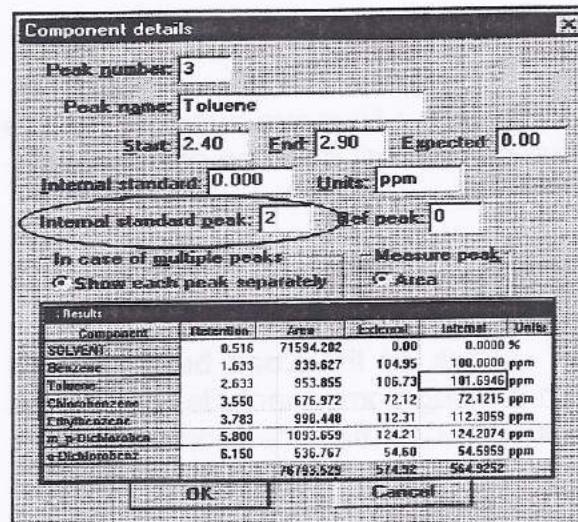
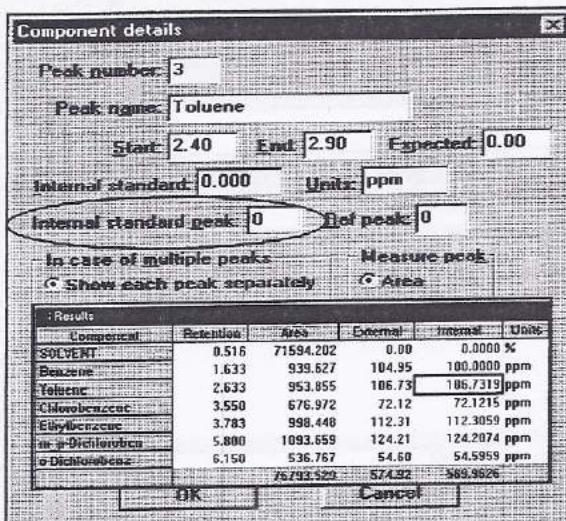


The EDIT-CHANNELS-COMPONENTS-DETAILS Screen (continued)

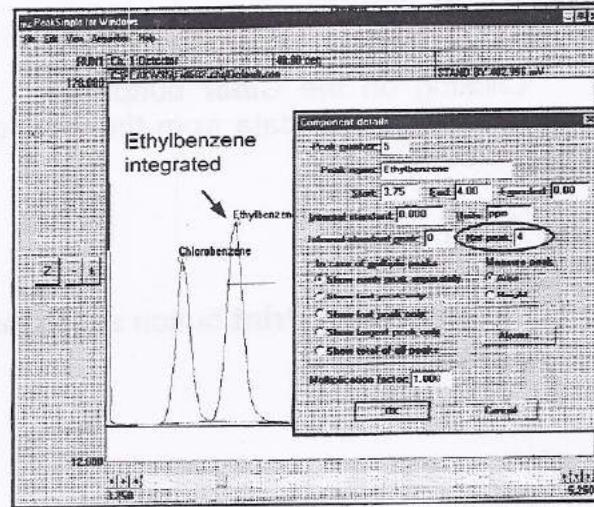
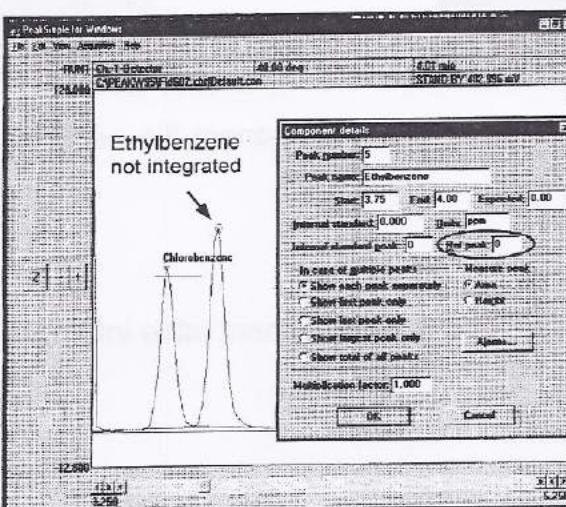
Internal Standard Peak

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

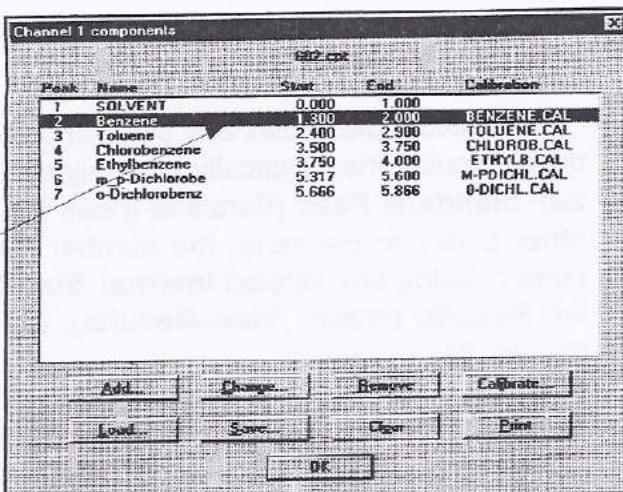
A **Reference Peak** is used to shift the retention windows of other peaks. In the example below, ethylbenzene eluted prior to its retention window so therefore it was not integrated. By entering a value of **4** in the **Reference Peak** box, ethylbenzene's retention windows are referenced to chlorobenzene, [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

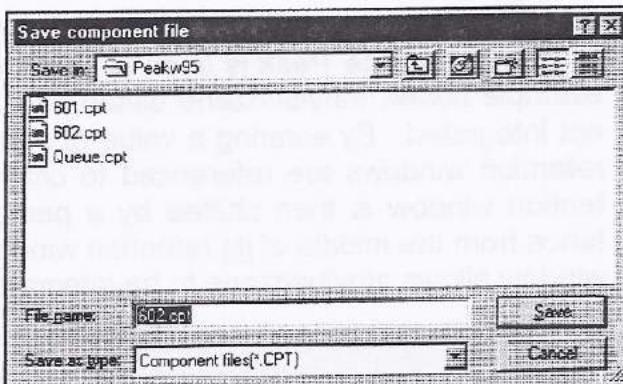
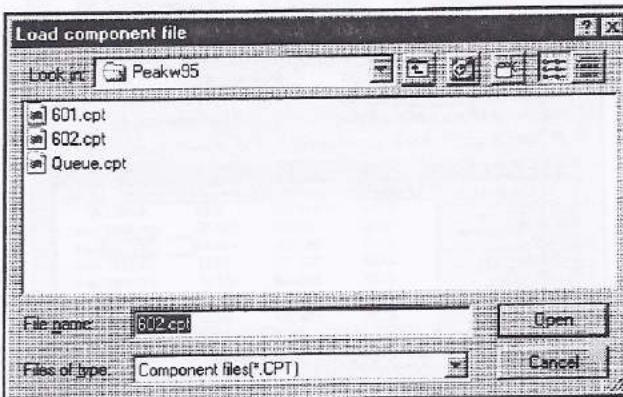
Click on the **Save** button to save a new component file, or to update an existing one. Remember to always use the .CPT extension when naming the component file. The saved file name appears at the top of the components window indicating the file in use.

