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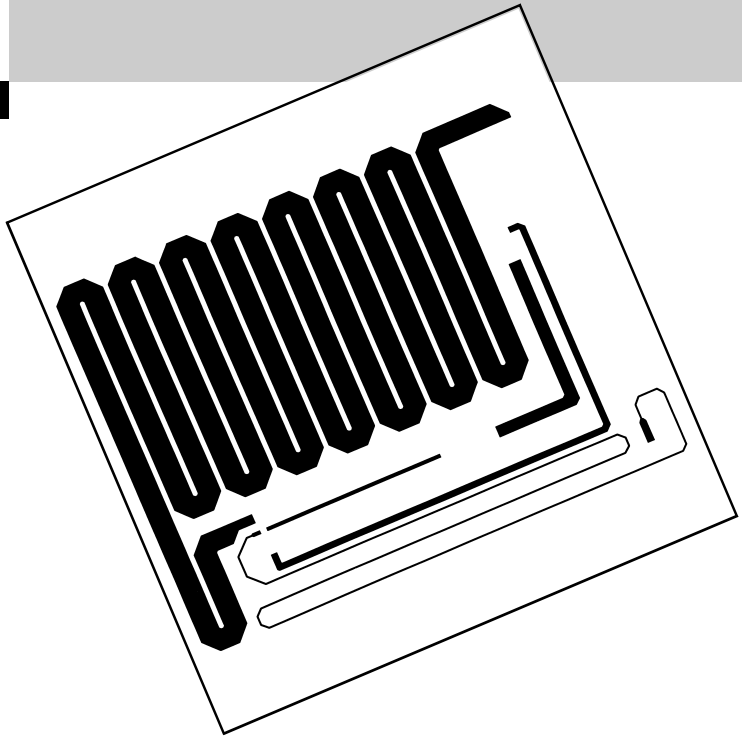

Agilent EZChrom
Chromatography
Data System

User's Manual

Agilent G2890/G2891 Micro GC

Agilent EZChrom Chromatography Data System

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Safety Symbols

This manual contains safety information that should be followed by the user to ensure safe operation.

WARNING

A warning calls attention to a condition or possible situation that could cause injury to the user.

CAUTION

A caution calls attention to a condition or possible situation that could damage or destroy the product or the user's work.

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1

Introduction

Introduction

General description

Welcome to the EZChrom Chromatography Data System, a complete gas chromatography (GC) data system designed to control Agilent Technologies micro gas chromatographs. EZChrom's compatibility with Microsoft® Windows™ 3.1 makes the data system extremely user friendly. With a simple click of a mouse button, you can initiate data collection, save or retrieve data, or begin data analysis of your chromatogram.

This manual covers the operation of both EZChrom 200, which controls the operation of the M/P series of GCs, and EZChrom 400, which controls the Quad series of GCs.

Whether you are using the EZChrom 200 or the EZChrom 400 Chromatography Data System, you will be able to reference this manual for operation and explanation of either software package. For this reason, the EZChrom Chromatography Data System will be referred to as EZChrom 200/400 throughout this manual. Instructions given in the manual will apply to either software package, with the following understanding of the differences between the two data systems.

There are several operational and display differences between EZChrom 200 and EZChrom 400; however, these differences are subtle and thus EZChrom 400 does not require a separate manual. With an understanding of the differences between the two software packages, you will be able to use this manual for operation of either EZChrom 200 (in conjunction with an M/P series Gas Chromatograph) or EZChrom 400 (in conjunction with a Quad series Gas Chromatograph).

The differences between the EZChrom 400 and EZChrom 200 Chromatography Data Systems are as follows:

- Four channel display: EZChrom 400 software has a four channel display window (Channels A, B, C, and D) rather than a two channel display window (Channels A and B, only) as found in EZChrom 200. EZChrom 400 collects, displays, and reports data for up to four (4) GC modules; whereas, EZChrom 200 does so for up to two (2) GC modules only. As a result, where there is reference to Channels A and B in this manual regarding the operation of EZChrom 200, the same instructions will apply for the operation of Channels C and D in EZChrom 400.
- Real time display: EZChrom 400 does not have real time display of data during a chromatographic analysis. Chromatogram displays go blank during an analysis and only an hour glass is displayed. Once data acquisition is complete, EZChrom 400 displays four chromatograms, one in each of the four window displays.

EZChrom 200/400 is designed for beginning chromatographers and experts alike and when interfaced with an Agilent gas chromatograph, performs routine gas analysis with minimal user input. For complex samples, a variety of commands are provided to simplify the quantitation and identification of gaseous fractions.

A personal computer is used as a sophisticated chromatography data analysis station and storage device. With EZChrom 200/400 running on a personal computer, you can:

- Set up, modify, catalog, recall, and print
  - Instrument settings
  - Peak identification tables
  - Calibration tables
  - Integration parameters
- Start and stop the instrument, to upload and download experimental conditions and to monitor instrument status.
- Catalog and recall chromatographic data.

- Integrate and identify observed peaks.
- Set up and run automated sequences.
- Perform calibration sequences.
- Graphically manipulate data displays via zooming functions, scroll bars and mouse (or keyboard) controlled zoom boxes and cursors.
- Graphically specify Timed Integration Events.
- Calculate, display, and print Area, Normalization and External Standard reports.
- Store integrated results in DIF or PRN format for direct input to spreadsheet applications such as Lotus® 1-2-3® and Microsoft Excel™.

How to use this manual

- This manual covers the installation and operation of the EZChrom 200 and EZChrom 400 Chromatography Data Systems.
- Chapter 2 discusses the installation of EZChrom 200/400.
- Chapter 3 describes how to get started with EZChrom's user interface. This includes selecting commands, graphic manipulation both with and without a mouse and text/numeric editing.
- Chapter 4 provides a step by step sample session that takes you through building a method, collecting data, optimizing integration parameters (timed events) and calibrating a method.
- Chapter 5 is a technical reference describing the theory behind topics such as data sampling, peak detection and peak identification.
- Chapter 6 describes the purpose, syntax and any relevant comment on each menu and command available in EZChrom 200/400.
- Chapter 7 focuses on more advanced skills such as method locking, multilevel calibration, print options and automated sequences.
- Chapter 8 describes how to modify or correct several GC instrument configuration parameters that are specific for a particular module.
- Chapter 9 describes how to verify that your GC firmware is in proper working order, to restore its configuration parameters, clear error conditions or recover a lost configuration record.
- Chapter 10 provides answers to your most common questions.

2

Installation

Installation

Chapter 2 describes how to set up your computer to install and operate EZChrom 200/400.

Requirements

Computer system requirements

1. Computer: Any Microsoft Windows 3.1 compatible system, minimum 20 MHz 386 processor.
2. System memory: Minimum 4 Mb of RAM.
3. Disk storage: 100 megabyte hard drive and 3.5 or 5.25 inch floppy drive
4. Ports: One free serial port (two serial ports if a serial mouse is used).
5. Operating System: MS-DOS® version 5.0 or higher.
6. Video Monitor: Any Microsoft Windows 3.1, 100% compatible, including EGA, VGA or SVGA.
7. Operating environment: Microsoft Windows Version 3.1.
8. Printer: Any Microsoft Windows 3.1, 100% compatible.

GC instrument requirements

Firmware version 17.4 or later.

Windows installation

Before installing the EZChrom 200/400 Data System, Windows 3.1 must be properly installed and running on your computer. If you are not familiar with Microsoft Windows, it is recommended that you acquaint yourself with Windows 3.1 (or later Windows versions being used with EZChrom 200/400). Refer to Chapter 1 of the Windows 3.1 manual for an excellent overview of basic Windows skills.

The HIMEM.SYS driver must be present in the CONFIG.SYS file for EZChrom to function properly.

EZChrom 200/400 installation

Introduction

Your Agilent GC is shipped with one diskette containing the following:

1. EZChrom 200/400: This diskette contains the chromatography data system.

Installing EZChrom 200/400

1. Make sure the computer is running Windows, and is at the Program Manager.
2. Insert the EZChrom 200/400 diskette into the appropriate floppy drive.
3. Click the left mouse button on **File** in the top menu bar; the **File** menu will appear. Then click the left mouse button on **Run**.

4. A command box will appear. In the command line:

   Type:

   a:\setup If your installation disk is in drive A.

   b:\setup If your installation is in drive B.

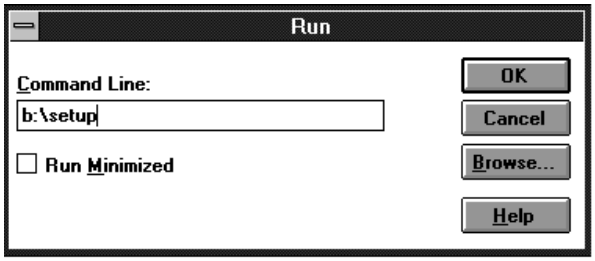


Figure 1 Program manager run window

5. The initializing window will appear (see below). EZChrom 200/400 will automatically load the software to C:\HP.

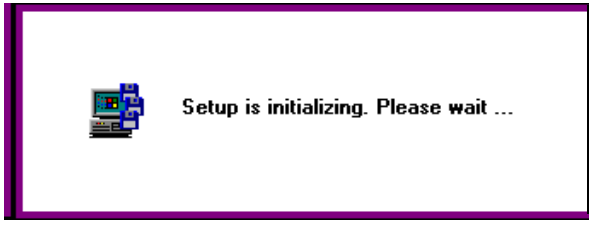


Figure 2 EZChrom 200/400 initializing window

6. Should you wish to use another drive or directory, type the location in the space provided. (see below) Click the left mouse button on **[OK]** or press **<Enter>**.

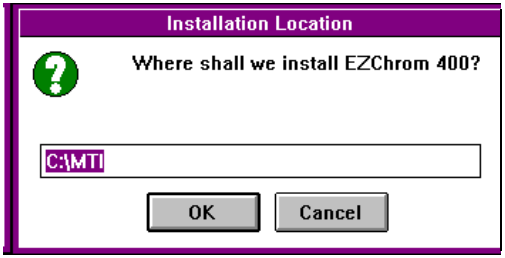


Figure 3 EZChrom 200/400 installation location window

7. After all the EZChrom 200/400 files have been copied, an HP Group window should appear with an EZChrom 200/400 icon.

Getting started

Starting EZChrom

In the Windows Program Manager

1. Locate the EZChrom 200/400 icon in the HP group window. Double-click on the EZChrom 200/400 icon.

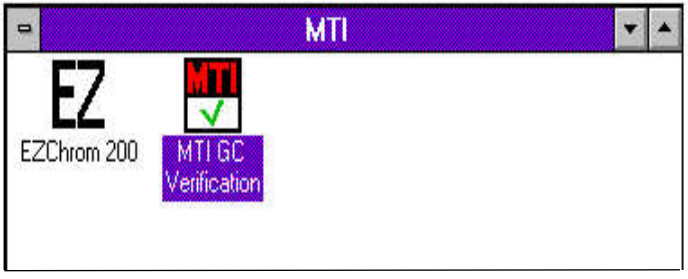


Figure 4 HP group window

2. When EZChrom 200/400 begins, a confirmation “window” will appear (see below). Press **<Enter>** or use your mouse to click on **[OK].**

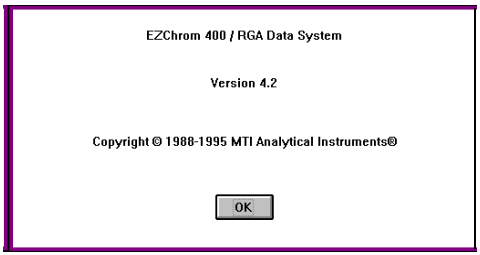


Figure 5 EZChrom 200/400 confirmation window

EZChrom 200/400 will then proceed to open and draw the windows which comprise the data system. Two display windows (four display windows, one each for Channels A, B, C, D will appear with EZChrom 400) will appear in EZChrom 200 (i.e., one each for Channels A and B).

At this time, a default method and data file named after your instrument serial number will be recalled and displayed (see Figure 6). When you access the window represented in Figure 6, the method and file names will be replaced by the serial number of your particular instrument (i.e., the method EXAMPLE.MET will read XXXXX.MET and the file name EXAMPLE.1 will read XXXXX.1, where XXXXX respresents the serial number for your instrument).

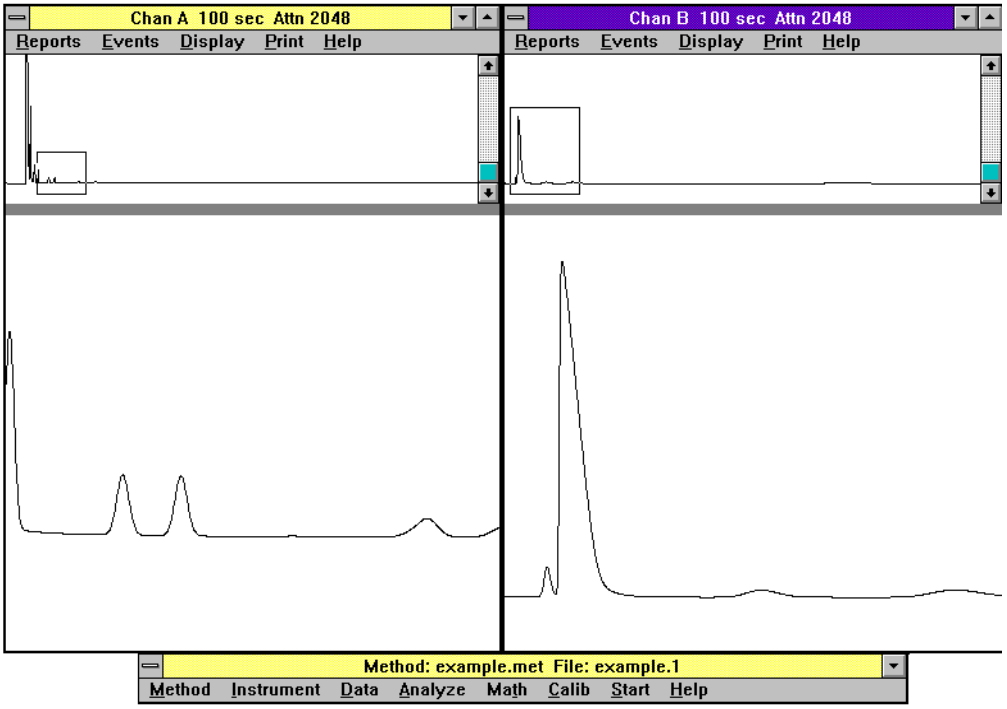


Figure 6 EZChrom 200/400 recalls EXAMPLE.MET and EXAMPLE.1

If EZChrom doesn't start

1. Verify that you have at least 4 Mb of RAM. Also check that your processor clock speed is at least 20 MHz. (Refer to your owner's manual.)
2. If the previous conditions have been met and EZChrom 200/400 still will not function, call Agilent Technologies for assistance.

Hardware setup

Setup your Micro GC as specified in your instrument manual (e.g., M200/M200H, P200/P200H). To connect your Micro GC to your computer do the following:

1. Connect the serial port of the computer (COM 1 when using a **bus** or no mouse, COM 2 when using a **serial** mouse) to the serial port of the instrument (back panel for M and Q series, front or back panel for P series) using the RS-232 serial cable provided. (See Figure 7 for mouse connections.)
2. Plug the mouse and printer (if you are using one) into the computer (see your computer owners manual). This completes the installation of the EZChrom 200/400 data system.

Figure 7 Mouse connections

Installation troubleshooting

See description of installation troubleshooting in the chapter addressing the GC Verification program found in the addendum of the instrument manual.

3

Getting Started

Getting Started

Selecting commands in EZChrom 200/400

Any command in EZChrom 200/400 may be selected via the keyboard or with a mouse-controlled cursor. To select a command with the keyboard:

1. Hold down the **<ALT>** key while **at the same time** pressing the **underlined** letter of the Menu you wish to select. A pull down menu will be displayed. Now all that is necessary is to press the underlined letter of any command listed in the pull down menu.

To select a command with a mouse:

1. Place the **single-headed arrow** cursor on the desired menu. The types and functions of the various cursors will be discussed in the next chapter.
2. Click the **left** mouse button once. When the pull down menu appears, place the arrow on the desired command and once again click the **left** button one time.

Graphic manipulation with a mouse

EZChrom 200/400 allows the selection of commands, the adjustment of zoom boxes and window sizes, and the setting and manipulation of reference cursors through use of the mouse. To perform these functions, four types of cursors are used: a **single headed arrow** (mentioned above), a **double headed arrow**, **two vertical lines** (only active in the channel A or B Windows), and a **crosshair**. This chapter describes the functions of each cursor and provides a short tutorial.

Single headed arrow

Use this cursor to select commands, draw and select zoom boxes, move whole windows in two dimensions, or to activate a window. To become familiar with this cursor and its functions, start EZChrom 200/400:

1. When EZChrom 200/400 is started, the cursor that is visible and active is the single-headed arrow cursor. Place this arrow just to the left and near the top of a peak of the chromatogram in the channel A window.

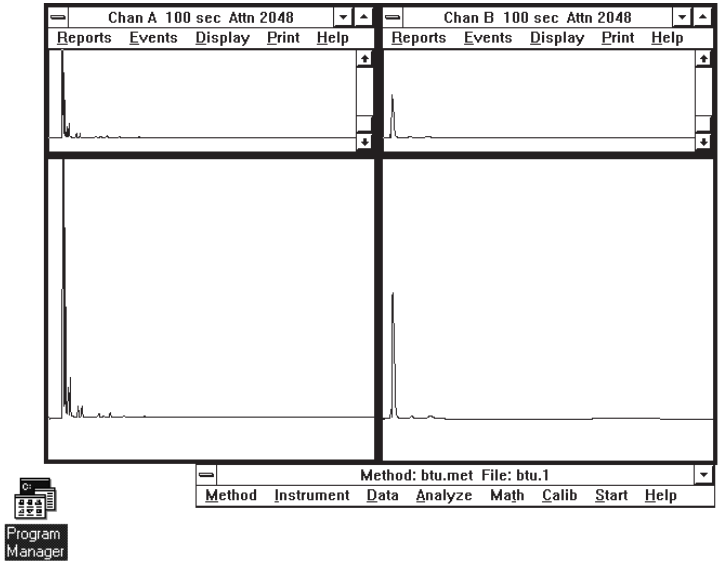


Figure 8 Drawing a zoom box

2. Press and hold the **left** mouse button and **at the same time** drag the cursor to the right and down, just below the baseline. This should draw a rectangular box around a portion of the chromatogram.
3. Release the mouse button. EZChrom 200/400 will now draw the area inside the zoom box so that it fills the lower screen (See Figure 9).

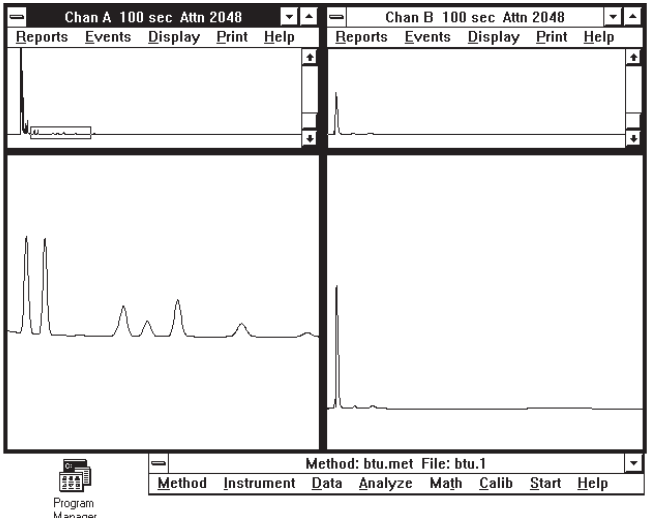


Figure 9 Zoom box display

4. Move the cursor to the top bar of the channel A window where the channel identification, run time, and attenuation are listed.

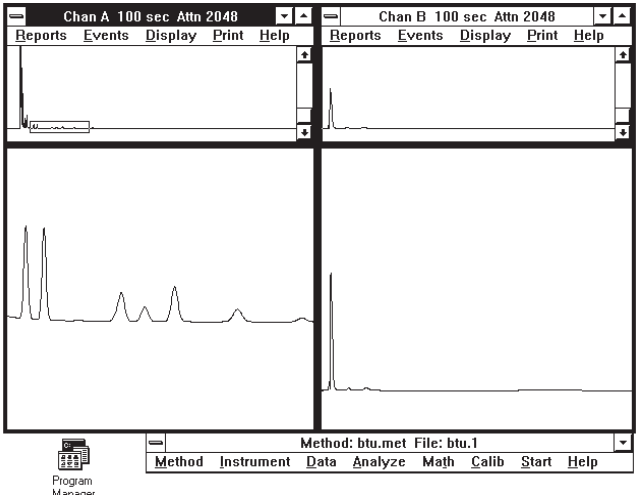


Figure 10 Moving channel A window

5. Click and hold the **left** mouse button while dragging the mouse to the right. This should move all of the channel A window to the right; partially covering the channel B window (See below). When about 50% of the channel B window is covered, release the mouse button. Movement in two dimensions is not restricted to channel windows. Any and all windows or icons in either EZChrom 200/400 or Windows may be moved in this way.

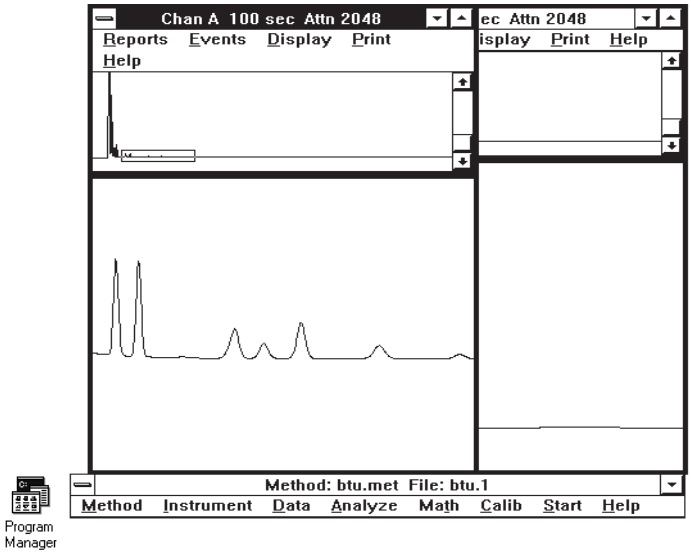


Figure 11 Channel A overlay

6. Now try to draw a zoom box in the channel B window. To do this, the channel B window must first be "activated". Place the cursor in an **uncovered** portion of the channel B window.
7. Click the **left** mouse button. This will cause the channel B window to be redrawn "above" the channel A window (See Figure 12). Zoom boxes may now be drawn as previously described.
8. Take a few minutes to become familiar with the single-headed arrow and its functions. When you are comfortable, move to the next chapter.

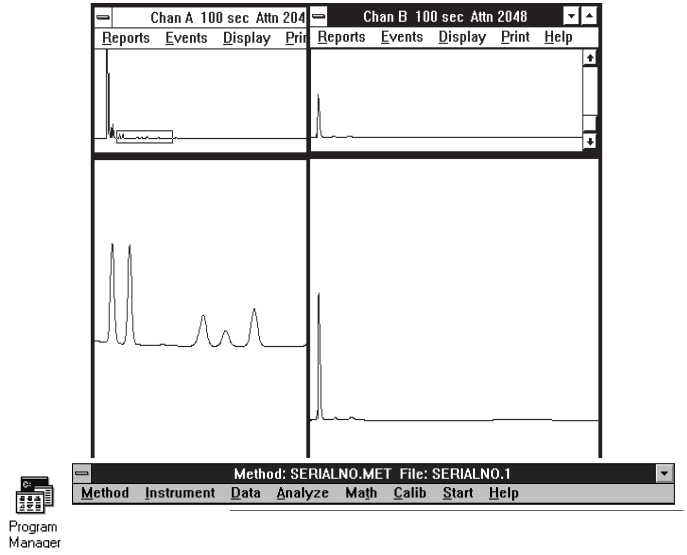


Figure 12 Channel B overlay

Double headed arrow

Use this cursor to enlarge or shrink any of the windows in EZChrom 200/400 or Windows.

1. Place the single-headed cursor on a vertical border of any window displayed on the screen. When the cursor rests on the border it will change from a single headed to a double headed arrow. (See Figure 13) This may take a bit of practice as the borders are narrow and easy to overshoot.

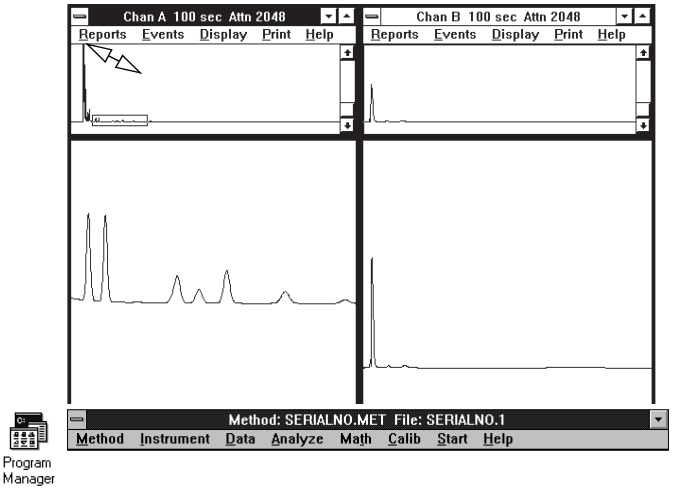


Figure 13 Using double headed arrow

2. Click and hold the **left** mouse button and drag the mouse to the left or right and release. (If a horizontal border was chosen, drag the mouse up or down.)
3. Place the cursor on any corner of a window border.
4. Click and hold the **left** mouse button and drag the mouse in any direction and then release. The corner border allows two dimensional growth or shrinkage of any window. (See Figure 14)
5. Practice placing the cursor on the borders of the various windows until it is routine.

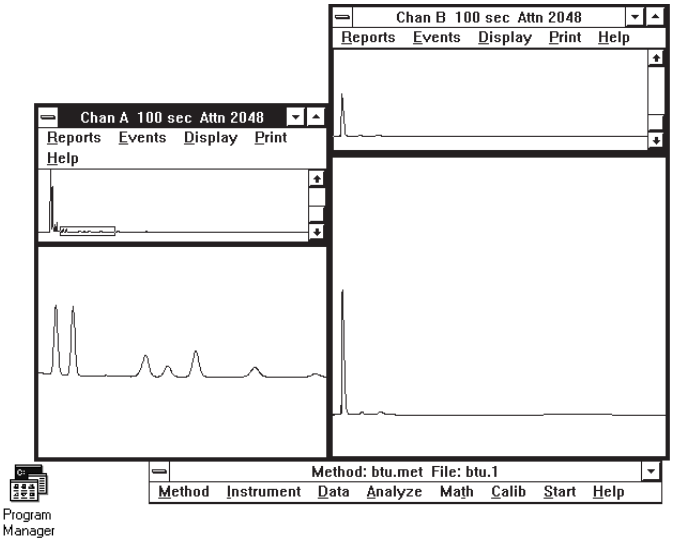


Figure 14 Reduced channel A window

Vertical lines

Use these cursors as mobile reference points to observe peak amplitude and retention times. They are also extremely useful in simplifying the selection and definition of integration parameters and in the peak identification routine.

1. Place the single-headed arrow cursor in either the channel A or B lower window.
2. Click the **right** mouse button. A single vertical line should appear. (See Figure 15) When the mouse is moved to the left or right, the vertical cursor should move accordingly.

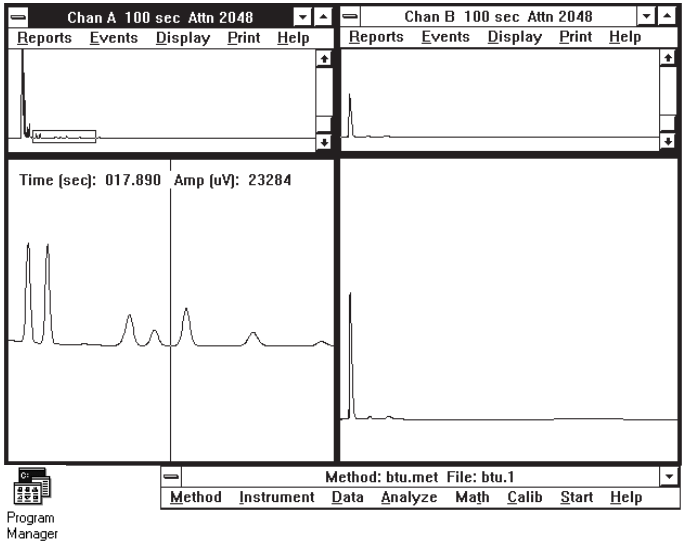


Figure 15 Using the single vertical cursor

When using a laptop computer, the mouse must be moved slowly for the vertical line to remain visible.

