GAS CHROMATOGRAPHY

CLARUS SQ 8 GC/MS

(Gas Chromatograph/Mass Spectrometer)

Tutorial

PerkinElmer®

Clarus SQ 8 GC/MS

Gas Chromatograph/Mass Spectrometer

Tutorial

Release History

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About This Tutorial

The *Clarus SQ 8 MS Tutorial* is your initial guide to using the TurboMass software with a Clarus SQ 8 T, Clarus SQ 8 C, or Clarus SQ 8 S mass spectrometer.

This tutorial is designed to teach you basic operating procedures by using typical examples. More specifically, this tutorial begins by showing you how to prepare the hardware. Next, it shows you how to start the TurboMass software, develop GC and MS methods, create and run a sample list, and analyze the data obtained.

After completing this tutorial, you will have the background information required to explore and use all of the features described in the *TurboMass Software User's Guide* (P/N 09931016).

In addition, this tutorial provides the basis for operating TurboMass for Environmental Reporting. For detailed operating procedures for Environmental Reporting, refer to the Clarus MS Environmental Tutorial – Basic Principles (P/N 09931008).

Prerequisites

Before operating the Clarus SQ 8, T, C, or S system you should:

- Thoroughly understand the recommended safety practices. Read the *Warnings and Safety Information* section in the *Clarus SQ 8 MS Hardware Guide* (P/N 09931017).

- Have a basic understanding of how to use the Clarus GC, computer, and Microsoft Windows. For details, refer to the corresponding manuals provided with each product.

This tutorial is presented with the assumption that you have a basic familiarity with Microsoft Windows operations. If you need a refresher about the basic principles of Microsoft Windows, such as opening and closing files, adjusting the window size or position, printing, or using the Windows Explorer, refer to your printed Microsoft Windows documentation, or online Help files, for details.

About the Clarus SQ 8 MS System

The Clarus Mass Spectrometer is a sophisticated benchtop mass spectrometric detector that provides you with the simple tools needed to perform routine gas chromatography/mass spectrometry (GC/MS) analyses as well as the sophisticated tools needed to perform the more complex analyses. Clarus MS runs analyses that best characterize your sample by using either the electron ionization (EI) mode or chemical ionization (CI) mode. Designed as a detector for the Clarus SQ8 GC/MS, this system produces positive identification and quantification of compounds separated by the Clarus GC, even those complex compounds that coelute. See Figure 1 and 2 for details.

The following Clarus SQ 8 Models are available:

Clarus SQ 8 T – Compatible with the Clarus 680 GC, with Electron Impact (EI) ionization and 255 L/sec turbomolecular pump.

Clarus SQ 8 C – Compatible with the Clarus 680 GC, with Electron Impact (EI) and chemical Impact (CI) ionization and 255 L/sec turbomolecular pump.

Clarus SQ 8 S – Compatible with either the Clarus 580 GC or Clarus 680 GC, with Electron Impact (EI) ionization and 75 L/sec turbomolecular pump.

The Gas Chromatograph Mass Spectrometer (GC/MS) is controlled and data analyzed through the TurboMass GC/MS software system.

Clarus SQ 8 MS System compatible with a Clarus 680

Clarus SQ 8 MS System compatible with a Clarus 580 GC

Figure 1 The Clarus SQ 8 MS system

The Clarus SQ 8 GC/MS is controlled by a personal computer (PC) based data system using the Microsoft Windows operating environment. The TurboMass software user interface contains color graphics and provides full user interaction by using either the keyboard or the mouse. TurboMass completely controls the GC/MS system from tuning and data acquisition (scanning or selected ion recording mode) through quantifying your results. Complete operating instructions of all TurboMass controls are in the *TurboMass Software User's Guide* (P/N 09931016) and TurboMass on-line help file, supplied with the system.

A high-performance, research-grade analytical quadrupole mass analyzer with a quadrupole prefilter assembly transmits only those ions having your selected mass-to-charge ratio. The prefilter assembly protects the analytical quadrupole rods from contaminating ion deposits.

Ions emerging from the quadrupole mass analyzer are detected by the electron multiplier detector system. The low noise electron multiplier typically operates with a gain of 10^5 that amplifies the ion current collected.

Vacuum Pump Options

The Clarus SQ 8 MS offers two different vacuum pump capacities. The turbomolecular pump options are designed to fit your applications, performance and budgetary needs.

Turbomolecular Pump

Clarus SQ 8 MS has two turbomolecular pump options in three configurations. Turbomolecular pumps are high-speed turbines which transport the sample and carrier gas molecules away from the mass spectrometer.

- **Clarus SQ 8 S** (compatible with the Clarus 580 and 680 GC) - The 75 L/sec turbomolecular pump supports Electron Ionization operation (EI) and has optional water cooling.
- **Clarus SQ 8 T** – All of the functions and options of the Clarus SQ 8 S with a 255 L/sec turbomolecular pump for higher column flow rates, pump-down time under three minutes, and lower detection limits
- **Clarus SQ 8 C** - All of the functions and options of the Clarus SQ 8 T with positive and negative Chemical Ionization (CI) operation.

Vacuum Gauge

The Clarus GC/MS systems use a single wide-range vacuum gauge that monitors the system pressure from atmosphere down to 10^{-9} Torr using a combined Pirani/Inverted Magnetron ionization sensor.

Normal operating pressure with 1 mL/min helium for the 255 L/sec turbomolecular pump is between $9x10^{-6}$ Torr and $2x10^{-5}$ Torr after pump-down and ion source bake-out. The 75 L/sec turbomolecular pump will operate at a somewhat higher pressure.

Basic GC/MS

As its name implies, this important analytical technique is composed of parts, gas chromatography (GC) and mass spectrometry (MS), which have been combined. Historically, both individual techniques are quite old. GC was developed as a means for separating mixtures into their component substances and provided a big step forward in the analysis of mixtures.

Figure 2 summarizes the GC process. It shows that by passing a mixture in a gas stream (the gas phase) through a long capillary column with the inside walls thinly coated with a liquid (the liquid stationary phase), the components of the mixture separate and emerge (elute) one after another from the end of the column.

In a simple GC instrument, the emerging components are either burnt in a flame for detection (the popular flame ionization detector, FID) or passed to atmosphere after traversing some other kind of detector. The detected components are recorded as peaks on a plot (the gas chromatogram). Information on the possible identity of the component is found by measuring the resulting area and height of a chromatographic peak. This corresponds with the amount of a component detected and the time it takes that component to pass through the column. The measured time to the peak maximum is the retention time. This identification is seldom absolute and is often either vague or not possible at all.

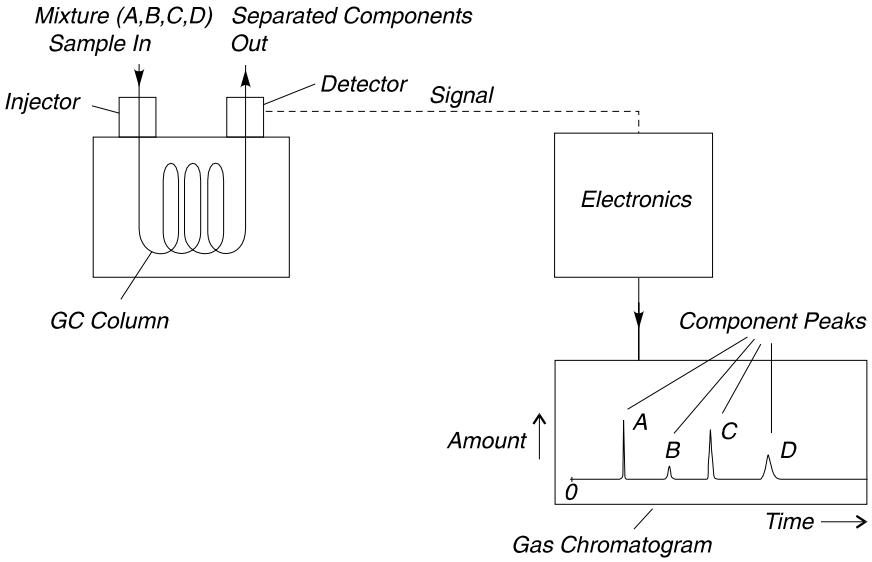


Figure 2 Schematic diagram of a gas chromatograph showing an injection of a mixture of four substances (A, B, C and D) onto a GC column, followed by their separation into individual components, their detection and the display (gas chromatogram) of the separated materials emerging at different times from the column

In complete contrast to a GC, a mass spectrometer is generally not useful for dealing with mixtures. If a single substance is put into a mass spectrometer, its mass spectrum can be obtained using a variety of ionization methods (Figure 3). Having obtained the spectrum it is then often possible to make a positive identification of the substance or to confirm its molecular structure. Clearly, if a mixture of substances were put into the MS, the resulting mass spectrum would be a summation of the spectra of all the components (Figure 4).

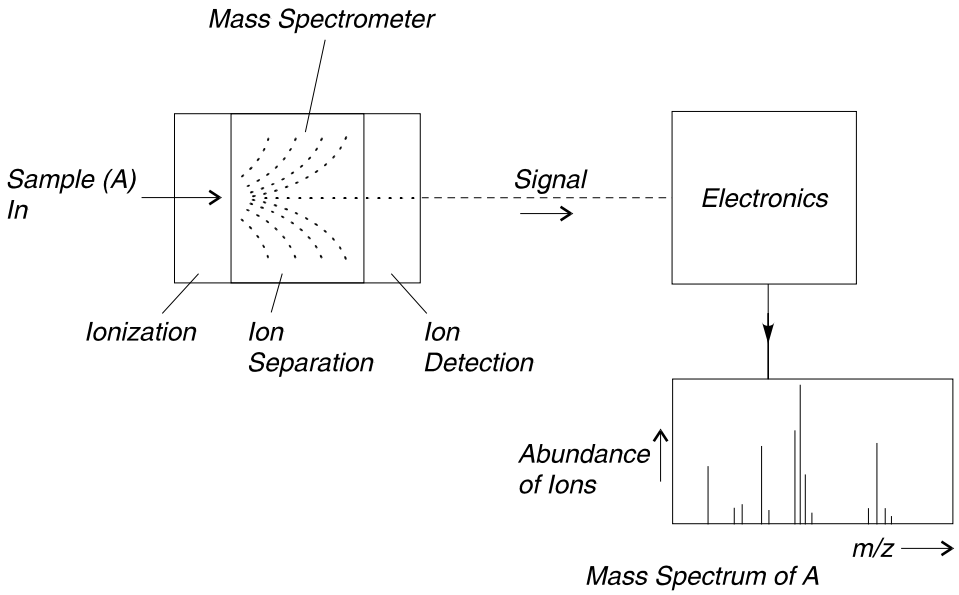


Figure 3 Schematic diagram of a mass spectrometer. After inserting a sample (A) it is ionized, the ions are separated according to their m/z value and, finally, the numbers of ions (abundance) at each m/z value are plotted against m/z to give the mass spectrum of A. By studying the mass spectrum, sample A will be identified

This spectrum could be extremely complex and it would be difficult or impossible to positively identify the various components. Thus, there is one instrument (the GC) that is highly efficient for separating mixtures into their components but is not good on identification and another instrument (the MS), which is efficient at identifying single substances, but is not good with mixtures. It is not surprising to find early efforts being made to combine the two methods into one system (GC/MS) capable of separating, positively identifying, and quantifying complex mixtures, provided these could be vaporized.

Combining GC and MS was not without its problems (see *The Connection between GC and MS* on page 13) but modern GC/MS is now a routinely used methodology in many, many areas, ranging from interplanetary probes to finding levels of dioxins in environmental dust samples. Further, the addition of GC to MS does not simply give a sum of the two alone; the information provided by combined GC/MS yields information that could not be extracted from the isolated techniques, an aspect which is discussed below.

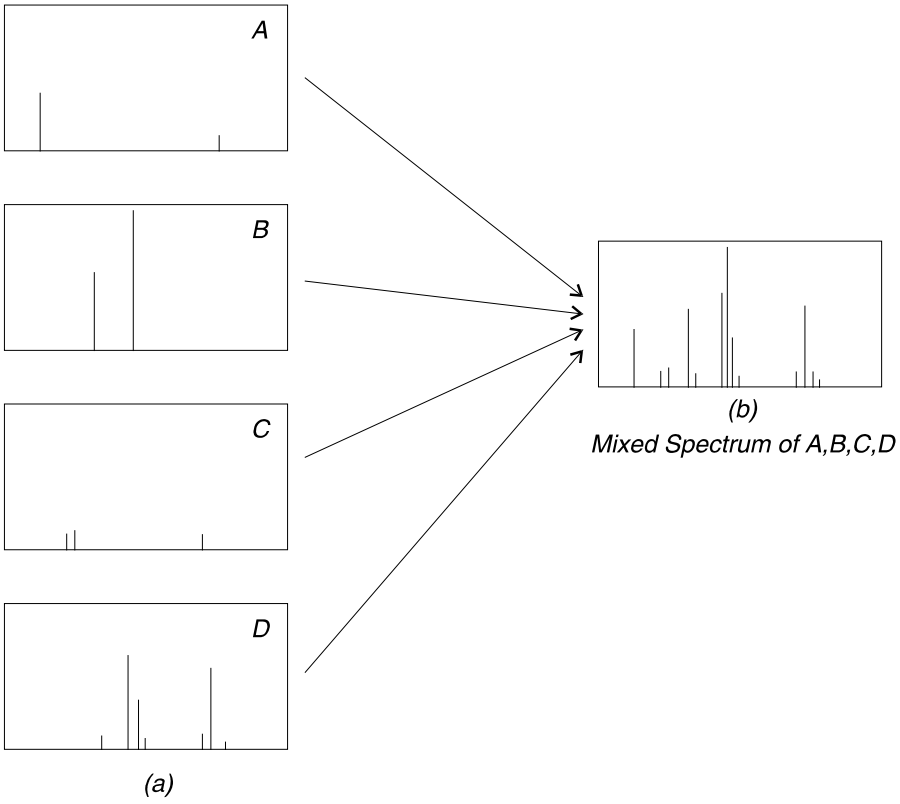


Figure 4 By way of illustration, very simple spectra for four substances (A, B, C, and D) are shown; (a) separately, and (b) mixed in unequal proportions. In the mixture spectrum it is virtually impossible to decode A, B, C and D if they are not know to be present

The Connection between GC and MS

As previously described, the mobile phase carrying mixture components along a gas chromatographic column is a gas, usually helium. This gas flows at or near atmospheric pressure at a rate of generally about 0.5 to 3.0 mL/min and flows out of the end of the capillary column into the ion source of the mass spectrometer. The ion sources in GC/MS systems normally operate at about 2×10^{-5} Torr with 1.0 mL/min helium flowing into the manifold for electron ionization to about 3×10^{-4} Torr for chemical ionization.

This large pressure change between the end of the chromatographic column and the inside of the ion source causes the gas to expand to a flow equivalent to several liters per minute. Therefore, large pumps are required to remove the excess of gas and maintain the vacuum inside the source near that which is optimum for ionization.

In modern GC/MS installations, the use of capillary chromatographic columns and high-speed pumps means that the end of the column can be literally right inside the ion source. In older GC/MS instruments, gas flow rates were much greater and it was necessary to have an interface between the end of the column and the ion source.

This interface or separator removed much of the GC carrier gas without also removing the eluting mixture components that traveled on into the ion source. A most popular form still to be found occasionally was the jet separator.

Recording Mass Spectra

As each mixture component elutes and appears in the ion source, it is normally ionized either by an electron beam (electron ionization) or by a reagent gas (chemical ionization) and the resulting ions are analyzed by the mass spectrometer to give a mass spectrum (Figure 5).

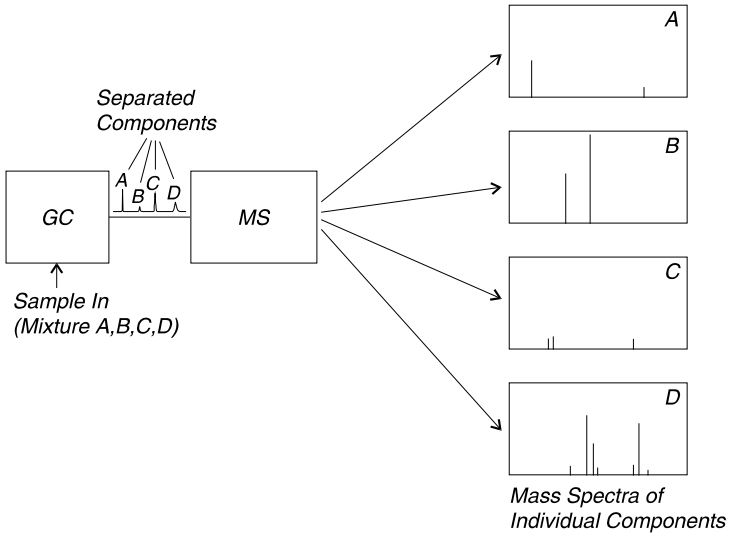


Figure 5 In a GC/MS combination, passage of the separated components (A, B, C and D) successively into the mass spectrometer yields their individual spectra

For capillary GC, separated mixture components elute in a short time interval, often only lasting a few seconds. Thus, the amount of any one component in the ion source is not constant as its mass spectrum is being obtained but, rather, it starts off as zero, rises rapidly to a maximum, and then drops rapidly back to zero.

If this passage through the ion source is faster than the mass spectrometer can scan the spectrum, then a true spectrum will not be found because the start of the scan will see less compound than at the middle of the scan and less again near the end. This changing concentration of the eluting component results in a distorted mass spectrum that might well not be recognizable (Figure 6). The answer to this problem is to scan the spectrum so fast that, in effect, the concentration of the eluting component has scarcely changed during the time needed for a spectrum.

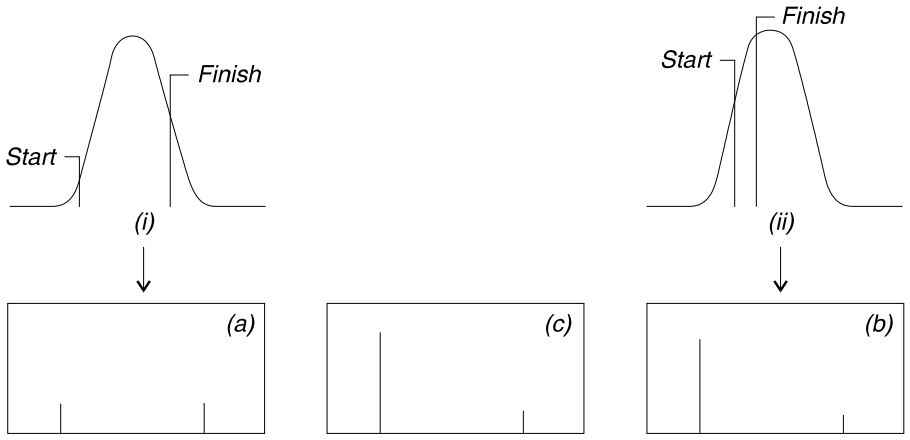


Figure 6 Slow scanning (i) of the mass spectrum over a GC peak for substance A produces a spectrum (a), but rapid scanning (ii) gives spectrum (b) which is much closer to the true spectrum (c)

For a quadrupole mass spectrometer this high rate of scanning is not difficult because it requires only simple changes in some electrical voltages and these changes can be made electronically at very high speed. This is one reason for quadrupoles being popular in GC/MS combinations. In the early days of magnetic sector mass spectrometers the required scanning speed was just not possible because of serious hysteresis effects in the magnets. With modern magnet technology, scanning can be done at high speed with insignificant hysteresis.

As described above, the concentration of an eluting component in the ion source goes from zero to zero through a maximum. Usually, the greater the amount of a substance in an ion source the better the resulting mass spectrum, within reason. This suggests that the best time for a scan will be near the maximum concentration (the top of a GC peak) and that the instrument operator must watch the developing chromatogram continually, trying to judge when best to measure a spectrum.

The answer has been to simply set the mass spectrometer to scanning continuously. As the mixture is injected onto the chromatographic column, repetitive scanning is instituted over a preset mass range (for example, 50 – 500 mass units) at a preset interval (for example, every ½ second). Thus, scan follows scan right through the chromatogram and, literally, hundreds of mass spectra may be recorded in a routine

GC/MS experiment (Figure 7). Data flows from the mass spectrometer at such a high speed that a microprocessor/computer is absolutely necessary to process it.

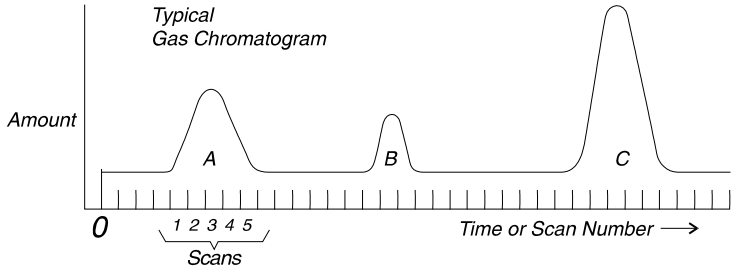


Figure 7 A typical gas chromatogram showing three components (A, B and C) emerging at different times. Continuous scanning for mass spectra is started at zero and scans are repeated regularly (small tick marks). Thus, peak A is scanned five times during its passage through the ion source. Because scanning is regular, the "time" axis can be replaced by a scan number axis

At the end of a GC/MS run all of the data are stored, usually on a hard disk in the computer, in the form of a mass spectrum for every scan that was done. These spectra can then be manipulated. For example, the scan corresponding to the top of a chromatographic peak can be selected (scan 3 or 4 in Figure 7 for example) and the mass spectrum displayed or printed out. As a routine, the computer adds up all the ion peaks in each mass spectrum to give a total ion current for the mass spectrum. These total ion currents are then plotted along an x-axis (time for elution) and a y-axis (amount of total ion current) to give a total ion current chromatogram (TIC) showing the elution of all the components of a mixture. Storage of all these data has other major advantages.

Manipulation of Scan Data

Some ways in which data can be utilized are described briefly below.

Background Subtraction

Gas chromatograms are often obtained by running the column at a sufficiently high temperature. This causes the stationary liquid phase on the column walls to volatilize to a noticeable extent and it too begins to elute in the mobile gas stream

from the end of the column. This effect is column bleed. Clearly, if the liquid phase is eluting all the time, it must reach the ion source when there is and even when there is not an eluting mixture component and, since the spectrometer scans continuously, the mass spectrum of the column bleed is recorded continuously. Therefore, the recorded spectrum for an eluting component will not be pure but will be mixed with that from the column bleed.

Fortunately, it is a fairly simple matter to get the computer to subtract the known column bleed spectrum from the mixed spectrum so as to obtain the desired pure spectrum of the eluting component. This process is called background subtraction and is usually carried out routinely before a library search (next item) or before the spectrum is printed out (Figure 8).

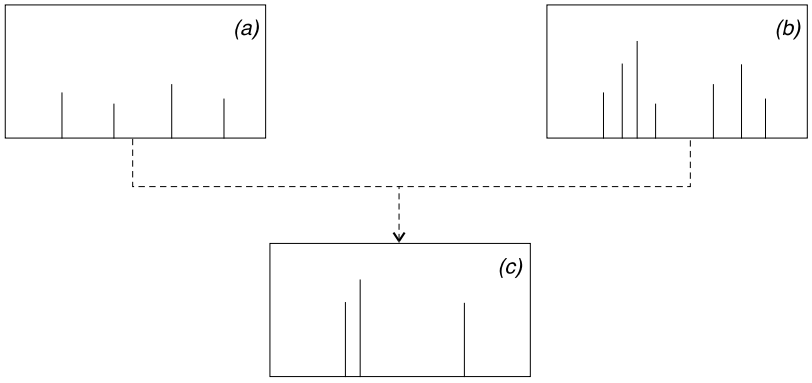


Figure 8 Column bleed will give a mass spectrum (a), which is mixed with that of an eluting component to give a complex spectrum (b). By subtracting (a) from (b) the true spectrum (c) of the eluting component is obtained

Identifying the Component

After obtaining a good pure mass spectrum from an eluting component, the next step is to try to identify the component either through the skill of the mass spectroscopist or by resorting to a library search. Most modern GC/MS systems with attached data station include a large library of spectra from known compounds (for example, the NIST library). There may be as many as 60,000 - 220,000 stored spectra, covering most of the known simple volatile compounds likely to be met in analytical work. By special search routines under the control of the computer, this huge database can

be examined very quickly, comparing the mass spectrum of the eluted mixture component with each of the library spectra. The computer then provides a short list of the best matches between the library spectra and the measured one. Often, from the goodness of match or fit and its chromatographic retention time, the eluted component can be identified.

Resolution Enhancement

Sometimes a single peak from a gas chromatogram may actually be due to not just one eluting substance but to two, three, or more substances all eluting very close together. By examining the scans across the peak and using a simple mathematical process, the computer is able to reveal the existence of more than one eluting component in a single peak and print out the mass spectra of the discovered components. This is a form of resolution enhancement. The GC/MS combination has been able to do what neither the GC nor the MS alone could do. In effect the mass spectrometer has improved (enhanced) the resolution of the gas chromatograph (Figure 9).

Figure 9 A single peak from an ordinary gas chromatogram (a) is revealed as two closely separated peaks by resolution enhancement (b)

Identifying a Series of Compounds

There could be a series of compounds, designated RX, in which the part R was the same for all but X was different. In such a series the part R can give a characteristic ion, that is, the mass corresponding to R is the same for every member of the series. When one member of such a series elutes from the chromatographic column its resulting mass spectrum will always contain one mass common to the whole series. Therefore, even if the series is mixed with other compounds, its members can be recognized from the characteristic ion. It is a simple process to get the computer to print out a chromatogram in which only those scans which contain the characteristic ion are used to draw the chromatogram. Basically, the output is blind to any component other than those containing an R group. Such a selected chromatogram is called a *mass chromatogram* and is useful for pinpointing where certain compounds elute without the need for examining all the spectra (*Figure 10*).

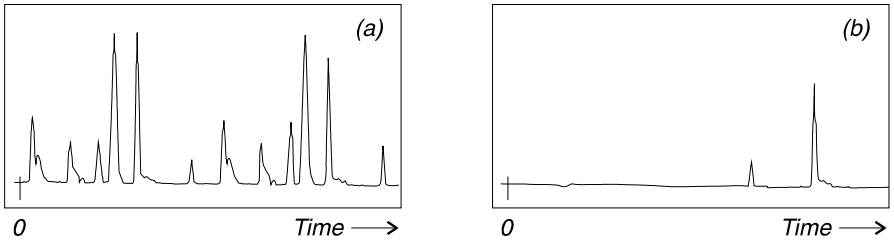


Figure 10 (a) A typical total ion current gas chromatogram showing many components, some of which have a part (R of characteristic mass, say 91). (b) A mass chromatogram based on m/z 91. The same data as used in (a) are plotted but now manipulated so that only ion currents corresponding to m/z 91 are shown. Note the decrease in complexity, making the desired identification much easier

Identifying Specific Compounds

In a process somewhat similar to that described above, the stored data can be used to identify not just series of compounds but specific ones. For example, any compound containing a chlorine atom is obvious from its mass spectrum because natural chlorine occurs as two isotopes, ^{35}Cl and ^{37}Cl in a ratio of 3:1. Thus, its mass spectrum will have two molecular ions separated by two mass units (35 + 2 = 37) in an abundance ratio of 3: l. It becomes a trivial exercise for the computer to print out

only those scans in which two ions are found separated by two mass units in the abundance ratio of 3:1 (Figure 11).

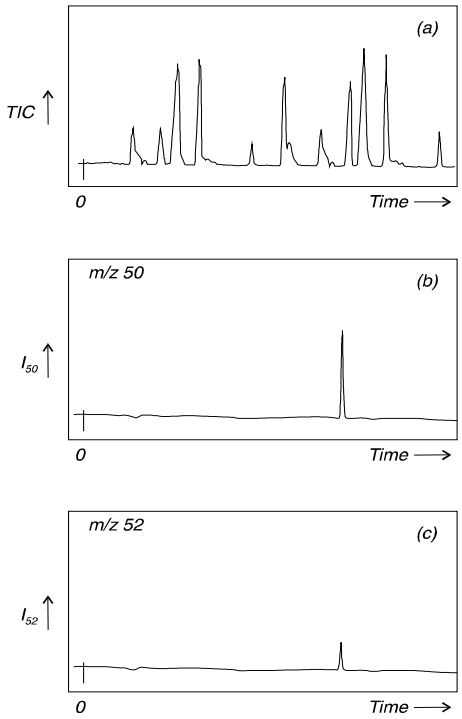


Figure 11 (a) A typical total ion current chromatogram showing many components of a mixture. The aim is to find if any methyl chloride (CH_3Cl; RMM = 50, 52) is present. To this end, the data (or a new chromatogram) are scanned at two specific positions: (b) at m/z 50 and (c) at m/z 52
Note how m/z 50, 52 both reach a maximum at the same scan and have a ratio in peak heights of 3:1 (^{35}CI:^{37}CI = 3:1). Such an experiment in selected ion recording identifies the suspected component against a complex background and can be very sensitive

This selection of only certain ion masses is called selected ion recording (SIR) or selected ion monitoring (SIM).

The ions selected for such recording can be one, two, three or more (multiple ion recording). In fact, through judicious choice of ions, the method can be so selective that a chosen component can be identified and quantified even though it could not even be observed in the original total ion current chromatogram. This very powerful technique is frequently used in examinations of extremely complex mixtures in which it is desired to identify small amounts of a particular substance in a mass of other things, as with detection of banned drugs in the body fluids of athletes or racehorses.

Conclusion

By connecting a gas chromatograph to a suitable mass spectrometer and adding a data system, the combined method of GC/MS can be used routinely for separation of complex mixtures into their individual components, identification of the components, and estimation of their amounts. This technique is in very widespread use.

Definition of Terms and Abbreviations

The terms and abbreviations used in this tutorial are described in the Glossary of Mass Spectrometry Definitions and Terms in Chapter 3 of the Clarus SQ 8 MS Hardware Guide (P/N 09931017). Additional Reading-Bibliography of GC/MS Books.

Basic Introductions to GC/MS

Message, Gordon M. *Practical Aspects of Gas Chromatography/Mass Spectrometry*. John Wiley & Sons, 1984.

Middleditch, Brian S. *Practical Mass Spectrometr*y. Plenum Press, 1979.

Watson, J. Throck. *Introduction to Mass Spectrometry*. Raven Press, 1997.

Davis, Reg, and Frearson, Martin. *Mass Spectrometry: Analytical Chemistry by Open Learning*. edited by F. Elizabeth Prichard. John Wiley & Sons, Pub., 1999.

Dawson, Peter H. (Editor). *Quadrupole Mass Spectrometry and Its Applications*. Amer Inst of Physics, 1995.

Harrison, Alex G. *Chemical Ionization Mass Spectrometry*. CRC Press, 1992.

Mass Spectrum Interpretation

McLafferty, F.W. *Interpretation of Mass Spectra*. University Science Books, 4th ed., 1993.

McLafferty, Fred W. and Venkataraghaven, Rengachari. *Mass Spectral Correlations* 2nd Ed. American Chemical Society, Advances in Chemistry Series #40, 1982.

Computer Requirements

Computer and System Software Requirements

To ensure that your system performs at the expected high level, your computer must be configured to the minimum capabilities indicated below.

These requirements may be updated as the requirements for TurboMass software and/or Microsoft Windows 7 are changed. Consult your PerkinElmer support engineer for current requirements.

NOTE: *This guide does not cover the installation and configuration of your computer. If you have purchased a complete system from PerkinElmer, the computer will already have been configured.*

PC Requirements

The TurboMass software is installed at PerkinElmer prior to shipment and tested using the following minimum PC system specifications. If you need to reinstall the software, verify that the PC meets the following minimum requirements:

- Lenovo ThinkCentre® M58p
- 3.0 GHz Intel® Core 2 Duo Processor
- 4 GB of Random Access Memory (RAM)
- Integrated video, Intel® GMA4500
- Hard disk with 2.0 GB free space
- 1 RS-232 port
- 2 RJ-45 10/100Base-T ports
- Lenovo USB Keyboard and Lenovo USB optical mouse with scroll

Operating System

Windows 7

Software

TurboMass Software

Instrument Firmware Versions

Internal dotLINK

Printers

HP LaserJet P4014 Printer Series (CB506A)

NOTE: *Using any printers other than the ones recommended above may not correctly display the Communiqué reports.*

Conventions Used in This Manual

Normal text is used to provide information and instructions.

Bold text refers to text that is displayed on the screen.

UPPERCASE text, for example ENTER or ALT, refers to keys on the PC keyboard. '+' is used to show that you have to press two keys at the same time, for example ALT + F.

Three terms (Note, Caution and Warning) are used in the text. Each one implies a particular level of observation or action as follows:

NOTE: *A note indicates additional, significant information that is provided with some procedures.*

CAUTION

*A caution indicates an operation that could cause **instrument damage** if precautions are not followed.*

WARNING

*A warning indicates an operation that could cause **personal injury** if precautions are not followed.*

Clarus GC

If you have a Clarus SQ 8 T or Clarus SQ 8 C you operate and control the Clarus 680 GC using the GC control section of the TurboMass software and/or the GC touch screen.

If you have a Clarus SQ 8 S, then you operate and control either a Clarus 580 GC or Clarus 680 C using the GC control section of the TurboMass software and/or the GC touch screen. The GC touch screen contains active areas that you touch to perform the required action.

You may find it more convenient to use a stylus or you may like to use your fingers to touch the active areas of the touch screen. **Do not use sharp, pointed objects**. A light touch is all that is needed. You do not need to press forcefully on an active area.

Please note that when you touch an active area, various processes are started in the instrument. Some of these processes may take several seconds. Be patient and wait for the process to complete before touching another active area on the touch screen.

When the GC is under the TurboMass software control, some functions are not available for use on the touch screen.

Touch Screen

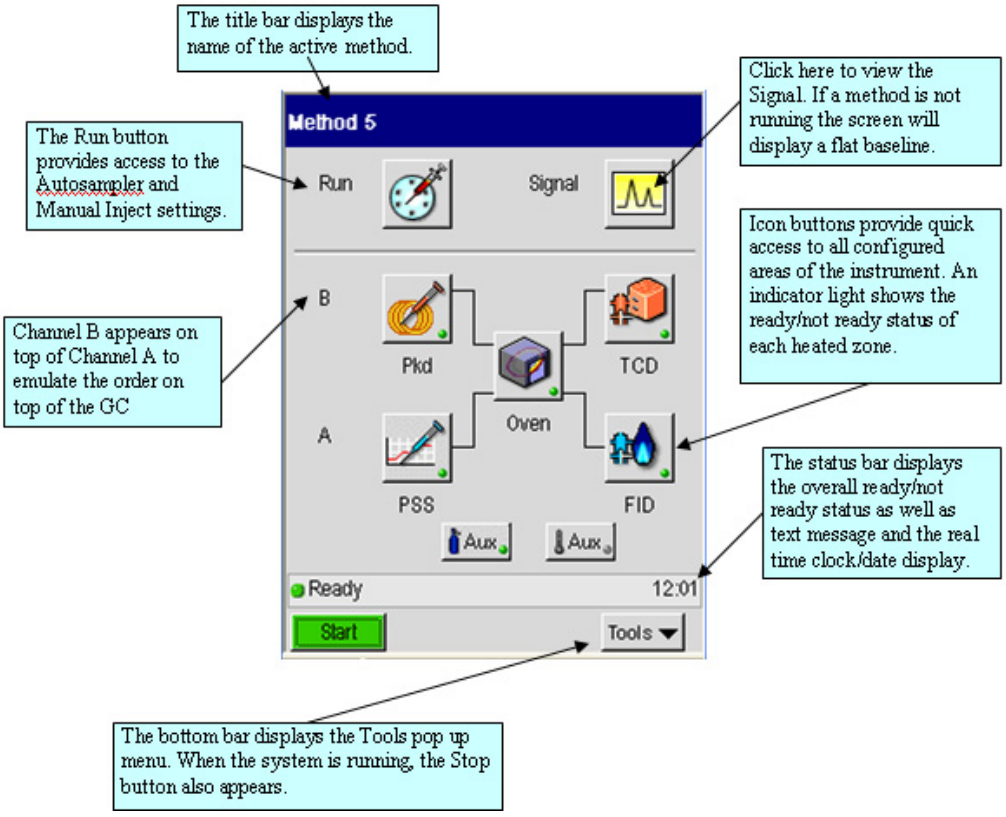
The main displays on the touch screen are termed screens. You use various screens to set up your Clarus GC to perform the analyses. These screens contain:

- **Entry fields** which allow you to make entries,
- **Buttons** that you touch to start or stop actions or to display a dialog,
- **Option Buttons** that you select an option from a list and
- **Boxes** that you use to switch functions on or off.

The Clarus GC is controlled by a collection of operating parameters called the Active Method. You can prepare and save up to five methods and make any one of them the Active Method. However, the fifth method is reserved for TotalChrom and may be overwritten.

The following Status screen shows the different sections of the user interface.

The Status Screen displays icons that provide quick access to major areas of the system. The injector and detector buttons show graphic representations of the devices for each channel. If an auxiliary zone is configured, the Aux button appears below the Oven button. The icon buttons that represent the heated zones (injectors, detectors, oven, and Aux if configured) include a light to indicate the ready/not ready status. A red blinking light indicates not ready and a steady green light indicates ready status.



2 How to Start the Clarus MS from a Cold State

Starting the Clarus MS from a Cold State

This chapter describes how to start the Clarus MS from a cold state. This implies that the system is off. Before starting the Clarus MS system, first verify that the hardware is properly connected. After verifying that the hardware is properly connected, you can start your Clarus MS system and bring it to the point where you are ready to create a GC/MS method.

The following steps summarize the procedure for staring the Clarus GC/MS system from a cold state:

1. Verify hardware connections.
2. Verify sufficient carrier gas to the Clarus GC.
3. Turn on all instruments.
4. Set the Clarus GC to communicate with the mass spectrometer.
5. Start Clarus GC and Clarus MS.

Verifying hardware connections

Before turning on any instrument, answer the following questions:

- Are the gas lines connected to the GC? Are they leak-free?
- Is the mass spectrometer vacuum line connected to the forepump?
- Are all AC line cords plugged-in to the proper AC voltage outlets?
- Is the proper source (EI or CI) installed for your analysis?
- Is the mass spectrometer forepump plugged into the outlet on the rear of the mass spectrometer and the power switch set to ON?
- Is one end of the GC start cable connected to the plug located in the lower right rear of mass spectrometer?

- Is the other end of the GC start cable connected to terminals 7 and 8 (Start Out) on TB1 in the Clarus GC?

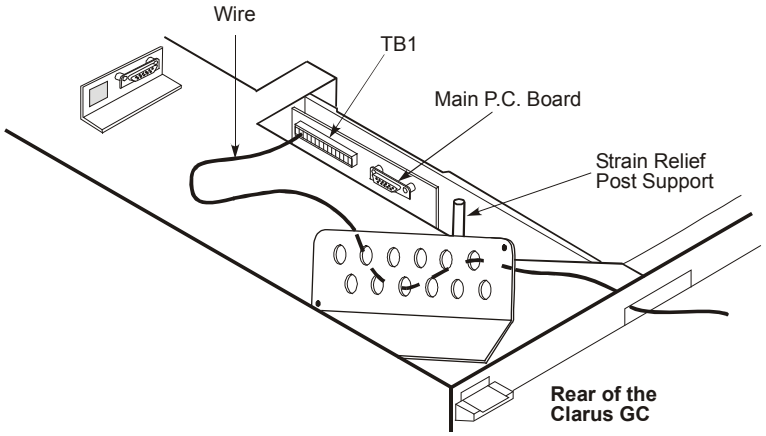


Figure 12 The location of TB1 in the Clarus 680 GC

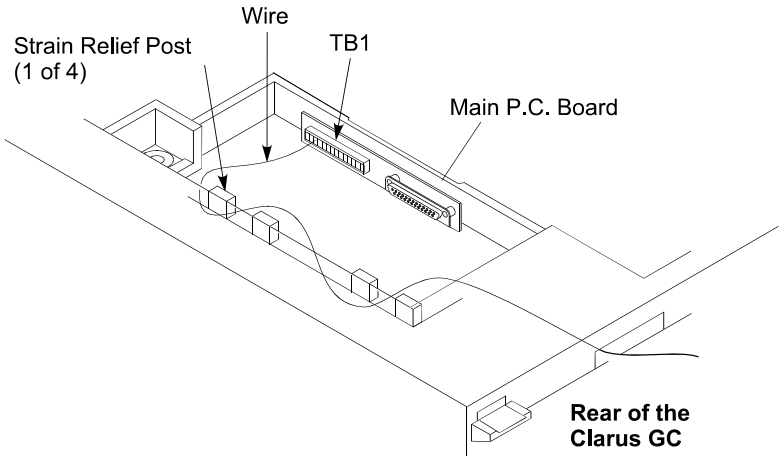


Figure 13 The location of TB1 in the Clarus 580 GC

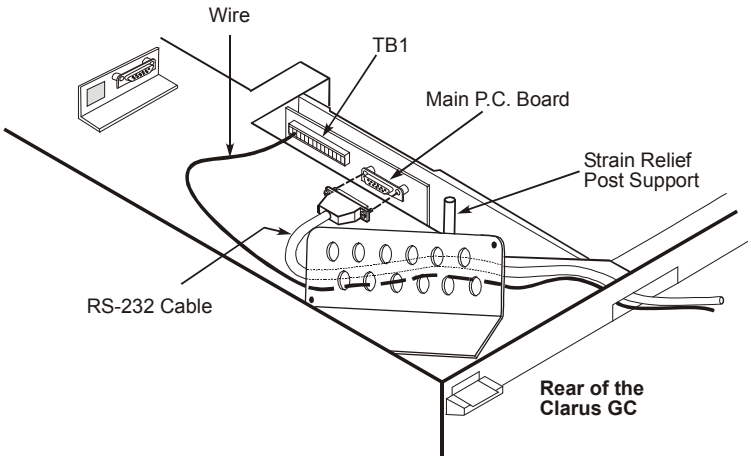


Figure 14 View of a Clarus 680 GC TB1 with a PC cable connected

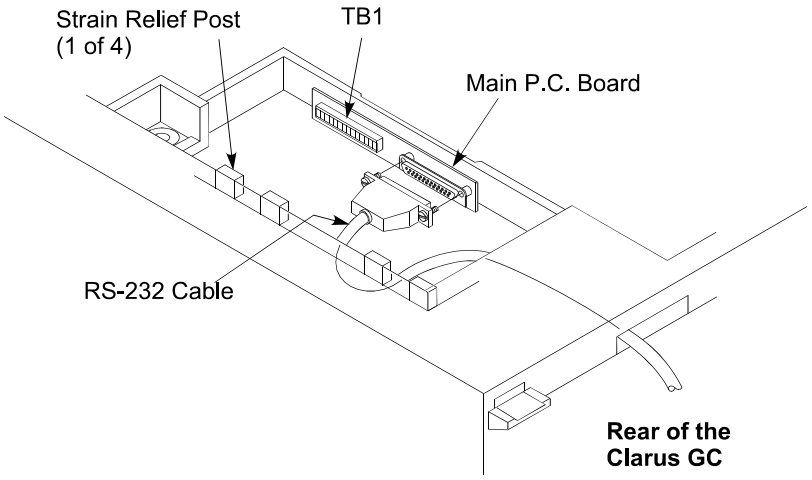


Figure 15 View of a Clarus 580 GC TB1 with a PC cable connected

- Is the Clarus cabling properly connected as shown below?

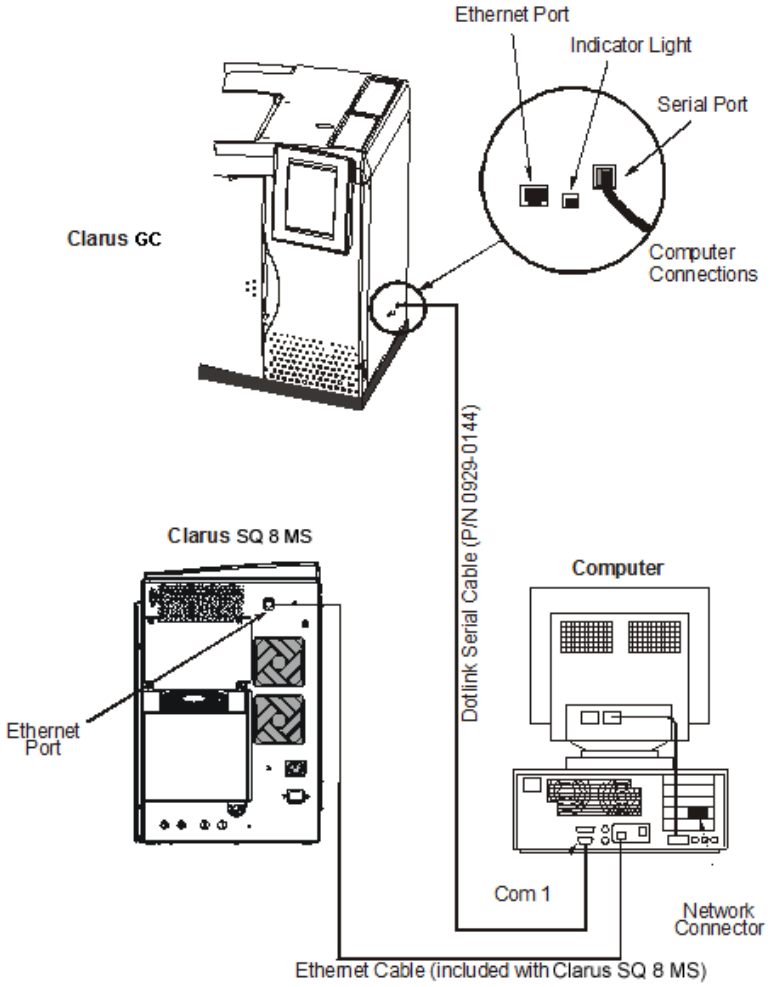


Figure 16 Connections between a computer, Clarus SQ 8 T, C, or S and Clarus 680 GC

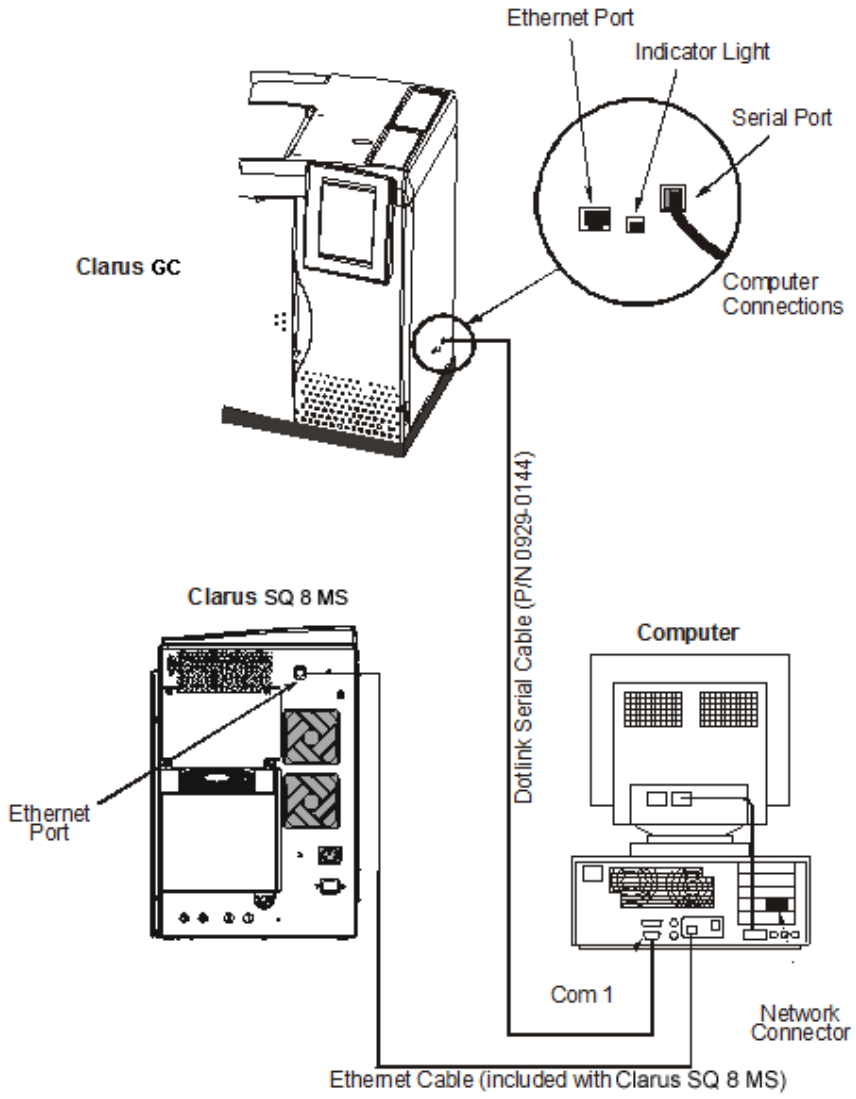


Figure 17 Connections between a computer, Clarus SQ 8 S MS and Clarus 580 GC

Verify sufficient carrier gas to the GC

Before turning on the GC power, verify that the carrier gas tank contains sufficient gas and that the main valve is turned on. Set the tank regulator to 90 psig (621 kPa) and open the regulator needle valve.

Turn on all instruments

CAUTION	*To prevent heating the GC column before turning on the carrier gas, verify that the GC oven door is open before turning on the GC.*

Turn on the power switches of each instrument in the following order.

1. Computer
2. Printer and additional peripherals
3. Clarus GC
4. Mass spectrometer

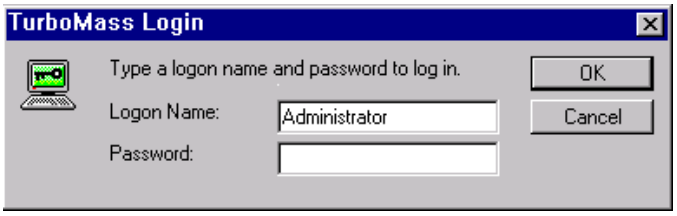
NOTE: *Wait for the Windows desktop to appear before powering on the mass spectrometer.*

Set the Clarus GC to communicate with the mass spectrometer

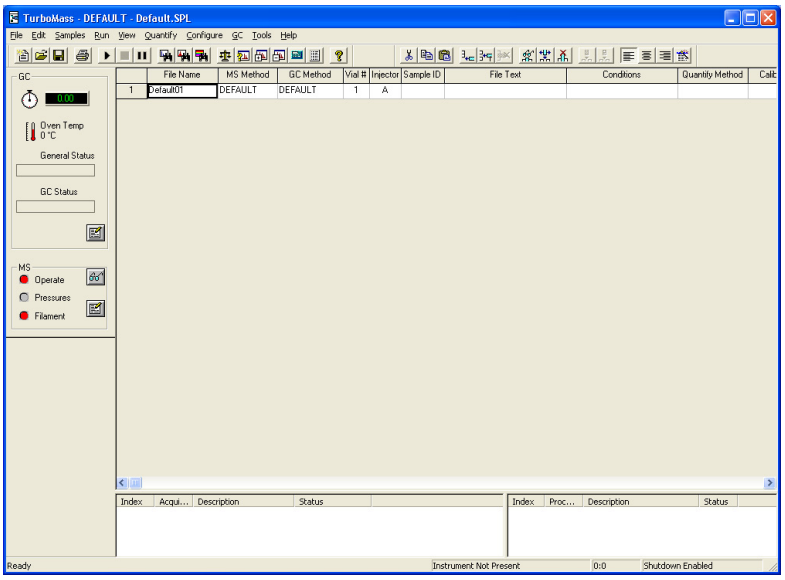
Unlike the AutoSystem XL GC, the Clarus GC is automatically ready to be controlled by the computer.

Starting TurboMass

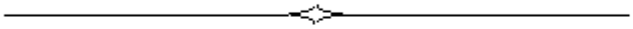
1. To start TurboMass double-click the TurboMass desktop icon.
   The *TurboMass login* dialog is displayed.



2. Enter the **Logon Name** and the **Password** (if any), then click **OK**.
   The initial TurboMass window is displayed.



If GC control has not been set up, an error message appears.
Set up TurboMass and the GC as described in the next section



3 How to Set Up TurboMass and the GC

Configuring TurboMass for GC Control

Before you begin to use TurboMass for the first time, you must first configure it (or verify that it is configured) to interact with the Clarus GC.

After establishing communication between the instruments in the system and creating your GC method, you can develop your TurboMass method.

The procedure used to configure the GC depends upon whether you are initially configuring TurboMass for GC control or are making front panel changes to the GC.

- **Initial GC Configuration:** To set up the dotLINK and GC for TurboMass control for the first time.

- **Reconfiguring the GC:** To make front panel or hardware changes to the GC without changing the LINK configuration. Reconfiguration is also required if you add an autosampler.

Configuring the GC for the First Time

The following steps summarize the procedure for configuring TurboMass for GC control the first time:

1. Display the top level (main) window.

2. Select an interface.

3. Select **Configure** from the *GC* menu.

4. Verify the Data Acquisition port.

5. Set the *LINK Configuration* Options.

6. Set the *GC Configuration* Options.

To configure the Clarus GC:

NOTE: *This example show screen references to a Clarus SQ 8 T system. If you have a Clarus SQ 8 S system with a Clarus 580 GC, then select the Clarus 580 GC.*

1. In the top-level window, **Select Inlet Interface** from the Configure menu.

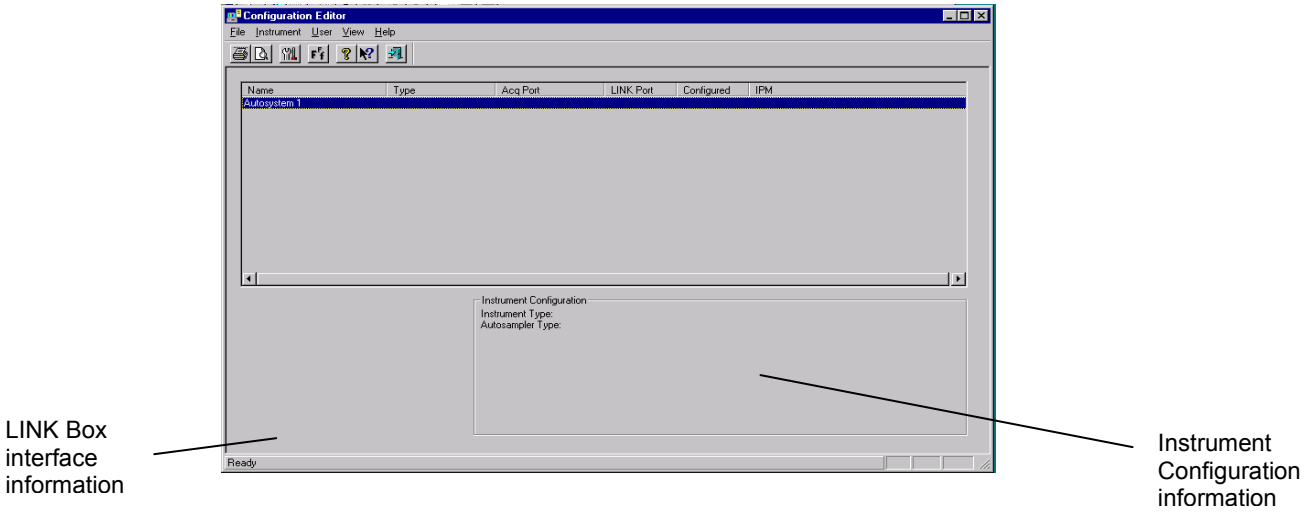
2. Select **Clarus 680** (or if you have the Clarus SQ 8 S system, you can select **Clarus 580**).

3. Click **OK**.

   All GC commands are accessible from the **GC** menu in the top-level window. From this menu you can set up your GC configuration, develop your GC method, work interactively with the GC, and execute all other GC related procedures.

4. From the *GC* menu, select **Configure**.

   The *Configuration Editor* summary window is displayed. This displays the GC information that will be defined during configuration.



After configuring the GC, the area below the Configuration Editor summary list displays key GC information. The box on the left contains information about the LINK interface, which includes the type (model number), EPROM

version number, memory size available in the interface (in bytes), and serial number. The box on the right contains a summary of the GC configuration.

Summary of information displayed on the Configuration Editor window:

Field	Description
Name	The name of the GC.
Type	The GC model or type.
Acq Port	The physical data acquisition port to which the LINK interface is connected.
LINK Port	The physical port in the LINK interface to which the GC is connected.
Configured	Displays **YES** if you provided all the information needed to configure the GC. Otherwise, **NO** is displayed.
IPM	Displays **YES** if the Instrument Personality Module (IPM) for the GC has been downloaded. The first time you open this window, the IPM will not have been downloaded.

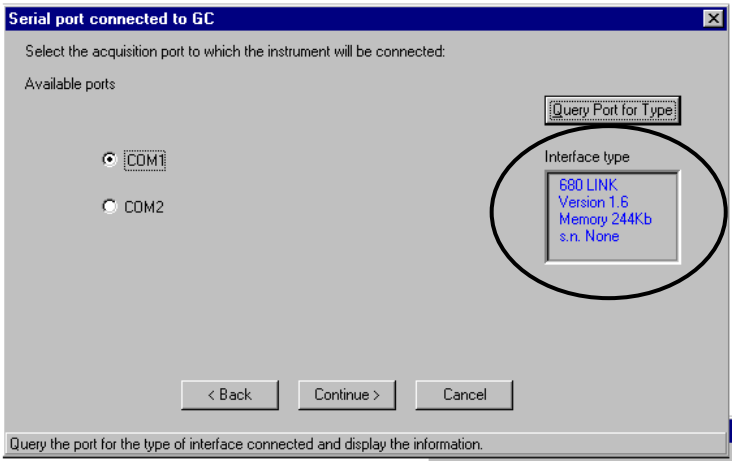
5. From the *Configuration Editor* window, select **Configure** from the *Instrument* menu.

