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Magnum GCMS
Training and Operating Instructions

02/07/2012 S.V.

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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 1238A, please wear all appropriate personal protective equipment. Aprons, safety glasses, and rubber gloves are available.

Properly dispose of waste solvents and glass pipettes 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.

I. Prerequisites for Training

The following prerequisites are required.

A. Completion of a graduate level course with significant mass spectrometry content.

B. A good understanding of gas chromatography acquired through experience or course work.

Exceptions to these prerequisites will be made on a case by case basis. The final decision will be made by CIF staff.

II. Overview of the Magnum GCMS Instrument and Software.

The Magnum GCMS is an ion-trap mass spectrometer coupled to a Varian 3400 gas chromatograph Both *electron ionization* (EI) and *chemical ionization* (CI) are possible, however EI is the normal operating mode. The mass spectrometer acquires data with "nominal mass" precision. The mass peak width at half-height is approximately 0.7 Daltons. When properly calibrated, mass measurements are accurate to about +/- 0.3 Daltons. If you require greater mass measurement accuracy, consider using the Waters GCT GCMS, also located in 1238 Hach Hall. NOTE: *If you do not understand the information in this paragraph, then you fail to meet training Prerequisite A, a basic understanding of mass spectrometry.*

The instrument-control and data acquisition software is called ***Vx Acquisition,*** and includes all of the programs required for tuning the instrument, acquiring data, and converting the data to formats suitable for processing. During the acquisition it is possible to view chromatograms and spectra, but it is not possible to process or print the data until the acquisition has finished.

T
hree software packages can be used for processing data. These include ***Varian MS Workstation***, ***Agilent Mass Hunter***, and ***Waters Mass Lynx***. Each requires that the acquired data be converted to the appropriate format. At the moment, we are only teaching the ***Mass Lynx*** software. However, if you are already familiar with and have access to ***Mass Hunter*** or ***MS Workstation***, you may prefer to use one of those packages. For each of these three packages, the availability of searchable mass spec libraries and the complexity of data conversion prior to processing is summarized in the table below.

Software Package	**Library Availability**	**Data Conversion Complexity**	**Other Instruments**
MS Workstation	Yes	No user action required	Saturn in undergrad lab
Mass Lynx	Yes	Two step conversion (automatic)	GCT in MS lab
Mass Hunter	Not yet	One user step required	QTOF in MS lab

III. Ion Trap Mass Spectrometer Considerations

Ion trap mass spectrometers have very good detection limits, but restricted dynamic range. If too many ions are present in the trap (i.e. your sample is too concentrated), the quality of the mass spectrum deteriorates. Through training, we will help you achieve the following goals:

Make sure you are getting good data. We have certain responsibilities related to instrument performance. *Your responsibility is to follow the operational procedures we specify.*

Make sure you know the difference between good data and bad data. Injecting *more* sample does NOT give better results, it gives worse results. You need to be able to recognize a poor quality spectrum when you see one.

Protect the capillary column from damage. The GC column is very expensive and can be damaged by acids, bases, oxidizing agents, water, derivatizing reagents, particulates, et cetera. Be sure your samples are neutralized and filtered.

Use important features of the software:

-Using single ion chromatograms to solve problems
-Averaging scans and subtracting background to achieve high quality spectra
-Adjusting library search parameters to give you the information you need

Use the "Method Page" properly. You have been assigned a default method page for data acquisition which can be easily modified and saved to meet your specific needs.

IV. User Responsibilites

ALWAYS make a proper entry in the logbook.

ALWAYS place a copy of the GC trace and the spectrum at the apex of the largest peak in the binder. Please write your name at the top of each page.

ALL GC METHODS must end with a "bakeout" segment that takes the column to at least 280 degrees and keeps it there for at least five minutes.

ALL GC METHODS must have an injector temperature no lower than 180 degrees or higher than 280 degrees. Use the default value of 260 degrees unless you have a good reason not to.

ALL ACQUISITION METHODS must have a filament/multiplier delay so that the mass spectrometer is not acquiring data on the solvent peak. The proper delay time is a function of the column length, GC temperature program and the solvent. A value of 2.5 minutes is about right for most situations.

If you are one of the *very few people* who must acquire data in the solvent region, you may reduce or eliminate the delay. Don't do it unless you have to!

ALL ACQUISITION METHODS should use a low mass of 35. The ion trap operates using a complicated set of pulse sequences, and as part of automatic gain control (AGC), the ion trap uses the region below mass 33 for other purposes. We are still learning how the **Vx** software works in that regard.

ADJUST YOUR SAMPLE CONCENTRATION. The ion trap works best with low nanogram amounts of sample. You may still get good data at higher levels, but let's use 2.5 nanograms as an example. Since you will be injecting one microliter of your mixture into the GC, and the injector is operating in "split mode" with a split ratio of approximately 40:1, ***the initial concentration of your solution should be about 100 nanograms/microliter***. This is the same as 0.1 mg/mL. This is roughly equivalent to a 0.01% solution. By way of comparison, the NMR solutions you use are probably about 1-2%. ***This means that an NMR solution should be diluted by at least a factor of 100 before being injected into the GCMS.***

V. Recognizing Good Spectra

Your ability to recognize and print out good spectra is important. We have already talked about one of the key aspects, reducing sample concentrations to give appropriate detector signal levels. Other important factors include:

-Selecting the correct scan from within the GC peak
-Performing an appropriate manual or automatic background subtraction
-Determining if certain masses in your spectra are "real", or if they are:
1) noise (very rare)
2) column or septum bleed (common)
3) a co-eluting or tailing GC peak (common)

The most useful method for determining the purity of your spectrum is to generate single-ion mass chromatograms, discussed later in the Mass Lynx data processing part of this document. Mass chromatograms allow you to display "single ion chromatograms" of interest over part of, or the entire, GC run. For example, if you have a GC peak that elutes at a column temperature of 200 degrees and a previous GC peak eluting a few seconds earlier, you might notice in the spectrum corresponding to the top of your GC peak that in addition to an expected molecular ion at m/z 150, there are masses at m/z 180 and m/z 207. You can investigate the nature of these ions by using "mass chromatograms".

In addition to displaying the GC Total Ion Current trace, you would want to display mass chromatograms for the ions present at m/z 150, 180, and 207. You might then see that the m/z 150 single-ion chromatogram trace clearly corresponds to the GC peak in the Total Ion Current display. However, the m/z 180 trace might clearly show that m/z 180 is the result of tailing from the GC

peak eluting prior to the peak you are investigating. The m/z 207 mass chromatogram will probably show a continuous or gradually rising baseline at m/z 207. This is a common column or septum bleed peak that increases as the temperature increases. There might be a small increase in the bleed at the exact point where the GC peak elutes; large GC peaks can sometimes "carry" a little bleed with them.

Mass chromatograms can also be used to determine if a GC peak is actually the combination of two or more components. As long as the maxima of each component differs by at least one scan (or there is a significant difference in the peak profiles - lead-in, tailing, et cetera) you can usually display some combination of mass chromatograms that will help you identify which masses belong to which GC components. A careful examination of mass chromatograms will provide the information necessary to properly average scans and subtract background in order to get the most representative spectrum possible.

VI. Recognizing the Molecular Ion

Sometimes there can be confusion about whether an ion is the molecular ion. Often it will seem to be at about the right mass, but may be one mass unit higher than you expected. You have been instructed during your training that as a consequence of how an ion trap works, "self-CI" can occur. This can result in an MH+ ion and adduct ions. Let's review this. The normal equations of ionization are:

1) $M + e^- \rightarrow M^{+*\cdot} + 2e^-$ (initial ionization event)
2) $M^{+*\cdot} \rightarrow M^{+\cdot}$ (internal stabilization)
3) $M^{+*\cdot} \rightarrow F1^{+\cdot} + N$ (neutral loss fragmentation)
4) $M^{+*\cdot} \rightarrow F2^{+} + R^{\cdot}$ (radical loss fragmentation)
5) $F1^{+\cdot} \rightarrow F3^{+} + R^{\cdot}$ (fragment ions also fragment)
6) $M + M^{+*\cdot} \rightarrow MH^{+} + (M-H)^{\cdot}$ ("self-CI")
7) $M + M^{+*\cdot} \rightarrow (M+A)^{+} + (M-A)^{\cdot}$ (formation of adduct ions)

Reactions 1 through 5 are unimolecular and occur rather quickly. Reactions 6 and 7, being bimolecular, are much slower. In other common types of mass analyzers such as quadrupole mass spectrometers, ions are formed continually in the ion source and extracted continually into the mass filter. The filter only allows ions of a specific m/z value to pass through to the detector, depending on the values of the RF and DC voltages. All other masses are discriminated against, and are "thrown away". A complete mass spectrum is achieved by ramping the mass filter voltages through the entire mass range in about one second. At any given moment in time, MOST of the ions formed in the source are being thrown away! Ions formed in the source are extracted, "filtered", and detected or eliminated, in just a few microseconds. In a quadrupole, ions only live for a few microseconds. There is not enough *time* for reactions 6 and 7 to significantly occur. (Actually, the amount of self-protonation that can occur is compound or "class" specific... there are some compounds that form an observable MH+ even in a quadrupole mass spectrometer.)

