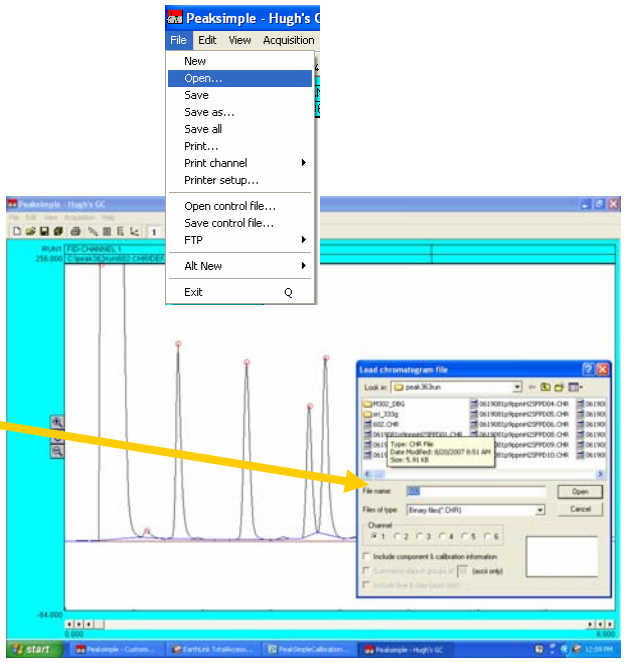
PeakSimple Calibration Tutorial
August 2008

Launch PeakSimple software. You do not need the A/D hardware connected, but it is OK if it is.

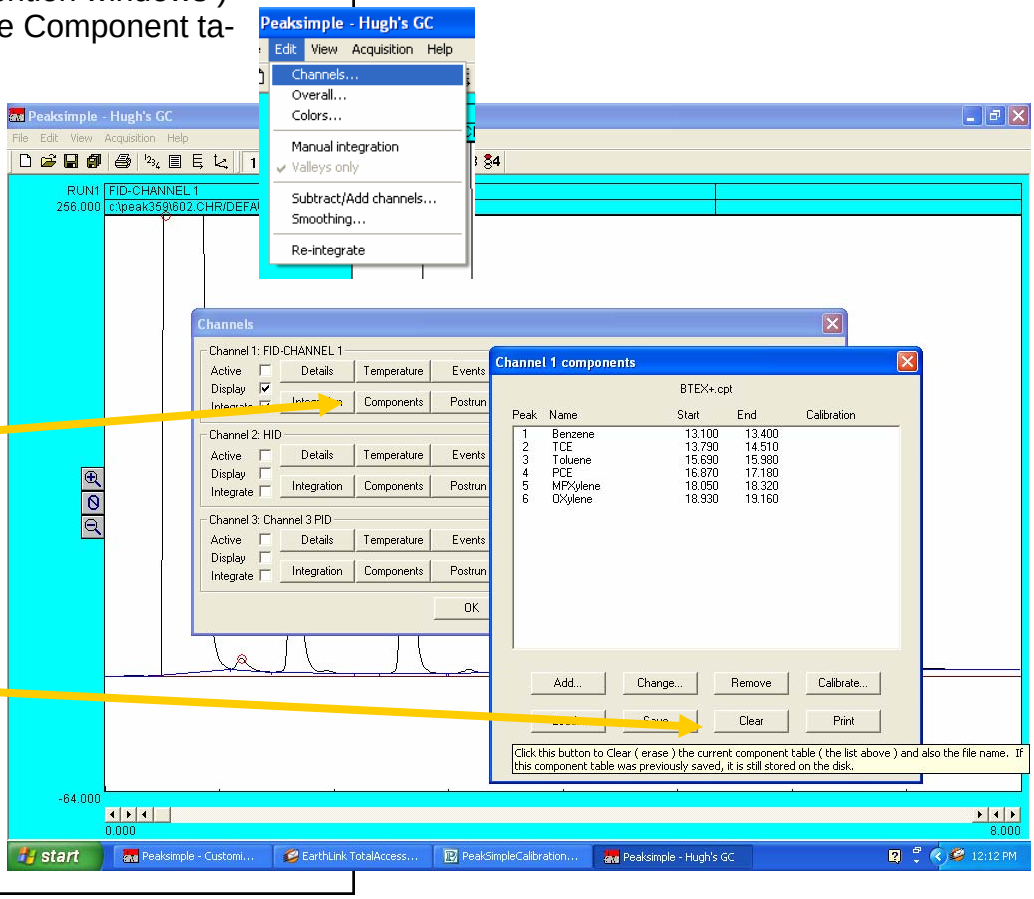
Load an example chromatogram from the PeakSimple folder. If you have installed PeakSimple version 3.65, then the PeakSimple folder will most likely be c:\peak365. To load the example chromatogram use your mouse to click File/ Open and then the filename. For this tutorial, load the file named 602.chr.



