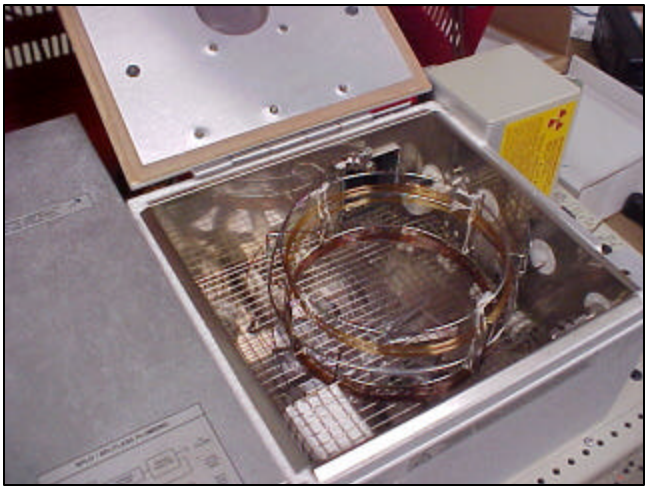
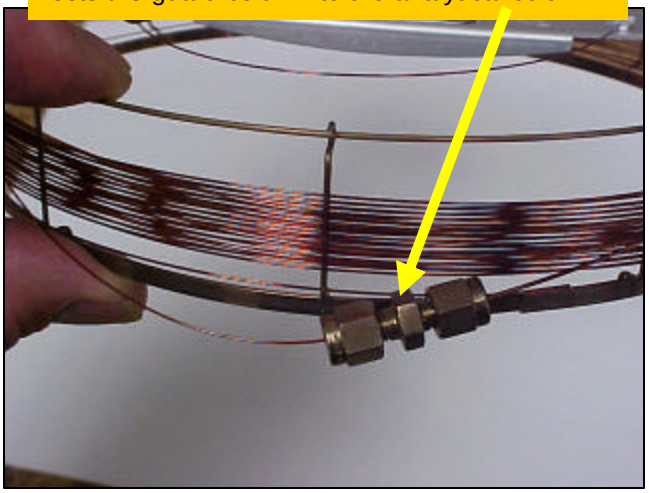
Performing ASTM 6584 free and total glycerin in BioDiesel using an SRI Gas Chromatograph and PeakSimple software

Install a capillary column in the oven of the SRI GC. The ASTM method suggests a 12 meter .32mm id narrow-bore column coupled with a 2.5 meter guard column but permits the use of any column which exhibits acceptable resolution of the glyceride analytes. For ease of use, SRI prefers a 15 meter fused silica lined metal capillary column commonly called a wide-bore MXT column. The ideal column has a thin film (.1-.25 microns thick) and a temperature rating of 380C or higher)
This type of column is **unbreakable** (unlike plain fused silica columns) and allows the injection syringe to deposit the sample directly into the bore of the column itself. This is important because heated or split/splitless injectors can discriminate against high boiling analytes like triglycerides. The ASTM 6584 method specifies cool-on-column injection like that found as standard equipment on all SRI gas chromatographs to avoid boiling point discrimination.

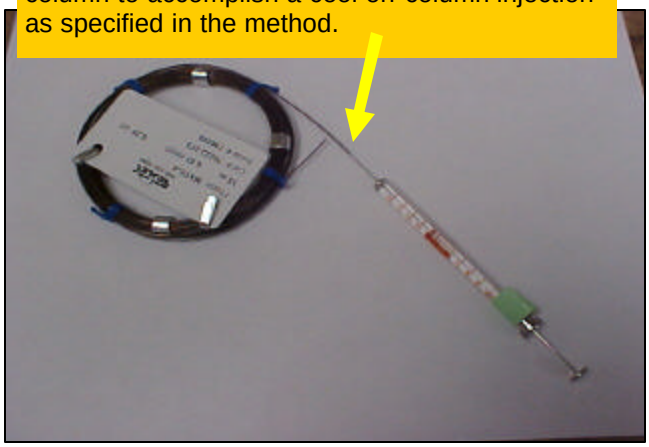


Chrompack HT5 .32mm id fused silca coupled with 2.5 meter .53mm id guard column. A 1/16'" stainless steel union with graphite ferrules connects the guard column to the analytical column.



Restek fused silica lined stainless steel MXT 5 .53mm id capillary column coiled to 3.5 inches

The 26 ga. Syringe needle fits inside the .53mm column to accomplish a cool on-column injection as specified in the method.



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Connect carrier gas, hydrogen and air to the GC. Helium is recommended as carrier gas, while the hydrogen and air are required for the FID (flame ionization detector) which is used to detect the sample molecules.

You can also use the optional SRI H2-40 hydrogen generator and "whisper quiet" built-in air compressor to provide all necessary gases without bulky gas cylinders

Note: Some pre-made calibration standards (Supelco 44918-U) are 10 times less concentrated, but the instructions specify adding 10 times more volume resulting in the same mass injected.

Prepare your calibration standards. You will need glycerin (500ppm), butanetriol (1000ppm), monoolien (10000ppm), tricaprin (8000ppm), diolien (5000ppm) and triolien (5000ppm) each dissolved in pyridine. You can buy these starting materials(stock solutions) from Supelco(part# 44898-U), Restek, or other sources. You will also need a derivitization reagent called MSTFA and Heptane (a common solvent).

The ASTM 6584 method specifies that for the highest calibration level (level 5), 100ul of each material (in pyridine) is added to a 10 ml vial along with 100ul of MSTFA. Allow 20 minutes for the reaction to occur, then add heptane to bring the final volume to 8ml.

The ASTM 6584 method describes making the calibration standards at 5 different levels, so the level 1 calibration standard is prepared using 10ul of each starting material instead of 100ul, but the procedure is otherwise the same.

You will need the starting materials plus MSTFA and Heptane. Some pyridine is also handy to have on hand.

A 100ul syringe is helpful for making the dilutions along with vials, and pipets.

