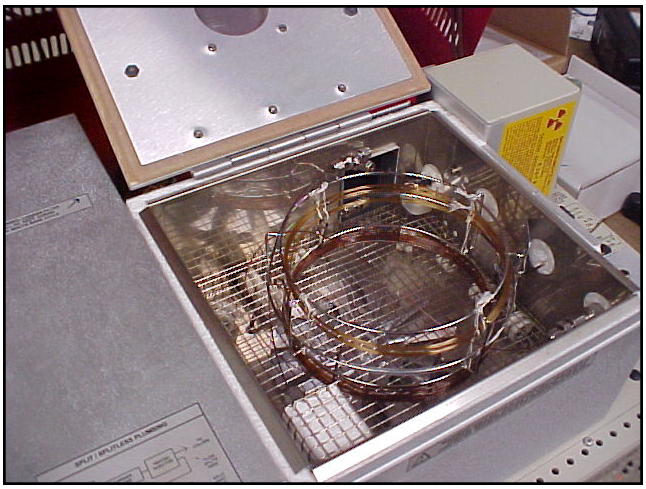
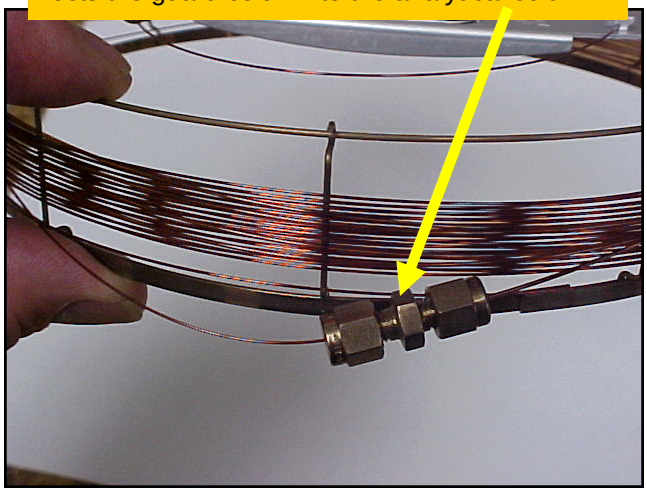
Performing ASTM 6584 free and total glycerin in BioDiesel using an SRI Gas Chromatograph and PeakSimple software version 3.67

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 (.16 micron thick) and a temperature rating of 380C or higher)

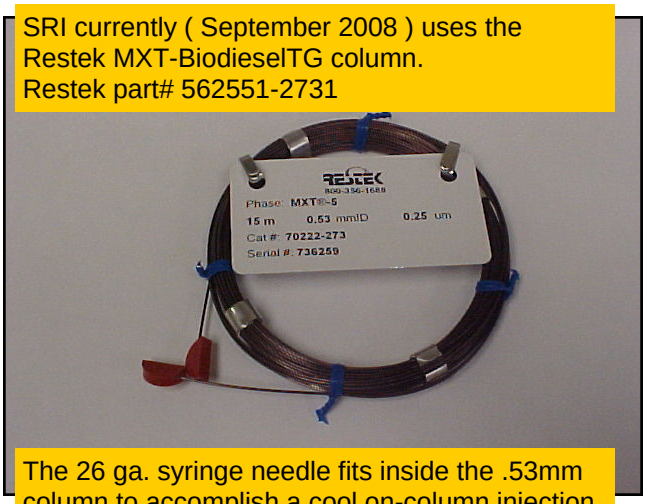
This type of column is **unbreakable** (unlike plain fused silica columns) and allows the injection syringe to deposit the sample directly into the bore of the column itself. This is important because heated or split/splitless injectors can discriminate against high boiling analytes like triglycerides. 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.



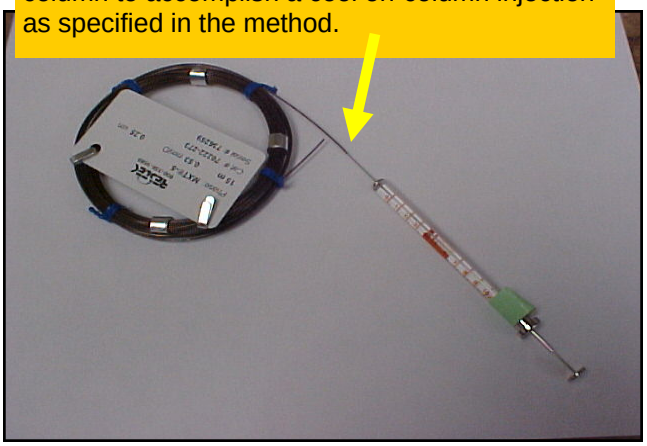
Chrompack HT5 .32mm id fused silca coupled with 2.5 meter .53mm id guard column. A 1/16'" stainless steel union with graphite ferrules connects the guard column to the analytical column.



SRI currently (September 2008) uses the Restek MXT-BiodieselTG column.
Restek part# 562551-2731



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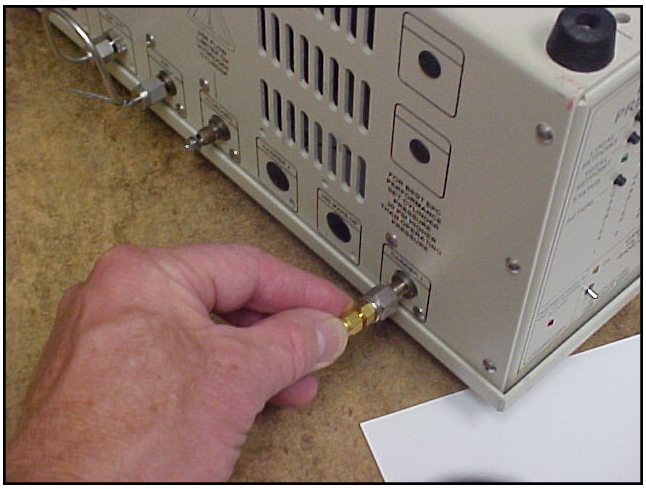
Connect carrier gas, hydrogen and air to the GC. Helium is recommended as carrier gas, while the hydrogen and air are required for the FID (flame ionization detector) which is used to detect the sample molecules.



