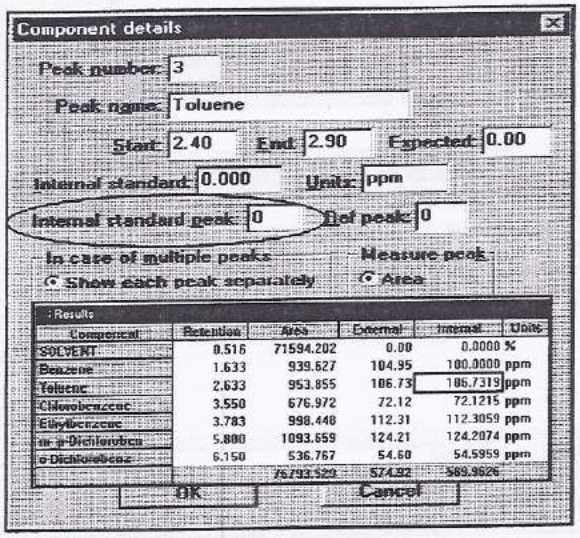
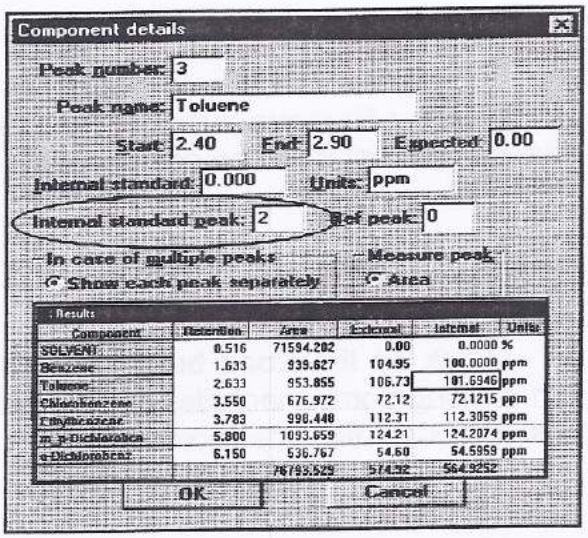
The EDIT-CHANNELS-COMPONENTS-DETAILS Screen (continued)

Internal Standard Peak

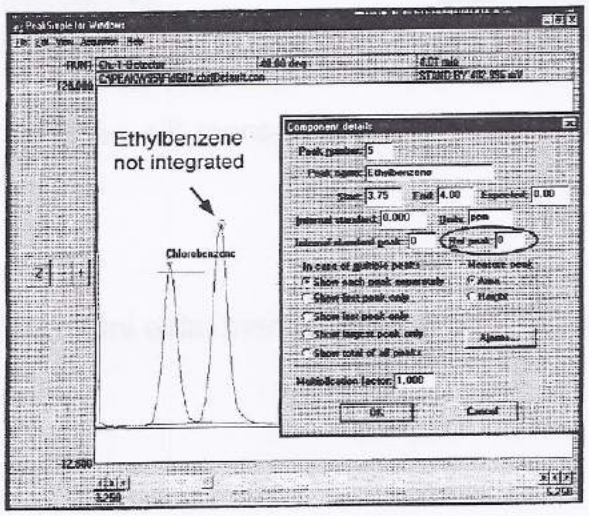
PeakSimple allows any peak to be referenced to any other peak for internal standard calculations. Typically all analyte peaks will be referenced against a single **Internal Standard Peak** (Benzene [peak #2] in the example shown below). To reference other peaks to Benzene, the number **2** must be entered 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.

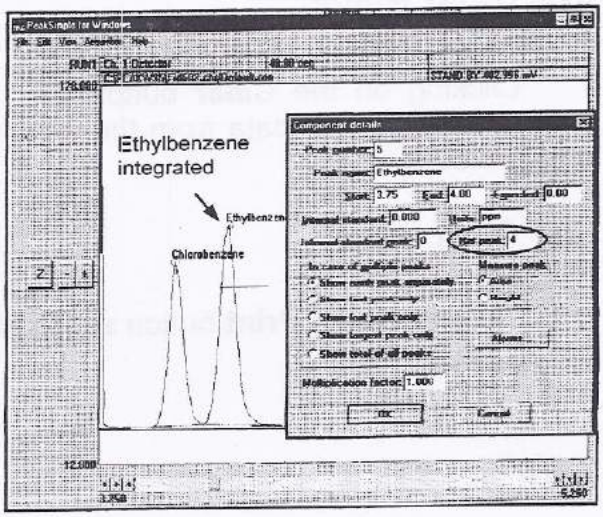




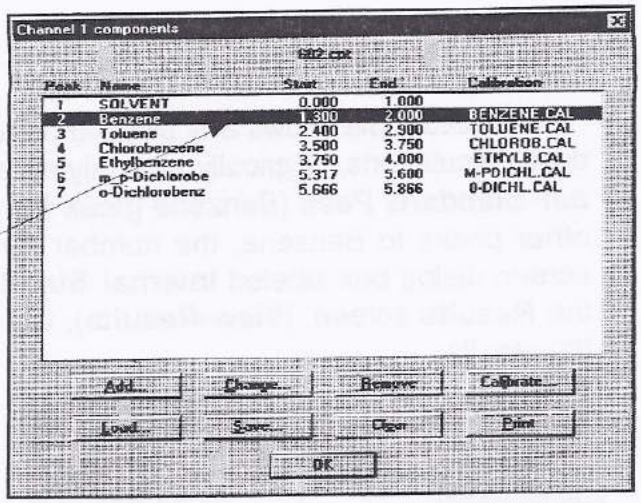
Reference Peak

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)*

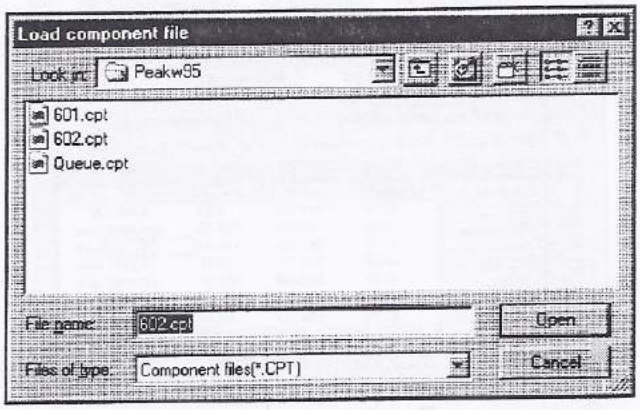


The **Change** Button

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.

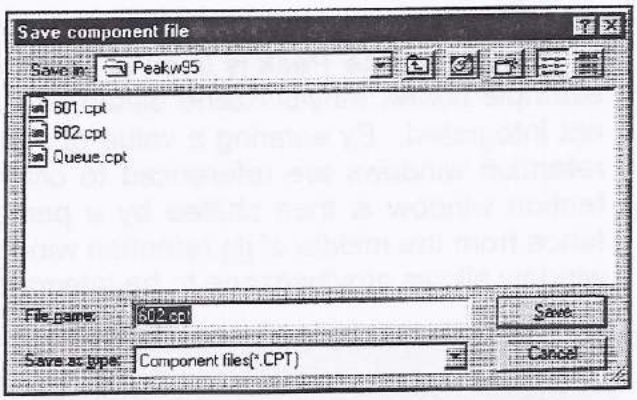


The **Load** Button

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)

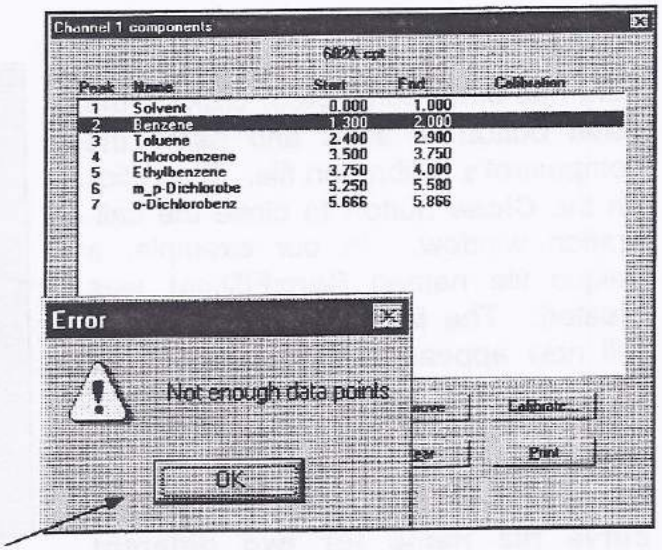
The Calibrate Button

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.

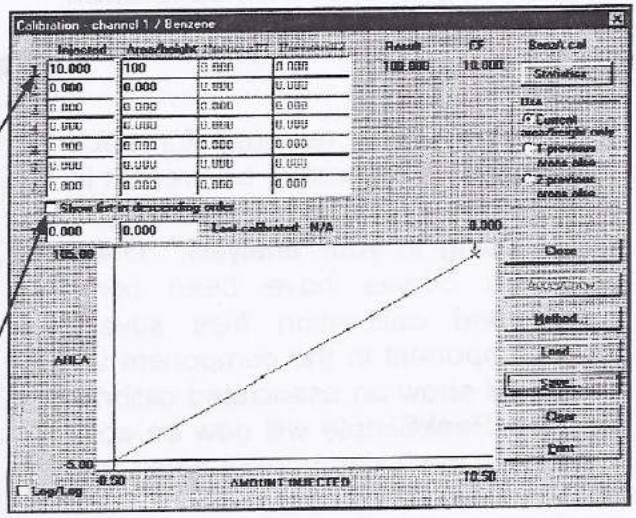
Inject a standard containing a known concentration of the component you wish to calibrate. Use a concentration higher than what you would expect to encounter in your analyses. Another few samples should be run at lower levels, using precise dilutions of your standard. Make note of the area counts or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

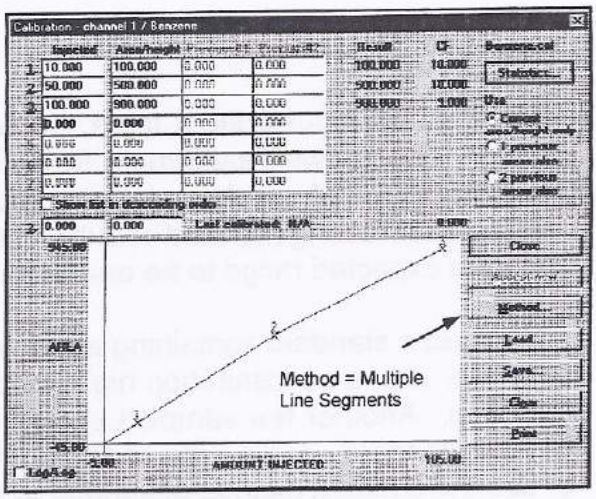


The **Calibration** window will open and allow you to enter the raw data that you previously obtained. In the example shown, data is entered into the table in the upper left corner of the calibration screen, beginning with the lowest concentration and ending with the highest concentration. If you wish 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.

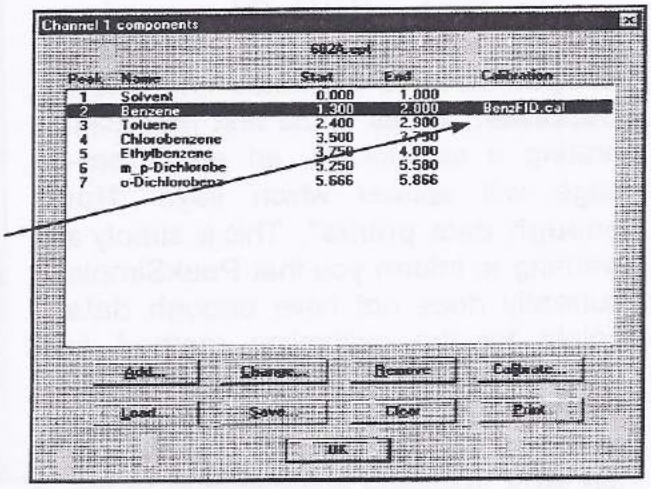


The Calibrate Button (continued)

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.

