

**Waters GCT GCMS Training Manual**  
**(Accurate Mass Version)**  
*02/06/2012 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.



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## **I. Prerequisites for Training**

Some or all of the following prerequisites are required, depending upon the level of GCT training you are requesting.

- 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.
- C. Proficiency using another GCMS system, for example the Magnum GCMS.

	<b>Training Level</b>	<b>A.</b>	<b>B.</b>	<b>C.</b>	<b>Other</b>
1.	Process GCT data	Required	Required		
2.	Acquire Nominal Mass Data	Required	Required	Required	
3.	Acquire Accurate Mass Data	Required	Required	Required	GCT Experience
4.	Acquire Chemical Ionization Data	Required	Required	Required	GCT Experience

Table 1. Prerequisites vs Training Requested

The final determination of the level of training offered to you will be made by CIF staff.



## **II. Overview of the GCT Instrument and Software.**

The GCT is an accurate mass time-of-flight (TOF) mass spectrometer coupled to an Agilent 6890 gas chromatograph equipped with an autosampler. Both *electron ionization* (EI) and *chemical ionization* (CI) sources are available. EI is the normal operating mode. The mass spectrometer always acquires data with accurate mass *precision*, but only acquires with accurate mass *accuracy* under certain instrumental conditions. By convention, you should have the software label mass peaks to one decimal place of precision unless the measurement was performed under mass accuracy conditions, in which case you should have the software label the peaks to three or four decimal places of precision.

The software is called **MassLynx**, and includes all of the programs required for acquiring data and for processing data, including two large mass spectral libraries used for search-match comparisons. In addition to the library search program, the software also facilitates compound identification by determination of the elemental composition (accurate mass measurements only), and theoretical isotope distribution comparisons. Complete quantitation software is also included.

## **III. Software Organization**

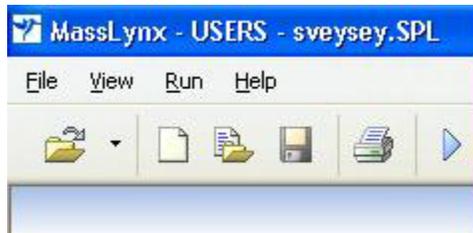
Achieving the proper software operational state is complicated. Therefore the MassLynx software package must already be running when you decide to use the GCT. This means that we cannot monitor use through individualized login accounts. You must manually record your use in the logbook.

Properly operating MassLynx software will show at least two programs running, although one or more may appear to be minimized on your desktop. The critical programs are **MassLynx** and **GCT**. The **GCT** program is used for direct control of the mass spectrometer including instrument tuning, calibration and manual injection data acquisition. Most of the functions of the GCT page should not be used by the student. Great care needs to be exercised or you could cause serious damage to the instrument. The **MassLynx** program is used to acquire data using the GC autosampler and for all aspects of data processing, including processing the data acquired using the **GCT** program.

The software is structured to allow multiple users working on different projects to maintain completely separate instrument control, acquisition, and processing parameter sets. While some aspects of this versatile structure are useful, some aspects can lead to confusion and increase the potential for instrument malfunction or damage. Therefore you must carefully follow the structured approach discussed below. Be aware that in a multi-user environment you will often find that the instrument is not configured as you require, and your first step must always be to load in the parameter sets and configuration files assigned to you. Unfortunately, there is no protection against modifying parameter sets belonging to someone else. While this is just a nuisance on the Magnum GCMS, it can have serious consequences when using the GCT. Zero tolerance will be allowed for mistakes of this nature.

**Projects** – This is the highest level. All users must use the project labeled USERS unless you receive special permission and staff assistance to create a new project. To load the project file,

first check whether the correct project is already loaded.



At the upper left corner of the MassLynx window the active project is identified. In this example it is “USERS”.

If the USERS project is not active, load the project:

*MassLynx -> File -> Open Project -> [yes] -> USERS.PRO*

**Sample Lists** – Load your sample list. You may have as many sample lists as you like, but they must all be named with your username and an incremental number if you have more than one, for example: “johndoe01.SPL”. Since I only use one sample list and edit it continually, my sample list in the example above is just “sveysey.SPL”. To open a sample list:

*MassLynx -> File -> Open...-> [select sample list] -> Open*

Queue Is Empty							
Spectrum Chromatogram Map Edit ▾ Samples ▾							
	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume	Control
1	sv-03-08-01	reference compound	ms1	GC1	1	1.000	
2	sv-03-08-02	sample 1	ms1	GC1A	2	1.000	
3	sv-03-08-03	sample 2	ms2	GC1A	3	1.000	
4	sv-03-08-04	sample 1 repeated	ms1	GC1B	2	1.000	
5	sv-03-08-05	sample 2 repeated	ms2	GC1B	3	1.000	
6	sv-03-08-06	reference compound repeated	ms1	GC1	1	1.000	

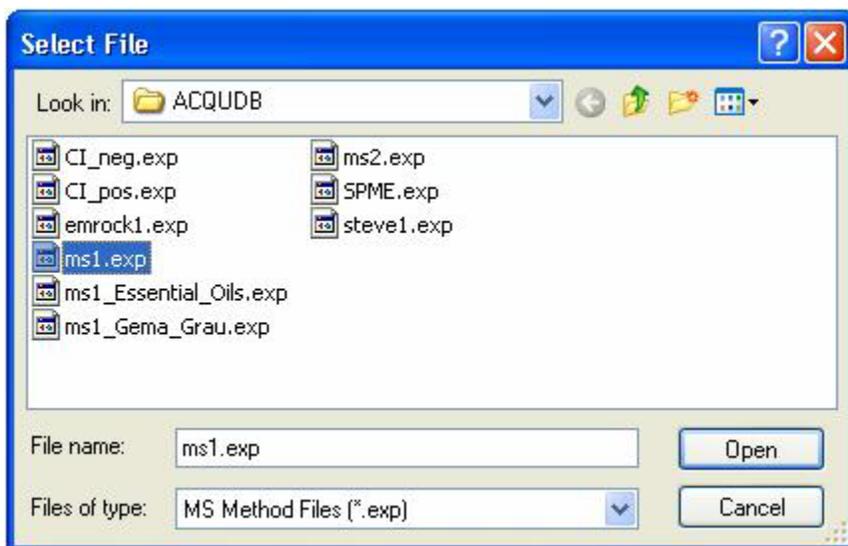
*A Typical sample list*

Notice that a “sample list” contains all of the information necessary for the GCT to automatically acquire data files for samples loaded in the GC autosampler. This includes file names, text describing the sample, pointers to the MS and Inlet (GC) parameter sets that should be used, sample locations in the autosampler, and the amount of each sample that should be injected.

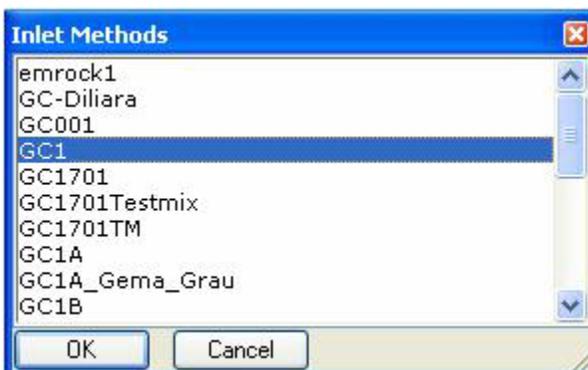


Use the Samples pulldown menu to add or delete lines in the Sample List.

**Method Files (GC and MS)** – Different mass spec parameter sets and GC parameter sets can be selected for each sample in your sample list. The parameter sets must already exist. To assign a GC or MS parameter set, double-click in the appropriate field in the table.



Double-clicking a field in the “**MS File**” column allows you to select the appropriate mass spec parameter set for each sample in the sample list.



Double-clicking a field in the “**Inlet File**” column allows you to select the appropriate GC parameter set for each sample in the sample list.

**Instrument Parameters** – These parameters are set from the **GCT** software window. There are only a few of them that you should customize for your measurement. Therefore all users **MUST** use the “users” instrument parameter set. During your training, you will be shown how to modify several of the parameters as necessary. The procedures are described later in this document. To load the instrument parameter set, first check whether the correct instrument parameter set is already active.

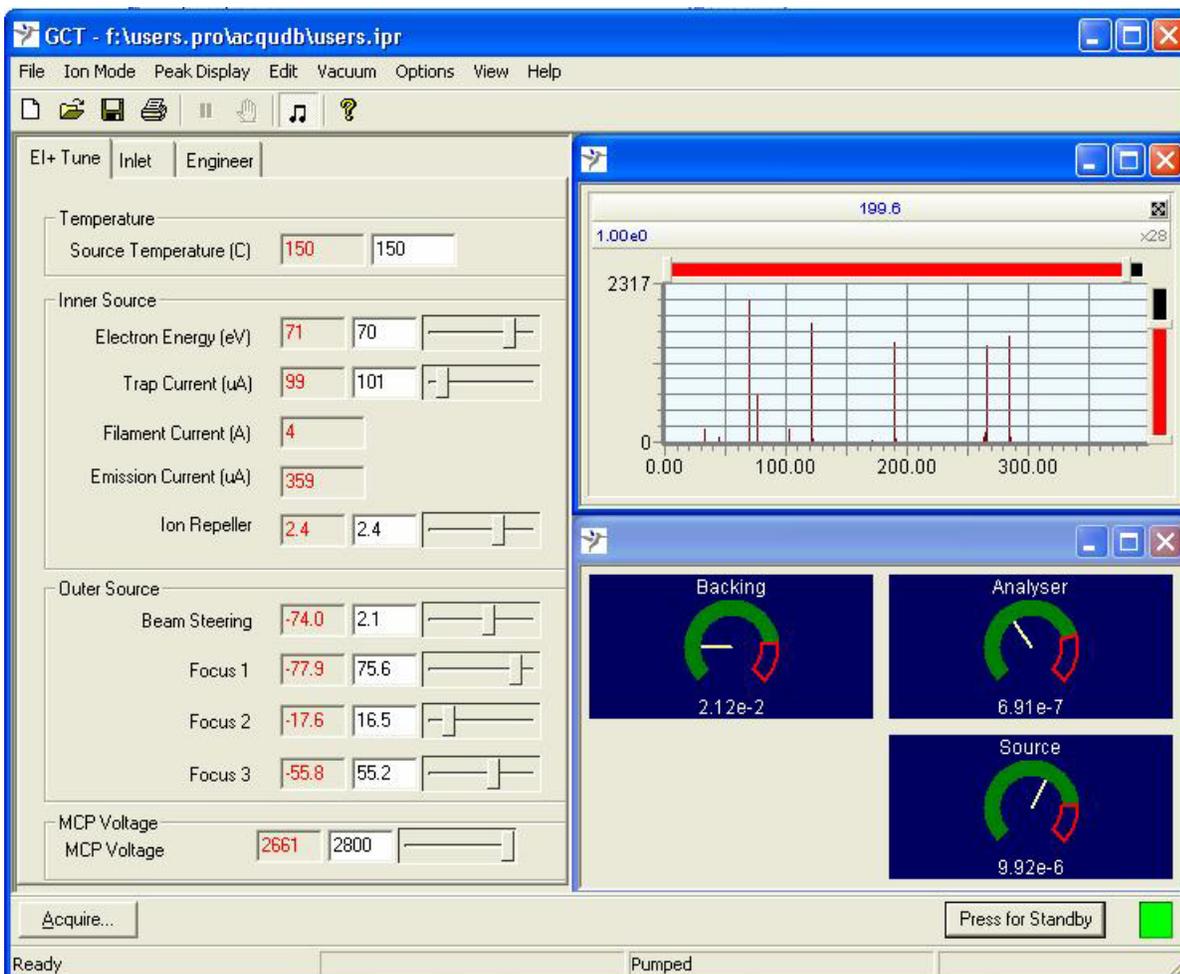


In this picture, “users.ipr” is the correct GCT instrument parameter set.

If “users.ipr” is not active, load the parameter set:

GCT -> File -> Open -> [users.ipr]

#### **IV. Tuning and Calibrating the GCT: Acquiring Data Manually**



*Typical GCT program display with filament ON and calibration compound present*

The GCT page contains real-time displays of the vacuum readings and a real-time mass spectral display window. The page also contains all of the mass spec control and tuning parameters organized on three tabs and in several pull-down menus:

**“EI+ Tune”** tab – The most common tuning parameters are on this tab, along with a display of the filament emission and trap current.

**“Inlet”** tab – This tab does NOT refer to the GC inlet parameters. There is nothing on this tab that you will need to adjust.

**“Engineer”** tab – Some of the less common tuning parameters are contained on this tab. Normally I would hide this tab from you, but there is one function that you will use on this tab.

**“Options”** menu – You will need to access the “TDC Settings...” feature from this pull-down menu in order to change between accurate mass precision acquisition (the nominal mass accuracy mode), and accurate mass accuracy acquisition. The Options menu is also used as part of the instrument calibration procedure.

Typical vacuum readings will be:

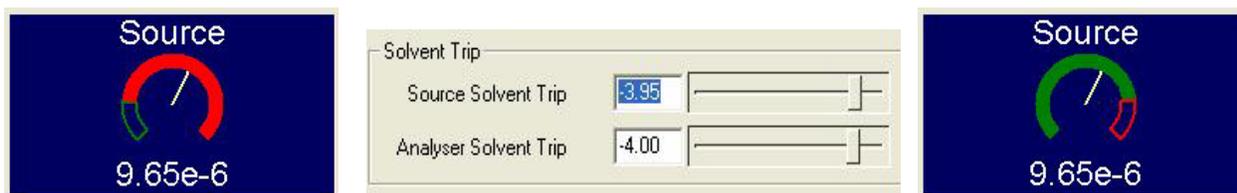
Backing: approximately 2 e-2 (+/- 1)  
Analyzer: approximately 7 e-7 (+/- 2)  
Source: approximately 9 e-6 (+/-2)

If any of the readings differ from these values by more than the tolerances indicated above, then contact staff immediately. DO NOT CONTINUE.

The status light located in the lower right corner of the window next to the “standby” button must be **green**. If it is **red**, contact staff immediately. DO NOT CONTINUE.

