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Chemical Instrumentation Facility

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Agilent 5973 GCMS Training Manual

Draft - 06/12/17 S.V.

Location: **1238 Hach Hall**

Contact: **Steve Veysey, 1234 Hach Hall; Kamel Harrata, 1236 Hach Hall**

Safety

All researchers working in 1238 Hach Hall must complete the EH&S courses: "*Fire Safety and Extinguisher Training", and "Lab Safety: Compressed Gas Cylinders".* When preparing samples in 1238 or 1238A, please wear all appropriate personal protective equipment. Aprons, safety glasses, and rubber gloves are available.

Properly dispose of all waste solvents, glass pipettes, plastic pipette tips, syringes, et cetera in the containers provided in 1238A. All of the data processing computers and many of the data acquisition computers in this lab have direct links from the desktop to MSDS sheets, the EH&S Laboratory Safety Manual and to the CIF Safety Manual.

Training Protocol

Request training from Steve or Kamel.

Beginning…

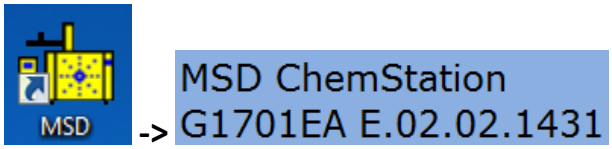
Login using your netID (username and password) established local to this computer, NOT your ISU active directory account. Your desktop should have the following program links.

Programs on Your Desktop

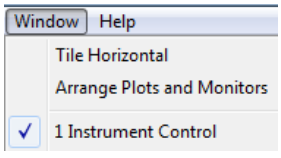
- *MSD* Starts the GC-MSD *ChemStation* data acquisition software
- *MSD Data Analysis* Starts the *ChemStation* data processing software
- *Qualitative Analysis* Starts *MassHunter* data processing software
- *GCMS Translator* Used to convert ChemStation data to MassHunter format
- *Word 2013* Starts Word; used to document errors and other problems
- *Errors Folder* Location of all Word documents discussing errors and problems
- *MassHunter PDF* Agilent guide to using MassHunter
- *ChemStation PDF* Agilent guide to using ChemStation
- *5973 MSD Guide PDF* This CIF guide for using the 5973 MSD, ChemStation, and MassHunter
- *Snagit 10* Starts screen-capture; capture images of errors to paste into Word

Data Acquisition

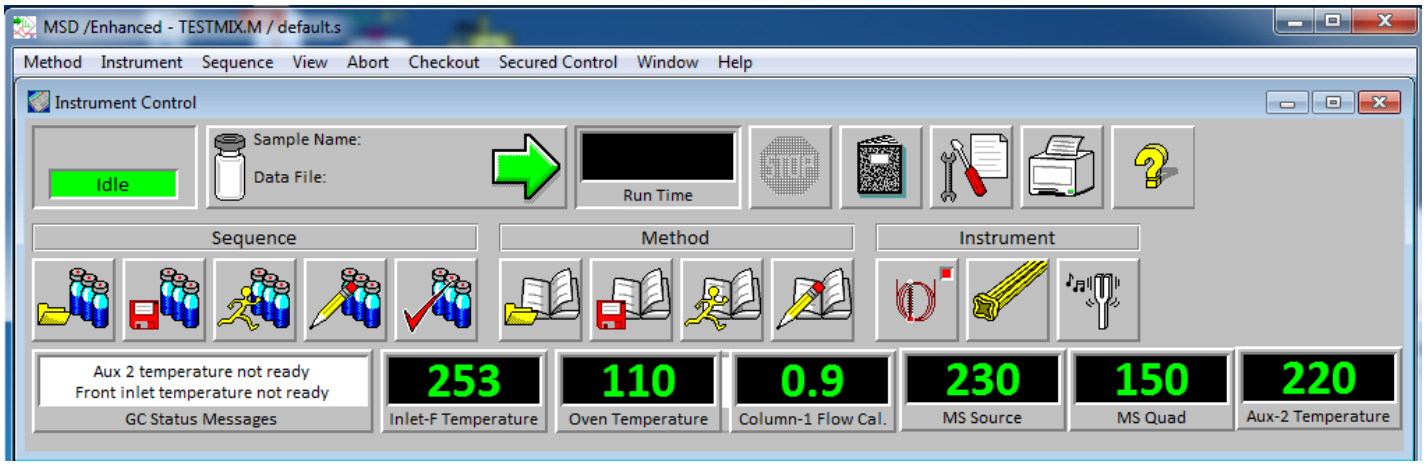
Click on the MSD icon to open the acquisition program. The software uses the concept of "tune files", "methods", and "sequences". As it is starting, you should see the *ChemStation* version information display briefly.



When the program opens, there will be several windows pertaining to *instrument control*, *GC and MS plots*, and *status monitors*. You may need to adjust and resize them so they fit on the screen properly. Use the *Windows -> Tile Horizontal* selection. I normally select *"Instrument Control"* to be the top view.



