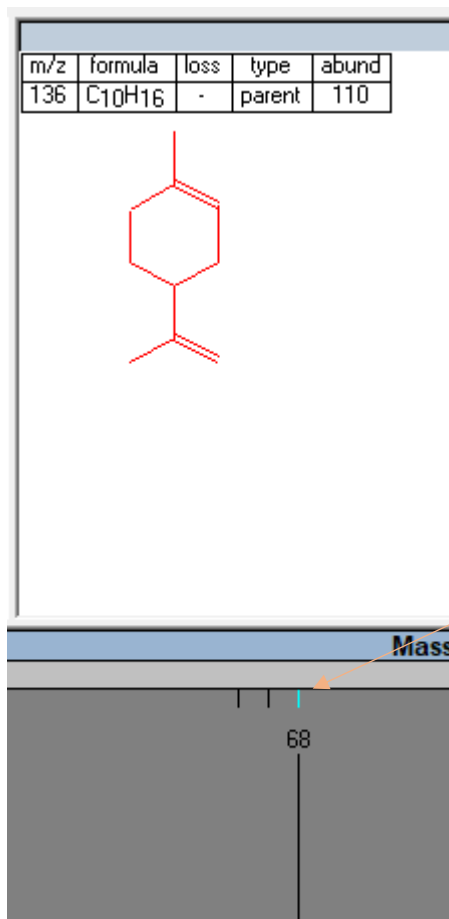
m/z formula loss type abund

136 C10H16 - parent 110

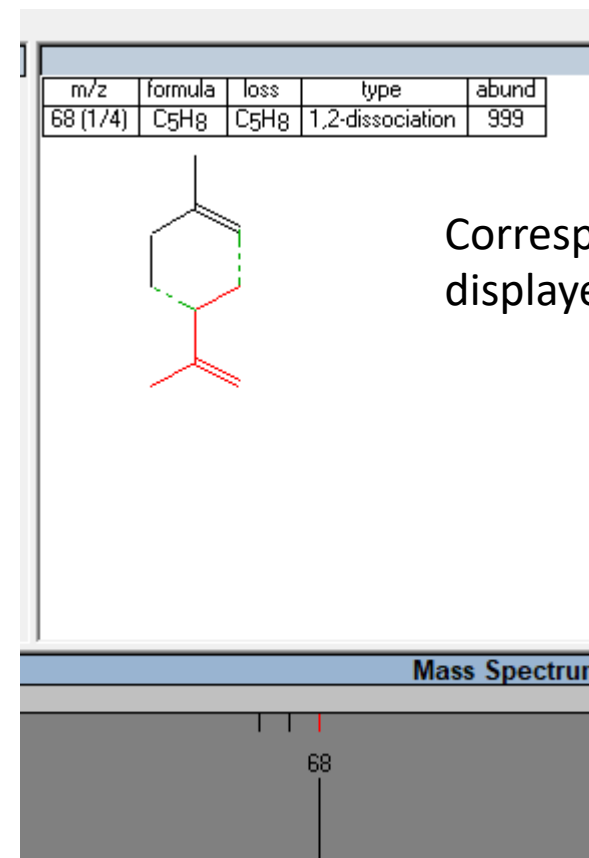
Structure, Maximum Dissociation = 99

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



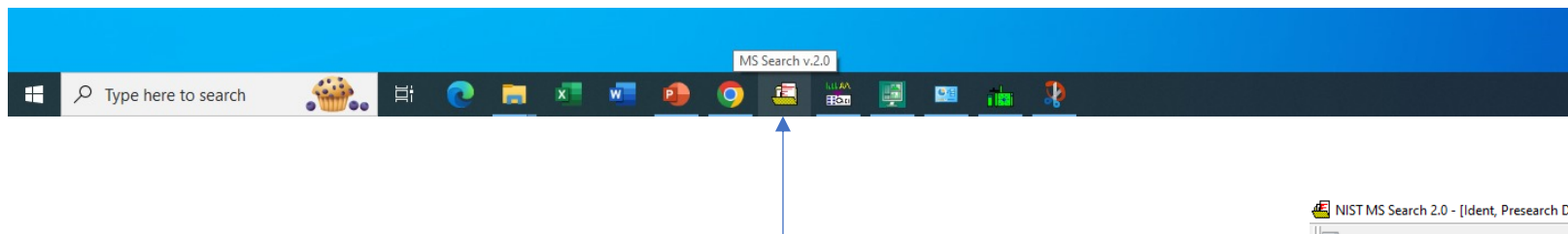
Left click the notch on top of the peak of interest, for example, the peak m/z 68