### Step 1. Turning on the Mass Spec

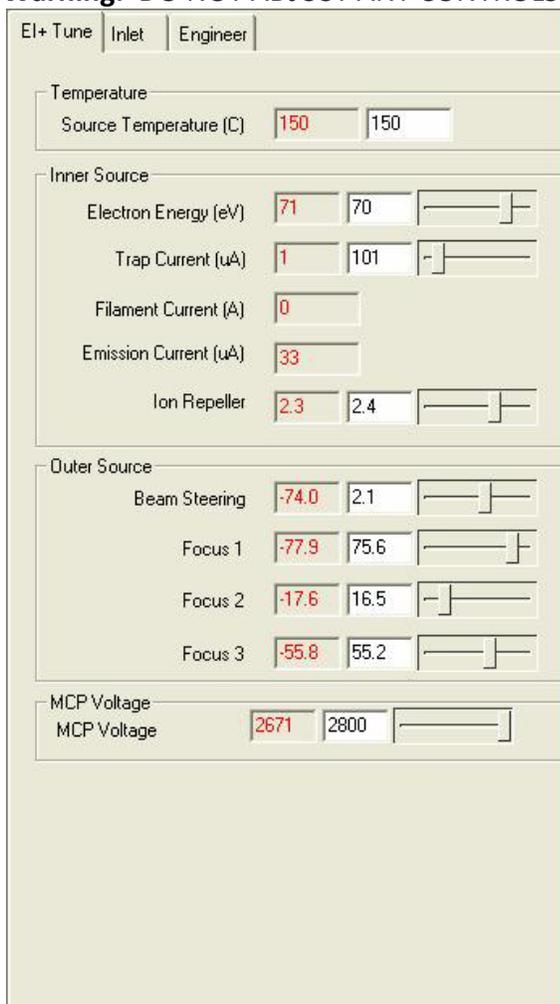
The visual display of the Source Trip Setting should initially be red, indicating that the filament is not turned on. Normally there will be no real-time peaks in the mass spectral display window if the Source Trip Setting is red. The filament and other ion source electronics are activated from the **Engineer’s Tab** by dragging the “Source Solvent Trip” slider bar to the right.



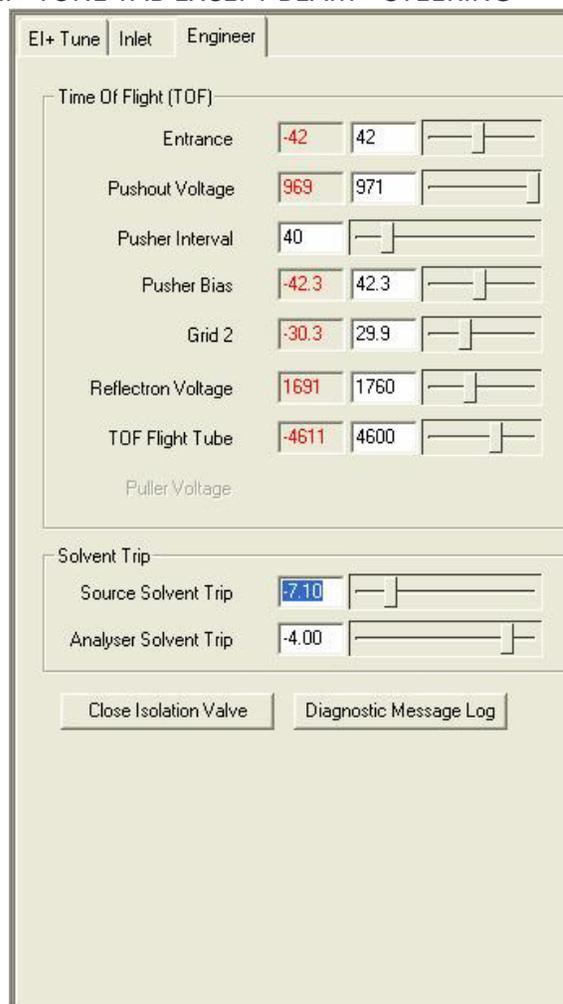
The Source Solvent Trip should be set to about -4 for filament ON operation, and about -7 for filament OFF operation.

**Warning:** DO NOT ADJUST ANY OF THE OTHER CONTROLS ON THE ENGINEER'S TAB.

**Warning:** DO NOT ADJUST ANY CONTROLS ON THE EI+ TUNE TAB EXCEPT BEAM STEERING



*Typical EI+ Tune Tab display*



*Typical Engineer's Tab display*

Typical values are shown for all of the tuning parameters controlled from the EI+ Tune Tab and the Engineer's Tune Tab. The actual values you see may be slightly different. Only CIF staff are authorized to change the tuning parameters with the exception of the Beam Steering parameter, which is user adjustable.

## Step 2. Verifying Mass Spec Functionality

When the filament is turned on, the ion source temperature will change slightly. Allow the source and electronics to stabilize for at least ten minutes. While you are waiting, this is usually a good

time to manually increase the GC oven temperature to about 280 Degrees to bake out the column for a few minutes. At the Agilent 6890 Keypad:

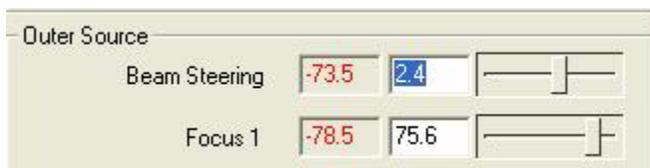
*Oven -> Temp -> 280 -> Enter*

Also while waiting, now is a good time to introduce the tuning and calibration compound, METRI. On the GCT, close the black valve labeled “reference pump out”. Using the 10 uL METRI syringe, draw up about 0.5 uL of METRI from the labeled vial, and inject into the reference inlet septum. The needle should insert completely into the septum. If you encounter resistance part way, withdraw the needle and try again. If the needle still fails to insert completely, contact staff. DO NOT CONTINUE.

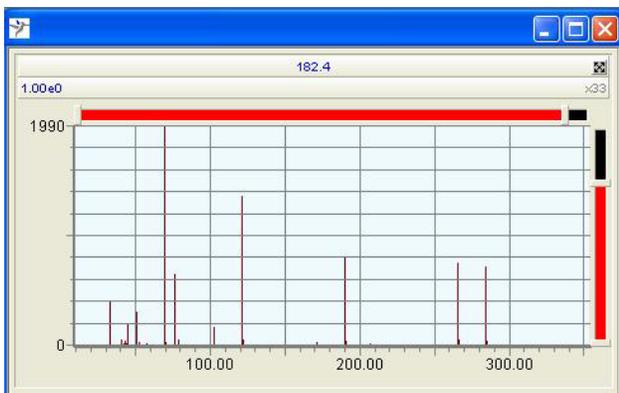


You should immediately see the METRI ions in the real-time display window. If not, check that the display is activated by toggling the “non-storage acquisition” button . Use the **Peak Display** red slider controls to display the mass range from 0 to 300 Daltons, with the Y-axis full-scale set to about 2000. While monitoring the spectrum, carefully open the “reference pump out” valve until all of the METRI ions are on scale.

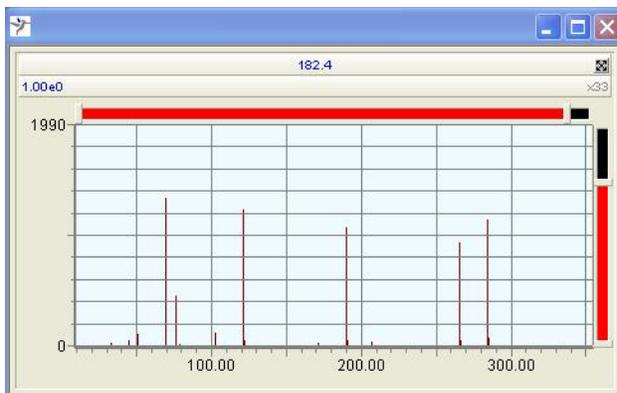
Activate the Beam Steering control by clicking on the slider. The target value will now be shown in blue. Adjust beam-steering until m/z 69 is about 10% larger than the other four major ions, which should all be approximately equal in height.



*Drag the slider to adjust the beam-steering value, or use the keyboard arrow keys for finer control.*

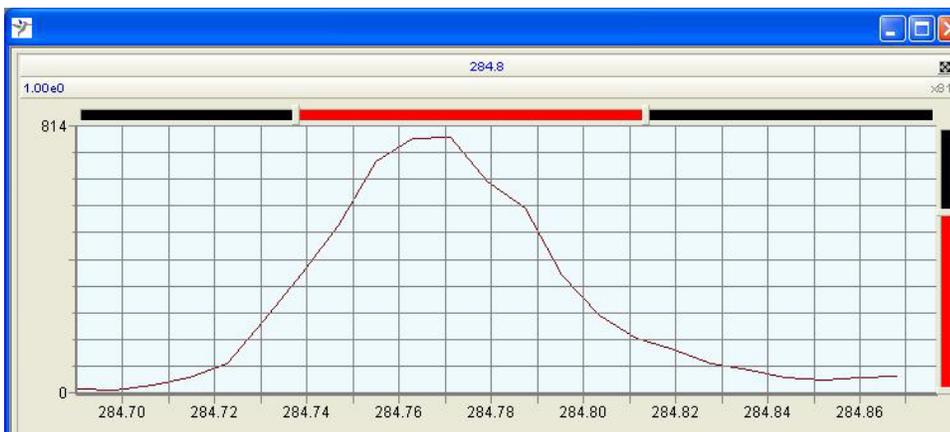


*METRI spectrum before BS adjustment*



*METRI spectrum after BS adjustment*

If you wish, you can now verify the mass spectrometer resolution. Use the red horizontal slider until only  $m/z$  285 is displayed (or left-click and drag the mouse across the  $m/z$  285 ion). Repeat the expansion until  $m/z$  285 occupies the entire display. Adjust the red vertical slider until the peak height occupies the entire display. Use the grid line scale to estimate the peak-width at half-height. Calculate the resolution by dividing 285 by the peak width. Resolution values are typically greater than 6000. If the resolution is less than 5000, contact staff. DO NOT CONTINUE.



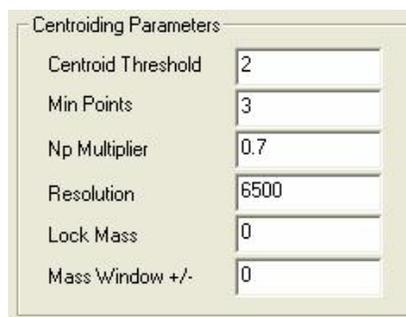
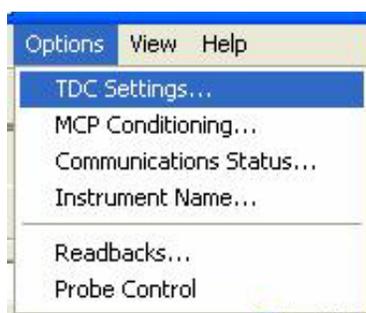
*In this example, resolution =  $285/.053 = 5400$ .*

### Step 3. Verifying Mass Accuracy

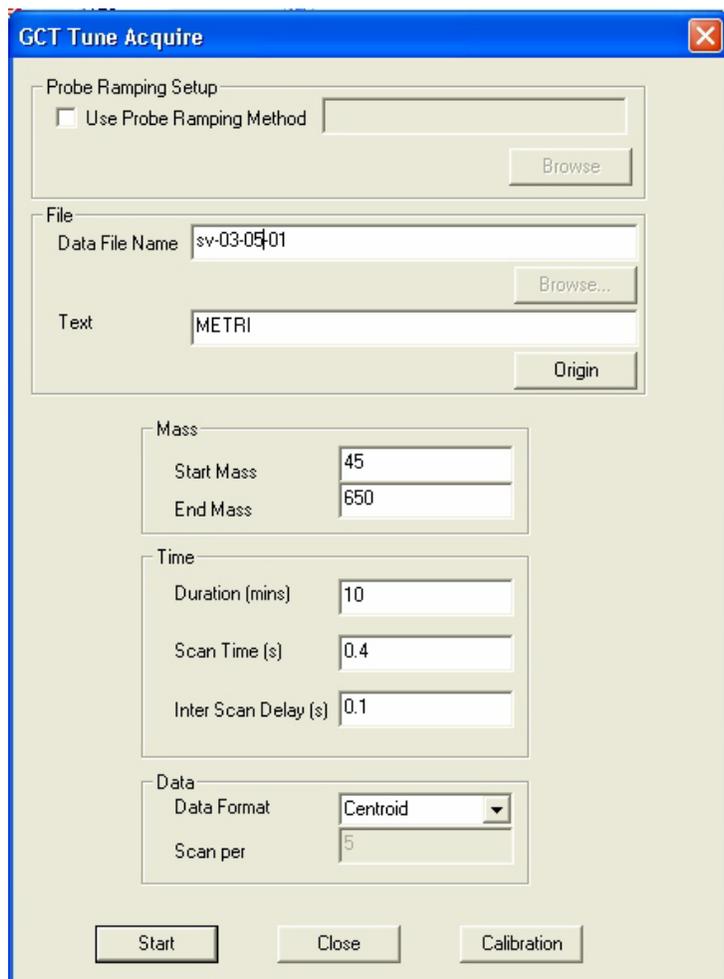
By now, ten or fifteen minutes have passed. Manually cool the GC oven back down to a reasonable “standby” temperature:

*Oven -> Temp -> 100 -> Enter*

At the GCT page, use the “Options” pull-down and select “TDC Settings...”. Verify that the **Resolution** setting is 6500; or you may choose to set it to the value you (optionally) measured. For all acquisitions except *accurate mass accuracy* acquisitions, the **Lock Mass** and **Mass Window** parameters must be set to zero. (Accurate mass accuracy acquisitions will be discussed later.) Click the OK button to exit from “TDC Settings”.



Next, open the **GCT Tune Acquire** page by clicking on the Acquire button located in the lower left corner of the **GCT** program display. 



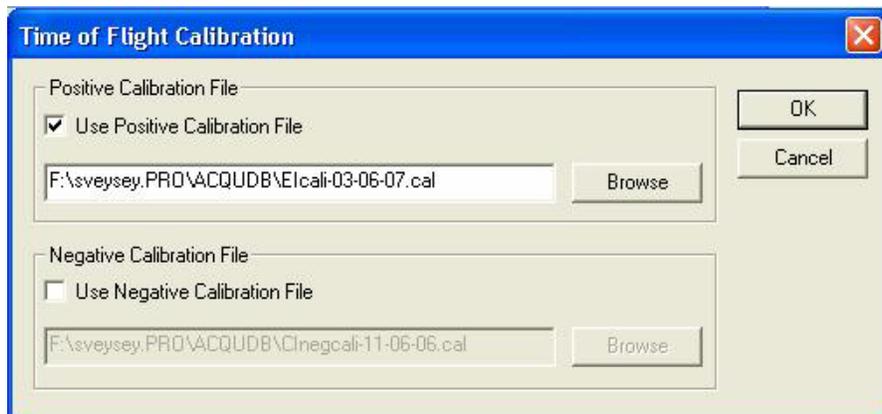
Note the file-naming convention.

The mass spectrometer is normally scanned from  $m/z$  45 to  $m/z$  650 for METRI acquisitions. It is only necessary to acquire a few minutes worth of data to verify instrument performance. We typically acquire two scans per second.

The data format should be "Centroid" unless you have a particular reason for needing to acquire "Profile" data.

*Typical GCT Tune Acquire page settings for METRI*

Before starting the acquisition, you should first open the Time-of-Flight Calibration page by clicking on the **Calibration** button.



Unless you are acquiring raw data to create a new calibration file (discussed later), be sure that “Use Positive Calibration File” is checked, and that the correct calibration file has been selected. Use the **Browse** button if necessary.