   The serial port connected to GC dialog is displayed.

6. Verify the serial port (in this example, COM1), LINK box connection, and firmware version.

NOTE: *If the mass spectrometer and your computer were configured for COM2, then COM2 is your serial port.*

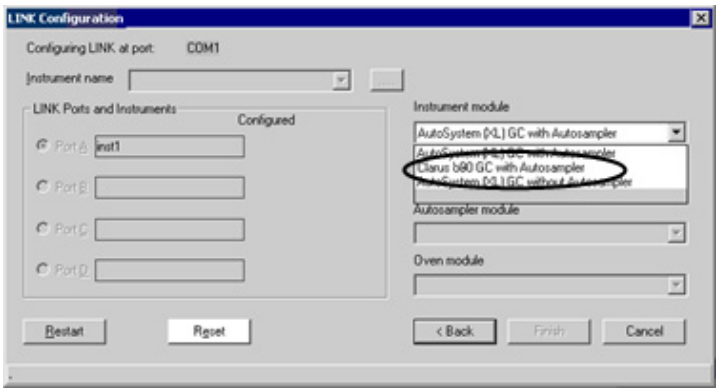
7. Click on the **Query Port** for *Type* button.
   The Interface type is displayed in the box.



8. Click the **Continue** button.
   The *LINK Configuration* dialog with the COM port and instrument name displays.

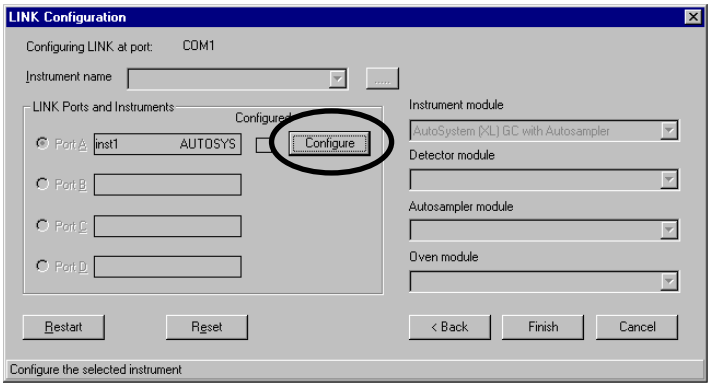
9. Select **Port A** from the **LINK Ports and Instruments** list:
   inst1 is displayed in the box to the right of Port A.

10. Select the **Clarus 680 GC (L) with Autosampler** (or if you have the Clarus SQ 8 MS system with a Clarus 580 GC, then select **Clarus 580 GC with Autosampler**) in the *Instrument Module* list.
    If your Clarus 680 GC has an autosampler, select **Clarus 680 GC with Autosampler** even if you won't be using the autosampler. You can change this setting from the *Method Editor*.



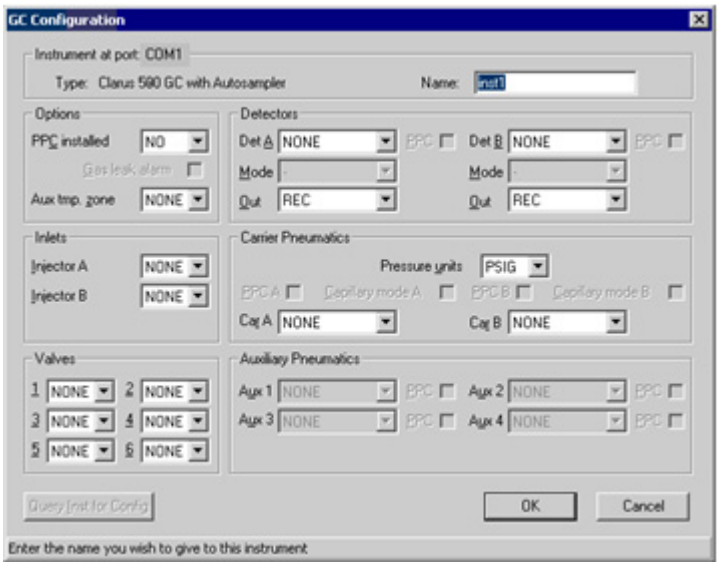
If you make a mistake, click **Restart**. This disconnects the GC and clears the LINK port. Clicking **Reset** clears all the changes to this dialog and returns it to the state it was in before you opened it.

When the software verifies this connection, a box appears under **Configured** and a **Configure** button appears.



11. In the *LINK Configuration* dialog, click the **Configure** button next to the port selection.

    The *GC Configuration* dialog is displayed.

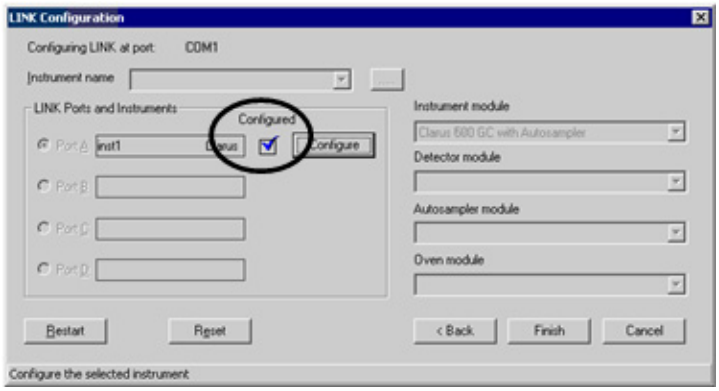


12. To rename the GC to something other than its default name (*inst1*), enter the new name in the *Name* text box.

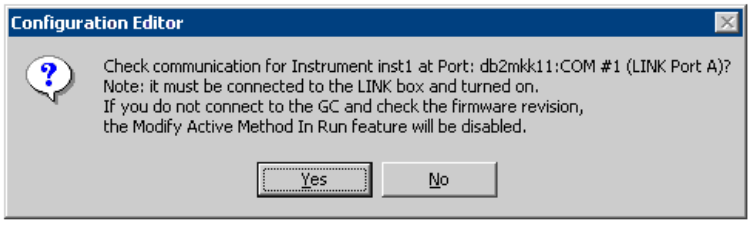
    This name will appear under the *Name* field in the *Configuration Editor* window.

13. Click **OK**.

    A check mark in the *LINK Configuration* dialog indicates that the GC has been configured.



14. Click **Finish**.
    When you first configure the GC, the following message may display:

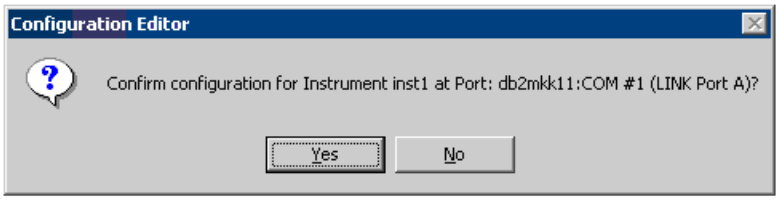


15. If the GC is not turned on, turn it on now and then click **Yes**.

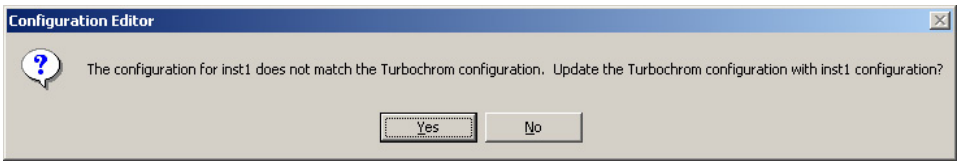
    OR

    If the GC is connected and turned on, click **Yes**.
    The following message is displayed:

16. Click **OK**.
    The following message is displayed:

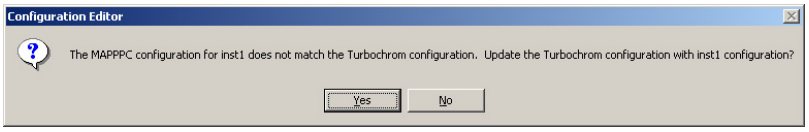


17. Click **Yes**.
    The following message is displayed:

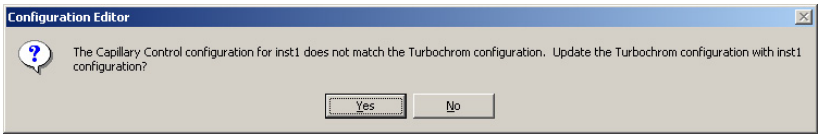


18. Click **Yes**.
    If your Clarus GC has PPC, the following message is displayed:

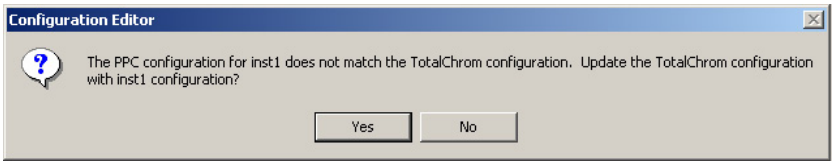
19. Click **Yes**.
    The following message is displayed:



20. Click **Yes**.
    The following message is displayed:

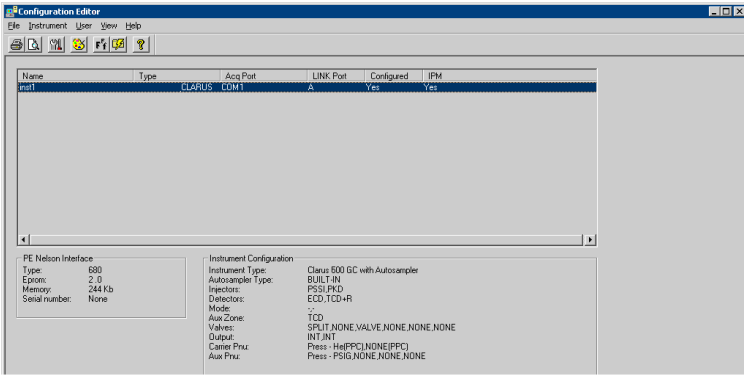


21. Click **Yes**.
    The following message is displayed:

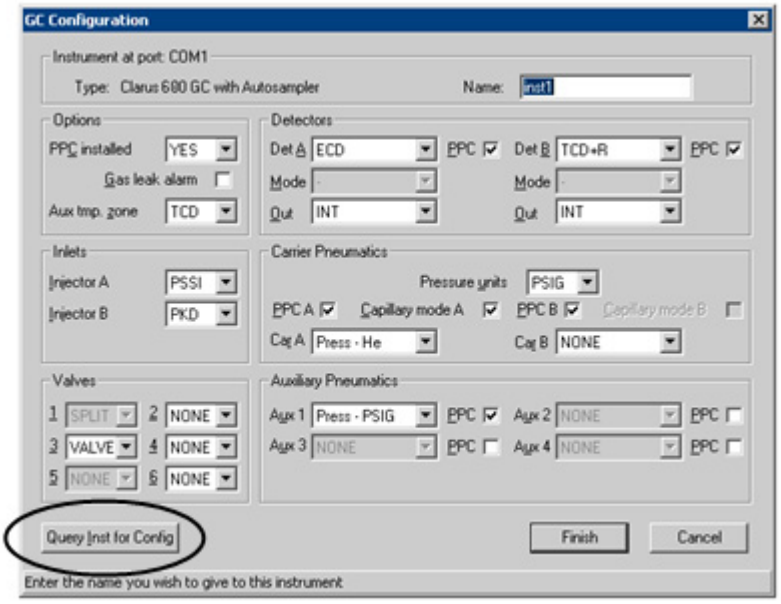


22. Click **Yes**.

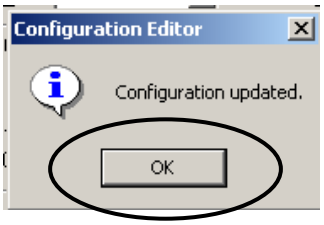
The following screen is displayed:



23. Select **Configure** from the *Instrument* menu.
The following screen is displayed.

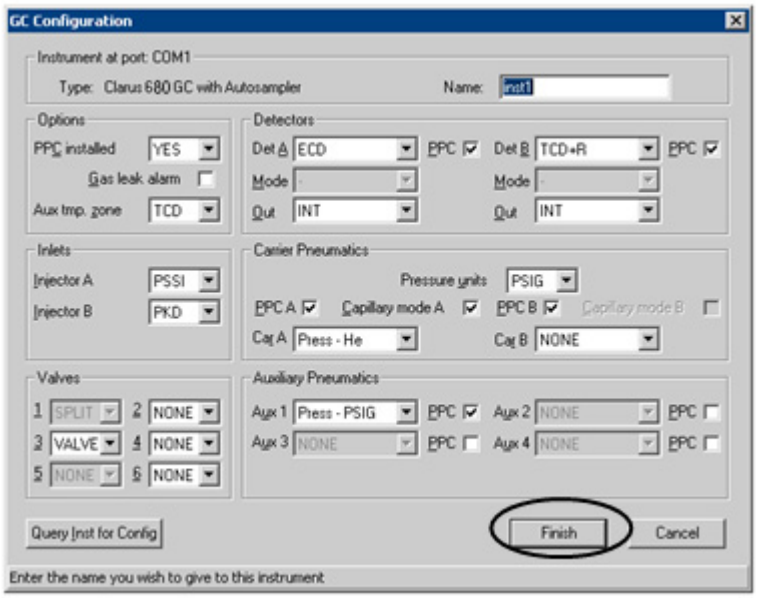


24. Click the **Query Inst for Config** button.
The following is displayed.

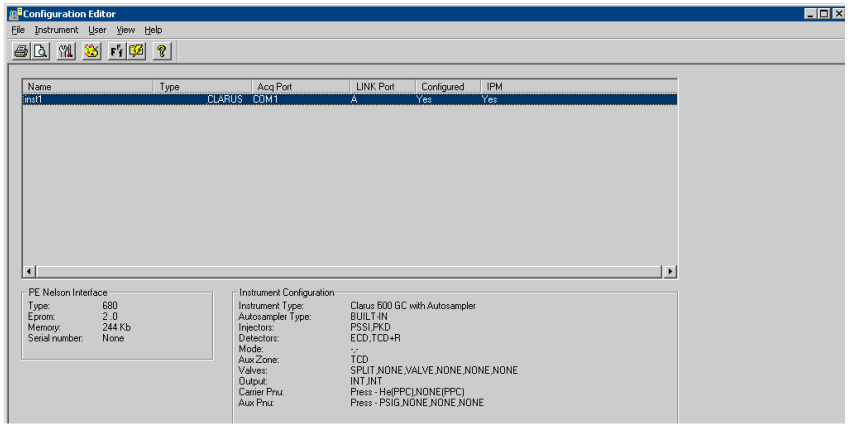


25. Click **OK**.

    The *Configuration Editor* window is updated and your instrument is now configured and the GC Configuration screen displays.



26. Click **Finish.**



27. Close the *Configuration Editor* by selecting **Exit** from the File menu.

How to Select and Install the GC Column 4

How to Select and Install the GC Column

Before searching for the proper column, you must understand your sample and the type of analysis you are about to perform. Gas chromatography (GC) provides the means for separating components in a mixture by eluting the sample with a carrier gas (helium) over a stationary phase contained within a column. Capillary columns are used with an (increasing) temperature program to ensure that all of the components have been eluted from the column. A series of quite narrow (several seconds wide) chromatographic peaks emerge and can be detected by the mass spectrometer (MS).

In a GC/MS analysis, the column is installed in the GC oven with one end connected to a GC injector and the other end passed through a heated interface (through the GC oven wall), where the column end is precisely positioned at the inner source of the mass spectrometer. The Clarus SQ 8 MS uses two types of inner sources: (1) a source for electron ionization (EI); and, (2) a source for chemical ionization (CI). The following steps summarize the procedure for selecting and installing the column.

Selecting the proper column

1. Consider the variables of a GC/MS analysis.
2. Consider five variables when choosing a GC column.

Installing the column

1. Prepare the system to install the GC column.
2. Remove an installed column.
3. Connect one end of the new column to the GC injector.
4. Condition the new column for use.
5. Connect the other end of the new column to the mass spectrometer.
6. Equilibrate the system.

Selecting the Proper GC Column

Consider the following variables when performing GC/MS analyses

Variable	Variable Options	Reason
GC column	Stationary phase Column length Column internal diameter Stationary phase film thickness	Depends on the sample type, sample concentration, and complexity.
GC injection port	On-column Split/splitless Temperature Split/flow	Depends on the sample volatility and concentration.
GC oven temperature program	Isothermal periods Ramp rates	Depends on the GC column, and on the sample, its volatility and complexity.
GC column gas pressure	Isobaric or programmed Isobaric periods Ramp rates	Depends on the GC column, and on the sample, its volatility and complexity.
MS ionization mode	EI CI+ CI–	Depends on the polarity of the sample and its tendency to fragment in EI, and also the type of information required (MW only or structural/ library searchable).

Consider the following five variables when choosing a GC column:

- The types of samples you will be analyzing.
- The stationary phase of the column.
- The internal diameter of the column.
- The thickness of the stationary phase.
- The column length.

Know the types of samples you will be analyzing. Are they volatile, semi-volatile, pesticides, solvents, etc. Next choose a stationary phase based on polarity of the sample. A very general rule in column selection is that "like dissolves like." Column polarity has the greatest effect on how the column separates the compounds of interest as the sample interacts with the stationary phase. There are different degrees of polarity from non-polar to very polar. When compounds are separated primarily on their boiling points, the phase is considered to be non-polar. Polar phases typically separate compounds based on the chemical interactions between the sample components and the stationary phase.

Many different types of stationary phase are available and the following list is by no means exhaustive. Consult recent manufacturers' literature for the particular application you have in mind.

Stationary Phase	Trade Names	Sample Type
100% dimethylpoly-siloxane	PE-1 MS, CPSil 5, SE 30, OV-1, OV-101, SP 2100, SF 96, DB 1.	General use; including, hydrocarbons, pesticides, steroids, alkaloids, and derivatized sugars.
5% phenyl, 95% methyl-polysiloxane	PE-5MS, CPSil 8, SE 52, SE 54, OV-73, SPB 5, DB 5.	Polycyclic hydrocarbons, di- and tri- glycerides, alcohols, fatty acids, pesticides, steroids, PCBs.
polyethylene glycol	CPWax 51, CPWax 57, Carbowax 20M, Carbowax 1000, SP 1000, OV-351.	Essential oils, fatty acids, flavors, esters, beverages, pheromones, alcohols, aldehydes, amines, acids, glycols.
chiral	Chirasil Val.	Optically active isomers

The stationary phase thickness depends on the amount of retention required. Choose thick films for the analysis of volatile compounds to retain them on the column for a longer time. Non-volatile compounds are better suited to thin films, otherwise their retention times will be too long. Remember, the thicker the film, the more column bleed one may expect. ***Whenever possible, always select a low-bleed column designed for GC/MS.***

The internal diameter of the column depends on the concentration of sample to be analyzed. Small diameter columns (0.25 mm i.d.) offer high efficiency and are useful for quantification of small amounts of sample, but are easily overloaded. Only small diameter columns (0.25 mm i.d. or less) may be used for the Clarus 680 S and Clarus 580 S because of carrier gas flow rate requirements. For on-column analyses 0.32 mm i.d. columns are recommended. The smaller the i.d., the lower the flow of helium gas required. The flow through a short 0.53 mm I.D. column may exceed the pumping capacity of some mass

spectrometers.

The column length is another consideration. In general, the more complex the sample, the longer the column length should be in order to retain and separate the various components. Typically 25 m and 30 m columns are the most suitable for general work. Shorter columns are used for simple separations and longer columns are used for the most challenging samples such as petroleum analyses and dioxin detection in complex matrices.

An Example of an EPA Analysis

There are two frequently followed EPA (environmental) methods that use very common columns. EPA method 625 (semi-volatile analysis) uses a non-polar column. The phase used is a 5% diphenyl 95% dimethylpolysiloxane (PE-5MS, RTx-5, Xti-5, DB-5, SPB-5). The length is 30 meters with an inside diameter of 0.25 mm I.D. The film thickness will be from 0.25 μm to 1.00 μm.

EPA method 624 (volatile analysis) uses an intermediate polarity column. The phase is a methyl cyanopropyl phenyl polysiloxane (PE-624, RTx-Volatiles, RTx-502.2, DB-624, VOCOL). The length can be 60, 75, or 105 meters (60 and 75 meter columns are the most common) depending if the GC has subambient cooling capability. The 105 meter column has the greatest separation power and the most desirable if a laboratory's budget allows. The inside diameter of the column is 0.53 mm I.D. with a film thickness of typically 2.0 μm or 3.0 μm.

This procedure outlines the steps required to properly position the end of the column in the Source and connect the column to the MS Transfer Line tube fitting inside the GC oven.

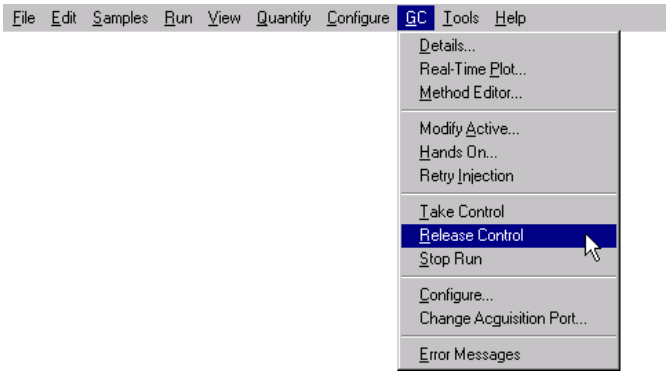
CAUTION	*To prevent contamination of the mass spectrometer, always wear clean, powder-free PVC or nitrile gloves when handling the part of the capillary column that will be inserted into the MS Transfer Line. Never touch these parts with ungloved (bare) fingers, as this will introduce contaminants into the system.*

Preparing the system to install the GC column

Before installing the column, check that the following actions have occurred, and if not, perform the procedures to make them occur.

1. Cool the GC oven by opening the GC oven door slightly.

2. Cool the GC injector by selecting **Release Control** from the top level *GC* menu.



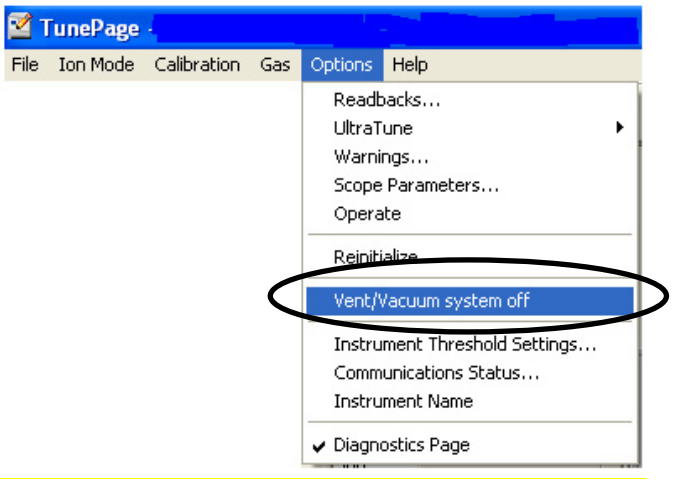
3. Select the appropriate injector icon on the Clarus GC touch screen.

4. On the injector screen select the **Heater Off** button or check box.

5. Display the *Tune Page* by clicking on 

6. Ensure that the mass spectrometer is not under a vacuum by selecting **Vent/Vacuum system off** from the *Options* menu.

NOTE: *Make sure to cool the source and transfer line to <100 °C before venting.*



7. The green light above the mass spectrometer power on/off switch should be flashing.

   A flashing green light above the power ON/OFF switch indicates a vented system. A steady green light indicates that the system is under a vacuum.

   - A **turbomolecular pump** takes approximately 5 minutes to slowdown and vent.

Tools and Items Required

NOTE: *Do **not** use a 100% Graphite ferrule to connect the column since it is a porous material that will allow air to diffuse into the MS system and prevent a high vacuum from being attained inside the system.*

- 9/16 inch wrench
- 1/4 inch wrench
- 5 mm wrench
- One 1/16 inch Column Nut
- One Graphite/Vespel ferrule appropriately sized for the capillary column i.d. that you will be using. (</= 0.25 mm i.d. columns require a 0.4 mm i.d. ferrule and >/= 0.32 mm i.d columns require a 0.5 mm i.d. ferrule.)
- Optional Plug Handle and Sight (P/N N6480380)

NOTE: *Please read the following guidelines in entirety prior to attempting the procedure for the first time. Following these guidelines at all times when performing this procedure will ensure that contaminants entering your MS system will be minimized, vacuum leaks will be minimized and detector response (sensitivity) will be maximized.*

NOTE: *The placement of the outlet end of the column relative to its position inside the source is a critical parameter for maximizing peak responses in your application. Follow this procedure closely to ensure that you achieve the correct column placement inside the source. Failure to do so may result in less than optimal detector response of target compounds in your analysis.*

CAUTION	*Prior to removing the source from the Clarus SQ 8 MS instrument (for example, to change a filament or clean the source), the column must be pulled back from its placement inside the source. This is conveniently achieved by loosening the large nut on the Transfer Line tube assembly so that the entire Transfer Line tube assembly can be pulled back from the source. It is not necessary to loosen the column nut as this may disrupt its proper positioning inside the source after the source is reinstalled. The source will not be able to be removed or reinstalled if the Transfer Line tube assembly is not pulled back first.*

Positioning and Connecting the Capillary Column

There are three different techniques recommended that may be used to position and connect the capillary column. Each of the three techniques are outlined in the following procedure. Use the technique that you are most comfortable with.

The three different techniques are:

1. Physical measurement outside the SQ 8MS
2. Alignment using the 10 mm gauge tool (supplied with the SQ 8 MS instrument)
3. Optical Column Alignment Using the Optional Plug Handle and Sight (P/N N6480380)

Physical Measurement Outside the SQ 8MS

CAUTION	*To prevent contamination of the mass spectrometer, always wear clean, powder-free PVC or nitrile gloves when handling the part of the capillary column that will be inserted into the MS Transfer Line. Never touch these parts with ungloved (bare) fingers, as this will introduce contaminants into the system.*

1. Slide an injector septum over the outlet end of the capillary column to use it as a positioning indicator aid.

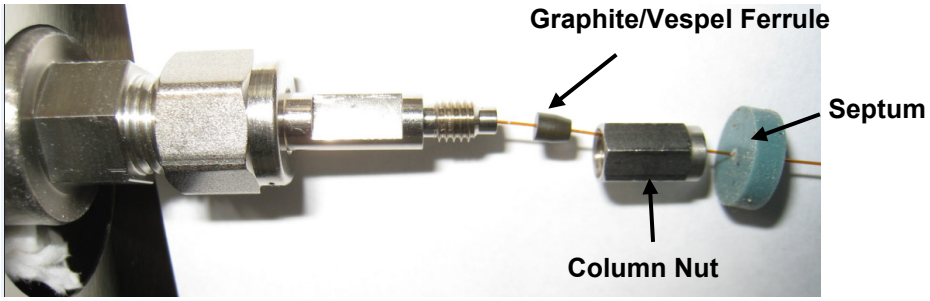


Figure 18 Location of septum on capillary column.

2. Slide a 1/16 inch column nut over the outlet end of the column.
3. With the tapered end facing towards the column nut, slide a graphite/vespel ferrule over the outlet end of the column.

4. Using the edge of a wafer scribe, score the outside surface of the column perpendicular to its length approximately 2 inches from the end and carefully break it off and discard the cut off piece of column. Jagged or angled cuts should be avoided. See Figure 19 for examples of good cuts and bad cuts.

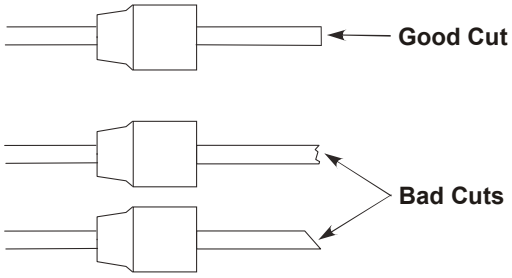


Figure 19 Good cuts and bad cuts

5. Use a lint-free wipe pre-soaked with a small amount of methanol to wipe the outside of the column a few times to remove surface contamination.
6. Place the column onto a clean, lint-free surface on the bench top. Measure exactly **34.4** cm from the outlet end of the column to the front side of the septum.
7. Confirm that the Source is fully installed in the instrument. If it is not, install the source into the instrument. See ***Removing and Returning the Source*** on page ***75***.
8. Insert the column into the MS Transfer Line tube and carefully slide it *partially* toward the source. Be careful not to move the septum.
9. With the column *partially* inserted, engage the threaded column nut onto the Transfer Line tube fitting until it is just finger-tight.

10. Slide the column into the Transfer Line tube until the septum is aligned at a distance exactly 10 mm away from the end of the column nut. See Figure 20.

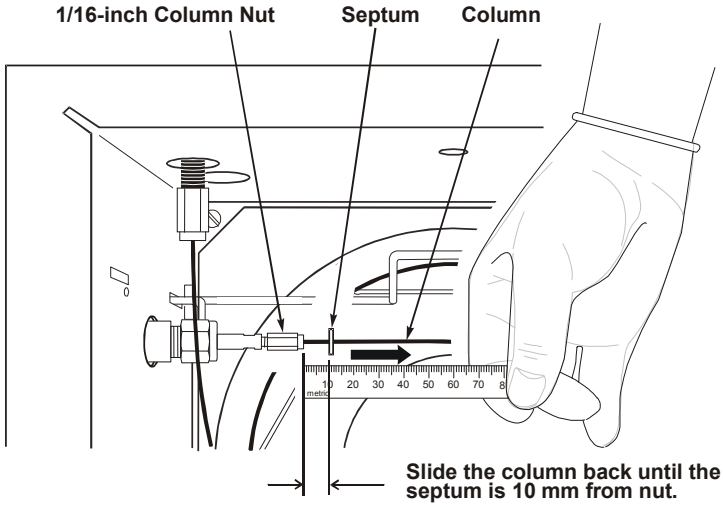


Figure 20 Measuring from the outlet of the column to the front of the septum.

11. Using the 5 mm and 1/4 inch crescent wrenches, tighten the column nut until the ferrule is crimped onto the column. See Figure 21.

Figure 21 Tightening the column nut.

12. The column is now installed the MS may be pumped down. Confirm that the proper vacuum level is reached (on the TurboMass software Tune Page).
13. After initially heating the GC oven through one or two analysis cycles, the column nut may loosen. Re-tighten the column nut. After re-tightening, the column nut should not loosen any more.

Alignment Using the 10 mm Positioning Gauge Tool

CAUTION	*To prevent contamination of the mass spectrometer, always wear clean, powder-free PVC or nitrile gloves when handling the part of the capillary column that will be inserted into the MS Transfer Line. Never touch these parts with ungloved (bare) fingers, as this will introduce contaminants into the system.*

NOTE: *The 10 mm positioning gauge tool is supplied with the instrument.*

1. Slide an injector septum over the outlet end of the capillary column to use it as a positioning indicator aid. Slide the septum approximately 40 mm up the column.
2. Slide a 1/16 inch column nut over the outlet end of the column.
3. With the tapered end facing towards the column nut, slide a graphite/vespel ferrule over the outlet end of the column. See Figure 18.
4. Using the edge of a wafer scribe, score the outside surface of the column perpendicular to its length approximately 2 inches from the end and carefully break it off and discard the cut off piece of column. Jagged or angled cuts should be avoided. See Figure 19 for examples of good cuts and bad cuts.
5. Use a lint-free wipe pre-soaked with a small amount of methanol to wipe the outside of the column a few times to remove surface contamination.
6. Confirm that the Source is fully installed in the instrument. If it is not, install the source into the instrument. See ***Removing and Returning the Source*** on page ***75***.
7. Insert the column into the MS Transfer Line tube and carefully slide it toward the source.
8. With the column partially inserted, engage the threaded column nut onto the Transfer Line tube fitting until it is finger-tight.
9. Carefully continue inserting the column into the Transfer Line tube until it hits the far side of the source. Be careful to avoid forcefully jamming the column into the side of the source. Doing so may damage the cleanly cut end of the column resulting in poor chromatography.
10. With the column positioned as described in Step 9, slide the septum toward the column nut until it touches the nut.

11. Pull the column back 10 mm.
12. Use the 10 mm positioning tool (Gauge) to set the column nut at this position by placing the slot in the 10 mm Gauge over the column between the nut and the septum. At this point be careful to avoid moving the septum from its position on the column. See Figure 22.

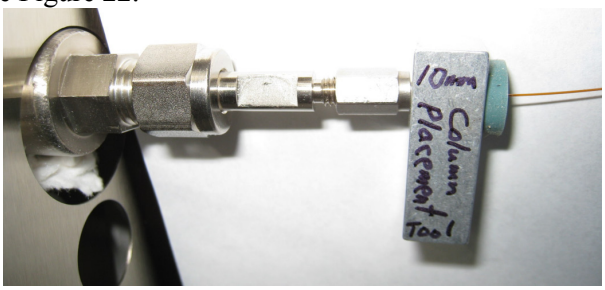


Figure 22 The 10 mm positioning tool (Gauge) in place.

13. Using the 5 mm and 1/4 inch crescent wrenches, tighten the column nut until the ferrule is crimped onto the column making sure that the 10 mm spacing is retained between the end of the column nut and the septum.
14. Remove the 10 mm Gauge.
15. The column is now installed and the MS may be pumped down. Confirm that the proper vacuum level is reached (on the TurboMass software Tune Page).
16. After initially heating the GC oven through one or two analysis cycles, the column nut may loosen. Re-tighten the column nut. After re-tightening, the column nut should not loosen any more.

Optical Column Alignment Using the Optional Plug Handle and Sight

CAUTION	*To prevent contamination of the mass spectrometer, always wear clean, powder-free PVC or nitrile gloves when handling the part of the capillary column that will be inserted into the MS Transfer Line. Never touch these parts with ungloved (bare) fingers, as this will introduce contaminants into the system.*

1. Loosen the large nut on the Transfer Line tube assembly using the 9/16 inch wrench and pull back the Transfer Line tube approximately 1 to 2 inches.
2. Remove the source from the Clarus SQ 8 MS instrument.
3. Insert the optional Plug Handle and Sight (Part No N6480380). Make sure to line up the red dots on the plug and instrument.
4. Turn the plug clockwise until the line on the plug and the lock symbol line up.

5. You will hear a click when the lock is in place.

Figure 23 Inserting the Plug Handle and Sight.

6. Re-insert the Transfer Line tube assembly and tighten the large nut.
7. Slide a 1/16 inch column nut over the outlet end of the column.
8. With the tapered end facing towards the column nut, slide a graphite/vespel ferrule over the outlet end of the column. See Figure 18.
9. Using a wafer scribe, score the outside surface of the column approximately 2 inches from the end and carefully break it off and discard the cut off piece of column.

10. Use a lint-free wipe pre-soaked with a small amount of methanol to wipe the outside of the column a few times to remove surface contamination.
11. Insert the column into the MS Transfer Line tube and carefully slide it toward the source.

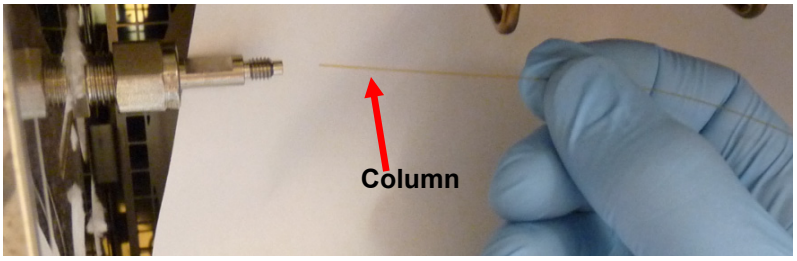


Figure 24 Inserting the transfer line.

12. With the column partially inserted, engage the threaded column nut onto the Transfer Line tube fitting until it is finger-tight.
13. Carefully continue inserting the column into the Transfer Line tube until it becomes visible inside the Plug Handle and Sight. Be careful to avoid inserting the column so far that it hits the far side of the MS vacuum chamber.
14. Position the outlet end of the column so it aligns with the edge of the engraved circle on the tip of the Plug Handle and Sight.

15. The correct position of the column is reached when it is aligned with the edge of the circle (in other words, at the "3 o'clock" position).

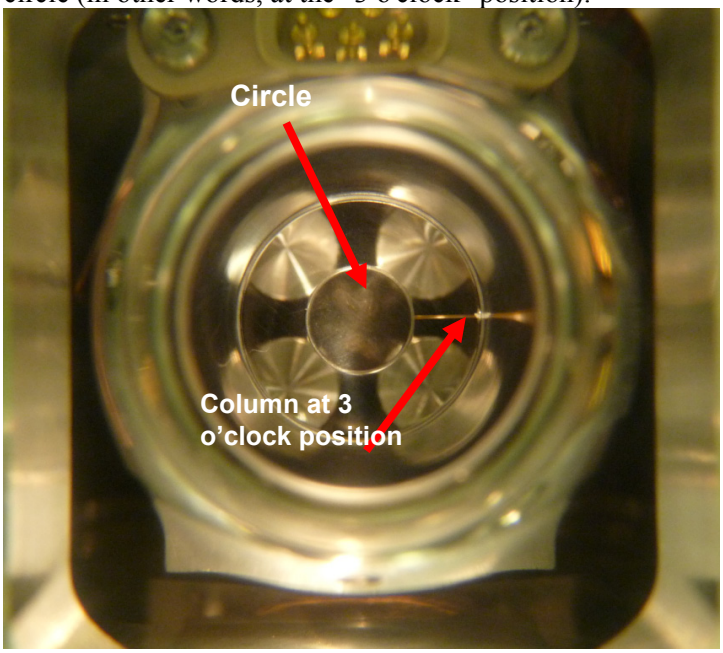


Figure 25 Viewing the Column through the Plug Handle and Sight.

16. Using the 5 mm and 1/4 inch crescent wrenches, tighten the column nut until the ferrule is crimped onto the column. Be careful to avoid moving the column from the set position. Confirm by gently tugging back the column. It should not move from the set position. Verify that the column placement is correct by visually inspecting its position on the circle inside the Plug Handle and Sight.
17. Loosen the large ¼ inch nut on the Transfer Line tube assembly using the 9/16 inch wrench and pull back the Transfer Line tube approximately 1 to 2 inches. See **Figure 26**.
18. Turn the Plug Handle and Sight counterclockwise to the unlock symbol on the instrument and remove it from the Clarus SQ 8 MS instrument.
19. Insert the source. See ***Removing and Returning the Source*** on page ***75***.
20. Re-insert the Transfer Line tube assembly and tighten the large nut.

21. The column is now installed and the MS is ready to be pumped down. Confirm that the proper vacuum level is reached (on the TurboMass software Tune Page).
22. After initially heating the GC oven through one or two analysis cycles, the column nut may loosen. Re-tighten the column nut. After re-tightening, the column nut should not loosen any more.

Equilibrating the system

After installing the column, equilibrate the system for a new column by following this procedure.

1. Cycle the GC through one temperature cycle.
2. Open the oven door and using a ¼ inch wrench, tighten the column nuts slightly.
3. Check for leaks.

Removing and Returning the Source

CAUTION	*To prevent contamination of the mass spectrometer, always wear clean, powder-free PVC or nitrile gloves before touching, removing or replacing parts. Hold the source by its handle only. Never touch these parts with ungloved (bare) fingers, as this will introduce contaminants into the system.*

Removing the Source

To remove the source, follow this procedure:

1. Prepare the mass spectrometer for maintenance as described in *Preparing the Clarus MS for Hardware Maintenance* in the *Clarus SQ 8 MS Hardware Guide*.
2. Open the GC oven door and locate the mass spectrometer transfer line.
3. Using a 9/16-inch wrench, loosen the ¼-inch nut on the transfer line.

WARNING

Risk of burns. *Never touch a heated mass spectrometer transfer line or a GC injector cap with unprotected (bare) fingers.*

4. Pull the inner transfer line tube back 25 mm (1 inch).

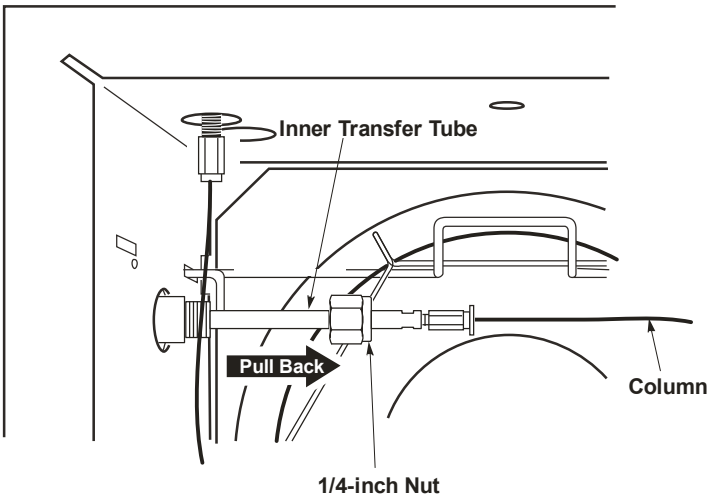


Figure 26 Pulling back the inner transfer tube.

5. Open the Clarus SQ 8 MS access door.
6. Hold the source by the edge and rotate it counter clockwise.

Figure 27 Rotating the source.

7. Carefully pull out the source.

Figure 28 Removing the source.

8. Place the source on a clean surface.
   Preferably place the handle end on a flat surface so that the source stands in an upright position.

Figure 29 The source placed in an upright position.

Returning the Source

CAUTION	*To prevent contamination of the mass spectrometer, always wear clean, powder-free PVC or nitrile gloves before touching, removing or replacing parts. Hold the source by its handle only. Never touch these parts with ungloved (bare) fingers, as this will introduce contaminants into the system.*

1. Carefully hold the source by its edges.
2. Align the red dot on the source handle with the red dot on the instrument panel and rotate the source clockwise until it locks into position.

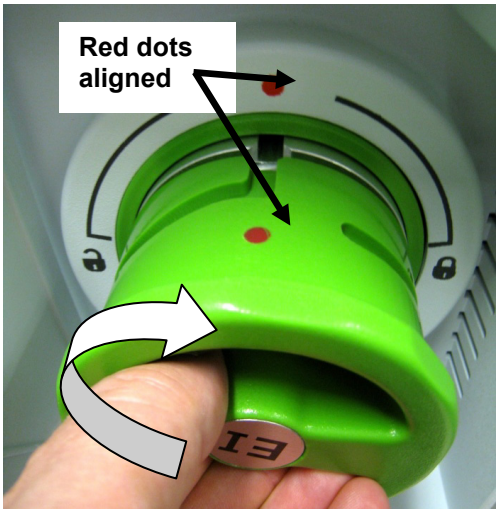


Figure 30 Lining up the red dots.

3. Push the inner transfer line tube back.
4. Using a 9/16-inch wrench, tighten the ¼-inch nut on the transfer line.
5. Close the Clarus SQ 8 MS access door.

How to Leak Check the System 5

Leak Checking

The lack of a good vacuum will severely hinder the performance of the mass spectrometer. To ensure that your analyses produce the expected results, it is good practice to Leak Check the system prior to running an analysis.

The following steps summarize the procedure for leak checking the system.

1. Ensure that all connections are made to the mass spectrometer.
2. If necessary, start the TurboMass software.
3. Display the Tune page.
4. If necessary, start the vacuum pumps.
5. Set the GC split flow to 50 mL/min.
6. Display the Tune Peak list.
7. Change the Tune Peak masses to 4, 18, 28, and 32 respectively.
8. Ensure the proper vacuum is maintained, and then turn on the filament.
9. If a leak exists, locate and fix it.

Checking for leaks is actually checking the integrity of the vacuum system. You observe masses 4 (helium), 18 (water), 28 (nitrogen), and 32 (oxygen).

Ensure that all connections are made to the mass spectrometer

1. Visually inspect the system to ensure that the mass spectrometer electrical and gas connections are made and there are no obvious disconnections.
2. Verify that you have carrier gas pressure to the GC.

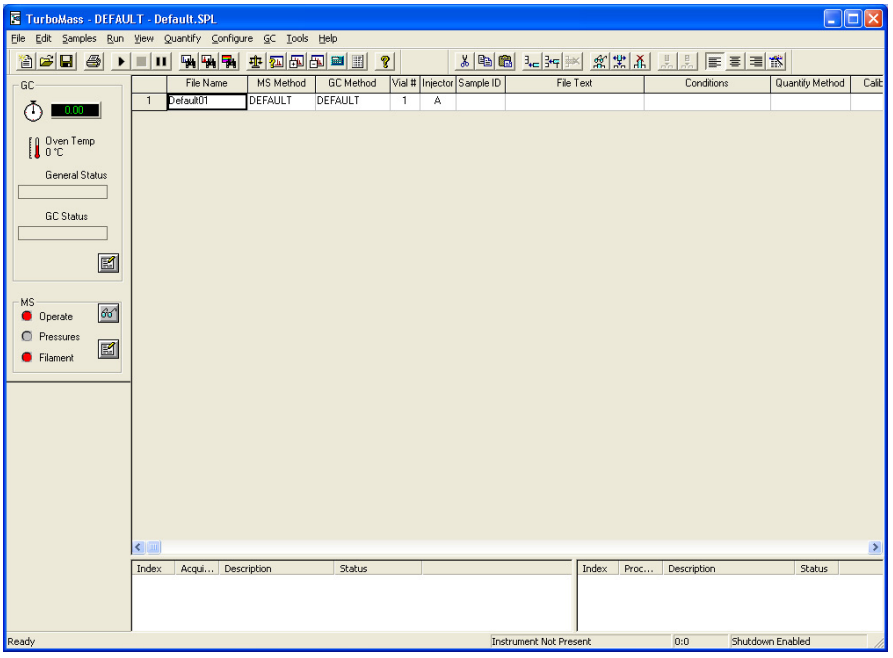
Starting the TurboMass software

1. Click the Windows Start button (at the bottom-left of the screen) and select **TurboMass** from the *Programs/TurboMass* path

   OR

   double-click the **TurboMass** desktop icon.
   The *TurboMass Login* dialog is displayed.

2. Enter the **Password** and click **OK**.
   The initial *TurboMass Sample List* window is displayed.

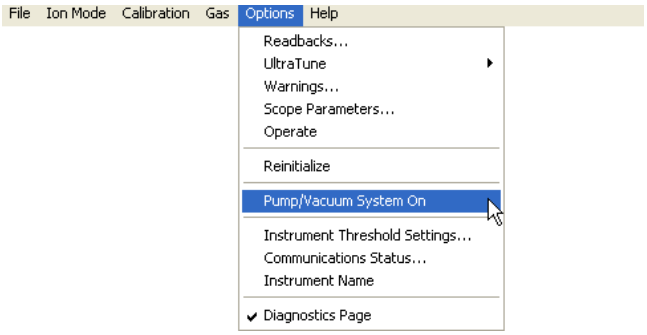


Displaying the Tune page

Click  to display the *MS Tune* window.

Starting the vacuum pumps

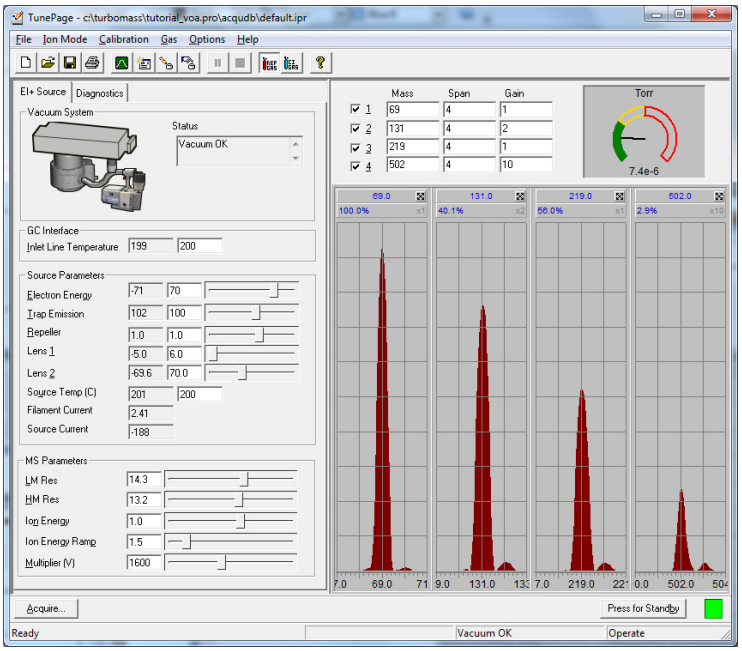
1. Select **Pump/Vacuum System On** from the *Options* menu.



   This starts the forepump and the high vacuum pump.

2. Allow about 5 minutes for the gauge to achieve 3.0 x 10^{-5} Torr with 1 mL/min of helium and a turbomolecular pump.

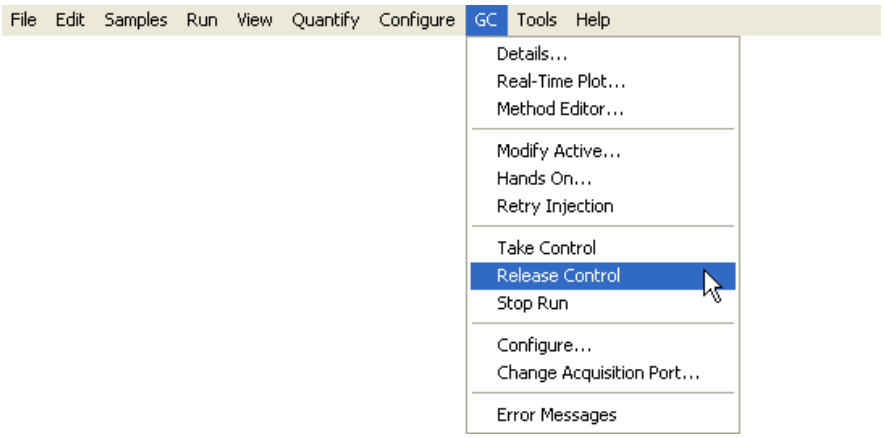
3. If your reference gas bulb has not been previously pumped down, select **Reference Gas On** from the *Gas* menu. Leave it this way for one hour, with **Operate** Off.



Setting the GC split flow to 50 mL/min for the CAP or PSS injector

If your mass spectrometer is under GC control, free it from GC control as follows.

1. Display the top-level (main) window and select **Release Control** from the **GC** menu.



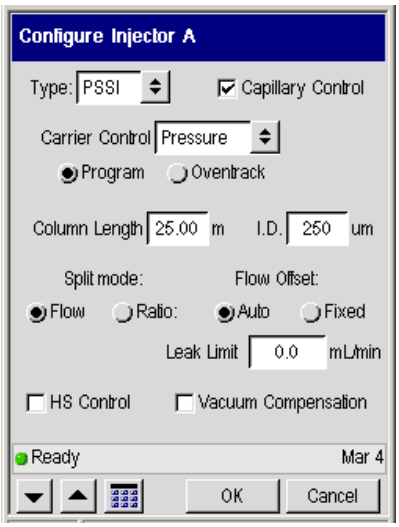
2. On the **GC** screen, touch **Tools** button and select **Configuration**.

   The Configuration screen appears.

3. Touch the **Injector** button for which you which to configure the split mode.

   The Configure Injector screen appears.

4. Touch the **Capillary Control** check box.



For **Split mode**, select either **Flow** or **Ratio**.

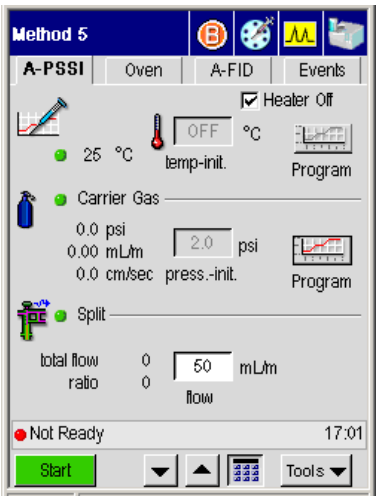
5. Touch **OK**.

   The **Configuration** screen appears.

6. Touch **Close**.

7. On the **System Status** screen, touch the Injector A button.

The active method screen appears.

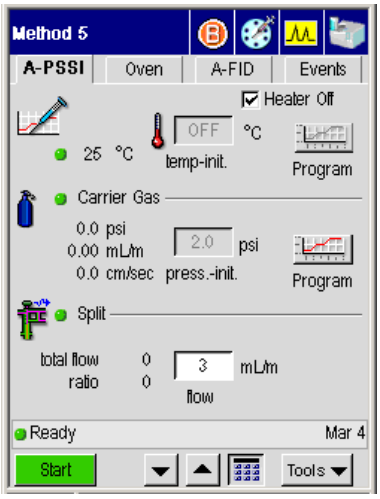


The screen above displays the Split mode configured for **Ratio**.

8. Touch the **ratio** field to modify the value.

   OR

   If you configured the split mode for **Flow**, the following screen appears:

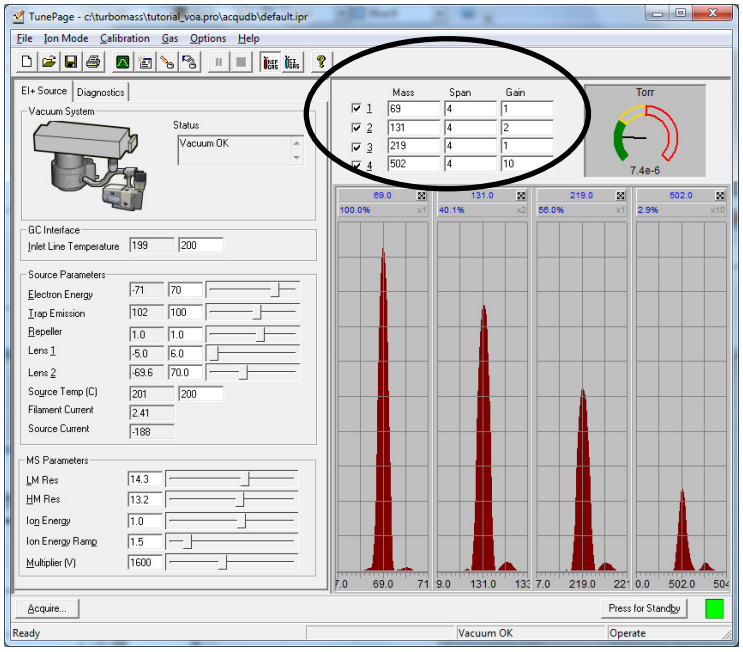


The **Total Flow** (split vent + septum purge + column) is displayed as a non-editable text field. The septum purge flow is factory preset to ~3.0 mL/min. It is not user adjustable. If you set the split flow to zero, the flow display shows column flow plus septum purge. Common places for leaks would be the septum cap, the O-ring seal, column connector, etc. See the Clarus Hardware Guide, Chapter 8 for further information.

NOTE: *If the displayed value is much greater than the total flow (split flow and septum purge + column flow), this indicates a leak in the pneumatics.*

Displaying the Tune Peak list

➢ Select **Peak Editor** from the *Options* menu.
The Peak Editor is displayed.



Changing the masses for Tune Peaks 1 through 4 to 4, 18, 28, and 32 respectively

1. Click in the *Mass* box for **Tune Peak 1**, enter **4** and press **Enter**.
2. Click in the *Mass* box for **Tune Peak 2**, enter **18** and press **Enter**.
3. Click in the *Mass* box for **Tune Peak 3**, enter **28** and press **Enter**.
4. Click in the *Mass* box for **Tune Peak 4**, enter **32** and press **Enter**.
5. Enter a *Span* of **4** for all four Tune Peaks.
6. Enter a *Gain* of **1** for all four Tune Peaks.
7. Click in the box to the right of *Multiplier*, enter 160**0**, and press **Enter**.

