



Report

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MF-9071

INSTRUCTION MANUAL

ChromGraph® Report

Bioanalytical
Systems, Inc
2701 Kent Avenue
West Lafayette
Indiana 47906

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SECTION 1. INTRODUCTION

ChromGraph is a sophisticated set of programs for the control of BAS instruments and the analysis of data generated by Liquid and Gas Chromatographs. The software runs in the Windows™ environment, thus providing all the flexibility and multitasking inherent in this operating system.

The data processing sections of ChromGraph (ChromGraph Report) include the following functions: peak identification, peak integration, comparison to internal and/or external standards and report generation. Reports may be output to printer, disk, or to special files configured for easy manipulation by spreadsheet programs. Special features include automatic updating of standards and superb graphics.

ChromGraph REPORT may operate independently in a batch-process mode for already collected data, or it may be linked to its sister program, ChromGraph CONTROL, for on-line processing of current data. Please refer to the ChromGraph CONTROL manuals (MF-9070 for the BAS-200B and MF-9005 for the DA-5) for details about instrument control and data collecting software.

This manual is intended to be a simple introduction to REPORT. Where there are several paths to accomplish the same task, usually only one will be described. For example, many operations can be carried out either by clicking on a pushbutton with the mouse, or by selecting items from a menu with the keyboard. Only the mouse operations are described, to keep the manual as simple and undaunting as possible. A list of keystroke alternatives to the mouse is provided in Section 10, page 64. Those who wish to explore other alternatives are encouraged to experiment, using the comprehensive Help screens and their knowledge of Windows.

Both ChromGraph REPORT and CONTROL run in the Windows operating system. Wherever possible, we have maintained the style of Windows in the “look” and “feel” of our software, including drop-down menus, list boxes, dialog boxes, radio buttons, etc. If you are already familiar with Windows, the transition to ChromGraph will be smooth. If Windows is new to you, we urge you to learn it first, using the tutorial provided with Windows.

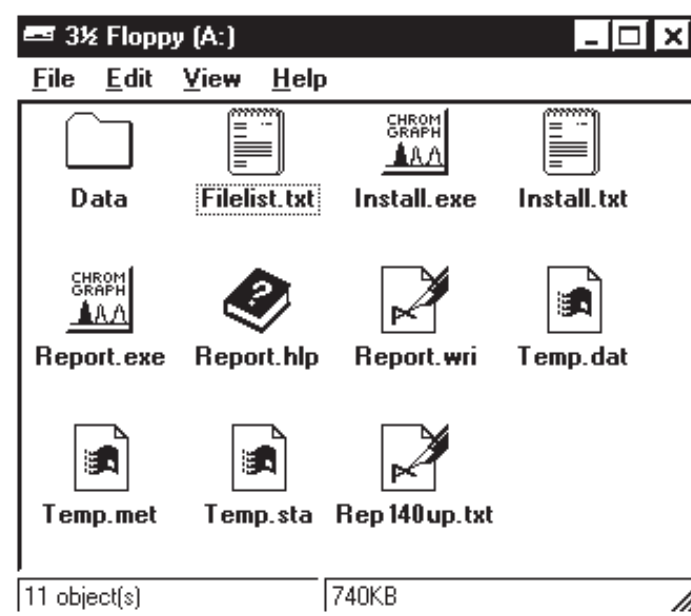
SECTION 2. INSTALLATION

NOTE: If you purchased your computer from BAS along with the software, this installation has been done for you. You may proceed to section three.

ChromGraph REPORT and CONTROL are complex programs that require a moderately powerful computer for optimal performance. In theory, any 80286 or larger computer that can run Windows will run ChromGraph. However, speed and multitasking ability will suffer with too small a computer. The minimum computer size we recommend is a 25 MHz 386SX, with a 4 MB RAM and an 80 MB Hard Drive. The Windows version should be 3.1 or higher. These instructions assume you are using Windows95.

Begin the installation of ChromGraph REPORT by inserting the disk into the A: drive of your computer. Double-click on the "My Computer" icon, and then on the "3½" floppy" icon. You should now see the folders on the A: disk (Figure 2.1).

Figure 2.1. Obtaining installation instructions



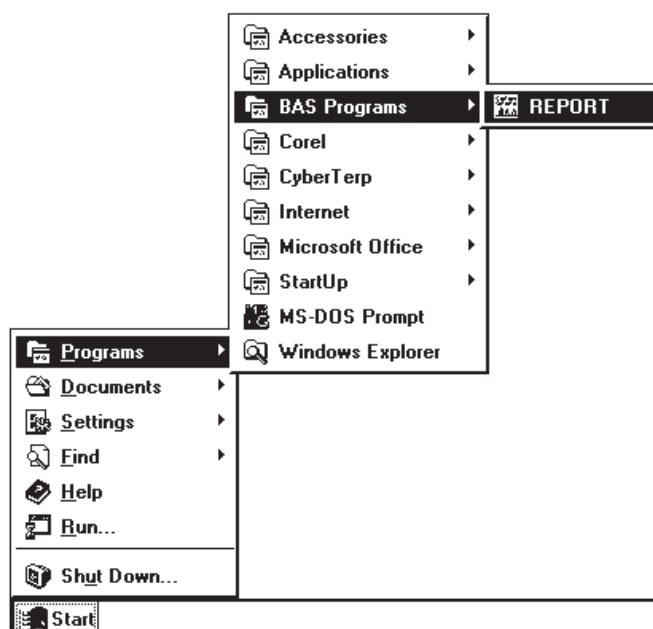
NOTE: Depending on how Windows95 is configured, you may not see the 3-letter extensions (e.g., .exe, .txt) following the folder names.

Double-click on the notebook icon labeled "INSTALL" or "INSTALL.TXT", which brings up complete instructions for installing REPORT. It is helpful to print out these instructions, using the PRINT command found in the FILES menu at the upper left of the screen.

Begin the installation by double clicking on the peaks icon labeled "INSTALL" or "INSTALL.EXE."

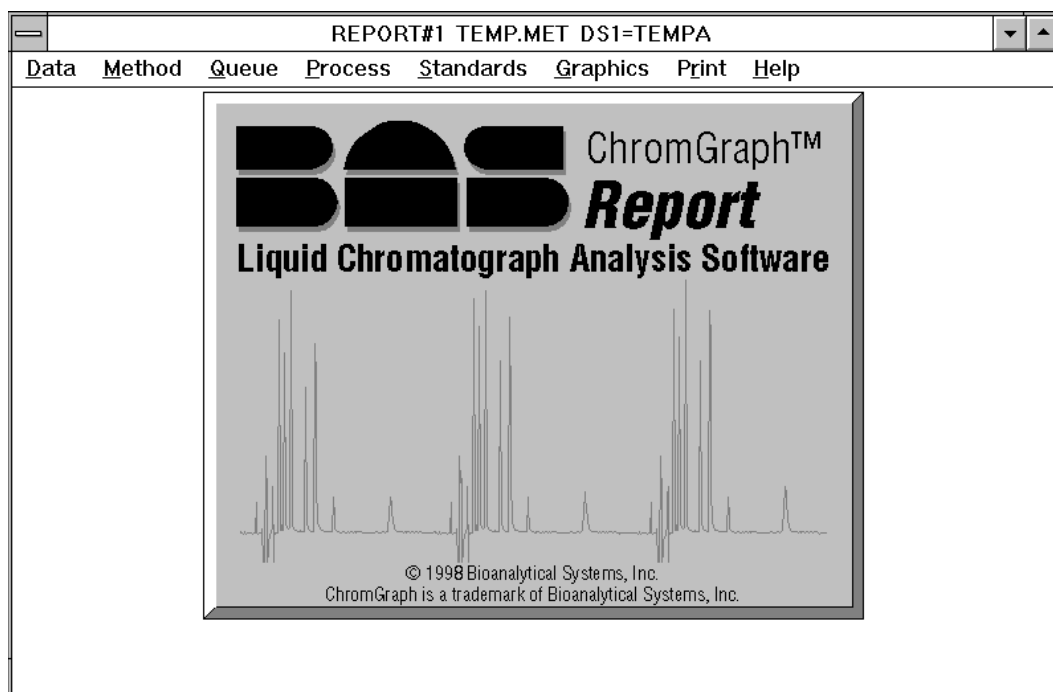
During the installation, REPORT will be installed into a subdirectory called C:\BAS. Demonstration data files and methods will be installed into C:\BAS\DATA. The Windows95 START Menu will be modified so that REPORT will appear in a new program group called BAS PROGRAMS (Figure 2.2).

Figure 2.2. BAS PROGRAMS group in Windows95.



NOTE: The demonstration data sets in C:\BAS\DATA can be deleted once you have learned the system. If you ever wish to see them again they can be easily copied from the installation disk.

To start the data processing section of ChromGraph, click on REPORT as in Figure 2.2. The initial screen for REPORT will appear (Figure 2.3). Notice that the title bar indicates that this is Report #1. This is to distinguish this running of REPORT (called an instance) from subsequent ones. Several instances can run concurrently, but this multitasking feature is limited by your computer's memory. To start another instance of REPORT, return to the START Menu and click on REPORT again.

Figure 2.3. Initial Report screen.

Complete the installation by selecting the SETUP OPTIONS in the DATA Menu (Figure 2.4). These options are tones, mouse positioning, field separators, and printer indent (Figure 2.5).

TONES enables a set of sounds associated with the various steps in processing data. They may be used as an audible cue to indicate analyses in progress.

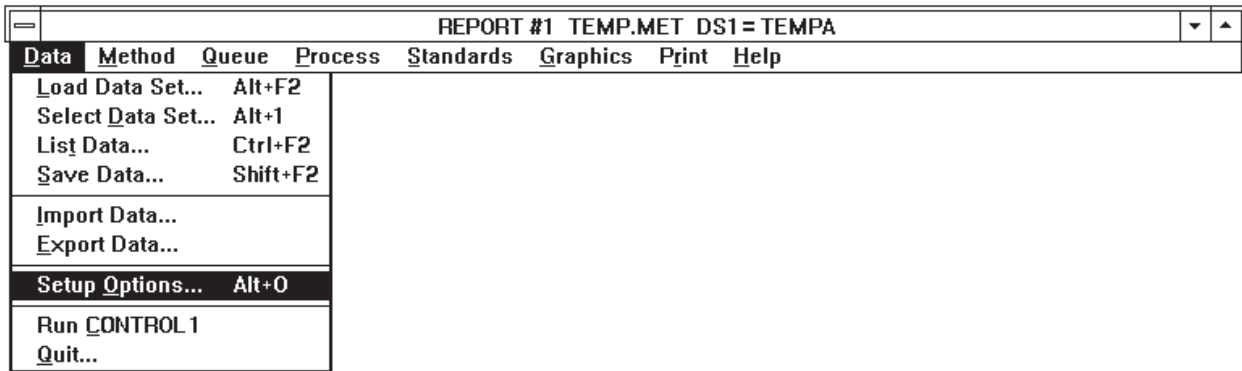
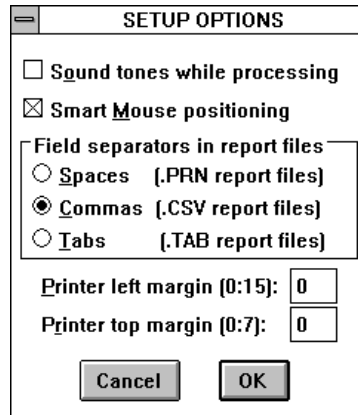
SMART MOUSE POSITIONING puts the mouse cursor at the most likely next step when screens change.

PRINTER MARGIN moves printing to the right by up to 15 spaces, and down by up to 7 lines, for better control of the appearance of printed output.

The FIELD SEPARATOR options determine the data format for export to spreadsheets (Section 6.2, page 28).

The SETUP OPTIONS will remain in effect throughout the session. They can be made permanent by saving the REPORT.INI file when exiting (Section 11, page 69).

If ChromGraph CONTROL software is to be used concurrently with ChromGraph REPORT, it may be started conveniently using the RUN CONTROL1 option provided in the Data Menu (Figure 2.4). This option will start ChromGraph CONTROL if it is not already running. If it is already running in the background or as an icon, taking this option will bring it to the foreground.

Figure 2.4. Use the Data Menu to reach Setup Options...**Figure 2.5.** Setup Options.

SECTION 3. HELP SCREENS

ChromGraph contains a complete on-line, context-oriented system of Help files. Information is provided for virtually any aspect of the software, and for any entry that must be made. Help information can be obtained in several ways which parallel the Help screens of Windows.

For general access to Help screens, select HELP from the MAIN MENU (the menu bar across the top of the screen, Figure 3.1). The INDEX option opens up a screen with all the main Help topics listed (Figure 3.2). Select whichever topic is of interest.

Figure 3.1. Accessing the Help Screens.

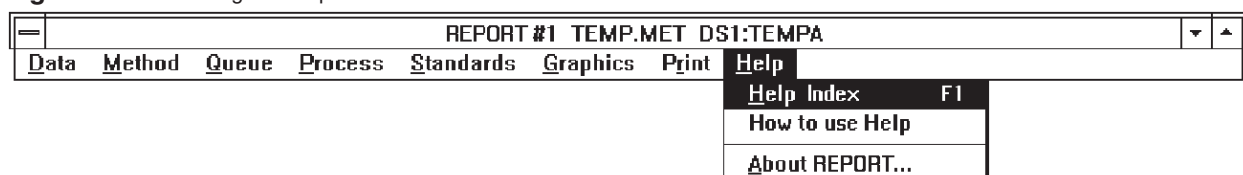
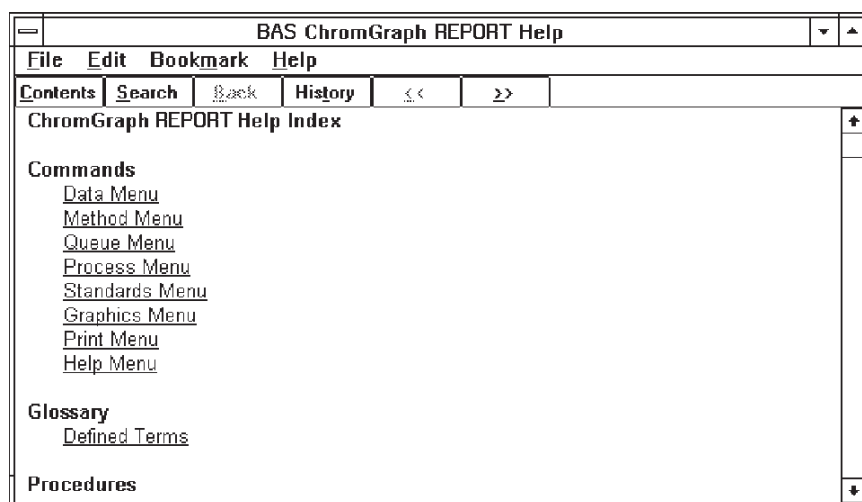


Figure 3.2. The Help Index.



For more specific help when using the software (e.g., when you're not sure what to enter for a Menu command or an option in the Method), use the context-oriented feature of the Help screens. Pressing the F1 key will bring information about whatever parameter is highlighted on the screen. Highlights can be moved around the screen using the tab and arrow keys.

A faster way to get information about any option is with the mouse. If the section of interest requires a text entry, simply click on it, then press F1. If the section of interest is a pushbutton that will cause immediate action, you must prevent that action from occurring in order to get help. Press the mouse button but do not release it; then press and release F1. Now you can release the mouse button; instead of activating the button, a Help screen will appear.

SECTION 4. CREATING A METHOD FILE

ChromGraph REPORT data processing Methods are concerned only with the analysis of aquired data. We will call this analysis PROCESSING. Data collection and instrument control are the provinces of ChromGraph CONTROL. To distinguish the two types of Methods, the extensions .MTH (CONTROL) and .MET (REPORT) are automatically appended to the Method names.

All the information necessary to process a Data file is contained within a REPORT Method. This information includes the sensitivity for discriminating peaks from baseline, the times during which to search for peaks, which set of standards to use, what items to include in the report, etc. We will go through the development of an entire Method here. This will rarely be needed, since in most cases an existing Method can be edited slightly to create new Methods.

4.1 Loading A Method

Begin creating a Method by loading an existing Method which we will modify. (Loading is not strictly necessary: a temporary Method is always loaded into RAM at startup, so a Method is always present. But this is a good place to learn how to load a Method.)

Select the METHOD MENU by clicking on the METHOD section of the MAIN MENU (Figure 4.1). Select the LOAD METHOD option, and a dialog box containing a directory of existing Methods in the default data location will appear (Figure 4.2). To load a Method, either double click on its name or highlight it and click the OK button.

Figure 4.1. The Method Menu.

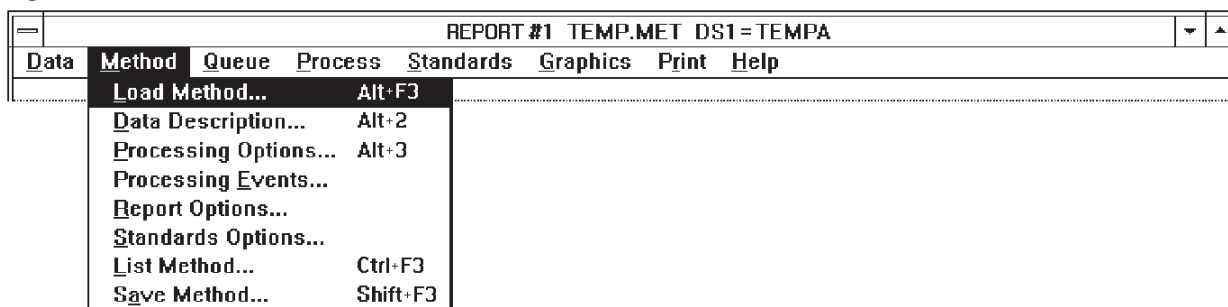
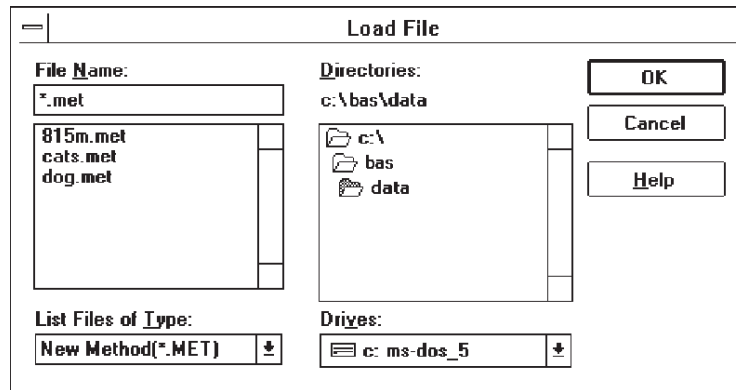


Figure 4.2. Loading a Method file.

Methods can be loaded from other locations by using the mouse to select other drives or subdirectories. For example, if you double click on C:\ in the Directories listbox (Figure 4.2) you will get a list of all the directories on the hard disk. Double-clicking on any one of these will select it as the current data directory. All the examples in this manual were taken from Methods and Data files in the C:\BAS\DATA directory.

If you load a file from a different directory, this new directory automatically becomes the default data location. The default data location will remain in effect throughout the session, unless changed by another LOAD or SAVE operation. When you finish your session and QUIT, saving the REPORT.INI file will save the new default data location for subsequent sessions. If you don't save the REPORT.INI file, the new data location will be forgotten (Section 11, page 69).

4.2 Opening The Method Screens

To edit or review the Method you must open the Method screens (technically they are called dialog boxes). Start by clicking on the METHOD section of the MAIN MENU. A list of options will appear (Figure 4.1). Except for the LOAD, LIST and SAVE options, each option will open a screen for a section of the Method. Simply click on the desired section with the mouse.

Figure 4.3. Data Description section of the Method.

DATA DESCRIPTION			
Method name =	CATS	Data Set 3 name =	CATS003A
Operator:	SOLOMON		
Conditions:	CATECHOLAMINES		
Run Notes:	MP-1 MOBILE PHASE		
Time of run =	09:44	Date of run =	03-29-91
Data name =	CATS	Run # =	3
Detector =	A = LCEC1	Vial # =	0
Start minutes =	0.00000	End minutes =	12.4800
Points per minute =	74.9998		
<input type="button" value="≤< Std. Opt."/> <input type="button" value="Cancel"/> <input type="button" value="OK"/> <input type="button" value="Save"/> <input type="button" value="Proc. Opt. >≥"/>			

4.3 Pushbuttons Found In Method Screens

At the bottom of each Method screen are five pushbuttons for subsequent action (Figure 4.3). These are:

OK accepts any changes and saves them to RAM. These changes are then used in subsequent runs of the Method. To permanently save these changes to disk, use the SAVE option.