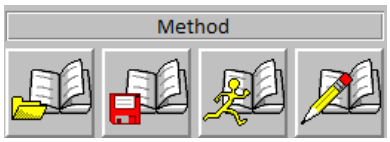
Unfortunately, there doesn't seem to be an easy way to save the layout once you have it adjusted. Let me know if you figure that one out!



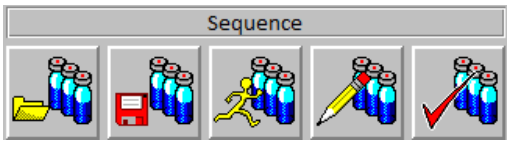
Instrument Control Panel

When you start *MSD Chemstation*, the program opens in the *Instrument Control* view with the last **instrument method** used, and with the last **autosampler sequence** used. Note in this example it is:

The various parameters and settings contained in the method are implemented immediately. If you wish to use a different method, load it as soon as the program starts. Note that *mouseover* does not reveal what each icon represents, however the icon protocol used by Agilent is generally self-explanatory.



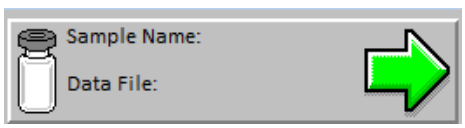
-Open a method
-Save a method
-Run a method
-Write a method



-Open a sequence
-Save a sequence
-Run a sequence
-Write a sequence
-Enable parts of a sequence

-Configure all GC and autosampler parameters
-Configure all mass spec related parameters
-Perform a quick tune or a full tune of the mass spec

It is also possible to run individual samples, bypassing the sequence functions.

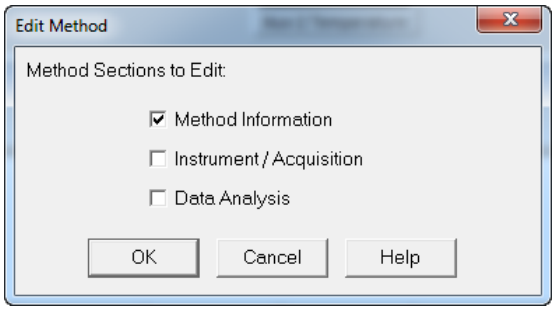


Selecting this option will open a brief dialog (filename, sample name, vial number, et cetera) and then allow you to run one sample. Acquisition is still controlled by all other relevant settings. In the *Total Ion and Spectrum* panes normally positioned below the *Instrument Control* pane,

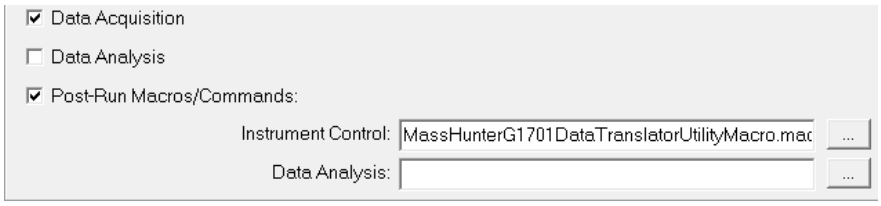
you will see the GC trace and the spectrum of each scan as data is acquired, but you won't be able to process the data here.

Converting Data Automatically for use in MassHunter

If you plan to process data using **MassHunter**, you must enable automatic conversion of the data file format as part of your Method. This will create a MassHunter compatible folder with appropriate files in the same location as the ChemStation data. To do this, choose *Edit Method*, and then select *Method Information*.



Check "*Post-Run Macros/Commands*". From the Instrument Control box, browse to *C:\msdchem\MSexe* and select: MassHunterG1701DataTranslatorUtilityMacro . Be sure to save the Method change.

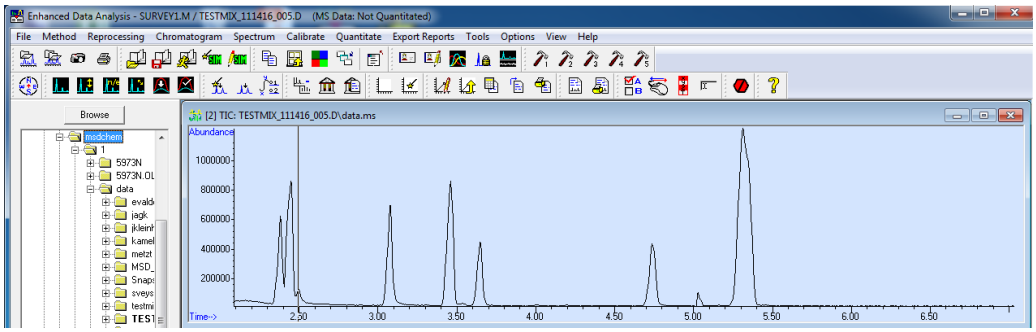


Data Processing - ChemStation

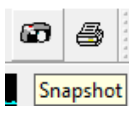
You may process your data using ChemStation or MassHunter. ChemStation instructions follow:

Click on the *MSD Data Analysis* icon to open the ChemStation processing program.