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

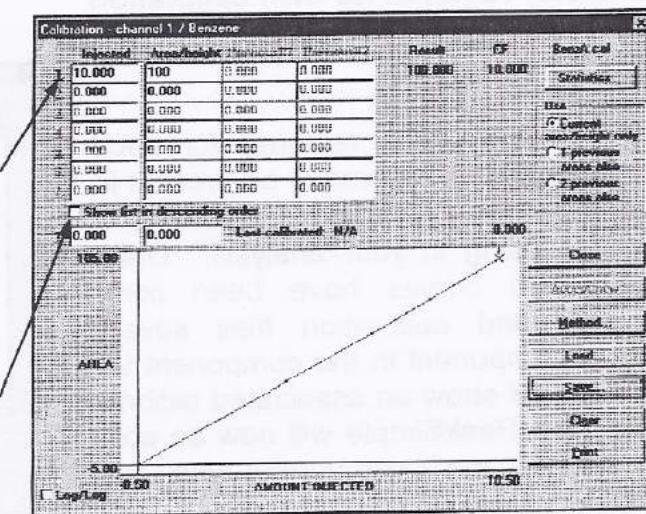
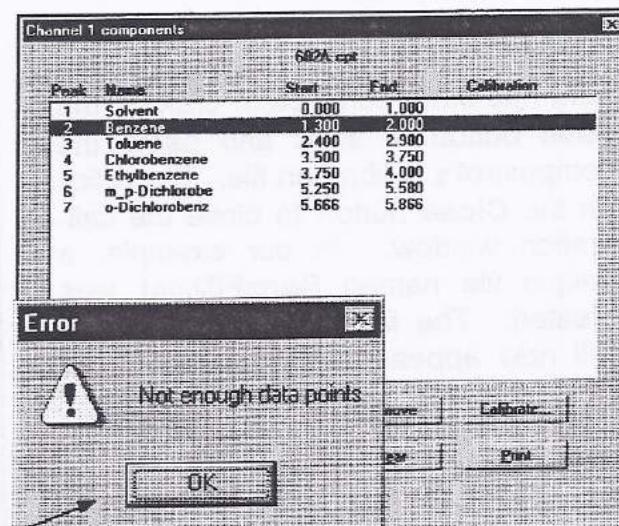
After creating a component table, each component in the table will need to be calibrated. This allows PeakSimple for Windows to not only identify each analyte peak, but also to quantify each peak using a calibration curve. The calibration curve is calculated from user-generated results obtained at several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

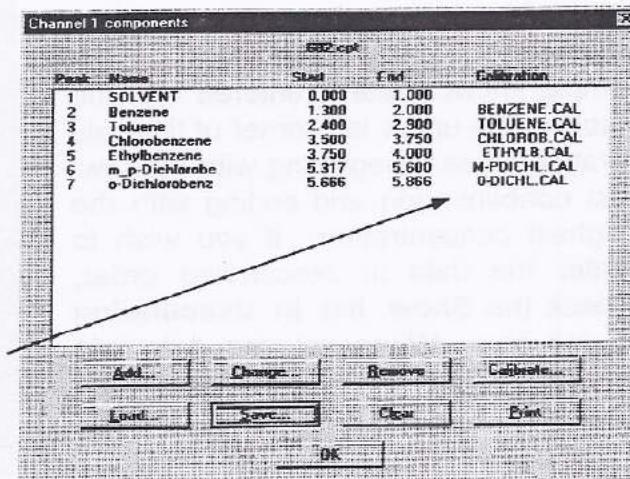
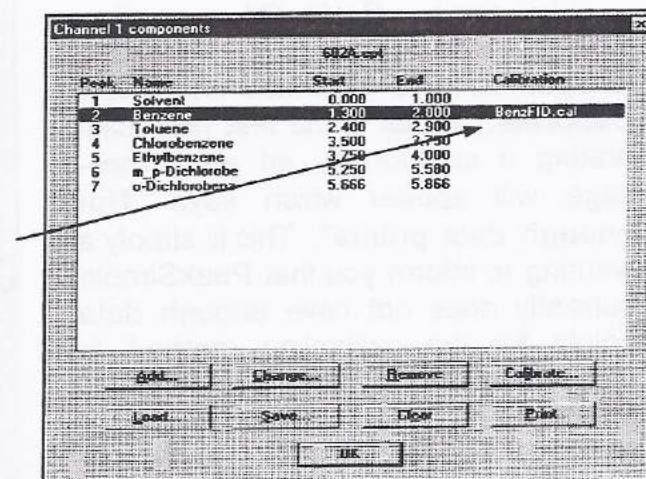
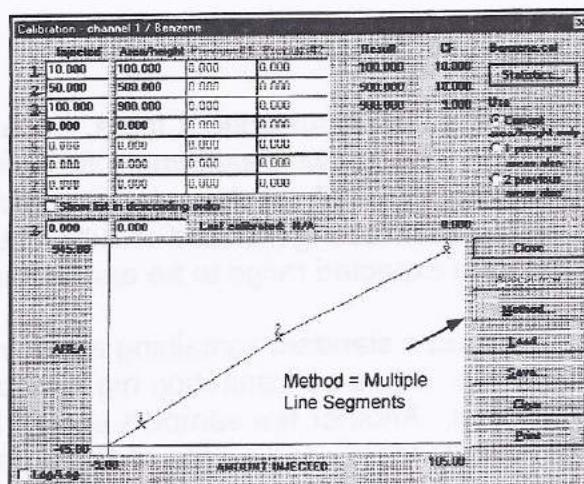
As data is entered for each concentration, a data point will be added to the calibration curve displayed in the lower section of the window. You may use as many as seven concentration levels for your calibration curve. In the fictitious example to the right, a Benzene standard was injected in concentrations 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.