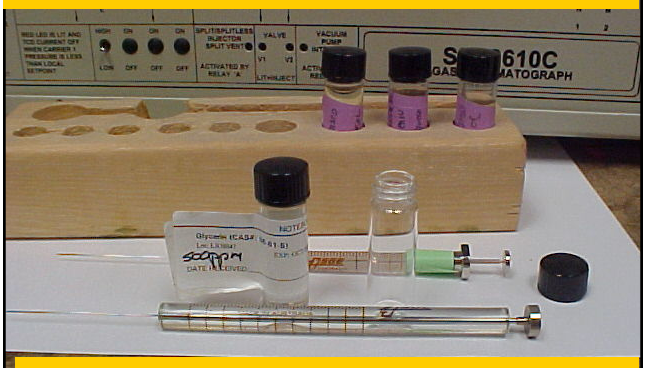
Prepare your calibration standards. The stock standards (diluted in Pyridine) can be purchased from Restek (800-356-1688) www.restek.com. They are also available from Supelco and other lab suppliers.
Unfortunately we have not found any one source for all the items.

You will need:
glycerin (500ppm) Restek# 33020
butanetriol (1000ppm) Restek# 33024
monoolien (10000ppm) Restek# 33021
tricaprin (8000ppm) Restek# 33025
diolien (5000ppm) Restek# 33022
triolien (5000ppm) Restek# 33023
MSTFA (Derivatization Reagent) Restek# 35601
Heptane (a common solvent) Sigma# 34873-1L
8ml vials Grace# 98862 or
Cole-Parmer# WU-08919-86
100ul syringe Grace# 85012
Disposable pipets Sigma# Z350613-400EA

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

The ASTM 6584 method specifies that for the highest calibration level (level 5), 100ul of each material (in pyridine) is added to an 8 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.

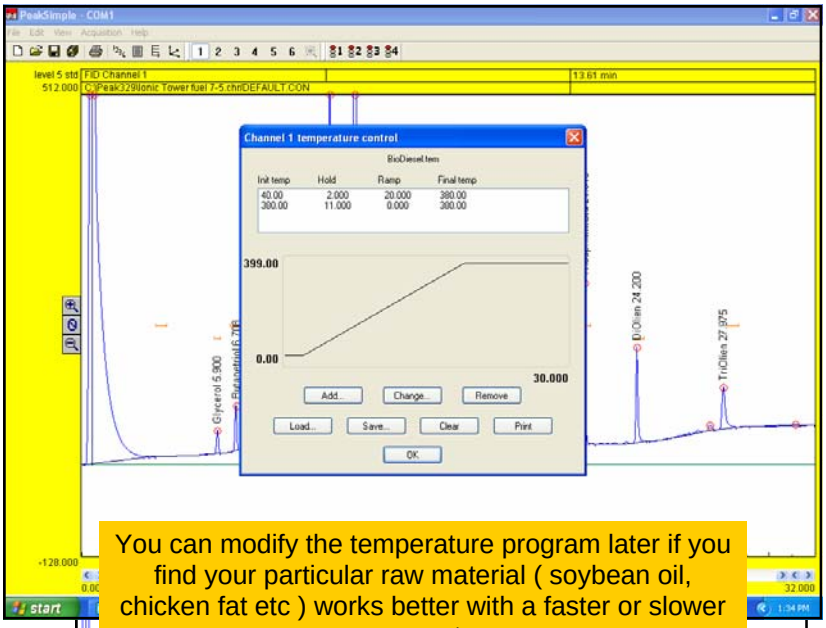
The ASTM 6584 method describes making the calibration standards at 5 different levels, so the level 1 calibration standard is prepared using 10ul of each starting material instead of 100ul, but the procedure is otherwise the same.



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

Performing ASTM 6584 free and total glycerin in BioDiesel using an SRI Gas Chromatograph and PeakSimple software version 3.67

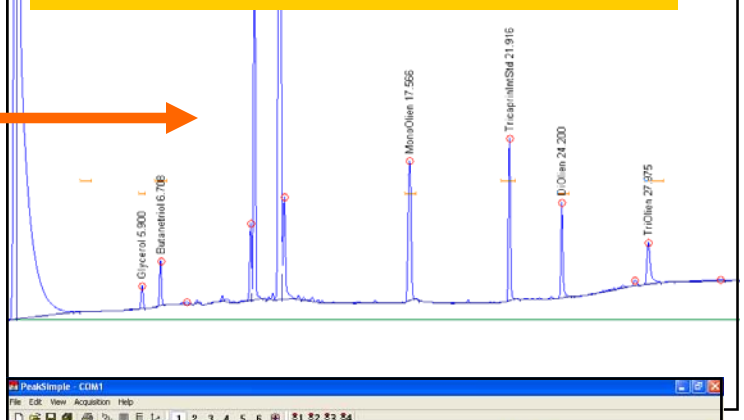
Set up a temperature program in the PeakSimple software (which comes free with every SRI GC) starting at 40 degrees, holding there for 2 minutes, then ramping at 20 degrees per minutes to 380 degrees, and holding there for 11 minutes. The ASTM 6584 method does not specifically recommend a temperature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



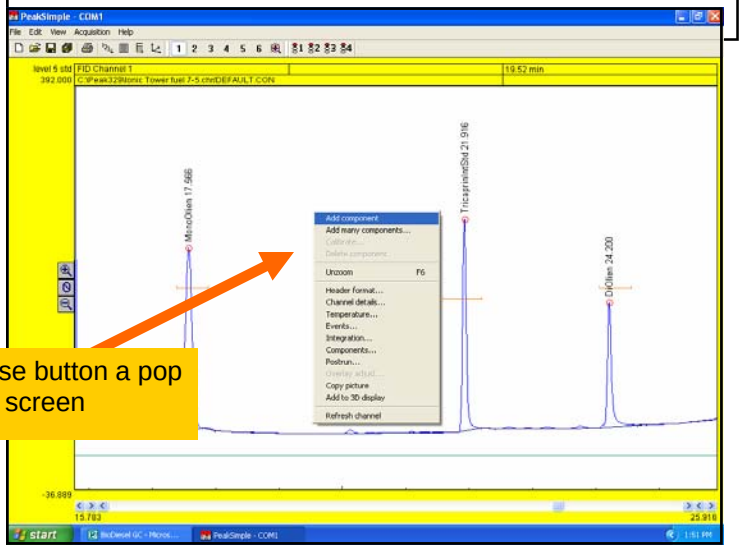
You can modify the temperature program later if you find your particular raw material (soybean oil, chicken fat etc) works better with a faster or slower ramp rate.