There may already be a Component table loaded with associated retention windows. For this tutorial we want to start from scratch (no pre-existing component table or retention windows) so let's delete the Component table.

Click with your mouse on Edit/ Channels. This will open the Channels screen. Click on the box labeled Components. This will open the Channel 1 Components screen.

Click the button labeled Clear. Then return to the main screen.



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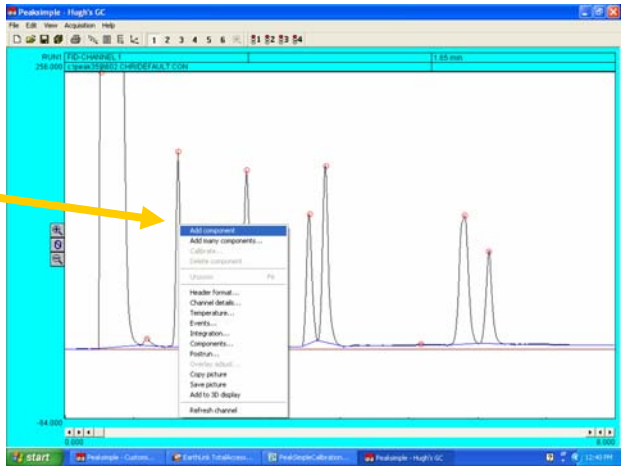
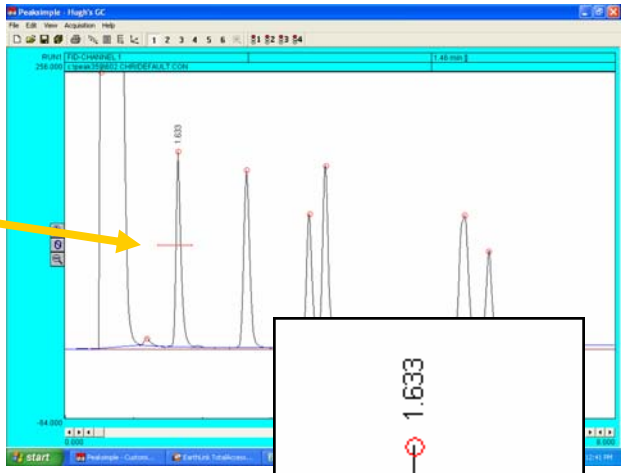
Point to the first analyte peak (not the large Solvent peak) with your mouse. This peak has a retention time of about 1.633 minutes.



Click the **RIGHT** mouse button and a small menu window will pop up on the screen.

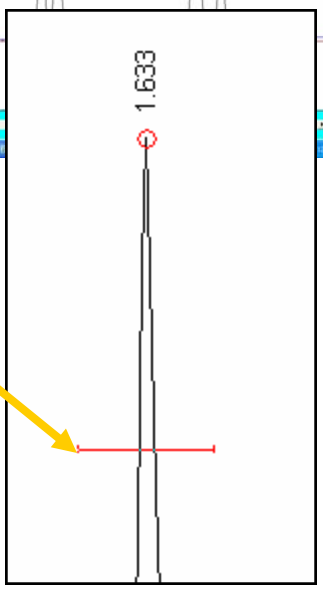
Point to the words Add Component and click with the **LEFT** mouse button.

This will result in a horizontal line centered where your mouse was pointing. Hopefully this horizontal line (called a retention window) is roughly centered on the first analyte peak.



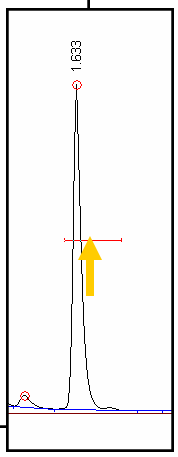
Don't worry if the retention window is not perfectly centered, we are going to adjust it in the following step.

Notice that the retention window bar has vertical uprights at each end of the bar.



Grab the vertical handle by pointing to the upright with your mouse and holding down the **LEFT** mouse button and dragging the handle left or right. Use this to make the retention window wider or narrower.

Next grab the retention window bar in the center of the line. Now you can move the entire bar left or right with your mouse.