3. Scan the cursor across the chromatogram and observe the string of text in the lower display window. You should see the time and amplitude vary. Dragging the mouse to the right should result in a larger time value. (This is because data is collected from time 0, left to right). The value of the amplitude is the actual instrument output in microvolts at the displayed time.
4. Click the **left** mouse button. This will freeze the first vertical cursor.
5. Drag the mouse to the right. The frozen cursor should remain, while a second, mobile cursor will appear (See Figure 16). The values for time and amplitude now displayed are **in reference to the first vertical cursor.** That is to say, the first vertical cursor now has been assigned a time and amplitude value of 0.

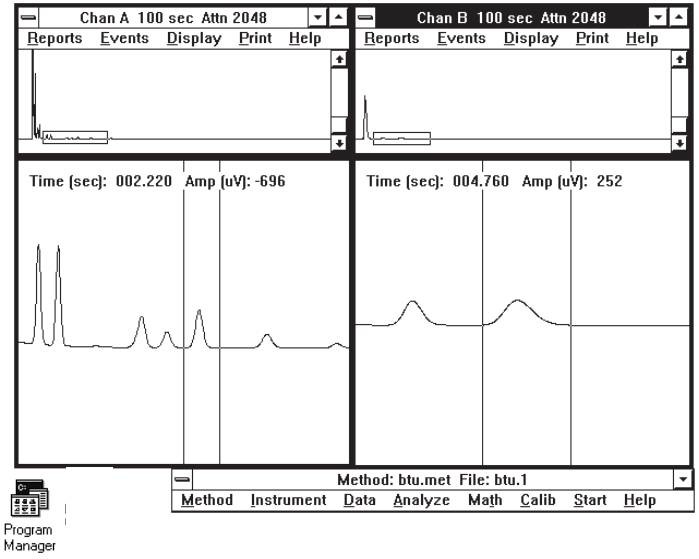


Figure 16 Using two vertical cursors

6. Click the **right** mouse button. This freezes the second vertical cursor. The region between the two vertical cursors is called the **selected region**. Being able to select specific regions in a chromatogram is essential in graphically setting up peak identification tables and integration parameters.
7. Click the **right** mouse button again. This unfreezes the second vertical cursor.
8. Click the **left** mouse button. This will cause the two vertical cursors to merge and be mobile (as in step 2).
9. Click the **right** mouse button. This will freeze the single vertical cursor remaining, while also reactivating the single headed cursor. The single headed cursor may now be used in any of the ways previously described.
10. To remove the vertical cursor from the screen, click on **Display** in the appropriate channel A or B window, then click on **CURSOR**.

11. Practice selecting various portions of the chromatogram. At first you may find it difficult to obtain the desired cursor in an appropriate frozen or unfrozen state. Don't get frustrated; after a short time the manipulation of the mouse clicks will become habit.

Crosshair

This cursor appears when a report is displayed and the single headed cursor is moved into the report window. No commands or manipulations are possible with this cursor.

1. Using the procedure described on page 16, select the **Reports** menu in either channel A or channel B (See Figure 17).
2. Select any of the reports available.

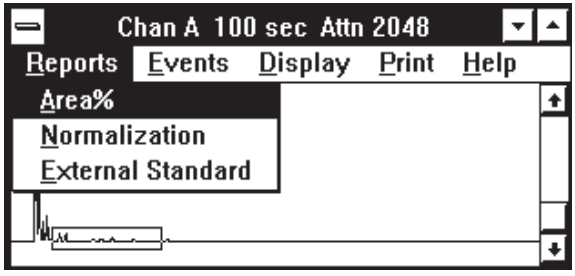


Figure 17 Reports menu

3. Move the double headed cursor into the report window. You should see the single headed arrow change to the crosshair cursor (See Figure 18).

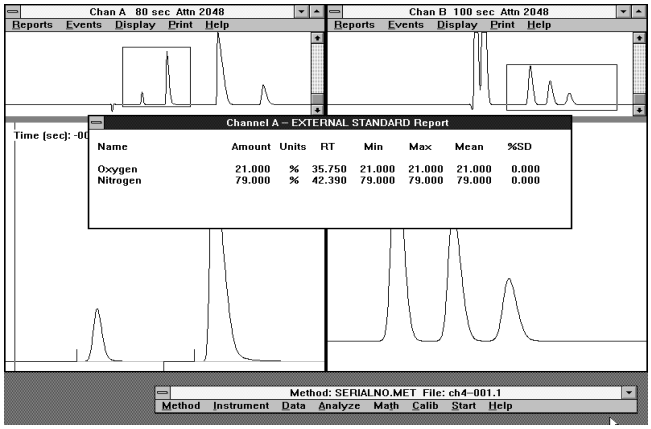


Figure 18 Crosshair cursor

Graphics manipulation without a mouse

While more time-consuming than using a mouse, all the manipulations described in the previous chapter can be made using the keyboard. In this case, the numeric **key 1** acts as the left mouse button and the numeric **key 3** acts as the right mouse button. The **arrow keys** on the number pad are used for moving the various cursors. Each arrow allows movement in a different direction.

Be sure the “Numbers Lock” is not engaged if you use the arrow keys.

Text and numeric editing

Occasionally it will be necessary to manually add, delete, or replace text and/or numbers during an EZChrom 200/400 session. For example you may want to add a compound to the peak identification table. The general procedure to manually edit text or numbers is the same and is as follows:

1. First select the **Method** menu and choose the **Open** command. A series of existing Methods will appear. Choose the default method Serialno.met

2. Call up the Peak Table (See Figure 19) by selecting the **Method** menu and then the **Peak Table** command.

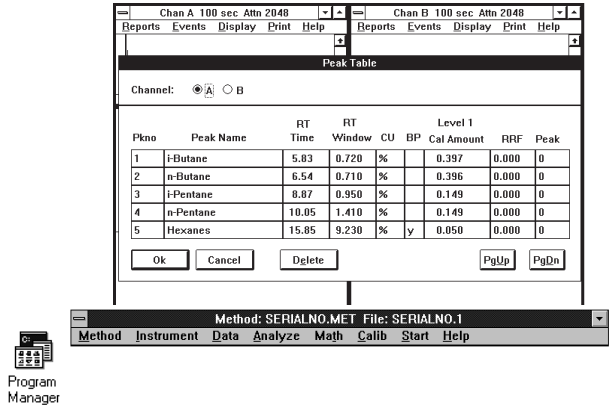


Figure 19 Peak table

3. Select Channel A, then place the single-headed arrow cursor in the first empty box under the heading, Peak Name, and click the left mouse button.
4. The flashing cursor which appears may be moved to the left or right, up or down using the arrow keys. Type the word “Example.” then place the cursor at the end of “Example.”
5. Press the **backspace** key repeatedly until the entry is completely deleted. To delete more easily, place the arrow cursor at the beginning or end of the entry. Then click and hold the left mouse button while dragging the mouse so that the whole entry is blackened. Now press the **delete** key to remove the entry. All text and numbers surrounded by a box, other then the values in the instrument status window, may be edited in this way.

4

Sample Session

Sample Session

Once you have mastered a few skills, running an Agilent gas chromatograph with EZChrom 200/400 is relatively simple. The system has been designed so that a wide variety of analyses can be handled quickly and easily. The purpose of the Sample Session is to provide you with the basic skills needed in a typical session with EZChrom. While it does not cover all the capabilities and features of EZChrom 200/400, all of the necessary steps required for data acquisition, analysis, and storage are discussed.

This sample session, run on ambient air, is meant to be followed in sequence; so please start at the beginning. If the chromatograph option you have purchased is unable to separate the N_2 and O_2 in air, the single composite peak you observe is sufficient to demonstrate the operating procedure. First, start EZChrom 200/400.

Building a method

The first step in using EZChrom 200/400 is to construct the proper Method. This is important because the Method not only controls the GC but also controls all the calculations and peak detection performed by EZChrom 200/400. When EZChrom 200/400 is first started, the last saved Method is automatically loaded (initially Serialno.met). This default Method is created using your column configurations. The method may or may not be appropriate for your sample analysis. The following instructions illustrate how to build and save a Method which is optimized for the analysis of air. Once mastered, constructing an optimized Method for your specific application(s) will be easy.

If at any point you wish to terminate the sample session, double click on the square above Method.

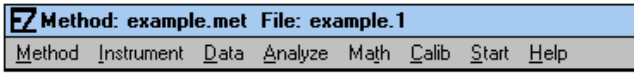


Figure 20 EZChrom 200/400 menu

A screen will appear asking if you wish to terminate EZChrom. Click on **[OK].** The screen will then ask if you want to save changes. Click on **[Save].** The command Save File Name As: will appear. Enter any eight character name appropriate for the file. (DOS illegal characters not accepted. See page 162 for a list of these.)

To build a method from scratch, do the following:

1. Select the **New** command which is listed in the **Method** menu (Figure 20). This resets the timed events, peak, and calibration tables. If you have been practicing cursor moves and want to begin a sample session, click on **Method**, then **New**, and the following window will appear (See Figure 21). Do not save changes to the default Method.

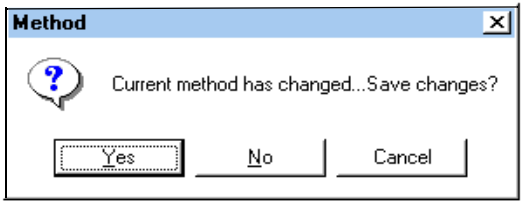


Figure 21 Save method changes window

2. Select the **Instrument Setup** command under the **Method** menu. A window will be displayed which lists the chromatograph instrument settings from the last stored Method (See Figure 22).

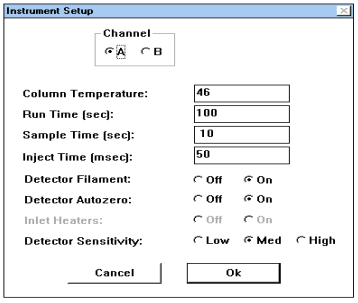


Figure 22 Instrument setup window

A description of the settings found in this window is as follows:

Column temp (°C)

This is the temperature at which the column and detector are kept during and after a run. When this setting is changed, time must be allotted for equilibration of the GC module (about 5 minutes over any temperature range). A large steady baseline drift indicates the system is not at equilibrium. Remember, the higher the column temperature the faster the elution. Therefore, analysis time can be reduced by increasing the column temperature. A balance must be struck however, as peak separation decreases with an increase in temperature and this can make accurate integration difficult. The allowed range is ambient to 180°C. (160°C for modules equipped with a HayeSep column). Columns in the two modules may have different temperature settings.

Run time (seconds)

The amount of time that data is collected from the detector. Initially this should be set to the maximum value of 160 seconds. Later, it should be set to a value slightly greater (~10 s) than the time at which the tail of the last sample peak has eluted. This parameter may be different for the two modules.

Sample time (seconds)

The time that the built-in vacuum pump is run prior to sample injection. The pump should be run long enough that the injector sample loop is flushed with sample. The maximum sample time is 255 seconds, but 15 to 20 seconds is usually sufficient. This parameter is the same for both channels.

Inject time (milliseconds) variable loop only

The amount of time the inject valve is open and sample is flushed onto the column from the sample loop. The range of values is 0-255 milliseconds. A practical minimum value is 20 milliseconds.

In most cases a value of 0 to 5 milliseconds will result in no injection.

Each channel can have a different inject time. Long injection times in conjunction with high detector sensitivity allow detection in the low ppm range (parts per million).

Detector filament

This toggles power to the GC detector, which must be on for the GC to collect data. A minimum column head pressure of 5 psi is required for the detectors to function. If carrier gas pressure falls below 5 psi, power to the detectors is turned off automatically. Allow a minimum of 5 minutes equilibration time between powering on detector and the first analysis.

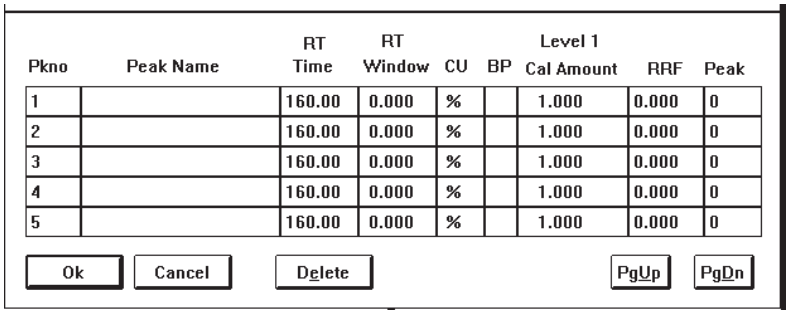
Detector autozero

This compensates for detector dc voltage offset when pure carrier gas is passed over both sample and reference filaments. Detector Autozero should always be left on.

Detector sensitivity

This controls the detector output gain. The low setting amplifies the detector output by a factor of 5. The medium and high settings each provide an additional factor of 10. Set these at the highest value which does not give flat-topped peaks when the display is fully unzoomed.

3. Increase the temperature settings by 10 degrees for channels A and B. To do this, click on channel A in the instrument setup window shown in Figure 22. Instrument settings for channel A should appear on the screen. Click on the "Column Temperature" box and enter a value 10 degrees higher than that shown. The settings for channel B are displayed by clicking the cursor in the small circle labeled B at the top of the instrument status window.
4. Change the run times for the two channels to 50 seconds. Air elutes quickly on all columns, so a long run time is unnecessary.
5. Change the sample time to 10 seconds. This needs to be done in only one channel; the other channel is automatically updated.
6. Reduce the injection times for both modules to 20 milliseconds (variable loop injectors only). The main components in air (O_2, N_2) are so concentrated that very little sample is needed to produce a large signal. Using a short inject time also reduces the risk of column overload.
7. Make sure the detector filaments and autozero are **ON** for both channels.
8. Set the sensitivity on both channels to **Low.** Again, N_2 and O_2 are present in such large amounts, amplifier overload is a concern.
9. Close the **Instrument Setup** window by clicking on **[OK]** or pressing **<Enter>.**
10. Send the revised instrument settings to your GC by selecting the **Send Current Method** command which resides under the **Instrument** menu. Click on **[Ok]** when the confirmation window appears. If an error message appears, click on **[Ok]** or press **<Enter>**. Then refer to Installation Troubleshooting (resetting the COM port) using the GC Verification program found in the addendum to the instrument manual.
11. Check to see that the increase in column temperature has been received and implemented by the GC. To do this, select the **Instrument Status** command under the **Instrument** menu. If the temperature is correct, click on **[Ok]**. If the temperature setting is <u>not</u> correct, return to **Instrument Setup**, confirm that your entries are correct and **Send Current Method** again.



Pkno	Peak Name	RT Time	RT Window	CU	BP	Level 1 Cal Amount	RRF	Peak
1		160.00	0.000	%		1.000	0.000	0
2		160.00	0.000	%		1.000	0.000	0
3		160.00	0.000	%		1.000	0.000	0
4		160.00	0.000	%		1.000	0.000	0
5		160.00	0.000	%		1.000	0.000	0

Figure 23 Peak table for a new method

12. Open the **Peak Table** which is listed under the **Method** menu. The Peak Table (See Figure 23) contains all the information on the types of compounds in a sample, and the amount of time it takes for these compounds to pass through the sample column. A table is provided for both channels. A brief description of the peak table begins with:

    Peak name

    The names of the compounds to be analyzed are entered in this portion of the table. The peak names for each component are entered by typing them in (maximum 20 characters). When an integrated peak falls in a defined retention time window, it is assigned the name linked to that window.

    Retention time

    Retention Time is the amount of time it takes for the crest of the named peak to elute from the column. The retention time can be entered graphically or with the keyboard. (In the Calibration Setup table there is an option to update the retention time at the end of a run. If this is selected and the retention time changes, then the retention time and the window will be updated.)

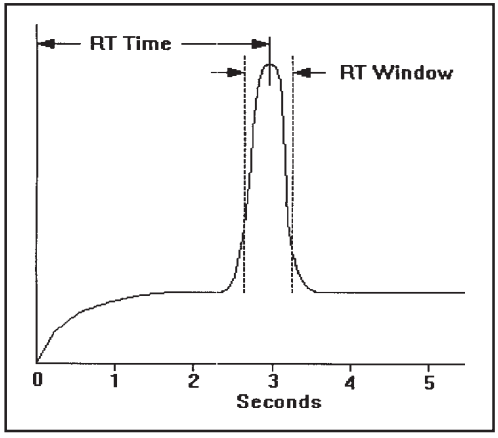


Figure 24 Retention time & retention time window

Retention time window

Retention Time Window is a sectioned length of time around the peak apex. If a peak apex falls within the defined window, even though the retention times of the peak width at its base may not exactly match the retention time window in the table, that peak will still be identified as the peak name listed. (See Figure 24 for a graphic representation of retention time and retention time window)

Concentration unit

This is a text entry which identifies the concentration units of the various compounds. All text entries are allowed up to a maximum of three characters. The unit is not a conversion factor and has no mathematical significance. Each component may have a different concentration unit. The concentration units are only used for external standard reports.

BP (bunched peaks)

Allows all peaks within a RT Window to be recognized as one component. Therefore, the peak area reported for the component will be the sum of all peaks found in the RT Window. Type **Y** for "Yes" to activate BP.

When BP is actuated, no Retention Time will be listed in the External Standard Report.

Level 1 cal amount

This is the amount of a compound that the level one calibration standard contains. When a calibration is run, EZChrom assigns the entered amounts to the areas of any integrated peaks which elute in the appropriate retention time window. Obviously, accurate calibration standards must be used and retention time windows must be properly set for EZChrom 200/400 to accurately quantify real samples. EZChrom 200/400 can use up to eight levels of calibration, but for routine analysis one level is usually sufficient. Each level corresponds to a standard concentration and a point on a calibration plot. (Calibration plots are discussed in **Advanced Skills,** Chapter 7.)

Relative response factor, peak

These advanced features deal with calibrating compounds which are not present in your calibration gas standard. A complete discussion is reserved for the **Advanced Skills** chapter of the manual.

13. In the channel A peak table, activate the text cursor in the PKNO 1 peak name box. Then type in:

    Air: If you have an OV-, HayeSep- or PoraPLOT-type column in module A

    Oxygen: If you have a Molecular Sieve column in module A

14. Proceed to fill in the peak names for channels A and B. For OV-type columns, you are already finished since these columns will not separate any of the percent level components in air. For a HayeSep or PoraPLOT column, enter: **Carbon Dioxide** in the PKNO 2 peak name box.

    For a Molecular Sieve column enter:

    Nitrogen in the PKNO 2 name box

15. Leave the **RT Time** and **RT Window** at default levels for the moment. They will be set graphically after the air sample is analyzed.

16. Enter the symbol % in the **CU** boxes.
17. Enter values in the **Level 1 Cal Amount** boxes for the various compounds. Air on an OV column is 100%. On a HayeSep or PoraPLOT, however, it will be 99.967% as CO_2 is separated from O_2 and N_2 on this type column and makes up approximately 0.033% of the atmosphere. On a Molecular Sieve, the amounts of O_2 and N_2 will be about 21% and 78%, respectively.

    Enter values as whole numbers since the CU has already been set to percent. For example, 21% should be entered as 21, not 0.21.
18. Leave the BP, RRF, and Peak boxes at default values and close the Peak Table by pressing **<Enter>** or clicking on **[Ok]**.

This completes the basics of building a peak table. At this stage, many of the commands found under the Method menu can be ignored. Because certain default values have been specified during EZChrom 200/400 installation, these features will function properly under normal conditions without a new user having to worry about them.

The one exception to this rule is the setting of optimized Timed Events. The Timed Events tables contain all the parameters which control peak detection and integration of the chromatograms collected on channels A and B. While it is possible to enter timed events manually (for example, as we made entries in the peak table), it is more accurate to graphically set these events on a real data set. At this point, it is time to collect an actual data set which will be stored, and for which optimized Timed Events tables will be constructed.

Collecting data

1. Select the **Start** menu. A run window should appear (See Figure 25).
2. Enter a **Run ID** for the air sample which is to be run. This may be a maximum of eight characters with no DOS control characters. (See page 162 for a list of DOS illegal characters.)

3. Enter 1 for the **Number of Runs**.

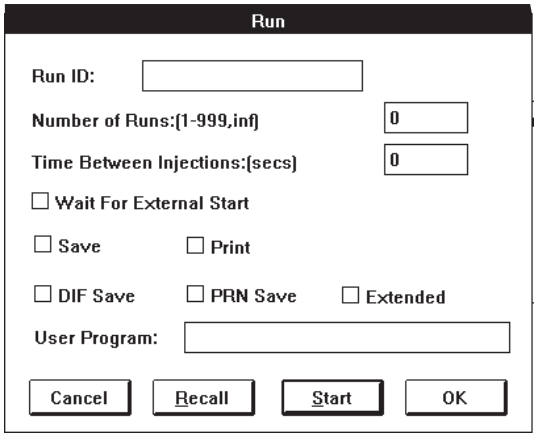


Figure 25 Run window

4. Select the **Save** box by clicking on it. (If any other boxes are selected (as evidenced by an X appearing in the box) you may deselect them by clicking on them.)
5. Click on **[Start]** or press **<Enter>** to begin data acquisition

After the RUN window disappears, the vacuum pump will start and run for the amount of time you specified in the Instrument Settings window. A small amount of air sample is injected onto the sample columns, then EZChrom 200/400 proceeds to draw the chromatograms for channels A and B. A run clock is provided at the top of the channel A and B windows. This clock displays the real time of the chromatographic analysis that has progressed through a run. It should count up to the run time specified in the Instrument Settings window.

When the run is completed, the data is first stored on the hard drive in the default directory c:\HP\ezchrom\200\chrom under the run ID which you provided. The data set is then automatically analyzed using the default timed events provided with the NEW method. At this point take the opportunity to carefully examine the analyzed chromatograms using the zooming and unzooming techniques you practiced in Chapter 3. During your examination, keep in mind the following questions:

Have all of the peaks been detected?

This is indicated by a baseline, set off by vertical hashmarks, drawn under each peak. (See Figure 26) Hashmarks which point upward indicate the start of integration while downward-pointing hashmarks define the end of integration.

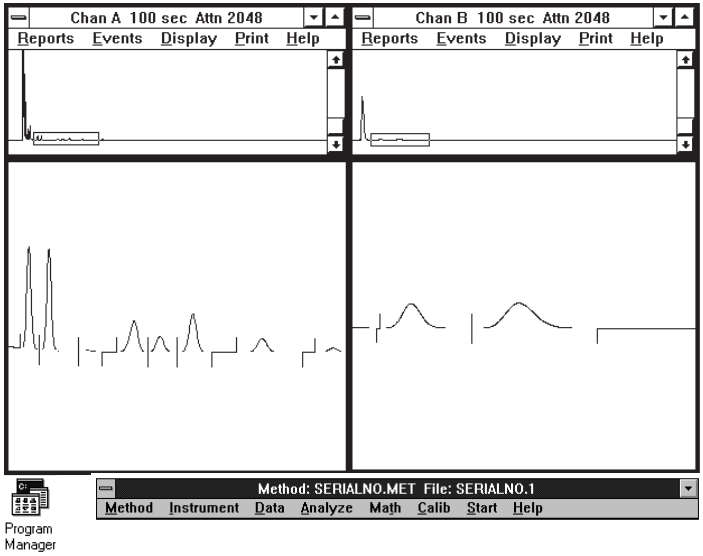


Figure 26 Integrated peaks

Are the peaks correctly integrated?

Even though a peak has been detected, perhaps only a small portion of the peak was actually integrated. Conversely, an integrated region may include areas on either side of a peak which should not be integrated. Be careful to zoom closely on the base of a peak to determine if it has been properly integrated. If the peak is properly integrated, the upward-pointing hashmark will appear at the beginning of the peak's upward slope and the downward-pointing hashmark will appear where the peak returns completely to the baseline. (See Figure 26)

If the answer to either of the questions was no, then the Timed Events tables **must** be optimized in order for an accurate analysis to be made. It may be that the default timed event parameters were sufficient to find and integrate the peaks in such a simple application. In general, though, this will not be the case. Therefore, please proceed to the next chapter which describes optimizing timed event parameters graphically.

Optimizing timed events

A Timed Event is a resetting of any of 10 calculation variables (see the Events menu) that are used to calculate the area under a peak. Any or all of these may be adjusted at various time points along the chromatogram. The purpose for setting Timed Events is to attain the best possible peak area integration.

In order for EZChrom 200/400 to perform an accurate analysis, it is essential that the proper parameters and values are entered into the Timed Events tables. Tables are provided for both channels because different columns will require different parameters to properly identify and integrate individual peaks. The most accurate way to build an optimized Timed Events table is to select regions of a real chromatogram with the vertical cursors and then evaluate the region with one of the four basic timed event parameters. EZChrom 200/400 then calculates the correct value for the event over the selected region and, if desired, adds the event with the calculated value to the Timed Events table.

1. If necessary, review the use of vertical cursors beginning on page 22. Familiarity with vertical cursor manipulations is critical for this stage of the sample session.
2. In the channel A window, activate a single vertical cursor and freeze it about 0.5 seconds to the left of the first peak (See Figure 27). Note the time where you froze the vertical cursor. Be careful that only one vertical cursor is visible in the window, otherwise the time and amplitude of the mobile cursor will be in reference to the frozen one. Should two vertical cursors appear, click the **right** mouse button, then the **left** mouse button and the two

cursors should merge. For further information on vertical cursors, see Chapter 3.

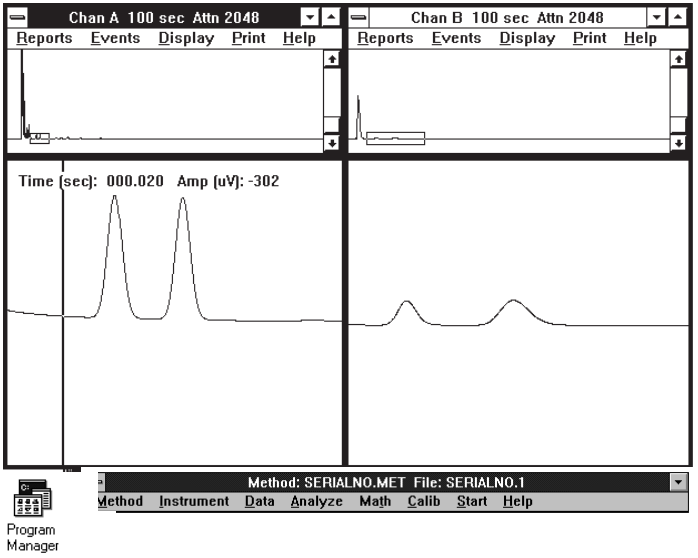


Figure 27 Using vertical cursor to set integration off

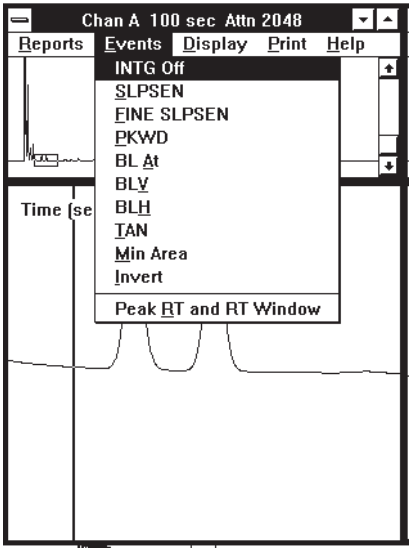


Figure 28 Events menu

3. Select the **Events** menu located in the channel A menu bar (See Figure 28).
4. Select the **INTG Off** command. This will place an integration off event in the channel A Timed Events Table. It will also set the time of the event to zero and the value to the time where the vertical cursor was frozen.