*Typical Calibration dialog box*

To begin the acquisition, press the **Start** button on the GCT Tune Acquire page. To end the acquisition before the specified time (10 minutes in the example above), use the Stop button

located on the **GCT** toolbar: 

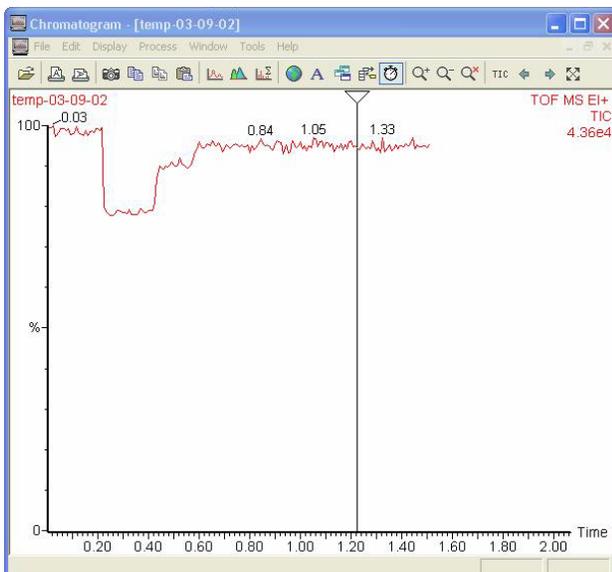
**Note:** THE FOLLOWING FILE-NAMING CONVENTIONS MUST BE USED

For temporary files that can be overwritten or deleted, use the format: **temp-mm-dd-nn**, where “mm” is the month, “dd” is the day, and “nn” is a two-digit incremental sample number. For example, **temp-03-06-01** would be an appropriate file name to use for a quick METRI acquisition occurring on March 6 to verify mass spectrometer performance and the accuracy of the current calibration file.

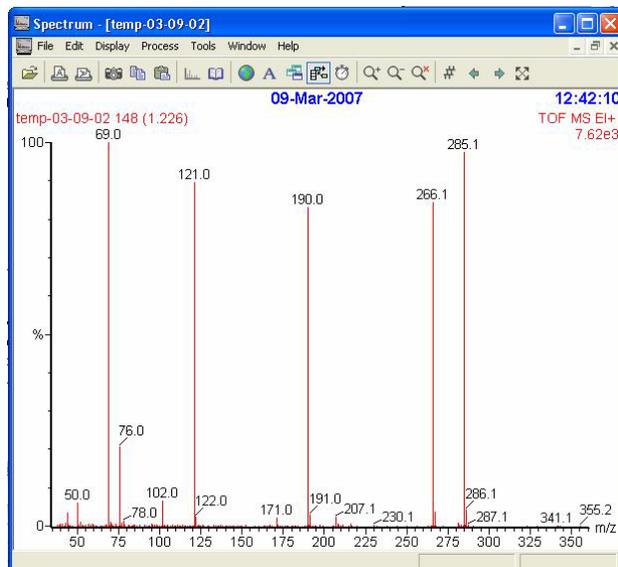
For data files acquired specifically for use as input to the calibration program, use the format: **cali-mm-dd-nn**. For example, **cali-03-06-01**.

For sample data files, use the format: **ii-mm-dd-nn**, where “ii” is typically the initials of your first and last name. For example, **sv-03-06-02** would be the second sample-related data file acquired on March 6. Note that the year is not coded in the file name. At the end of each calendar year the files are archived by year in a different directory on this computer.

The MassLynx **Chromatogram** and **Spectrum** programs are normally used to view the data as it is being acquired. A more detailed discussion of how to use these programs is given later in this document. It is only necessary to acquire a few scans in order to check instrument performance and calibration accuracy.



Typical "chromatogram" display of METRI



Typical spectrum of METRI

Note that the METRI signal level in the Chromatogram display shows variation. This occurs when the **Beam Steering** parameter on the **GCT EI Tune+** page is adjusted on the fly to set the correct relative intensities of the METRI peaks as observed in the Spectrum display. It is normal to re-adjust Beam Steering in this manner. For nominal mass work, the five METRI peaks should have the following mass assignments: 69.0, 121.0, 190.0, 266.0, and 285.0. All assignments should be accurate within a tolerance of +/- 0.1 Daltons. NOTE: *If any of the assignments are outside this tolerance window, or if you plan to acquire accurate mass accuracy data, then you must create a new calibration file.*

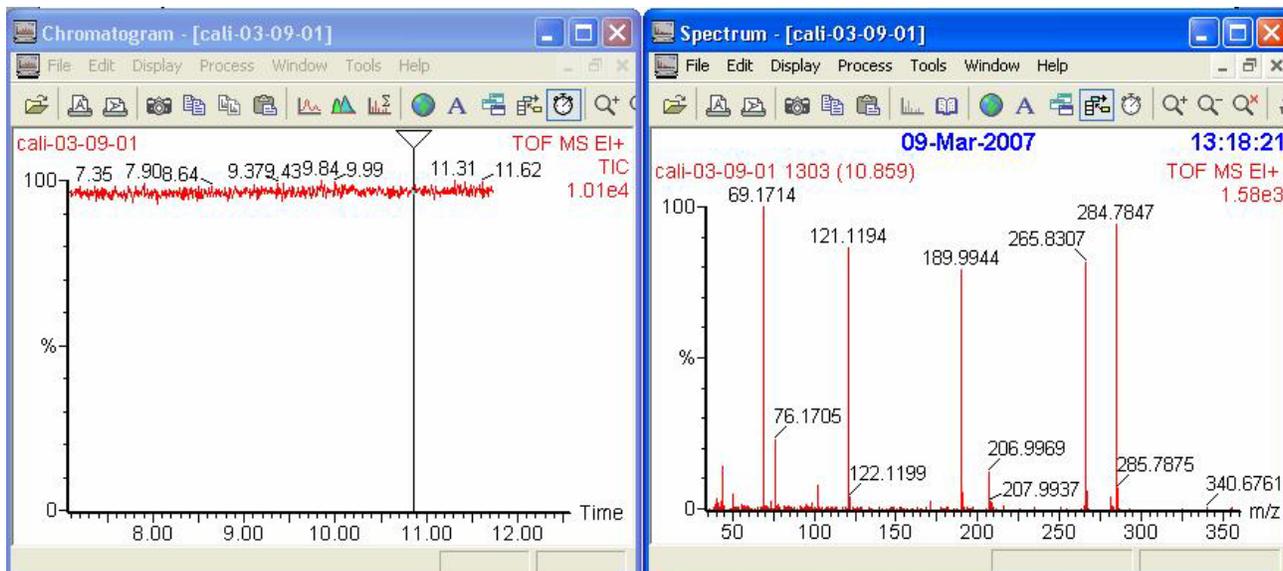
If the assignments are acceptable, then you are ready to begin acquiring sample data files through the **MassLynx** program. **You may ignore Step 4.** Instead, open the "reference pump out" valve located on the GCT. Reference compound should not be present when acquiring sample data with nominal mass accuracy. Repeat: *Ignore Step 4.*

#### Step 4. Recalibrating the Mass Spectrometer

This step only needs to be performed if the mass accuracy on the METRI peaks is not within the +/- 0.1 tolerance window necessary for acquiring nominal mass accuracy data OR if you wish to acquire data with accurate mass accuracy.

Verify on the **GCT -> Options -> TDC Settings** page that the **Lock Mass** and **Mass Window** parameters are set to zero. On the **GCT Tune Acquire** page, set the file name using the proper naming convention for a calibration data file, for example "cali-06-09-01". On the Time-of-Flight Calibration page (accessed from the GCT Tune Acquire page), uncheck both the "Use Positive Calibration File", and "Use Negative Calibration File" boxes. Click the Start button to begin collecting data.

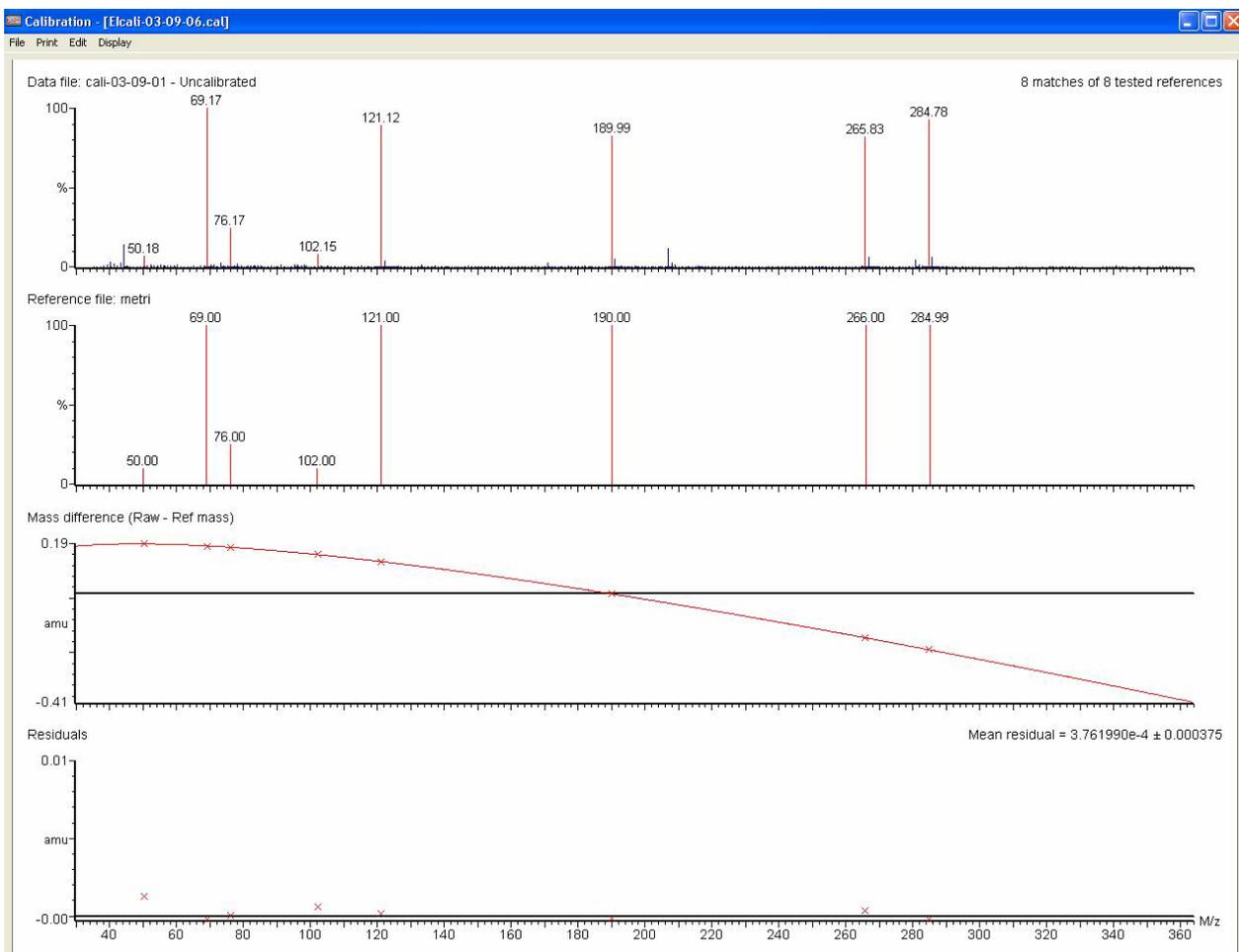
Open the **MassLynx** Chromatogram and Spectrum windows. Using the reference pump out valve, adjust the METRI level so that the peaks in the Spectrum display are in the range of  $1e3$  to  $2e3$ . This will correspond to a Chromatogram display reading of approximately  $1e4$ . Collect at least one minute of data at the proper signal level.



**NOTE:** The mass accuracy will initially be well outside the normal tolerance because Cali data files are acquired without a calibration file correction. Also note that a "Spectrum -> Display" parameter has been modified to display the masses with four decimal places of precision.

To form a new calibration file, perform the following:

- 1) In the Chromatogram window, right-click-drag the mouse to average about a minute of data.
- 2) In the Spectrum window, pull down the Tools menu and select Make Calibration.
- 3) In the "Make new calibration" pop-up window, scroll down and select "metri.ref" as the theoretical reference file. Click OK.
- 4) A full screen Calibration window will open displaying a comparison of the uncalibrated data file and the "metri.ref" reference file. Plots of "Mass difference" and "Residuals" will also be displayed. Focus on the Residuals plot. The residual for each of the reference masses (indicated by X's) should be less than  $\pm 0.001$  amu. (It is acceptable if one or two of the residuals are larger, as long as they are less than  $\pm 0.002$  amu). The "mean residual" shown in the lower right corner of the plot should be less than  $5e-4$ .



*Typical plot. All residuals are less than 0.001 amu except for one. Mean residual is 3.7e-4.*

- 5) If the calibration is acceptable, use the Print menu to print a copy. Put it in the Tuning and Calibration Plot binder.
- 6) Use the *File -> Save As* menu to save the new calibration file. Use the proper naming convention: Elcali-mm-dd-yy. For example, **Elcali-03-09-07**. Note that we do include a year code when naming calibration files.
- 7) File -> Exit. You will be asked a confusing question: "Accept this Calibration?" Actually the new calibration file has already been saved. Answering "yes" to this question would then apply the calibration to every scan in the raw (uncalibrated) data file you just acquired. This effectively prevents you from trying to create a better calibration file, perhaps by using scans over a different intensity range. So the correct answer to this question is "no". A pop-up box will then ask if you wish to "Make a new calibration". Just press cancel.

### Step 5. Nominal Mass

If you plan to acquire your data with nominal mass accuracy (usually +/- 0.1 amu) then nothing more need be done except to pump away the METRI by opening the black reference pump-out

valve on the GCT. Keep in mind however that you will have to select the calibration file you just acquired using the **GCT Tune Acquire** page. This step is discussed later.

## Step 6. Accurate Mass

If you plan to acquire your data with accurate mass accuracy (usually +/- 0.002 amu), perform the following steps. **Note: This represents a higher level of training, and you must be approved before attempting these measurements.**

- 1) On the GCT -> Options -> TDC Setting page, set the "Lock Mass" parameter to 284.9949, and the "Mass Window" parameter to 0.3.
- 2) On the GCT -> GCT Tune Acquire -> Time-of-Flight Calibration page, check the "Use Positive Calibration File" box, and browse to select the calibration file you just acquired.
- 3) From GCT Tune Acquire, acquire a short "temp" file of METRI. Use the MassLynx Chromatogram and Spectrum windows to view the data being acquired. Expand the spectrum window to look at the m/z 285 ion.
- 4) Carefully open the reference pump-out valve on the GCT until the intensity of m/z 285 is reduced to approximately 500 counts. This establishes an optimum level of the m/z 285 "lock mass" that must be present at all times during your sample data acquisitions to enable mass assignment with accurate mass accuracy.