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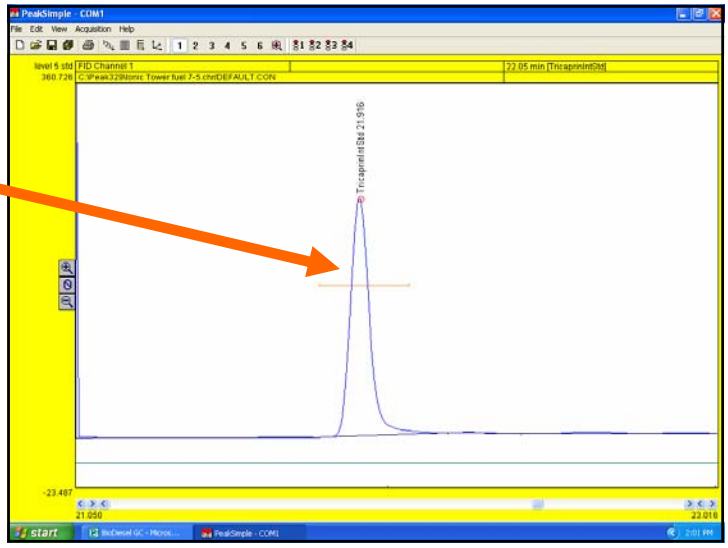
Adjust the retention window (the red horizontal line which appears) so that it is centered on the peak. Adjust the width of the retention window so it is just a little wider than the peak. Grab the middle of the H-bar with your mouse to move the window side to side, or grab the vertical ends to narrow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

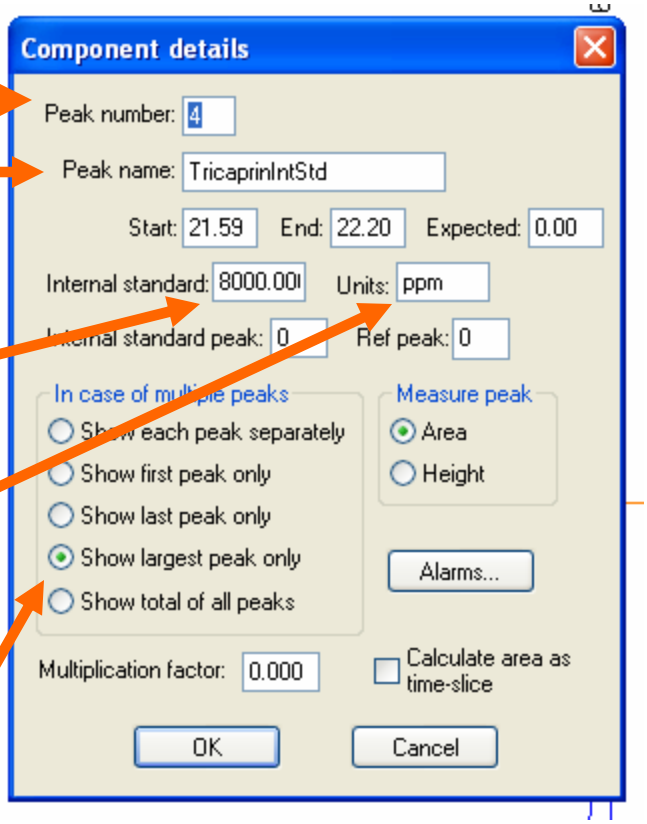
Give each peak a different peak number.

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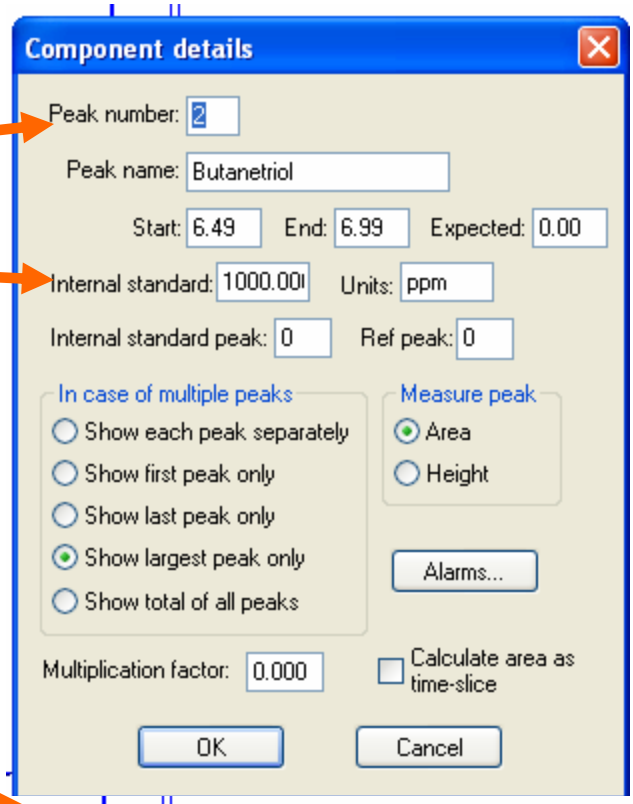
Do the same thing for the other internal standard peak, Butanetriol.

Make sure to use a different peak number.