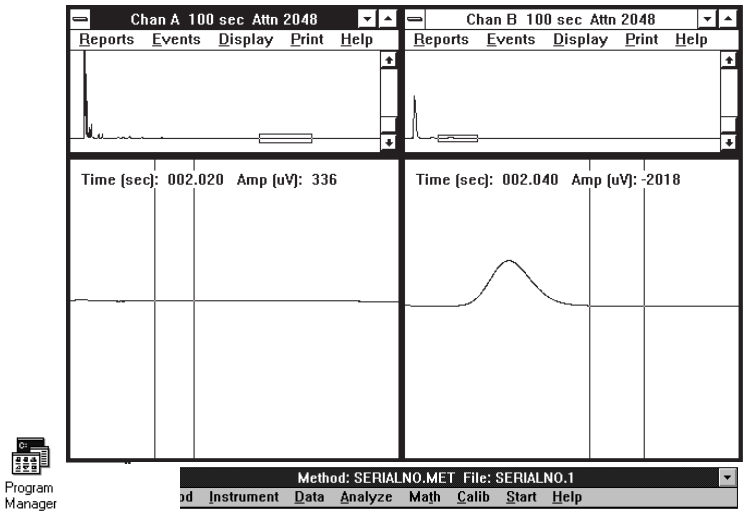


Figure 29 Using vertical cursors to determine noise level

5. Again using the vertical cursors, select a small region (about 2 seconds) on the baseline of the channel A chromatogram which does **not** contain any portion of a peak (See Figure 29). This region will be used to determine the noise level in the chromatogram.

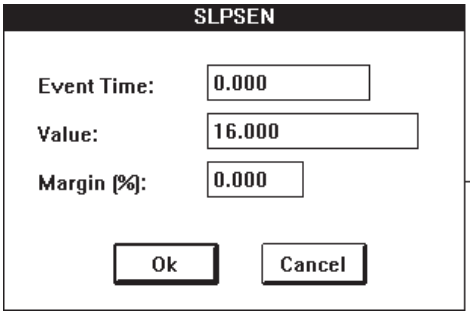


Figure 30 SLPSEN Event window

6. Select the **SLPSEN** command under the **Events** menu. A window will appear which displays a calculated value for the SLPSEN event (See Figure 30). Change the Event Time to 0.000.
7. Click on **[Ok]** to add the SLPSEN event to the timed event table.
8. Using the same region of baseline as was defined in Step 5, select the **Events** menu again, but this time add a **FINE SLPSEN** event to the timed events table for channel A. Change the Event Time to 0.000.

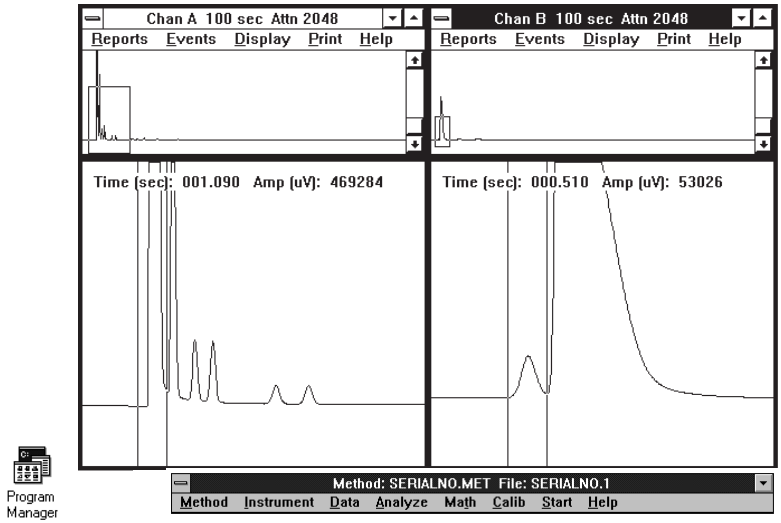


Figure 31 Using vertical cursors to set peak width

9. Next, place the vertical cursors on either side of the first peak (See Figure 31). **Be sure that the cursors are frozen at the very beginning and end of the peak;** zooming may be required to get the clearest view.
10. Select the **PKWD** event under the **Events** menu. A window with a calculated peak width value for the selected peak will be displayed. Click on **[Ok].**
11. Repeat steps 9 and 10 for any remaining peaks in channel A.
12. Open the Timed Events table for channel A and confirm that all of the timed events you selected (INTG Off, SLPSEN, FINE SLPSEN and PKWD) are present in the table.
13. Select **[Ok]** or press **<Enter>** to close the Timed Events table.
14. Construct an optimized Timed Events table for channel B by repeating steps 2 through 13.

    Changing the time values for **SLPSEN, FINE SLPSEN,** and **PKWD** to zero allows EZChrom 200/400 to begin its peak search with the proper sensitivity from time zero.

Setting retention times graphically

The next step in constructing a complete and optimized application-specific method is to enter the retention times and retention time windows for each of the compounds in the peak table. The most accurate way to do this is to graphically select each peak in a real chromatogram with the vertical cursors.

1. Place the vertical cursors around the first peak that elutes on channel A. You may use the vertical hashmarks as a guide in placing the vertical cursors. (For an explanation of vertical hashmarks, see the peak detection and peak integration questions on page 40.)
2. Select the **Peak RT and RT Window** command which is found under the **Events** menu for channel A. A window will appear which lists the compounds you previously entered in the channel A Peak Table (See Figure 32).

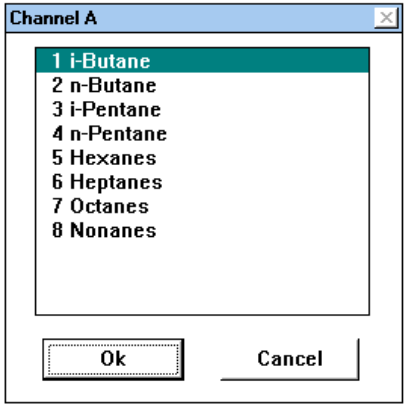


Figure 32 Compound list window

3. Click on the compound name of the peak in the window. The compound name will depend on the type of column you have in module A (See number 13 and 14 on page 37). Click on **[Ok]** or double click on the highlighted peak name.

4. Repeat the procedure for each successive peak in the channel A chromatogram, choosing the appropriate name from the list.
5. Repeat the procedure for the channel B chromatogram.
6. Select the **Analyze** command from the lower menu bar. EZChrom will analyze the chromatogram according to the parameters just set. Zoom in closely on each peak to assure that the entire peak is within its predetermined hashmarks.

   If the peak does not have an upward hashmark at its beginning and a downward hashmark at its end, it has not been integrated.

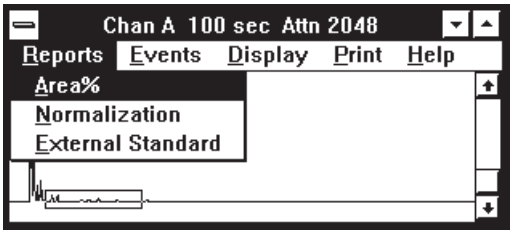


Figure 33 Reports menu

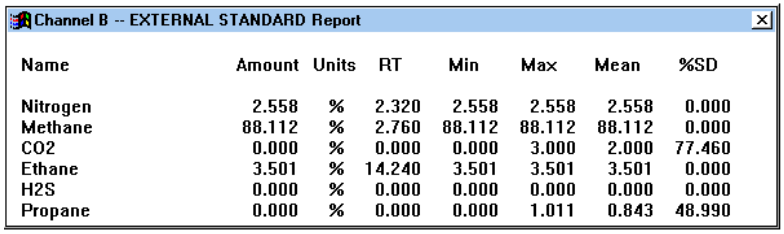
Channel B -- EXTERNAL STANDARD Report

Name	Amount	Units	RT	Min	Max	Mean	%SD
Nitrogen	25.576	%	2.320	25.576	25.576	25.576	0.000
Methane	88.118	%	2.760	88.118	88.118	88.118	0.000
CO2	30.012	%	8.560	30.012	30.012	30.012	0.000
Ethane	35.033	%	14.240	35.033	35.033	35.033	0.000
H2S	0.000	%	0.000	0.000	0.000	0.000	0.000
Propane	10.111	%	71.520	10.111	10.111	10.111	0.000

Figure 34 Uncalibrated external standard report

7. Open the External Standard reports for channels A and B by clicking on Reports, then on External Standard (See Figure 33). They should display the peak names in the order of elution time and have large amount values for all of the peaks listed in the reports. This is because the method is not yet calibrated (See Figure 34).

8. If any of the peaks in the report have an amount of zero (See Figure 35), and has an integration baseline drawn underneath it, the retention time has been incorrectly set. Repeat steps 1-3.



Channel B -- EXTERNAL STANDARD Report

Name	Amount	Units	RT	Min	Max	Mean	%SD
Nitrogen	2.558	%	2.320	2.558	2.558	2.558	0.000
Methane	88.112	%	2.760	88.112	88.112	88.112	0.000
CO2	0.000	%	0.000	0.000	3.000	2.000	77.460
Ethane	3.501	%	14.240	3.501	3.501	3.501	0.000
H2S	0.000	%	0.000	0.000	0.000	0.000	0.000
Propane	0.000	%	0.000	0.000	1.011	0.843	48.990

Figure 35 External standard report with incorrect retention time

9. When all the names you have added to the Peak Table are present in the External Standard report with a non-zero value, proceed to the next chapter.

Calibrating the method

The last step in building an optimized application Method is calibration. This step is necessary when creating a Method in order to generate a response factor on a known sample so you can, at a later time, perform quantitative analysis on an unknown sample. EZChrom allows a Method to contain up to eight calibration levels (eight different calibration gas concentrations) at one time.

In the great majority of cases, however, only a level one calibration is required. (Peak area = 0, concentration = 0 is the assumed second value of the peak).

1. Select the **Calib** menu on the EZChrom command bar.
2. A window will appear which says “Calibration Level” and has a number highlighted (See Figure 36). Enter **1** and click on **[Ok]**.

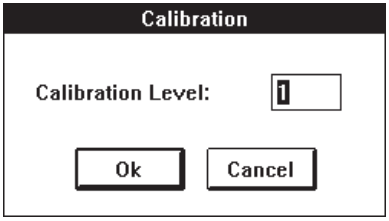


Figure 36 Calibration window

3. The EZChrom bar will then display “Calibration Level 1” and “Number of Runs Left=1”. For purposes of this sample session, a level 1 calibration is all that is necessary.
4. Select **Analyze** from the lower command bar. EZChrom will perform an analysis and assign the peak areas to the amount values you entered into the Level One Cal Amount spaces in the peak table.
5. Again select the **Reports** menu and open the External Standard reports for channels A and B. They should display the peak names in order of elution time, but the values should now be readable as standard numbers.
6. Select the **Method** menu and the **Save As** command. There will be a prompt (See Figure 37) which requires you to enter a name for the method you have just constructed. Remember, only eight characters may be used in an ID, and DOS illegal characters are forbidden.

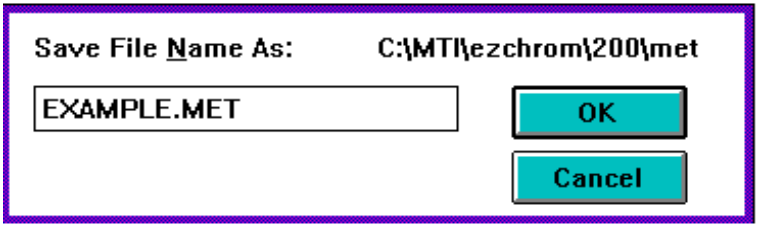


Figure 37 Save file name as

Use the extensions ".met" when naming method files to distinguish them from data and instrument ID files.

This concludes the EZChrom 200/400 sample session. The examples in this sample session have been provided to illustrate the ease of use of the EZChrom 200/400 software. The integration timed events used when setting up a particular Method may need to be changed for different applications. When you need more information regarding operation or use of specific features in the software, please refer to Chapter 6 for the menu reference.

5

Technical Reference

Technical Reference

Data sampling

The analog signal generated by the detector of the gas chromatograph is digitized by an A/D converter in the GC. The A/D is a 24 bit precision voltage-to-frequency converter.

The detector voltage is digitized at a rate of 100 points per second. Each data point requires 4 bytes of disk space if stored. A 60 second run for a dual module unit will generate 12,000 data points and requires 48,000 bytes of disk space for storage.

60 sec/channel × 100 pts/sec × 2 channels × 4 bytes/pt. = 48,000 bytes

The digital data is made available through the serial RS-232 port. Data transfer occurs as it is collected.

Peak detection

Peak width

The data output rate for an Agilent micro-GC is 100 points per second per channel. For most peaks in a chromatogram, this is far more data then is required to get an accurate integration. To reduce processing time, data points are therefore averaged into data bunches by using the PKWD timed event. Peak detection is then performed on the bunched points. In EZChrom 200/400, the number of bunched points is divided by 5 to produce a peak width value. Example: 4 bunched points divided by 5 equals 0.8 PKWD.

As a general rule, sharp, early eluting peaks are best detected with PKWD values of 0.2. Late eluting, broad peaks may require PKWDs of 0.4, 0.8, or even 1.6. See Chapter 7 for details of properly setting PKWD values. Setting a PKWD event

graphically may generate non-integer values. These non-integer values are rounded down to the nearest multiplier of 0.2 when EZChrom 200/400 analyzes a chromatogram.

Slope sensitivity

The slope of the signal (the rate of change of the detector voltage) is used by EZChrom 200/400 to detect peaks. A slope value, or first derivative, is calculated one bunched point to the next. The PKWD determines how frequently the slope is calculated. Narrow peaks need small bunch sizes so that abrupt changes in slope can be quickly detected. Wide peaks, however, require bunches large enough to allow small changes in amplitude to be detected. Peaks are detected when the slope value between two data bunches is greater than the SLPSEN (slope sensitivity) value defined in the Timed Events table. The optimum slope sensitivity usually occurs when it is equal to twice the background noise level.

Fine slope sensitivity

Fine slope sensitivity (FINE SLPSEN) is related to slope sensitivity; it is the second derivative of the chromatogram displayed. The fine slope sensitivity value corresponds to the change in the slope. It is, therefore, a threshold for the change in steepness of a chromatogram. This threshold is used to determine the start and stop of peaks in conjunction with slope sensitivity.

Baseline correction

Up to this point, only peak detection has been discussed. Besides finding the beginning and end of a peak, the peak's baseline needs to be defined so that a peak area can be calculated. The area is calculated by subtracting the trapezoid under the baseline correction from the raw uncorrected baseline.

EZChrom 200/400 constructs separate baseline segments from the beginning of the first peak to each of several successive valleys. The baseline that has the least positive slope is selected to correct the peaks included in that baseline. This method works well for almost all cases of baseline drift.

Peak identification

The next step in the process is peak identification. The peaks are identified by using the retention time and retention time window specified in the Peak Table. EZChrom 200/400 tries to match the retention times of peaks it has found with retention times listed in the Peak Table. If a matching retention time is found, the peak is given the name specified for that window. If more than one peak is found within a given window, the retention time of the largest peak in the window is used in the Normalization or External Standard reports.

Retention times can be updated at the end of each run as long as the peaks are detected and identified. This is particularly handy if the retention times are shifting. To activate this feature:

1. Select **Method**.
2. Select **Calibration Setup**.
3. Check **Update Retention Time After Run**.

The retention time can also be updated after each calibration by checking the appropriate box.

Peak quantitation

The external standard calculations are performed by multiplying the raw area of an identified peak by its response factor:

Component area × Response factor = External standard amount

The response factor is usually generated from a calibration plot. The calibration plots are displayed in the Peak Calibration portion of the Method. The type of calibration plot which is to be used (point-to-point or linear) is determined in the Calibration Setup portion of the Method.

Point to point calibration plots

A point-to-point calibration plot uses calibration gas standard amounts (y axis) and their raw peak areas (x axis) as Cartesian coordinate pairs. These pairs are used as points on a calibration plot. In the simplest case (a one level calibration), a line is drawn from the origin through the single point. The slope of the line is equal to the **Response Factor**. Real sample amounts are then calculated by multiplying the area of the sample components by the slope of the line (response factor) in the calibration plot.

For a multi-level point-to-point calibration, there are several lines which define the calibration plot. Each response factor corresponds to the slope of a segment on the calibration plot.

Linear calibration plots

If the linear option is selected, a single line is calculated using a least squares approximation.The points used to generate the fit are the same amount/area pairs as those in the point-to-point plots. The slope of this line is used as the response factor for subsequent quantitations.

6

Menu Reference

Menu Reference

This chapter of the manual describes the menus and commands available in EZChrom 200/400. They are categorized in levels according to the screen menus in which they appear. Within a menu, they are alphabetized for ease of reference.

EZChrom 200/400 menus and commands

The EZChrom 200/400 Data System main menu contains all of the commands necessary to set up, run, calibrate, and analyze data collected on an Agilent gas chromatograph, as well as store and catalog all Methods and data files. This menu is located in the menu bar at the lower right of the screen.

Analyze

(Main menu)

Purpose

Analyzes the current data file using the current analysis Method (i.e., timed events, retention times, and calibration tables). Reports and baselines are updated accordingly.

Syntax

Select **Analyze** or type **<Alt> A.**

Comments

During analysis a series of messages are displayed in the menu bar starting with “Analyzing Channel A Chromatogram.” Upon completion of the analysis, the chromatograms will be displayed with baselines and hashmarks drawn under integrated peaks.

Calib

(Main menu)

Purpose

Sets up EZChrom for a two channel calibration. It also provides the capability of selecting a calibration level (from 1-8) for users who desire a multilevel calibration.

Syntax

1. Select **Calib** or type **<Alt> C**
2. Enter a number between 1 and 8 to select a calibration level.
3. Select **[OK]**. To stop a calibration prior to analysis, select **[Cancel]**
4. Select **Analyze**. This will take the calculated peak areas and assign them to the appropriate peak name and amount value.

Comments

Calibration does not begin until data is actually collected or stored data is analyzed. Level 1 is all that is required for normal use. Higher levels are used to construct linear or nonlinear (point-to-point) calibration plots.

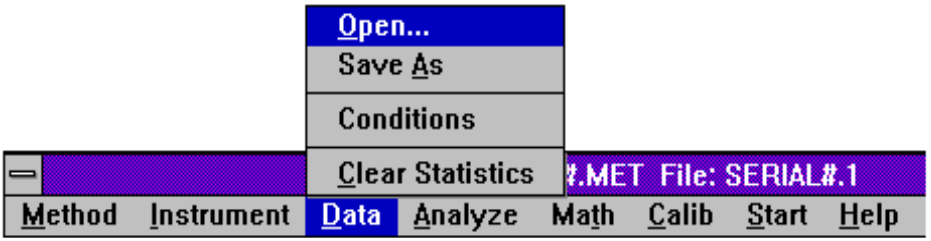


Figure 38 Data menu

Data

(Main menu) (See Figure 38)

Purpose

Allows raw chromatograms to be accessed or stored. Also allows the statistical records of the External Standard or Normalization reports to be cleared.

Syntax

Select **Data** or type **<Alt> D.**

Clear Statistics

(sub Data)

Purpose

Resets the statistics (MIN., MAX., MEAN, %RSD) in the External Standard and Normalization reports.

Syntax

Click on **Clear Statistics** or type **C**.

Comments

After the next analysis, the values for MIN., MAX., MEAN and %RSD will be reset in both the External Standard and Normalization reports.

Conditions
(sub Data)

Purpose

Displays and prints the run conditions of a stored chromatogram.

Syntax

Select **Conditions**.

Comments

The conditions window is tied to the instrument ID file which was resident in memory at the time the chromatogram was stored. The ID file contains information on carrier gas and column configuration under which the sample was run. Make sure that the proper instrument ID file is resident before data is collected.

Open
(sub Data)

Purpose

Allows the retrieval of a data file by highlighting a name from a scrolled list or by entering a path and filename in the space provided.

Syntax

1. Select **Open** or type **O**
2. Choose a data file from the list given. Use the scroll bar to access files which are not immediately visible.
3. Select **[Open]**.

Comments

Select the (..) symbol or a drive letter to access alternate directories or drives. The **Open** command can also be used to delete files that are no longer needed. When the list of data files is shown on the screen, highlight the file you wish to delete by clicking on it. (This should blacken the file name.) If

you wish to delete several files in a row, press the left mouse button over the first file and hold it down while scrolling down the list. Release the mouse button when you reach the last file to be deleted. The entire list of files to be deleted should be blackened. Now press the **<Delete>** key.

Save As

(sub Data)

Purpose

Allows raw chromatograms to be saved under user-specified names.

Syntax

1. Click on **Save As** or type **A**.
2. Enter a filename.

Comments

The file will be stored in the directory listed at the top of the popup window unless another directory is specified in the filename dialog box. The specified name must be eight characters or less and may not contain any spaces or illegal DOS characters.

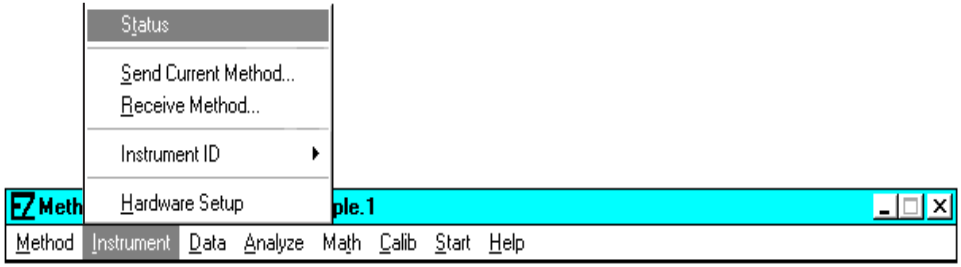


Figure 39 Instrument menu

Instrument

(Main menu) (See Figure 39)

Purpose

Allows the current state of the M200 or P200 to be monitored and updated. Commands for method sending, receiving, and status monitoring are located here.

Syntax

Select **Instrument** or type **<Alt> I.**

Hardware Setup

(sub Instrument)

Purpose

Allows changes to be made to the COM port designation for the GC.

Syntax

1. Select **Hardware Setup** or type **H**
2. Click on the down arrow until the desired COM port number is in the highlighted box.
3. Click on **[OK]**.

Comments

With this capability, no longer must EZChrom be reinstalled if the original GC COM port designation is incorrect.

Instrument ID

(sub Instrument)

Purpose

Provides a table in which column and carrier gas information can be stored.

Syntax

1. Select the **Instrument ID** command.
2. Select the **Edit** option.
3. Enter an instrument ID along with the column types and carrier gas used in your GC.
4. Select **[OK]**.
5. Select the **Instrument ID** command again.
6. Select the **SAVE** option.
7. Enter an ID for the file just created.

Comments

The Instrument ID file last opened will be automatically loaded when EZChrom 200/400 is restarted. The Instrument ID file is tied to report and condition window printouts. The Instrument ID, column type, and carrier gas sections of these printouts will display the information you entered into the Instrument ID file. The file is also attached to all raw chromatograms stored while the file was loaded in memory.

Receive Method

(sub Instrument)

Purpose

Loads the Method from the GC to EZChrom 200/400 and makes it the current EZChrom 200/400 Method.

Syntax

Select **Receive Method** or type **R**.

Comments

The current Instrument Setup parameters (column temperature, run time, inject time, etc.) will be written over and lost unless saved. The method name displayed in the EZChrom title bar will **not** be changed. To check for a successful received operation, open the Instrument Setup table located under the Method menu.

Send Current Method

(sub Instrument)

Purpose

Sends the current Method to the GC.

Syntax

Select **Send Current Method** or type **S.**

Comments

A method is sent automatically when Start is selected. Therefore, the GC will always have the current Method in memory before it starts to run. However, if the column temperature has been changed, it is important to send the Method a short time prior to a run so that the GC module can equilibrate.

Status

(sub Instrument)

Purpose

Allows monitoring of certain instrument settings between runs.

Syntax

Select **Status** or type **T**.

Comments

Status can only be monitored during idle time. While the GC is in a run sequence, the data collection rate is too high to allow status monitoring, as well as, data acquisition.

Math

(Main menu)

Purpose

Subtracts a user-selected chromatogram from the currently displayed chromatogram. After analyzing, the reports and graphical display are updated.

Syntax

1. Select **Math** or type **<Alt> T**.
2. Select **Catalog**, or enter a path and file name to select a chromatogram. This chromatogram will be subtracted from the currently displayed data set.
3. Select **[OK]** to initiate the subtraction.

Comments

This feature could allow subtraction of large solvent peaks allowing easy identification and integration of low level, overlapping peaks.

Method

(Main menu) (See Figure 40)

Purpose

Allows access to the tables and settings which control GC operation and data analysis.

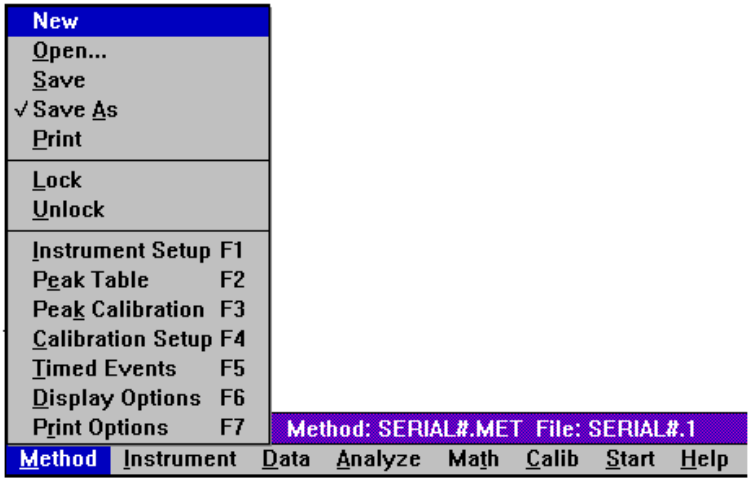


Figure 40 Method menu

Syntax

Select **Method** or type **<Alt> M**.

Calibration Setup

(sub Method)

Purpose

Allows customization of the calibration sequence.

Syntax

Select **Calibration Setup** or type **C**.

Comments

The following options are available:

Channel
Selects the module whose calibration sequence is to be modified. The module chosen will be indicated by a bullet in the circle.

Peak Attribute
The choices are Area and Height. This parameter forces EZChrom 200/400 to calibrate on either peak area or maximum peak amplitude.

Calibration Fit
The choices are Point and Linear. This allows point-to-point or linear extrapolations between different calibration levels.

Number of Runs (A & B)
Allows the user to specify the number of runs for each calibration level. For multi-run calibrations, an average value for the peak area or amplitude is calculated and entered into the peak calibration table.

Uncalibrated Peaks RF
Assigns a single user-defined response factor to all unidentified peaks in the concentration reports.

Multiplication Factor
Multiplies the external standard amounts by a user-defined value. Allows compensation for preconcentrators or diluters.

Update Retention Time After
This allows EZChrom 200/400 to update the peak retention times contained in the peak table after a calibration or after each data analysis.

Display Options

(sub Method)

Purpose

Allows the user to select a boot-up set of display criteria. Every time the system restarts, the screen will appear in the configuration that was specified.

Syntax

Select **Display Options** or type **D**.

Comments

The menu items are defined as follows:

Channel
Selects the module for which the display will be altered.

Attenuation
Sets the display magnification. The range of values is 1 to 2048, with 1 being the greatest magnification.

Start Time
The time (in seconds) of the first displayed data point.

Stop Time
The time (in seconds) of the last displayed data point.

Start Amp
The minimum voltage (in microvolts) which will be displayed.

Stop Amp
The maximum voltage (in microvolts) which will be displayed. The absolute maximum value is 12,000,000 microvolts.

Display
Allows the user to choose which window will be displayed when EZChrom 200/400 is started.

Instrument Setup
(sub Method)

Purpose

Contains the instrument conditions for data acquisition.

Syntax

Select **Instrument Setup** or type **I**.

Comments

Valid entries for the instrument parameters are:

Parameter	Range
Column temperature (°C)	30–180°*
Run tme (seconds)	0–160
Sample time (seconds)	5–255
Injection time (milliseconds)	5–255
Detector filament	On/off
Detector autozero	On/off
Inlet heaters	On/off
Detector sensitivity	Low, med., high

* For heated inlet GCs (M/P 100H or 200H), column temperature range is 40–180°C. The increased minimum temperature results from the transfer of heat from the heated inlet and injector portions of the module to the column heater.

Remember to set parameters for both channels. The Sample Time is set for both channels at once.

Lock/ Unlock

(sub Method)

Purpose

Protects a method from alteration.

Syntax

1. Select **Lock** or type **L**.
2. At the prompt, enter a password.

Comments

Any locked method can be temporarily modified **during** a session. Locking prevents these changes from being stored to the hard disk (i.e., when quitting EZChrom or opening another method) unless the appropriate password is given.

New

(sub Method)

Purpose

Creates a method called New. This clears the peak, timed event, and calibration tables, and sets the instrument setup and calibration setup tables to their default values.

Syntax

Select **New** or type **N**.