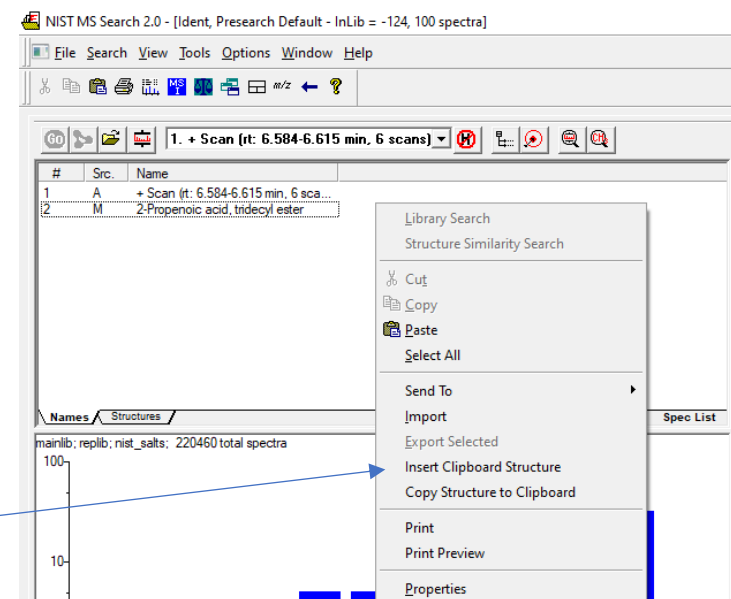
Corresponding fragments are displayed in red

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.

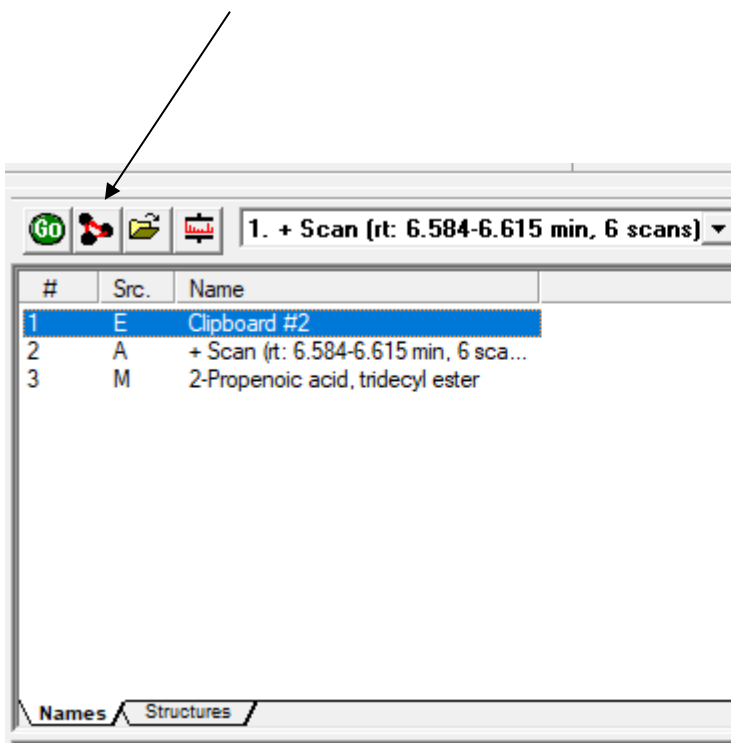


2. Copy a structure that you are interested in from ChemDraw.
Right Click Spec list window of NIST > Insert Clipboard Structure >
OK