Ensuring the proper vacuum is maintained, and turning on the filament

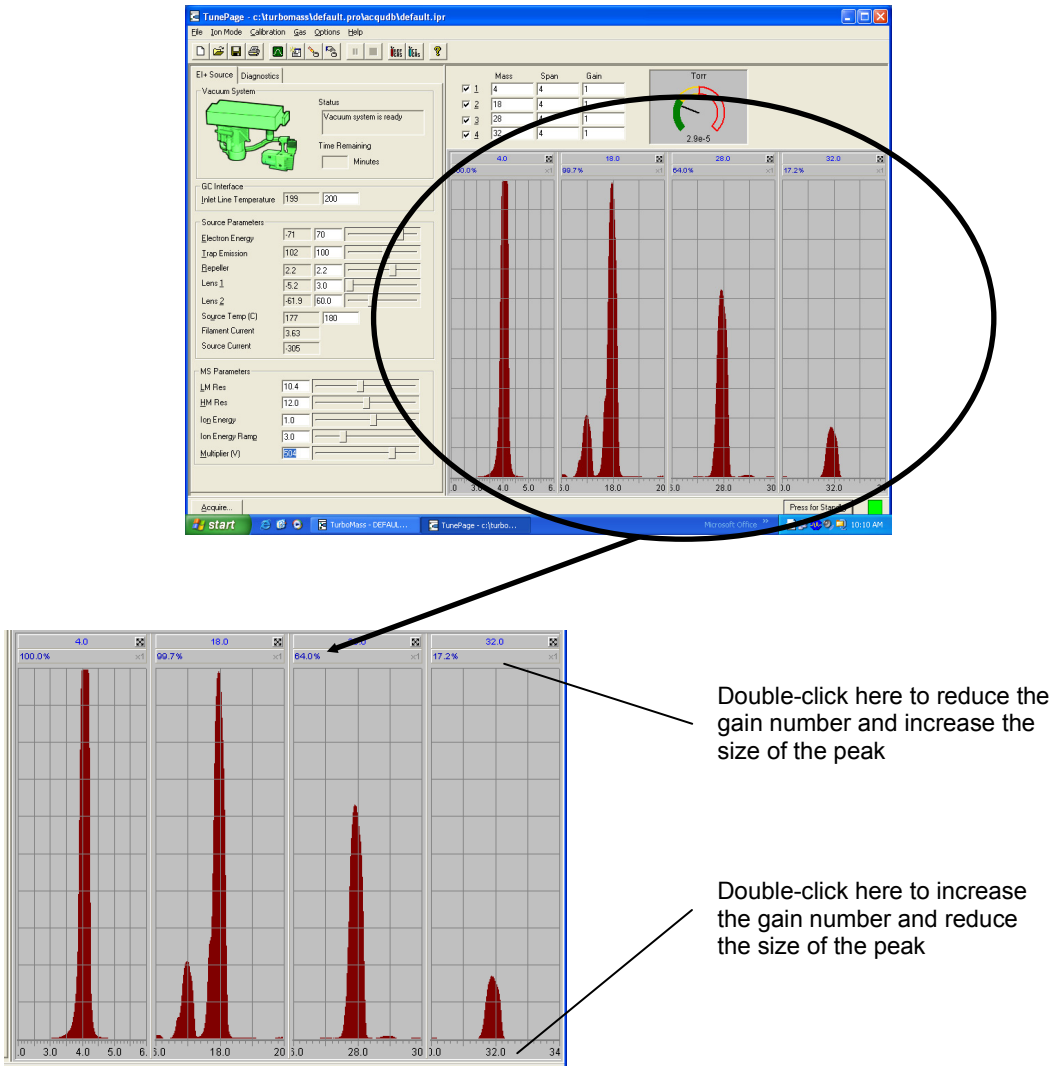
CAUTION	*Filament damage may occur if you turn on the filament by clicking the* **Press for Operate** *button if the vacuum is above 1 x* 10^{-4} *Torr.*

1. Ensure the proper vacuum is maintained.
2. Click **Press for Operate** to turn the filament on.
3. Observe that mass 4 (helium) should be much larger than mass 18 (water), which should be larger than mass 28 (nitrogen), which should be about 4 times larger than mass 32 (oxygen).
4. If necessary, increase or reduce the displayed peak size by adjusting the Multiplier voltage and/or entering a higher Gain number.

   OR

Double-click in the box where the gain is displayed above the peak.

See the following example:



CAUTION	*If the nitrogen (28) and oxygen (32) are larger than helium (4) and water (18), this indicates an air leak that could damage the filament. Immediately click the* **Press for Operate** *button to turn off the filament.*

Locating and fixing any vacuum leaks

Check for leaks around fittings and all parts of the system that are under a vacuum.

Determining if a vacuum leak exists

The following table indicates the types of spectra that may be generated from leaks in the GC/MS system.

	m/z				
Type of Leak	**4**	**18**	**28**	**32**	**40, 44**
None (Leak free)	base	< 4	< 18	< 25% of 18	<< 32
Small leak	base	< 4	≥ 18	< 25% of 18	<< 32
Medium leak			> 18	> 25% of 18	<< 32
Large leak				> 18	<< 32
Very large leak				very wide	
Enormous leak	0	0	0	0	0

All of the above ratios assume that you are using high-purity helium for your carrier gas. The gasses should have a 99.998% purity or higher, and the gas supply lines should have water and oxygen filters installed as close to the GC as possible. If a split/splitless (CAP) or programmed split/splitless (PSS) injector is used, the split vent should be opened to 50 mL/min flow of helium to prevent any air or water diffusing through the septum being confused with a leak.

It is always good practice to print the background spectrum just before changing a tank to be sure that the new tank is not contaminated. The tank should be changed at about 300 psig, so there is enough left for a few days if the new tank is contaminated.

Leak-Free System

Water at m/z 18 will be much smaller than helium at m/z 4. Nitrogen at m/z 28 will be smaller than water, and oxygen at m/z 32 will be < 25% of the nitrogen height.

NOTE: *A leak-free system may not achieve these ratios for several hours if the system has been open to the air for some time and the air is humid.*

Small Leak

A small leak is indicated if the nitrogen peak at m/z 28 is equal to or larger than the water m/z 18 peak in height, but oxygen at m/z 32 is < 25% of the nitrogen height. These ratios are also often observed just after the GC/MS is pumped down after venting. They can also occur if the MS has been under vacuum for a long time and the residual water has been nearly pumped away. For this reason it is a good idea to periodically print a copy of the background spectrum and store it in your instrument log book.

Medium Leak

If oxygen at m/z 32 is higher than 25% of the nitrogen peak at m/z 28, this indicates an air leak. The oxygen concentration is high enough so it begins to “cannibalize” the charge from the nitrogen. This leak should be fixed now. Prolonged operation in this condition will burn out the filament.

Large Leak

If oxygen at m/z 32 is higher than nitrogen at m/z 28, this indicates a significant air leak. The filament is now at risk of burning out. The leak must be fixed immediately. Keep **Operate** on only when necessary.

Very Large Leak

The leak is so large that oxygen at m/z 32 has broadened out to wider than 1 Da at base because of the high pressure in the MS. Water and nitrogen peaks may be small or absent. Turn **Operate** on for no longer than a few seconds at a time while trying to locate the leak to avoid burning out the filament.

Enormous Leak

There is so much air in the MS the ions cannot traverse the distance from the ion source to the detector. No peaks are seen, although there may be a slight increase in the height of the baseline. Turn **Operate** off immediately.

Locating Air Leaks

Once determining that you have an air leak, finding the leak can be time-consuming and frustrating. You can save days of effort by using the correct tools.

Necessary Tools

- Electronic (thermal conductivity) leak detector. Never use soap solution.
- Argon, carbon dioxide, or Freon.
- Acetone or methanol.

The first thing to check is the part of the system you most recently worked on. If nothing else has changed, this is probably the source of your leak.

Starting at the Beginning

Assuming you have no idea where the leak is, you need to test everything. Start at the tank or gas supply and work your way towards the MS.

1. If you have a Clarus GC with PPC (programmed pressure control) make sure the power is on and the thermal zones are cold.

   Turning the power off turns off the carrier flow, and may allow air to diffuse into the GC pneumatics.

2. Check the oil level in your mechanical pump.
   The oil should be between the high and low marks on the sight glass.

3. Check that the hose fittings on the mechanical pump are tight (do not overtighten).

4. Check that the valve on the gas supply tank is open.

5. Check that the high-purity, stainless steel, dual-stage regulator is set to 90 psig (6.3 kPa) and the needle valve is open.

6. Turn on your electronic leak detector and allow it warm up. Exhale briefly into it to verify a negative deflection from the carbon dioxide in your breath.
   Use the leak detector to check all tank and regulator fittings. Make sure that you keep your fingers away from the probe - your finger oils may cause a positive deflection, just like helium.

7. Work your way towards the GC, checking all fittings carefully.

8. Check the GC injector septum.

9. Open the GC oven door and check the injector fittings.

10. Check any other column connections inside the oven.

11. Set the *Multiplier* to 1300 V and set the *Gain* to a value where you can see baseline noise at about 5%.

12. Using a very light stream of argon (m/z 40), carbon dioxide (m/z 44), or Freon (m/z is usually 69 and 83, but depends on the Freon used - look up the spectrum in the NIST library), check the transfer line connection while observing the Tune page.

CAUTION

If you have a large or worse leak, only keep **Operate** *on long enough to complete the test. Large leaks can destroy a filament.*

Argon is the best choice, if available, because it is most effective at getting in through small leaks. Freon is the most convenient, as it can be found in small spray cans at electronic and photographic stores. Make sure the Freon is a "zero residue" grade.

13. Open the front door of the mass spectrometer. Use the gas to test the O-ring by spraying around the front of the manifold.

14. Remove the mass spectrometer top chassis cover. Test around the manifold top O-ring by spraying around the edge of the manifold lid.

15. If you have a very bad leak, carefully spray acetone or methanol around both O-rings and look for an increase in pressure.

WARNING

Spray a small amount of acetone or methanol with extreme caution - both liquids are flammable.

16. Spray gas around the brown PEEK reference gas and CI reagent gas manifold connections on the top of the manifold.

17. Spray gas around the transfer line connection to the manifold.

18. Close the *Tune page* and exit the TurboMass software.

19. Replace the column with a no-hole (solid) ferrule.

20. Restart the TurboMass software, pump-down the mass spectrometer, then Tune the mass spectrometer.

21. Check the spectrum. If the leak is gone, recheck the carrier gas and the GC.

Advanced Leak Checking for Properly Trained Service Engineers

WARNING

There are high voltages and hot surfaces present.

The following procedure should only be performed by a properly trained user or PerkinElmer Service Engineer.

1. Remove the GC.
2. Remove the right-hand side panel from the mass spectrometer.
3. Spray argon or Freon gas around all flanges.
4. Spray argon or Freon gas in the RF coil housing and around the high vacuum feed-throughs.
5. Tape a plastic bag around different portions of the manifold and fill it with gas to attempt to locate the general area of the leak.

CAUTION

If not properly cooled, the turbomolecular pump will be damaged.

The RF box will overheat if sealed off too long.

How to Create a Project for your Analysis 6

Creating a Project for your Analysis

TurboMass groups related sets of data together into Projects. This provides you with the convenience of finding the data for post run analysis and for archiving data. The TurboMass file structure is designed with the top-level directory as the project name under which all data and information about that analysis are stored. TurboMass comes with several predefined projects, including DEFAULT.PRO, QUANTIFY.PRO, and TUTORIAL.PRO. All data is stored in DEFAULT.PRO until you create a new project.

NOTE: *The Created and Modified date and time reported are taken from Microsoft Windows. When you import an *mdb you may see in the properties of the *.mdb file that the displayed Creation date is newer then the Modified date. This is because the Created date changes and is set to the date you imported the file where the Modified date remains unchanged as the date the file was last modified.*

The following steps summarize the procedure for creating a new project.

1. Display the *Sample List* page and select **Project Wizard** from the *File* menu.
2. Enter a **Project name** and **Description**.
3. Save the settings by clicking **Next**.
4. Select **Create using existing project as a template**.
5. Select **DEFAULT.PRO** and click **OK**.
6. Save the Project by clicking **Finish**.

About Project Directories

The TurboMass data file architecture is organized around the concept of a Project. This is a subdirectory, with an extension of .pro, which contains several other subdirectories. The benefit is that keeping all of this information together makes archiving and backup easier.

The subdirectories under Project are:

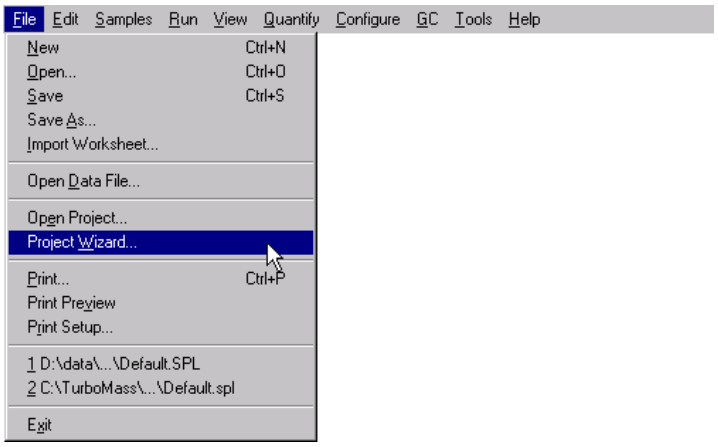
Subdirectory	Files Under the Data Subdirectory	Description
Acqudb		Acquisition defaults, saved tune settings, GC and MS methods, etc.
	*.cal	Mass Calibration
	*.ipr	MS Tune Conditions
	*.exp	MS Acquisition Methods
	*.mth	GC Acquisition Methods
CurveDB	*.cdb	Chromatographic calibration databases
Data	Contains the raw data files. Raw data files are subdirectories with an extension of .raw, which contain several other data files, as follows:	
	_expment.inf	Data on the MS conditions
	_inlet.inf	Data on the GC conditions
	_func00n.ee	Temporary file used for processing
	_func00n.dat	The MS data. “n” is usually 1 , but can go up to 32, depending on the number of data acquisition functions
	_func00n.idx	An index file for the MS data

Subdirectory	Files Under the Data Subdirectory	Description
	_proc001.dat	First processed data file
	_proc001.idx	An index file for the first processed data file
	_tcfunc0.raw	A Turbochrom/TotalChrom .raw file from the first GC detector (if present)
	_tcfunc1.raw	A Turbochrom/TotalChrom .raw file from the second GC detector (if present)
	_functns.inf	Information on the data acquisition functions
	_header.txt	A brief ASCII summary of MS conditions
	_history.inf	A short history of post-run processing
MethDB	*.mdb	Chromatographic quantification methods
	*.rme	Report Method Editor files
	*.qlm	Qualitative Method files
	*.tpl	Template files
PeakDB	*.pdb	Peak List data. Files of integrated peaks in chromatograms
SampleDB	*.spl	Acquisition sequences, or “Sample Lists”

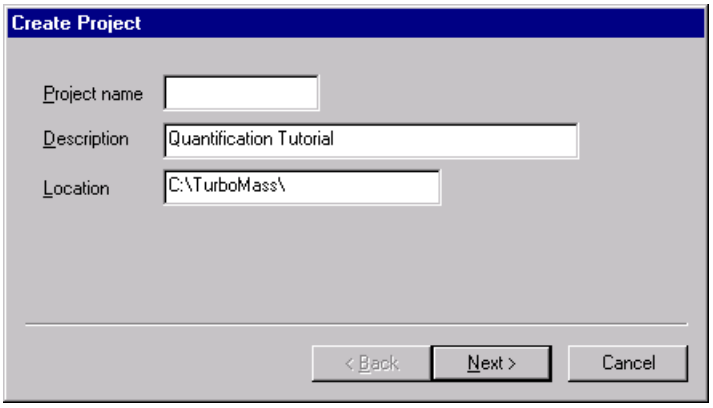
NOTE: *The Created and Modified date and time reported (or displayed) are taken from Microsoft Windows. When you import an *.mdb you may see in the properties of the *.mdb file that the displayed Creation date is newer then the Modified date. This is because the Created date changes and is set to the date you imported the file where the Modified date remains unchanged as the date the file was last modified.*

Creating a New Project

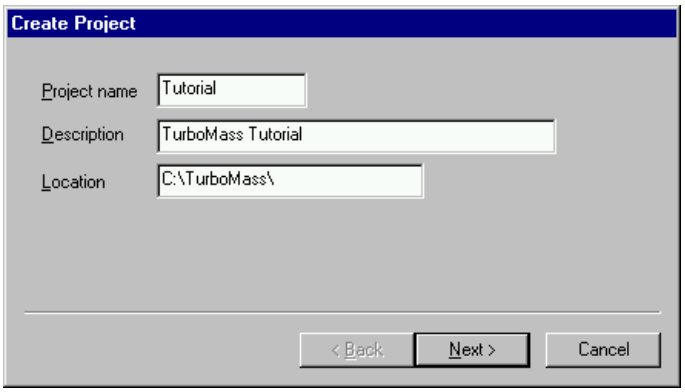
1. From the **File** menu, select **Project Wizard**.



The Create Project dialog is displayed.

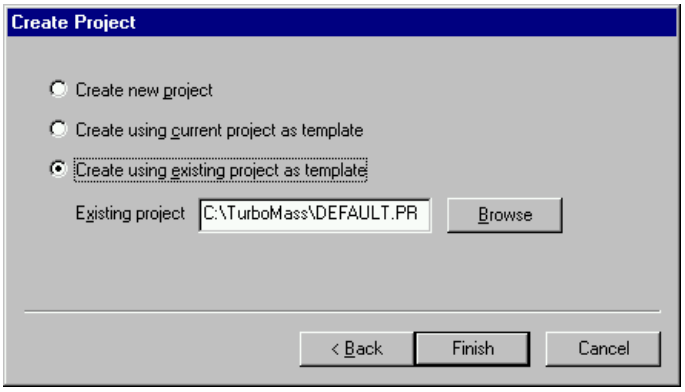


2. Enter a **Project name** and **Description**.
   For this example, enter **Tutorial** for the *Project name* and **TurboMass Tutorial** for the *Description*.



NOTE: *The project Tutorial is located in* C:\TurboMass\.

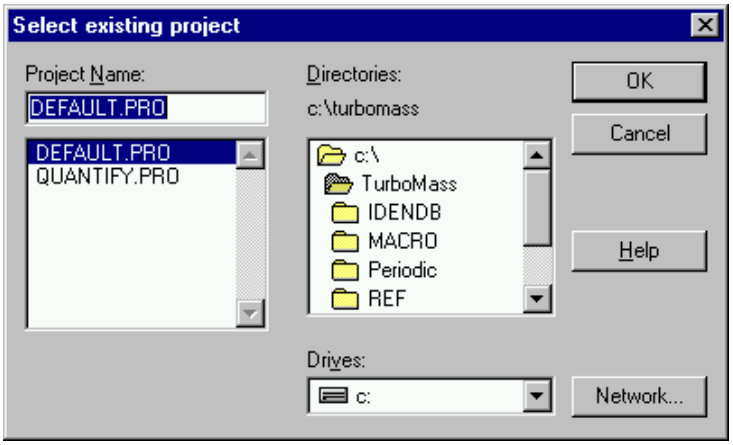
3. Save the settings by clicking **Next**.
   The *Create Project* dialog is displayed.



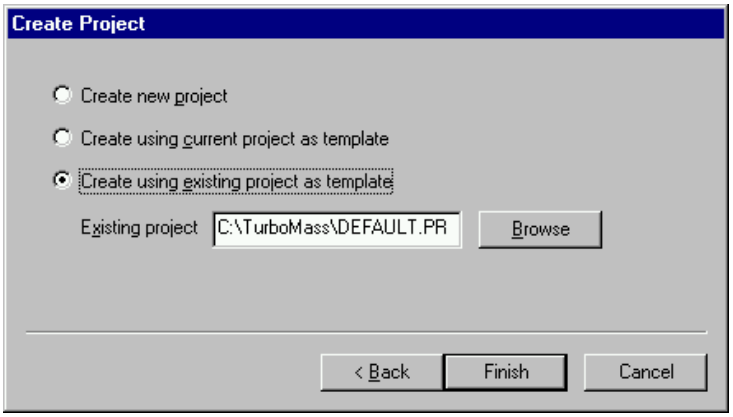
4. Select **Create using existing project as template**.
   A recommended existing project to select is *DEFAULT.PRO*.
   Creating a project using the current project as a template or an existing project as a template, copies the methods and calibration files from those projects, leaving the data behind. This enables you to use your newly created project as is, or delete the files you no longer need.

Creating a new project creates the project with blank subdirectories. You must manually copy over the required information.

5. If *DEFAULT.PRO* is not displayed, click **Browse**.
   The *Select existing project* dialog is displayed.

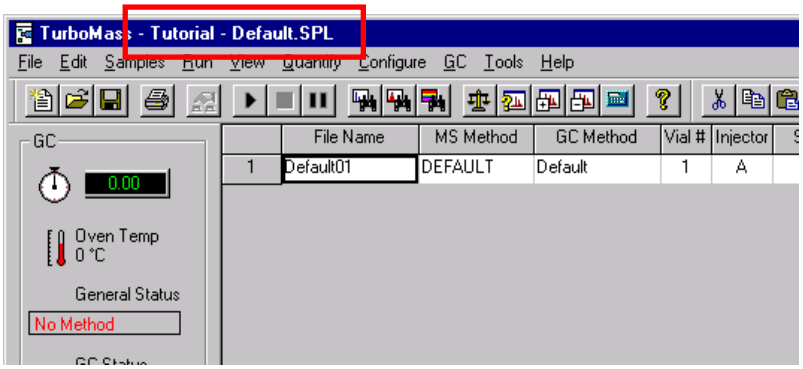


6. Select **DEFAULT.PRO** and click **OK**.
   The *Create Project* dialog is re-displayed.



7. Save the project by clicking **Finish**.

NOTE: *The top line of the window contains your project name (*Tutorial*) and the default Sample List (*Default.spl*).*



How to Tune 7

About Tuning the Mass Spectrometer

After determining that no leaks exist and before acquiring data, you may need to check the mass spectrometer tuning conditions, and if necessary, modify one or more of the tuning parameters. The mass spectrometer can be UltraTuned either automatically (Standard Tune) or manually (Custom Tune) from the *Tune page*. This procedure shows you how to tune automatically using Standard UltraTune.

TurboMass software can automatically tune the mass spectrometer using UltraTune with an EI ion source. UltraTune ramps the settings for the tuning parameters until they are optimized for intensity, resolution, and peak shape.

The goal of Standard UltraTune is not to give the absolute "best" tune, but one that will provide good library-searchable spectra with reasonable sensitivity. High or low concentration range samples may require you to decrease or increase the electron multiplier voltage for best concentration dynamic range.

Standard UltraTune determines the optimum settings for Lens 1, Lens 2, Low Mass Resolution, High Mass Resolution, Ion Energy, and the Repeller. You should set the Ion Energy Ramp to 1.0 for a clean ion source and 3.0 for a dirty one.

Reference Gas and Filament Control

Since reference gas is always required for the Standard UltraTune process, the TurboMass software will assume control for switching the flow on and off.

Tune History

A tune history file is maintained that records the tune parameters following a successful UltraTune. It is saved in an ASCII, comma–separated format, with one tune record per line. The file is located in the main 'TurboMass' directory. Each new successful UltraTune will append a new line to the end of the file.

If the tune history file does not exist when a record comes to be written to it, it will be created. The first line of the file will contain names for each field in the record, which may be used as column headings when the data are imported into Excel. The tune history file is named "UltraTuneHistory.CSV" and it is located in the primary TurboMass path (C:\TurboMass\).

Each line (record) of the tune history contains:

- The name of the logged–on user
- The date the record was written to the file (format as defined in Regional Settings)
- The time of day the record was written to the file (format as defined in Regional Settings)
- Three sets of tune parameters (peak widths at half height and required peak intensity ratios). If only one set of values was generated ('custom' tune or no alternates requested for 'standard' tune) then the second and third sets will contain blank values.
- The multiplier voltages used during UltraTune. The history record will contain places for five values, although not all will be populated. The multiplier voltage used for acquisition will always appear. If you specified a final value to be set following UltraTune (in the UltraTune Setup dialog) then that will appear. Alternatively, if you did not specify a final value to be set then up to three "optimized" values will be recorded (the number depends on the specific tune parameters used).
- Set values and readbacks for the parameters not set by UltraTune (Inlet Line Temperature, Electron Energy, Trap Emission, Source Temperature, Ion Energy Ramp, and Pressure Gauge)
- Values generated by UltraTune for Lens 1, Lens 2, Low Mass Resolution, High mass Resolution, Ion Energy and Repeller.

NOTE: *If you cancel UltraTune during the data acquisition phase, the instrument will be restored to the state it was in prior to the start of UltraTune (i.e., previous tune parameters, reference gas off, etc).*

The following steps summarize the procedure for autotuning the mass spectrometer.

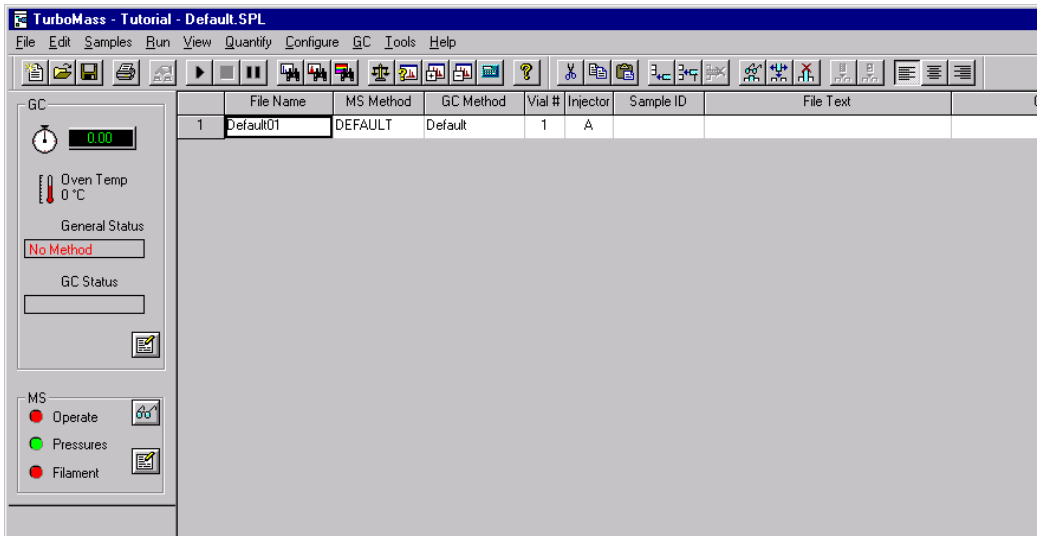
1. Display the *Tune Page*.
2. Start *UltraTune*.
3. Setup *UltraTune*.

4. Accept the setup parameters.
5. Start *tuning TurboMass*.
6. Clear the *UltraTune dialog* box.
7. Start the mass calibration process.
8. Enter the *Automatic Mass Calibration* process.
9. Start the mass calibration.

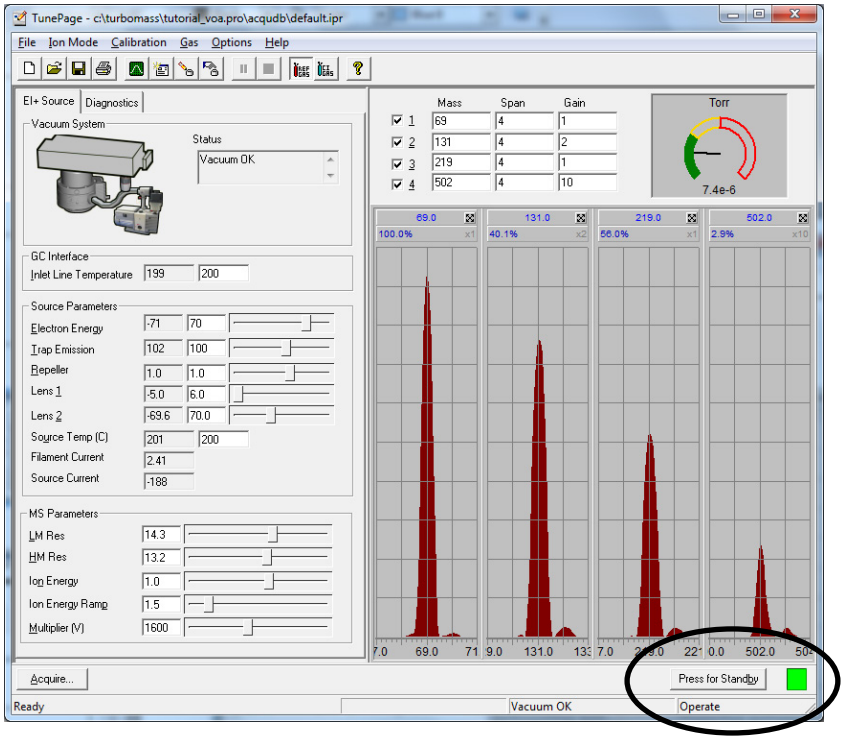
UltraTuning the Mass Spectrometer

The default setup parameters for EI UltraTune are suitable for tuning with Heptacosa (also called FC-43, PTA, PFTBA, and heptacosatributylamine). There is no need to alter the Tune Mass or Peak Width parameters if you are using Heptacosa. In a Standard UltraTune (intended to meet DFTPP/PFB tune criteria), you can select whether just the primary set of results from UltraTune will be generated or whether two alternate sets will also be generated.

Before you start tuning, display the initial sample list window.



1. Click  to display the *Tune window*.



2. Turn on the filament and high voltages by clicking **Press for Operate** at the bottom center of the window.

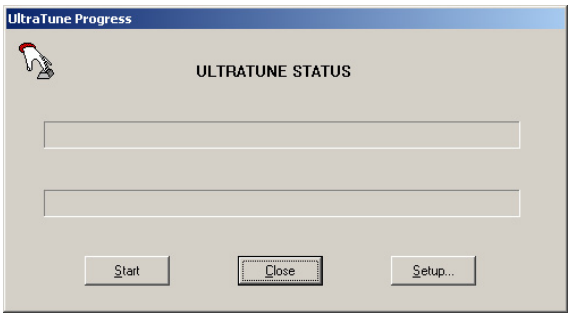
   The box to the right of ***Press for Operate*** turns green to indicate that it is on.

3. Turn on the *Reference Gas* by clicking 

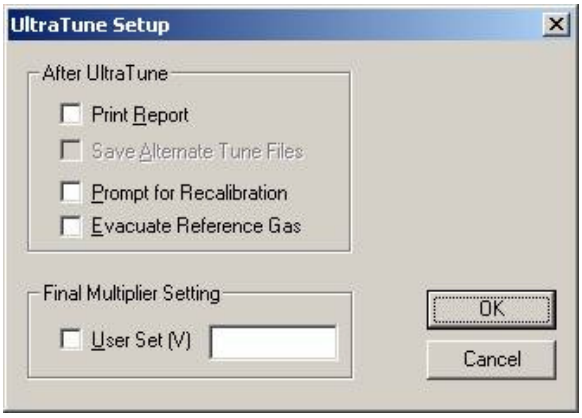
.

4. Click  to start *UltraTune*.

   You will hear a click when the reference gas solenoid valve opens and *UltraTune* begins. The first page of *UltraTune* is displayed.



5. Click **Setup** to display the *UltraTune Setup* dialog.



   The default tune parameters (Full Width Half Max **(FWHM)** = 0.55 for masses 69, 131, 219, and 502) for EI UltraTune are suitable for tuning with Heptacosa. There is no need to alter the Tune Mass or Peak Width parameters if you are using Heptacosa.

   SIR sensitivity may be improved by choosing a number larger than 0.6, but care must be taken to avoid nearby eluting peaks with ions one m/z above or below the target ion.

6. Select your **After UltraTune** settings:

   Print Report - Automatically prints a tune report after a successful UltraTune.

   Save Alternate Tune Files - Saves the top three UltraTune result sets as tune files rather than just the top one.

   Prompt for Recalibration - Prompts you to perform a mass calibration after a successful UltraTune.

   Evacuate Reference Gas - Automatically pumps out the reference gas following UltraTune. Not required if your reference gas valve is mounted on the vacuum manifold lid.

7. Set the Final Multiplier Setting.

   User Set (V) - Check this box if following UltraTune, you want to set the multiplier voltage to the value you enter in the field.

8. When you are satisfied with the **UltraTune Setup** parameters, choose **OK** to exit this dialog.

9. Choose **Start** to start UltraTune and the following occurs:

   - If the source is not at the set temperature (within normal tolerance) then the message "Waiting for the source temperature to equilibrate" is displayed in the UltraTune Status dialog.

   - Once the source reaches the set temperature, if Operate is Off, Reference Gas is Off, the message "Waiting for Reference Gas pressure to stabilize" will be displayed.

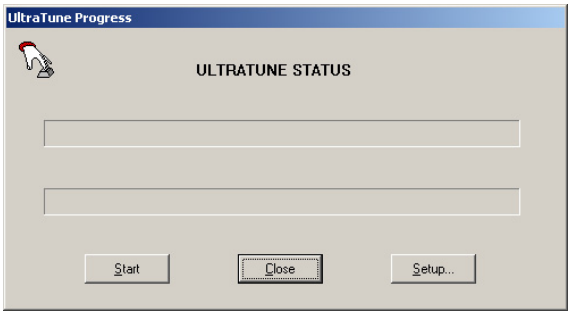
   - If Reference Gas is Off it will be turned on, followed by a delay of 100 seconds. The "Waiting for Reference Gas pressure to stabilize" message will then be removed.

   - If Operate is Off it will be turned on, followed by a delay of 5 seconds. A check is then made to determine if the Electron Energy and Trap Emission current are at the set values (within ±7 and ±10 respectively). If either (or both) readbacks are out of tolerance the error message is displayed is "Electron energy not at the set

value." And/or "Trap emission current not at the set value" and the UltraTune procedure stopped.

The *UltraTune status* dialog is displayed.

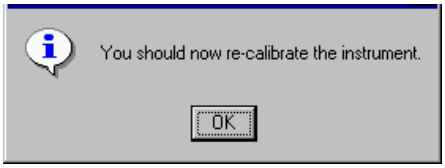
The status dialog displays the part of the tuning process that is currently occurring. The UltraTune status bar is updated to show the progress of UltraTune.



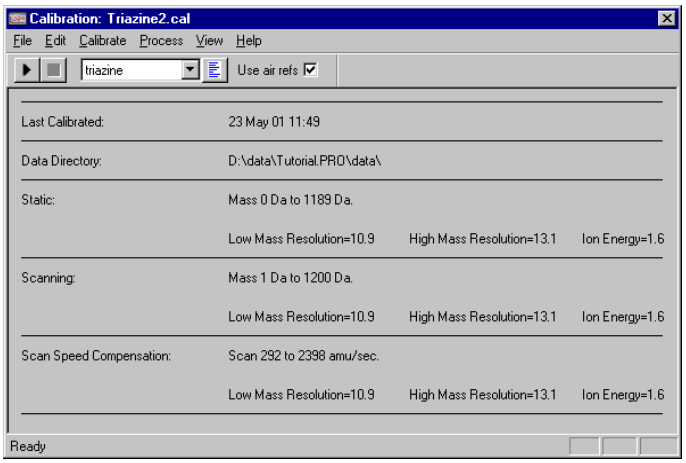
When *UltraTune* has finished, the UltraTune completed message is displayed.

10. Click **OK**.
    The tuning parameters determined by *UltraTune* will be saved to the *current tune parameter file*.

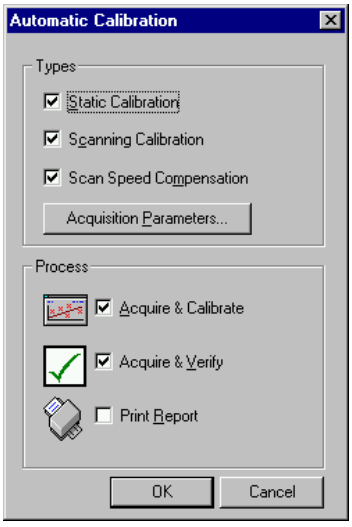
11. If the *Prompt for Recalibration* box was checked in the *UltraTune Setup* dialog, the following message is displayed:



12. Click **OK** to start the mass calibration process.
    The *Calibrate* dialog is displayed:

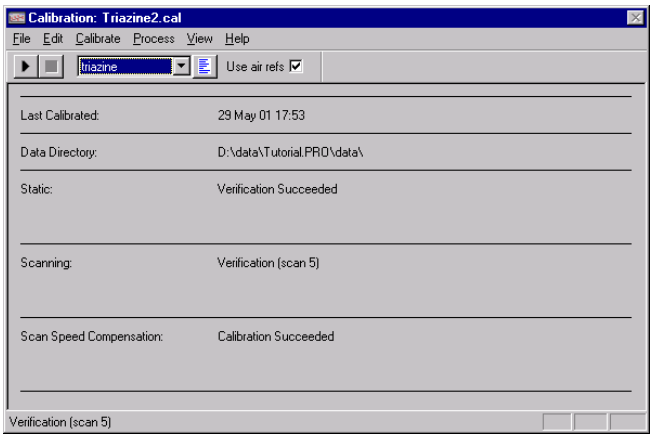


13. Click  to start the automatic mass calibration process.
The *Automatic Calibration* dialog is displayed.

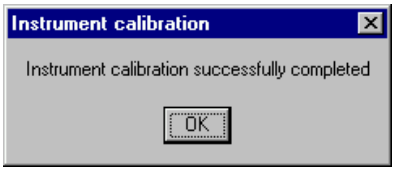


14. Ensure that the boxes are ticked as shown above.

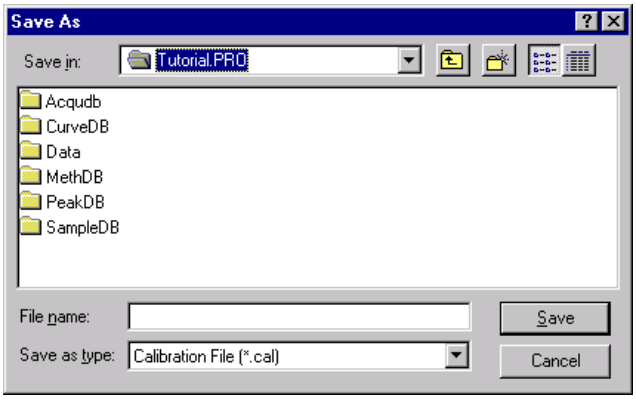
15. Click **OK** to start the mass calibration.
As TurboMass calibrates, observe the *Calibration Status* portion of the dialog.



16. Upon completion the following message is displayed:



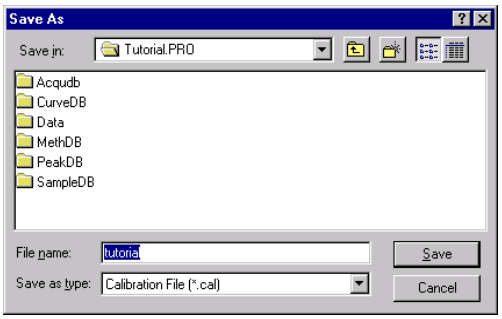
17. Click **OK** to clear the message.
    The *Save Instrument Calibration* dialog is displayed.



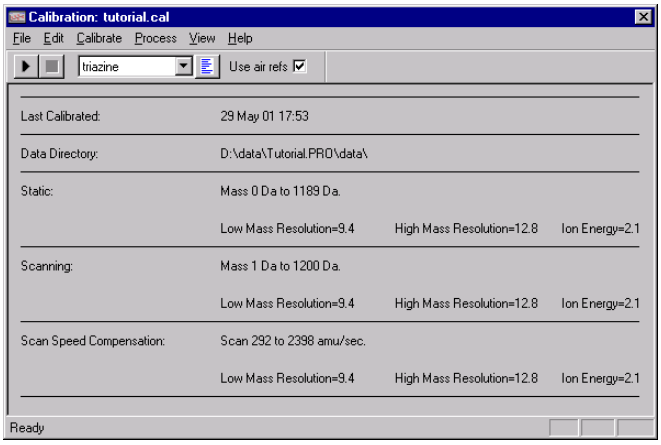
    As shown above, the file will be saved in the *Tutorial.PRO* directory.

18. Enter a file name for your calibration file.

In this example, the file is named tutorial.cal.

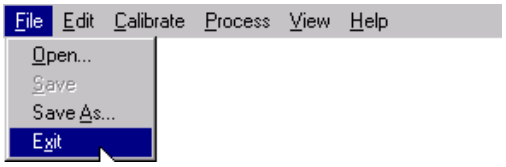


19. Click **Save** to save the file.
    The **Calibrate** dialog is displayed.



After closing the mass calibration window, if you selected Evacuate reference Gas in the UltraTune Setup dialog, UltraTune turns off the Reference Gas and the pumps out the reference gas.

20. Select **Exit** from the *File* menu.



How to Develop a GC/MS Method 8

Developing GC/MS Methods

The GC method is created in the GC Method Editor and it controls the GC operating parameters and data acquisition. For additional information, refer to Chapter 7 in the *TurboMass Software User's Guide*.

The MS method controls the way TurboMass scans. The MS method is created in the Function List Editor. Here you set up the functions that a mass spectrometer uses to scan during an acquisition. The function list can contain a single scanning technique, or a mixture of scanning techniques arranged to run sequentially or concurrently during an acquisition (run). For additional information, refer to Chapter 8 in the *TurboMass Software User's Guide*.

Developing a GC method

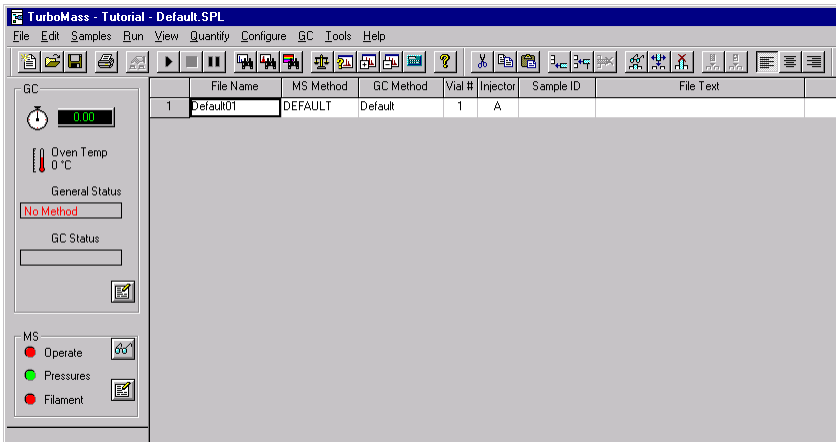
1. Select **Method Editor** from the *GC* menu.
2. Select **default.mth**.
3. Display the GC parameters by selecting **Control Options** from the *Instrument* menu.
4. Set the *Autosampler*.
5. Set the *Oven/Inlets*.
6. Set the *Carrier gas*.
7. Set the *Detectors*.
8. Set the *Instrument Timed Events*.
9. Save the *GC method*.

Developing a MS method

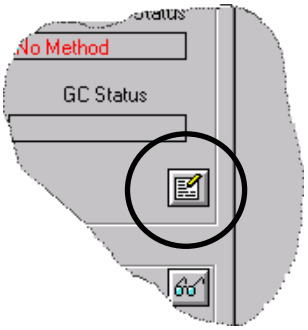
1. In *sample list*, right click on **MS Method**.
   Scan Functions window is displayed.
2. Select **MS scan** or **SIR** from the *Functions* menu.
3. Enter the *method values*.
4. Enter a *Solvent Delay Time*.
5. Save the *MS method*.

Developing a GC Method

This example shows how to develop a GC method by manually injecting the test mix. The starting point is the *Sample List* window.



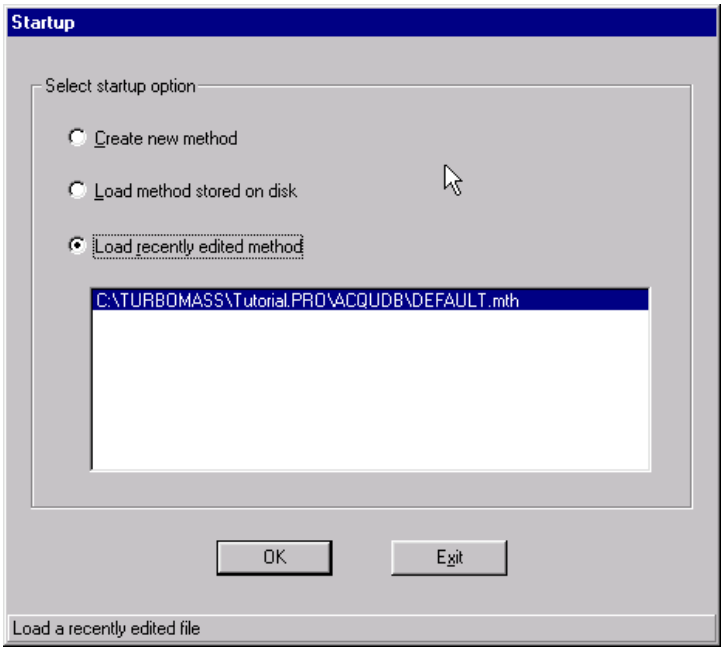
1. Click  in the *GC* panel.



OR

Select **Method Editor** by right clicking the method name shown in the *Sample List* window for a pop-up menu, and selecting **Open**.

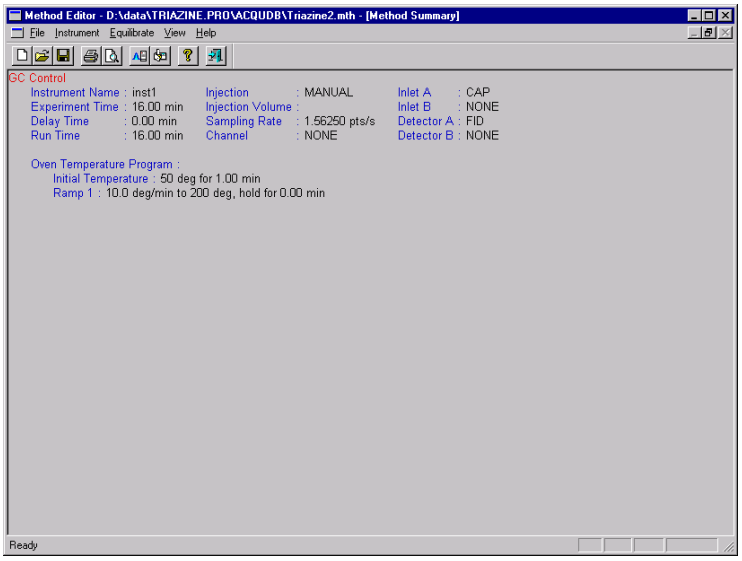
The *Startup* dialog is displayed.



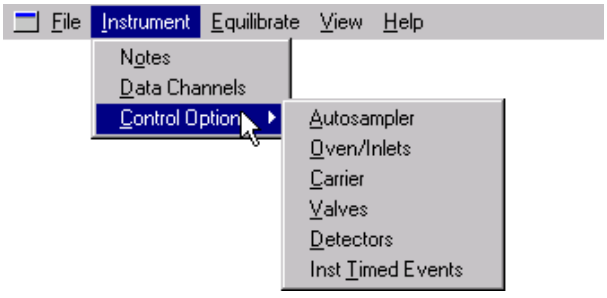
2. Select **Load recently edited method** and select **Default.mth** from the displayed path.

   For example, Default .mth is in the C:\TurboMass\Tutorial.pro\acqudb\default.mth path.

3. Click **OK** to display the *Method Editor* window.



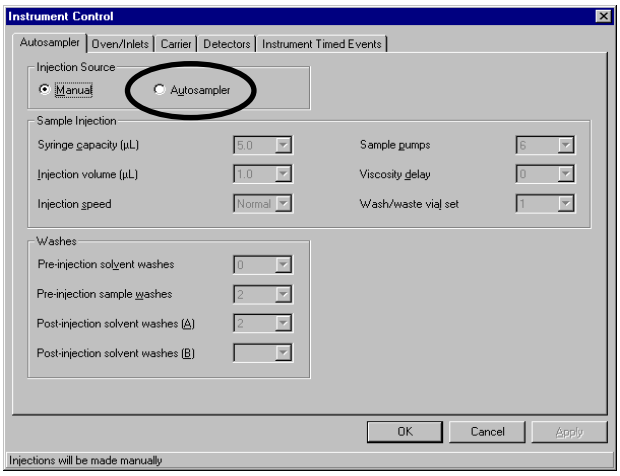
4. Display the *GC parameters* by selecting **Control Options** from the *Instrument* menu.



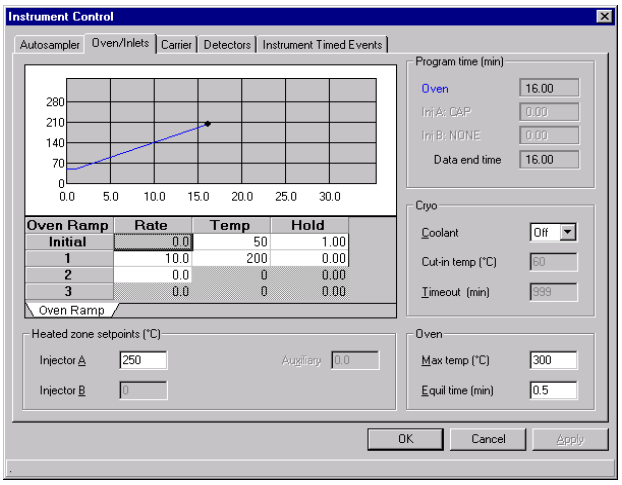
The *GC parameters* are:
Autosampler, Oven/Inlets, Carrier, Valves, Detectors, and *Inst Timed Events.*

5. Select **Autosampler**.

6. On the *Autosampler* tab, select **Manual** as the *Injection Source* for this tutorial. If you are using the Clarus 680 GC (or Clarus 580 GC) autosampler to make the injection, select **Autosampler**.

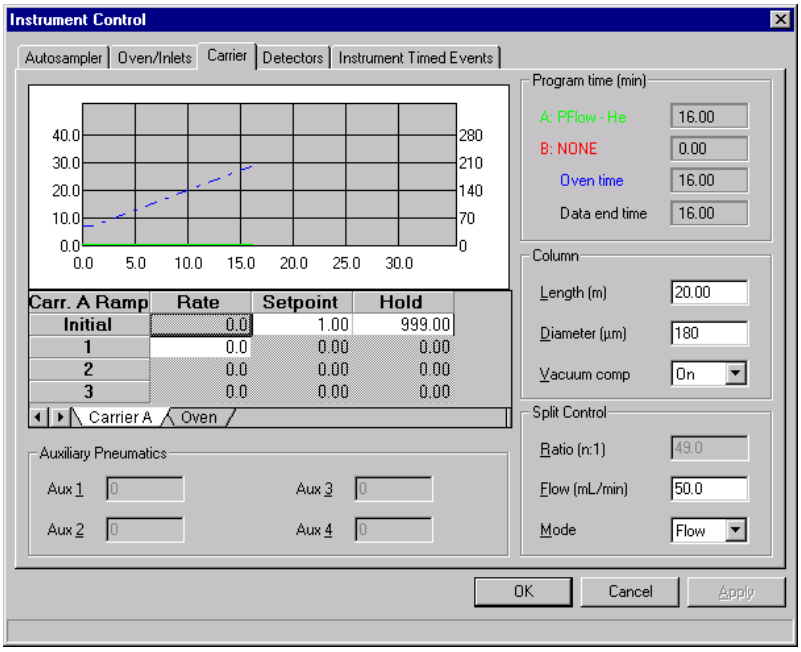


7. Select the **Oven/Inlets** tab and enter the parameters for the *GC oven temperatures* and the *GC injector temperatures* as shown below.



The above window contains the appropriate values for this tutorial. After entering the values, the window visually shows you oven program temperatures.

8. Select the **Carrier** tab and set the values as shown in the following window.



NOTE: *Be sure to enter the correct values in the Column and Split Control sections.*

9. If you have *PPC injector parameters*, enter the parameters for the GC injector carrier gas.

   The above window shows the appropriate values for this tutorial.

10. After entering the values, the window visually shows you carrier gas program pressures.

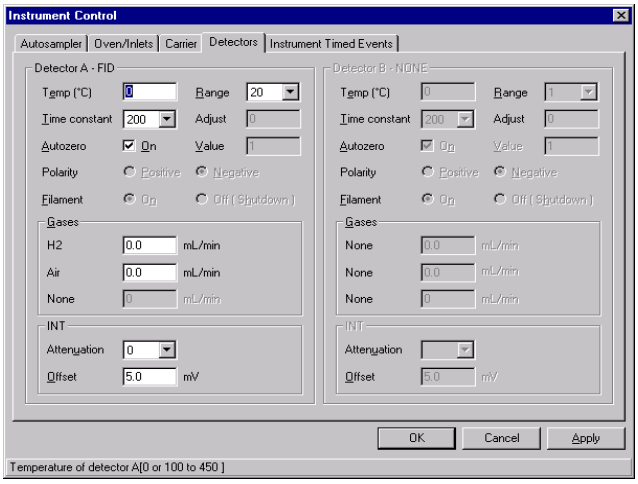
    The above window shows that the analysis consists of the following:

    - Injector A has helium gas flowing.
    - *Program time* of **15 minutes** for the analysis. This includes the *Oven time* and the *Data end time*.
    - *Column Length* of **20 m**, *Diameter* of **180 µm**, and *Vacuum comp* is **On.**

      For the PPC pneumatics to properly function, these values must be set correctly.
    - *Split Control Flow* of **50 mL/min.**

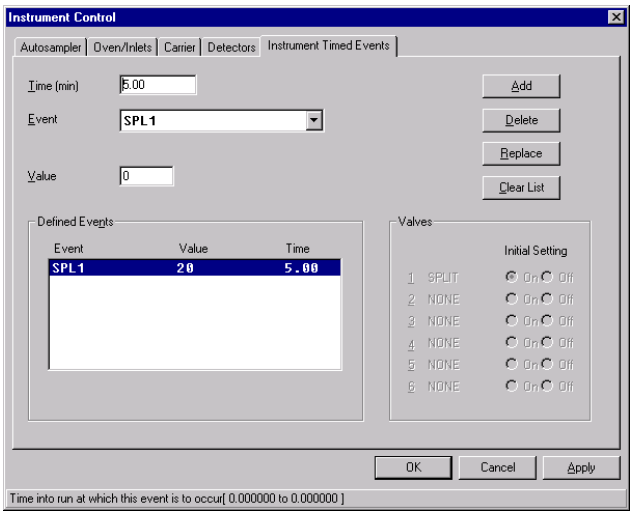
11. Select the **Detectors** tab. If no GC detectors are installed, go to next step.

    As an example, the following window shows that an FID is installed on the GC.



If no detectors are installed or in use, set the *Gases* (*H2* or *Air*) to **0.0**, the *Temp* to **0.0** and the *FID Range* to **0.0**.

12. Select the **Instrument Timed Events** tab and enter the values as shown in the following window.

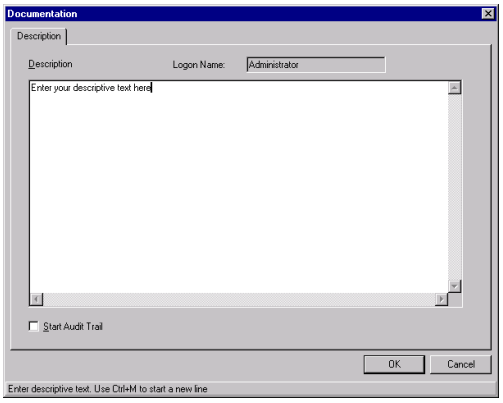


These settings are for a splitless injection. They turn the split flow off just before the injection and then reopen the split at one minute.

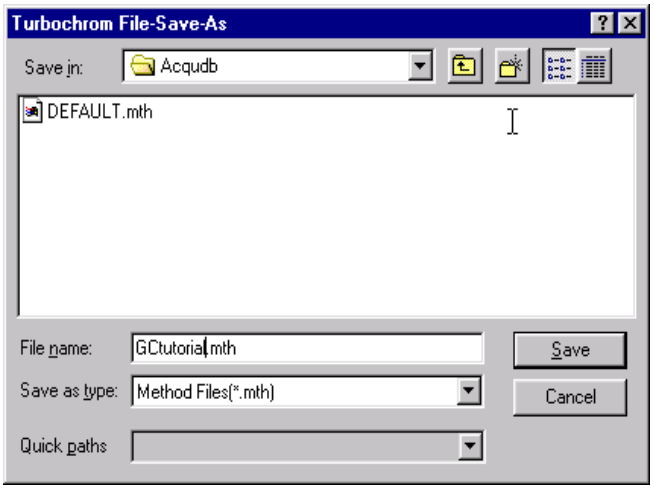
13. Click **OK** to display the main Method Summary window.

14. Save the GC by selecting **Save As** from the *File* menu.

The *Documentation* dialog is displayed.



15. Enter a method description if required and click **OK**.
    The *File Save As* dialog is displayed.



16. Enter a **File name** ending in **.mth** for your *GC method*, then click **Save**.

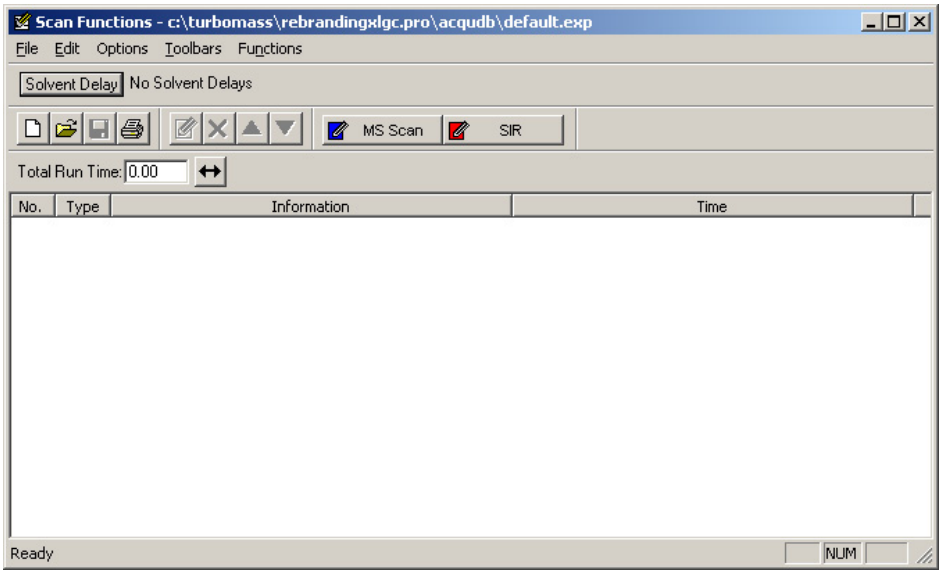
17. Select **Exit** from the *File* menu.

Developing a MS Method

1. Click  in the **MS** panel.

   OR

   Select *Method Editor* by right clicking the **method name** shown in the *Sample List* window for a pop-up menu, and selecting **Open**.
   The *Startup* dialog is displayed.