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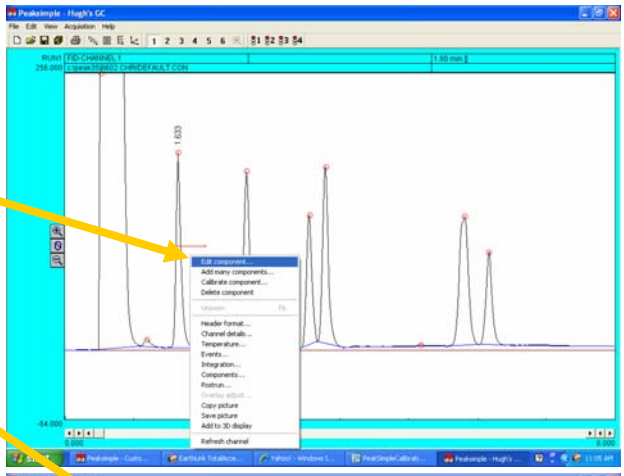
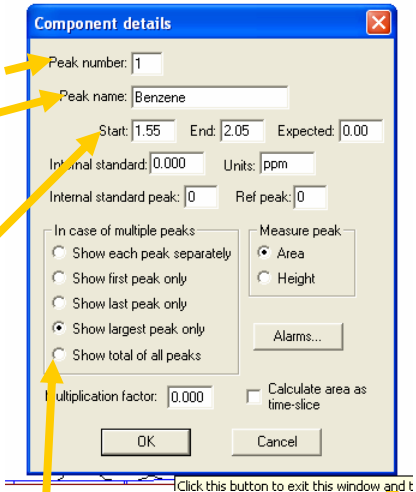
Point to the peak again with your mouse and **RIGHT** click to pop up the menu. Click the words Edit Component with the **LEFT** mouse button. (Short Cut=double click under the peak)

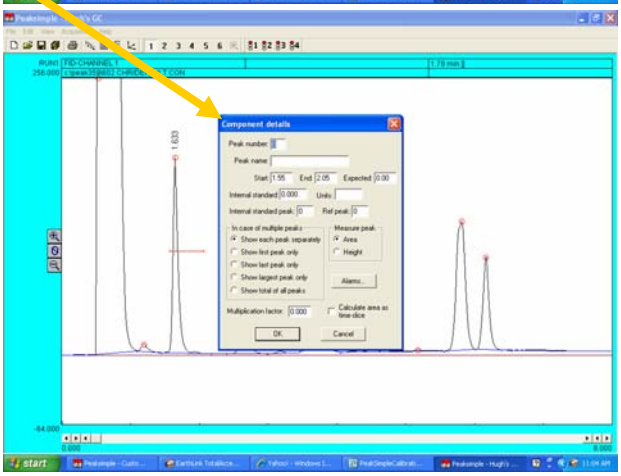


The Component Details menu then appears.



Enter a peak number and Peak name.

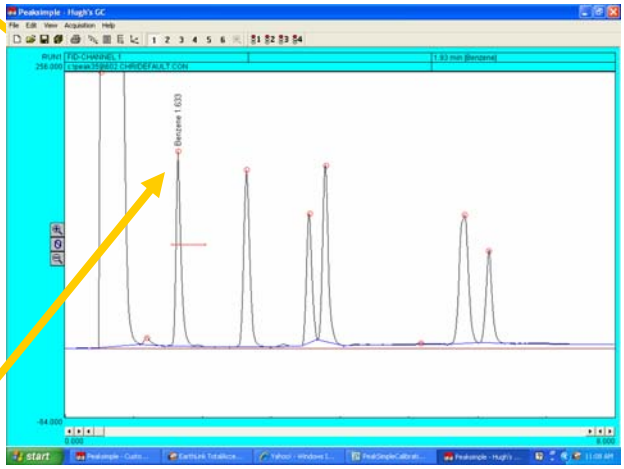
Notice that the start and end of the retention window is already entered from your dragging the uprights around.



Click the radio button labeled “Show Largest Peak Only” to select the biggest peak whose apex falls into the window. This avoids having a small noise peak erroneously identified as Benzene.

Enter “ppm” or “%” or “mg/kg” or any other unit in the Units box.

When you click OK, the peak on the screen is now labeled Benzene



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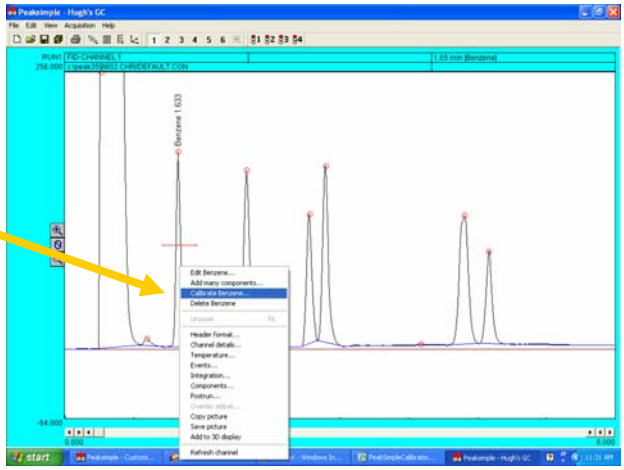
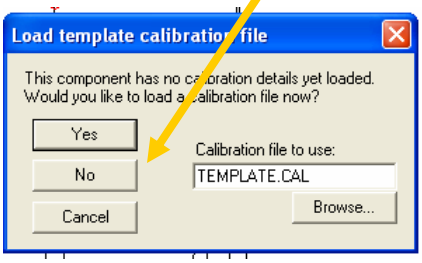
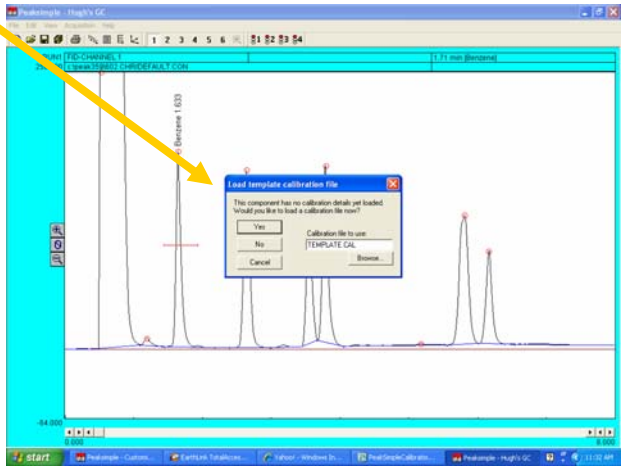
Point to the Benzene peak and click with the **RIGHT** mouse to pop up the menu. Click with the **LEFT** mouse button on the words Edit Benzene.