Comments

This command should be selected when first using the GC, or when a new application is being developed.

It is usually easier to modify an existing method to compensate for slight variations in sample conditions, as this is much faster than starting from scratch.

Open

(sub Method)

Purpose

Allows the user to recall any Method file and make it the current Method.

Syntax

1. Select **Open** or type **O**.
2. Enter the filename or select one from the list.
3. Select **[Open]**.

Comments

Scroll bars on the right of the file box enable the complete list to be examined.

Peak Calibration

(sub Method)

Purpose

The peak calibration table contains the area counts and corresponding amount values for all compounds listed in the peak table. A separate page exists for each compound listed in the Peak Table.

Syntax

1. Select **Peak Calibration** or type **K**.
2. Enter the area and amount values for each compound in ascending order. Up to eight area /amount pairs may be entered.
3. Select **[Next]** or **[Previous]** to access the calibration tables of the other compounds to be calibrated.

Comments

Typically, this procedure is done automatically by using the Calib command. Thus, manual entry is usually not required. If multiple levels are being used, amount/area pairs should be entered in ascending order. This allows the software to correctly plot the calibration data.

Peak Table

(sub Method)

Purpose

Contains compound names and elution times which allows EZChrom 200/400 to properly identify and quantify integrated peaks. It also provides the option of grouping multiple peaks which elute in a user-specified window. User-defined response factors can be defined and referenced here.

Syntax

Select **Peak Table** or type **E**.

Comments

In most cases, the only information that needs to be entered in this table are peak names, concentration units and Level 1 Cal Amounts. Retention times can be entered graphically. The Peak Table contains the following information:

Peak Name
In this portion of the table the names of the compounds which are to be analyzed are entered. The peak names are entered for each component by typing them in. When an integrated peak falls in a defined retention time window, it is assigned the name linked to that window.

Retention Time
This is the amount of time it takes for the apex of a named peak to elute from the column. The retention time can be entered graphically or with the keyboard. (In the Calibration Setup table there is an option to update the retention time at the end of a run. If this is selected and the retention time changes, then the retention time and the retention time window will be updated).

Retention Time Window
If a peak apex lies within a defined region of time, even though the retention time of the peak may not match the retention time listed, it will be identified as the compound defined by the preset retention time window.

Concentration Unit (CU)
This is a text entry which identifies the concentration units of the various compounds. Any three character text entries are allowed. The unit is not a conversion factor and has no mathematical significance. Each component may have a different concentration unit. The concentration units are only used for external standard reports.

Level 1 Cal Amount
This is the amount of a compound that the level one external calibration standard contains. When a Level 1 calibration is run, the entered amounts are assigned to the areas of any integrated peaks which elute in the appropriate retention time windows. Obviously, accurate calibration standards must be used and retention time windows must be properly set for EZChrom 200/400 to accurately quantitate real samples. EZChrom 200/400 can use up to eight levels of calibration but for routine analysis one level is usually sufficient. Each level corresponds to a gas standard concentration and a point on a calibration plot.

Relative Response Factor (RRF)
When an external standard is unavailable, an estimated response factor may be specified. Enter a number which will be multiplied by a calibrated response factor.

Peak
This entry is used to reference the RRF discussed above to a particular peak in the Peak Table. Choose a compound which has a similar thermal conductivity to the uncalibrated peak.

Print
(sub Method)

Purpose

Prints the current Method.

Syntax

1. Click on **Print** or type **P**.

Comments

A method printout includes the following items for both channels: Instrument Setup, Peak Table, Calibration Setup, Peak Calibration Tables, Timed Events, Print Options, Display Options

Print Options
(sub Method)

Purpose

Allows the user to choose which reports and chromatograms will be printed after a single run or during the time between runs in an autorun.

The Print option under the Start window must also be selected for this option to be active.

Syntax

Select **Print Options** or type **R**.

Comments

The following choices are available:

Full scale chromatogram
Zoomed chromatogram
Area Report
Normalization Report
External Standard Report

Check or uncheck any or all entries to get the desired set of printed reports. Be aware that printing large datasets, such as full scale chromatograms, is time consuming. If printing must be done between runs in an autorun, sufficient time must be allotted for printing to be completed. A standard IBM dot matrix printer will require approximately 3 minutes to print a single 100 second chromatogram.

Save

(sub Method)

Purpose

Saves the current Method under the current Method filename.

Syntax

Select **Save** or type **S**.

Comments

If the current Method is locked, the method password must be entered before this action can be completed.

Save As

(sub Method)

Purpose

Saves the current Method under a user-defined filename.

Syntax

1. Select **Save As** or type **A**.
2. Enter the new filename for the current method using a maximum of eight characters in DOS format.
3. Click on **[OK]**.

Timed Events

(sub Method)

Purpose

Establishes integration parameters for each data analysis.

Syntax

Select **Timed Events** or type **T**.

Comments

Other than the **Spike** event, Timed Events are described in this chapter under Events. **Spike** is a filtering mechanism. Specify the time that filtering should start and the smallest absolute spikes that should be filtered as the time and value, respectively. **Spike** will continue to smooth the data each time the data is analyzed.

Reports may change when data that has a Spike Timed Event is reanalyzed.

Spike is not available via graphics as a timed event. All the other timed events are available via graphics.

Start

(Main menu)

Purpose

Opens the run sequence window, from which the GC can be started, and various run time options specified.

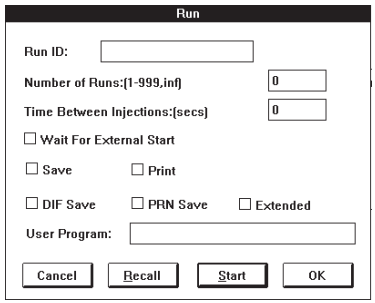


Figure 41 Run window

Syntax

1. Select **Start** or type **S.**
2. Fill in the Run Setup screen (See Figure 41).
3. Select **[Start]** to run the GC or **[Recall]** to reanalyze a stored dataset.

Comments

The **Print** or **Save** functions can be turned on or off globally, but the items that are to be printed must be activated in the Print Options menu.

Cancel

Cancels any alterations made to the Start window, and returns the user to the EZChrom 200/400 menu.

DIF/PRN Save

Saves the peak name, amount, and run number from the External Standard Report to ASCII files read by Excel or Lotus respectively. File extensions will be .DIF (Excel) or .PRN (Lotus).

Extended
Causes the peak areas and retention times to be added to DIF and PRN files.

Number of Runs
Enter the number of runs you wish EZChrom 200/400 to collect during an autorun. Entering 0, 999, or INF will cause EZChrom 200/400 to run continuously. If the Save option has been selected while EZChrom is running continuously, the thousandth saved file will overwrite file number one, and so on.

OK
Allows the Start window to be altered without actually starting a run.

Print
Will cause all selections in the Print Options menu to be printed in the time between the end of a run and the beginning of the next. If a full-scale chromatogram is printed, the interval between runs must be at least 3 minutes, as this type of printing takes a long time to complete.

Recall
Allows batch reprocessing of data files. To use this option, enter the names of a sequential set of datafiles in the Run ID window. Then, enter the number of files which are to be reanalyzed in the Number of Runs window. Selecting the Recall command will cause EZChrom 200/400 to retrieve and reanalyze as many files as you entered in the Number of Runs window. Only datasets which have sequential extensions beginning with 1 can be "Recalled". It may be necessary to rename a data file to generate sequential extensions (.1, .2, .3, etc.).

The Recall function can be used with Print and/or PRN and Save options in the start window.

Run ID
Will save data files automatically under the filename specified. The run number is appended to the Run ID as a file extension.

Save
Saves raw chromatograms to the hard disk or a user-specified drive or directory. The chromatogram is stored under the Run ID.

Time Between Injections
The time from the beginning of one run to the beginning of the next run. The interval time must be long enough to allow for run time, saving data and report printing. Example: 100 seconds (Run Time) + 5 seconds (save data) + 60 seconds (interval) = 165 seconds (time between injections)

User Program
Enter a path and program name to run an external program between runs in an autorun. The external program **must** terminate prior to the next data acquisition.

Caution

EZChrom 200/400 is not multi-tasking! Attempts to multi-task may cause a system crash.

Wait for External Start
This feature allows EZChrom and an Agilent GC to be started from an external source. If this box is checked when the START command is given, EZChrom will pause until pin 2 on the GC **ANALOG** port is grounded. Details of the GC IO port pin connections can be found in the M200/M200H or P200/P200H operations manuals.

Channel A/B commands

This chapter describes the commands and features available in the display windows of EZChrom 200/400. Zooming, printing, and graphical selection of timed events are all controlled by selecting the appropriate command. The command menus are located at the top of the channel A and B windows.

DISPLAY

(Main menu) (See Figure 42)

Purpose

Controls basic display features of zooming, splitting the screen, and displaying the baseline.

Syntax

Select **DISPLAY** or type **<Alt> D**.

Comments

Be sure that Channel A or Channel B windows are activated. Activate a window by clicking in the window.

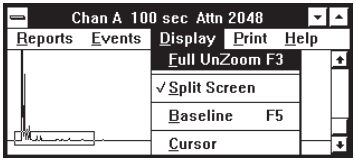


Figure 42 Display menu

Baseline

(sub Display)

Purpose

Draws or removes the baseline on the lower display.

Syntax

Select **Baseline** or type **B**.

Comments

Removing the baseline from the display also removes it from a printout of the chromatogram.

Cursor

(sub Display)

Purpose

Turns the vertical cursors, time, and amplitude readouts on or off.

Syntax

Select **Cursor** or type **C**.

Comments

This option is most useful when operating without a mouse, or when the cursors and/or readouts overlap an interesting portion of the chromatogram. A check will appear next to the Cursor command when the cursor is activated. Simply click on the command bar to activate or deactivate.

Full Unzoom

(sub Display)

Purpose

Unzooms the lower portion of the window to a full-scale chromatogram.

Syntax

Select **Full Unzoom** or type **F**.

Comments

The fully unzoomed chromatogram is displayed at the attenuation setting shown at the top of the channel A or B window.

Split Screen

(sub Display)

Purpose

Splits/unsplits the screen. An unsplit screen will display only the lower or zoom portion of a channel window.

Syntax

Select **Split Screen** or type **S**.

Comments

Removing the upper display can give better vertical resolution of high amplitude peaks.

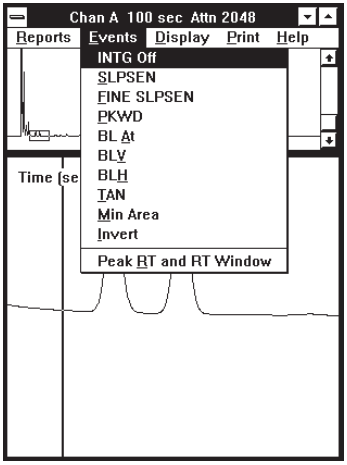


Figure 43 Events menu

Events

(Main menu) (See Figure 43)

Purpose

Allows most Timed Events to be set graphically.

Syntax

1. Select a region or point of interest with the vertical cursor(s).
2. Select **Events** or type **<Alt> E**.
3. Select the appropriate Event.
4. Inspect the time and value given in the confirmation window and make any necessary alterations.
5. Select **[OK]** to add the event to the timed events table.

Comments

Refer to page 22 to page 25 for instructions on selecting a region of a chromatogram with the vertical cursors.

BL AT

(sub Event) (Baseline At)

Purpose

Resets the peak search from a user-selected point.

Syntax

1. Choose a start point by freezing a single vertical cursor just to the left of a peak where you wish the peak search to reset.
2. Select **BL AT** or type **A**.

Comments

For this timed event, only the **left** cursor needs to be set. BL AT only requires a start time.

BLH

(sub Event) (Baseline Horizontal)

Purpose

Forces the baseline to be drawn horizontally from the start time selected until the stop time selected.

Syntax

1. Choose a baseline segment with the vertical cursors.
2. Select **BLH** or type **H**.

Comments

The horizontal baseline is drawn forward only, not backward.

BLV

(sub Event) (Baseline Valley)

Purpose

This command forces the integration baseline to be drawn to the lowest point in the valley between two peaks.

Syntax

1. Choose the two peaks whose baseline is to be identified.
2. Place the vertical cursors around this region.
3. Select **BLV** or type **V.**

Comments

Two peaks that are separated by a valley must be selected. The baseline will be drawn from the start of the first peak to the valley between the peaks. Then it will continue from the valley between the two peaks to the end of the second peak.

FINE SLPSEN

(sub Event) (Fine Slope Sensitivity)

Purpose

Defines a threshold value for the second derivative of the detector voltage. The second derivative is used in peak processing to determine peak start and stop.

Syntax

1. Choose a 2-second segment of the baseline with **no** peaks.
2. Select **FINE SLPSEN** or type **F**.
3. Review/adjust the event values and choose **[OK]**.

Comments

Generally you should adjust the time for FINE SLPSEN to zero seconds. This starts the peak search with the correct FINE SLPSEN value from time zero. When the change in the slope between three data bunches is greater than the threshold FINE SLPSEN value, a peak start is defined. When the change in slope falls below the threshold FINE SLPSEN value, a peak end is defined.

INTG OFF

(sub Event) (Integration Off)

Purpose

Turns integration off for a specified period of time.

Syntax

1. Choose a region of the chromatogram as described for Events.
2. Select **INTG Off**.

Comments

This event is used to prevent integration over user selected regions (i.e., the baseline rise immediately after injection or a large solvent peak).

Invert

(sub Events)

Purpose

Allows the user to invert any portion of a chromatogram.

Syntax

1. Choose the region to be inverted with the vertical cursors as described in Chapter 3.
2. Select **Invert** or type **I**.

Comments

This event is used when a negative peak is present in a chromatogram. An inversion will only occur when Invert is selected or added to the Timed Events (or the first time a dataset is analyzed with a method which includes an Invert event). When Invert is selected, an analysis is done automatically.

Min Area

(sub Events) (Minimum Area)

Purpose

Defines the smallest integrated area that should be identified as a peak.

Syntax

1. Select a peak with the vertical cursors
2. Select **Min Area** or type **M**.
3. Confirm the time and value for the minimum area then press **[OK]**.

Comments

Allows small peaks or baseline defects to be ignored during peak search.

Peak RT and RT Window

(sub Events)

Purpose

Used to set retention times and retention time windows for peaks listed in the Peak Table.

Syntax

1. Select a peak with the vertical cursors.
2. Select **Peak RT and RT Window** or type **R**. A popup window listing valid peak names will then be displayed.
3. Double click on the appropriate peak name on the list, or single click on a peak name and select **OK**.

Comments

The popup window automatically highlights the last peak chosen. Asymmetric retention time windows can be created by placing the vertical cursors asymmetrically about the selected peak.

PKWD

(sub Events) (Peak Width)

Purpose

Used to set the data bunching rate.

Syntax

1. Select a peak with the vertical cursors.
2. Select **PKWD** or type **P.**
3. Review/adjust the event values and choose **[OK]**.

Comments

Peak width adjusts the bunching of data points to optimize peak detection. This mechanism, combined with slope sensitivity and fine slope sensitivity, acts as a noise filter.

SLPSEN

(sub Events) (Slope Sensitivity)

Purpose

Defines the average slope of the baseline in microvolts per unit of time.

Syntax

1. Choose a segment of the baseline with no peaks.
2. Select **SLPSEN** or type **S**.
3. Review/adjust the event values and choose **OK**.

Comments

Generally you should adjust the time for the first SLPSEN event to zero seconds so that the peak search starts with the correct SLPSEN value from time zero. When the slope between two data bunches is greater than the threshold SLPSEN value, a peak start is defined. When the absolute value of the slope between two data bunches falls below the threshold SLPSEN value, a peak end is defined.

TAN

(sub Events) (Tangent Skim)

Purpose

Forces a baseline tangent from the start of overlapping peaks to their tails.

Syntax

1. Select a region for which the tangent baseline is to be drawn.
2. Click on **TAN**.

Comments

If there is a peak valley that is higher than the tangent baseline, a perpendicular is dropped to the baseline from the lowest point in the valley. This perpendicular defines the peak start for the second peak.

Help

(Main menu) (See Figure 44)

Purpose

To provide access to an interactive help menu.

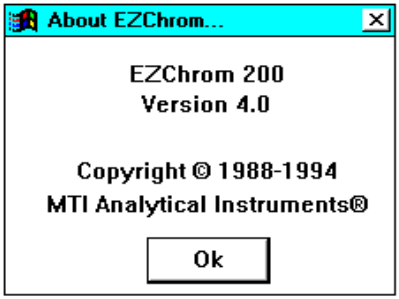


Figure 44 Help window

Syntax

Select Help, or type H.

Comments

Future versions of EZChrom 200/400 will have help routines similar in format to those used by Windows. At the present time, Help will only display a screen showing the current version of EZChrom.

Print

(Main menu) (See Figure 45)

Purpose

Allows the user to print individual reports or chromatographic displays.

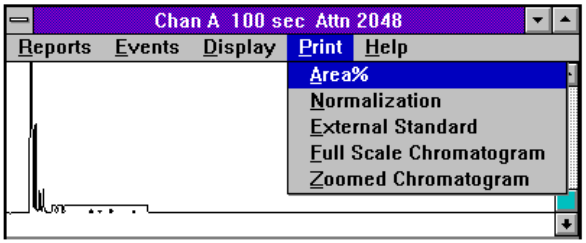


Figure 45 Print menu

Syntax

1. Click on **Print** or type **<Alt> P**.
2. Click on the desired type of report or display that is to be printed.

Comments

Multiple print jobs may be selected with the print command. Disabling the Windows Print Manager will decrease the amount of time required to print; however, multiple print jobs cannot be selected.

Reports

(Main menu) (See Figure 46)

Purpose

Reveals the menu of data reports available for display.

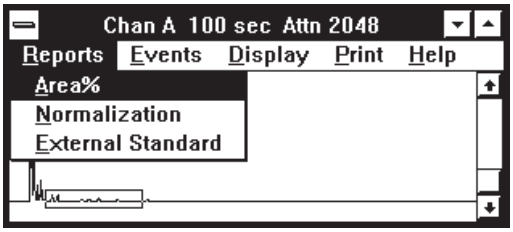


Figure 46 Reports menu

Syntax

Click on **Reports** or type **<Alt> R**.

Comments

To close a report, double click on the top left corner of the report display or type **<Alt> spacebar** and type **C.** Reports may be left on the screen during data collection allowing the user to monitor data analysis during an autorun.

Area % report

(sub Reports)

Purpose

Displays a report showing the number of peaks and their retention times, raw areas, and percent of total area.

Syntax

Click on **Area**.%.

Normalization report

(sub Reports)

Purpose

Displays a report of peak names, retention times, normalized amounts, units, and the percent standard deviation.

Syntax

Click on **Normalization.**

Comments

Amounts are normalized over the channel selected. Min., Max., Mean, and %RSD are a record of the repeatability of sample analysis over a set of runs.

External Standard report

(sub Reports)

Purpose

Displays a report of peak names, retention times, amounts and units.

Syntax

Click on **External Standard**.

Comments

Amounts are calculated relative to the calibrated External Standard. Min., Max., Mean, and %RSD are a record of the repeatability of sample analysis over a set of runs.

7

Quick Reference

Quick Reference

This chapter of the manual provides greater detail concerning the features available in EZChrom 200/400. It is geared to those users who are already familiar with basic EZChrom operation, and should not be used until prior chapters are fully mastered. Due to the presumed sophistication of the user, certain procedures are presented in other than strict chronological order of operation. In certain cases, the reader will be asked to refer to other chapters of the manual for further explanation or for review. This chapter also presumes an extensive knowledge of EZChrom menus. You should be thoroughly familiar with Chapters 4, 5, and 6 before attempting the procedures described in this chapter.

Refer to Appendix C. for several quick reference flowcharts describing the following:

Setting Up a Run
Developing a Method
Calibrating a Method

Quick and easy method development

The first step in optimizing the performance of EZChrom is to build a good Method. This chapter provides a step-by-step sequence which simplifies the Method construction procedure outlined in Chapter 4. The following is an outline of the procedure that should be followed whenever a new application is to be run.

1. Set up the instrument parameters.
2. Fill out the Peak Table.
3. Run the GC.
4. Set up the Timed Events Table.
5. Adjust the instrument parameters, and rerun the GC.
   (This step is optional and frequently unnecessary.)

6. Optimize the Timed Events Table.
7. Set the peak retention times and retention time windows graphically.
8. Calibrate and save the method.

The details of each of these steps are reviewed below.

To exit EZChrom 200/400 at any time, double click on the box above Method in the main menu.

Steps for the method construction procedure

1. Open the Instrument Setup window (See Figure 47) and set the column temperature, run time, inject time, and detector sensitivity to appropriate values.

   For most applications, columns temperatures of between 50 and 80 degrees will give acceptable peak resolution.

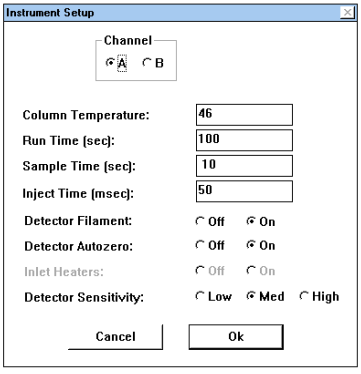


Figure 47 Instrument setup window

   Start with the maximum run time (160 seconds) and later reset it to 10 seconds longer than the tail of the last eluting peak.

   Use a value of 50 milliseconds for the inject time, unless sample gas concentrations are in the high % range (in which case use 20 milliseconds) or low ppm range (in which case use over 100 milliseconds).

For concentration levels between 10 and 100%, use **Low** sensitivity. For levels from 500 ppm to 10 %, use **Medium** sensitivity. For levels less than 500 ppm, use **High** sensitivity.

Set the column head pressures for the individual modules. Pressures of between 15 and 20 psi are reasonable in most applications for the majority of columns.

2. Open the Peak Table (See Figure 48) and enter the names of the compounds for which you wish to screen. If you are unsure of which column is appropriate for analysis of a given compound, call Agilent Technical Support for assistance.

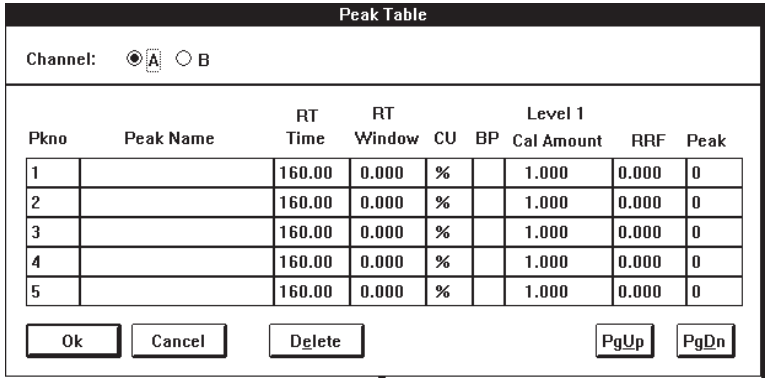


Figure 48 Peak table

In the **Level One Cal Amount** boxes, enter the concentrations of each compound in your first external calibration gas standard.

3. Attach your sample or calibration gas to the sample inlet and run the GC. As the data is being acquired, observe the number, resolution, and amplitude of the peaks in the chromatogram.

4. Open the **Timed Events** tables (See Figure 49) and add a **SLPSEN**, **FINE SLPSEN**, and **INTG Off** event to both the channel A and B tables. The *time* parameter should be zero for all these events. The *value* for the INTG Off event (in seconds) should be set to 1.5 seconds. This turns the integration off during the first part of the aquisition, where baseline artifacts are

observed due to sample injection. The *values* for the SLPSEN and FINE SLPSEN events are dictated by the detector sensitivity for a particular channel.

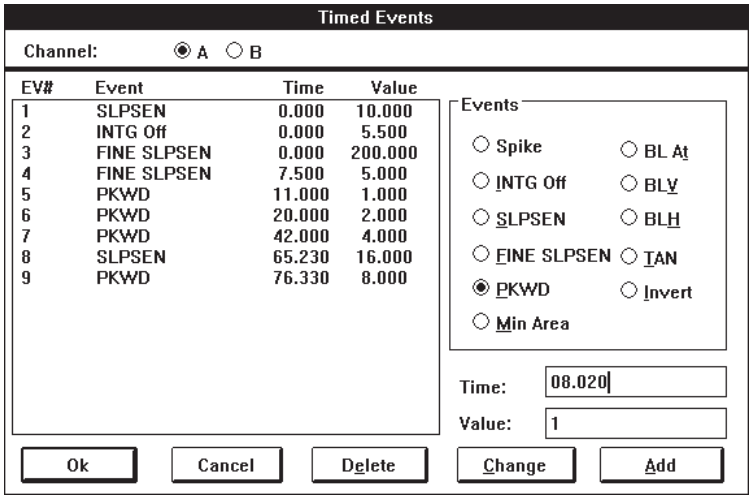


Figure 49 Timed events table

The most accurate way of setting SLPSEN and FINE SLPSEN is graphically (see Chapter 5).

The following table provides suggested initial values for SLPSEN and FINE SLPSEN.

Sensitivity	SLPSEN value	FINE SLPSEN value
Low	10	5
Med	50	30
High	100	75

5. If necessary, adjust the instrument setup parameters and column pressures so all of the desired peaks are well resolved and on scale. Detector saturation is evidenced by the tops of peaks rising above the upper limits of the channel window display. (See page 117 through page 120 respectively for explanations of how to unzoom and how to adjust attenuation.)

   After adjusting the instrument parameters, run the GC again. If the analysis is still unsatisfactory, repeat this step.

   Be careful not to be fooled by the display. Always set the display **Attenuation** to 2048, and **Fully Unzoom** the lower display when checking for detector saturation.

6. Once the instrument settings have been optimized, the integration parameters tables should be adjusted. During the course of completing Step 5, you may have noticed that some of the peaks in the chromatogram were not integrated, or were integrated incorrectly. To correct this deficiency, **PKWD** events must be added to the **Timed Events** tables.

   Observe the last analyzed dataset on the screen. Note the first peak which is not properly integrated (the calculated baseline does **not** extend from the true beginning of the peak to its true end).

   Using a **Vertical Cursor,** determine the time at which the missed peak begins to elute.

   Now, add a **PKWD** event, with a *value* of 0.2, and a *time* slightly less (approximately 0.5 seconds) than the beginning of the missed peak, to the appropriate **Timed Events** table (See Figure 49).

   After the **PKWD** event has been added, **Reanalyze** the data.

   The peak which was previously not integrated should now be properly integrated. If it is not, double the **PKWD** value to 0.4 and reanalyze. Eventually, the peak will be successfully integrated. Repeat the above steps for both channels and all subsequent peaks which are still incorrectly integrated, remembering to double the value for each successive **PKWD**. (See page 52 for additional information on the adjustment of **PKWD**.)

At this time, you may also wish to extend the **Intg Off** event to include solvent or composite peaks which are not of interest.

7. Set the **Retention Times** and **Retention Time Windows** graphically (as described in Chapter 4) for all peaks of interest in both channels. If you now reanalyze the data, the External Standard report should display the names and area counts for all integrated peaks in the chromatogram.
8. Select the **Calib** command from the lower menu bar and run the GC. Data will be acquired, and a calibration will be done. Inspection of the External Standard reports should show all compounds which were in the calibration gas, and at the correct concentrations.

   The Method is now complete. Save the Method to the hard drive and, if necessary, lock it.

Method locking

Locking a Method protects it from being inadvertently modified. A locked Method may be modified during a session, but these changes will not be stored at the end of the session unless a password is given.

To Lock a Method:

1. Select **Method**.
2. Select **Open**.
3. Select the method that is to be locked.
4. Select **Method**.
5. Select **Lock**.
6. Enter a password (maximum of eight characters).
7. Save the Method, entering your password at the prompt.

To Unlock a Locked Method:

1. Open the Method that is locked.
2. Select **Unlock** in the Method menu.
3. Enter the password.
4. Changes in Method parameters will now be accepted.

Multi-level calibration

EZChrom 200/400 will handle calibrations with up to eight gas standard concentration levels. Each calibration level corresponds to a point on a calibration plot. Each component for a particular channel must be calibrated with the same type of calibration method (i.e., point-to-point or linear). That means that if a point-to-point calibration is used for one component, the same must be used for all of the other components. However, the number of calibration levels may be different for each component. The procedure for initiating a multi-level calibration (or single level for that matter) begins with:

- **Calibration setup**

  In addition to allowing up to eight levels of calibration, EZChrom 200/400 software can calibrate either on peak height or peak area. Also, the calibration plot can be either point-to-point or linear. For a linear plot, at least two levels of calibration are required. When a new method is being built, EZChrom defaults to a set of values for the Calibration Setup. In most cases the default values will suffice. In certain cases, however, values other than the defaults may be appropriate. To access and alter the Calibration Setup table:

  1. Select **Method**.
  2. Select **Calibration Setup**.
  3. Make any necessary changes in the table.
  4. Select **OK** to close the table.