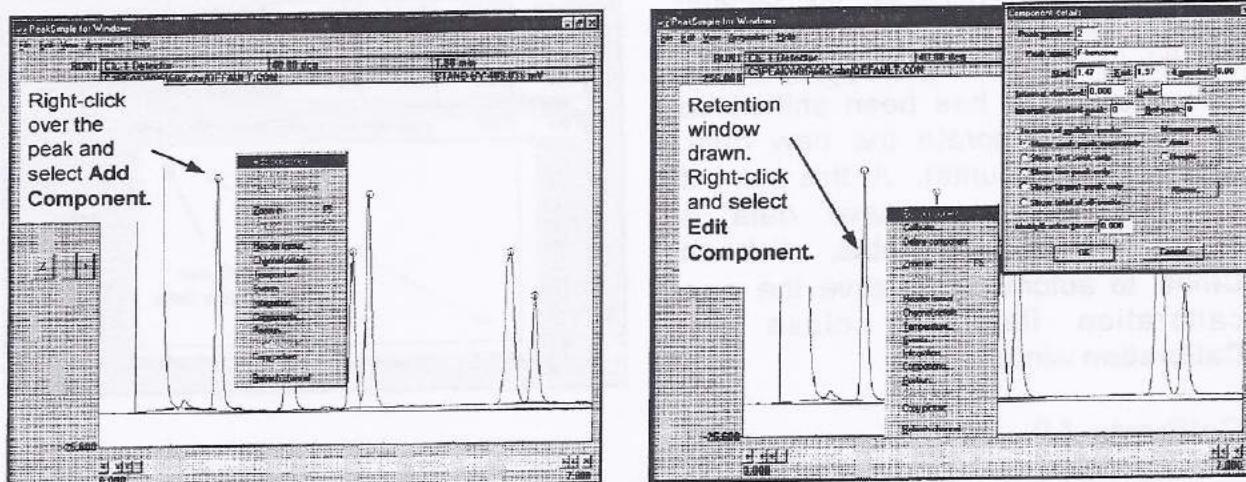


Calibration Screen Shortcuts

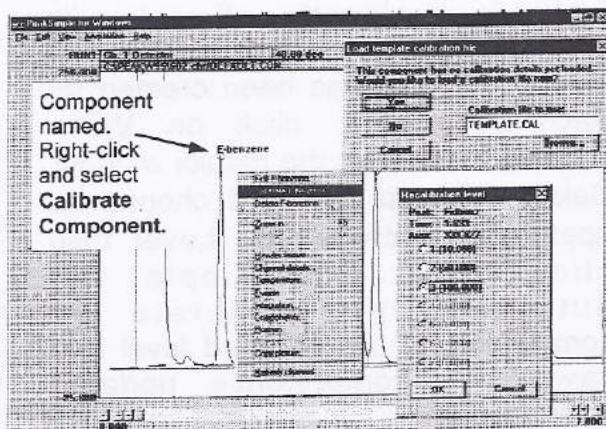
As an added convenience, PeakSimple for Windows offers shortcuts to commonly used screens. These shortcuts may be accessed by pointing to the desired channel and **clicking once on the right mouse button**. The following pages describe the shortcuts available to set up calibration tables and calibrate 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 calibration file name is derived from the first eight letters of the component name. The F-benzene calibration file would be named F-benzen.cal.



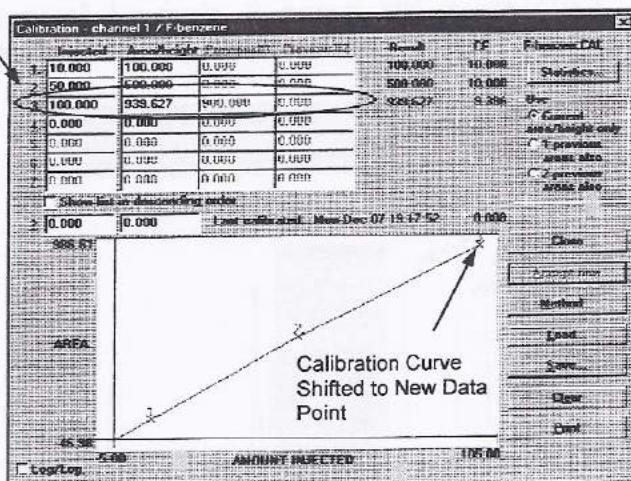
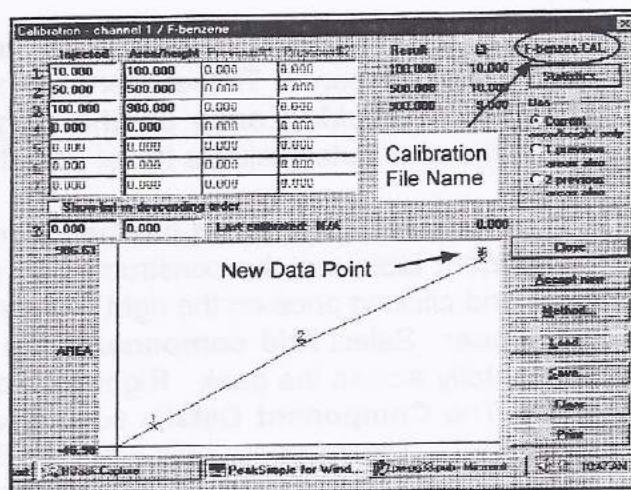
Right-click over the peak again and select **Calibrate**. If no calibration curve exists for the peak, a window will open asking if you would like to use a calibration file. PeakSimple offers a template calibration file aptly named **TEMPLATE.CAL**. Click yes to use the default **TEMPLATE** calibration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

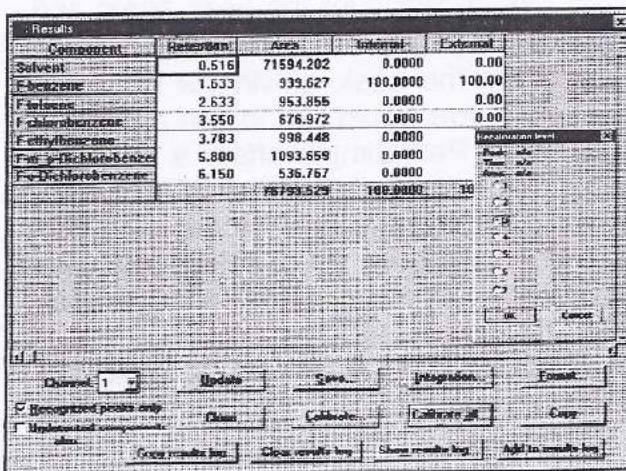
Click **OK** to accept the **Recalibration Level**. The Calibration screen will open and a flashing asterisk (*) will appear along the existing calibration curve depicting the new data point. Notice that the calibration curve has been named **F-benzen.CAL**. If the new calibration data point is acceptable,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