->



Files are selected from the browser pane by *left-double-click*, or *right-click -> select*. The chromatogram view will open. In this program, *mouse-over* DOES reveal the nature of each icon. *NOTE: It is possible to view and process the current data acquisition by using the Snapshot icon located next to the printer icon on the tool bar.*



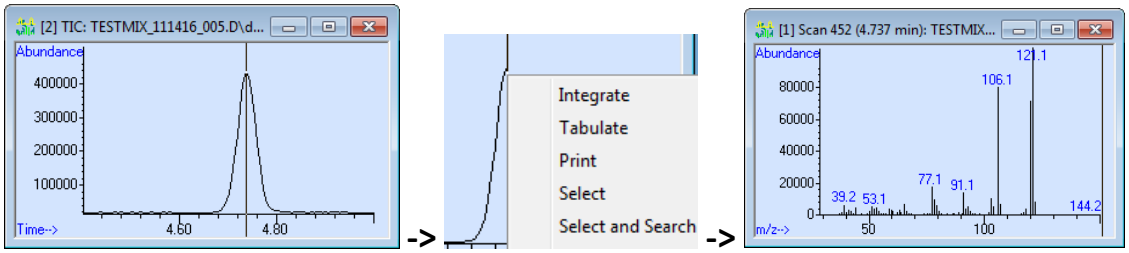
As the run progresses, periodically update the snapshot.

When processing data, there are two modes available for mouse action. These modes are toggled by selecting the *mouse icon* on the toolbar. Additional attributes of each mouse mode are selectable using the *data analysis options* icon located next to the mouse icon.

 &  The action of the LEFT mouse button is used to move the cursor and to *zoom* or *unzoom,* and is the same in each mode; the action of the RIGHT mouse button toggles from *selection* mode to *menu* mode. Selection mode is faster for initial spectral viewing (*right-dbl-clk* opens the spectrum at the cursor position), but you will need to be in menu mode to manually use the library search program. In menu mode, actions are *right-clk-select*.

For this example, toggle the mouse icon until you are in menu mode.

Using the LEFT mouse button, zoom in on a peak using *left-clk&drag*. Note that *left-dbl-clk* unzooms. Position the cursor over the peak and select the scan by using *right-clk-select*. The spectrum pane will open.

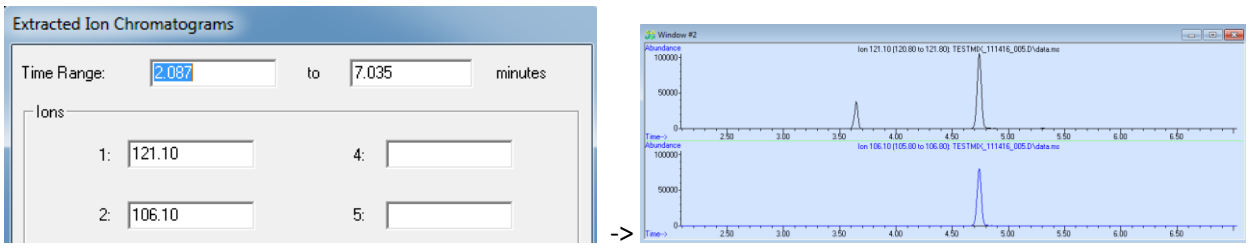


The left mouse button can be used to zoom or un-zoom the spectrum view.

Extracted Ion Chromatograms

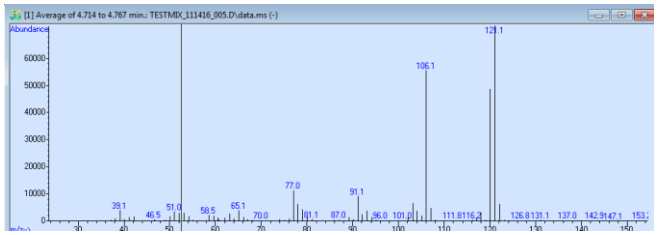
Obtaining the highest quality spectrum from your data generally requires subtraction of a baseline spectrum, or addition of several scans followed by subtraction of several baseline scans. It is often useful to extract single-ion-chromatograms from the TIC data to assist with the selection of sample and baseline scans, especially if the GC peak might be a mixture of components.

Zoom the spectrum to show the m/z 121-122 region. Then *right-clk-select* Extracted Ion Chromatogram. If you only want to select one EIC, then answer YES to the pop-up dialog, otherwise choose NO, and select additional masses from your spectrum for extraction. Extract as many ions as necessary to help you evaluate the peak purity of the GC peak and to locate suitable scans for averaging and baseline subtracting.