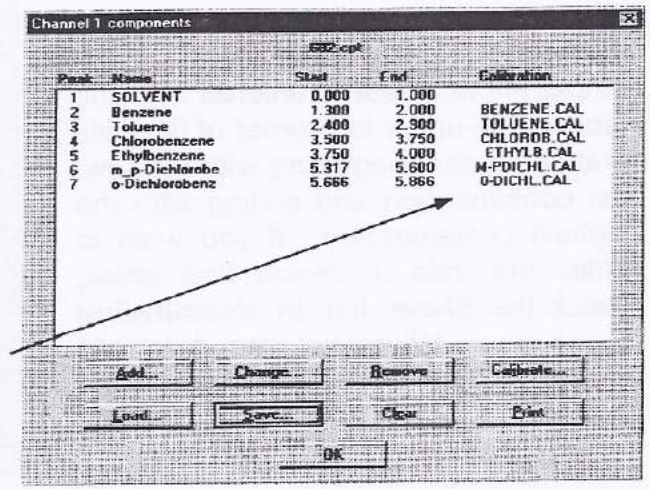


When calibration for each component has been completed, click on the **Save** button to save and name the component's calibration file. Then click on the **Close** button to close the calibration window. In our example, a unique file named **BenzFID.cal** was created. The **BenzFID.cal** file name will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

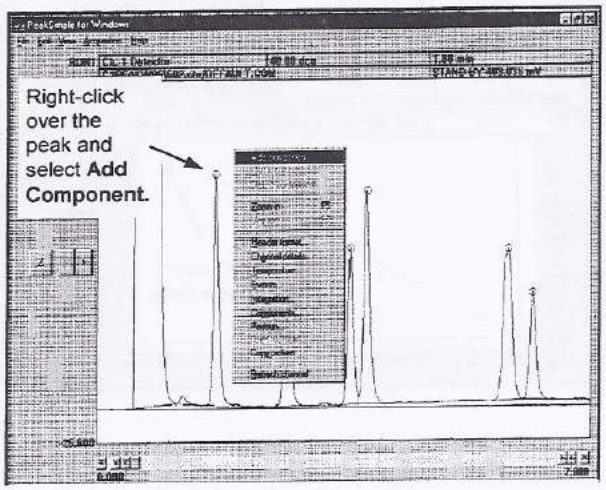


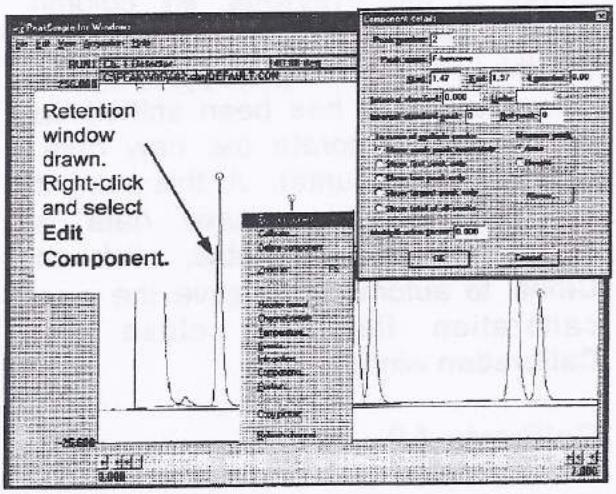
Calibration Screen Shortcuts

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.

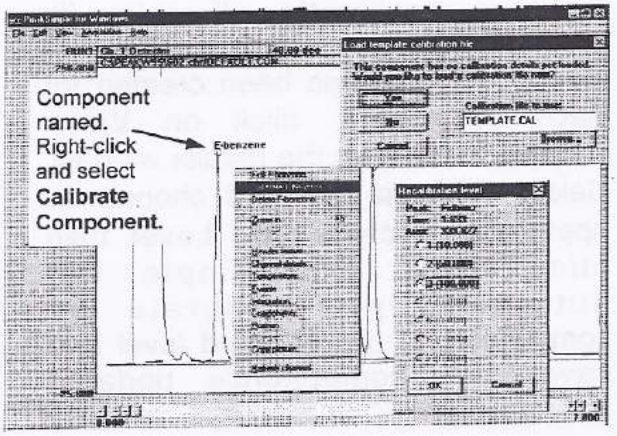
After a known standard has been run and the peaks have been identified, a new component table may be constructed by simply positioning the mouse pointer over a peak and clicking once on the right mouse button, ("right-clicking"). The shortcut menu will appear. Select **Add component** from the menu. 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.



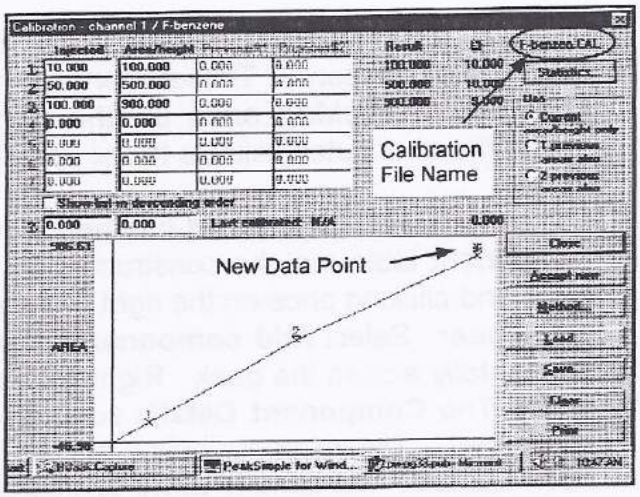


Right-click over the peak again and select **Calibrate**. If no calibration curve exists for the peak, a window will open asking if you would like to use a calibration file. PeakSimple offers a template calibration file aptly named TEMPLATE.CAL. Click yes to use the default TEMPLATE calibration file 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.

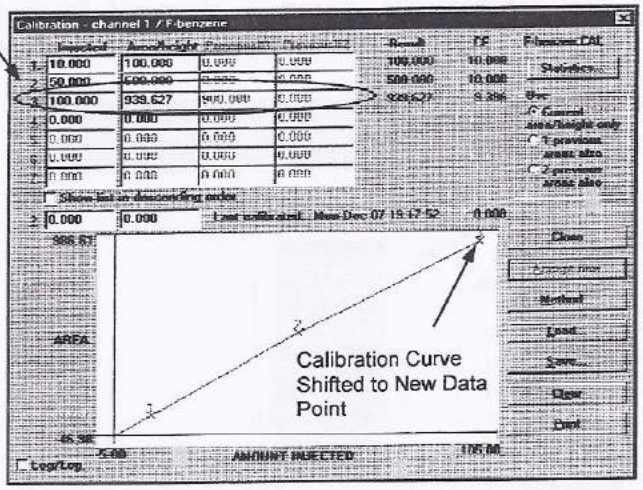


Calibration Screen Shortcuts (continued)

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.

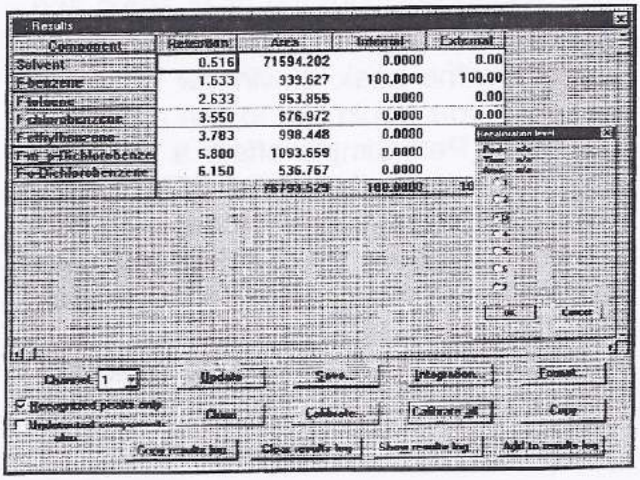


In the example to the right, the updated **F-benzen.CAL** calibration table reflects the new area count of **939.627** at the concentration level of **100** ppm. (The previous calibration data of 900 area counts at 100 ppm is shown in the **Previous #1** column which is 'grayed out'). Notice also that 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.

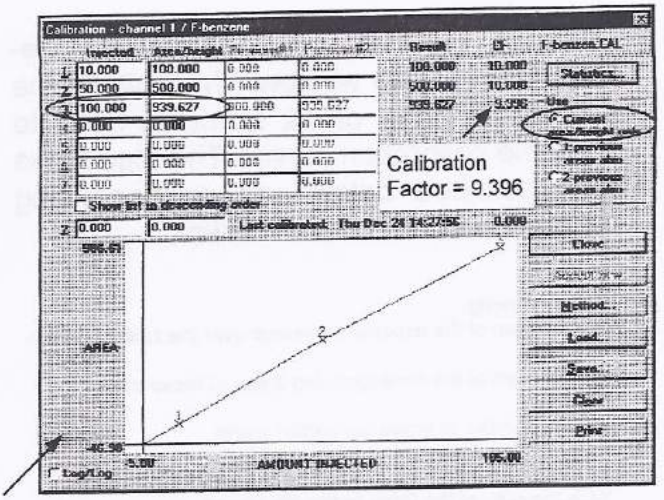


Calibrate All

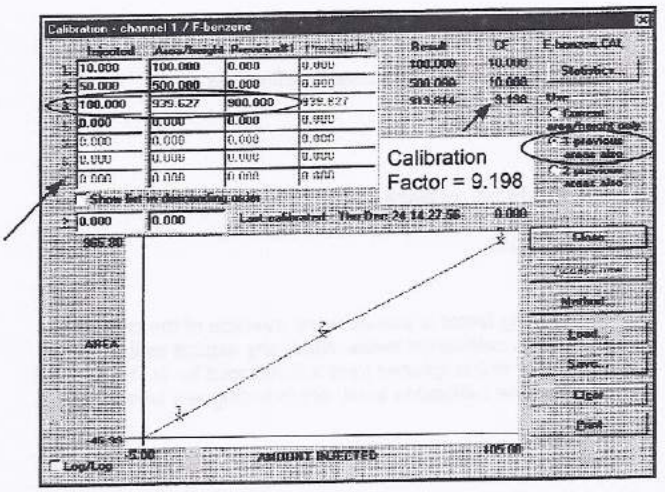
PeakSimple offers a time-saving feature for **recalibrating all peaks** with just one mouse click. After a calibration curve has been created for each component, click on **View-Results** to bring up the results window. Select **Calibrate All** and choose an appropriate **Recalibration Level**, then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



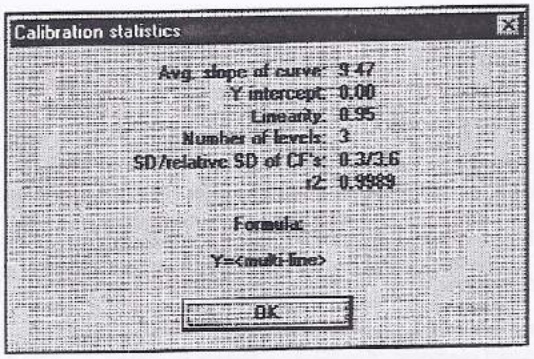
Calibration Screen – Use and Statistics Radial Buttons



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)

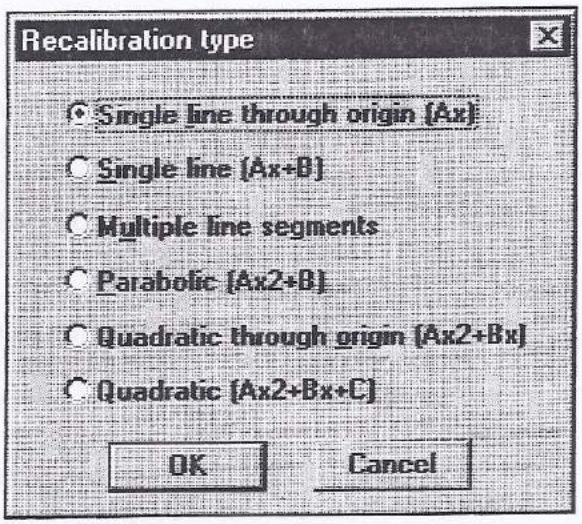


Setting **Use** to **2 Previous Areas Also** will average the last three areas to derive the calibration factor.

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.

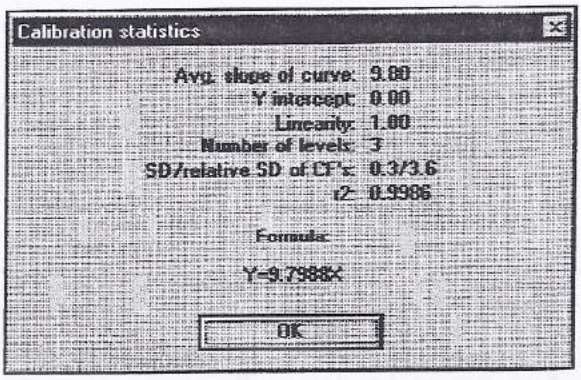
Single line through origin:

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:

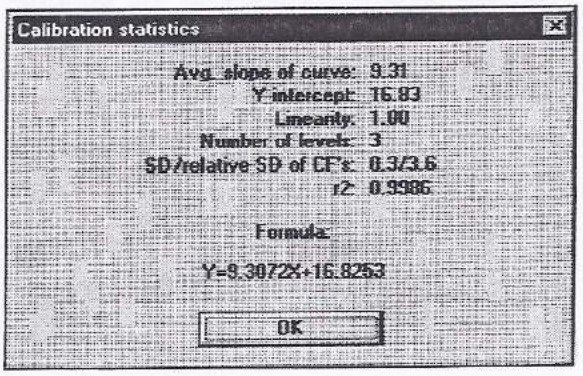
The resulting calibration curve is defined as

$y=Ax+B$

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

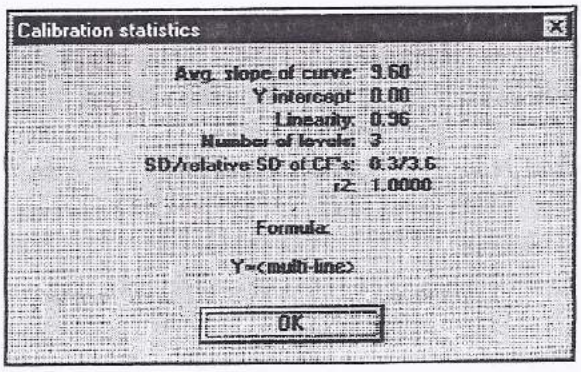
Notes:
This is a least squares fit algorithm over the calibration levels. A point at (0,0) is also assumed (by incrementing n) unless there is already a value at x=0, or if [Statistics]R2IncludeZero is set to 0 in the PEAKWIN.INI file. There must be at least 2 calibration levels.