<< (destination) and (destination) >>. These have the same function as OK, except they automatically bring up the previous (<<) or next (>>) screen of the Method.

CANCEL closes the dialog box without accepting any changes that were made in the box. The original information is thus preserved.

SAVE opens a dialog to save the Method with a name and location of your choice. Valid Method names are discussed in Section 4.10, page 19.

4.4 Data Description

Choosing the DATA DESCRIPTION option under METHOD (Figure 4.1) brings up the edit screen for the first section of the Method (Figure 4.3). The Data Description section shows information about the Data file currently loaded; as a consequence, it is not a permanent part of the Method and will change as new Data files are loaded.

The OPERATOR, CONDITIONS and RUN NOTES are user-inserted comments entered into the ChromGraph CONTROL software Method at the time of data collection. This information is saved along with every Data file generated by CONTROL. REPORT imports this information and includes it in the final processing report that it generates. By using these fields when you collect data, you will always be able to identify any particular Data file.

TIME and DATE of run indicate when the data were collected. VIAL# refers to the position of the sample in the BAS Sample Sentinel, if used. These items are automatically entered as part of the Data file by ChromGraph CONTROL.

DATA NAME, RUN# and DETECTOR also are entered automatically by CONTROL at the time of data collection. They identify the name of the Data file, its number in the sequence of samples, and which detector was used. These items are used to create a unique name for each Data file.

The Data file name begins with the data name, which can have up to four numbers and letters (the last position must be a letter, to avoid confusion with the run number, which follows). Run numbers (three digits, up to 255) and a letter (A through D, for detector 1 through 4) are automatically appended to the data name by the data-acquisition software. When saved to disk, the name is given the extension .DAT. Thus, CATS003A.DAT is the third run in a series called cats, and contains data from detector A.

The information above will print out with the report. Three remaining items do not print out: START and END minutes indicate the time from the start of the run during which data were collected, and POINTS PER MINUTE is the rate of data collection.

A slash (/) followed by a number in the RUN NOTES field indicates a volume adjustment for the sample. Volume adjustments are generally attached during data collection by ChromGraph Control (see the ChromGraph Control Manual). Volume adjustments allow the results to be reported on a per mass or per volume basis when the weight or volume of the original sample is known. When a volume appears here, the amount reported for this sample in the final report will be the calculated amount divided by the indicated volume.

4.5 Processing Options

This next section of the Method can be accessed by either choosing it from the METHOD MENU (Figure 4.1) or by clicking the << or >> buttons from other screens. The options in the PROCESSING OPTIONS screen (Figure 4.4) define how peaks are detected and specify some aspects of the report.

Figure 4.4. Processing Options section of the Method.

INITIAL SLOPE THRESHOLD is the sensitivity of the peak-finding algorithm. A small value is very sensitive, whereas a large value is very insensitive. Set this option so the important peaks are detected, while small extraneous peaks are not. Try the AUTO option initially. AUTO will decide on an appropriate value and insert that value into the INITIAL SLOPE THRESHOLD box. AUTO will switch off after one Data file has been processed. You can then observe the peaks and modify the threshold if necessary (Section 5.2, page 21).

SMOOTHING WIDTH compensates for noisy baselines. Use a small value for smooth baselines, and a larger value if baseline noise is being detected as peaks. Peak detection takes longer with large values than with small values. Try the AUTO option initially. AUTO will decide on an appropriate value for SMOOTHING WIDTH. AUTO will switch off after one Data file has been processed. You can then observe the peaks and modify the Smoothing Width if necessary (Section 5.2, page 21). A Smoothing Width of 9 is a good general-purpose setting.

TIME UNITS selects minutes or seconds as time units in tables and graphs of the report.

MINIMUM PEAK AREA enables you to reject peaks below a certain area during peak detection. Use this option to exclude small peaks that are of no interest, but still large enough to be detected. Unless you are thoroughly familiar with your data, use a value of 0 so that all detected peaks appear in the report.

LIST PEAKS allows one of the intermediate steps of peak detection, the finding of start and stop points for the peaks, to be listed to the screen during manual data processing (Section 5.2, page 21).

REVIEW BASELINE causes a graph of the peaks to automatically appear on the screen after manual peak detection. This box need not be checked to see the graph: Review Baseline can be selected from the PROCESS menu any time after the peaks have been detected (Section 5.2, page 21). Review Baseline is automatically disabled during automatic processing.

The SAVE BASELINE checkbox causes an actual copy of the chromatogram to be saved to the default data location each time a run is processed. The file will have a name composed of the data name and run number, followed by a .bln extension. These files take up a lot of disk space, and are redundant in that the chromatogram can be easily redrawn at a later time from the raw data. However, if the regulations you work under require the archiving of the original chromatogram, check this box.

Figure 4.5. Processing Events section of the Method.

Time	Event	Status
0.00	Detect_Peaks	OFF
4.00	Detect_Peaks	On
4.02	Perpend._Drop	OFF

4.6 Processing Events

This section of the Method can be reached by taking the PROCESSING EVENTS option from the Method Menu (Figure 4.1) or by clicking the << or >> buttons from other screens. The left side of this section contains a listbox of instructions that determine how peaks are detected (Figure 4.5). These instructions are called events because they can be activated or disabled at specified times. (In this manual, time will always refer to elapsed time from the start of a chromatographic run.) The following events are available:

DETECT PEAKS (ON or OFF) determines the times during which ChromGraph searches for peaks. All Methods must have at least one Peak Detect ON (usually at time 0.0) in order to process data. A more sophisticated use is to turn Peak Detect OFF at time 0.0 to ignore the baseline disturbance at the beginning of the run, and turn it ON before the first peak of interest (Figure 4.5).

NEGATIVE PEAK (ON or OFF) may be useful in vacancy chromatography. It serves to reverse a negative-going peak so it can be processed like a normal peak. Most users can safely ignore this option.

PERPENDICULAR DROP (ON or OFF) determines how the baseline is drawn under two peaks that are not completely resolved. ON draws a straight baseline between the start of the first peak and the end of the second, and drops a perpendicular divider from the valley between the two peaks to the baseline (Figure 4.6). OFF draws the baseline for each peak independently, resulting in a "tangent skimming" approach that is appropriate for quantitating small peaks on the shoulders of larger ones (Figure 4.7). The default state for Perpendicular Drop is ON.

Figure 4.6. Perpendicular drop is ON. Note how the baseline is drawn under the valley at 20 minutes.

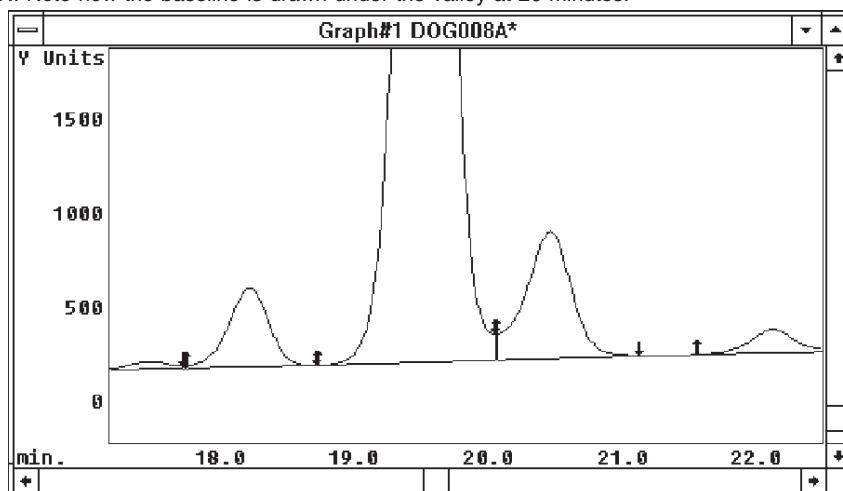
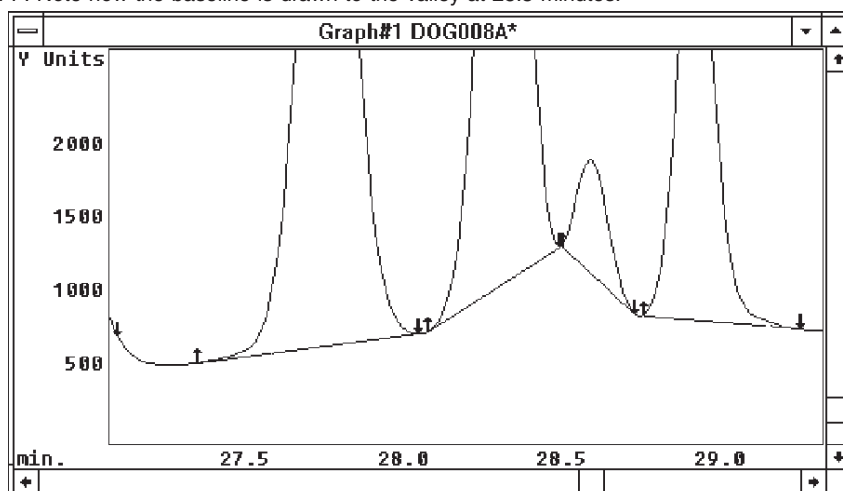


Figure 4.7. Perpendicular drop is OFF. Note how the baseline is drawn to the valley at 28.5 minutes.



FIX BASELINE forces the software to accept the baseline value at any given time as a true baseline. Occasionally the baseline before the first peak has such a steep slope that the software won't recognize it as baseline, and therefore can't recognize the first peak. Inserting a Fix Baseline command before the first peak will solve this problem.

THRESHOLD VALUE allows the Initial Slope Threshold (Section 4.5, page 10) to be changed at a given time. This feature can be used to increase the sensitivity for late-eluting, broad peaks. In most cases, however, the Initial Slope Threshold will be sufficient to detect all the peaks.

To edit an existing event, click on it and it will appear in the edit boxes on the right side of the screen (Figure 4.5). Make the appropriate changes and click CHANGE. To insert a new event, enter the time and event in the edit boxes, then click INSERT. To delete an event, highlight it and click DELETE. Processing events also can be edited graphically, using the mouse to point to sections of the baseline (Section 8.3.5, page 60).

4.7 Report Options

The REPORT OPTIONS screen is accessed through the Method Menu (Figure 4.1) or by clicking the << or >> buttons from other screens. This screen customizes the report of the analysis, detailing which output devices are to be used and which items are to be included (Figure 4.8).

Figure 4.8. Report Options section of the Method.

Output can be routed to the SCREEN and the PRINTER. The remaining output options are intended to transfer reports to spreadsheets or word processors:

PRN, CSV and TAB files are ASCII files, formatted so their information can be readily imported into spreadsheets (Section 6.2, page 28). To select PRN, comma-delimited (CSV) or tab-delimited (TAB) files, see the SETUP OPTIONS dialog (Figure 2.5, page 5). In manual mode (Section 5, page 20) requesting a PRN, CSV or TAB file will generate one file for each Data file (each run). The file will have the Data file name followed by .PRN, .CSV or .TAB (e.g., CATS005A.PRN). In automatic mode (Section 6, page 26) you can specify one file to which reports for all Data files will be sent. As ASCII files, these files contain only text (not graphs).

REP files also are ASCII files. These are exact images of the report that is generated on the screen or printer. One REP file will be generated per Data file, and named with the data file name followed by .REP (e.g., CATS002A.REP). Only text can be stored in a .REP file.

CLIPBOARD sends an image of the report that is generated on the screen or printer to Clipboard, Windows' internal message board. From Clipboard the image can be pasted to other Windows programs. Since Clipboard can hold only one report or image at a time, reports from subsequent Data files will overwrite those of earlier Data files.

On the remainder of this screen, check the items you wish printed in the final report. The screen and printer have only a finite width; if more items than can fit on a line are selected, the line will wrap around and print on a second line. For neatness, we suggest you select only the items that are important for your analysis.

The available items include the following:

DATA DESCRIPTION is the identifying information about the chromatographic run (Section 4.4, page 9).

GRAPH OF RUN plots the chromatogram, baseline and peak markers (Section 8, page 52).

PEAK NUMBER is a sequential numbering of the peaks in the chromatogram, ordered by retention time.

RETENTION TIME is the elution time of the apex of each peak. Time units are minutes or seconds, as selected in Processing Options (Figure 4.4, page 10).

PEAK TYPE describes how well a peak was resolved from its neighbors. The first letter of this two-letter code indicates whether the peak started at the baseline (B) or in a valley (V) between peaks, and the second letter indicates the same for the end of the peak. Thus, a BV peak is one that began at the baseline, but was not well separated from the following peak.

SKEW is a number from 0 to 2 that represents the symmetry of the peak. A symmetrical peak has a skew of 1. Peaks with skews below 1 have a majority of their area before the apex, while those with skews greater than 1 have a majority of their area after the apex.

WIDTH is the peak width at half height, in the time units selected in Processing Options (Figure 4.4, page 10).

HEIGHT is the height of the peak.

% HEIGHT is the percent contribution made by any one peak to the sum of the heights of all peaks in the chromatogram.

AREA is the area delimited by the peak and the baseline drawn under it.

% AREA is the percent contribution made by any one peak to the sum of the areas of all peaks in the chromatogram.

4.8 Standards Options

The STANDARDS OPTIONS screen is reached through the Method Menu (Figure 4.1) or by clicking the << or >> buttons from other Method screens. STANDARDS OPTIONS define how the peaks are to be quantitated, and which items will appear in the report (Figure 4.9).

Figure 4.9. Standards Options section of the Method.

STANDARDS OPTIONS

Standards filename:

Time zero minutes: Time scale factor:

Dilution multiple: Standard additions

Type of Standard Curve

Multiline Linear Quadratic Cubic Quartic

Items included in report

Peak name Calculated Amt. Standard Error

Standard # Ret. Time Error Group Number

Degree of Polynomial Unidentified Peaks

<< Report Opt. Cancel OK Save Data Desc. >>

STANDARDS FILENAME is the name of the Standards file to which the sample results are to be compared (Section 7, page 35). When a new Standards file is created and SAVED, its name will automatically be inserted here. To skip the standards-comparison step of data processing, delete the name from this box.

TIME ZERO minutes (or seconds) compensates for data whose injection time does not correspond to the injection time of the standard. Time units are those specified in Processing Options (Figure 4.4, page 10). TIME SCALE FACTOR compensates for runs in which elution was faster or slower than the standard. The Time Zero Offset is subtracted from the retention time of each peak, and the result is multiplied by the Time Scale Factor, to make the correction. These options will rarely be used, and should be set at 0 and 1, respectively.

DILUTION MULTIPLE corrects calculated amounts for any known dilutions of the samples. Thus, if you diluted your samples in half before analyzing, a dilution multiple of 2.0 will double the calculated amounts so they are corrected to the original sample. The default value of 1.0 eliminates the correction. The correction is applied equally to all samples. See Section 4.4 for a discussion of how to adjust for differences in volume or weight among samples.

The STANDARD ADDITIONS checkbox indicates whether you have used standard additions, or endogenous, calibration. This occurs when a pooled matrix (such as plasma or urine) containing an unknown but constant amount of an endogenous analyte is spiked with known amounts of the analyte to create a series of calibrators. In this situation the size of the peak is determined by both the endogenous amount and the spiked amount. The endogenous amount is represented by the y-intercept of the regression equation, and the spiked amounts by the slope. When you check this box the y-intercept will be dropped from the formula, correcting the calibrators so the endogenous amount is ignored.

TYPE OF STANDARD CURVE: Choose MULTILINE (point-to-point), LINEAR, QUADRATIC, CUBIC or QUARTIC. Most standard curves are linear (if there are three or more levels of standards) or multiline (two or fewer levels).

The following items will be included in the report if checked. Since there are too many items to fit neatly across the width of a standard page, we suggest that you select only those that are useful in the report.

PEAK NAME is the name assigned to a peak with a given retention time. Names are entered when creating a Standards file (Section 7.1, page 35).

CALCULATED AMOUNT is the result of comparison of a sample component to internal and/or external standards.

STANDARD ERROR is the standard error of the calculated amount.

STANDARD # is the sequential number of the peak with this retention time in the Standards file.

RET. TIME ERROR is the difference in time between the unknown peak and the standard peak. Time units are those selected in Processing Options (Figure 4.4, page 10).

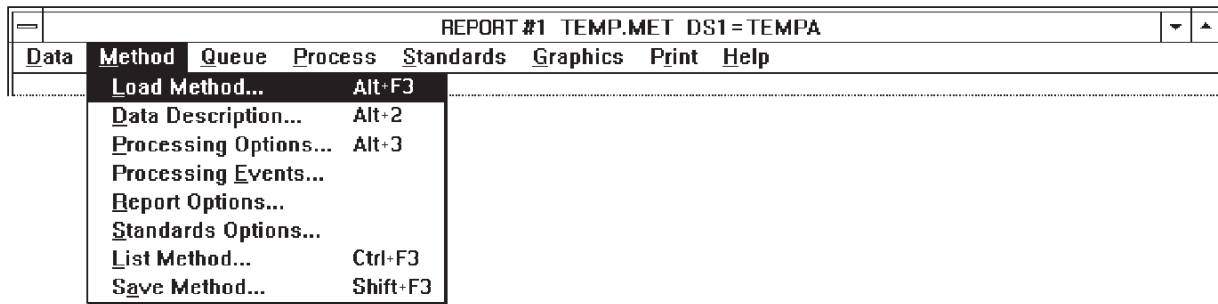
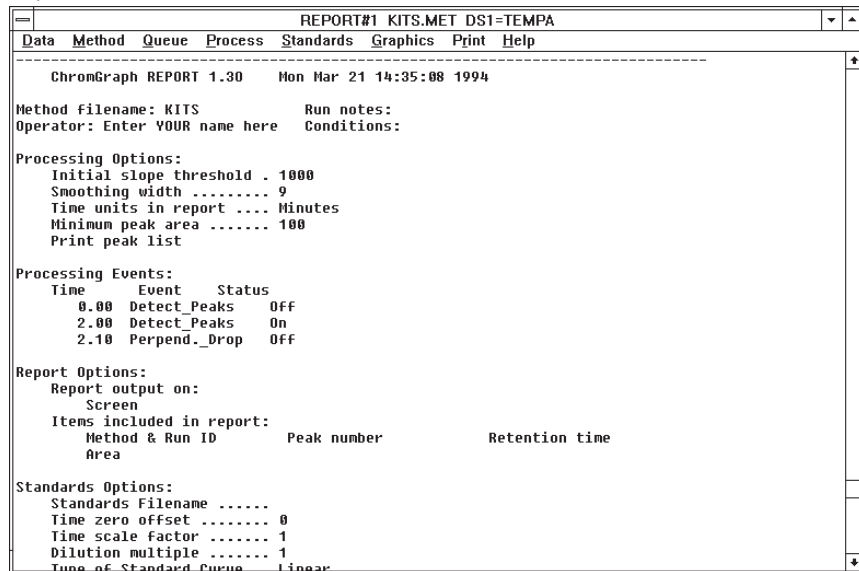
GROUP NUMBER allows the standards to be assigned to categories for pooled area reports. Standard peaks are assigned to categories in Section 7.1, page 35).

DEGREE OF POLYNOMIAL (0 = multiline, 1 = linear, etc.) is the actual degree used for quantitation. This may be automatically lowered from the degree requested above in Type of Standard Curve, if some standard peaks or levels are missing.

UNIDENTIFIED PEAKS are those which do not match any peak in the Standards file. They may be included in the report by checking this option.

4.9 Listing A Method

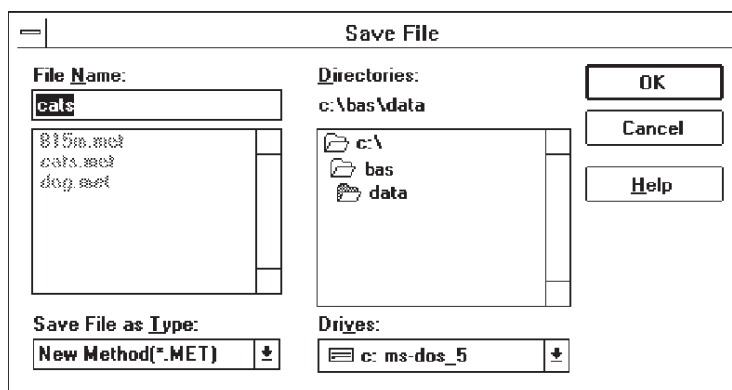
To review the Method, you may scroll forward or back through all the screens using the << and >> buttons, or choose LIST METHOD from the Method Menu (Figure 4.10). Methods may be listed to the screen, printer, disk and Clipboard. If listed to the screen, they scroll along the rearmost section of the screen, the OUTPUT WINDOW. After the listing is finished, you may scroll through the Method by using the scroll bars on the right side of the Output Window (Figure 4.11).