  The features available in the Calibration Setup table (See Figure 50), and their default values are described below:

Peak Attribute (Area or Height)

Determines whether the calibration and quantitation are to be based on peak height or peak area. The default is **Area**.

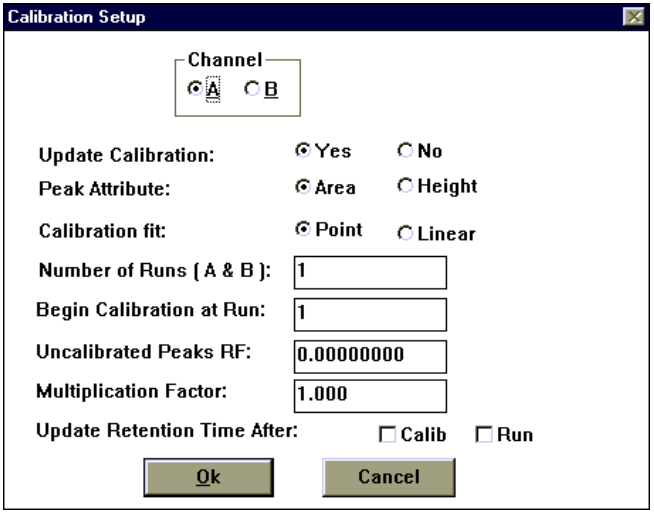


Figure 50 Calibration setup table

Calibration Fit (Point or Linear)

Determines whether the calibration plot is to be based on a point-to-point plot or a linear plot. The default is **Point**.

Number of Runs (A & B)

This corresponds to the number of calibration runs that will be performed at each calibration level. The integrated areas for each peak are then averaged, and an average response factor is generated. The default is **One**.

Begin Calibration at Run

This allows the instrument to stabilize before calibration. The default is **One.**

Uncalibrated Peaks RF
This option allows the assignment of a blanket response factor to all unidentified peaks. This response factor is multiplied by the area of the unknown peaks to give an amount value. The default is **Zero**.

Multiplication Factor
This is a multiplication factor that is applied to all quantitated peaks, both in calibration standards, as well as, samples. This feature is useful when a concentrator or dilutor is used in the sampling system. The default is **One**.

Update Retention Time After (Calib and/or Run)
This allows the user to update the retention times of the components listed in the peak table after each calibration and/or run. The default is **No**.

- **Entering calibration amounts**

  1. To enter the concentrations from your calibration standard gases into your Peak Calibration Table (See Figure 51):
  2. Select **Method**
  3. Select **Peak Calibration**.
  4. Select Channel A or B
  5. Enter the amount information for each level using **Page Down** as necessary. The calibration amounts **must** be entered in order from least to most concentrated. A level one calibration will require only one entry per compound.
  6. To switch compounds, click on **[Prev]** or **[Next]**.
  7. The area information can also be entered now, manually, or later while performing a calibration on stored or newly acquired data.
  8. To plot the new calibration points, click on the **Plot** button. The slope of this plot defines the response factor for that compound.

- **Calibrating the instrument**

  Once the Calibration Setup and Peak Calibration Tables have been completed, the instrument is ready to be calibrated.

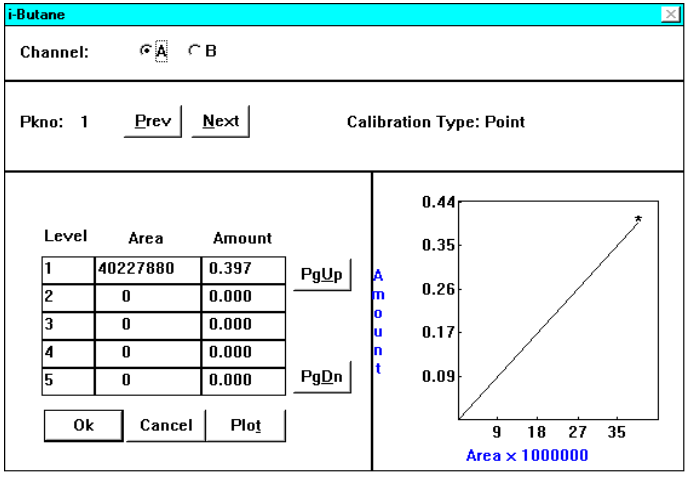


Figure 51 Peak calibration table

Calibration can be performed with stored data or during an acquisition sequence. Follow directions for Stored Calibration or Run Calibration, depending on which option is preferred. Any combination of these options can also be performed.

- **Stored calibration**

  First, clear the statistics for a clean start.

  1. Select **Data**.
  2. Select **Clear Statistics**.
  3. Select **[OK]**.

  Now select the stored data file to use as a calibration standard.

  1. Select **Data**.
  2. Select **Open**.
  3. Enter the data file name manually, or double click on the data file name.

  Now set up to calibrate.

  1. Select **Calib**.
  2. Enter the Calibration Level (The calibration level refers to which calibration gas dataset is being analyzed: first, second, third, etc.).
  3. Select **[OK]**.

  To finish the calibration:

  1. Select **Analyze**.
  2. Repeat the preceding steps for each calibration gas standard dataset.

- **Run calibration**

  1. Attach the first cal gas standard.
  2. Select **Calib**.
  3. Enter the Calibration Level.
  4. Select **[OK]**.
  5. Start the GC.

  Calibration will begin immediately. Prior to sampling, the EZChrom menu title bar will display the following message:

  Calibration level X – Runs left Y

  Where:

  X = the calibration level selected under Calib.

  Y = the number of calibration runs entered in the Calibration Setup.

  Repeat the preceding steps for each calibration gas standard.

  It is critical to remember that the cal gases **must** be calibrated in order of increasing concentrations. This is necessary, not only to match the previously entered Calibration Amounts, but also to allow the plotting algorithm to function properly (the plotting routine does not automatically sort area/amount pairs).

  For multi-component cal gases, it may be impossible to calibrate all compounds in order of least to most concentrated. In this case it will be necessary to manually sort the area counts in the Peak Calibration table.

Print options

To print reports and/or chromatograms during an autorun sequence, the Print Options table (See Figure 52) must be filled in. By checking the **Print** option in the **Start** window, the selected material will be printed during the time between runs. Remember, enough time must be allowed for the printing process to be completed prior to the next run.

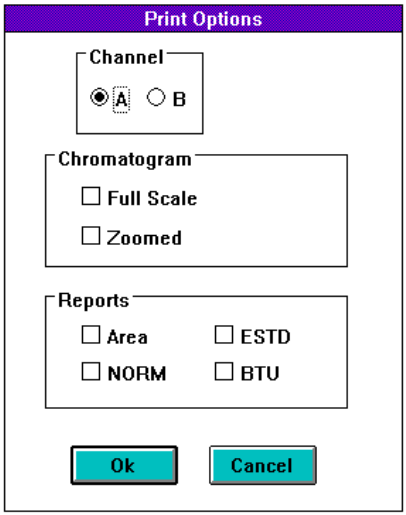


Figure 52 Print options window

Caution Chromatograms generate many data points that occupy a lot of memory on your storage media. If you are printing multiple chromatograms simultaneously, you may overload the printer memory and lock up your system.

1. Select **Method.**
2. Select **Print Options**.
3. Check any of the reports you wish to print, at the completion of the analysis, for both channels.
4. Select **[OK]**.

Remember to check the **Print** box in the **Start** window prior to beginning a run. The available options are:

Chromatogram (full scale and/or zoomed)

Hard copies of the chromatograms can be generated for channels A or B. If the Zoomed option is selected, the zoomed chromatogram shown in the lower display will be printed.

Reports (area percent, normalized percent and/or external standard)

Any or all of the three available reports for channels A and B can be printed between autoruns.

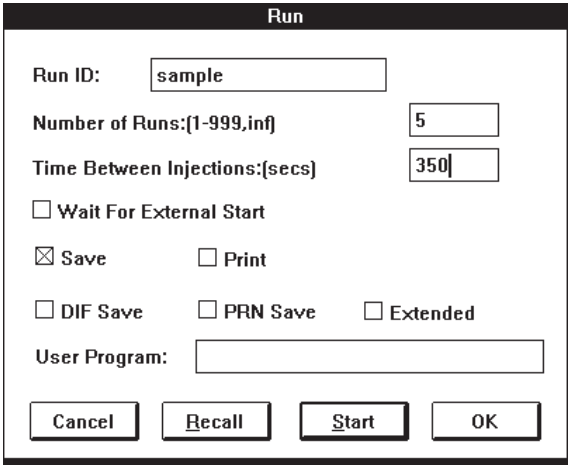


Figure 53 Run window

Running the GC

Running the GC and obtaining accurate results is a simple task once a good Method has been developed.

1. Select **Start**.
2. A run window will appear which prompts for information regarding the automated run sequence (See Figure 53).
3. In the **Run ID** space, enter a name for the data which is to be collected and stored. This may be a maximum of eight characters and DOS illegal characters are forbidden (See page 162 for a list).
4. Specify the **Number of Runs**. If the number of runs is greater than one, the run number is appended to the Run ID as a data file extension (for example: run.1, run.2, etc.).
5. Specify the **Time Between Injections** in **seconds**. Note what the delay interval is between injections; make sure this interval is long enough to accommodate the run time, time between runs, and any necessary printing time.
6. Check if the data is to be **Saved** and/or **Printed** after each run. Make sure that printing selections have been made in **Print Options** before selecting **Print** here.
7. Check if the data is to be output in a **DIF** or **PRN** format.

   The DIF format saves external standard report peak name and amount information as ASCII characters delimited by tabs (EXCEL format). The filename used is the Run ID followed by the extension .DIF.

   The PRN format saves the peak name and amount report information as ASCII characters delimited with quotes and numbers delimited by commas (Lotus format). The filename used is the Run ID followed by the extension .PRN.

   Check **Extended** if you would like to include the area and retention time in the information saved to DIF or PRN files.

8. Select **[Start]** to begin a run.
9. After completion of the run, the chromatogram is automatically analyzed.

Analyzing the data

All of the data collected by EZChrom 200/400 is kept in an active buffer and saved to a mass storage device at the user's request. All of the analysis and reporting activities are handled according to the current Method parameters. The analysis process does not alter the raw chromatographic data. Methods may therefore be altered and adjusted, and chromatograms reanalyzed until an optimal Method is constructed. An analysis is always done when a chromatogram is first acquired. To reanalyze a stored dataset:

1. Select **Data**.
2. Select **Open**.
3. Double-click the left mouse button on the desired datafile.
4. Select **Analyze**.

Upon completion of the Analyze step, the chromatograms will be displayed in the split screen labeled Channel A and Channel B. All reports are updated and, if specified, will be printed and saved. Now the graphics can be manipulated, reports can be displayed, the Method can be adjusted and data can be reanalyzed until the results are satisfactory.

Automated sequences

Autorun

An Agilent GC can be set up to run automatically from the **Start** window. The chromatograph can be set to run continuously (i.e., for process control applications), or for a specified number of times. The procedure for initiating an autorun is as follows:

1. Select the **Start** menu. The Run window will appear. (See Figure 54)
2. Enter the number of times you wish the GC to run (for continuous operation enter a 0, 999, or INF).

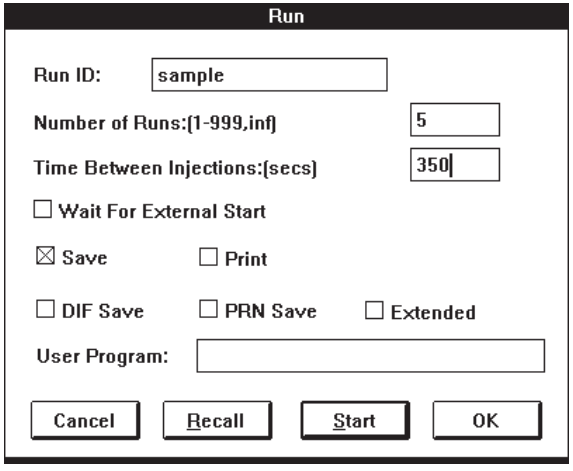


Figure 54 Run window

3. Enter a value for the time between injections (in seconds). This time should be long enough so that any highly retained compounds can elute from a column prior to the next run, and all specified print jobs can be completed.

An autorun sequence can be aborted at any point by pressing the **<Esc>** key, or paused by pressing the **<F1>** key while the EZChrom command bar is active. Each file saved in an autorun is given the Run ID plus a numeric extension. The

extension of the last collected file will be the same as the number of runs you entered.

Batch processing

EZChrom 200/400 can be instructed to reanalyze, print, and display sets of stored data by using the **Recall** command in the **Start** window. To use this feature:

1. Select the **Start** menu. (See Figure 54)
2. Enter the **Run ID**, minus the extension, of the series of files to be reanalyzed.
3. Enter the number of files to be reanalyzed.
4. Set the time between injections to 0 seconds.
5. Select the **[Recall]** command.

EZChrom will then begin recalling and reanalyzing the specified set of chromatograms. The program always starts with the first file extension.

If the .1 file or any file between .1 and the number of runs you entered is missing, an error message will be displayed and RECALL will abort. During a RECALL, all selected operations in the START window can be active (i.e. Print, Save).

Transferring methods

Once a valid Method has been established, it is important to give this information to the GC. EZChrom automatically sends the current Method to the instrument when Start is selected.

To guarantee that the columns have reached the correct operating temperature prior to a run, it is best to transfer the Method well before the next analysis.

To send an updated method to the GC:

1. Select **Instrument**.
2. Select **Send Current Method**.
3. Select **[OK]**.

In order to check the current instrument setup in the GC, Methods can be received fromt he GC.

1. Select **Instrument**.
2. Select **Receive Method**.
3. Select **[OK]**.

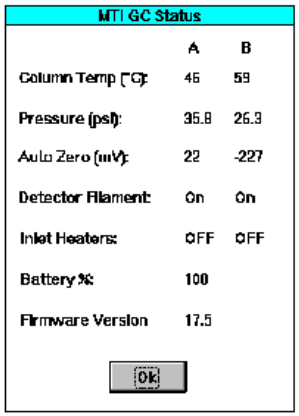


Figure 55 Instrument status window

Once a Method has been sent, it is a good idea to check the instrument status to determine when column temperatures have stabilized. This is done with the **Status** command. To check the instrument status:

1. Select **Instrument**.
2. Select **Status**.
3. Observe the display window (See Figure 55) which lists the current temperature, pressure, autozero voltage, detector filament status and remaining battery charge (P200 only) in the GC.
4. Select **[OK]** to close the Status window.

Graphical manipulation of chromatograms

Once the data has been analyzed and the chromatograms have been displayed, there are a wide variety of options available for manipulating the display and setting up new analysis parameters (See Figure 56). Note that the upper portion of the channel windows has a complete display of the chromatograms and the capability to adjust the display attenuation. Using the mouse or keyboard, a zoomed portion of the chromatogram may be displayed in the lower portion of the screen.

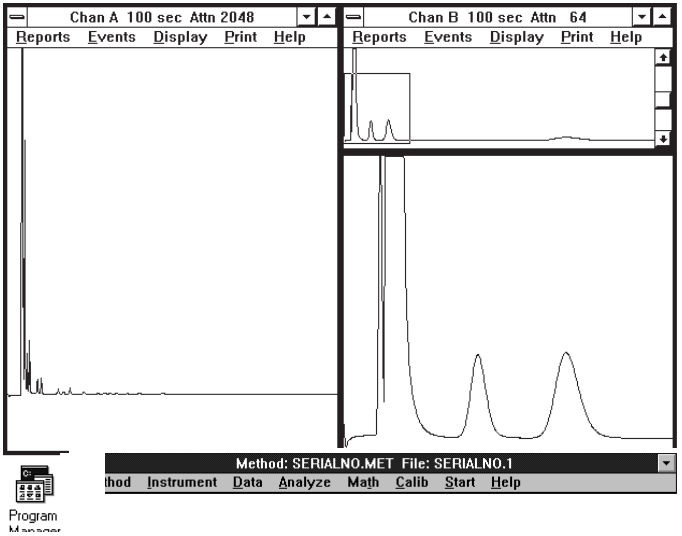


Figure 56 Full unzoomed and split screen windows

Full unzoom

This command returns the lower display to a full-scale chromatogram at the current display attenuation.

1. Select **Display.**
2. Check **Full Unzoom**. The lower portion of the window will return to a full-scale chromatogram.

Split screen

This command either removes or restores the upper display.

1. Select **Display**.
2. Check **Split Screen**. This removes the upper display.
3. To regain the upper display, select **Split Screen** again.

Baseline

This command either removes or restores the calculated integration baselines and hashmarks.

1. Select **Display**.
2. Select **Baseline**. No baseline will be displayed.
3. To regain the baselines and hashmarks, select **Baseline** again.

Cursor

This command enables or disables the vertical cursors, as well as, the time and amplitude windows in the lower display.

1. Select **Display**.
2. Check **Cursor**. A mobile vertical cursor will appear in the lower portion of the window.
3. To disable the vertical cursor and regain the arrow cursor, press the number **3** key or click the right mouse button.

Remember, all these mouse functions can be performed with the keyboard by using the arrow keys for direction, and the numeric 1 and 3 keys as the left and right mouse buttons respectively. The **Cursor On** command **must** be selected (will show a check mark next to the Cursor command) to operate the cursor with the keyboard.

Defining a zoom box

A zoom box may be defined in the upper or lower portion of the channel A or B window. The zoomed image will always appear in the lower portion of the window and the zoom box will appear on the full-scale chromatogram displayed in the upper window (See Figure 56). To draw a zoom box:

1. Place the cursor at the top left of the desired zoom area.
2. Hold the left mouse button down and, at the same time, drag the mouse down and to the right until the displayed box encompasses the portion of the chromatogram to be enlarged.
3. Release the left mouse button.

Defining a reference cursor

Once the Cursor On has been selected, it is possible to freeze the first vertical cursor. This first cursor can then be used as a reference point for a second vertical cursor.

1. Check **Cursor** and move the cursor to the desired reference point.
2. Click **once** on the **left** mouse button. Notice that the cursor becomes frozen and that the values for time and amplitude are both zero. This is the reference cursor.
3. Move the mouse; a second cursor will appear. Note that the time and amplitude values change relative to the reference cursor.
4. Click once on the **right** mouse button to freeze the second cursor and regain the arrow cursor.

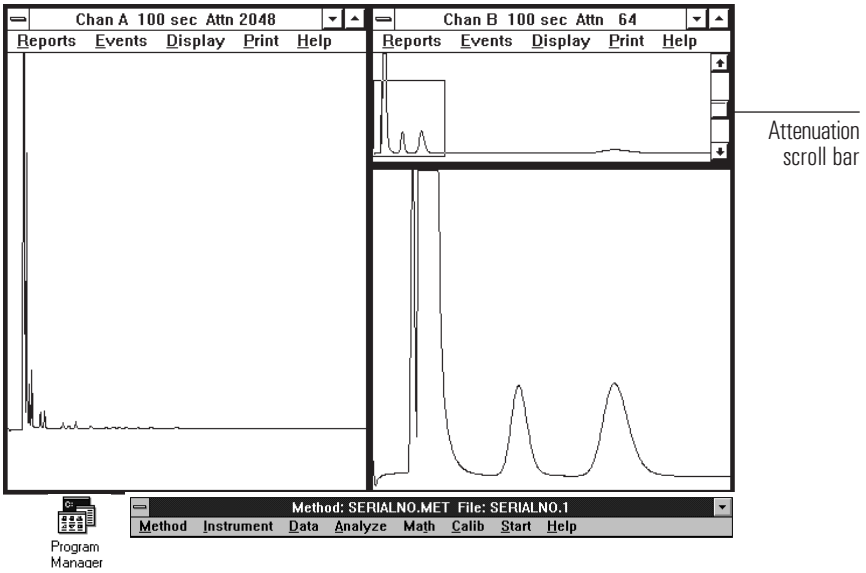


Figure 57 Attenuation scroll bar

Adjusting the attenuation

The full-scale chromatogram displayed in the upper portion of the window may be adjusted by scrolling the display attenuation. To adjust the full- scale magnification:

1. Move the cursor onto the scroll bar at the right side of the upper chromatogram display (See Figure 57).
2. Select the **up** or **down** arrow of the scroll bar to change the attenuation value. (The **up** arrow increases magnification, the **down** arrow reduces it).
3. Observe the change in the upper display.

8

The GC Module Change Tool

The GC Module Change Tool

The GC Module Change Tool is provided to allow you to modify or correct several GC instrument configuration parameters that are specific for a particular module (i.e., injector, column, and/or column heater specific parameters). With this program, you can alter or swap GC module parameters in both the GC firmware and in the computer's configuration record (i.e., HP.INI). The configuration parameters that can be modified through this Program include the following items:

- Column temperature offset
- Column temperature scale
- Maximum column temperature limit
- Fixed or variable loop (volume) injector

When shipped from our factory, your GC was loaded with the GC configuration file appropriate for the particular GC modules installed in your instrument. However, it may be necessary to change a GC's configuration, and thus use this Module Change Tool, when you do any of the following:

1. Install a new GC module or swap positions of existing modules whose configuration record is known.
2. Need to correct an error resulting from installing a GC module.
3. Are asked to alter the GC module configuration by Agilent Technical Support.

You will use the GC Module Change Tool whenever any of the above situations occur and there is a need to update or correct the GC's configuration parameters.

Although these instructions are written for an M/P200 instrument, Quad instrument-specific instructions can be found in Chapter 3 of this manual.

Installing GC Module Change Tool

GC Module Change Tool is automatically installed when you run SETUP.EXE from the GC Tools diskette shipped with your instrument. At the time of installation, the configuration record for your GC is also installed in your PC as the HP.INI file. Since the GC Module Change Tool relies upon the existence of the configuration record, you should run **SETUP.EXE** on each computer that you will want to use with a given GC.

You may install GC Module Change Tool on as many computers as you will use with your GC. No icon is created in the HP Program Group Window for the Module Change Tool, but the file is installed as C:\HP\UTILITY\Change.EXE on your computer system.

Starting GC Module Change Tool

The easiest way to start the GC Module Change is via the Windows Program Manager. From program Manager, select **File\Run.** (See Figure 58.)

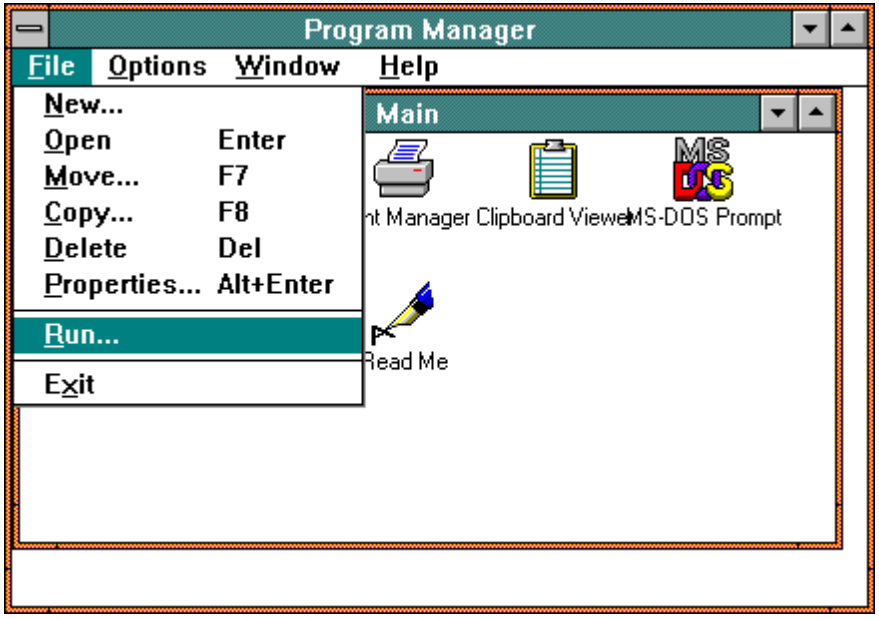


Figure 58 Program manager window

When you select **[Run...]**, you will see a window like Figure 59.

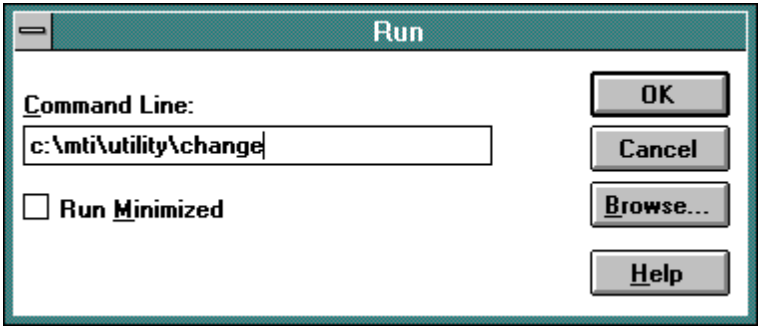


Figure 59 Run window

Type in the complete path and file name of the Module Change Tool as shown in Figure 59 and click **[OK]**. If you've typed in the correct name, the Module Change Tool will search your serial ports for a connected GC and then display a window similar to Figure 60, showing the current module configuration record:

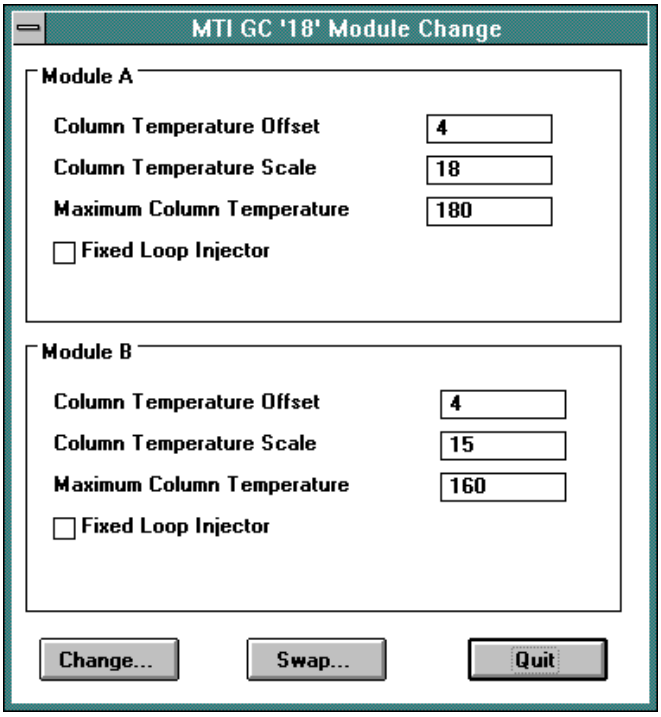


Figure 60 Agilent GC 18 * Module change window

The path shown ("C:\HP\UTILITY\") assumes that you have installed the GC Tools diskette in the standard directory, "C:\HP\". If you have installed the Tools program in any directory other than C:\HP, you will have to enter "**<dir>:" "\UTILITY\CHANGE**", where <dir> is the directory into which you have installed this tool. You can use the **[BROWSE]** button from the Run window (Figure 59) to search your disk for this directory (see Figure 61).

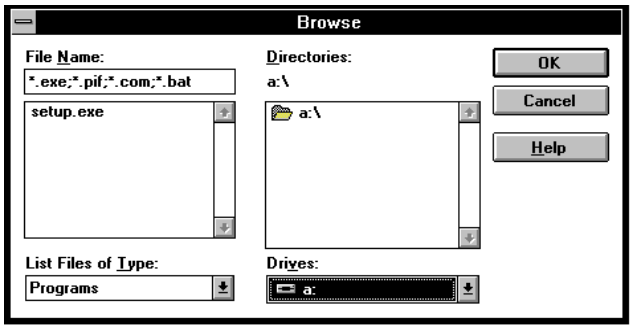


Figure 61 Browse window from run window

If there is no available GC, you will see an error message as shown in Figure 62.

Figure 62 Change window

A GC may be "unavailable" if another program is using it. Windows™ does not allow programs to share serial ports.

If you see the window shown in Figure 62, click **[OK]** and check the following:

1. Check that the GC is connected and that it is ON.
2. If the GC has a front panel, check that the GC is in Remote mode.
3. Check that there are no other programs running in your computer that are trying to use the GC. The safest policy is to make sure that no other GC programs are running (e.g., EZChrom 200/400).