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)

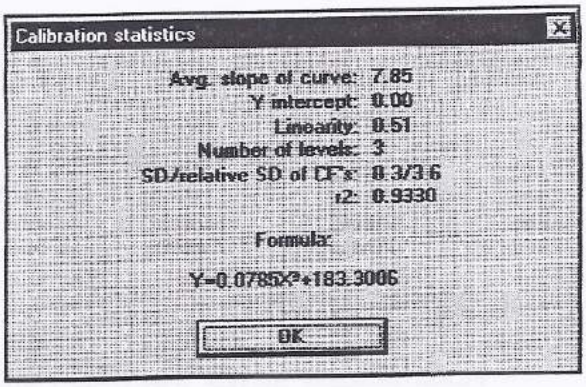
Parabolic:

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).



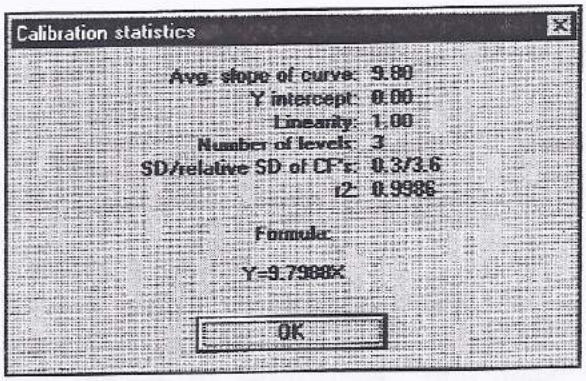
Quadratic through origin:

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:

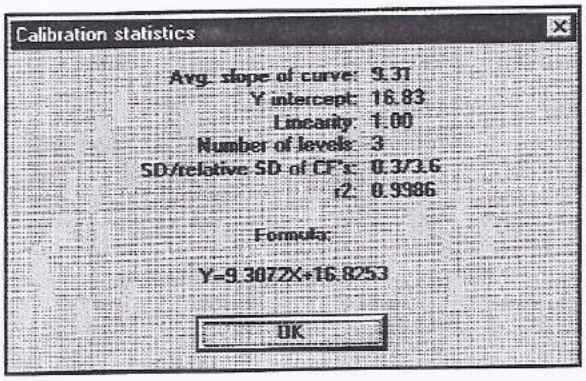
The resulting calibration curve is defined as

y=Ax2+Bx+C

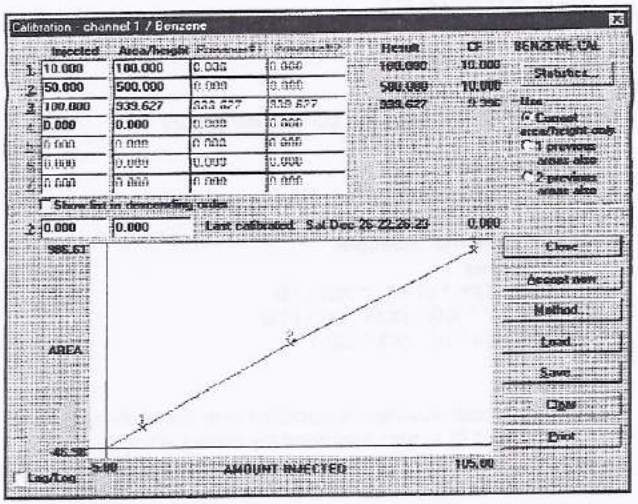
where:
x is external measure
y is area

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)



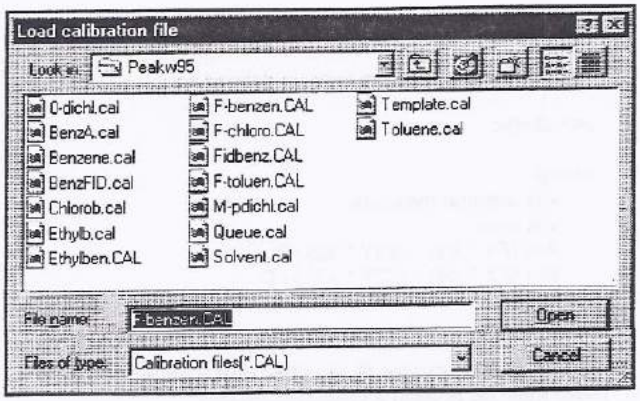
The **Accept New** Button

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.

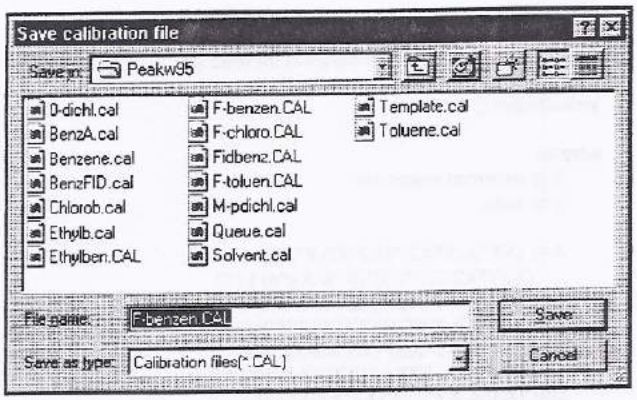
The **Load** Button



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

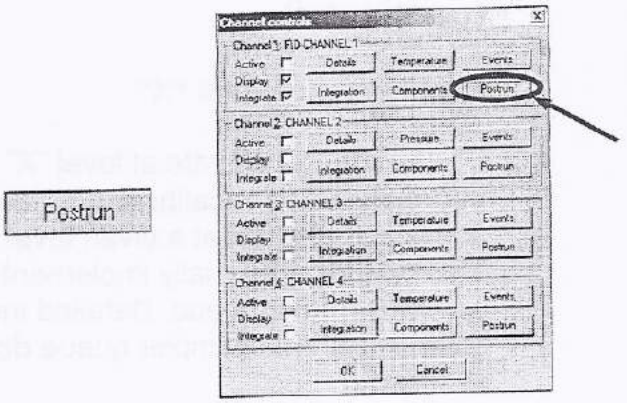
Clicking on the **Clear** button deletes all calibration data from the calibration window. The calibration file name is also removed.

The **Print** Button

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

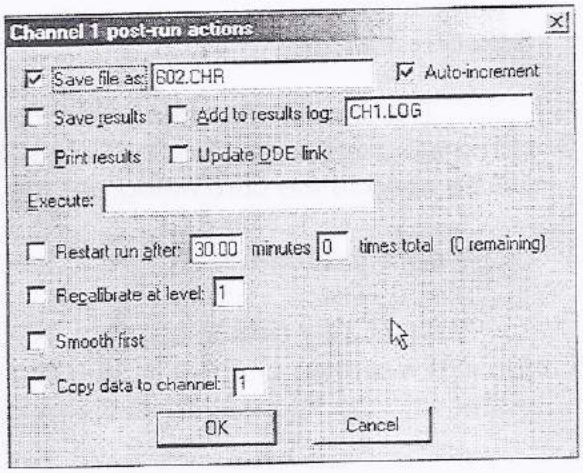
The Edit-Channels-Postrun Window

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.

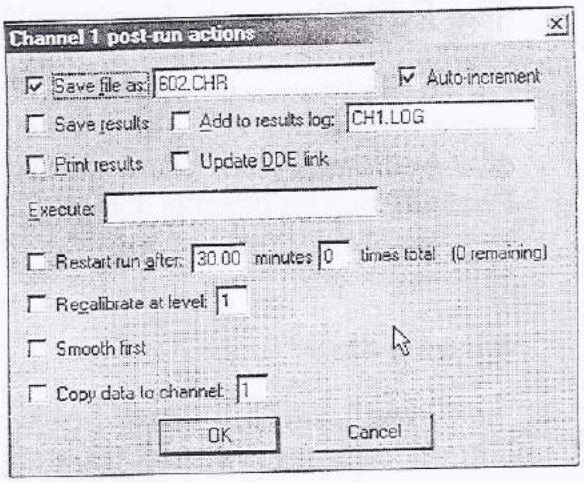
Execute "X"

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.

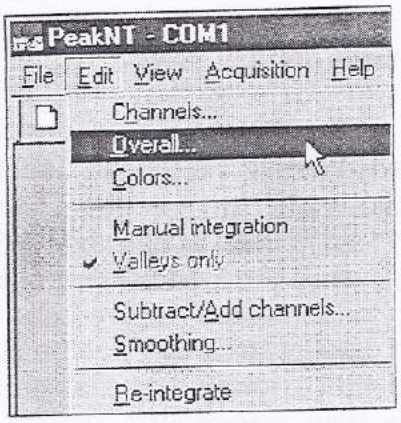
Smooth first

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.

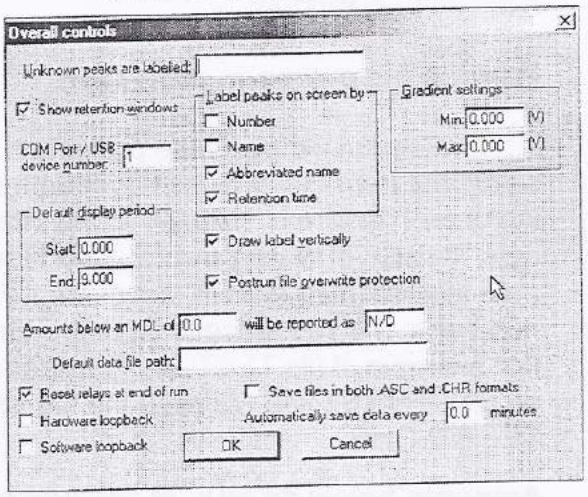
Copy data to channel "X"

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.



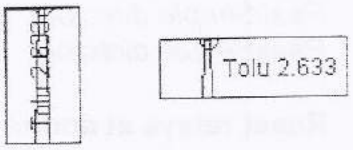
Unknown peaks are labeled "X"

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.

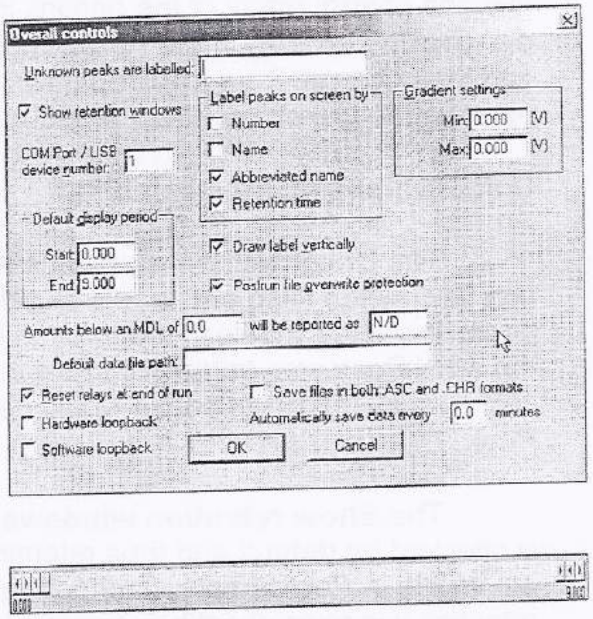


Gradient settings

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.

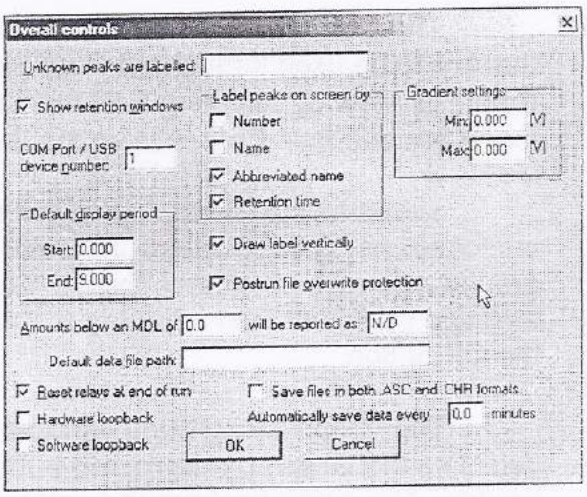
The Edit-Overall Window (continued)

Save files in both .ASC and .CHR formats

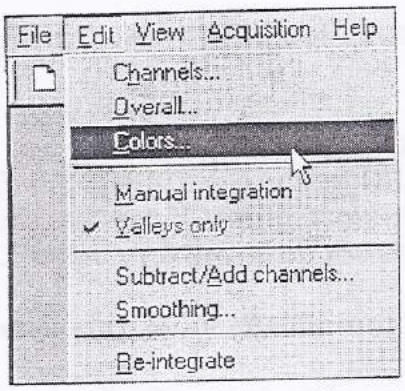
The Save files in both .ASC and .CHR formats checkbox when selected saves files in the .ASC format (ASCII) and the .CHR format (chromatogram). If the checkbox is not selected files will be saved only in the .CHR format.

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.

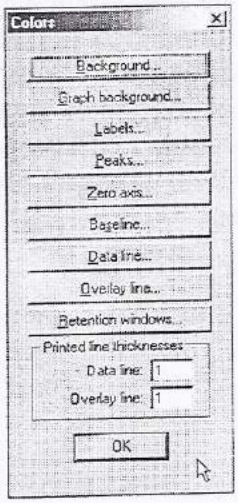


The Edit-Colors Window



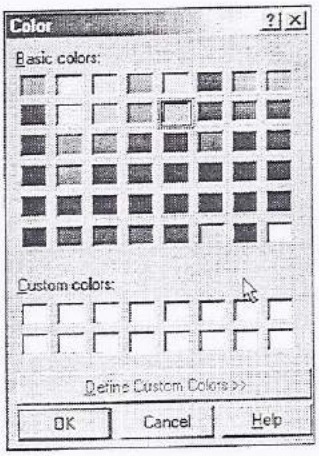
The Colors window determines the color schemes that are to be used throughout PeakSimple. Open the Colors window by selecting **Edit** from the PeakSimple menu bar and then **Colors** from the list of options.