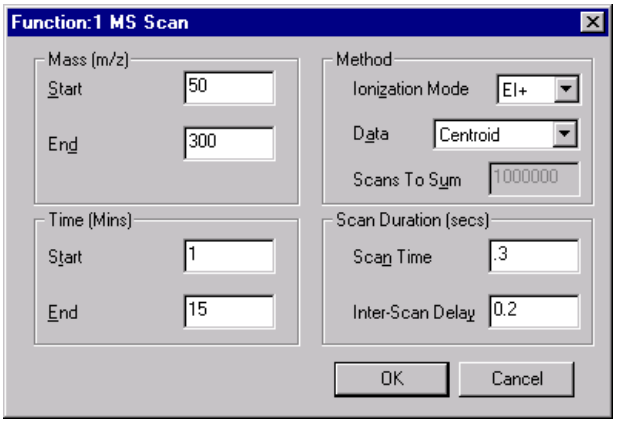


2. Display the *Functions* dialog by selecting **MS Scan** from the *Functions* menu.

   OR

   Click on the **MS Scan** button.
   The *Function* dialog is displayed.

3. Enter the values shown in the following dialog and click **OK**.

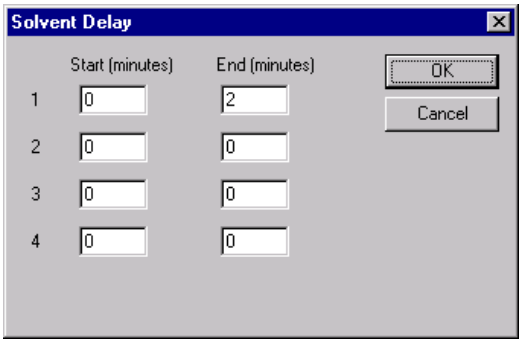


Parameter	Description
Start Mass	The mass at which the scan starts.
End Mass	The mass at which the scan stops.
Ionization Mode	The mode and polarity used during the acquisition. This must match the type of source installed and the active Tune.
Data	Specifies the type of data collected and stored on disk. Centroid - Stores data as centroided, intensity, and mass assigned peaks. Data are stored for every scan. This is the normal mode for GC/MS. Continuum - Stores data regularly to give an analog intensity picture of the data being acquired. Data are acquired continuously, even when no peaks are being acquired. Multi-Channel Analysis (MCA) - Summed continuum

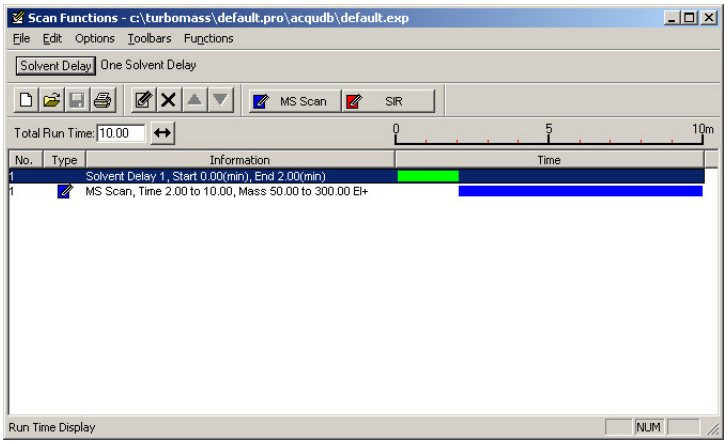
Parameter	Description
	with only one intensity-accumulated scan stored to disk for each experiment.
Repeats	The number of repeats of a particular function. Only relevant for experiments having more than one function.
Retention Window Start Time	The retention time in minutes at which data acquisition starts for the scan function.
Retention Window End Time	The retention time in minutes at which TurboMass stops acquiring and storing data for the scan function.
Scan Time	The duration of each scan in seconds.
Inter-Scan Delay	Time in seconds between a scan finishing and the next scan starting. During this period data is sent from the MS to the computer.

4. Click the **Solvent Delay** button.

   The multiple Solvent Delay dialog appears:

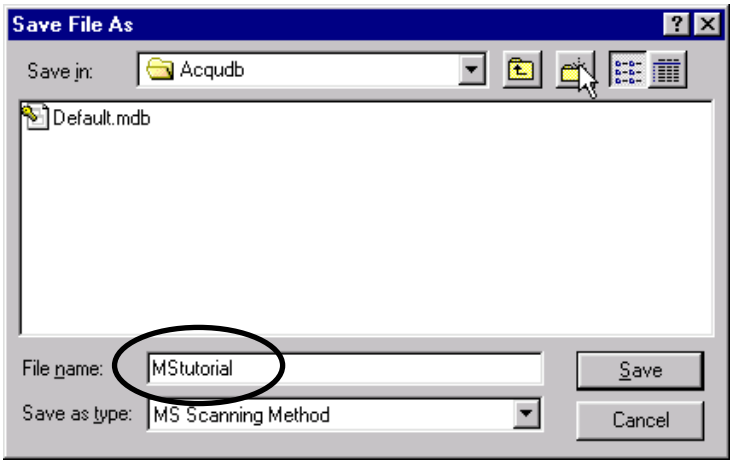


5. Leave the number 1 **Start** time at 0 and enter an **End** time for the solvent delay. Leave the remaining solvent delay lines at 0 and click **OK.**



6. Select **Save As** from the *File* menu.

7. Enter a **nam**e for your f**ile,** ending with **.mdb**, and click **Save**.



In this example we entered MStutorial. Notice that the name appears to the right of Scan Functions in the dialog header.

8. Select **Exit** from the File menu.

How to Perform Various Types of Injections 9

Good Manual Syringe Technique

Good manual syringe technique is fundamental to obtaining reliable and reproducible results, especially in quantitative work. Poor injection techniques account for many of the problems encountered with capillary columns.

Consistency is perhaps the most important factor in achieving reliable results.

The choice of syringe can affect results. For 1 or 2 microliter injections, a 5 or 10 microliter syringe is best. For injections less than 1 microliter, a 1 microliter syringe should be used.

The plunger is withdrawn only a short distance and is unlikely to be damaged during the injection. It is quite easy to bend a fully withdrawn plunger when making the injection.

Keeping syringes clean and in good condition is also important. A common cause of confusing results is contamination due to sample accumulating in the syringe.

The following injection procedure is not intended to be definitive, but should form the basis for the development of a consistent technique.

Rinsing a Syringe

Before and after an injection is made, the syringe should be rinsed as follows:

1. Pour a few mL of solvent into a clean beaker or autosampler vial.
2. Draw up into the barrel 4 or 5 microliters of solvent and inject it out onto a tissue or into another autosampler vial.

   Care should be taken when using flammable or toxic solvents.
3. Repeat steps 1 and 2 several times.
4. Pump the syringe with the needle remaining in the solvent.
5. Draw up a few microliters and once again inject the solvent onto a tissue.
6. Wipe the needle with the tissue taking care not to draw any solvent out of the needle by capillary action.

   This method wets the barrel of the syringe and leaves the needle full with pure solvent.

Loading a Syringe

To load the syringe:

1. Draw up into the syringe about 1.0 microliter of air.
2. Draw up excess sample.
3. Position the plunger for the required amount of sample to be injected.

   Always examine the quantity by viewing the syringe at eye level. Parallax can be the cause of misleading measurements.
4. Remove the syringe needle from the sample, wipe the needle with a laboratory tissue and draw up the plunger until the slug of sample can be seen inside the syringe barrel: this action will help prevent the loss of sample through evaporation.
5. Smoothly inject the needle through the septum while keeping a finger on the plunger to prevent blow-back. Guide the needle into the injector with a clean laboratory wipe (for example, a Kimwipe). Avoid touching the injector needle with your fingers.
6. Fully insert the plunger and then press it firmly and quickly.
7. Leave the needle in the injector for a few seconds. Usually five seconds is enough.

   If the needle is withdrawn straight after injection, then the increase in the pressure in the injector, due to the evaporating solvent, may cause the sample to blow out through the hole in the septum.

   Sometimes slow injections are required to concentrate the sample slug at the end of the column. Conversely, when thermally labile samples are being handled, a faster injection is required to prevent thermal degradation in the injector. Your technique should be modified accordingly.
8. Rinse the syringe as described in *Rinsing a Syringe* on page 141.

NOTE: *Cleaning is especially important when changing samples to avoid cross contamination. Build-up of organic material can be removed by washing the syringe and plunger in an ultrasonic bath. Alternatively, draw through the syringe a dilute chromic acid solution followed by a thorough rinse with water and then methanol (or other water-miscible solvent). Most syringe suppliers will advise on cleaning, and many offer a syringe reconditioning service.*

Sometimes small fragments of septa lodge inside the needle and may block the bore. Silicone rubber may absorb some of the sample causing loss of sensitivity and cross-contamination between samples. Needles can be cleaned out with fine wire, usually supplied with the syringe.

Split Injection

The sample capacity of thin film 0.25 mm i.d. capillary columns is approximately 200 ng per component before you experience GC peak distortion. Larger column diameters or thicker films have higher capacities.

When operating in the split mode, only a small portion of the injected sample actually enters the column thus preventing column overloading. The remaining sample is carried out by the carrier gas through the split vent. The ratio of inlet flow to column flow, commonly referred to as the split ratio, ranges from 10:1 to 500:1, depending on the column being used and the sample being analyzed. A split of 25:1 is common for fused silica columns. When operating in the split mode, ensure that the correct liner is installed in the injector. Refer to the *Clarus 680 GC Hardware Guide* (P/N 09936781) or the *Clarus 580 GC Hardware Guide* (P/N 09936625) for the procedure to set up split ratios.

Although the technique of split injection is fairly easy to master, it does have two major disadvantages:

- During trace analysis the total amount of sample is severely limited. The analyst cannot afford to waste most of the sample through the vent.
- Split injection is a flash vaporization technique and there is a possibility of sample discrimination when injecting wide boiling range mixtures.
- To overcome these disadvantages, splitless injection is used.

Splitless Injection

The splitless injection technique allows virtually all of the sample to be concentrated at the head of the column, behind the condensed solvent band. In view of the beneficial solvent effect and much improved discrimination of wide boiling range mixtures, this technique is widely used in trace analysis work.

Concentration of the sample at the head of the column is achieved by stopping the gas flow through the split vent. None of the sample is vented away. After a short interval the vent flow is restarted. In this mode larger quantities of solvent are used to provide the solvent effect. Component levels should be less than 50 ng to avoid overloading the column.

When operating in the splitless mode:

1. Ensure that the correct injector liner is fitted.
2. Cool the oven to the recommended temperature for the solvent being used (see below).
3. Refer to the GC method and set the valve timings such that the split valve is closed at injection and opens one minute later.
4. Inject the sample.

   For large quantities, inject over a period of 5 - 10 seconds.

5. Start the run at the required temperature.

Recommended Initial Oven Temperatures	
Dichloromethane	10-30 °C
Chloroform	25-50 °C
Carbon Disulfide	10-35 °C
Diethyl Ether	10-25 °C
Pentane	10-25 °C
Hexane	40-60 °C
Iso-octane	70-90 °C

Attention to fine detail is essential to obtain reproducible results and the following points should be noted:

- The inside of the injector must be kept clean.
- The capillary column inlet must be properly positioned in the injector. Refer to Start the Clarus MS from a Cold State on page 29.
- The injection port temperature must be sufficient for efficient vaporization of the sample; 200 - 300 °C is usually adequate.
- Solvent quantities of 1 - 3 microliters are required to obtain the solvent effect.
- Initial temperature of the column should be 10 - 20 °C below the boiling point of the solvent to obtain the solvent effect.
- To prevent the solvent tailing, purge the injector by opening the splitter 60 - 100 seconds after injection with 50 to 100 mL/min split flow.

How to Build a Sample List 10

Building a Sample List

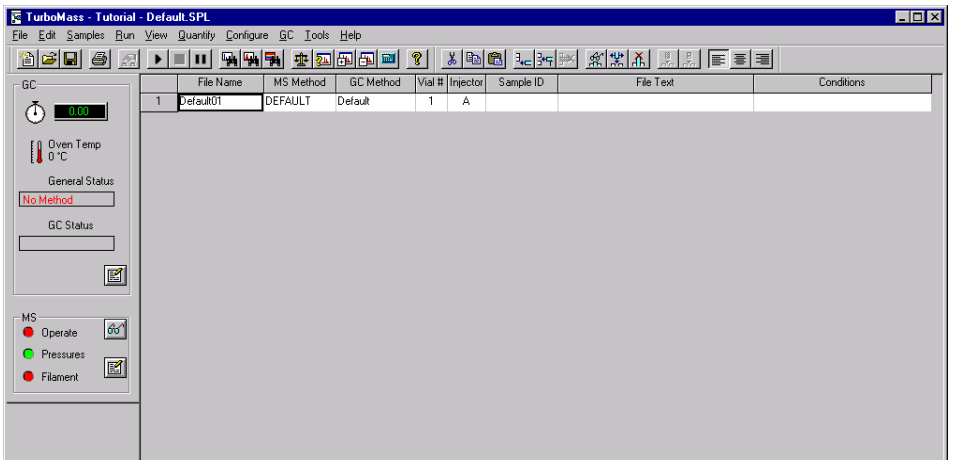
Before acquiring mass chromatograms, you need to build a sample list. This is simply a list that links GC methods, MS methods, and autosampler vials for a particular analysis. Each line (row) in the list is a separate analysis. This gives you the ability to set up for unattended operation.

The following steps summarize the procedure for building a sample list:

1. Enter a unique File Name for your analysis.
2. Select the MS Method.
3. Select the GC Method.
4. Enter the vial number of your sample.
5. Select the GC injector position.
6. Enter a sample I.D.
7. Enter File Text.
8. Enter a Quantify Method
9. Enter a Calibration File
10. Enter a Qualitative Method
11. Enter a Report Method
12. Save the sample list file.

In this example we will edit an existing sample list for an analysis. You can keep the list simple or make it complex by adding categories as columns.

NOTE: *You can also build a sample list using the Sample List Wizard from the Samples menu. The Sample List Wizard is described in the Clarus MS Environmental Tutorial.*



This sample list consists of **File Name**, **MS Method, GC Method, Vial #, Injector, Sample I.D., File Text**. These are the only entries you need to enter. You can also enter a **Qualitative Method, Calibration File, Quantify Method, and/or Report Method**.

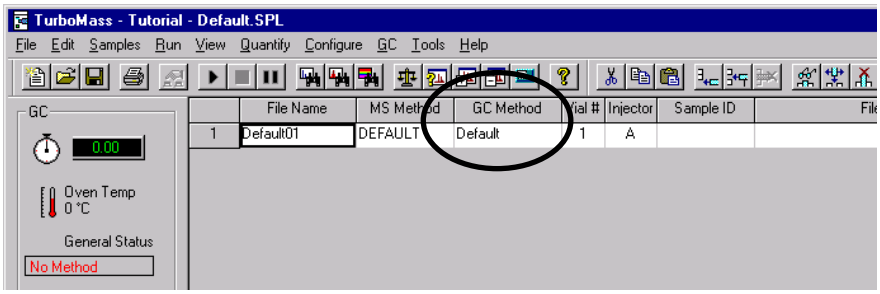
1. Click in the **File Name** field and enter a unique file name.

2. This file name becomes the subdirectory name under which all data for this analysis is stored. In this example, we entered Tutorial01. The subdirectory is *c:\Turbomass\Tutorial.pro\Tutorial01.raw*

3. Select the MS Method by double-clicking in the lower right part of the **MS Method** field and selecting a mass spec method from the drop-down list.

4. In this example, we selected MSTUTORIAL.

5. Select the GC Method by double-clicking in the lower right part of the **GC Method** field and selecting the **GC method** from the drop-down list.



In this example, we selected GC tutorial.

6. If you have a Clarus 680 GC (or Clarus 580 GC) with an autosampler, click **Vial #** and enter the number corresponding to the sample vial location in your GC autosampler.

   If you do not have this autosampler, this is just a text entry field.

7. If you have a Clarus 680 GC (or Clarus 580 GC) with autosampler, enter the letter that corresponds to the *GC Injector*. Injector **A** is the front position and Injector **B** is the rear position.

   In this example, we selected Injector A.

8. Enter a **Sample ID**.

   In this example, we entered Fid test mix.

9. Enter **File Text**.

   Enter descriptive text that will be used to describe this sample.
   In this example, we entered Fid test mix C.

10. Select the Quantify Method by double-clicking in the lower right part of the **Quantify Method** field and selecting the **method** from the drop-down list.

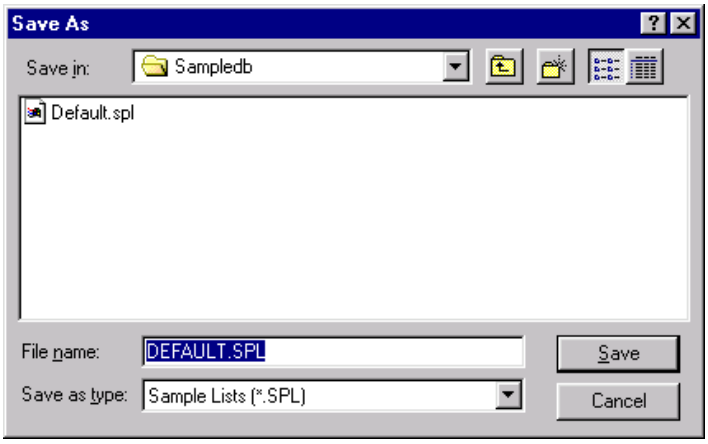
11. Select the Calibration Curve file by double-clicking in the lower right part of the **Calibration Curve** field and selecting the **file** from the drop-down list.

12. Select the Qualitative Method by double-clicking in the lower right part of the **Qualitative Method** field and selecting the **method** from the drop-down list.

13. Select the Report Method by double-clicking in the lower right part of the **Report Method** field and selecting the **method** from the drop-down list.

14. Select **Save As** from the *File* menu and enter a name for this *Sample List*.

15. The ***.spl** extension is added to the name and the file is stored in the *TUTORIAL.PRO Project directory*.



How to Acquire Mass Chromatograms 11

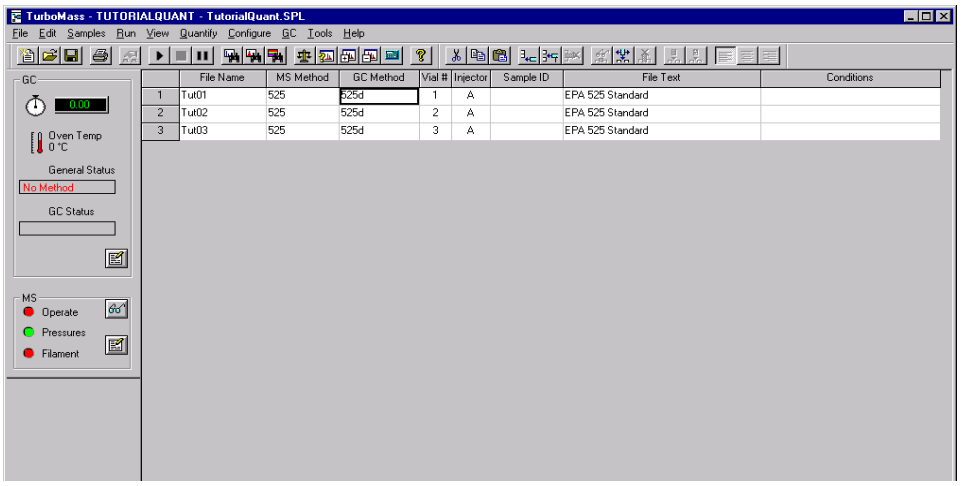
After the instrument has been tuned and calibrated, the GC and MS methods created, and the sample list built, it is time to run an analysis and acquire mass chromatograms of your sample. Once the mass chromatograms are acquired, you can perform different post-run analyses on the acquired data as discussed later in this tutorial.

The following steps summarize the procedures for acquiring mass chromatograms.

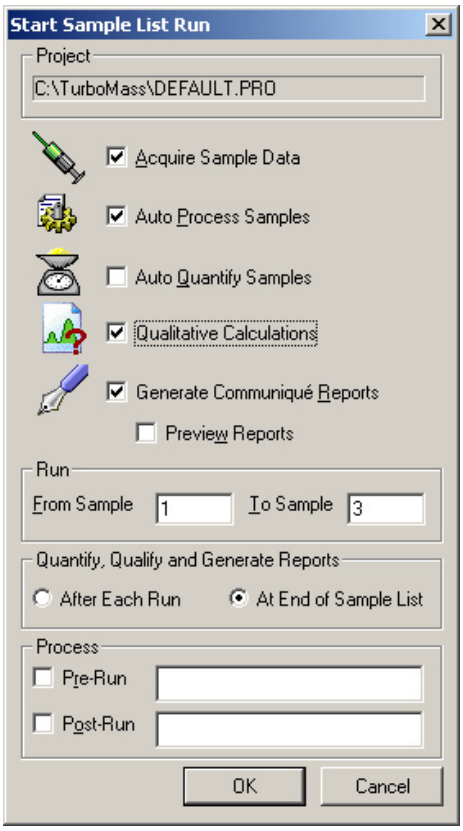
1. Display the *Sample List page*.
2. Click **Run**.
3. Click **OK**.
4. Inject your sample and click **Run**.

Acquiring a Mass Chromatogram

1. Display the *Sample List* window.



2. Click **Run**  to display the *Start Sample List Run* dialog.

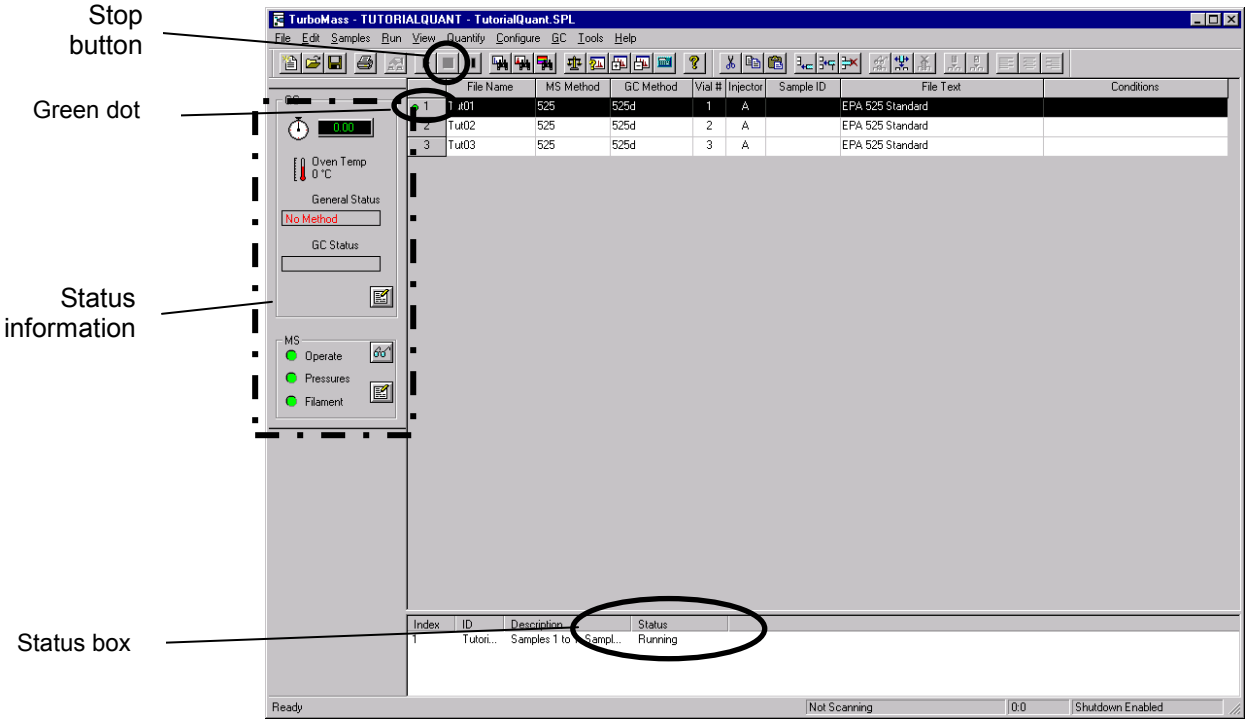


3. Click **OK** to start the analysis.

   When the analysis begins, notice the following actions in the sample list window:

   - The stop button illuminates red.
   - A green dot appears next to the number of the sample list you are running.
   - Status Information appears in the GC box.

   When both **General Status** and **GC Status** are ready, you can make an injection.



4. Press the **Run** key on the GC touch screen and inject 1.0uL of sample.

   Observe the status box on the bottom of this window. The *Waiting for injection* status changes to *Running*.

How to Display Mass Chromatograms 12

Displaying Mass Chromatograms

While running samples, TurboMass saves the collected sample information (mass chromatograms) as .RAW files in the Data directory of your Project. In this example, the data is stored in the Tutorial Project. You can view and manipulate the mass chromatograms in real time during data acquisition or afterwards (post-run).

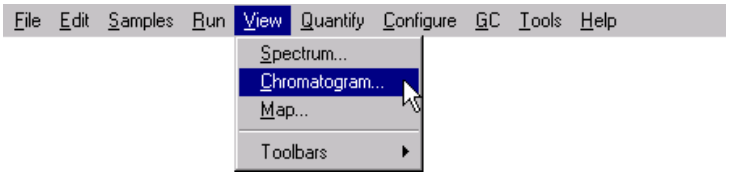
The following steps summarize the procedures for displaying mass chromatograms:

1. Select **Chromatogram** from the *View* menu on the *Sample List* window.
2. Select **Open** from the *File* menu.
3. Click **Replace** so that only one chromatogram is displayed.
4. Select the raw chromatogram file from the *Chromatogram Data Browser*.
5. Click **OK**.

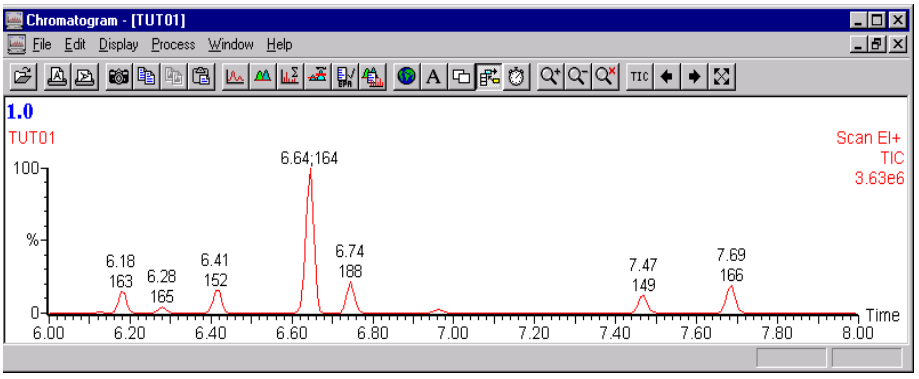
Finding a Specific Mass Chromatogram

You can open and view any mass chromatogram through the *Chromatogram Data Browser.*

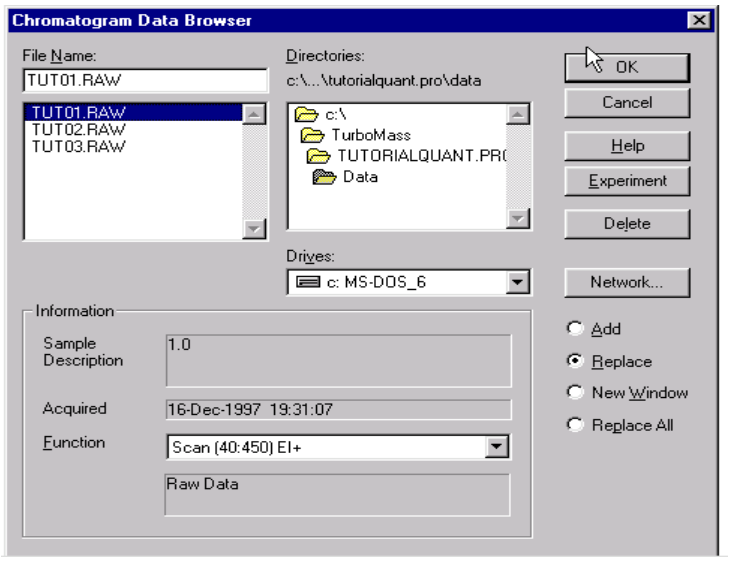
1. Select **Chromatogram** from the *View* menu on the *Sample List* window.



The *Chromatogram* dialog is displayed.



2. Select **Open** from the *File* menu to display the *Chromatogram Data Browser* dialog.



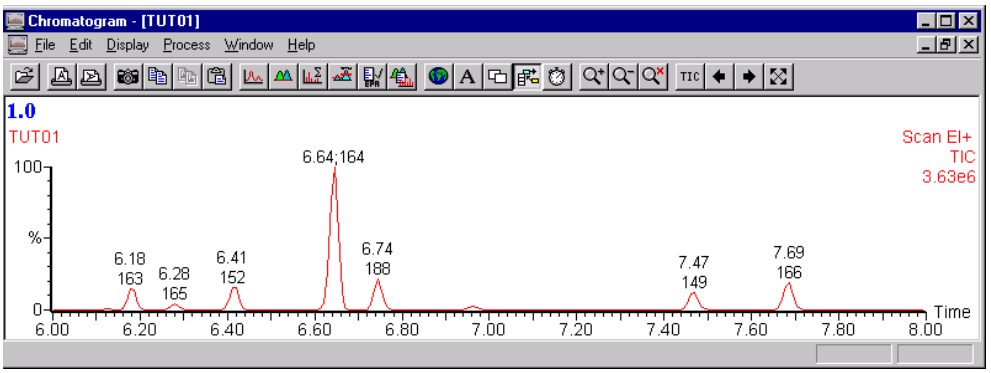
This example shows the three .RAW files of the chromatograms in this tutorial.

3. Select **Replace,** so that only one chromatogram is displayed.

4. Select the **.RAW** chromatogram file from the *Chromatogram Data Browser*. In this example, we selected the .RAW chromatogram TUT01.RAW.

5. Click **OK**.

The chromatogram of TUT01.RAW is displayed.



To view a particular peak or change the viewing area of the chromatogram, click and hold the left mouse button, drag it across the area, then release it.

For additional information on displaying mass chromatograms, refer to the *TurboMass Software User's Guide* .

How to Search a Library to Identify Unknowns 13

Searching a Library to Identify Unknowns

The following steps summarize the procedure for searching a library to identify unknowns.

1. Display the mass chromatogram of the unknown.

2. Display the unknown peak of interest.

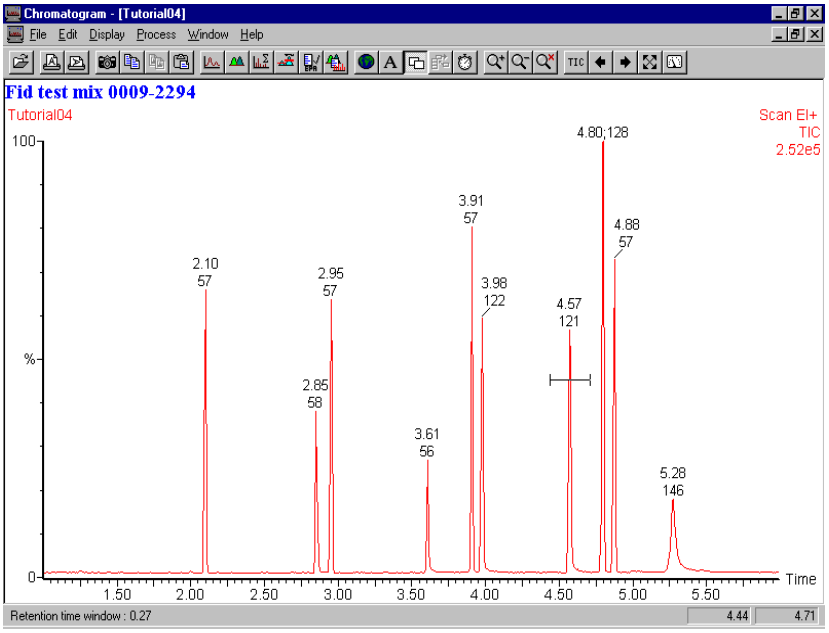
3. Select **Combine Spectra** from the *Process* menu to subtract out the background information.

4. Perform a library search.

Library Searching

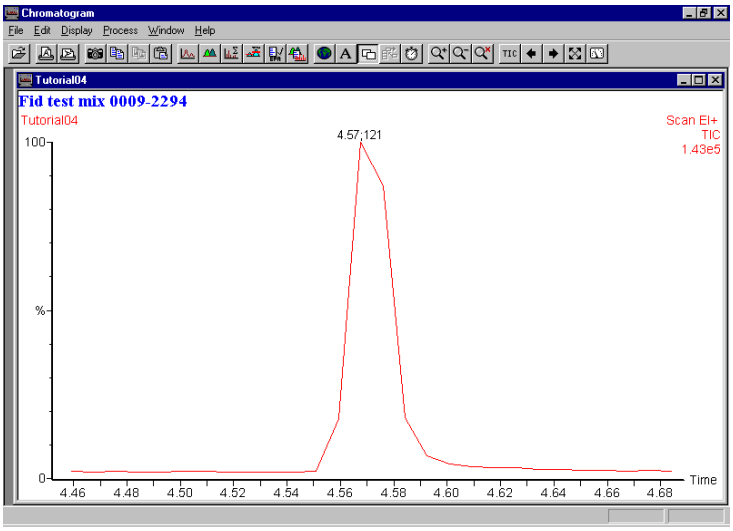
1. Display the mass chromatogram of the unknown.

2. Display the unknown peak of interest.
   Locate the peak of interest in the following window.

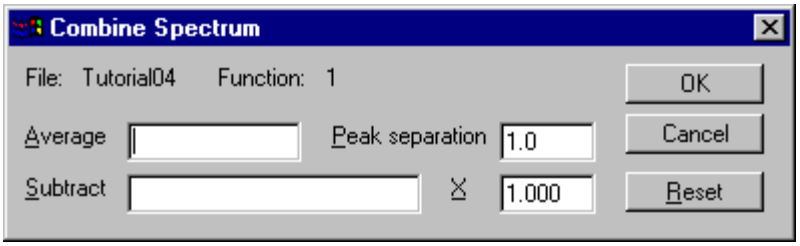


To view a particular peak or change the viewing area of the chromatogram, click and hold the left mouse button, drag it horizontally, then release it.

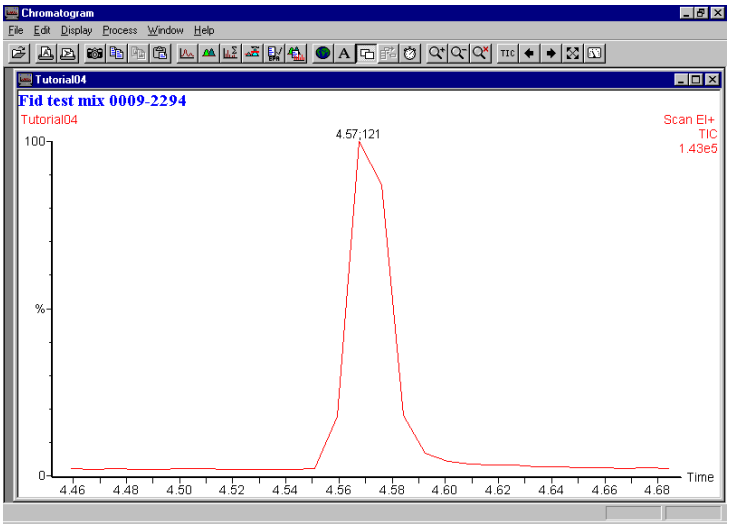
For example, when you do this across the peak shown at 4.57 minutes, a window similar to the following appears:



3. Select **Combine Spectra** from the *Process* menu to display the *Combine Spectrum* dialog.



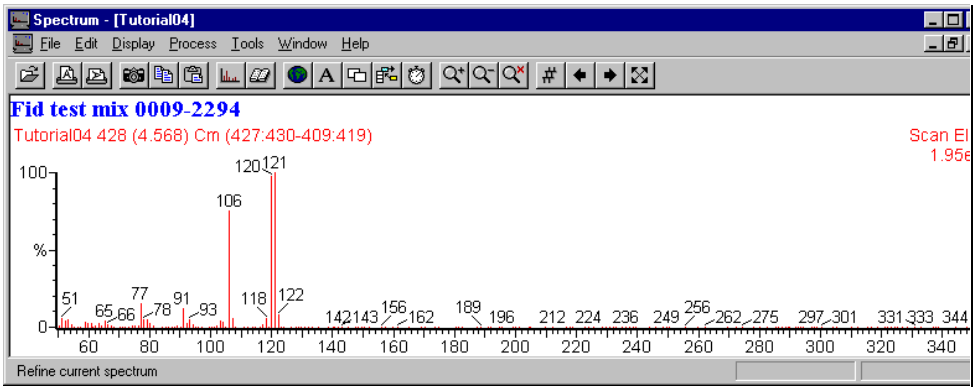
At about half peak height, use the right mouse button and click on one side of the peak and drag the mouse to the other side of the peak, then release the button. The selected scans appear in the *Average* text box.



Place the cursor over an area of the baseline, click and hold the right mouse button, and drag it over a portion of the baseline, then release it. The selected scans appear in the *Subtract* text box.

4. Click **OK**.

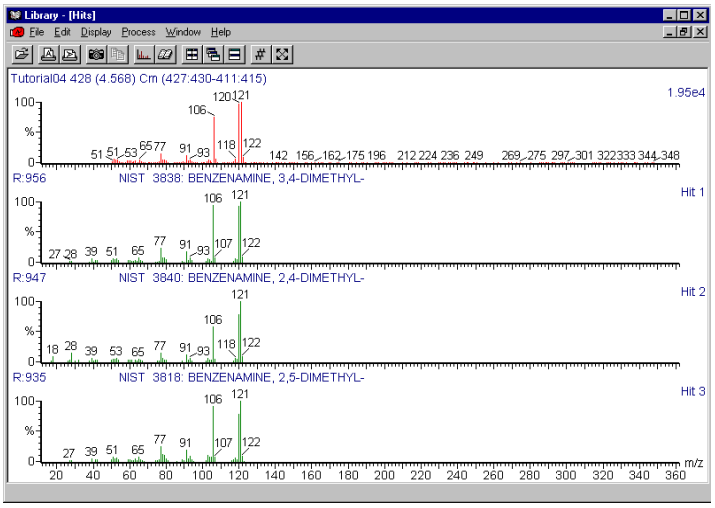
   The following background subtracted Spectrum is displayed.



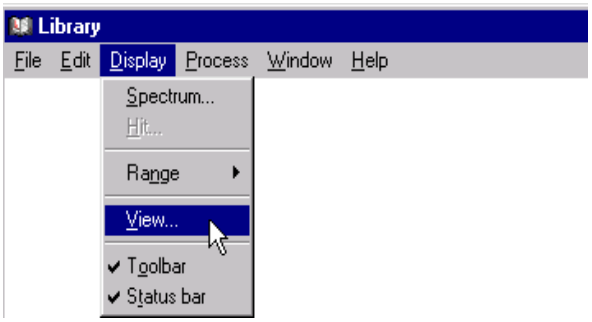
Background subtraction removes residual background peaks and column bleed from the spectrum. This produces a “purified” spectrum that is more representative of the eluting compound.

5. Click  (the book button) to perform a library search.

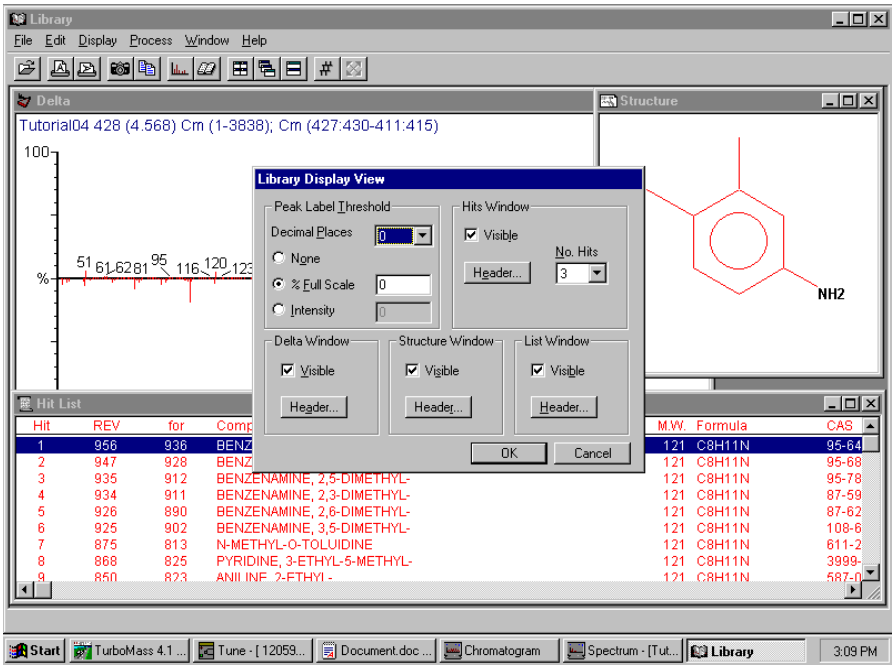
   A window similar to the following appears with the library search results.



6. You can change the library information by selecting **View** from the *Display* menu in the *Library* window.



A window similar to the following appears.



How to Build a Quantification Method 14

Quantification Methods

Since Quantification is analyzing data to determine the concentration of each sample using an internal or external standard, you need to acquire data from more than one sample. The previous example in this tutorial showed how to set up and run TurboMass using one sample. This quantification example uses a data set of previously obtained data stored in the TutorialQuant Project.

This chapter describes how to use TurboMass to perform quantitative assays. In Quantify you view the summary of Quantify results, calibration curves, and lists of integrated chromatography peaks.

TurboMass enables you to form Quantify calibration curves using Standard samples containing compounds of known concentrations. The calibration curves can then be used to calculate the concentrations of compounds in Analyte samples. In the Quantify Summary window, you can view the Quantify results. For example, you can view the calibration curves on screen and produce a number of Quantify reports. You can also copy Quantify information to the Windows Clipboard so that it can be used by other Windows applications.

Automated quantification provides a simple way of quantifying large numbers of samples within an analysis. Data can be acquired, processed, and reports printed without user intervention. This process is controlled from the Sample List Editor, which is a very important part of the Quantify system.

The following steps summarize the procedure for building a quantification method.

1. Create the GC method, MS method, and MS Tune.

2. Create a sample list for this analysis.

3. Create a Quantify method.

About Internal and External Standards

Before you begin, you need to decide whether you will use internal or external standards for your quantitative method. This section provides information on both.

In an internal standard method, a known and constant quantity of a compound that is not one of the analytes is added to the sample; this is the internal standard. The ratio of its retention time to the retention times of the analytes has been established. The ratio of its peak area to the peak areas of the analytes is determined for various concentrations of analytes. The unknown concentrations of the analytes in samples are then calculated, using the area of the internal standard peak as a reference. Response factors are included in the calculation to compensate for differences in the sensitivity of the detector to different analytes.

Analysis by an internal standard is the preferred technique whenever practical because it corrects for errors in sample preparation and variations in the amount of sample injected. The concentrations reported for the peaks of interest are affected only by the quantities of the various components and the quantity of internal standard added.

Internal standards should be of the same family as the target compounds (for example, phenols) but their retention times should generally not overlap those of the targets. An exception is isotopically labeled internal standards.

In an external standard method, known amounts of analytes are run in separate analyses (the standard runs), and the resulting peak areas are used to obtain calibrated response factors. In subsequent analyses of samples with unknown concentrations, the concentrations of the analytes are calculated by applying the response factors obtained from the standard runs.

Since, in an external standard method, there is no standard peak whose area changes with variations in injection size, the sample injection size must be reproducible from run to run. Therefore, external standard methods are best used with an autosampler. They are not recommended for manual injection.

Terminology

The software and the procedures in this chapter frequently use the following words and expressions:

Target compound

Analyte: a compound whose concentration is to be determined.

Quantification

Quantitative analysis: determining the concentration of a target compound from mass spectral data.

Internal Standard

An internal standard is a compound that is added, in known and constant concentration, to the sample. The ratio of its retention time to the retention times of the target compounds must be established, and the ratio of its peak area to the peak areas of target compounds must be determined for various concentrations of the target compounds. The software uses this information to determine the unknown concentrations of the target compounds in the sample. Multiple internal standards may be present in a chromatogram.

External Standard

Known amounts of analytes are run in a separate analysis, a standard run, and the resulting peak areas are used to obtain calibrated response factors that are stored in a calibration library. In later runs, these response factors are used to calculate analyte concentrations.

Response Factor Curve

This is a plot of peak area versus concentration for a given target compound. The software uses response curves to compensate for differences in the sensitivity of the detector to different compounds.

Calibration

In the Quantification software, the process of generating points on the response curve using scan files in which the analytes and internal standards (if any) are present in known concentrations.

Calibration Library

Calibration Library is a file that contains retention times, mass peak intensities, and concentrations for the internal standards, if any, and the target compounds. This file constitutes, in effect, a method for quantitative analysis.

Creating a GC and MS Method

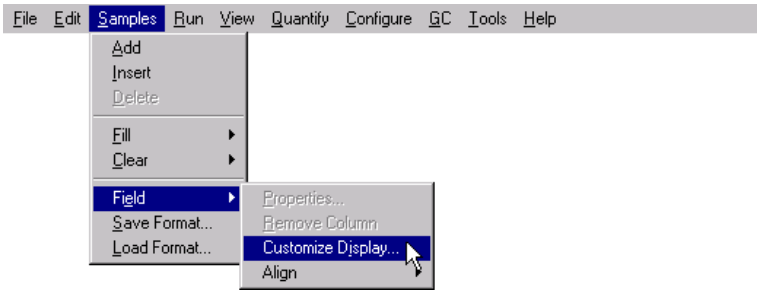
If you wish to run your own analysis, create and save a method to control the GC for your analysis, and create and save a method to control the mass spectrometer as described earlier in this tutorial. The method shown earlier in this tutorial was created specifically to analyze the mass spectrometer sensitivity test mix. You will need to create methods in a similar way for your own quantification analysis.

NOTE: *The example in this tutorial assumes you have run an analysis and collected data. To illustrate this, we will use a sample data set in the TutorialQuant project containing three mass chromatograms. Proceed to* Create the Quantify Method *on page 183.*

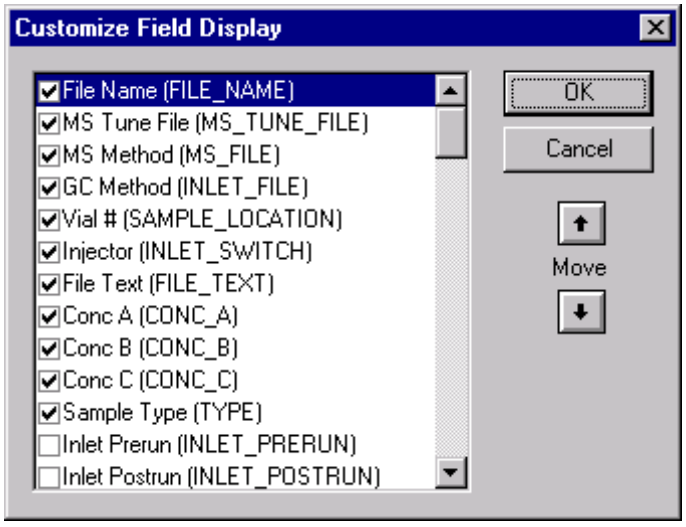
Creating a Sample List

After building the GC and MS method and Tuning and calibrating, you can create a Sample List to perform the analysis. Earlier in this tutorial you created a simple one-line sample list. Now you will create a multi-line sample list. These samples can be run (acquired) manually, but are more easily acquired automatically using the Clarus GC autosampler.

1. You can customize your sample list to add the categories necessary for quantification. To do this select **Customize Display** from the *Field* selection in the *Samples* menu.



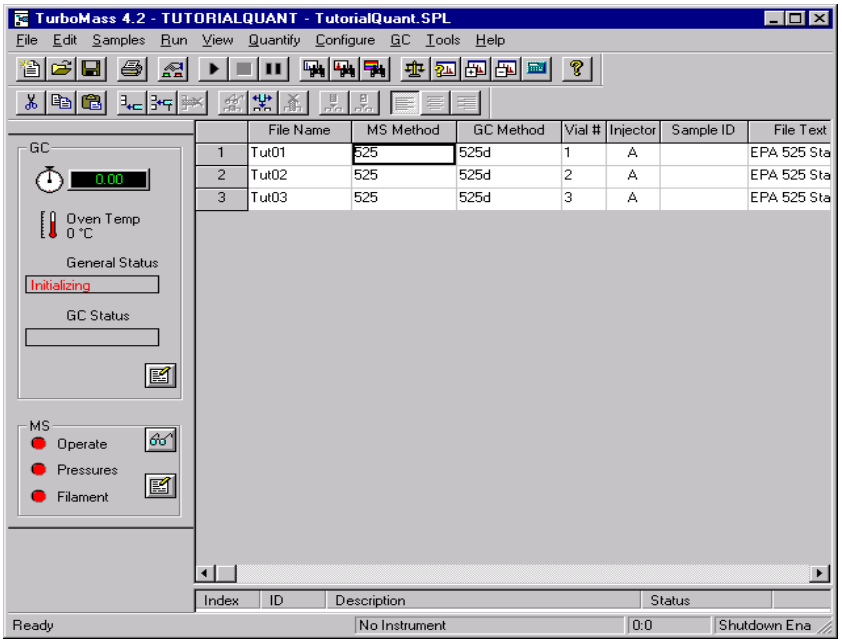
The *Customize Field Display* dialog is displayed.



2. Select the categories to display as columns in the *Sample List* and click **OK**. A √ indicates a selected category.

NOTE: *You may want to save this customized display for use with other analyses. To save this display, select* **Save Format** *from the* **Samples** *menu and enter a name for the file. This file will reside under the TurboMass directory as a "xxxx.fmt" file.*

The *Sample List Editor* now has columns such as **File Name**, **Vial # and Sample Type** that can be filled in for each sample. Each sample is displayed as one row in the *Sample List*. The following sample list contains three samples.

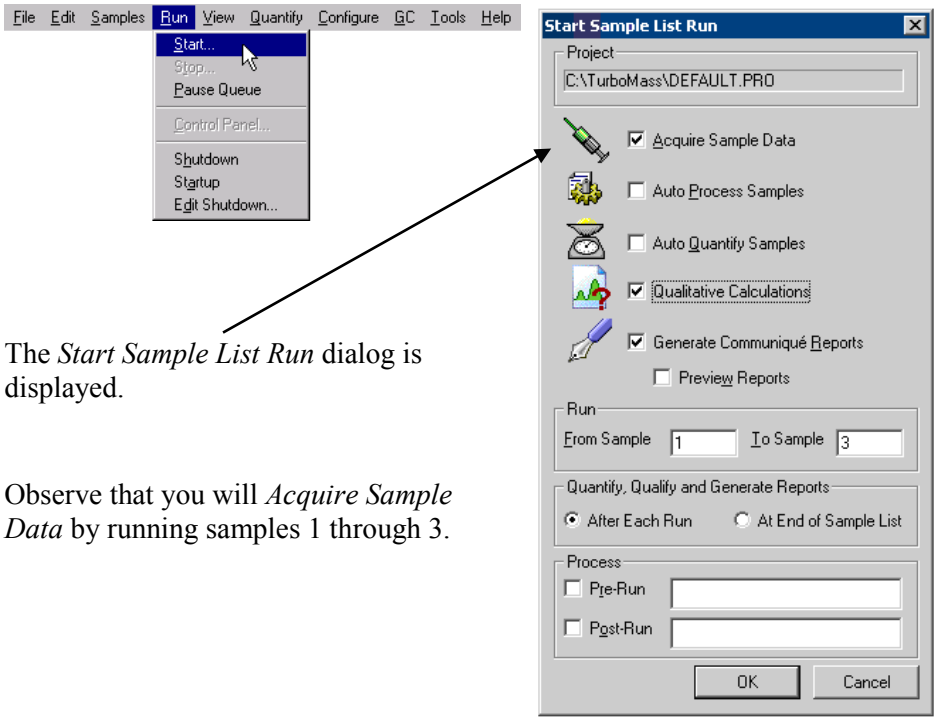


You need to tell TurboMass everything it needs to know about the samples in the list in order for it to perform a complete analysis. First, describe what each of the vials in the autosampler contains, that is, whether it contains a standard, an analyte, a blank or a QC sample, how to acquire it, and its concentration(s), if it is a standard. In addition, you must specify the name of the file in which to store the data. You may also want to add some management information such as Sample ID, the submitter's name, or a sample description.

Running the Samples

As this tutorial contains previously acquired data, you do not need to run samples. However, if you are running an analysis of your samples using this tutorial as a guide, then you need to follow this procedure.

1. Select **Start** from the *Run* menu.



The *Start Sample List Run* dialog is displayed.

Observe that you will *Acquire Sample Data* by running samples 1 through 3.

NOTE: Check **Preview Reports** to display Communiqué reports in a preview window prior to being printed

2. Click **OK**.

   The sequence of samples begins. A green dot appears next to the line of the sample list that is currently running.

Creating the Quantify Method

Before performing Integration or Quantification, you will create a Quantify Method using the Quantify method editor. By selecting a method from within the Method Editor this method becomes the current system method file and is used when performing Quantify operations. Changes made to the method are not permanent until they have been saved to disk. Consequently, you must save the method before it can be used to perform quantification by selecting Save from the File menu to update the current method file, or Save As from the File menu to save to a new method file.

Each Sample List may have only one Quantify method.

The Quantify method describes how a data file is processed to produce calibration curves and quantitative information. Details must be entered into the method for each of the compounds being used in the analysis.

The Quantify Method specifies information for performing the following tasks:

- Integration of a chromatogram trace to obtain peak information.
- Location of the chromatogram peak relating to a specific compound from the list of detected peaks.
- Calculation of a response factor for the located peak.
- Formation of a Quantify calibration curve.

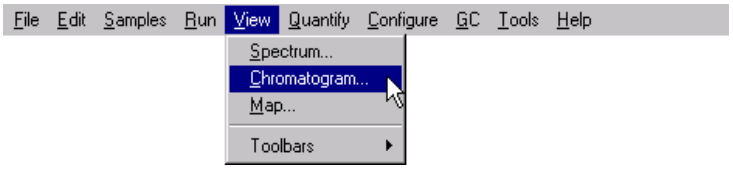
This procedure shows you how to build a method.

Identifying a Compound

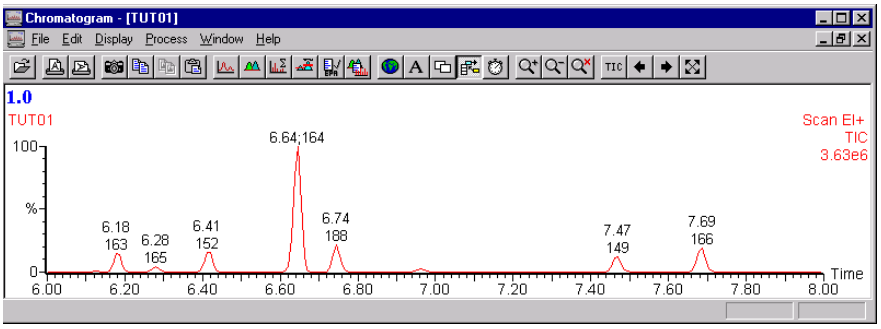
After running samples, TurboMass saves the collected sample information as a .RAW file in the Data directory of your Project. In this example, the data is stored in the Tutorial project. You will now select the .RAW data file and identify the compound from the RAW data. The first compound we will identify is dimethyl phthalate with a characteristic ion at 163 m/z.

We typically try to use the strongest, highest mass in the spectrum for quantification as it provides the best selectivity and sensitivity.

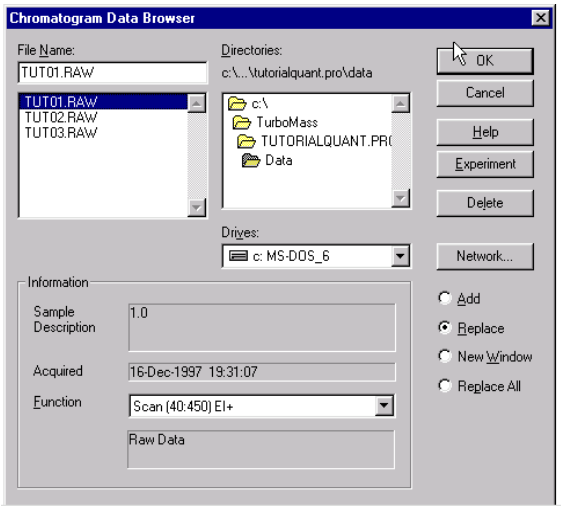
1. Select **Chromatogram** from the *View* menu on the *Sample List* window.



The *Chromatogram* window is displayed.

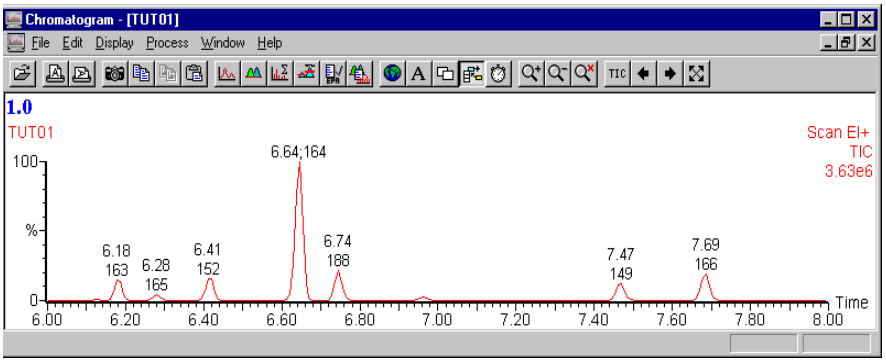


2. Select **Open** from the *File* menu to display the *Chromatogram Data Browser* dialog.

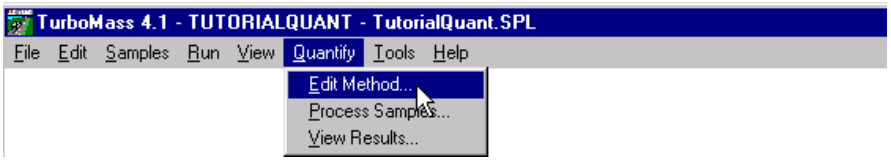


3. Select the **.RAW** chromatogram file from the *Chromatogram Data Browser*.
   In this example the .RAW chromatogram is TUT01.RAW.