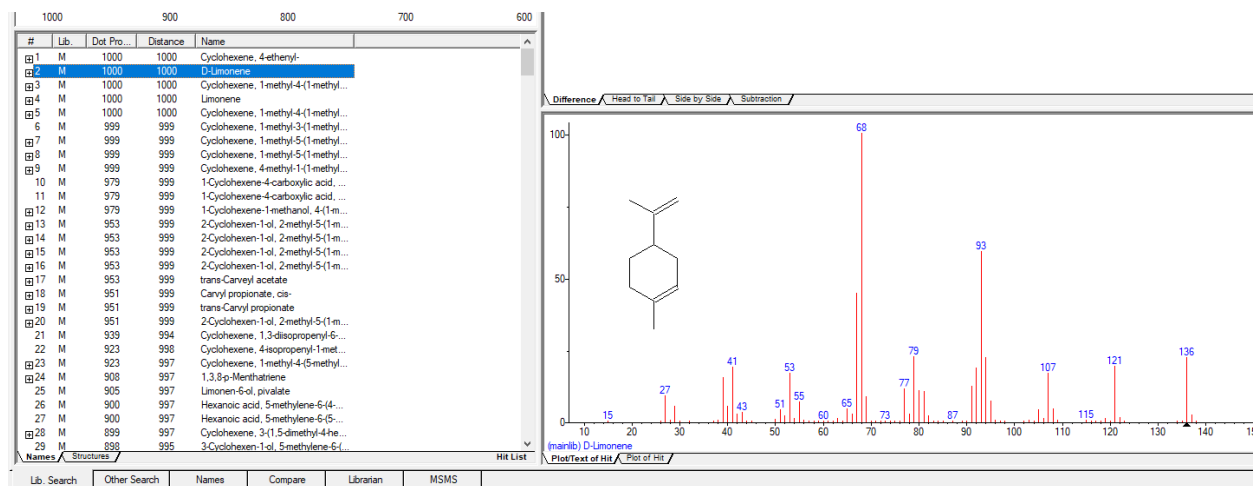


Structure search

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



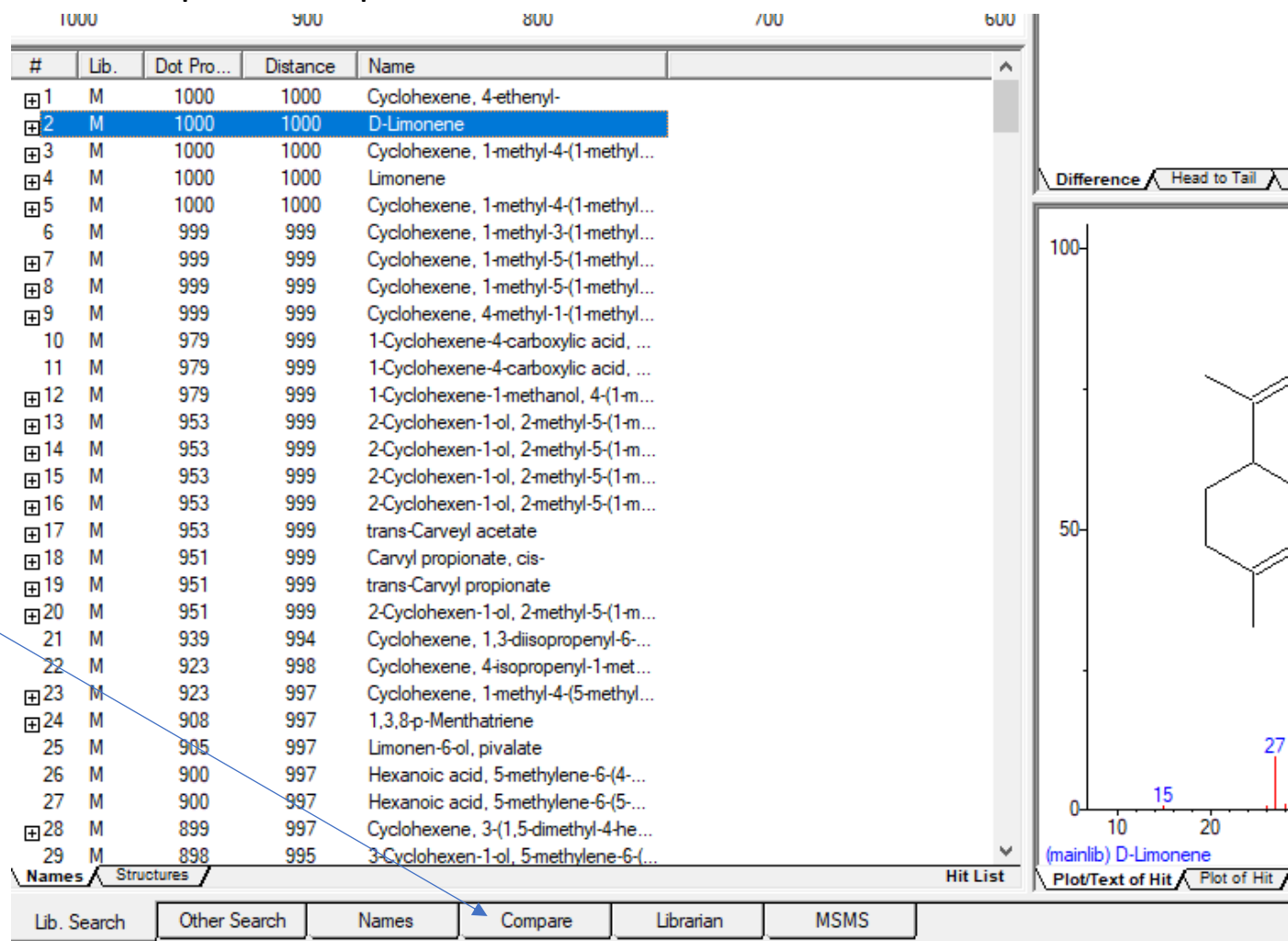
#	Src.	Name
1	E	Clipboard #2
2	A	+ Scan (rt: 6.584-6.615 min, 6 scans)
3	M	2-Propenoic acid, tridecyl ester



