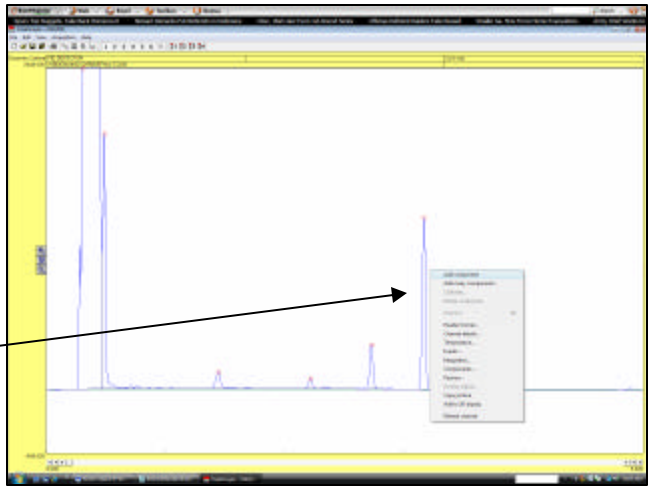
Performing Internal Standard Calibration using PeakSimple version 3.51

For this example, we will create a 5 point calibration curve for Glycerin using Butanetriol as the Internal Standard.

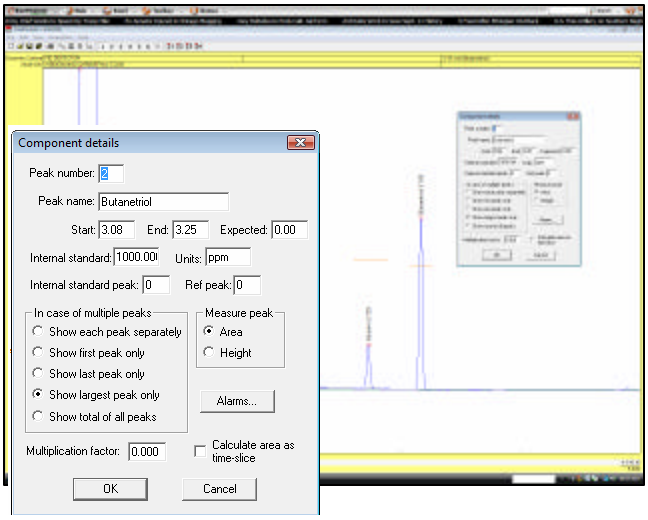
Load the level 1 chromatogram into channel 1. (File/Open)

Identify the Butanetriol Internal Standard peak by using the mouse to point to the Butanetriol peak. Click on the right mouse button, then click Add Component.

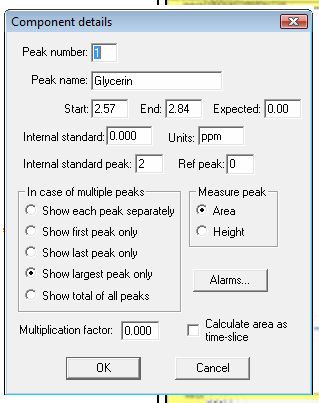


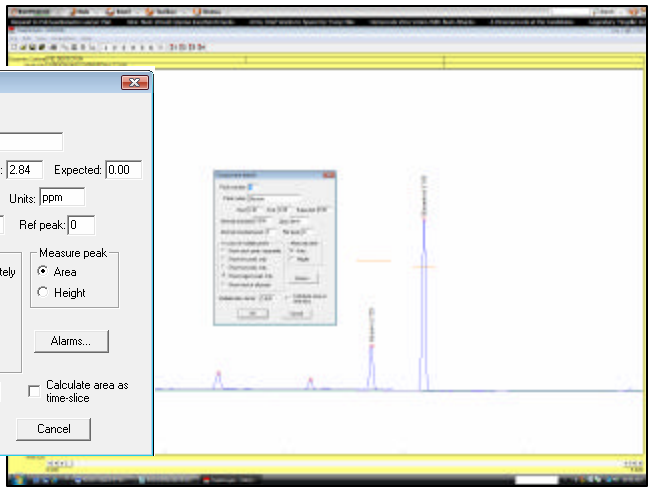
Point to the Butanetriol peak again, right click, then select Edit Component (you can just double-click the peak as a shortcut).