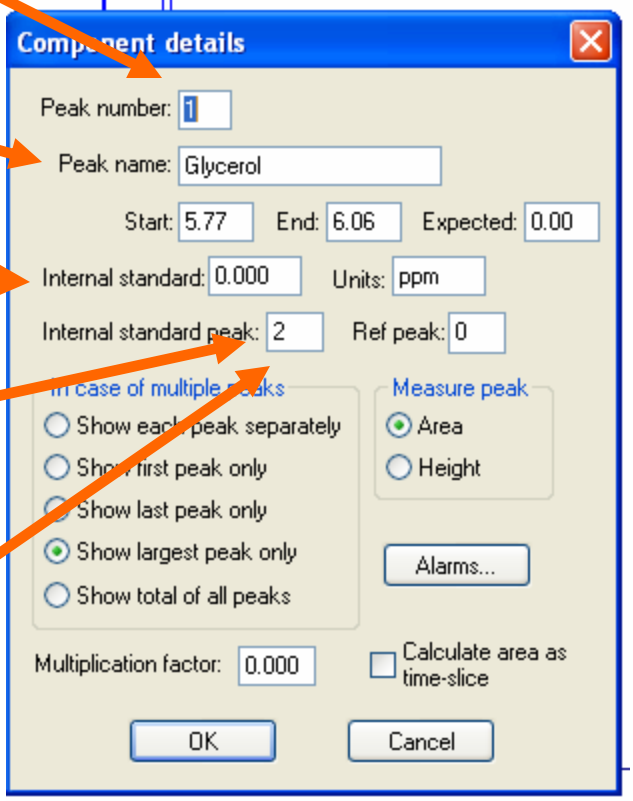
Enter the concentration in the stock solution (1000ppm)



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 (4) since tricaprin is the internal standard for mono, di and triolien.

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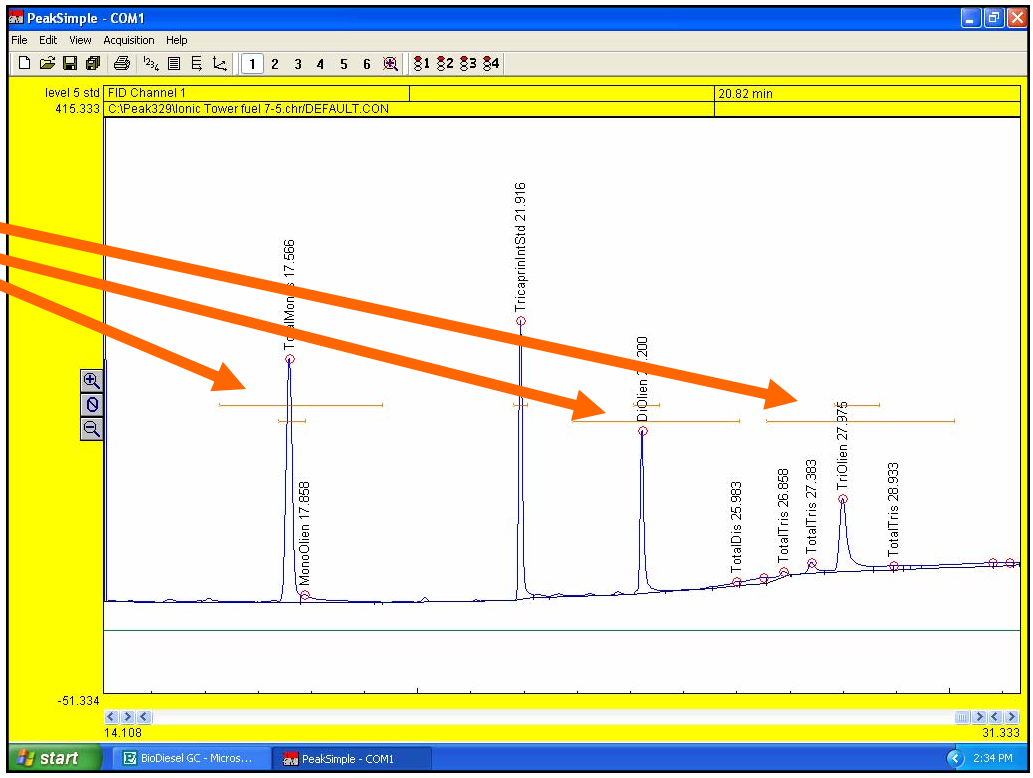
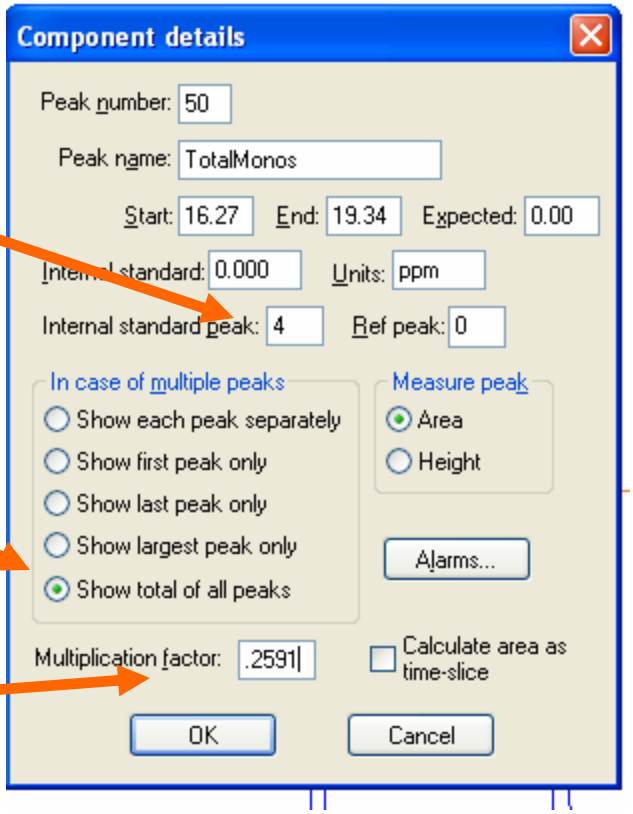
Create three additional retention windows called Total MonoGlycerides, Total DiGlycerides, and Total Triglycerides. These windows should be wider than the monoolien, diolien and triolien and should overlap the individual calibration compounds. The function of the Total Mono, Di and Tri windows will be 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.