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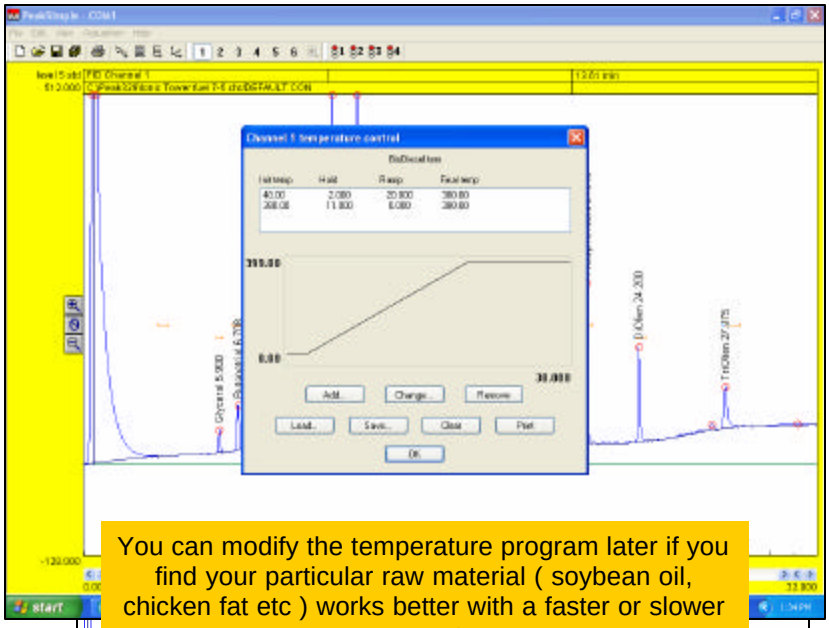
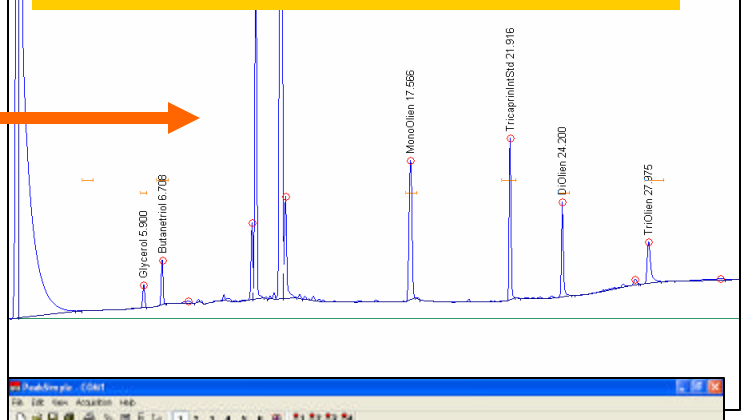
Set up a temperature program in the PeakSimple software (which comes free with every SRI GC) starting at 40 degrees, holding there for 2 minutes, then ramping at 20 degrees per minutes to 380 degrees, and holding there for 11 minutes. The ASTM 6584 method does not specifically recommend a temperature program so long as the peaks are well separated from each other and from any interfering peaks.



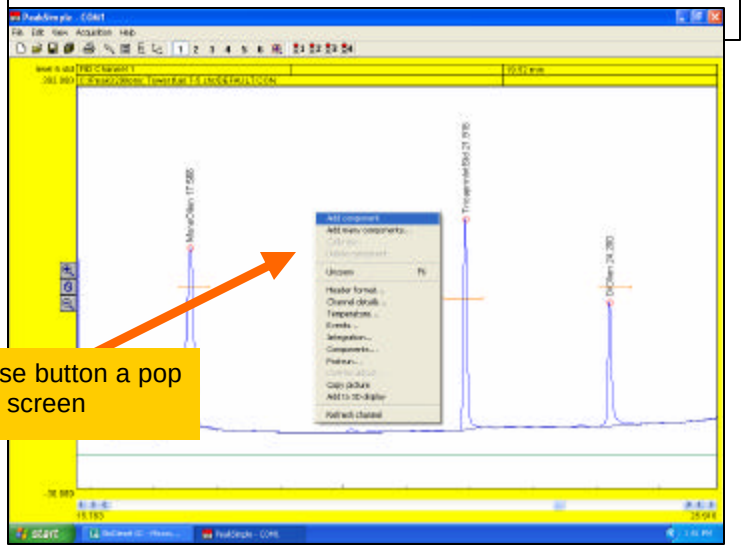
You can modify the temperature program later if you find your particular raw material (soybean oil, chicken fat etc) works better with a faster or slower ramp rate.

Inject each of the 5 calibration standards, saving the data file under a unique name each time (level1cal.chr, level2 cal.chr etc).

A typical level 5 calibration is shown to the right.



Create a retention window for each of the 6 peaks by pointing to the peak with your mouse, clicking on the right hand mouse button and then left clicking on “add component”



When you click the right hand mouse button a pop up window appears on the screen

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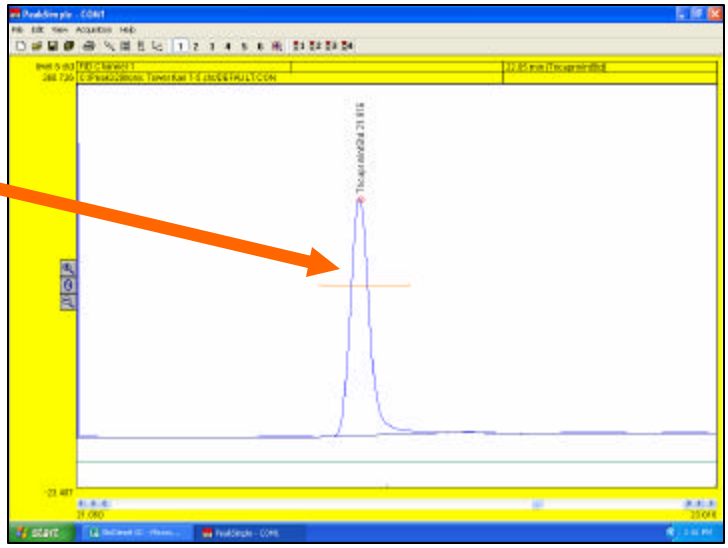
Adjust the retention window (the red horizontal line which appears) so that it is centered on the peak. Adjust the width of the retention window so it is just a little wider than the peak. Grab the middle of the H-bar with your mouse to move the window side to side, or grab the vertical ends to narrow or widen the window.



Double click on the retention window or right click then select Edit Component. This brings up the Component Details screen shown at right.

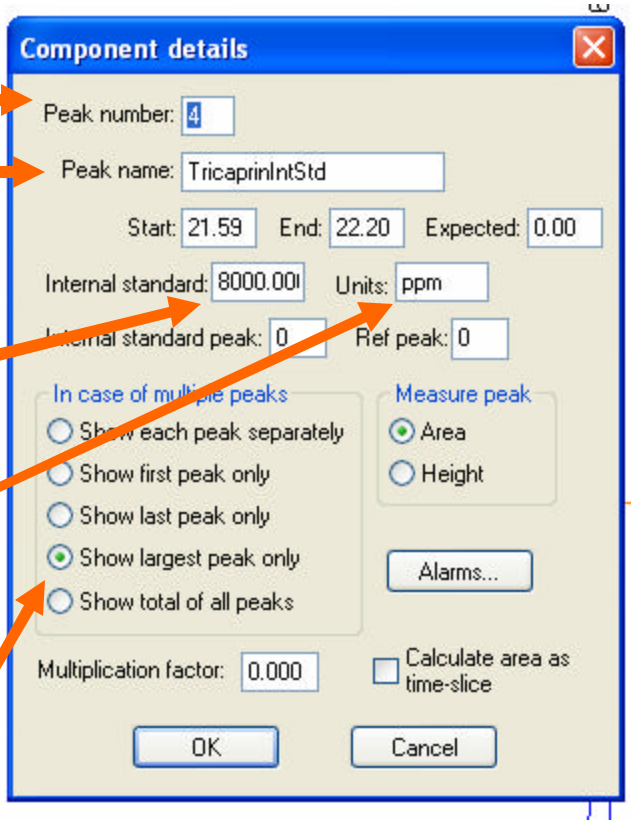
Give each peak a different peak number.