If you are still unable to communicate with the GC after checking all the above conditions, contact Agilent Technical Support for assistance.

Changing a module's configuration record

After you install the new module (as described in the instrument User's Manual), connect the GC to both electrical power and the computer's serial port, turn the GC on, start the GC Module Change Program (described in Chapter 2) and click [**Change...**] button A (as shown in Figure 60). The program will ask you which module you have affected with the change of position as shown in Figure 63.

Figure 63 shows a top view of the GC (with the top cover removed) displaying the position of modules A and B.

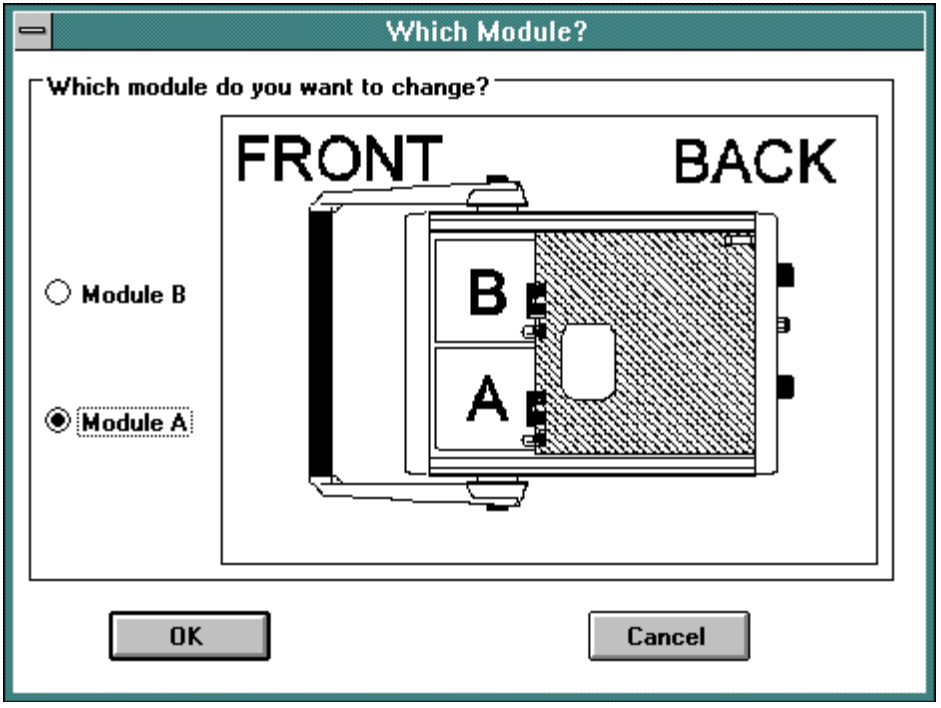


Figure 63 Which module? Window

Click the **[Module A]** or [**Module B]** button depending upon which module's location has altered and then click **[OK]**. Use the diagram on the right-hand side of the window to determine which module you have replaced and wish to update with the correct configuration file. You can also click on the diagram of the GC–clicking on the upper half selects module B and clicking on the lower half selects module A.

When using GC Module Change Tool for a Quad instrument, make sure only one "side" of the GC is connected. The program will "see" the GC as an M200/P200 having modules A and B. If the C/D "side" is connected, module "A" represents module C and Module "B" represents module D.

GC Module Change Tool for a Quad instrument

The GC Module Change Tool allows you to alter or swap GC Module parameters in the GC and the HP.INI file ("configuration record") when a physical GC module is replaced.

The Module Change program can also be used to assist in changing the configuration record for modules in a Quad GC, but the procedure outlined below should be followed:

1. Terminate all software (e.g., EZChrom) that is using the GC and disconnect all serial cables between the GC and your computer.
2. Determine which module you will/have replace(d) (i.e., A, B, C or D).
3. If module A or B is replaced, find the *RS232* (DB-9) connector labeled "A and B" on the right-hand side of the GC (on your left, if you're looking at the back panel). If module C or D, find the *RS232* (DB-9) connector labeled "C and D" on the left-hand side of the GC (on your right, if you're looking at the back panel).
4. Connect a serial cable from the connector that you located in step 2 to your computer's serial port (it does not matter which serial port, but make sure that no other GCs are connected and that there is no other connection to

your Quad). If there is only one serial cable connected to your computer, you're OK.

5. Run the Module Change Tool. To change module A or C, select **[Module A]** in the Module Changer; to change module B or D, select **[Module B]** in the Module Changer.

You will then see a window that allows you to edit the GC's module configuration record. (See Figure 62). Click on [**Cancel**] if you want to quit without making changes.

Caution It's a good idea to write the original configuration down somewhere before changing anything. That way, if you make a mistake, you can recover from it by clicking the [**Change...**] button again, selecting the same module and reentering the original configuration from your notes.

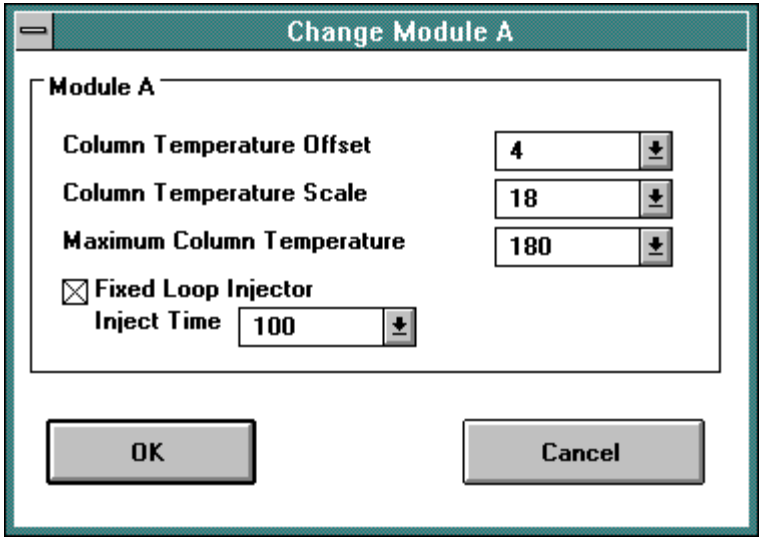


Figure 64 Change module "A" window

The new module configuration parameters can be found on the label on the module cover.

Change the configuration by selecting items from the drop-down lists and/or checking the **Fixed Loop Injector** check box. Be sure that you enter the data correctly from the module's label.

When you click [**OK**], the GC's configuration will be updated and the Change Module window will disappear. Click [**Cancel**] if you want to quit without making changes.

The Inject Time selector will only be shown when the Fixed Loop injector is checked.

If you are swapping module positions

If you have swapped modules (i.e., you have simply moved module A to the module B position, and module B to the module A position), you will not need to manually enter any data. Simply click the **[Swap...]** button (as shown in Figure 60). After asking if you're sure of this choice, the program will update the GC's configuration file to reflect the swap. If you make a mistake here, you can always click the [**Swap...**] button again to get things back to their original configuration parameters.

If you are using a Quad GC, you may only swap modules A and B or modules C and D and use the **[Swap..]** button. You cannot swap any other combination of modules and use the **[Swap..]** button. However, if you choose to swap any other combination of modules (other than A and B or C and D), you should write down the current configuration parameters for the two modules on a notepad and enter each module's configuration record to the other's record.

9

The GC Verification Tool

The GC Verification Tool

Your GC was shipped with a GC Tool diskette which contains a record of the proper instrument configuration (operational parameters) for your particular GC and two GC Tools programs; GC Verification and GC Module Change.

The GC Verification Tool is used to verify and/or restore your instrument configuration record.

You can use GC Verification at any time to verify that your GC firmware is in proper working order, to restore its configuration parameters, clear error conditions or recover a lost configuration record.

You should run GC Verification whenever any of the following conditions pertain:

1. You have just unpacked a new chromatograph and want to verify that it is in good condition.
2. EZChrom, or other software, reports that the GC has an error condition.
3. The main controller board is changed.
4. The EPROM (U32) is changed.
5. The GC configuration record is changed.
6. The GC battery-backed RAM is zeroed or otherwise compromised.

GC Verification requires no specialized knowledge of a GC beyond its serial number. It will “walk you through” whatever procedures you can perform to establish and/or restore a proper configuration.

GC Verification only verifies that the embedded computer system firmware in the GC is OK. It does not check the columns, or other chromatography apparatus, nor does it perform any special hardware checks.

Installing GC Verification Tool

GC Verification is automatically installed when you run SETUP.EXE from a GC Tools diskette. At this time, the configuration record for your GC is also installed in your PC. Since GC Verification relies upon the existence of the configuration record, you should run SETUP.EXE on each computer that you will want to use with a given GC.

You may install GC Verification on as many computers as you will use with your GC.

Starting GC Verification Tool

To start GC Verification, double-click upon the GC Verification icon (the one with a green check mark on it) in the HP Program Group Window as shown in Figure 65.

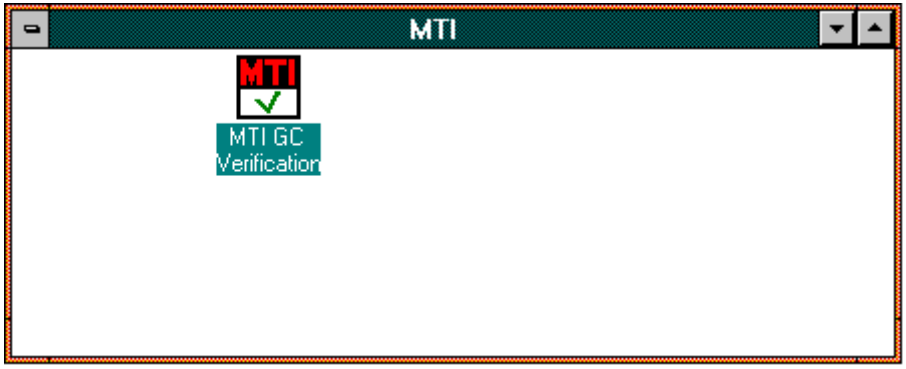


Figure 65 HP program group window

The first window you will see is the Introduction Window as shown in Figure 66.

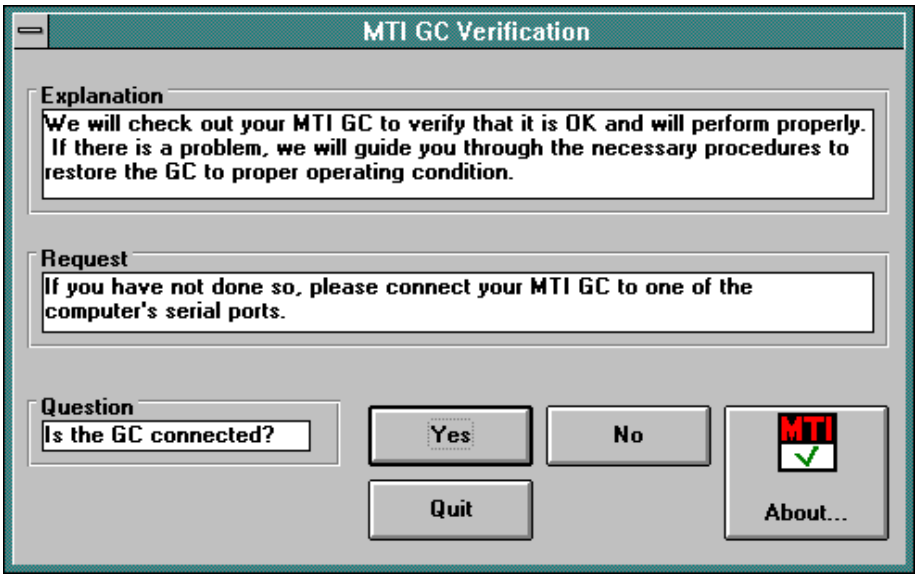


Figure 66 Agilent verification introduction window

In general, GC Verification windows will have three text items:

Explanation A short explanation of the purpose or use of a particular window

Request If you are expected to do anything in particular, the **Request** item tells you what the program wants you to do.

Question If GC Verification needs any information from you, a **Question** item will pose a question which you answer by clicking a button in the window.

This window asks you to verify that a GC is connected to your computer. Click **[Yes]** when you have connected your GC to the serial port of your computer and are ready to proceed. If you click **[Quit]**, GC Verification will terminate.

If you click **[No]**, GC Verification Tool will tell you that it can not proceed without a GC as shown in Figure 67.

Figure 67 GC verification window

If you click **[About...]**, GC Verification will tell you about itself as shown in Figure 68

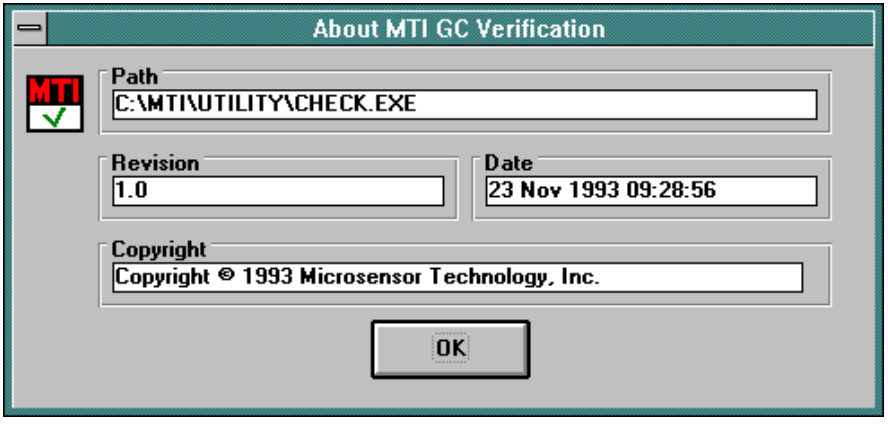


Figure 68 About GC verification window

It does not matter which serial port you use. GC Verification will automatically search the serial ports available to it for a connected GC.

Typical sessions

In general, there are two types of sessions you will encounter when you are using GC Verification. If your GC is OK, there is a simple sequence of windows which will be displayed as the condition of the GC is verified. If there is a problem, there are a large number of possible window display sequences, depending on the type of problem which has occurred. In this section we will illustrate cases when your GC is OK and when a GC is experiencing problems.

What happens if your GC is OK

The first window is the introduction window as shown in Figure 69.

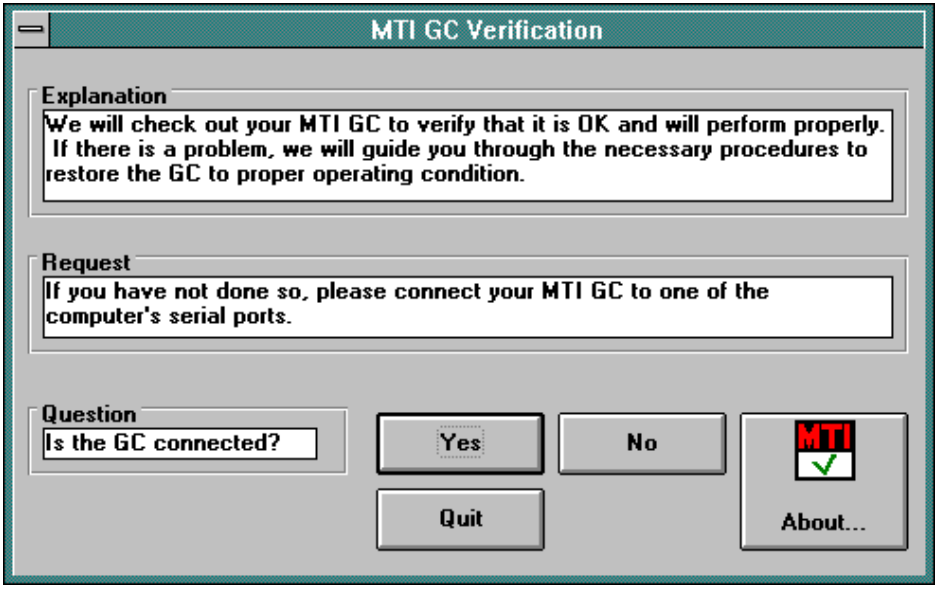


Figure 69 Introduction window

Connect your GC and click **[Yes]**. GC Verification checks each serial port on your computer system for an M200. During this time a window like Figure 70 is displayed:

Figure 70 Searching for a GC window

If no GC is found, an error message is displayed in the status box as shown in Figure 71. When you click **[OK]**, GC Verification returns to the Introduction Window.

Figure 71 Searching for a GC window

If a GC is found, the window displays its serial number in the Status box and, when you click **[OK]**, GC Verification proceeds with checking the GC. In Figure 72, the '18' represents the serial number of the GC. Your Status box should display the serial number of your particular GC.

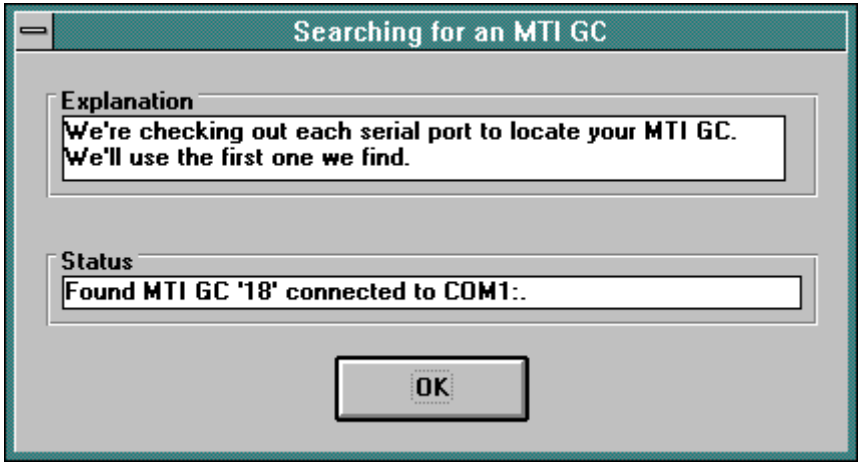


Figure 72 Searching for a GC window

If your GC is OK, the next (and final) window you will see is as shown in Figure 73.

Figure 73 GC verification successful window

If you get to this window, the GC has been verified and is ready for use. Click **[OK]** to terminate GC Verification.

What happens if your GC has problems

If your GC has problems, there are several possible scenarios, but they all lead to one of two outcomes: either GC Verification will be successful or it will not be successful.

What happens if your GC has problems that can be fixed

Here is what a successful error fixing session would look like. First you get the introduction as shown in Figure 74.

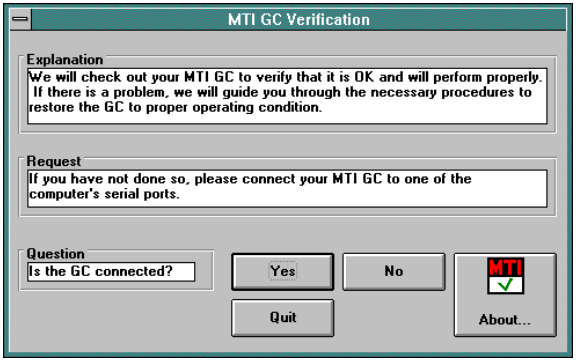


Figure 74 GC verification window

Click **[Yes]** and GC Verification establishes a connection to the GC as shown in Figure 75.

Figure 75 Searching for GC window

The first indication that there is a problem is that the GC name is indicated as ‘’, which implies that it is empty as shown in Figure 75. Click **[OK]** and GC Verification confirms that there are problems as shown in Figure 76.

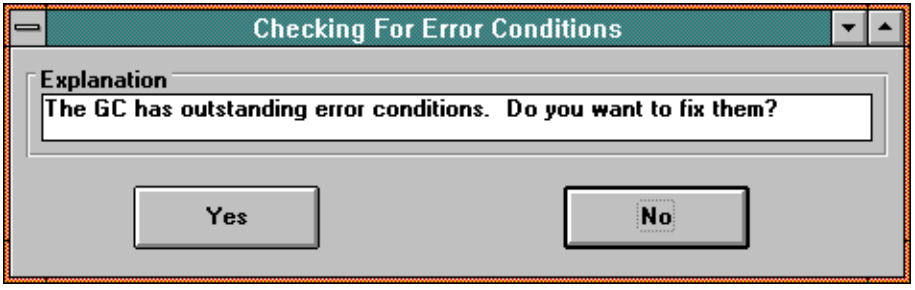


Figure 76 Error condition window

Since you want a functioning GC, click **[Yes]**. The next window (Figure 77) advises you that the GC has forgotten its name (i.e., serial number).

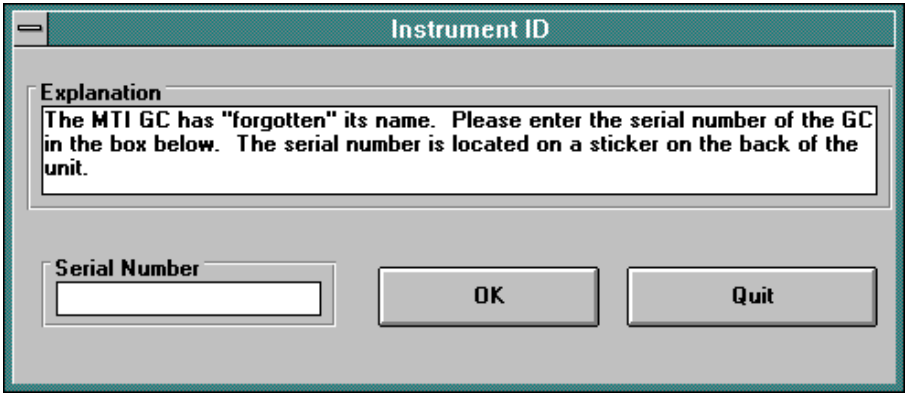


Figure 77 Instrument ID window

Type in the serial number (which is found on a sticker on the rear panel of the GC) and click **[OK]**. If you have typed in the serial number correctly, GC Verification will restore its configuration as shown in Figure 78.

Figure 78 Status window

After the configuration is restored, you will probably have to cycle the power to the GC. GC Verification Tool automatically detects when this is the case and walks you through this process.

First you will have to turn the GC OFF as requested in Figure 79.

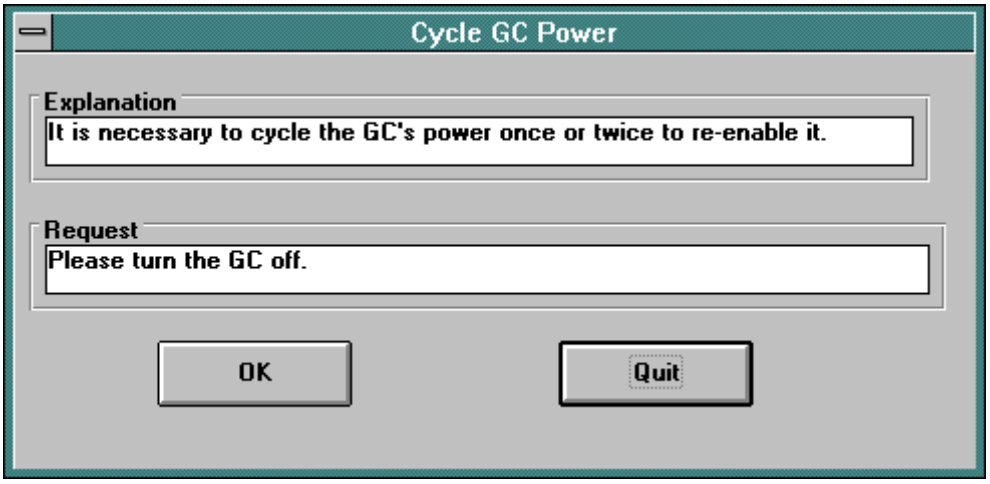


Figure 79 Cycle GC power window

Unplug the GC first and then click the **[OK]** button. Now you need to turn the GC back ON as requested in Figure 80.

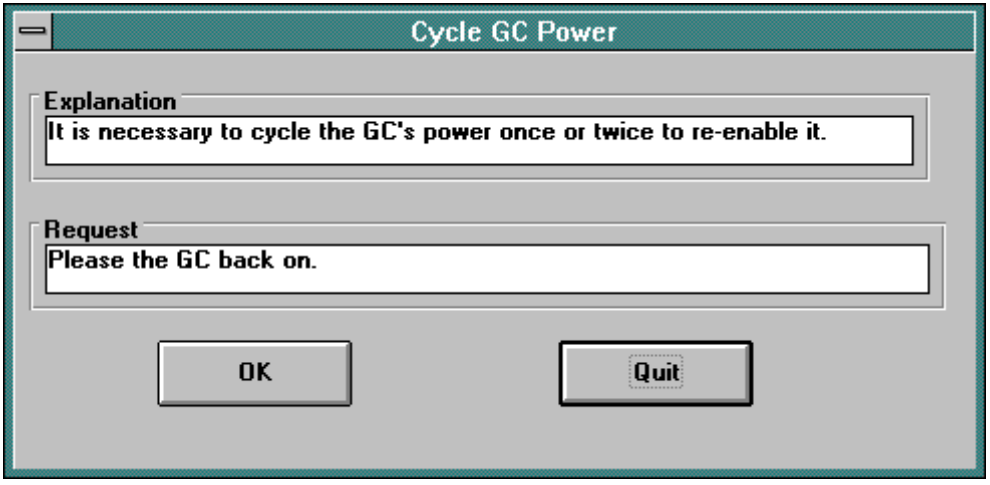


Figure 80 Cycle GC power window

Plug the GC back in and then click the **[OK]** button.

Because of the way certain options are implemented, it will probably be necessary to cycle the power again. GC Verification will know if this is the case and, if so, it will ask you to turn the GC OFF and then back ON again.

When the sequence is done, you will see the window illustrated in Figure 81.

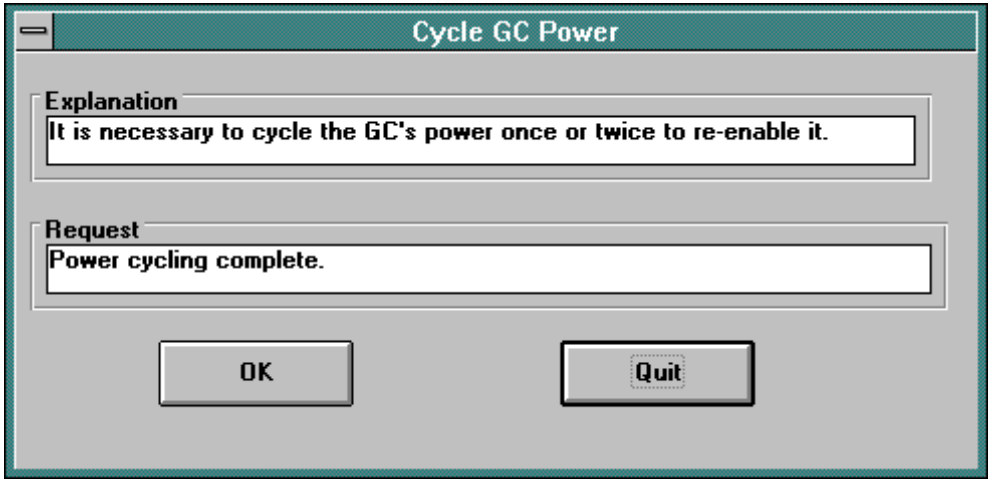


Figure 81 Cycle GC power window

Click **[OK]**.

If there were problems with memory that might have compromised the column head pressure (CHP) offset data stored in the memory, you will see the window displayed in Figure 82.

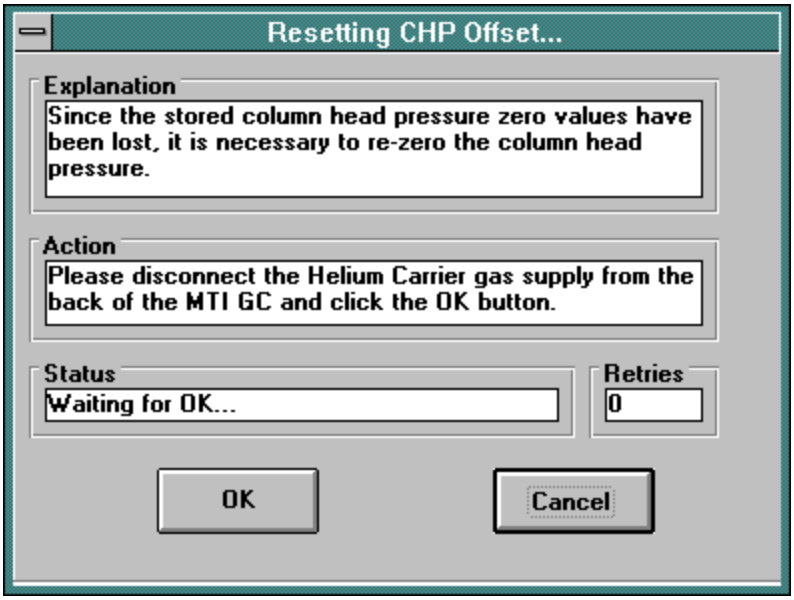


Figure 82 Resetting CHP offset window

Disconnect the helium carrier from the back of the GC and click the **[OK]** button. GC Verification will repeatedly send a command to set the CHP zero and then check that the CHP actually is zero. Each time through this process, the number of retries is incremented and displayed in the Retries box. The process should be complete within one to five retries; if it goes longer, there may be a problem with the carrier gas plumbing. If the number of retries becomes excessive (more than 10 or so), click **[Cancel]** and call Agilent Technical Support.