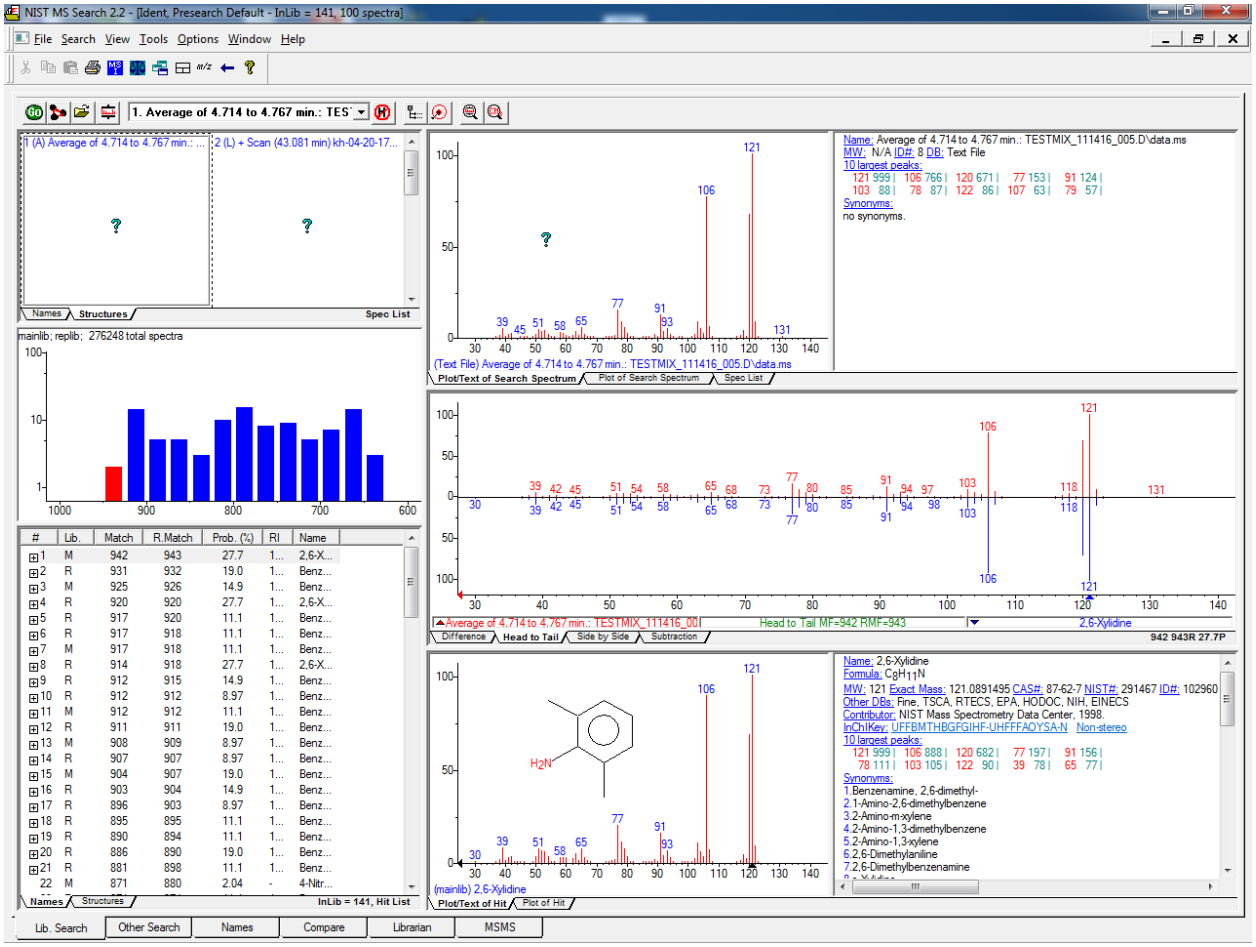
Spectral Subtraction

Averaged scans are stored in registers which can be subtracted. In the Chromatogram View, *right-clk-drag* to sum scans across a GC peak. The average scan is shown in the Spectrum View. Then *right-clk-drag* to sum a region of baseline scans. That average is now shown in the Spectrum View. From the Spectrum pulldown, select Subtract. Then subtraction resultant is then shown in the Spectrum View.



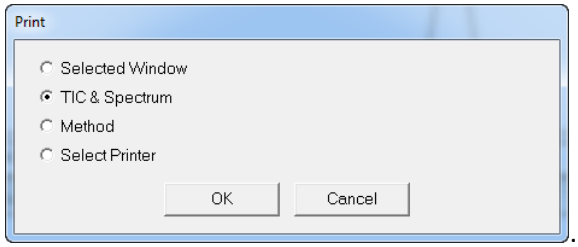
Library Search

In the Spectrum View, *right-double-click,* or *right-clk-select Search*. The NIST Search program will be initiated.



Printing

If the Mouse toggle is set to give *right-clk* menus, select Print to just print the view you are in. Otherwise,

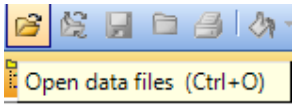
-> 

. Note that the *File -> Printer Setup…* is used to set portrait or landscape as the default.