Fill in the peak's name.

For the tricaprin and butanetriol internal standard peaks **ONLY**, enter the concentration in the stock solution. This is how PeakSimple knows the concentration of the internal standards.

Enter the units you prefer to calibrate in (ppm or percent). *Note: one million ppm=100%, 100,000ppm=10%, 10,000ppm=1%, 1000ppm=.1%, 100ppm=.01%, 10ppm=.001%, 1ppm=.0001%.*

Select the largest peak only radio button so PeakSimple finds the largest peak in the window as tricaprin, not a small noise peak



Don't fill in any of the other fields in this screen at this time

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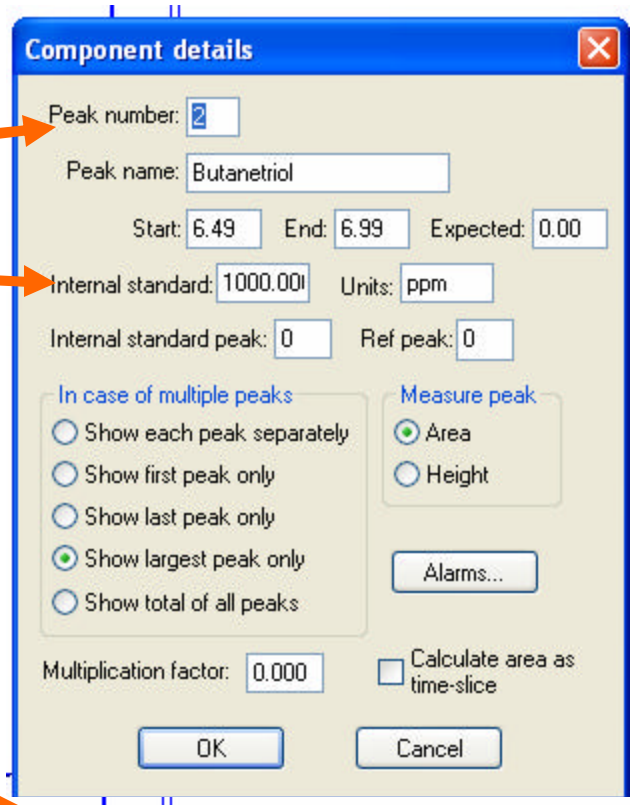
Do the same thing for the other internal standard peak, Butanetriol.

Make sure to use a different peak number.

Enter the concentration in the stock solution (1000ppm)



Then do the same thing for the 4 remaining peaks, Glycerin (the free glycerin), monolien, diolien and triolien.

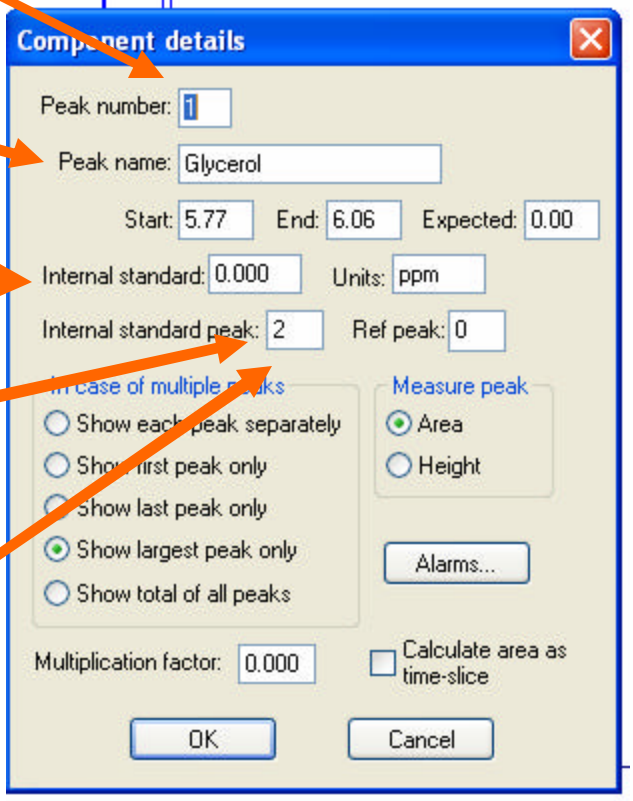
Use a different number for each peak.

Glycerol is the same thing as glycerin, you can use either name.

Do **NOT** enter a number in the Internal Standard box, because this peak is **NOT** an internal standard peak.

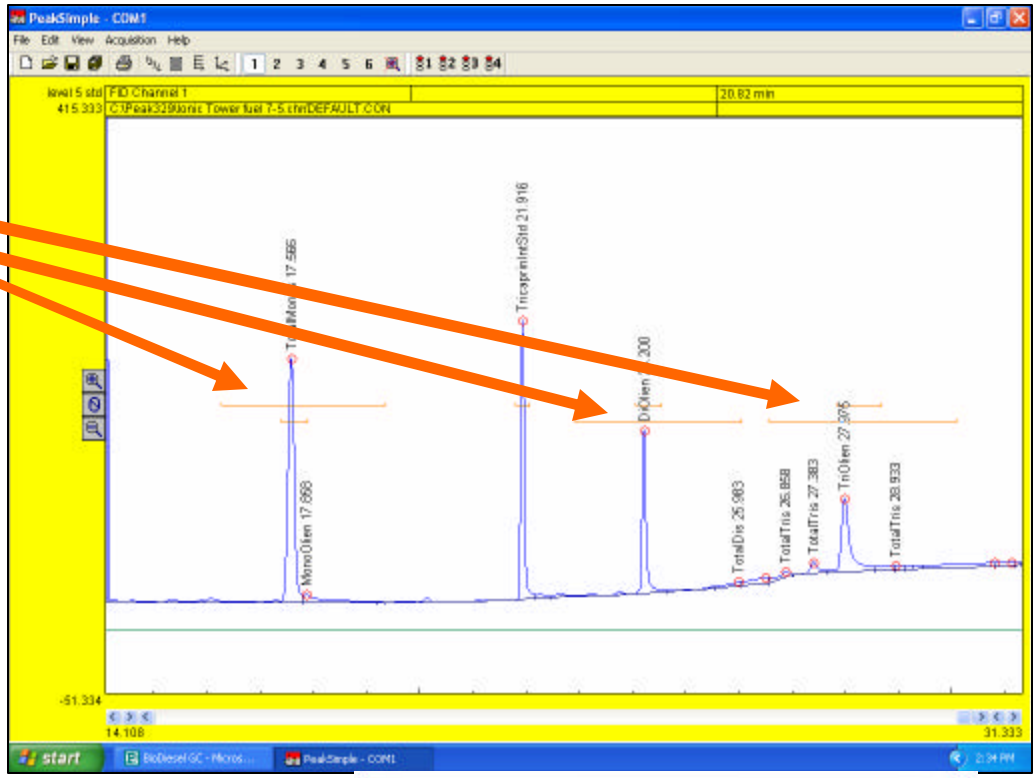
Enter the peak number of the Butanetriol internal standard. This is how PeakSimple knows to use Butanetriol as the internal standard for Glycerol.