In an ion trap, ALL the ions formed during the ionization pulse (variable from 16 microseconds to 24,999 microseconds) are collected in the trap. None are thrown away. Once the optimum number of ions has been collected, mass filtering occurs through a mass-dependant destabilization process. Ions are ejected along a defined trajectory to the detector. A complete mass spectrum is achieved by ramping the ejection criteria over a period of about 200 milliseconds for a complete scan. The entire sequence is called a microscan. You should now understand two things:

-Why the ion trap is so sensitive *(no ions are thrown away!)*

-Why reactions six and seven can occur *(ions live in the trap for hundreds of milliseconds.)*

Although some "self-CI" may occur, the spectrum is still interpretable using all the classical rules for El spectra, because almost all the fragmentation originates from the $M^{+\cdot}$ ion. When investigating whether an ion is MH^+, first determine if the ion is actually part of the compound. Use "mass chromatograms" as explained previously. Next, consider whether you have any nitrogen in your sample. If there is no nitrogen in the molecule, the molecular weight cannot be at an odd mass. Therefore, the ion must be an MH^+, or the true molecular ion is at a higher mass and fragments so readily that it is not observed. In this case, you may need to do CI (chemical ionization) to determine the molecular weight of the compound.

VII. User Accounts

Only properly trained students will be given user accounts on the Magnum. Accounts that are not used for more than six months are usually deleted. Occasionally instrument access may be suspended for one or more users for a short period of time if problems have been detected pertaining to their usage of the instrument. Access is granted as soon as the difficulty has been resolved.

Your username for this computer is the same as your ISU email address, minus the @iastate.edu extension. For example, my username is **sveysey.** For billing purposes, the computer automatically records the length of your session based upon your login and logout times. Be sure to log out of the computer when you are finished.

All of your methods MUST be named with your username, followed by a one-digit number, e.g. *sveysey1*. All of your data files MUST be named with the first five letters of your username, followed by a three digit number, e.g. *sveys001.* NO EXCEPTIONS.

VIII. Computer Etiquitte

1) We may have loaded various software packages on this computer to serve the needs of different users. Only use the software that you have been trained and authorized to use.

2) The **Vx** and **Mass Lynx** software packages require that all users have "administrator" privileges. Do NOT make any changes to the software packages or the system settings.

3) This computer is on the internet to facilitate data archival and billing. Do NOT use this computer to browse the web, view your e-mail, or otherwise amuse yourself. Do NOT download any files of any sort to this computer.

IX. Acquiring Data Using Vx

Start the **Vx** Acquisition program by left-double-clicking the icon 

. The program will open to show three panes:

1) Explorer pane (left) in tree-structure: Used to easily move between parts of the program.
2) Messages pane (bottom): Displays the status of program actions and alerts you to problems.
3) Program pane (center): Initially displays the Welcome page; normally displays program tabs.

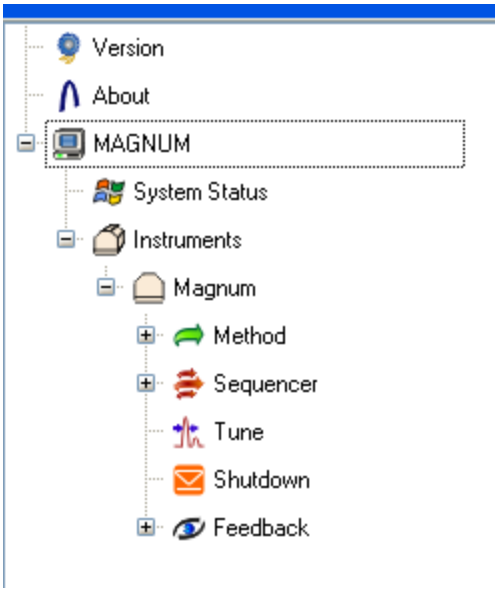
NOTE: Both Vx and MassLynx can be placed in your start-up file; both programs would then open automaticvally when you log onto the computer.

The Explorer pane (shown on the next page) shows that the name of this PC is "MAGNUM", and that the only instrument definition is also called "Magnum". For the Magnum instrument, there are five major software branches:

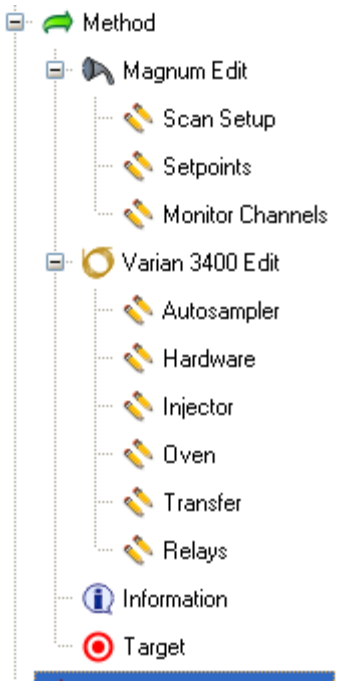
Method (with branches)	-used to set all GC and MS acquisition parameters
Sequencer (with branches)	-not used; controls GC auto-sampler if present
Tune	-used by CIF staff to tune and calibrate the mass spec
Shutdown	-used by CIF staff to initiate a controlled shutdown
Feedback (with branches)	-displays chromatogram and/or spectra during acquisition

Left-clicking on any of the icons will open the program in the Program pane. The normal entry point is, of course, "Method". This is where you will call up your own unique "acquisition page", edit the appropriate GC and mass spec parameters, choose a filename, and start the data collection.

IMPORTANT: Use only YOUR own methods!



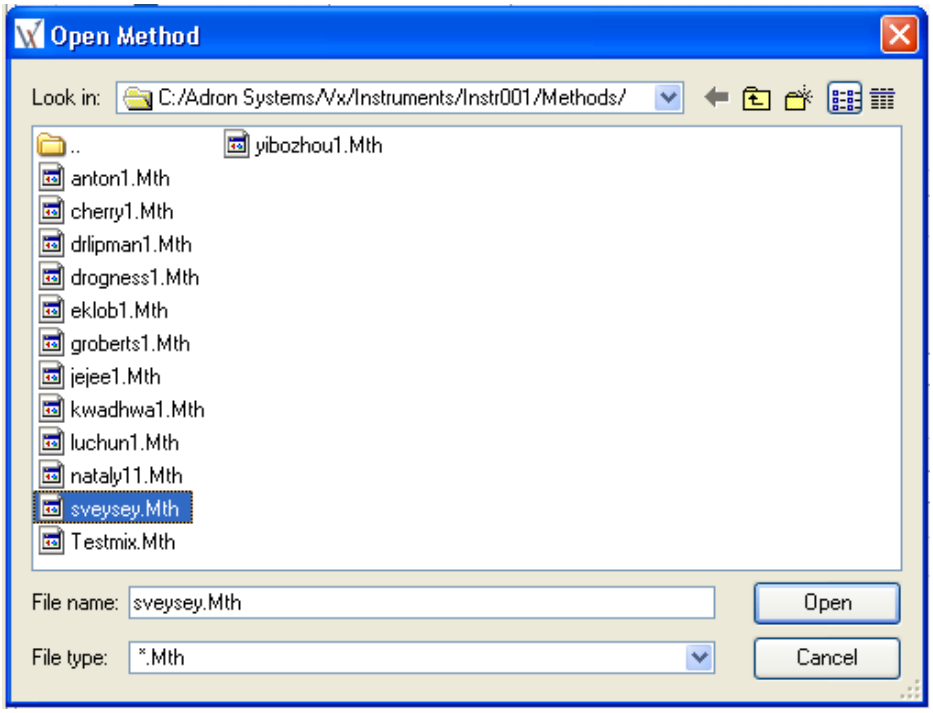
Software tree



"Method" branch expanded

"Feedback" branch expanded

<u>Step One – Open Your Method</u>

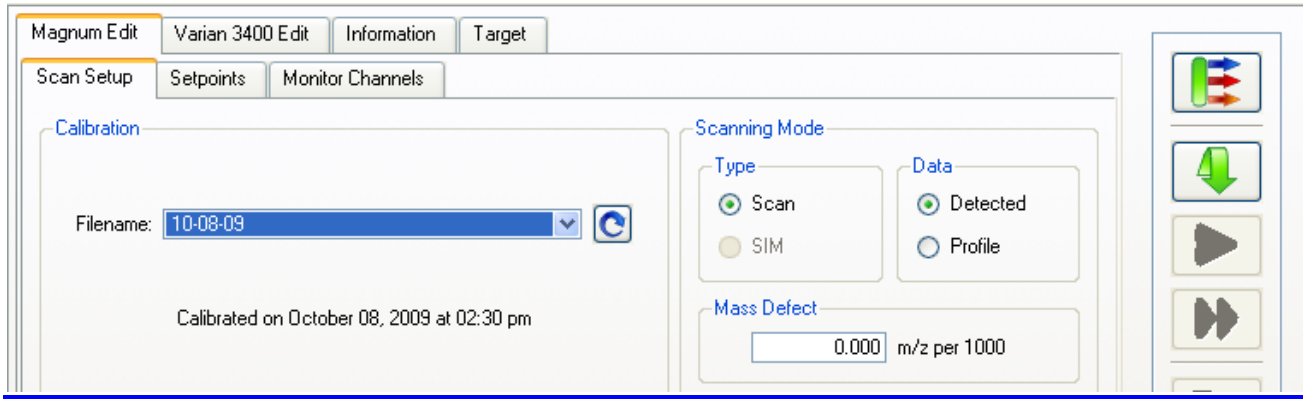


From the task bar choose "Method -> Open…" A page of method files will appear. Left-click to highlight one of your methods, and then click on "Open".