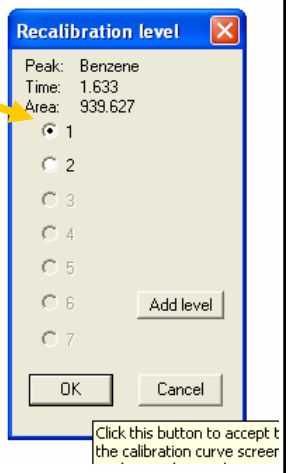


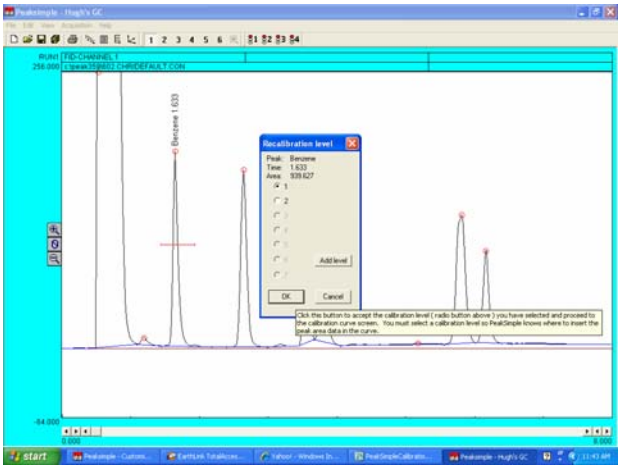
A menu pops up wanting to know if you wish to copy an existing calibration curve. This is a convenience if you are calibrating many similar peaks. Click the No button for this tutorial.





The Recalibration Level menu then appears. Leave the radio button selected for Level 1





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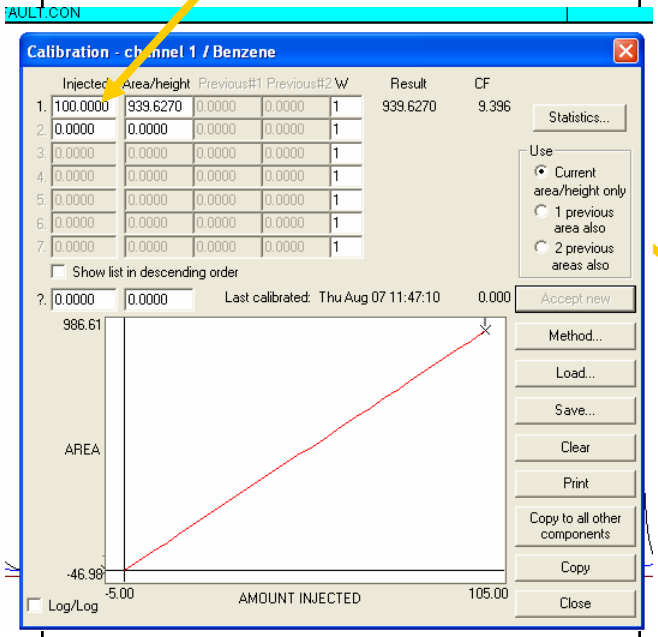
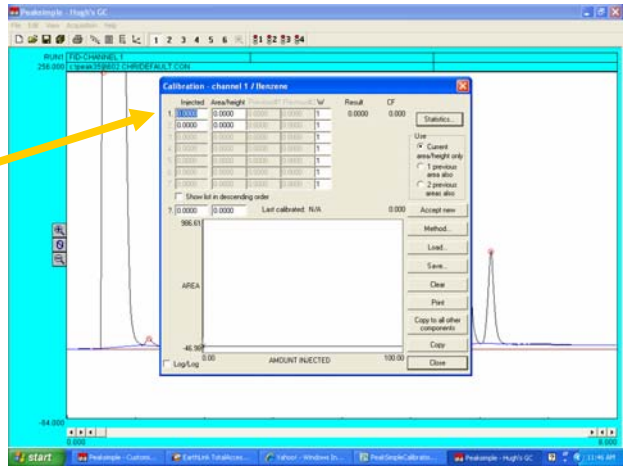
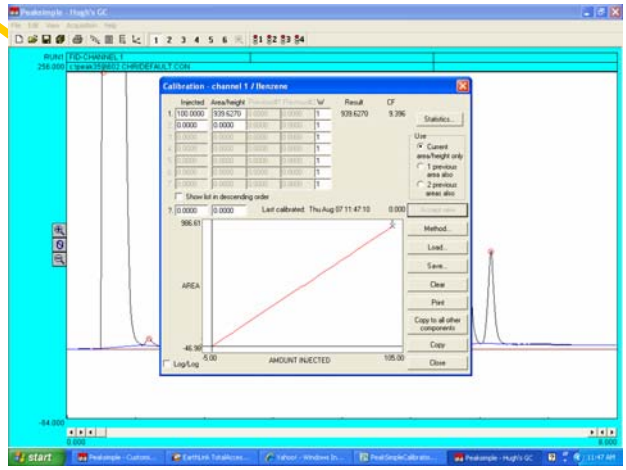
This takes you to the blank calibration curve.

