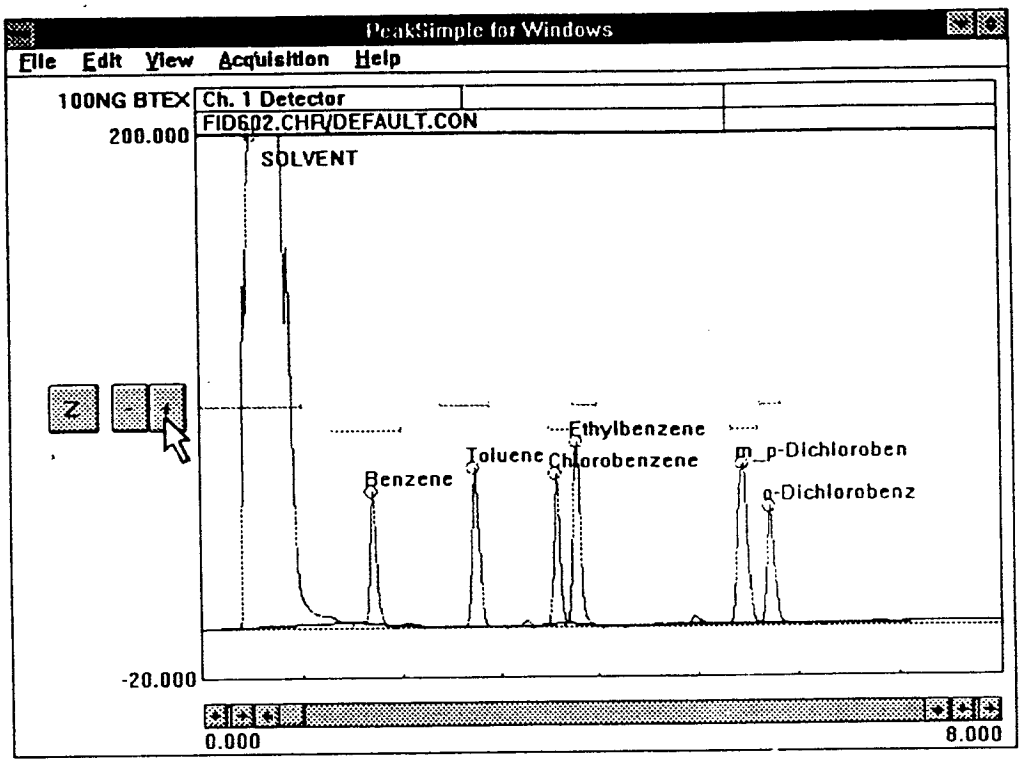
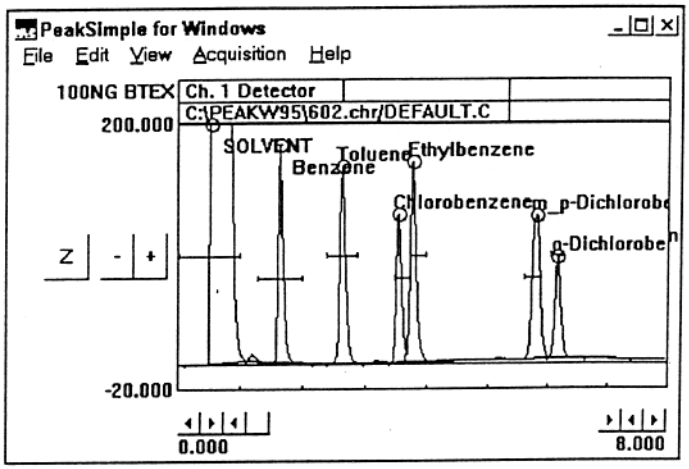
Peaksimple For Windows



Installation and Operation Manual

Getting Started

In this section, we will cover the basic information needed to set up proper communication with your G.C. or Data System hardware.
The Windows version of PeakSimple requires the use of the serial port interface that is built into most 8610-C and Model 310 gas chromatographs. This data acquisition and interface unit permits you to acquire up to four separate channels of data simultaneously without the need for additional hardware or acquisition boards.



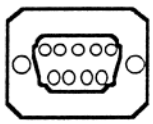
The earlier IBM PC-compatible ISA expansion bus data acquisition cards (AD100 and AD110) used by PeakSimple II and PeakSimple III data systems are *not supported* by PeakSimple for Windows. However, all chromatograms acquired using DOS-based PeakSimple II and PeakSimple III continue to be compatible with this Windows version and may be imported as native files.

Identifying Your COM Port

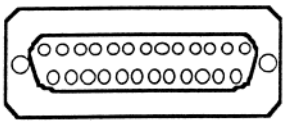
Before attempting to establish communication between your G.C. and the serial data system interface, be sure to check that all the necessary electrical connections have been made, including the connection of any optional remote start cables.

Select an unused serial port on your PC and identify the COM port number assigned to it. It is important that this port NOT SHARE AN INTERRUPT with any other device used in your computer. Typical PCs are equipped with two COM (or serial) ports. COM 1 is typically used by the mouse or some other pointing device. COM 2 may be open (unused) or shared with another device, such as a fax modem, scanner or other peripheral. Determine which COM port you will use and remove any other device that may be in contention with that specific COM port number. Refer to your PC's hardware manual for instructions on changing COM port addresses and device drivers.

Most COM ports are provided with DB-9 connectors (nine pins configured in two rows - 5 pins over 4 pins - within a D-shaped plug or chassis connector). If your PC has a DB-25 serial port (25-pin connector), you will require a DB-25 to DB-9 adapter.



DB-9
Serial Port
Connector



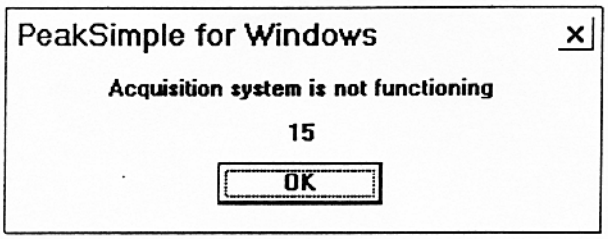
DB-25
Serial Port
Connector

Establishing Communication

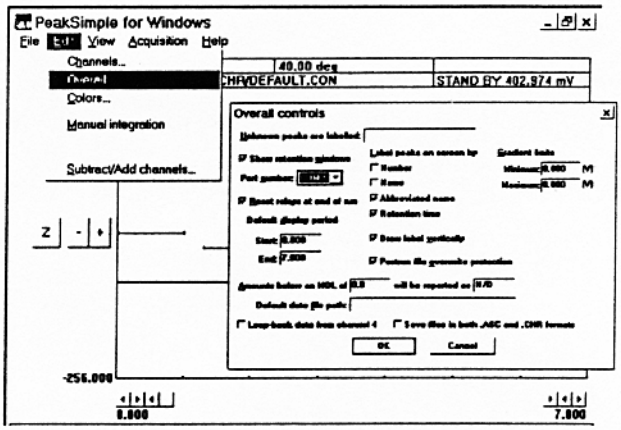
The cable provided with your G.C. or data system has a male DB-9 plug on one end and a female DB-9 connector on the other end. Plug one end of this cable into your available computer COM port and plug the other end into the G.C. or Data System DB-9 connector and tighten the retaining screws.

Plug your G.C. or Data System into an approved GFCI protected outlet and turn the power switch to the 'ON' position. Start PeakSimple by double-clicking on the PeakSimple icon (or by clicking 'Start', 'Programs', 'PeakSimple' in Windows 95). When PeakSimple loads, it will automatically attempt communication with your G.C. or Data System using *COM 1 as the default COM port.*

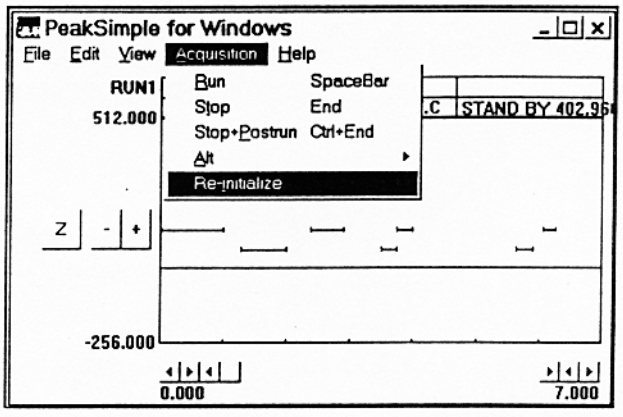
If the serial port interface does not respond, you will see the following error messages appear on the screen: **"Can't wake- check power and cable"** followed by the message **"Acquisition system is not functioning"**.



These messages indicate that your computer cannot communicate with your G.C. or Data System through the default COM port, COM 1. You will need to set up the correct COM port in PeakSimple. To do this, click on the **EDIT** pull-down menu and select **OVERALL**. Change the **PORT NUMBER** to the COM port into which you chose to plug your interface cable. Click **O.K.**.



If at anytime you wish to force PeakSimple to reinitialize communication, click on the **ACQUISITION** pull-down menu and select **RE-INITIALIZE**. If the COM port information is correct and communication errors still appear when the computer attempts to activate the serial port interface, check the serial port connections at both ends of the interface cable for loose connections. Also, visually check the serial cable for nicks or cuts.



It is important to understand that in order for PeakSimple to communicate with your G.C. or Data System, at least ONE channel must be **ACTIVE**. To determine which channels are active, click on the **EDIT** pull-down menu and select **CHANNELS**. A channel is active if the box next to **ACTIVE** is marked with a checkmark. The **EDIT-CHANNELS** menu is described in greater detail in the **EDIT** section in this manual.

7. Connect Power to the Model 302

The Model 302 is provided with a power cord which plugs into a standard 110 (or 220) volt outlet. Plug the Model 302 into the wall outlet. Turn ON the power switch and verify that the POWER LED on the front of the Model 302 is lit.

The power LED is lit when the Model 302 is connected to a power source & switched ON.

8. Install PeakSimple Chromatography Software

8-1. Locate your copy of PeakSimple, which is shipped inside the front cover of your manual. Insert the CD or floppy disk(s) into your computer's appropriate drive.

8-2. Open the appropriate drive through My Computer, then double click on "Setup.exe" and follow the instructions. By default, the setup program places the PeakSimple application directory on the hard drive: c:\peak2000. If you put the application directory elsewhere, take note of the path as you may have to enter it in a dialog box during the USB driver installation procedure.

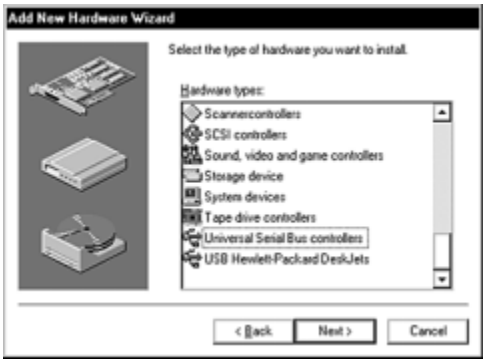
9. Install the USB Drivers

There are three important files saved to the PeakSimple application directory at the conclusion of the software installation: LL_USB.inf, LL_USB.sys, and LL_USB2K.sys. These files are required for Windows to recognize the A/D board connected to the computer's USB port.

9-1. Double-click on the My Computer icon on your desktop, then on Control Panel, then on Add New Hardware, which should open the Add New Hardware Wizard.

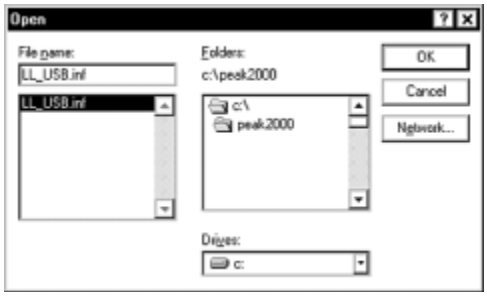
9-2. Click the Next button twice, until you get to the screen that gives you a choice between letting Windows find the new hardware, or selecting it yourself from a list. Click the radio button to choose the hardware from a list and click the Next button.

Model 302
Six Channel USB PeakSimple Data System

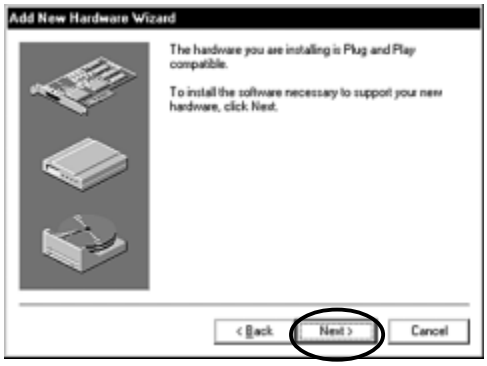


9-3. Scroll down the hardware list, click on Universal Serial Bus controllers, then click Next. From the following screen click the Have Disk button.

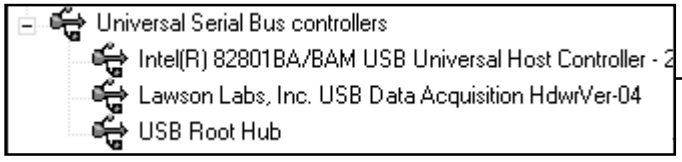
9-4. Click Browse and navigate to the PeakSimple application directory, or type in the path ("c:\peak2000" or the name you have chosen). The Wizard should find the LL_USB.inf file. When you click OK, the Wizard will verify that you want to copy files from the PeakSimple directory ("Copy manufacturer's files from: c:\peak2000").

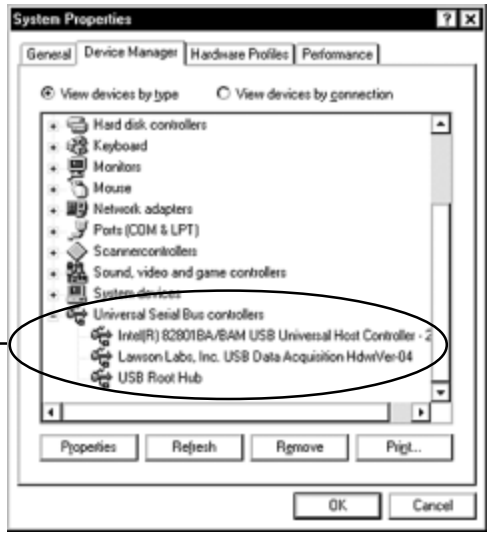


9-5. When you click OK again, the Wizard will confirm that the drivers are for Lawson Labs. Click Next on this screen and the following screen, and Windows will finish installing the software for the Model 302. Click Finish.



9-6. Restart your computer (you MUST restart your computer before the drivers will work). Open the Control Panel again, then System, then click on the Device Manager tab. If the USB drivers have been successfully installed, the Universal Serial Bus controllers section will list "Lawson Labs, Inc. USB Data Acquisition HdwrVer-04."

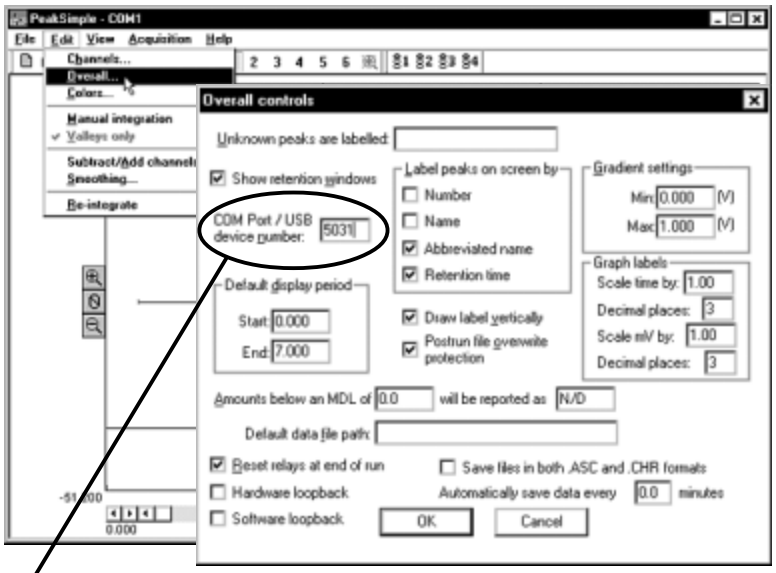




10. Launch PeakSimple

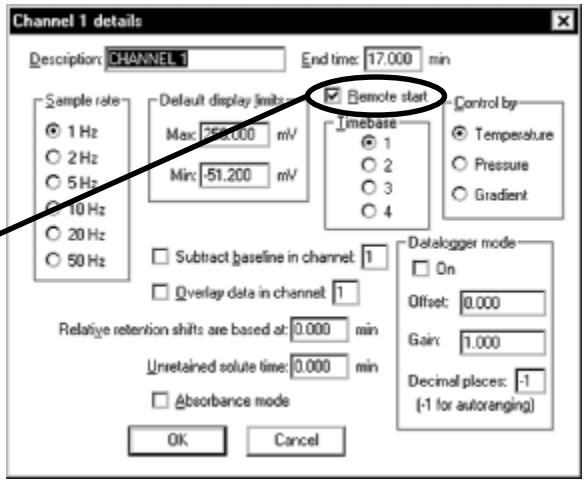
10-1. Double-click on the PeakSimple icon to launch the program. Verify that communication has been established between your computer and the Model 302. An error message will appear if communication is not established. This is normal until you complete the following step.

10-2. Each SRI USB data system has a unique 4-digit USB device number beginning with "5" (5031, 5032, etc.). This I.D. number is printed on the back of your Model 302, and on your PeakSimple disk. Open the PeakSimple Edit menu and choose Overall. Enter your Model 302 I.D. number in the box labeled "Com port / USB device number." Click OK, and PeakSimple will attempt to "wake-up" the data system. Click the Save All icon so you don't have to re-enter the USB device number.



Enter the 4-digit USB device number here

10-3. For the remote start option:
Open the Edit menu and choose Channels. Click on the Details button for channel 1. Verify that Remote start is enabled (the box should be checked). Repeat this step for channels 2-6 if necessary.

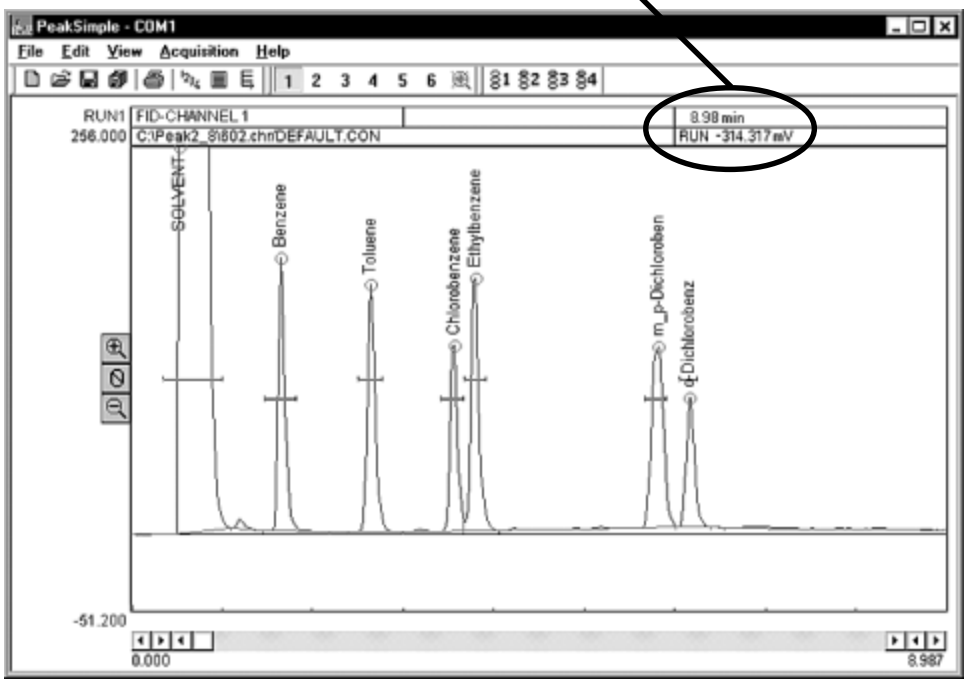


10-4. For information about using Event tables, manual Relay activation, etc., see the "PeakSimple Tutorials" and the "PeakSimple Software" sections in the manual (and online at www.srigc.com—click on the "Download Our Documents" button on the homepage).

Model 302
Six Channel USB PeakSimple Data System

11. Starting an Analysis

10-1. The upper right corner of the PeakSimple chromatogram window contains real-time information pertinent to your analysis in progress. The status of the run (STAND BY, RUN) is displayed in capital letters next to the millivolt (mV) reading, underneath the amount of time into the run.



11-2. Hit your computer keyboard spacebar to begin the run, and the data is plotted onscreen in the chromatogram window.

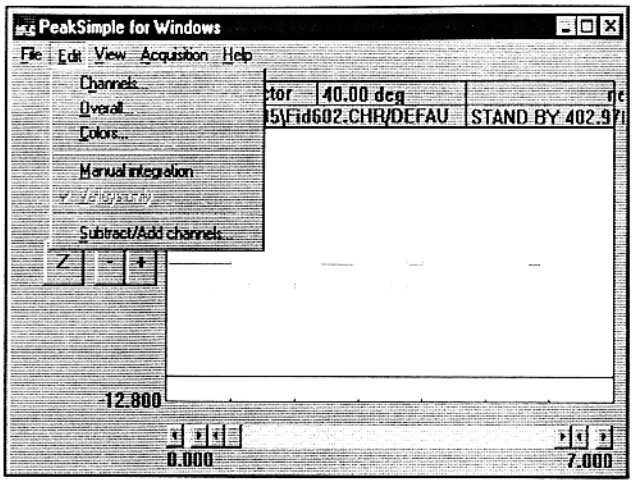
11-3. Hit the End key on your computer keyboard to stop the run.

Technical Support:

If you have questions or problems, call SRI for free technical support at 310-214-5092, 8am - 5pm California time.

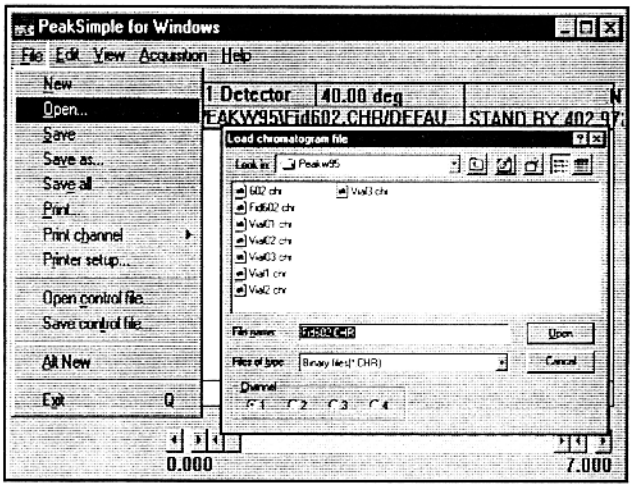
Using PeakSimple: Menus

Operation of Menu Bar Pull-Down Menus



All PeakSimple for Windows features may be accessed from pull-down menus. When you click on a menu bar item, a pull-down group menu will open to permit navigation to specific group features. These pull-down menus may also be opened by pressing the **<ALT>** key and the letter key corresponding to the **underlined** letter in the menu bar item name. For example, to open the **EDIT** menu press **<ALT>** and the letter "**E**" (This is not case sensitive).

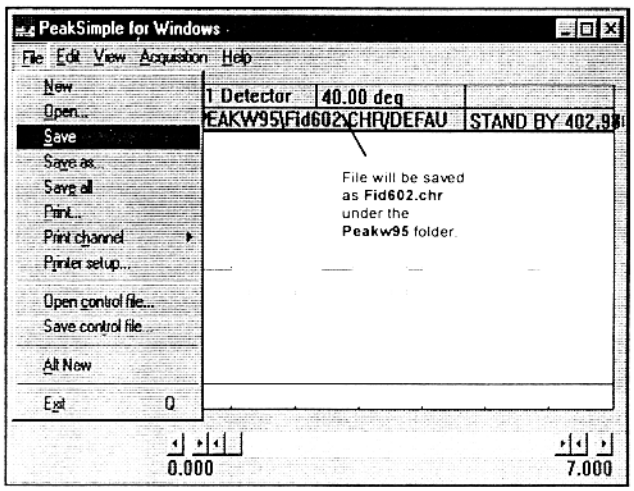
The FILE Pull-Down Menu



The **FILE-NEW** feature will clear the display of all active channels in the **Main** timebase without starting a new chromatographic run.

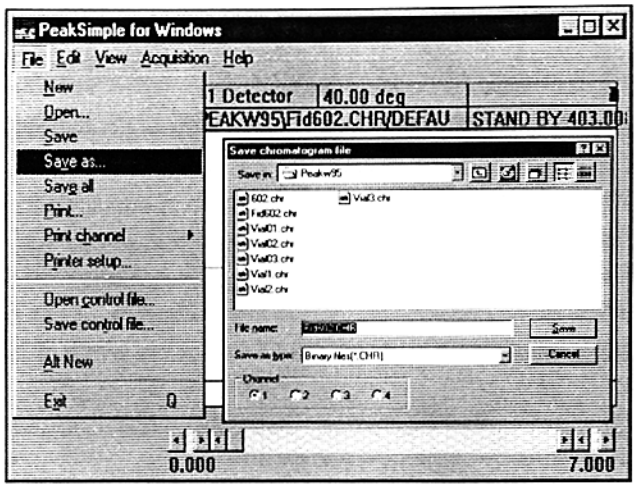
To open a previously saved chromatogram file, select **FILE-OPEN**. A **LOAD CHROMATOGRAM FILE** screen will appear which will allow you to select any file from any directory (folder) on your system. Choose the channel (1-4) in which you wish to display your saved chromatogram and than click **OPEN**.

FILE SAVE



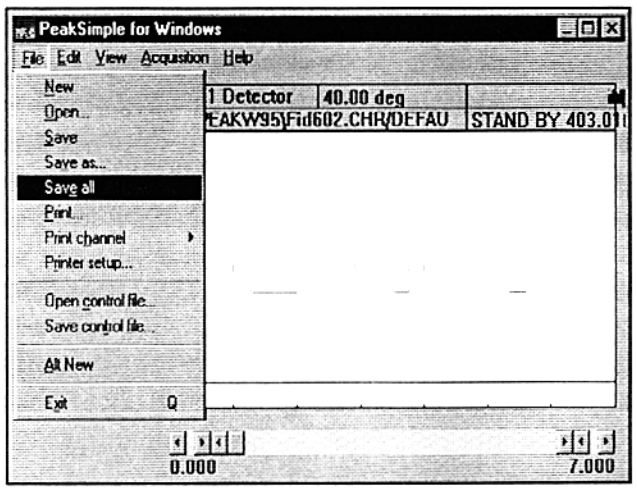
The **FILE-SAVE** feature saves the displayed chromatograms of all active channels. The name given to the file(s) is the same name that is displayed in the Data Boxes below the menu bar and will be given the default **.CHR** extension. This file name is editable by the user by changing information in the **EDIT-CHANNELS-POSTRUN** pull-down menu. See the **EDIT** section for more information.

The FILE-SAVE-AS Pull-Down Menu



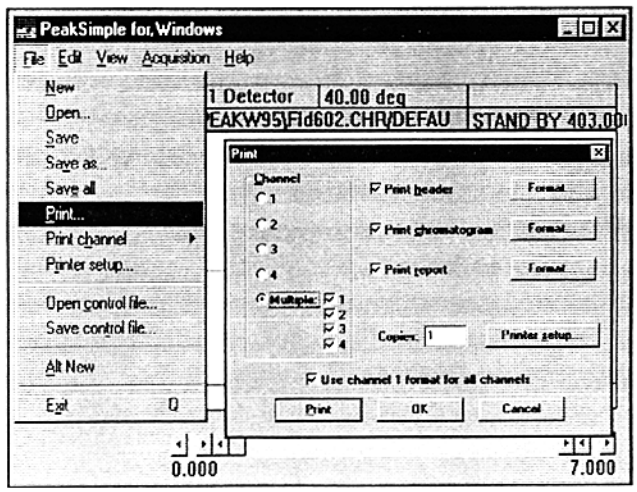
To save a newly created chromatogram file, select **FILE-SAVE AS**. A **SAVE CHROMATOGRAM FILE** screen will appear which will allow you to save the file in any directory (folder) on your system. Type a name up to eight characters into the **File** Name box and choose which channel (1-4) you wish to save and than click **SAVE**. The file will be saved as a **binary file** by default, with a **.CHR** extension. You may also select to save the file in **ASCII** format with a **.ASC** extension.