In the Program window, you will now see a series of tabs corresponding to the four parameter pages associated with setting up the GCMS for data acquisition.

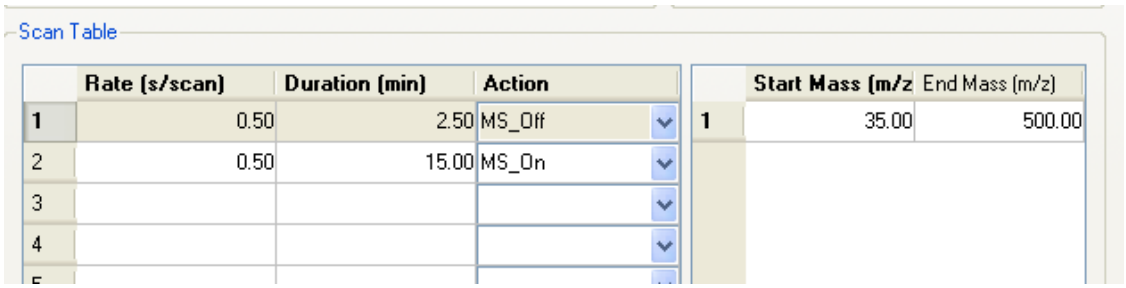
Magnum Edit (Scan Setup tab)	-scan speed, mass range, acquisition time, calibration
Varian 3400 Edit (Oven tab)	-GC temperature program
Information	-sample information (non-critical)
Target	- filename, file type, conversion macro (critical)

Step Two - Magnum Edit



Default parameters were selected for you when your method was initially set up. The only Magnum Edit tab you may need to change is the "Scan Setup" tab. The top portion is shown in the figure above. Note that a calibration file acquired on October 8, 2009 has already been selected. As newer calibration files are acquired by CIF staff, you will need to select the most recent calibration file.

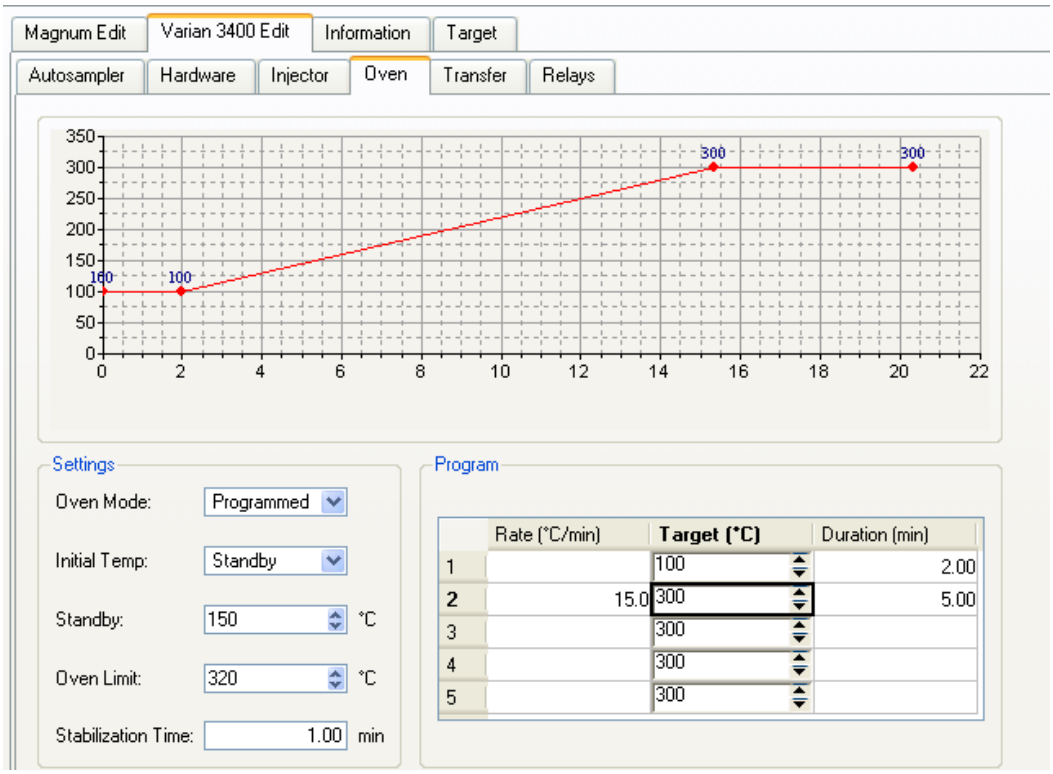
The bottom half of the Scan Setup tab contains the "scan table". In the example below, note that the first scan segment specifies that the mass spectrometer will be "off" for the first 2.5 minutes. This is the equivalent of the "filament / multiplier delay" you are familiar with from the old Magnum software. The second scan segment specifies that after 2.5 minutes, the mass spectrometer will turn on and acquire data for 15 minutes at two scans per second. The scan range in this example is from m/z 35 to m/z 500. Recall that the maximum m/z allowed is 650, and the minimum allowed is 35.



	Rate (s/scan)	Duration (min)	Action		Start Mass (m/z	End Mass (m/z)
1	0.50	2.50	MS_Off	1	35.00	500.00
2	0.50	15.00	MS_On			
3						
4						

Step Three – Varian 3400 Edit

Once again, default parameters were selected when the method was established. You should normally only have to edit the parameters on the Oven tab.



Leave the "standby" temperature at 150 degrees. As you adjust the various segments of the GC temperature program, the graphical temperature display automatically changes. As with the old Magnum software, be sure that your program includes a "bake-out step" of at least 5 minutes at a minimum of 280 degrees.

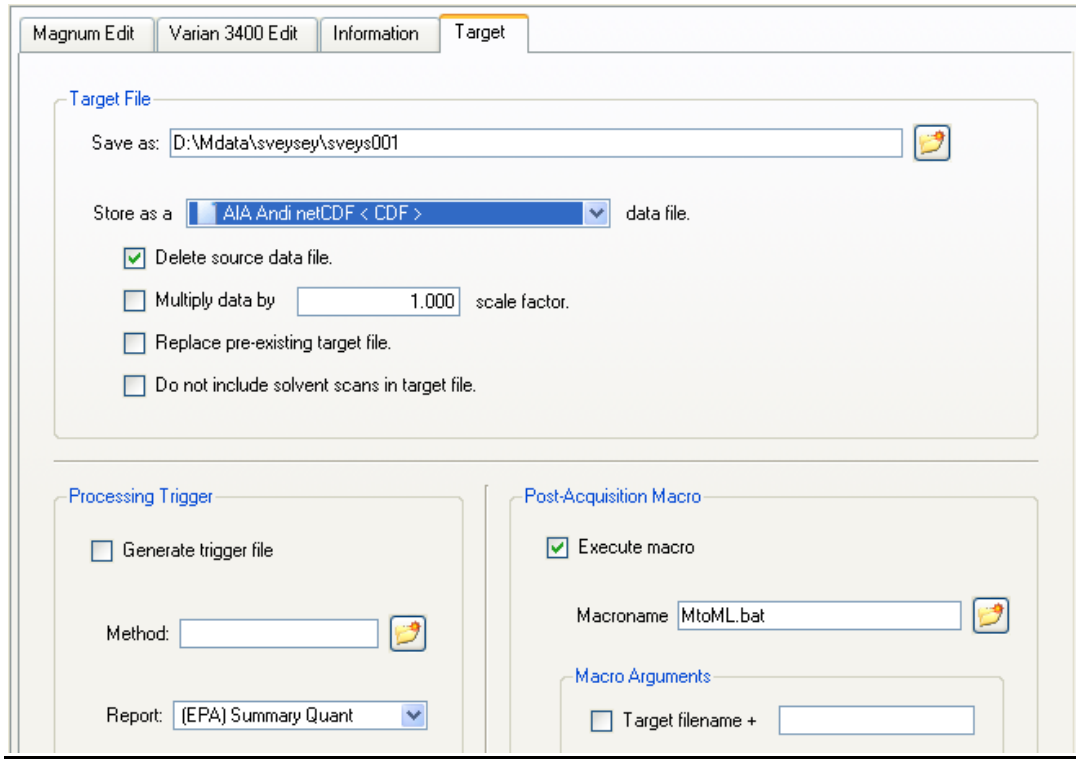
Step Four – Enter Sample Information (optional)

The information entered on this page will be kept in the electronic log file for the sample, but is not actually used by any aspect of instrument control or data acquisition.