Do the same thing for the monolien, diolien and triolien peaks EXCEPT use the peak number of tricaprin since tricaprin is the internal standard for mono, di and triolien.



Performing ASTM 6584 free and total glycerin in BioDiesel using an SRI Gas Chromatograph and PeakSimple software

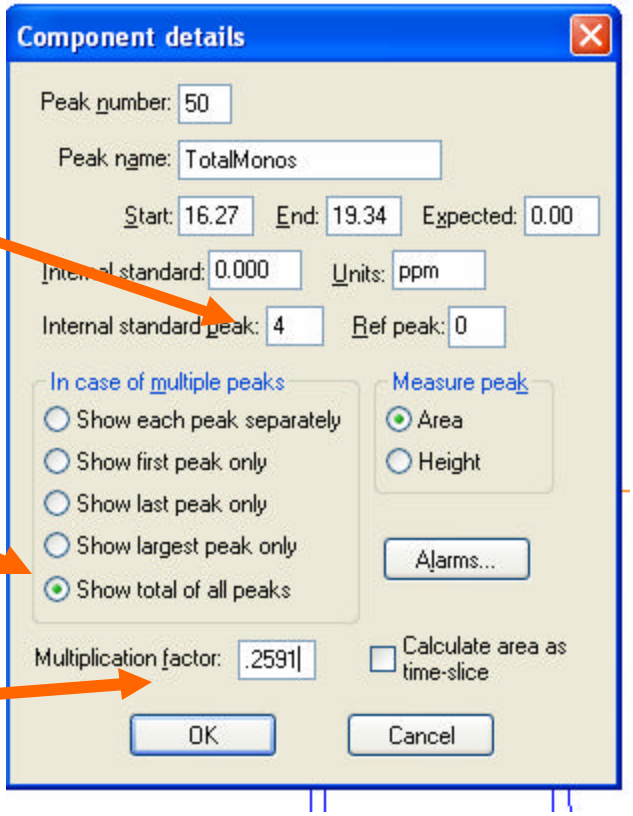
Create three additional retention windows called Total MonoGlycerides, Total DiGlycerides, and Total Triglycerides. These windows should be wider than the monoolien, diolien and triolien and should overlap the individual calibration compounds. The function of the Total Mono, Di and Tri windows will be to sum up all the mono, di and tri glycerides to arrive at an answer for total bound glycerin.



Enter the peak number for tricaprin since we are still using tricaprin as the internal standard for the Total windows.

Select the radio button labeled Show total of all peaks.

Enter the factor specified in the ASTM 6584 method. Monoglycerides=.2591 Diglycerides=.1488 Triglycerides=.1044 This factor accounts for the percentage of the molecule which is bound glycerin.



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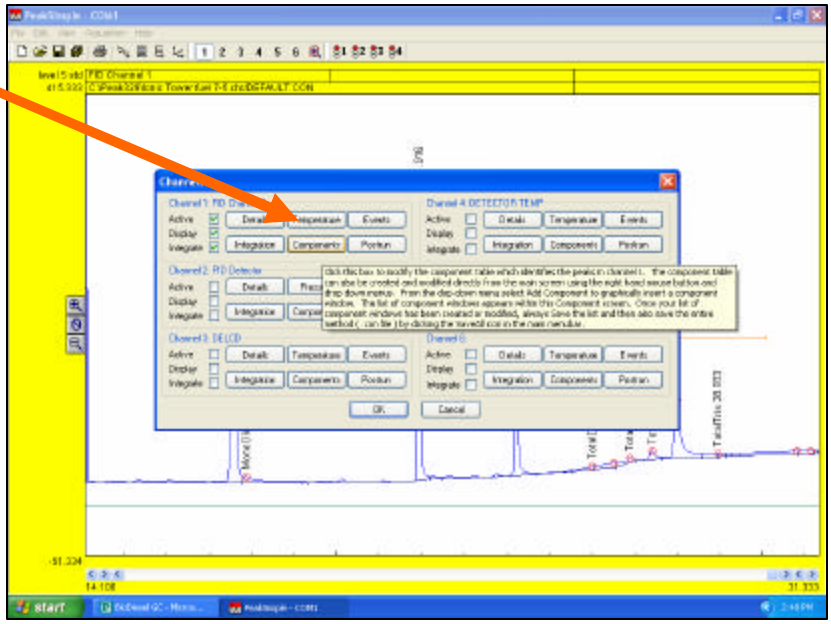
Navigate to the Component screen for Channel 1.