Figure 4.10. Choosing List Method...**Figure 4.11.** A Method listed to the Output Window.

4.10 Saving A Method

Choose the SAVE button from any of the Method screens, or SAVE METHOD from the Method Menu (Figure 4.10). A SAVE screen will appear, showing the default Method name and the location to which it will be saved (Figure 4.12). A listbox will show the existing Methods in the default data location. Change the Method name or data location as appropriate and click on the OK button to save it. A Method name can have up to 8 characters. The extension .MET will automatically be appended to the name you choose. If a file of the same name already exists, a warning will appear.

Figure 4.12. Saving a Method.



ChromGraph CONTROL Methods have the extension .MTH to distinguish them from ChromGraph REPORT Methods. They can therefore be given the same name, to associate them. For example, Methods to run and process catecholamine samples can be named CATS.MTH and CATS.MET, respectively, while those to run and process amino acids can be named AMINO.MTH and AMINO.MET.

SECTION 5. MANUAL DATA PROCESSING

Data processing includes three main functions: peak detection, integration and quantitation. During peak detection the software determines where peaks start and stop. During integration, which can only occur after peak detection, the areas under the peaks are determined. These peak areas are compared to the areas of known standards during quantitation, which estimates the amount of each analyte in the injection.

ChromGraph REPORT can rapidly process an almost unlimited number of Data files in the fully automatic mode (Section 6, page 26). A slower manual approach is useful, however, when developing a Method, and when learning to use the software.

5.1 Loading Data

Begin the manual processing mode by loading the Data files to be processed. Take the LOAD DATA option from the Data Menu (Figure 5.1). The LOAD DATA SETS dialog box appears, with information about the Data files already in RAM (Figure 5.2).

Figure 5.1. The Data Menu.

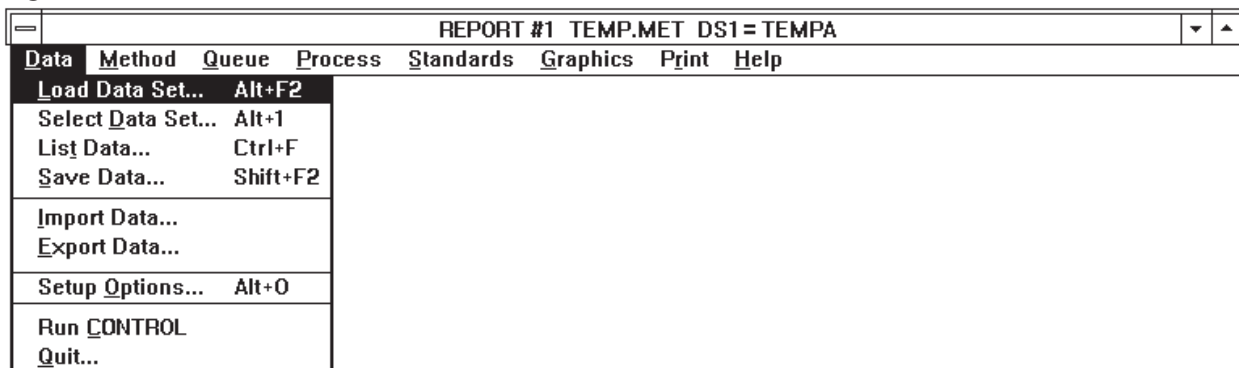
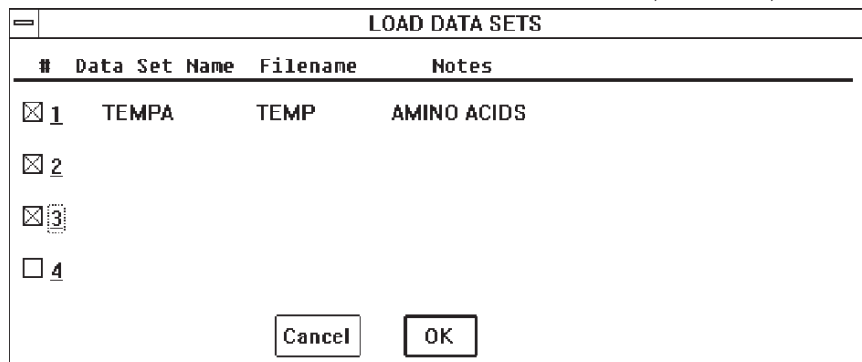


Figure 5.2. Load Data Sets dialog box. The checked boxes indicate that we wish to load three data files, into slots 1, 2 and 3.



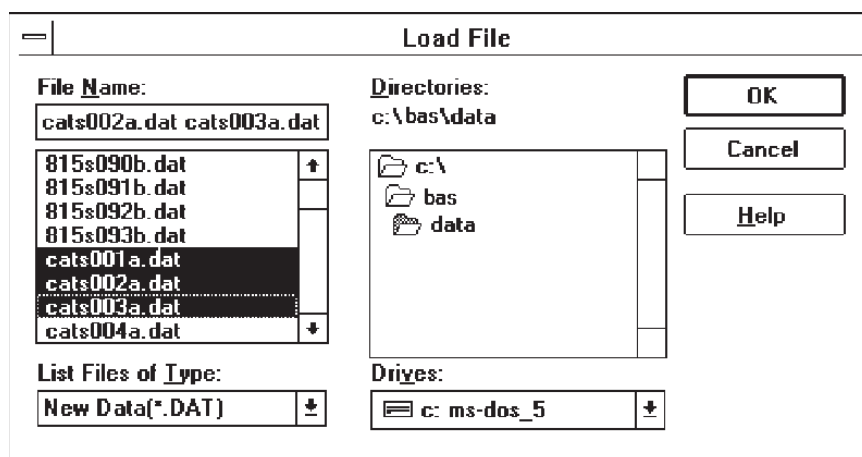
There are four slots for Data files in RAM. In Manual mode, four Data files (runs) can reside in RAM simultaneously. The Load Data Sets dialog box gives the name, slot number, and

the Run Notes for each Data set. To load new Data files, click on up to four of the slot numbers. Data files already residing in clicked-on slots will be overwritten.

Click on the OK button in the Load Data Sets dialog box and the LOAD FILE dialog box appears (Figure 5.3). Click on the Data files you wish to load, using the Windows convention of CTRL-click to highlight more than one file. To follow along in this section, load the files indicated in Figure 5.3. These will be present on your disk if you followed the installation instructions (Section 2, page 2). Click on OK and the files will be sequentially loaded into the previously designated slots (Figure 5.2). Also load the appropriate Method for these Data files, CATS.MET, following the instructions in Section 4.1, page 7).

When you load multiple Data files, the last-loaded file automatically becomes the focus for subsequent operations. You can change this focus with the SELECT DATA SET option (Figure 5.1, page 20) or the PROCESS DATA SET option (Section 5.5 page 25).

Figure 5.3. Loading Data files.



Data or Method files can be loaded from other locations by using the mouse to select other drives or subdirectories. Such a selection automatically becomes the new DEFAULT DATA LOCATION. The default location will remain in effect throughout the session, unless changed by a LOAD or SAVE operation. When you finish your session and QUIT the software, saving the REPORT.INI file will save the new default data location for subsequent sessions. If REPORT.INI is not saved, the new data location will be forgotten; the original default location will be used for subsequent sessions (Section 11, page 69).

5.2 Detecting Peaks

The first step in manually processing a Data file is to identify the peaks that are present. In this step the software determines what a normal baseline is (a consecutive set of data points whose values don't change significantly) and then determines where peaks start and stop (consecutive sets of data points whose values change markedly). The Method options involved in these decisions are the INITIAL SLOPE THRESHOLD, SMOOTHING WIDTH, and PROCESSING EVENTS (Sections 4.5 and 4.6, pages 10 and 11).

Select the DETECT PEAKS option from the Process Menu (Figure 5.4), and select the screen as LIST DEVICE when prompted (peaks will be listed only if the List Peaks option in Processing Options is turned on – Figure 4.4, page 10). As peak start and stop points are detected, a list of times and baseline values will be posted to the output window (Figure 5.5). These can be reviewed by using the scrolling feature of the Output Window.

Figure 5.4. Select the Detect Peaks option.

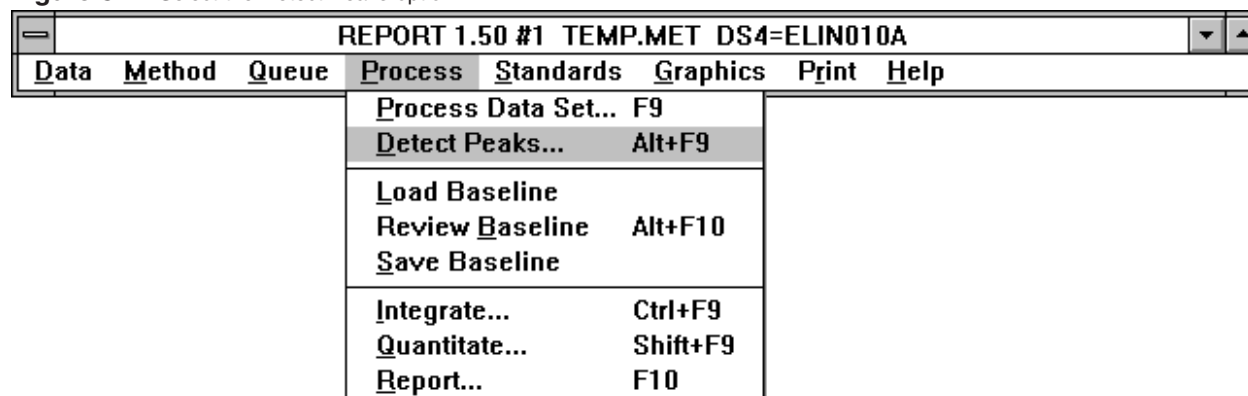
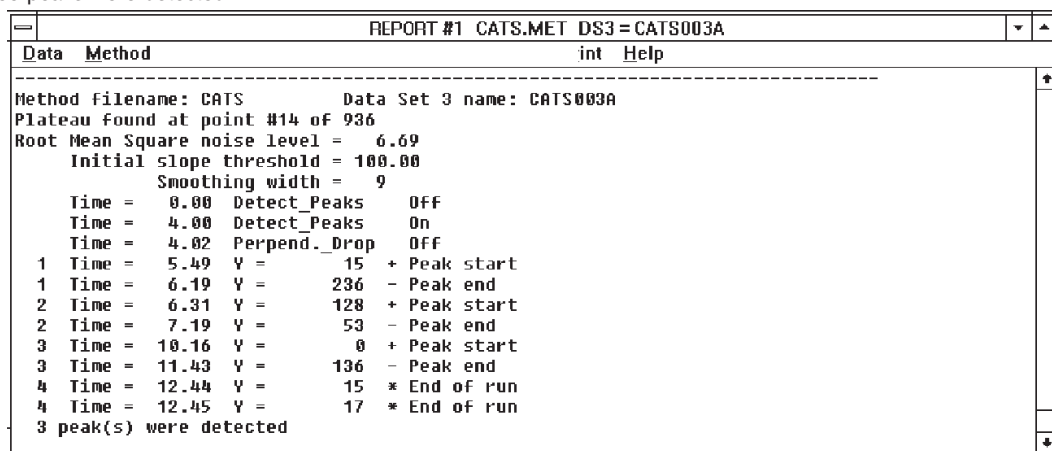
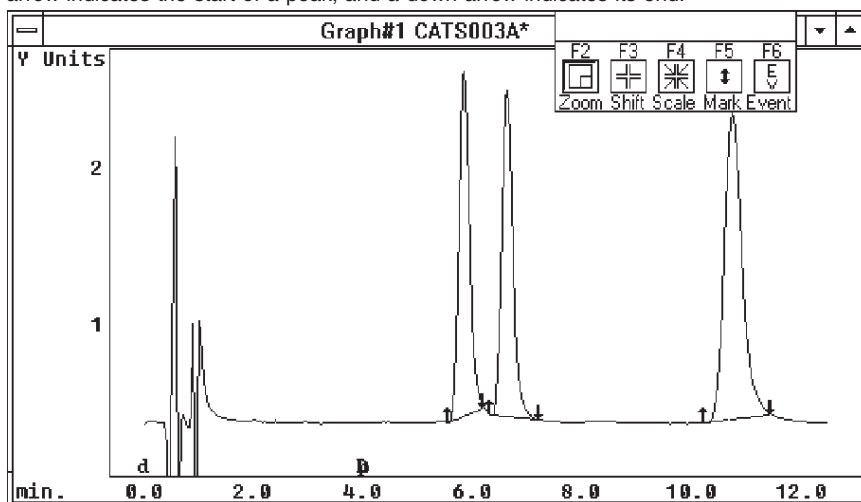


Figure 5.5. Three peaks were detected.



If you wish to see the chromatogram at this point, take the REVIEW BASELINE option from the Process Menu (Figure 5.4). The graph shows the chromatogram, with up- and down-arrows indicating the start and stop points of peaks (Figure 5.6). All the interactive features of ChromGraph graphics are available in this screen (see Using The Graphics Menu, Section 8, page 52).

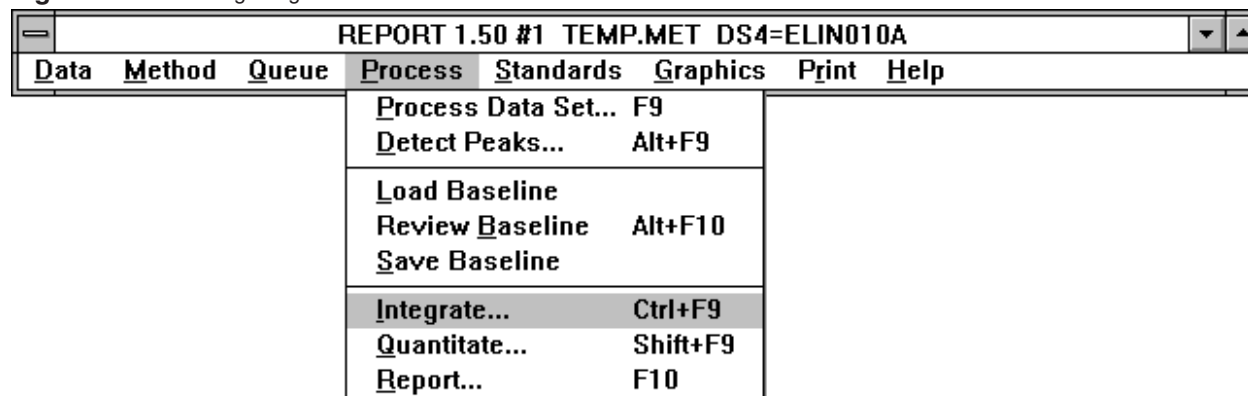
You can also LOAD a BASELINE, which brings up a choice of previously saved baseline (.bln) files. Baseline files can be saved here as well, or automatically saved whenever a data set is processed (Section 4.5). Baseline files take up a lot of disk space, and can easily be recreated from the raw data using the REVIEW BASELINE option. Therefore, we recommend that they only be saved if you are required by regulation to archive the original chromatogram.

Figure 5.6. Review Baseline. An up arrow indicates the start of a peak, and a down arrow indicates its end.

It's likely that the first attempt at peak detection for an unknown Data file will not provide entirely satisfactory results. There may be peaks that have not been detected. Or every little blip in the baseline may have been interpreted as a peak. If this is the case, return to the PROCESSING OPTIONS and PROCESSING EVENTS sections of the Method (Sections 4.5 and 4.6, pages 10 and 11), adjust the relevant parameters, and detect the peaks again. Feel free to enter extreme values to see what will happen; it's a good way to gain familiarity with the software.

5.3 Integration

Integration can proceed only after the peaks have been detected. The integration function determines the area under each peak between the start and stop points. To integrate the peaks, select INTEGRATE from the Process Menu (Figure 5.7), and choose the screen as LIST DEVICE when prompted.

Figure 5.7. Selecting Integrate from the Process Menu.

A list of sequentially numbered peaks, with retention times, heights and areas, will be posted to the Output Window (Figure 5.8). These may be reviewed with the scrolling feature of the Output Window.

Figure 5.8. The integrated data.

REPORT #1 CATS.MET DS3=CATS003A			
Data	Method	Queue	Process Standards Graphics Print Help
Peak	Minutes	Height	Area *
1	5.81	5537	70967
2	6.60	5227	70305
3	10.71	4920	107665
		15684	248937 #

Small peaks of little interest may be deleted from the list of peaks by raising the MINIMUM PEAK AREA (Figure 4.4, page 10).

5.4 Quantitation

Quantitation is the comparison of the integrated data to a pre-defined set of integrated standards, called a STANDARDS FILE. The Standards file must be created from a Data file generated by one or more injections of standards (see Using Standards, Section 7, page 35).

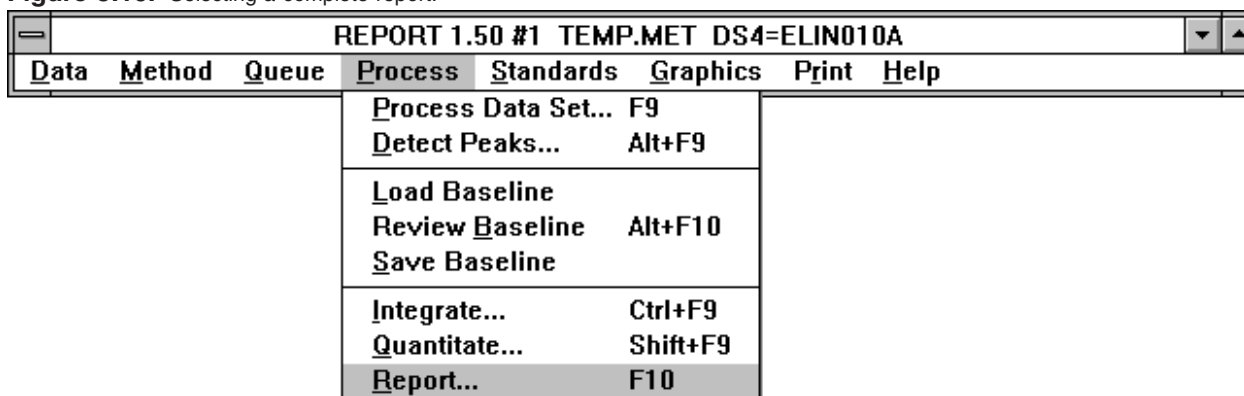
In order to quantitate in the manual mode, you must have integrated the current Data file and have a Standards file in RAM. Standards files may be loaded through the Standards Menu. If a Standards file is specified in the Method (Figure 4.9, page 16) it will automatically be loaded when the Method is loaded. Load the Standards file named CATS.STA for this example (LOAD STANDARDS option, Figure 7.1, page 35).

To quantitate, select QUANTITATE from the Process Menu, and choose the screen as LIST DEVICE when prompted. A sequential list of peaks, and the results of the quantitation, will appear on the Output Window (Figure 5.9). The items that appear in the list are those checked in the REPORT OPTIONS and STANDARDS OPTIONS sections of the Method (Figures 4.8 and 4.9, pages 14 and 16). The column under the asterisk (*) is a comment column: a question mark (?) here indicates that the calculated amount was extrapolated more than 10% from the standard curve, and may be questionable. A pound sign (#) indicates that a peak present in the standards file was not found in this sample.

Figure 5.9. Results of Quantitate

REPORT #1 CATS.MET DS3=CATS003A					
Data	Method	Queue	Process	Standards	Graphics Print Help
Peak	Minutes	Area *	Peak-Name	pg	dTime
1	5.81	70967	epinephrine	753.67	0.00
2	6.60	70305	norepinephrine	753.43	0.00
3	10.71	107665	dhba	760.56	0.00
		248937 #	Totals	2267.66	

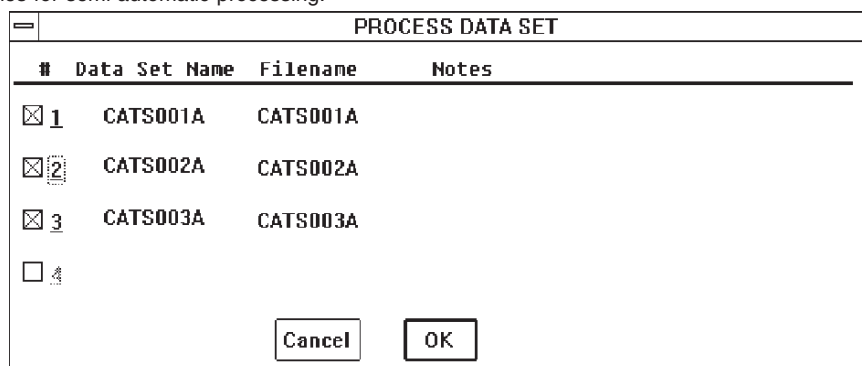
A complete report, including a descriptive header and all items (including the graph) selected in the Method, can be obtained by selecting the REPORT option in the Process Menu (Figure 5.10).