Step Five – Target (this is the critical stuff!)

This is where you will specify the path and filename for the data you plan to acquire. The data format is also specified on this page. All data is initially acquired by **Vx** in one default format (Teknivent - .tkf). The data is then converted to one of several formats recognized by common mass spec processing software packages. In our case, we wish to save the data in a format compatible with **Mass Lynx**. Unfortunately **Vx** does not provide this conversion option. Instead, we have set up your page to save the data in "netCDF" format, a common file interchange protocol. We have also written a macro program to pass this file to a part of the **Mass Lynx** software package that converts the data from netCDF format to Mass Lynx format.

All of this should happen without any intervention on your part. However, it is necessary that you strictly follow the path and file naming protocols we have established.



Do not make any changes to the settings on this page other than the filename for the data you are about to acquire. Use the "browse folders" button to set the path to your folder, and then change the filename. The filename MUST start with the first five letters of your username, and end with three numbers. In the example above, the username is "sveysey". The acquisition filename selected is "*sveys001*".

Step Six – Connect to the Instrument and Start Data Collection

Data acquisition requires a sequence of three steps. First, there is a "handshake" that must be made between the PC and the instrument. The **Vx** software uses "Connect" and "Disconnect" commands not only to make the handshake, but also as a way of clearing any unexpected hardware errors that might occur. Once the PC and instrument are "connected", the GC and mass spec methods you established need to be downloaded. Finally, the acquisition must be started either by "arming" the Start button on the Varian 3400 GC front panel, or by "starting the acquisition" from **Vx**.

Connect / Disconnect button (shown disconnected – press to connect)

Download button (grayed out until the instrument is "connected")

Arm Acquisition (allows the Start Button on the Varian 3400 to trigger acquisition)

Start Acquisition immediately

Add 5 minutes to the run time

Abort the run (data file is NOT saved!!!)

The actual instrument status is reflected by the state of the action buttons shown above, and by the system status display buttons. There are various configurations; the "armed and ready" state is shown below.

Mass Spec armed and waiting for Start

GC armed and waiting for Start

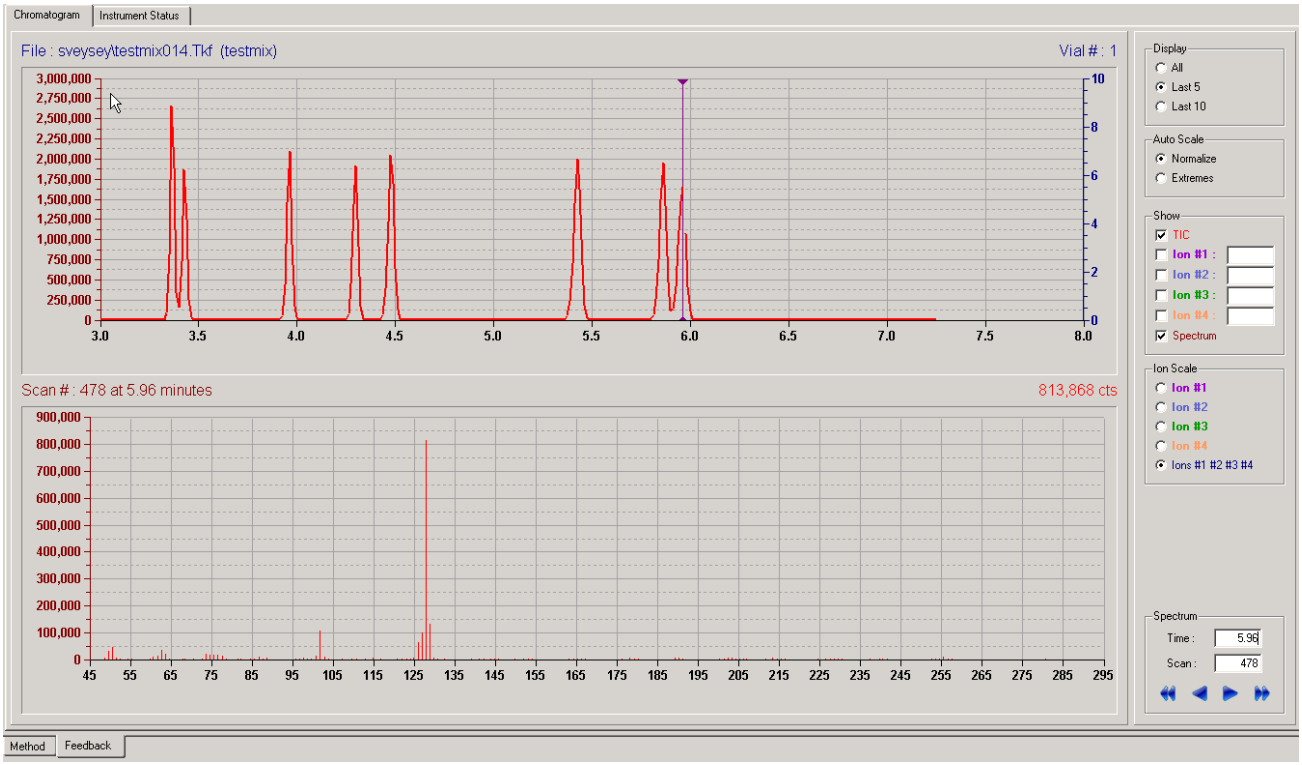
Autosampler not present

System ready and waiting for Start

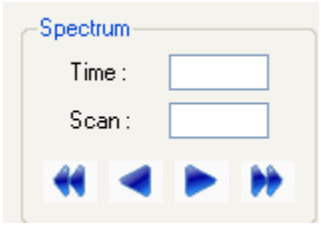
In this configuration, simply inject your sample and press the “Start” button on the Varian 3400. Both the GC program and the mass spec data acquisition will start.

Step Seven – Feedback: Viewing the Chromatogram and Mass Spectra

Double-click the “Feedback” tab in the Explorer pane. **Vx** software allows you to view the GC trace as it is being acquired, and allows you to view the mass spectrum of any GC peak that has been acquired. You may also view “mass chromatograms”. However, in this release of software you cannot interact with either display. All data processing and printing must be done post-run.



The mass spectrum selection cursor in the chromatogram view can only be moved by typing in a “Time” or “Scan#”, or by using the mouse to click on the blue arrow keys.



The mass spectrum selection cursor moves when a time or scan number is entered, or when the mouse is used to click on the blue arrow keys.

At the end of the run (runtime as specified in Magnum Edit -> Scan Table) the real-time display will go blank. The data file will be automatically converted to “netCDF” format and stored in the

D:\MData\[username]\ directory. A copy of the "netCDF" file is then automatically converted to Mass Lynx format, and stored in a communal data directory: D:\MLdata\[filename]\.

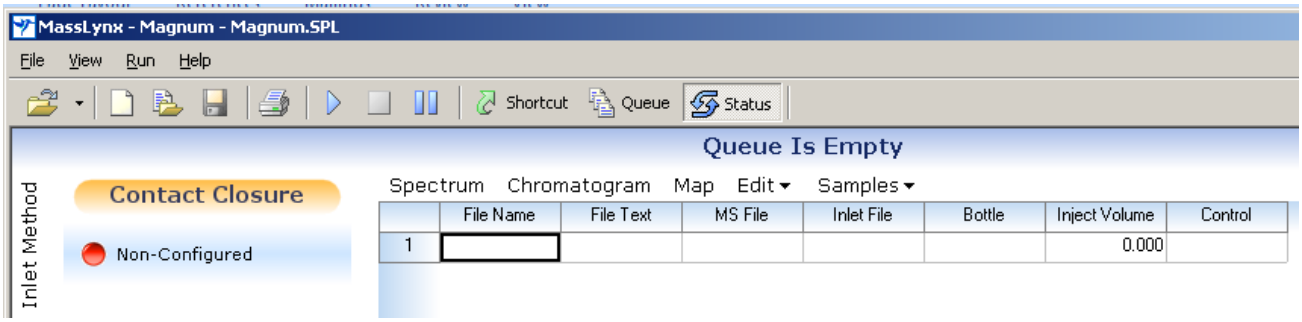
The data file can now be opened from within the **Mass Lynx** data processing software package as described in the next section. The **Mass Lynx** program may be started prior to, during, or after your use of **Vx**.