Data Processing – MassHunter

MassHunter **Qualitative Analysis** is newer and more powerful processing software. It is the same software used with the Agilent 6540 QTOF LCMS system. Because the acquired ChemStation data files must be translated to MassHunter format at the end of the acquisition, it is not possible to use MassHunter to process "snapshots" of the data as it is being acquired. As discussed previously, your acquisition method must have the correct Post Run Macro enabled to convert the data.

Start Qualitative Analysis. Using the Desktop icon, start MH Qualitative Analysis. Open a data file and then open the method file **MSD_Default.**



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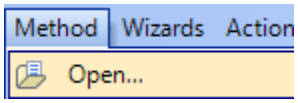

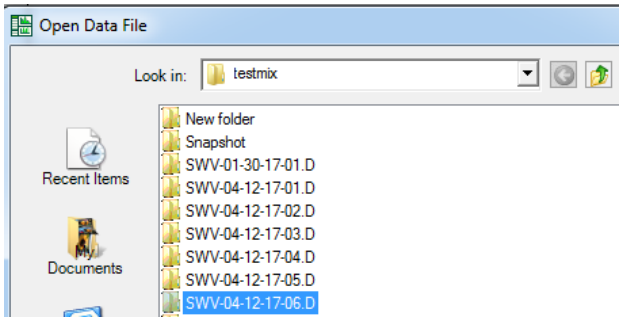

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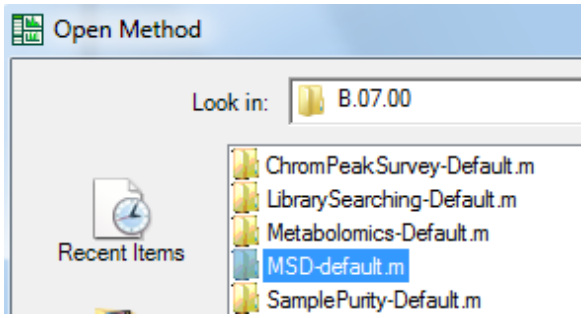
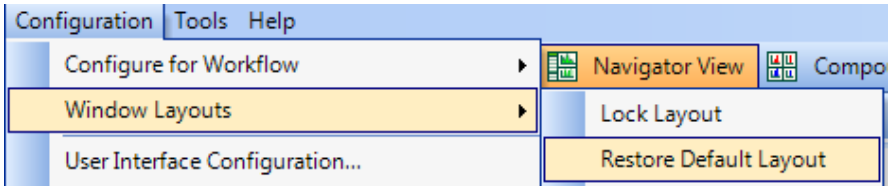
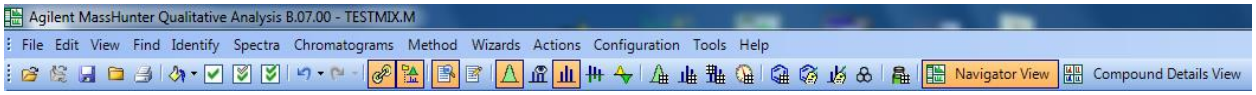


Finally, restore the default layout for the various windows that the method opens:

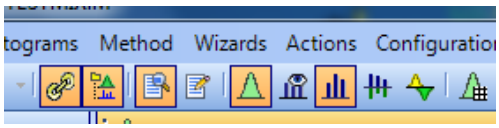


Qualitative Analysis Overview



The workspace in Qualitative Analysis is highly customizable. The method you just opened, "MSD_Default", has already been configured as a reasonable presentation of the program components, default settings, and workspace layout appropriate for processing GCMS data from the Agilent MSD. In the "MSD_Default" workspace, **Navigator View** is the default mode (not Compound Details View), and the screen layout, as indicated by selected icons in the ribbon bar, initially contains sections for:

- **Data Navigator** -a list of the open data files and related chromatograms, spectra, etc.
- **Chromatogram Results** -the total-ion-chromatogram (TIC) and extracted ion chromatograms (EIC's)
- **MS Spectrum Results** -individual mass spectra, averaged spectra, and background subtracted spectra
- **Method Explorer** -portals into many customizable analysis protocols and parameter settings



In both the Chromatogram Results and MS Spectrum Results panes a number of icons representing commonly used features are presented; **right-click** brings up extensive contextual menus to further process or modify the displayed data.