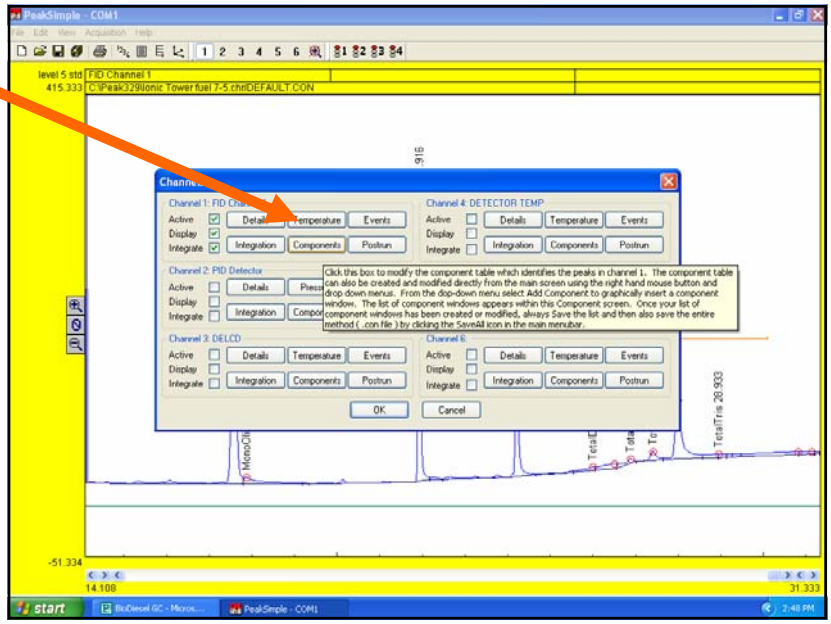
Select the radio button labeled Show total of all peaks.

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.



Performing ASTM 6584 free and total glycerin in BioDiesel using an SRI Gas Chromatograph and PeakSimple software version 3.67

Navigate to the Component screen for Channel 1.



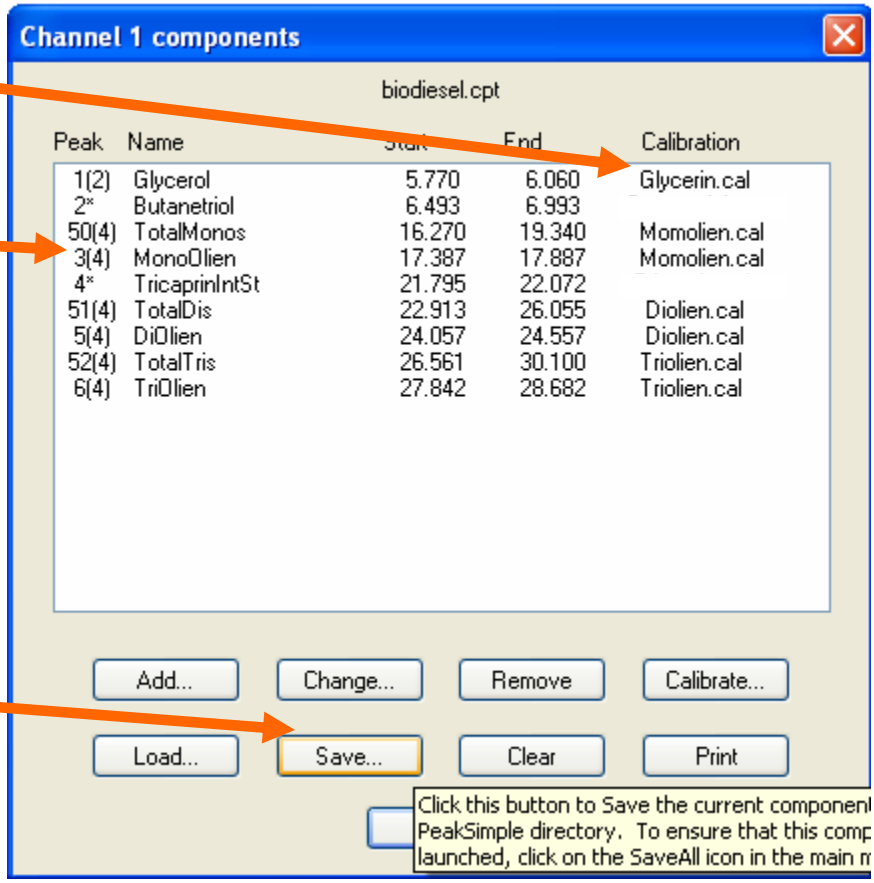
The Component screen should look like the one to the right except that the names of the calibration curves for each component will not yet have been entered.

The internal standard peaks (butanetriol and tricaprin) do not get calibration curves.

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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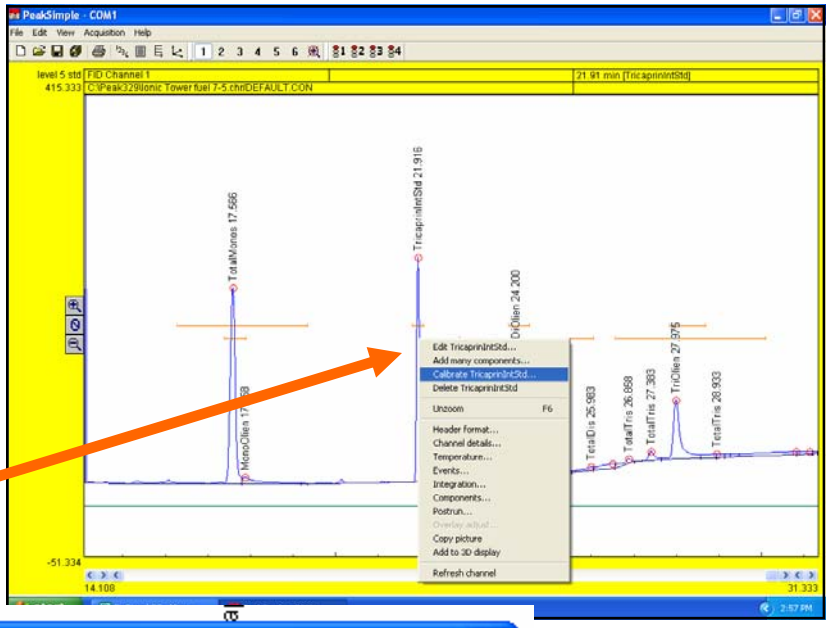
Now that all the components have been identified they can be calibrated.