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.



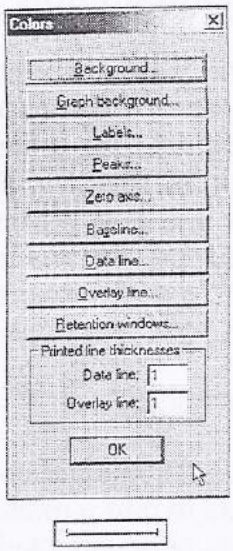
The peak color is the color of the circle at the top of each identified peak and is determined by the Peak color window which is opened up by selecting the **Peak** button in the Color window. Select the desired peak color and then click on the OK button to close the window and affirm the change.

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.

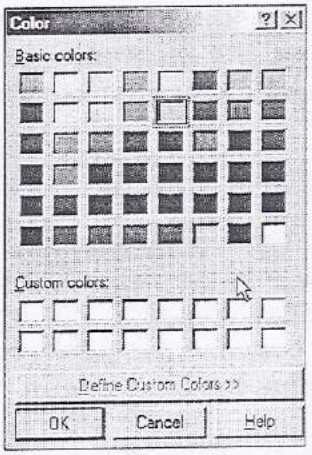
The data line is the signal line that makes up the peaks in PeakSimple and its color is defined by selecting the **Data line** button in the Colors window and then selecting a color from the Data line colors window. Once the desired color is selected apply the color change by clicking on the OK button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

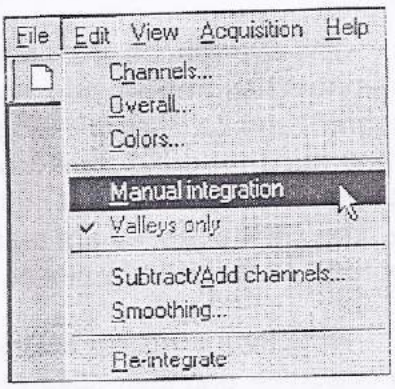
Retention windows are the horizontal bars that appear onscreen and their color can be changed by clicking on the **Retention windows** button in the Colors window and then selecting the desired color in the Retention windows colors window. To apply the color changes click on the OK button to close the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



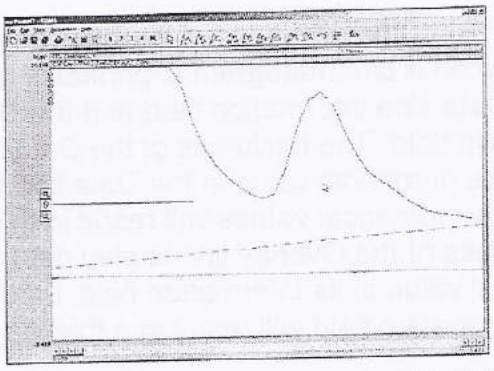
The manual integration tools are used to manually draw in the baseline in a PeakSimple chromatogram. The manual integration toolbar is opened up by selecting **Edit** from the PeakSimple menu bar and then clicking on the **Manual integration** option. The manual integration toolbar appears to the right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

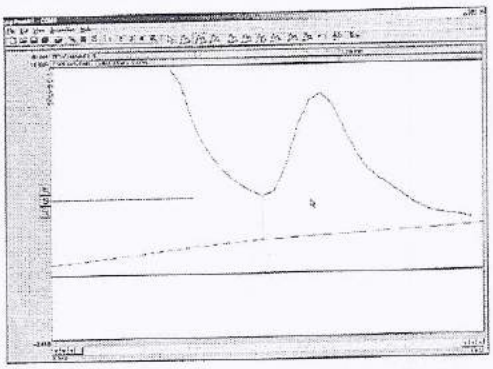
None Integration Tool 

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.



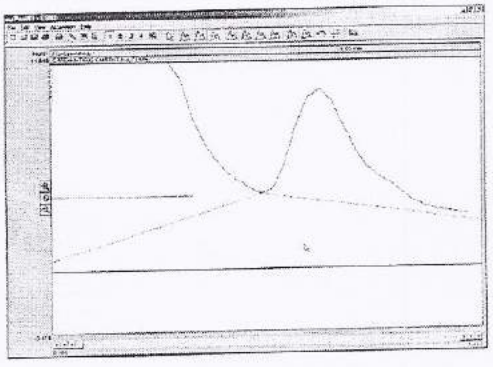
Drop Integration Tool 

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 

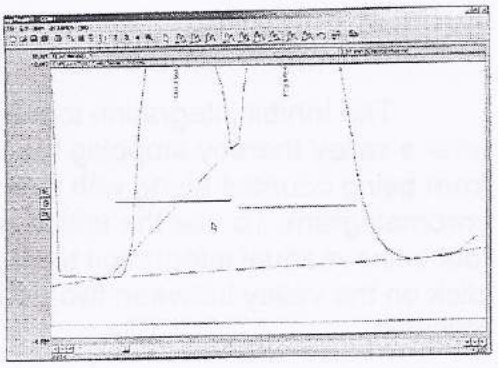
The Based integration tool raises the baseline to a valley between two specified peaks. To change the baseline select the Based tool and click on a peak with the mouse cursor to raise the baseline up to the valley.



Manual Integration (continued)

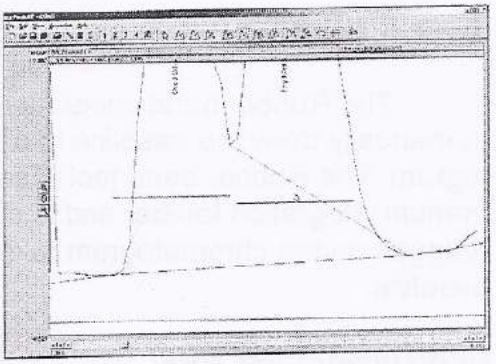
Lead Skim Integration Tool 

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.



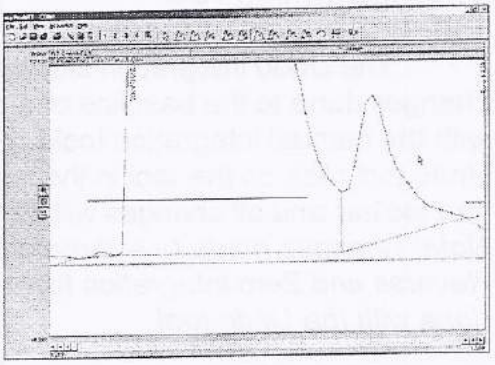
Trail Skim Integration Tool 

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.



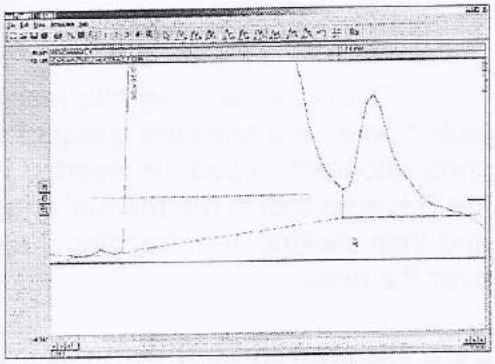
Lead Horizontal Integration Tool 

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 

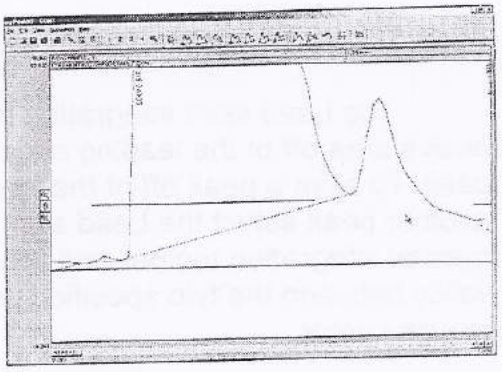
The Trail horizontal integration tool draws the baseline horizontally for the trailing peak while the leading peak's baseline stretches from the horizontal line to the previous valley in the chromatogram. The Trail horizontal tool is used by selecting the Trail horizontal tool in the manual integration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



Manual Integration (continued)

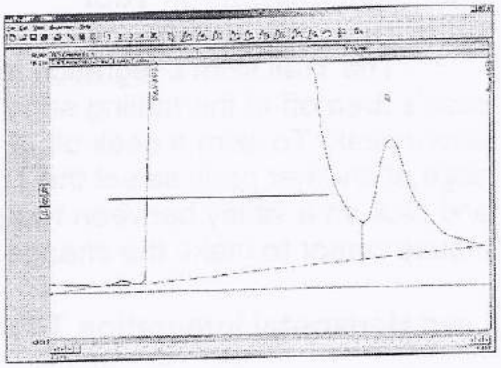
Inhibit Integration Tool

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.



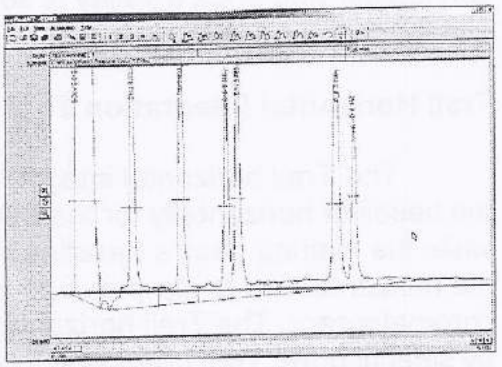
Rubber Band Integration Tool

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.



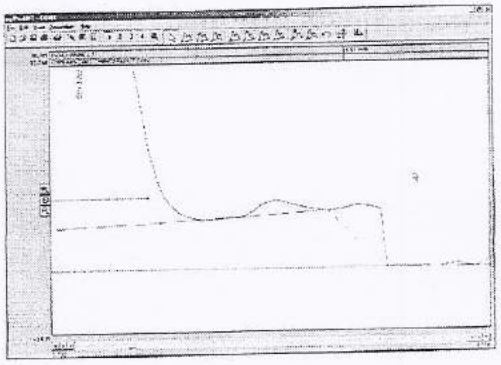
Undo Integration Tool

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

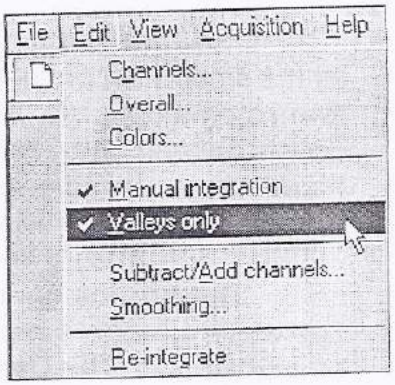
The Reverse integration tool inverts a selected peak or a selected group of peaks in a chromatogram. A peak is inverted by selecting the Reverse tool in the manual integration toolbar and then clicking and dragging the mouse cursor over the peak.



Zero Integration Tool

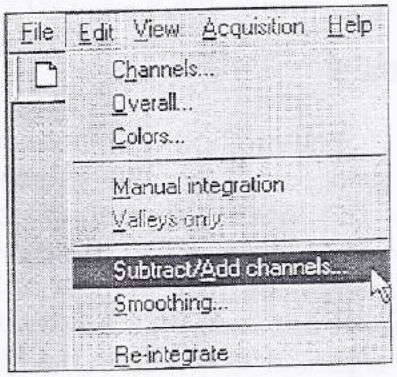
The Zero integration tool sets the value of the data line at zero starting at a selected point. To zero the data line at a given point select the Zero tool from the manual integration toolbar and click on the data line with the mouse cursor.

The Edit-Valleys Only Option



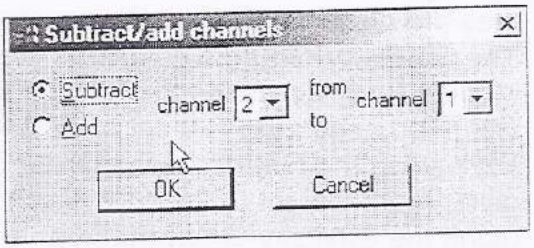
The Valleys only option is available only when the Manual integration toolbar is open in PeakSimple. The Valleys only option can be selected by opening up the Manual integration toolbar in the Edit menu and then selecting the Valleys only option immediately below Manual integration in the drop down menu. 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



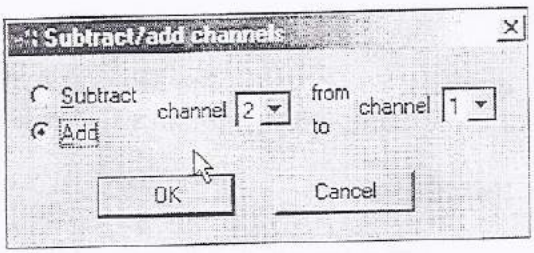
The Subtract/Add channels menu removes or adds the analog data signal from/to one channel in PeakSimple from/to another channel. The Subtract/Add channels menu is opened by selecting the Edit menu and then by clicking on Subtract/Add channel in the drop down menu.