## V. MassLynx Data Acquisition

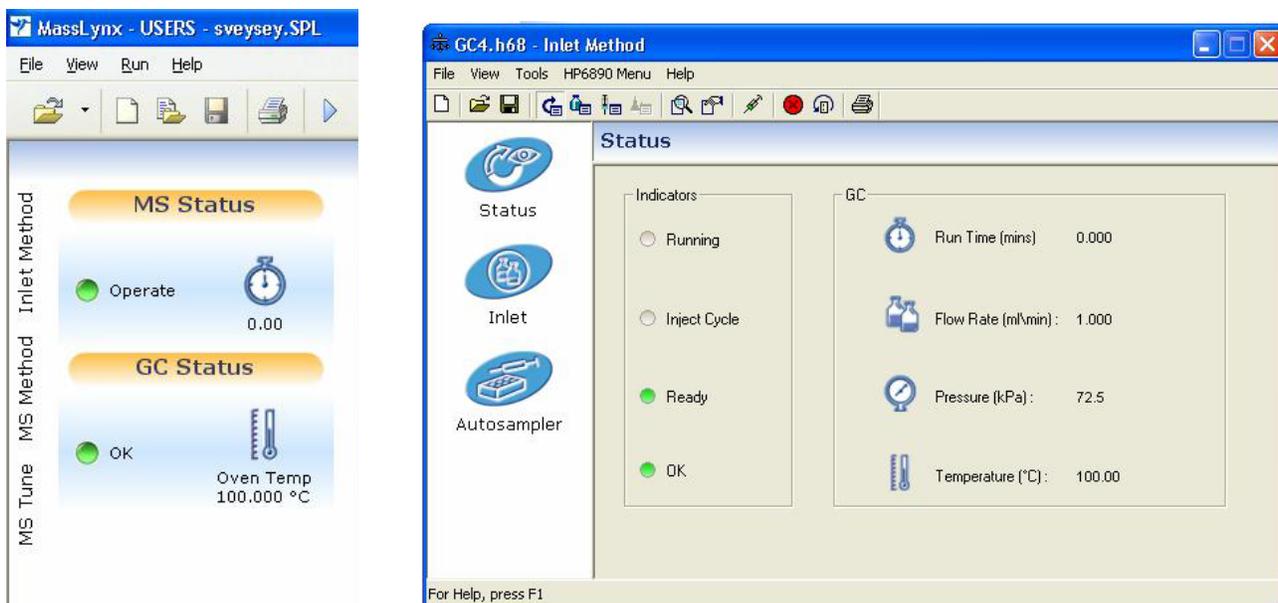
Queue Is Empty							
Spectrum Chromatogram Map Edit ▾ Samples ▾							
	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume	Control
1	sv-03-08-01	reference compound	ms1	GC1	1	1.000	
2	sv-03-08-02	sample 1	ms1	GC1A	2	1.000	
3	sv-03-08-03	sample 2	ms2	GC1A	3	1.000	
4	sv-03-08-04	sample 1 repeated	ms1	GC1B	2	1.000	
5	sv-03-08-05	sample 2 repeated	ms2	GC1B	3	1.000	
6	sv-03-08-06	reference compound repeated	ms1	GC1	1	1.000	

As discussed earlier, most data files will be acquired using the Sample List feature of the **MassLynx** program. Each sample is assigned a filename, descriptive text, a Mass Spec parameter set, an Inlet (GC) parameter set, a location in the autosampler, and an injection volume. Procedures for establishing and modifying the Sample List have already been discussed. You have also been shown how to select an MS File (mass spec parameters) and an Inlet File (GC parameters). We will now discuss these parameter sets in more detail.

## Editing the GC parameters

Parameters can be modified easily, sometimes unintentionally, by you or other users. You should always verify the content of the MS and Inlet File parameter sets you have selected before instigating the sample list data acquisition. If modifications are necessary, change the GC parameters (Inlet File) first.

On the left-hand side of the MassLynx page, click on Inlet Method.

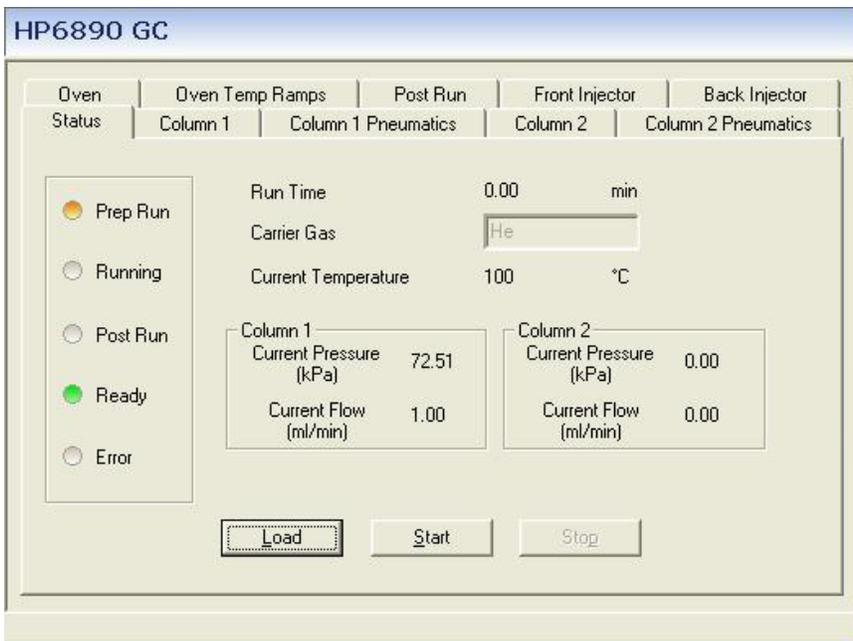


*Clicking on “Inlet Method” will bring up the Inlet Method Editor page*

All GC methods have the extension “.h68”. Note in the example above that “GC4” is the method open for editing. Also note that the full path is not given for the file. You might actually be viewing a file named “GC4” that is in a *different project*. This can happen! To select the proper file for editing, use File -> Open, and be sure you are selecting the file USERS.PRO / ACQADB / xxxx.h68.

When the method editor opens, the Status page is displayed first. If the software is communicating correctly with the GC, both the Ready and OK lights should be green. At this point, don't worry if they are not. Normally click first on the Inlet icon on the left of the Inlet Method page.

Note: Use the Help pulldown at any time to clarify the various parameter settings. In many respects, the on-line help is actually much better than the information in the GCT or MassLynx printed manuals.



Column 1 You must correctly specify details of the column being used. The “injection pressure mode” should be set to “constant flow”. Other options are available, but discuss with staff before using.

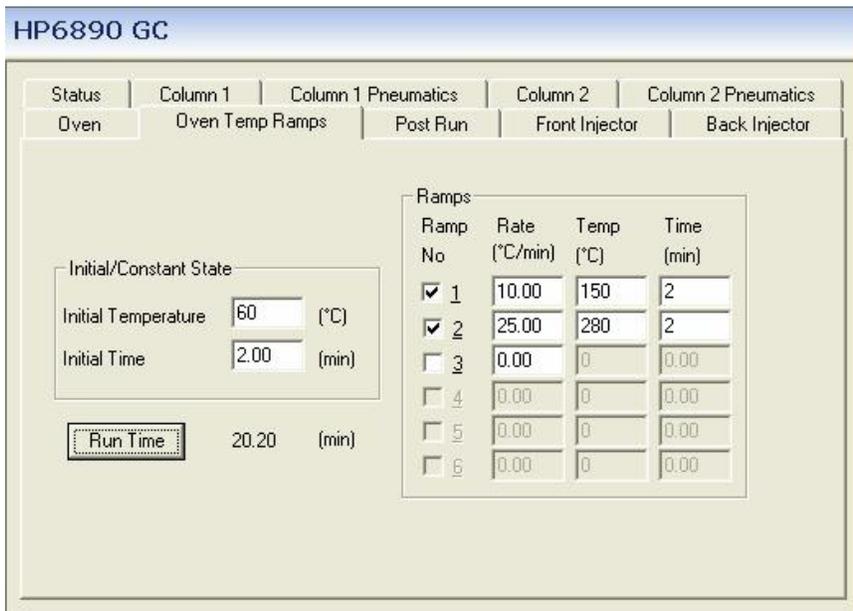
Column 1 Pneumatics: The initial flow for a 30 meter column with an internal diameter of 0.25 mm should be set to 1.0 mL / minute. Flow ramps are available, but discuss with staff before using.

Column 2: N/A

*Inlet Method Editor GC tabs are largely self-explanatory*

Column 2 Pneumatics: N/A

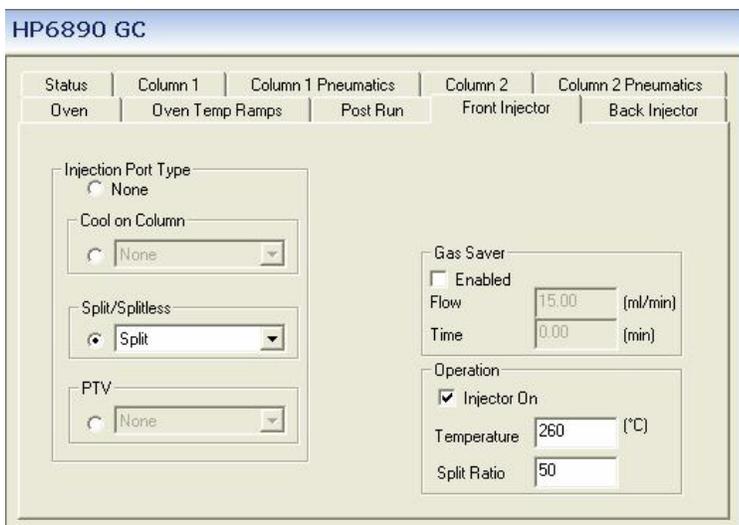
Oven: The maximum oven temperature for a DB-5 column should be set at 320 degrees. In general, set the maximum temperature at least 10 degrees below the vendor-specified upper temperature limit of the column. The GC is not currently equipped with cryocooling hardware.



Oven Temp Program: Set as appropriate. Be sure to select Run Time to show you the total run time after you have set all of the temperature ramps. You will need to know this value when adjusting the MS File parameters.

Post-Run: Normally this should not be enabled. Discuss with staff before using.

*Typical multi-ramp temperature program.*

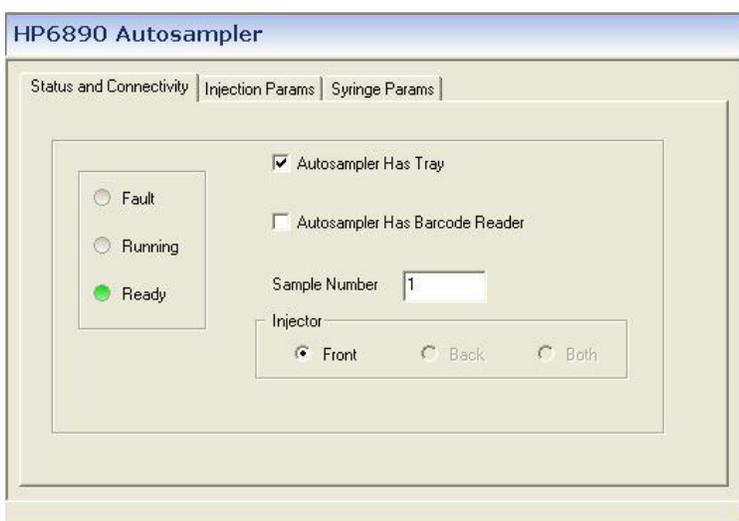


**Front Injector:** The Split/Splitless radio button must be selected. Choose “split” or “splitless” as appropriate. Note: Normally the split liner is in the GC injector. This liner can also be used for splitless injections, with some small performance loss. We also have dedicated splitless liners and SPME liners. Discuss with staff before using.

**Back Injector:** N/A.

*Typical injector settings for a split injection*

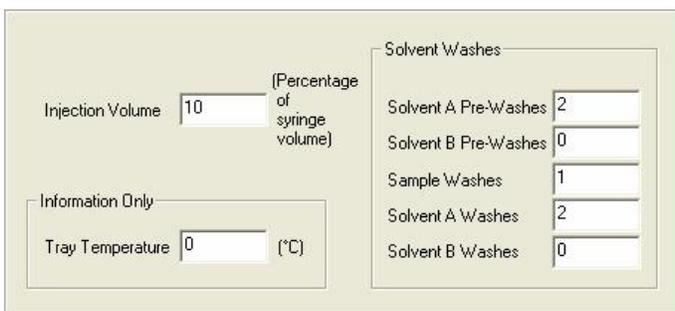
After setting the Inlet [GC] parameters, click on the Autosampler icon to verify settings.



Normally the autosampler ready status light will be green. If not, there is a problem which must be corrected.

Sample Number (position in the tray) must always be set to 1 on this tab. The actual sample position used is specified on the MassLynx sample list.

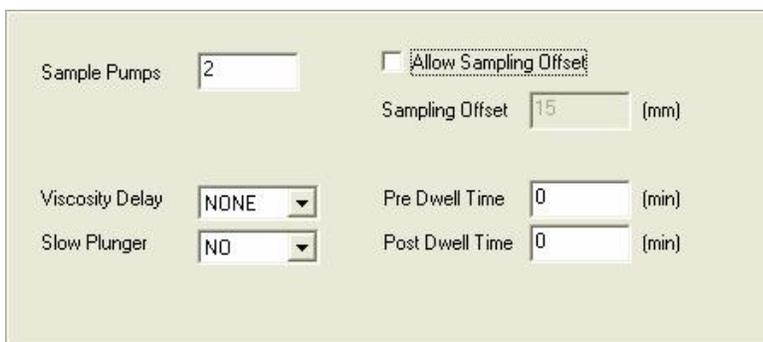
*Autosampler Status and Connectivity Tab*



The injection volume may be set as a percentage of the syringe volume. Normally a 10uL syringe is mounted, so 10% represents a 1uL injection.

The syringe can be rinsed with the solvents located in the A or B position of the autoinjector tower.

*Autosampler Injection Parameters Tab*



For a normal split/splitless, injection, the Slow Plunger setting should be “No”.

Normally “Allow Sampling Offset” should be disabled; however it can be used to sample from a specific point in a multi-layer solution, or to perform headspace analysis.

### Autosampler Syringe Parameters Tab

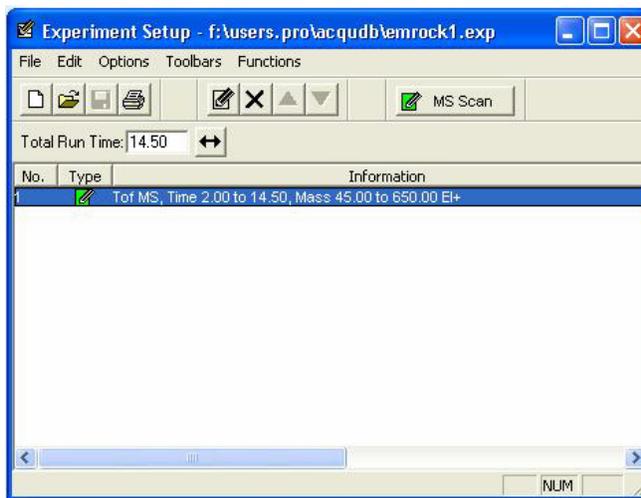
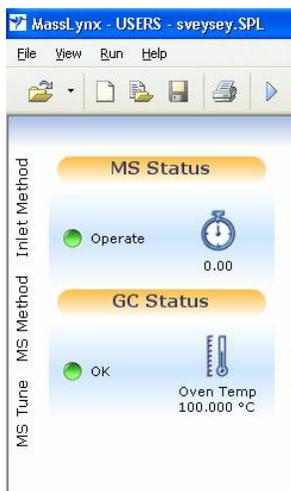
“Sample pumps” is used to remove trapped air from the syringe needle and should be left as shown.