Enter the Component Details especially note that Peak Number is 2 and the Internal Standard is 1000. The Internal Standard is the amount of Butanetriol which you know to be in the sample. In this case it is 1000 ppm.



Go through the same process for Glycerin. Note that the peak number is 1, that the Internal Standard box is left at 0.00, but the Internal Standard Peak box is set to 2. By entering the 2, PeakSimple knows that peak number 2 should be the Internal Standard for this peak (Glycerin).





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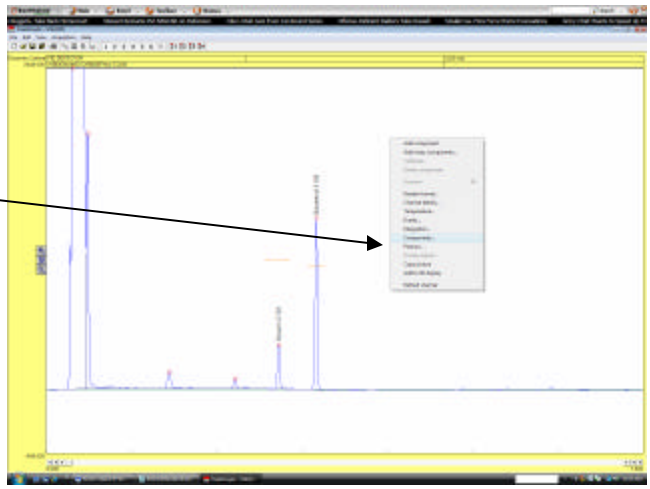
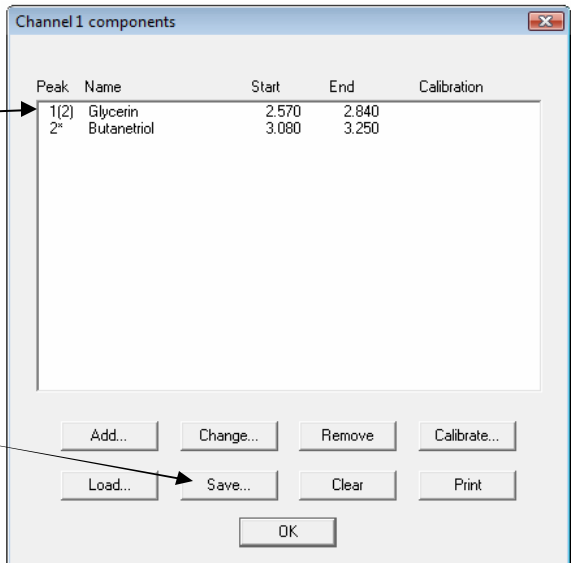
Right click anywhere on the chroma-togram screen and select Components



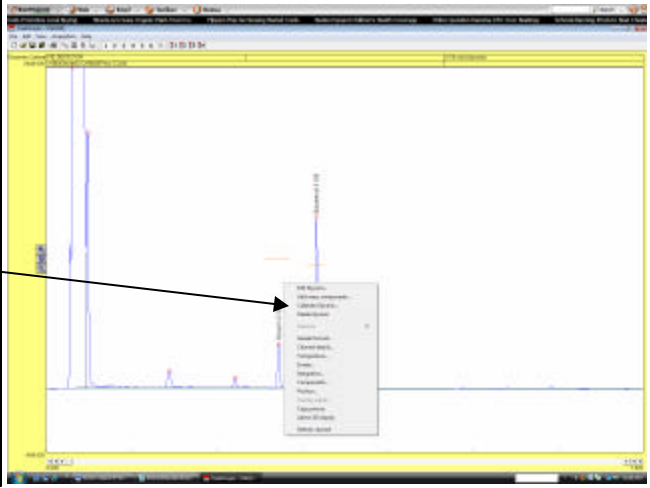
Notice in the Components window that the number 2 is listed in parentheses next to the peak number 1.