Subtracting a Channel



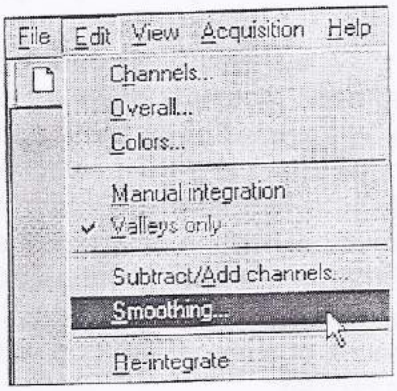
To subtract one channel from another channel click on the Subtract radio button with the mouse cursor and select the channel that is to be taken away in the first dialogue box. In the second dialogue box select the channel that is to have the first selection taken away from. Click on OK with the mouse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

Adding a Channel



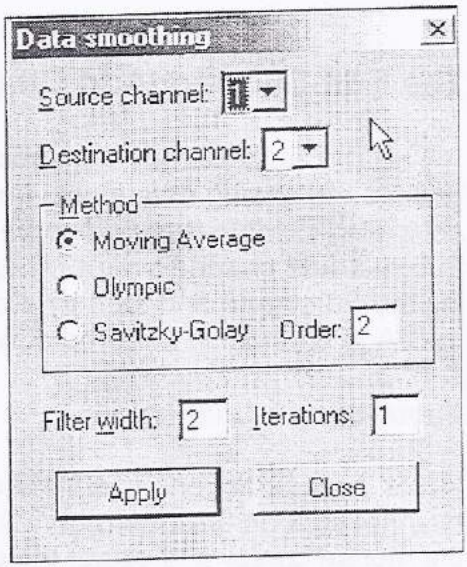
To add one channel to another channel select the Add radio button in the Subtract/Add channels menu. Select the channel that is to be added by selecting a number in the first dialogue box and then choose the channel that it is to be added to by selecting a number in the second dialogue box. All changes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

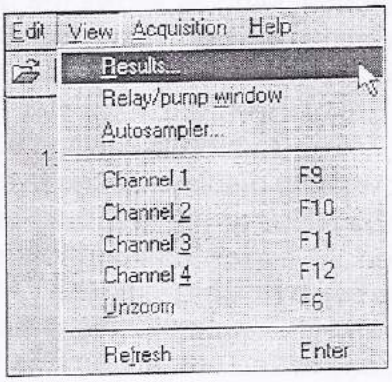
The Filter width dialogue box controls the number of samples that are to be taken into account when using the Moving Average smoothing method. A filter width of 2 means that 2+1+2 samples are taken while a filter width of 5 means that 5+1+5 samples are taken.

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.

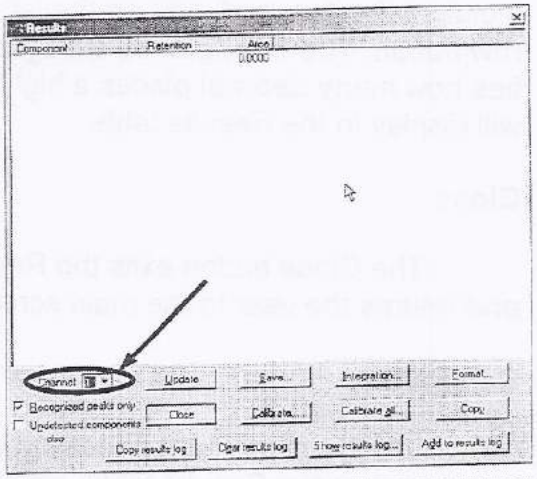
The Re-Integrate Option

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.



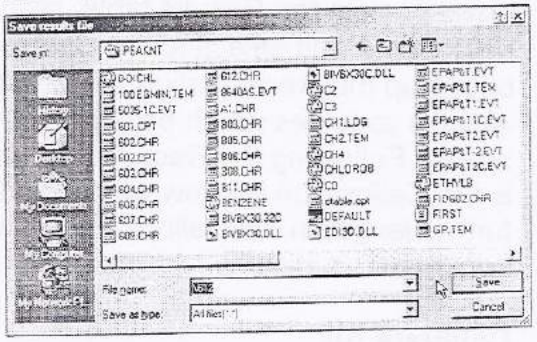
The **Channel** option scrollbar specifies which of the four channels the results data should be displayed for. When the **Recognized peaks only** checkbox is selected only the results for named peaks will be displayed. **The Undetected components also** checkbox displays the results for the undetected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

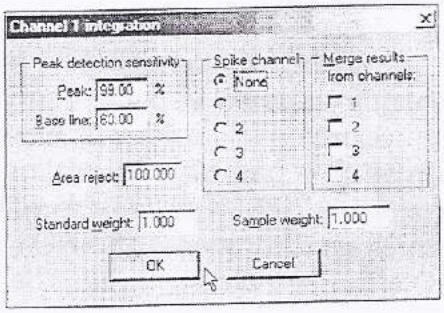
Save

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.

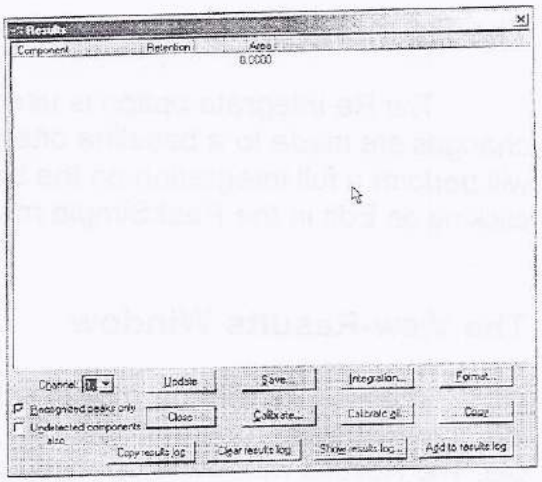


Integration

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.

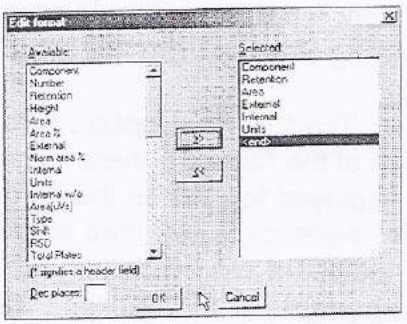


The View-Results Window (cont.)



Format

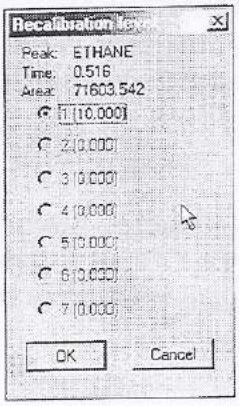
Selecting the Format button in the Results window opens up the Edit format window. The Edit format window allows the user to specify the information that is to be included in the Results table.



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.

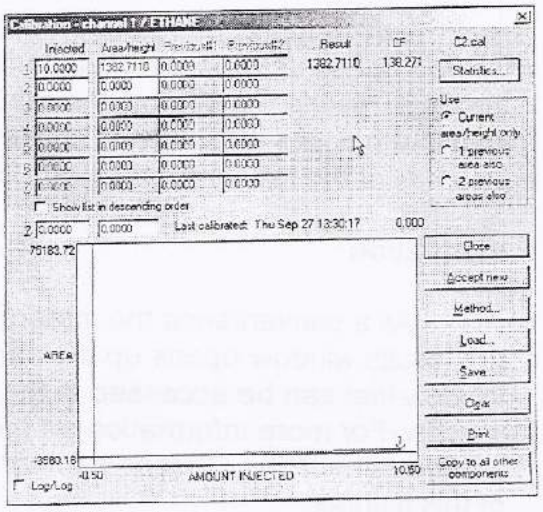
Close

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

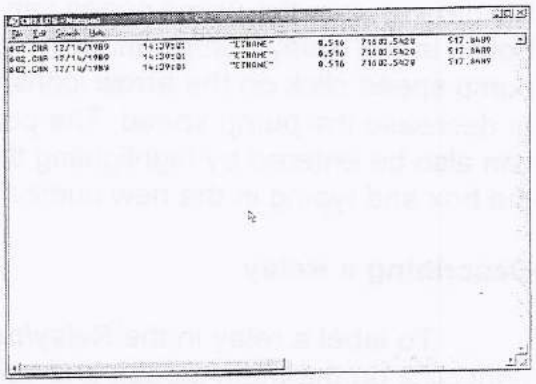
The Copy results log button copies the .log file for the results to the Clipboard. This log file can be pasted into any Windows program. A certain number of lines in the results log will always be copied, by default the number is 20. If more than 20 lines are needed for an application the user must modify 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.

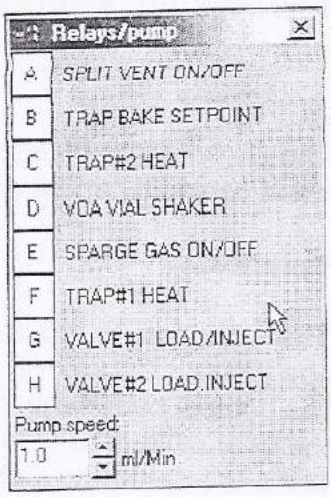


Add to Results Log

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.

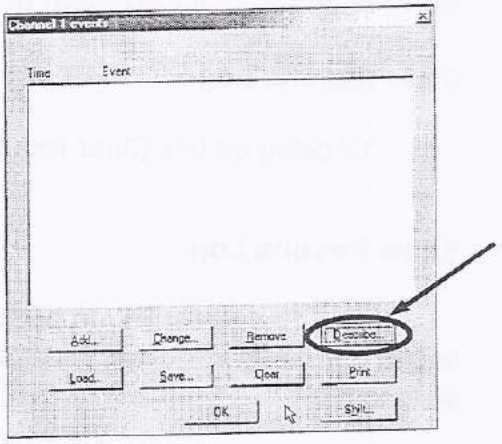


Selecting/Deselecting a Relay

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.

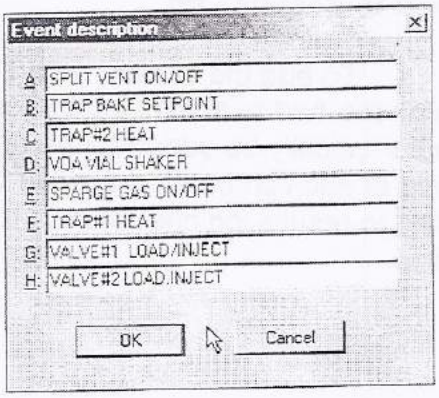
Pump Speed

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.

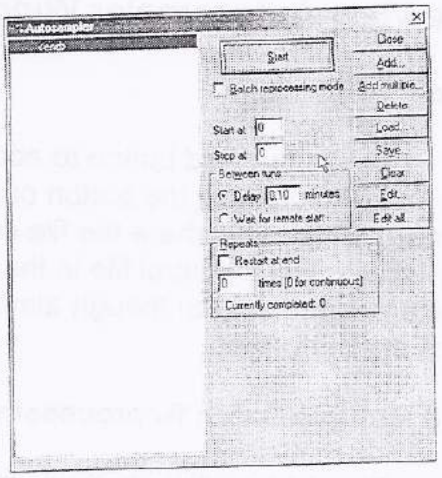


Describing a Relay

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.

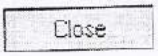
The **Start at** dialogue box specifies which control file number to begin operation at first. If no number is entered the autosampler will begin at the first control file. The **Stop at** dialogue box specifies the last control file to be run before operations of the autosampler cease. If no number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

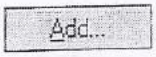
Close

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

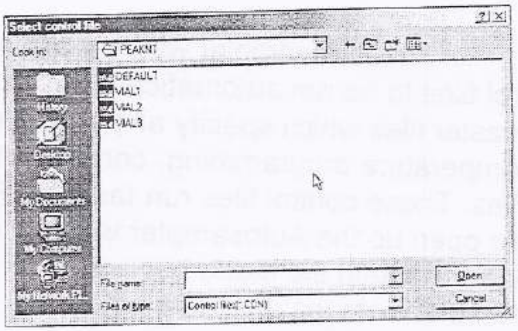


The View-Autosampler Window (cont.)

Add

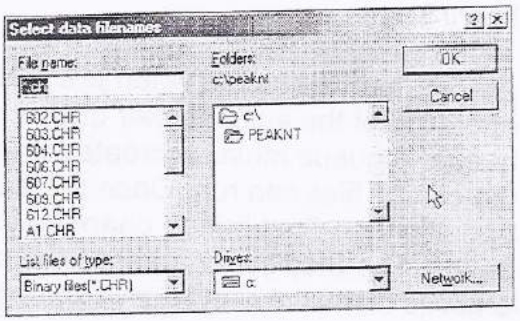


Select the Add button to add a control file to the queue. Selecting the button opens up the Select control file window where the file can be loaded into the list box. Each control file in the queue must have a different name even though almost identical actions are performed.



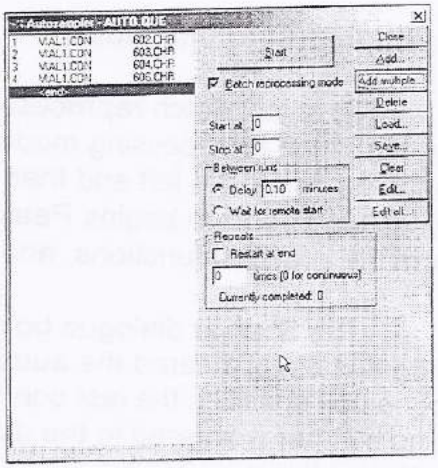
Add Multiple/Batch Reprocessing

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.