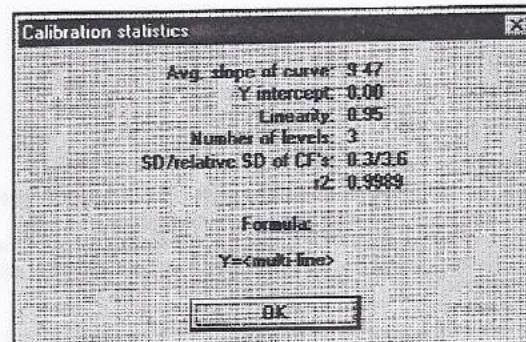
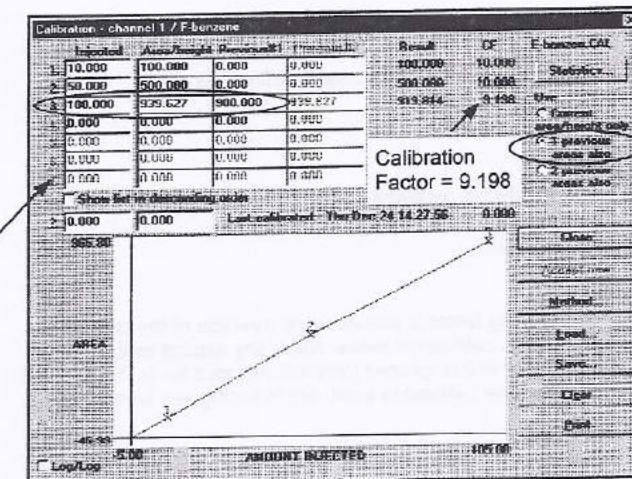
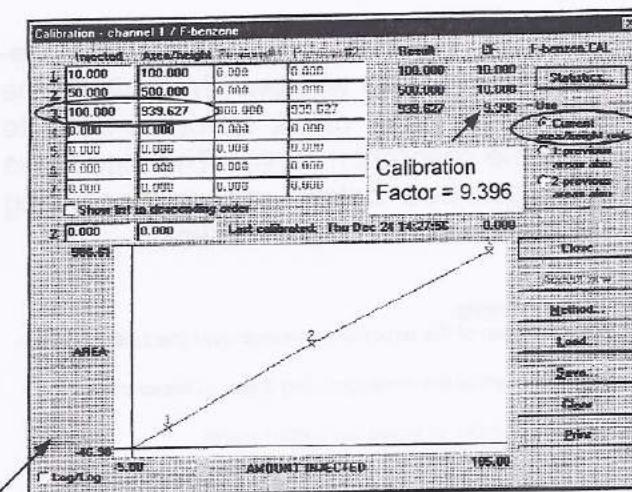
Calibration Screen – Use and Statistics Radial Buttons

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

$$A = ((XY * n) - (X * Y)) / D$$

$$B = ((X * Y^2) - (XY * X)) / D$$

$$D = (X^2 * n) - (X * X)$$

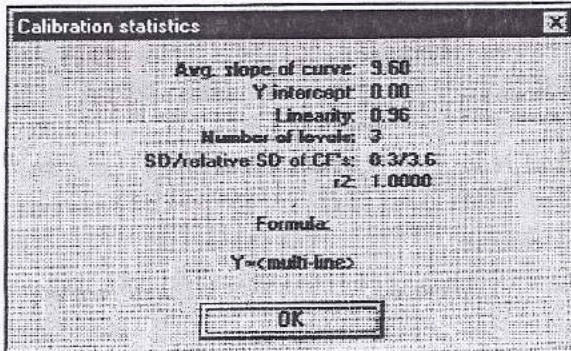
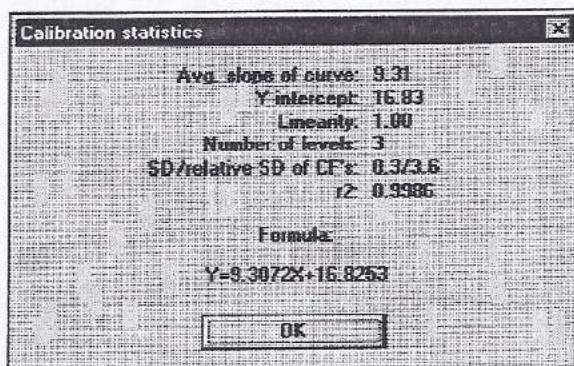
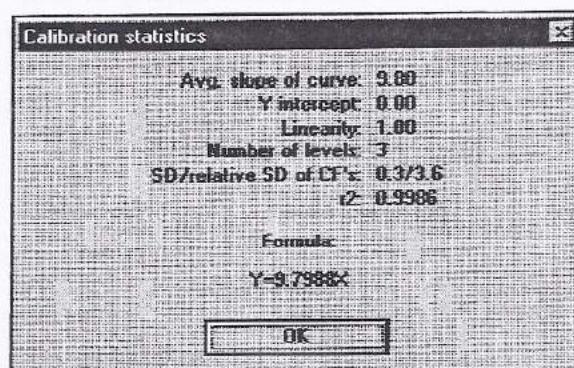
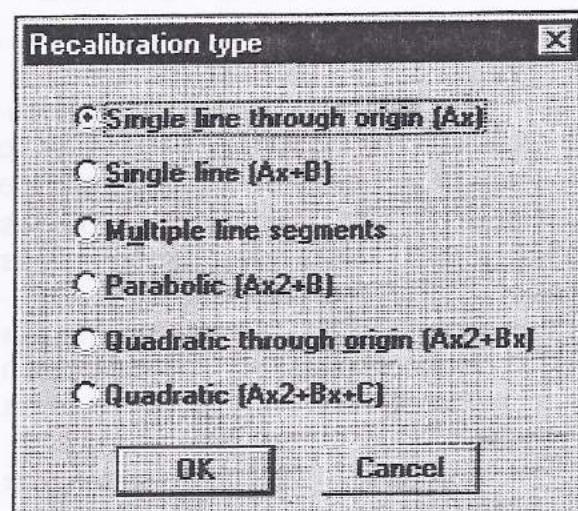
Notes:

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

$$y = Ax^2 + B$$

where:

x is external measure

y is area

$$A = (X_2 Y * n) - (Y * X_2) / D$$

$$B = (Y * X_4) - (X_2 Y * X_2) / D$$

$$D = (X_4 * n) - (X_2 * X_2)$$

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

$$y = Ax^2 + Bx$$

where:

x is external measure

y is area

$$A = (XY * X_3) - (X_2 Y * X_2) / D$$

$$B = (XY * X_4) - (X_2 Y * X_3) / D$$

$$D = (X_3 * X_3) - (X_4 * X_2)$$

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

$$y = Ax^2 + Bx + C$$

where:

x is external measure

y is area

$$A = (XY * X - Y * X_2) * (X_2 * X_2 - X * X_3) - (X_2 * Y * X_2 - XY * X_3) * (X * X - X_2 * n) / D$$

$$B = (XY * X_2 - Y * X_3) * (X_2 * X_3 - X * X_4) - (X_2 * Y * X_3 - XY * X_4) * (X_2 * X_2 - X * X_3) / E$$

$$C = (XY * X_2 - Y * X_3) * (X_3 * X_3 - X_2 * X_4) - (X_2 * Y * X_3 - X * X_4) * (X_2 * X_2 - X * X_3) / F$$

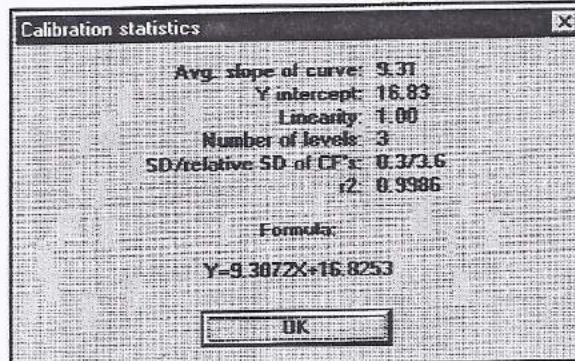
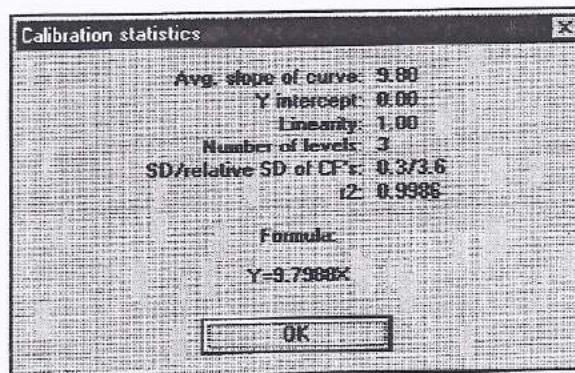
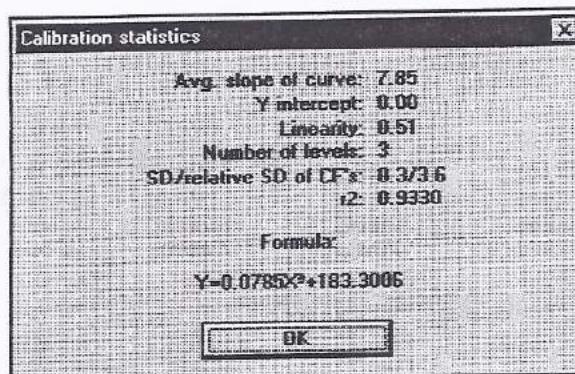
$$D = (X_3 * X - X_2 * X_2) * (X_2 * X_2 - X * X_3) - (X_4 * X_2 - X_3 * X_3) * (X * X - X_2 * n)$$

$$E = (X_2 * X_2 - X * X_3) * (X_2 * X_3 - X * X_4) - (X_3 * X_3 - X_2 * X_4) * (X * X_2 - X * n)$$

$$F = (X * X_2 - X_3 * n) * (X_3 * X_3 - X_2 * X_4) - (X_2 * X_3 - X * X_4) * (X_2 * X_2 - X * X_3)$$

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).



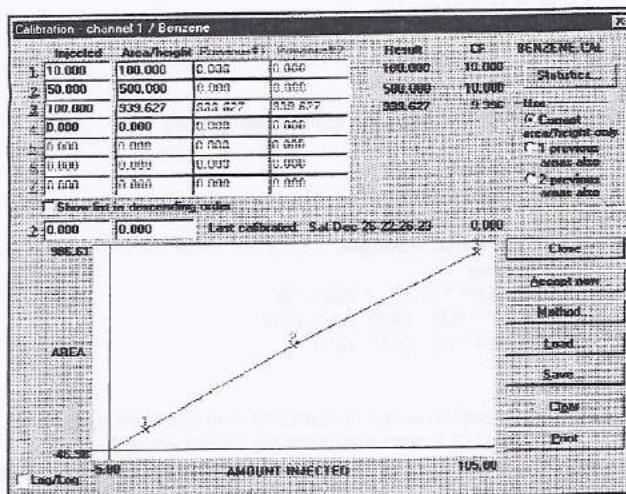
The Calibration Window (continued)

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

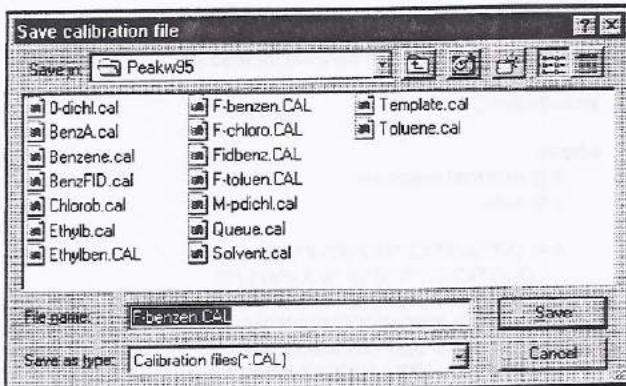
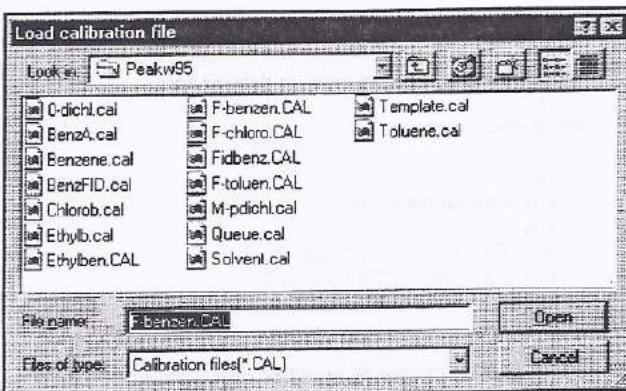
Click on the **Save** button to save a new calibration file, or to update an existing one. Remember to always use the .CAL extension when naming the calibration file. The saved file name appears at the top of the calibration window indicating the file in use.

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.