Enter the amount of Benzene injected in the top left cell of the spreadsheet

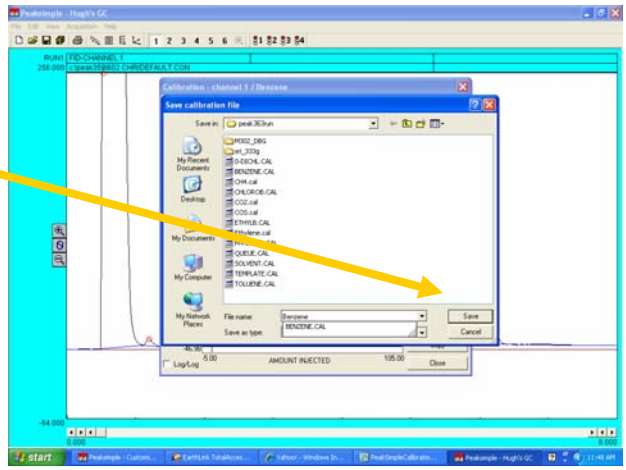






Then click the Accept New button. The peak area for the Benzene peak is then automatically inserted into the adjacent cell in the spreadsheet. The graph is also drawn on the screen.

Save the calibration curve by clicking the Save button and then entering a name for the calibration curve. Benzene.cal would be a logical choice but any name is OK. Then click the Close button.



Once you have named the calibration curve it is saved AUTOMATICALLY when you exit the calibration curve screen, so you do not have to Save each time.