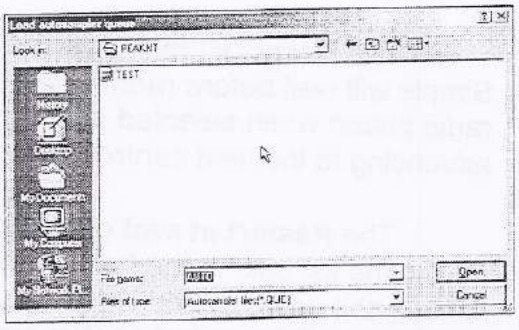
Delete

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

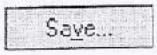


Load

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.

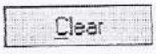


Save



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.)

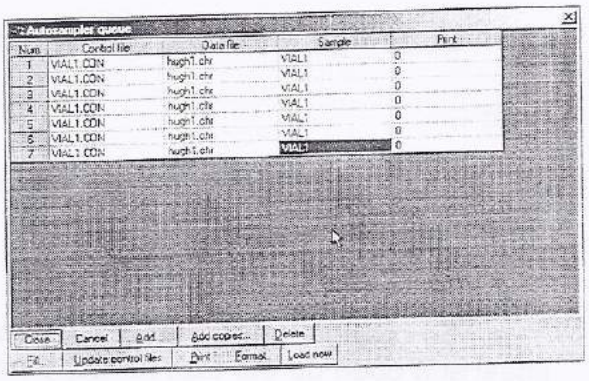
Edit

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.

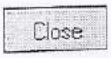
Edit All

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.

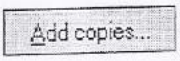
Cancel

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

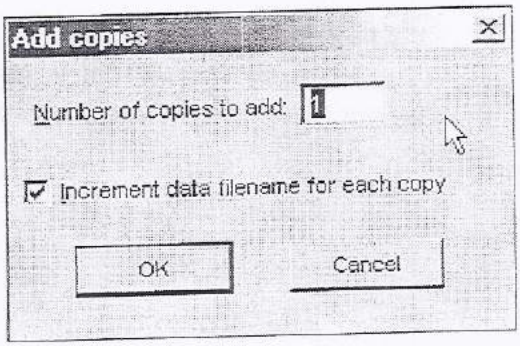
Add

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.



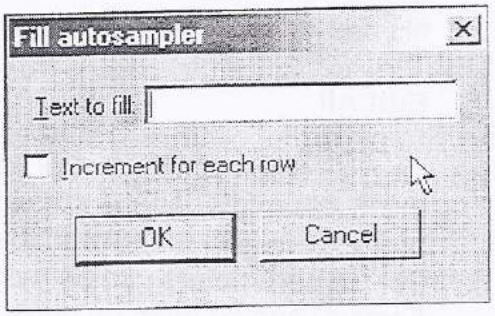
Autosampler Queue Window (cont.)

Delete

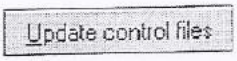
The Delete button deletes a highlighted control file off the list. If no file is highlighted then the last file will be deleted from the queue.

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

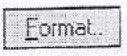


Selecting the Update control files button saves all changes to the control files in the list.

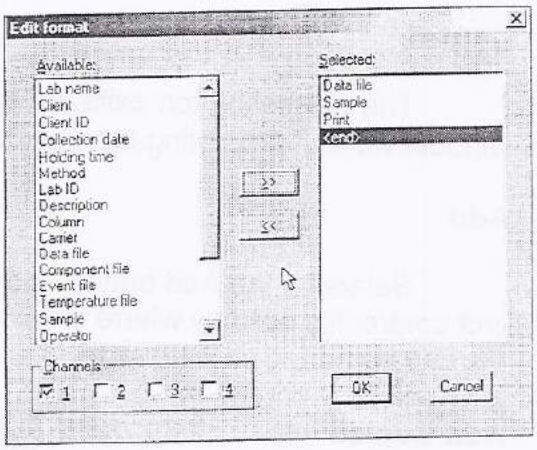
Print

The Print button prints the queue spreadsheet.

Format



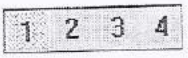
To change the format of the queue spreadsheet and open up the Edit format window select the Format button. In the Edit format window a format type can be added by selecting it in the Available window and then hitting the right facing arrow button. To remove a format type from being displayed in the spreadsheet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

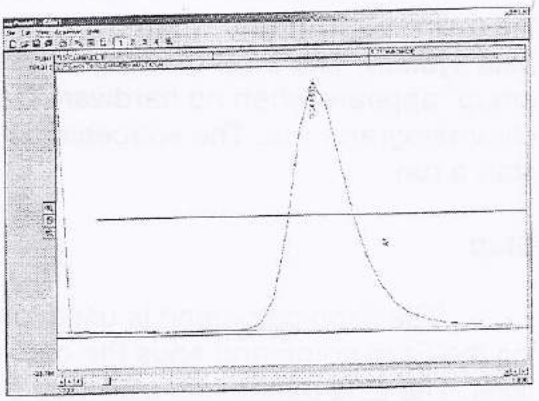
The View-Channel "X" Options



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.

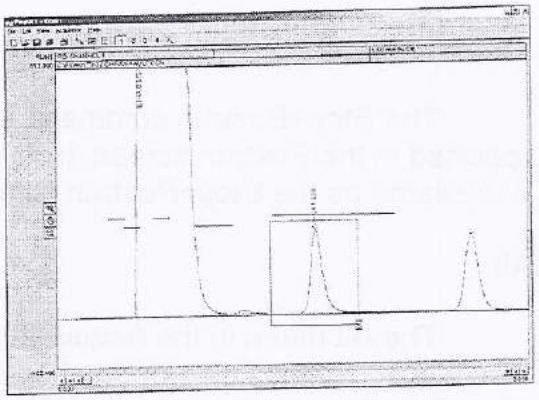
Unzoom

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

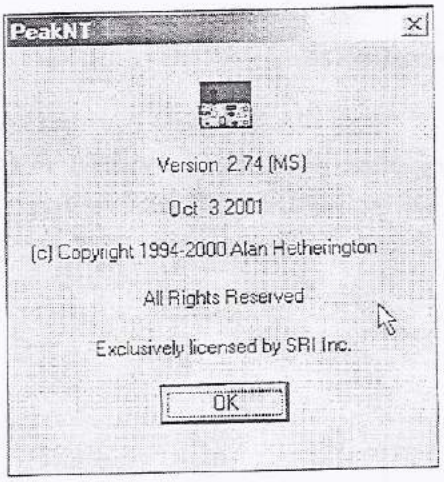
The Refresh tool in the View menu redraws the chromatogram screen to fix any glitches or resolve an error. Pressing Enter on the keyboard also refreshes the screen.



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.

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.

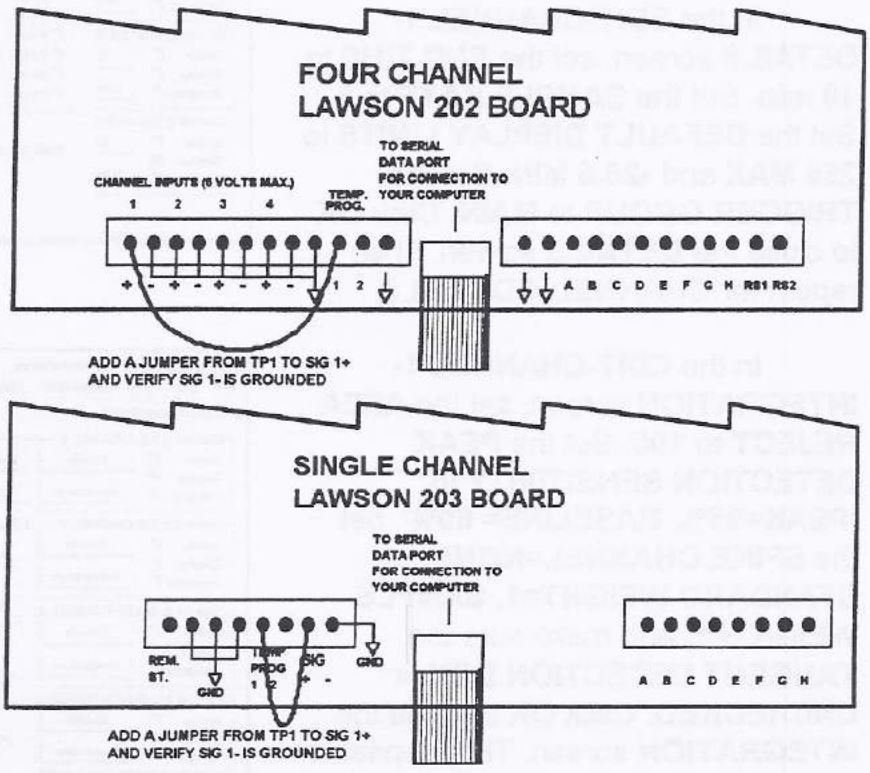
Setting Up The Hardware:

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.

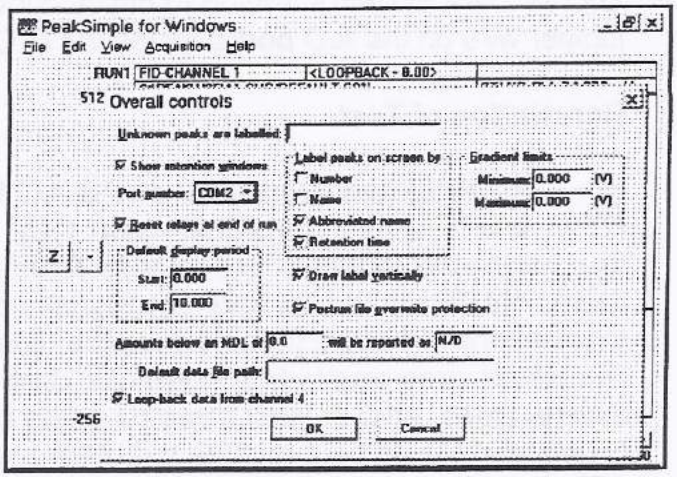


Loopback Test: (continued)

Setting Up The Software:

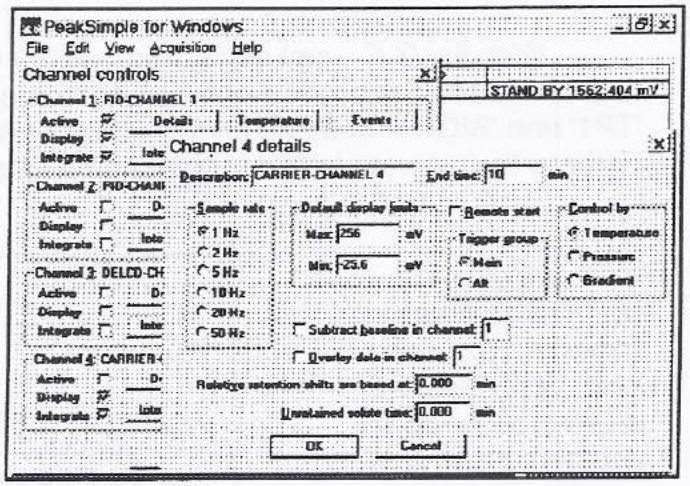
Re-attach the bottom cover and plug the G.C. back in. Turn the G.C. power on and start PeakSimple. Verify that the computer is communicating properly with the G.C..

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.

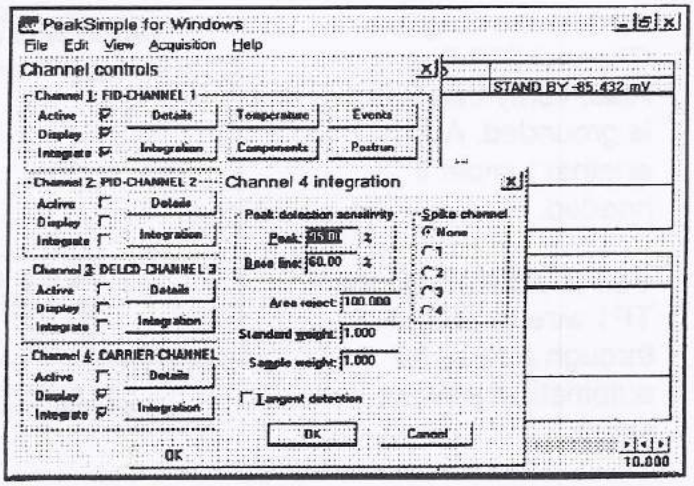


In the **EDIT-CHANNELS** screen, check the **ACTIVE, DISPLAY** and **INTEGRATE** boxes for channel 1. And check the **DISPLAY** and **INTEGRATE** boxes for channel 4.

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**.

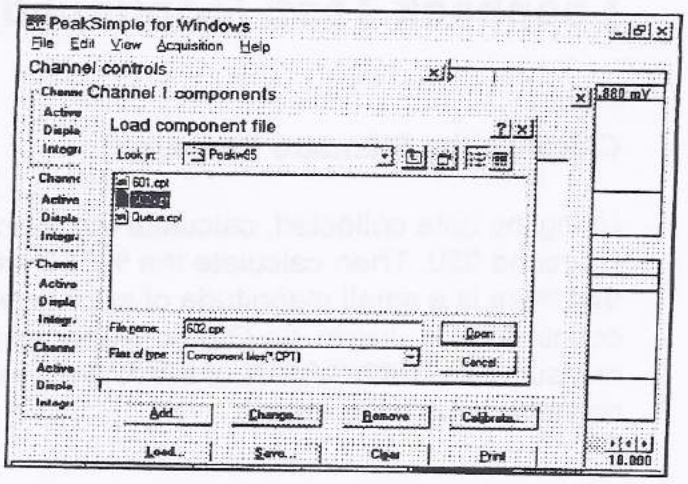


Loopback Test: (continued)

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**.