Figure 5.10. Selecting a complete report.

Once the data analysis and reporting options are properly set, be sure to SAVE the updated version of the Method (Section 4.10, page 19).

5.5 Semi-Automatic Processing

From one to four runs can be processed fairly rapidly, once a satisfactory Method has been developed. Select PROCESS DATA SET from the Process Menu. The Process Data Set dialog box will appear, allowing the selection of any (or all) Data files in RAM for processing (Figure 5.11). Click on the Data files to be processed, and then on OK. The Data files will be processed sequentially according to the options specified in the Method.

Figure 5.11. Selecting three Data files for semi-automatic processing.

SECTION 6. QUEUES: AUTOMATIC PROCESSING

6.1 Using A Queue

A QUEUE is a list of Methods and Data files to be processed. It is used for automatic operation, and for linking with ChromGraph CONTROL (see the ChromGraph CONTROL manual, MF-9070).

Queues may be accessed through the QUEUE MENU (Figure 6.1). To begin setting up a Queue, take the DEFINE OR EDIT QUEUE option. This opens the DEFINE QUEUE dialog box (Figure 6.2).

Figure 6.1. The Queue Menu.

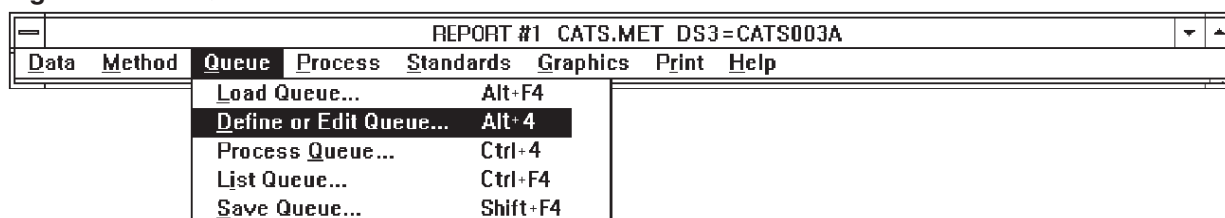


Figure 6.2. Define Queue dialog box.

Define Queue

Select a Queue item to edit...

#	Method	file	Data	Det	First	Last	Unknown/Standard	Level
1	CATS		CATS	A	1	6	Unknown	

#	Method	Data	Det	First	Last	Unknown/Standard	Level
1	CATS	CATS	A	1	6	<input checked="" type="radio"/> Unk <input type="radio"/> Bpl <input type="radio"/> Avg	

Buttons: Delete, Change, Insert

Data Path: _____

.PRN file: _____ Erase .PRN file Cycle detectors

Buttons: Cancel, OK, Save, Process Queue >>

The Define Queue dialog box contains a listbox of up to 20 lines. Each line directs ChromGraph to use a specific Method to process specific Data files. The Queue is not really limited to just 20 lines: you can chain Queues together by entering the name of a subsequent Queue as the Method name in the last line of any Queue.

Queue instructions are edited in the row of edit boxes across the middle of the screen, just above the row of DELETE, CHANGE and INSERT buttons. To edit an existing line in the Queue, first click on the line in the listbox. That line will then appear in the edit boxes. Make the appropriate changes, then click on the CHANGE button. The modified line will then be posted to the listbox.

To insert a new line in the Queue, first enter the information in the edit boxes. Then click the INSERT button.

To delete a line from the Queue, click on the line, then click DELETE.

The following items appear in the lines of the Queue:

is the sequential line number, 1-20.

METHOD is the ChromGraph REPORT Method to use to process the data.

DATA is the Data file name (4 characters, must end with a letter).

DET. is the detector letter code, A-D.

FIRST and LAST define the sequence of run numbers to be processed.

UNKNOWN/STANDARD indicates whether these Data files are from standards or unknowns. If they are standards, indicate whether the old standards are to be replaced (RPL) by the new data, or averaged (AVG) with them. For standards, also indicate which level (concentration) of standards is being processed. Section 7.4, page 46 contains detailed information on automatically updating Standards files using a Queue.

The options at the bottom of the screen provide general instructions for processing the Queue:

DATA PATH can be used to specify a non-default location from which to get Data files. Leave this box blank to get data from the default data location. The Method files must always be in the default data location.

.PRN FILE allows you to specify a name for the PRN, CSV or TAB file to which output from the entire Queue will be sent. Output to a PRN, CSV or TAB file also must be specified in the Report Options section of the Method (Figure 4.8, page 14), and PRN, CSV or TAB files must be specified in SETUP OPTIONS (Figure 2.5, page 5).

ERASE .PRN FILE box allows you to automatically erase any existing PRN, CSV or TAB file of the same name at the start of Queue processing. If not checked, and a PRN, CSV or TAB file of the same name exists, the report will be added to that file.

CYCLE DETECTORS can be useful if more than one detector was employed. If there are 20 runs for detector A on the first line of the Queue, and 20 runs for detector B on the second line, the Queue will usually process all the runs for detector A before going on to B. If the CYCLE DETECTOR box is checked, however, ChromGraph REPORT will process run 1 detector A, then run 1 detector B, then run 2 detector A, and so on.

Once editing is complete, the Queue may be SAVED for future use with the SAVE QUEUE option (Figure 6.1) or the SAVE button (Figure 6.2). A Queue name can have up to eight characters; the extension .QUE will automatically be appended. ChromGraph CONTROL Queues have the extension .BAQ to distinguish them. To save time when working with a series of runs, the ChromGraph CONTROL Queue can be loaded directly into ChromGraph REPORT (some editing will be necessary).

To begin processing the Queue, click on PROCESS QUEUE in the Define Queue dialog box or in the Queue menu. As each run is processed, the FIRST entry in the current line of the Queue is incremented by one. If you wish to restart the Queue from the beginning, either change FIRST back to its original number or load the SAVED version of the Queue.

If a Data file specified in the Queue is not present in the Default Data Location (or in the Data Path, if specified), the message REPORT QUEUE AWAITS, with the complete Data file name, will appear. This is normal behavior if ChromGraph REPORT is linked to ChromGraph CONTROL: when the run is completed, the DATA file will be passed to REPORT and processing will begin. If REPORT is not linked to CONTROL, the message indicates that the specified Data file cannot be found. Cancel the Queue and check for errors in the name or location of this Data file.

6.2 Using a PRN File

The PRN/CSV/TAB option allows you to easily import your data into spreadsheet programs such as Lotus 123™, Quattro Pro™, or Excel™. These spreadsheets are designed to import delimited ASCII files with, depending on the spreadsheet and version, a “PRN” and perhaps a “CSV” ASCII data format. Some applications may offer the option of importing tab delimited files as well.

6.2.1 Decide which file type to import.

Consult your application's documentation to determine which type of field delimited file it can import. Individual data fields in a PRN style file are typically separated by a comma, space, or semicolon, with character strings contained within quotation marks. Each line in a PRN file begins with an apostrophe (Figure 6.3). Since the PRN file format is the oldest, the importation of PRN files is supported by a wide variety of applications. As the name might suggest, TAB delimited files contain data fields which are separated by the Tab character. This format may be most widely used by database programs. CSV indicates a third type of field delimiter. CSV stands for “Comma Separated Values” (Figure 6.4). This format separates the contents of each data field with a comma, and leaves it up to the application to determine if characters in a field are text or numeric data (recent applications are usually quite good at such decisions). If your application supports importing comma separated

values, this is usually the best choice. In the unlikely event your application is mis-identifying whether data are text or numeric, then try the PRN format. Regardless of the type of field delimiter, some applications may default to expect any importable file to use a PRN filename extension, while others distinguish field delimiter types by filename extension. When ChromGraph Report creates a spreadsheet importable file, the delimiter format used is indicated by either a PRN, CSV, or TAB filename extension

6.2.2 Tell ChromGraph which file type to create.

Select DATA at the ChromGraph Report pull-down menu, then select SETUP OPTIONS (Figure 2.4). Check the PRN, CSV or TAB option as appropriate (Figure 2.5).

NOTE: The Setup Option, and occasionally this documentation, uses the term "PRN" files to generically refer to spreadsheet importable files which may actually be either CSV or TAB delimited files or classic PRN style files.

Once you have configured Report to create either PRN, CSV, or TAB files, you must then specify that you want the selected file type created by the current method. To do so, go to REPORT OPTIONS in the Method Menu and check .PRN.CSV.TAB FILE in the Report Output box (Figure 4.8).

6.2.3 Creating PRN/CSV/TAB Importable Files.

In Manual mode (Section 5, page 20) ChromGraph will create one importable PRN, CSV or TAB file for each run. If you have lots of samples, this can be somewhat cumbersome. In contrast, automatic processing using Report's QUEUE function creates just one PRN/CSV/TAB file for the whole series of runs. In this case, the data from each sample will be added to the PRN/CSV/TAB file sequentially, one run at a time. Thus it is much more efficient to automate run processing via a Queue, since only one all-inclusive importable file needs to be imported into the spreadsheet.

Figure 6.3. A typical PRN formatted file.

```

** Peak      Minutes      Height      Area      *
** 0000      00000000      000000000000 *
** 1          5.82          1963        24255      epinephrine      253.67      0.01      CATS      1      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 2          6.61          1884        25130      norepinephrine   253.43      0.01      CATS      1      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 3          10.72         1744        37524      ddba              260.56      0.01      CATS      1      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
**
**          5591        86908      0          Totals           767.66      CATS      1      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 1          5.82          3686        46584      epinephrine      492.67      0.00      CATS      2      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 2          6.61          3496        46786      norepinephrine   493.13      0.00      CATS      2      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 3          10.72         3247        68151      ddba              478.89      0.00      CATS      2      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
**
**          10429       161522     0          Totals           1464.69     CATS      2      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 1          5.81          5537        70967      epinephrine      753.67      0.00      CATS      3      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 2          6.60          5227        70305      norepinephrine   753.43      0.00      CATS      3      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 3          10.71         4920        107665     ddba              760.56      0.00      CATS      3      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
**
**          15684       248937     0          Totals           2267.66     CATS      3      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 1          5.81          1776        22070      epinephrine      230.27      0.00      CATS      4      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 2          6.60          1676        22269      norepinephrine   221.77      -0.00     CATS      4      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 3          10.71         1576        33891      ddba              234.66      -0.00     CATS      4      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
**
**          5028        78230     0          Totals           686.71      CATS      4      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 1          5.81          3627        45991      epinephrine      486.32      -0.00     CATS      5      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 2          6.60          3445        46164      norepinephrine   486.25      -0.00     CATS      5      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 3          10.70         3251        70381      ddba              494.78      -0.01     CATS      5      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
**
**          10323       162536     0          Totals           1467.35     CATS      5      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 1          5.81          5491        70390      epinephrine      747.48      -0.00     CATS      6      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 2          6.60          5176        69441      norepinephrine   743.87      -0.01     CATS      6      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
** 3          10.70         4899        106180     ddba              749.97      -0.01     CATS      6      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*
**
**          15967       246010     0          Totals           2241.32     CATS      6      A      CATS      SOLOMON      CATECHOLAMINES      CATS      1.00000      0.00000      1.00000*

```


Figure 6.4. A typical CSV delimited file.

```

Peak,Seconds,Height,Area,*,Peak-Name, pg, Std, Data, Run, D, Method, Operator, Conditions, Standard, Dilution, TimeZero,
1,98.5,68811,961244,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
2,162.1,5238,110279,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
3,201.3,5182,52024,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
4,220.8,9014,88442,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
5,294.9,9886,56826,,DA,2.36,4,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
6,310.4,9727,93976,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
7,328.8,9741,49995,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
8,343.7,6419,40225,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
9,372.1,9758,60759,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
10,532.3,7856,47570,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
11,622.5,8166,43556,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
12,711.8,14193,176100,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
13,750.0,6405,47850,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
14,754.5,8686,44396,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
15,884.9,8610,62520,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
16,902.7,11267,85835,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
17,964.0,9558,54661,,,,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
,,208516,2076258,0,Totals,2.36,,RCVH,1,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
1,99.4,67202,815050,,,,,RCVH,2,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
2,135.8,27701,198644,,EPI,9.09,2,RCVH,2,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
3,190.7,13428,131018,,DOPAC,7.51,3,RCVH,2,B,RCVH_B,JG,CSV file sample data,RCVH_B,1.00000,0.00000,
[...]
```

6.2.4 Selected Examples of Importing PRN/CSV/TAB Files.

With the variety of different importation formats and varying capabilities of different spreadsheets, it is beyond the scope of this manual to provide detailed instructions on importing files into every spreadsheet. However, several examples follow which may provide general guidelines. Most all spreadsheets incorporate the same basic functions given here, though their specific command structure may differ somewhat. These instructions presume that you are proficient with your spreadsheet program. If not, consult the documentation from your particular application for specific instructions.

Example I: Importing CSV files to Excel * 5.0 .

- 1) Open the spreadsheet into which you wish to import a ChromGraph CSV file.
- 2) Press the Home key to ensure that the cursor is in cell A1.
- 3) Select FILE→OPEN from the pull down menu.
- 4) Select the "*.PRN, *.TXT, *.CSV" file type in the Open File dialog box.
- 5) Navigate to the directory containing the file you want to import.
- 6) Double click on the listed CSV file you want to import.
- 7) Your CSV file should now be imported and properly parsed into the appropriate data fields.

Example II: Importing CSV files to Quattro Pro * 5.0.

- 1) Open the spreadsheet into which you wish to import a ChromGraph CSV file.
- 2) Press the Home key to ensure that the cursor is in cell A1.
- 3) Select DATA→IMPORT from the pull down menu.
- 4) Select the "Only Commas" option in the Import dialog box.
- 5) Navigate to the directory containing the file you want to import.
- 6) Change the filename extension box to read "*.CSV" and press OK.
- 7) Double click on the listed CSV file you want to import.
- 8) Your CSV file should now be imported and properly parsed into the appropriate data fields.

Example III: Importing files to older versions of Lotus 123.

As noted earlier, if your spreadsheet offers the option of importing CSV delimited files, use that option. CSV files, coupled with automatic parsing makes importing Report data files simple and quick. Recent versions of 123 support importing CSV files and automatic parsing. If your version of 123 does, select that option. The classic Lotus 123 for DOS, and 123 for Windows up to version 4.0, supported importing PRN but not CSV files. Instructions for importing a ChromGraph PRN file into 123 (for DOS) follow below in Example IV. Lotus 123 for Windows provides pull-down menu options for performing these functions. However, it also fully supports the backslash style of Lotus for DOS commands. If you are learning to import PRN files, we suggest that 123 for Windows users use the backslash style commands described in Example IV, and then adapt those instructions to the pull-down menu.

Example IV: Importing PRN files.

PRN formatted files are supported by a wide variety of applications. The instructions which follow illustrate importing PRN files into Lotus 123 for DOS. If you are using a different application which only supports importing PRN formatted files, adapt these instructions as necessary.

A description of the specific commands, as they appear in the Lotus menu, plus the standard Lotus command nomenclature of a slash followed by the first letter of each command, is provided below (e.g., /FIN for File, Import, Numbers). Use the “slash[command]” approach described below both with Lotus for DOS and Lotus for Windows. 123 for DOS users should recognize this nomenclature. 123 for Windows users should find that they have this same command structure available as soon as they press the slash (“/”) key.

- 1) Open a 123 spreadsheet and do a /File Import Numbers (/FIN) command, selecting the name (and directory if necessary) of the file containing your data. This will import the PRN file into the empty worksheet. The data is imported as long strings of text. The subsequent steps tell how to parse the imported data into text and data fields to prepare them for later processing.
- 2) Press the Home key to ensure that the cursor is in cell A1.
- 3) Do a /Data Parse Format-Line Create (/DPFC) command to create a format line for parsing the label line (in row 1) into column labels. The original label line will now move to row 2, and a parsing command line for those labels will be placed in row 1.
- 4) Select Input-Column (/DPI) to parse both the row of column labels imported with the file and the parsing format line created from the previous operation. Highlight cells A1 . . . A2.
- 5) Select Output-Range (/DPO). For the output range, specify column AA1 to the right of the imported data.
- 6) Select Go (/DPG).

You have now parsed the column labels into their individual fields. You must now parse the data itself into its individual fields. As described below, you will use essentially the same commands to do so.

The various data fields may consist of either text or numbers. To ensure that your spreadsheet can properly identify what fields should be parsed as text and numbers, ChromGraph includes a parsing instruction line in PRN style files. That line should now be in cell A3. The line consists of strings of zeros interspersed with some of the text from the line above it.

- 7) With your cursor in cell A3, do a /Data Parse Format-Line Create (/DPFC) command to create a format line for parsing the data. The formatting line will be placed in row 3, with all subsequent rows moving down one position.

- 8) Select Input-Column (/DPI) to parse both the new parsing format line created from the previous operation and all the rows of data beneath that line to the end of the imported data (cells A3 . . . An).
- 9) Select Output-Range (/DPO). Specify cell AA2 as the output range.
- 10) Select Go (/DPG).
- 11) Select /Range Erase (/RE) and erase the unparsed data that were originally imported into the spreadsheet (starting at cell A1 down).
- 12) Select /Range Erase (/RE) and erase cells (AA2 . . . AR2) to eliminate the parsing instruction line inserted by ChromGraph.
- 13) Your PRN file should now be imported and properly parsed into the appropriate data fields.

6.2.5 Arranging the spreadsheet data.

Your Report PRN or CSV file is now imported into your spreadsheet and parsed into the appropriate columns. Sequentially, the rows contain all the peaks from run one, followed by all the peaks from run two, followed by all the peaks from run three, etc. This is probably not the format you want your final data in. You must now arrange your data into the desired format. Different investigators will have specific requirements for the final format of their spreadsheet data. To accommodate these differing needs, ChromGraph also includes the identifying information associated with each peak. This information is on the same line as the peak's values (Figure 6.3, Figure 6.4). This way, investigators can use standard spreadsheet commands (sort, cut, paste, etc.) to manipulate their data into the desired format, without risk of losing the data's identifying information in the process. For example, to arrange the data into a table showing the calculated amount of each analyte for each run (Figure 6.5):

- 1) Erase all but the Peak Name, Units Of Amount, Run, and * fields (Note, an asterisk or question mark in this field indicate that the validity of the corresponding calculated value may be suspect. Thus it is important to keep this field to call your attention to any questionable data).
- 2) Sort the data by Peak Name and Run Number as the primary and secondary sort keys.
- 3) Copy one set of the Run Numbers into an empty row of cells.
- 4) Copy or type the Peak Names into the following rows (thus placing them orthogonal to the Run Numbers to form a table of Run Numbers X Peak Names).

- 5) Move the data for each peak beside the corresponding Run Number and Name. The data are now organized by Analyte and Run Number.
- 6) Add the necessary annotation.

Figure 6.5. Final spreadsheet excerpt of Report data after importing and sorting into run number vs. analyte format.

A1: READY								
	A	B	C	D	E	F	G	H
1								
2	Data Set: Cats							
3	Operator: Solomon							
4	Metod: Cats							
5	Conditions: Catecholamines							
6								
7	—— picograms of: ——							
8	Run #	Norepi	Epi	DHBA				
9	1	253.43	253.67	260.56				
10	2	493.13	492.67	478.89				
11	3	753.43	753.67	760.56				
12	4	221.77	230.27	234.66				
13	5	486.25	486.32	494.78				
14	6	743.87	747.48	749.97				
15								
16								
17								
18								
19								
20								

SECTION 7. USING STANDARDS

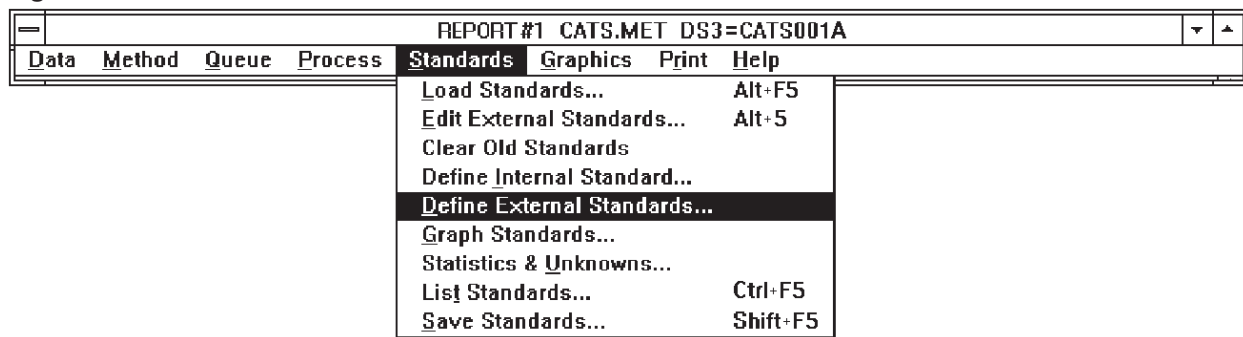
A STANDARDS FILE is simply a set of peak names, retention times, peak areas and amounts (or concentrations) used to quantitate unknown samples. A typical Standards file will have at least two concentrations of standards, generally a high and a low, to bracket the expected concentrations of the samples. A one-level Standards file cheats somewhat by assuming the origin (0 concentration, 0 area) as the lower limit. This works mathematically, but is subject to extrapolation errors. We recommend using at least a two-level Standards file.