This is how PeakSimple reminds you that peak number 2 (Butanetriol) is the Internal Standard for peak number 1 (Glycerin).

It is a good idea at this point to save the Component table. Click Save and enter a filename (BioDiesel.cpt for example)



Return to the Chromatogram screen, point to the Glycerin peak with your mouse and right click. Select Calibrate Glycerin from the menu.



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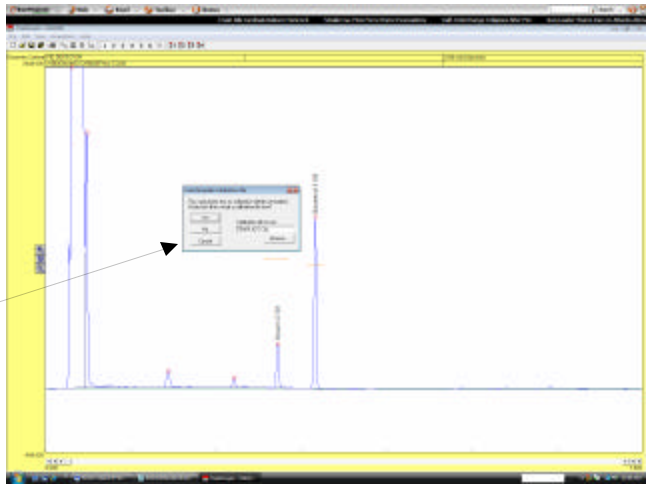
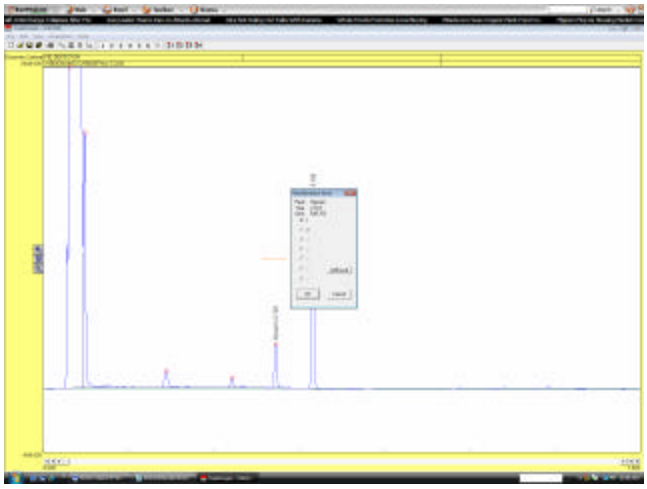
A screen will appear offering to let you copy a Template Calibration file. This is a convenience when creating many calibration curves since you can make a Template calibration curve once and avoid typing in the same information over and over for each similar curve.

We don't need to do this for this example, so just click No.