X. Processing Data Using Mass Lynx

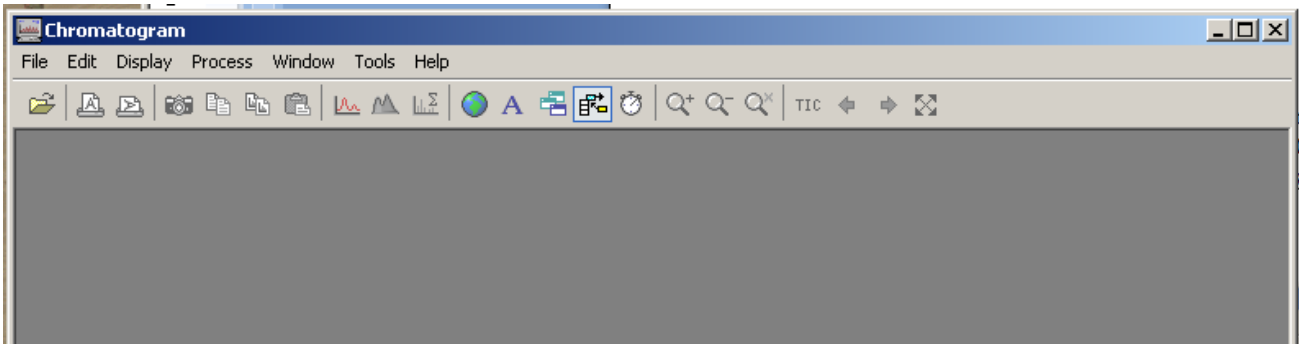
Starting Mass Lynx

Start the **Mass Lynx** software by clicking on the desktop icon. 

After about twenty seconds, you should see the following:

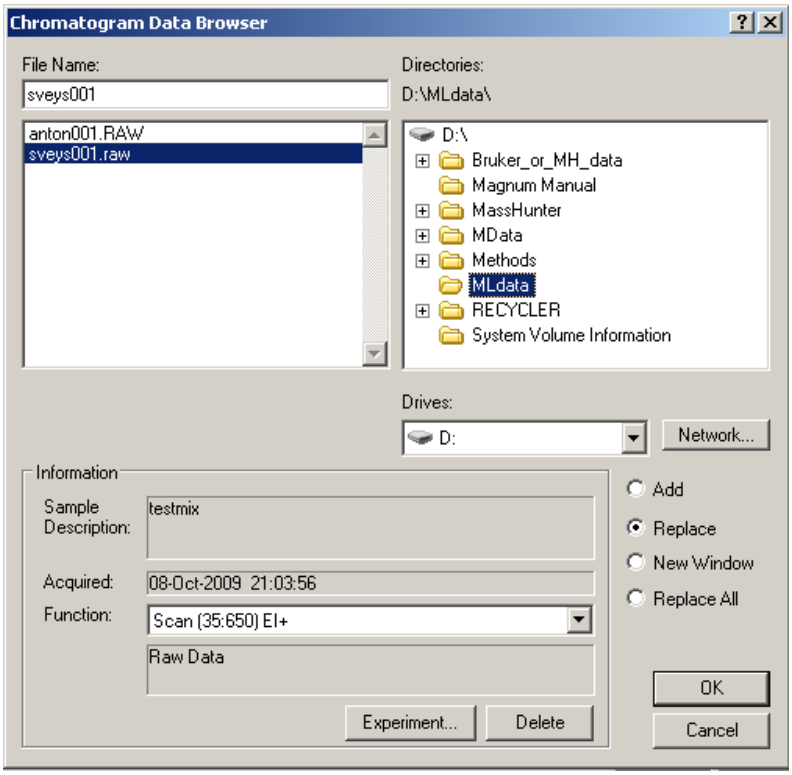


As with the previous Magnum GCMS software, the normal entry point for data processing is the Chromatogram program. Single-left-click on the "Chromatogram" heading and a window should now appear. It may be empty, or it may show the last data file you processed.

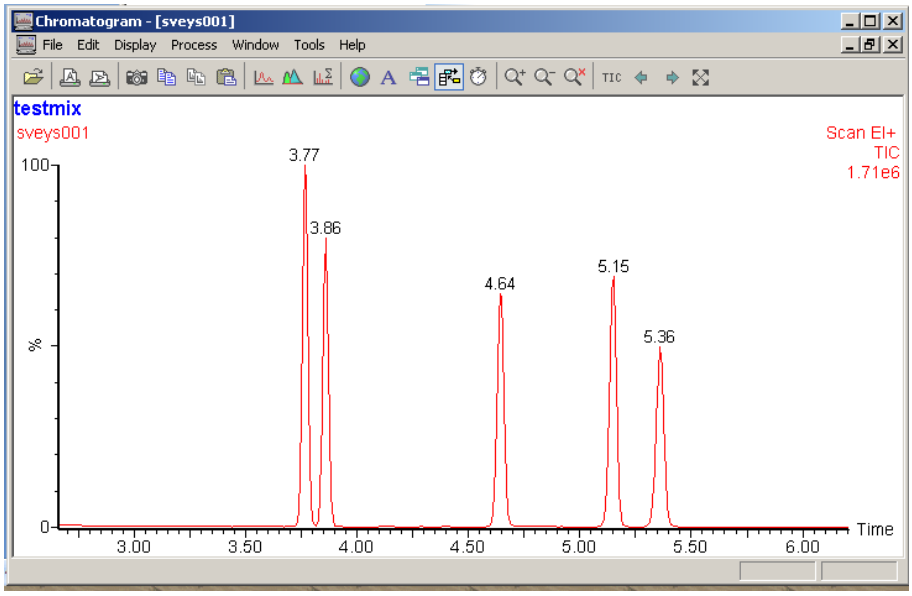


Retrieving Data Files

In the Chromatogram window, pull down the File menu and select "Open…" This will bring up the file browser tab. At the moment, all converted data files are stored automatically in the directory D:\MLdata. Note that you can use this browser to preview the sample description, acquisition date, and acquired mass range. Additional information is displayed in a separate text window by selecting the "Experiment…" button.

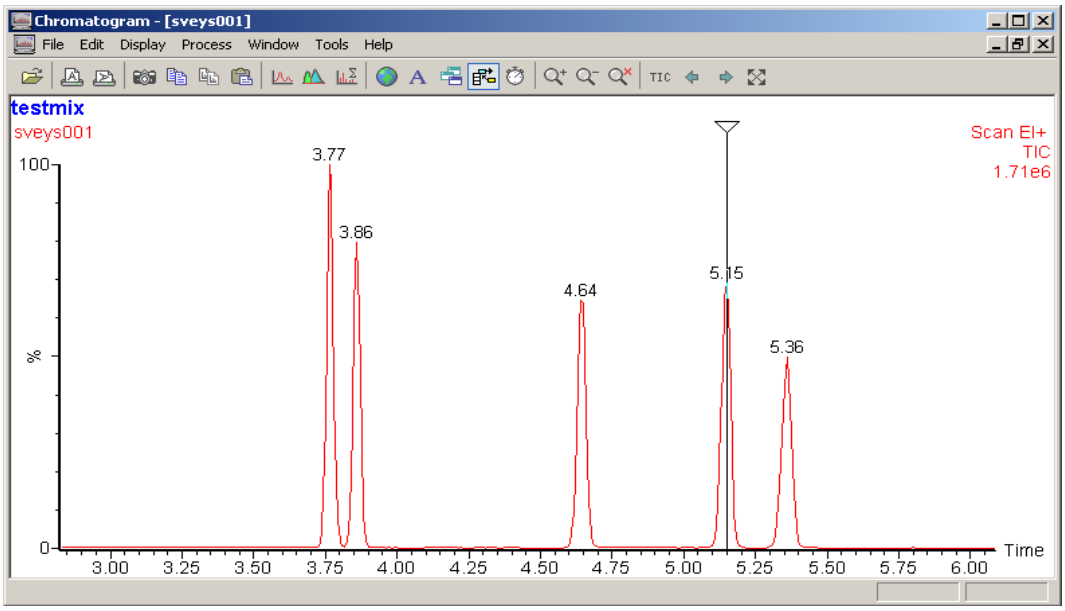


Select the file you wish to process, choose "Replace", and select "OK". Your data file will replace whatever was previously in the Chromatogram view.