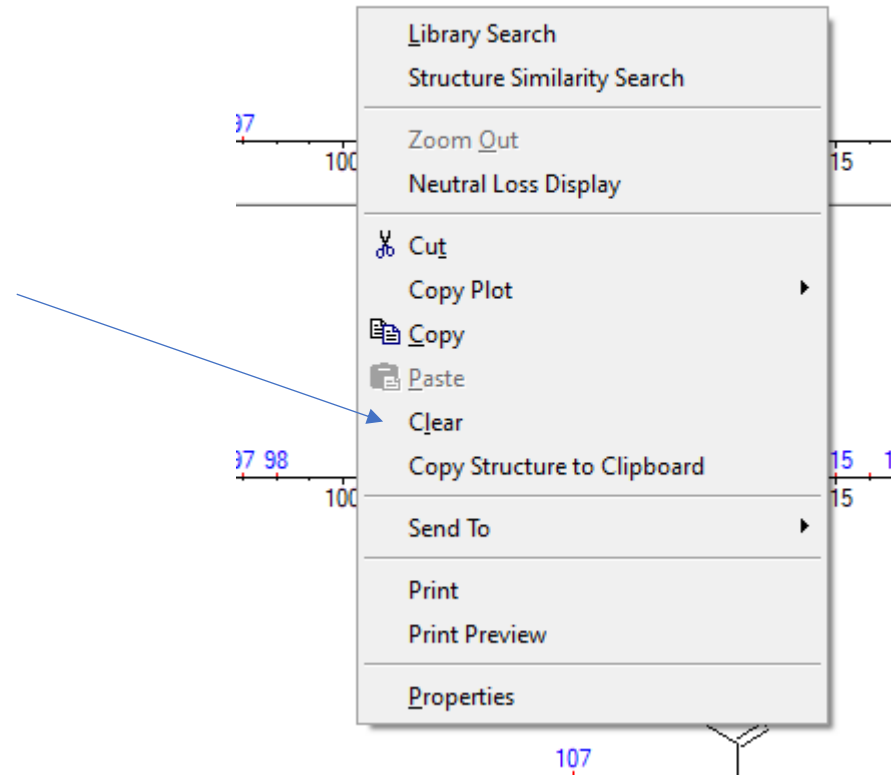
Structure search

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.