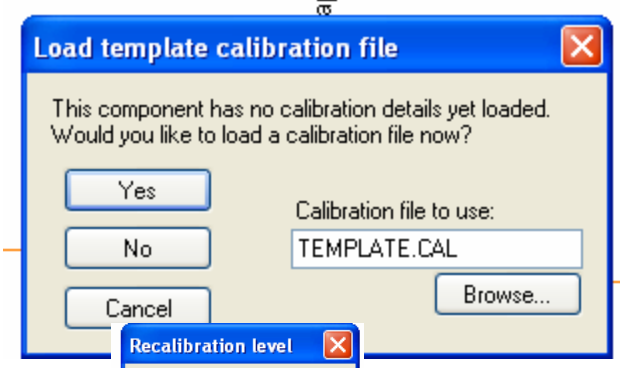
Load the previously run Level 1 chromatogram

Point to each of the 4 standard peaks (glycerin, mono, di and triolein) and go through the following sequence for each peak in turn. **Do not calibrate the internal standard peaks butanetriol and tricaprin.**

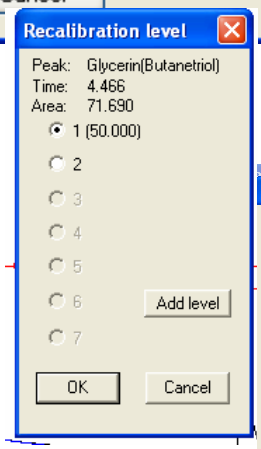


Point to the 1st peak and click on the right hand mouse button. This brings up a menu from which you select “calibrate component” (calibrate glycerol for example).

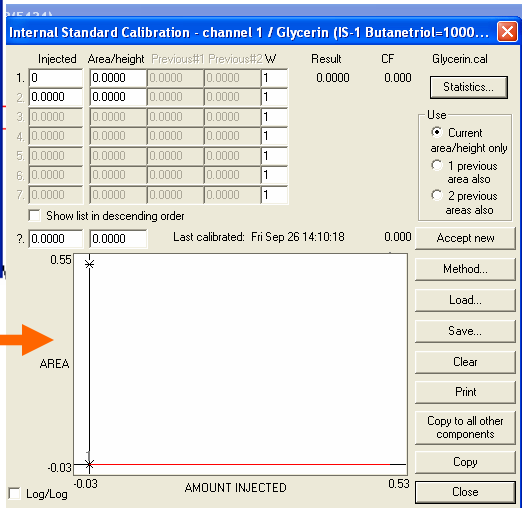
Since no calibration curve currently exists, PeakSimple asks if you want to copy a template curve. This is a convenience when calibrating many peaks, but for now just say NO.



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



This takes you to a blank calibration curve screen.



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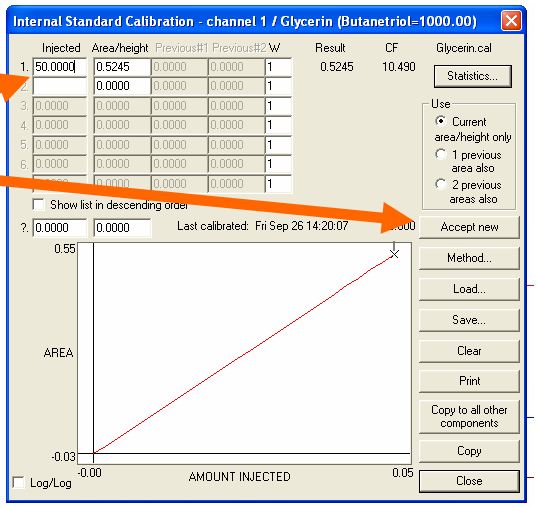
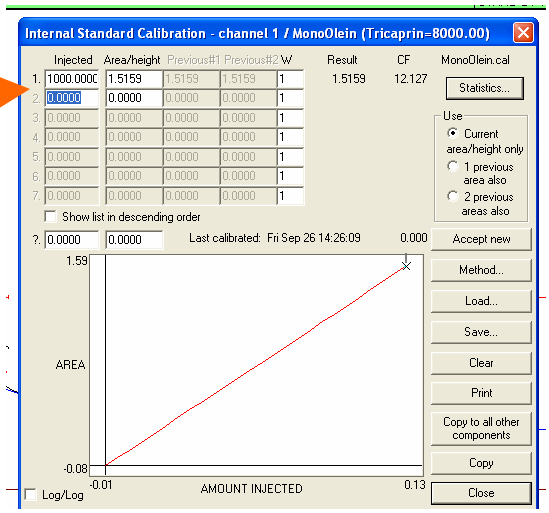
For Glycerin the Level 1 amount is 50, so enter the number 50 in the top left cell of the calibration spreadsheet.
Then click the Accept New button on the right of the screen. A number which is the ratio of the Glycerin peak area divided by the area of the Butanetriol internal standard peak will be automatically calculated and entered in the next cell to the right.



Go through the same procedure with the Monoolein, DiOlein, and TriOlein peaks.
The MonoOlein calibration screen is shown at right. Note that the Level 1 amount for MonoOlein is 1000. For DiOlein and TriOlein- the Level 1 amount is 500.

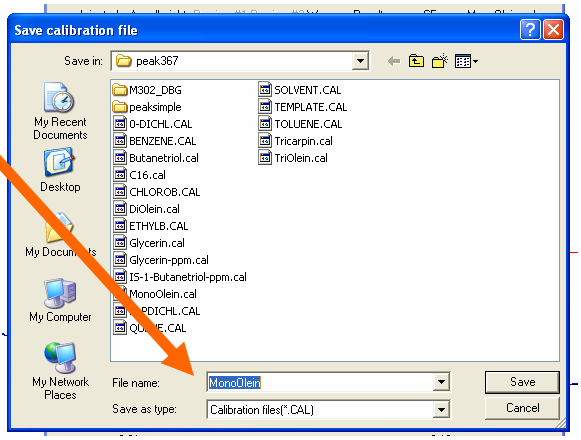


The table below taken from Table 3 of ASTM D6584-00 shows the calibration level amounts for the 4 standard peaks.