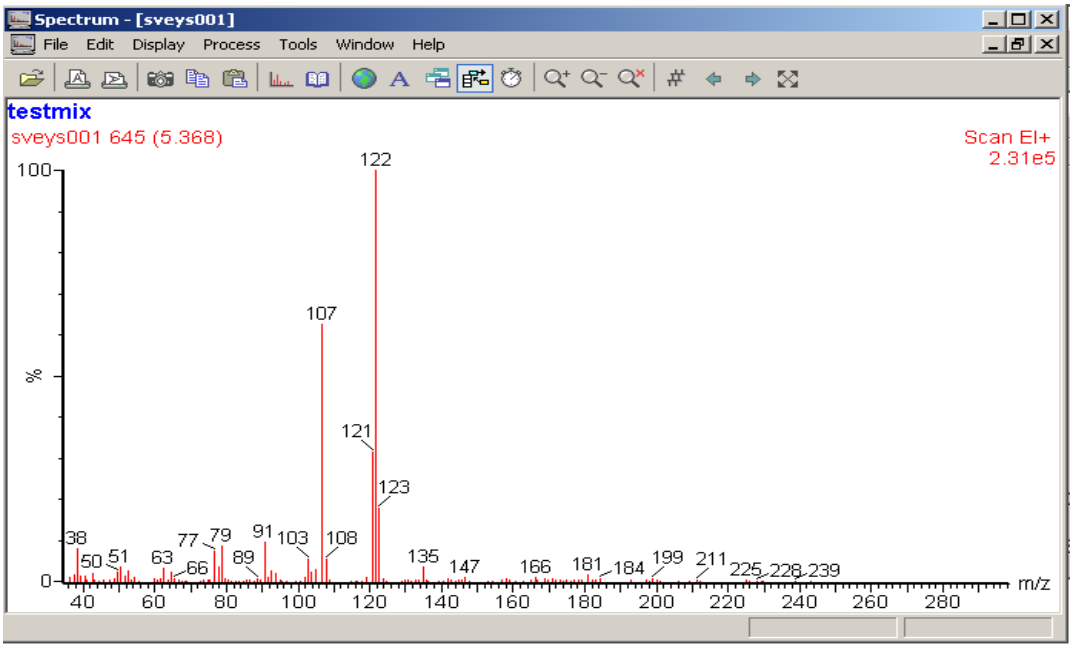


*NOTE: In this example, the filename is sveys001, but carries with it the description "testmix" as assigned on the "Information" tab during **Vx** acquisition.*

The “Spectrum” program can now be started by left-double-clicking anywhere within the Chromatogram window. Note that in Chromatogram, a vertical selection bar will now be present. You may now browse the chromatogram and view spectra simply by using the left mouse button (left-click-drag) to grab the top triangle of the bar and move it within the Chromatogram view.



The spectrum header shows both the scan number, and the retention time.



Chromatogram and Spectrum Views

Both Chromatogram and Spectrum provide access to other programs. Each program view and subprogram view can be modified through the use of the pull-down menus and/or the button bar located along the top of the view. Most of the actions and settings are easily reversible if you happen to choose an inappropriate setting; others can cause problems that are harder to recover from. You will need to spend some time investigating which features are important to you and which features are either useless or dangerous.

I I I I I I I_ paste clipboard contents onto the display
I I I I I I_ copy peak integration list to clipboard [use in Excel]
I I I I I_copy list of chromatogram data points to clipboard [use in Excel]
I I I I_copy snapshot of the view to the clipboard [use in Word]
I I I_print the view in landscape mode
I I_print the view in profile mode
I_open the file browser to select a data file

I I I I I I_ toggle between add/replace trace
I I I I I_ toggle add/replace processing window
I I I I_ annotate text into the view
I I I_ co-add spectra and subtract background
I I_ integrate the chromatogram peaks; show peak areas
I_ generate single ion (mass) chromatograms

Increase magnification range _____ I I I I I I I
Decrease magnification range_____ I I I I I I
Delete magnification range _____ I I I I I
Show just the Total Ion Current (GC) trace _____ I I I I
Decrement scan in spectrum display _____ I I I
Increment scan in spectrum display _____ I I
Reset display (normalize in X and Y) _____ __ I

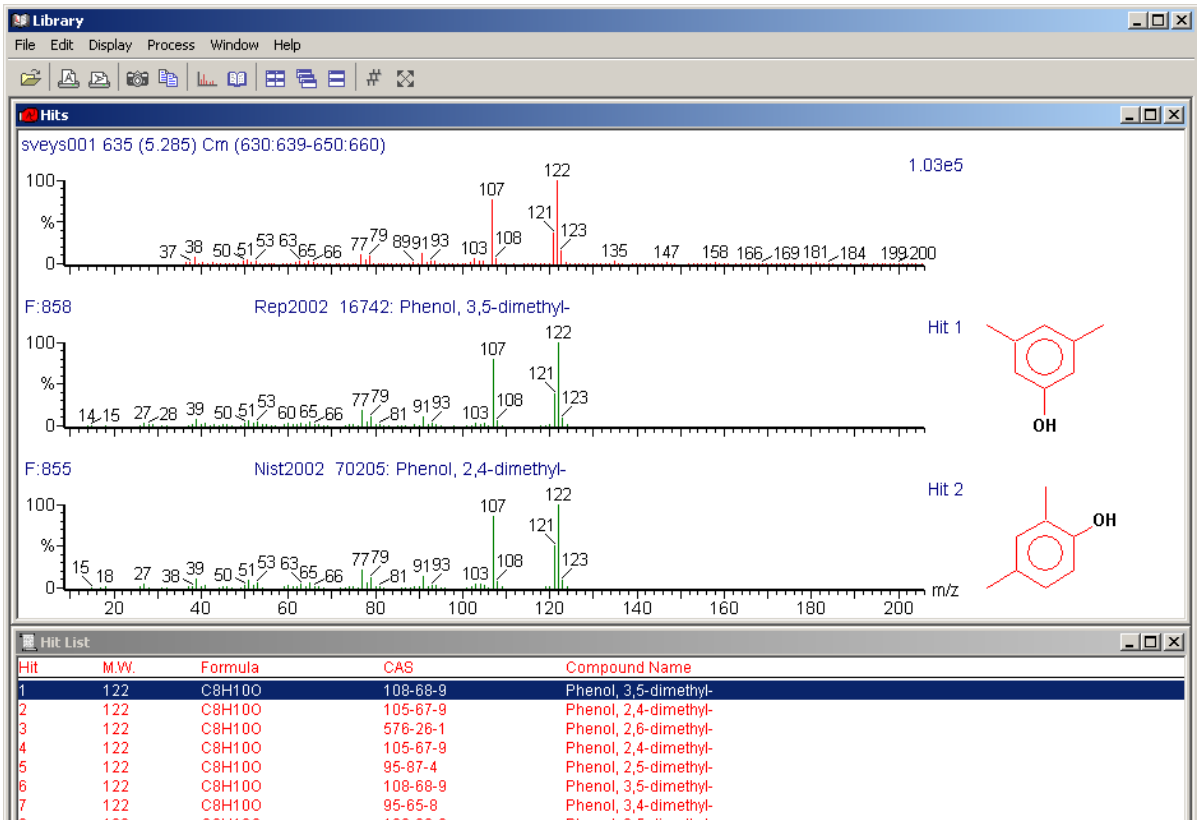
Many of the actions in Spectrum are the same as in Chromatogram. An additional program, Library Search, can be called from the Spectrum toolbar:

l__ Library search button

A “Molecular Weight / Isotope Pattern” calculator can be called from the Tools pull-down menu, but is not available from the toolbar.

Library Search

The spectrum displayed in the Spectrum view can be searched against two large computer libraries by clicking on the Search button, or by selecting “Tools -> Library Search”. For best results, be sure to average the appropriate scans in the GC peak, and subtract representative background scans. The Search / Match page can be configured many different ways. It is often useful to display the unknown spectra and the two or three best matches (including structures for those library entries containing them) and a list of the twenty best matches.



Typical Library Search Results view

"Chro, Spec and Libr" are the three processing programs that you will be using most often. However, MassLynx software contains many other features that may be helpful to you. Several of the most useful include:

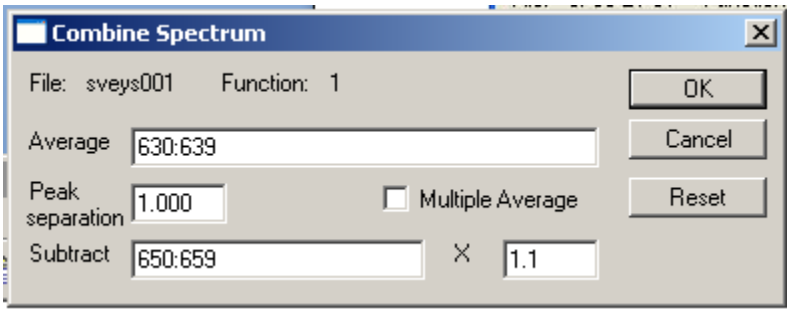
-Averaging Scans and Subtracting Background
-Mass Chromatograms
-Integration Reports and Quantitation
-Theoretical Isotopic Abundance Reports
-Elemental Composition Reports (useful with "accurate mass" data; NOT Magnum data)