The FILE-SAVE-ALL Pull-Down Menu



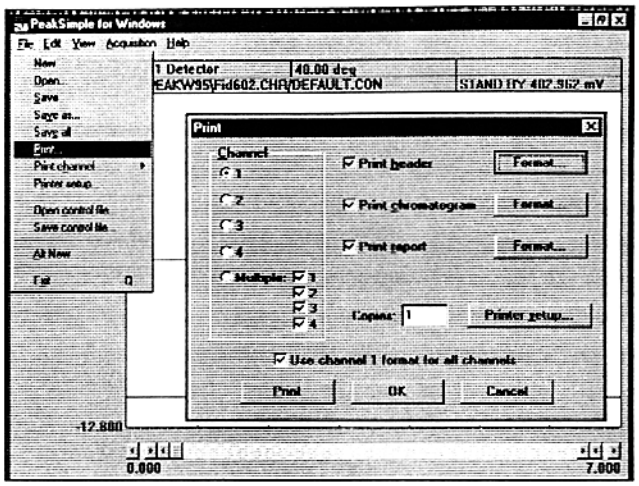
The **FILE-SAVE-ALL** feature will automatically save your chromatogram as a **.CHR** file; your temperature program as a **.TEM** file; your component table as a **.CPT** file; your event table as a **.EVT** file and then saves them all under a control file (**.CON** file). **DEFAULT.CON** will be used if no other name for the **control file** is specified using the **SAVE-CONTROL FILE** feature. All print information is also saved when you save a **control file**.

The FILE-PRINT Pull-Down Menu



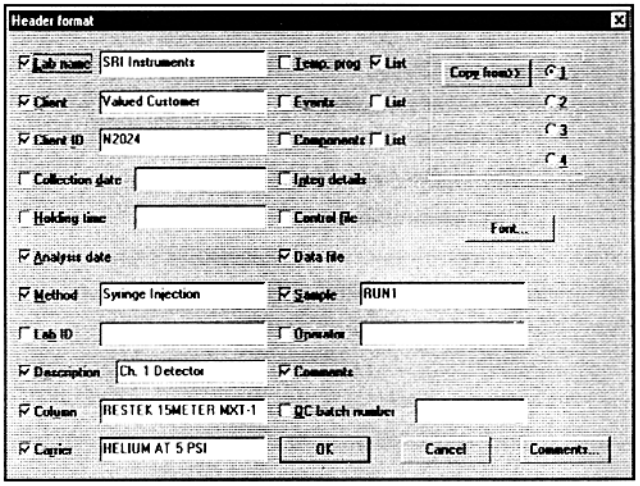
Numerous fields are available for print information. When you access the **FILE-PRINT** pull-down menu you will notice that any combination of one to four channels can be printed out on a single sheet of paper simply by marking the circle next to the channel number. Print information concerning the **header, chromatogram** and **report** can be easily edited.

The FILE-PRINT Pull-Down Menu (CHANNEL 1)



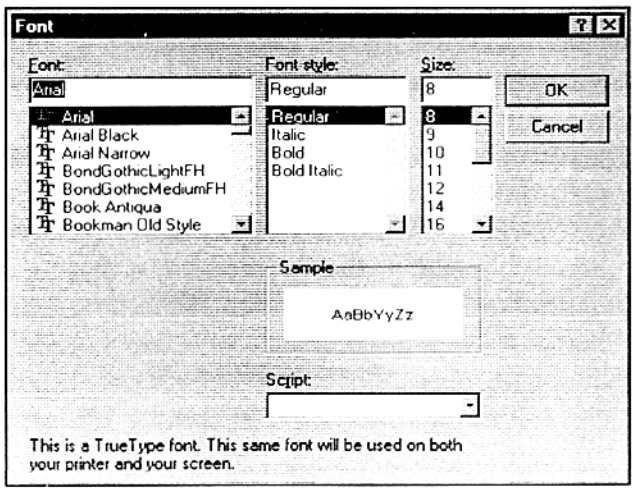
When you access the **FILE-PRINT** pull-down menu you will notice that you can select to print any combination of **multiple** channels by clicking on the circle next to the word **multiple.** You may also choose to print individual channels by clicking on the circle next to the desired channel. **Click on Channel 1** to edit the **Channel 1** information in the **Print Header, Print Chromatogram and Print Report Format** fields. Rather than enter unique information for all four channels, you may wish to check the **Use channel 1 format for all channels** box.

PRINT HEADER FORMAT

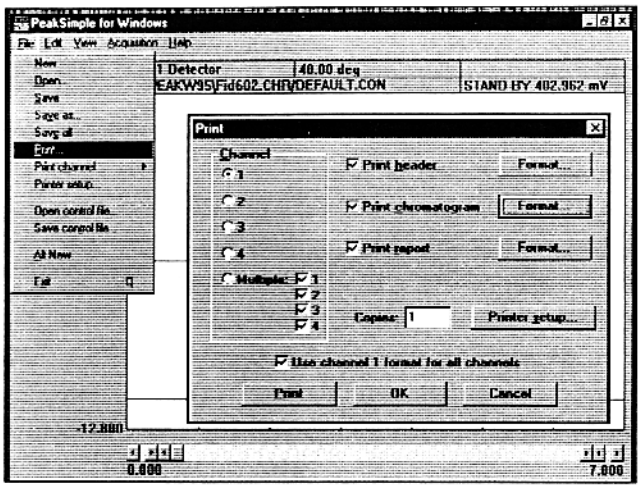


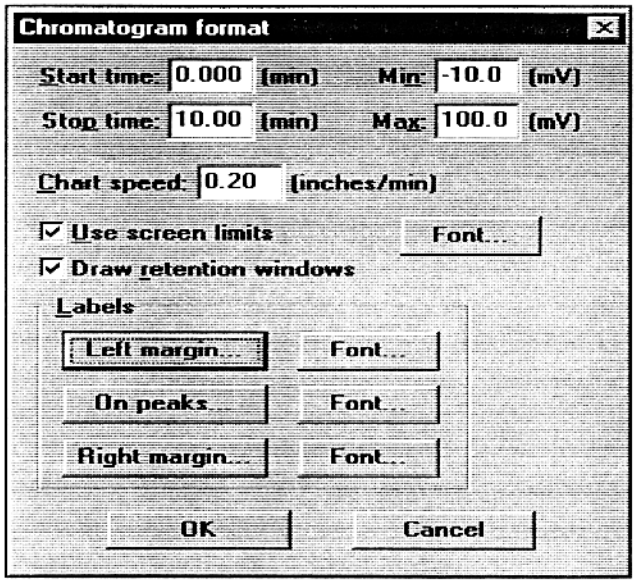
Clicking on the **Print Header FORMAT** button will allow you to customize the appearance of your printed chromatogram header. Input your **Laboratory name, Analysis method, Sample type, Column, etc** and check the box next to each field. **Analysis date** prints the date in your PC's BIOS.

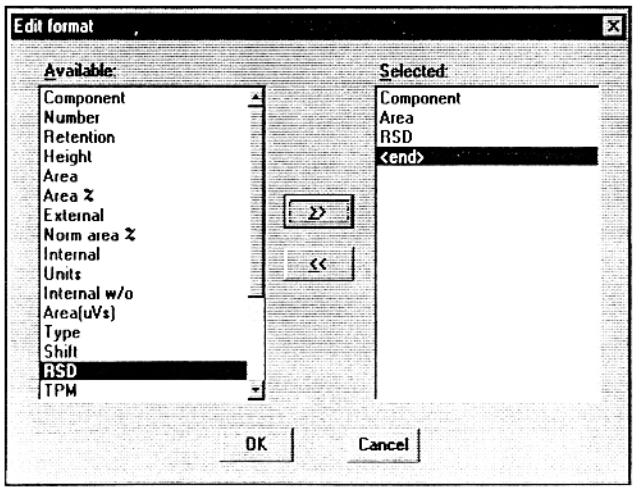
Print out **Temperature Programs, Events and Components** file names by checking their boxes; or click on **List** to print the complete Temperature Program, Event Table or Component List. **Copy from:** selects which channel will provide the **List** information. Check the **Comments** box and click on **Comments...** to enter customized information about your analysis. You can change the **Font,** style and size of your printed text by clicking on the **Font** box. Select a size that will provide readable text while still leaving room for your chromatogram and report.



PRINT CHROMATOGRAM FORMAT







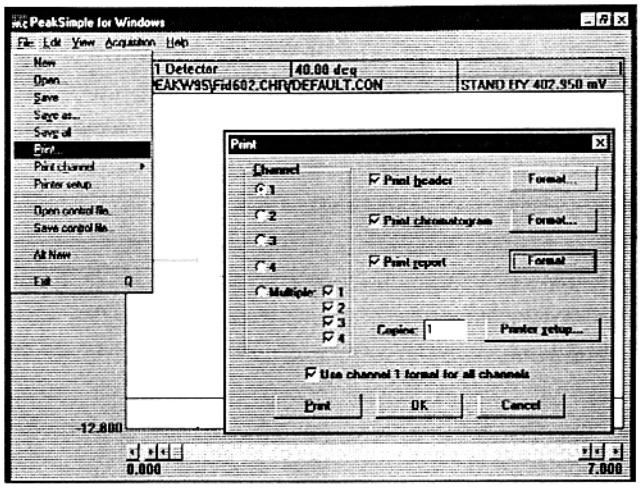
You can also edit the chromatogram print parameters when you access the **FILE-PRINT** pull-down menu. Check the **Print Chromatogram** box and select **Format**. The **Chromatogram format** screen allows editing of the chromatogram **Start time** and **Stop time** and the **Min** and **Max** millivolt levels.

The **Chart speed** setting will determine the size of the chromatogram section of your printout. A setting of **1.0 inches/minute** for a 5 minute chromatogram will produce a **5 inch** chromatogram print. You may need to experiment with this setting to fit your **header, chromatogram and report** information all on one printed page. When the **Use screen limits** box is checked only the displayed section of a chromatogram will be printed. The **Draw retention windows** box allows for retention windows to be printed as well.

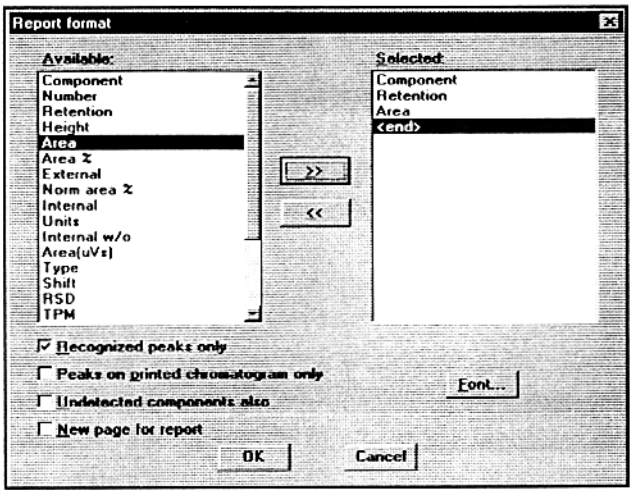
The **Labels** section of the screen lets you select what useful information will be printed along the borders of the chromatogram, and above the peaks. Clicking on **Left margin**, for example, will bring up the **Edit format** screen which will allow you to select from a list of measurements which will automatically be calculated and printed in the left margin of your chromatogram. To choose **RSD**, for example, click on **RSD** from the left column and then click on the right arrows (**>>**). **RSD** will now appear in the **selected** column on the right. Click **OK** to close the window. Edit **On peaks** and **Right margin** in the same manner.

PRINT REPORT FORMAT

A report may be printed along with your chromatogram to summarize component retention time, area counts or other data. Clicking on the **View** pull-down menu and selecting **Results** will show a preview of your report.

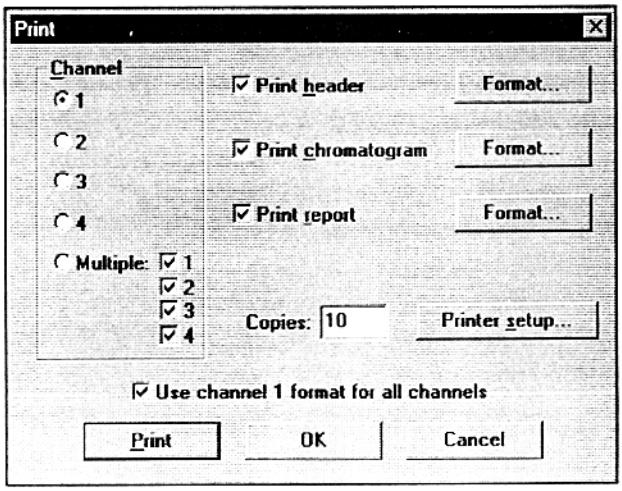


Click on the **Print Report** box and select **Format**. The **Report Format** screen will appear which will allow you to select from a list of measurements which will automatically be calculated and printed on the bottom of your chromatogram. To choose **AREA**, for example, click on **AREA** from the left column and then click on the right arrows (**>>**). **AREA** will now appear in the **Selected** column on the right.

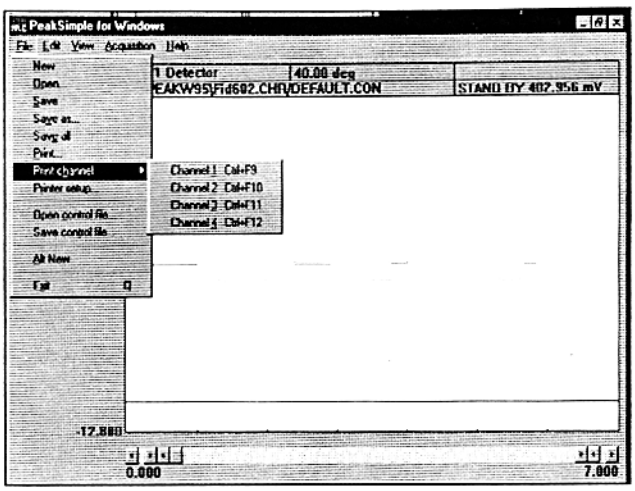


Clicking on the box next to **Recognized peaks only** will place a check mark in the box and only those peaks which integrate properly within named retention windows will be printed in the report. Checking the **Peaks on printed chromatogram only** box will allow the report to show only those peaks defined by the **Chromatogram format– Start time and Stop time.** This feature allows you to set up your report to ignore all peaks that appear outside your window of interest.

Checking the **Undetected components also** box will report information about all named peaks even if no peak is present within the retention window. Checking **New page for report** will print all report information on a separate page. Click **OK** to close the **Report format** window. You may print out as many Chromatogram **Copies** as you need by entering a number in the **Copies** box and selecting **Print**.

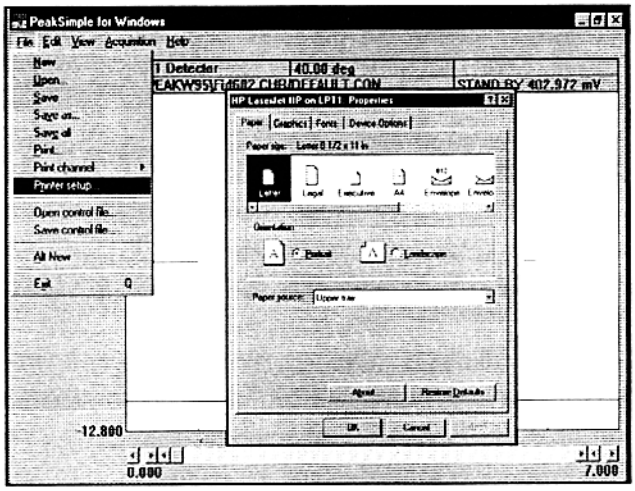


The FILE-PRINT CHANNEL Pull-Down Menu



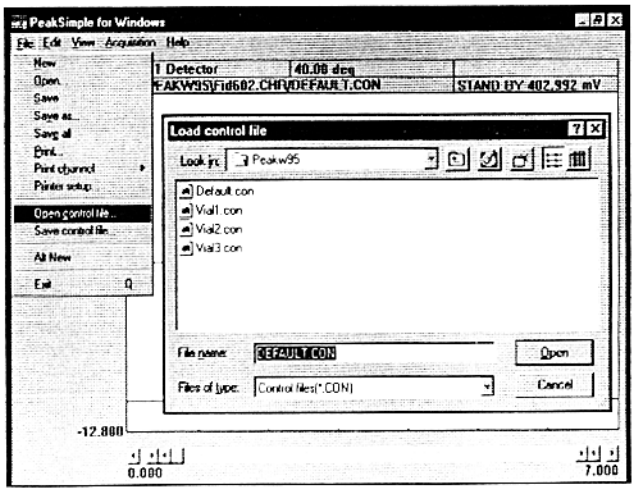
After all **Print** parameters have been set up, the easiest way to print out a chromatogram is to use the **File-Print Channel** quick keys. Hold down the **Ctrl** (control) key and then press **F9** (function #9) to instantly print the **Channel 1** chromatogram. Press **Ctrl F10** to print **Channel 2, Ctrl F11** for **Channel 3** or **Ctrl F12** for **Channel 4**. Of course you may also select these commands from the pull-down menu.

The FILE-PRINTER SETUP Pull-Down Menu



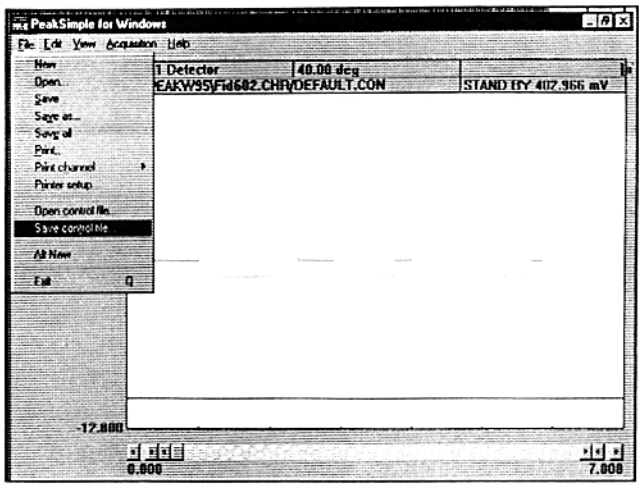
Selecting **Printer setup** from the **FILE** pull-down menu will allow you to enter the Printer Properties screen for your specific printer. This screen is similar to Windows Printer Properties screen that is accessible from the Windows Control Panel. Typically, using your printer default settings with **portrait** orientation will produce a visually appealing printout.

The FILE-OPEN CONTROL FILE Pull-Down Menu

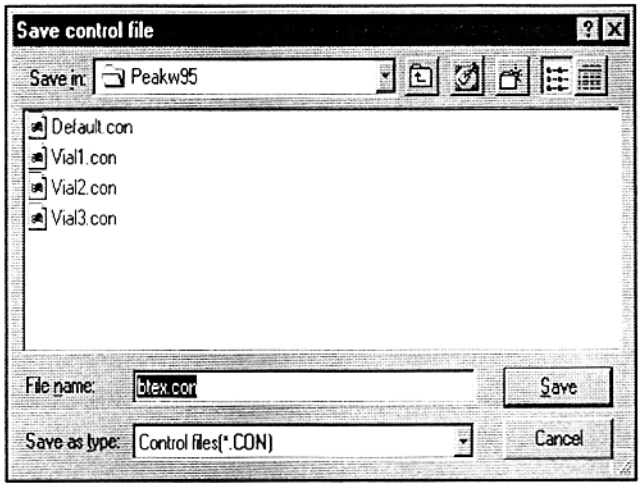


PeakSimple for Windows uses **Control Files**, identified with the **.CON** extension, to save the operating settings of specific methods. To load a **Control File**, drop down the **FILE** menu and select **OPEN CONTROL FILE**. A window will open which will allow you to use standard Windows navigating tools to select from a list of **.CON** files, located on the **Drive** or **Directory** of your choice. Click on the desired **File Name** and then click **O.K.**

The FILE-SAVE CONTROL FILE Pull-Down Menu



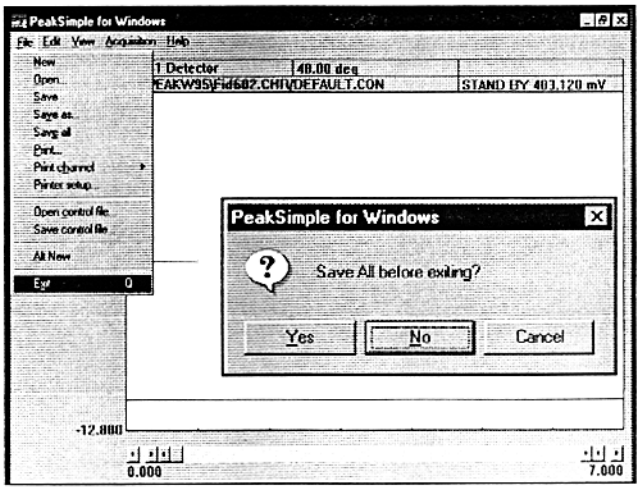
Once you have set up all of the user-definable parameters within PeakSimple for Windows that meet the requirements of your system and/or your specific analytical method, it is wise to save these settings for future use. PeakSimple uses **control files**, identified with a **.CON** extension, to save the operating settings of specific methods, this includes the event table, temperature program, component table, print information, calibration table, etc.



A **control file** is like a photocopy of your operating settings that you can reload for use at any time. When using **control files**, you only need to set analysis parameters once and then save them using a descriptive filename, followed by the **.CON** extension, (for example, **BTEX.CON**). To save the **control file**, drop down the **File** menu and select **Save control file**. Enter the name for your file in the **File name** box and click **O.K.**. If you want these current settings to be loaded by default each time you start PeakSimple, name the control file **Default.con**.

FILE-ALT NEW

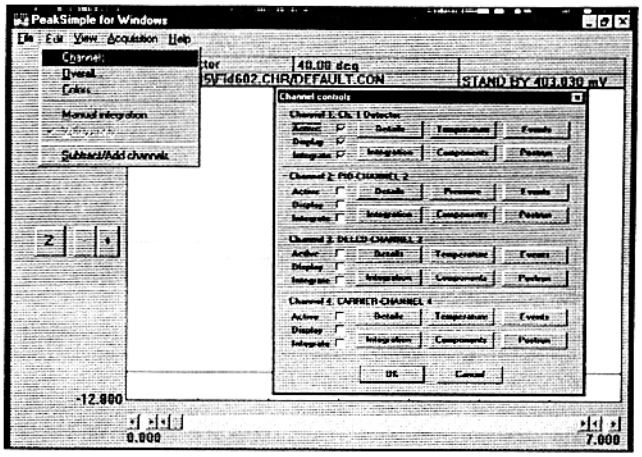
The **FILE-ALT NEW** feature will clear the display of all active channels in the **Alt**ernate timebase without starting a new chromatographic run.



FILE-EXIT

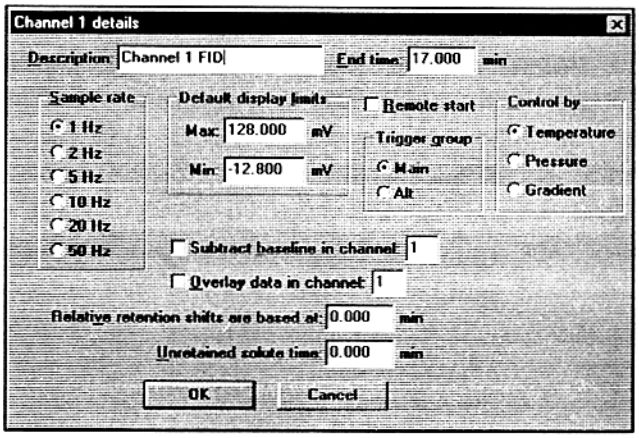
Exits PeakSimple for Windows. Click **Yes** to save any changes made to your **control file** parameters.

The EDIT-CHANNELS Pull-Down Menu



The **EDIT** pull-down menu allows you to modify most of the operating parameters for your specific application. Selecting **EDIT-CHANNELS** will bring up a screen which will enable you to select which of the four channels are **active, displayed and integrated**. Each channels' operating parameters such as **Details, Temperature, Events, Integration, Components and Postrun** information can be easily modified.

The EDIT-CHANNELS-DETAILS Screen



Clicking on the **Details** box for **Channel 1** will bring up a screen where you can enter a **Description** of your analysis. **End Time** displays the length of the chromatographic run in minutes. By default, the **End Time** is determined by the length of the temperature program but you may modify this field to end the chromatographic run at any time.

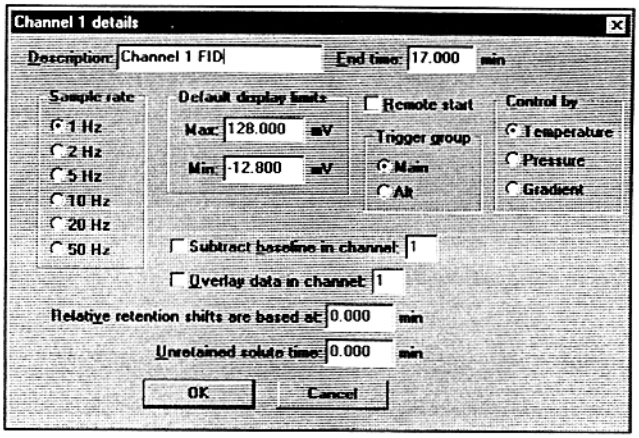
The **Sample Rate** should be set to a rate sufficient to ensure that 20 data samples are collected for each peak. For example: A **Sample Rate** of 1 Hz will allow the collection of 20 data points from a peak 20 seconds wide from base to base. And a **Sample Rate** of 10 Hz will allow the collection of 20 data points from a peak 2 seconds wide from base to base. The analog to digital converter is limited in its ability to sample high rates when many channels are active. The limits are: 50 Hz with one channel active, 10 Hz with two channels active and 5 Hz with three or four channels active.

The **Default Display Limits** can be adjusted to view data above and below the 0 mV baseline. A minus (-) setting for **minimum** will display negative going peaks. The ratio of **min./max.** display limits is maintained when you click on the Display minus and plus buttons in the main data acquisition screen.

The **Remote Start** feature allows the user to start a chromatographic run using an external signal such as a footswitch. Check the box to enable **Remote Start.** (There must be an internal connection made to the A/D board in order for this option to work.)

The EDIT-CHANNELS-DETAILS Screen (continued)

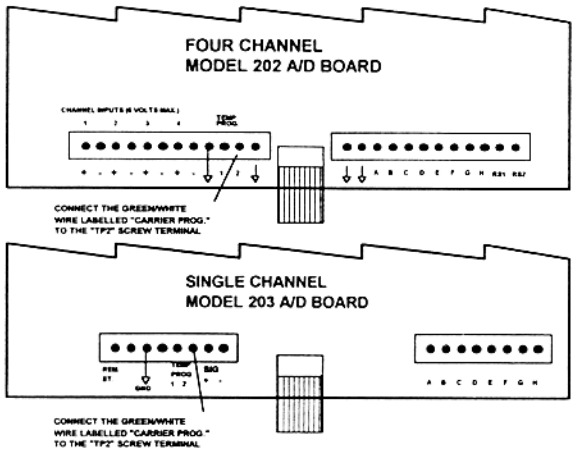
Unretained Solute Time



If resolution has been selected to be printed in the chromatogram report, then a **Unretained Solute Time** value needs to be entered to ensure correct resolution calculations. Enter the number of minutes an **Unretained Solute** takes to pass through the column. This value is used in the determination of peak resolution statistics.