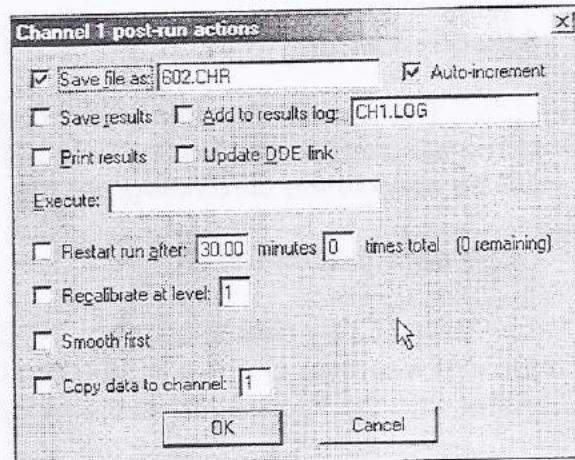
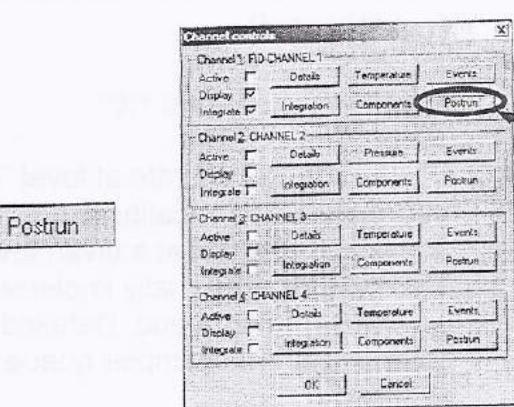
The Save results checkbox when selected will save the data in the results screen to disk after a chromatogram run (*Note: This is not the raw data but instead is the ASCII results*). The Add to results log "X" checkbox adds the results of a run to the results log specified in the information field to 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"

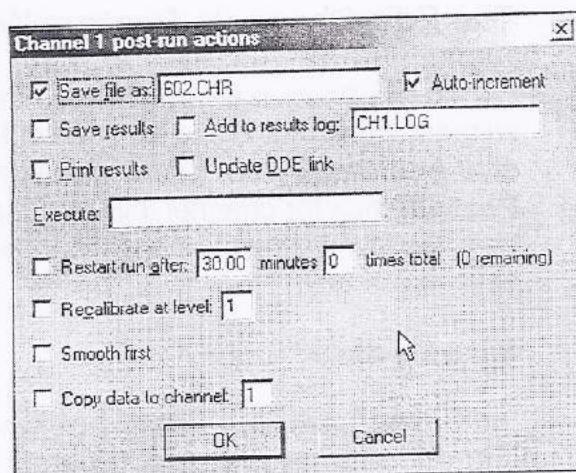
The Restart run after "X" checkbox and information field restarts a chromatogram run after an inputted delay time. The delay time is inputted in minutes and can be repeated as many times as is entered into the times total information field. *Note: If 0 is entered into the times total information field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

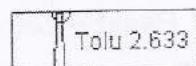
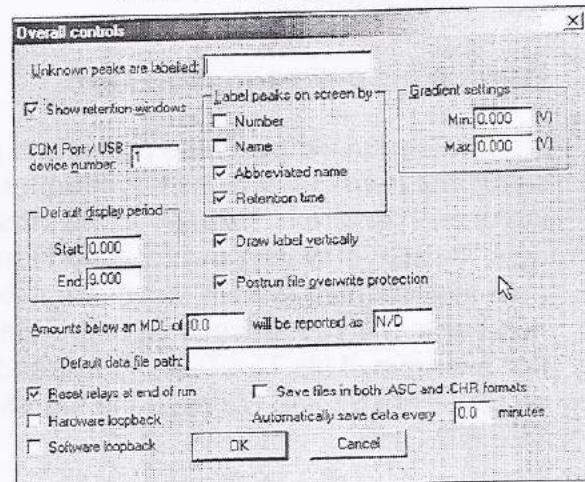
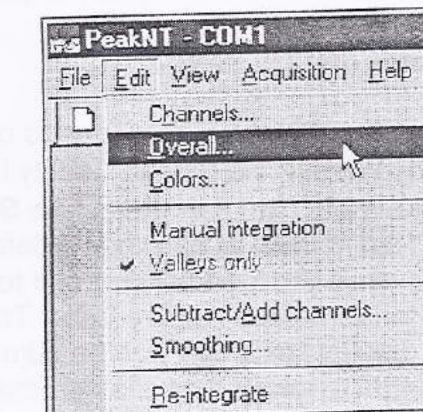
Unknown peaks are labeled "X"

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 **Name** checkbox labels all peaks with their peak number. 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.

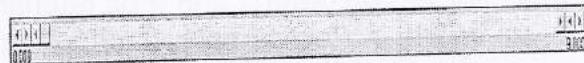
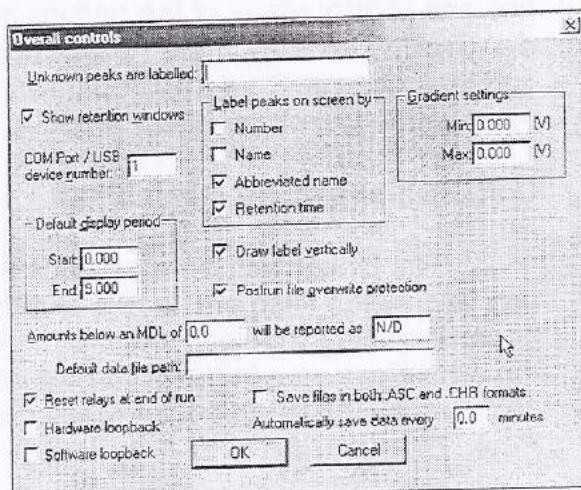
Default data file path

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.



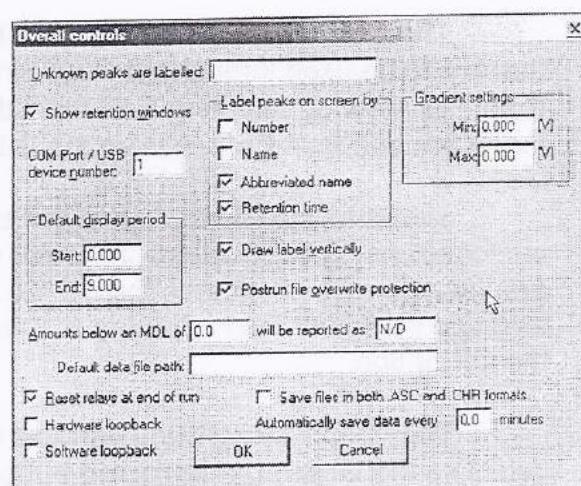
The Edit-Overall Window (continued)

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.