Averaging scans and subtracting background

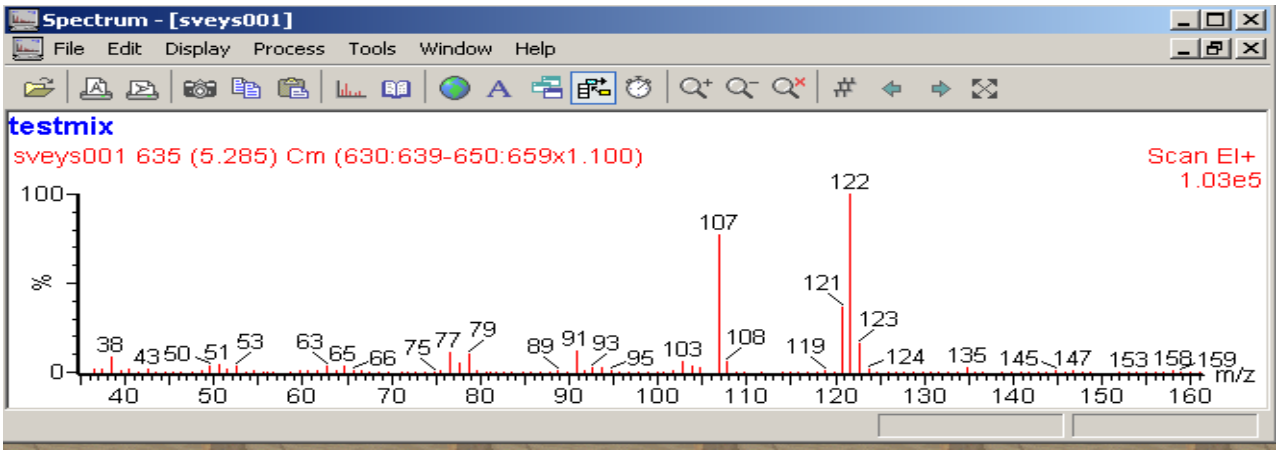
Averaging several scans and subtracting several background scans can improve the quality of your spectrum, and improve the performance of the Library search program. However, you must be aware of the potential problems and the actual limitations of spectrum averaging. Before averaging scans you must answer two questions. First: Is the GC peak "pure"? Am I averaging scans from the same component, or are some of the scans coming from a co-eluting GC peak? Proper use of the Mass Chromatogram tool will help you answer that question. Second: Is the signal level of the individual scans appropriate for averaging? If quantitation is important, be sure that none of the scans are saturating the detector.

There are two ways to average scans. The first method requires that you simply right-click and drag the mouse over the scans you wish to average. The second method involves use of the Combine Spectra icon. This program also allows you subtract a representative background.

Click on the Combine Spectra button.  The following dialog box will open:



Click in the "Average" field. Right-click and drag the mouse over the scans in the chromatogram you wish to co-add. Then click in the "Subtract" field. Right-click and drag over an equal number (or slightly larger number) of scans in the baseline that you would like to subtract. Notice that you can specify a degree of "over-subtraction" to be sure that all of the background ions are removed. A value of 1.1 is typical.



A typical background-subtracted spectrum

Notice that the spectrum will now show the averaging and subtraction information.

Mass chromatograms

This is certainly one of the most useful tools at your disposal. It can be used:

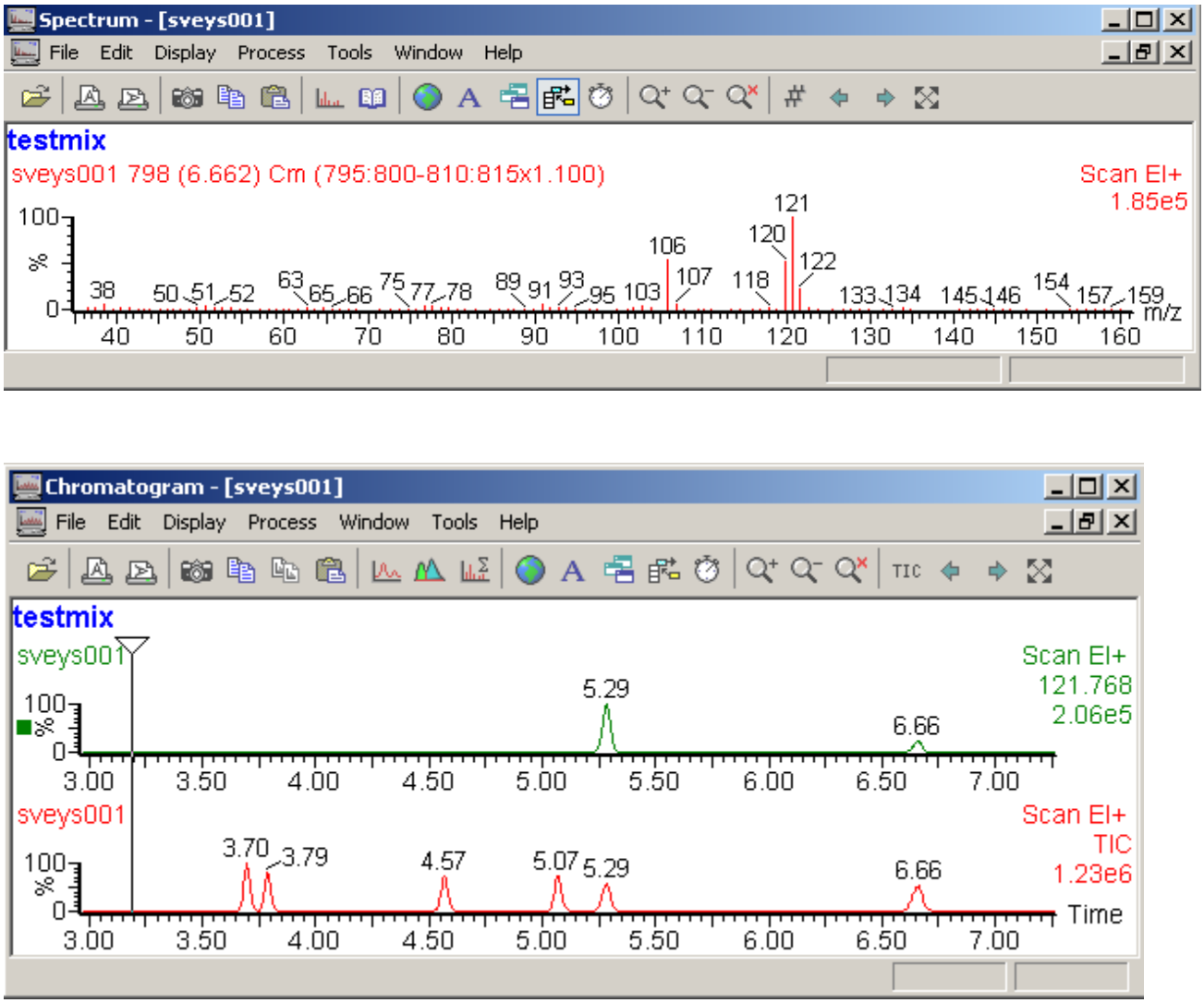
a) To find the "needle in the haystack" by searching for a specific mass that you expect to appear in one or more GC peaks.

b) To find all related compounds in a complex mixture by looking for fragmentation ions known to be representative of a class of compounds.

c) To determine whether a GC peak is pure or is a mixture of more than one component.

d) To determine whether a specific mass in a spectrum is from the sample, from background, or from the tail of a closely eluting peak.

The program can be initiated in three ways. The easiest method is to simply double-click on the mass of interest in the spectrum display. In this example, we are creating a single ion chromatogram for m/z 122. Note in the results below that m/z 122 is a significant ion in the GC peaks at retention time 5.29 minutes and 6.66 minutes. Inspection of representative spectra leads to the conclusion that m/z 122 is probably the molecular ion for the compound eluting at 5.29 minutes, but is probably the C13 isotope peak (with a contribution from MH+) for the compound eluting at 6.66 minutes.