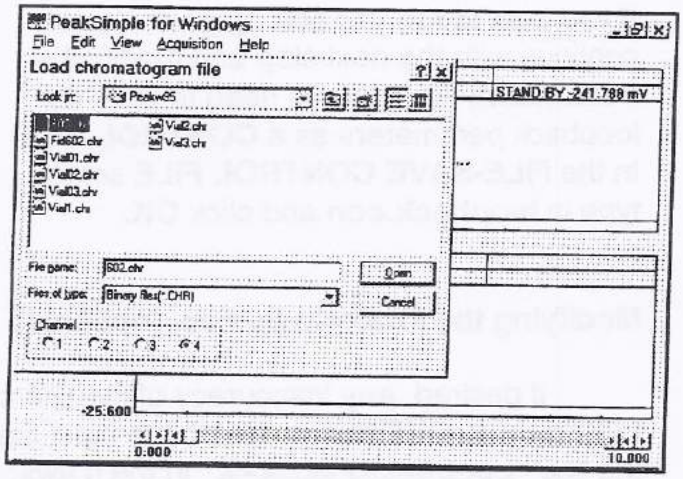
In the **FILE-OPEN** screen, select **CHANNEL 4** at the bottom of the window and then highlight the **602.chr** sample chromatogram file and select **OPEN**.



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:

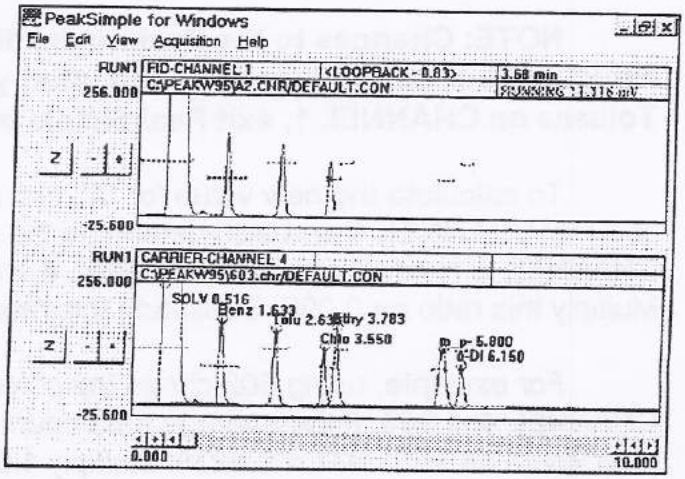
Auto-zero channel 1 by clicking the **'Z'** button. Depress the **SPACEBAR** and the chromatogram will start running. The data on **CHANNEL 1** should appear to be an exact replica of the data that was fed into **CHANNEL 4.**



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.



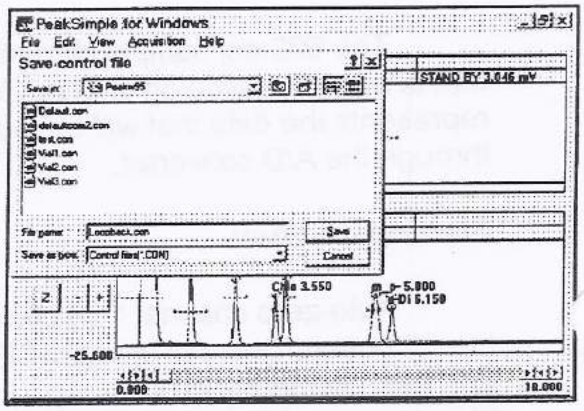
Loopback Test: (continued)

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'.

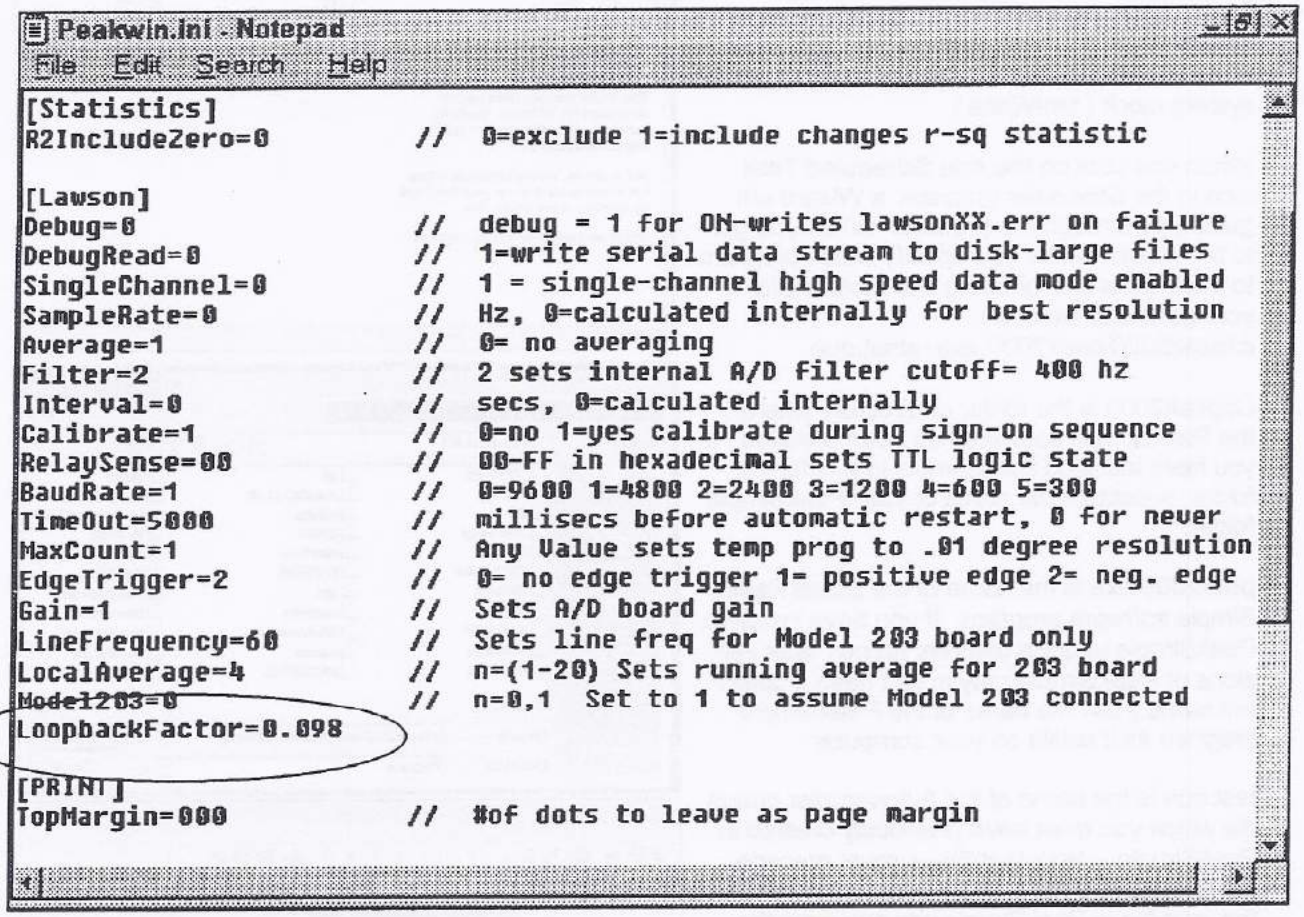
To calculate the new value for **'X'**, first determine the average area counts of a specific peak for **CHANNEL 1** and also determine the area count of the corresponding peak on **CHANNEL 4.** Next, divide the **CHANNEL 4** area count by the **CHANNEL 1** area count. Multiply this ratio by 0.098. Substitute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

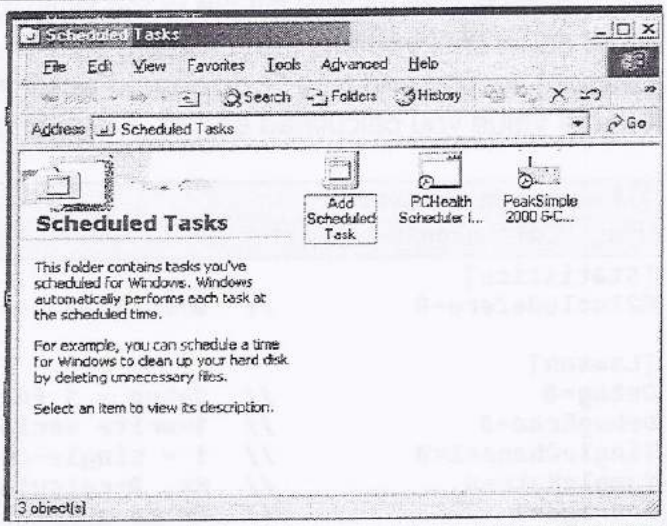
When you click on the Add Scheduled Task icon in the Scheduler program, a Wizard will guide you through the process. When you get to the screen where you specify which program to start, enter the following line modified for your particular situation:
c:\peak2000\peak2000.exe -atest.que

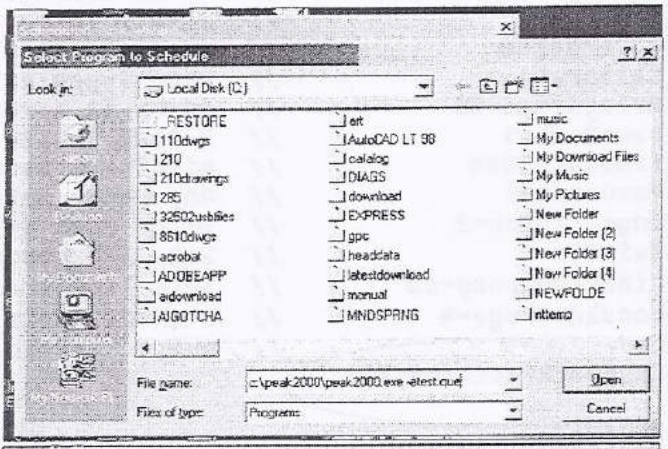
C:\peak2000 is the folder or directory where the PeakSimple software has been installed. If you have installed PeakSimple in a different folder, substitute the name of your PeakSimple folder.

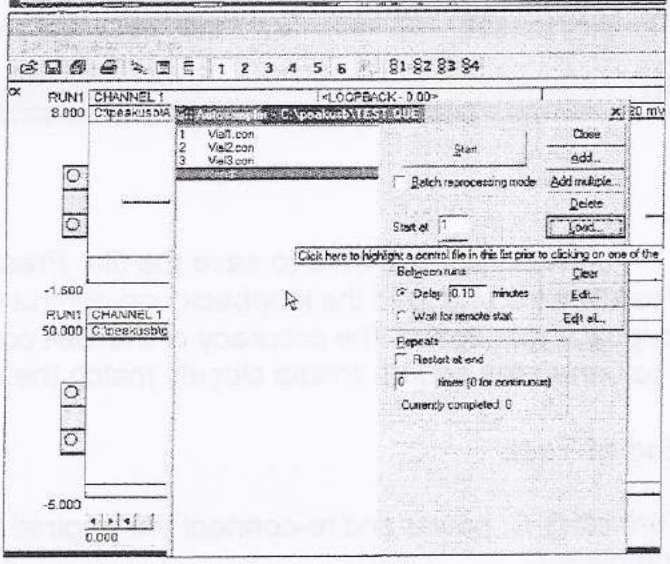
peak2000.exe is the name of the actual PeakSimple software program. If you have installed PeakSimple under a different name (later versions of PeakSimple may in fact have a different name) use the name of the PeakSimple program as it exists on your computer.

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.







GPC From PeakSimple Data Acquisition

Introduction

The following is an outline of how PeakSimple data acquisition software/hardware can be used to acquire and analyze (in conjunction with an appropriate spreadsheet) gel permeation chromatography data. At this time, two different version of PeakSimple software were required for successful analysis. Version 2.08 was used to collect the data and obtain result tables for narrow polymer standard chromatograms, while version 2.09 was used to obtain the peak slice information for broad unknown polymers. That is, using 2.09, the voltage difference between the detector output and the subsequently drawn baseline was obtained for each data point and saved as an ASCII file, which was then imported into Excel for in-depth GPC analysis. Ultimately, it would be preferred to use only one version of PeakSimple. However, 2.09 (the latest version) was not stable while acquiring data. The program would crash after approximately 5 minutes. Furthermore, the time display in the upper right hand corner did not appear to work and retention windows were not visible on the screen although a component file was active. Thus, 2.09 was used only for obtaining slice information with non-active channels.

To illustrate how PeakSimple can be used for GPC analysis, I have included 3 narrow polystyrene standard chromatograms (4 standards per chromatogram) and two broad unknown polymer chromatograms. Chromatograms were obtained using a Waters 510 pump (U6K injector), an ethyl acetate mobile phase (1 mL/min), a series of Ultrastyrogel® columns (Waters 10^6, 10^4 and 500 Å) and Waters 2410 refractive index detector. All polymers were pre-dissolved in ethyl acetate and chromatograms were collected at 1 Hz. Polystyrene standard concentrations were 0.1 % w/w or less (50 µL injection volume) while broad unknown polymers were approximately 1 % w/w (75 µL injection volume). Also included are component files, containing the standard identities and expected retention windows, an event file for integration, and two ASCII data files containing slice information for the broad unknown polymers, and an Excel file with in-depth GPC analysis.