Be sure that you actually disconnect the carrier at the fitting on the rear panel of the GC. It is not enough to simply turn off the carrier supply, it must be allowed to fall to atmospheric pressure. When the pressure measured at the pressure transducer remains steady for greater than one second, the measured pressure value will be taken as zero psig, regardless of whether it is atmospheric pressure or the pressure remaining in the attached carrier gas line. If the measured pressure is anything other than atmospheric pressure, this will cause an error in the pressure readings equivalent to the difference.

If the CHP reset is successful, the window display will read as shown in Figure 83.

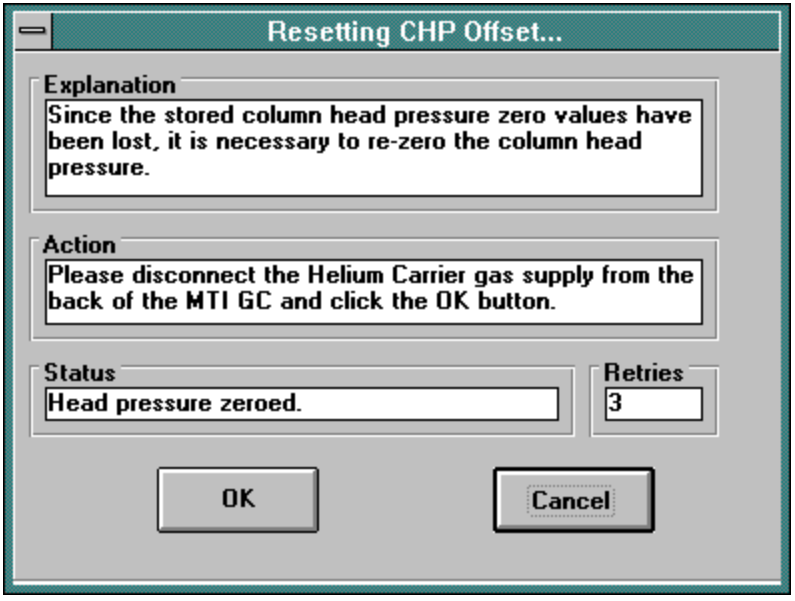


Figure 83 Resetting CHP offset window

Click **[OK]** to continue.

Usually this will fix any problems and you will see the GC Verification Successful window as shown in Figure 84.

Figure 84 GC verification successful window

What happens if your GC has problems that cannot be fixed

If a problem arises that GC Verification can not solve, you will see a window as shown in Figure 85.

Figure 85 Call technical support window

If you get a window as shown in Figure 85, the problem is probably not one that you can fix. Call Agilent Technical Support and tell them what message is displayed in the **Subject** box so that they will know how to help you. The contents of the **Subject** box will vary with the precise nature of the problem, so it is probably a good idea to contact Agilent Technical Support while the window is on your screen so that you can read it back verbatim.

What happens if your GC is OK, but the configuration record is missing

This can happen if you use different computers with your GC or if the setup disks that came with your GC have been lost.

The first window is the Introduction Window shown in Figure 86.

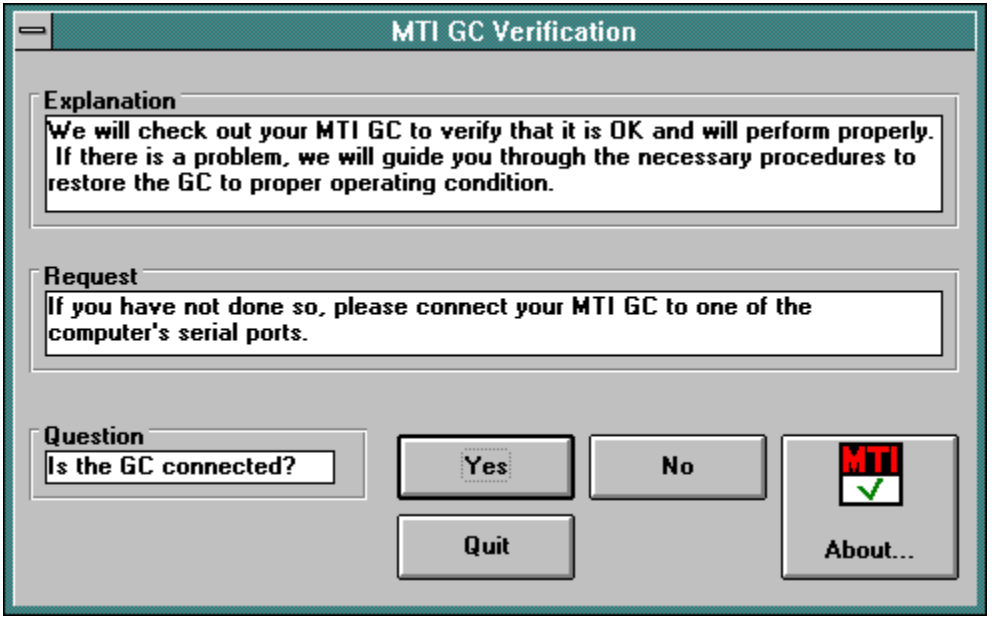


Figure 86 GC verification window

Connect your GC and click **[Yes]**. GC Verification checks each serial port on your computer system for an M200. During this time, a window like Figure 87 is displayed:

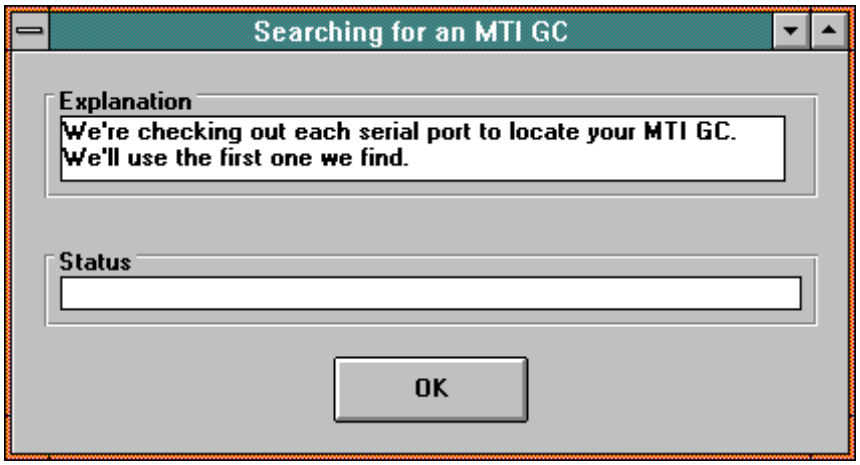


Figure 87 Searching for a GC window

If no GC is found, an error message is displayed in the status box and, when you click **[OK]**, GC Verification returns to the Introduction Window shown in Figure 88.

Figure 88 Searching for a GC window

If a GC is found, the window displays its serial number in the Status box and, when you click **[OK]**, GC Verification proceeds with checking the GC (Figure 89).

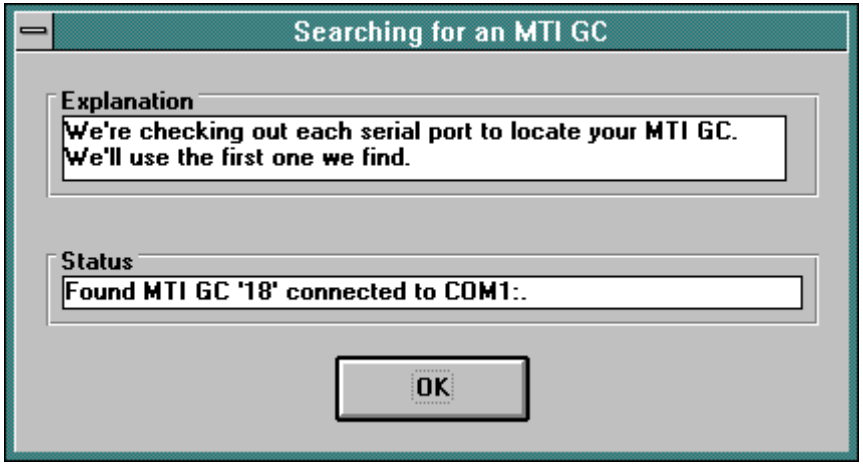


Figure 89 Searching for a GC window

If your GC appears to have no errors but the verification program can not locate a configuration record for the GC, you will see a screen like the one in Figure 90.

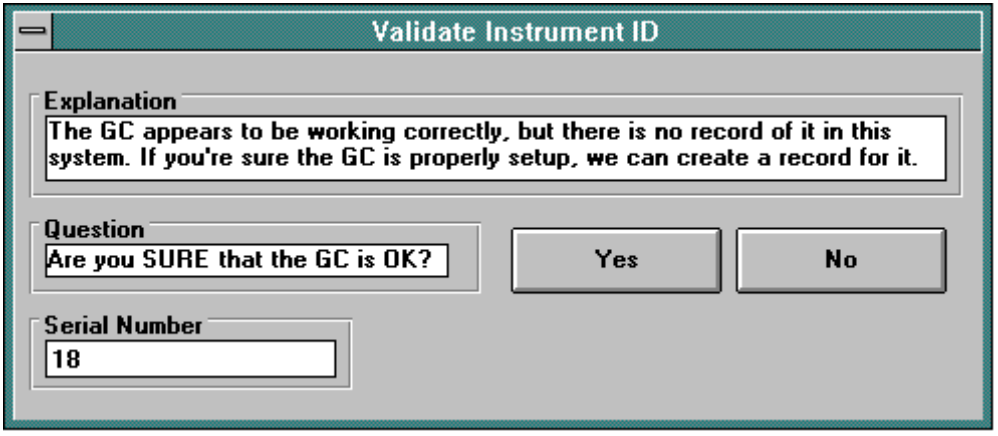


Figure 90 Validate instrument ID window

At this point, it is important to realize that the actual configuration record of the GC cannot have been verified because the configuration record is missing. The GC, however, does appear to be working properly and there are no errors in the GC's memory where it has a stored copy of the configuration record. If you know of no other problems with the GC and you have not performed any replacement or swapping of modules since the configuration record was last verified, click **[Yes]**. The Verification Tool will create a new configuration record for the GC in your computer system and continue with the verification process.

If you are unsure whether your GC is properly configured, you can call Agilent Technical Support to find out how to manually verify the configuration. In this case, click **[No]** and you will see the screen in Figure 91.

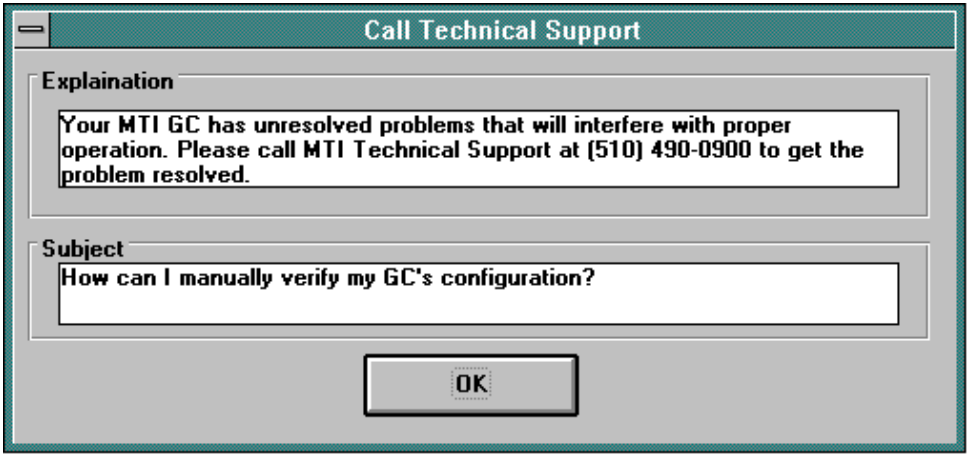


Figure 91 Call technical support window

Terminating GC Verification Tool

GC Verification terminates automatically when you click **[OK]** in an GC Verification Successful (Figure 9) or Call Technical Support Window. Some windows, such as the Introduction Window (Figure 5), have **[Quit]** buttons that can also be used to terminate GC Verification Tool.

Explanation of GC Verification windows

The display windows of the GC Verification Tool are alphabetically listed by window title.

About GC verification

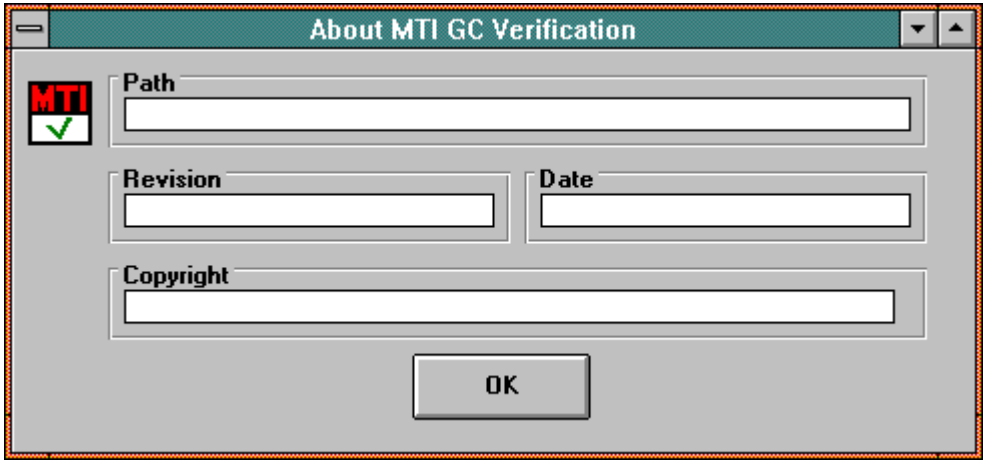


Figure 92 About GC verification window

This window is shown when you click the **[About...]** button in the GC Verification main window. It displays the path name of the executable file, its revision, the date the program was created and the owner of the copyright.

Call Technical Support

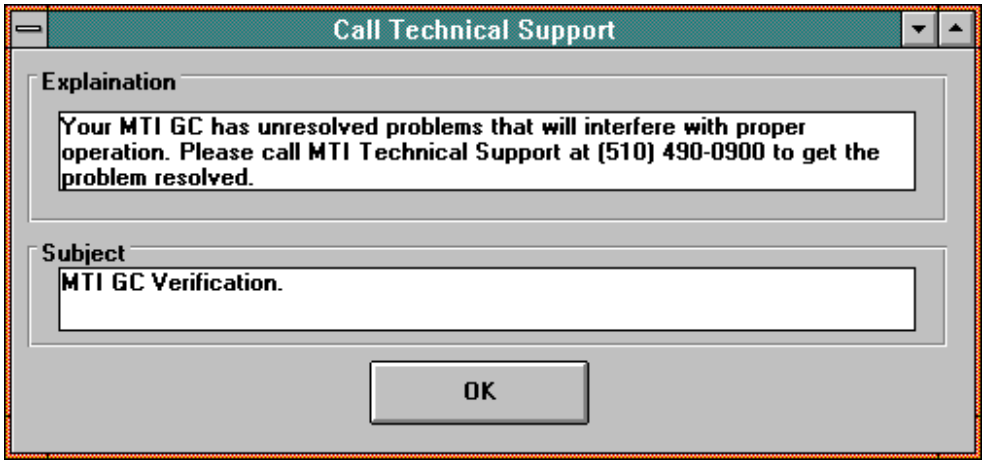


Figure 93 Call technical support window

A window like Figure 93 is drawn whenever GC Verification encounters a problem that it cannot fix. Call Agilent Technical Support and give them, verbatim, the message displayed in the **Subject** box; the message will vary with the nature of the problem.

Checking for error conditions

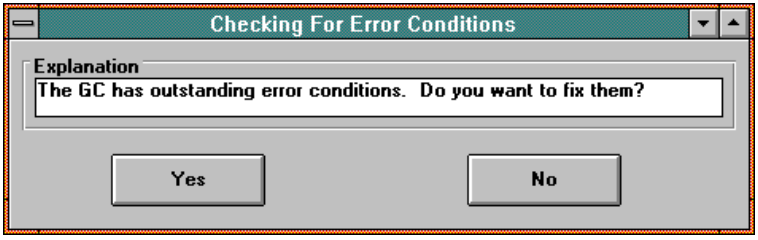


Figure 94 Checking for error conditions window

The window shown in Figure 94 is displayed if GC Verification finds that the GC has error conditions. Click **[Yes]** to attempt to fix the errors, or **[No]** to terminate GC Verification.

Cycle GC power

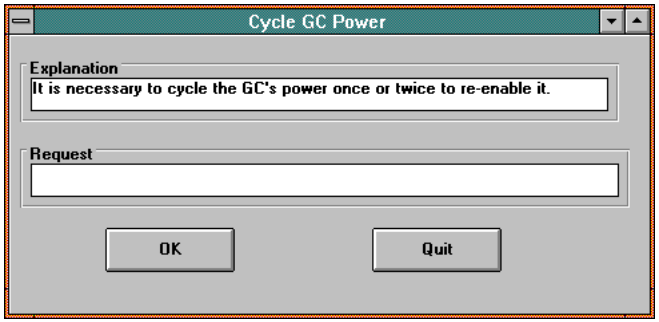


Figure 95 Cycle GC power window

When it is necessary to cycle the GC's power (i.e., turn it on and off), the window in Figure 95 will be displayed. First perform the requested action (turn power on or off), then click **[OK]**. Click **[Quit]** to terminate GC Verification. Pay close attention to the text in the **Request** box.

Have HP GC setup disk?

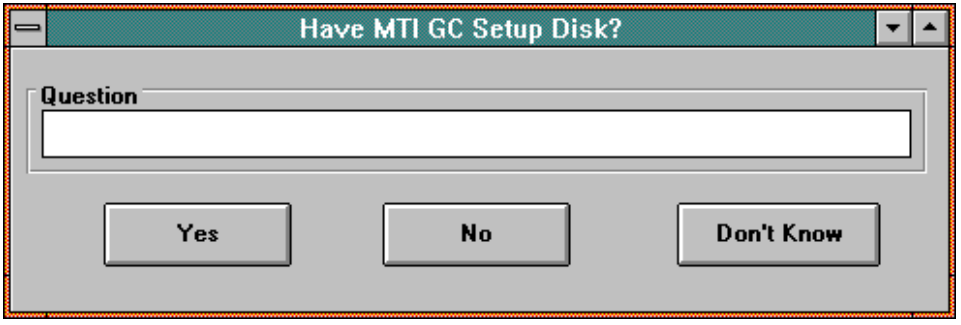


Figure 96 Have GC setup disk? Window

If a configuration record for a GC cannot be located on your computer, GC Verification will ask if you still have the setup disk. Click **[Yes]** if you do, **[No]** if you don't or **[Don't Know]** if you are unsure. GC Verification will tell you what you should do next.

Instrument ID

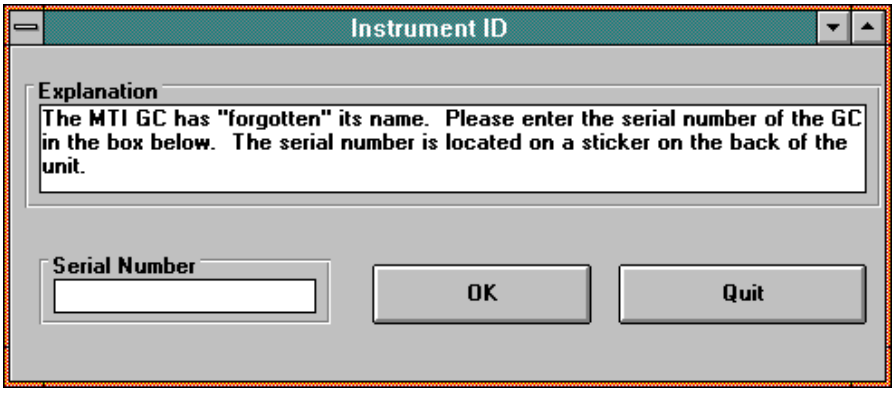


Figure 97 Instrument ID window

If the GC has lost its serial number, GC Verification will display this window. Type in the serial number as it appears on the label on the back of your GC and click **[OK]**. Click **[Quit]** to terminate GC Verification.

Invalid or unknown GC ID

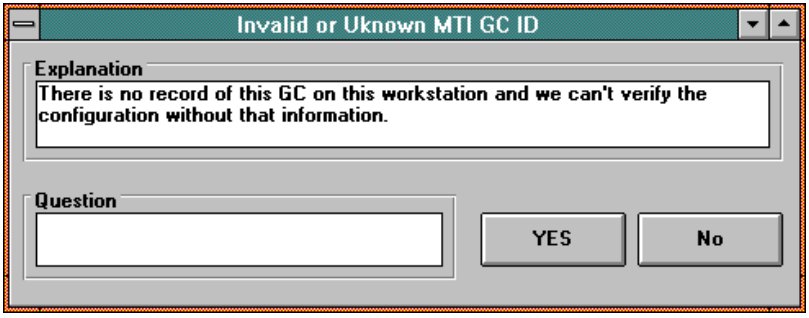


Figure 98 Invalid or unknown GC ID window

If the serial number you enter does not match any numbers on file, GC Verification will display this window, asking you to double-check the serial number. Click **[Yes]** if you are sure it is the right one, or **[No]** if you want to re-enter the number.

GC verification

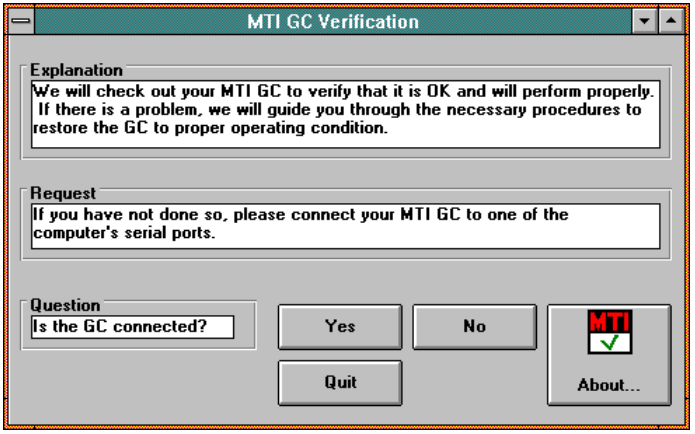


Figure 99 GC verification window

Figure 99 shows the first window that GC Verification displays. Click **[Yes]** if your GC is connected, **[No]** if it is not, **[Quit]** to terminate GC Verification or **[About...]** to find out which version of GC Verification Tool you are using.

GC verification successful

Figure 100 GC verification successful window

Figure 100 shows the window that will be displayed when your GC is OK and either there were no problems or all problems have been fixed. Click **[OK]** to terminate GC Verification.

(Re)Install the GC support software

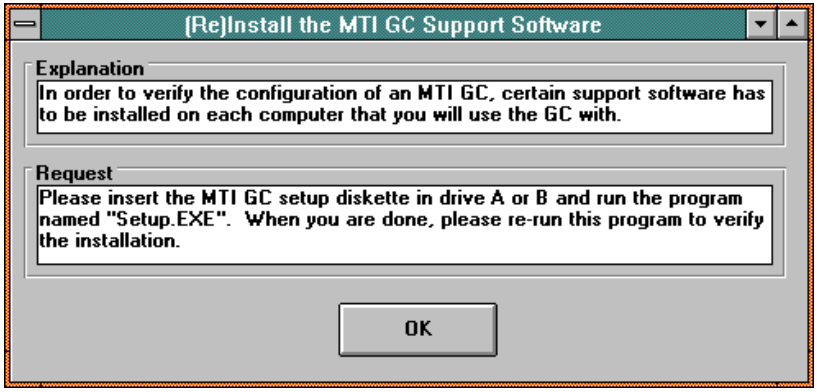


Figure 101 (Re)Install the GC support software window

If there is no configuration record on file but you have an GC Tools diskette for the GC, GC Verification will display this window to ask you to set up the GC on the computer you are currently using.

Resetting CHP Offset

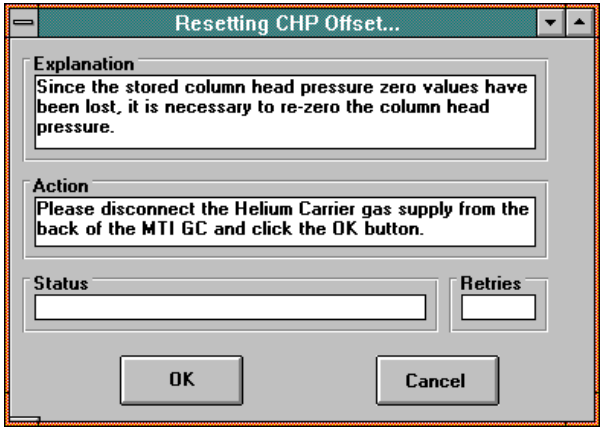


Figure 102 Resetting CHP offset window

If necessary, the window in Figure 102 walks you through resetting the column head pressure transducers. Disconnect the helium carrier at the fitting on the rear panel of the GC (do not just turn it off at a valve) and click the **[OK]** button. If the number of retries becomes excessive (more than 10), click **[Cancel]**.

Searching for a GC

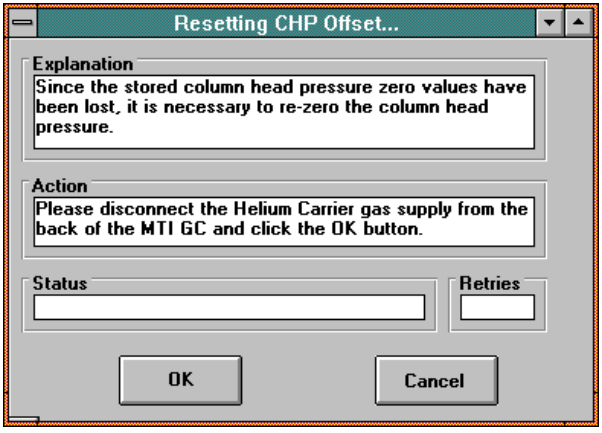


Figure 103 Searching for a GC window

GC Verification automatically locates the first GC connected to your computer by checking each serial port, in turn, for a device that behaves like a GC. The window shown in Figure 103 is displayed to let you monitor that process. Click **[OK]** to continue.

Status: Restoring GC configuration

Figure 104 Status window

If the GC's configuration needs to be restored from the saved configuration record, the message shown in Figure 104 will be displayed.

Validate Instrument ID

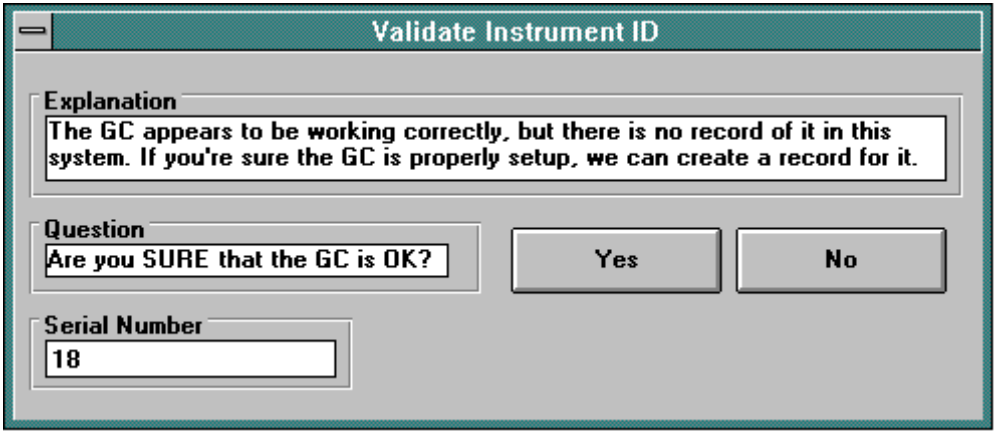


Figure 105 Validate instrument ID window

Your GC appears to be OK, but the configuration record is missing from your system. Click **[Yes]** to (re)create the configuration record, **[No]** if you are unsure about whether or not the GC is properly configured.