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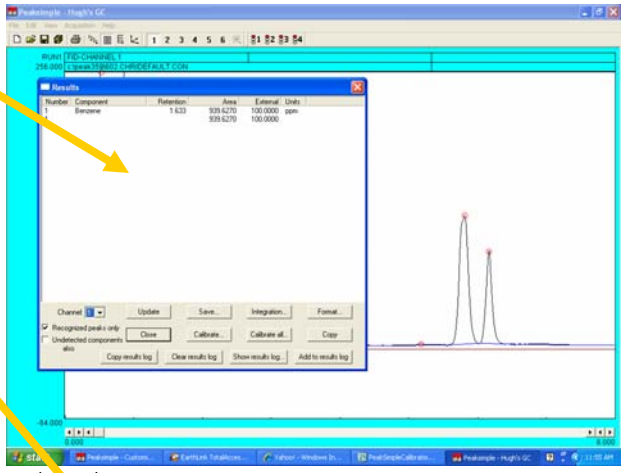
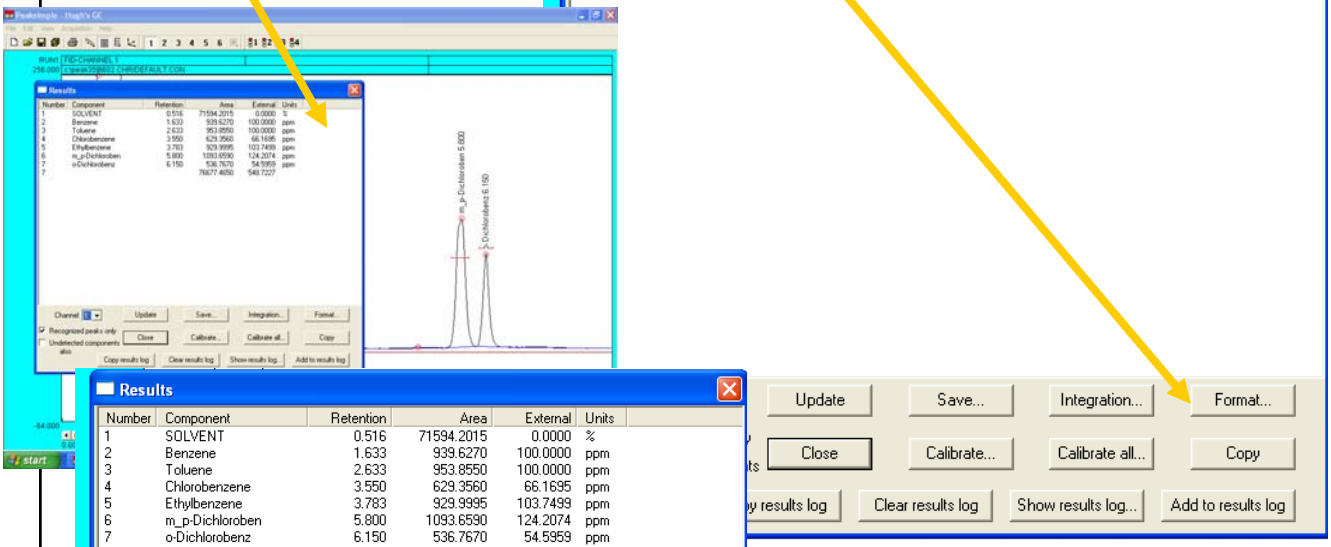
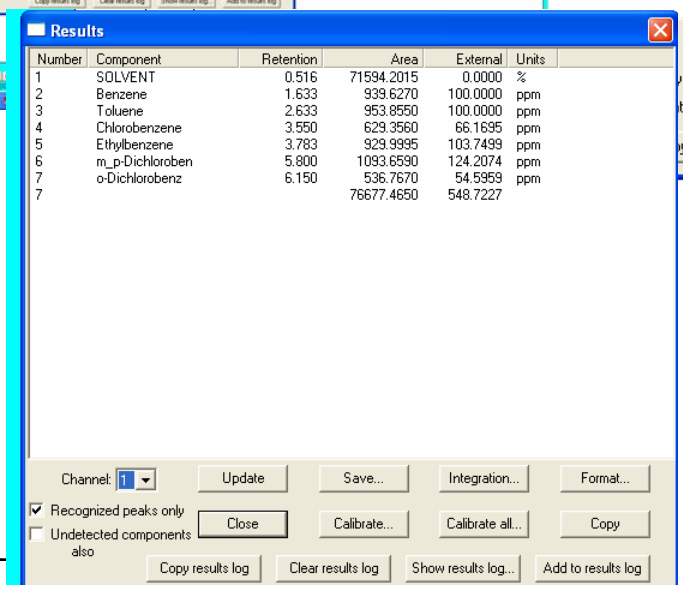
We have now identified and calibrated the Benzene peak. If you click View/ Results the Results window appears.

The Peak number, Peak name, retention time, peak area, External standard result calculated from the calibration curve and Units appear on the report. The report can be formatted differently by clicking on the Format button.

Once you repeat this process for **each peak** in the analysis, the Results screen will display a report which includes multiple lines. One for each peak.







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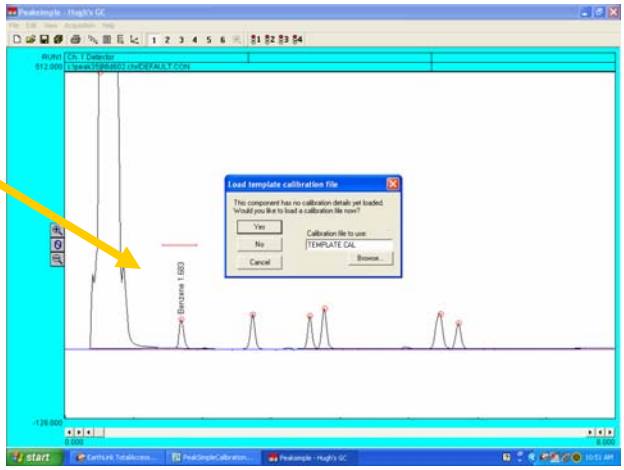
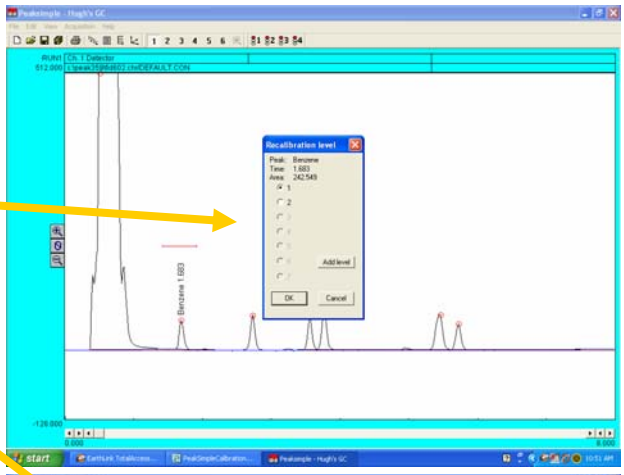
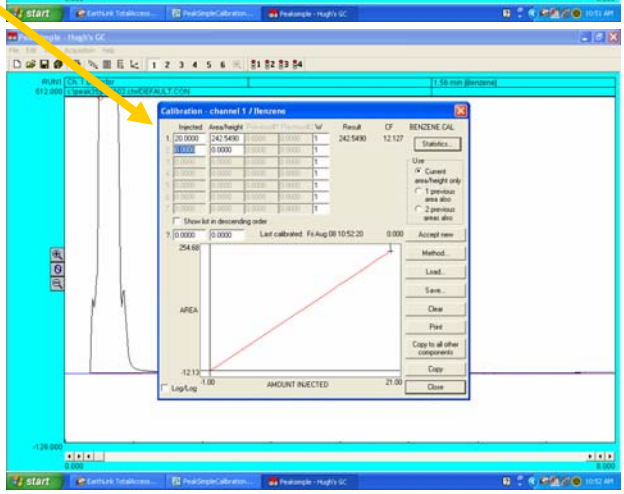
To create a **Multi-Level Calibration Curve,** load the chromatogram which is your Level 1 (lowest concentration) standard. You can also just inject the Level 1 standard and perform the calibration once the run is ended.
Click on the peak and go through the same steps as described previously.