ChromGraph allows you to construct a Standards file of up to ten levels, including both external and internal standards, and to update this Standards file automatically. The Standard Addition method for endogenous analytes is supported (Section 4.8).

7.1 Creating A One-Level External Standards File

A Standards file is constructed from processed data (retention times, peak heights, peak areas), so before creating the Standards file, load the appropriate Data file and process it. (CATS001A.DAT for this example.)

Figure 7.1. The Standards Menu.



Next choose the DEFINE EXTERNAL STANDARDS section from the Standards Menu (Figure 7.1). The DEFINE EXTERNAL STANDARDS dialog box will appear (Figure 7.2).

Figure 7.2. Define External Standards.

DEFINE EXTERNAL STANDARDS

Standards Filename Quantitate by
 Area Height

Current results from Data Set # 1 CATS001A
 Number of Standard Peaks: 0 Number of Levels: 0

Standard level:

Default amount: *

Units of amount:

Default % toler:

Update retention times

Action on standard level
 Insert new results
 Average with old results
 Replace old results
 Insert zeroes
 Delete this level

<< Clear Old Cancel OK Save Edit Ext. Std. >>

Since this will be a new Standards file, click on the << CLEAR OLD button, to erase any old Standards file that may be in RAM. Next enter the following information (consult Figure 7.2 for the appropriate entries for this data set):

STANDARD LEVEL should be 1, since this is the first level (concentration) of a one-level file.

DEFAULT AMOUNT. If all peaks in the standard injection have the same amount or concentration, enter it here. This is a convenience, as this amount will be brought up automatically in the EDIT STANDARDS section. If all peaks have different concentrations, just ignore this section.

The *-box to the right of Default Amount makes it easier to add new levels to Standard files whose peaks have different concentrations. See Section 7.2, page 39.

UNITS OF AMOUNT. This is a label for the final results. Enter whatever is appropriate (e.g., pg, μm).

DEFAULT % TOLERANCE is the window in time within which a sample peak must fall in order to be identified. For example, if peak 1 in the Standards file has a retention time of 5.0 minutes and a tolerance of 5%, any unknown peak between 4.75 and 5.25 minutes (5 minutes $\pm 5\%$) will be identified as peak 1. Default % Tolerance must be greater than zero in order to identify peaks.

UPDATE RETENTION TIMES is used during manual or automatic updating (Section 7.4, page 46). It allows the retention times of the new standard injection to be incorporated into the Standards file, and should generally be turned on.

QUANTITATE BY (choose area or height). This option becomes fixed after the first standard is entered; all components of the Standards file must use the same criterion.

ACTION ON STANDARD LEVEL selects the current operation. In this case we will INSERT NEW RESULTS. When updating an existing Standards file with a new injection, use REPLACE OLD RESULTS. DELETE THIS LEVEL is useful when you've accidentally added an extra level, or to clear all levels without losing peak names.

To enter the specified information into the Standards file, click the EDIT EXT. STD. >> button. (OK also enters the information, but does not proceed to the next section.) Information on the level-one standards is automatically entered from the most recently processed Data file, but must be edited. The EDIT EXTERNAL STANDARDS dialog box appears next (Figure 7.3).

Figure 7.3. Edit External Standards.

Ret.Time		Peak name
5.82		
6.61		
10.72		

Level	Amount	Peak Area

Ret.Time:	Peak Name:	Level:	Amount:	Peak Area:
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Group:	% Tolerance:			
<input type="text"/>	<input type="text"/>			

<input type="button" value="Delete"/>	<input type="button" value="Change"/>	<input type="button" value="Insert"/>	<input type="button" value="Change level"/>
---------------------------------------	---------------------------------------	---------------------------------------	---

<input type="button" value=" << Define Std."/>	<input type="button" value=" Cancel"/>	<input type="button" value=" OK"/>	<input type="button" value=" Save"/>	<input type="button" value=" List Std. >>"/>
--	--	------------------------------------	--------------------------------------	--

Although the EDIT EXTERNAL STANDARDS dialog box has many options and features, for most purposes the only thing that has to be done here is to name the peaks and perhaps enter an amount. Click on the retention time for the first peak that you wish to incorporate into the Standards file: the retention time will appear in the edit boxes below, and the amount and area will appear on the right. Enter the peak's name in the edit box, and press CHANGE to post it to the listbox. If you used a Default Amount in the Define External Standards dialog, then AMOUNT should be correct. If not, click on it and enter the correct amount in the edit boxes on the right. Click CHANGE LEVEL to post the corrected amount to the listbox.

Continue to name each peak of interest. If there are other peaks present, they may be deleted by highlighting their retention times and clicking the DELETE button. (If you accidentally delete a peak, press the INSERT button immediately to recall it.) The completed dialog box should look like Figure 7.4.

Figure 7.4. Editing of external standards is complete.

EDIT EXTERNAL STANDARDS		
Select a Peak...		Then, select a Level...
Ret.Time	Peak name	Level Amount Peak Area
5.82	EPINEPHRINE	1 250.00000 37524
6.61	NOREPINEPHRINE	
10.72	DHBA	
Ret.Time: Peak Name:		Level: Amount: Peak Area:
10.72	DHBA	<input type="text"/> <input type="text"/> <input type="text"/>
Group: 0	% Tolerance: 5.0	
<input type="button" value="Delete"/>	<input type="button" value="Change"/>	<input type="button" value="Change level"/>
<input type="button" value="Insert"/>		
<input type="button" value=" << Define Std."/>	<input type="button" value=" Cancel"/>	<input type="button" value=" OK"/>
<input type="button" value=" Save"/>	<input type="button" value=" List Std. >>"/>	

As a final check that all is correct, click the LIST STD. >> button for a listing of your completed Standards file (Figure 7.5). Verify that each peak of interest is included in the list, with accurate amounts and areas or heights. Then take the SAVE STANDARDS option from the Standards Menu (Figure 7.6) to name and save the Standards file. Standards file names can have up to eight characters, and the extension .STA will automatically be appended to the name you choose. The name of the Standards file will automatically be recorded in the Method, under STANDARDS OPTIONS (Figure 4.9, page 16).

Figure 7.5. List the completed Standards file to verify.

REPORT #1 CATS.MET DS3=CATS001A						
Data	Method	Queue	Process	Standards	Graphics	Print Help
ChromGraph Fri Jul 09 08:32:30 1993						
Standards (Area):						
< External Standards >						
Level	Data file					
1	CATS001A					
Std#	Minutes	+/-%	Peak_name	Group	pg	Area
1	5.82	5.0	EPINEPHRINE	0	250.00000	24255
2	6.61	5.0	NOREPINEPHRINE	0	250.00000	25130
3	10.72	5.0	DHBA	0	250.00000	37524

Figure 7.6. Take the Save Standards option to permanently save the completed Standards file.

REPORT #1 CATS.MET DS3=CATS001A					
Data	Method	Queue	Process	Standards	Graphics Print Help
<ul style="list-style-type: none"> Load Standards... Alt+F5 Edit External Standards... Alt+5 Clear Old Standards Define Internal Standard... Define External Standards... Graph Standards... Statistics & Unknowns... List Standards... Ctrl+F5 Save Standards... Shift+F5 					

7.2 Creating A Multi-Level External Standards File

Building a multi-level Standards file is a stepwise extension of building a single-level Standards file. Editing or adding subsequent levels is easier, however, since the peak names have already been entered. (If you've just built the one-level standards file of section 7.1, skip the next paragraph.)

Begin by loading and processing the Data file for the first level. (Most people find it easier to build the Standards file in ascending order of concentration, so we will adopt that convention.) Then build a one-level Standards file, exactly as in Section 7.1. Save the Standards file, as a precaution.

Next load and process the Data file for the second level. To follow along here, load and process CATS002A.DAT. Then select DEFINE EXTERNAL STANDARDS from the Standards Menu.

IMPORTANT: DO NOT CLEAR THE OLD STANDARDS FOR THE SECOND AND SUBSEQUENT LEVELS! WE WANT TO ADD TO THE PREVIOUS LEVEL, NOT REPLACE IT.

Figure 7.7 shows the options for the second level. There are only two changes from the first level: STANDARD LEVEL changes from 1 to 2, indicating that this will be the second level, and DEFAULT AMOUNT (if used) changes to reflect the concentration of standards in the second level.

Figure 7.7. Defining the second level of standards.

The screenshot shows a dialog box titled "DEFINE EXTERNAL STANDARDS". It contains the following fields and options:

- Standards Filename:** (empty text field)
- Quantitate by:** Radio buttons for Area and Height.
- Current results from Data Set # 2 CATS002A:**
 - Number of Standard Peaks: 3
 - Number of Levels: 1
- Standard level:** Text box containing the number 2.
- Default amount:** Text box containing 500, followed by a small square checkbox and an asterisk (*).
- Units of amount:** Text box containing pg.
- Default % toler:** Text box containing 5.00.
- Update retention times**
- Action on standard level:** Radio buttons for Insert new results, Average with old results, Replace old results, Insert zeroes, and Delete this level.

At the bottom of the dialog are five buttons: "<< Clear Old", "Cancel", "OK", "Save", and "Edit Ext. Std. >>".

The *-box to the right of Default Amount makes adding levels easier when 1) peaks within levels have different amounts, and 2) all peak amounts in a subsequent level are a constant multiple of amounts in the immediately preceding level. To use this feature, click on the *-box and enter the multiple as the Default Amount. For example, assume level one has four peaks with the following amounts: 100, 50, 250, 75ng. Clicking the *-box and entering 2.0 as Default Amount causes the following amounts to be automatically entered for the four peaks in level two: 200, 100, 500, 150 ng. This feature should not be checked for the tutorial example.

When the Define External Standards section is completed, click on the EDIT EXT. STD. >> button to add the second level and switch to the editing dialog. The EDIT EXTERNAL STANDARDS dialog box will appear much as it did in level one (Figure 7.8). But when you click on one of the peak names, both levels of amounts and areas will appear on the right side of the screen. As in level one, if the peaks comprising level two do not have the same concentrations you may have to do some editing of the amounts. When this is completed, click the LIST STD. >> button. The listing should look somewhat like Figure 7.9. Again, save these intermediate standards as a precaution.

Figure 7.8. Editing the second level of standards.

Select a Peak...		Then, select a Level...		
Ret. Time	Peak name	Level	Amount	Peak Area
5.82	EPINEPHRINE	1	250.00000	24255
6.61	NOREPINEPHRINE	2	500.00000	46584
10.72	DHBA			

Ret. Time: 5.82 Peak Name: EPINEPHRINE
 Group: 0 % Tolerance: 5.0
 Level: Amount: Peak Area:
 Change level

<< Define Std. Cancel OK Save List Std. >>

Figure 7.9. Listing of completed two-level Standards file.

REPORT #1 CATS.MET DS3 = CATS002A							
Data	Method	Queue	Process	Standards	Graphics	Print	Help
Standards (Area):							
< External Standards >							
Level	Data file						
1	CATS001A						
2	CATS002A						
Std#	Minutes	+/-%	Peak_name	Group	pg	Area	
1	5.82	5.0	EPINEPHRINE	0	250.00000	24255	
					500.00000	46584	
2	6.61	5.0	NOREPINEPHRINE	0	250.00000	25130	
					500.00000	46786	
3	10.72	5.0	DHBA	0	250.00000	37524	
					500.00000	68151	

Subsequent levels can be added exactly as level two was added. For a three-level Standards file, load and process CATS003A.DAT. Choose DEFINE EXTERNAL STANDARDS from the STANDARDS MENU. Change STANDARD LEVEL to 3 and DEFAULT AMOUNT to 750. Then click the EDIT EXT. STD.>> button. The EDIT EXTERNAL STANDARDS dialog box will look like Figure 7.10, and LIST STD.>> will produce a listing like Figure 7.11. Be sure to save the final version of the Standards file.

Figure 7.10. Editing a three-level Standards file.

Figure 7.11. Listing of completed three-level Standards file.

REPORT #1 CATS.MET DS3=CATS003A						
Data	Method	Queue	Process	Standards	Graphics	Print Help
Standards (Area):						
< External Standards >						
Level	Data file					
1	CATS001A					
2	CATS002A					
3	CATS003A					
Std#	Minutes	+/-%	Peak_name	Group	pg	Area
1	5.81	5.0	EPINEPHRINE	0	250.00000	24255
					500.00000	46584
					750.00000	70967
2	6.60	5.0	NOREPINEPHRINE	0	250.00000	25130
					500.00000	46786
					750.00000	70305
3	10.71	5.0	DHBA	0	250.00000	37524
					500.00000	68151
					750.00000	107665

7.3 Using An Internal Standard

An internal standard is used to correct for variable recovery during an extraction step in sample preparation. (If you're not using an extraction step, an internal standard may not be necessary.) A fixed amount of an internal standard is added to each sample before extraction, and to the standards. During processing, the area (or height) of each peak in every run is divided by the area (or height) of the internal standard for that run, to produce a ratio. This process automatically corrects for run-to-run variation in extraction efficiency

and chromatographic response (Snyder, L.R. and J.J. Kirkland. 1979. Introduction to modern liquid chromatography. Wiley- Interscience, NY).

For the standard runs, these ratios are fitted to a regression with amount as independent variable. For the samples, the ratios are used to predict amount from the regression. The report from an analysis using an internal standard will contain a column for HeightCF or AreaCF which contains these ratios (times 10,000, for precision).

Begin defining an internal standard by loading the data sets for the standards. For this example, load KITS001A.DAT through KITS004A.DAT (Figure 7.12) and KITS.MET.

Next take the CLEAR OLD STANDARDS option from the Standards Menu (Figure 7.13). Then process the KITS001A.DAT Data file. When the report appears, take the DEFINE INTERNAL STANDARD option from the Standards Menu (Figure 7.13).

Figure 7.12. Data files for the internal standard example. The NOTES section indicates amounts for the three peaks.

PROCESS DATA SET				
#	Data Set Name	Filename	Notes	
<input checked="" type="checkbox"/> 1	KITS001A	KITS001A	E = 100 PG NE = 100 PG DHBA = 400	
<input type="checkbox"/> 2	KITS002A	KITS002A	E = 200 PG NE = 200 PG DHBA = 400	
<input type="checkbox"/> 3	KITS003A	KITS003A	E = 300 PG NE = 300 PG DHBA = 400	
<input type="checkbox"/> 4	KITS004A	KITS004A	E = 400 PG NE = 400 PG DHBA = 400	

Figure 7.13. The Standards Menu

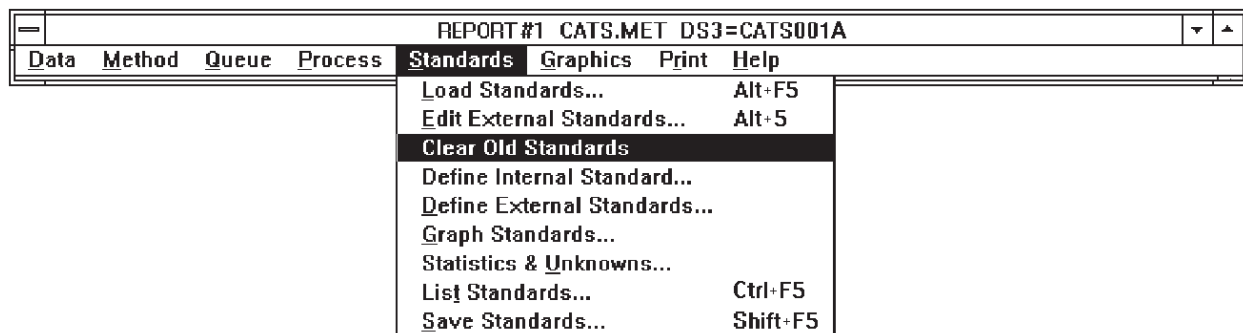


Figure 7.14. Selecting options in the Internal Standard dialog box.

DEFINE INTERNAL STANDARD		
Ret. Time	Height	Area
2.88	48148	304806
5.54	23217	247734
7.03	113718	1492695

Select a peak and then Edit below

Retention time:

% Tolerance:

Int. Std. Amount:

Expected area:

Minimum area:

Maximum area:

Quantitate by

Area **Height**

In the INTERNAL STANDARD dialog box (Figure 7.14) click on the retention time of the internal standard (7.03 minutes in this example). Select AREA or HEIGHT for quantitation. As before, % TOLERANCE is the window of time within which the software will accept a peak as being the internal standard (retention time \pm %).

INT. STD. AMOUNT should be set at zero. This tells the software that amounts are to come from external standards, and that the internal standard is to be used for calculating peak ratios within runs.

EXPECTED AREA (or height) is a correction factor that can be used to format the AreaCF (or HeightCF) column of the report. Set this at 10000, and the AreaCF column will report RATIO x 10000. As a verification that the standards have been set up properly, the internal standard identified in each run will have an AreaCF or HeightCF of exactly 10000 (read this as 1.0000).

MINIMUM and MAXIMUM AREAS (or heights) serve both to reject extraneous peaks and to warn you if there was an error in the extraction procedure. The software will not identify a peak in a sample as being the internal standard unless it 1) falls within the window of tolerance, 2) is no larger than the maximum area and 3) is no smaller than the minimum area.

Set the MAXIMUM AREA (or height) slightly greater than the area of the internal standard peak. If the internal standard peak in a sample is larger than this, it indicates an error in the extraction procedure, or a coeluting contaminant. The message "Internal standard was not found" will appear above the report.

Set the MINIMUM AREA (or height) to a value corresponding to a minimally acceptable extraction efficiency. For example, if you consider anything less than 50% extraction efficiency to indicate an error in the extraction procedure, set the MINIMUM AREA at 50% of the area of the internal standard peak in the first standard. If the internal standard peak in a sample is smaller than this, the message "Internal standard was not found" will appear above the report.

After defining the internal standard, press the SAVE button and name the standard. This saves the standard and, more importantly, enters its name in the Method (Figure 4.9, page 16) to enable the ratio calculations to proceed.

Since the level-one standard was not processed against the internal standard, this Data file must be processed again. After KITS001A.DAT is reprocessed, the report will contain an AreaCF column (Figure 7.15). Remember that these values are ratios x 10000, so the value for the internal standard (peak 3) is read as 1.0000, and the value for peak 1 is read as 0.2042.

Figure 7.15. Report of the level-one standard processed with the internal standard method.

REPORT#1 KITS.MET DS1=KITS001A							
Data	Method	Queue	Process	Standards	Graphics	Print	Help
ChromGraph REPORT 1.30 Tue May 10 09:08:09 1994							
Data filename: KITS	Run number: 1	Detector: A=EC1					
Uial number: 0	Data Set 1 name: KITS001A						
Time of run: 14:40	Date of run: 02/21/94						
Method filename: KITS	Run notes: E = 100 PG NE = 100 PG						
Operator: solomon	Conditions: catecholamines						
Standards (Area): KITS	Dilution multiple: 1						
Time zero offset: 0	Time scale factor: 1						
Int. Std. Time = 7.03	Area =1.4927e+006						
Expected Area = 10000	Correction Factor=0.00669929						
Peak	Minutes	AreaCF	×	Peak-Name	Amount		
1	2.88	2042					
2	5.54	1660					
3	7.03	10000					
				13702 0 Totals	0.00000		

Next you must build the external standard file as in Section 7.2. Choose DEFINE EXTERNAL STANDARDS from the Standards Menu (Figure 7.13).

IMPORTANT: DO NOT CLEAR OLD STANDARDS NOW

For the level-one standard, change STANDARD LEVEL to 1, DEFAULT AMOUNT to 100, UNITS OF AMOUNT to pg, DEFAULT % TOLERANCE to 5, and click on UPDATE RETENTION TIMES and INSERT NEW RESULTS (Figure 7.16). Then press the EDIT EXTERNAL STD. >> button.

Figure 7.16. Define External Standards dialog box for the level-one standard.