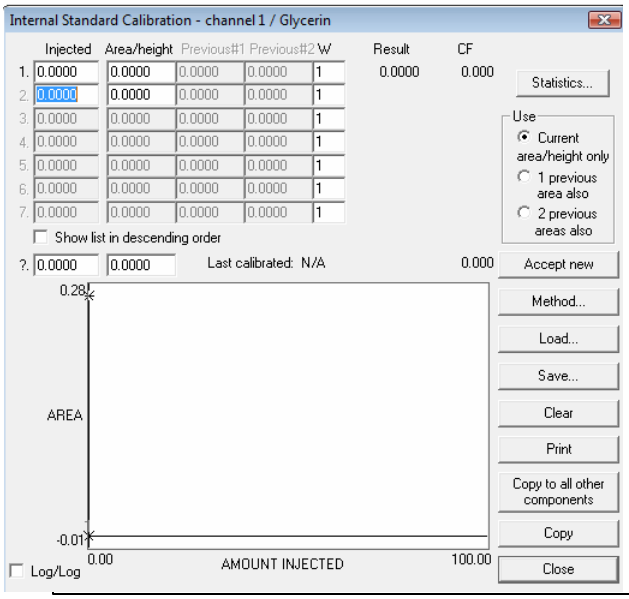


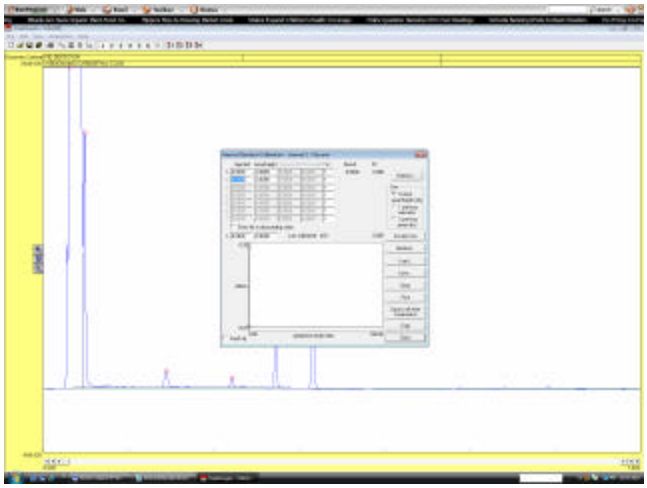
Another screen appears which allows you to specify the calibration level (i.e. Level 1, Level2, Level 3 etc.).

Since we do not as yet have a curve, click the radio button for Level 1 and then OK.



PeakSimple then displays the blank calibration curve





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Enter the amount of Glycerin in the Level 1 standard (the chromatogram on the screen). In this example, the Glycerin is at 100ppm, so enter 100 in the top left cell of the table.

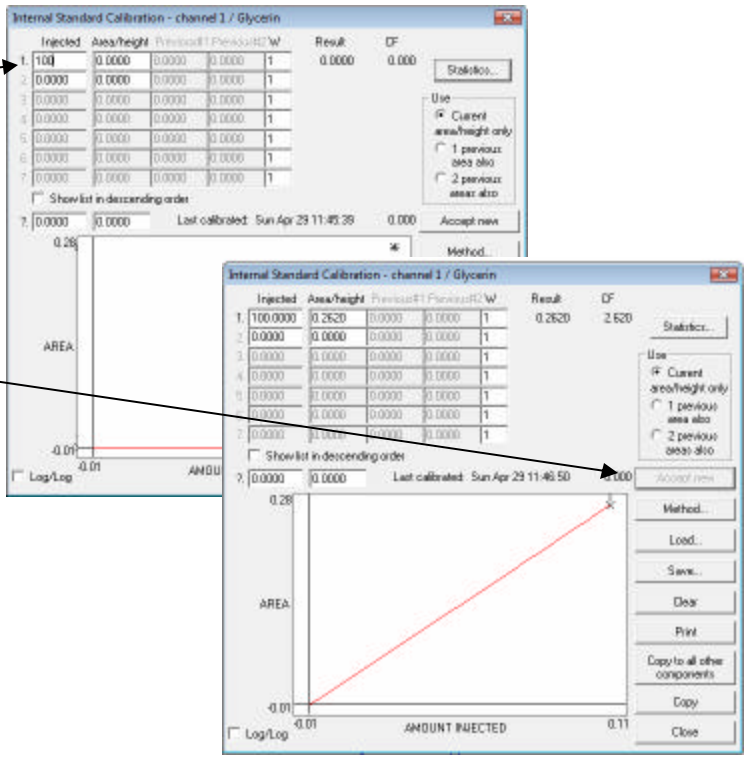
Then click on the button labeled Accept New.