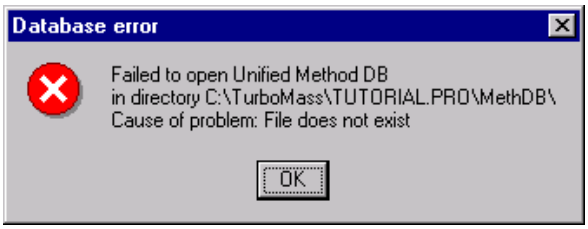
4. Select **Replace** to display only one chromatogram, then click **OK**.
   The Chromatogram of TUT01.RAW is displayed.



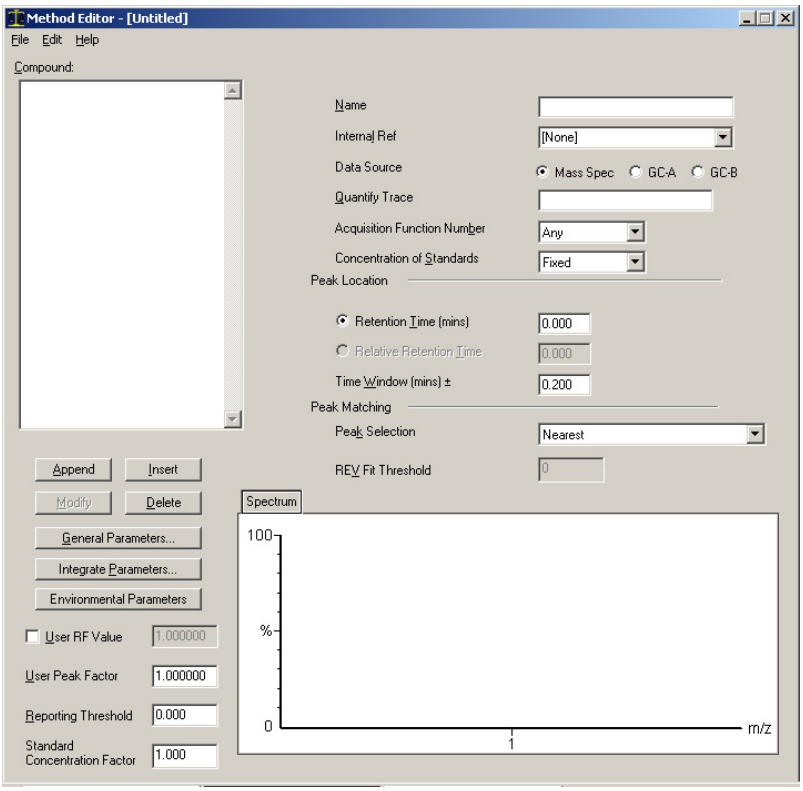
5. Select **Edit Method** from the *Quantify* menu on the *Sample List* window.



If no method currently exists, the following message is displayed:

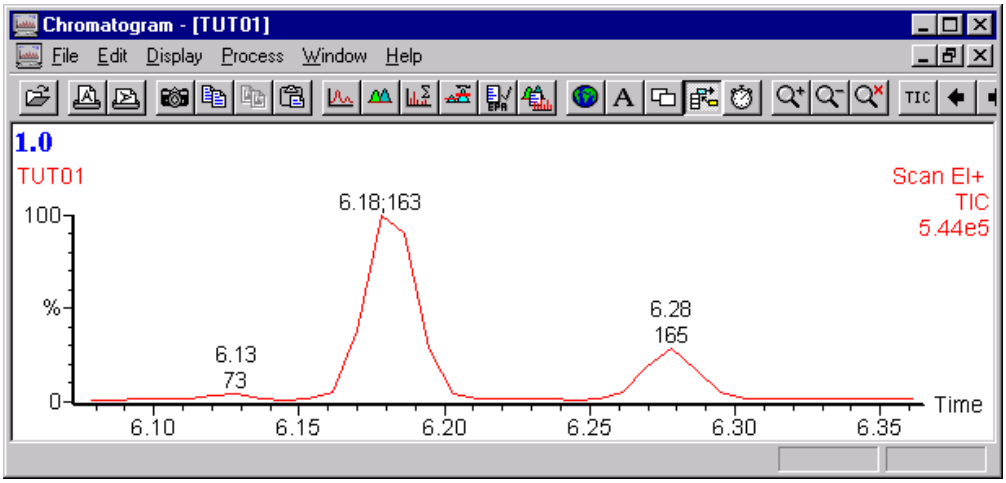


6. Click **OK** to clear the message.
   The following *Method Editor* dialog is displayed.

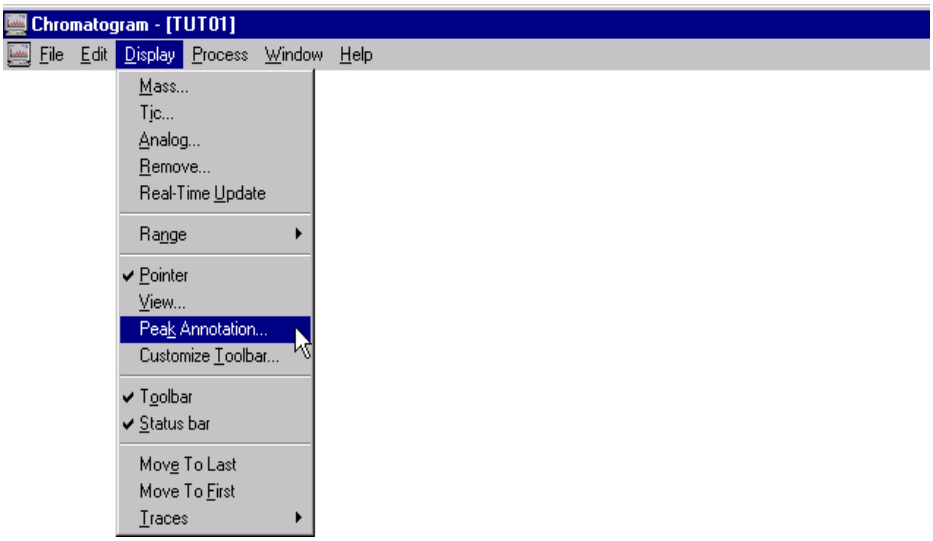


7. Display the chromatogram and expand it around the 6.18 minute mass 163 peak.
   Position the cursor at the 6 minute mark, press and hold the left mouse button, drag the mouse to the 6.35 minute mark (as indicated by a drawn line), and release the mouse button.

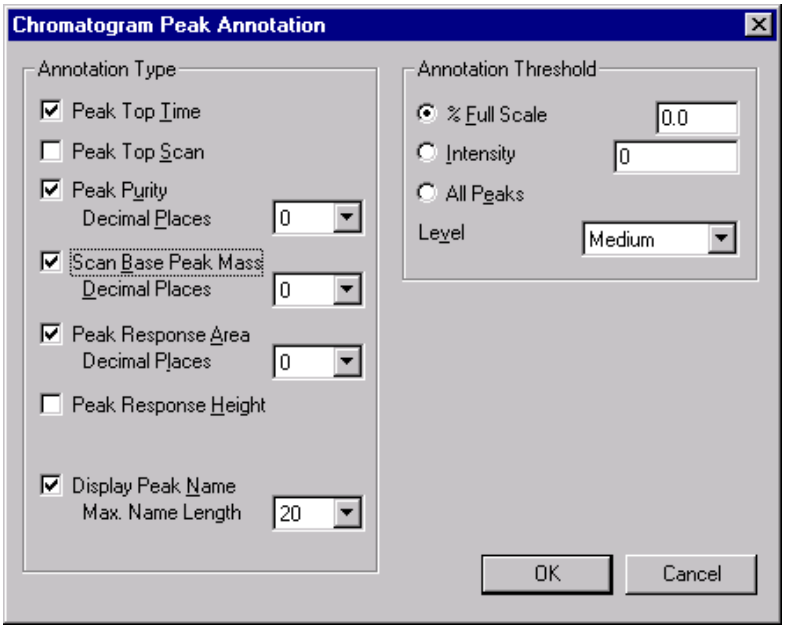
The following Chromatogram is displayed.



8. Select **Peak Annotation** from the *Display* menu.



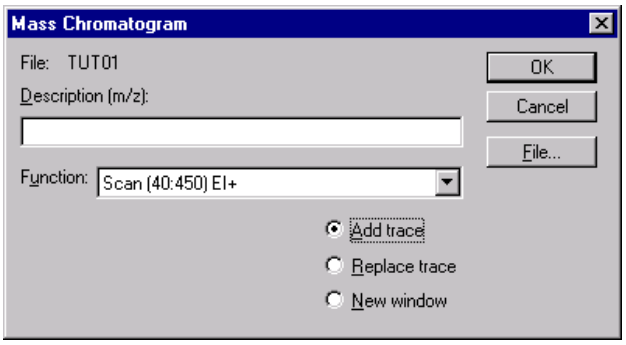
The *Chromatogram Peak Annotation* dialog is displayed.



9. Check that the boxes are checked as shown above. If not, select the boxes to check them.

10. Click **OK**.

11. Select **Mass** from the *Display* menu on the *Chromatogram* window.
    The *Mass Chromatogram* dialog is displayed.

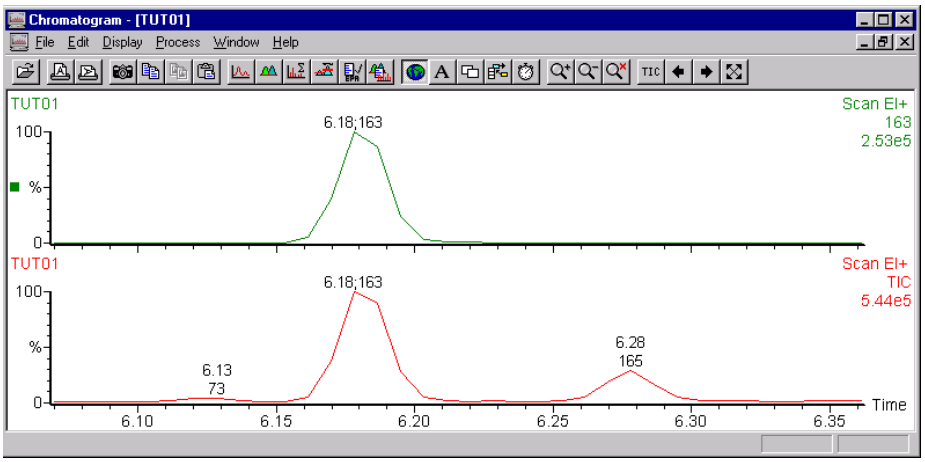


12. Make the following entries:

    - Position the cursor in the *Description (m/z)* text box and enter **163**.
    - Select **Add trace.**
    - Click **OK**.

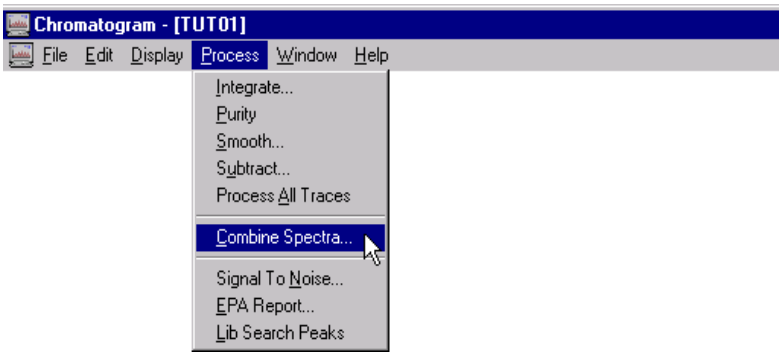
    The following Chromatogram is displayed.

    If necessary, expand the chromatogram around the 6.18 minute peak by positioning the cursor at the 6 minute mark, press and hold the left mouse button, drag the mouse to the 6.35 minute mark (as indicated by a drawn line), and release the mouse button.

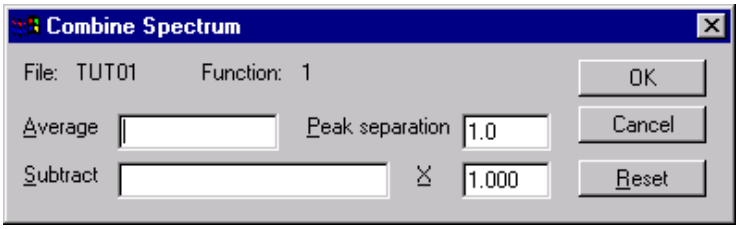


Observe how the m/z 163 trace has much less interference and a much better signal-to-noise ratio, even though it sacrifices some signal intensity.

13. From the *Process* menu, select **Combine Spectra**.

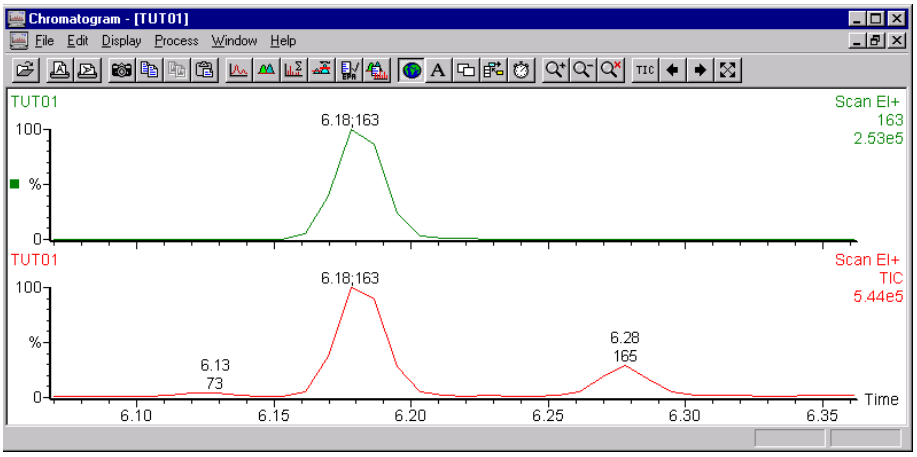


The *Combine Spectrum* dialog is displayed.

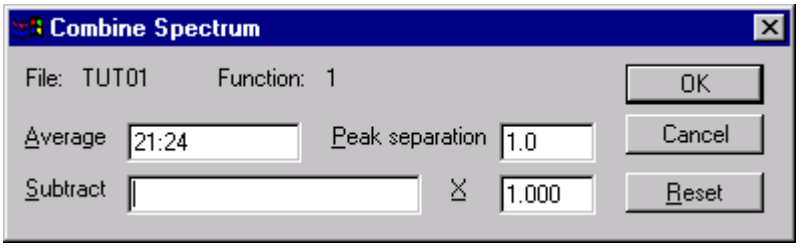


14. Enter the **Average** scans.

    In the upper Chromatogram window shown below, position the cursor on the middle of the left side of the 163 mass peak apex, press and hold the right mouse button, drag the mouse across the peak (as indicated by a drawn line), and release the mouse button.



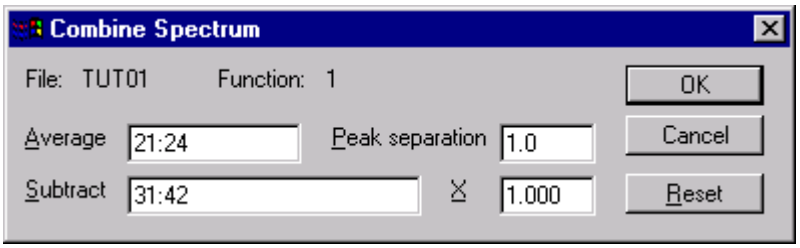
    This enters the selected scans in the *Average* text box in the *Combine Spectrum* dialog.



15. Enter the **Subtract** scans.

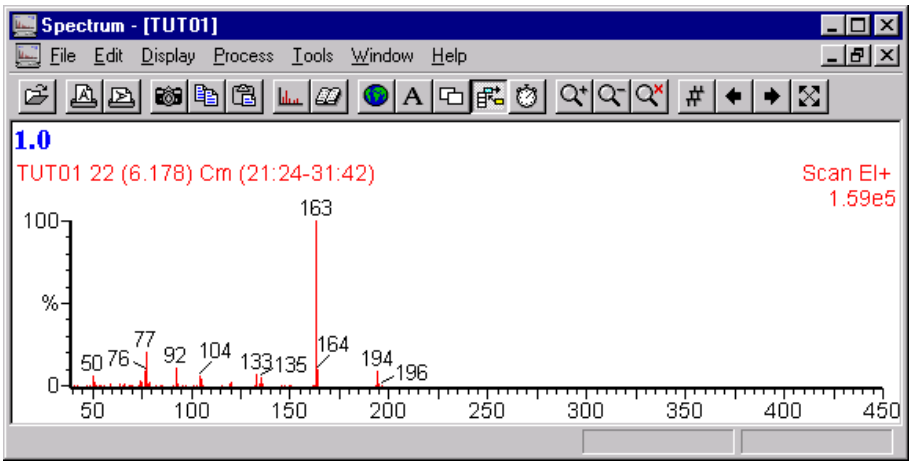
    Put the cursor in the *Subtract* text box. Then in the *Chromatogram* window, position the cursor to the left of the 163 mass peak, press and hold the right mouse button, drag the mouse across an area above the baseline, but not across the peak (indicated by a drawn line), and release the mouse button.

    This enters the selected scans in the *Subtract* text box in the *Combine Spectrum* dialog.



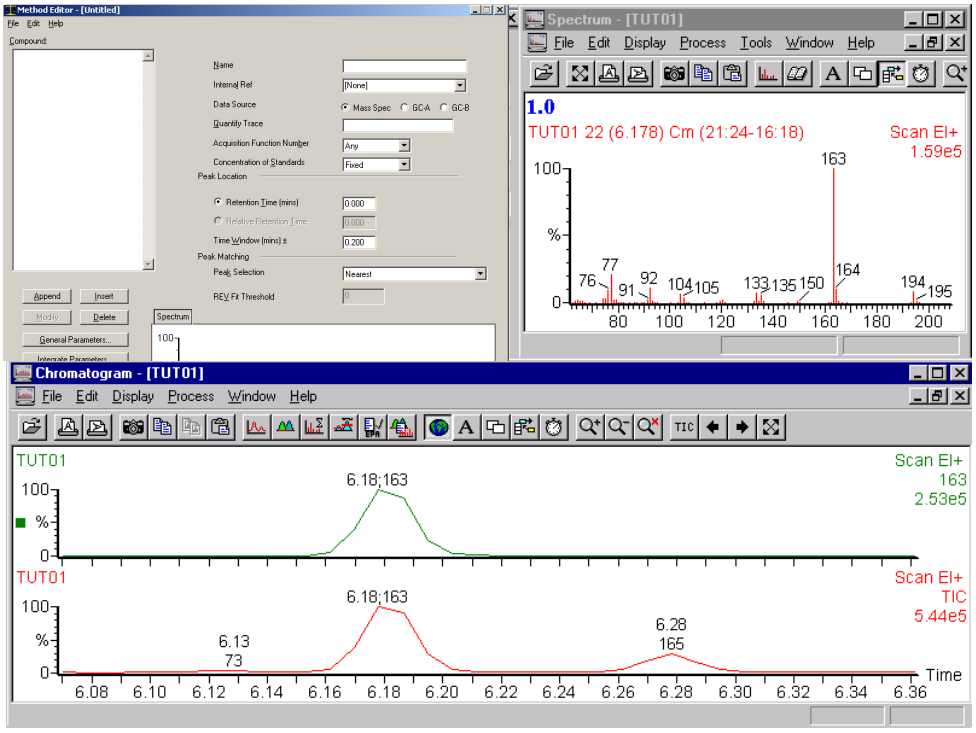
16. Click **OK**.

    The following Spectrum window is displayed.



This produces a purified spectrum of dimethyl phthalate by removing residual background contaminants, partially eluting peaks, and column bleed from the spectrum.

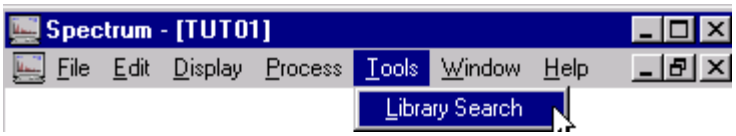
17. Arrange your windows as shown below.



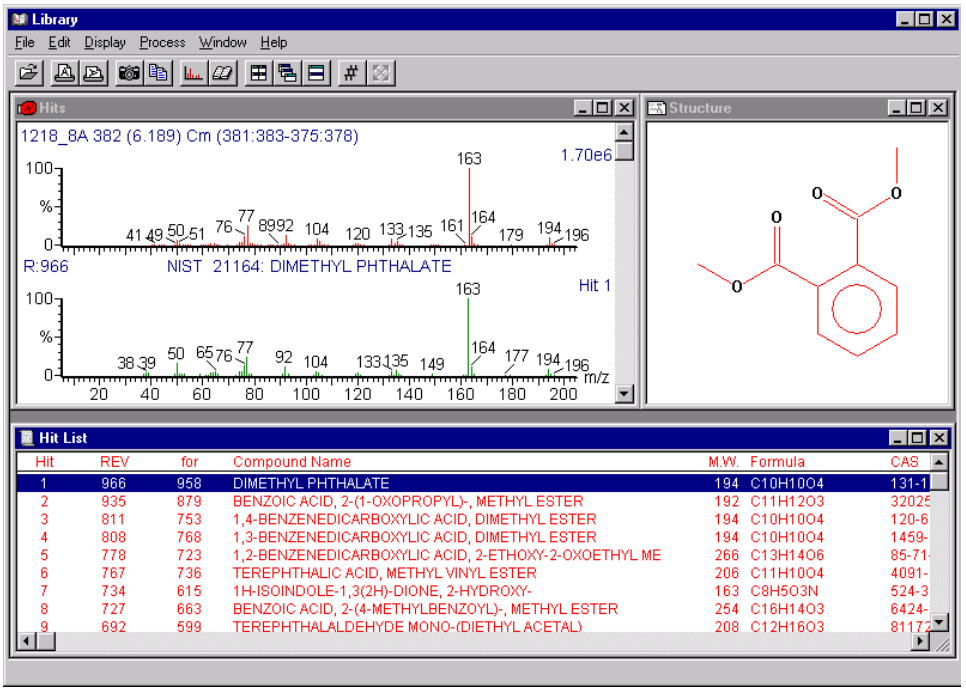
18. Perform a Library search on this peak by selecting **Library Search** from the *Tools* menu on the *Spectrum* window

OR

Click  .



A library search of the compound is performed and the *Library* window is displayed:



The library search has identified this peak as dimethyl phthalate.

19. Click the **close** button in the upper right corner of the window (in the *Library dialog* header) to close.

Entering Parameters into the Quantify Method (For Expert Users)

Summary of the procedure:

1. Open the chromatogram of the calibration run of interest. Have the method editor open at the same time.

2. In the chromatogram screen, select the peak of interest and click to get the spectra window open.

3. In the spectra window, double-click the mass you want to quantify on. This will open another chromatogram with that extracted mass of interest.

4. Right-click and drag a retention time window in the extracted mass chromatogram, and notice that in the message bar it say "retention time window XX where XX is minutes.

5. Then right click on the extracted ion peak of interest.

6. Finally copy the spectrum list from the spectra window.

7. Toggle back to the method, and observe that all the values are in the right place.

8. Paste the spectrum, add the name, and append

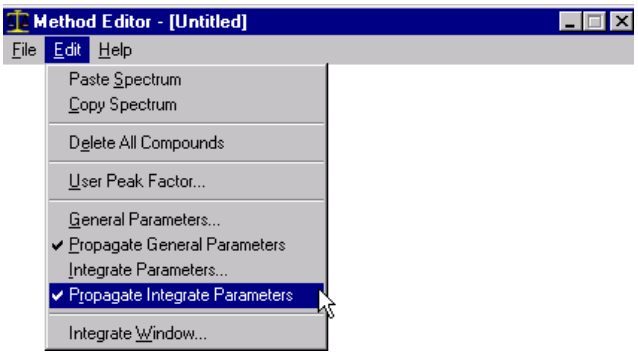
Identifying and Entering the First of Three Compounds

Each compound in the Sample List must be identified and entered in the Method Editor to properly quantify the results. This example illustrates how to enter three compounds.

1. Select **Edit** from the *Quantify Method Editor* window.

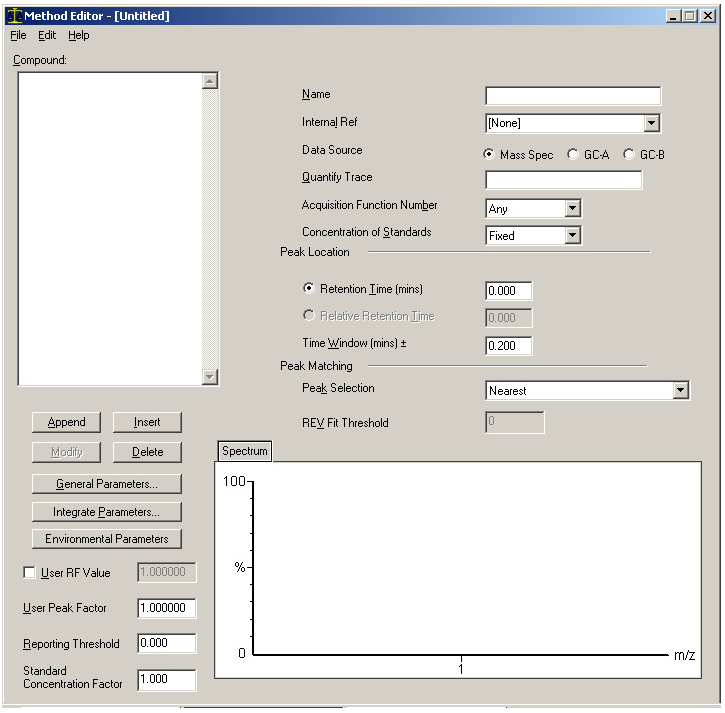
   Make sure the following two items are checked in the *Edit* menu. If the item is not checked, clicking on the item displays the checkmark.

   - Propagate General Parameters.
   - Propagate Integrate Parameters.



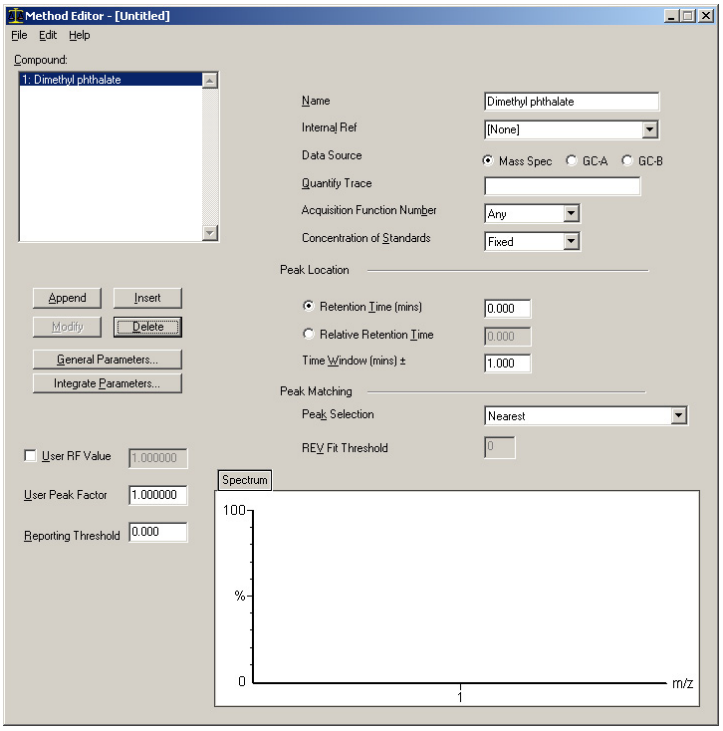
These selections ensure that various peak detection and integration parameters are used for all compounds. If these items are not checked each compound may have its own parameters.

2. Display the *Method Editor* dialog.



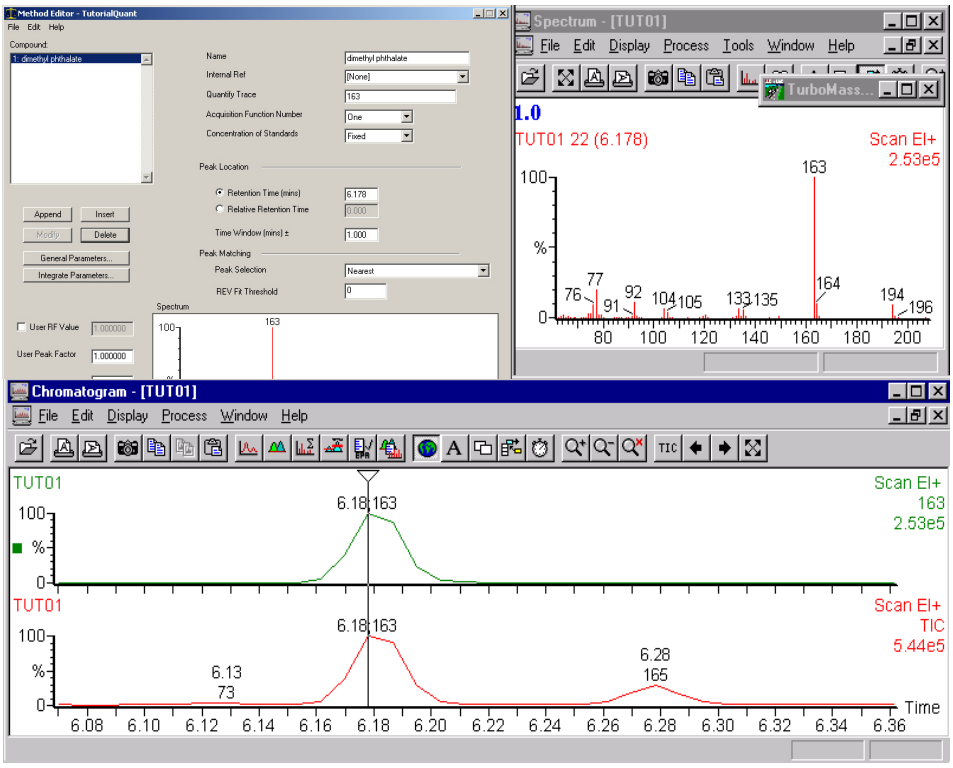
3. Type **Dimethyl phthalate** in the *Name* text box.

4. Click **Append**.
   Dimethyl phthalate appears in the *Compound* list.



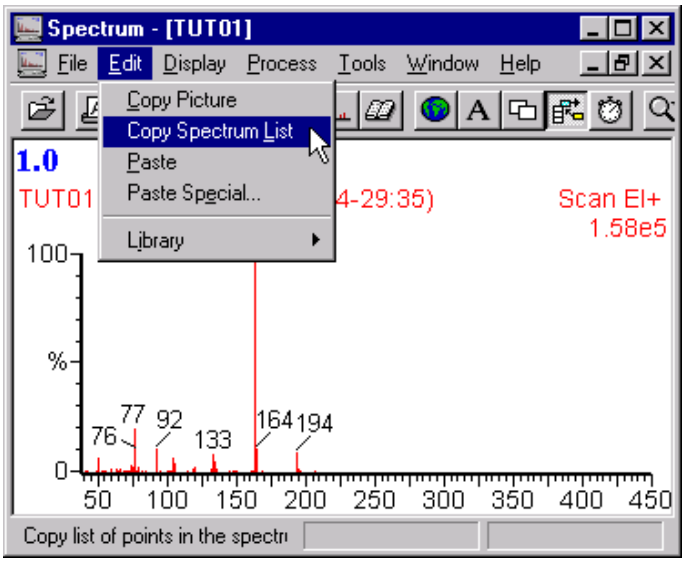
5. Enter the **Quantify Trace** and chromatogram's **Retention Time** values for m/z 163 by positioning the cursor at the peak apex and clicking the right mouse button.

A line is drawn through the peak to show where the apex is selected.



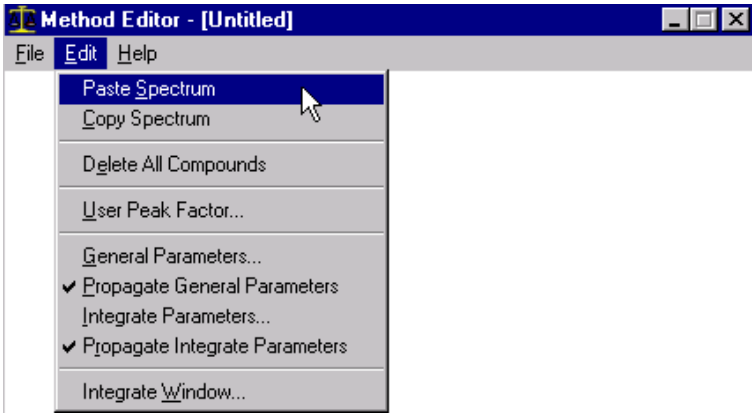
The **Quantify Trace** and **Retention Time** values for m/z 163 are now entered in the *Method Editor* window.

6. To enter the Spectrum, select **Copy Spectrum List** from the *Edit* menu on the *Spectrum* window.

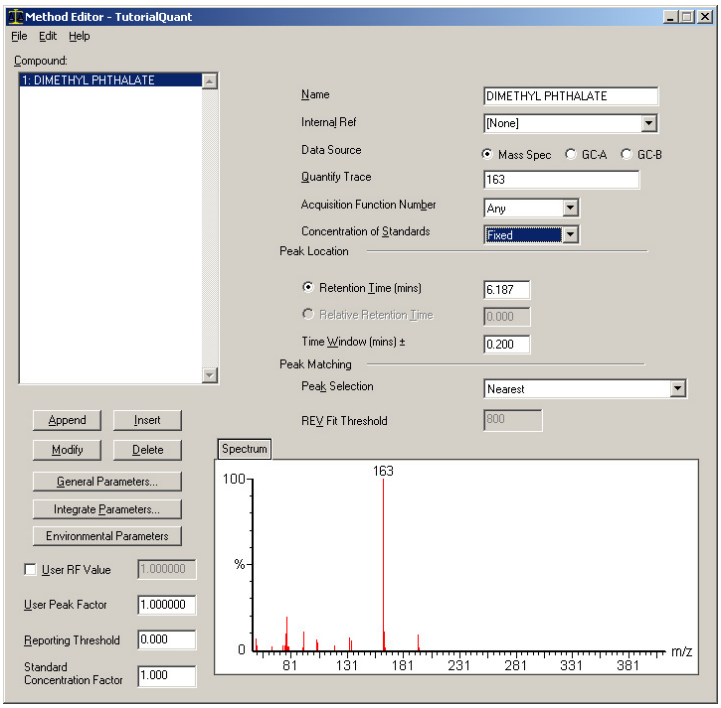


This copies all information for this spectrum.

7. Select **Paste Spectrum** from the *Edit* menu on the *Method Editor* window.



The spectrum information is now pasted in the Method Editor with the spectrum in the bottom window.

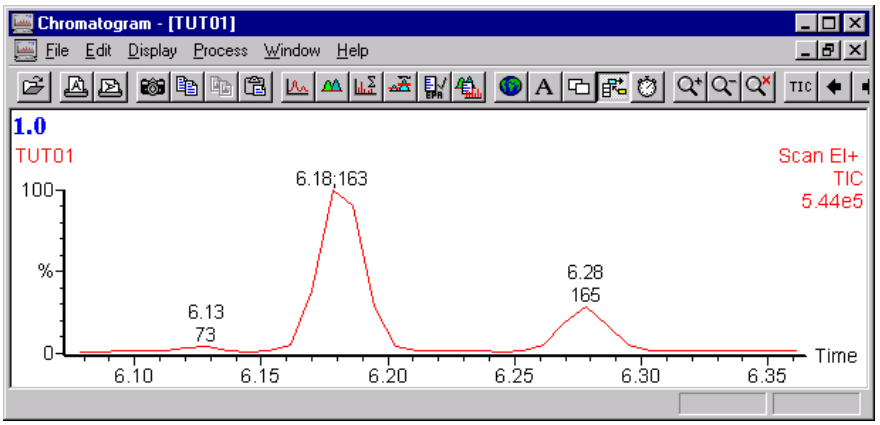


8. Select **Conc A** from the *Concentration of Standards* drop-down list.

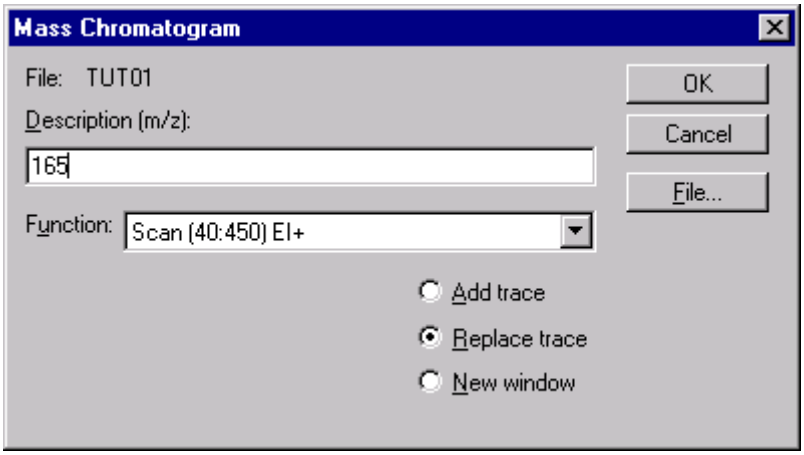
9. Click **Append** to save this information in the method.

Identifying and Entering the Next Compound

1. Display the Chromatogram.



2. Select **Mass** from the *Display* menu.
   The *Mass Chromatogram* dialog is displayed.

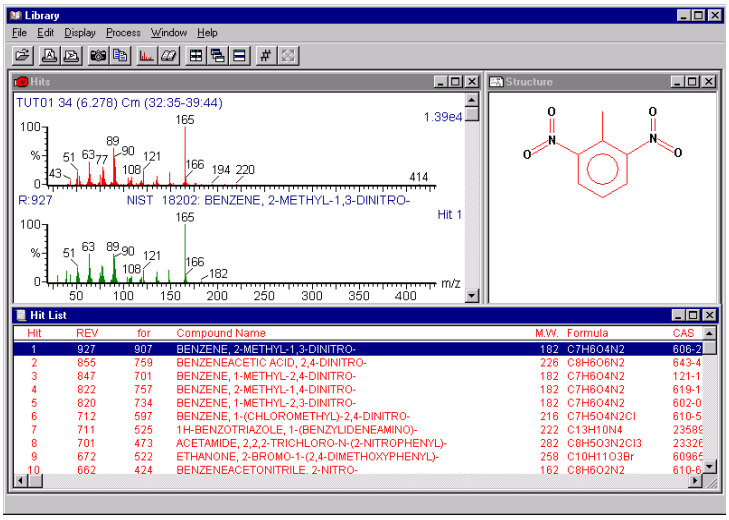


3. Enter **165** for the *Description (m/z)* of your next peak.

4. Select **Replace trace**, and click **OK**.

5. Repeat the following procedures as described for the first compound.

   - Perform Combine Spectra and Background subtract.
   - Perform a Library Search to identify the compound (2,4–dinitrotoluene).



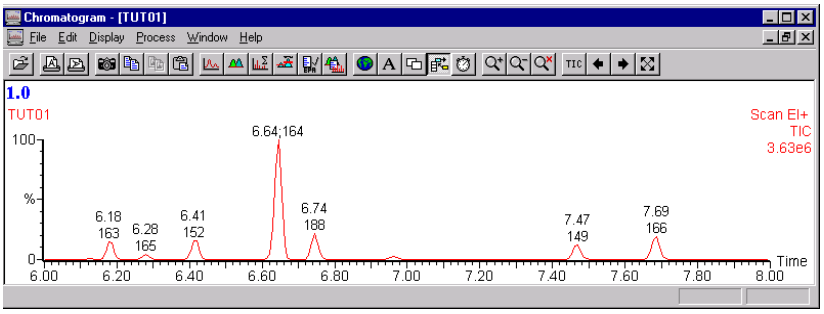
   - Enter 2,4–dinitrotoluene in the **Name** text box on the *Method Editor* window.
   - All of the dinitrotoluenes generate almost identical spectra.
   - Click **Append** to enter 2,4–dinitrotoluene to the list of compounds.

NOTE: *After typing* **2,4–dinitrotoluene** *in the* Name *field, click* **Append** *to add this name to the list of compounds. Do not click* **Modify** *since this will overwrite the current entry.*

   - Enter the **Quantify Trace** and **Retention Time** values for m/z 165 by positioning the cursor at the peak apex and clicking the right mouse button.
   - Copy and paste the spectrum information into the *Method Editor*.
   - Select **Conc A**.

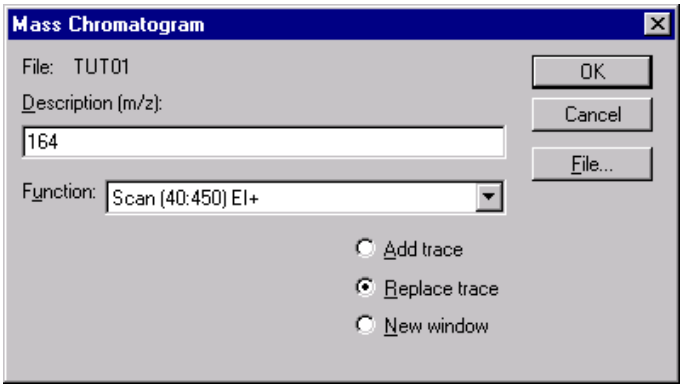
Entering the Internal Standard

➢ Display the Chromatogram.



The internal standard is mass 164.

1. Select **Mass** from the *Display* menu.
   The *Mass Chromatogram* dialog is displayed.



2. Enter **164** for the *Description (m/z)* of your next peak, select **Replace trace**, and click **OK**.

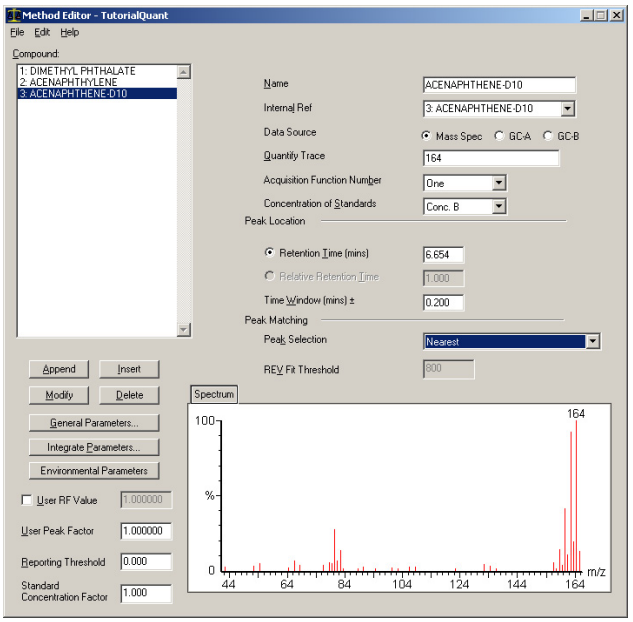
3. Repeat the following procedures described above.

    - Perform Combine Spectra and Background subtract.
    - Perform a Library Search to identify the compound (Acenaphthene-d10).
    - Enter **Acenaphthene-d10** in the *Name* field on the *Method Editor* window.

NOTE: *After typing Acenaphthene-d10 in the* Name *field, click* **Append** *to add this name to the list of compounds. Do not click* **Modify** *as this will overwrite the current entry.*

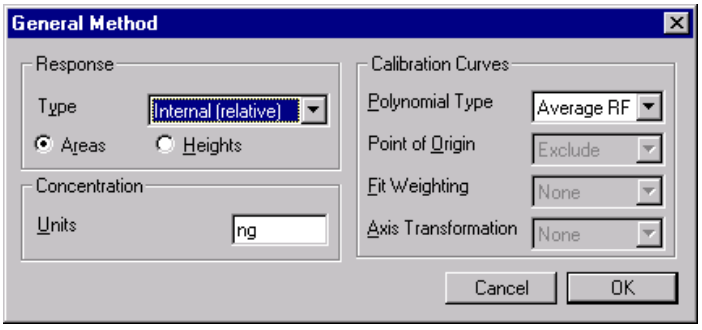
    - Enter the **Quantify Trace** and **Retention Time** values for m/z 163 by positioning the cursor at the peak apex and clicking the right mouse button.
    - Copy and paste the spectrum information into the *Method Editor*.
    - Select **Conc B**.
    - In this data set the analytes have one set of concentrations indicated by "A" and the Internal Standards have a different, fixed concentration indicated by "B" in the *Sample List* window.
    - Select **Acenaphthene-d10** as the *Internal Ref.*
    - Click **Append** to enter Acenaphthene-d10 to the list of compounds.

4. Select each of the two previously entered compounds, select **Acenaphthene-d10** as the *Internal Ref*, and click **Modify** after each selection.



5. Click **General Parameters** and verify that the *Type* is set to **Internal (relative)**.

6. If *Type* is not set to **Internal (relative)** select it from the drop-down list and click **OK**.



7. When you have entered your last compound, select **Save As** from the *File* menu and name the *Quantify method* Tutorial.

8. Close all windows except the *Sample List* and proceed to *Processing Quantification Results* on page 209 to process your data.

How to Process Quantification Results 15

Processing Quantification Results

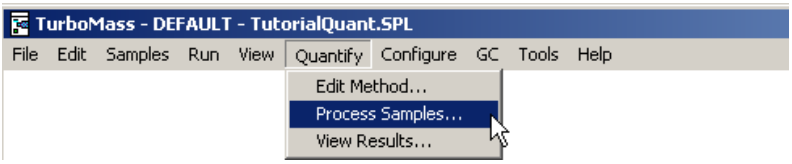
After acquiring your data and creating your quantification method, your next step is to process and view the results. This chapter shows how to process results, view calibration curves, and print reports.

The following steps summarize the procedure for processing quantification results.

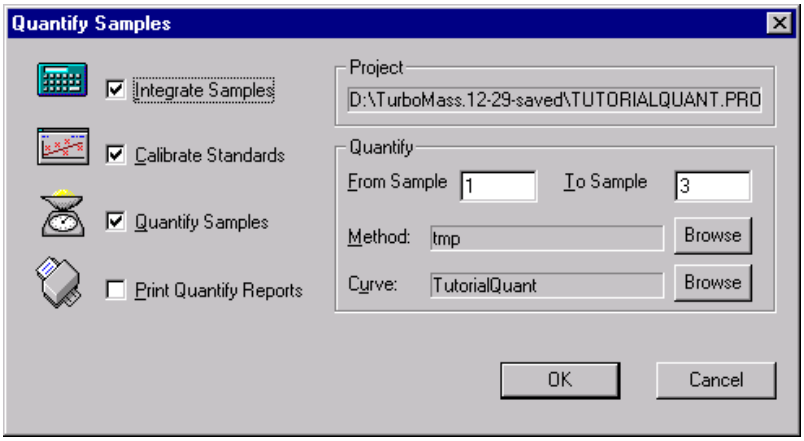
1. Select **Process Samples** from the *Quantify* menu.
2. Verify the proper *Quantify Method* file.
3. Create the calibration curve file.
4. Run *Quantify*.
5. Select **View Results** from the *Quantify* menu.
6. Select **View** from the *Display* menu to set the way you want your results displayed.
7. View the integrated peak.
8. Print the results.

Processing Samples

1. Select **Process Samples** from the *Quantify* menu.



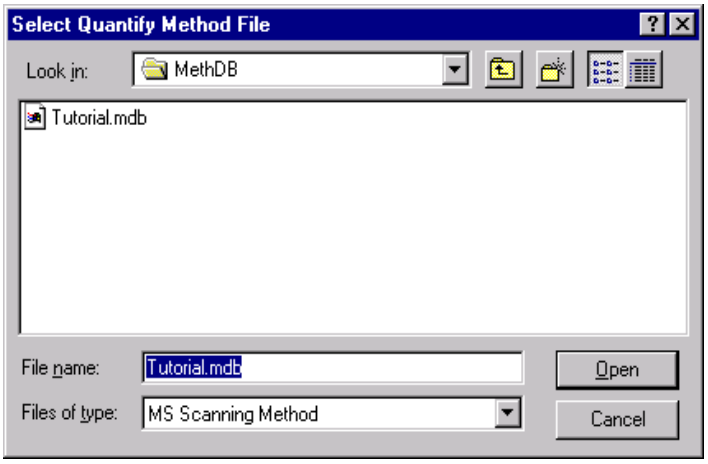
The *Quantify Samples* dialog is displayed.



Make sure that the proper boxes are checked, that you are quantifying the proper samples (for example, **From Sample** 1 **To Sample** 3), and that you are quantifying with the proper *Method* and calibration *Curve*.

2. Verify the proper *Quantify Method* file by clicking **Browse** next to the *Method* text box.

   The Select Quantify Method File dialog is displayed.

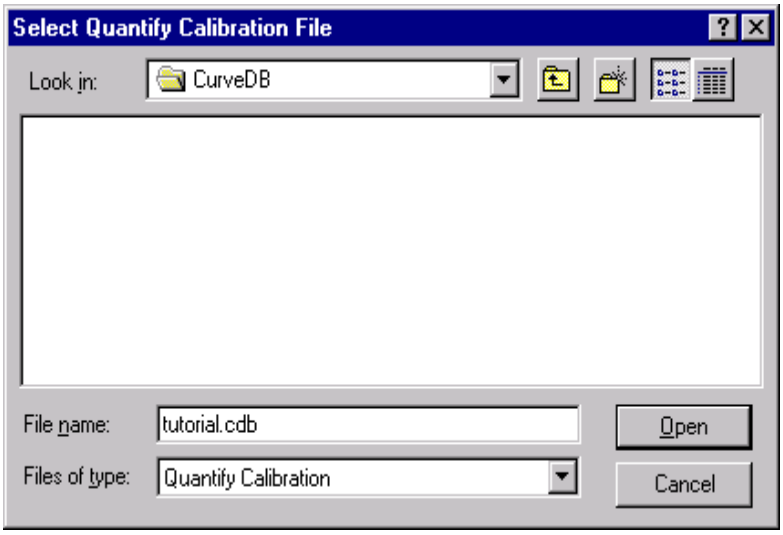


   The *Quantify Method* file in this example is Tutorial.mdb.

   If necessary, select Tutorial.mdb and click **Open**.

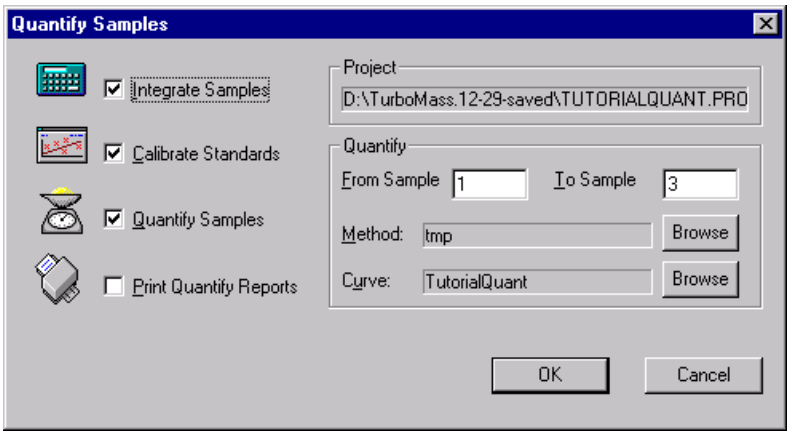
Calibrate the method by creating the *Curve* file as follows:

1. Click **Browse** next to the *Curve* text box.
   The Select Quantify Calibration File dialog is displayed.

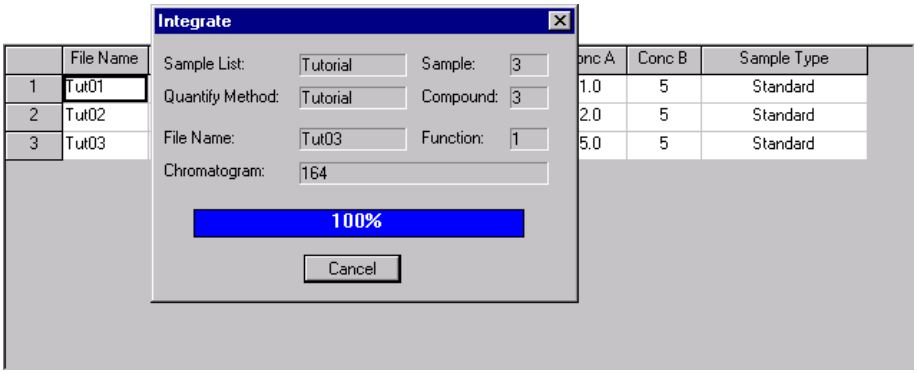


2. Enter a unique **File name**.
   In this example a unique file name is tutorial.cdb.

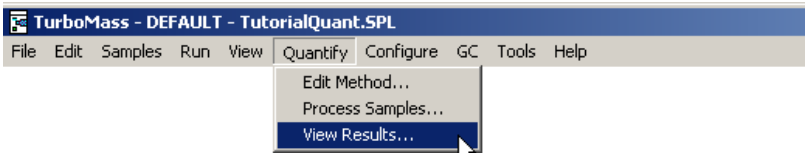
3. Click **Open**.
   The *Quantify Samples* dialog is displayed.



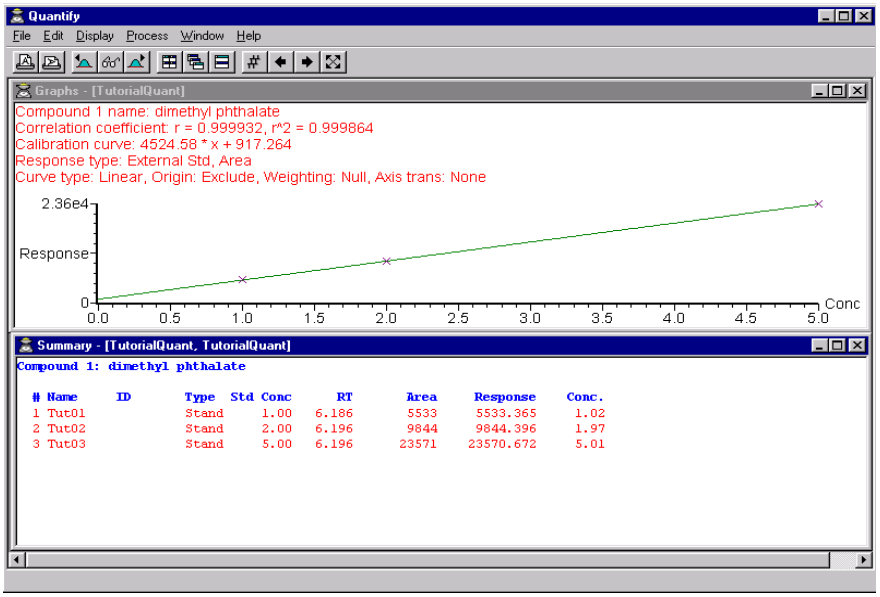
4. Click **OK** to quantify.
   The following dialog is displayed as the quantification process progresses.



5. Select **View Results** from the *Quantify* menu.

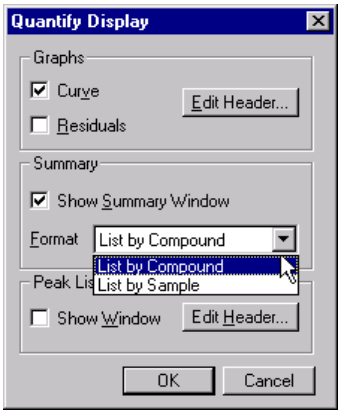


The following Quantify window is displayed.



6. To set the way results are displayed, select **View** from the *Display* menu.

   The *Quantify Display* dialog is displayed.



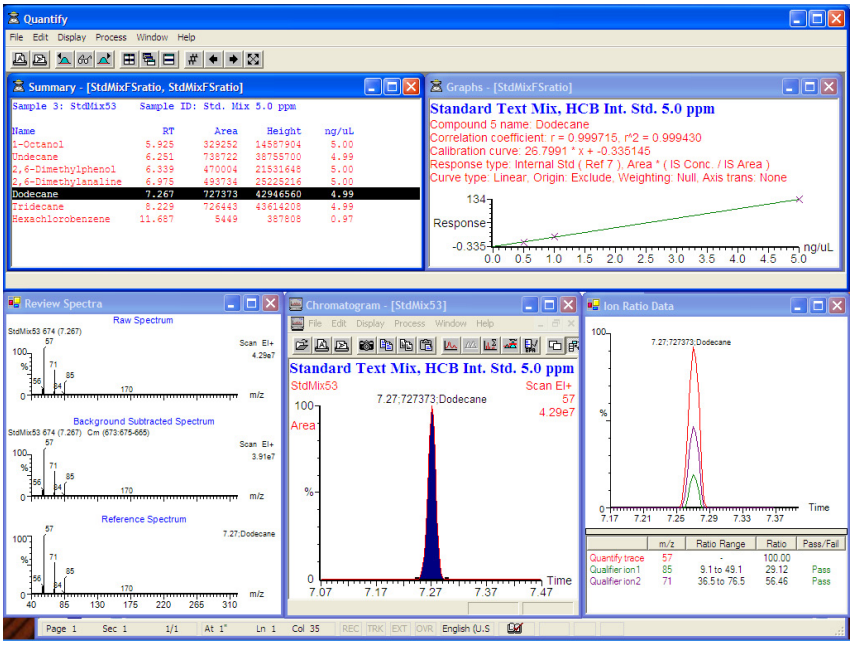
   This dialog enables you to set the way you want your results displayed. You can select **List by Compound** to show the results for all chromatograms in the *Sample List* for that compound, or ***List by Sample*** to show all of the compounds for that sample.