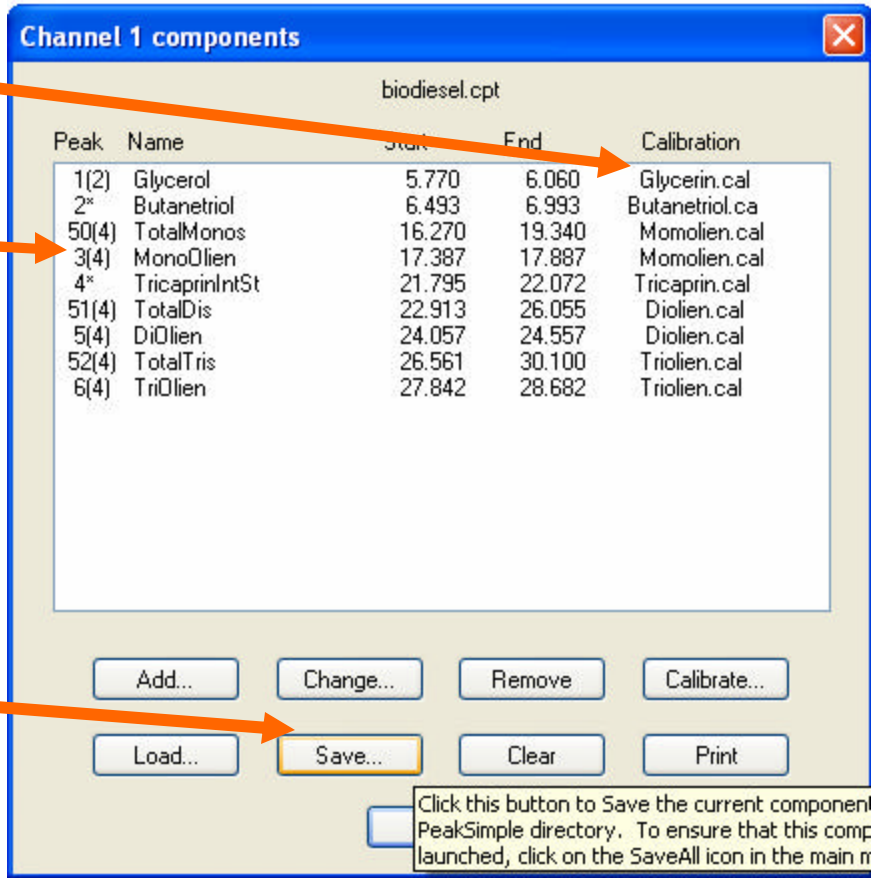


The Component screen should look like the one to the right except that the names of the calibration curves for each component will not yet have been entered.

Notice that the peak number is followed by a number in parentheses.

The number in parentheses indicates which peak is the internal standard. So Monolien which is peak#3 uses tricaprin (peak#4) as its internal standard. Glycerol which is peak#1 uses butanetriol (peak#2) as its internal standard.

Save the Component table so you don't have to enter this information over again.



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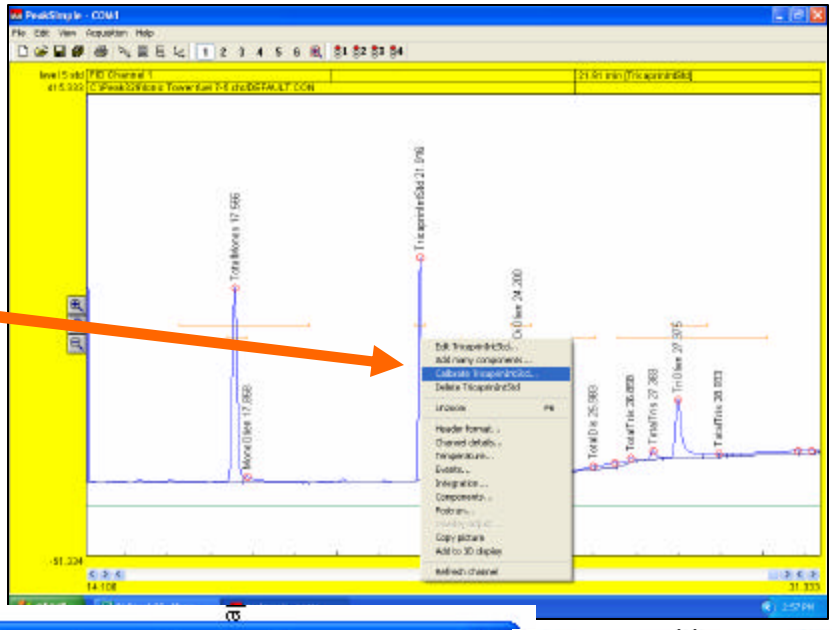
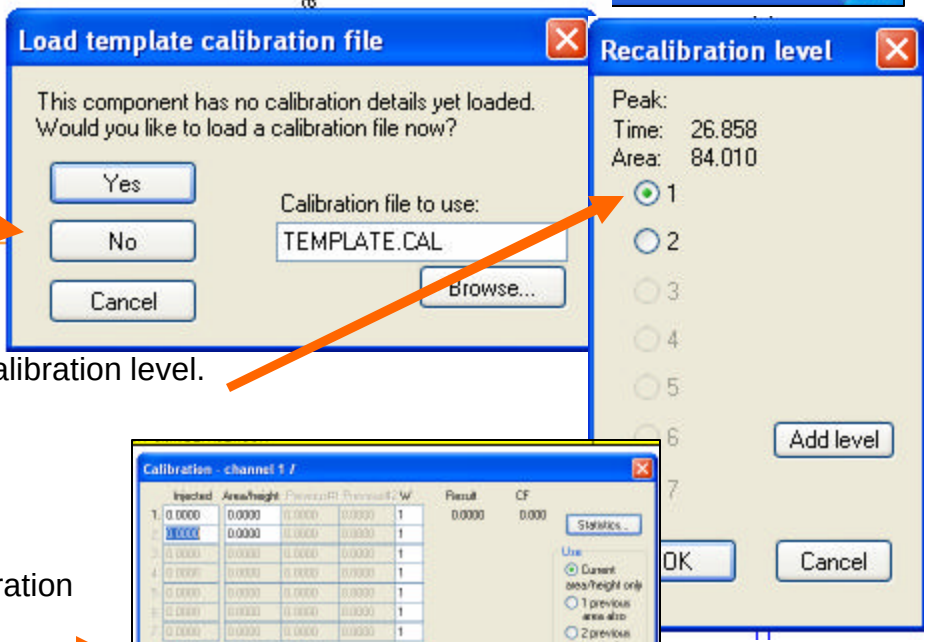
Now that all the components have been identified they can be calibrated. Point to each of the 6 peaks plus 3 Totals peaks and go through the following sequence for each peak in turn.



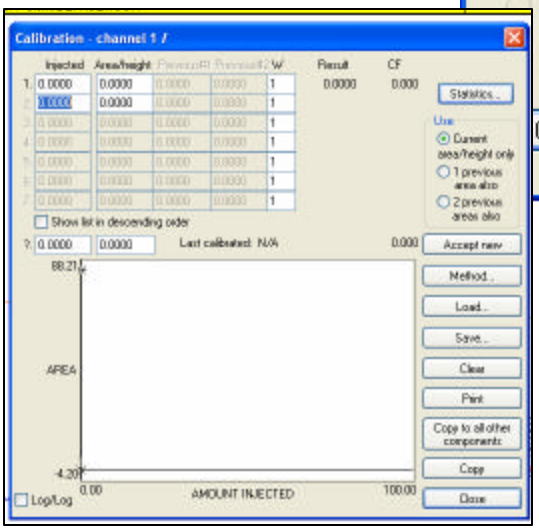
Point to the peak and click on the right hand mouse button.
This brings up a menu from which you select “calibrate component” (calibrate glycerol for example).

Since no calibration curve currently exists, PeakSimple asks if you want to copy a template curve. This is a convenience when calibrating many peaks, but for now just say NO.



The next screen asks for the calibration level.
Select level 1.

This takes you to a blank calibration curve screen.