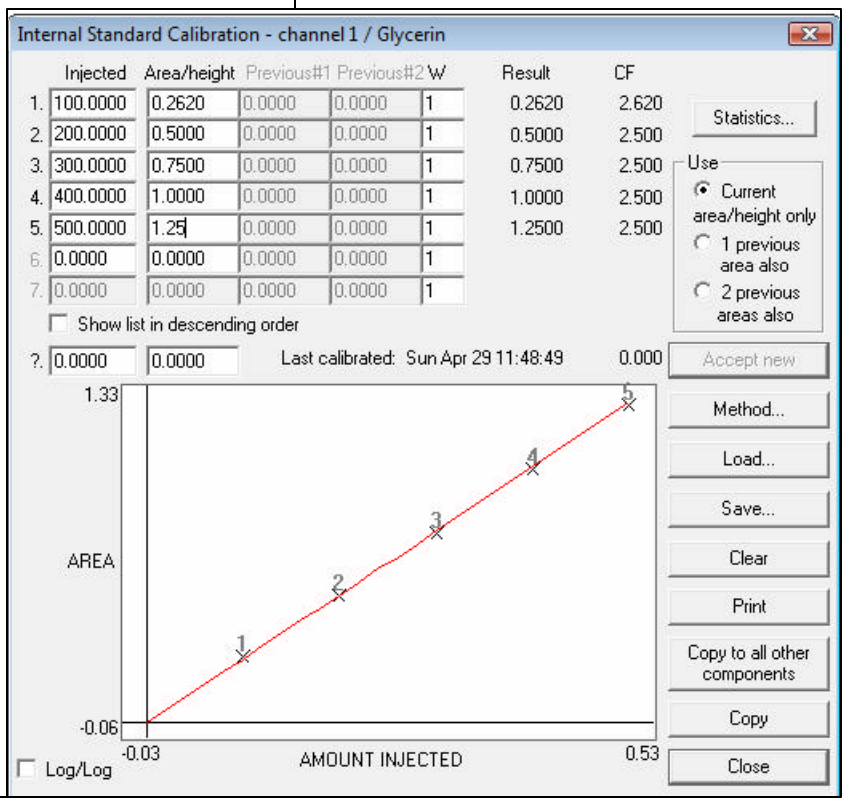
The ratio of the Glycerin peak area divided by the Butanetriol peak area in then entered automatically in the cell immediately to the right of the 100. A single point curve is also drawn on the graph.

It will save time if you enter the remaining Level 2, Level 3 , Level 4 and Level 5 amounts along with estimated ratios. These are just approximately correct numbers and will be replaced when we actually calibrate Level2 through Level 5.

As you do this you will see the multiple points plotted on the graph.

Save the curve by clicking Save





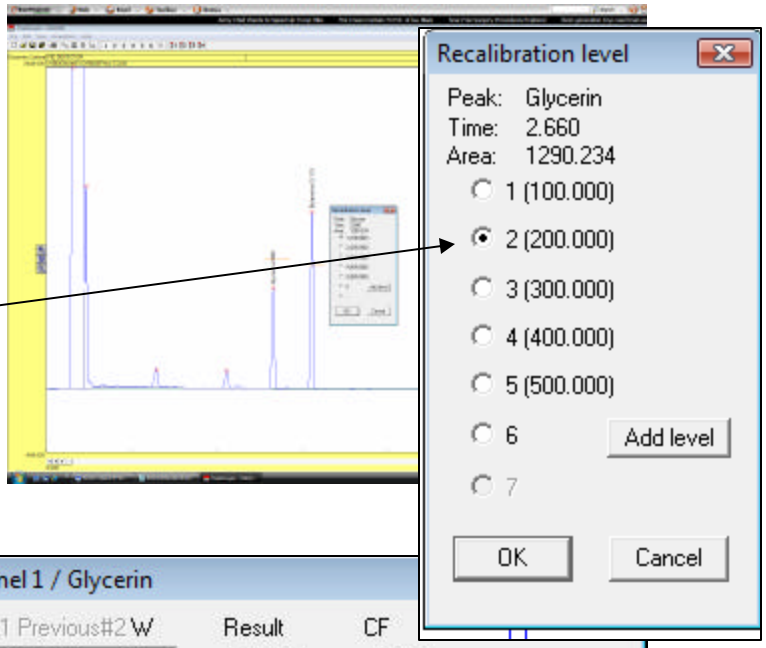
Performing Internal Standard Calibration using PeakSimple version 3.51