   This example shows that **List by Compound** was selected.

7. View the integrated peak by double-clicking on the compound name in the *Summary List* or double-clicking on the plotted point in the graph.

   You can interactively review the data using a view similar to the following:

NOTE: *The different windows appear and you will have to arrange them for your particular need as shown below.*



If the peak isn't scaled properly, click  .

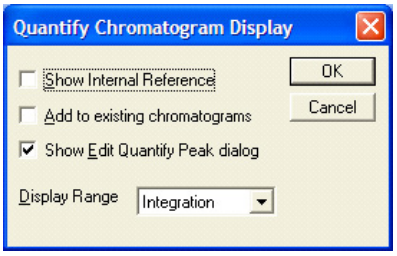
You can also drag the small boxes at the base of the peak to perform manual integration of that peak.

8. To view data for the next (or previous) peak click the toolbar button: 

   (or )

To modify peak data:

1. Double-click on the compound name in the Summary window you wish to manually integrate.
2. If the Edit Quantify Peak dialog is not displayed, select Chromatogram from the Display menu in Quantify and make sure the Show Edit Quantify Peak dialog is selected. Then try step 1 again.



3. Perform manual reintegration, if necessary.
4. Click OK to accept the changes made and have the results display updated.

How to Run a Qualitative Analysis 16

About the Qualitative Method

A Qualitative Method defines the parameters required for generating reports that only optionally make use of quantitative results. You can specify a Qualitative Method in each row of the Sample List. The method parameters fall into two main categories:

- Defining the parameters for generating a peak data set
- Setting library search parameters

The Qualitative Method Editor may be accessed by one of the following two ways:

- By selecting the Qualitative Method cell in the Sample Table, right-clicking and choosing the Open command from the context menu. If no method name is specified in the cell, or if there is currently no qualitative method of that name in the project directory, then the Qualitative Method Editor will open in the 'New Method' state. If the cell contains the name of an existing Report Method then Qualitative Method Editor will open in the *Edit Method* state.

- By choosing the Qualitative Method Editor command from the TurboMass Tools menu. The default method will be the most recently used. If there is currently no qualitative method of that name in the project directory (that is the METHDB subdirectory in the project hierarchy), then the Qualitative Method Editor will open in the *New Method* state.

The Qualitative Method Editor window has three tabs; General, Search Parameters and Library Settings tab. Each of these tabbed sub-windows has various controls associated with them.

The Qualitative Method is initiated and run by the TurboMass application from the Sample List upon completion of the sample or the Sample List.

NOTE: *The names in the Qualitative Results may come from the Library Search results, or from the Quantify results (if they exist).*

Also, if a peak has both Qualitative and Quantify results, the Quantify name is used. The NIST Library Search results are displayed in upper case since Quantify displays both upper and lower case.

The Qualitative Process

There can be one or two stages to the Qualitative process:

Stage 1: Integration and peak selection:

This is the integration of one of more defined portions of the chromatogram to generate a list of chromatogram peaks. This step is always carried out if a Qualitative Method is specified in the Sample List. The processing steps are:

1. Integrate the chromatogram using the parameters specified in the Qualitative Method.
2. Sort the peaks in retention time order.
3. If more than one peak maximizes within ±2 scans, eliminate all but the one with the largest area.
4. If 'Exclude target compounds' is set in the method, remove peaks that maximize within ±2 scans of the actual retention time of the target compound taken from previous quantification results. This setting is ignored if no quantification results are available. If this option is not checked and quantification results exist then these results (including compound name, area, height and concentration) will be associated with the appropriate peak in the data source. However, Area% and Norm% results will always come from the qualitative processing.
5. Sort in descending area order.
6. Eliminate all but the largest n peaks (where n is the 'Largest peaks' parameter from the qualitative method).
7. Re–sort in retention time order.
8. Calculate Area% and Norm% values for each peak
9. Add the peak data to the data source.

   The output of this process will be a collection of peak data which can be made available to Communiqué as a data source for Qualitative Reports and/or used as input to the automatic library search.

Stage 2: Library search process:

The library search process is the automatic library searching of the chromatographic peak spectra and reporting of potential 'hits'. The n largest peaks found by the qualitative peak integration process described in the previous section will be library searched using the NIST library search algorithms. The spectrum passed to the search will use the same mass defect correction as currently applied in Spectrum. The number of peaks to be searched, spectrum background treatment, and search criteria are defined by the Qualitative Method.

- Exclude target compounds: If the Qualitative Method parameter 'Exclude target compounds' is selected then target compounds (that is peaks identified as compounds as a result of quantification) will not be in the list of n largest peaks. Hence they will not be library searched or available for the subsequent library search reports.

  If the Qualitative Method parameter 'Exclude target compounds' is not selected then target compounds will be library searched and available for the subsequent library search reports. Since the target compounds will already have been quantified, the associated quantitative information will be available (e.g., Name, Area, Area%, Norm%, RT, etc).

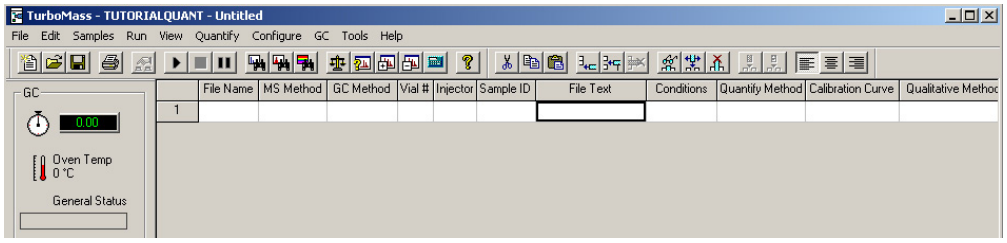
A Step-by-Step Qualitative Method Summary

1. Create a Sample List
2. Create a Qualitative Method
3. Put the Qualitative Method in the Sample List
4. Start the Analysis

1. Create a Sample List

The first thing that you must do is to create a list of samples that you want to use to perform the analysis. These samples can be acquired manually, but more often they will be acquired automatically using an autosampler. The Sample List Editor has various columns such as Filename, vial or bottle Number and Sample Type that can be filled in for each sample. Each sample is displayed as one row in the Sample List. The Sample List Editor is part of the TurboMass top-level menu.

You need to tell TurboMass everything that it needs to know about the samples in the list in order for it to perform a complete analysis. You must describe to the system what each of the vials in the autosampler contains, i.e., whether it contains a standard, an analyte, a blank or a QC sample, how to acquire it, its concentration(s), if it is a standard or has internal standards. In addition, you must specify the name of the file in which to store the data. You may also want to add some management information such as Sample ID, the submitter's name, or a sample description, and the Report Method template used.



For more information on how to create a Sample List, see How to Build a Sample List on page 147.

2. Create a Qualitative Method

A Qualitative Method is required for most Communiqué reporting (an exception is when you just report the chromatogram plot or acquisition conditions).

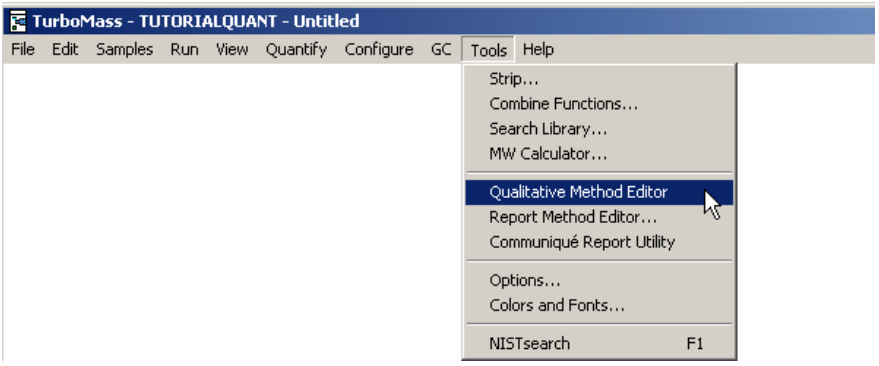
NOTE: *The Quantify Method is required for Calibration and Quantification curves.*

The Qualitative method describes how a data file is processed to produce calibration curves and qualitative information. Details must be entered into the method for each of the compounds being used in the analysis. The Qualitative Method specifies information for performing the following tasks:

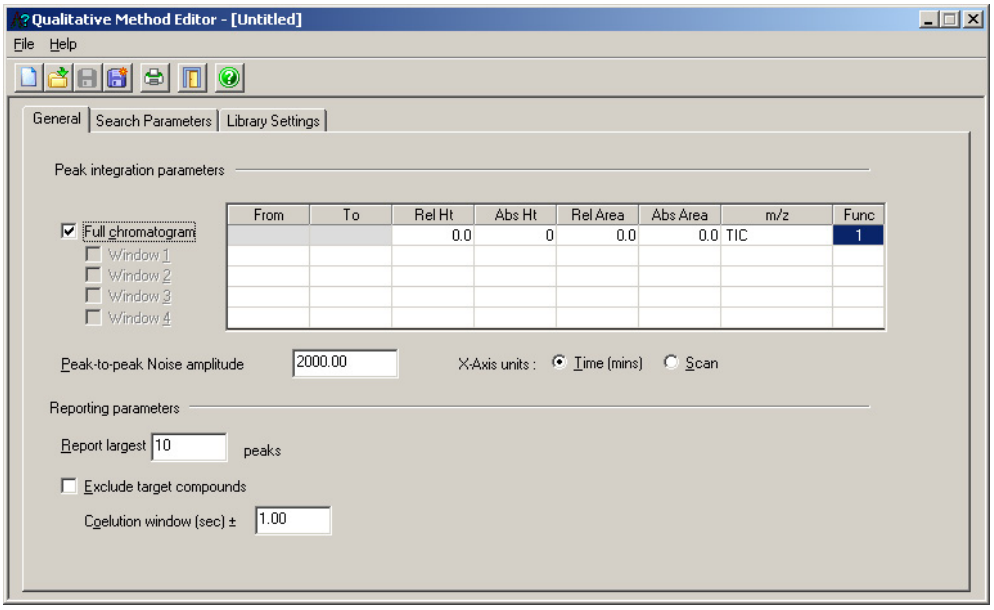
- Qualitative Peak integration and Reporting parameters.
- Library Search parameters (Spectrum treatment, search type, search options and limits; molecular weight constraints; and reporting parameters).
- Library Selections.

To create a Qualitative Method, follow this procedure:

1. Select **Qualitative Method Editor** from the *Tools* menu



The *Qualitative Method Editor* appears:



The **General** parameters are needed for all qualitative reports that require a peak data set. Since **Search Parameters** and **Library Settings** are only required when a library search is to be performed, these have been placed on secondary tabs.

2. Specify the chromatographic data sets which are to be subject to peak detection and integration.

   The options are: The full chromatogram **From** start **To** end.

   OR

   One to four segments (**Windows 1-4**) with specified start and end times.

NOTE: *Enter the peak integration parameters in the same manner as you have done in Chromatogram or Quantify.*

3. Enter a **From** value. A blank cell indicates the chromatogram segment will start from the beginning of the data. The **From** value must be less than the **To** value (unless either is blank).

4. Enter a **To** value. A blank cell indicates the chromatogram segment will end at the end of the data. The **To** value must be greater than the **From** value (unless either is blank).

5. Enter a Relative height threshold (**Rel Ht**) of the peak in the chromatogram. (default is 0).

6. Enter an Absolute height threshold (**Abs Ht**) of the peak in the chromatogram (default is 0).

7. Enter a Relative area threshold (**Rel Area**) of the peak in the chromatogram (default is 0.0).

8. Enter an Absolute area threshold (**Abs Area**) of the peak in the chromatogram (default is 0.0).

9. Enter a mass **(m/z**)or mass function (**Func**) for the segment (enter TIC, a selected ion, or a valid mass chromatogram equation).

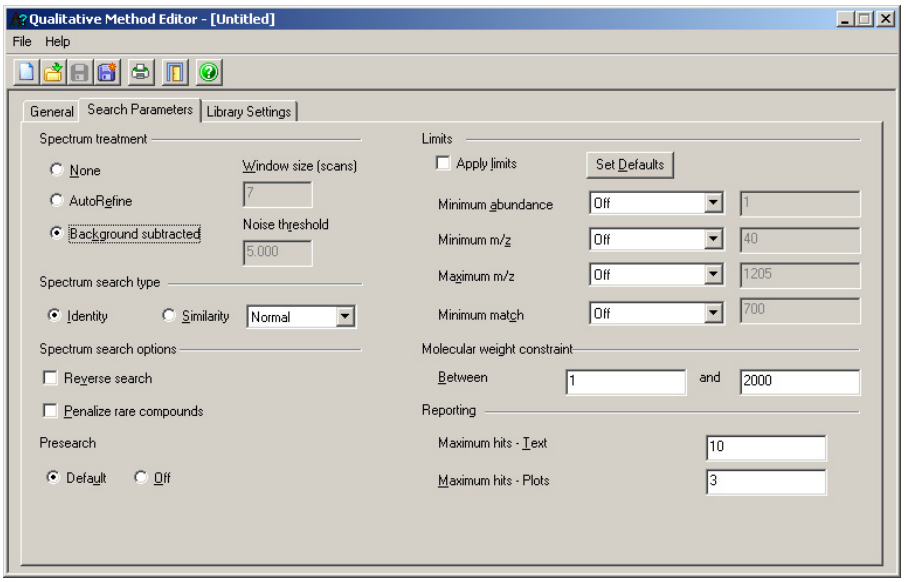
10. Enter the **Peak-to-peak Noise amplitude** value for integration.

11. Select the **X-Axis units Time (min)** or **Scan**.
    Time - Selects Time units for setting the 'From' and 'To' values in the grid.
    Scan - Selects Scan numbers for setting the 'From' and 'To' values in the grid.

12. Enter the number of largest peaks you want to report.

13. Set the 'Coelution window (sec) ±' to set a time window for peaks to maximize.
    Use this parameter to ensure reliable identification of target compounds within the qualitative chromatograms. Peaks identified in the quantitative results can be missed in the qualitative report if their retentions do not exactly match those found by the qualitative report method. This can occur because two specific masses in a peak may maximize one or two scans away from each other (or the TIC) due to noise and scan-rate induced "spectral skewing".

14. Click on the **Search Parameters** tab.



15. Select the **Spectrum Treatment** to be applied to the spectrum before initiating the search. For example, **Background Subtracted**.

16. Select the thoroughness of the search (the **Spectrum search type**) and its options. Keep in mind that more thorough searches take longer.

17. You can select the **Reverse search** option to improve the matching capability when there are contaminant peaks in the mass spectrum.

18. You can leave the rest of the settings as their default values or optimize as needed.

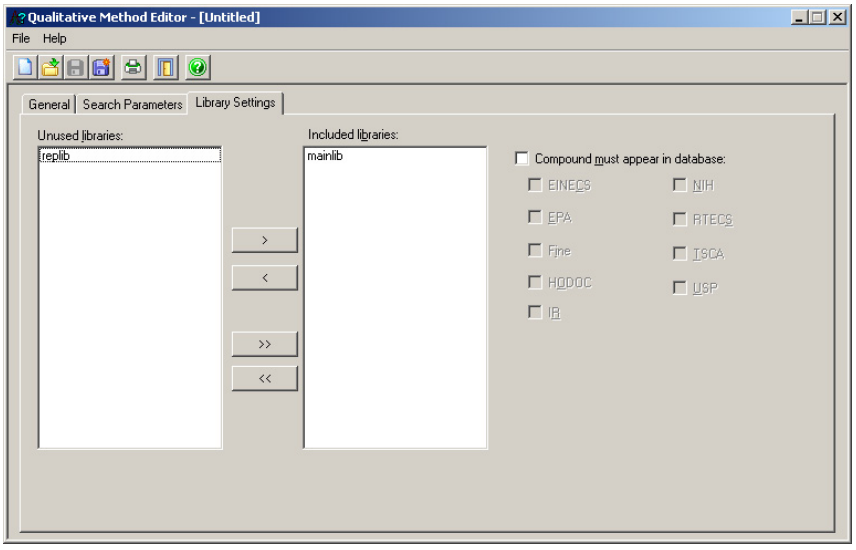
19. Click the **Library Settings** tab.

    You may have several mass spectral libraries on your computer. Some are from NIST (mainlib, the main library, and replib, a smaller library of replicate spectra (subset of compounds, different spectra) which can improve the chances of finding the right compound (Refer to the NIST Software Manual on the NIST CD). There can also be other commercial libraries (e.g., the Pfleger-Mauer-Weber drug library) or user-created libraries.

This tab allows you to select which libraries to search, and in what order. Restricting the number of libraries (spectra) searched increases speed in a linear fashion. Searching libraries which have only the target compounds with few "unlikely" matches also increases the chance of a correct match. For example, someone doing drug analyses might search a drug library, but would not want to search a flavors and fragrances one.

Compounds in mainlib (the primary NIST library) have flags indicating which databases they originally came from or in which industry-standard lists of compounds they occur. If the unknown compound is expected to be in one of these databases or lists, the speed and accuracy of the search can be improved by restricting results to members of those databases or lists.

NOTE: *If no libraries are selected, no library search will be performed. This is the default method for only picking Qualitative Peaks.*



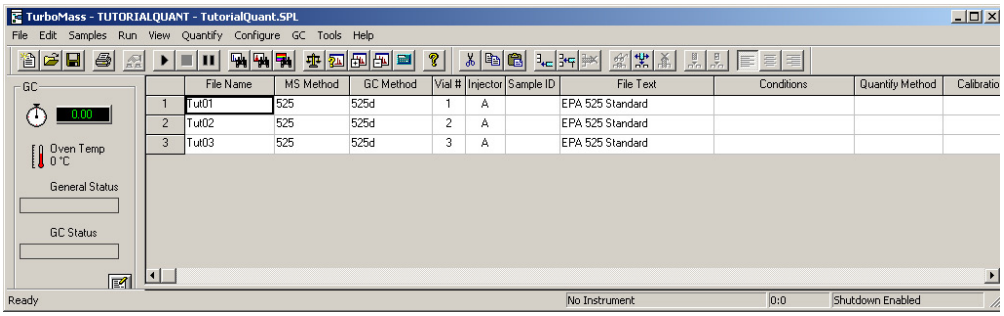
20. Click **Save** or **Save As** from the File menu, then name and save the method.

    This method name will now be available in the **Sample List** under the **Qualitative Method** column.

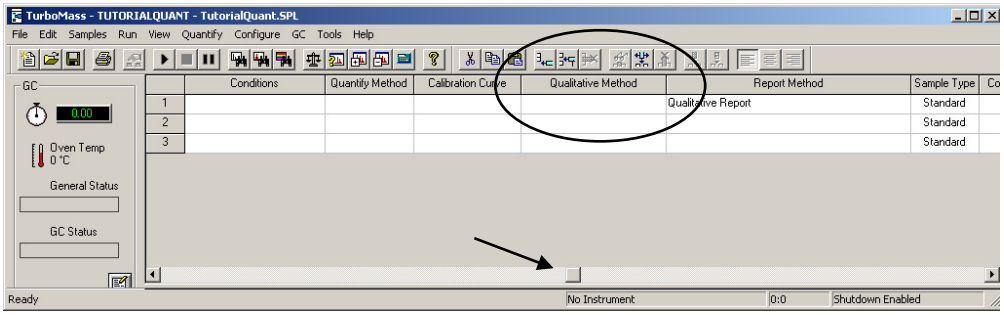
3. Put the Qualitative Method in the Sample List

After creating and saving a Qualitative Method, enter it in your Sample List.

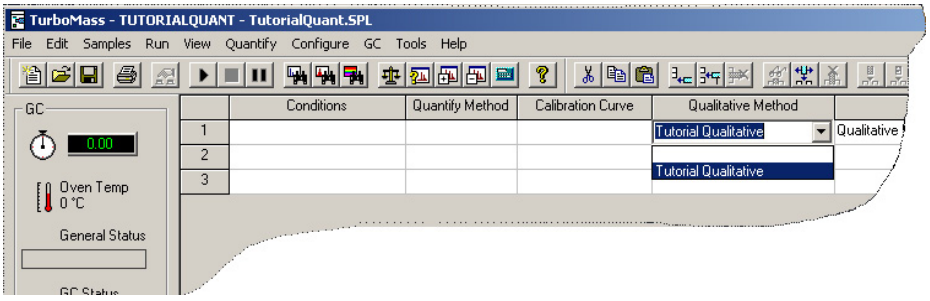
1. Display your **Sample List**.



2. Move the slider on the bottom of the Sample List window to the right until you can view the **Qualitative Method** column.



3. Double-click in the cell and select your **Qualitative Method**.



4. Start the Analysis

Before starting an analysis save any changes made to the Sample List by selecting **Save** or **Save As** from the Sample List *File* menu.

1. To begin acquiring data or perform post-run reporting:
    - Choose **Start** from the TurboMass top-level *Run* menu

      OR
    - Choose  to open the Start Sample List Run dialog box.

   The Start Sample List Run dialog appears.

2. Enter values or check boxes in this dialog. (The order in this dialog indicated the order of execution. For Example, you **Acquire Sample Data**, then **Auto Process Samples**, **Auto Quantify Samples**, perform **Qualitative Calculations**, and **Generate Communiqué Reports**.)

   Project - The name of the current project appears in this text box. To acquire data to a different project, choose OK or Cancel exit this dialog box, open another project, and restart data acquisition.

   Acquire Sample Data - Selecting this option will acquire data for the specified samples in the list.

   Auto Process Samples - Selecting this option will process the acquired data as specified in the Process column of the Sample List.

   Auto Quantify Samples - Selecting this option will automatically enable sample quantification.

   Qualitative Calculations - Selecting this option will enable Qualitative Method processing

   Generate Communiqué Reports - Selecting this option will enable Communiqué Report generation.

Preview Reports - Check this box to specify that the Communiqué reports generated during processing will be displayed in a preview window prior to printing (or saving to a file or database).

NOTE: *The options above allow you to acquire and immediately process and quantify data as desired. Or, you may choose to process or quantify data at a later time.*

Run: From Sample n To Sample m - Sets the range of samples in the sample list which will be acquired/and or analyzed. If you highlight a range of rows before starting the analysis, the first and last rows of the highlighted region will be displayed here.

Quantify, Qualify and Generate Reports: After Each Run - Indicates specified processing will occur after each row in the Sample List.

At End of Sample List - Indicates specified processing will occur only after the sample list is complete.

Process: Pre-Run - Specify the name of the process that will be run before the acquisition of the files in the Sample List.

Post-Run - Specify the name of the process that will be run after the acquisition of the files in the Sample List. For example, to switch the instrument out of the operate mode and turn off various gases.

NOTE*: If you want to run a process after each sample in the Sample List has been acquired, format the Sample List to display Process and enter the name of the process to be run for each of the samples. If you want the process to automatically operate on the data file that has just been acquired, select Options from the Sample List Tools menu, then deselect the Use Acquired File as Default parameter on the System tab.*

3. When all are entered, click **OK**.

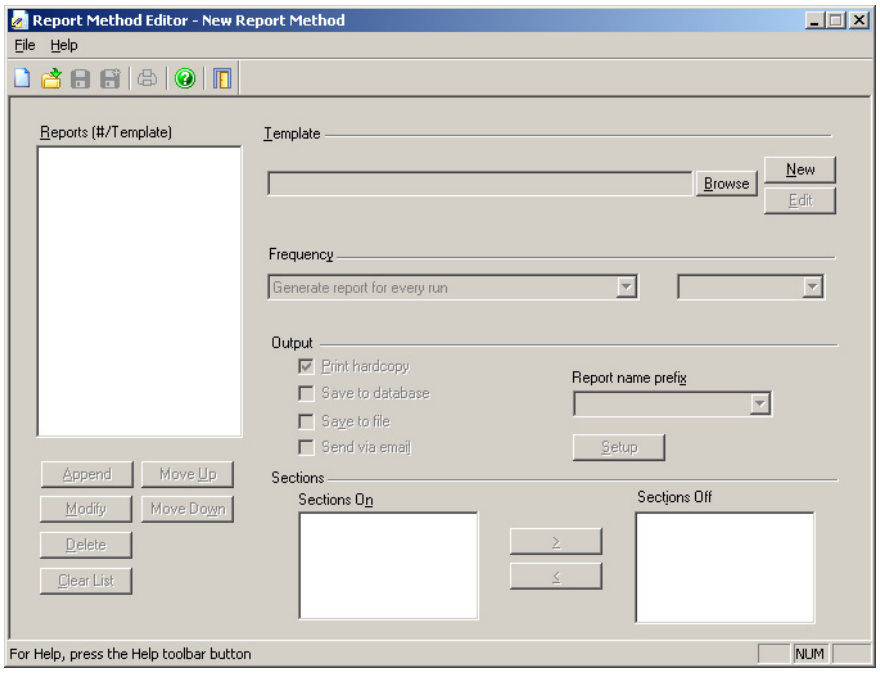
How to Set Up Communiqué Reports 17

About the Report Method Editor

The Report Method Editor is an extension to the TurboMass software. It enables you to specify a collection of report definitions (Communiqué report templates and related parameters) that are printed sequentially. The Report Method is a dataset that you can specify in each row of the Sample List. It defines the reports to be generated following the analysis data or at the end of the Sample List. The Report Method will consist of a list of report templates and associated parameters, namely:

- When the report will be generated (for all runs, for runs of a specific type, for only the last run in the sample list)
- The output destination(s) of the report (print, save to database, save to file, send via email)
- The printer (if printed) and/or the file name (if saved)
- The email address(es) and message text (if emailed)
- Sections of the report template to be printed or suppressed

NOTE: *Only a single report method can be loaded in the Editor at a time and only a single instance of the Report Method Editor can be run. If you edit a Report Method while the Editor is already open (e.g., by choosing the Open command from the context menu when the Report Method cell is selected in the sample table) then the new report method will replace the existing one, provided it is not in a modified state. If the current method is in a modified state the 'Do you want to save the changes to "<report method name>"?' warning dialog will be displayed.*

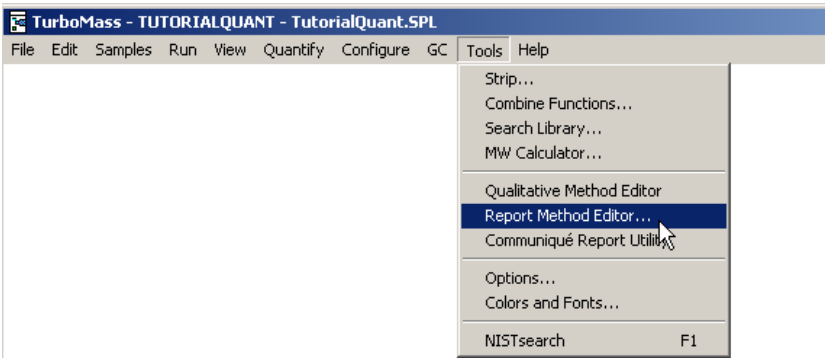


You open the Report Method Editor in one of two ways:

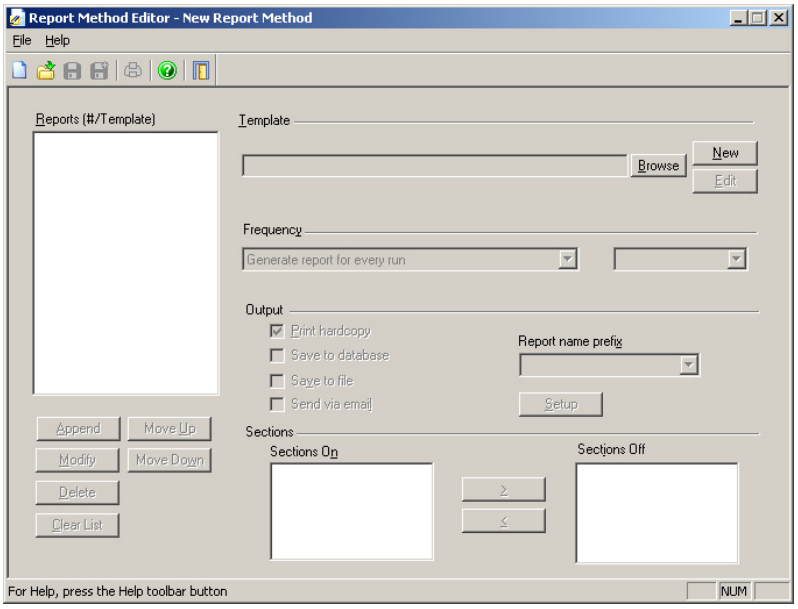
- By choosing **Open** from the context menu when the Report Method cell is selected in the Sample List. If no method name is specified in the cell, or if there is currently no report method of that name in the **Project** directory, then the Report Method Editor will open in the 'New Method' state. If the cell contains the name of an existing report method then Report Method Editor will open in the 'Edit Method' state.
- By choosing the **Report Method Editor** command from the TurboMass *Tools* menu. The default method will be the method most recently used. If there is currently no report method of that name in the project directory (that is the METHDB subdirectory in the project hierarchy), then the Report Method Editor will open in the 'New Method' state.

Selecting an Existing Template

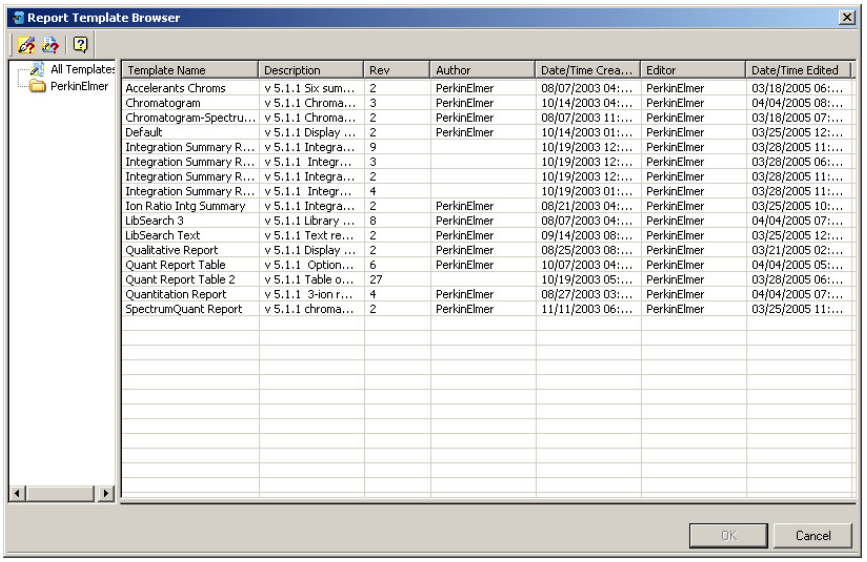
1. Select **Report Method Editor** from the *Tools* menu.



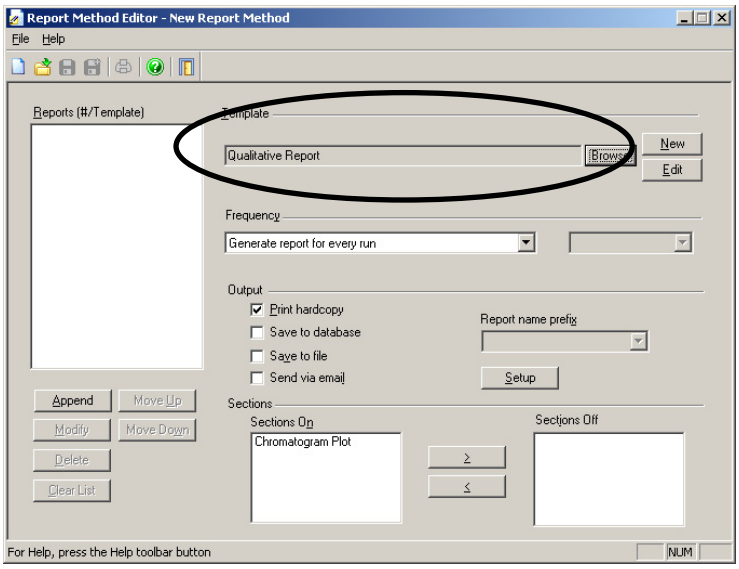
The Report Method Editor screen appears:



2. Click the **Browse** button to the right of the Template field. The Report Template Browser appears:



3. Select a **Template** name and click **OK** (for example, click Qualitative Report). This now appears in the Report Method Editor dialog:



4. Select the **Frequency** of reporting. The options available are:
   - **Generate report for every run**
   - **Generate report for every run of specified type**
     Selecting this option enables a second drop-down list. Here you select the type of run from the available options.
   - **Generate report only for final row in the sample list**

5. Select a desired **Output** type.

6. Click the **Append** button to add this template to the current method.

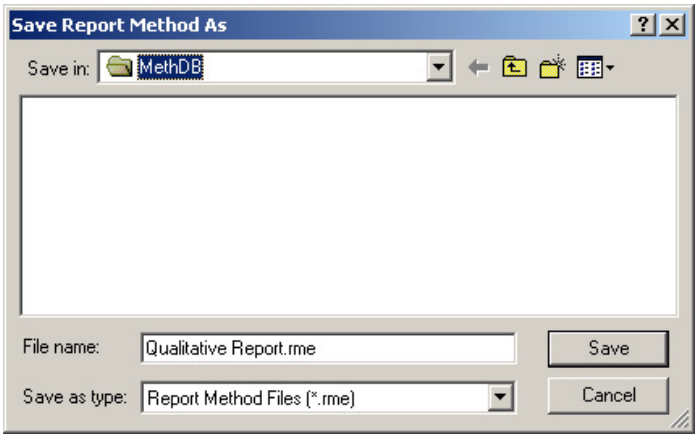
   This template appears in the **Report (#/Template)** field This is a display of the reports defined for the current method.

   When the list is empty (New Method, after **Clear List** or after all reports have been **Deleted)** the controls on the right will be set to default values. In this way the Append button is always enabled and valid.

   Using the **Move Up** and **Move Down** command buttons reorders the Reports in the list. Reports will be processed by report number (i.e., in the order in the list).

7. Select **Save As** from the Report Method Editor **File** menu.

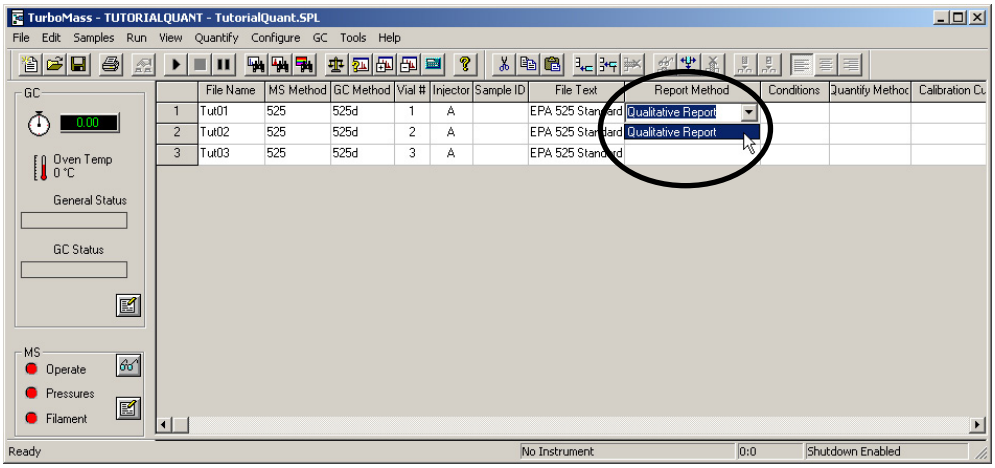
   The **Save Report Method As** dialog appears:



8. Type a File name for your Report Method (for example, Qualitative Report) then click **Save**.

This Report Method is now available for the **Report Method** column of the **Sample List**.

9. Double-click in the **Report Method** field and select **Qualitative Method** from the drop-down list.



10. Close the Report Method Editor by clicking the **Close** box (in the upper-right hand corner of the screen).

How to Use the Communiqué Report Designer with TurboMass 18

About the Report Method Editor

In this section we will show you how to use the Report Method Editor and Communiqué to modify an existing report template and create a new report template.

A vital aspect to the flexible reporting capabilities of the TurboMass software will be the data model. This defines the data that will be made available to Communiqué for design of the template and generation of the report. Most TurboMass data is available through the data model. This includes:

- All existing quantitative data generated by the TurboMass Quantification process (including Area% and Norm% values).
- The chromatograms and spectra defined by the Qualitative method and its processing.
- Values associated with the multiple ion ratio identification process (ratios, pass/fail, etc.)
- Peak plots associated with multiple ion ratio processing (for target compounds and internal standards).
- Calibration curve plots associated with target compounds.
- Library search spectral plot data and text results.
- Quantification and Qualitative method parameters.

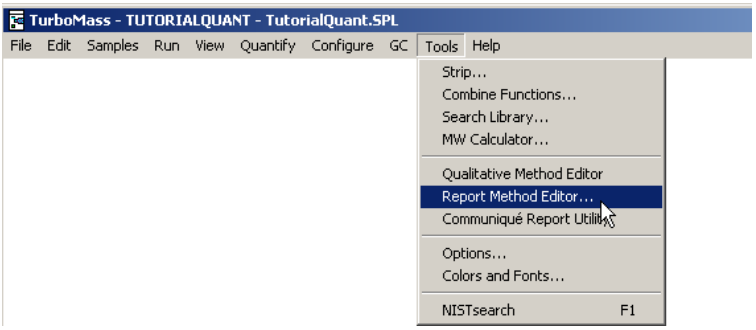
TurboMass creates a collection of Data Objects that appear in the Communiqué Report Creator.

In the **Communiqué Report Creator**, note that ***collections*** (in the Communiqué sense of the word) are indicated by parentheses '( )' following the collection name for example Samples( ).

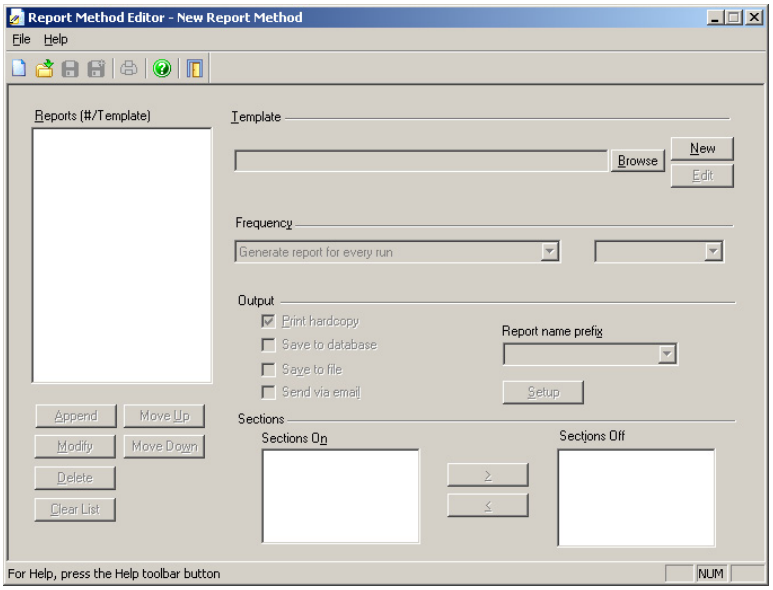
Modifying a Report Template

To modify an existing report template, follow this procedure:

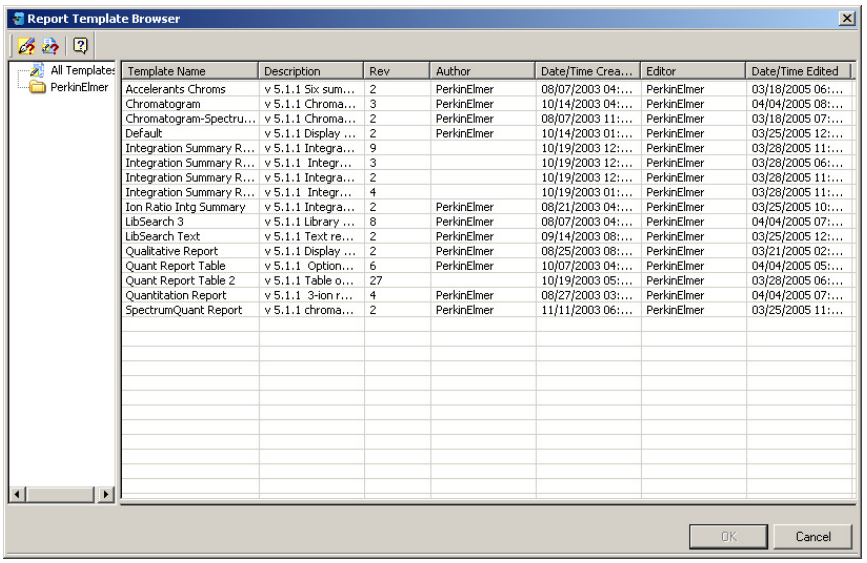
1. Select **Report Method Editor** from the *Tools* menu.



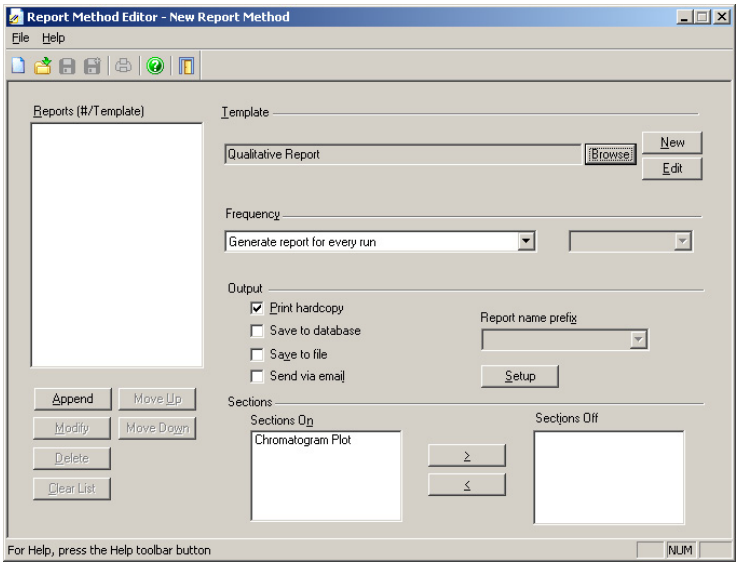
The Report Method Editor screen appears:



2. Click the **Browse** button to the right of the **Template** field. The Report Template Browser appears:

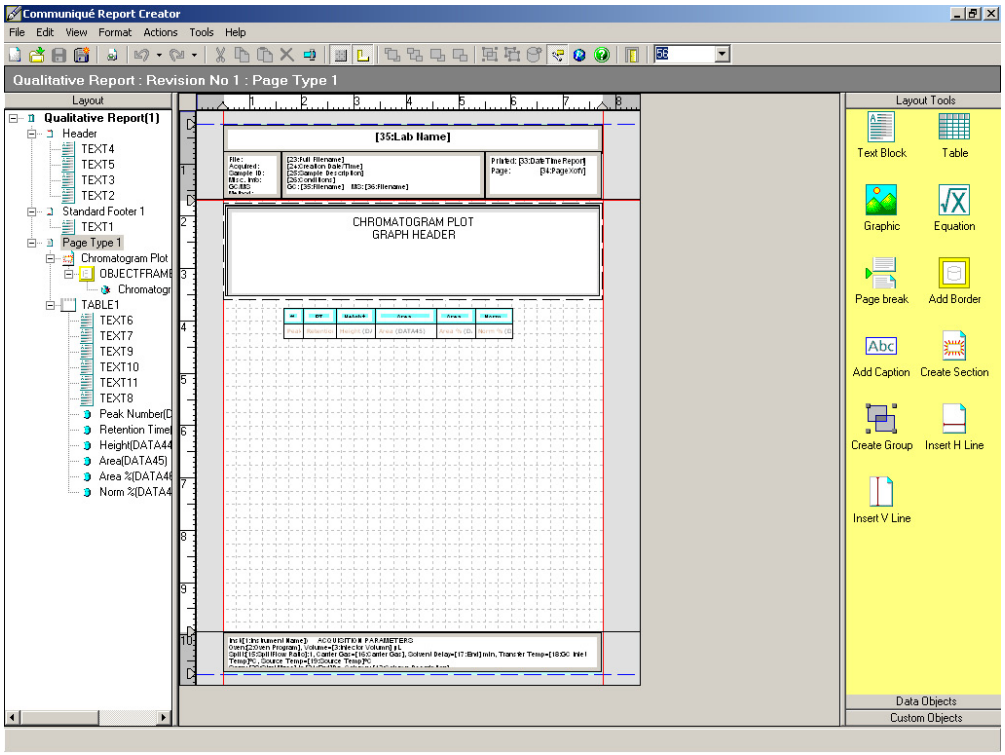


3. Select a template name and click **OK** (For example, click Qualitative Report). This now appears in the Report Method Editor dialog:



4. Click the **Edit** button.

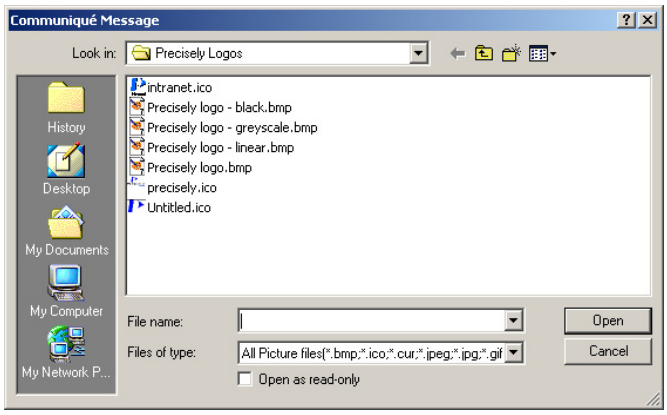
This launches the Communique Report Creator.



Add a Graphic to the Template

To add a graphic to the template, follow this procedure:

1. Click **Graphic** in the **Layout Tools** toolbox.
   A dialog similar to the following appears:

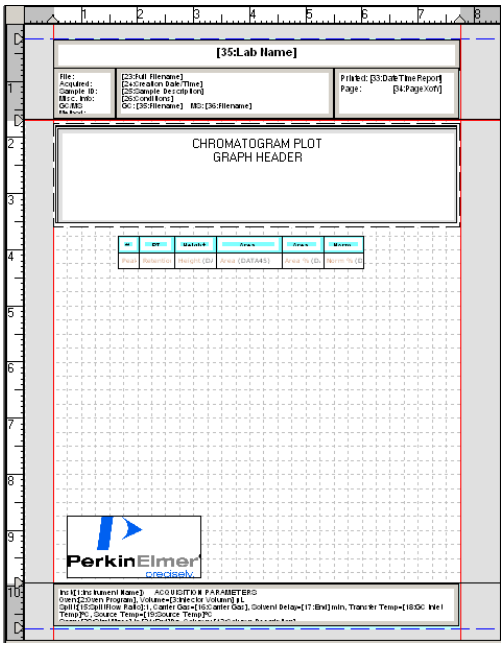


2. Browse to the directory where the graphic is stored and select it by clicking on it.

3. Move your cursor to the design page and click. The cursor changes to the following cursor:

4. Move your cursor to a desired spot on the page. Click the left mouse button, drag the mouse to scale the graphic to a desired size, then release the mouse button.

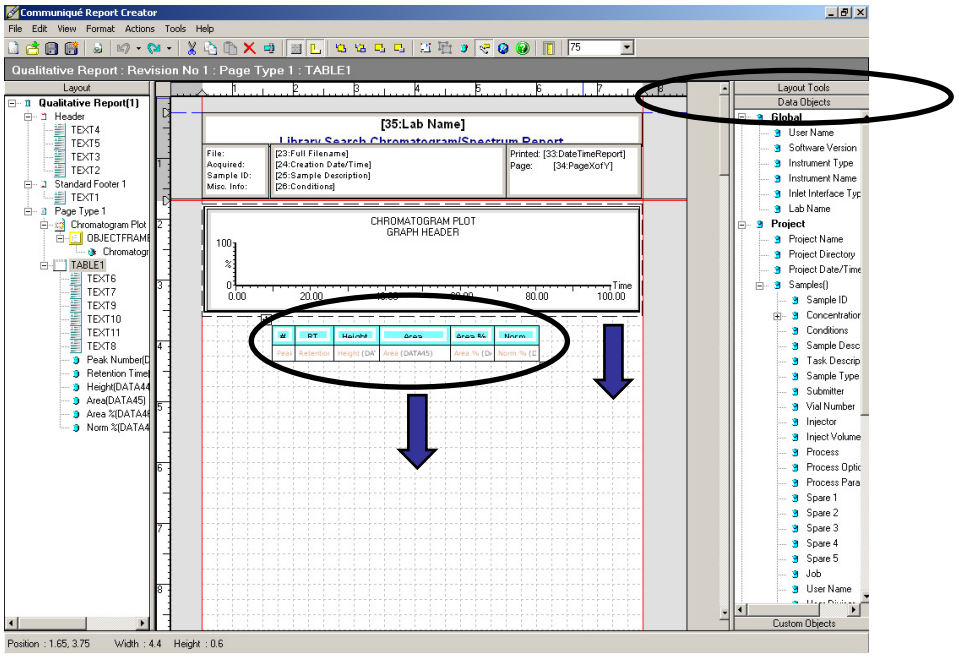
   The graphic appears on the page.



Adding a Data Object to the Template

To add a data object to the template, follow this procedure:

1. Click on **Data Objects** to display the available data objects.

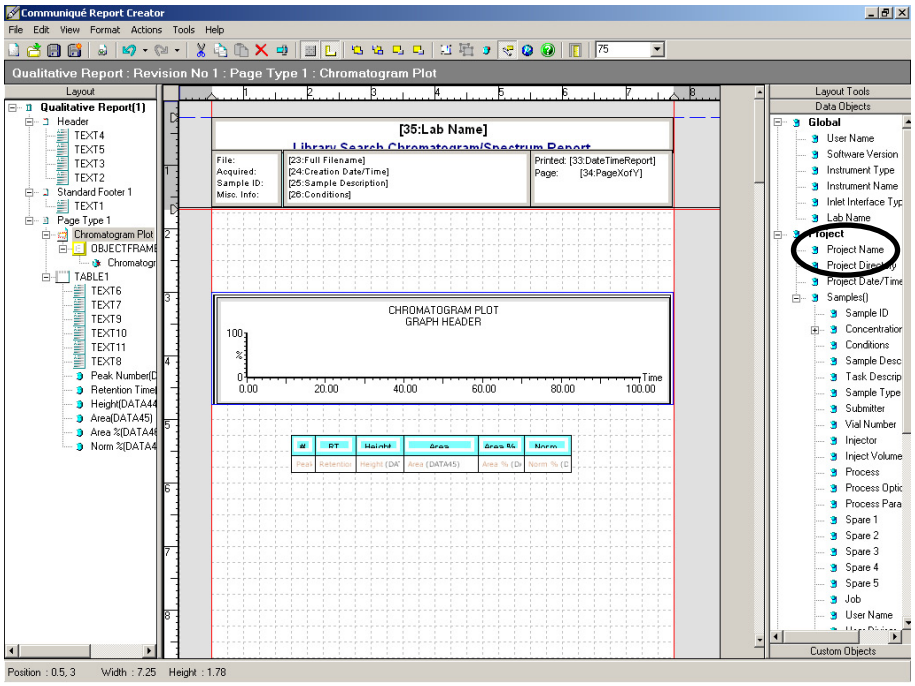


2. Move the *table* (see above) and then the *Chromatogram Plot* down the page to make room at the top of the Chromatogram Plot.

To move the table and Chromatogram Plot, move your cursor over the table.

It turns into ✥ Now hold the left mouse button down and move your mouse to move the table.

Next move the Chromatogram Plot the same way.

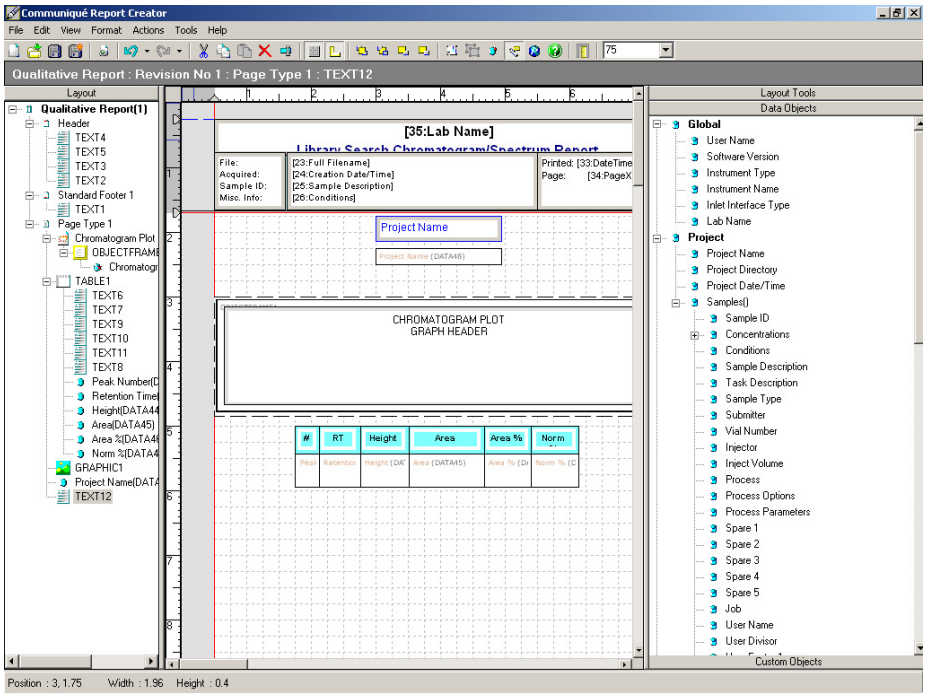


3. Click on **Project Name**, move your cursor to the designer page, the click where you want the Project Name to appear and drag it to the desired size.

4. Click on **Layout Tools** to display the Layout Tool selections, click on **Text Block**, move your cursor to a position on the designer page, click and drag to put the text block on the page. Position the cursor on an edge of the **Text Block** and hold the left mouse button down and drag the edge to the proper size.

5. Click in the **Text Block** and type (for example, Project Name).

6. Highlight the text and right click to display the text formatting options.



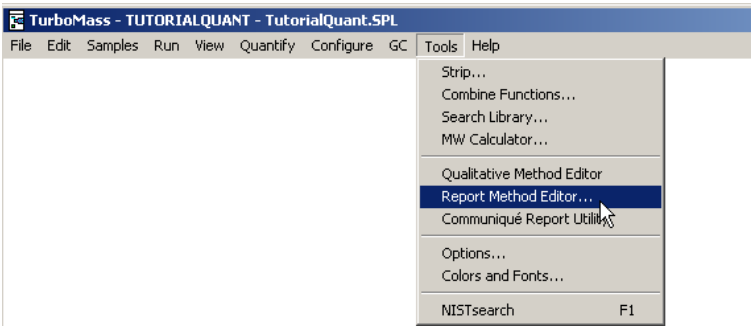
7. Click **Save As** from the *File* menu, name and save your file.

8. Exit the Report Designer.

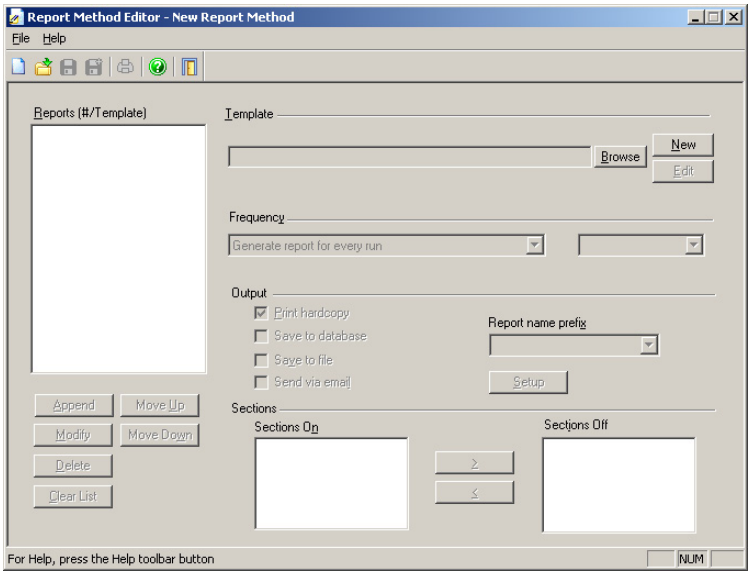
Creating a New Report Template

To create a new report template, follow this procedure:

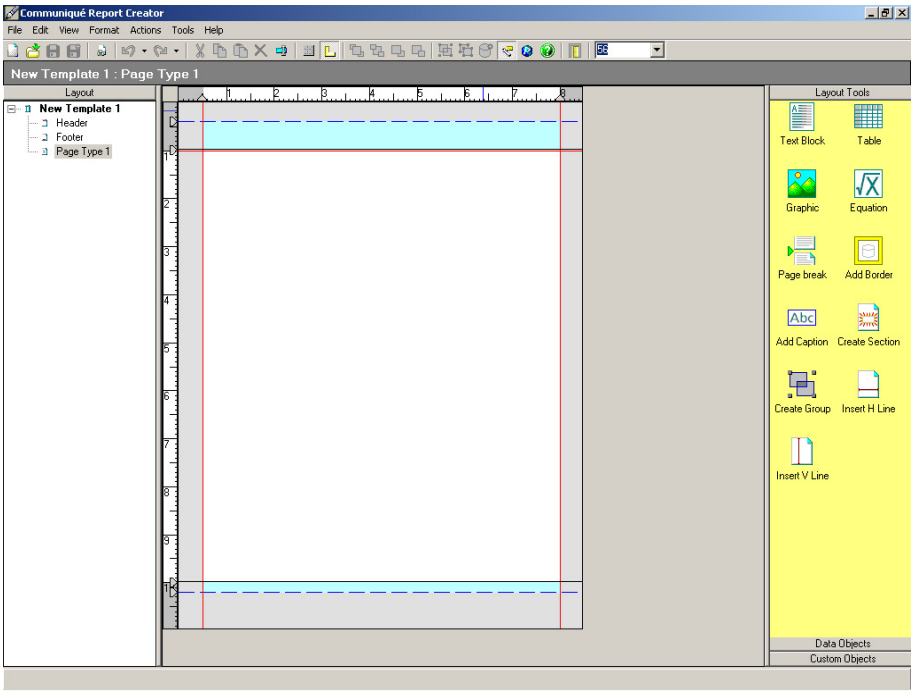
1. Select **Report Method Editor** from the *Tools* menu.