After setting all of the Inlet and Autosampler parameters, be sure to save your parameter set before exiting from Inlet Method Editor:

Inlet Method -> File -> Save As -> [username#]

### Editing the MS parameters

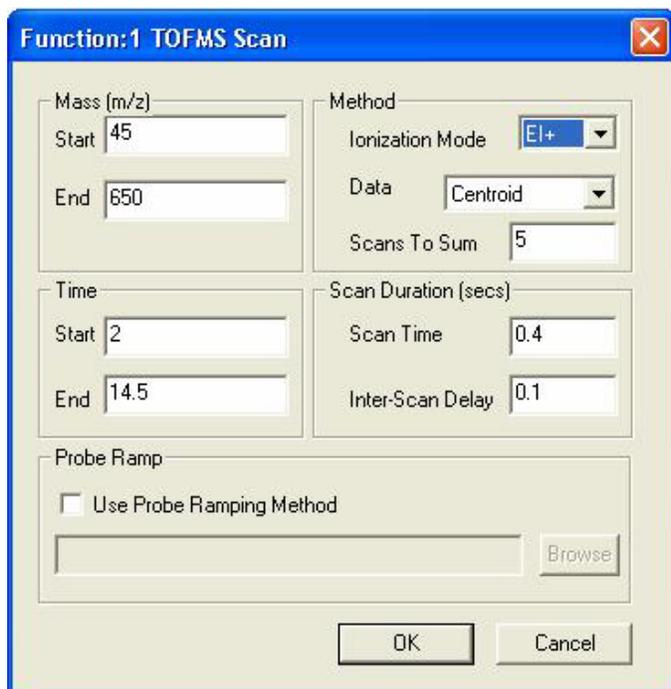
On the left-hand side of the **MassLynx** page, click on MS Tune to review or change the mass spec scanning parameters, select the proper calibration file, and to set the data acquisition period.



*Clicking on “MS Tune” will bring up the Experiment Setup page*

Note that in this program the entire path to the experimental method is properly displayed in the header. If the appropriate file is not displayed, use File -> Open to select the correct file, just as you did when you were using the Inlet Method editor program.

To make changes, double-click on the blue highlighted ms scan function. Note that an experiment can use different scan functions for different parts of the GC run, but this is normally not necessary. If you feel you need to create multiple scan function experiments, discuss with staff before using.



In this example, the mass range from 45-650 Daltons will be scanned in 0.5 seconds, giving two complete scans per second.

Ionization Mode should be "EI". It is possible to do "CI" with special training.

Data should normally be acquired in "centroid" mode.

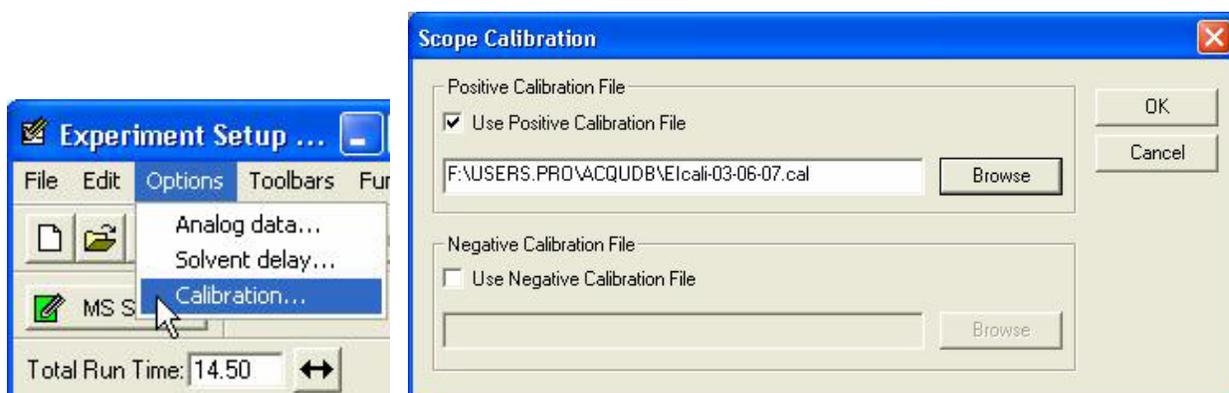
Leave "Scans to Sum" at five.

"Start Time" should incorporate a solvent delay, expressed in minutes. **DO NOT ACQUIRE THE SOLVENT PEAK.**

"End Time" should match the length of the GC run, expressed in minutes.

*Double-clicking on the blue highlighted function in Experiment Setup opens the MS Function Page*

Finally, two additional settings need to be checked on the Experimental Setup "Options" pulldown menu.



*The correct calibration file must be selected using the Options pulldown menu*



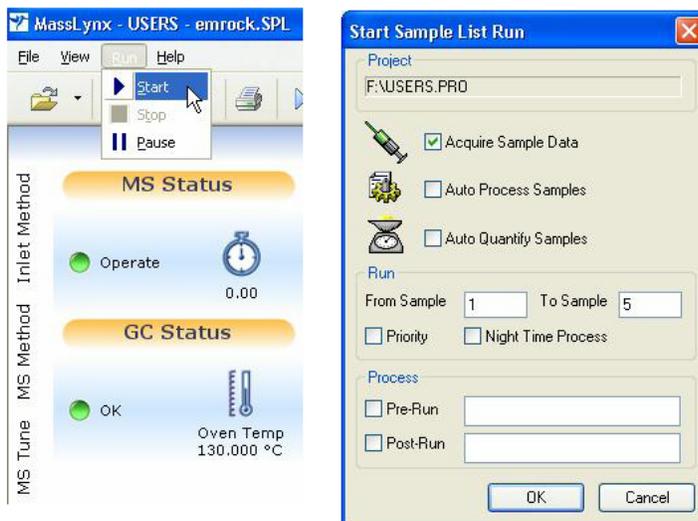
Because of a bug in the software, all values in “Solvent Delay” must be set to zero. We accomplish a solvent delay by delaying the start time in the TOFMS scan function, as discussed previously.

### Acquiring Data

Everything should now be correct in the sample list displayed on the MassLynx page. You think that are ready to proceed with data acquisition. However...

***Before starting the autosampler run you must make sure that all of your sample vials are properly loaded in the proper spaces in the autosampler tray, and that the appropriate solvents are in the Solvent A and Solvent B bottles!!!***

To begin the run, select Start from the **MassLynx** -> Run pulldown menu.



Only the “Acquire Sample Data” box should be checked. “Auto Processing” and “Auto Quantify” are more advanced features not normally selected. Discuss with staff before using.

Select the numerical range of samples in your sample list that you want to run.

*Use MassLynx -> Run -> Start to open the “start Sample List Run” window.*

The GC program selected for the first selected sample will now be downloaded to the GC. It may take a minute or two for the oven and/or injector temperatures to reach the proper values. You should then observe the autosampler arm pick up the appropriate vial from the autosampler tray, perform the requested rinses, and inject the sample.



*Autosampler arm in action*

The status bar along the bottom of the MassLynx page will show you the system action that is occurring. You may now view and process the data file as it is being acquired.

## **VI. Data Processing**

You will normally use two major data processing programs, *Chromatogram* and *Spectrum*, both accessed from the **MassLynx** program. As with the Magnum GCMS, the normal entry point for data processing is the Chromatogram program.

Queue Is Empty								
	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume	Control	
1	er-03-21-01	dp085	emrock1	emrock1	1	1.000		
2	er-03-21-02	dp104	emrock1	emrock1	2	1.000		
3	er-03-21-03	stio2.4mrc.010	emrock1	emrock1a	3	1.000		
4	er-03-21-04	stio2.4mrc.011	emrock1	emrock1a	4	1.000		
5	er-03-21-05	stio2.4mrc.014	emrock1	emrock1a	5	1.000		

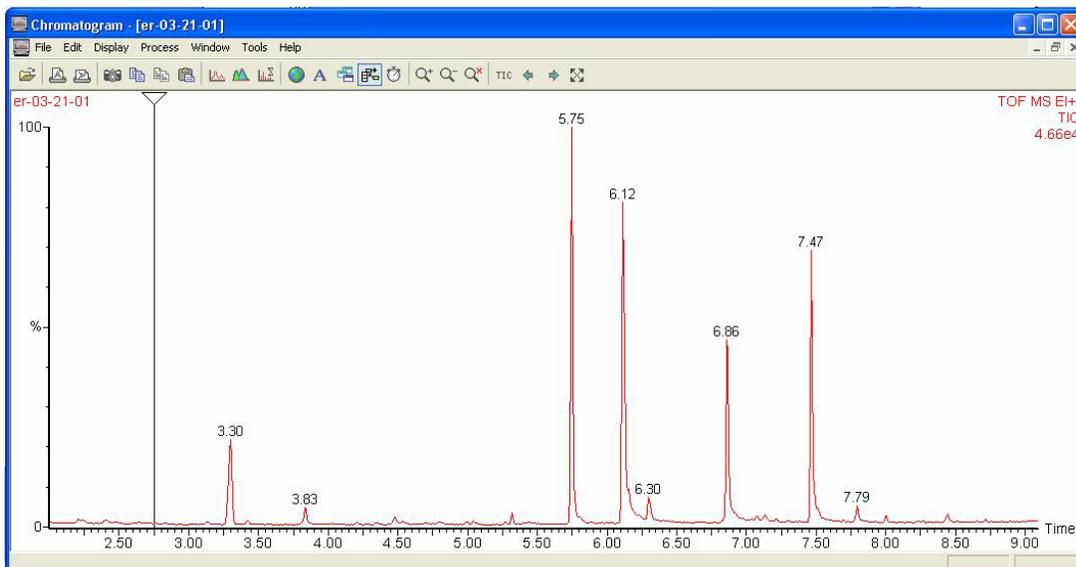
*Data processing normally begins by opening the Chromatogram window.*

### **Chromatogram, Spectrum, and Library overview**

Both Chromatogram and Spectrum provide access to other programs. For example, you have already seen that the calibration function is a subset of the Spectrum program; and it should be no surprise that the peak integration function is a subset of the Chromatogram program.

Each program and subprogram view can be modified through the use of the pulldown menus and the button bar located along the top of the view. Many of the actions and settings controlled by

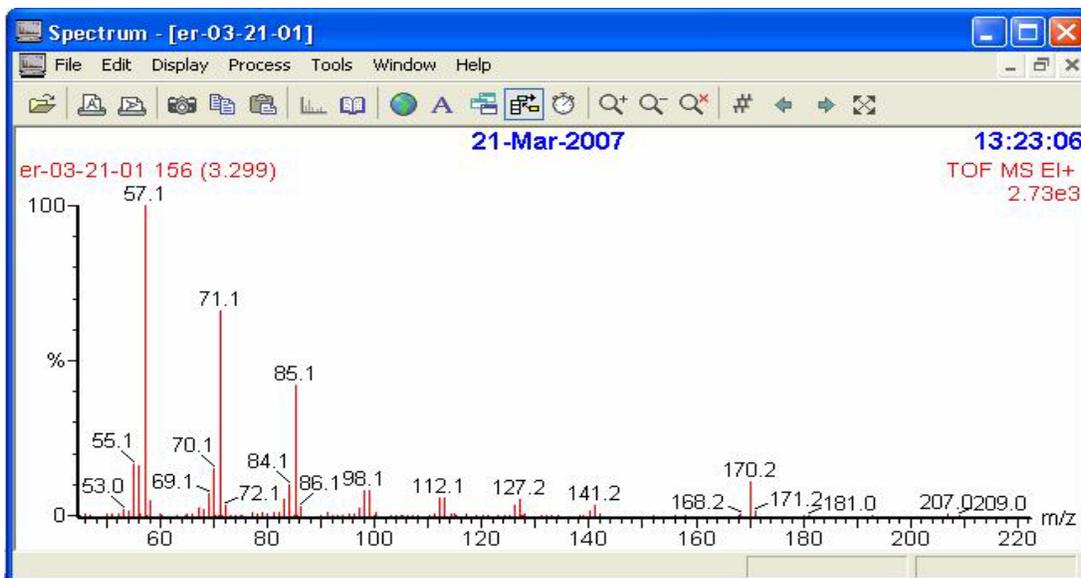
the pull-downs and buttons are straight-forward and intuitive; others are not. Most of the actions and settings are easily reversible if you happen to choose an inappropriate setting; others can cause problems that are harder to recover from. You will need to spend some time investigating which features are important to you and which features are either useless or dangerous. The button functions are all described in the Appendix.



Typical Chromatogram view.

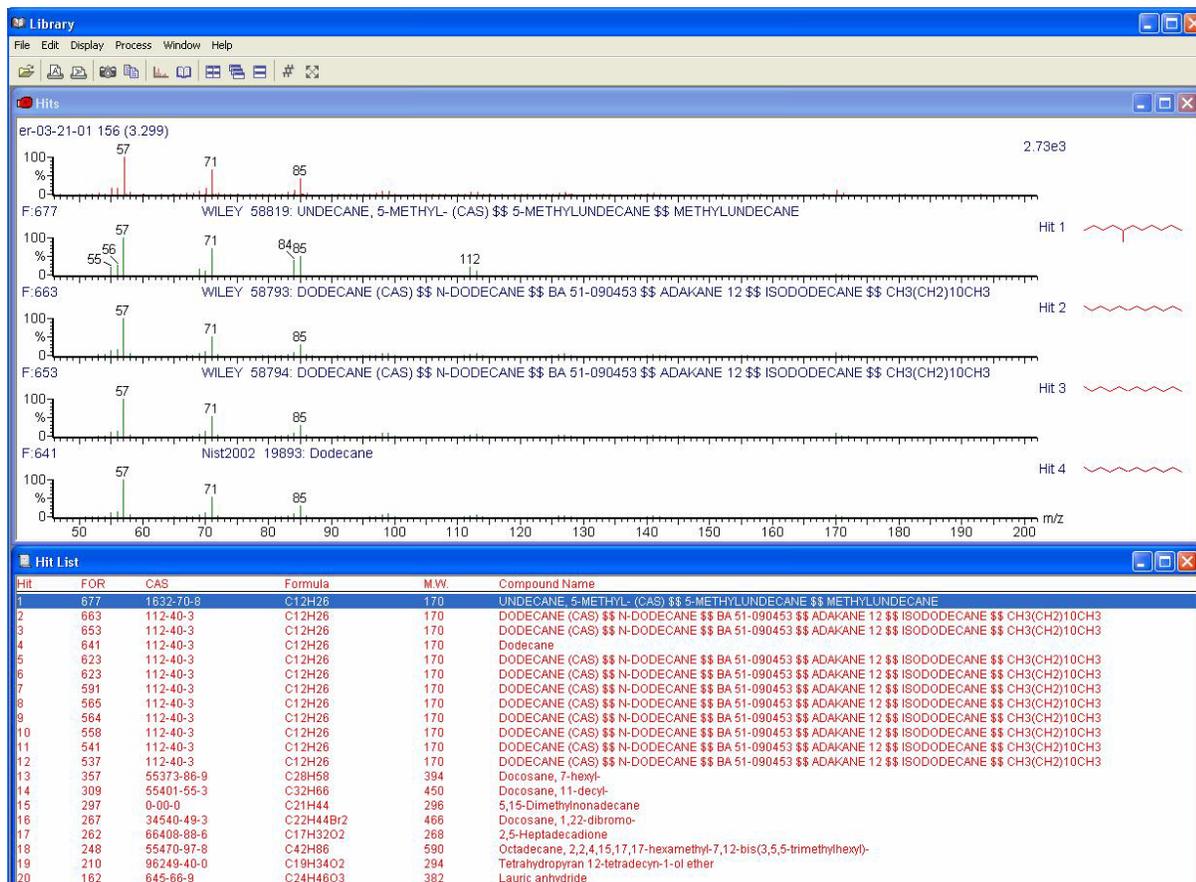


Double-click anywhere in the Chromatogram view to open the Spectrum view. Left-click and drag the triangular top of the slider bar to select a spectrum. As you move the slider bar across the chromatogram, you will see the corresponding spectrum displayed in the Spectrum view.