Level	*Glycerin*	*Mono*	*Di*	*Tri*
1	*50*	*1000*	*500*	*500*
2	*150*	*2500*	*1000*	*1000*
3	*250*	*5000*	*2000*	*2000*
4	*350*	*7500*	*3500*	*3500*
5	*500*	*10000*	*5000*	*5000*

Don't forget to save the curves using a unique file name for each one.

Once the calibration curve has been saved the first time, it will be saved automatically thereafter when you hit the Close button.



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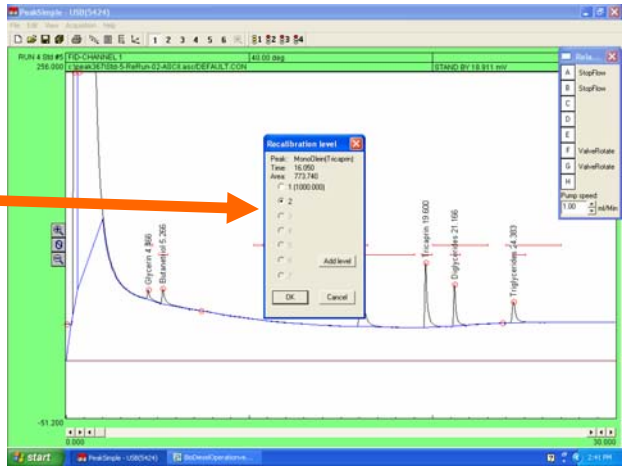
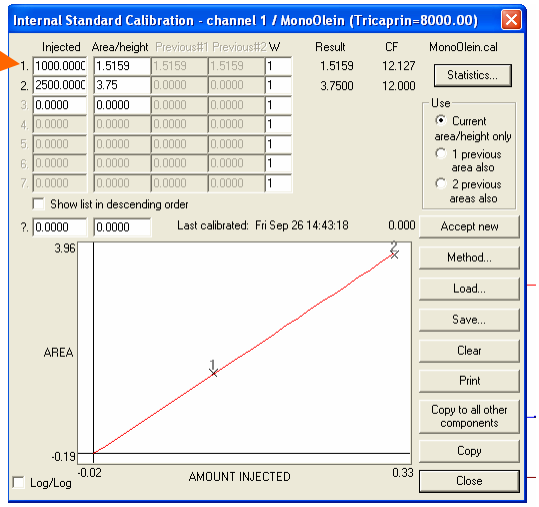
Once all four peaks (Glycerin, Mono, Di and Tri) have been calibrated at Level 1, Load the previously run and saved Level 2 chromatogram. Go through the same procedure except at Level 2. Note that the Re-calibration window requires you to select Level 2.



At the Calibration window, enter the Level 2 amount (2500 for MonoOlein), then hit the Accept New button. Again the ratio of the MonoOlein peak area divided by the area of the Tricaprin internal standard peak is calculated and entered into the cell just to the right of the Level 2 amount.

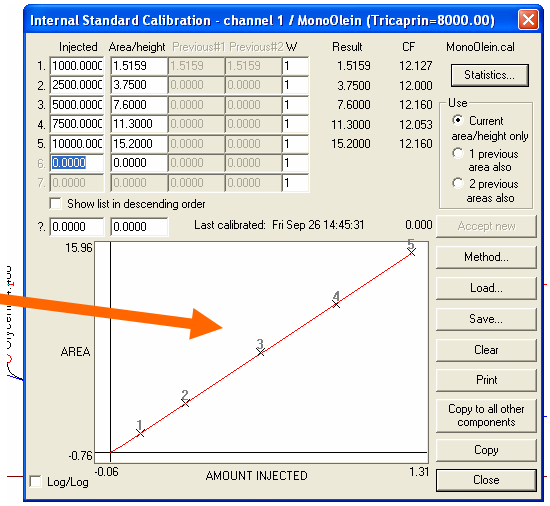


Do the same procedure for Glycerin, DiOlein and TriOlein.

Then Load the Level 3 chromatogram and repeat the calibration process for Level 3 for each of the 4 peaks.

Repeat for Level 4 and Level 5.

After calibrating all five levels, the calibration curve for each peak should look like the MonoOlein example shown at right. Note that the points on the curve should form a straight line.



Some analysts (who do not have to follow the ASTM Method 6584 exactly) elect to save time and just calibrate at Level 5 (instead of the 5 levels). Since the curve is linear anyway, a single point calibration at Level 5 will produce the same curve as plotting the 5 levels and take much less time.

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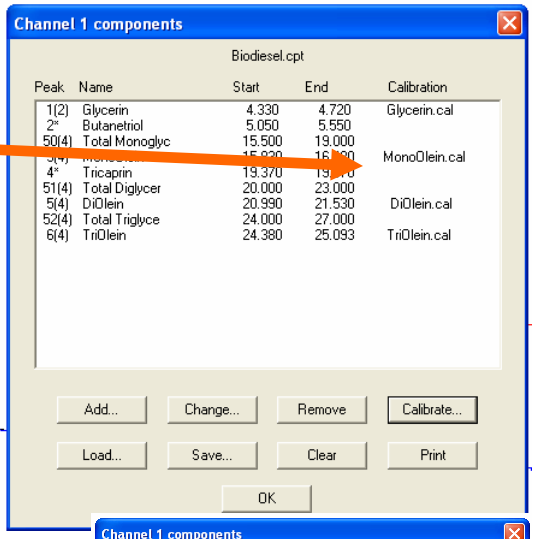
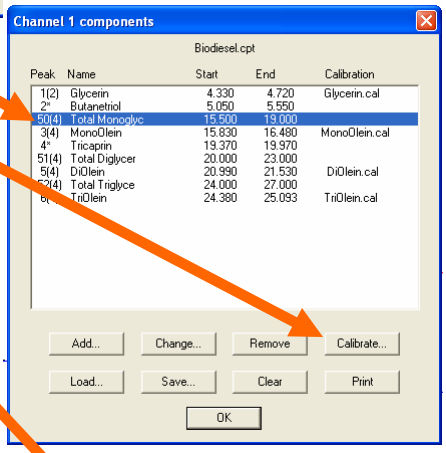
The Component Table should now look like the one shown at right. Note that only the four standard peaks (glycerin, Mono, di and Tri) have calibration curves attached.