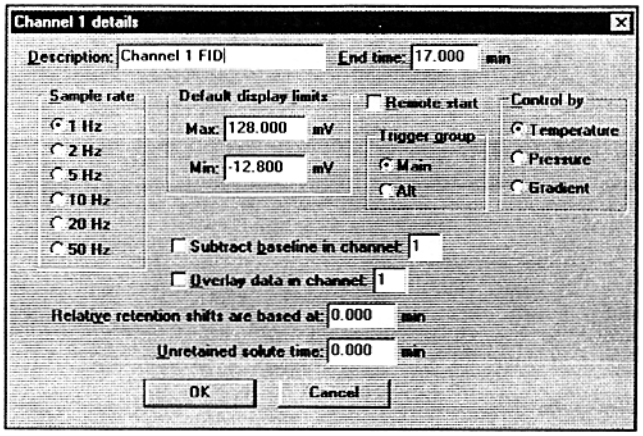
Control By

The A/D Board that is built into the SRI gas chromatograph includes two digital-to-analog converters or DACs. DAC1 is primarily used to control the column oven #1 **temperature** ramp by introducing 10mV / °C to the oven heating circuit and is programmable by editing the **Channel 1–Temperature** control window. DAC2 is primarily used to control the column oven #2 **temperature** ramp. Carrier gas E.P.C. pressure is also programmable by editing the **Channel 2–Temperature/Pressure** control window. The DACs may be used to control **Pressure** by following the procedure described below and then selecting **Pressure** in the **Control By** window of the **Edit-Channels-Details** screen.



To avoid startup difficulties, the Carrier E.P.C. is shipped disabled. To enable the use of the DACs to set up a **Pressure Program**, only a single wire needs to be moved inside the G.C.. Unplug the G.C. and remove the six screws which secure the bottom cover. Tilt the G.C. onto its back and remove the bottom cover. The A/D Board is green in color and is mounted on the right-hand side of the G.C. chassis. Locate the Green wire with a White stripe on the A/D Board. This is the **Carrier Program** wire. Normally this wire is connected to a ground (GD) screw terminal. Unscrew the **Carrier Program** wire and connect it to the temperature/pressure #2 (TP2) screw terminal also on the A/D Board. Re-attach the bottom cover, connect power and re-establish communication between the G.C. and the computer. Select **Pressure** in the **Control By** window of the **Edit-Channels-Channel 2-Details** screen. A pressure program ramp set up in **Channel 2** will now control the Carrier Gas E.P.C. pressure by introducing 10mV for every P.S.I.. Turn the Carrier 1 **Local Setpoint** to zero. This is necessary since the Local setpoint is added to the programmed E.P.C. input in determining the Carrier 1 **total setpoint**.

The EDIT-CHANNELS-DETAILS Screen (continued)



Trigger Group

The **Trigger Group** selection assigns the channel to the **Main** or **Alt** trigger group. The picture at right shows the **Channel 1 Details** screen with the **Main Trigger Group** selected.

Any **Channel** with the **Main** trigger group selected will start running when the SPACEBAR is pressed and end when the END key is pressed. Any **Channel** with the **Alt** trigger group selected will start running when the + (plus) key is pressed and end when the – (minus) key is pressed. When acquiring four detector signal inputs from one gas chromatograph; verify that all four channels' **Trigger Group** is set to **Main**. This ensures that all four channels are acquiring data synchronously by using the same timebase. If two channels of data are coming from an SRI gas chromatograph, and you also wish to acquire two channels from an external input device such as an HPLC, then select the **Alt** trigger group for channels 3 and 4. This allows for asynchronous data collection.

Subtract Baseline In Channel "X"

Checking **Channel 1's** box for **Subtract Baseline In Channel "X"**, where **"X"** is 1,2,3 or 4, will cause the chromatogram in **Channel 1** to subtract the baseline stored in **Channel "X"**, while running in real-time. Load the baseline to be subtracted into an inactive channel to ensure that the data is not deleted by the start of a new run on that channel. (Uncheck the **active** box, see **Edit-Channels**). Baseline subtraction can also be performed using PeakSimple's **Edit-Subtract/Add Channels** feature, however, this is not a real-time function, but a post-run function, done at the end of the chromatographic run.

Overlay Data In Channel "X"

Checking **Channel 1's** box for **Overlay Data In Channel "X"**, where **"X"** is 1,2,3 or 4, will overlay the data stored on **Channel "X"** onto **Channel 1** using contrasting colors. The channel selected for overlay can be either an active or inactive channel. When the overlay channel is active then the overlay will be seen in real-time.

Relative Retention Shifts Are Based At "X" Minutes

Relative Retention Shifts Are Based At "X" Minutes. Enter into this box the time, in minutes, that the sample is actually injected onto the column. This is done to ensure that relative retention times are correctly calculated. See the **EDIT-CHANNEL-COMPONENTS** section of this manual for more details.

The EDIT-CHANNELS-DETAILS Screen (continued)

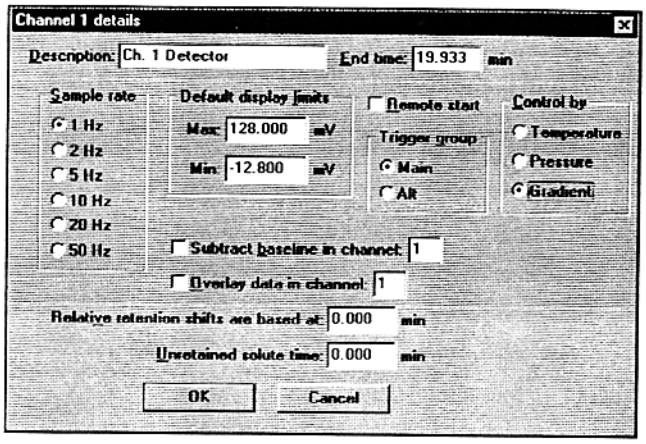
Setting Up Gradients for Liquid Chromatography

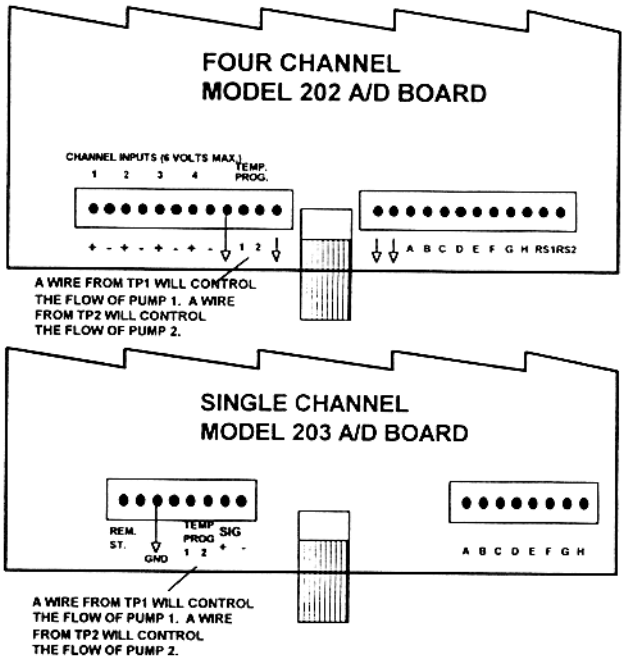
Data System users may wish to use the A/D Board DACs for setting up an HPLC solvent gradient. PeakSimple for Windows allows the user to control the flow of two pumps, provided they operate from a zero to five volt (0-5V) ramp input.

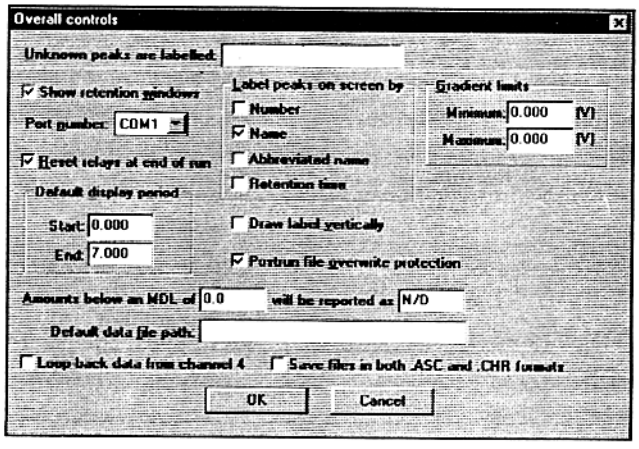
To operate the pumps, several internal connections must be made between the HPLC and the Data System. Unplug the Data System and remove the two screws which secure the top cover. Route the Pump A and Pump B control wires from the HPLC to the Data System and connect the Pump A control wire to TP1 and the Pump B control wire to TP2. Re-attach the top cover, connect power and re-establish communication between the Data System and the computer.

Set up the **Gradient** ramp on **channel one** (TP1) to control the flow of Pump A into the system (10mV / %) and the **Gradient** ramp on **channel two** (TP2) to control the flow of Pump B. Modifying the **Gradient** ramp program on **Channel 1** to rise from 10% to 90% will automatically create a **Gradient** ramp program on **Channel 2** that decreases proportionately from 90% to 10%.

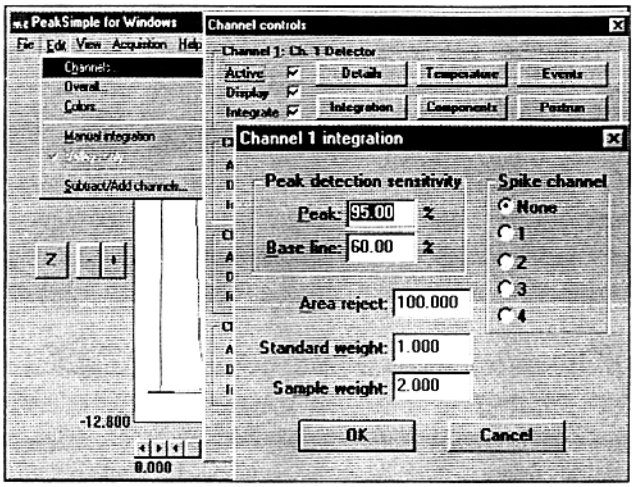
Gradient Limits Zero and **Span** may be scaled in the **Edit-Overall** screen to account for any offsets. PeakSimple allows for a voltage offset and scaling factor in these fields to calibrate the voltage output to match the pump's requirements.







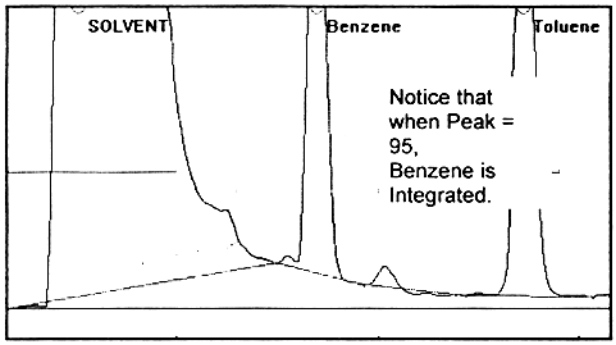
The EDIT-CHANNELS-INTEGRATION Screen

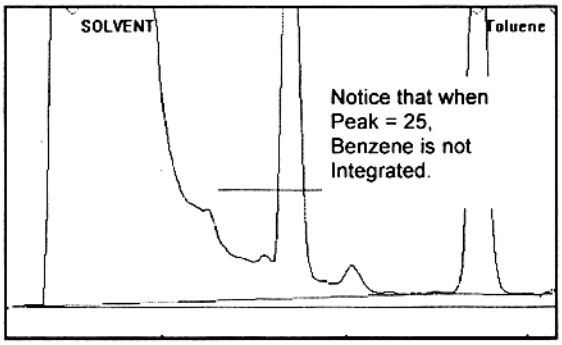


PeakSimple for Windows allows you to define specific integration parameters necessary for the proper analysis of your sample data, such as peak and baseline sensitivity and area reject. Any of the **Integration** parameters described below may be modified either before or after data collection. Pressing the **ENTER** key will update the report and the results of the chromatogram currently being displayed.

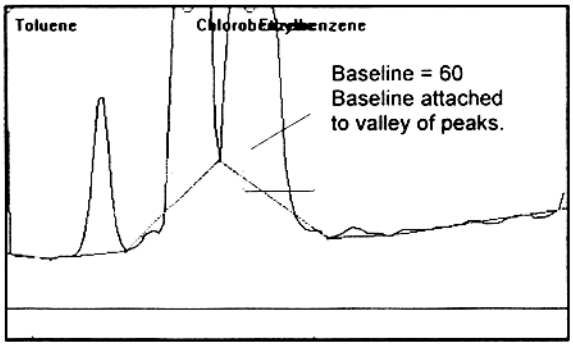
Peak Detection Sensitivity

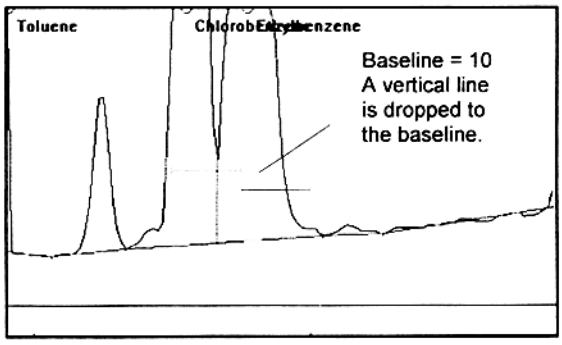
The **Peak** sensitivity setting determines how PeakSimple detects the beginning and end of a peak. A high **Peak** number requires only a small slope change to initiate the start or end of a peak. A low **Peak** number requires a very large slope change to initiate the start or end of a peak.



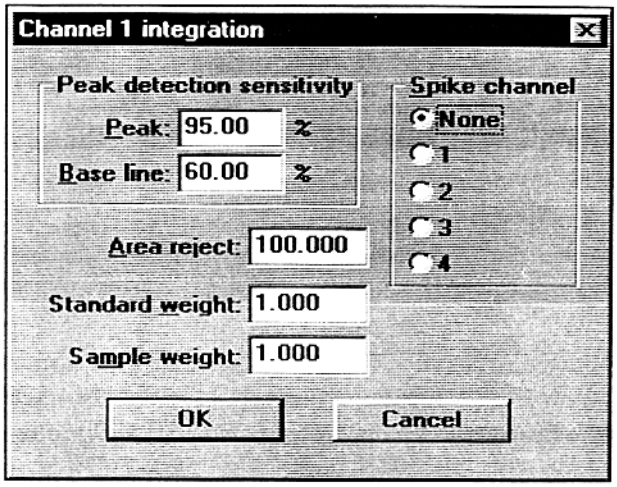


The **Baseline** sensitivity setting determines how PeakSimple attaches the baseline to the data line. The larger the **Baseline** number; the more likely PeakSimple will draw the baseline to a valley between two peaks. The smaller the **Baseline** number; the more likely PeakSimple will drop a vertical line from a valley to a horizontally constructed baseline below the peak.





The EDIT-CHANNELS-INTEGRATION Screen (continued)



Area Reject

If a chromatogram contains peaks whose area counts fall below the threshold defined by the **Area Reject** for that channel, the peak will be ignored and no integration will occur. If the peak area is of interest, you can lower the **Area Reject** value until the peak in question is integrated. Integrated peaks are marked with a circle at the top of the peak.

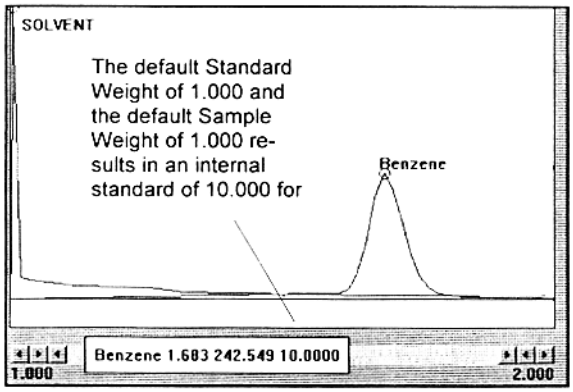
Standard Weight

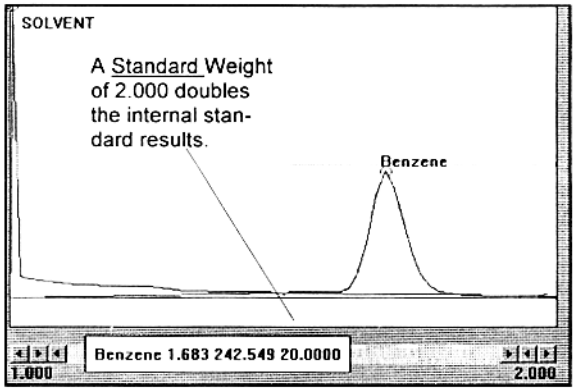
PeakSimple for Windows determines the internal or external standard results by the ratio of the STANDARD divided by the SAMPLE.

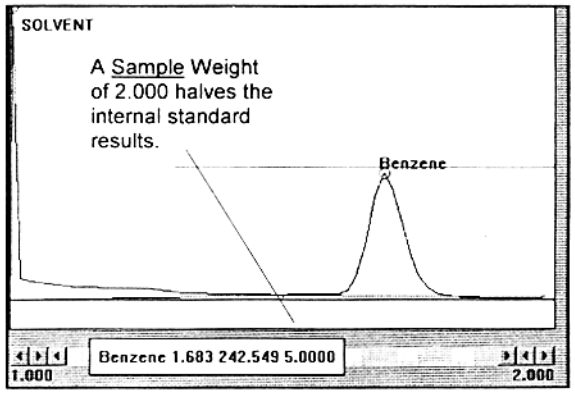
The **Standard Weight** setting may be changed to adjust the channel's quantification, affecting internal or external peak results by the factor entered. For instance: A setting of 2.000 will double the weight of the standard thereby doubling the internal or external standard results. (Increased to 20.000 in the example shown.)

Sample Weight

The **Sample Weight** setting may also be changed to adjust the channel's quantification, affecting internal or external peak results by the factor entered. For instance: A setting of 2.000 will double the weight of the sample thereby halving the internal or external standard results. (Decreased to 5.000 in the example shown.)



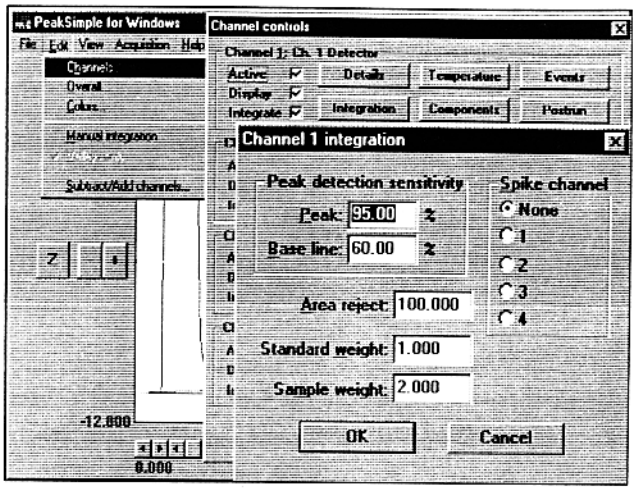




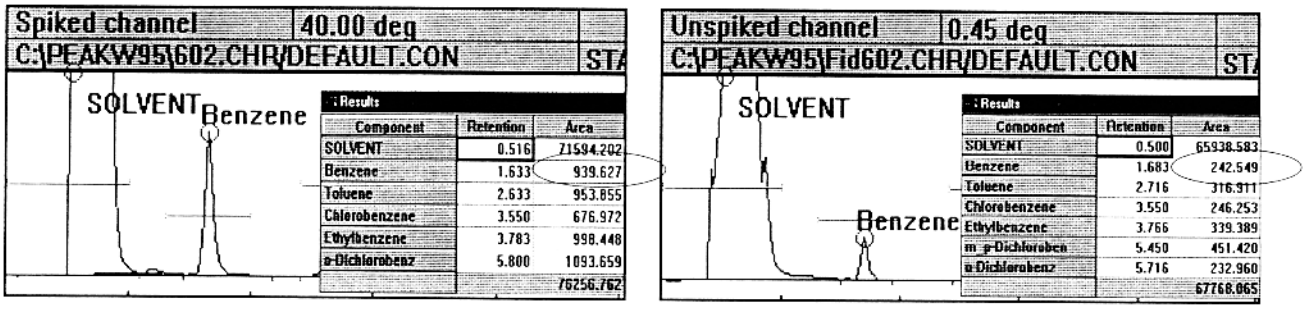
The EDIT-CHANNELS-INTEGRATION Screen (continued)

Spike Channel

Another feature of PeakSimple for Windows allows you to display the results of a matrix **Spike Channel** subtraction. The example shown below demonstrates the peak area counts of a unspiked channel being subtracted from the area counts of a spiked channel.

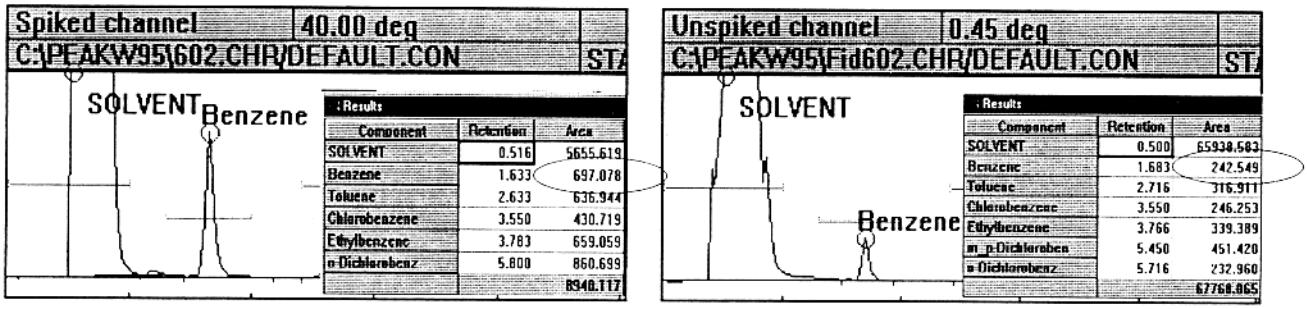


Before Spike Channel Subtraction



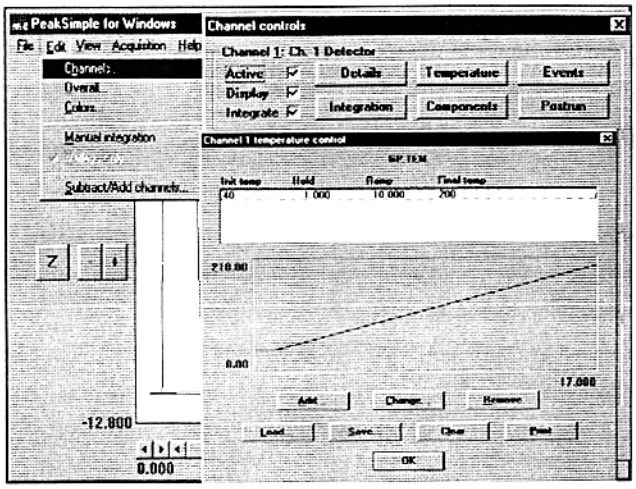
Notice that the area counts for Benzene are 939 on the spiked channel, and 242 area counts on the unspiked channel.

After Spike Channel Subtraction



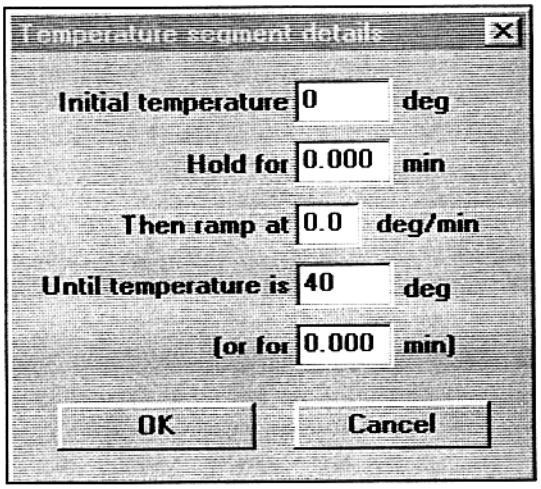
After selecting channel 2 as the **Spike Channel**, the area counts for channel 2 are subtracted from channel 1 to equal 697, (939 – 242 = 697). The difference of 697 indicates the area counts of the amount of sample spiked into channel 1.

The EDIT-CHANNELS-TEMPERATURE Screen



PeakSimple for Windows features temperature-programming of the G.C 's column oven(s). Access the **Edit-Channel 1-Temperature screen** to specify the temperature parameters to be used during the analytical run. The temperature program is capable of executing an unlimited number of temperature ramp and hold periods during the analysis as well as maintaining a single temperature throughout the run for isothermal operation.

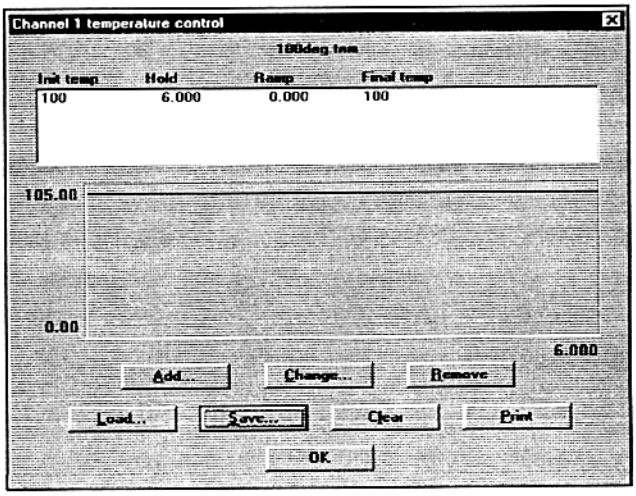
The Temperature Segment Details Screen



The **Add** Button

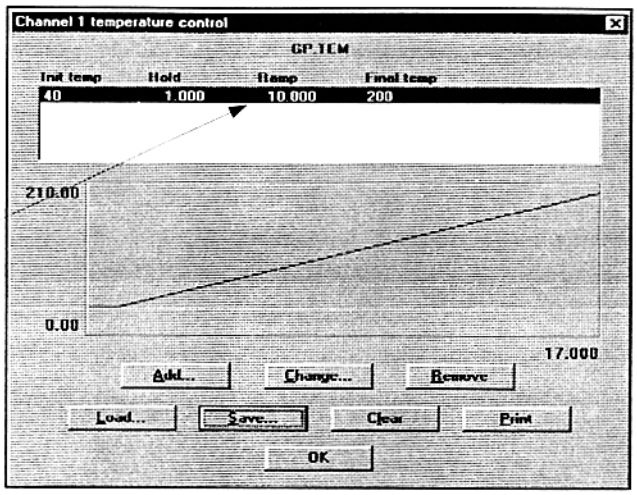
Click on the **Add** button from a blank **Channel 1 temperature control** window to create a new temperature program for Column oven #1. (Use the **Edit-Channel 2-Temperature** screen for controlling column oven #2). Type in the required data in the following fields; **Initial temperature**, the **Hold** period in minutes, the **Ramp** rate in ºC / min, and the final **Temperature**, or the duration of the Ramp.

The length of the run is automatically calculated by PeakSimple based on the information provided in these fields, and is also displayed in the **Edit-Channels-Details End Time** field. Additional ramp segments may be added by clicking the **Add** button again.



In isothermal operation, the **Initial** and the final temperature are the same, so a **Ramp** rate of 0.000 is entered. The **Hold** period determines the length of the analytical run.

The EDIT-CHANNELS-TEMPERATURE Screen (continued)

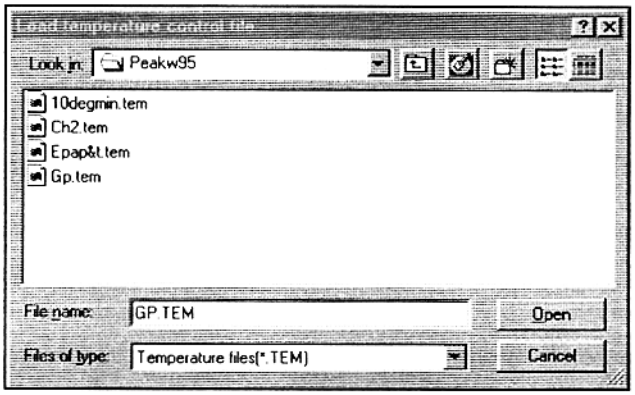


The **Change** Button

Click on an existing temperature program segment to select it. Click on the **Change** button to change the parameters of the segment.

The **Remove** Button

Click on the **Remove** button to remove the segment from the current program.