Typical Spectrum view.

The spectrum can be searched against two large computer libraries by selecting “Tools -> Library Search” from the Spectrum view.



Typical Library Search Results view

As with the Magnum, “Chro, Spec and Libr” are the three processing programs that you will be using most often. However, the 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

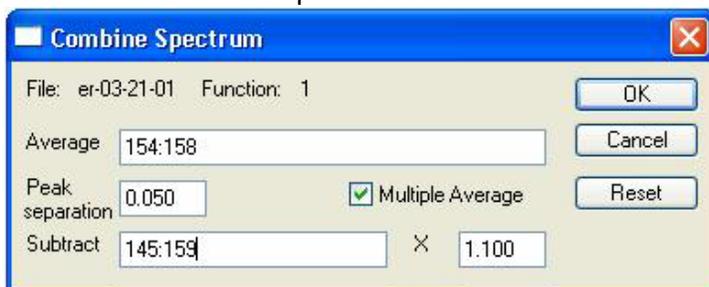
### Averaging scans and subtracting background

Averaging several scans and subtracting several background scans can improve the quality of your spectrum, and improve the performance of the Library search and Elemental Composition programs. However, you must be aware of the potential problems and the actual limitations of

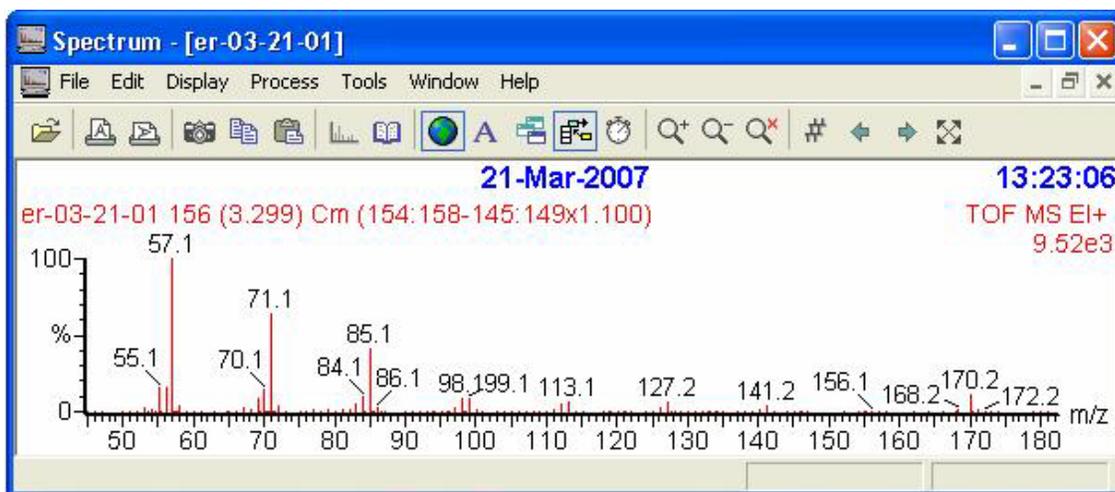
spectrum averaging. Before averaging scans you must answer two questions. First: Is the GC peak “pure”? Am I averaging scans from the same component, or are some of the scans coming from a co-eluting GC peak? Proper use of the Single Ion Chromatogram program will help you answer that question. Second: Is the signal level of the individual scans appropriate for averaging? If quantitation is important, then be sure that none of the scans are saturating the detector. If elemental composition (accurate mass accuracy) is important, then be sure that the signal levels of all of the scans fall within the proper intensity window, particularly for the ions of interest.

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*

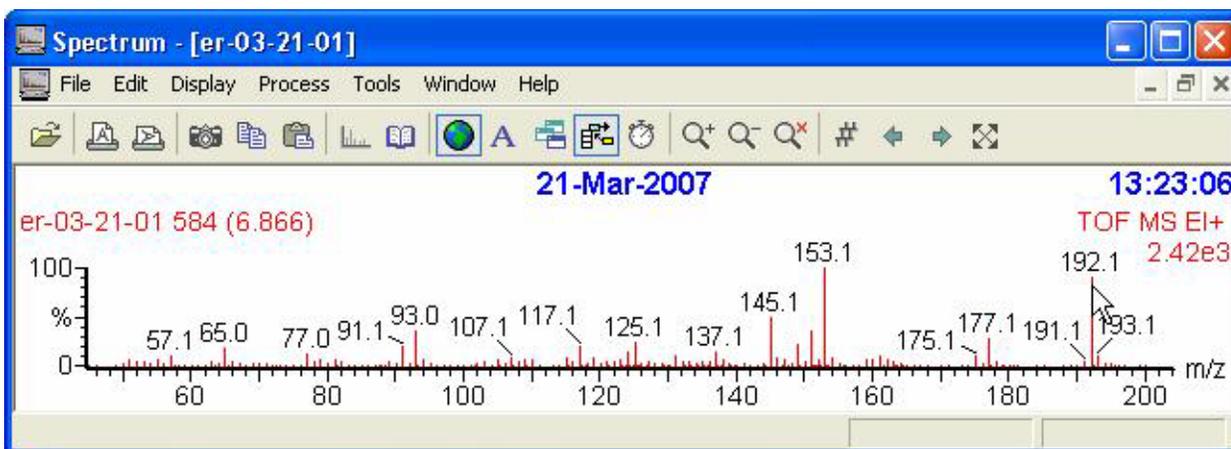
Notice that the spectrum will now show the averaging and subtraction information.

## Mass chromatograms

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

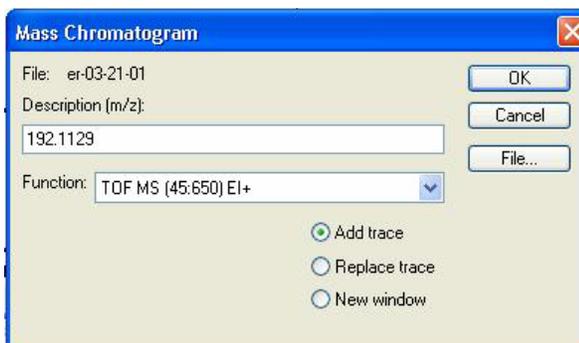
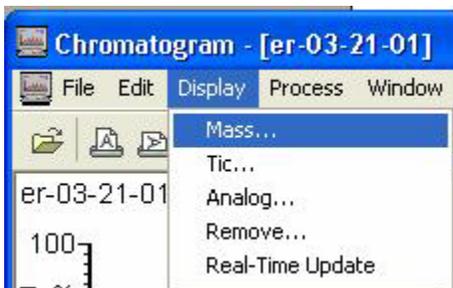
- To find the “needle in the haystack” by searching for a specific mass that you expect to appear in one or more GC peaks.
- To find all related compounds in a complex mixture by looking for fragmentation ions known to be representative of a class of compounds.
- To determine whether a GC peak is pure or is the mixture of more than one component.
- To find scans meeting certain intensity and mass precision criteria for accurate mass accuracy averaging.
- To determine whether a specific mass in a spectrum is from the sample, from background, or from the tail of a closely eluting peak.

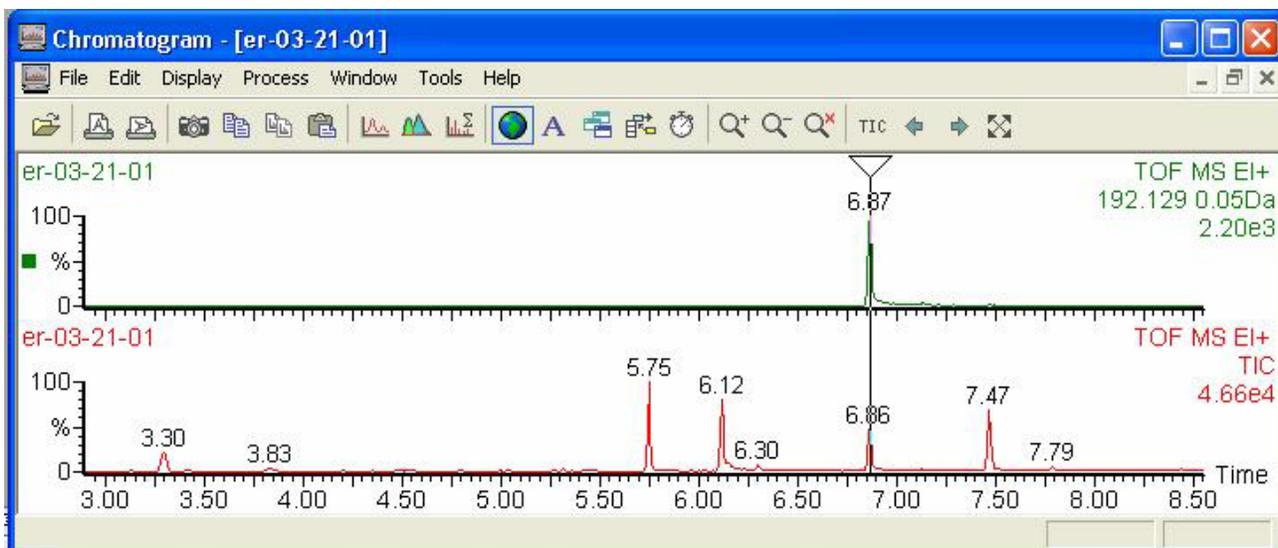
The program can be initiated in two ways. The easiest method is to simply double-click on the mass of interest in the spectrum display.



Depending upon the toggle state of the Add/Replace button , This will open a new window or replace the contents of the existing window to show the mass chromatogram of the mass selected.

The second way to specify a mass chromatogram is from the Display pull-down menu. Simply type in the mass of the ion you would like displayed.





*Typical mass chromatogram display*

Notice in this example that the tolerance for the single ion display is a window of 0.05 Daltons (+/- 0.025 Daltons). This is a very narrow mass window by most mass spec standards, but perfectly fine to use with the GCT because of the inherent precision of the instrument. The tolerance is set in a rather obscure place in the **MassLynx** program. To change the tolerance, you must first change the MassLynx view from “Status” to “Shortcut”. Then select the “Options” button. Then enter a different tolerance for the mass chromatogram.



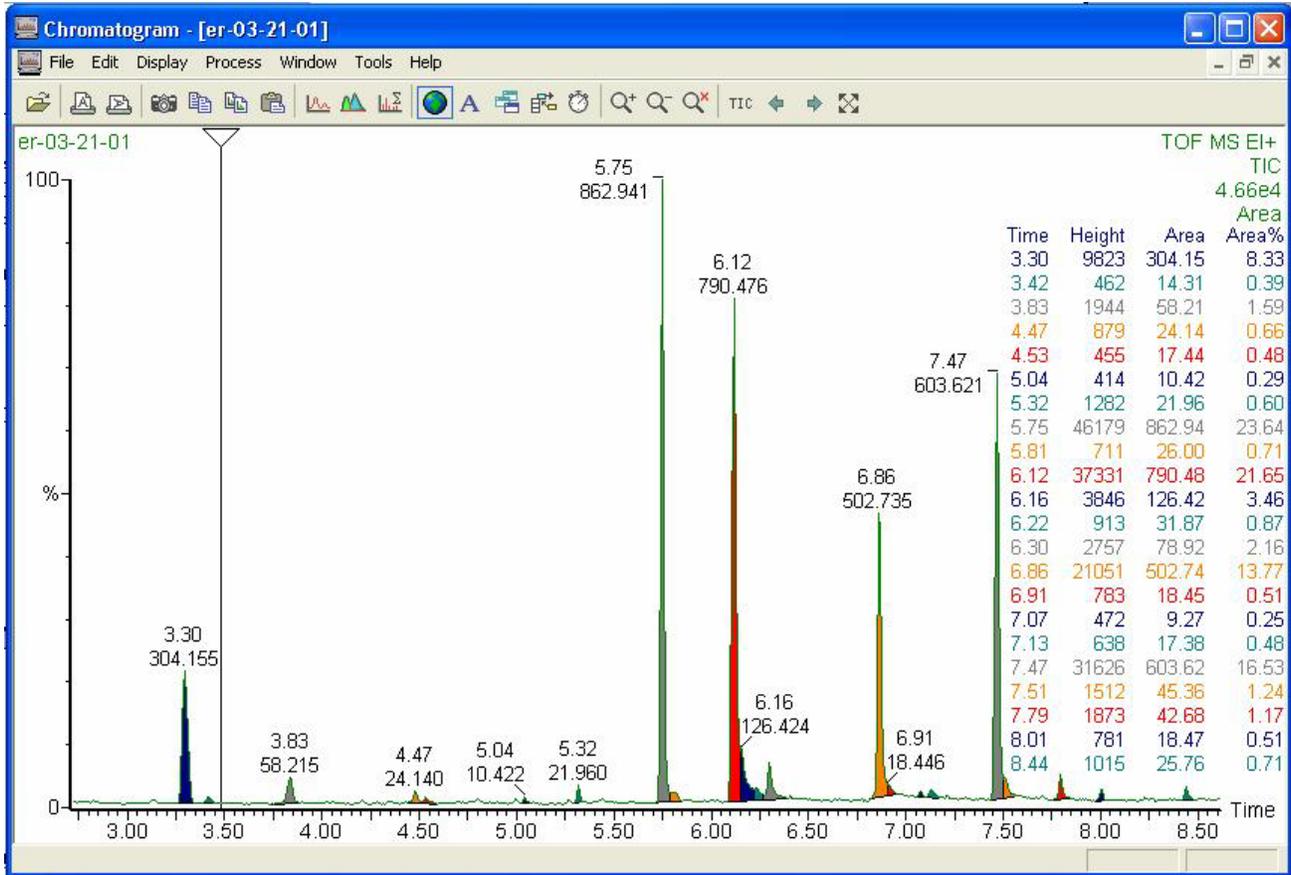
*Process for changing the mass chromatogram tolerance*

If you are trying to find a particular GC peak in a complex mixture, you would normally calculate the molecular weight of the component (perhaps by using the MassLynx Isotope Ratio program) and then enter that value in the Mass Chromatogram dialog box. If the instrument has just been calibrated, or you are acquiring in accurate mass accuracy mode, you can easily set the tolerance window as tight as 0.01Da. However, if you are acquiring in nominal mass accuracy mode (the normal situation), then set the tolerance window to 0.5 Da until you find the peak.