DEFINE EXTERNAL STANDARDS	
Standards Filename	KITS
Quantitate by <input checked="" type="radio"/> Area <input type="radio"/> Height	
Current results from Data Set # 1 KITS001A	
Number of Standard Peaks: 0	Number of Levels: 0
Standard level: <input type="text" value="1"/>	Action on standard level <input checked="" type="radio"/> Insert new results <input type="radio"/> Average with old results <input type="radio"/> Replace old results <input type="radio"/> Insert zeroes <input type="radio"/> Delete this level
Default amount: <input type="text" value="100"/> *	
Units of amount: <input type="text" value="pg"/>	
Default % toler: <input type="text" value="5"/>	
<input checked="" type="checkbox"/> Update retention times	
<input type="button" value=" << Clear Old"/> <input type="button" value=" Cancel"/> <input type="button" value=" OK"/> <input type="button" value=" Save"/> <input type="button" value=" Edit Ext. Std. >>"/>	

In the EDIT EXTERNAL STANDARDS screen (Figure 7.17), click on the retention time of each peak of interest, enter its name in the edit boxes, and change the % tolerance or amount if necessary. Delete any peaks of no interest, including the internal standard. Then press the OK button.

Figure 7.17. Edit External Standards

EDIT EXTERNAL STANDARDS																					
<table border="1"> <thead> <tr> <th colspan="2">Select a Peak...</th> <th colspan="3">Then, select a Level...</th> </tr> <tr> <th>Ret.Time</th> <th>Peak name</th> <th>Level</th> <th>Amount</th> <th>Peak Area</th> </tr> </thead> <tbody> <tr> <td>2.88</td> <td>norepinephrine</td> <td>1</td> <td>100.00000</td> <td>2042</td> </tr> <tr> <td>5.54</td> <td>epinephrine</td> <td></td> <td></td> <td></td> </tr> </tbody> </table>		Select a Peak...		Then, select a Level...			Ret.Time	Peak name	Level	Amount	Peak Area	2.88	norepinephrine	1	100.00000	2042	5.54	epinephrine			
Select a Peak...		Then, select a Level...																			
Ret.Time	Peak name	Level	Amount	Peak Area																	
2.88	norepinephrine	1	100.00000	2042																	
5.54	epinephrine																				
Ret.Time: <input type="text" value="2.88"/>	Peak Name: <input type="text" value="norepinephrine"/>	Level: <input type="text"/>	Amount: <input type="text"/>	Peak Area: <input type="text"/>																	
Group: <input type="text" value="0"/>	% Tolerance: <input type="text" value="5.0"/>	<input type="button" value="Change level"/>																			
<input type="button" value="Delete"/> <input type="button" value="Change"/> <input type="button" value="Insert"/>																					
<input type="button" value=" << Define Std."/> <input type="button" value=" Cancel"/> <input type="button" value=" OK"/> <input type="button" value=" Save"/> <input type="button" value=" List Std. >>"/>																					

Process each subsequent level of the standards, and insert each into the standards file via the DEFINE EXTERNAL STANDARDS dialog box. The run numbers and amounts are given in Figure 7.12. When the last level is entered, save the standards file and list it. The listing should look like Figure 7.18.

Figure 7.18. Final listing of four-level standards file with both internal and external standards

REPORT#1 KITS.MET DS4=KITS004A							
Data	Method	Queue	Process	Standards	Graphics	Print	Help
REPORT 1.30 Tue May 10 09:13:44 1994							
Standards (Area): KITS							
< Internal Standard >							
Retention time	7.03	% Tolerance	5.0	Int. Std. Amt.	0.00000		
Expected value	10000	Minimum value	1000000	Maximum value	1600000		
< External Standards >							
Level	Data file						
1	KITS001A						
2	KITS002A						
3	KITS003A						
4	KITS004A						
Std#	Minutes	+/-%	Peak_name	Group	pg	Area	
1	2.88	5.0	norepinephrine	0	100.00000	2042	
					200.00000	4129	
					300.00000	6064	
					400.00000	8242	
2	5.53	5.0	epinephrine	0	100.00000	1660	
					200.00000	3437	
					300.00000	5239	
					400.00000	7126	

You can now load and process KITS005A.DAT and KITS006A.DAT, which are simulated unknowns. One has about 150 pg norepinephrine and 350 pg epinephrine, and the other has the opposite amounts.

7.4 Manual And Automatic Standards Updating

Once set up, even multi-level Standards files containing internal and external standards may be easily updated with information from fresh standard injections. To manually update a Standards file, first do the relevant chromatographic runs, then load the Data files into RAM. Process the first-level Data file, then take the DEFINE EXTERNAL STANDARDS option in the Standards Menu (Figure 7.19).

Figure 7.19. Manually updating the first level of a Standards file.

DEFINE EXTERNAL STANDARDS	
Standards Filename	CATS Quantitate by <input checked="" type="radio"/> Area <input type="radio"/> Height
Current results from Data Set # 1 CATS001A	
Number of Standard Peaks: 3	Number of Levels: 3
Standard level: <input type="text" value="1"/>	Action on standard level <input type="radio"/> Insert new results <input type="radio"/> Average with old results <input checked="" type="radio"/> Replace old results <input type="radio"/> Insert zeroes <input type="radio"/> Delete this level
Default amount: <input type="text" value="250"/> *	
Units of amount: <input type="text" value="pg"/>	
Default % toler: <input type="text" value="5.00"/>	
<input checked="" type="checkbox"/> Update retention times	
<input type="button" value="← Clear Old"/> <input type="button" value="Cancel"/> <input type="button" value="OK"/> <input type="button" value="Save"/> <input type="button" value="Edit Ext. Std. >>"/>	

IMPORTANT: DO NOT CLEAR THE OLD STANDARDS!

Change the STANDARD LEVEL to 1, and click on REPLACE OLD RESULTS. Click the OK button and level one is done. All the peak names, amounts, and tolerances from the old Standards file are carried into the new file. Only areas (or heights) and retention times are updated. For updating to work properly, the retention times of the new standards must fall within the % Tolerance of the old standards. Information about the internal standard does not require updating.

Repeat the updating procedure for the remaining levels, remembering to first process the Data files. Be sure to indicate the appropriate level of each update. After the last level is entered, LIST the new Standards file to check for errors, then SAVE it.

Automatic updating of standards can be accomplished with a Queue (Section 6, page 26). The Queue can be set up so the first few Data files update the Standards file, and the remaining Data files are quantitated against the updated Standards file (Figure 7.20).

Figure 7.20. Automatically updating a three-level Standards file with a Queue.

Define Queue

Select a Queue item to edit...

#	Method file	Data	Det	First	Last	Unknown/Standard	Level
1	CATS	CATS	A	1	1	Standard_Replace	1
2	CATS	CATS	A	2	2	Standard_Replace	2
3	CATS	CATS	A	3	3	Standard_Replace	3
4	CATS	CATS	A	4	100	Unknown	

#	Method	Data	Det.	First	Last	Unknown/Standard	Level
4	CATS	CATS	A	4	100	<input checked="" type="radio"/> Unk <input type="radio"/> Rpl <input type="radio"/> Avg	

DO files Data Path:

BAS200.D0 .PRN file: Erase .PRN file Cycle detectors

The Queue in Figure 7.20 is set up to update a three-level Standards file, then process 97 samples. For clarity, use a separate line in the Queue for each standard level to be updated. For each level, indicate the Data file name and run number, and which level is to be replaced, then click on the RPL (replace) button. Click the UNKnown button when entering lines for unknowns.

7.5 Standards For Multiple Detectors

There are often several detectors in use, and therefore several channels of data, for each sample. These may be different types of detectors (e.g., electrochemical, ultraviolet and fluorescence) or the same type of detector at different gains. In either case, response will differ among detectors, so you need a separate Standards file for each detector.

In order to make a separate Standards file for each detector, simply build each one as before (Sections 7.1 and 7.2). Start with detector A, since it's easier to build the Standards files in a logical sequence. Load the Data files for detector A, optimize the Processing Options and Processing Events for these files, process the Data files and build the Standards file.

Both the Standards file for detector A, and the Method for detector A, must be differentiated from those for the remaining detectors. The Standards file must be separated because response differs among detectors. The Methods must be separated because 1) processing must be optimized for each detector individually, and 2) since the Method specifies which Standards file to use, the only way to ensure that the data from each detector are compared to the appropriate standards is to use a different Method for each detector.

Use an easily remembered code to differentiate Methods and Standards files by detector. For example, after building the Standards file for detector A, save it with a name associated with detector A, let's say GOATA. This automatically enters its name in the Standards Options section of the Method (Figure 4.9, page 16). Now save the Method with the name GOATA.

Next load the Data files for detector B, optimize processing for this detector, and build a Standards file. Save this file as GOATB, and save the Method file as GOATB. Do the same for detector C, calling the relevant Standards and Method files GOATC.

There will now be three Method files on disk: each one is optimized for processing the output of its associated detector, and each one will call the appropriate Standards file for quantitation. To start processing, simply define a Queue listing each Method and the appropriate Data files for each Method to process (See Section 6, page 26).

To add a level of complexity to this, Figure 7.21 shows a Queue designed to automatically update a three-level Standards file for each of two detectors, and then process sample Data files.

Figure 7.21. A Queue to automatically update Standards files and process sample data from two detectors.

Define Queue

Select a Queue item to edit..

#	Method	file	Data	Det	First	Last	Unknown/Standard	Level
1	GOATA		GOAT	A	1	1	Standard_Replace	1
2	GOATA		GOAT	A	2	2	Standard_Replace	2
3	GOATA		GOAT	A	3	3	Standard_Replace	3
4	GOATB		GOAT	B	1	1	Standard_Replace	1
5	GOATB		GOAT	B	2	2	Standard_Replace	2
6	GOATB		GOAT	B	3	3	Standard_Replace	3
7	GOATA		GOAT	A	4	100	Unknown	
8	GOATB		GOAT	B	4	100	Unknown	

#	Method	Data	Det.	First	Last	Unknown/Standard	Level
<input type="checkbox"/>	<input type="text"/>	<input type="text"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>	<input checked="" type="radio"/> Unk <input type="radio"/> Rpl <input type="radio"/> Avg	

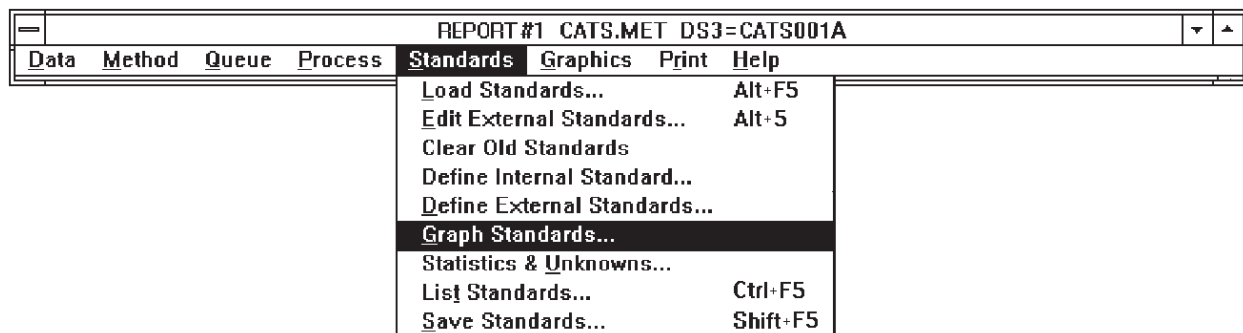
.DQ files Data Path:

BAS200.D0 .PRN file:
 Erase .PRN file
 Cycle detectors

7.6 Utilities For Standards

The Standards Menu (Figure 7.22) contains several options that facilitate working with Standards files. LOAD, LIST and SAVE have the usual meanings. GRAPH STANDARDS and STATISTICS & UNKNOWNNS allow you to interactively work with the Standards file.

Figure 7.22. The Standards Menu.



GRAPH STANDARDS opens a dialog box to graph the completed Standards file (Figure 7.23). This is used as a visual check for linearity or errors. Click on up to 5 peaks, select the line options, and click on OK. A sample graph is shown in Figure 7.24.

Figure 7.23. Graph Standards option.

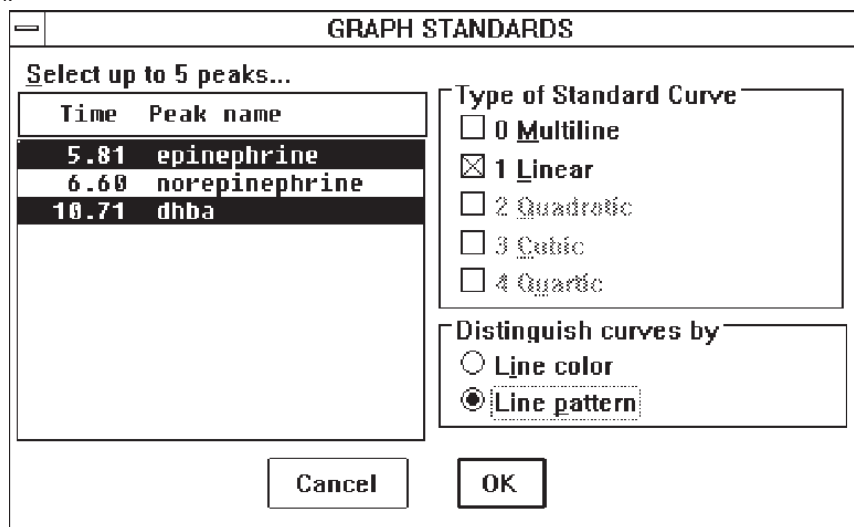
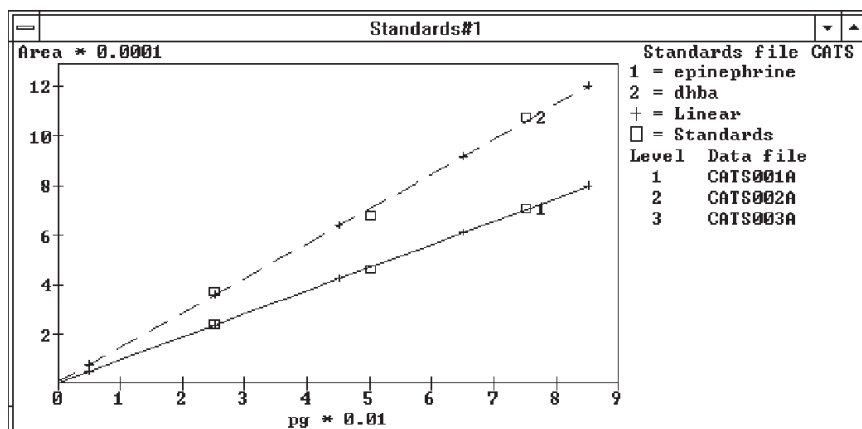


Figure 7.24. A graph of the standards.

STATISTICS & UNKNOWNNS opens a dialog box that reports statistical information about the standard curve for each peak, and allows you to enter areas or heights of unknowns and obtain calculated amounts (Figure 7.25). Simply click on the peak of interest to obtain statistics on the standard curve. To estimate the amount of an unknown, enter its height or area in the edit box, and click EVALUATE>>.

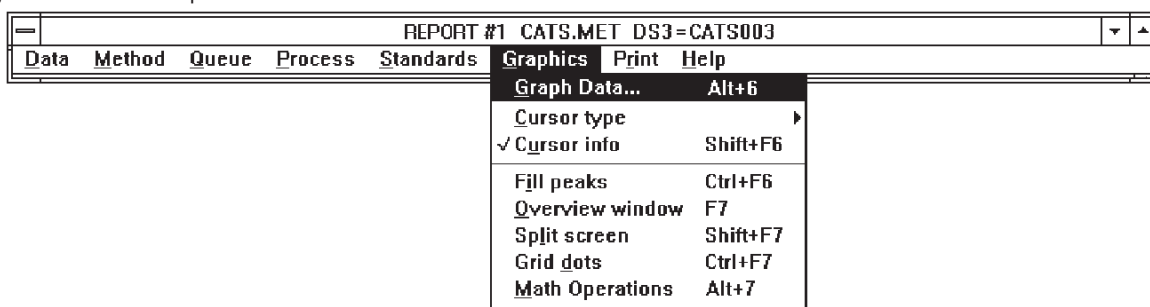
Figure 7.25. Statistics for the regression of epinephrine area on amount.

STANDARDS STATISTICS & UNKNOWNNS	
Select a peak...	
Time	Peak name
5.81	epinephrine
6.60	norepinephrine
10.71	dhba
Type of Standard Curve <input type="radio"/> 0 Multiline <input checked="" type="radio"/> 1 Linear <input type="radio"/> 2 Quadratic <input type="radio"/> 3 Cubic <input type="radio"/> 4 Quartic	
$Y = a+bX+cX^2+dX^3+eX^4$ a 556.798 d b 93.424 e c	
Coeff. of Determ. = 0.999356 Coeff. of Correl. = 0.999678 Standard Error = 838.71	
Evaluate Unknowns	
Measured: 75000	>>Evaluate>>
Amount	796.832
+/-Error	8.97849
<input type="button" value="Cancel"/> <input type="button" value="OK"/> <input type="button" value="List all >>"/>	

SECTION 8. USING THE GRAPHICS MENU

The GRAPHICS MENU (Figure 8.1) allows you to control the appearance of the graph, including which Data files are graphed, labels, colors, scaling, etc. In addition, a MATH OPERATIONS option is provided, which lets you manipulate the data (e.g., baseline subtraction).

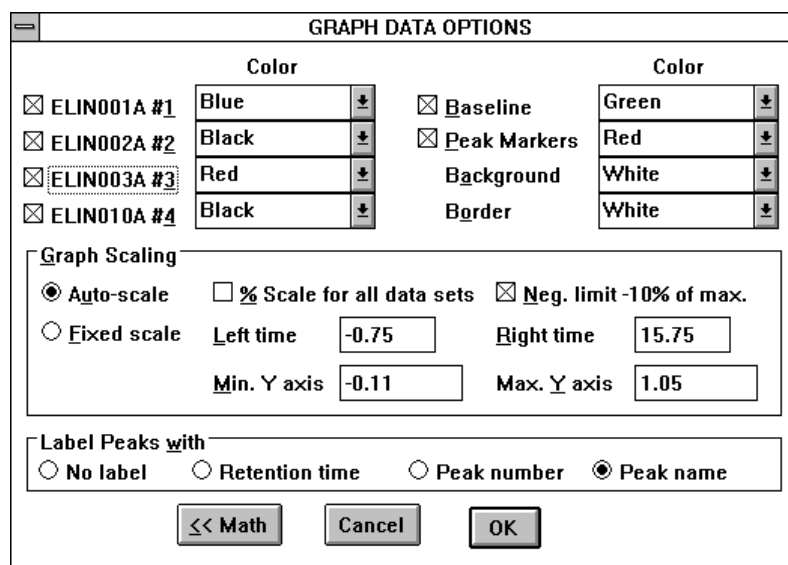
Figure 8.1. The Graphics Menu.



8.1 Graph Data

The GRAPH DATA option opens a dialog box with options for controlling the appearance of the graph (Figure 8.2). Each Data file in RAM will be listed. Simply click on the check box to request that one or more of the Data files appear in the graph. A drop-down listbox allows selection of a unique color for each Data file.

Figure 8.2. Options for graphing.



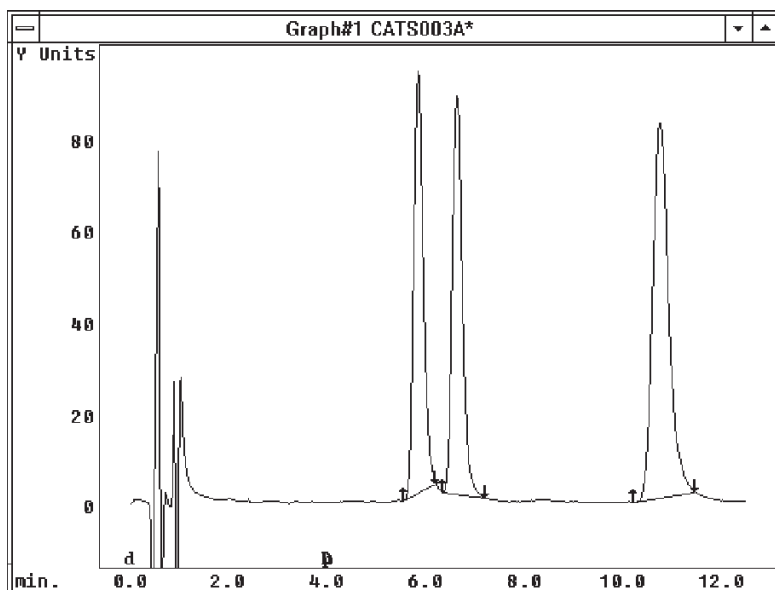
Color selection also is provided for other aspects of the graph: you may select whether or not the calculated baseline is drawn underneath peaks, and whether or not peak start and stop markers are included, and the colors for these options. Background and border colors also may be selected.