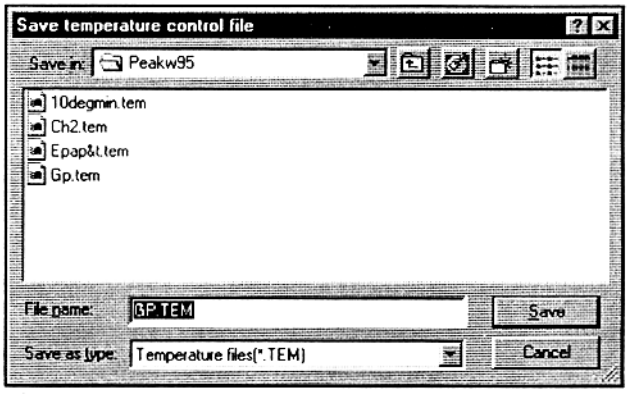


The **Load** Button

Click on the **Load** button to load an existing temperature control file, designated with the **.TEM** file extension.

The **Save** Button

Click on the **Save** button to save a new temperature control file, or to update an existing one. Remember to use the **.TEM** extension when naming the temperature control file. The saved file name appears at the top of the temperature control window indicating the file in use.



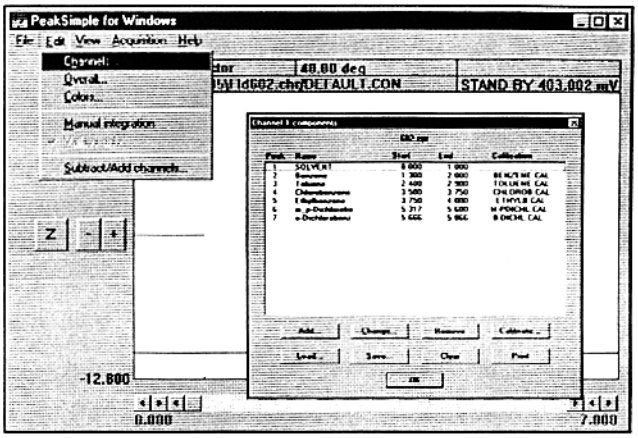
The **Clear** Button

Clicking on the **Clear** button deletes all temperature data from the temperature control window. The temperature program name is also removed.

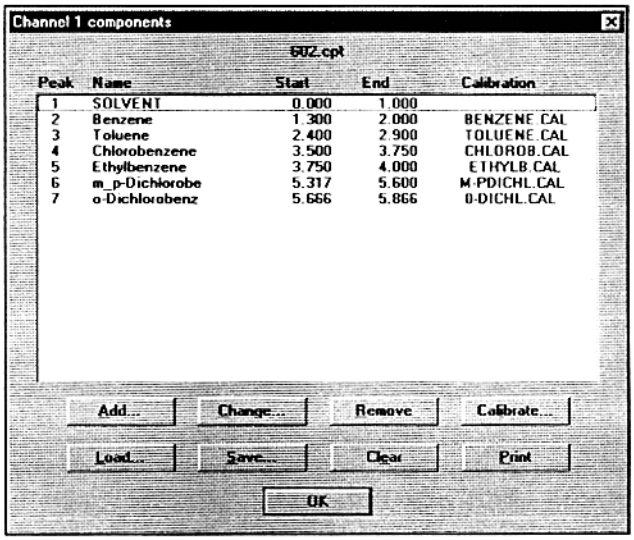
The **Print** Button

Clicking on the **Print** button sends the file data and temperature program profile to the printer.

The EDIT-CHANNELS-COMPONENTS Screen

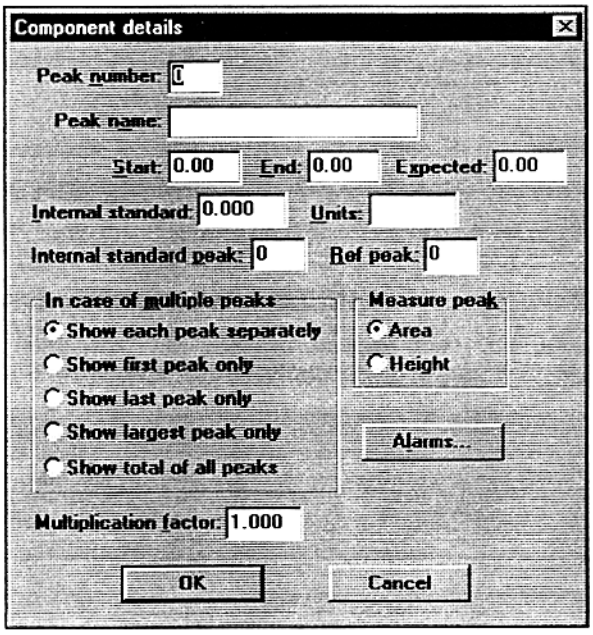


PeakSimple for Windows can identify and quantify sample components through the use of a component table. The component table enables PeakSimple to recognize each peak by its retention time and compare the area counts against the calibration curve to produce actual concentration data. The user can edit the component table for each channel by accessing the **Edit-Channels-Components** screen.



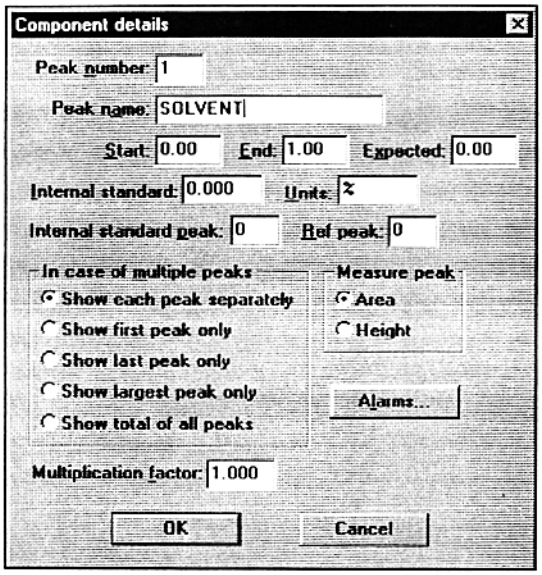
When a component table is loaded, the table will show each component by its peak number, peak name, the start time for the retention window, the stop time for the retention window, and the associated calibration file name. Different component tables may be used for each active channel and any component table can be saved as a component file for future use. Component files are designated with a **.CPT** extension. The component file-name appears at the top of the **Components** screen.

COMPONENT DETAILS



Select **Add** to add a new component to a blank or existing component table. The Component Details screen will open allowing the user to input specific peak parameters. As a minimum, enter the **Peak Number**, **Peak Name**, **Start** time and **End** time. Other optional parameters are the **Expected** peak time, the concentration **Units** to be reported, any **Internal Standard** or **Reference** peak information, peaks measured by **Area** or **Height**, handling of **Multiple Peaks**, the **Multiplication Factor** and **Alarm** parameters.

The EDIT-CHANNELS-COMPONENTS-DETAILS Screen (continued)



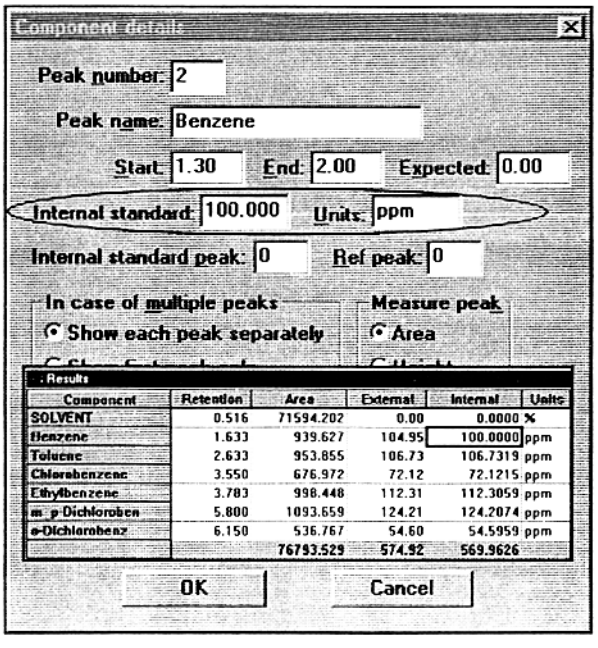
Peak Number, Peak Name, Start and End

A blank **Component Details** screen is opened by selecting the **Add** button. Enter a unique **Peak Number** for each component, typically starting with **1** and incrementing for each additional peak. Then enter a unique **Peak Name** for each component. **Start** and **End** define the beginning and ending of the retention windows, which is used to identify the peak. The width of the retention window should be set wide enough so that small fluctuations in the peak's retention time will still allow for proper integration.

Internal Standard and Units

Internal Standard calculations are used to correct for injection size variations, or to compensate for changes in detector sensitivity. An internal standard peak is added to the sample prior to injection at a known concentration. The internal standard peak is calculated just like any other peak using a calibration curve, typically a single point calibration. The known concentration of the internal standard peak is entered into the **Internal Standard** dialog box of the **Component Details** screen. In the example shown below, Benzene has been chosen as the internal standard peak. The known concentration of Benzene is entered as **100**, and **ppm** is entered in the **Units** dialog box. When a chromatogram is integrated and a report is produced, the external calculation yields a result which is the **peak area x calibration factor** (slope of the calibration curve) **= external standard result**.

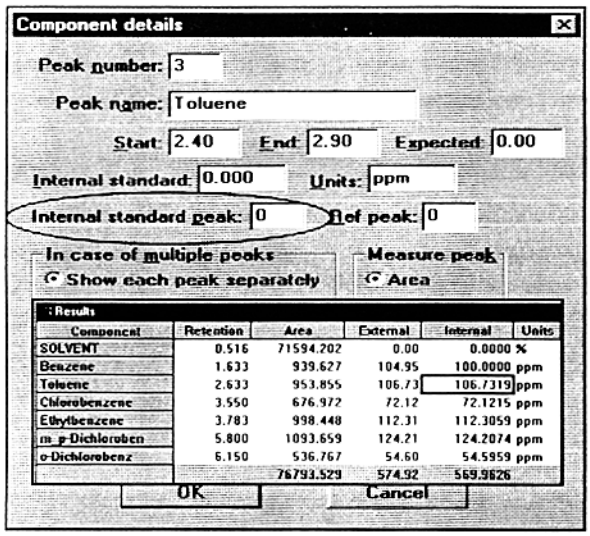
The **internal standard** calculation yields a result which is the **external result times the ratio of the known concentration of the internal standard peak divided by the external result for the internal standard peak**. As shown in the example to the right, note that while the external result for Benzene yields 104.95, the internal result yields exactly 100 (the known concentration) as a result of the calculation **104.95 x 100/104.95**. In the same way, the internal result for every analyte peak which is referenced to Benzene is calculated as **external result x 100/104.95 = internal standard result**.

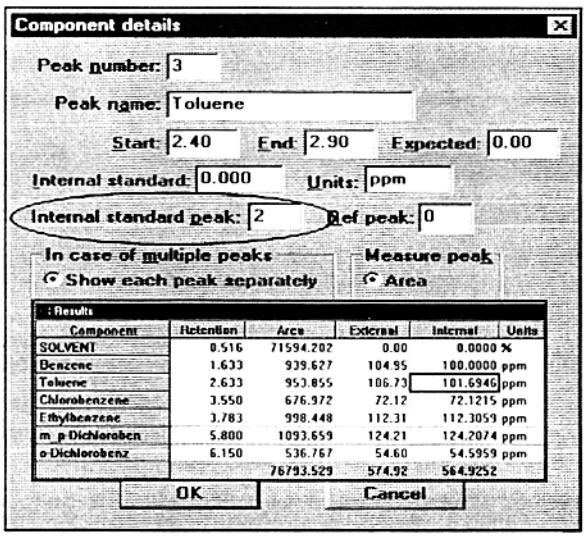


The EDIT-CHANNELS-COMPONENTS-DETAILS Screen (continued)

Internal Standard Peak

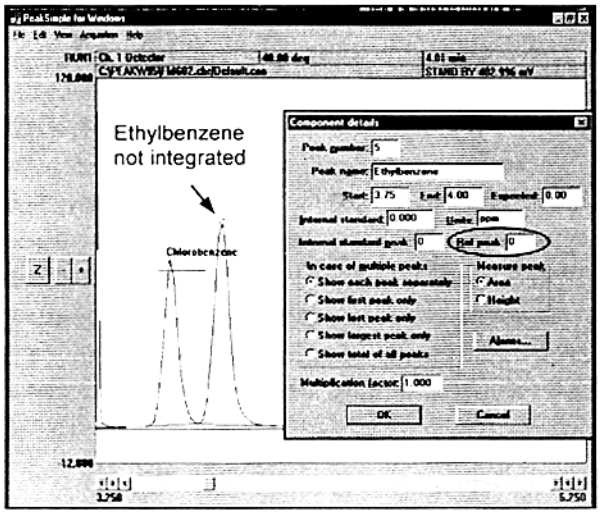
PeakSimple allows any peak to be referenced to any other peak for internal standard calculations. Typically all analyte peaks will be referenced against a single **Internal Standard Peak** (Benzene [peak #2] in the example shown below). To reference other peaks to Benzene, the number **2** must be entered in the **Component Details** screen dialog box labeled **Internal Standard Peak** for each analyte peak. Notice that the **Results** screen, (**View-Results**), will reflect the new value for all the peaks' internal results.

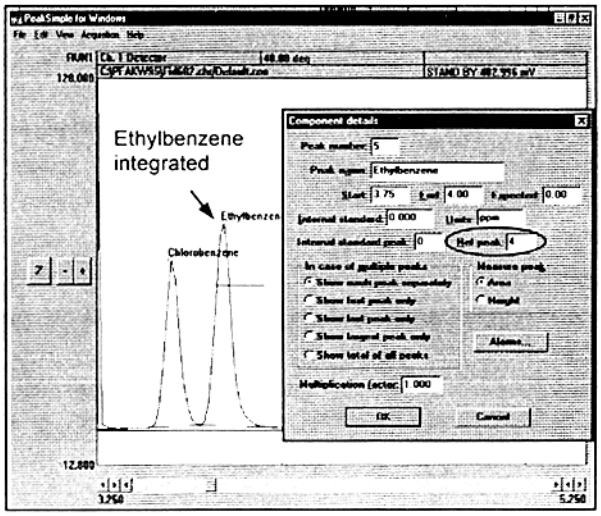




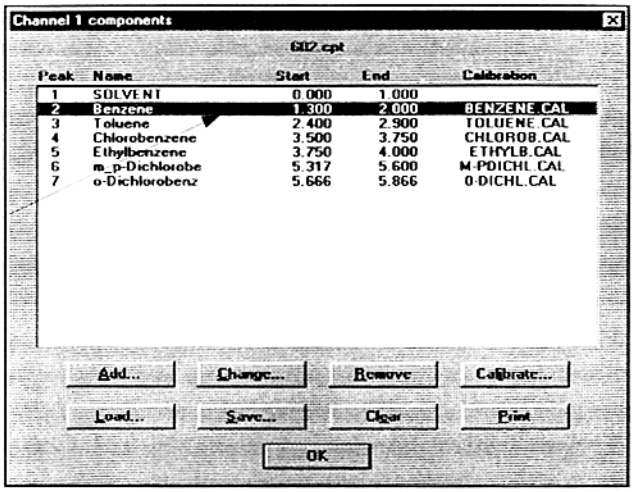
Reference Peak

A **Reference Peak** is used to shift the retention windows of other peaks. In the example below, ethylbenzene eluted prior to its retention window so therefore it was not integrated. By entering a value of **4** in the **Reference Peak** box, ethylbenzene's retention windows are referenced to chlorobenzene, [peak #4]. Ethylbenzene's retention window is then shifted by a percentage equivalent to chlorobenzene's distance from the middle of its retention window. This shift in the ethylbenzene retention window allows ethylbenzene to be integrated.





The EDIT-CHANNELS-COMPONENTS Screen (continued)

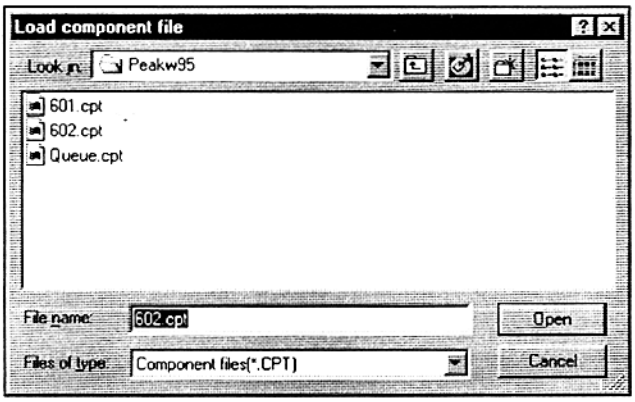


The **Change** Button

Click on an existing component to select it. Click on the **Change** button to change the parameters of the component.

The **Remove** Button

Click on the **Remove** button to remove the component from the component table.

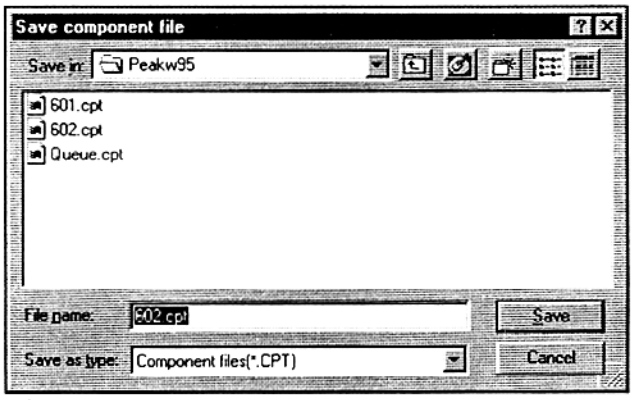


The **Load** Button

Click on the **Load** button to load an existing component file, designated with the **.CPT** file extension.

The **Save** Button

Click on the **Save** button to save a new component file, or to update an existing one. Remember to always use the **.CPT** extension when naming the component file. The saved file name appears at the top of the components window indicating the file in use.



The **Clear** Button

Clicking on the **Clear** button deletes all component data from the component window. The component file name is also removed.

The **Print** Button

Clicking on the **Print** button sends the file data and the component table information to the printer.

The EDIT-CHANNELS-COMPONENTS Screen (continued)

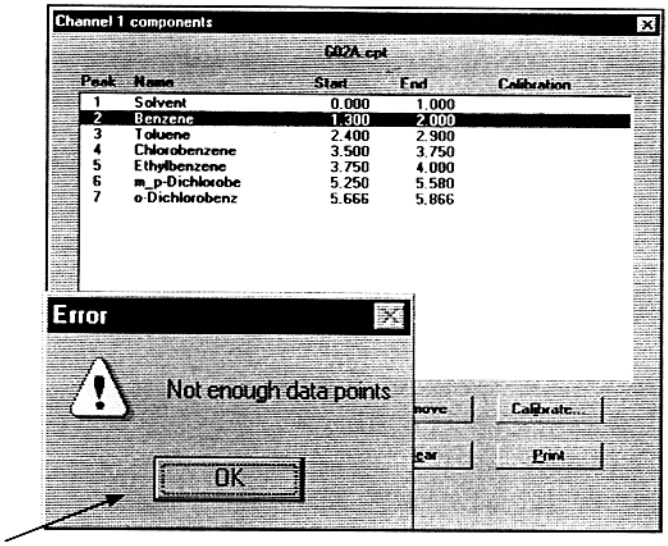
The Calibrate Button

After creating a component table, each component in the table will need to be calibrated. This allows PeakSimple for Windows to not only identify each analyte peak, but also to quantify each peak using a calibration curve. The calibration curve is calculated from user-generated results obtained at several different concentrations that span the expected range to be encountered in actual samples.

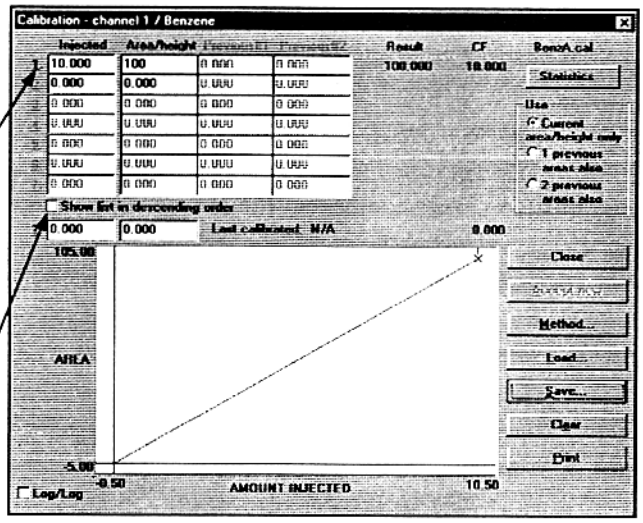
Inject a standard containing a known concentration of the component you wish to calibrate. Use a concentration higher than what you would expect to encounter in your analyses. Another few samples should be run at lower levels, using precise dilutions of your standard. Make note of the area counts or peak height at each concentration or use the shortcut method described in the next section.

The Calibration Window

In the **Edit-Channels-Components** screen, highlight the component to be calibrated and select **Calibrate**. If this is the first time calibrating a component, an error message will appear which says "**Not enough data points**". This is simply a warning to inform you that PeakSimple currently does not have enough data points for the calibration method in use. Once enough data is entered for the calibration curve, this message will no longer appear. Click **OK** to bypass the error message and continue to the calibration window.

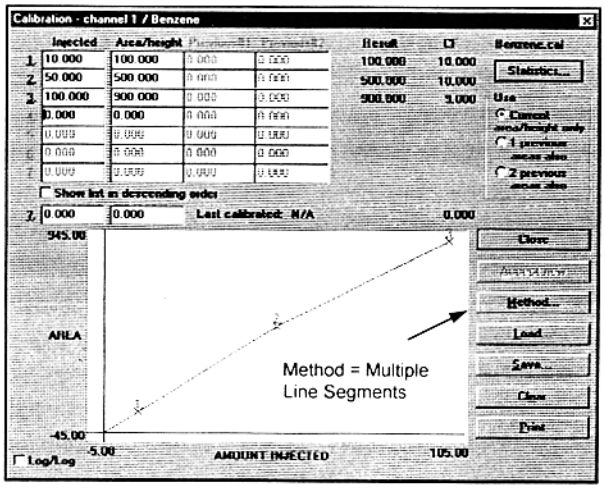


The **Calibration** window will open and allow you to enter the raw data that you previously obtained. In the example shown, data is entered into the table in the upper left corner of the calibration screen, beginning with the lowest concentration and ending with the highest concentration. If you wish to enter the data in descending order, check the **Show list in descending order** box. When entering data into the table, first enter the concentration injected, then the area count or peak height obtained.

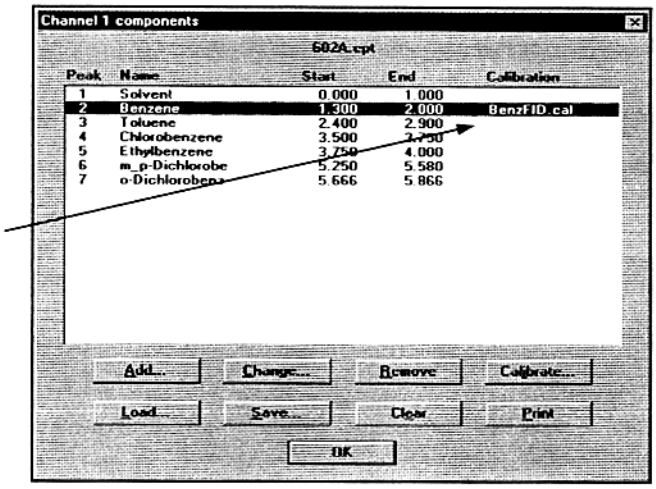


The Calibrate Button (continued)

As data is entered for each concentration, a data point will be added to the calibration curve displayed in the lower section of the window. You may use as many as seven concentration levels for your calibration curve. In the fictitious example to the right, a Benzene standard was injected in concentrations of 10 ppm, 50 ppm, and 100 ppm. The area counts from the FID detector were 100, 500 and 900, respectively. Notice the three corresponding data points on the newly created calibration curve.

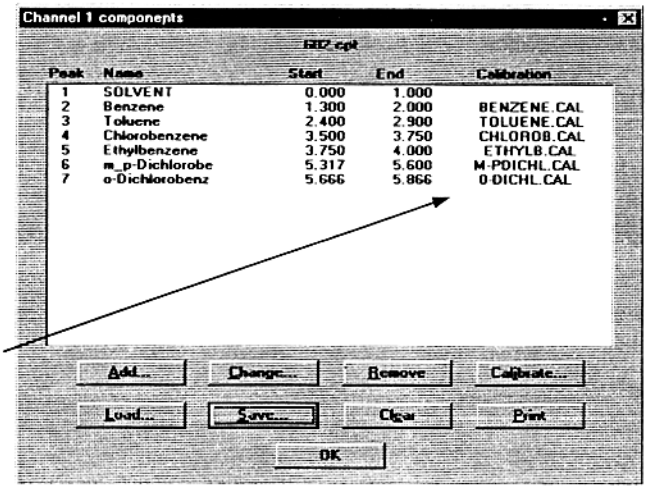


When calibration for each component has been completed, click on the **Save** button to save and name the component's calibration file. Then click on the **Close** button to close the calibration window. In our example, a unique file named **BenzFID.cal** was created. The **BenzFID.cal** file name will now appear in the **Components** window next to Benzene.



WARNING:
Do not use the same calibration curve file name for two different channels or detectors since each detector requires its own calibration curve. (ie BenzFID.cal; BenzPID.cal; etc)

Calibration is required for each component you expect to be present in your sample, and for each detector you will be using in your analysis. Once calibration curves have been completed, and calibration files saved, every component in the component table should show an associated calibration file. PeakSimple will now be able to quantify each component when actual samples are injected.

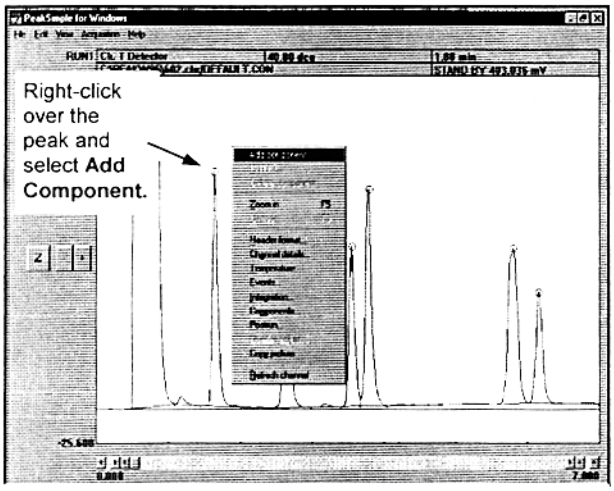


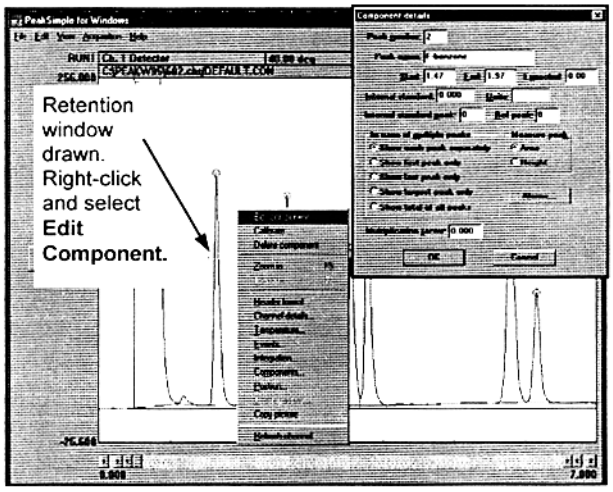
Calibration Screen Shortcuts

As an added convenience, PeakSimple for Windows offers shortcuts to commonly used screens. These shortcuts may be accessed by pointing to the desired channel and **clicking once on the <u>right</u> mouse button**. The following pages describe the shortcuts available to set up calibration tables and calibrate components.