Common Button Actions Used in Chromatogram and Spectrum Results Views

 Reset display

 Reset display without normalizing

 Normalize display

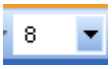
 Toggles previous display

 Autoscale Y-axis during zoom

 Set Anchor display

 Separate displays

 Overlaid displays

 Set number of displays

 Range select

 Peak select

 Manual integration

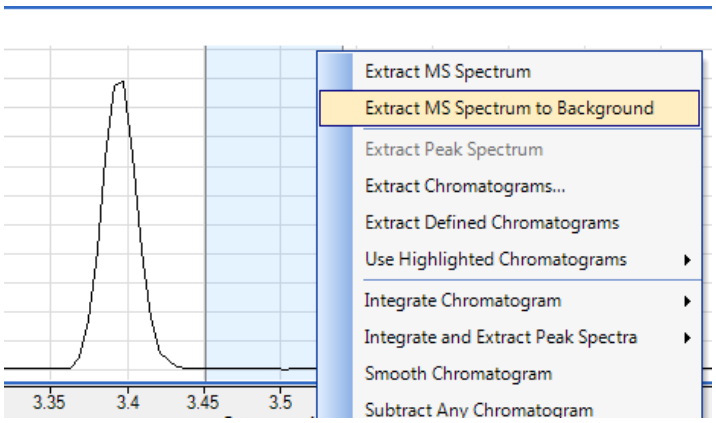
 Walk chromatogram

 Print chromatogram display

Spectra and Background Subtraction

The mouse controls in **Qual Analysis** are the exact opposite of **ChemStation**! In the Chromatogram Results view, use *right-clk-drag* to zoom, and then use *left-clk-drag* to highlight a region of scans to be averaged into one spectrum. A representative background spectrum is normally created first.

Once a region of the baseline is highlighted, *right-click* to select "Extract MS Spectrum to Background" from the context menu. Your selection will now show up in "Data Navigator" and will be automatically subtracted from each MS spectrum you subsequently extract from the chromatogram.



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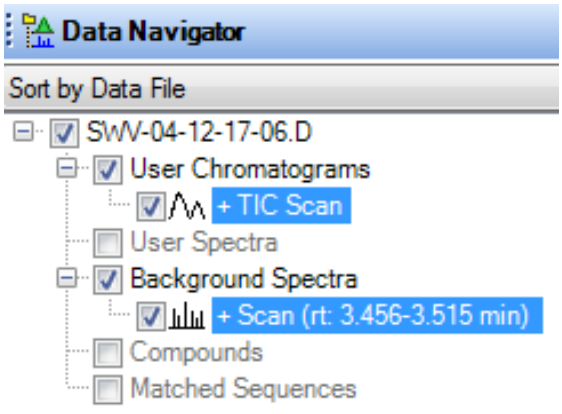
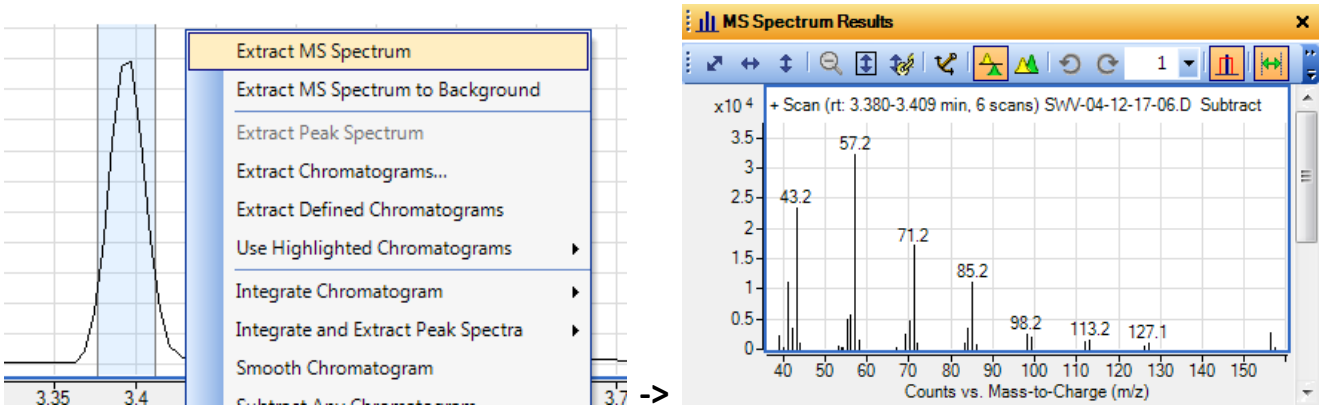
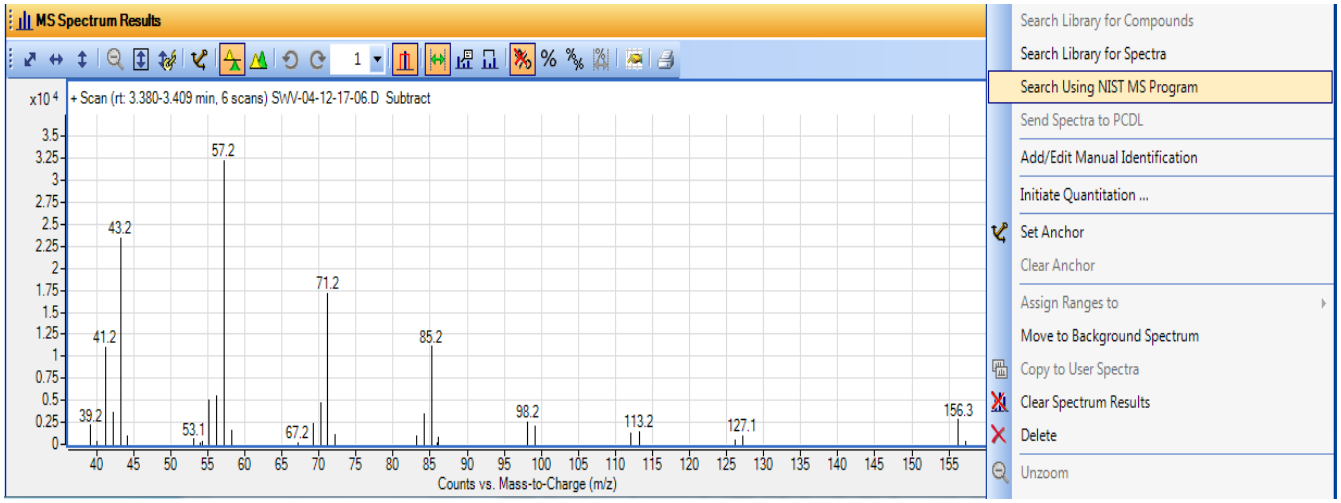