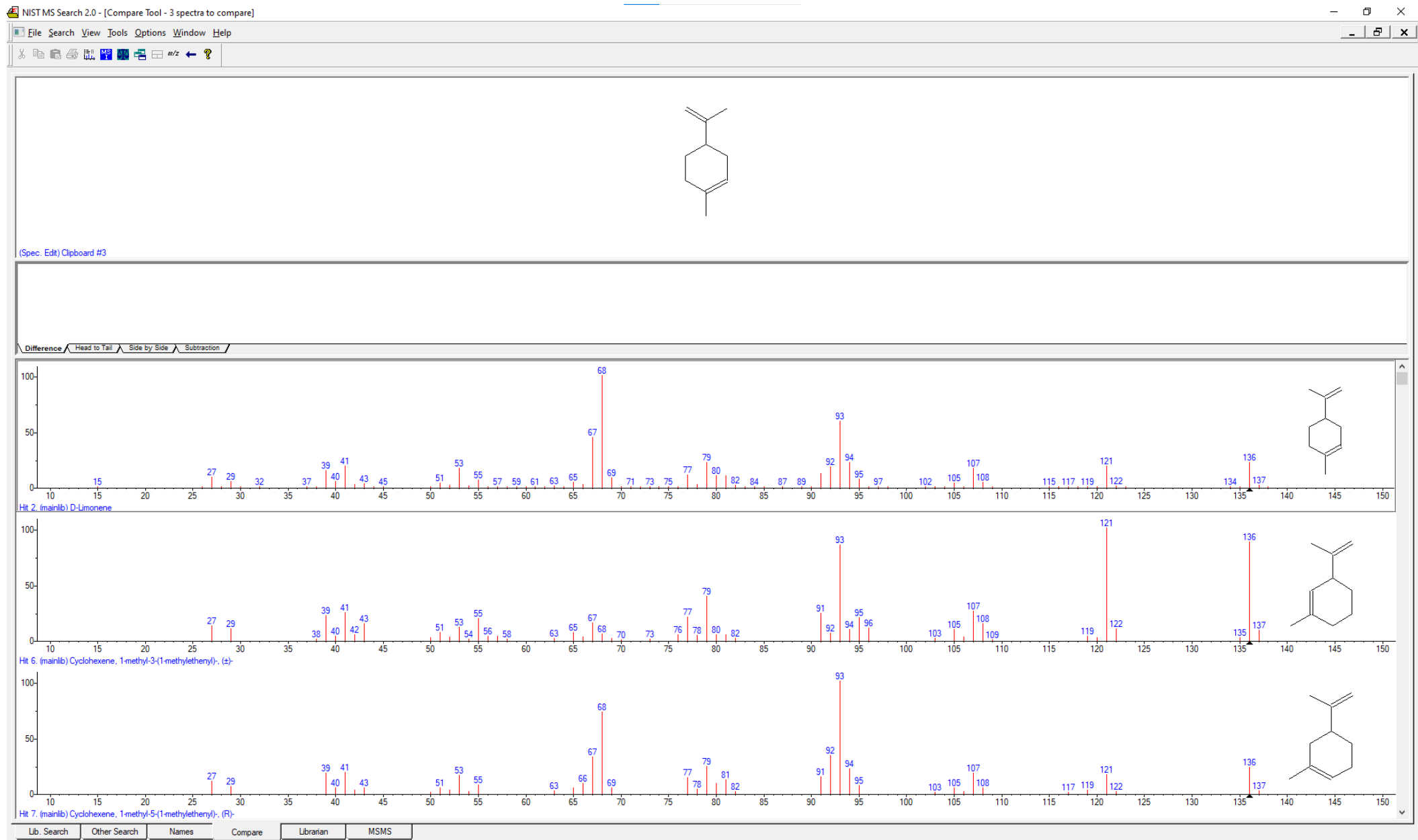
The result is as shown in next slide.

The screenshot shows a software interface with a table of chemical compounds. A right-click context menu is open over the table, and a sub-menu is open under the 'Send To' option.

#	Lib.	Dot Pro...	Distance	Name
1	M	1000	1000	Cyclohexene, 4-ethenyl-
2	M	1000	1000	D-Limonene
3	M	1000	1000	Cyclohexene, 1-methyl-4-(1-methyl...
4	M	1000	1000	Limonene
5	M	1000	1000	Cyclohexene, 1-methyl-4-(1-methyl...
6	M	999	999	Cyclohexene, 1-methyl-3-(1-methyl...
7	M	999	999	Cyclohexene, 1-methyl...
8	M	999	999	Cyclohexene, 1-methyl...
9	M	999	999	Cyclohexene, 4-methyl...
10	M	979	999	1-Cyclohexene-4-carbo...
11	M	979	999	1-Cyclohexene-4-carbo...
12	M	979	999	1-Cyclohexene-1-metha...
13	M	953	999	2-Cyclohexen-1-ol, 2-m...
14	M	953	999	2-Cyclohexen-1-ol, 2-m...
15	M	953	999	2-Cyclohexen-1-ol, 2-m...
16	M	953	999	2-Cyclohexen-1-ol, 2-m...
17	M	953	999	trans-Carveyl acetate
18	M	951	999	Carvyl propionate, cis-
19	M	951	999	trans-Carvyl propionate
20	M	951	999	2-Cyclohexen-1-ol, 2-m...
21	M	939	994	Cyclohexene, 1,3-diiso...
22	M	923	998	Cyclohexene, 4-isoprop...
23	M	923	997	Cyclohexene, 1-methyl...
24	M	908	997	1,3,8-p-Menthatriene
25	M	905	997	Limonen-6-ol, pivalate

The context menu options are:

- Library Search
- Structure Similarity Search
- Copy
- Select All
- Close All Replicates
- Export Selected
- Send To
 - Spec List
 - Compare List
 - MS Interpreter
 - Default Structure Editor
- Copy Structure to Clipboard
- Print
- Print Preview
- Properties



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