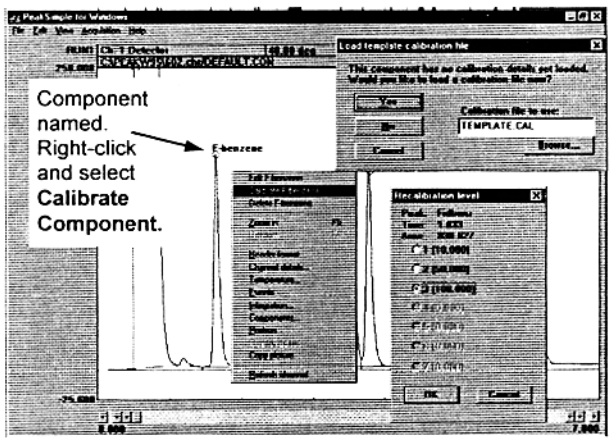
After a known standard has been run and the peaks have been identified, a new component table may be constructed by simply positioning the mouse pointer over a peak and clicking once on the right mouse button, ("right-clicking"). The shortcut menu will appear. Select **Add component** from the menu. A retention window will be drawn horizontally across the peak. Right-click again over the peak and select **Edit component**. The **Component Details** screen will open allowing the peak to be named and numbered. The example below shows Benzene as peak #2. The component has been named F-benzene to avoid confusion with a benzene peak from another detector such as a PID.

Note: It is important that you choose the component name carefully since the <u>calibration file name</u> is derived from the <u>first eight letters</u> of the <u>component name</u>. The F-benzene calibration file would be named F-benzen.cal.



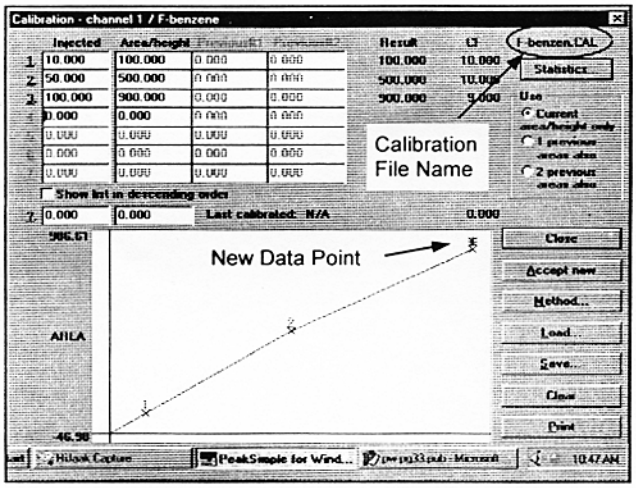


Right-click over the peak again and select **Calibrate**. If no calibration curve exists for the peak, a window will open asking if you would like to use a calibration file. PeakSimple offers a template calibration file aptly named TEMPLATE. CAL. Click yes to use the default TEMPLATE calibration file or select your own by clicking **Browse**. This example uses the template calibration file. Another window will open asking you to select the **Recalibration Level**. Select **100** for 100 ppm standards, **50** for 50 ppm, etc.

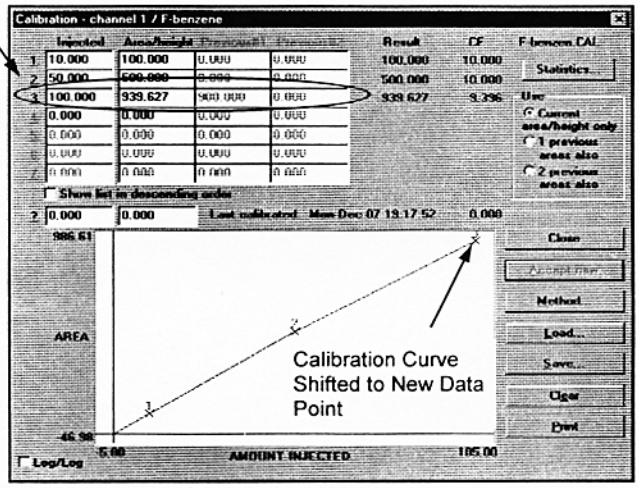


Calibration Screen Shortcuts (continued)

Click **OK** to accept the **Recalibration Level**. The Calibration screen will open and a flashing asterisk (*) will appear along the existing calibration curve depicting the new data point. Notice that the calibration curve has been named **F-benzen.CAL.** If the new caliabration data point is acceptable, click **Accept New** to update the calibration curve data.

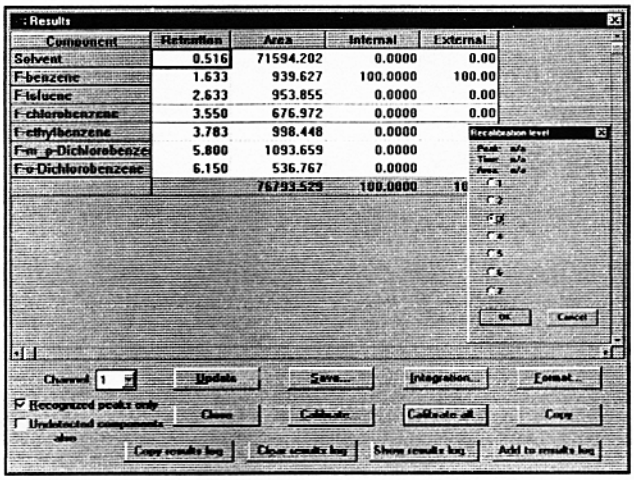


In the example to the right, the updated **F-benzen.CAL** calibration table reflects the new area count of **939.627** at the concentration level of **100** ppm. (The previous calibration data of 900 area counts at 100 ppm is shown in the **Previous #1** column which is 'grayed out'). Notice also that the third data point (100 ppm) in the calibration curve has been shifted up slightly to incorporate the new data, (939.627 area counts). At this point, if the new calibration curve data is deemed to be acceptable, click on **Close** to automatically save the new calibration file, and close the **Calibration** window.

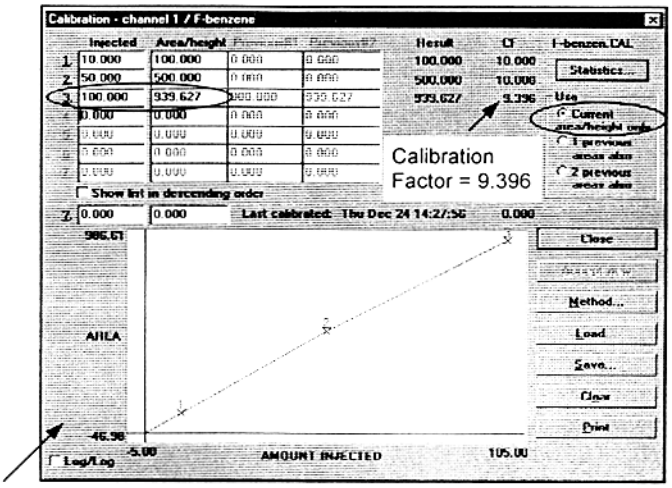


Calibrate All

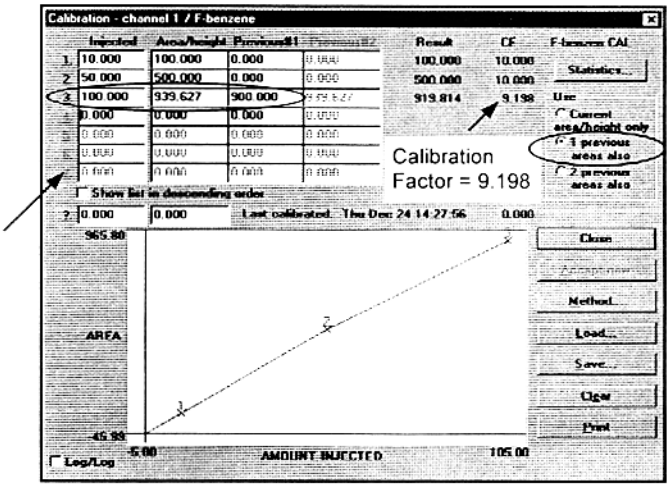
PeakSimple offers a time-saving feature for **recalibrating all peaks** with just one mouse click. After a calibration curve has been created for each component, click on **View-Results** to bring up the results window. Select **Calibrate All** and choose an appropriate **Recalibration Level**, then click **OK**. PeakSimple will automatically recalibrate all components at the selected level and save each component's updated calibration file.



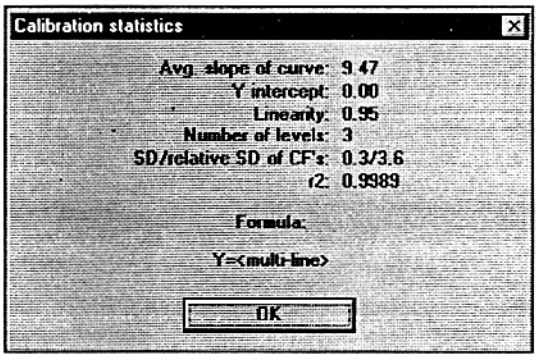
Calibration Screen – Use and Statistics Radial Buttons



To improve the calibration accuracy, chromatographers may prefer to average the areas of 1, 2 or 3 replicate injections. The **Use** radio button allows the user to select how many injections are used in the calculation of calibration factors, (CF). Calibration Factors are used to construct the calibration curve using the formula: CF = area count divided by the amount injected. The example to the right shows the calibration data at the 100 ppm concentration level, (circled), with the **Use** button set to the default setting of **Current Area / Height Only**. This setting uses only the latest calibration data to calculate the calibration factor for the #3 data point. (CF = 939.627 / 100 = 9.396)



This next example shows how the calibration curve is changed when the **Use** button is set to **1 Previous Areas Also**. This setting averages the last two areas to derive the average calibration factor. Notice that the calibration factor is now 9.198 when the two area counts are averaged together. (939.627 + 900.000 / 2 = 919.814 average area counts. The CF is calculated as: CF = 919.814 / 100 = 9.198)

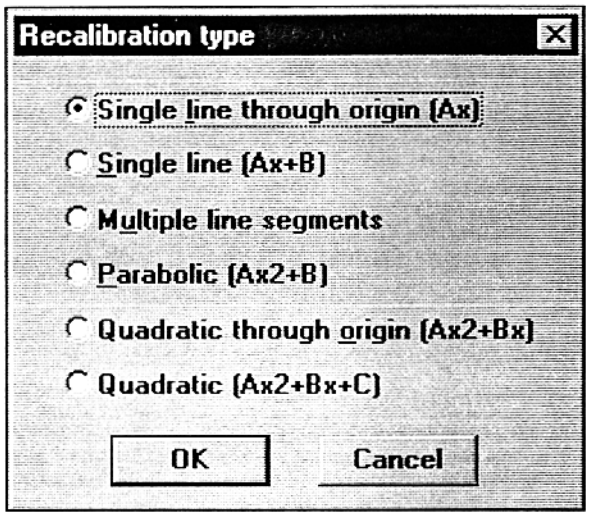


Setting **Use** to **2 Previous Areas Also** will average the last three areas to derive the calibration factor.

The **Calibration Statistics** screen shows calibration curve details such as the **Average Slope of the Curve**, the **Y Intercept**, the **Linearity** of the curve, the **Number of (calibration) Levels**, the **Standard Deviation and Relative Standard Deviation of Calibration Factors**, the **R2** and the **Formula** used which is based on the **Method** selected.

Calibration Window– Methods

The **Method** button opens the **Recalibration Type** window which allows the selection of one of six formulas used to draw the calibration curve. The algorithms are described below and corresponding calibration statistics are shown.



In the following:
X is the sum of the external measures over the calibration levels
Y is the sum of the corresponding areas at those calibration levels
n is the number of active calibration levels
Several other sums are used, for instance:
X2 is the sum of the squares of the external measures
Y4 is the sum of the (area to the 4th power)
XY is the sum of the (external measure * area)
X2Y is the sum of (external measure squared * area)
Y|X is the sum of the (area / external measure) etc.

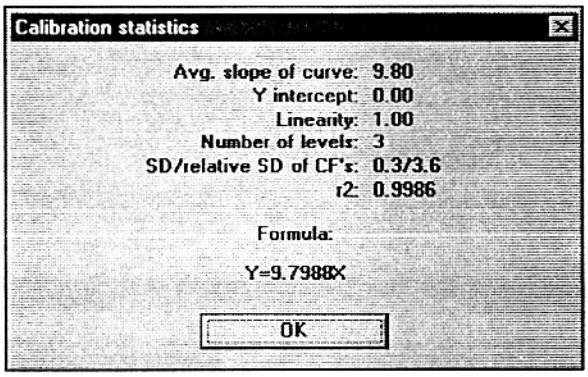
Single line through origin:

The resulting calibration curve is defined as

$y=Ax$

where:
x is external measure
y is area
$A=(Y|X)/n$

Notes:
The resulting factor is therefore the average of the calibration factors at the calibration levels. Note: any explicit calibration level point at x=0 is ignored (and n is reduced by 1). There must be at least one calibration level, not including any level at x=0.



Single line:

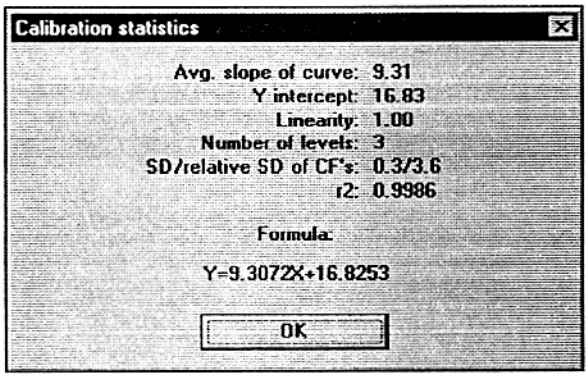
The resulting calibration curve is defined as

$y=Ax+B$

where:
x is external measure
y is area
$A=((XY * n) - (X * Y)) / D$
$B=((X * Y2) - (XY * X)) / D$
$D=((X2 * n) - (X * X))$

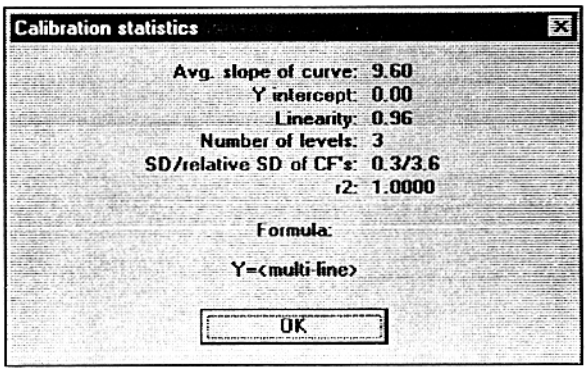
Notes:
This is a least squares fit algorithm over the calibration levels. A point at (0,0) is also assumed (by incrementing n) unless there is already a value at x=0, or if [Statistics]R2IncludeZero is set to 0 in the PEAKWIN.INI file. There must be at least 2 calibration levels.

EPA rules allow the use of Single Line Fit provided that the standard deviation of calibration factors is <20%.



Multiple line segments:

There is no resulting formula here, just interpolation between the levels, and the origin. There must be at least one calibration level.



Calibration Window– Methods (continued)

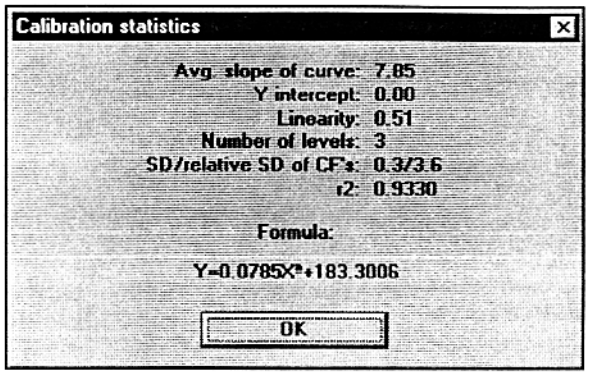
Parabolic:

The resulting calibration curve is defined as

y=Ax2+B

where:
x is external measure
y is area
A=((X2Y * n) - (Y * X2)) / D
B=((Y * X4) - (X2Y * X2)) / D
D=((X4 * n) - (X2 * X2))

Notes:
This is a least squares fit algorithm over the calibration levels. A point at (0,0) is also assumed (by incrementing n) unless there is already a value at x=0, or if [Statistics]R2IncludeZero is set to 0 in the PEAKWIN.INI file. There must be at least 2 calibration levels (3 if the origin is not assumed).



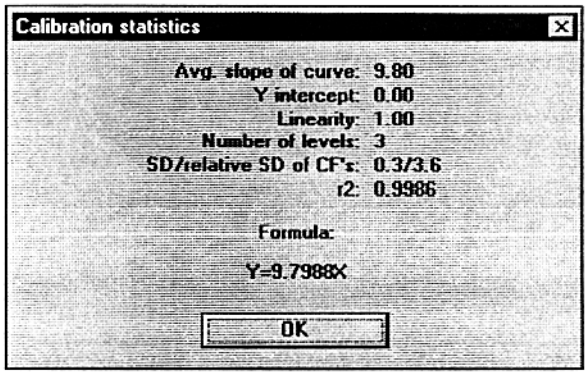
Quadratic through origin:

The resulting calibration curve is defined as

y=Ax2+Bx

where:
x is external measure
y is area
A=((XY * X3) - (X2Y * X2) / D
B=((XY * X4) - (X2Y * X3)) / D
D=((X3 * X3) - (X4 * X2)

Notes:
This is a least squares fit algorithm over the calibration levels. There must be at least 2 calibration levels.



Quadratic:

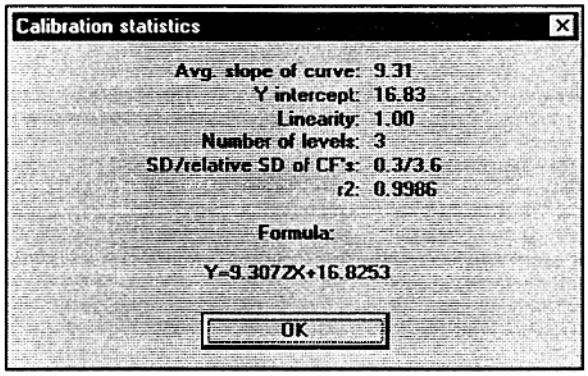
The resulting calibration curve is defined as

y=Ax2+Bx+C

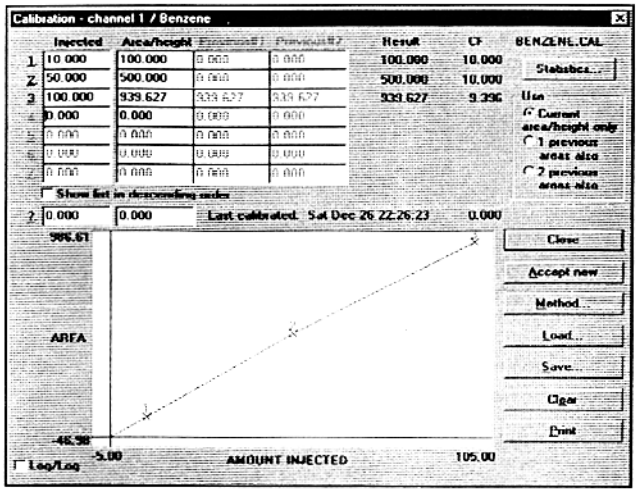
where:
x is external measure
y is area

A=((XY*X-Y*X2)*(X2*X2-X*X3) -
(X2Y*X2-XY*X3)*(X*X-X2*n)) / D
B=((XY*X2-Y*X3)*(X2*X3-X*X4) -
(X2Y*X3-XY*X4)*(X2*X2-X*X3)) /E
C=((XY*X2-Y*X3)*(X3*X3-X2*X4) -
(X2Y*X3 - X*X4)*(X2*X2-X*X3))/F
D=((X3*X-X2*X2)*(X2*X2-X*X3) -
(X4*X2-X3*X3)*(X*X-X2*n))
E=((X2*X2-X*X3)*(X2*X3-X*X4) -
(X3*X3-X2*X4)*(X*X2-X3*n))
F=((X*X2-X3*n)*(X3*X3-X2*X4) -
(X2*X3-X*X4)*(X2*X2-X*X3)

Notes:
This is a least squares fit algorithm over the calibration levels. A point at (0,0) is also assumed (by incrementing n) unless there is already a value at x=0, or if [Statistics]R2IncludeZero is set to 0 in the PEAKWIN.INI file. There must be at least 2 calibration levels (3 if the origin is not assumed).



The Calibration Window (continued)

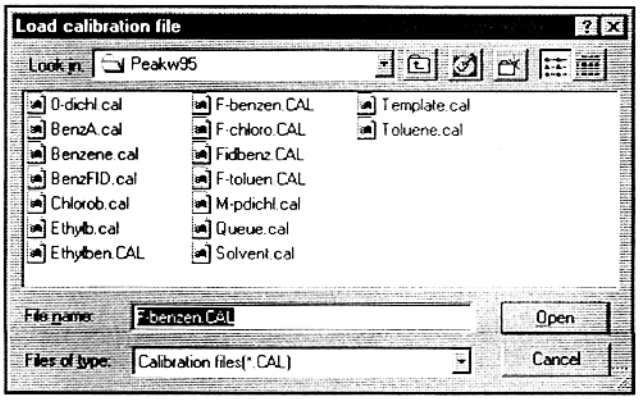


The **Accept New** Button

If the new calibration data is acceptable, Click **Accept New** to update the calibration curve data.

The **Close** Button

Automatically saves the new calibration file and closes the Calibration window.

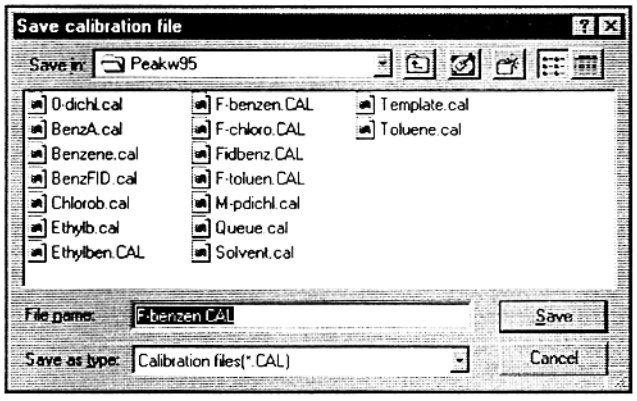


The **Load** Button

Click on the **Load** button to load an existing calibration file, designated with the **.CAL** file extension.

The **Save** Button

Click on the **Save** button to save a new calibration file, or to update an existing one. Remember to always use the **.CAL** extension when naming the calibration file. The saved file name appears at the top of the calibration window indicating the file in use.



The **Clear** Button

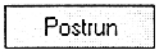
Clicking on the **Clear** button deletes all calibration data from the calibration window. The calibration file name is also removed.

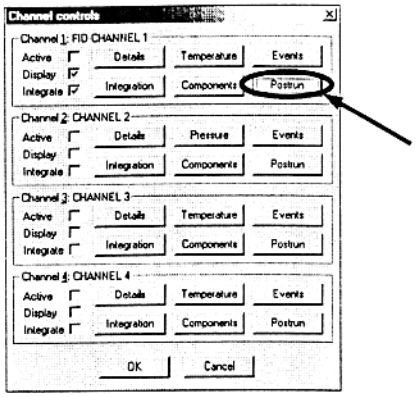
The **Print** Button

Clicking on the **Print** button sends the file data and the calibration curve information to the printer.

The Edit-Channels-Postrun Window

The Postrun Screen is used to determine all the actions that are to be done in PeakSimple after a chromatogram run. Clicking on the **Postrun** box for channel 1 in the Channel controls window will open up the Channel 1 post-run actions window.



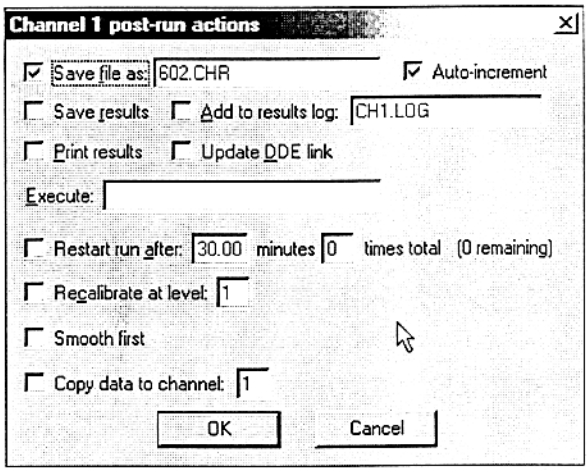


Save file as "X"

The Save file as checkbox, when selected, automatically saves a chromatogram file to disk after a run is completed. The file will be saved under the file name and path entered in the information field to the right of the checkbox.

Auto-increment

When selected, the Auto-increment checkbox will incrementally add a numerical digit to the entered filename after each run. For example, a chromatogram run saved as RUN.CHR would be saved as RUN1.CHR after the second run and RUN2.CHR after the third run.



The **Save results** checkbox when selected will save the data in the results screen to disk after a chromatogram run (*Note: This is not the raw data but instead is the ASCII results).* The **Add to results log "X"** checkbox adds the results of a run to the results log specified in the information field to its right. It will be saved under the same filename as the raw data but with the extension .RES, for example 602.RES. The **Print results** checkbox will print whatever is specified to be printed in the Print format window, this might include the chromatogram and its results data. The **Update DDE link** checkbox when selected will automatically update the Dynamic Data Exchange link once the run is completed.

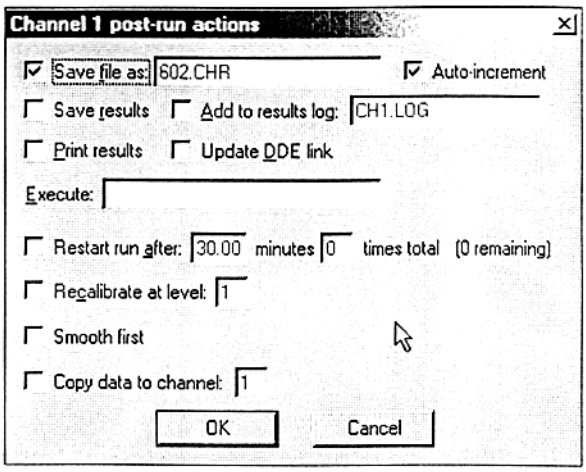
Execute "X"

The Execute information field opens any executable file (.exe, .bat, .bas) after the chromatogram run is completed. *Note: Be sure to include the full filename and path for the executable file.* Control is returned to PeakSimple when the called application closes.

Restart run after "X"

The Restart run after "X" checkbox and information field restarts a chromatogram run after an inputted delay time. The delay time is inputted in minutes and can be repeated as many times as is entered into the times total information field. *Note: If 0 is entered into the times total information field then the run will be restarted an infinite number of times.*

The Edit-Channels-Postrun Window (continued)



Recalibrate at level "X"

The Recalibrate at level "X" checkbox and information field recalibrates all identified peaks at the end of a run at a given level from 1 to 7. This feature is normally implemented as part of an autosampler queue. Detailed instructions are given in the Autosampler queue documentation section.

Smooth first

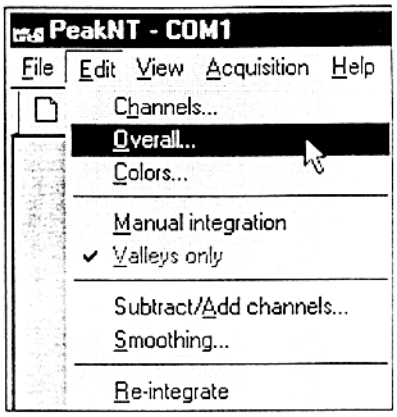
The Smooth first checkbox runs the smoothing algorithm as it was last applied to the chromatogram before the final integration is done. If the box is left unchecked no smoothing will be done to the chromatogram run.

Copy data to channel "X"

The Copy data to channel "X" checkbox and information field inputs the chromatogram run into whatever channel is selected in the information field. Only the values 1 to 4 can be inputted into the information field as there are four chromatogram channels in PeakSimple.