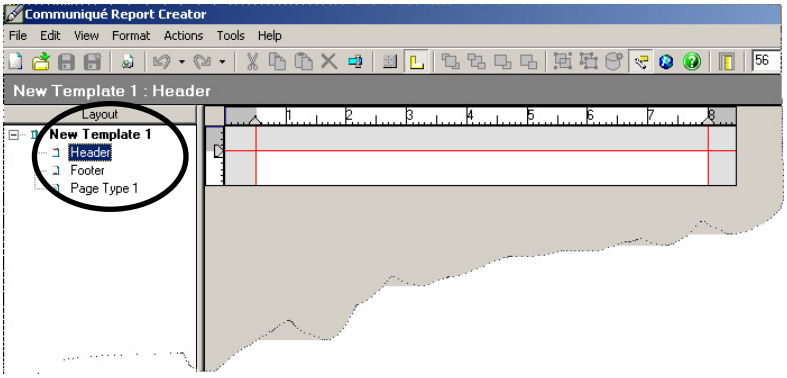
The Report Method Editor screen appears:



2. Click the **New** button to the right of the Template field. The *Communiqué Report Creator* appears:

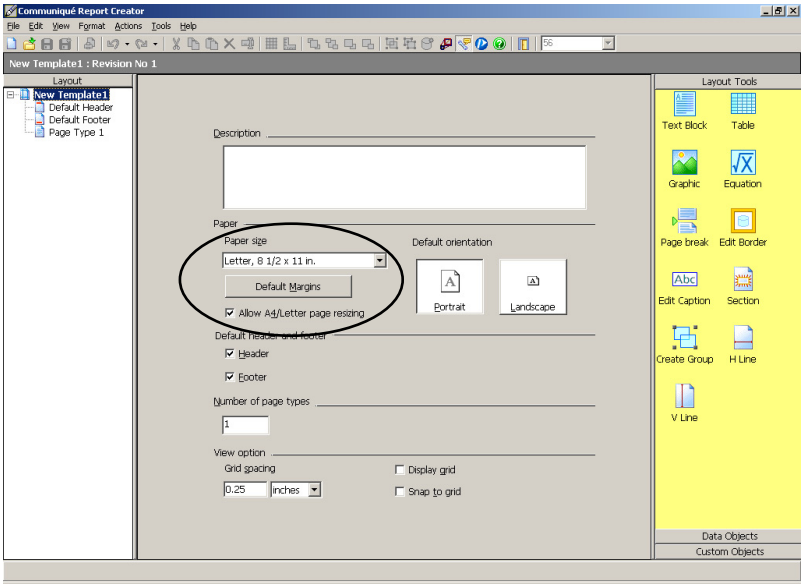


3. Click on **Header** in the **Layout** toolbox on the left of your screen.
   The Header appears:



If you click on **New Template** the template description will be displayed. Here you can allow for A4/Letter resizing of your template. To properly use the non-A4 templates as the only set, the **Allow A4/Letter page**

resizing option in each template's description page should be selected. In all cases the Paper drop-down list on the template description page should always be set to Letter so that the page size appears properly in the template editor.

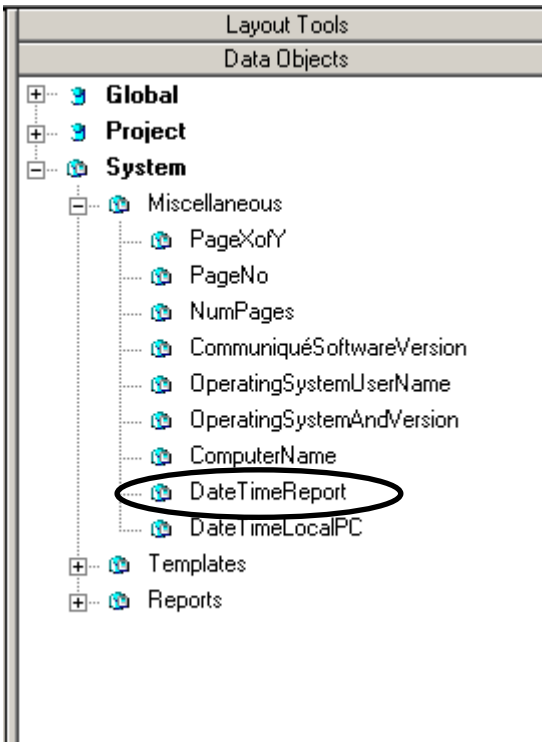


4. Drag a **Text Block** into the Header. Label it "Qualitative Report Test1." Then format the text as follows:

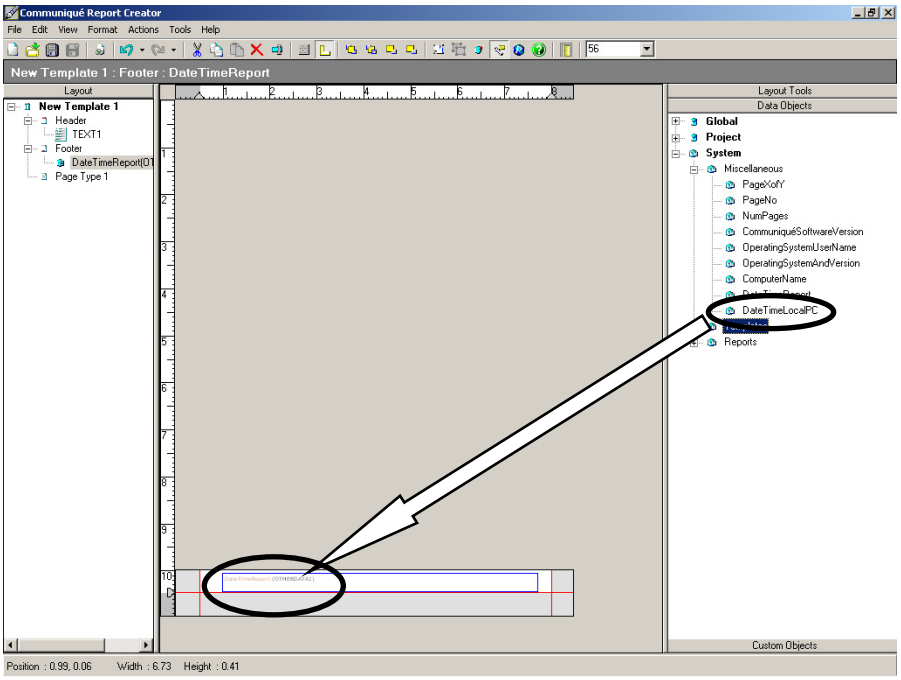
   - Highlight text and right-click to select **Format**.
   - Select **Font** tab.
   - Set to **Blue, 20 pt. Ariel, Bold**.
   - Set **Paragraph** tab, alignment **Horizontal/Center**.
   - Click **OK**.
   - Resize the **Text Block** so that you can see the text.

5. Click on **Footer** in the **Layout** toolbox on the left of your screen. The footer appears.

6. From the **Data Objects** toolbox, left click **DateTimeReport**.

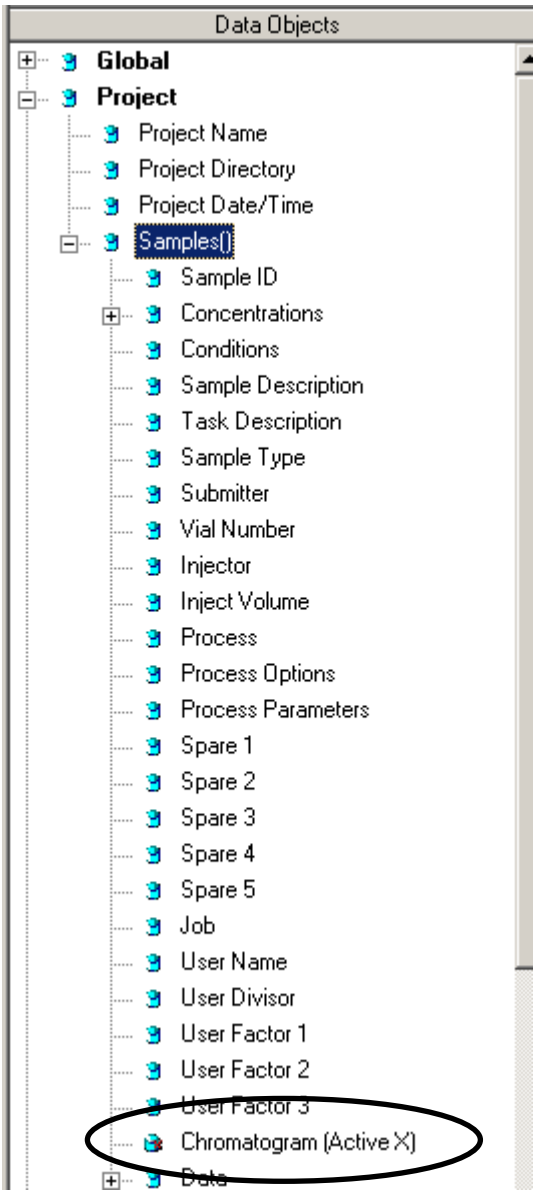


7. Click in the footer, then size **Date Time Report** into the Footer to full width.



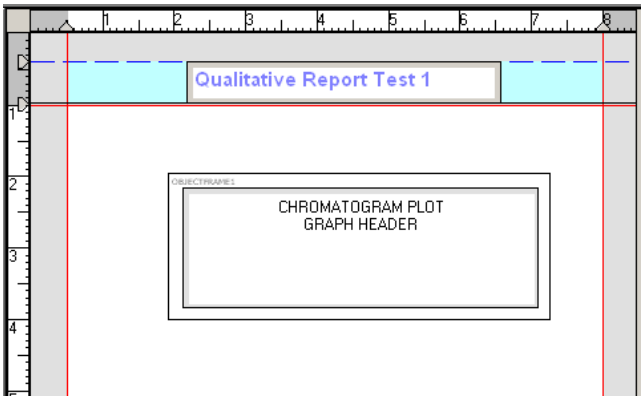
8. Click on **Page Type 1** in the **Layout** toolbox on the left of the screen. This will display the main body of the template.

9. Click on **Chromatogram (Active X)** in the Project > Samples() directory of the **Data Objects** toolbox (Graphic Data Objects have a small red “x” as their icon.



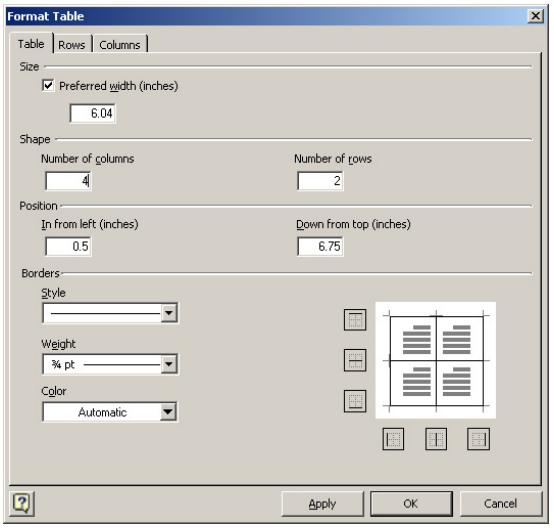
10. Left-click on **Chromatogram (ActiveX)**, move the cursor to the center of the design page, then left click and hold the mouse as you drag the mouse to

expand the box. The box displays as **CHROMATOGRAM PLOT GRAPH HEADER**.



11. From **Layout Tools**, left click on **Table** then move your cursor to the page and left click. A four column by two row table appears.

12. Select the table. Right-click Format.
    The Format Table dialog appears:

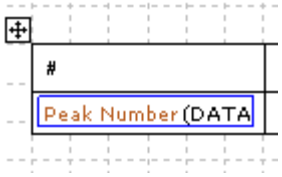


13. Change the Number of columns from **4** to **5** then click **OK**. If necessary, click on the **Columns** tab and change the Column width to 0.5.

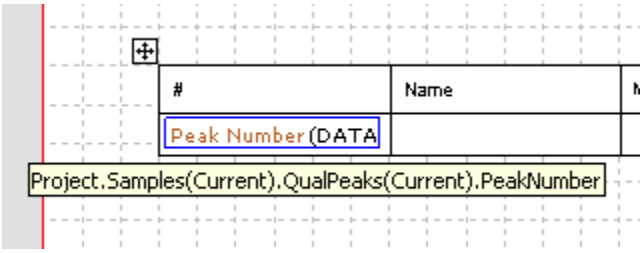
14. Add text blocks to the top row of the table. Label each cell as follows; **#, RT, Name, Match Factor, and Area%.**

NOTE: *Remember to first put a Text Block (from the Layout Tools) in the table so that you can enter a label.*

15. Under "#" put **Peak Number**, from the Project / Samples() / Qualitative Peaks () path in the **Data Objects** toolbox.



If you move your cursor over **Peak Number (DATA)** the following appears:

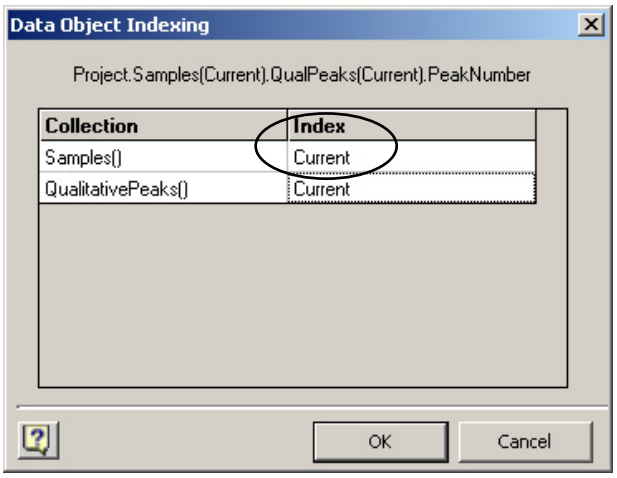


This information **Project.Samples(Current).QualPeaks(Current).PeakNumber** is the source string of the data. In this example, it will report the peak number of the current QualPeak in the current Sample.

About Samples; Current versus Last: The template is used to pull information from the data source during report creation. From the point of view of the data source, the most recent data (e.g., the current line on the Sample List) is the last data entered into the data source. Therefore, to view data from the current line of the Sample List, use **Samples(Last)**.

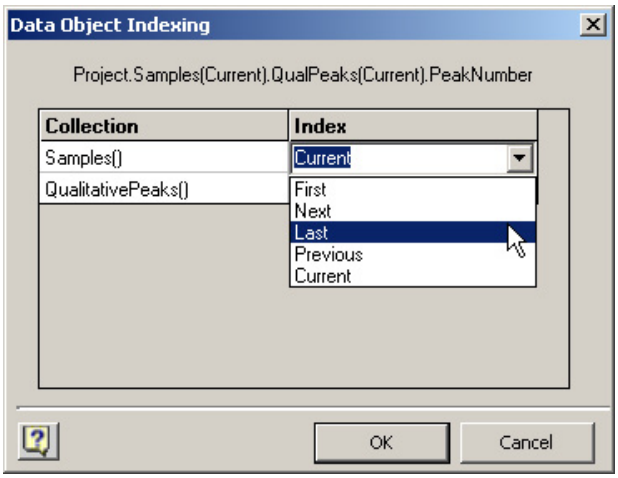
To report the most recent line in the Sample List we need to change the Indexing of the Peak Number. Right click on **Peak Number (DATA)** and select Indexing from the menu.

The *Data Object Indexing* dialog appears:



Double click on **Current** in the Samples Index cell.

The following **Index** dropdown menu appears:



Select **Last** then click **OK**.

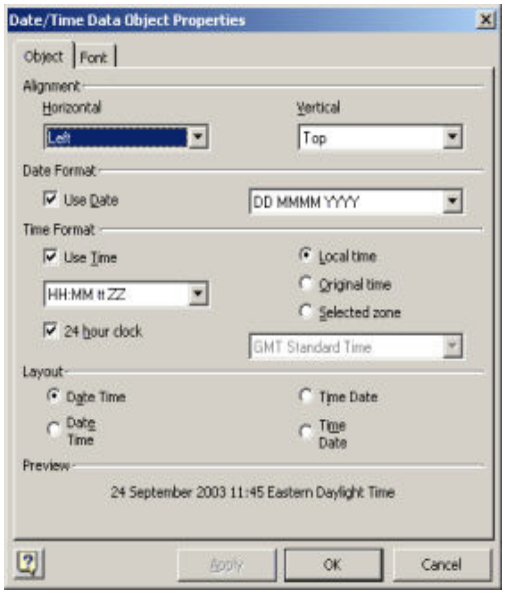
16. Under "RT" put **Retention Time**, from the Project / Samples() / Qualitative Peaks () path in the Data Objects toolbox. Set to Samples (Last).

17. Under "Name" put **Name**, from the Project / Samples() / Qualitative Peaks () path in the Data Objects toolbox. Set to Samples (Last).

18. Under "Match Factor" put **Match Factor**, from the Project / Samples() / Qualitative Peaks () / Text Hits / path in the Data Objects toolbox. Set to Samples (Last).

19. Under "Area%" put **Area%**, from the Project / Samples() / Qualitative Peaks () / path in the Data Objects toolbox. Set to Samples (Last).

20. Check your **Date/Time Data Object Properties** for the correct **Time Format**.

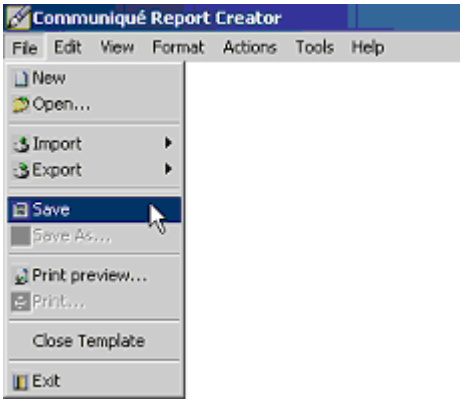


The default Communiqué setting for **Date/Time Data Objects** is "**Local time**." In order to ensure that the correct time is reported on your computer, right-click the **Date/Time Object** in your template, select Object Properties from the pop-up menu and in the following dialog select "**Original time**" to set the Time Format, then click **OK**.

NOTE: *When setting the Numeric Data Object Properties in your Communique template, increase the number of significant figures from the default of 4 to 6 in order to see*

the reported number with no rounding applied. Otherwise Communiqué does not report 6 figures but instead applies rounding.

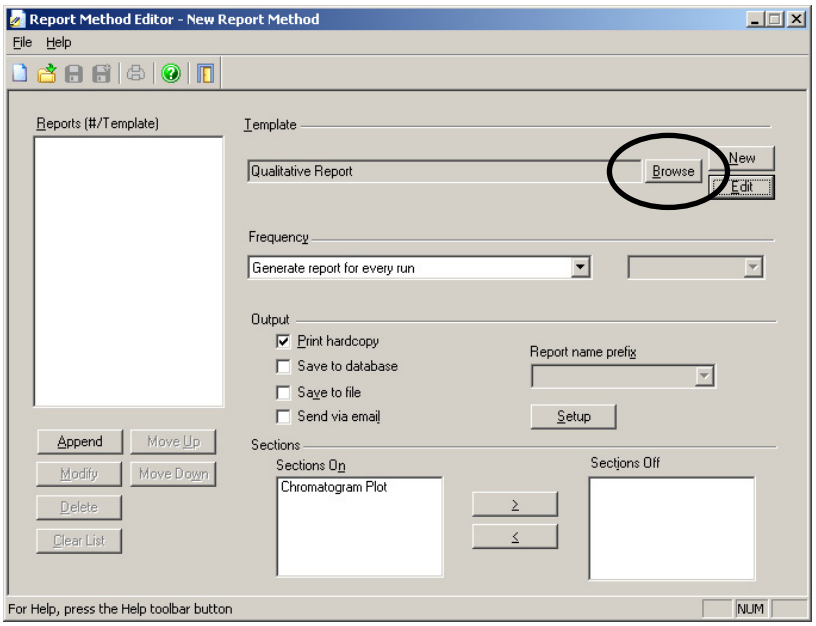
21. Select **Save** from the Communiqué Report Creator *File* menu and name the file (for example, Mod Qualitative Report).



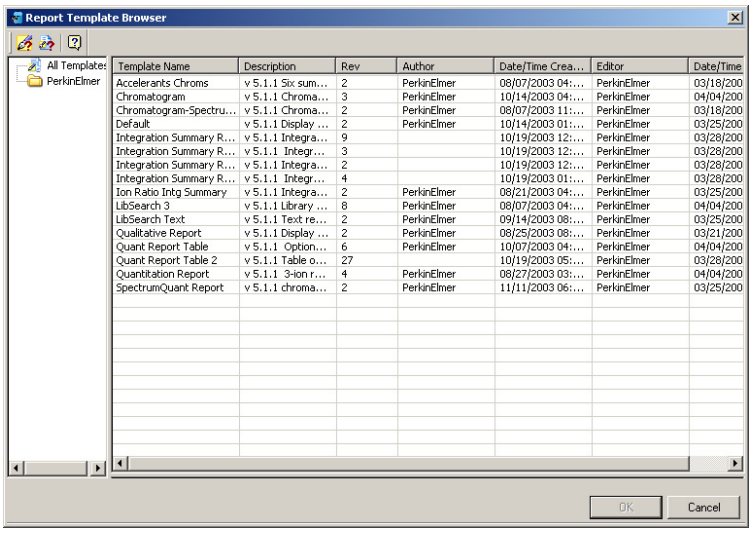
22. Close *Communiqué Report Creator* by clicking **Exit** from the *File* menu.

Select the Template in the Report Method Editor

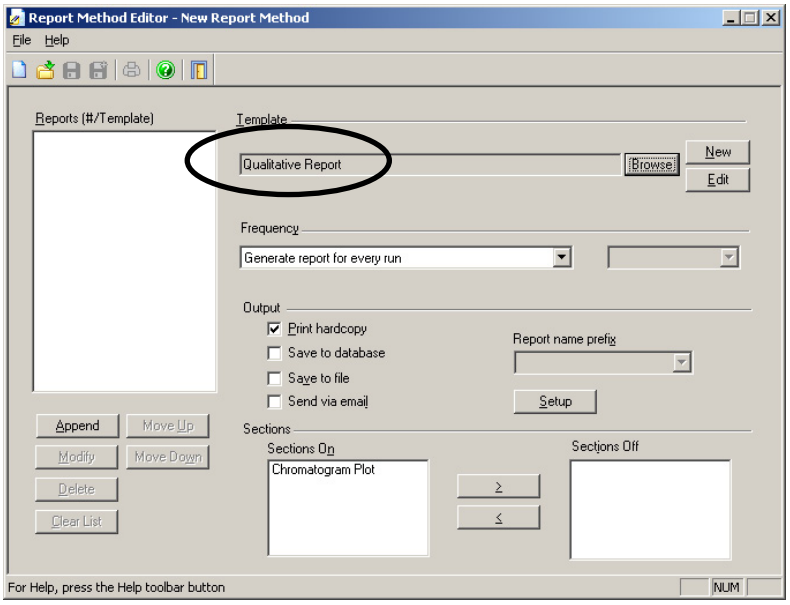
After closing the *Communiqué Report Creator* the Report Method Editor appears:



1. Click the **Browse** button.
   The following dialog appears:

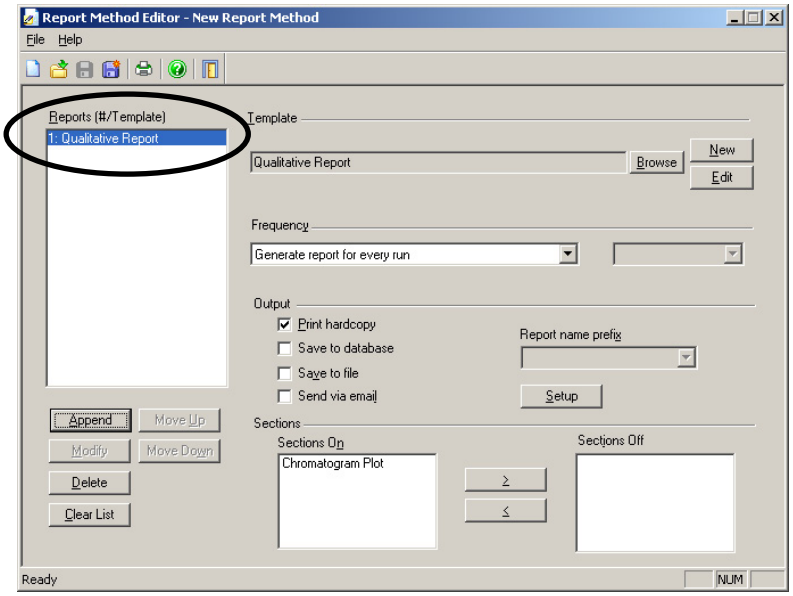


2. Select your template (for example, Qualitative Report) and click **OK**. Qualitative Report appears as shown below:



3. Click the **Append** button.

This template appears in the **Report (#/Template)** field This is a display of the reports defined for the current method.



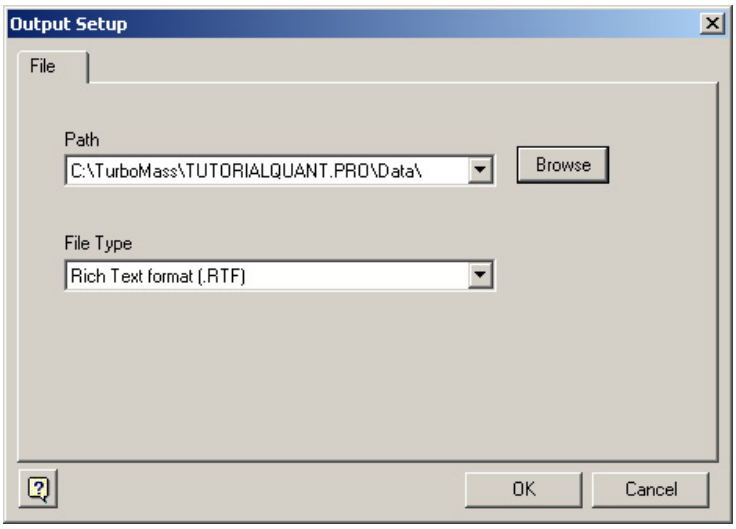
When the list is empty (New Method, after **Clear List** or after all reports have been **Deleted)** the controls on the right will be set to default values. In this way the Append button is always enabled and valid.

Using the **Move Up** and **Move Down** command buttons reorders the Reports in the list. Reports will be processed by report number (i.e., in the order in the list).

4. Set the other options on this screen. For example, set the **Frequency** to one of the following:
   - Generate report for every run.
   - Generate report for every run of specified type. (Analyte, Blank, QC, or Standard)
   - Generate report only for final row in the sample list.

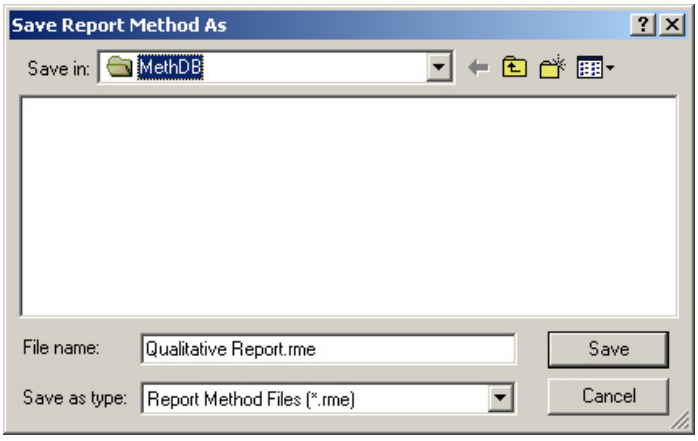
5. Set the **Output** options and specify a Report name prefix if it is required by the Output type selected. (For example, if you select **Save to file**, select a Report name prefix of <Sample Name>, and click the **Setup** button.

   The following dialog appears:



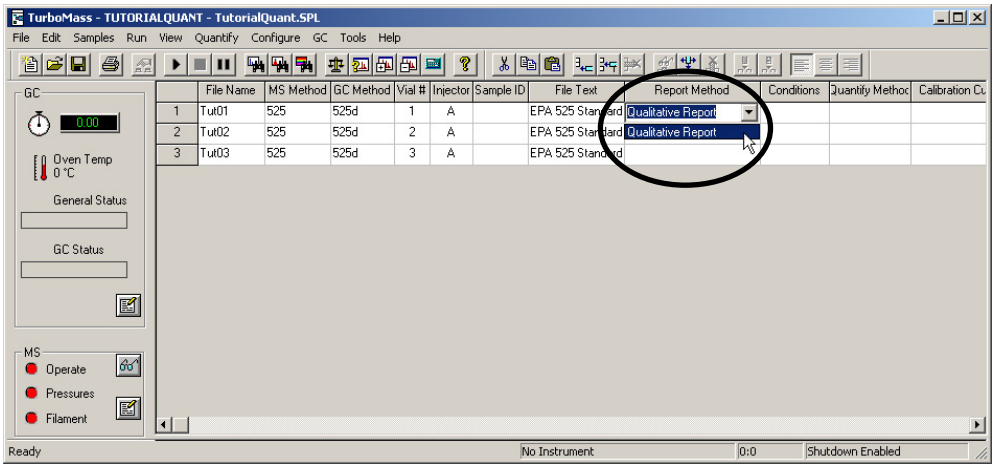
6. Click **OK**.

7. Select **Save As** from the Report Method Editor **File** menu.
   The **Save Report Method As** dialog appears:



8. Type a File name for your Report Method (for example, Qualitative Report) then click **Save**.
   This Report Method is now available for the **Report Method** column of the **Sample List**.

9. Double-click in the **Report Method** field and select **Qualitative Method** from the drop-down list.



10. Close the Report Method Editor by clicking the **Close** box (in the upper-right hand corner of the screen).

How to Shut Down the Clarus MS 19

Shutting Down Clarus MS

There are two types of shutdown procedures:

- Turning off the filament (and, if in the CI mode, the reagent gases)
- Turning off the mass spectrometer vacuum (usually for a long-term shutdown)

You can shut down Clarus MS either manually or automatically.

Manual shutdown is simply turning off the filament. TurboMass also features a user-settable shutdown function that automatically shuts down Clarus MS after it completes the analysis defined in the Sample List. The shutdown process works as follows:

1. TurboMass turns off the filament and switches to the Standby mode.
2. The Autoshutdown Tune file opens and applies its parameters.

The following steps summarize the procedure for shutting down the Clarus MS:

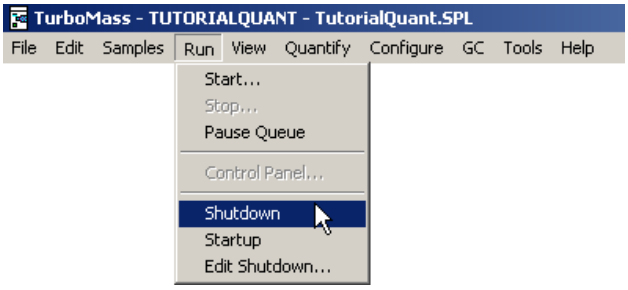
1. Determine if you want to manually or automatically shut down Clarus MS.
2. Determine the type of shutdown you wish to accomplish: overnight, weekend, or long term.
3. If automatically shutting down Clarus MS, create the shutdown file.

Types of Shutdown

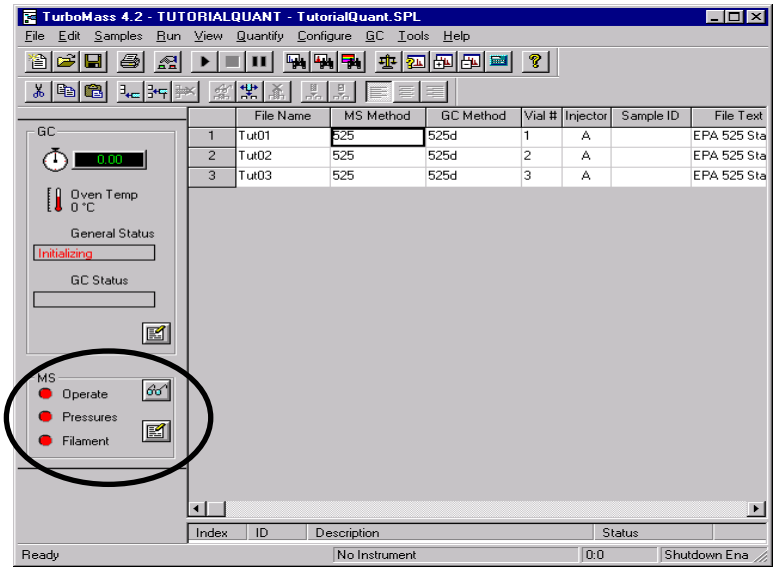
Overnight

This is the most commonly used shutdown procedure.

1. Turn off the filament by selecting **Shutdown** from the **Run** menu on the Sample List window.



2. Check the **MS** section of the Sample List window and ensure the **Operate** light is off.



This ensures that the filament is off.

If the **Operate** or **Filament** lights are on (green), display the **Tune** page and turn **Operate** off.

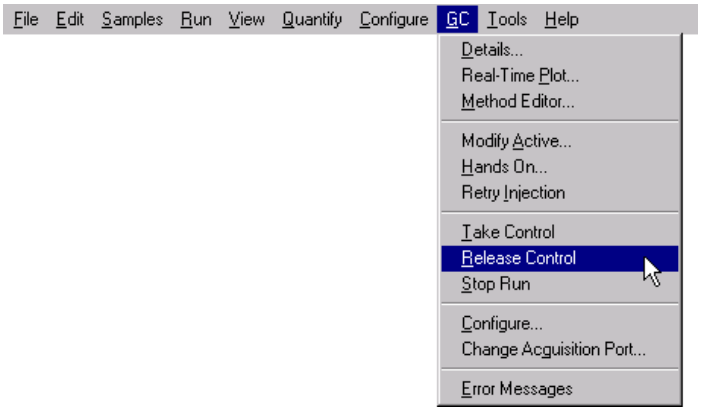
3. If you are using a split/splitless injector, turn off the split flow or set it to below 10 mL/min.

   Turning off the splitter conserves carrier gas.

If TurboMass is under GC control, free it from GC control as follows:

For PPC Pneumatics

1. Select **Release Control** from the top-level **GC** menu.



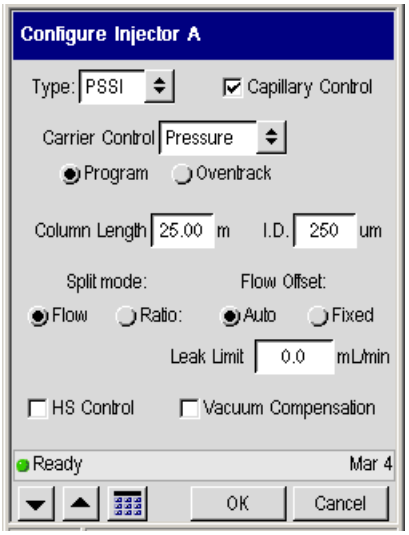
2. Touch the **Tools** button and select **Configuration**.

   The **Configuration** screen appears.

3. Touch the **Injector** button for which you which to configure the split mode.

   The **Configure Injector** screen appears.

4. Touch the **Capillary Control** check box.



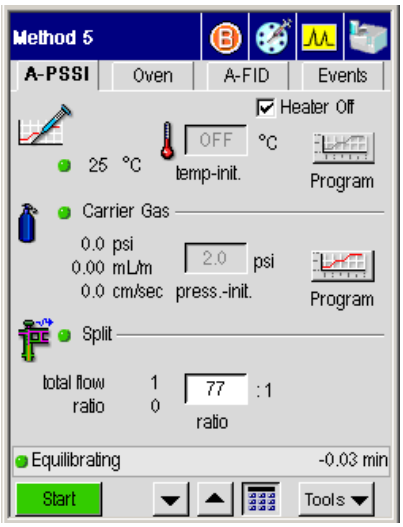
For the **Split mode** select either **Flow** or **Ratio**.

5. Touch **OK**. The **Configuration** screen appears.

6. Touch **Close**.

7. On the **System Status** screen, touch the Injector button.

   The active method screen appears.

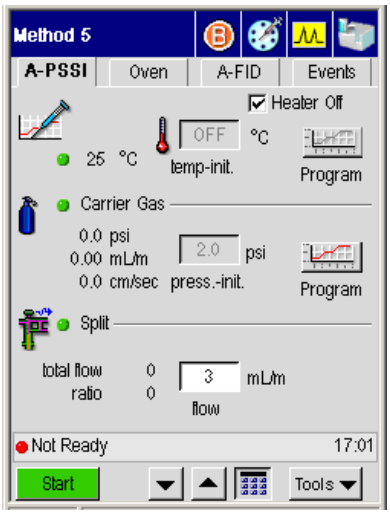


The screen above displays the Split mode configured for **Ratio**.

8. Touch the **ratio** field to modify the value.

   OR

   If you configured the split mode for **Flow**, the following screen appears:



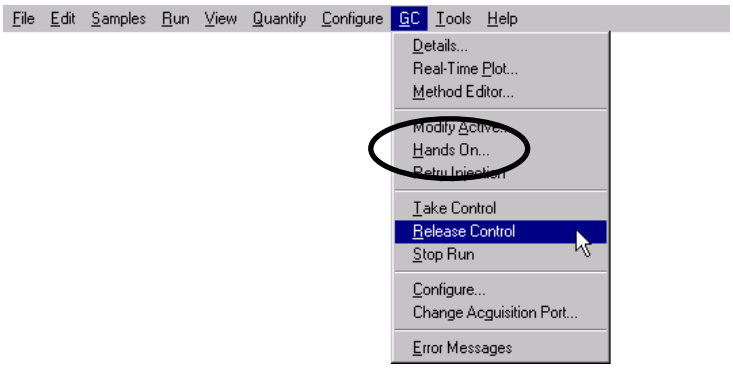
The **Total Flow** (split vent + septum purge + column) is displayed as a non-editable text field. The septum purge flow is factory preset to ~3.0 mL/min. It is not user adjustable. If you set the split flow to zero, the flow display shows column flow plus septum purge. Common places for leaks would be the septum cap, the O-ring seal, column connector, etc. See the Clarus Hardware Guide, Chapter 8 for further information.

NOTE: *If the displayed value is much greater than the total flow (split flow and septum purge + column flow), this indicates a leak in the pneumatics.*

For non-PPC Pneumatics

1. Click  to display the Acquisition Control Panel dialog.

2. Select **Hands On** from the top-level GC menu.



3. Change the appropriate split valve to **OFF**.
4. Click **Set valves**.
5. Click **Close**.
6. Turn off your computer monitor to prevent burning the display into the screen.

Weekend

1. Perform the Overnight shutdown procedures.
2. Turn off the GC oven heater by slightly opening the oven door.

Long-Term

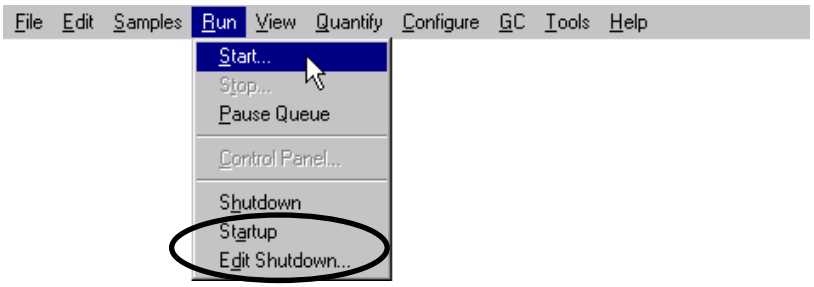
1. Perform the Overnight and Weekend shutdown procedures.
2. Vent the vacuum system.
    1. Cool the source and inlet to below 100 °C.
    2. Select **Vent/Vacuum System Off** from the **Options** menu on the Tune page, and confirm that you want to vent the system.

Automatic Shutdown

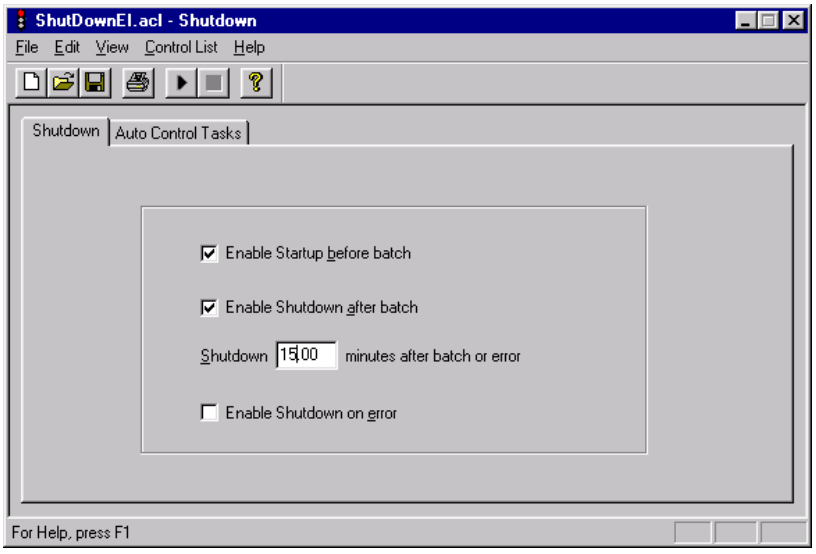
Automatic shutdown turns off the filament (and, if running CI, the reagent gases) at the end of a run (batch). For additional information about Automatic Shutdown, refer to the *TurboMass Software User's Guide*).

Turning off the Filament

1. Select **Edit Shutdown** from the **Run** menu



The following Shutdown dialog is displayed.



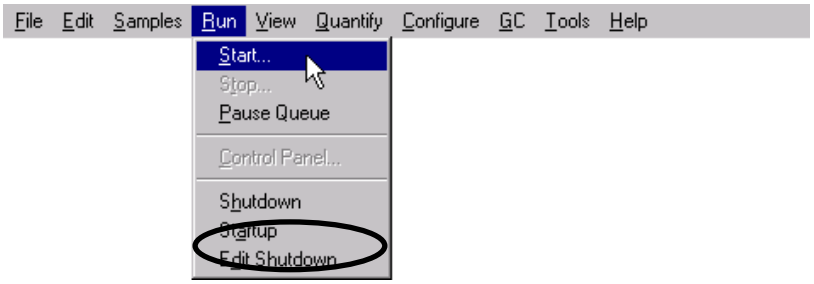
2. Select **Enable Shutdown after batch** and enter a time in minutes.

3. Select **Save** from the **File** menu.

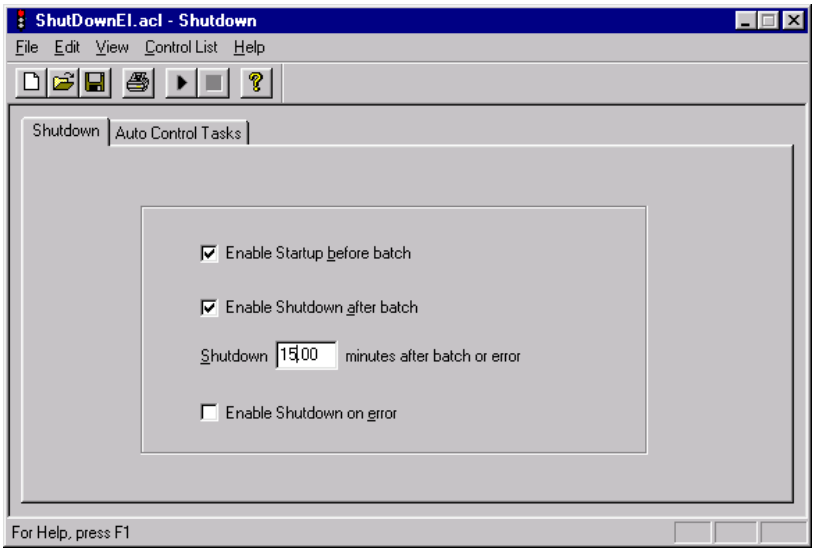
4. Select **Exit** from the **File** menu to close this window.

Auto Control Tasks

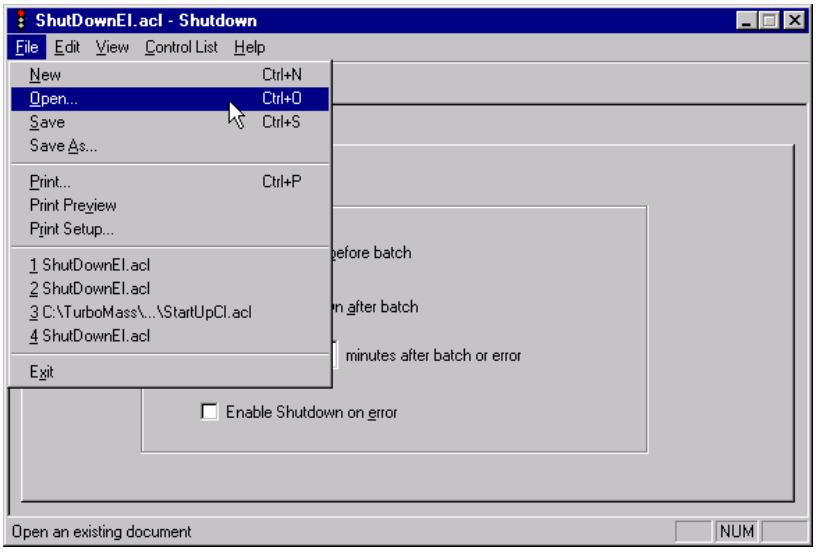
1. Select **Edit Shutdown** from the **Run** menu.



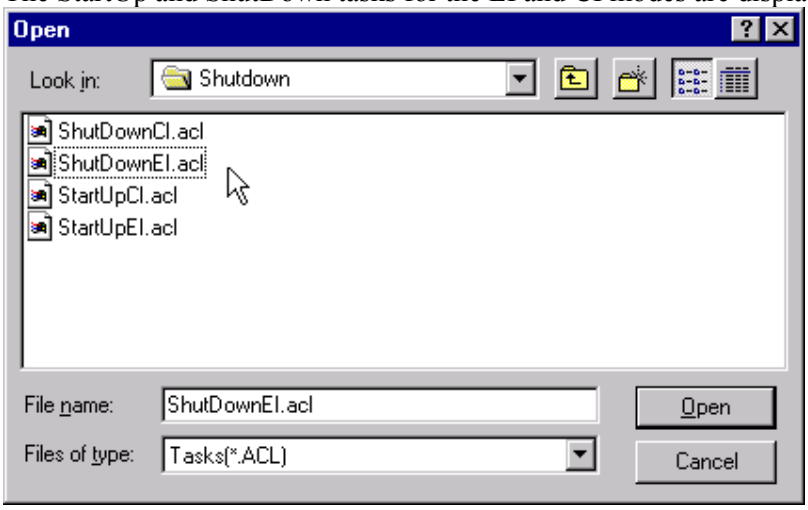
The following dialog is displayed.



2. Select **Open** from the **File** menu in the Shutdown dialog.



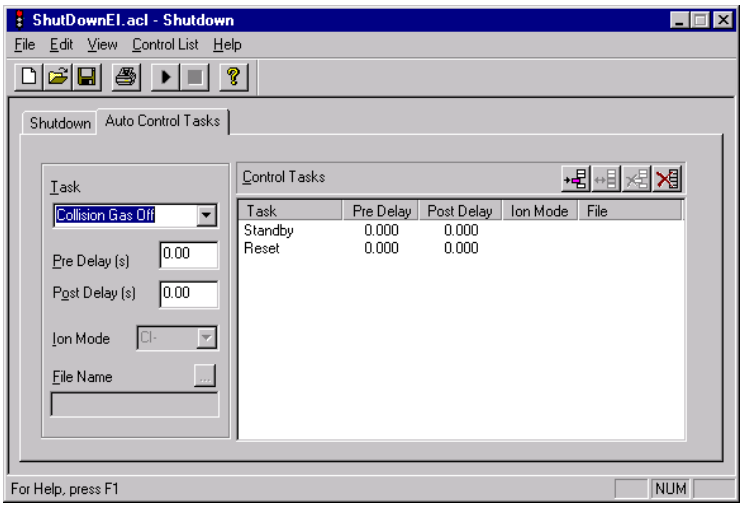
The StartUp and ShutDown tasks for the EI and CI modes are displayed.



3. Select a file and click **Open**.

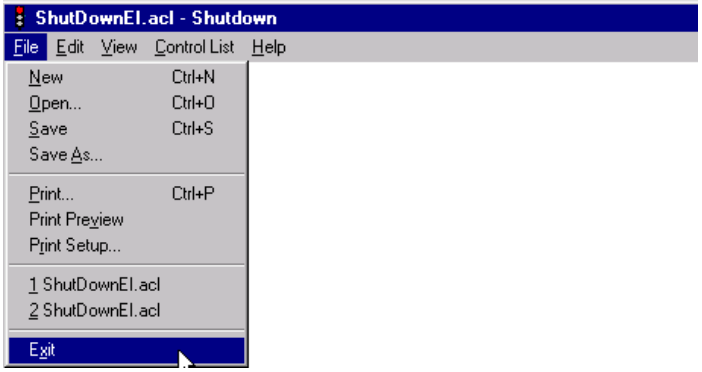
   This loads the selected **StartUp** or **ShutDown** file. In this example, ShutDownEI.acl was selected.

4. Select the **Auto Control Tasks** tab and observe the **Control Tasks**.



CAUTION	*Do not edit the contents of these lists unless you have thoroughly read the TurboMass Software Guide and understand how the control tasks function.*

5. Select **Exit** from the **File** menu.



How to Set Up and Run Chemical Ionization 20

Chemical Ionization

Chemical Ionization (CI) uses an inner ionization source different from that of electron ionization (EI). The primary difference is that the CI source does not have repeller and trap electrodes. This is because when the electron beam is emitted from the filament, very few electrons are likely to reach the trap (due to the high source pressure). The filament current is therefore regulated using the total emission current (the source current).

Also, the electron entrance and ion exit apertures in the CI source are smaller to restrict gas flow and maximize the pressure inside the ion volume. When a reagent gas such as methane, iso-butane, or ammonia is introduced into the ion chamber of the source, it is bombarded with the electron beam thereby producing reagent gas ions. By producing a higher source pressure (than EI) in the CI ion chamber, reactions occur where these ions lead to the protonation of the sample. In CI+ the MH+ sample ions are usually detected, and in CI-negative sample ions are detected, for example M^-.

The following steps summarize the procedures for setting up and running Chemical Ionization.

1. Connect the CI reagent gas.
2. Leak check.
3. Tune for EI.
4. Set the CI parameters.
5. Adjust the CI reagent gas.

About the EI and CI Inner Ion Sources

The mass spectrometer EI ion source works on the principle of electron ionization, whereby a heated filament produces a beam of electrons as shown in Figure 31. The electrons are directed at the electron trap that is held in the ion block. This process is helped by focusing (or collimating) using a pair of attracting magnets. Any vaporized sample molecules in the ion block are liable to collide with the energetic electrons and be ionized. The sample ions are repelled out of the ion block through the lens assembly towards the m/z analyzer by the ion repeller, which runs typically at a voltage of 0 to 1 V. The source is heated by a heater in the ion block.

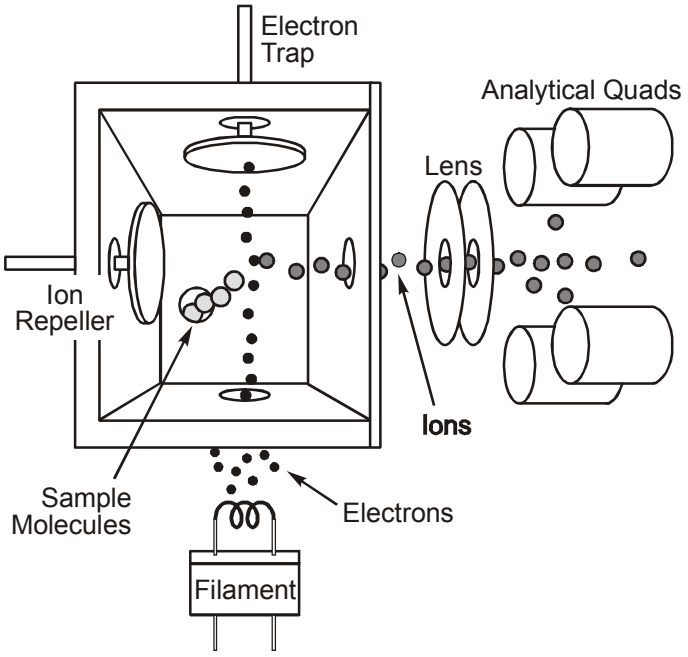


Figure 31 EI Ion Source

Before analyzing samples, the source should be tuned and calibrated by continuously passing an appropriate reference material (through the reference gas inlet) into the source as you tune and calibrate.

The mass spectrometer UltraTunes in the EI mode, but does not UltraTune in the CI mode. To set up the mass spectrometer for CI, perform UltraTune in the EI mode with the EI inner source. After Tuning vent the system, remove the EI inner source, install the CI inner source, and optimize the tuning parameters for CI operation. Then inject a known sample through the GC column to evaluate the GC/MS performance and sensitivity. The following table lists the suitable reference and test materials.

Ionization	Reference Material	Reference File	GC Test Compound
EI	Heptacosa	Hepta.ref	octafluoronaphthalene or hexachlorobenzene
CI+	(n.b. use EI calibration)		benzophenone
CI-	Heptacosa	HeptaNeg.ref	octafluoronaphthalene

Typical EI Parameters

The following table lists the typical EI parameters:

Parameter	EI Value and Comments
ion energy	For example, 0.5 to 1.5 (set ion energy with a low repeller and set repeller later.)
trap emission	50 to 200 μA (leave as low as possible.)
Repeller	0 to 2.5-- V (keep as low as possible.)
resolution (LM/HM)	For example, 10/12; varies with the mass spectrometer; (need a minimum 50% valley between $^{12}C/^{13}C$ isotopes.)
multiplier	1300 V to 2300 V (Typical values for a new multiplier. It can operate up to 4000 V max as multiplier ages; however, **leave the Voltage as low as possible** to preserve multiplier life.)
source temperature	150 to 220 ºC (lower temperatures minimize fragmentation.)
filament current	2.0 A to 4.0 A
source current	Twice the emission current or higher.

NOTE: *If the trap is dirty or the filament misaligned, the source current will read high, while at the same time the trap current will read low.*

Sample ion beam stability is related to electron current stability and so for electron ionization (EI) the source is trap (emission current) regulated, at a level of 50 to 200 μA. This usually requires a filament current of 2.0-- to 4.0 A. If the filament is operating any higher than this value, it is likely to burn out. The electrons that do not reach the trap are monitored as the source current, and the "source: emission" current ratio is also critical. A ratio of 3 : 1 or less is appropriate, and implies that the filament is correctly aligned and the source is clean. A ratio of above 3:1 indicates that the source requires cleaning or that the filament is misaligned.

NOTE*: If the inner source is clean, the repeller reading will be close to zero. When the source is becoming dirty, a higher repeller voltage is required to force the ions out of the source. The inner source should be cleaned when 4 or 5 volts is required.*

Changing from EI to CI Mode

Changing modes consists of the following:

- Connecting the CI gas.
- Changing the source and instrument control mode.
- Leak-checking.
- Setting-up CI.

Connecting the CI Gas

WARNING

Hazardous gas vapors. *When using ammonia gas when running in the chemical ionization (CI) mode, it is necessary to vent the mass spectrometer effluent from the forepump exhaust into a fume hood or charcoal trap.*

WARNING

Explosive Hazard. *If the hydrogen, methane or iso-butane is turned on without a column attached to the injector and/or detector fittings inside the oven, the gas could diffuse into the oven creating the possibility of an explosion.*

If the mass spectrometer is not under vacuum, hydrogen, methane or iso-butane can fill the vacuum chamber thereby creating an explosive hazard.

To avoid possible injury, do not turn on the hydrogen, methane or iso-butane unless a column is attached, all joints have been leak-tested, and the mass spectrometer is under vacuum with the forepump exhaust properly vented to a fume hood.

Recommended Gases

Reagent gases used in chemical ionization (CI) are ammonia, and the hydrocarbon gases methane and iso-butane at 99.95 % or better purity.

Methane and isobutene require a gas delivery pressure of 15 psi (104 kPa) to the bulkhead fitting on the back of the mass spectrometer. A two-stage stainless steel diaphragm, high purity regulator is. A single-stage stainless steel diaphragm, high purity, rated for corrosive service is required for ammonia. Clean tubing must be used. It must be solvent-washed and nitrogen-dried. The bulkhead connector at the rear of the instrument is a 1/8 inch Swagelok fitting.

WARNING

If ammonia is used for chemical ionization, all fittings and tubing must be stainless steel to avoid corrosion. Also, the forepump must be vented to a fume hood or trap.