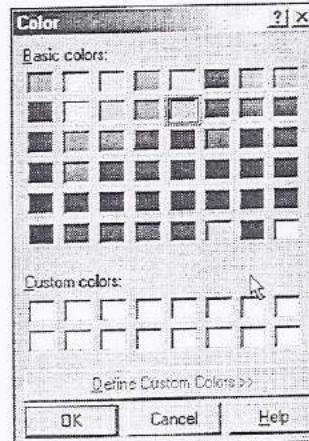
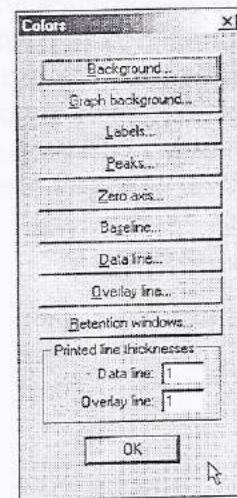
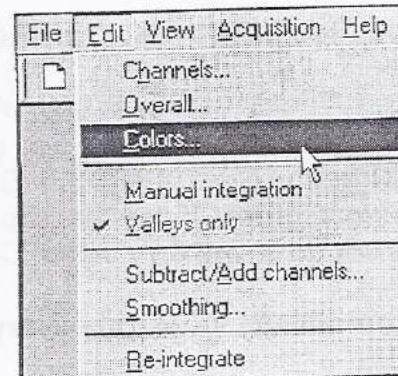
The color of the labels controls the color of the words that belong to the peaks. The color of the labels is changed by selecting the **Labels** button to open up the Labels color window. In the Labels color window select a color and then press on the OK button to make the change to the labels color.

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.



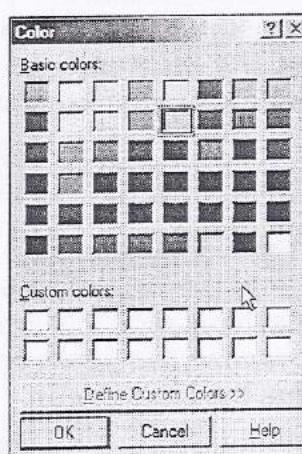
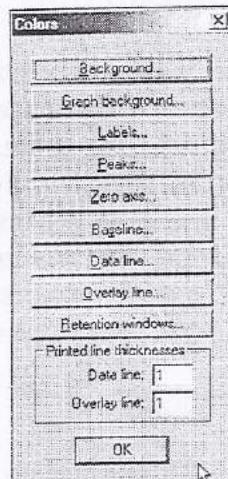
The Edit-Colors Window (continued)

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

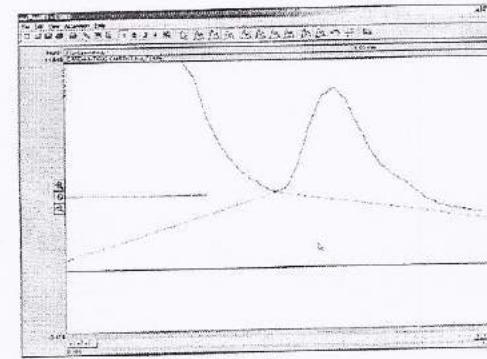
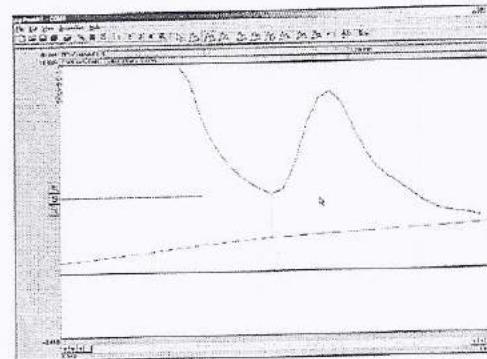
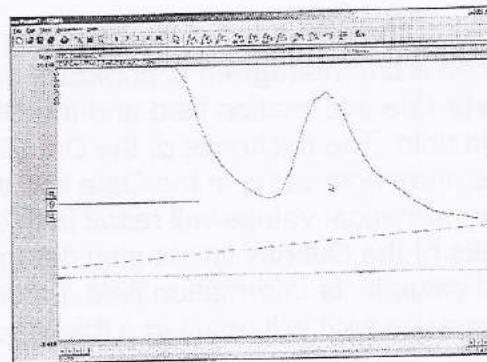
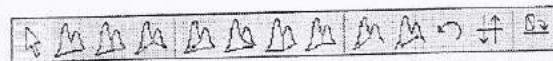
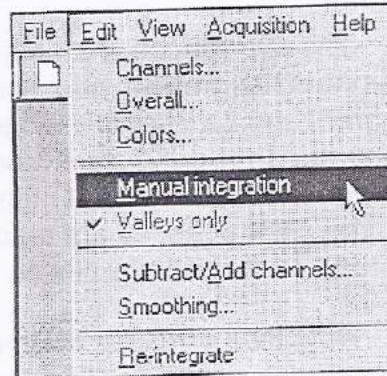


The Drop integration tool drops the baseline between two peaks straight down onto an existing baseline. The Drop integration tool is used by selecting the Drop tool in the manual integration toolbar and then clicking on a valley between two peaks to change the baseline.

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.

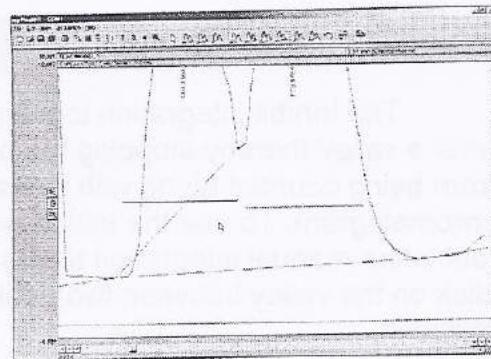


Manual Integration (continued)

Lead Skim Integration Tool



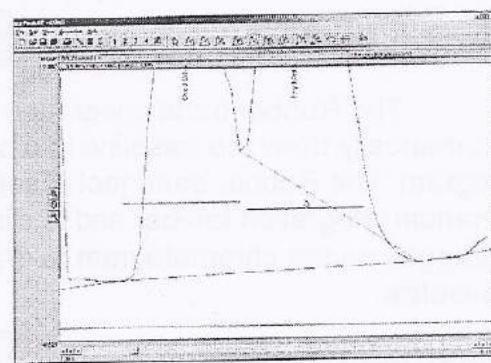
The Lead skim integration tool skims a peak's area off of the leading edge of an adjacent peak. To skim a peak off of the leading edge of another peak select the Lead skim tool from the manual integration toolbar and then click on the valley between the two specified peaks with the mouse cursor.