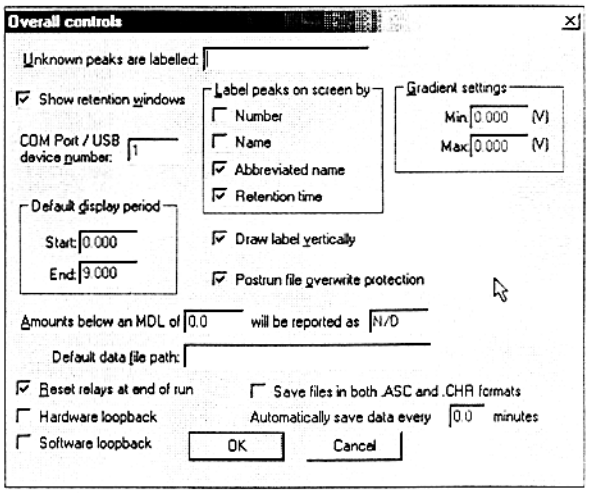
The Edit-Overall Window

The Overall controls window is used to define and control many of the options in PeakSimple. Clicking on **Edit** in the PeakSimple menu bar and then **Overall** from the drop down menu will open up the Overall controls window.



Unknown peaks are labeled "X"

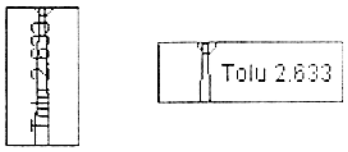
The Unknown peaks are labeled information field, when filled out, labels all unknown peaks the value that is in the information field. If the word Peak was entered into the information field then all unknown peaks would be labeled Peak.



The **Show retention windows** checkbox is checked by default and thus retention windows are visible in PeakSimple; unchecking the Show retention windows checkbox removes the retention windows from sight. The **COM Port / USB device number "X"** information field specifies the COM port or USB device number that is to be used for the connection between PeakSimple and hardware. The COM port number is typically 1 or 2 while the USB device number is typically between 5000 and 9999.

Label peaks onscreen by

The Label peaks onscreen by options box enables a peak to be labeled by as many as four options. The **Number** checkbox labels all peaks with their peak number. The **Name** checkbox labels all peaks with their full name. The **Abbreviated name** checkbox labels all peaks with a shorter, four character abbreviated name while the **Retention time** checkbox labels peaks with their retention times. The **Draw label vertically** checkbox specifies whether peaks should be labeled horizontally or vertically on the chromatogram screen. When the box is checked the peaks labels will be drawn vertically when it is deselected they will be drawn horizontally.



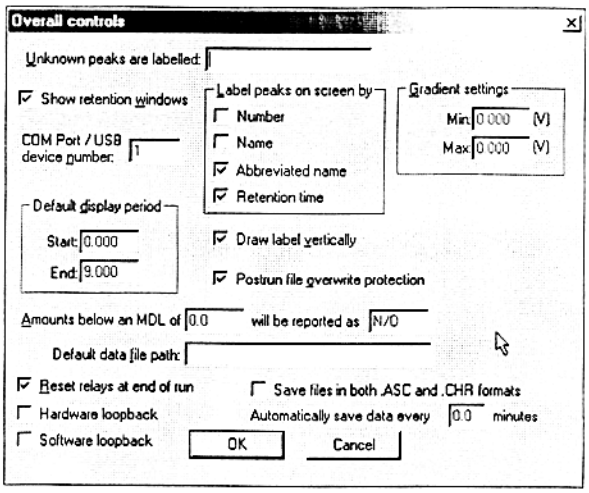
Gradient settings

Gradient settings are only used when PeakSimple is controlling an SRI HPLC Pump. The **Min** and **Max** voltage settings are used to calibrate the Pump.

The Edit-Overall Window (continued)

Default display period

The default display period options box is used to define the default display limits for a PeakSimple chromatogram. The **Start** information field is used to specify the default beginning limits while the **End** field is used to specify the end to the default display limits. The start and end display limits can also be adjusted by the left and right arrows below the chromatogram in the main display window.



Postrun file overwrite protection

Postrun file overwrite protection protects a saved file from being written over when the auto-increment feature is selected in the Postrun window. Instead of writing over a used filename an auto-incremented run will select the next unused number in the sequence to save the file to disk. For example, if file TEST02.CHR already exists on disk PeakSimple will save the file as TEST03.CHR.

Amounts below an MDL of "X" will be reported as "Y"

Peaks with a value below a specified Minimum Detection Level or MDL will be reported as whatever is specified in the second information field, typically N/D or not detected. The number that is below the MDL will not be reported, only the entry in the second information field will be seen.

Default data file path

Typically all PeakSimple files are saved to the PeakSimple directory but by entering a full directory path into the Default data file path information field another directory can be selected to save files to. *Note: It is recommended that users save all PeakSimple files to the PeakSimple directory. If necessary export files to a different directory after saving them to the PeakSimple directory.*

Reset relays at end of run

The Reset relays at end of run checkbox when selected turns off all relays (A-H) at the end of a chromatogram run. If the box is left unselected the relays will not be shut off after a chromatogram run.

Hardware loopback and **Software loopback** are used for system validation and will be discussed in further detail in the Loopback test section.

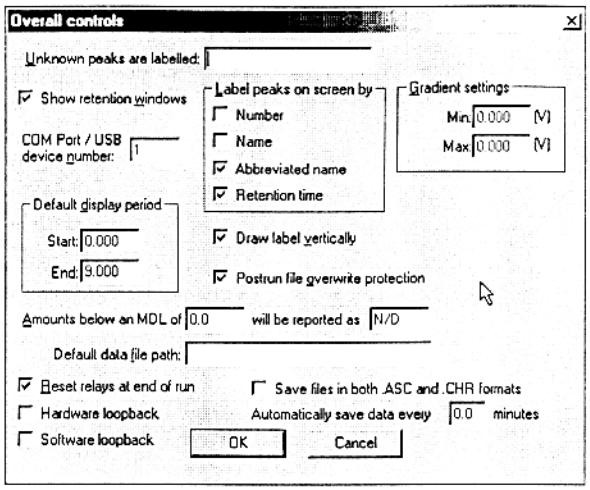
The Edit-Overall Window (continued)

Save files in both .ASC and .CHR formats

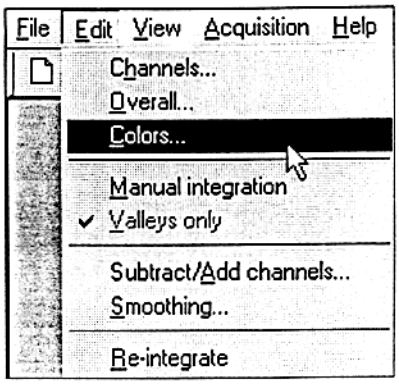
The Save files in both .ASC and .CHR formats checkbox when selected saves files in the .ASC format (ASCII) and the .CHR format (chromatogram). If the checkbox is not selected files will be saved only in the .CHR format.

Automatically save data every "X" minutes

The Automatically save data every "X" minutes checkbox and information field when selected saves the data during a chromatogram run at intervals specified by the information in the information field. This feature is useful for runs where power outages are frequent and data cannot be lost.

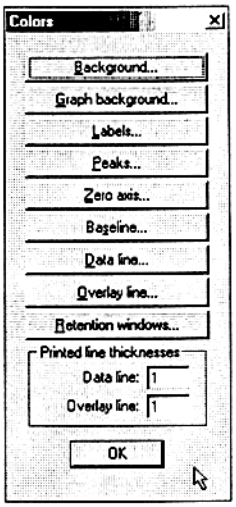


The Edit-Colors Window



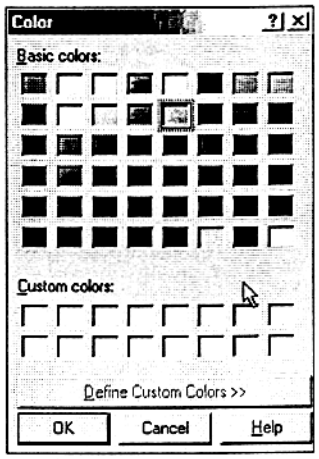
The Colors window determines the color schemes that are to be used throughout PeakSimple. Open the Colors window by selecting **Edit** from the PeakSimple menu bar and then **Colors** from the list of options.

Selecting the **Background** button with the mouse cursor opens up the Background color window. The background color can be chosen from a set of 48 colors by selecting a color and then affirming the choice by clicking on the OK button.



The Graph background window is opened up by selecting the **Graph background** button in the Colors window. The graph background color is changed by selecting a color and then clicking on the OK button to make the color change.

The color of the labels controls the color of the words that belong to the peaks. The color of the labels is changed by selecting the **Labels** button to open up the Labels color window. In the Labels color window select a color and then press on the OK button to make the change to the labels color.



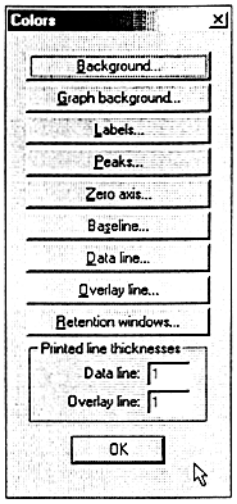
The peak color is the color of the circle at the top of each identified peak and is determined by the Peak color window which is opened up by selecting the **Peak** button in the Color window. Select the desired peak color and then click on the OK button to close the window and affirm the change.

The color of the zero axis is chosen by clicking on the **Zero axis** button and then selecting a color from the Zero axis color window. Clicking on the OK button closes the window and makes the change to the color of the zero axis. Don't set the Zero axis color to the same color as the Graph background because they won't be distinguishable from each other.

The baseline is the line that runs along the bottom of the peaks and its color is changed by selecting the **Baseline** button and then choosing a color from the Baseline color window. The change is made once the OK button is selected and the window is closed.

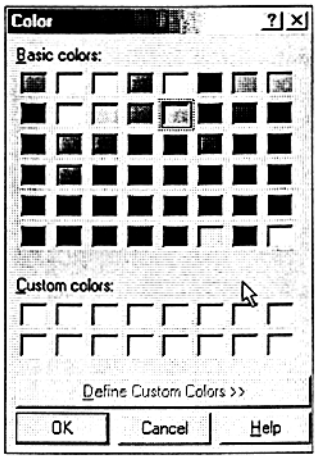
The data line is the signal line that makes up the peaks in PeakSimple and its color is defined by selecting the **Data line** button in the Colors window and then selecting a color from the Data line colors window. Once the desired color is selected apply the color change by clicking on the OK button to close the window.

The Edit-Colors Window (continued)



The overlay line is a data line from a chromatogram that has been overlaid on top of an existing chromatogram and its color is changed by selecting the **Overlay line** button in the Colors window and then selecting a color with the mouse cursor in the Overlay line colors window. The color changes are made once the OK button is selected and the window closes.

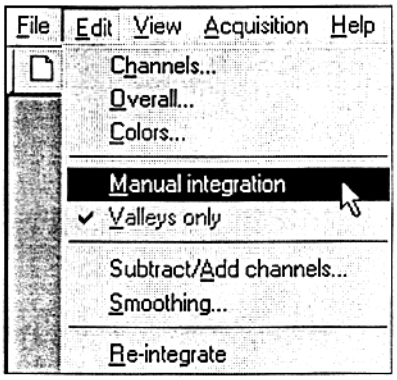
Retention windows are the horizontal bars that appear onscreen and their color can be changed by clicking on the **Retention windows** button in the Colors window and then selecting the desired color in the Retention windows colors window. To apply the color changes click on the OK button to close the window.



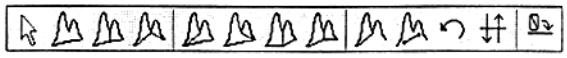
Printed line thickness

The thickness of the Data line and the Overlay line when a chromatogram is printed is determined by the **Data line** information field and the **Overlay line** information field. The thickness of the Data line is determined by the numerical value in the Data line information field, larger numerical values will result in thicker lines. The thickness of the Overlay line is also determined by the numerical value in its information field. Larger numbers in the information field will result in a thicker overlay line.

Manual Integration



The manual integration tools are used to manually draw in the baseline in a PeakSimple chromatogram. The manual integration toolbar is opened up by selecting **Edit** from the PeakSimple menu bar and then clicking on the **Manual integration** option. The manual integration toolbar appears to the right of the PeakSimple toolbar in the upper right hand corner of the screen.

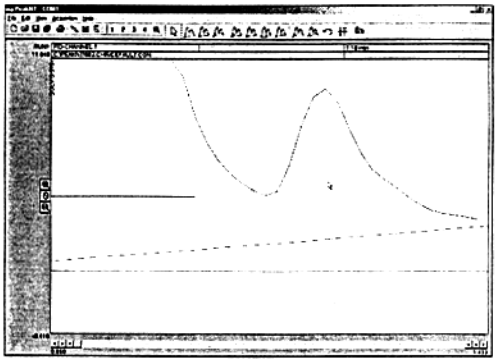


Off Integration Tool 

The Off integration tool or the mouse cursor is used to end a manual integration mode once it has been selected. When the mouse cursor icon is selected no more changes to the baseline of a chromatogram can be performed until another manual integration tool is selected.

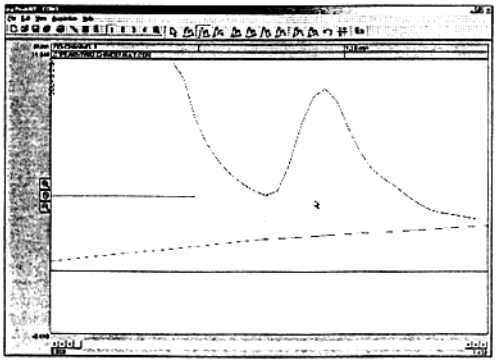
None Integration Tool 

The None integration tool adds the area of one peak to the area of an adjacent peak. Once the None integration tool is selected click on a valley between two peaks with the mouse cursor to change the baseline.



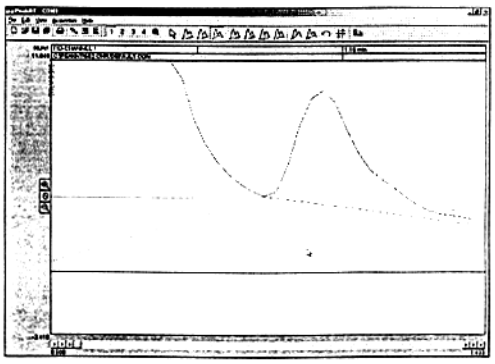
Drop Integration Tool 

The Drop integration tool drops the baseline between two peaks straight down onto an existing baseline. The Drop integration tool is used by selecting the Drop tool in the manual integration toolbar and then clicking on a valley between two peaks to change the baseline.



Based Integration Tool 

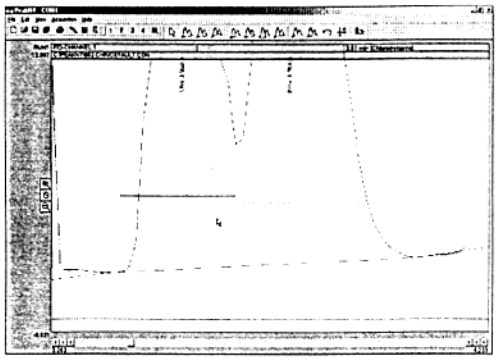
The Based integration tool raises the baseline to a valley between two specified peaks. To change the baseline select the Based tool and click on a peak with the mouse cursor to raise the baseline up to the valley.



Manual Integration (continued)

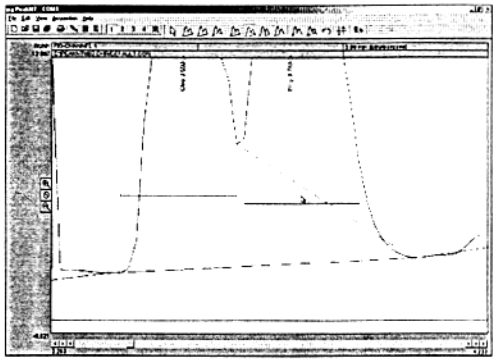
Lead Skim Integration Tool 

The Lead skim integration tool skims a peak's area off of the leading edge of an adjacent peak. To skim a peak off of the leading edge of another peak select the Lead skim tool from the manual integration toolbar and then click on the valley between the two specified peaks with the mouse cursor.



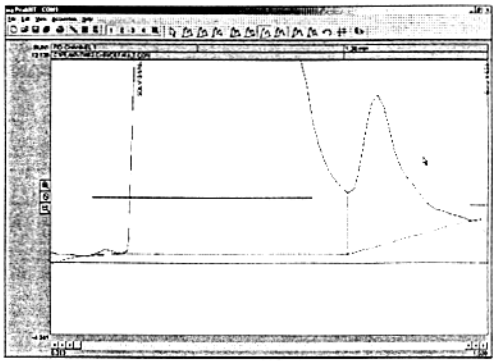
Trail Skim Integration Tool 

The Trail skim integration tool skims a peak's area off of the trailing edge of another, adjacent peak. To skim a peak off of the trailing edge of another peak select the Trail skim tool and click on a valley between two peaks with the mouse cursor to make the change.



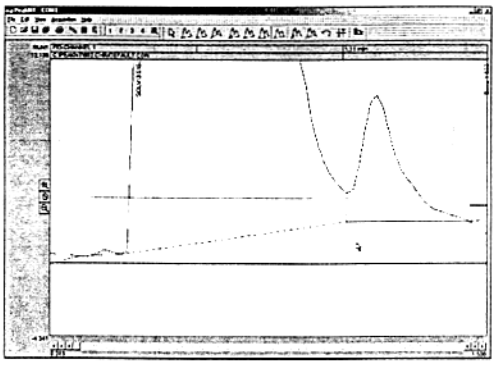
Lead Horizontal Integration Tool 

The Lead horizontal integration tool draws the baseline horizontally for the leading peak while the trailing peak's baseline stretches from the horizontal line to the next valley. The Lead horizontal tool is selected in the manual integration toolbar and once a valley is selected the change to the baseline is made.



Trail Horizontal Integration Tool 

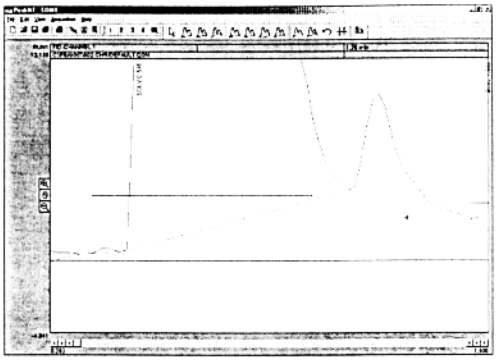
The Trail horizontal integration tool draws the baseline horizontally for the trailing peak while the leading peak's baseline stretches from the horizontal line to the previous valley in the chromatogram. The Trail horizontal tool is used by selecting the Trail horizontal tool in the manual integration toolbar and then clicking on a valley with the mouse cursor to make the change.



Manual Integration (continued)

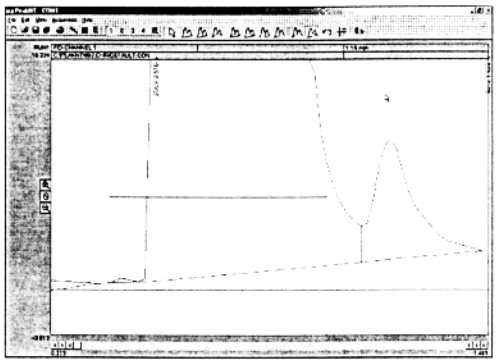
Inhibit Integration Tool

The Inhibit integration tool ends a baseline after a valley thereby stopping the peak's area from being counted along with the rest of the chromatogram. To use the Inhibit tool select the tool in the manual integration toolbar and then click on the valley between two peaks to end the baseline.



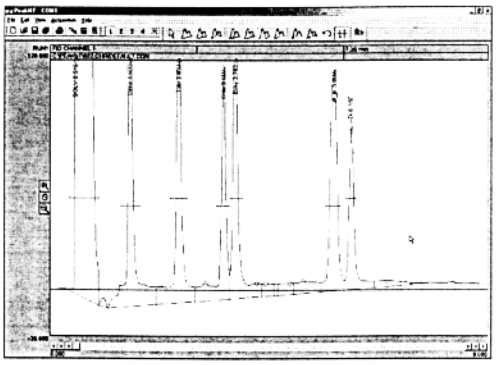
Rubber Band Integration Tool

The Rubber band integration tool is used to manually draw the baseline in a chromatogram. The Rubber band tool is selected in the manual integration toolbar and is clicked and dragged on the chromatogram to draw in the baseline.



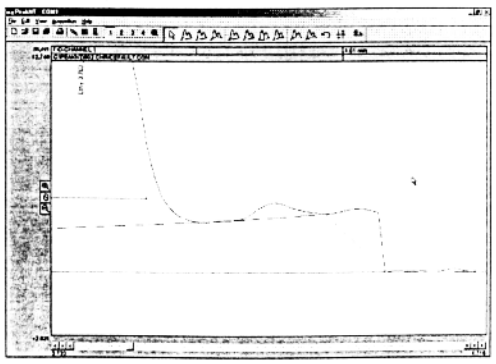
Undo Integration Tool

The Undo integration tool removes all changes done to the baseline of a chromatogram with the manual integration tools. To use the Undo tool click on the tool in the manual integration toolbar and all changes will be undone. *Note: Changes made to a chromatogram with the Reverse and Zero integration tools cannot be undone with the Undo tool.*



Reverse Integration Tool

The Reverse integration tool inverts a selected peak or a selected group of peaks in a chromatogram. A peak is inverted by selecting the Reverse tool in the manual integration toolbar and then clicking and dragging the mouse cursor over the peak.

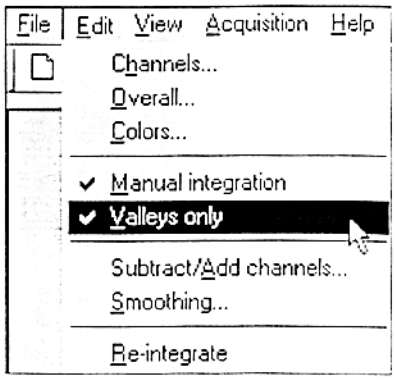


Zero Integration Tool

The Zero integration tool sets the value of the data line at zero starting at a selected point. To zero the data line at a given point select the Zero tool from the manual integration toolbar and click on the data line with the mouse cursor.

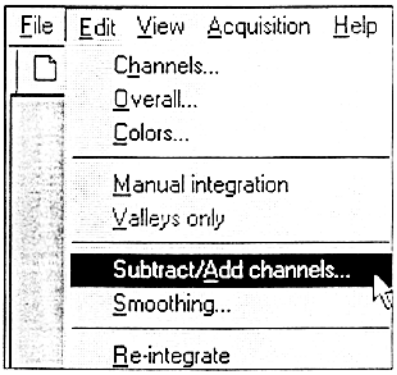
The Edit-Valleys Only Option

The Valleys only option is available only when the Manual integration toolbar is open in PeakSimple. The Valleys only option can be selected by opening up the Manual integration toolbar in the Edit menu and then selecting the Valleys only option immediately below Manual integration in the drop down menu. When the Valleys only option is selected all changes made to the baseline of a chromatogram will snap only to the valleys of the chromatogram. When the Valleys only option is turned off changes made to the baseline of a chromatogram will go to wherever the mouse cursor was clicked.



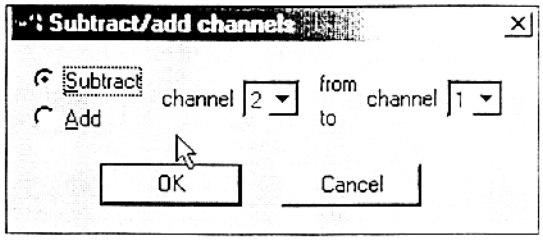
The Edit-Subtract/Add Channels Menu

The Subtract/Add channels menu removes or adds the analog data signal from/to one channel in PeakSimple from/to another channel. The Subtract/Add channels menu is opened by selecting the Edit menu and then by clicking on Subtract/Add channel in the drop down menu.



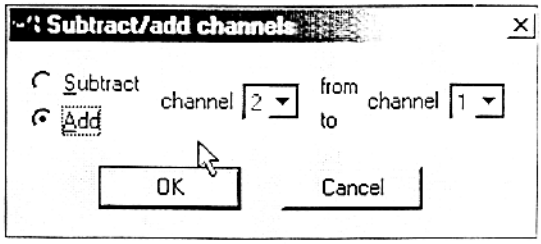
Subtracting a Channel

To subtract one channel from another channel click on the Subtract radio button with the mouse cursor and select the channel that is to be taken away in the first dialogue box. In the second dialogue box select the channel that is to have the first selection taken away from. Click on OK with the mouse cursor to effect the changes.

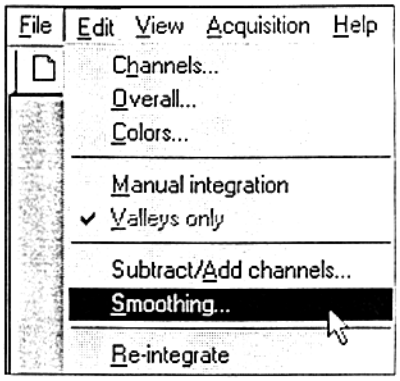


Adding a Channel

To add one channel to another channel select the Add radio button in the Subtract/Add channels menu. Select the channel that is to be added by selecting a number in the first dialogue box and then choose the channel that it is to be added to by selecting a number in the second dialogue box. All changes are made once the OK button is selected.

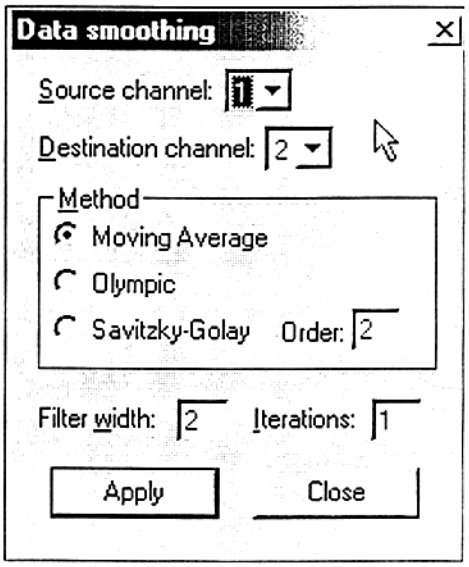


The Edit-Smoothing Window



The Data smoothing window determines all the smoothing options that are to be performed on a data line. The Data smoothing window is opened up by selecting Edit from the PeakSimple menu bar and then selecting Smoothing from the list of options.

The **Source channel** dialogue box specifies which channel the data line that is to be smoothed is in. The **Destination channel** is the channel that the smoothed data line from the source channel will be displayed in.



Method

The method of smoothing is determined by the smoothing algorithm selected in the Method box. The **Moving Average** algorithm sets each sample to the average of the samples around it including itself. The number of samples taken into account depends on the Filter width. The **Olympic** algorithm is similar to the Moving Average but the highest and lowest values in the set of samples are discarded before the average is taken. The **Savitsky-Golay** algorithm is similar to the Moving Average but each of the samples is weighted according to a set of weighting factors. Increasing the number in the **Order** dialogue box gives more weight to the central samples when using the Savitsky-Golay method.

Filter Width

The Filter width dialogue box controls the number of samples that are to be taken into account when using the Moving Average smoothing method. A filter width of 2 means that 2+1+2 samples are taken while a filter width of 5 means that 5+1+5 samples are taken.

Iterations

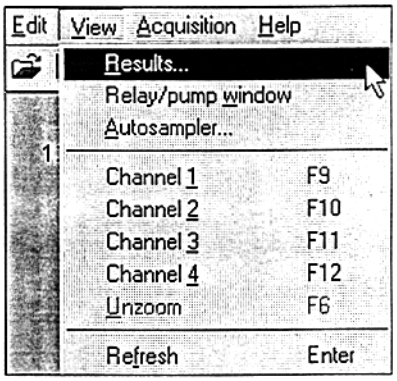
The Iterations dialogue box controls the number of times a smoothing method is to be applied to a chromatogram peak. Every iteration smoothes the data line more than the previous iteration eventually making the data line flat.