To prepare the mass spectrometer for CI:

- Obtain the CI gas cylinder for your analysis.
- Connect the gas line to the CI Gas connector on the rear of the mass spectrometer.
- Ensure that mass spectrometer is at the proper vacuum level.
- Turn on the CI gas and set the delivery pressure to 15 psi (104 kPa).
- Leak-check all connections.

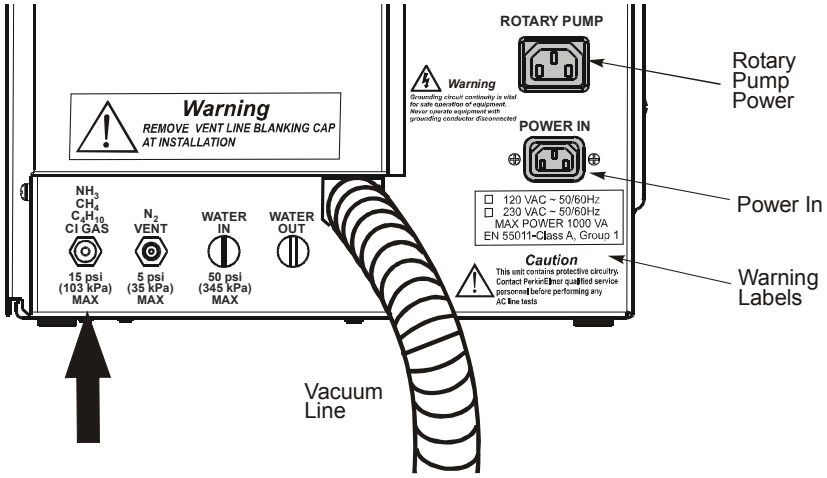
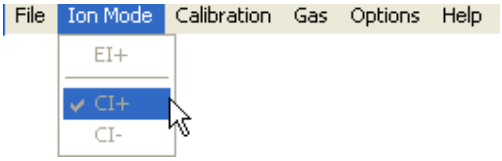


Figure 32 CI Gas connection on the rear panel of the MS

Changing to CI

To change from the EI to the CI mode:

1. Remove the EI inner source by following the procedure described in *this tutorial*.
2. Properly cover and protect the EI source and put it in a safe place.
3. Install the CI source by following the procedure described in *this tutorial*.
4. Select **CI+** from the **Ion Mode** menu.



5. Select **Pump/Vacuum System On** from the **Options** menu.
   This starts the forepump and the turbomolecular pump.

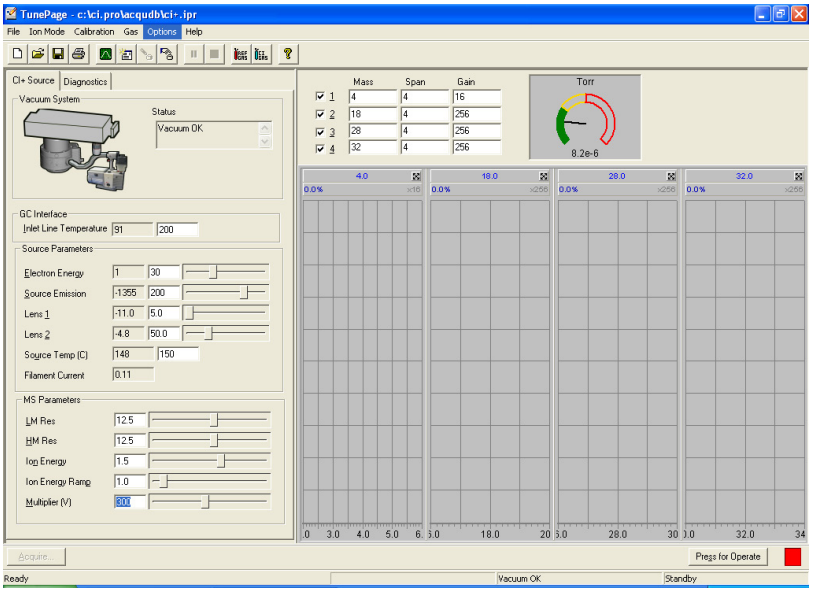
6. In the **Vacuum Pressure Gauge** area of the window, observe the Pressure gauge. Wait about 5 minutes until the gauge achieves about 2.5 x 10^{-5}.

Leak Checking

Before running in the CI mode, confirm that the column is properly installed and the system is leak-free. The best way to check this is by running CI without the reagent gas.

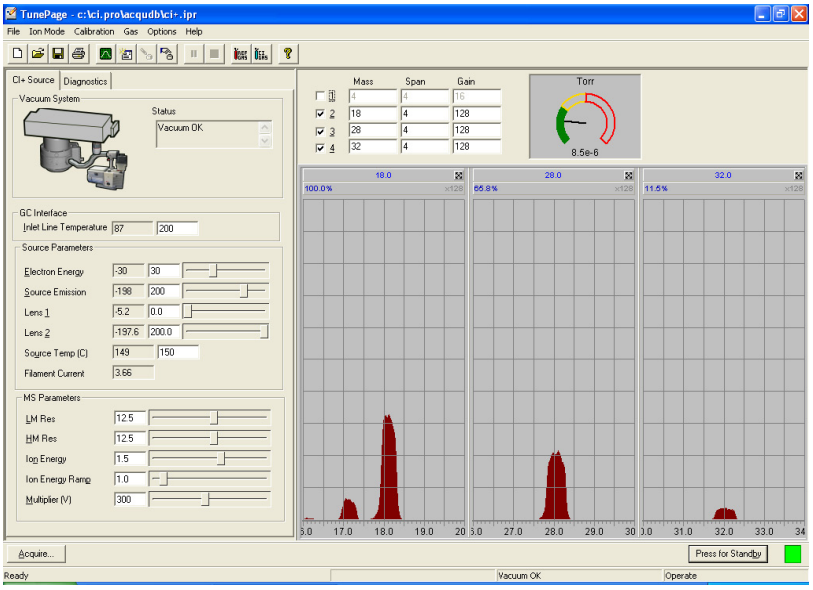
To leak-check the system:

1. Display the Tune page.



2. Select **CI+** from the **Ion Mode** menu

3. Click **Press for Operate** and observe the air/water masses.



The CI source running in the CI mode without reagent gas to produce an EI emission similar to the EI mode but with reduced sensitivity. You will leak-check your system this way.

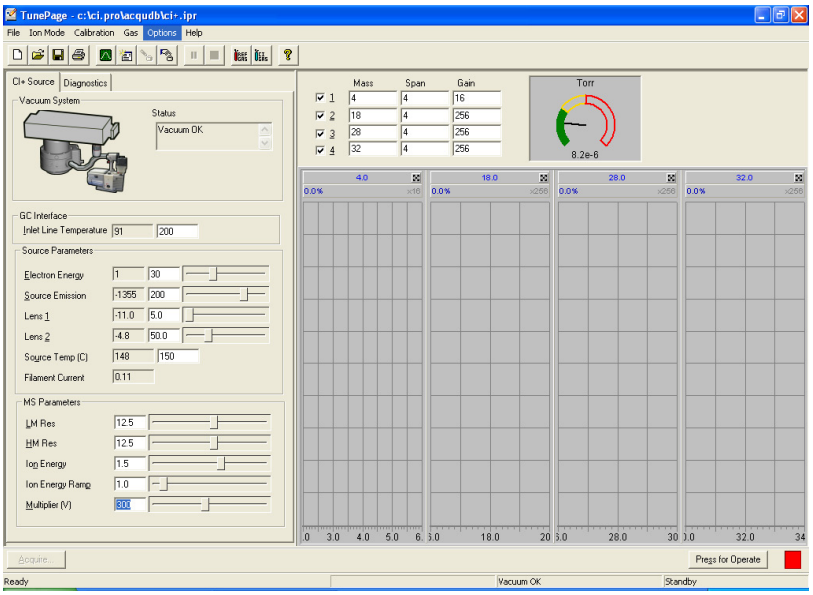
If mass 28 is larger than mass 18, you have a leak. Determine the source of the leak and correct it. For example, leak-check all fittings and connections.

Setting-Up CI

After verifying that no leaks exist, you can proceed to set up the CI mode for an analysis.

Setting the Parameter Values

1. Display the following CI window:



2. Set the values as shown in the following table.

Parameter:	**CI+ Values and Comments:**
Electron energy	30 eV
Emission	Should be below 200 mA, although 200 to 300 mA is acceptable. (Above 200 mA may cause hydrocarbon "cracking" patterns with methane and iso-butane.) Emission measures the real emission current, i.e. the source current from the source block, there is no trap "source current" in CI.
Lens 1 and 2	The tuning of these lenses may be different from the optimum values set for EI, since the source pressure is much higher in CI.
Multiplier	1300V to 1600V
Ion Energy	Approx. 1. Similar to EI.
Source temperature	150 ºC

Adjust the Reagent Gas for CI+

When running in the CI+ mode with reagent gas off, the resulting EI spectra have about 10x lower sensitivity than with the EI source.

- If using methane reagent gas, the reagent ions at m/z 17 (CH_5^+) and 29 ($C_2H_5^+$) should be of approximately equal intensity. Maximize the m/z 29 intensity. With m/z 29 maximized, the ion at m/z 16 should be about 1% of the m/z 17 peak height. (Higher indicates a leak at the transfer line/inner source connection.) Operate slightly to the low-pressure side of the maximum to minimize gas load on the MS. (The pressure gauge reading will be $1.5x10^{-4}$ to $5x10^{-4}$ Torr.)
- If using ammonia reagent gas, reagent ions at m/z 18 (NH_4^+) and 35 [$(NH_3)_2H^+$] should be present and the ions at m/z 35 should be optimized.
- If using iso-butane reagent gas, the reagent ions at m/z 43 ($C_3H_7^+$) and 57 ($C_4H_9^+$) should be tuned in the approximate ratio of 1:2.

The following example uses methane reagent gas.

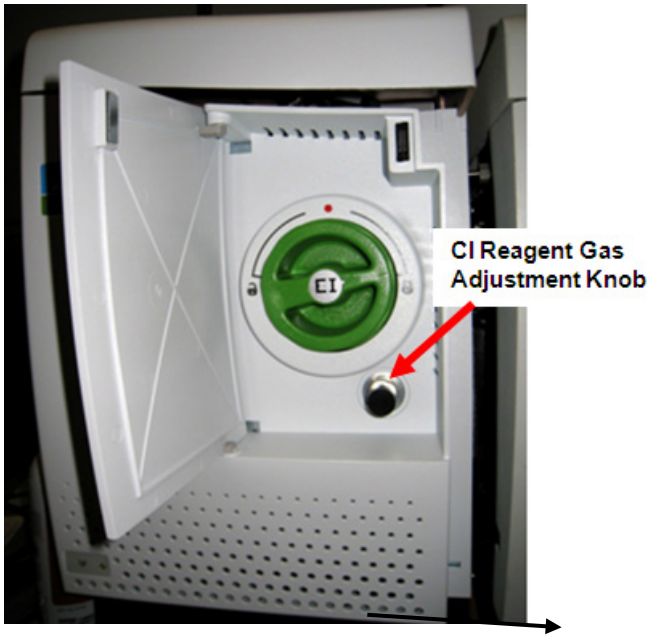


Figure 33 CI reagent gas adjustment knob

1. Carefully turn the delicate CI Gas adjustment knob fully clockwise until you feel it stop.

NOTE: *The CI gas adjustment knob controls a delicate needle valve. To avoid damaging the needle valve, do not overtighten it. Always use the CI gas button on the screen to turn off the CI gas.*

2. Select **CI Gas On** from the Gas menu. A check mark appears next to the option.

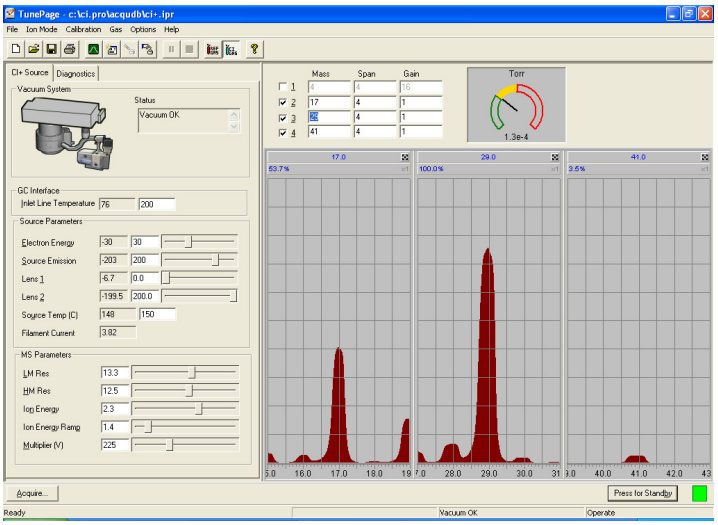
NOTE: *Always turn on the CI gas before Operate to avoid a pressure surge hitting the filament.*

3. Click **Press for Operate** and monitor the pressure gauge as you adjust the CI gas. Observe that mass 16 initially grows larger. As pressure increases in the ion chamber of CI source, the mass 29 peak will begin to grow. Keep the pressure below $5x10^{-4}$ Torr.

4. When using methane gas, carefully turn the CI adjustment knob counterclockwise until m/z 16 is low or non-existent, and m/z 29 is maximized. As you turn the knob, reduce the multiplier voltage to keep the peaks on scale.
   - A typical multiplier value is 1235.
   - m/z 17 and 29 will typically be 80 – 100%.

5. Continue to turn the knob counterclockwise. Observe that the pressure increases and mass 41 will start to grow. Stop when mass 29 is at 100%.

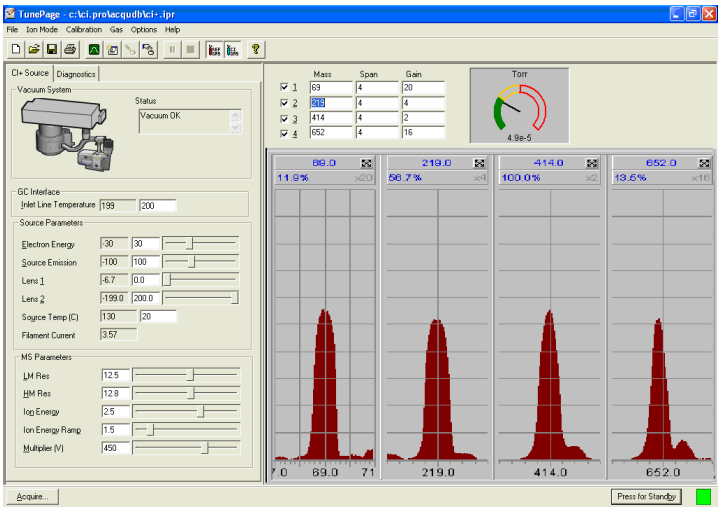
6. Turn the knob to maximize the intensity of mass 29. Also verify that mass 16 is small (< 1.0% of the height of the peak at mass 17).



If mass 16 does not appear as a small peak, STOP. You probably have a gas leak at the transfer line/inner source connection. Locate and correct the leak.

7. After you have maximized the peak, slightly decrease the reagent gas by turning the knob clockwise 1/8 turn.

8. Tuning may be optimized on the m/z 69, 219, 414, and 652 ions of the heptacosa reference gas.



9. Click **Press for Standby** to turn off Operate, followed by the CI gas.
   You are now ready to run your CI+ analysis.

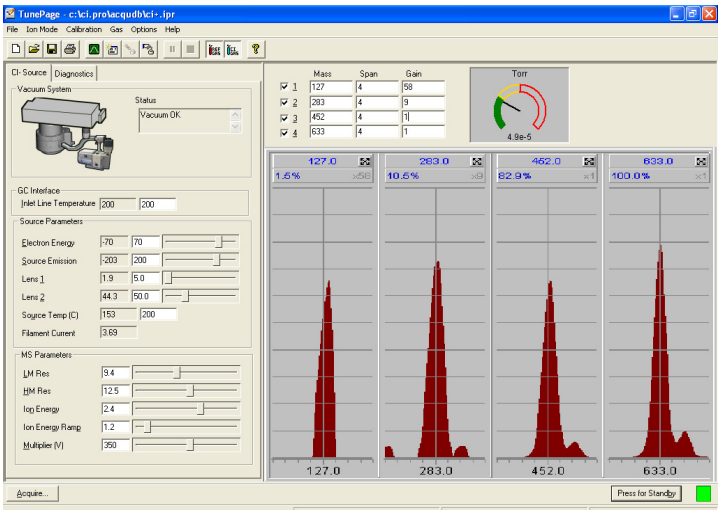
Adjust the Reagent Gas for CI-

1. Open the CI gas inlet by selecting **CI Gas** from the **Gas** menu. Wait at least 10 seconds before clicking **Press for Operate**.

Parameter:	CI– value and comments:
Electron Energy	30 to 70 eV (This parameter should be optimized.)
Emission	200 to 300 μA is acceptable (Emission measures the real emission current, i.e. the source current from the source block, there is no separate measurement of source current in CI.)
Lens 1 and 2	The tuning of these lenses may be different from the optimum values set for EI, since the source pressure is much higher in CI.
Multiplier	1300V to 1600V
Source temperature	150 °C is standard. Higher temperatures keep the source cleaner, but may increase fragmentation. For example, down a little from EI to minimize fragmentation. 120 °C is the practical lower limit.
Ion Energy	Approx. 1 or 2

2. Optimize the amount of reagent gas flowing into the source by using two heptacosa ions, m/z 452 and 633, which usually produce relative intensities of 65 – 85% and 95% respectively.
   Heptacosa can be used to calibrate the m/z range for negative ion CI analyses.

3. Maximize the peak intensities, then slightly decrease the reagent gas by turning the knob clockwise 1/8 turn.

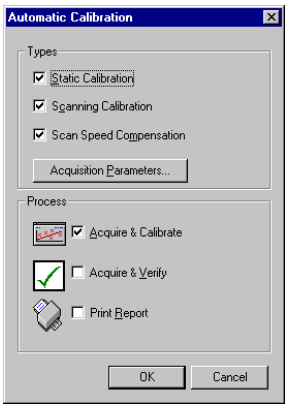


Optimize the tuning parameters for maximum intensity.

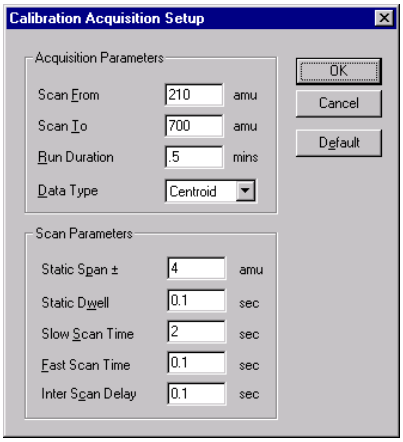
4. Save the Tune page parameters by selecting **Save As…**from the **File** menu.
5. Select **Calibrate Instrument** from the Tune page **Calibration** menu.
6. Select **heptaneg.ref** from the drop-down menu.

CAUTION	*Make sure the "Use Air Refs" check box is not selected.*

7. Click the **Start** button to display the following dialog box.



8. Click **Acquisition Parameters** and enter the following values.



9. Click **OK** to begin calibration.
   You are now ready to run CI analysis.

How to Create SIR Methods 21

SIR Methods

Selected Ion Recording (SIR, also called Selected Ion Monitoring or SIM) is primarily used to maximize sensitivity and may also be used to minimize the space required for recorded data.

SIR enhances sensitivity because only the target ions of interest are monitored so that no time is spent recording non-target ions. Spending more time on the target ions increases the signal-to-noise (S/N) ratio by the square root of the increase in time spent monitoring the ion. For example, changing from a full scan acquisition of m/z 50 to 450 in 0.5 seconds to monitoring a single ion for the same period of time will increase the S/N by approximately the square root of 400, or 20. This can also be viewed as lowering the detection limit by a factor of 20.

The following steps summarize the procedure for creating SIR methods.

1. Acquire the data in full scan.

2. Identify the target compounds.

3. Determine the ions to monitor for each target compound.

4. Enter these ions into the SIR Scan Functions in the MS method.

Creating SIR Methods with Multiple Functions

Acquiring the data in Full Scan

The following example uses a Grob-style capillary column test mix. Data were acquired from m/z 40 to 200.

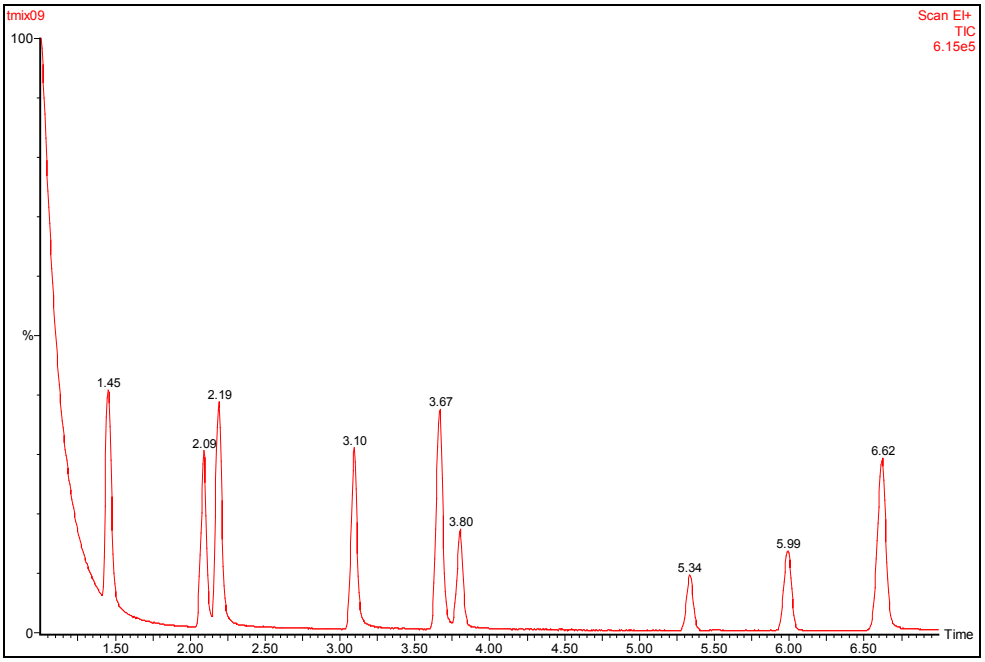


Figure 34 Data acquired using a Grob-style capillary column test mix

Identifying the Target Compounds

Examining the spectra provides us with ions characteristic of each compound for quantification. These are shown below as mass chromatograms:

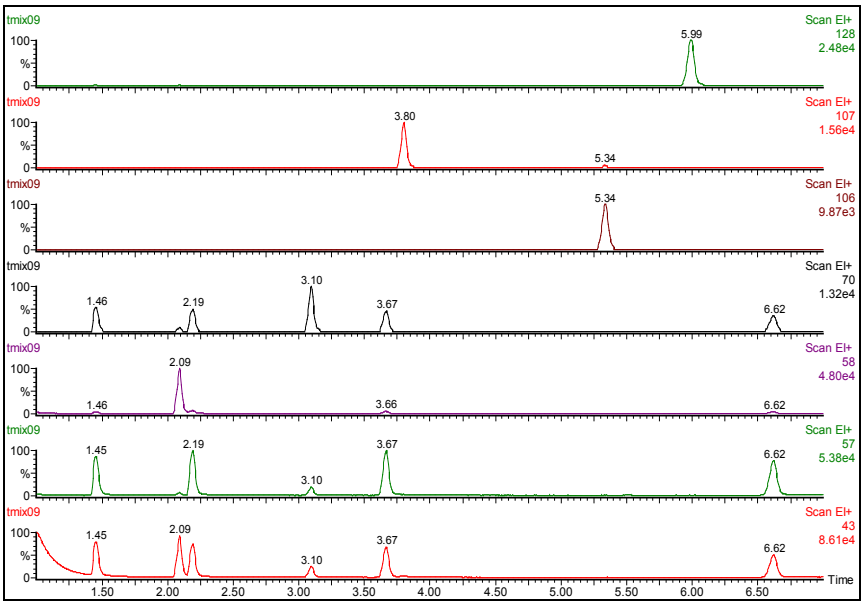


Figure 35 Mass chromatograms of characteristic ions

Determining the ions to monitor for each target compound

When possible we try to monitor ions characteristic of the compound, as high in mass and intensity as possible. High mass is desirable to minimize the probability of chemical interference. High intensity is desirable for sensitivity. We also try to choose ions with low baselines for these same reasons.

From the above chromatogram, we will monitor the following ions over the specified time intervals. The fewer ions we monitor at any particular time, the more time we spend on each ion, and the better its detection limit. Although not done in this example, it is common practice to monitor at least three ions per compound. This allows later compound confirmation by ion ratio. Entering these ions into the SIR Scan Functions in the MS method

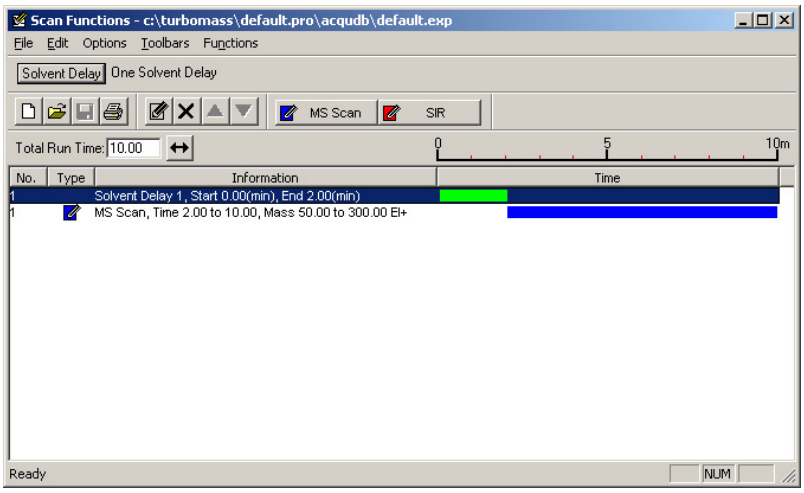
Create Scan Functions 1 through 7 by entering data from the following table.

Function	Start (min)	End (min)	Ions	Dwell (sec)
1	1.30	1.70	57	0.08
2	1.90	2.50	43, 58	0.10, 0.10
3	2.80	3.40	70	0.25
4	3.50	4.10	57, 107	0.10, 0.10
5	5.20	5.60	106	0.25
6	5.65	6.25	128	0.25
7	6.40	7.00	43	0.25

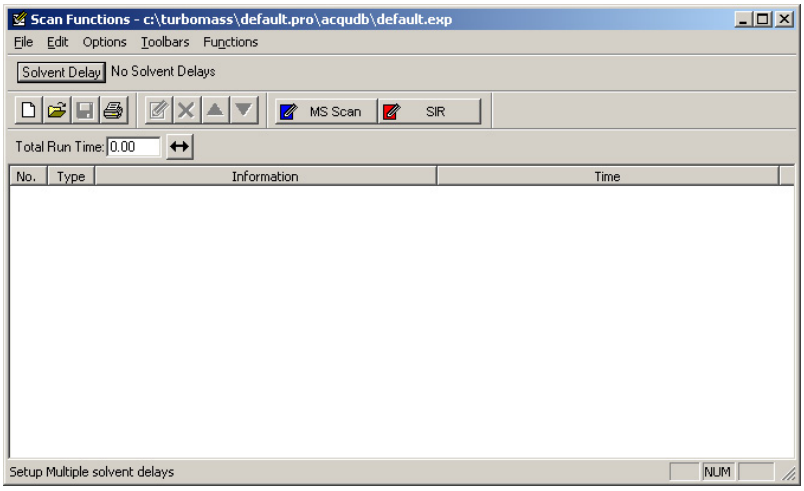
Creating Scan Function 1

To create the following seven scan functions, follow this procedure:

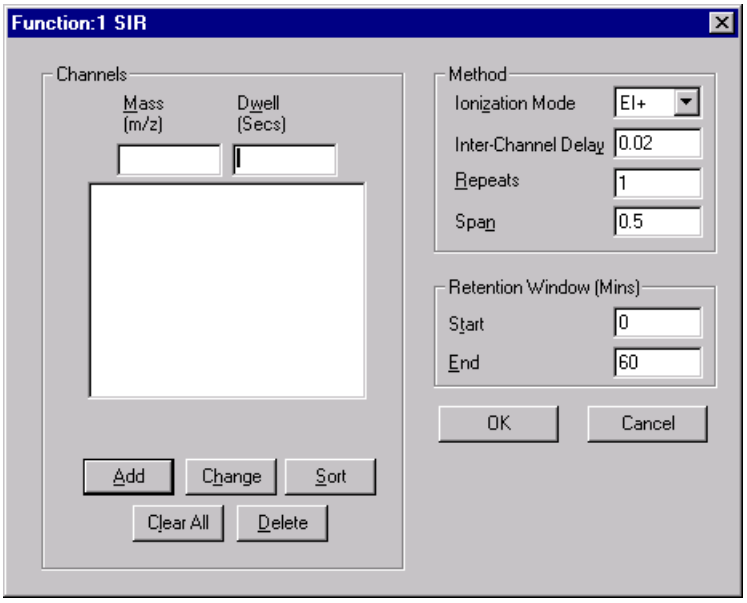
1. Right-click on MS Method in sample list. From drop-down menu select **Open**.



2. Select **New** from the **File** menu.
   The following dialog is displayed.



3. Create a SIR scan function by clicking **SIR**.
   The SIR dialog is displayed.



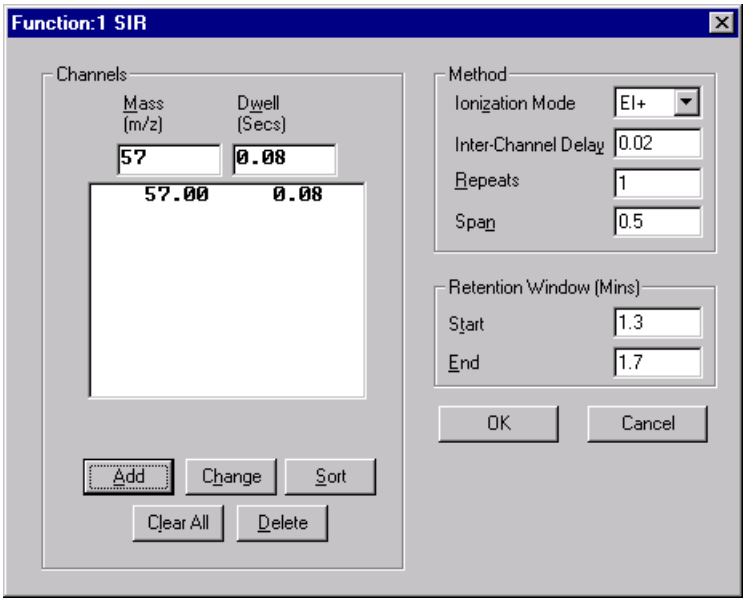
4. Enter the **Retention Window Start Time** and **End Time** for Function 1 from the table on page 304.

5. Enter 57 for **Mass** and 0.08 for **Dwell**.
   The Dwell time is the length of time this ion is monitored. This is normally set so that the sum of all the Dwell times for all the target ions gives about 10 scans across the GC peak.

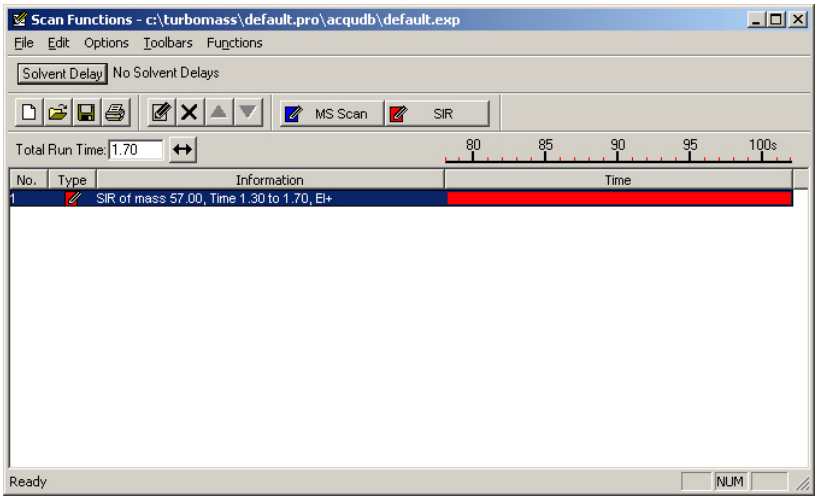
6. Click **Add** to enter this ion into the scan table.

7. To avoid being on the edge of the mass peak Gaussian curve, enter a **Span** of 0.5 for the mass spectrometer to scan from m/z 56.75 to 57.25.
   The **Repeats** text box specifies the number of times we wish to execute this Function per pass. For example, if we had two Functions defined by their Start Time and End Time to execute simultaneously, and the first Function has Repeats = 1, while the second has Repeats = 3, then the second Function would execute three times for each time the first Function executed once. With non-

overlapped Functions, better detection limits will be obtained by increasing the Dwell time rather than the number of Repeats.

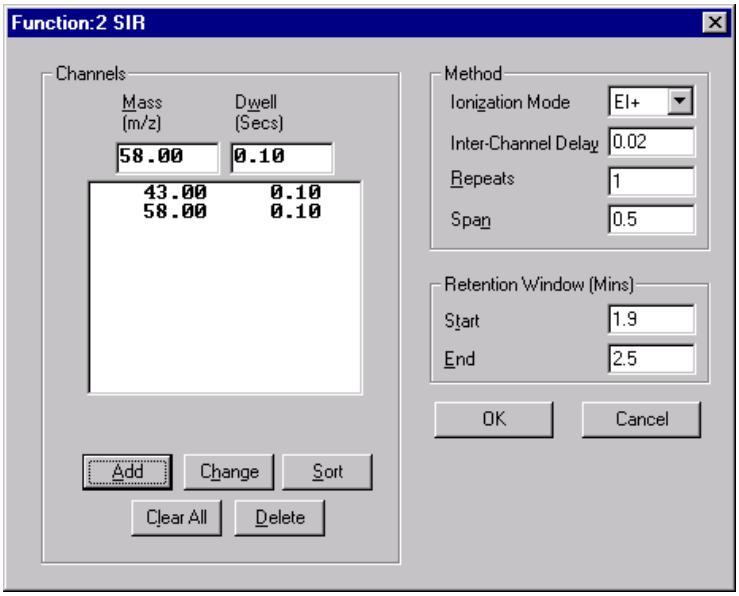


8. Click **OK** to enter this function into the MS Method.
   The following window is displayed.



Creating Scan Functions 2 through 7

1. Click **SIR** and add the next line of data from the table on page 304.

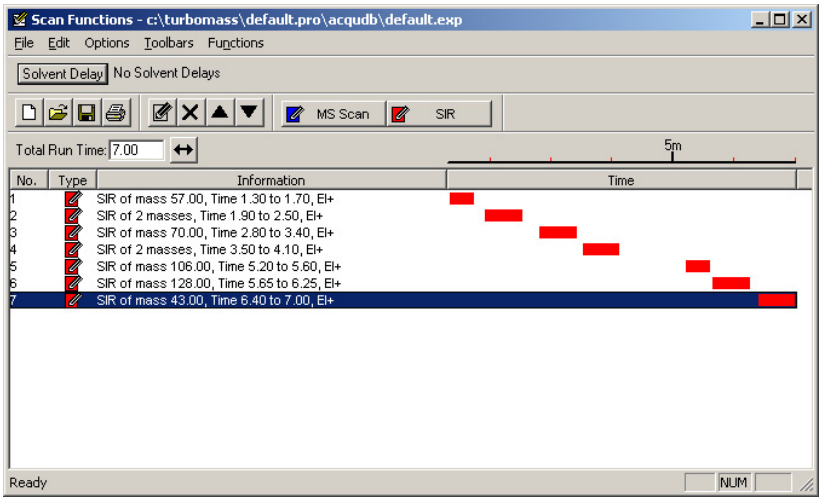


2. Enter Masses 43 and 58 sequentially (in the previous example we entered Mass 57).

3. Click **OK** to save this Function to the MS Method.

4. Continue making entries in this manner until all of the Functions have been added.

   A screen similar to the following is displayed:

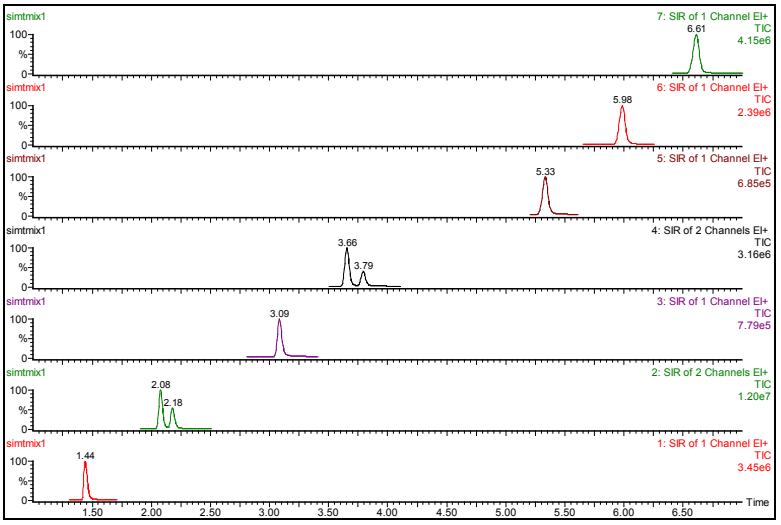


5. Click the **Solvent Delay** button and enter a **Solvent Delay** of 1.0 minute until the filament is turned on.

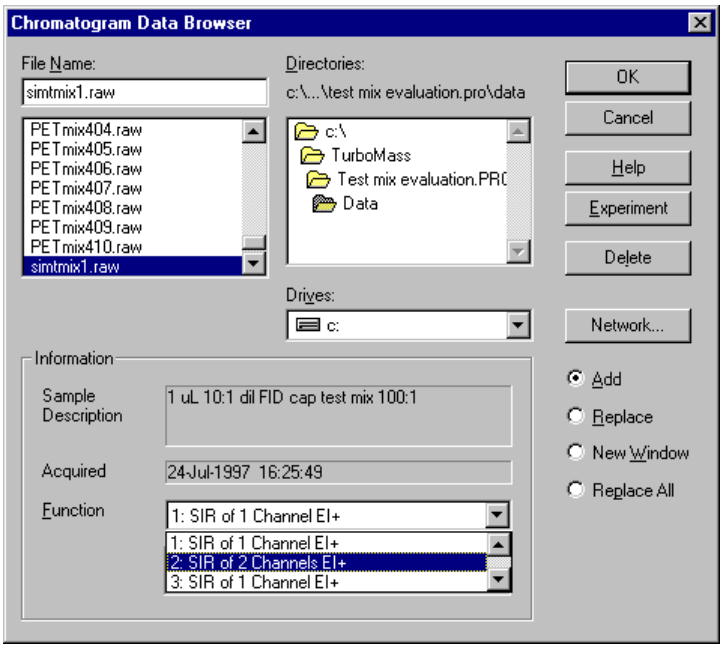
6. Select **Save As** from the **File** menu and give this function a name.

7. You may now enter this SIR MS Method into the Sample List for data acquisition just as a Full Scan function.

Acquiring data with this MS Method gives the following chromatogram.

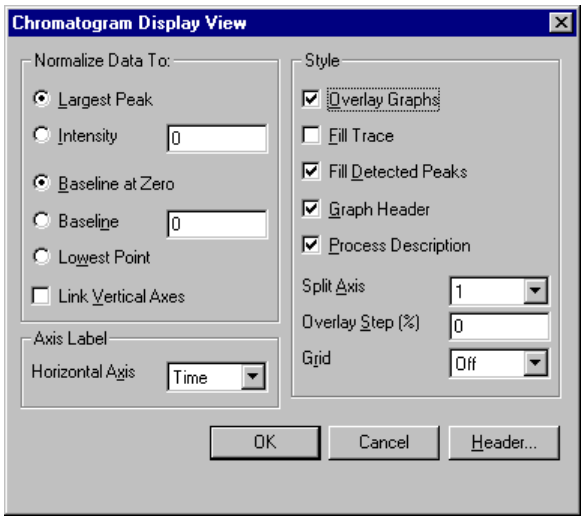


Observe that there are now 7 discontinuous chromatogram Functions, as specified by the method. If you go into the Chromatogram window and open a chromatogram you will get only the first (bottom) trace. To load all of them you need to open all of the functions individually. You do this by selecting the down arrow next to the Function description. This will drop down a scrollable list of the available functions.

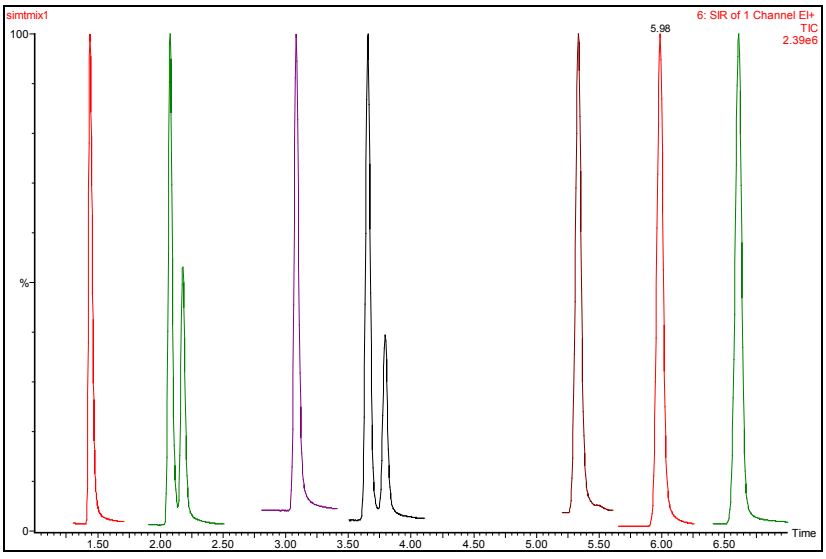


8. Select the **Function** you want and click **OK**.
   Doing this for all of the functions produces the chromatogram shown above.

9. You may also overlay the traces by selecting the **View** entry from the Chromatogram window's **Display** menu item, and selecting the **Overlay Graphs** style.



This generates the following overlaid chromatogram.



How to Optimize SIR Sensitivity 22

About Optimizing SIR Sensitivity

SIR (Selective Ion Recording) is the most sensitive operational mode for a mass spectrometer. It is often used for trace analysis. This chapter discusses strategies for optimizing sensitivity and discusses some of the tradeoffs.

The first thing you need to determine is if your analysis requires any further optimization. If the current Tune gives you sufficient sensitivity for your SIR analysis you have no need to go further.

If you need better detection limits you have two choices:

- Optimize the tuning while retaining the same mass resolution.
- Reducing mass resolution in trade for even better sensitivity.

Your ability to take the second option depends on your chromatography. If you have no background interferences from the vacuum system or nearby eluting GC peaks at adjacent m/z values, you can use the second.

To optimize SIR sensitivity:

The following steps describe how to maximize your TurboMass SIR sensitivity. They are arranged in order of increasing effort. You do not need to do all of them, just enough to get the sensitivity you need.

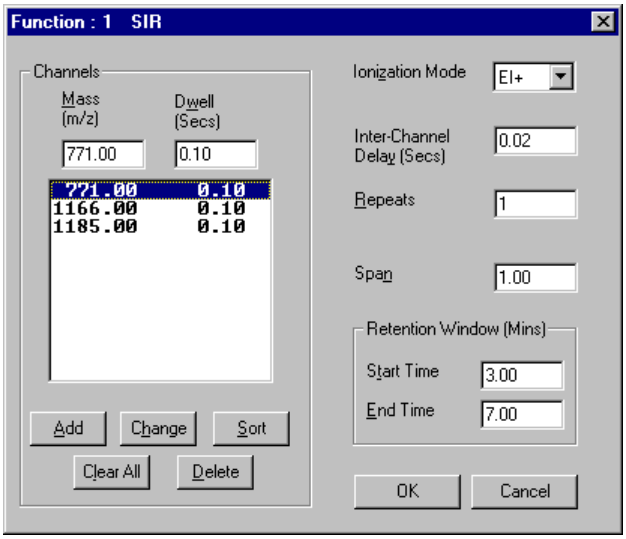
Before you begin, read over the list and decide what approach is most appropriate for your needs.

The last portion of this chapter summarizes the "normal" Tune guidelines for EI and CI. SIR may vary from these guidelines in the pursuit of maximum sensitivity.

1. Optimize the MS Method
2. Optimize Lens 1 and Lens 2
3. Optimize Repeller (EI only)
4. Optimize Mass Resolution
5. Optimize Ion energy
6. Optimize Multiplier voltage
7. Optimize Emission
8. Optimize Electron Energy
9. Optimize Ion Source Temperature
10. Empirical Calibration

1. Optimize the MS Method.

SIR sensitivity depends on the MS Method used. Several approaches can be taken to maximize sensitivity.



1. Choose the best ions for detection. In general, the higher the intensity and higher the mass the better. Use the Chromatogram display of Full Scan data to look for the best S/N ions.

2. Select the best **Ionization Mode** for your compound. Different compounds may be more sensitive in one mode than another, and background interferences may be better or worse.

3. Minimize the number of ions in a scan function. A single ion will give the best performance, while 2 to 3 per compound may be desirable for compound identification. At 10 or more ions, you may be better off with Full Scan.

4. Maximize the **Dwell** time for each ion.

   Arrange you dwell times so that the sum of all the ion dwell times is about one-tenth the GC peak width at base. This will give you about 10 points across the GC peak – optimal for SIR sensitivity and GC peak area precision. Fewer

points across the peak give better sensitivity at the expense of peak area precision. More give lower sensitivity and do not significantly improve precision. If you are monitoring ions of significantly different intensities, you may wish to assign a longer **Dwell** time to the less intense ion, because S/N increases proportionally to the square root of the **Dwell** time.

5. Set the **Peak Width at Half Height** for **Low** and **High Mass** in *UltraTune* to a value higher than the 0.6 default (e.g., 0.8 to 1.0) if your analysis permits. If this does not provide enough sensitivity, then proceed to the following steps.

2. Optimize Lens 1 and Lens 2.

The lenses will affect peak shape and intensity. They will also affect the relative intensities across the mass range. To optimize the lens:

1. Enter the **Tune** environment.
2. Turn on the Reference gas and **Operate**.
3. Locate a peak (or peaks) in the Reference gas close to your target ion(s) of interest.
4. Adjust **Lens 1** to maximize the intensity of your chosen peak(s).
5. Adjust **Lens 2** to maximize the intensity of your chosen peak(s).
6. Repeat steps 4 and 5 until there is no further improvement.

3. Optimize the Repeller (EI only).

The Repeller electrode “pushes” the ions out of the EI source. Increasing its voltage can improve sensitivity, but it may also distort peak shape.

To optimize the repeller:

1. Continue from the last set of parameters.

2. Increase the Repeller voltage until you begin to see a significant change in peak shape, paying particular attention to the starting and trailing edges of the peak.

3. If your sample permits you to reduce mass resolution, increase the Repeller voltage until the signal maximizes.

4. Optimize the Mass Resolution.

The **LM Res** (Low Mass Resolution) and **HM Res** (High Mass Resolution) control the width (resolution) of the ion peaks.

To optimize the mass resolution:

1. Continue from the last set of parameters.

2. Increase the **Gain** on you peak(s) until you can see the isotope ion (one mass unit higher than the Reference gas ion) at about one-third of full scale intensity. Observe the depression, or *valley* between the two peaks. In the picture below, the valley is about 20% of the height of the smaller isotope peak.

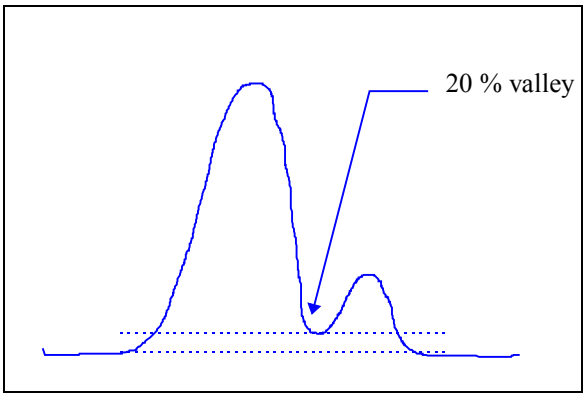


Figure 36 Resolution of ions

3. Lower the **HM Res** and **LM Res** until you see the valley increase to between 70 and 80% of the isotope peak. **HM Res** has a stronger effect at high mass, and **LM Res** at low mass, but both influence across the mass range.

4. If your mass resolution requirements permit, you can reduce the resolution to a 80 or 90% valley, and even go further and eliminate the valley entirely until the peaks become broader. At some point, however, you may find that you cease to gain useful intensity while continuing to lose resolution.

5. Optimize the Ion Energy.

The **Ion Energy** and **Ion Energy Ramp** control the acceleration of the ions out of the ion source. In graphical terms, the **Ion Energy** is the y-axis intercept and the **Ion Energy Ramp** is the slope of the line that defines this accelerating voltage.

The effects of the two controls are interrelated. If the **Ion Energy** is fairly low (e.g., below 1.0 V) increasing the **Ion Energy Ramp** will tend to increase the intensity of high mass ions relative to those at lower mass. If the **Ion Energy** is high to begin with little affect may be seen.

To optimize the ion energy:

1. Continue from the last set of parameters.

2. Increase first the **Ion Energy Ramp** and then the **Ion Energy** to increase signal intensity. Pay attention to the peak width at base. If your mass resolution requirements permit, you may allow it to broaden.

6. Optimize the Multiplier.

The Multiplier voltage is a key factor in sensitivity and dynamic range. Too low, and sensitivity can be severely reduced. Too high and the baseline is raised, lowering the dynamic range.

We will optimize it for sensitivity by setting it to a value which maximizes the signal-to-noise ratio of a peak near the baseline, and dynamic range by keeping the baseline noise as low as possible.

To optimize the multiplier:

1. Set the **Multiplier** voltage to 1300, and select the **Reinitialize** option from the **Other** menu, or press the  icon to reinitialize the baseline.

2. Look in the residual vacuum spectrum and find a small mass peak near your target mass of interest. It should be near the noise level. If you do not see one, let in the Reference gas and look at its spectrum. An example is m/z 495 in the reference gas.

3. Increase the **Multiplier** voltage in 20 V steps until you see peaks appear above the noise. **Reinitialize** after each voltage step. The signal will increase, but so will the noise. You will need to decrease the Gain of the display to keep the peaks at about the same height.

4. Continue doing this until you find that increasing the **Multiplier** voltage no longer increases the ratio of the peak signal to the surrounding noise. That is your optimal **Multiplier** voltage. Increasing the voltage will not gain you any sensitivity, only decrease you dynamic range. Typical values are 1300 to 1600 V.

7. Optimize the Emission.

The **Emission** setting controls the amount of electrical current flowing through the filament. Adjusting this can significantly change your signal intensity. The trade-off is filament lifetime. In general, for a rhenium filament, try to keep the **Filament Current** under 3.6 to 3.8 Amps. For a Marathon filament, the filament current is low enough that this is not a concern.

To optimize the emission:

EI and CI- often can give improved performance at higher **Emission** settings. Methane and isobutane CI+ may not because of increased noise related to hydrocarbon fragmentation patterns.

1. Continue from the last set of parameters.

2. Increase or decrease the **Emission** to maximize signal-to-noise ratio. **Reinitialize** the baseline level after each step. Keep the current under 3.5 Amps.

8. Optimize the Electron Energy.

The **E Energy** (Electron Energy) is the potential energy of the electrons striking the analyte (or reagent gas, for CI). For EI, a value of 70 eV is normally used. However, the value for optimum sensitivity is compound and ion-dependent.

To optimize the electron energy:

1. Save your new Tune method.
2. Create and save a series of new methods at 10 V increments.
3. Shut down Tune.
4. Add the **MS Tune File** column to your Sample List spreadsheet.
5. Acquire a series of injections of your analyte specifying the different Tune files you just created.
6. Compare the S/N ratios of chromatographic results for the target ions, and select the MS method with the Electron Energy that gives the best results.

9. Optimize Ion Source Temperature.

The **Source Temperature** can have an effect on relative ion intensity. In general, a lower temperature will give more intense high-mass ions, particularly the molecular ion. The tradeoff is that the source may become contaminated more rapidly, requiring more frequent cleaning. Very high boiling point compounds may exhibit GC peak tailing at low ion source temperatures.

A temperature of 150 ºC is probably close to the lowest practical temperature setting, since the filament and GC transfer line will make it difficult to keep the temperature stable at a lower value.

To optimize the source temperature:

1. Save your new Tune method.

2. Create and save a series of new methods with the **Source Temperature** at 10 ºC increments.

3. Shut down Tune.

4. Add the **MS Tune File** column to your Sample List spreadsheet.

5. Acquire a series of injections of your analyte specifying the different Tune files you just created. Allow enough time between each injection for the temperature to equilibrate (perhaps 20 minutes).

6. Compare the S/N ratios of chromatographic results for the target ions, and select the MS method with the **Source Temperature** that gives the best results.

10. Empirical Calibration.

The normal operation of SIR is to set the **Span** to m/z 0.25 to 1.0 so that the MS sweeps this over this range for each SIR ion. (For example m/z 100 with a **Span** of m/z 0.5 would sweep from 99.75 m/z to 100.25 m/z). This ensures that the MS peak top is covered in the sweep. The cost is that less time is spent at the maximum intensity point of the peak. Reducing the **Span** to 0.0 will improve sensitivity *if* the target **Mass** is exactly on the top of the peak.

To make sure the target **Mass** is exactly on the top of the peak we use the technique of *Empirical Calibration*. This is done in the following manner.

1. For each target **Mass**, create a SIR Function in the MS Method which scans at least five ions over the expected mass (calculated with the MW Calculator on the Sample List *Tools* menu as the **Mass**). If, for instance, this value were 100.0 Da, then the SIR ions would be m/z 99.6, 99.8, 100.0, 100.2, and 100.4. (You may do this for multiple compounds in the same Function).

2. Acquire the chromatogram.

3. Plot the resulting selected ion traces, specifying to mass to m/z 0.05.

4. Choose the plot with the highest intensity as the **Mass** for your method.

11. Typical Operation Parameters

Parameter	EI Value	CI+ Value	CI– value
Electron Energy	70 eV	30 eV	30 eV to 70 eV
Emission (trap current for EI)	50 to 200 μA (Leave as low as possible.)	< 200 mA, although 200 to 500 mA is acceptable.	
Filament current (Rhenium filament)	3.0A to 4.2A (At 5 A the filament will have a reduced lifetime.)	same	same
Filament current (Marathon filament)	2.0A to 2.8A	same	same
Ion energy	0.0 to 2.5	same	same
Ion Energy Ramp	~ 1.0 to 3.0 eV	same	same
Lens 1 and 2		The tuning of these lenses may be different from the optimum values set for EI, since the source pressure is much higher in CI. Optimal values change with pressure and mass.	
Multiplier	1300V to 2300V (Typical values for a new multiplier. It can operate up to 4000 V max as multiplier ages; however, leave the Voltage as low as possible to preserve multiplier life.)	same	same
Repeller	0 to 1.5V (Keep as low as possible.)	0 V (Repeller not present in the CI source.)	
Resolution (LM/HM)	~ 10/12; varies with the mass spectrometer; (Need a minimum 50% valley between $^{12}C/^{13}C$ isotopes.)	same	same
Source current (emission)	Twice the emission current or higher.	N/A	N/A
Source temperature	150 to 220 °C (Lower temperatures minimize fragmentation.)	150 ºC	150 ºC

Appendix 1
Common Vacuum System Contaminants

Common Vacuum System Contaminants

M/z	Species	Common Source
12	C^+	Organic contamination
13	CH^+	Organic contamination
14	N^+	Air leak, contaminated gas tank
14	CH_2^+	Organic contamination
15	CH_3^+	Organic contamination
16	O^+, CH_4^+	Air leak, contaminated gas tank
17	OH^+	Air leak, contaminated gas tank, water
18	H_2O^+	Air leak, contaminated gas tank, water
19	H_3O^+	Air leak, contaminated gas tank, water
19	F^+	Heptacosa reference gas
20	Ne^+, HF^+	Contaminated gas tank
23	Na^+	Human fingerprint oils
26	$C_2H_2^+$	Organic contamination
27	$C_2H_3^+$	Organic contamination
28	N_2^+, CO^+	Air leak, contaminated gas tank
28	$C_2H_4^+$	Organic contamination

M/z	Species	Common Source
29	$^{14}N^{15}N^+$	Air leak, contaminated gas tank
29	CHO^+, $C_2H_5^+$	Organic contamination
31	CF^+	Heptacosa reference gas
32	O_2^+	Air leak, contaminated gas tank
39	K^+	Human fingerprint oils
40	Ca^+	Human fingerprint oils
40	Ar^+	Air leak, contaminated gas tank
40	$C_3H_7^+$	Organic contamination
44	CO_2^+	Air leak, contaminated gas tank
44	$C_3H_8^+$	Organic contamination
69	CF_3^+	PTA calibration gas
73	Me_3Si^+	GC column or septum bleed
149	Phthalate	Plasticizer from plastics

Ion Series	Common Sources
73, 147, 207, 221, 281, 295, 355, 429	Methyl silicone – septa and columns
19, 31, 69, 131, 219, 264	Heptacosa reference gas
43, 57, 71, 85	Alkane series
41, 55, 69, 83	Alkene series
41, 43, 55, 57, 69, 71, 83, 85	Human fingerprint oils

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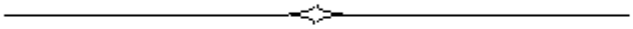

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