Depending on the hardware used during data collection, the graph might already be scaled in appropriate detector units. Alternatively, the graph might be scaled in arbitrary Y-units. The units of the Y-scale are purely esthetic — they have no effect on quantitation. However, you may change the units and the axis label to whatever seems appropriate. Press the MATH button (Figure 8.2), and change FULL SCALE Y and LABEL Y UNITS on the subsequent screen (Figure 8.13) to something suitable for the detector that was used.

The size of the peaks within the graph may be scaled in several ways. For temporary rescaling that only affects the current Data file, see SCALE CURSOR, Section 8.3.3, page 60. For rescaling that affects all the Data files to be processed, use the GRAPH SCALING options in the GRAPH OPTIONS dialog box (Figure 8.2).

AUTOMATIC SCALING is the simplest method of formatting the graph. In general terms, automatic scaling fits the length of the Data file into the X (time) axis, offsets the baseline about 15% above the bottom of the graph, and puts the highest peak near the top of the graph. This generally produces an acceptable graph (Figure 8.3). The size of the graph on the screen (and printer) may be adjusted by dragging its borders in or out with the mouse.

Figure 8.3. An automatically scaled graph.

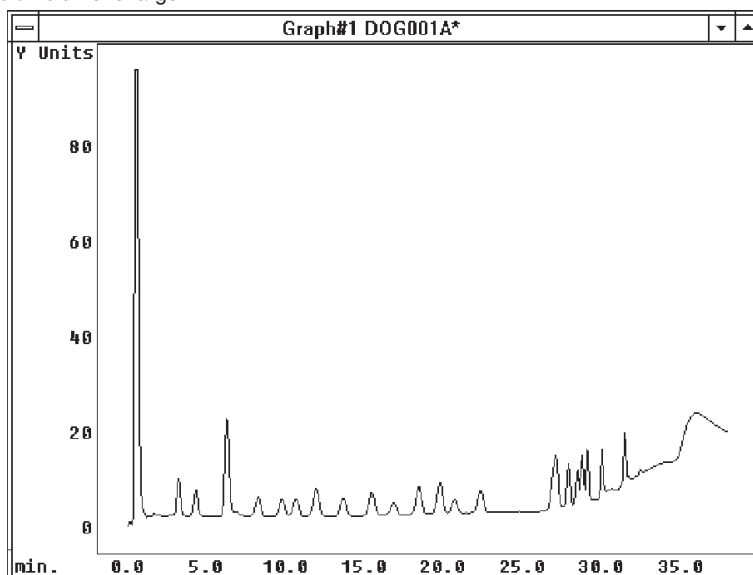


If the baseline is very negative and offscale, uncheck the Neg.Limit-10% box (Figure 8.2). The box is usually checked, to prevent large negative excursions of the baseline (e.g., the injection disturbance) from affecting scaling.

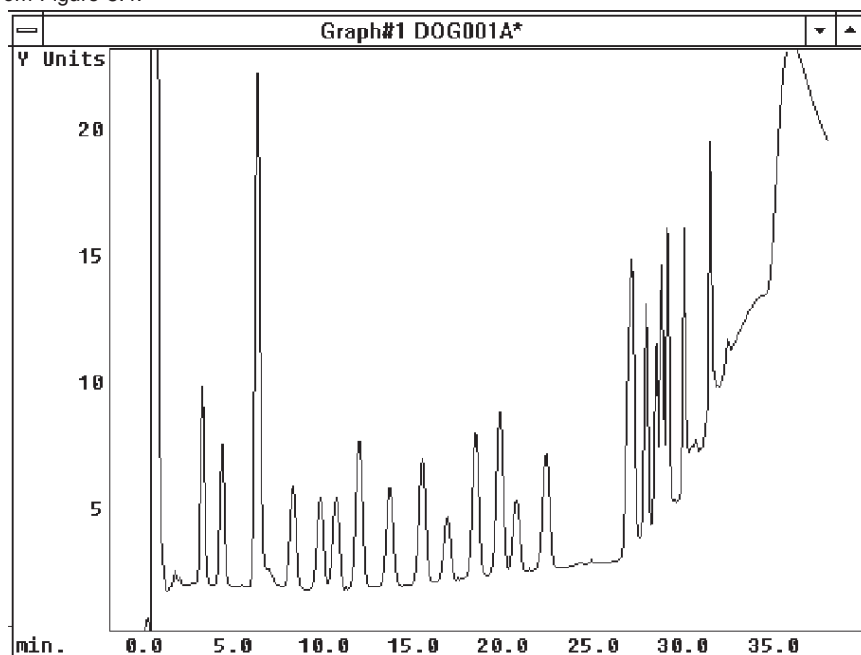
There are two drawbacks to automatic scaling. First, since each graph is separately scaled, it's hard to do visual comparisons of several Data files processed in batch: as each is processed and graphed, it will be maximized to fit the graph. (NOTE: peak heights, areas, and calculated amounts from the report are unaffected by scaling. These numbers may be used for comparing Data files.)

The second drawback to automatic scaling is that the scaling algorithms cannot distinguish between the void-volume response and a peak. At sensitive gains, the detector may show a full-scale deflection in response to the injection, while the true peaks may be quite small. Automatic scaling will fit the void-volume response to the graph, and consequently the peaks may be tiny (Figure 8.4).

Figure 8.4. Automatic scaling when the void volume is large.



Fixed scaling allows you to specify the upper and lower limits for the Y (and less commonly, the X) scale. Figure 8.5 shows an improved graph of the Data file from Figure 8.4, achieved by using fixed scaling. The fixed scale is retained in RAM, so every subsequent Data file will be graphed on the same scale. The fixed scale can be saved for subsequent use by Quitting with the REPORT.INI box checked (Section 11, page 69).

Figure 8.5. Fixed scaling of graph from Figure 8.4.

To use fixed scaling, first load the Data file with the largest peaks from your series of runs (this is generally the high standard). Process it with automatic scaling, then observe the graph. Use the Y-scale on the automatically scaled graph to estimate the appropriate upper and lower limits for the fixed scale. Then select GRAPH DATA (Figure 8.2). Click on the Fixed scale option, and enter the Min. and Max. Y-axis values. Click on OK to see the results, and if necessary, further refine the upper and lower limits. If necessary, the x-axis can be scaled using the Left and Right time boxes (Figure 8.2).

The final option in GRAPH DATA (Figure 8.2) allows the peaks to be labeled with their retention time, number (sequential number from the integration report) or name (from the Standards file). These options are only available when the Data file has been Processed (Section 5, page 20). Unprocessed data may be graphed but not labeled.

8.2 Graph Formatting Options

The formatting options include the following choices for controlling the appearance of the graph. They can be turned on or off by clicking on their names in the Graphics Menu (Figure 8.6).

FILL PEAKS colors in the area under each peak (Figure 8.7). Peaks will be filled with the line color selected for that Data file (Figure 8.2). If several Data files are graphed simultaneously, only the most recently processed Data file will have filled peaks.

OVERVIEW WINDOW enables a second window which always shows the entire chromatogram (Figure 8.7). When a section of the main graph has been enlarged, the enlarged section is indicated on the Overview Window by a rectangle (see Zoom Cursor, Section 8.3.1, page 58). The relative sizes of the Overview Window and the main graph can be adjusted by dragging on the border between them with the mouse.

SPLIT SCREEN divides the screen horizontally so that Data files will graph separately rather than overlap (Figure 8.7).

GRID DOTS overlays a grid on the graph, to facilitate visual estimations of peak height and retention time (Figure 8.7). For more accurate estimates, enlarge the peak of interest so the grid switches to a finer scale (see Zoom Cursor, Section 8.3.1, page 58).

Figure 8.6. Formatting options.

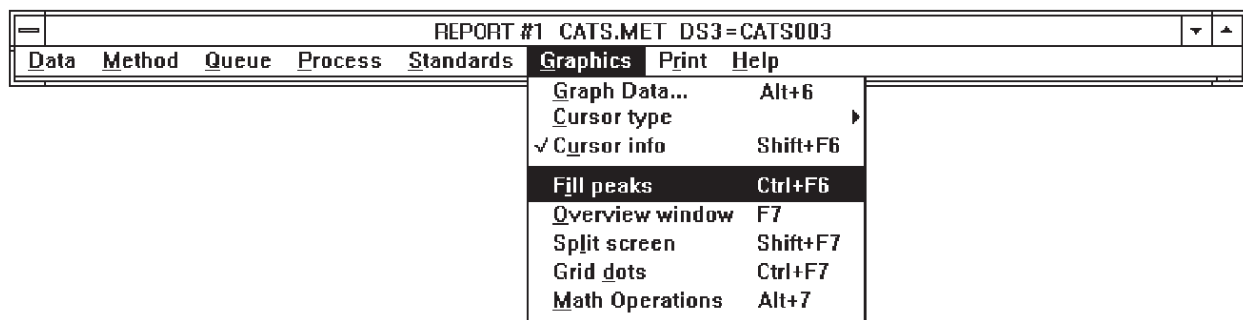
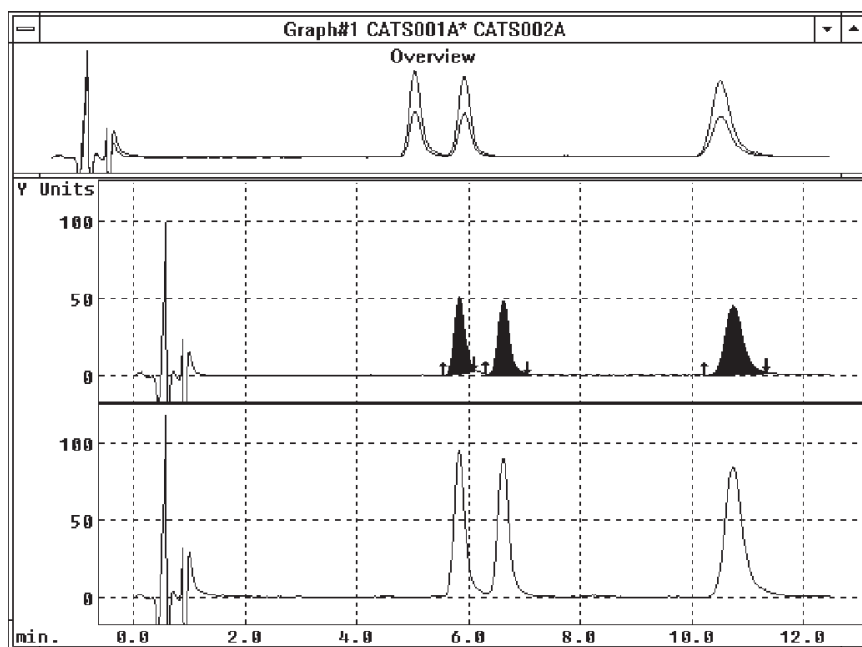


Figure 8.7. Graph with Split Screen, Overview Window, Fill Peaks and Grid Dots turned on.



8.3 Cursor Options

Five cursor types allow interactive manipulation of the graph. These are ZOOM, SHIFT, SCALE, PEAK MARK and EVENT. The cursor type may be selected from the Graphics Menu (Figure 8.8). Alternatively, if CURSOR INFO is checked in the Graphics Menu, cursor type may be chosen from a selector box that appears near the graph (Figure 8.9). Each cursor type has a distinctive shape when positioned on the graph. In addition to allowing cursor selection, this box provides a continuous display of the cursor's position on the X and Y axes. To select a cursor type, click on the appropriate button in the selector box.

Figure 8.8. Selecting Cursor Type from the graphics

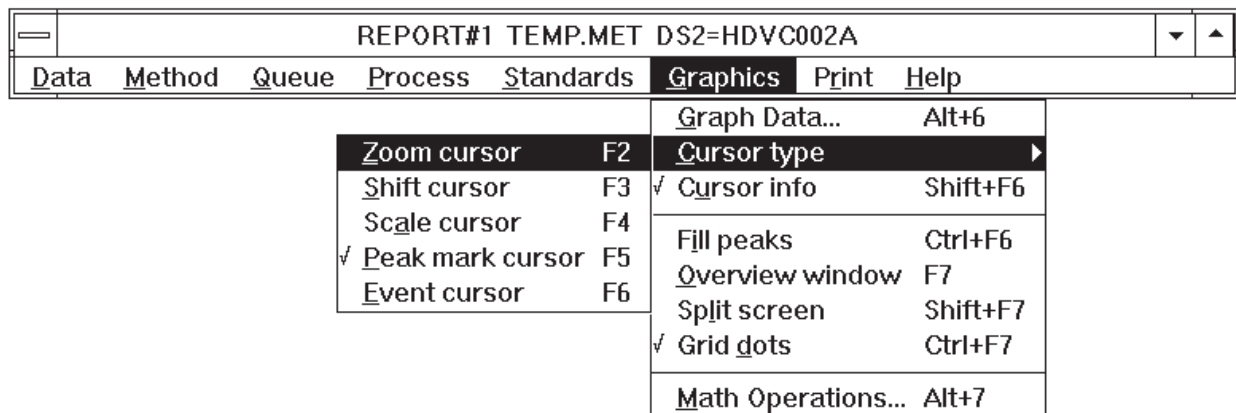
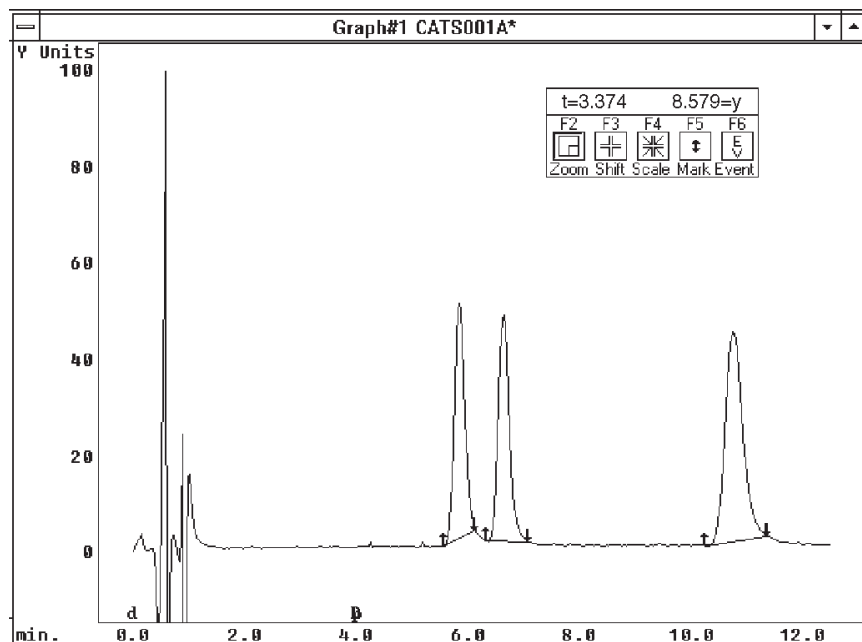


Figure 8.9. The Cursor Selector Box.



8.3.1 Zoom Cursor

Parts of the graph may be enlarged using the Zoom Cursor, which is the default cursor type. You can select the Zoom Cursor, if necessary, by clicking on its button (Figure 8.9). To use the zoom feature, simply click and hold the left mouse button on a section of the graph, drag the cursor to another section (thus making a rectangle) and release the button. The rectangle can be drawn either on the main graph or on the Overview Window, but only the main graph shows the enlargement. Figure 8.10 shows the outline of the rectangle, and Figure 8.11 shows the resultant graph. Notice that the Overview Window retains the original graph, with a rectangle delineating the enlarged area. This rectangle can be dragged along the Overview Window to enlarge other sections of the graph. To return from an enlarged graph to the previous view, position the cursor on the main graph and click the right mouse button.

Figure 8.10. Rectangle indicating section of chromatogram to be enlarged.

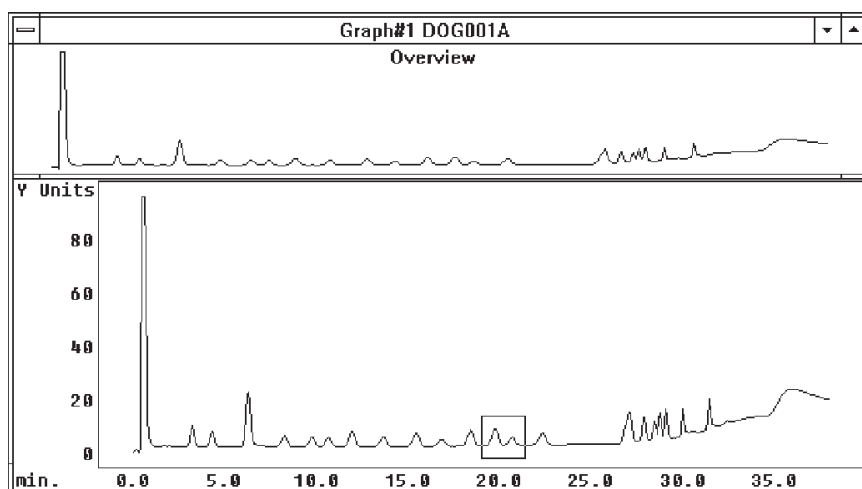
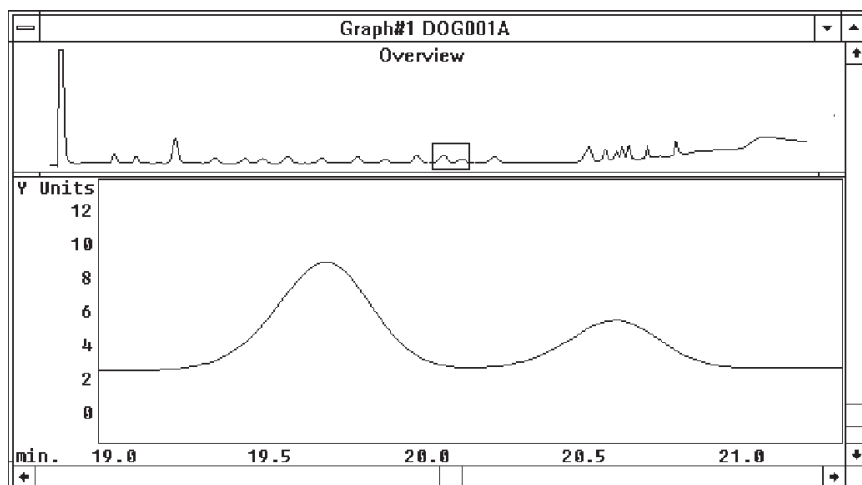


Figure 8.11. Enlarged section of chromatogram. Overview Window indicates location.



8.3.2 Shift Cursor

The Shift Cursor may be used to move the most recently processed chromatogram to the right or left, or to line up peaks on two chromatograms. A shift to the right or left actually changes the retention times of the data in RAM. To use the Shift Cursor, first select it by clicking on its button (Figure 8.9). Then click and hold the left mouse button anywhere on the graph. Drag the cursor to make a line in the desired direction of movement, then release the mouse button. If the shift affects the time axis, a warning message will appear, indicating that the data must now be reintegrated. Reintegration is needed only if you wish to analyze the modified data; it is not needed for replotting and visual examination. Click on OK and the new graph will be drawn. A shift cannot be easily undone. If this becomes necessary, reload the original Data file.

8.3.3 Scale Cursor

The Scale Cursor allows the most recently processed chromatogram to be stretched or shrunk in any direction. Select this cursor by clicking on its button (Figure 8.9). To rescale the chromatogram, click and hold the left mouse button anywhere on the graph, then drag a line in the desired direction. The length of this line is proportional to the amount of enlargement or reduction. Lines drawn up and to the right enlarge, while those drawn down and to the left reduce. When the mouse button is released the new graph will be drawn. If the scale operation occurs along the time axis, a warning that the data must be reintegrated appears. Reintegration is needed only if you wish to analyze the modified data; it is not needed for replotting and visual examination. The most common use of the Scale Cursor is to enlarge the peaks prior to printing the graph. A change in scale is not easily undone. If this becomes necessary, reload the original Data file.

8.3.4 Peak Mark Cursor

The Peak Mark (Mark) Cursor allows interactive adjustment of peak start and stop markers on the most recently processed chromatogram. To move a peak marker, first select the Mark Cursor by clicking on its button (Figure 8.9). Then simply click and hold the left mouse button on a marker, drag it to another location, and release the mouse button. You must move the cursor to the precise location in the horizontal direction. Vertically, however, it will jump to the baseline when you release the mouse button. A warning that the data must now be reintegrated will appear. Reintegration is needed only if you wish to analyze the modified data; it is not needed for replotting and visual examination.