Next, highlight a region of data scans to average and select "Extract MS Spectrum".



Library Search

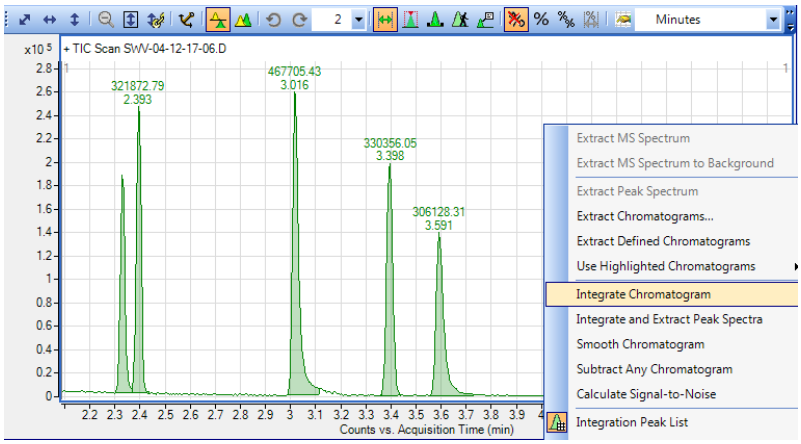
In the MS Spectrum Results view, *right-click* to select "Search Using NIST MS Program".



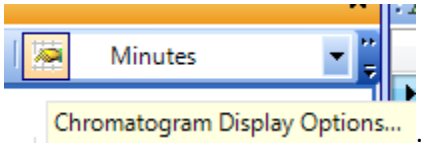
This will call the same NIST search algorithm used by the ChemStation processing program. You may also select "Search Library for Spectra" which uses the Agilent probability-based-matching (PBM) algorithm.

Peak Integration

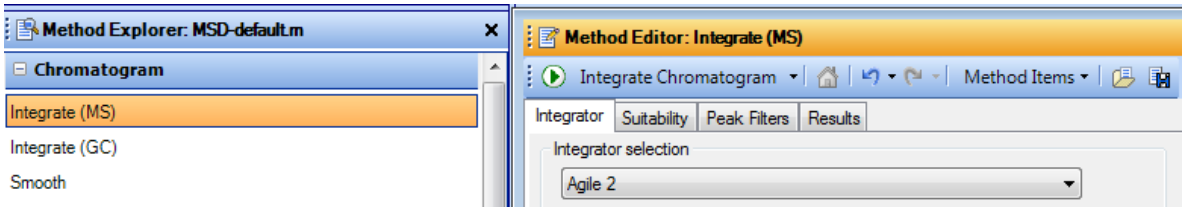
Simply right-click in the Chromatogram Results view and select Integrate Chromatogram.



Labeling choices are made using the Chromatogram Display options button.