Compatibility and computer system requirements

GC Verification requires that your computer meet the following specification:

- 80386 or better
- At least 2 Mb RAM
- At least 2Mb free Disk Space (before installation)
- At least 1Kb free disk space (for new configuration records)
- Windows Version 3.1 or greater

10

Common Questions

Common Questions

I have a printer connected to my computer, but I cannot get it to work with the EZChrom 200/400 Data System.

1. Make sure the printer is configured on, and connected to LPT1. Check Windows Control Panel - Printers for setup.
2. Make sure the correct printer driver has been installed during Windows installation.

I can't figure out how to delete data and/or method files.

1. To delete Method files, select the Method menu and the Open command. A list of method files will appear on the screen. Click the left mouse button on the file, or files, to be deleted. They will be highlighted, as evidenced by the file name being blackened. Press the **Delete** key to remove the files.
2. To delete Data files, select the Data menu and the Open command. A list of data files will appear on the screen. Click the left mouse button on the file, or files, to be deleted. They will be highlighted, as evidenced by the file name being blackened. Press the **Delete** key to remove the files.
3. To delete EZChrom files, you can also use the File Manager program provided with Windows.
    a. Open the File Manager executable located in the Main group of the Program Manager.
    b. Files are deleted by selecting File and Delete and then entering the fully qualified file name that is to be deleted.

I can't communicate with the GC.

1. Press **<ESC>** if EZChrom 200/400 is hung up.
2. Make sure the proper COM port is currently attached to the instrument (see Installation troubleshooting, page 14).

One of my applications closes itself and disappears from the screen. What is happening?

1. This phenomenon usually occurs when there is not enough RAM available to open EZChrom's three or four program windows. This can happen if you have tried to start EZChrom directly from Windows (using the EZSTART icon) while having other Windows applications opened. These other applications require memory which your computer may not be able to spare. Therefore make sure all Windows applications besides EZChrom are closed before you start EZChrom with EZSTART.
2. You do not have the required 4 MB of free RAM required to run EZChrom. If you are using a RAMDRIVE or a software RAM cache, you may have to decrease its capacity to free up the required RAM memory.

I am having trouble integrating peaks. What do I do?

If the peak is not being integrated:

1. Check the PKWD graphically at the base of the missed peak and make sure it corresponds in time and value to a PKWD event in the Timed Events table.
2. Check the SLPSEN and FINE SLPSEN graphically on a representative section of baseline without peaks and make sure they are set correctly.
3. Check to see if there are any INTG OFF periods that overlap any portion of the peak. If there are, delete or change them.
4. Check that the baseline has not been turned off.

If the peak is found, but the baseline is too high on the peak:

1. The SLPSEN and/or the FINE SLPSEN values are too high. Check and reset them graphically. (See page 99 for suggested values.)
2. The PKWD is too small, try dou*bling it.*

If too many peaks are found:

1. The SLPSEN and/or FINE SLPSEN value(s) is too low. Reset it on a region of baseline. (See page 99 for suggested values.)

If peaks are identified graphically, but the reports are empty:

1. Reset the retention time and retention time windows graphically.

   (See page 46 for a description on how to set retention times graphically.)

2. Make sure the multiplier in the Calibration Setup is not equal to zero. (See page 67 and Figure 50 for further explanation.)

After analysis the calibrated results in the External Standard report do not match the calibration amounts I entered:

1. Make sure that you select the CALIB menu and then the proper calibration level prior to analyzing the data.

My data and/or method files are not saved or can not be copied with the File Manager. What is happening?

1. The hard disk or storage device is full.
2. You have put illegal characters or spaces in your filename. Illegal characters are . " / \ { } : < > + = ; , ?

Appendix A

Error Number Description

Error Number Description

EZChrom 200/400 checks for communication errors while transferring data to and from the gas chromatograph. If an error occurs, you are prompted with a dialog box that displays the error number. Refer to the list below for a description of each possible error number.

Note that more than one of these errors can occur at one time. In such a case, the error number reported will be the sum of the numbers of the errors that occurred. For example, if error 1 and 2 both occurred, then the error number reported would be their sum, or 3.

Errors 1 and 2 usually occur when the computer is not fast enough to keep up the communication at the required speed. Running other programs in the background can cause these error to occur. All of the other errors are usually caused by some sort of communications failure in the hardware, either at the computer or at the chromatograph.

1	Receiving queue overflowed. There was either no room in the input queue or a character was received after the end-of-file character was received.
2	Character was not read from the hardware before the next character arrived. The character was lost.
4	Hardware detected a parity error.
8	Hardware detected a framing condition.
16	Hardware detected a break condition.
32	CTS (clear-to-send) timeout.
64	DSR (data-set-ready) timeout.

128	RLSD (receive-line-signal-detect) timeout.
256	Transmission queue was full when a function attempted to queue a character.

Appendix B

Dynamic Data Exchange (DDE)

Dynamic Data Exchange (DDE)

EZChrom is now extensible through the use of Microsoft's Visual Basic. You can write a program in Visual Basic that can control EZChrom 200/400. This gives you the ability to generate custom reports from analyzed data, or to create something as complex as on on-line process monitoring system. Agilent Technologies does not provide programmable applications but the framework on which to build them.

Overview

Features

A small, but powerful, set of commands are available to the Visual Basic programmer. EZChrom 200/400 will perform the following functions at the request of a Visual Basic program.

1. Acquire a new data file from the GC and return the results of the analysis.
2. Perform a calibration.
3. Recall a run from disk and return the results of the analysis.
4. Recall a method from a disk.
5. Return the status of the GC.
6. Return the conditions under which the current sample was acquired.

Example program

An example program is included with the EZChrom 200/400 data system in the form of a normalized report generator. It demonstrates how to use of some of the features described above. It is intended to be a simple example that can be used as a starting point for application development.

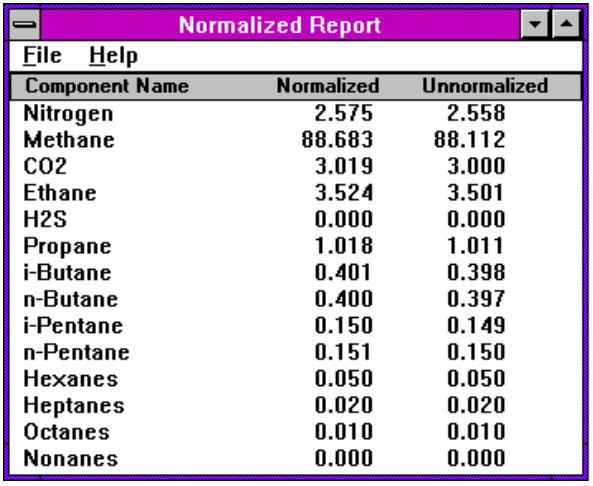


Figure 106 Normalized report window

The window above shows a typical output from the example program.

After the program is started it looks for the EZChrom 200/400 DDE server. If it finds the server it establishes a DDE conversation. Note that this implies EZChrom 200/400 must be loaded before the example program in order for it to work properly. Once the conversation is established the example program will wait for a message from EZChrom 200/400. When EZChrom 200/400 has finished analyzing data it sends a message to the example program to let it know that there is new data.

When there is new data the example program will copy the data into private storage and then perform a normalization on the data. The normalized and unnormalized data is then displayed on the screen. The example program then goes back to waiting for a message from EZChrom 200/400.

EZChrom DDE server

The EZChrom 200/400 control interface is comprised of a Dynamic Data Exchange(DDE) server and a companion Dynamic Link Library(DLL). EZChrom 200/400 accepts commands from the DDE client and returns analysis results through the DLL.

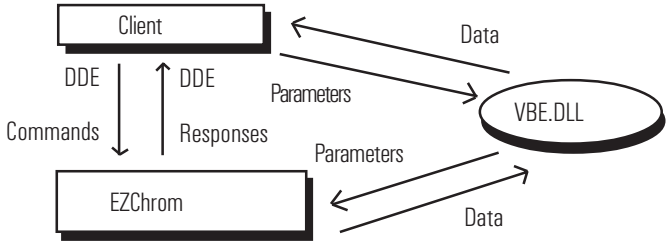


Figure 107 Server support

The DDE server supports a single item. The application name is “EZChrom”, the topic name is “Execute”, and the item name is “Run”. All communication takes place over this single link. Commands are poked to the server by the client. The server responds to the command by sending the response back to the client. Command parameters are passed through the DLL. Furthermore the results of the chromatographic analysis are stored in the DLL for retrieval by the client. The DLL contains all the functions necessary to access the results. Functions also exist for setting command parameters.

Commands

Name: OpenMethod

Parameter: Method name.

Remarks: This command will cause EZChrom 200/400 to open the method file that is passed as the parameter. The method name can be either a fully qualified path to a method file, or if only the name is given the file is assumed to reside in the current method directory.

Name: Sample
Parameter: Sample Name
Remarks: Get new data from the GC using the parameter as the run id. Sets the number of runs to one. If the parameter is not the empty string then the data is saved in the file specified by the parameter. Print, DIF, PRN, Ext, and User Program are not supported with this command.

Name: Calib
Parameter: Level
Remarks: Setup a calibration for the level specified by the parameter. The parameter must be between 1 and 8 inclusive. Note this command does not do any analysis with the GC.

Name: Analyze
Parameter: Data file name
Remarks: Opens the data file specified by the parameter and analyzes it. If the parameter is the empty string then the current data file is analyzed.

Name: GetMethName
Parameter: None
Remarks: Get the fully qualified path to the current EZChrom 200/400 method file.

Name: GetDataName
Parameter: None
Remarks: Get the fully qualified path to the current EZChrom 200/400 data file.

Name: GetCycleTime
Parameter: None
Remarks: Returns the maximum of the run times for all of the channels plus the sample pump run time.

Name: GetCalLevels
Parameter: None
Remarks: Returns the number of calibration levels.

Name: GetCalRuns
Parameter: None
Remarks: Returns the number of calibration runs per calibration level.

Name: GetInstStatus
Parameter: x
Remarks: Returns the current instrument status for channel x.

Name: GetRunCond
Parameter: x
Remarks: Returns the run conditions for channel x.

Return value formats

All of the following commands return their data via the DDE link. DDE uses text strings to pass data, therefore the data from some of these commands will need to be decoded. The format of the data that is returned is described below.

Name: GetMethName
Format: The path to the current method is returned. For example C:\HP\EZCHROM 200\200\METHODS\EXAMPLE.MET.

Name: GetDataName
Format: The path to the current data file is returned. For example C:\HP\EZCHROM 200\200\CHROM\EXAMPLE.1.

Name: GetCycleTime
Format: The cycle time is returned as a string. For example, if 120 is the cycle time "120" will be returned. Note the return value will have to be converted to a number before it can be used.

Name: GetCalLevels
Format: The number of calibration levels are returned as a string. For example, if there are 4 calibration levels "4" will be returned.

Note the return value will have to be converted to a number before it can be used.

Name: GetCalRuns
Format: The number of calibration runs per levels is returned as a string. For example, if there are 5 calibration runs per levels "5" will be returned. Note the return value will have to be converted to a number before it can be used.

Name: GetInstStatus

Format: The current instrument status for the specified channel is returned as a tab delimited string. The format is as follows:

Column Temperature<TAB>
Column Head Pressure<TAB>
Detector Auto Zero Voltage<TAB>
Detector Status<TAB>
Battery %<TAB>
Firmware Version

For example, the value returned may look like

60<TAB>15.1<TAB>27<TAB>On<TAB>100<TAB>17.5

Name: GetRunCond

Format: The conditions under which the current sample was acquired for the specified channel are returned as a tab delimited string. The format is as follows:

Acquisition Date<TAB>
Instrument ID<TAB>
Column Type<TAB>
Carrier Gas<TAB>
Column Head Pressure<TAB>
Column Temperature<TAB>
Detector Gain<TAB>
Sample Time<TAB>
Inject Time<TAB>
Run Time

For example, the value returned may look like

Feb 21, 1994 14:54:19<TAB>BTU<TAB>HSA<TAB>
Helium<TAB>17.383<TAB>52<TAB>LOW<TAB>10<TAB>
50<TAB>100

EZChrom 200/400 VBE.DLL

DLL Format

The peak data in the dynamic link library is stored in tab delimited strings. There is a limit of 100 characters per string and 50 strings in the library. These strings can be accessed through function calls made by the client program. The GetPeak function is used to access the data for the "current" peak in the DLL. The current peak is referred to by an index number which the DLL keeps track of. Each time the GetPeak function is called this index is incremented by the DLL. The SetPeakIndex function allows the client program to change the index number in the DLL. The GetPeakIndex function will return the current peak index number.

The DLL also serves as a place to place command parameters for commands that are sent to EZChrom 200/400 via DDE. The SetCmdParam function is used to place a parameter into the DLL for retrieval by EZChrom 200/400. The command parameter is stored as a string.

Peak Data Format

The following datum are stored for each peak. Peak name, peak area, concentration, retention time, peak number, and channel. The proceeding are stored in a string with the fields delimited by tabs.

Functions

Name: GetPeak

Prototype: int FAR PASCAL GetPeak(LPSTR);

Description: This function is used to access the peak data that is stored in the DLL. The data is stored in the strings that is passed as a parameter to this function. This function increments the peak index as a side effect.The function returns TRUE if the peak index is less than 50, FALSE otherwise.

Name: GetPeakIndex
Prototype: int FAR PASCAL GetPeakIndex(void);
Description: This function is used to retrieve the current peak index in the DLL. The index is returned by the function.

Name: SetPeakIndex
Prototype: int FAR PASCAL SetPeakIndex(int);
Description: This function is used to set the peak index. The function returns TRUE if the index is in the range (0-50), FALSE otherwise.

Name: SetCmdParam
Prototype: int FAR PASCAL SetCmdParam(LPSTR);
Description: This function is used to place a command parameter in the DLL. The command parameter is passed as an actual parameter to this function. This function always returns TRUE.

Appendix C

Dynamic Data Exchange (DDE)

Dynamic Data Exchange (DDE)

Quick Reference Flowcharts

This Appendix provides several simplified flowcharts on how to perform several chromatographic functions with EZChrom 200/400. The assumption made in developing these flowcharts is of an established understanding of the function and operation of the EZChrom chromatography data system. The intent of this Appendix is to serve as a quick reference to allow you to quickly perform these chromatographic functions with little explanation or description of what is being done. If you should find a need for further explanation as to the operation or definition of the functions referenced herein, please refer to the earlier chapters of this manual which address these issues in greater detail.

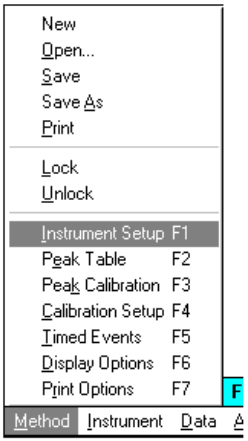
In this Appendix, you will find flowcharts describing the following chromatographic operations :

- Setting Up a Run
- Developing a Method
- Calibrating a Method.

Setting up a run

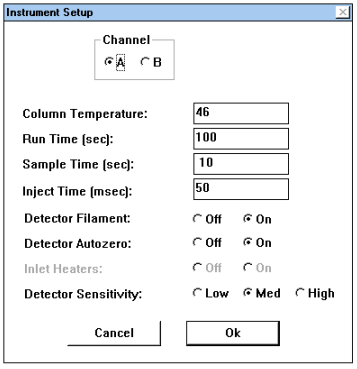
Method menu

1. In the **Method** menu, choose **Instrument Setup** to manipulate instrument settings.



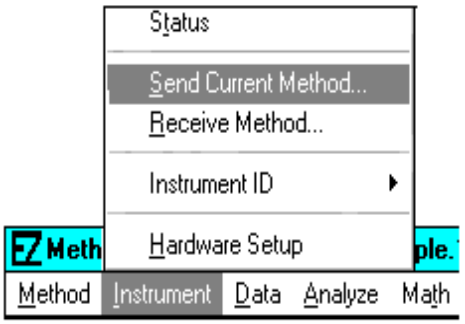
Instrument Setup Window

2. Adjust instrument parameters for optimal peak separation, for both Channel A & B. Click **[OK]** when adjustments are complete.



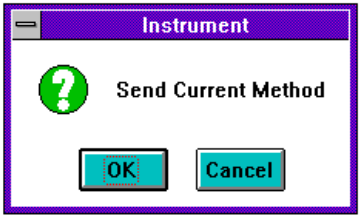
Instrument menu

3. In the **Instrument** menu, choose **Send Current Method** to implement recent changes.



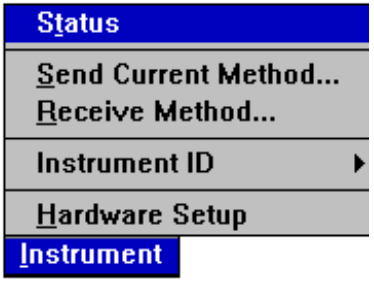
Send current method window

4. Click **[OK]** to **Send Current Method**.



Instrument menu

5. Choose **Status** in the **Instrument** menu to observe temperature and pressure stabilization.



Start menu

6. Choose **Start** to setup a run.

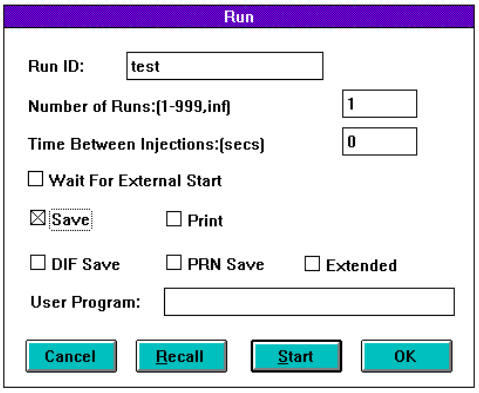
Run window

7. Name the data file and define run parameters to begin analysis.

   To save settings without starting a run, click **[OK]**.

   To begin run, click on **[Start].**

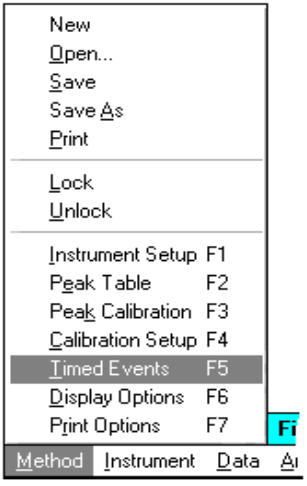
   Repeat steps 1 through 7, altering instrument parameters, until optimal peak separation is achieved.



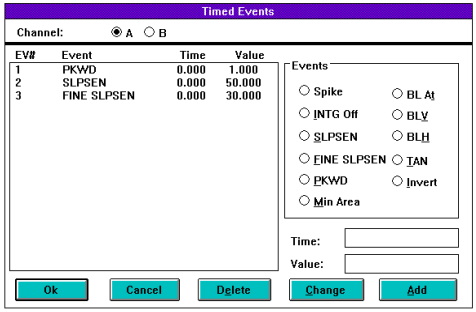
Developing a method

Method menu

1. After optimization of instrument settings, set integration parameters by choosing **Timed Events** in the **Method** menu.



Timed Events window



2. Begin with 4 basic events per channel:

   SLPSEN, **FINE SLPSEN**, **INTG OFF**, and **PKWD**, starting each at a **Time** 0.000.

   SLPSEN and **FINE SLPSEN** values depend on detector sensitivity settings. Start with:

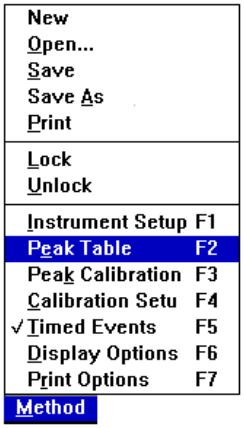
	Low	Med	High
SLPSEN	10	50	100
FINE SLPSEN	5	30	75

   INTG OFF: Start with a value of 1.5. Increase value if necessary.

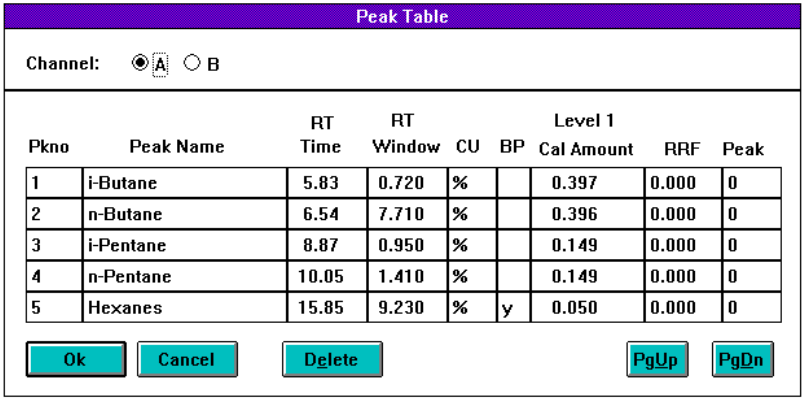
   PKWD: Start with a value of 1. Add additional pkwds, if necessary.

Method menu

3. To name integrated peaks, choose **Peak Table** from the **Method** menu.



Peak Table

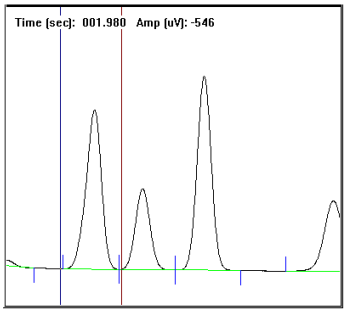


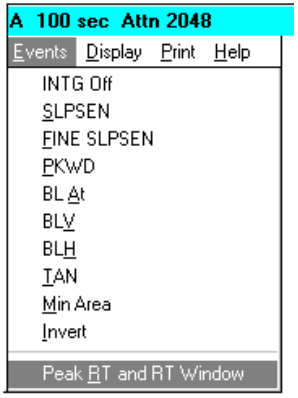
4. Enter **Peak Names**, **RT Times**, **RT Windows**, **CU**, and **BP** for each channel manually or with vertical cursors. If setting **RT Time** and **RT Window**s with vertical cursors, continue to steps 5 and 6.

   Click **[OK].**

   Then **Save** the method under the **Method** menu.

Peak RT and RT Window menu



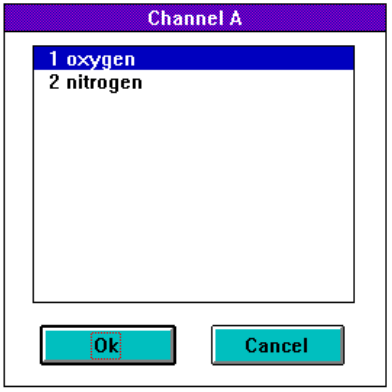


5. To set **RT Time**s and **RT Window**s graphically, set vertical cursors on either side of the peak of interest. Then, choose **Peak RT** and **RT Window** from the **Events** menu for the appropriate channel.

Channel A

6. From a list of components, darken the name of the peak that has been graphically chosen. Click **[OK]**.

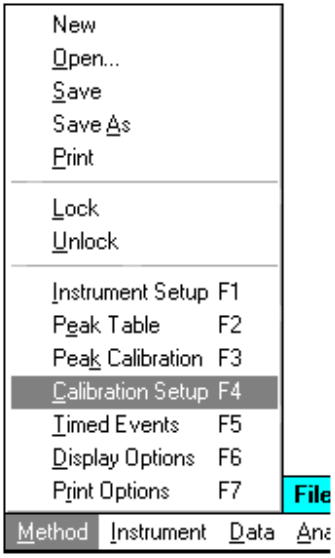
   Repeat steps 5 and 6 until all peaks have been named. After all peaks have been identified, **Save** the method in the **Method** menu.



Calibrating a method

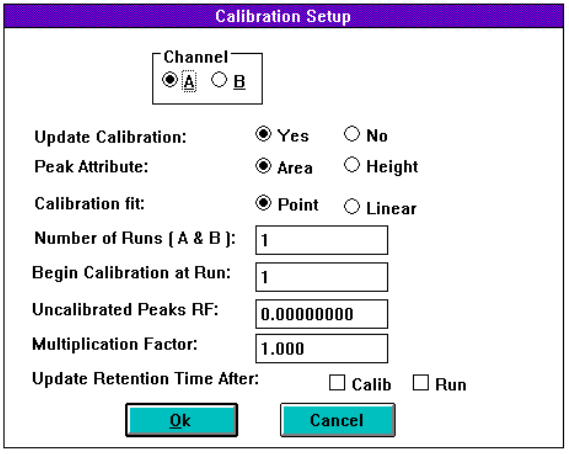
Method menu

1. Choose **Calibration Setup** in the **Method** Menu.



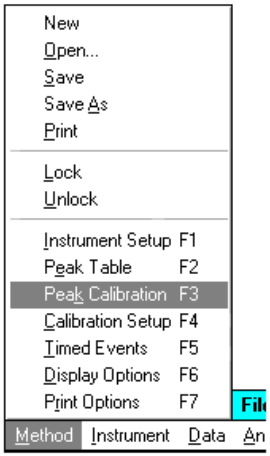
Calibration Setup window

2. Define calibration parameters for each channel. Specifying calibration fit and number of runs is usually sufficient. Click **[OK]**.



Method menu

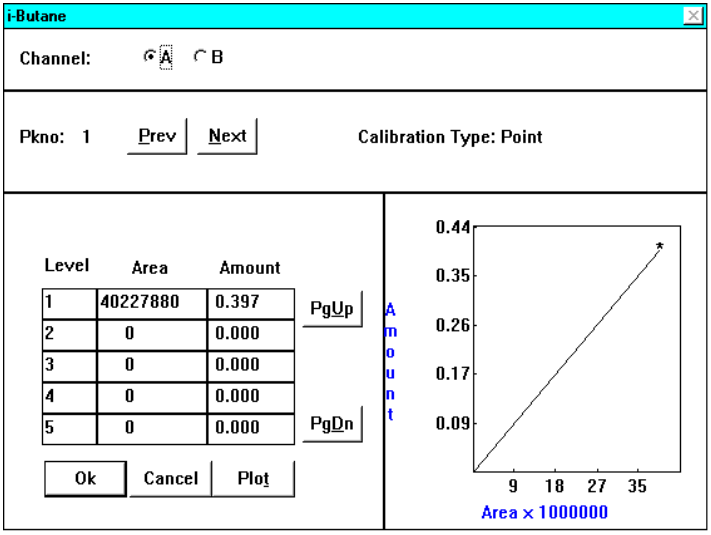
3. Choose Peak Calibration under the **Method** menu to enter calibration standard amounts.



Peak Calibration window

4. For each component, enter calibration standard **Amount**, in ascending order, for each level of calibratiion.

   The calibration **Amount** can also be entered in the **Peak Table** under the **Method** menu.

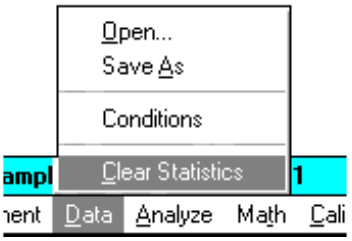


   Area will automatically be inserted at Step 7.

Data menu

5. To begin calibration, choose **Clear Statistics** in the **Data** menu to erase MIN., MAX., MEAN, and %RSD.

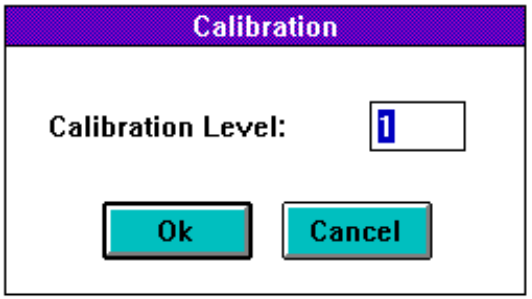
   Click **[OK]**.



Calibration menu and window

6. Choose **Calib**. Enter the calibration level.

   Click **[OK]**.



Analyze and Start menus

7. If calibrating with stored data, click **Analyze**.

   If calibrating with data to be collected, click **[Start]** to begin run.

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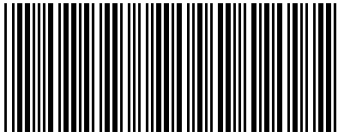

Manual Part No.
DOC-2049