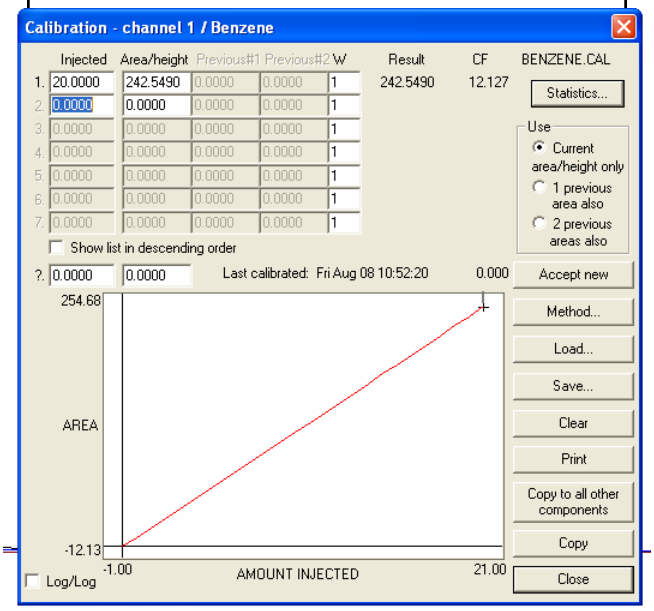


Select Level 1 in the Recalibration Screen.



Enter the Level 1 amount injected (20 in this example),
and then click Accept New.





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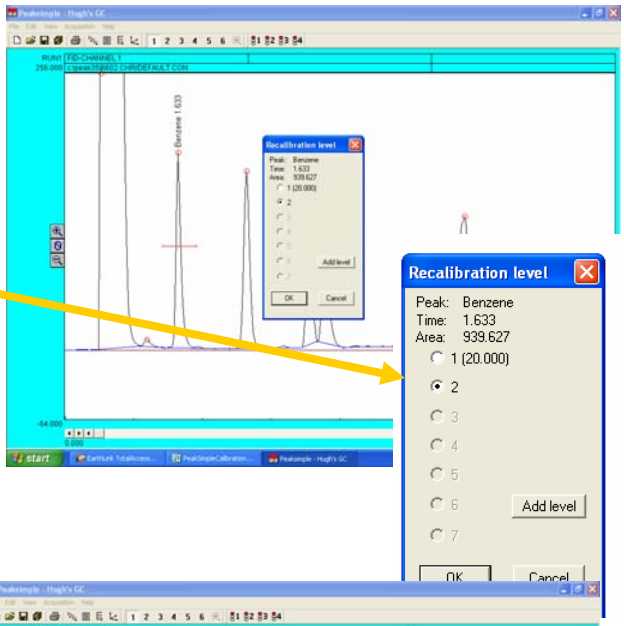
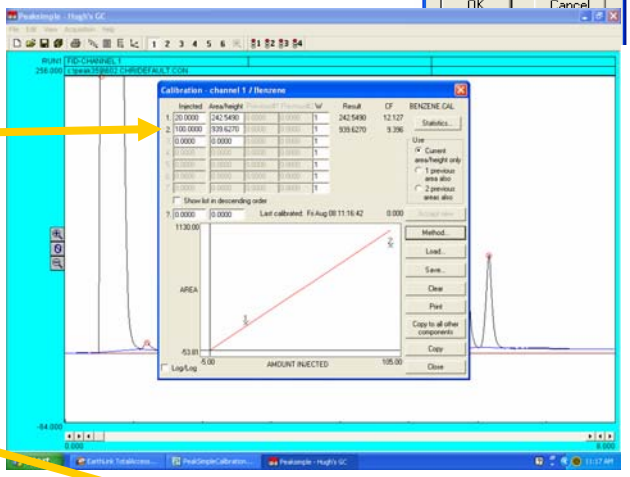
To calibrate at Level 2, load or run the Level 2 calibration standard. Click on the peak and select Level 2 in the Re-calibration screen.