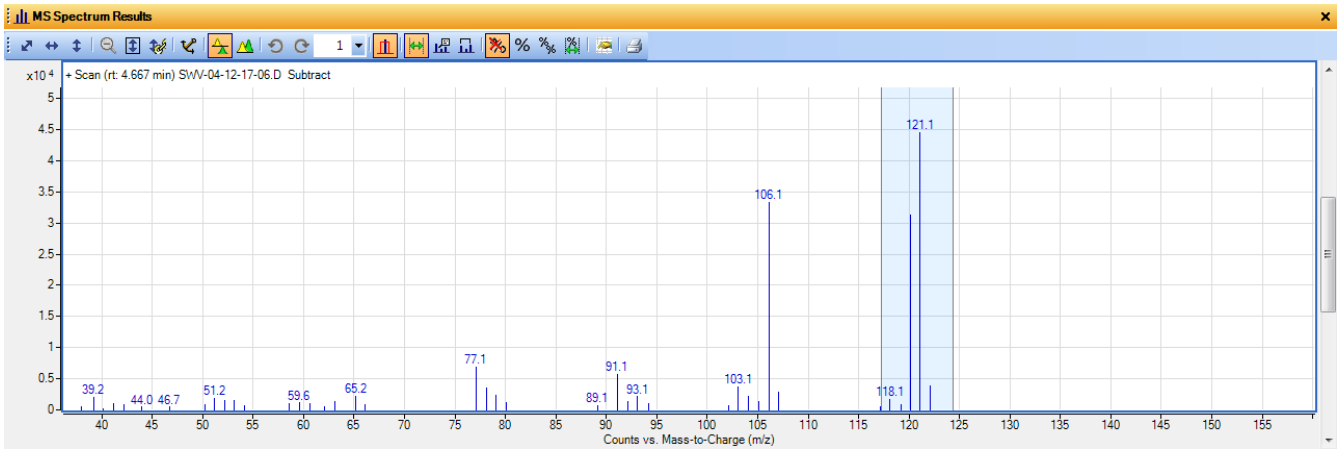


As with all of the tools, many other choices related to integration can be accessed in the Method Explorer.

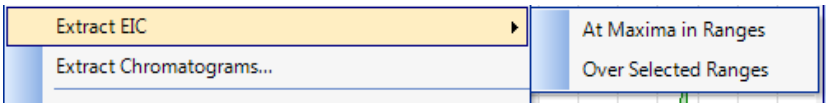


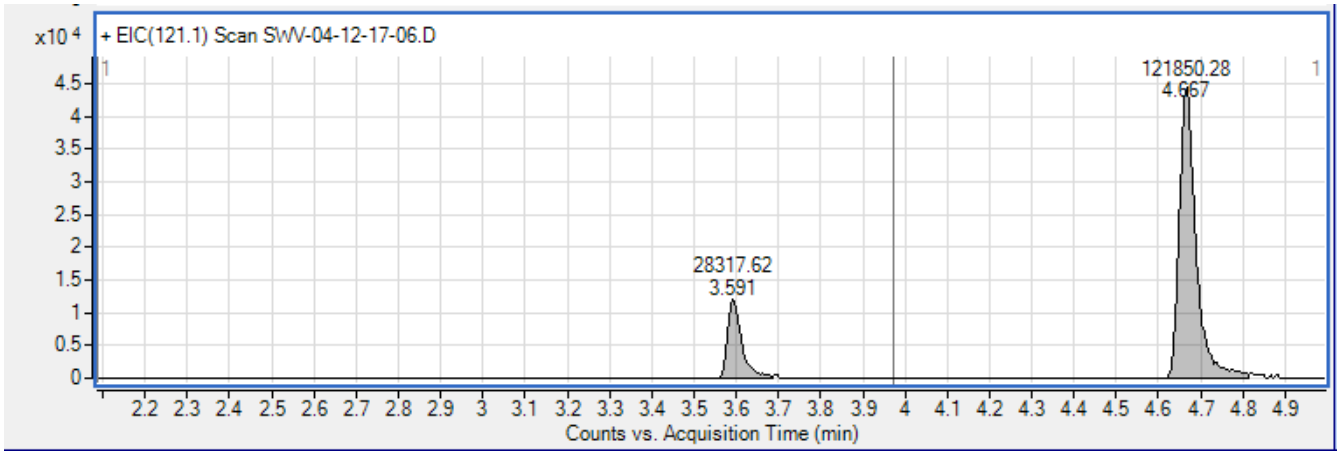
Extracted Ion Chromatograms

Within the MS Spectrum Results view, use the cursor *(left-clk-drag*) to select the region of the spectrum containing the ion or range of ions you wish to extract.



Right-clk to see the context menu, and select from the Extract EIC choices. Typically you would choose "At Maxima in Ranges". You can also integrate the EIC if you wish.

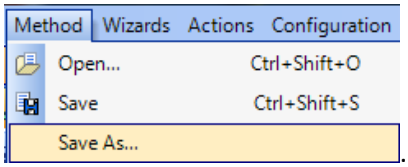




Conclusions

Clearly MH **Qualitative Analysis** offers many complex capabilities and you will need to do some exploring on your own to learn all of the features that are available. For additional guidance, use the Help tools provided by the software, and consult with the **Qual Analysis** training materials that Kamel has prepared for students using the QTOF.

NOTE: Do not save changes to the MSD_Default method!!! If you make changes you wish to preserve, choose Method -> Save As…



Similarly, if you wish to save changes to the layout of views on the screen, choose *Save Layout…* from the *Configuration* dropdown. This is also where you can "Restore Default Layout" if things get out of hand.