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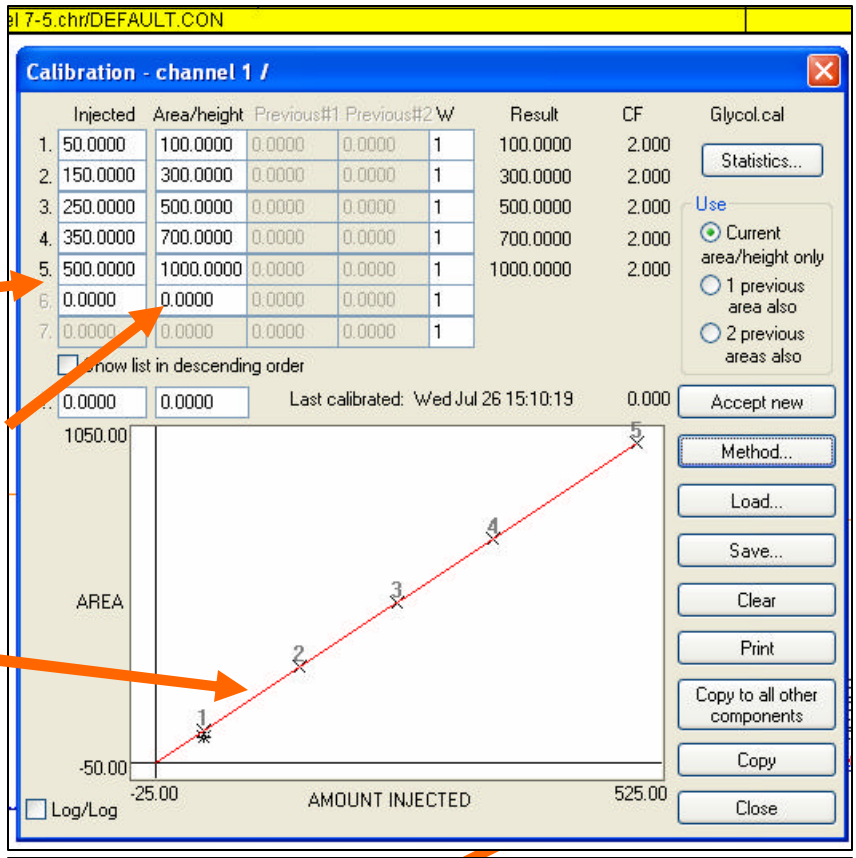
For Glycerin, the 5 point curve corresponds to 50, 150, 250, 350 and 500ppm injected. Enter these numbers in the Injected column of the spreadsheet .

In the Area/Height column enter the area reported for the Glycerin peak for each of the five levels. Take this data from the printout for each of the calibration runs previously performed.

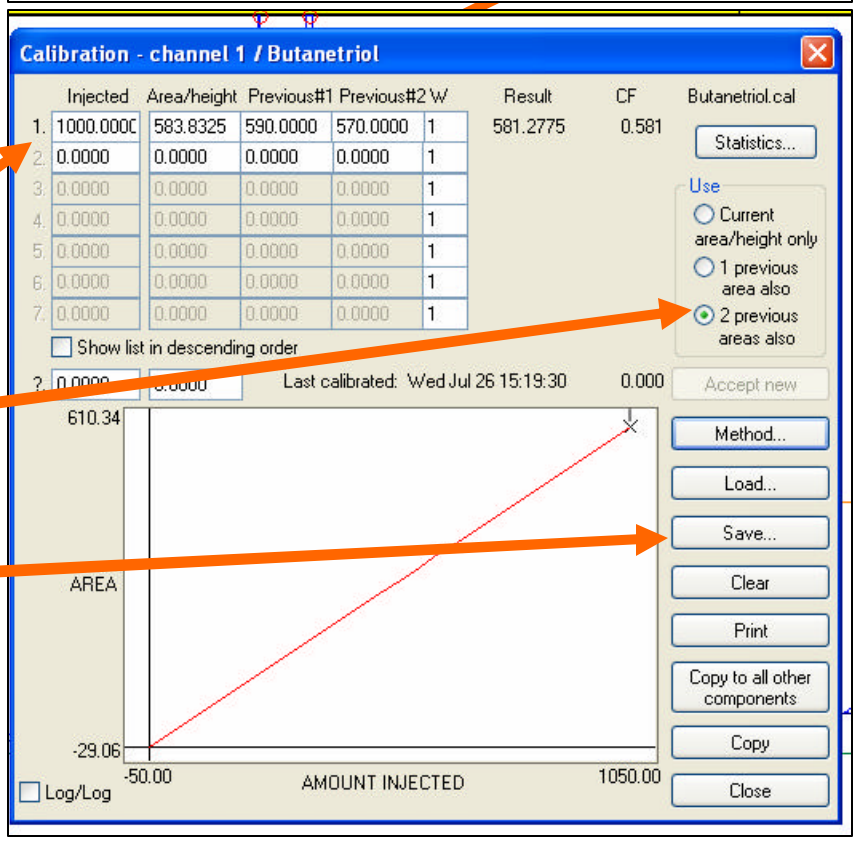
This should result in a relatively straight line calibration curve using a single line best fit .



The Butanetriol curve is only a single point since the Butanetriol internal standard is always present at the same amount (1000ppm) regardless of whether you inject a level1,2,3,4,5 standard or the unknown Biodiesel itself.

You can average up to 3 injections by clicking the “use 2 previous areas also” button.