To remove a pair of peak markers, position the cursor between the start (up arrow) and end (down arrow) markers and press the delete key. To insert a pair of markers, position the cursor a hair's breadth above the baseline, near where you want them. Press the insert key to insert the pair of markers, then move them with the mouse. It is helpful to enlarge the chromatogram with the Zoom Cursor before moving baseline markers.

A peak marker may not be moved across another peak marker. For example, an up-arrow (peak start) may not be moved to the right of its down-arrow mate, nor to the left of any previous markers.

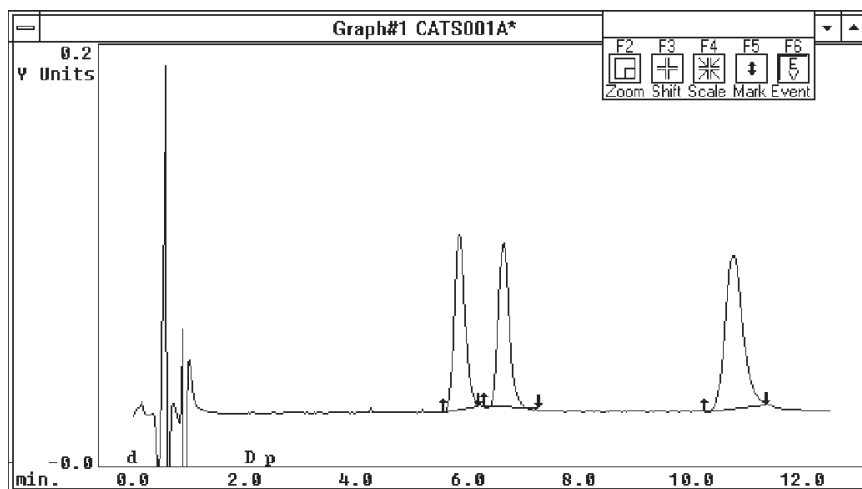
Moving the baseline markers does not change either the Data file or the Method. The DETECT PEAKS option will restore the original placement of the markers.

8.3.5 Event Cursor

The Event Cursor allows peak detection events (Section 4.6, page 11) to be interactively inserted and deleted. Existing events are indicated by letters along the lower margin of the graph (Figure 8.12). A corresponding letter over the cursor indicates the type of event to be edited:

- E (e) The initial state (can delete any event)
- D Peak detect on
- d Peak detect off
- N Negative peak on
- n Negative peak off
- P Perpendicular drop on
- p Perpendicular drop off
- F (f) Fix baseline
- T (t) New threshold

Figure 8.12. Graph showing the following events: Detect Peaks OFF (0 min), Detect Peaks ON (2 min) and Perpendicular Drop OFF (2.4 min).



To use this feature, first click the Event button (Figure 8.12). Then press the appropriate key so the letter code for the function of choice appears above the cursor. Position the cursor on the baseline, then press the insert key to insert an event, or the delete key to remove an existing event. The events are inserted or deleted from the Method in RAM, and any changes will appear in the Processing Events dialog box (Figure 4.5, page 11). The Data file must be processed (Section 5, page 20) for the changes to be put into effect. Subsequent Data files also will be processed with the changed events. SAVE the Method to make the changes permanent (Section 4.10, page 19). If it becomes necessary to restore the original Events, do not save the changed Method: instead, reload the original Method.

8.4 Math Operations

A number of mathematical operations are available through the MATH OPERATIONS section of the Graphics Menu. These include addition and subtraction of Data files, normalization, smoothing, derivatization, etc. Most of these are beyond the scope of this introductory manual, and additional information about them can be found in the Help screens. One feature of more general interest is subtraction, which can be used to remove from the samples any regular and predictable disturbances in the baseline. These disturbances may include slope (e.g., from gradients) or peaks from contaminants or derivatization reagents.

To perform a baseline subtraction, first perform the chromatography and collect the data. You should have a sample (or standard) run, and a relevant blank run. Next load the two runs into data slots in ChromGraph. Select the Math Operations section of the Graphics Menu (Figure 8.13). Designate the sample Data file as Source 1 and the blank Data file as Source 2. Select subtract (-) as the Operation and any unoccupied data slot as the Destination Data file. Press OK and the operation will be performed. The new Data file will be called 1Sub2, and can be processed like a typical Data file.

Figure 8.13. Subtracting a baseline (source 2) from a Data file (source 1) and putting the results in slot 3.

MATH OPERATIONS

Source 1 Data Set

None 1 2 3 4

Source 2 Data Set

None 1 2 3 4

Operation

None + - * / Smooth Derivative

Normalize **Offset Y +** **Scale Y ***

Destination Data Set

None 1 2 3 4

Cursor Operations

Enable shift or scale of

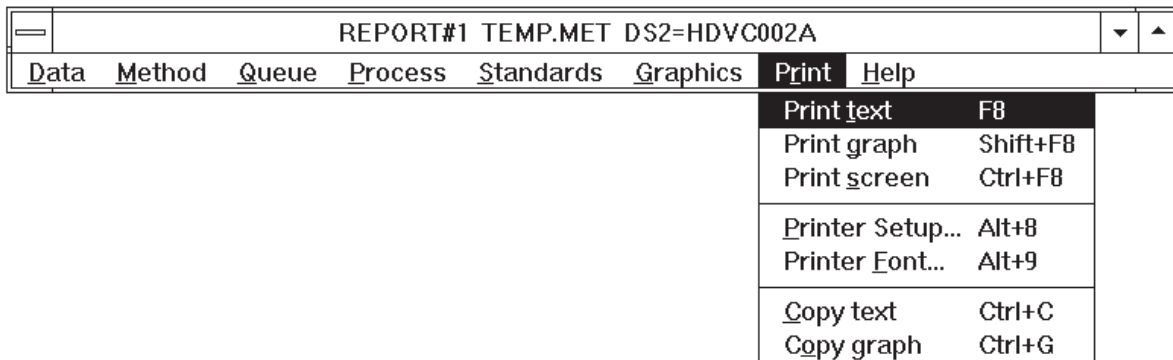
Data Sets: 1 2 3 4

Data Set →	NULL003B	#2	#3	#4
Number of Points	900	900	900	0
Start time, sec.	0.00	0.00	0.00	
End time, sec.	360.00	360.00	360.00	
Baseline Offset Y	0.00	0.00	0.00	
Full Scale Y	50.00	50.00	50.00	
Max. Raw Y Value	2501	2501	2501	
Label Y Units	nAmps	nAmps	nAmps	

SECTION 9. PRINTING

ChromGraph offers various options for printing. For example, via the Method you can instruct ChromGraph to send all output to the printer. Also, various other menu options employ LIST DEVICE boxes which permit you to send that task's output to the printer. However, occasionally you may want to print or capture only the screen currently displayed. The first four options in the PRINT MENU (Figure 9.1) afford that opportunity. The later two options provide printer configuration options.

Figure 9.1. The Print Menu.



Text on the output screen can be sent either to Windows' Clipboard, for pasting to other Window's programs, or to a printer. First highlight the text by dragging the mouse over the lines of interest. Then click on either COPY TEXT (to send it to Clipboard) or PRINT TEXT (to send it to the printer). To print graphics, select PRINT SCREEN to send the entire screen (text and graphics) to the printer, or PRINT GRAPH to send only the graphics. To send only the graph to Clipboard, then press COPY GRAPH. To send the entire screen to Clipboard, press the PRINT SCREEN key on the keyboard.

PRINTER SETUP opens a dialog for choosing among several printers and selecting certain options. This need not be done if a single printer has been configured under the Windows system.

PRINTER FONT opens a dialog for selecting printer font type and size. We have found the True Type (TT) fonts to work well under all conditions.

HINT: If the graphs print slowly and the labels are very small, reconfigure your printer under Windows for less resolution. Use the Printers section of Control Panel.

SECTION 10. KEYSTROKE ALTERNATIVES TO THE MOUSE

Keystrokes, as well as the mouse, can control all ChromGraph operations. Often, it is quick and convenient to enter a menu command or select a dialog box option by pressing a key with your left hand while keeping your right hand on the mouse.

10.1 Window Keys

Most windows have a system box in their upper left corner; pressing <Alt+Space> or clicking the system box opens a menu including these operations:

RESTORE returns a minimized or maximized window to normal size.

MOVE changes the cursor to a four-headed arrow; use the arrow keys to move the window in the desired direction.

SIZE changes the cursor to a four-headed arrow; use the arrow keys to first select the desired border and then to move the window in the desired direction.

MINIMIZE changes a window into an icon.

MAXIMIZE changes a window into a full-size window.

CLOSE or <Alt+F4> deletes a window; for ChromGraph's main menu window, the program ends without asking whether you want to save the current method, standards, and initialization files. Use the Quit command in the Data menu if you wish to save these files.

SWITCH TO or <Ctrl+Esc> opens the Task List and lets you choose which window will become the active window.

To shift focus to any window, including another Windows application, press <Alt+Tab> or <Alt+Shift+Tab>; then, when the desired window is selected, release the <Alt> key. The <Alt+Tab> key is also useful for exposing a window that is covered by another window. When you use <Alt+Tab> to shift focus to an iconized window, it opens at normal size.

10.2 Menu Keys

Pressing <Alt+Key> or <F10> generally selects an item in the main menu. However, if the Graph or Standards window is active (i.e., its caption is highlighted), you will hear a beep the first time you press a menu key and then the main menu window will become active; press <Alt+Key> again to select a main menu item.

In all ChromGraph menus, typing an underlined letter (ignoring upper- or lower-case) immediately performs the selected command. Within a dropdown menu, the <Up Arrow> and <Down Arrow> keys move the highlight up or down, and pressing <Enter> selects the

highlighted command. While any menu is highlighted, you can switch to an adjacent main menu item by pressing <Left Arrow> or <Right Arrow>. To leave a menu without selecting a command, press <Esc>.

Some commands in the Graphics and Print menus have “hot-keys” that allow you to execute the command without even going to the menu. These hot-keys are indicated on the right side of the menu; for example, in the Graphics menu, the Overview window command shows F7 on the right side; thus, pressing F7 immediately adds an Overview window in the Graph window.

The “Cursor type” command in the Graphics and Print menu has a small arrowhead in the right margin, which signifies that selecting this item opens a sub-menu that lets you select a particular type of cursor.

To receive Help on any highlighted menu command, press <F1>. To obtain Help on the next menu item that you select (rather than executing the command), press <Shift+F1>; the cursor then changes to a question mark.

10.3 Dialog Box Keys

Within a dialog box, the <Tab> or <Shift+Tab> key moves the cursor (or active focus) from one item to the next or previous item, which may be a pushbutton, a listbox, an edit field, a radio button, or a checkbox. Generally, the <Shift> key reverses the direction of the shift. While any dialog box item is active (or highlighted), pressing <Space> selects that option.

Most text labels in dialog boxes include an underlined letter. Typing this letter generally activates the specified option; however, if the currently active option is an edit box, you must press <Alt+letter> in order to distinguish the hot-key letter from a letter to be inserted in the edit box.

Within edit boxes, the following keys allow you to copy, cut, and paste text from any Windows application:

<Ctrl+C> or <Ctrl+Ins> copies the selected letters to the clipboard.

<Ctrl+X> or <Shift+Del> deletes the selected letters and copies them to the clipboard.

<Ctrl+V> or <Shift+Ins> copies the clipboard to the cursor position.

For check boxes, typing the underlined hot-key toggles the indicated check box on or off. For radio buttons, typing the hot-key selects the given option. In cases where the underlined hot-key letter occurs in the title of the group box surrounding a group of radio buttons, pressing the hot-key highlights the current choice; pressing an <Arrow> key then selects one of the adjacent choices within the group.

When a listbox is active, a dotted rectangle surrounds the current line. Pressing <Space> selects that line; if multiple selections are allowed, press <Ctrl+Space> for the second and

subsequent lines. Use the <Arrow> keys to move to a different line. <Home> moves to the first item in the list, and <End> moves to the last item. <PgUp> and <PgDn> can be used to page through long lists.

To receive Help on any highlighted dialog box item, press <F1>. If the dialog box contains a Help pushbutton, selecting Help will provide general help for all items in the dialog box.

If a system box is present in the upper left corner of a dialog box, pressing <Alt+Space> opens the system menu and allows you to Move or Close the dialog box using the keyboard.

Press <Enter> (same as OK) to accept all current selections in the dialog box or press <Esc> (same as Cancel) to reject all changes and close the dialog box.

10.4 Graphics Keys

The following graphics hot-keys duplicate commands in the Graphics and Print menus:

<F2> selects the Zoom cursor, which lets you zoom in by clicking and dragging over any region of the Zoom or Overview window.

<F3> selects the Shift cursor, which can shift selected data curves in any direction. Keys <1> to <4> select which data curves are enabled for Shift or Scale, as indicated by asterisks (*) in the caption.

<F4> selects the Scale cursor, which allows you to expand or shrink selected data curves in any direction. Keys <1> to <4> select which data curves are enabled for Shift or Scale, as indicated by asterisks (*) in the caption.

<F5> selects the Baseline cursor, which enables you to move, insert, or delete baseline markers.

<F6> selects the Event cursor, with which you can insert or delete any type of peak processing event.

<Shift+F6> turns the Cursor info dialog box on or off. This small box shows the cursor coordinates and lets you choose a cursor type by clicking on an icon.

<Ctrl+F6> toggles the Fill peaks option on or off. When Fill peaks is on, vertical lines are drawn from each data point to the baseline.

<F7> turns the Overview window on or off. The Overview window always shows the entire chromatogram, with a box indicating the region enlarged in the Zoom window.

<Shift+F7> toggles Split screen mode on or off. If multiple data curves are being graphed, each is shown in a separate frame when Split screen is on; otherwise, all data curves are superimposed within a single frame.

<Ctrl+F7> turns Grid dots on or off. Grid dots extend from numeric labels on the axes to the opposite side of the frame.

<Shift+F8> prints the current data graph at printer resolution. If the Standards graph is present and isn't iconized, the Standards graph is printed.

<F9> prints the entire screen at screen resolution.

<PrtSc> copies the entire screen, at screen resolution, to the clipboard.

<Alt+PrtSc> copies only the active window, at screen resolution, to the clipboard.

To shift the focus from ChromGraph's menu window to the Data Graph window, press <Tab> or <Shift+Tab>. While working in the Graph window, press <Ctrl+Tab> or <Ctrl+Shift+Tab> to move the cursor from the X-scroll bar to the Zoom window to the Overview window.

When the X-scroll bar is present (after a Zoom operation), the <Arrow> keys move the view (window) left, right, up, or down. The data curve moves in the opposite direction. <Home> pages the view one frame to the left, <End> pages right, <PgUp> page s up, and <PgDn> pages down. The same keys work when the Overview window is active, and the selection box moves in the indicated direction.

While the Zoom or Overview window has the focus (cursor), the <Arrow> keys move the cursor left, right, up, or down. For convenience, the rate of cursor movement accelerates as you hold a key down; release the key and press it repeatedly for slow movement. Press <Home> to start a selection (like clicking in the upper right corner for a zoom operation), move the cursor, and then press <End> to end the selection.

After a Zoom operation, press <Z> to zoom out to the previous size and position or press <Ctrl+Z> to zoom out to full size.

10.5 Text Output Keys

The Text Output window is the lower part of ChromGraph's main menu window, where results are listed. The following keys allow you to scroll the text up or down, select lines, print them, or copy them to the clipboard:

<Arrow> keys scroll the list up or down (since there is no horizontal scroll bar, it may be necessary to widen the window in order to see text beyond the righthand margin).

<PgUp> and <PgDn> move the list up or down by one page.

<Home> and <End> move to the beginning or end of the listing. Normally, 700 lines of output are saved; however, if memory is low, a warning message informs you that only the most recent 200 lines are being saved.

<Shift> with any of the above keys selects (highlights) lines. If a non-contiguous line is selected, all intervening lines are also selected.

<Ctrl+Shift> with any of the above keys selects (highlights) non-contiguous lines, retaining previously-selected lines.

<Ctrl+Ins> copies the selected (highlighted) lines to the clipboard.

<F8> prints the selected (highlighted) lines.

10.6 Tab Key

Within a dialog box, the <Tab> or <Shift+Tab> key moves the cursor (or active focus) from one item to the next or previous item, which may be a pushbutton, a listbox, an edit field, a radio button, or a checkbox. Generally, the <Shift> key reverses the direction of the shift.

Press <Tab> repeatedly to cycle the focus from ChromGraph's menu to the text Output window, then to the Standards Graph window, then to the Data Graph window and finally, back to the menu. <Shift+Tab> cycles through the windows in the opposite sequence. While working in the Graph window, press <Ctrl+Tab> or <Ctrl+Shift+Tab> to move the cursor from the X-scroll bar to the Zoom window to the Overview window.

To shift focus to any window, including another Windows application, press <Alt+Tab> or <Alt+Shift+Tab> repeatedly; when the desired window is selected, release the <Alt> key. The <Alt+Tab> key is also useful for exposing a window that is covered by another window. When you use <Alt+Tab> to shift focus to an iconized window, it opens at normal size.

SECTION 11. EXITING CHROMGRAPH REPORT

To exit REPORT, choose QUIT from the DATA section of the Main Menu (Figure 11.1). You will be given a choice of saving the TEMP.MET, TEMP.STA and REPORT.INI files (Figure 11.2). The TEMP files are backup copies of the Method and the Standards files currently in RAM. If you click these boxes, these files will be automatically loaded at the next startup.

The REPORT.INI file contains information on how you've set up the screen. Such things as the size and location of windows, the size and color of the graph, and the default data location are stored here. Click this box if you want all the current settings, including any changes you made during this session, to become the default. If you leave this box blank, the previous default settings will be used at the next session.

Figure 11.1. Exiting ChromGraph.

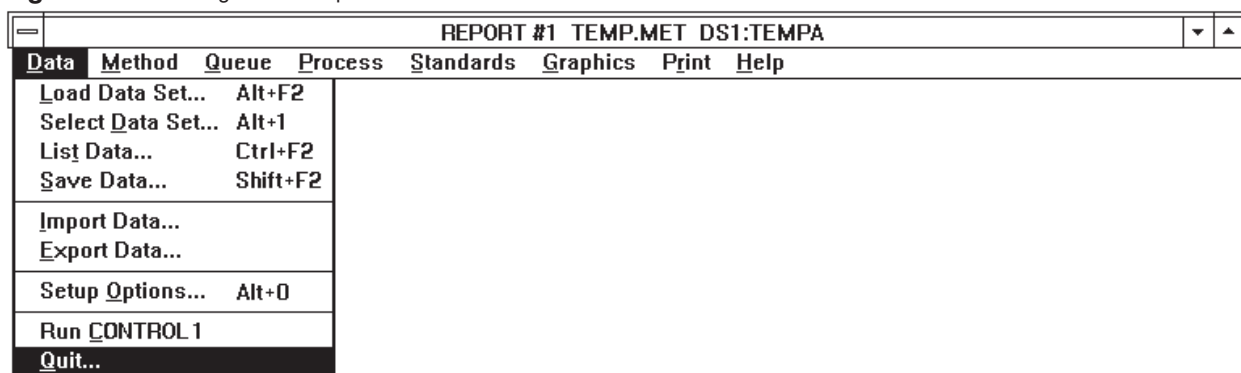
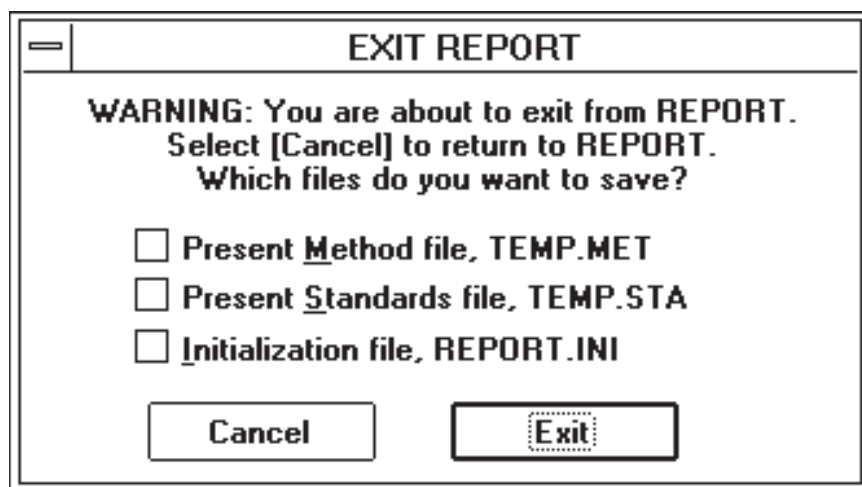


Figure 11.2. Check the boxes if you want the current Method and screen settings to be recalled at your next session.



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