Trail Skim Integration Tool



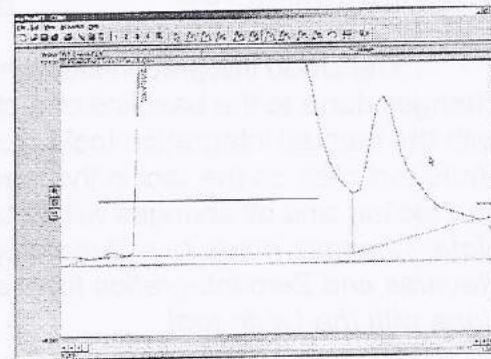
The Trail skim integration tool skims a peak's area off of the trailing edge of another, adjacent peak. To skim a peak off of the trailing edge of another peak select the Trail skim tool and click on a valley between two peaks with the mouse cursor to make the change.



Lead Horizontal Integration Tool



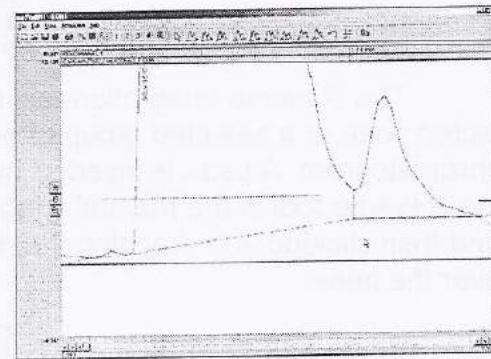
The Lead horizontal integration tool draws the baseline horizontally for the leading peak while the trailing peak's baseline stretches from the horizontal line to the next valley. The Lead horizontal tool is selected in the manual integration toolbar and once a valley is selected the change to the baseline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

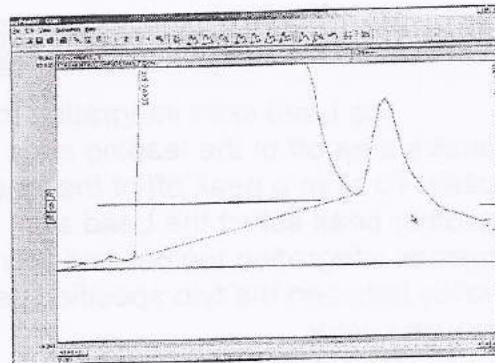


Manual Integration (continued)

Inhibit Integration Tool



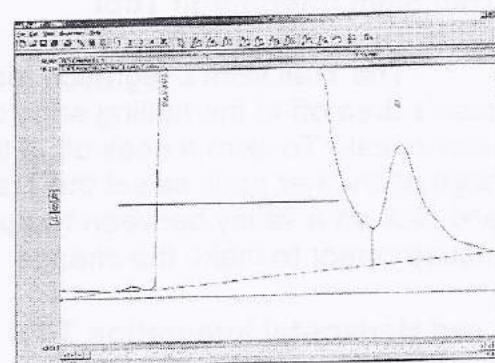
The Inhibit integration tool ends a baseline after a valley thereby stopping the peak's area from being counted along with the rest of the chromatogram. To use the Inhibit tool select the tool in the manual integration toolbar and then click on the valley between two peaks to end the baseline.



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.

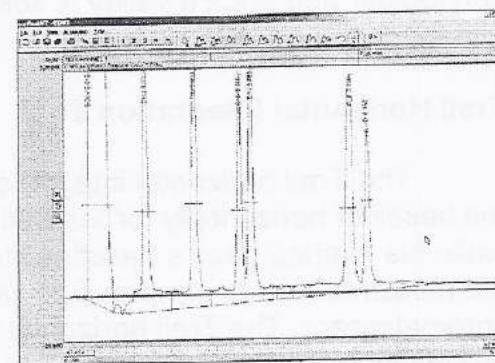


Undo Integration Tool



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.

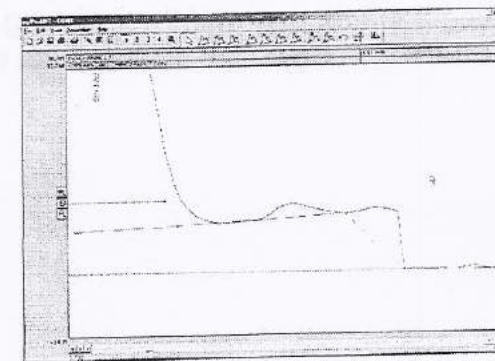
Note: Changes made to a chromatogram with the Reverse and Zero integration tools cannot be undone with the Undo tool.



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.



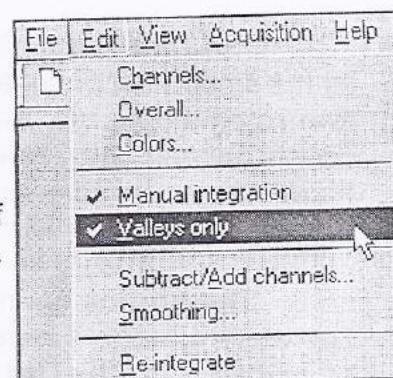
Zero Integration Tool



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.

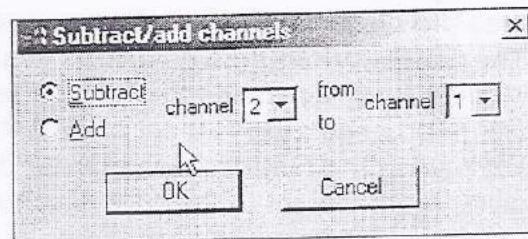
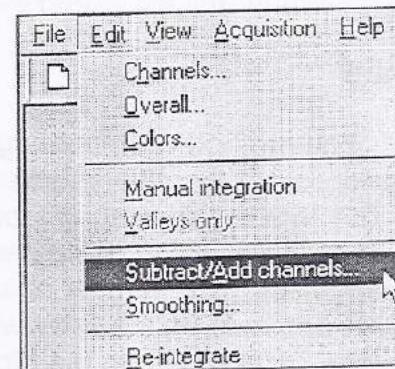


The Edit-Subtract/Add Channels Menu

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.

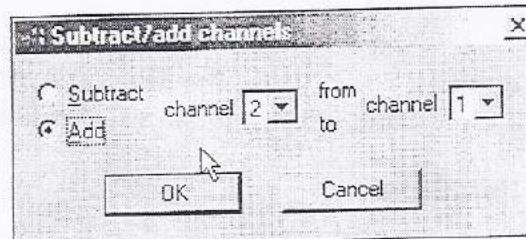
Subtracting a Channel

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.