Don’t forget to save the curves using a unique name for each one



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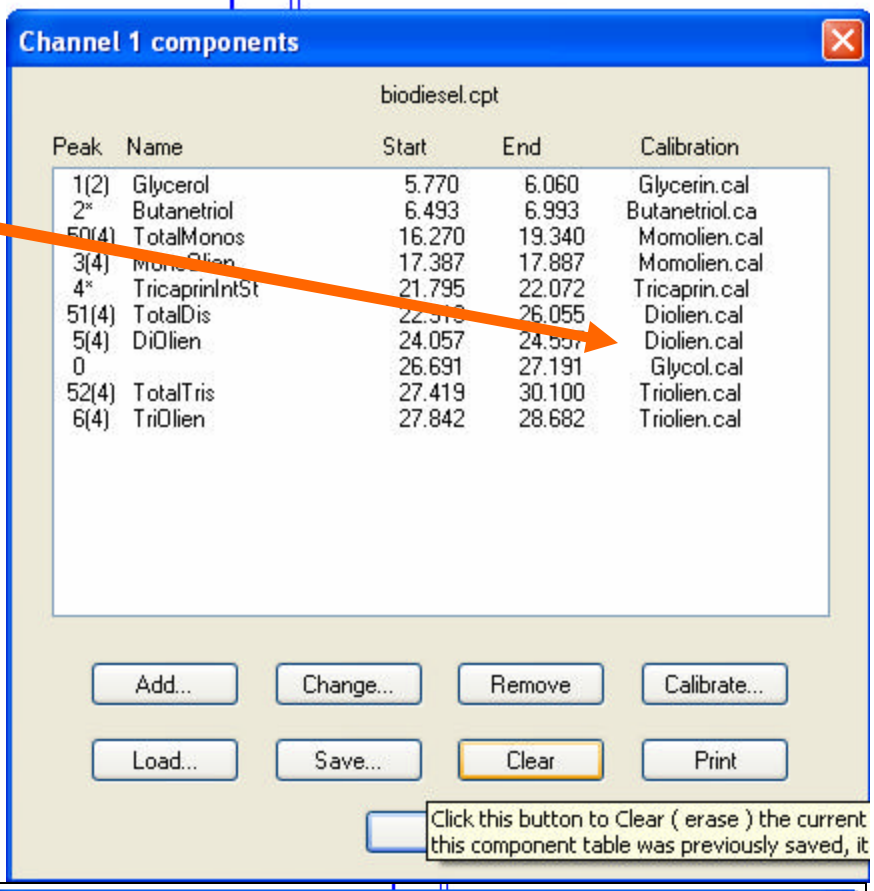
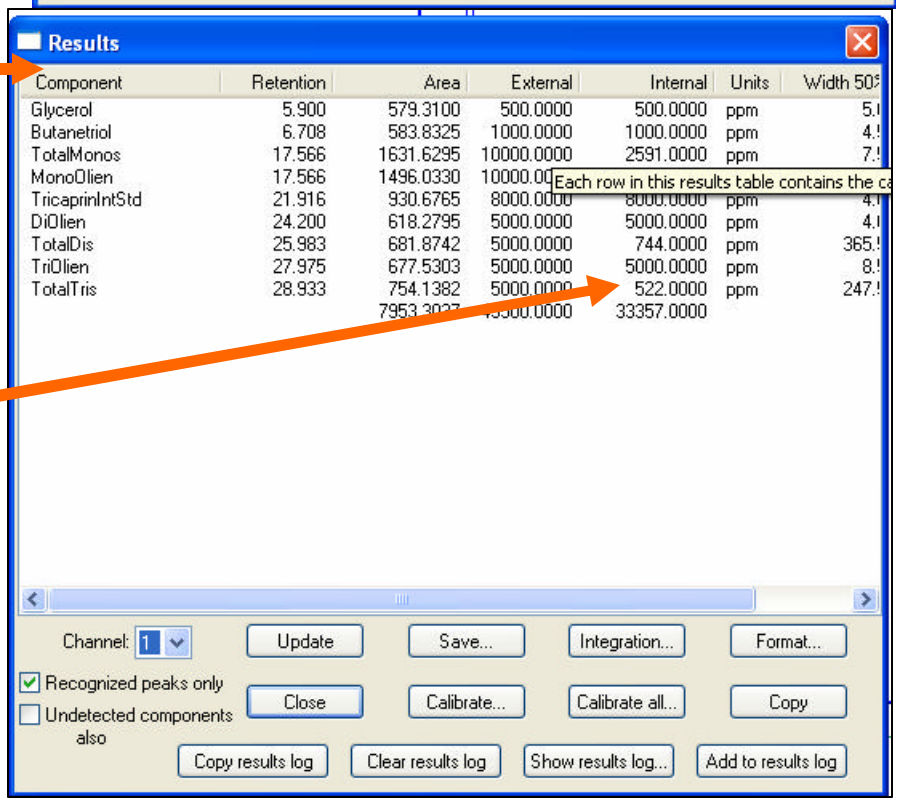
Once all the peaks are calibrated, the component table should look like the one to the right. Notice that the Total Mono, Total Di, and Total Tri components use the same calibration curves as the individual Mono, Di and Triolien peaks. This makes sense because these compounds are chosen in the ASTM 6584 method to be representative of the range of glyceride compounds which may be found in actual samples.



Navigate to the Results screen and verify that the results look like the screen to the right. (for a level 5 calibration standard) Notice that the internal standard result for the Total Mono, Di and Tri is adjusted by the factor (,2591, .1488 and .1044 respectively)



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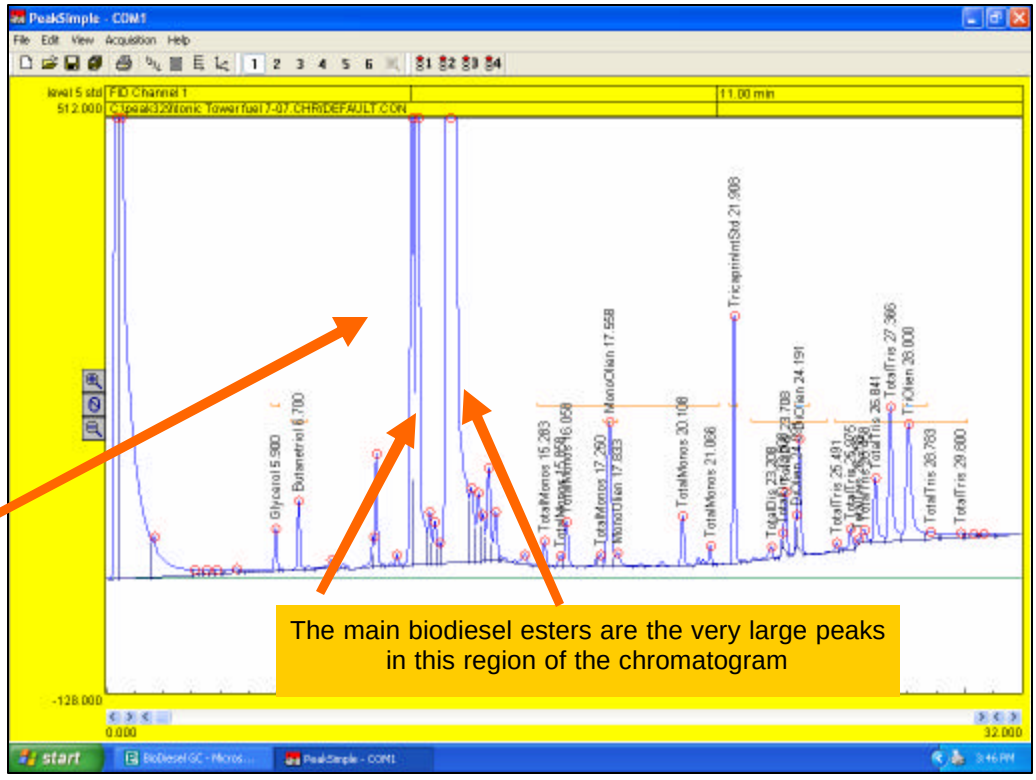
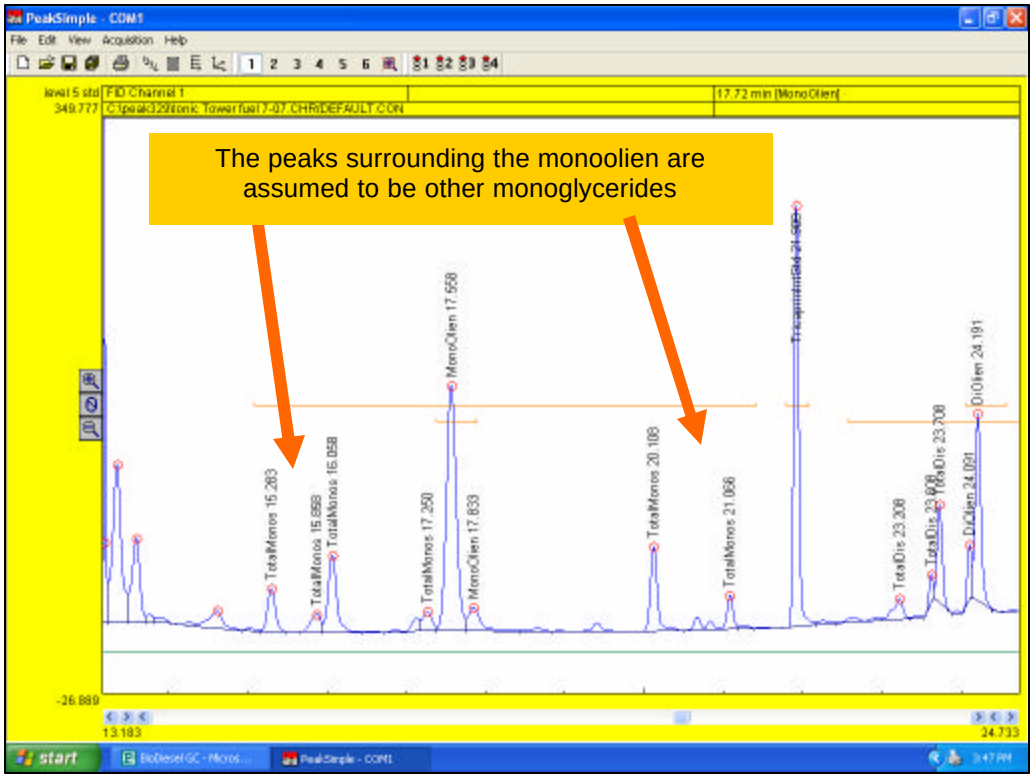
Now that the system is calibrated, prepare an actual BioDiesel sample by placing 100ul of BioDiesel, 100ul of Tricaprin in pyridine, 100ul of Butanetriol in pyridine, and 100ul of MSTFA in a vial. Allow 20 minutes for the reaction then dilute to 8ml with heptane.