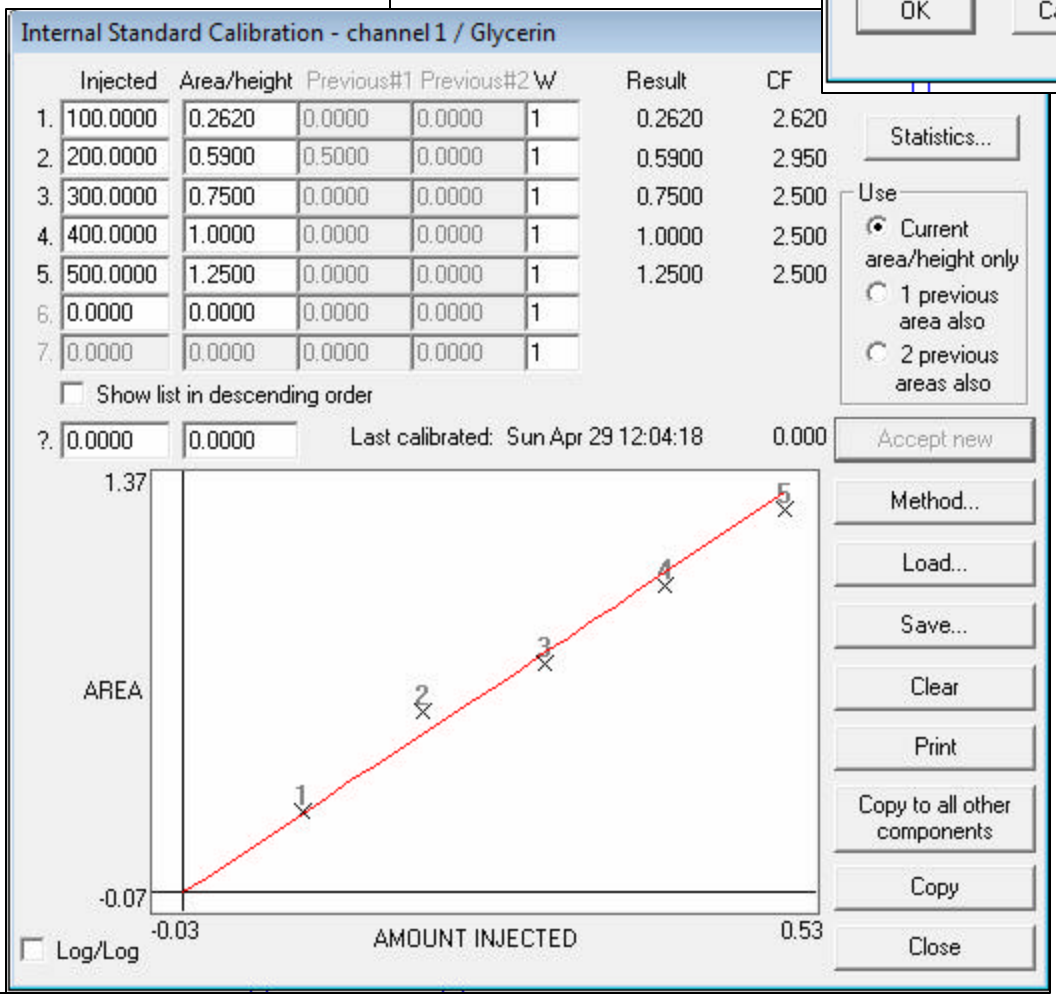
Open the Level 2 chromatogram (File/ Open) or inject the Level 2 standard and allow the run to end. (you can't calibrate until the run is over).
Point to the Glycerin peak and right click. From the menu select Calibrate Glycerin.

This time when the Recalibration screen appears, select the Level 2 radio button.

When the Calibration curve screen appears, click Accept New and the ratio between the Level 2 Glycerin and Butanetriol Internal Standard will be automatically inserted in the Level 2 cell.

Note that the old value (the estimated value we entered previously) is not lost but is shifted one cell to the right.





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Follow the same procedure for Level 3, 4 and 5.

The actual ratios replace the previously entered estimated values to produce a real 5 point calibration curve

Next inject a real sample (not a calibration standard) and verify that the Result screen displays a logical answer under the Internal column.

Note that the Butanetriol Internal Standard peak is not calibrated and shows a 0.00 result.