On the other hand, if you want to display a mass chromatogram by double-clicking on an ion currently displayed in the spectrum view, the tolerance window can easily be set as tight as 0.01Da. This will be discussed further in the Elemental Composition section.

## Integration reports and Quantitation

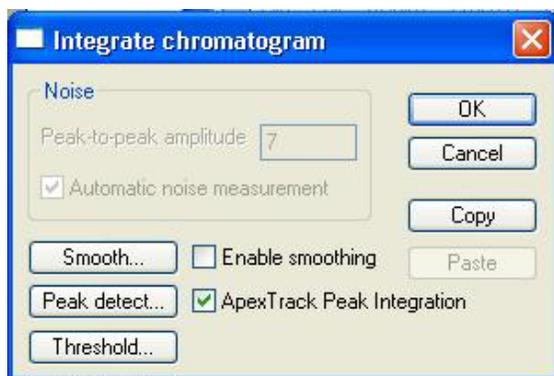
**MassLynx** includes a complete quantitation package. Quantitation is an advanced topic and will not be discussed here. However, a simple integration report can be easily generated just by using the Chromatogram Peak Integration button . The integration report is printed over the chromatogram. Depending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



*Typical chromatogram display after performing peak integration*

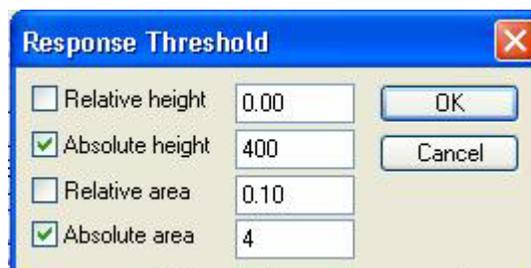
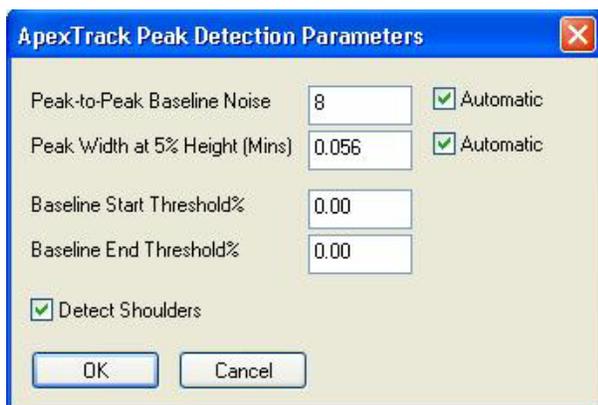
The integration report can also be sent to the clipboard by using the Detected Peaks button . The report can then be pasted into Excel or Word. When using the Peak Integration button, be aware that the integration is performed using parameters probably set by a previous user. Users have full control over the peak detection and integration algorithm through the Chromatogram -> Process -> Integrate pull-down menu.

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...

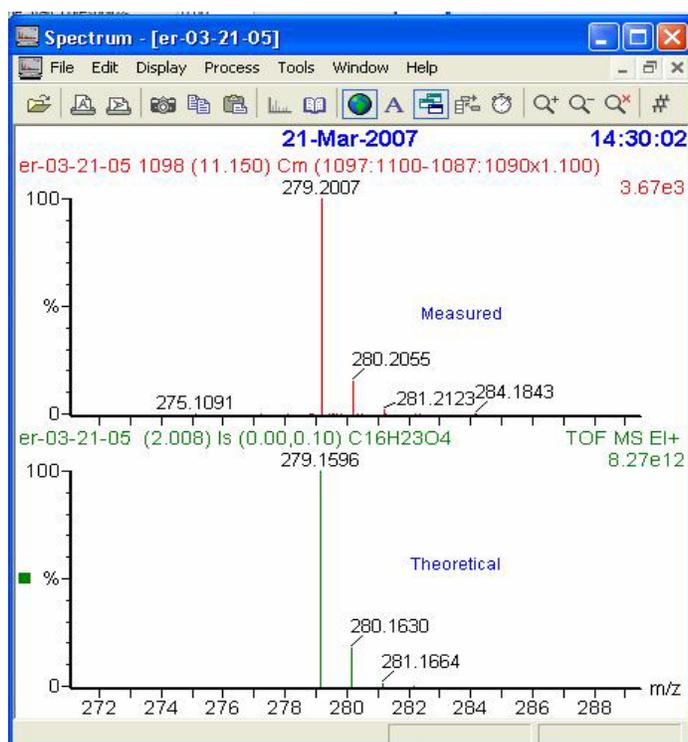
*Normally choose Apex Track Peak Integration*



*Typical settings for Apex Track peak detection and integration*

Occasionally you might find it necessary to disable Apex Tracking and use the more advanced features of integration in order to get the results you need.

### 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. Access: MassLynx -> Spectrum -> 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.



No	Mass	Inten	%BPI	%TIC	No
1:	279.15968	27e12	100.00	82.83	
2:	280.16301	47e12	17.80	14.74	
3:	280.16381	26e10	0.15	0.13	
4:	280.16592	85e10	0.35	0.29	
5:	281.16396	63e10	0.80	0.66	
6:	281.16641	23e11	1.48	1.23	
7:	282.16721	18e10	0.14	0.12	

### Accurate Mass Accuracy review

Before acquiring *accurate mass accuracy* data for use with the Elemental Composition program, you must follow Tuning Step 4 “Recalibrating the Mass Spectrometer” and Tuning Step 6 “Accurate Mass” discussed previously. Verify the following:

- 1) You have updated the MS Method File selected in the Sample List Queue with the calibration file you acquired in Tuning Step 4.
- 2) You have specified the *lock mass* as 284.9949 and the *mass window* as +/- 0.3 on the TDC settings page. Access: GCT -> Options -> TDC Settings
- 3) METRI calibration compound is present with an intensity of 400-600 counts for m/z 285.

Proceed to acquire and process data normally. There will be a constant background level from the METRI which will require you to subtract the background in order to get a high quality spectrum.

NOTE: *Very small GC peaks may be masked by the level of background, requiring you to use a mass chromatogram search to properly locate them.*

### Elemental Composition Program

The Elemental Composition program will be of no value to you unless you have acquired your GCMS data under “accurate mass accuracy” conditions as discussed previously. Assuming that to be the case, two steps are required to get a proper elemental composition report.

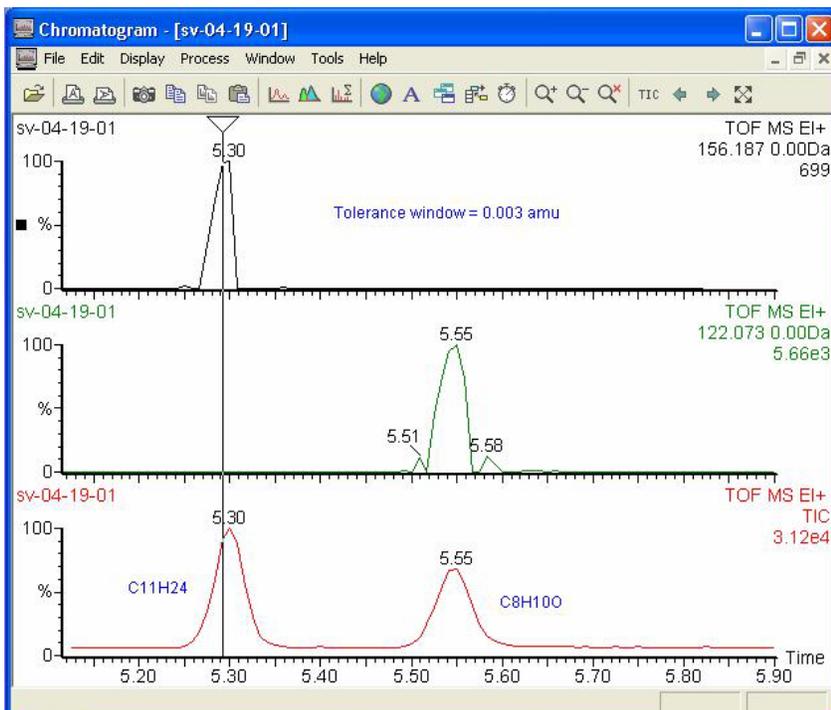
- 1) You must select the appropriate scan (or scans) as input to the program.
- 2) You must select reasonable elements and tolerances within the Elemental Composition program.

Accurate mass assignment is possible to within +/-10 ppm error on **unperturbed ions** with **appropriate intensities** within an **appropriate mass range**. *Not all of the ions in the spectrum or all of the scans comprising the GC peak will meet those criteria.* In order to avoid disappointment and

false conclusions, you must understand the limitations associated with accurate mass assignment before using the Elemental Composition program.

First of all, the ion signal for a particular mass has a peak shape as well as a peak position. You saw this earlier when you were evaluating mass resolution during the initial instrument setup. The software has the ability to assign the centroid mass very precisely. However, this centroid position will be an accurate representation of the mass of the ion only if the peak shape is not distorted by *saturation, detector dead time effects, poor ion statistics, or contributions from other isobaric ions* (ions of different elemental formula but with the same integer mass).

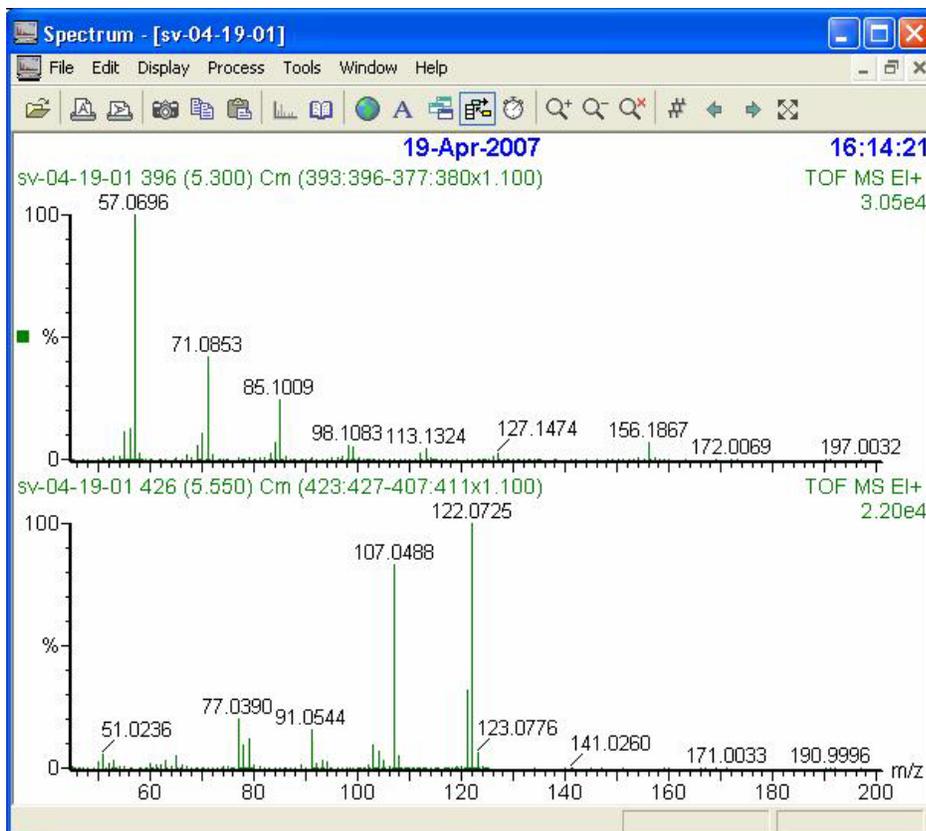
- If isobaric ions are contributing to the peak shape, there is no solution. That particular ion can never be measured with accurate mass accuracy; choose a different ion.
- If the intensities of the ions are below 100 counts for each scan being averaged, the peak shape will be noisy and the error in the centroid assignment may be large.
- Detector dead time effects occur long before detector saturation. As the intensity of the ion signal increases, the centroid of the peak will shift to an increasing lower mass. The software attempts to correct for this effect, but in practice ions with intensities greater than approximately 2000 counts will skew the centroid assignment to lower mass. The intensity at which this effect actually impairs the mass assignment will depend upon calibration, tuning, and other instrumental factors and is best evaluated empirically.



It is often useful to use the mass chromatogram program described previously to find suitable scans for accurate mass accuracy averaging. To do this effectively, it is best to set the tolerance window to 0.003 Daltons, or even tighter in some circumstances.

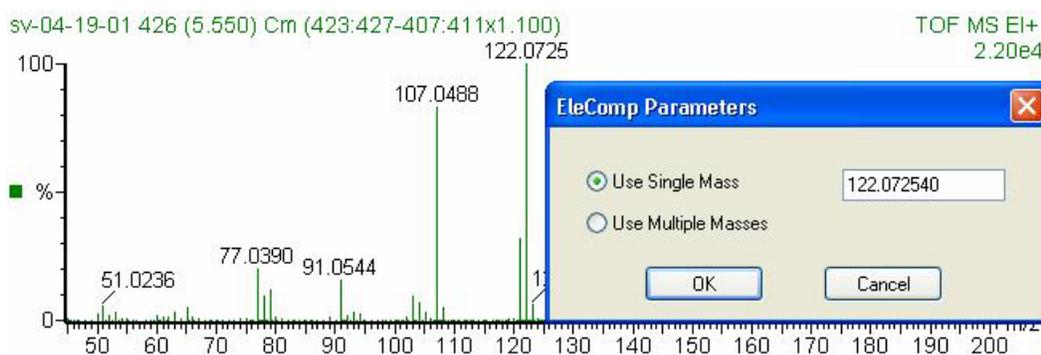
*Accurate mass chromatograms displayed for the expected molecular ions for two GC peaks in the mixture. Display tolerance is set to 0.003 amu in order to find contiguous scans suitable for accurate mass accuracy averaging.*

You may notice that only scans in the GC peak that fall within a rather narrow range of intensities will show mass agreement within this tolerance. For more intense GC peaks, these scans often are part of the leading and trailing edges of the peak. This is the result of the dead time effect just described. For less intense GC peaks, these scans might just be in the center of the peak where the intensities (ion statistics) are good enough to allow the centroid to be properly assigned. As long as you are sure the GC peak is homogeneous, it is perfectly acceptable to average just the contiguous scans that fall within a defined intensity range.