Inject 1ul to generate a chromatogram similar to the one at the right



Zoom in to the region surrounding the mono, di and triolien. Stretch the total mono, di and tri component window so it includes the small peaks surrounding the monoolien, diolien and triolien. The ASTM 6584 method does not precisely define which peaks to include or exclude, so there is some judgement required on the part of the operator.



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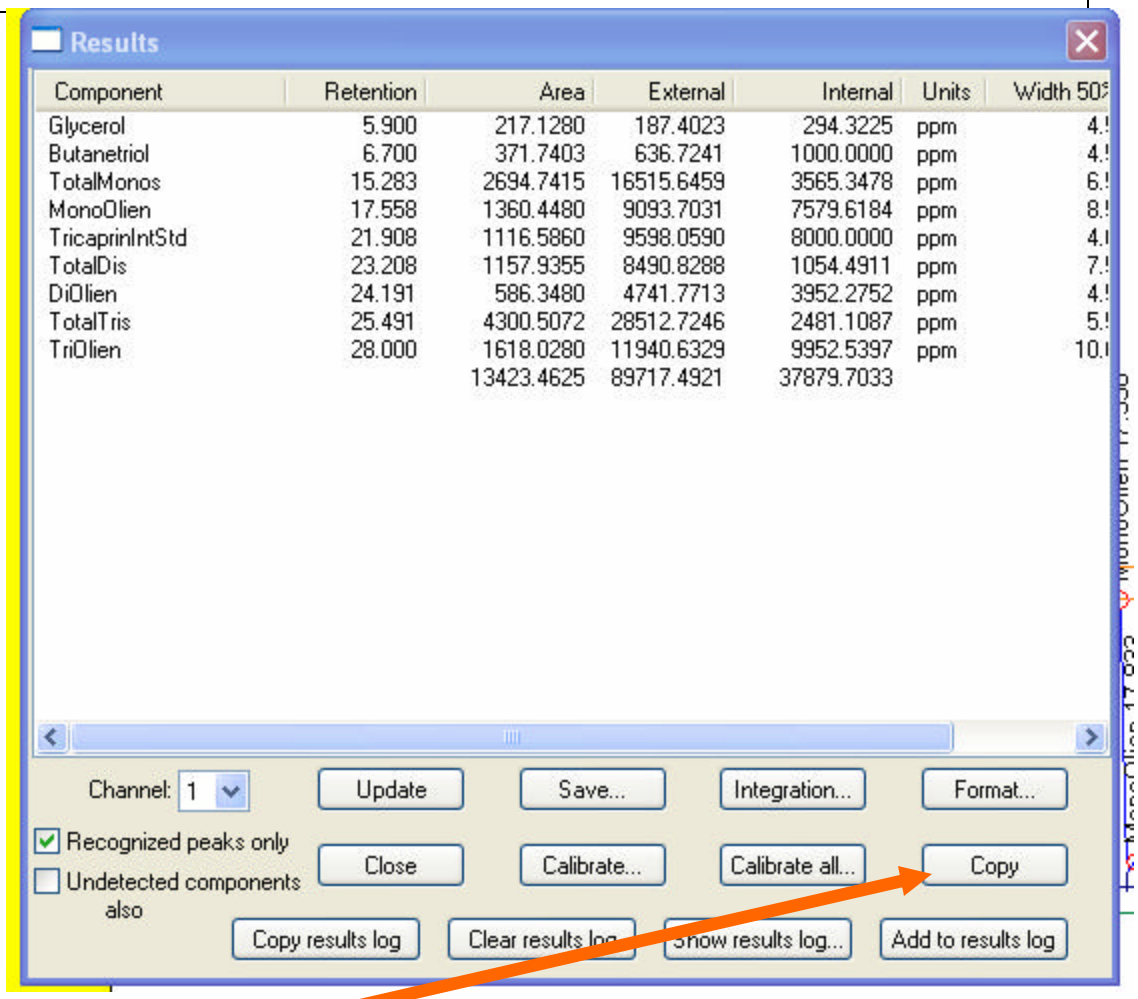
The Results screen now displays the calculated results for the Biodiesel sample. The result for Glycerol is the free glycerin and the sum of the Total Mono, Total Di and Total Tris are the bound glycerin.



Click the Copy button, then Paste the Results into Excel for further calculations or to produce a report for your client