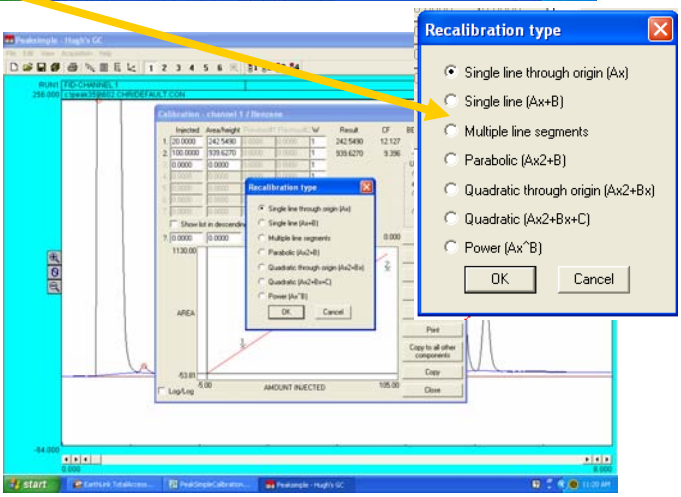


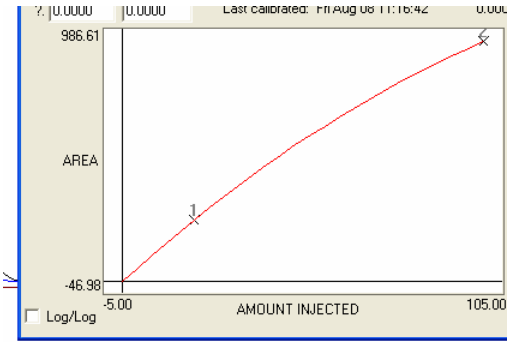
This takes you to the calibration curve. Enter the Level 2 amount injected in the second row, first column of the spreadsheet. Then click the Accept New button.



The graph is drawn to show the two level calibration.

With two or more calibration levels, you now have a choice of calibration methods (models). Click the Method button to display your choices. If you click Quadratic through Origin for example, the graph shows a curve instead of a straight line.





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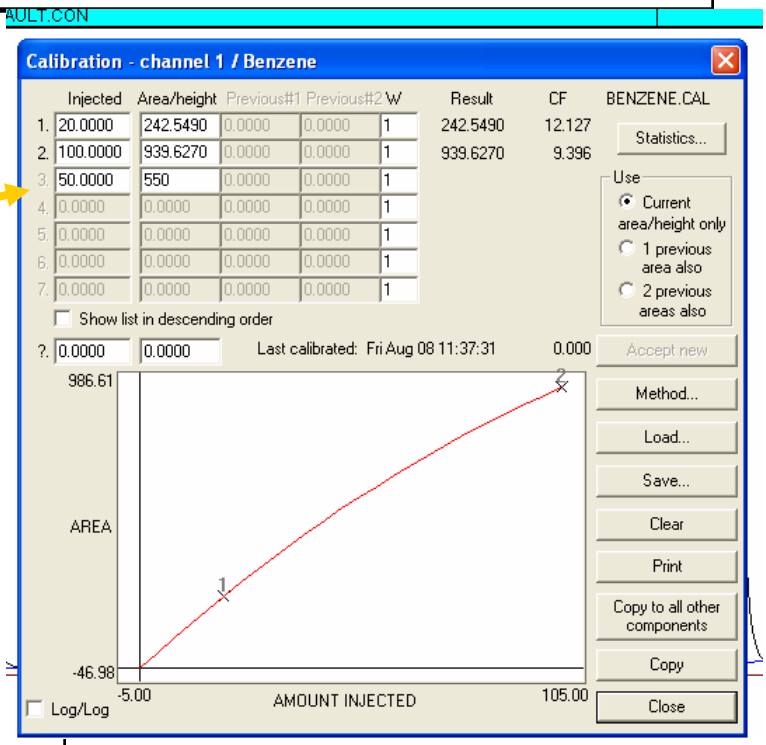
You MUST enter the calibration data in ASCENDING or DESCENDING order. If you try to enter a calibration point out of order as shown at right, PeakSimple will not let you proceed. The Level 3 amount (50) is less than the Level 2 amount (100) so PeakSimple will not allow you to enter this).



The Area/Height must also be greater for a Level 3 than for a Level 2. The example at right shows the Level 3 area (550) is less than the Level 2 area (939.6270). PeakSimple will not accept this.