The Re-Integrate Option

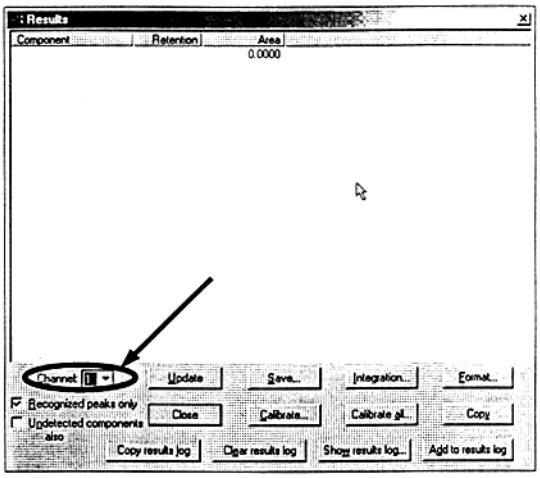
The Re-integrate option is used to fully re-integrate a baseline in PeakSimple. When changes are made to a baseline often a partial integration will occur, selecting Re-integrate will perform a full integration on the baseline. The Re-integrate option can be selected by clicking on Edit in the PeakSimple menu bar and then Re-integrate from the list of options.

The View-Results Window

The Results window displays the results of the chromatogram runs performed in PeakSimple. The Results window is opened up by clicking on View in the PeakSimple menu bar and then selecting Results from the list of options.



The **Channel** option scrollbar specifies which of the four channels the results data should be displayed for. When the **Recognized peaks only** checkbox is selected only the results for named peaks will be displayed. The **Undetected components also** checkbox displays the results for the undetected components as well as the detected components in the chromatogram run when the option is selected.

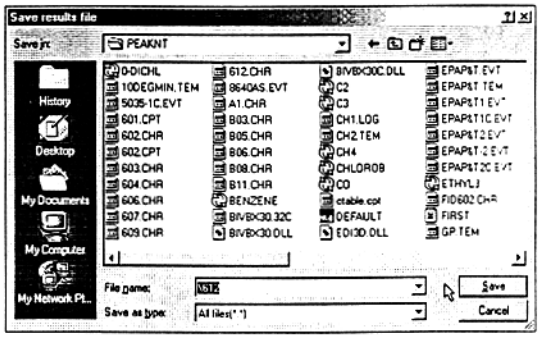


Update

The Update button in the Results window updates the DDE link between the Results data and the DDE host program (typically Excel).

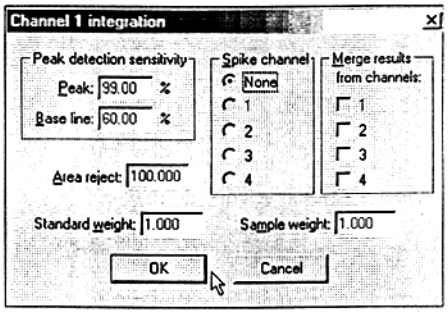
Save

Selecting the Save button in the results window opens up the Save results file window. In the Save results file window the results file is saved with a .res extension. The file is an ASCII file and not the raw chromatogram data.

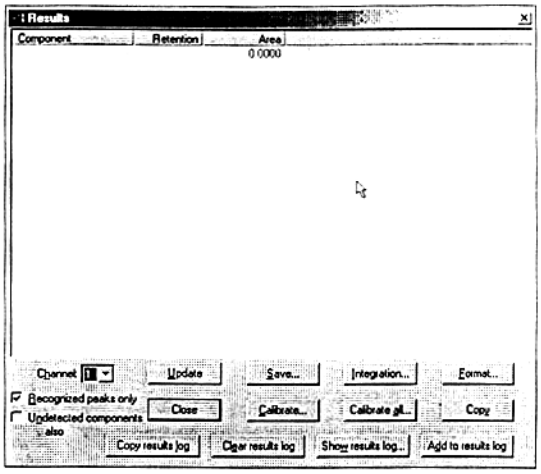


Integration

As a convenience the integration button in the results window opens up the same Integration window that can be accessed in the Channels window. For more information on the Integration window consult the Channels-Integration portion of this manual.

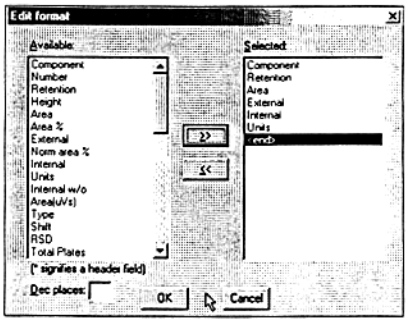


The View-Results Window (cont.)



Format

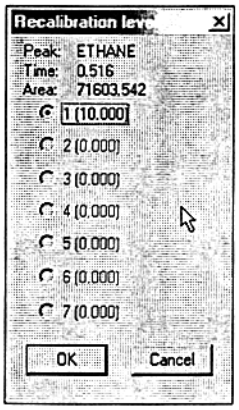
Selecting the Format button in the Results window opens up the Edit format window. The Edit format window allows the user to specify the information that is to be included in the Results table.



The **Available** options box in the Edit format window displays all the available options that can be included in the results but that aren't selected. An option is added to the **Selected** options box by highlighting the item in the Available box and clicking on the right facing arrow button. To deselect an option from the Selected box highlight the item and click on the left facing arrow button. The **Dec. places** dialogue box specifies how many decimal places a highlighted unit will display in the Results table.

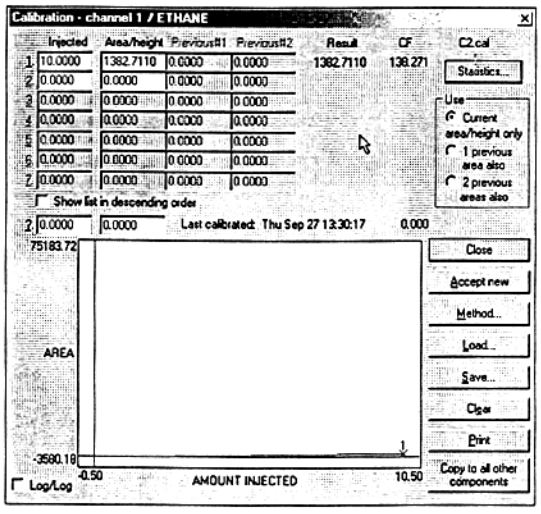
Close

The Close button exits the Results window and returns the user to the main screen.



Calibrate

The Calibrate button recalibrates a recognized peak in the Results table. Highlighting a peak name and selecting the Calibrate button opens up the Recalibration Level window. The window specifies which peak level should be calibrated. Following the Recalibration level window is the Calibration window which is discussed at further length in the Calibration section of this document.



Calibrate All

The Calibrate all button recalibrates all the recognized peaks at once. The Calibrate all button calibrates all peaks with existing calibration curves on a particular calibration level. If named peaks are in the results table without calibration curves an error message, (NOT ENOUGH DATA POINTS), will be displayed. The calibration will

The View-Results Window (cont.)

Copy

The Copy button in the results window copies the results report to the Clipboard. Once the report is copied it can be pasted into other programs i.e. Excel.

Copy Results Log

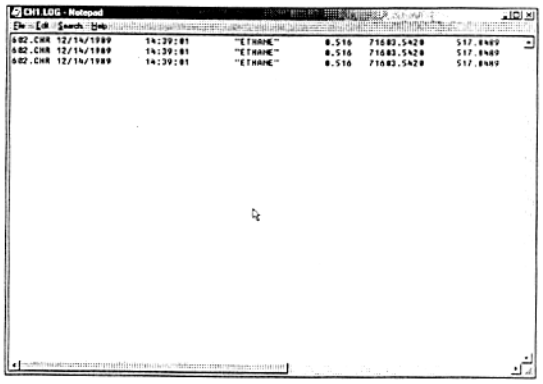
The Copy results log button copies the .log file for the results to the Clipboard. This log file can be pasted into any Windows program. A certain number of lines in the results log will always be copied, by default the number is 20. If more than 20 lines are needed for an application the user must modify the peakwin.ini file located in the Windows folder. The default entry in the file is (SpareLines=20), delete the number 20 and insert the number of lines that are needed (up to a maximum of 100).

Clear Results Log

Clicking on the Clear results log button erases the results log file.

Show Results Log

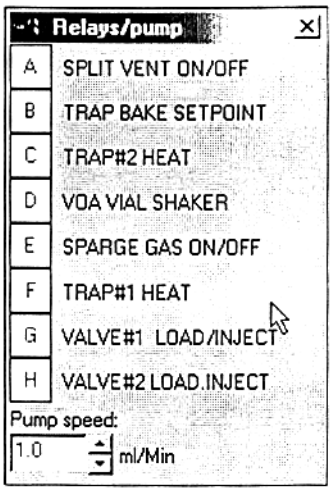
The Show results log button when selected opens up Windows Notepad to view the results log.



Add to Results Log

To add the current report to the results log click the Add to results log button. The report can automatically be added to the results log at the end of each chromatogram run by checking the Add to results log checkbox in the Postrun window.

The View-Relay/Pump Window



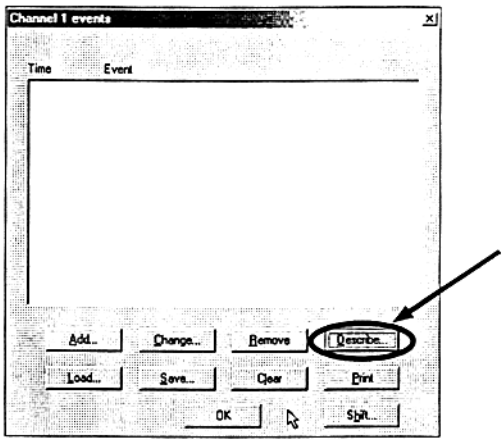
The Relay/pump window manually controls the actions of the relays in PeakSimple. The Relay/pump window is opened up by opening the View menu and then selecting Relay/pump window from the list of options.

Selecting/Deselecting a Relay

To manually activate a relay click on the letter next to the relay label to make the button dark. To deactivate a relay select the specified lettered button to turn it black. Pressing the control button and the letter corresponding to the relay together also selects/deselects the relay.

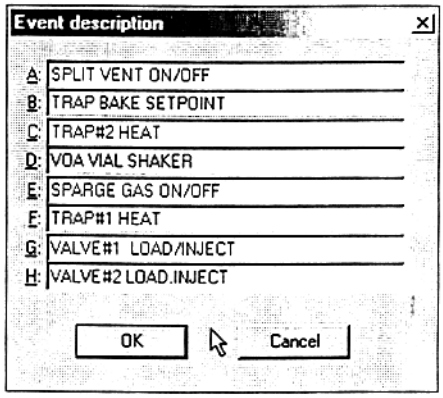
Pump Speed

When an SRI HPLC pump is connected to the data system the pump speed can be controlled in the Relay/pump window. To change the pump speed click on the arrow icons to increase or decrease the pump speed. The pump speed can also be entered by highlighting the value in the box and typing in the new number.

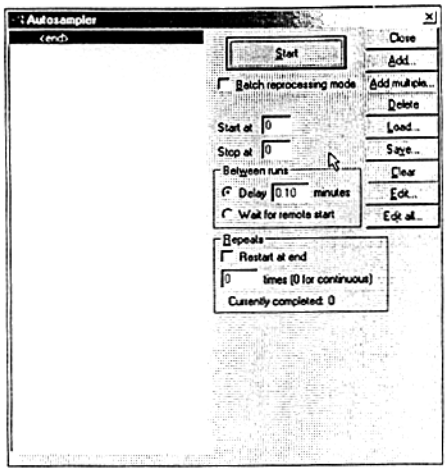


Describing a Relay

To label a relay in the Relay/pump window right click on the main screen and select Events from the list of options. Once the Events window is opened up clicking on the Describe button opens up the Event description window. To enter a relay description click on the specified relay's dialogue box and type in the information. The description of the relays has no effect on the relay function and will not affect hardware.



The View-Autosampler Window



The Autosampler window allows a list of control files to be run automatically. Control files are the master files which specify all parameters including temperature programming, component, and event files. These control files run tasks in PeakSimple. To open up the Autosampler window click on the View menu in the menu bar and then select Autosampler from the available options.

Start/Stop

The Start button when pressed begins the operation of the autosampler queue or reprocessing queue. A queue must be created or loaded before the control files can run. Once the autosampler is in operation the Start button changes into the Stop button. The Stop button ceases the autosampler operations that were previously running.

Batch Reprocessing Mode

To select Batch reprocessing mode click on the check box to the options left. While using the Batch reprocessing mode the user loads a list of previously stored chromatogram files in the list box to the left and then selects a control file which will reprocess the data files. When the operation begins PeakSimple will load each data file in the list into channel 1, perform the specified functions, and then increment to the next data file in the list.

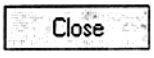
The **Start at** dialogue box specifies which control file number to begin operation at first. If no number is entered the autosampler will begin at the first control file. The **Stop at** dialogue box specifies the last control file to be run before operations of the autosampler cease. If no number is entered in the dialogue box the autosampler will end after the last control file in the list is run.

The **Delay "x" minutes** radio button when selected specifies how many minutes PeakSimple will wait before running the next control file in the list box. The **Wait for remote start** radio button when selected instructs the autosampler to wait for a remote start signal before advancing to the next control file.

The **Restart at end** checkbox restarts the queue after getting to the end of the control files in the list box. In the **"x" times** the user enters the number of times the control files in the list box should be cycled if the Restart at end checkbox is selected. If the value 0 is selected the queue will be cycled continuously.

Close

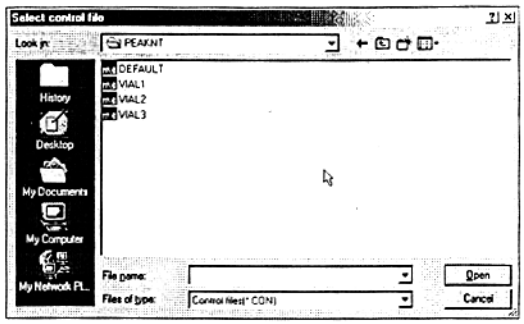
The Close button closes the Autosampler window when it is selected.



The View-Autosampler Window (cont.)

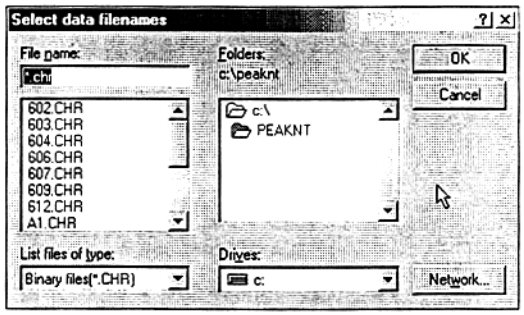
Add

Select the Add button to add a control file to the queue. Selecting the button opens up the Select control file window where the file can be loaded into the list box. Each control file in the queue must have a different name even though almost identical actions are performed.



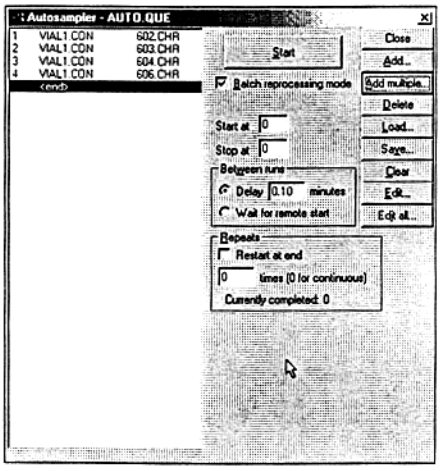
Add Multiple/Batch Reprocessing

The Add multiple button allows the user to load multiple data files into the list box. Click on the button to open up the Select control file window and then click on a control file name to open up the Select data filenames window. Select as many data files as needed by pressing the shift button and clicking with the mouse cursor and then click on OK to load them into the queue. The Add multiple button is only useful for use with the Batch reprocessing mode.



Delete

After highlighting a control file in the list box to the left select the Delete button to remove that control file from the queue.

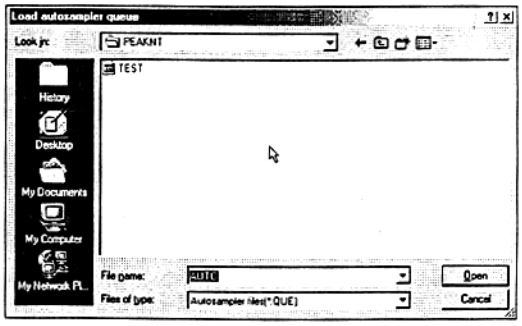


Load

Select the Load button to open up a previously saved queue file. Clicking on the Load button opens up the Load autosampler queue window where the queue file can be selected and loaded.

Save

Selecting the Save button opens up the Save autosampler queue window. Save the queue in the file box by naming the file and selecting save. It is recommended that all files be saved to the PeakSimple directory.



Clear

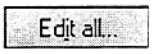
The Clear button erases the entire queue.

The View-Autosampler Window (cont.)

Edit

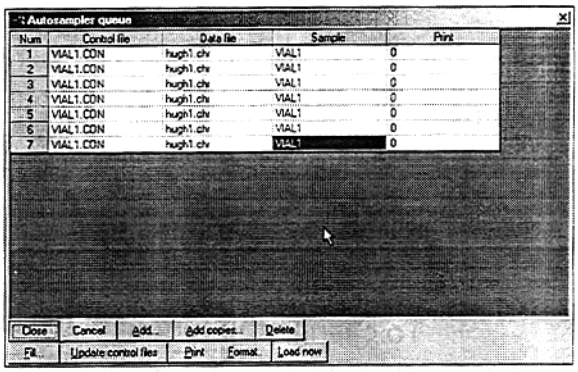
After highlighting a control file select the Edit button to modify that control file. Selecting the Edit button loads the control file on the PeakSimple main screen. To make any changes click on the main screen, do all modifications, and then select Save all from the PeakSimple file menu.

Edit All

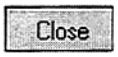


To edit all the control files in the queue at once click on the Edit all button to open up the Autosampler queue spreadsheet. Many of the commonly adjusted control file parameters are displayed in the spreadsheet enabling the user to input changes to the queue. Not all control file parameters can be modified using Edit all (only the parameters that are selected in Format) and so must be done individually with the Edit function.

Autosampler Queue Window



Close



The Close button exits the window after prompting the user to save the spreadsheet.

Cancel

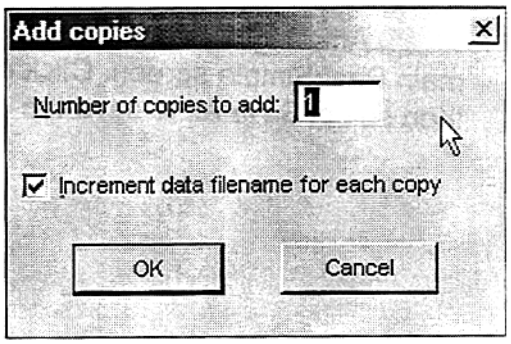
The Cancel button exits the spreadsheet window without prompting the user to save.

Add

Selecting the Add button opens up the Select control file window where an existing control file can be added to the queue.

Add Copies

After highlighting a control file in the spreadsheet select the Add copies button to add copies of the file to the list. Once the Add copies window pops up input the number of copies to be made in the dialogue box and specify whether the file names should be incremented. The Add copies button is useful for creating a queue from scratch with a single control file.

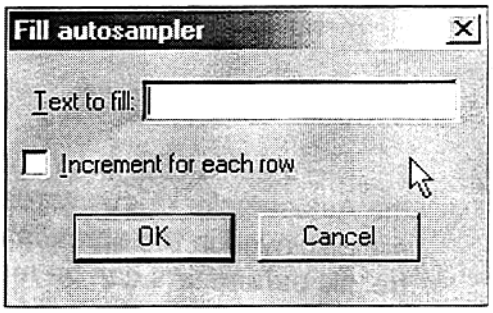


Autosampler Queue Window (cont.)

Delete

The Delete button deletes a highlighted control file off the list. If no file is highlighted then the last file will be deleted from the queue.

Fill



The Fill button fills a spreadsheet column, row, or cell with selected text. Once the desired cells are highlighted clicking the Fill button opens up the Fill autosampler options box. Input the text to fill in the information field and specify whether the text should be incremented for each row.

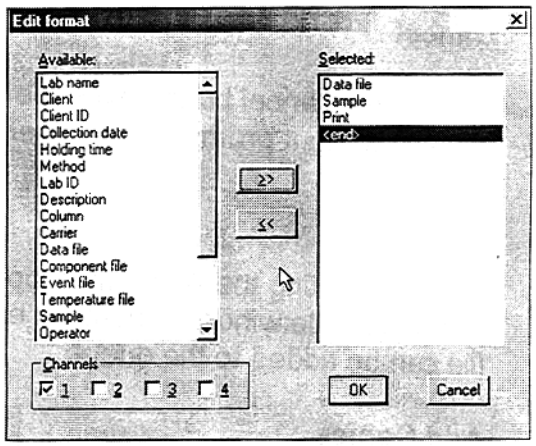
Update Control Files

Selecting the Update control files button saves all changes to the control files in the list.

Print

The Print button prints the queue spreadsheet.

Format



To change the format of the queue spreadsheet and open up the Edit format window select the Format button. In the Edit format window a format type can be added by selecting it in the Available window and then hitting the right facing arrow button. To remove a format type from being displayed in the spreadsheet highlight the format type in the Selected box and click on the left facing arrow.

Load Now

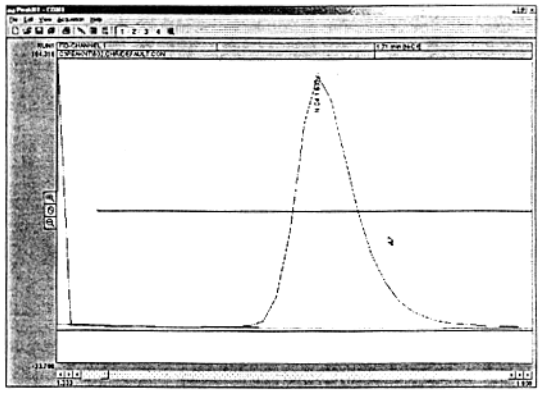
After highlighting a control file select the Load now button to load that control file to the main PeakSimple screen. Click on the screen and make any changes to the control file and then select Save all to save the changes.

The View-Channel "X" Options

To view a specified chromatogram channel open the View menu in the PeakSimple menubar and select a channel to be viewed; either 1, 2, 3, or 4. Keyboard shortcuts can also be used to alternate viewing between chromatogram channels. Hitting F9 displays channel 1, F10 displays channel 2, F11 displays channel 3, and F12 displays channel 4. Furthermore the numerical icons in the PeakSimple toolbar can be used to toggle between chromatogram channels.

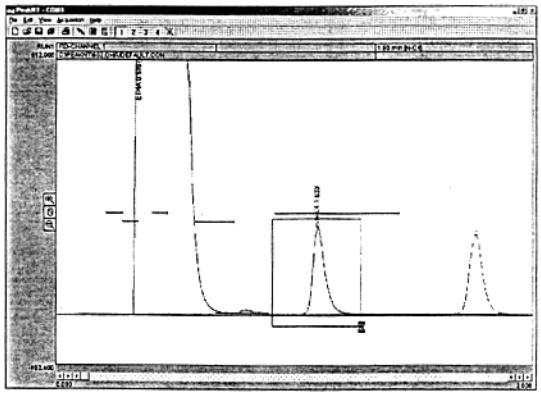
Unzoom

To unzoom from a close up view of a chromatogram select the Unzoom tool from the View menu or hit F6. PeakSimple will zoom out to the first level with the soriginal display units of the chromatogram when the Unzoom tool is used. The Unzoom button in the PeakSimple toolbar can also be used to unzoom a chromatogram or F6 on the keyboard.



Refresh

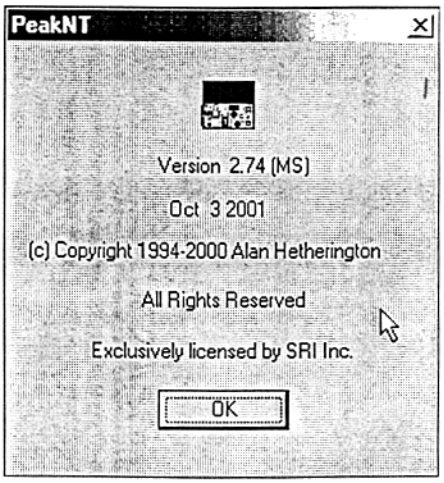
The Refresh tool in the View menu redraws the chromatogram screen to fix any glitches or resolve an error. Pressing Enter on the keyboard also refreshes the screen.



The Help Menu

About PeakNT

To view program information about PeakSimple click on the About PeakNT option in the Help menu. The PeakNT window will pop up and display the information.



Show Tooltips

The Show tooltips option in the Help menu toggles the PeakSimple tooltips off or on. When Show tooltips is checked a helpful text tip will appear when the mouse cursor is held over a tool or button in PeakSimple. The tooltips provide relevant information to the operation and use of the PeakSimple data system.

The Acquisition Menu

The Acquisition menu contains the commands to run a chromatogram run when hardware is connected to the PeakSimple data system. All Acquisition menu commands have corresponding keyboard hotkeys for convenience.

Run

The Run command begins a chromatogram run on the main trigger group when hardware is connected to the data system. The error message "No active channels in group" appears when no hardware is available to make a chromatograph run. The spacebar can also be used to start a run.

Stop

The Stop command is used to end a chromatogram run once it has been started. Using the Stop command ends the chromatogram run without running any of the Postrun operations. The End button can also stop a chromatogram run.

Stop+Postrun

The Stop+Postrun command ends a chromatogram run and executes the operations specified in the Postrun screen. Holding the Control button and pressing End on the keyboard is the same as the Stop+Postrun command.

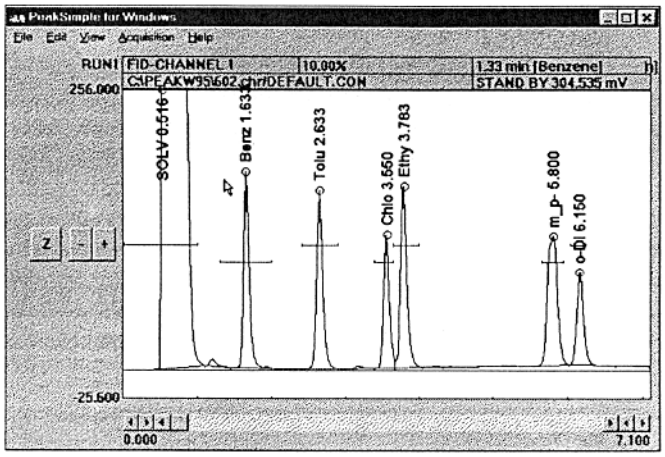
Alt

The Alt menu in the Acquisition menu controls the acquisition commands for the alternate trigger group. The + button begins the alternate trigger chromatogram run, the - button stops the alternate trigger run, and the / button on the keyboard stops the run and begins the Postrun operations for the alternate trigger group.

Re-initialize

The Re-initialize command reestablishes the connection between the hardware and the PeakSimple data system. A connection between hardware and the data system has to exist for re-initialization to occur.

PeakSimple for Windows Software and Chromatography Data System Validation Statement



February 1, 1999

1) PeakSimple for Windows software and Chromatography Data System (PeakSimple) is written, manufactured and maintained by SRI Instruments, Inc. a Nevada Corporation.

2) PeakSimple for Windows software has been under continuous development since 1994. Periodic testing of the software is performed by SRI employees.

3) PeakSimple software is designed to be self-validating to enable quick verification by customers that PeakSimple is functioning consistently, reliably and according to specifications under actual operating conditions.

4) Self-validation is performed by configuring PeakSimple into the "Loopback mode" (see loopback instructions in manual). In this mode, an actual user generated chromatogram which is loaded into channel 4 is re-played (like a tape recorder) through the TP2 output channel, and then re-acquired and processed through any one of the remaining input channels. This is done 7 or more times to insure that data is being processed consistently and reliably.
The results from multiple loopback analyses are used to calculate the percent relative standard deviation (precision) of each peak in the chromatogram. Chromatographic data is highly variable, and the precision obtained is dependent on many factors including the peak shape, signal to noise ratio, interferences, co-eluting peaks, data acquistion rate and customer selected integration parameters. For this reason, self-validation is more valid than factory validation, since self-validation takes into account the exact chromatographic conditions and user specified parameters in effect for the particular application whereas factory validation can not.