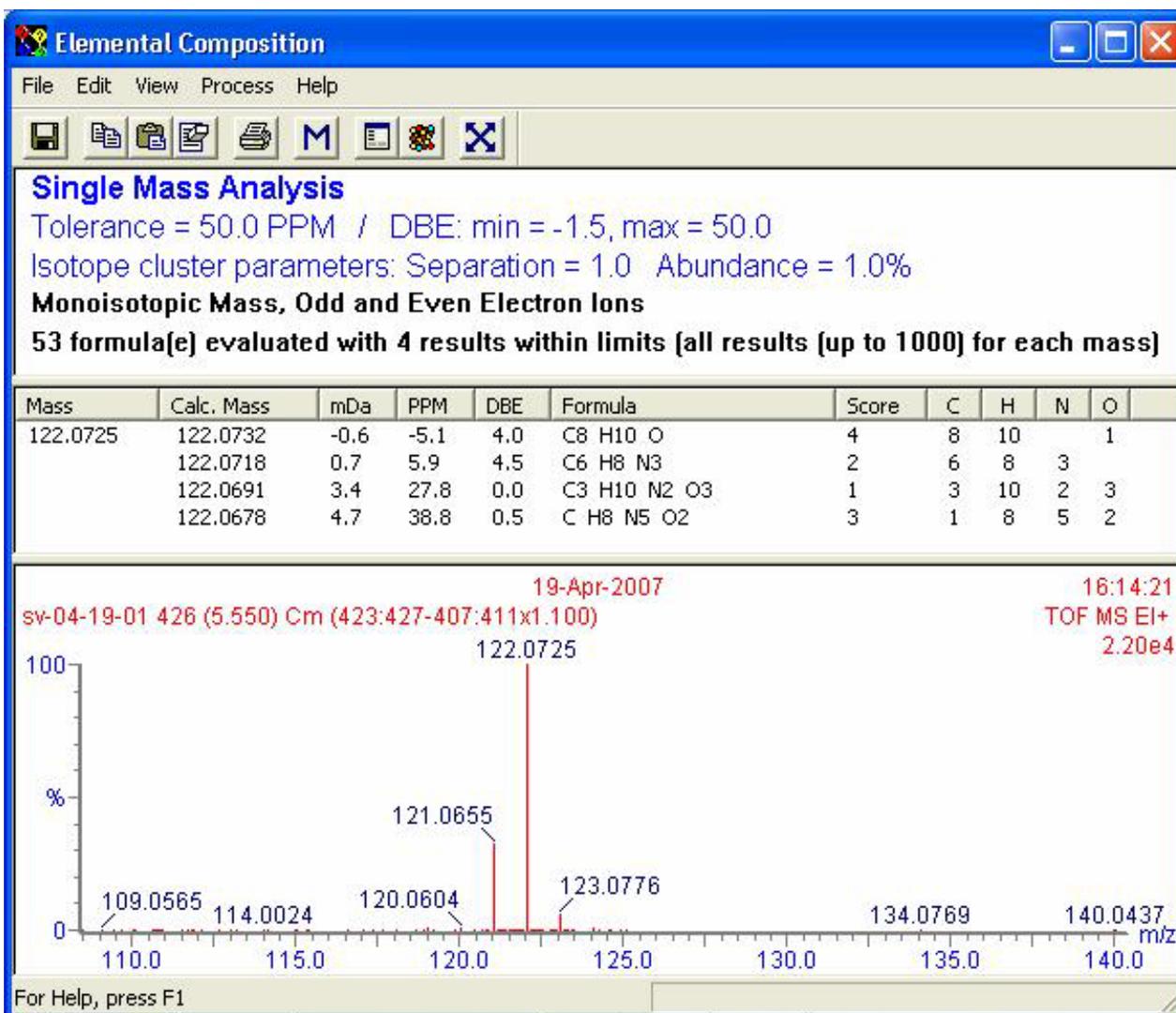


*Averaged spectra for the two GC components under investigation for elemental composition.*

Using Spectrum -> Tools-> Elemental Composition brings up a dialog box allowing you to select either a Single Mass search or a Multiple Mass search.



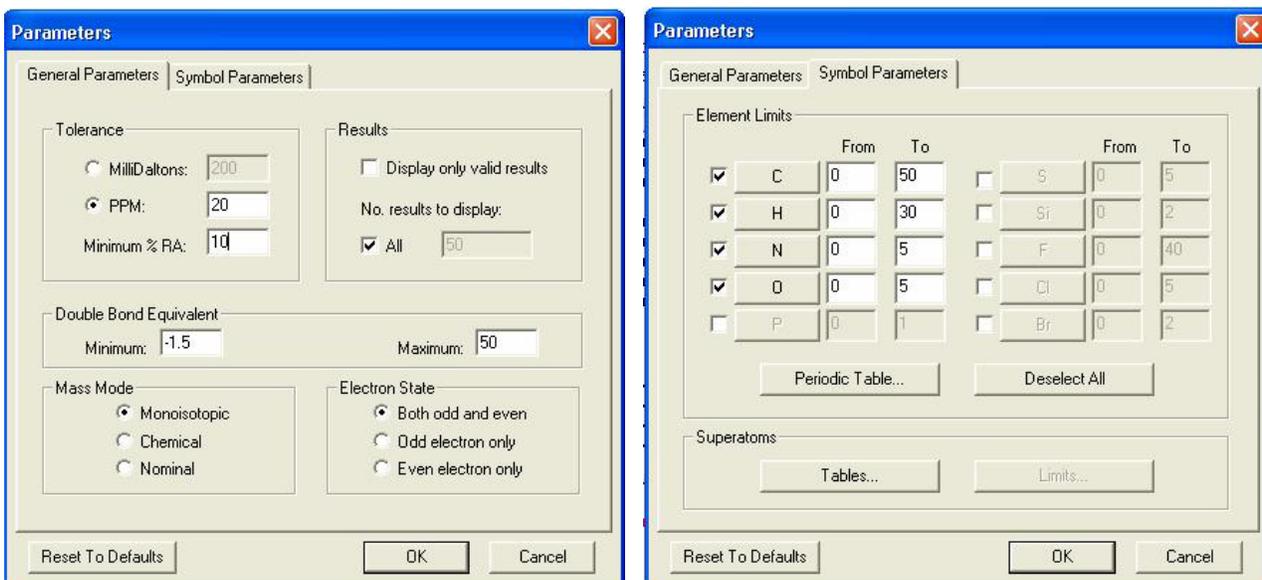
To define a single mass, simply click on the mass peak in the spectrum.



Typical Elemental Composition search result for a single mass.

Note that the program returns with several possibilities for the elemental composition of the mass selected. The number of possibilities will depend upon element parameters and tolerances selected. In this example, the best match happens to be the correct match, C<sub>8</sub>H<sub>10</sub>, with an agreement error of 5 parts per million. The validity of the elemental composition search is very dependant upon the conditions you select for the search. The program is meant to be highly interactive.

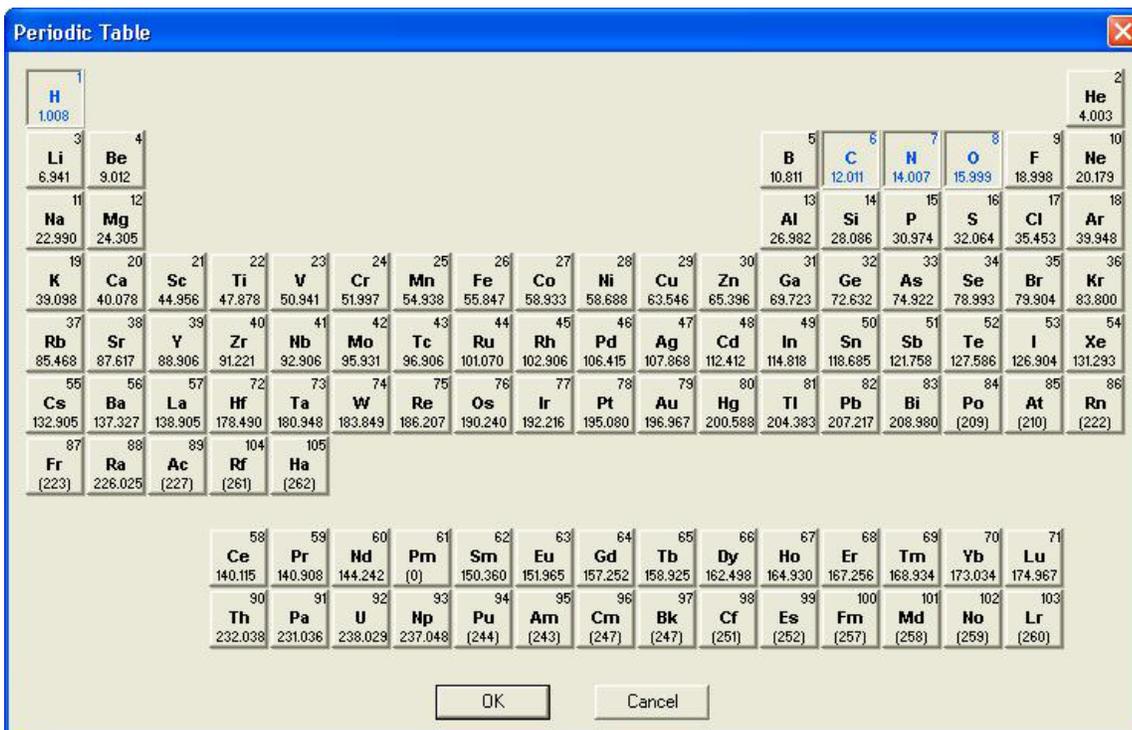
Click the parameters button  to modify the search.



Typical General parameter and Symbol parameter settings

On the General Parameters tab, the peak threshold (for multiple mass searches) and matching tolerances are set. Other parameters should be left as shown above. On the Symbol Parameters tab, the elements to be considered in the search can be selected. The ten most commonly used elements can be selected or deselected from this view.

Additional elements (and isotopes) can be selected by using the Periodic Table button.

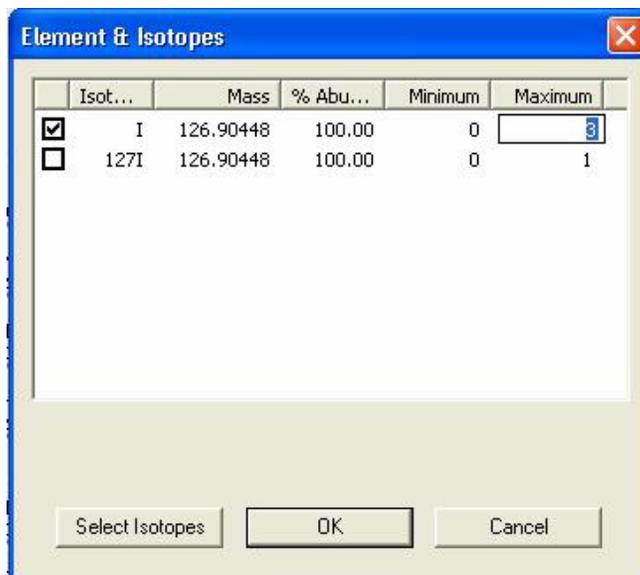


The periodic table will show the elements that have been selected from the Symbols Tab

**NOTE: Be aware that the Elemental Composition program has some quirks and pitfalls, especially if you wish to use elements or isotopes other than those directly selectable from the Symbol Parameters tab.**

Three scenarios will be discussed.

- 1) All of the elements you want to use are directly selectable from the Symbol Parameters tab.  
If you wish to perform a normal search without including  $^{13}\text{C}$  possibilities or other heteroatoms, then just check the boxes for the elements you wish to include in the search, and set reasonable minimum and maximum numbers for each element. Click OK and the search will be performed. Do NOT click on the "Reset to Defaults" button!
- 2) You wish to include elements other than those selectable from the Symbol Parameters tab.  
Enter the Periodic Table subroutine AFTER enabling elements, including min and max values as directed above. In the periodic table, click on the additional element you wish to be considered in the search. The Element & Isotopes dialog box will open. The Minimum and Maximum fields can be edited by clicking on them.



*Typical Elements & Isotopes dialog box*

- 3) You wish to include one  $^{13}\text{C}$  in the search possibilities.  
Enter the Periodic Table subroutine AFTER enabling elements, including min and max values as directed above. In the periodic table, click on Carbon. Enable the specific elements required with proper limits [eg.  $^{12}\text{C}$ , 0,50;  $^{13}\text{C}$ , 0,1. At the Symbols Parameter tab you will notice that the Carbon element is no longer selected! Ignore this seemingly incorrect setting and click OK to do the search.

## Appendix A

### Button Bar Actions Available in the Chromatogram View

-  \* Select a data file. Opens the data file browser program.
-  Print chromatogram display in portrait mode. [Opens the Windows printer dialog program.]
-  \* Print chromatogram in landscape mode. [Opens the Windows printer dialog program.]
-  Copy a picture of the chromatogram to the Windows clipboard. [Import into Word.]
-  Copy the data points forming the chromatogram to the clipboard. [Import into Excel.]
-  \* Copy list of detected peaks, and peak areas, to the clipboard. [Import into Excel.]
-  Paste clipboard contents onto the display.
-  Select mass chromatograms to display. [Normally done from Spectrum view.]
-  \* Perform peak integration. Uses integration parameters selected from the “Process” pulldown.
-  \* Combine spectra. Allows scan averaging and background subtraction.
-  Toggle processing of all traces in the current window “on/off”.
-  \* Allow text to be entered on the chromatogram display. [Used to annotate the display.]
-  Toggle opening of new window for process results “on/off”.
-  \* Toggle add OR replace new chromatogram.
-  \* Toggle real-time chromatogram update. [Used to view the data as it is being acquired.]
-  \* Increase magnification of current range. [Expand the display in the Y-axis.]
-  \* Decrease magnification of current range. [Contract the display in the Y-axis.]
-  Delete current magnification range. [Autoscale the X and Y axes.]
-  \* Reset the display to the TIC trace. [Reset to show just the chromatogram.]
-  Decrement the scan in the Spectrum display. [Show the previous scan in the data file.]
-  Increment the scan in the Spectrum display. [Show the next scan in the data file.]
-  \* Reset the display range back one zoom level (first click); reset to full display (second click).

\* Indicates commonly used buttons.

## Button Bar Actions Available in the Spectrum View

	Select a data file. Opens the data file browser program.
	Print spectrum display in portrait mode. [Opens the Windows printer dialog program.]
	* Print spectrum in landscape mode. [Opens the Windows printer dialog program.]
	Copy a picture of the spectrum to the Windows clipboard. [Import into Word.]
	Copy the data points forming the spectrum to the clipboard. [Import into Excel.]
	Paste clipboard contents onto the display.
	Unknown.
	* Perform library search for current spectrum.
	Toggle processing of all traces in the current window “on/off”.
	* Allow text to be entered on the spectrum display. [Used to annotate the display.]
	Toggle opening of new window for process results “on/off”.
	* Toggle add OR replace new spectrum.
	Toggle real-time spectrum update. [Used to view the data as it is being acquired.]
	Increase magnification of current range. [Expand the display in the Y-axis.]
	Decrease magnification of current range. [Contract the display in the Y-axis.]
	Delete current magnification range. [Autoscale the X and Y axes.]
	Select scan number.
	Decrement the scan in the Spectrum display. [Show the previous scan in the data file.]
	Increment the scan in the Spectrum display. [Show the next scan in the data file.]
	* Reset the display range back one zoom level (first click); reset to full display (second click).

\* Indicates commonly used buttons.