Typical mass chromatogram display

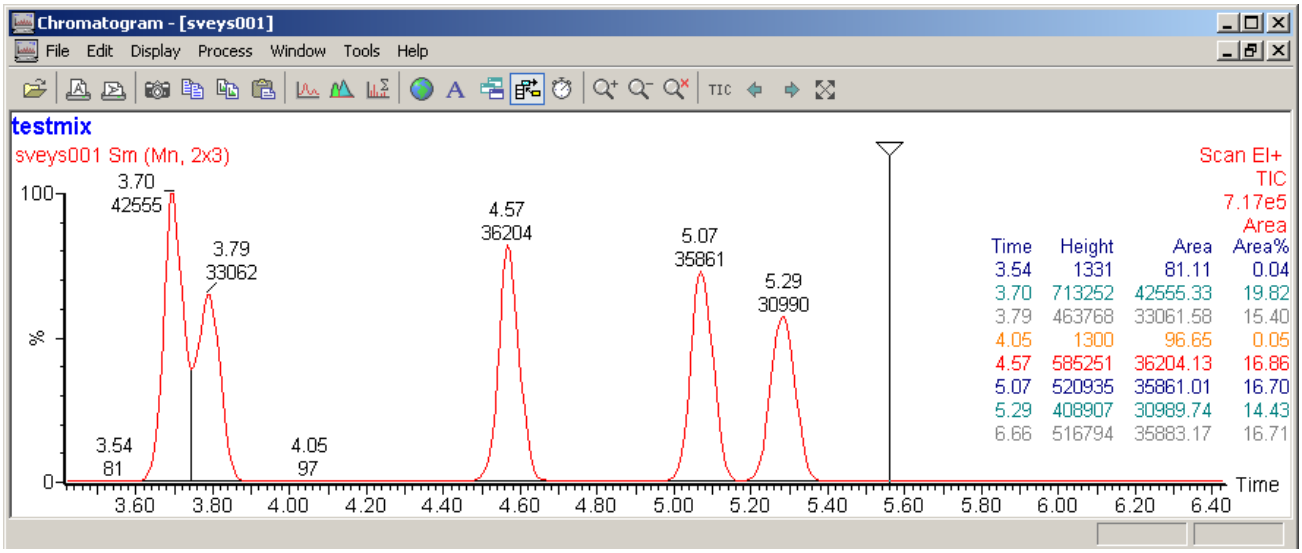
Depending upon the toggle state of the Add/Replace button  in the Chromatogram view, a new window is opened to show the mass chromatogram of the mass selected, or the contents of the existing window are replaced.

The second and third ways to specify a mass chromatogram are by using the  button on the toolbar, or by selecting “Mass…” from the Display pull-down menu. Simply type in the mass of the ion you would like displayed. In this example the tolerance for the single ion display is a window of 0.5 Daltons (+/- 0.25 Daltons). This is appropriate for the precision of the “nominal mass” data acquired by the Magnum.

If you are trying to find a particular GC peak in a complex mixture, you would normally calculate the molecular weight of the component (Spectrum view -> Tools -> Isotope Model…) and then enter that value in the Mass Chromatogram dialog box.

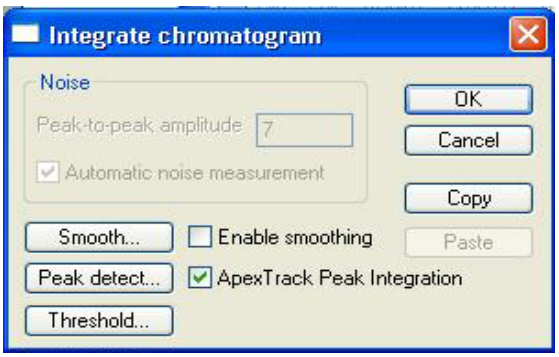
Integration reports and Quantitation

Mass Lynx includes a complete quantitation package. Quantitation is an advanced topic and will not be discussed here. However, a simple integration report can be easily generated just by using the "Chromatogram Peak Integration" button  . The integration report is printed over the chromatogram. Depending upon display settings, the chromatogram peaks are also annotated with retention times and areas.



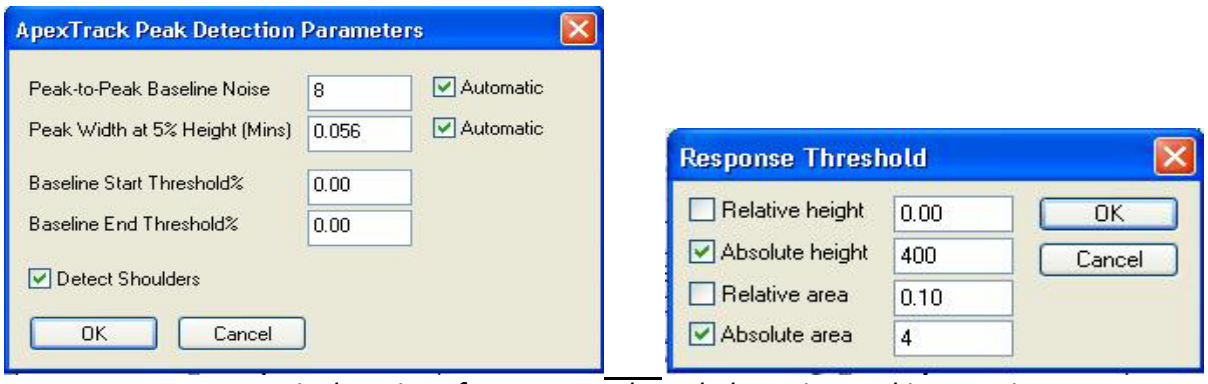
Typical chromatogram display after performing peak integration

The integration report can also be sent to the Windows clipboard by using the "Detected Peaks" button  . The report can then be pasted into Excel or Word. When using the "Peak Integration" button, be aware that the integration is performed using parameters set from the pull-down menu (Chromatogram view -> Process -> Integrate…) and these parameters may need to be modified to meet your needs. Users have full control over the peak detection and integration algorithm. Probably too much control!



A reasonable approach is to start with Apex Track Peak Integration enabled, and Smoothing disabled. This allows the software to make most of the hard decisions for you. However, you should still make sure that reasonable values are set in Peak Detect… and in Threshold…

Normally choose Apex Track Peak Integration

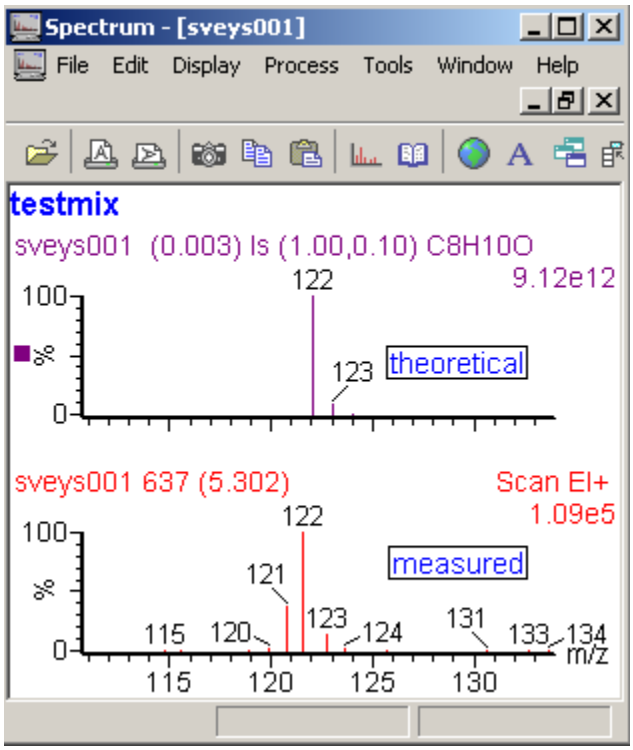


Typical settings for Apex Track peak detection and integration

Theoretical Isotopic Abundance Reports

Occasionally it is helpful to compare the measured isotopic distribution to the theoretical distribution. This is especially helpful if you suspect your compound contains hetero-atoms like silicon, sulfur, bromine or chlorine. Be aware that attention to sample concentration and careful selection of scans to co-add is necessary because of the propensity for MH+ formation in ion-trap mass spectrometers.

Access: MassLynx -> Spectrum view -> Tools -> Isotope Model



Note: This program also calculates the accurate masses in addition to the theoretical isotope distributions. This can be used as input to the Mass Chromatogram program.

Use "Display -> List Spectrum" to show a list of the masses and intensities.

XI. Archiving and Exporting Data

Adobe Acrobat Writer is loaded on this PC. Therefore, any "view" which can be printed can be saved as a PDF file. Simply use the "File -> Print" command to select Acrobat Writer as the "printer".

The "Snapshot" button available in Chromatogram, Spectra, and Library can be used to screen capture the active window and store it on the Windows clipboard. It can then be pasted into a Word document and saved.

More sophisticated screen capture software called "Snag-It" is loaded on this PC. See me if you would like to use this software.

The actual chromatogram or spectra data points can be sent to the clipboard and then pasted into an Excel spreadsheet. Similarly, the results of the Peak Integration program can be sent to the clipboard and then pasted into an Excel spreadsheet.

There is additional software in the Mass Lynx folder (Start -> Programs -> Mass Lynx -> Data Bridge) that can be used to convert your Magnum files (previously converted to Mass Lynx format) into ASCII format.

The data files acquired by **Vx** are stored in your personal directory (D:\MData\[Username]\) in netCDF format. These files are also present in the communal Mass Lynx data directory (D:\MLdata\) in Mass Lynx format.

All data files on this PC are automatically archived each day to our network storage device.

You may archive your own data to a USB memory stick or to a CD / DVD.

XII. In Conclusion

We have attempted to present the fundamentals of both acquisition software (Vx) and the processing software (Mass Lynx) in enough detail to allow you to immediately begin analyzing your GC mixtures. We have presumed that you have a sound knowledge of the principles of gas chromatography and mass spectrometry. If you are weak in either of those areas, you will need to educate yourself before you will be able to use this instrument safely and effectively.