Loopback Test: For Data Validation

A loopback test may be performed if you are required to validate the precision of the G.C. or Data System's analog to digital conversion. This test requires the user to install a jumper wire on the A/D board inside the G.C. or Data System.

Description of Test:

A jumper wire is installed on the A/D board between 'temperature program one', (TP1), and 'channel one signal input', (Sig. 1+). A data file is then loaded into channel four. When the 'loopback' mode is selected in PeakSimple, the data on channel four is routed out TP1 to the channel one signal input. When a chromatogram run is started, channel one will begin to reproduce the data loaded into channel four. After the run has completed, area counts from a specific data peak may be collected and the run repeated several times. After at least three runs, the user may then calculate the average area counts and the percent relative standard deviation,(%RSD) and thus the precision of the A/D converter. Less than 0.5% RSD is typical for the SRI Model 202 and 203 A/D boards.

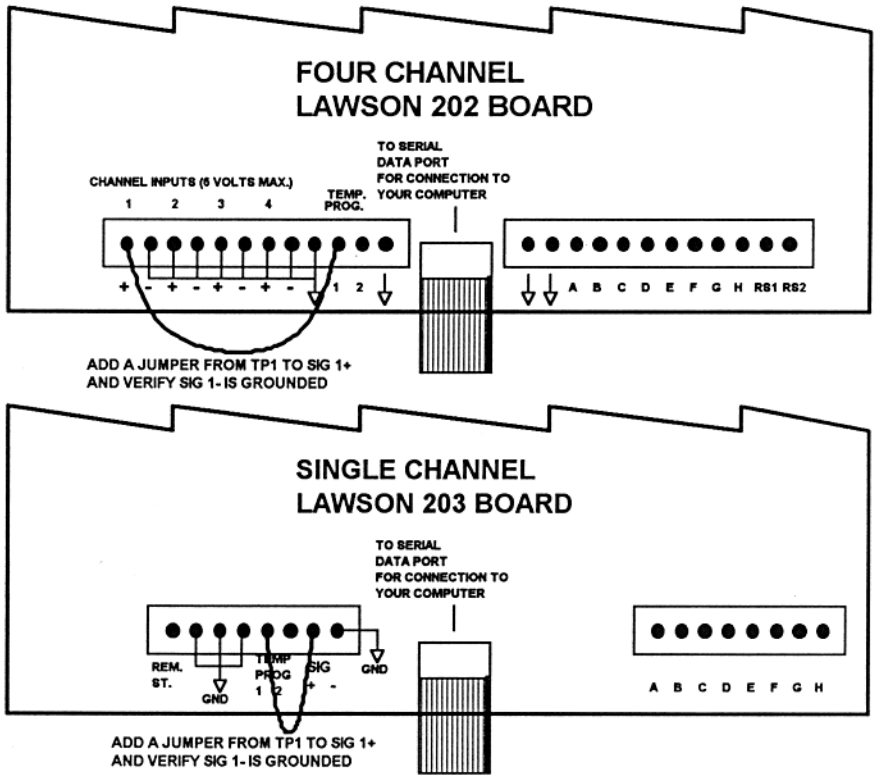
Setting Up The Hardware:

With the G.C. unplugged, remove the six screws securing the bottom cover. Flip the G.C. on its back and locate the A/D board on the right-hand side. Remove any wires from 'TP1' and 'SIG 1+' and add an insulated 22 AWG wire between TP1 and SIG 1.
Refer to the diagram below for jumper placement.

Most systems will contain the Four Channel 202 Board. Also, verify that SIG 1- is grounded. Add another jumper if needed.

Some systems will contain the Single Channel 203 Board. Also, verify that SIG 1- is grounded. Add another jumper if needed.

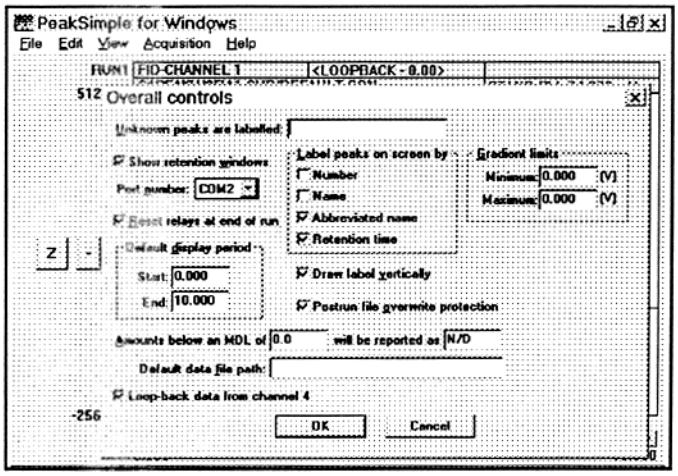
You could also run the TP1 wire to SIG 1+ through a relay for automatic hardware setup.



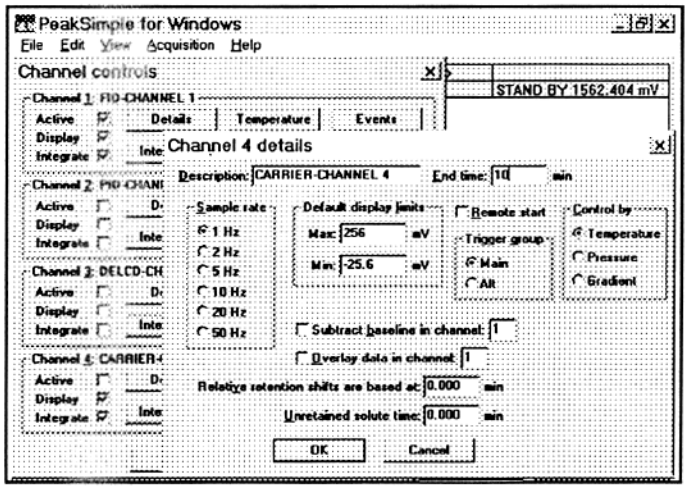
Loopback Test: (continued)

Setting Up The Software:

Re-attach the bottom cover and plug the G.C. back in. Turn the G.C. power on and start PeakSimple. Verify that the computer is communicating properly with the G.C..

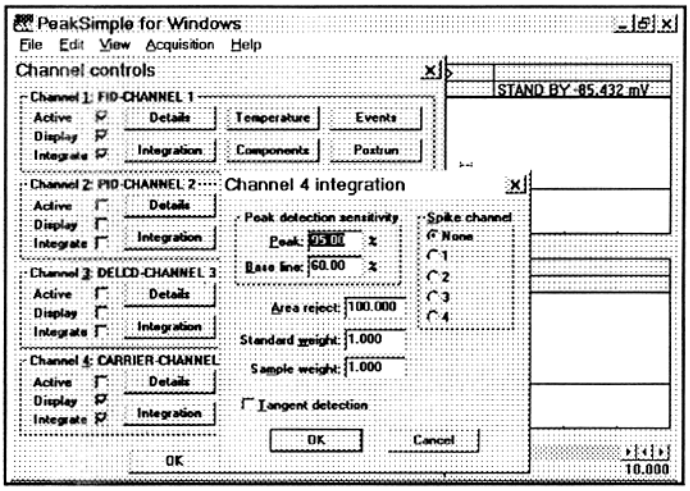


In the **EDIT-OVERALL** screen, check the **LOOPBACK** box. Set the **START TIME** to 0 minutes and the **END TIME** to 10 minutes. Also verify that the **SHOW RETENTION WINDOWS** box is checked.



In the **EDIT-CHANNELS** screen, check the **ACTIVE, DISPLAY** and **INTEGRATE** boxes for channel 1. And check the **DISPLAY** and **INTEGRATE** boxes for channel 4.

In the **EDIT-CHANNEL 1-DETAILS** screen, set the **END TIME** to **10 min.** Set the **SAMPLE RATE** to **1**. Set the **DEFAULT DISPLAY LIMITS** to **256 MAX** and **-25.6 MIN.** Set the **TRIGGER GROUP** to **MAIN**. Click **OK** to close the **DETAILS** screen. Then repeat for **CHANNEL 4-DETAILS**.

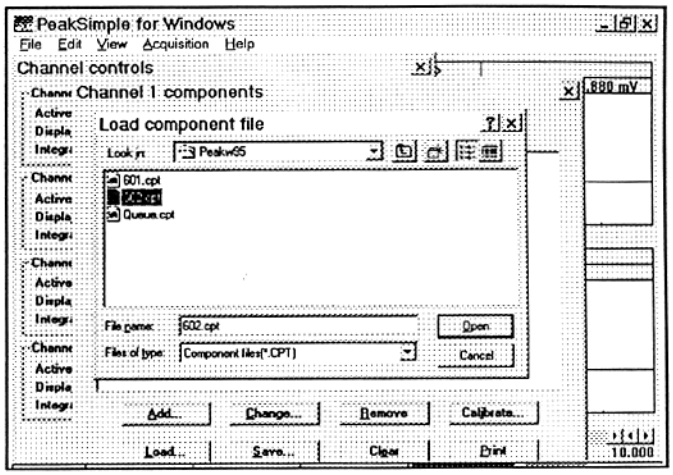


In the **EDIT-CHANNEL 1-INTEGRATION** screen, set the **AREA REJECT** to **100**. Set the **PEAK DETECTION SENSITIVITY** to **'PEAK=95%, BASELINE= 60%'**. Set the **SPIKE CHANNEL=NONE, STANDARD WEIGHT=1, SAMPLE WEIGHT=1,** and make sure the **TANGENT DETECTION BOX** is **UNCHECKED**. Click **OK** to close the **INTEGRATION** screen. Then repeat for **CHANNEL 4-INTEGRATION**.

Loopback Test: (continued)

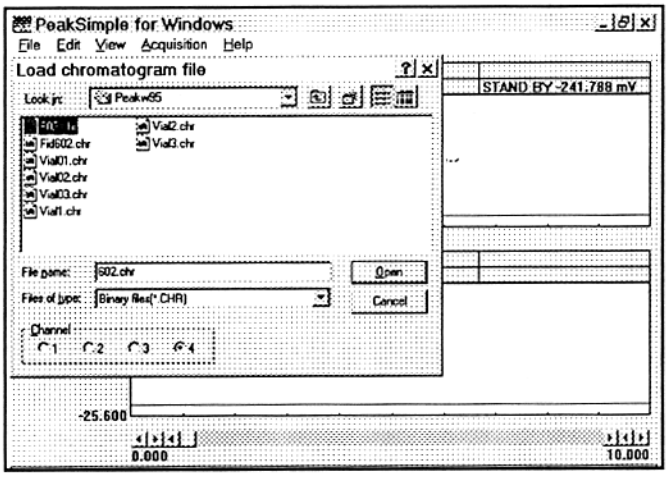
Software Setup: (continued)

In the **EDIT-CHANNEL 1-COMPONENTS-LOAD** screen, highlight the **602.cpt** sample components file and click **OPEN**. Click **OK** again to close the **COMPONENTS** screen. Then repeat for **CHANNEL 4-COMPONENTS**.



In the **FILE-OPEN** screen, select **CHANNEL 4** at the bottom of the window and then highlight the **602.chr** sample chromatogram file and select **OPEN**.

The 602.chr sample chromatogram that is now displayed on channel four represents the data that will be fed back through the A/D converter.



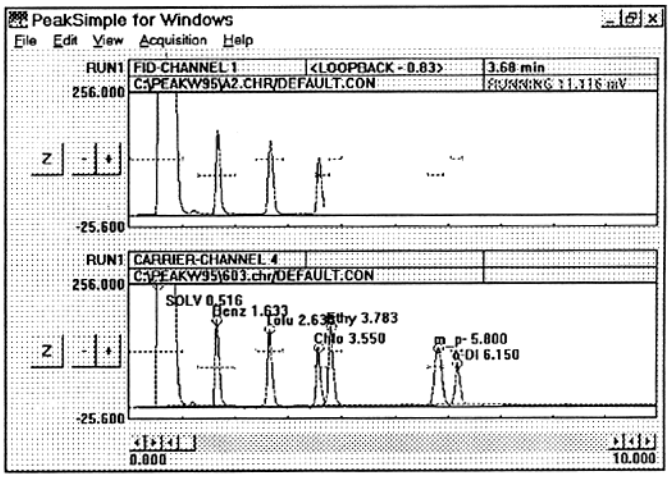
Starting the Run:

Auto-zero channel 1 by clicking the **'Z'** button. Depress the **SPACEBAR** and the chromatogram will start running. The data on **CHANNEL 1** should appear to be an exact replica of the data that was fed into **CHANNEL 4.**

Collecting the Data:

After the run has completed, make note of the area counts of one of the peaks by left-clicking on one of the peaks. Toluene, for example, may have an area count of 931.

Repeat the run three or more times; for each run, record the area counts of the same peak. Once the data has been collected from at least three runs; an average area count can be calculated as well as the percent relative standard deviation.



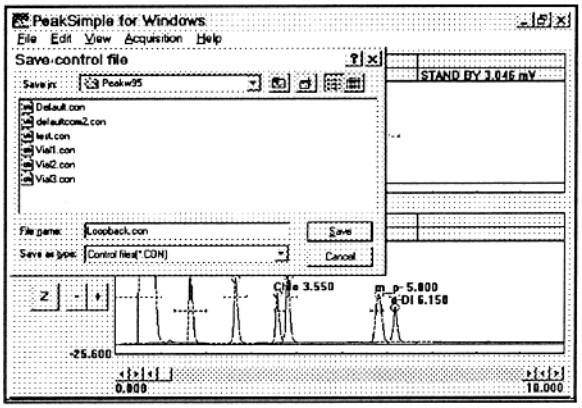
Loopback Test: (continued)

Calculate the Standard Deviation:

Using the data collected, calculate the average area counts for Toluene. Typically this value is around 950. Then calculate the %RSD which is usually less than 0.5%. You may notice that there is a small magnitude of error between the **CHANNEL 1** and **CHANNEL 4** area counts. This is due to the D/A converter and not the A/D converter. Since the loopback test measures the **PRECISION** of the A/D converter and not its **ACCURACY**, this minor discrepancy is insignificant.

Save Your Loopback Test as a CONTROL FILE:

If you wish to run this test again or if you continue with the next step and modify the **Peakwin.ini** file, you will need to save the loopback parameters as a **CONTROL FILE.** In the **FILE-SAVE CONTROL FILE** screen, type in **loopback.con** and click **OK.**



Modifying the Peakwin.ini File, (optional)

If desired, any inaccuracy of the D/A converter can be adjusted by attenuating the **LOOPBACK OUTPUT** to match the input signal. This adjustment can be made by entering the line **"LoopbackFactor=X"** in the [Lawson] section of the **PEAKWIN.INI** file located in the **WINDOWS** directory. The default value of **'X'** is **0.098**.

NOTE: Changes to the Peakwin.ini file will not be recognized unless the PeakSimple application is restarted. After you have obtained the average area count for Toluene on CHANNEL 1, exit PeakSimple by pressing 'Q', then 'Y'.

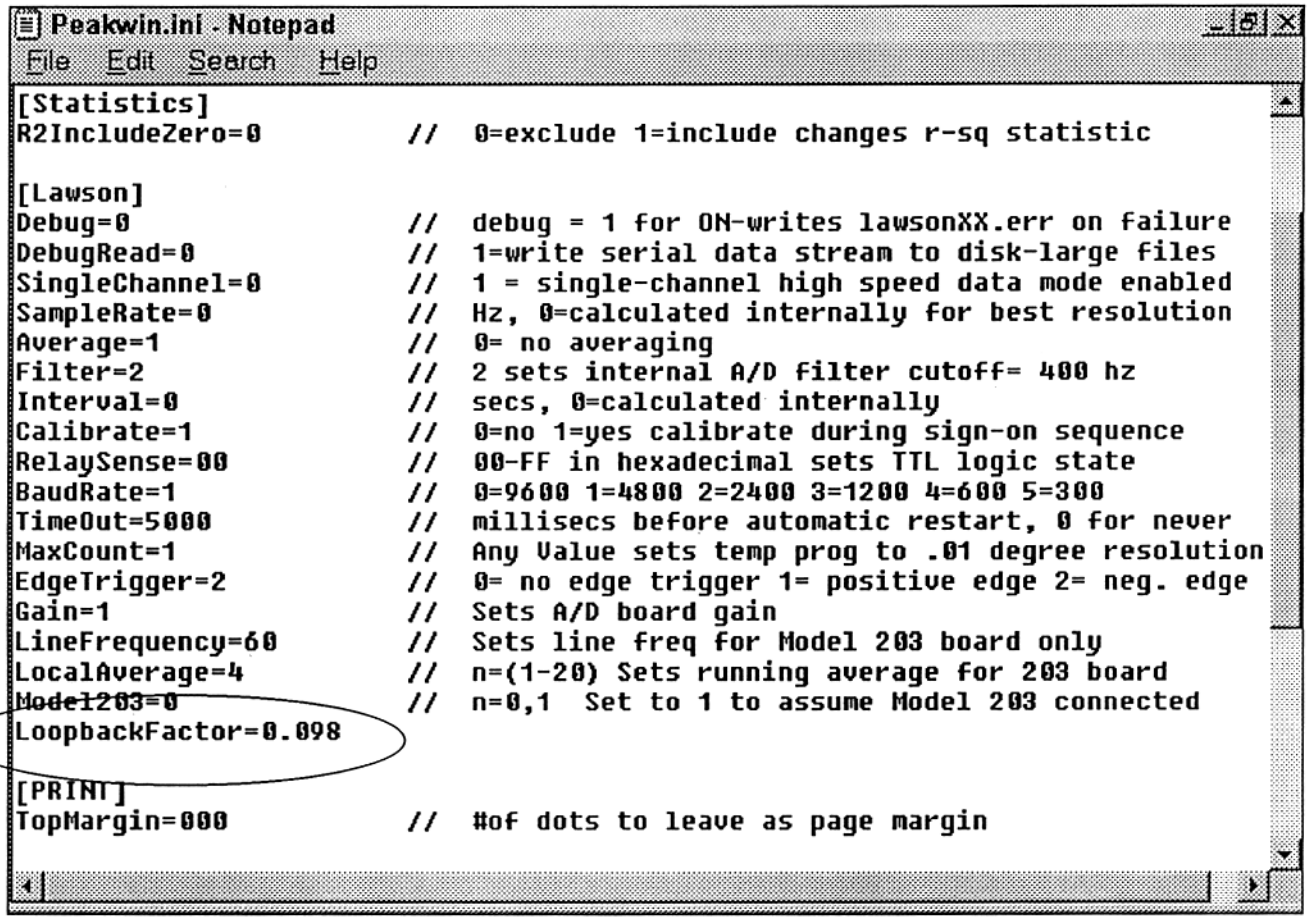
To calculate the new value for **'X'**, first determine the average area counts of a specific peak for **CHANNEL 1** and also determine the area count of the corresponding peak on **CHANNEL 4.** Next, divide the **CHANNEL 4** area count by the **CHANNEL 1** area count. Multiply this ratio by 0.098. Substitute this new value for **'X'**.

For example, using 602.chr as the chromatogram file, the toluene area count for **CHANNEL 4** is **953**. If the average area count of toluene on **CHANNEL 1** is **931** then the ratio would be **953 / 931 = 1.0236**. Multiply **1.0236 x 0.098**. the answer is **0.1003128**. Round this new value for **'X'** to 0.1003.

Loopback Test: (continued)

Modifying the Peakwin.ini File, (continued)

Find the **PEAKWIN.INI** file in the **WINDOWS** sub-directory. Double-click to open it. Scroll down until you find the [Lawson] section. Place the cursor at the last line of the [Lawson] section and type **"LoopbackFactor=X"**, and then press **ENTER**.
X is the value you calculated earlier. For example **"LoopbackFactor=0.1003"**.



Press **ALT-F** then **S** to save the file. Press **ALT-F** then **X** to exit. Restart **PeakSimple** and load the loopback.con control file you saved earlier. Run the loopback test again. The accuracy of the D/A converter should be improved. (Channel 1 toluene area counts should closely match the channel 4 toluene area counts).

End of Test:

Turn off G.C. power and re-connect the original A/D board wiring. The loopback test is completed.

Chapter: PeakSimple

Topic: Using the Windows Scheduler program to trigger PeakSimple's Autosampler queue

The Windows Task Scheduler program is supplied with the Windows operating system. It is found under Programs/Accessories/System Tools. The Scheduler allows you to trigger a PeaKSimple Autosampler Queue or a specific control file at a scheduled time and date, or on a regular repeating basis using the computer's system clock (time/date).

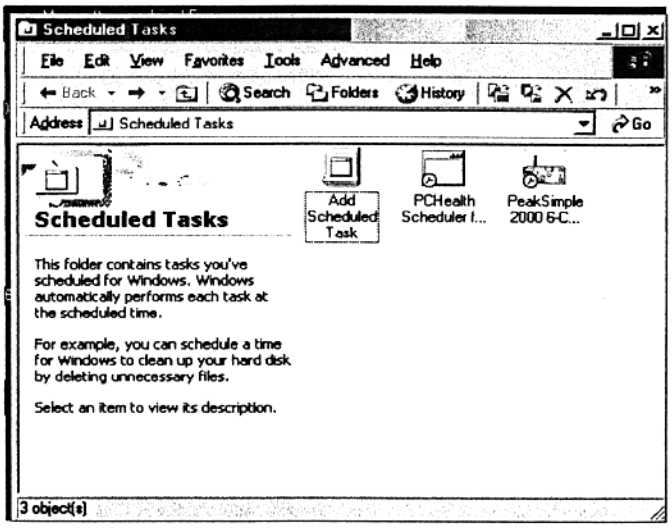
When you click on the Add Scheduled Task icon in the Scheduler program, a Wizard will guide you through the process. When you get to the screen where you specify which program to start, enter the following line modified for your particular situation:
c:\peak2000\peak2000.exe -atest.que

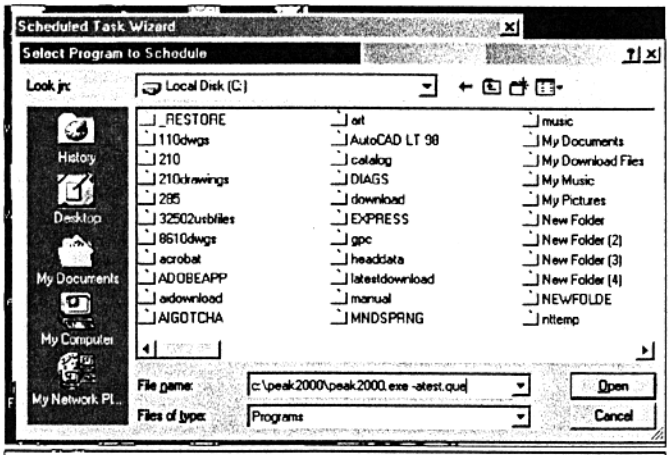
C:\peak2000 is the folder or directory where the PeakSimple software has been installed. If you have installed PeakSimple in a different folder, substitute the name of your PeakSimple folder.

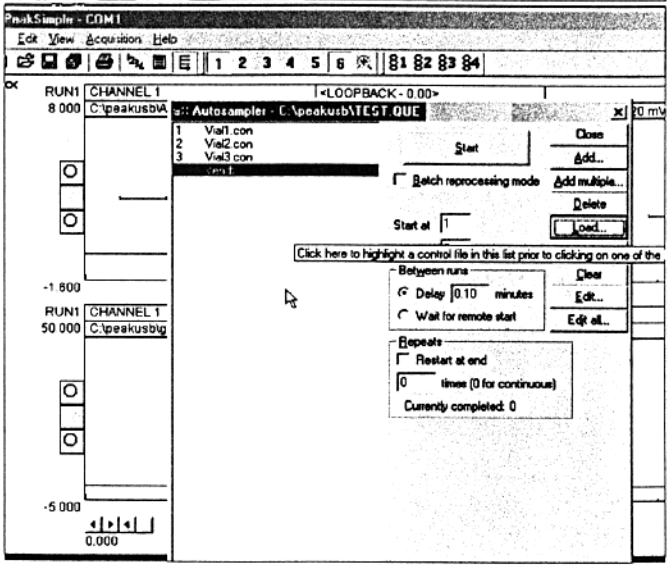
peak2000.exe is the name of the actual PeakSimple software program. If you have installed PeakSimple under a different name (later versions of PeakSimple may in fact have a different name) use the name of the PeakSimple program as it exists on your computer.

test.que is the name of the Autosampler queue file which you must have previously created in PeakSimple. Note that the -a must precede the name of the .que file. When you create the .que file in PeakSimple you can save the que under any name you want. The -a is a Windows programming convention and must precede the name of the que file you want to run.

When the Scheduler starts PeakSimple, the specified queue will begin. At the end of the queue, PeakSimple will wait for the delay time specified in the queue, and then PeakSimple will Close automatically.



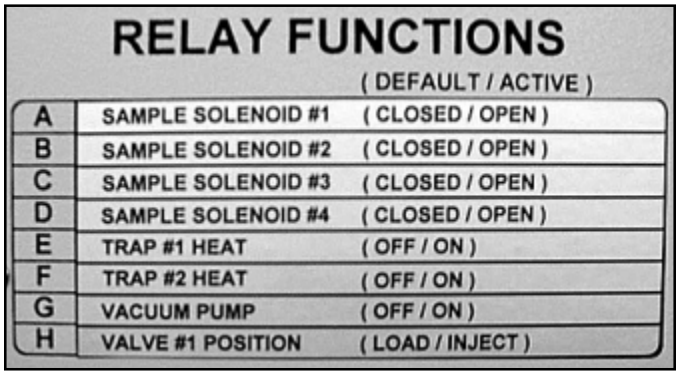




Events

PeakSimple gives you control of up to eight independent external events, or hardware events. A hardware event is the operation of a device external to the data system but integral to the analytical run. A hardware event can be a valve rotation, opening or closing a split vent, activation of an autosampler sequence, or operation of an electrical switch at a precise moment during the run. PeakSimple also gives you control over a comprehensive list of non-hardware events, such as integration events, data system signal control events (zero, reverse), and DOS command events. Events are controlled automatically with **Event tables** that use the system clock, which starts at 0:00 with each run. Using PeakSimple **Event tables** enhances the reproducibility of the resulting chromatograms by ensuring repeatable actuation of devices from run to run.

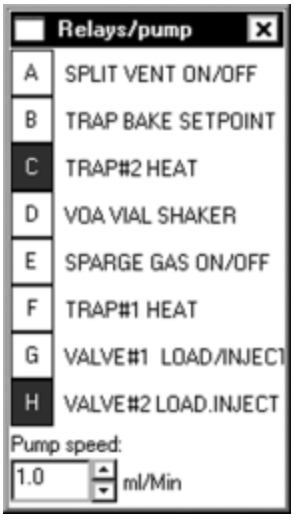
The eight timed event output signals are called relays, and are named A-H. For example, when an SRI GC is equipped with a 10-port gas sampling valve, its rotation/actuation is controlled by a relay: relay OFF = valve in the LOAD position; relay ON = valve in the INJECT position. The relay assignments for any given instrument are printed on the side panel (right hand side for GCs, left-hand side for HPLCs).



RELAY FUNCTIONS

		(DEFAULT / ACTIVE)
A	SAMPLE SOLENOID #1	(CLOSED / OPEN)
B	SAMPLE SOLENOID #2	(CLOSED / OPEN)
C	SAMPLE SOLENOID #3	(CLOSED / OPEN)
D	SAMPLE SOLENOID #4	(CLOSED / OPEN)
E	TRAP #1 HEAT	(OFF / ON)
F	TRAP #2 HEAT	(OFF / ON)
G	VACUUM PUMP	(OFF / ON)
H	VALVE #1 POSITION	(LOAD / INJECT)

This list of assigned relay functions is printed on the side of a TO-14 GC customized with 4 sample solenoid valves.



Users may manually control any relay event, either during the run or while in stand-by mode, by using the **Relays/pump** window. Click on View and choose **Relay/pump window**. In this window are eight buttons representing the relays with the appropriate letter. Activate a relay by clicking on its letter; it becomes highlighted to show its ON status. You can also toggle the relays from the keyboard by holding the control key (Ctrl) while pressing the letter of the relay: Ctrl+C, Ctrl+H, etc.