Obtaining a Calibration Curve

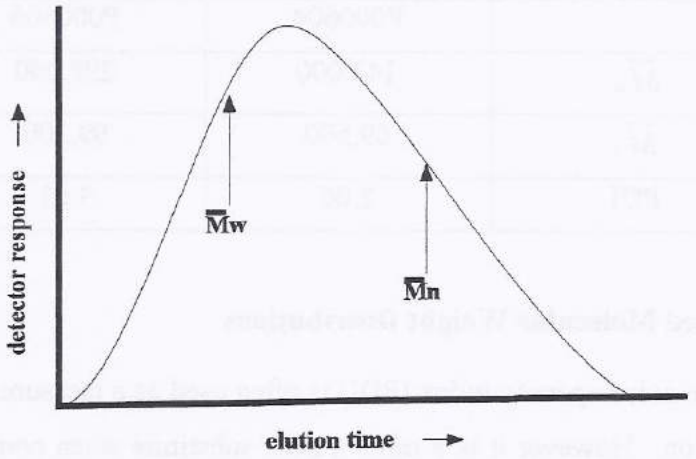
Polydisperse polymers in solution are fractionated according to size or hydrodynamic volume during GPC, which is also known as size exclusion chromatography. Molecular weight is related to the hydrodynamic volume. In GPC a dilute polymer solution is injected into a solvent stream which then flows through a series of columns packed with porous gel beads. Smaller molecules pass through and around the beads while larger molecules are excluded from all but the largest pores. Thus fractionation occurs with the largest molecules eluting first. The molecular weight of an eluting polymer molecule varies exponentially with eluting volume, the latter of which is proportional to time under constant flow rate conditions. To obtain molecular weight data and convert the GPC chromatogram into a molecular weight distribution, the relation between molecular weight and elution time is obtained from a series of polymer standards of known molecular weight. The calibration curve is thus obtained from a plot of the logarithm of molecular weight versus time. Given that GPC is a comparison of hydrodynamic volumes, unknown molecular weight determinations will be relative to the calibration standards. For a good introductory reference to polymer science, see R. J. Young and P. A. Lovell, Introduction to Polymers.

Using PeakSimple 2.08, the result table for each of the three polystyrene standard chromatograms was copied using DDE into Excel. The natural logarithm of molecular weight versus time was plotted and a best fit analytical approximation to the curve was obtained from a third order polynomial, $P(t_e)$. This is the calibration curve relating molecular weight to elution time.

Obtaining Molecular Weight Averages

The most common and convenient way to characterize a distribution of molecular weights making up a polymer sample is using molecular weight averages such as, number average molecular weight ($\overline{M}_n$), and weight average molecular weight ($\overline{M}_w$), as shown in the following figure for a typical polymer chromatogram. $\overline{M}_n$ is defined as a sum of products of the molecular weight of each fraction multiplied by its mole fraction. That is: $\overline{M}_n = \Sigma X_i M_i$ where X_i is the mole fraction of molecules of molecular weight mass M_i. The weight average molar mass is

defined as a sum of the products of the molecular weight of each fraction multiplied by its weight fraction, w_i. That is: $\overline{M}_w = \sum w_i M_i$. Additionally, it can be shown that the number average molecular weight, in terms of weight fraction, is equal to: $\overline{M}_n = 1/\sum(w_i/M_i)$. The ratio $\overline{M}_w/\overline{M}_n$ is known as the polydispersity or polydispersity index (PDI). The PDI is often used as a measure of the breadth of the molecular weight distribution. Polymers that are monodisperse (i.e. all chains have the same molecular weight) would have a PDI of 1.



A typical polydisperse polymer molecular weight distribution showing the approximate locations of $\overline{M}_n$ and $\overline{M}_w$.

Using PeakSimple 2.09, polymers p000604 and p000606 were integrated (using the GPC event file) and the results saved in ASCII files. The ASCII files were imported into Excel and the corresponding sample times were added as a third column of data starting at time equal to zero. Only slice and time data corresponding to the major peak of interest were retained (columns A,B and J,K respectively). For each time slice, a corresponding molecular weight, M_i, was calculated using the analytical equation fitted to the calibration curve (columns C and L, respectively). Note that extrapolation of a few minutes outside of the last standard (MW = 1,000,000) is usually not a problem. Furthermore, the refractive index response of the detector is proportional to the weight concentration of eluting polymer, independent of molecular weight. Thus, the weight fraction, w_i, of polymer in any slice is equal to the detector voltage response or height (baseline subtracted) divided by the sum of detector voltage responses for each polymer

elution slice (i.e. $w_i = \text{height}_i / \sum \text{height}_i$, columns D and M respectively). $\overline{M}_w$ was obtained by multiplying w_i and M_i and summing the appropriate columns (see bottom of columns E and N) $1/\overline{M}_n$ was obtained by dividing each w_i by M_i and summing the appropriate columns (see bottom of columns F and O). Thus, the molecular weight averages for the two polymers were obtained and are summarized in the following table.

Polymer Molecular Weight Averages

	P000604	P000606
$\overline{M}_w$	143,000	299,000
$\overline{M}_n$	69,500	99,300
PDI	2.06	3.01

Obtaining Normalized Molecular Weight Distributions

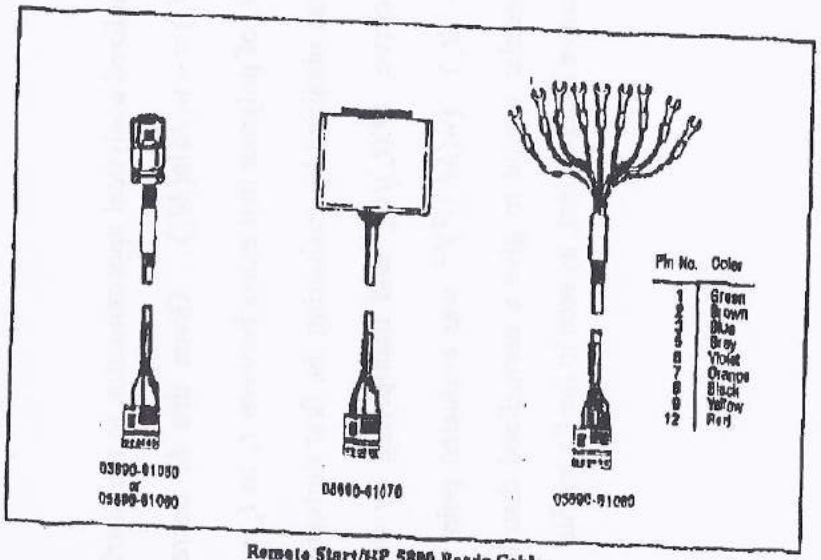
As mentioned the polydispersity index (PDI) is often used as a measure of the breadth of the molar mass distribution. However it is a often a poor substitute when compared to a graphical representation of the complete molecular weight distribution curve, especially when comparing polymer distributions. To a first approximation, the raw chromatogram (a graph of detector response, $f(t_e)$, versus elution time, t_e) is a graphical representation of the distribution. However, the chromatogram height is injection concentration dependent, making comparisons difficult, and t_e is often non-linear with $\ln(M)$, as evidenced by a third order calibration curve.

A normalized molecular weight distribution function is given by $w(M) = -\mathrm{d}w/\mathrm{d}\ln(M)$. Conversion of $f(t_e)$ versus t_e to a normalized molecular weight distribution plot (i.e. $w(M)$ versus M or $\ln(M)$), is obtained by considering that the weight fraction, $\mathrm{d}w$, of polymer which elutes between t_e and $t_e + \mathrm{d}t_e$ is given by: $\mathrm{d}w = f(t_e)\mathrm{d}t_e \Big/ \int_0^\infty f(t_e)\mathrm{d}t_e$ where the integral in the denominator is simply the area under the chromatogram. Thus, an analytical approximation of dw at the i^{th} slice is w_i, the weight fraction of polymer

A normalized analytical approximation to the distribution function, $w(M_i)$, is thus obtained from: $w(M_i) = -w_i / \mathrm{d}\ln(M_i)$. Given that M decreases as t_e increases, the same weight fraction, $\mathrm{d}w$, of polymer that exists between t_e to $t_e + \mathrm{d}t_e$ also exists between $\ln(M) - d\ln(M)$. $\mathrm{d}\ln(M_i)$ was obtained by evaluating the first derivative of the analytical equation fitted to the calibration curve, $\mathrm{d}P(t_e)/\mathrm{d}t_e$ and multiplying by the time interval (i.e. the 1 Hz sampling frequency ~ $\mathrm{d}t_e$). $(w(M_i))^2)^{1/2}$ was evaluated point by point (columns H and Q) and plotted against molecular weight to give a normalized distribution that is injection concentration and calibration curve independent, as seen in the Excel file.

Remote Start/HP 5890 Ready Cables

The REMOTE receptacle provides a function used primarily to start an integrator or data system when an HP 5890 run begins, and also provides ready information.

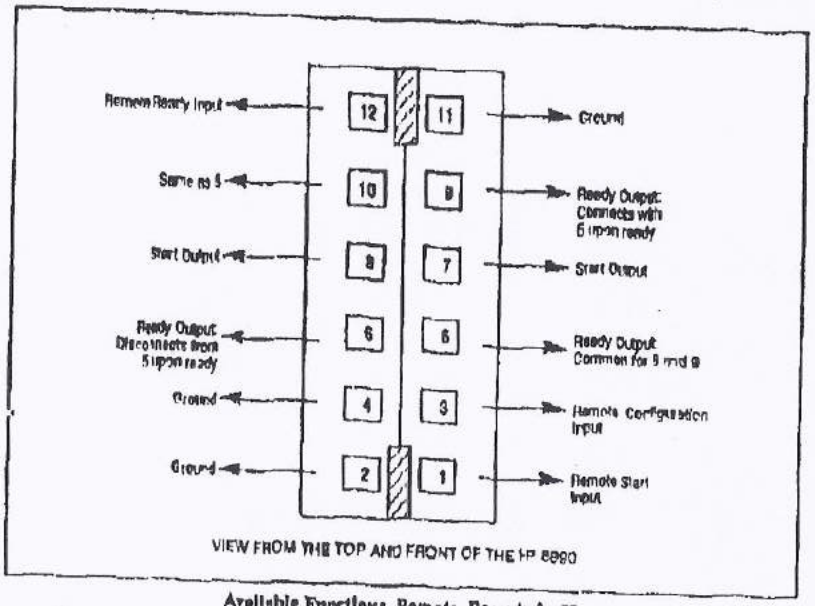


Remote Start/HP 5890 Ready Cables

Available Remote Start/HP 5890 Ready Cables		
Part No.	Use	Length
09394-60560	HP 3394/96A Integrator/Controller	2 m
05890-61060	HP 3392A Integrator/Controller	2 m
05890-61050	HP 3390A Integrator	2 m
05890-61070	HP 3350 Series LAS	2 m
05890-61080	General Purpose	2 m

Remote Receptacle

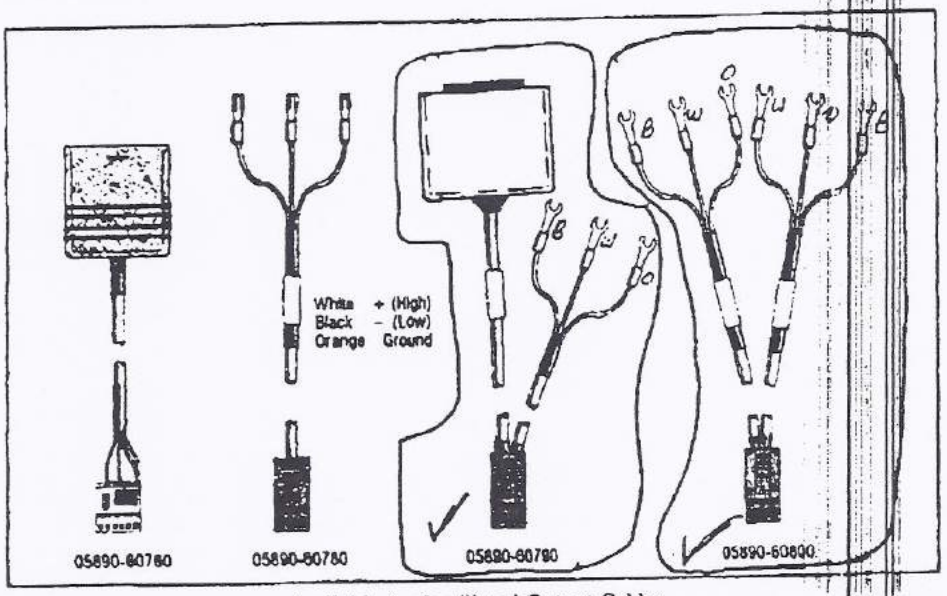
The 12-pin REMOTE receptacle provides a variety of functions, depending upon connections made via the cable. The figure below and the table following identify the function at each pin.



Available Functions, Remote Receptacle J2

Analog Signal Output Cables

The following figure and table show cables available to connect an HP 5890 ANALOG output channel (variable DC signals, +1 V or +1 mV maximum) to a recorder, integrator, and/or A/D converter for a computer system. If a second output channel is installed, a second cable is also required.



Available Analog Signal Output Cables

Note that the **general purpose** and **HP 3350 Series LAS** analog output cable assemblies consist of two independent cables, terminating together at a common, single, **female** plug at the HP 5890. One cable is labelled 1 mV, the other +1 V output. In general, the +1 V cable is connected to an integrating device or A/D converter, and the 1 mV cable is connected to a chart recording device.

3 11