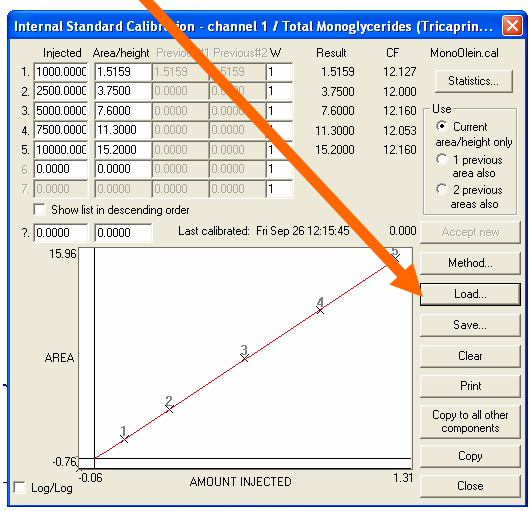
The two internal standards (butanetriol and tricaprin) do not have calibration curves. Also the 3 total peaks (Total Monoglycerides, Total Diglycerides and Total Triglycerdies do not as yet have calibration curves attached to the components windows.

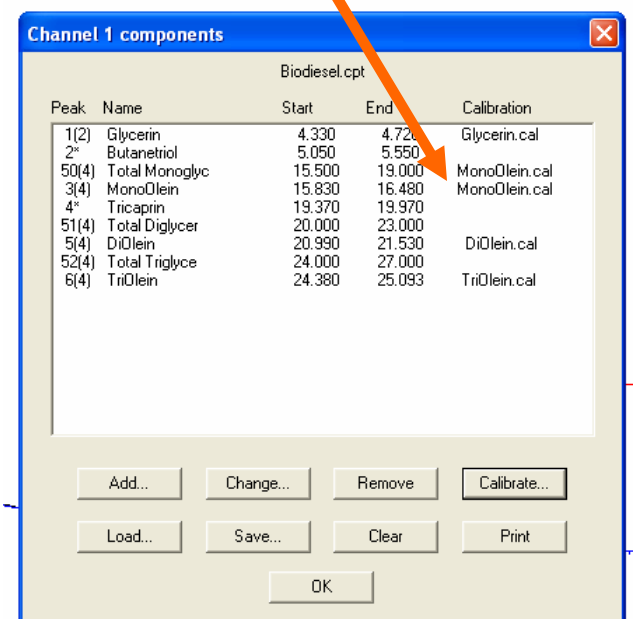
Highlight the Total Monoglycerides componenet by clicking it with your mouse, Then click the Calibrate button on the lower right of the screen.



A blank calibration curve window will appear. Click the Load button and then select the same calibration curve which you created for MonoOlein.

When you return to the Component table you will see that the Total Monoglycerides componenet now has the same calibration curve attached as the MonoOlein component.





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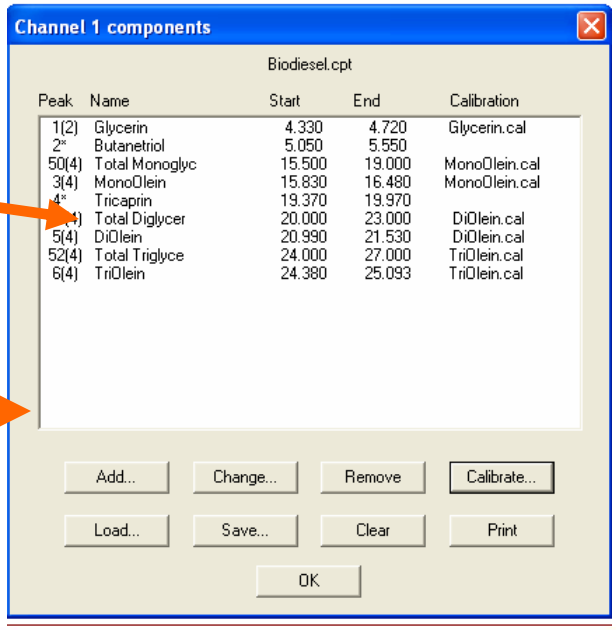
Do the same thing for the Total Diglycerides and Total Triglycerides peaks. Highlight the peak, click the Calibrate button and then load The DiOlein calibration curve for the Total Diglycerides window.

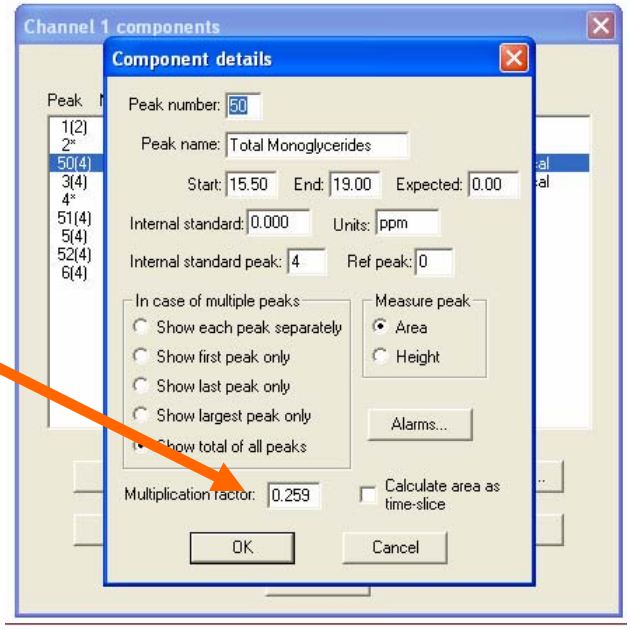
Do the same for the Total Triglycerides window, except load the TriOlein calibration curve.

The Component table should now look like the one at right.



Double check that the Total Mono, Total Di and Total Tri component windows have the correct multiplication factors inserted.

Be sure to click File/Save All to ensure that all the work you have done so far is saved.



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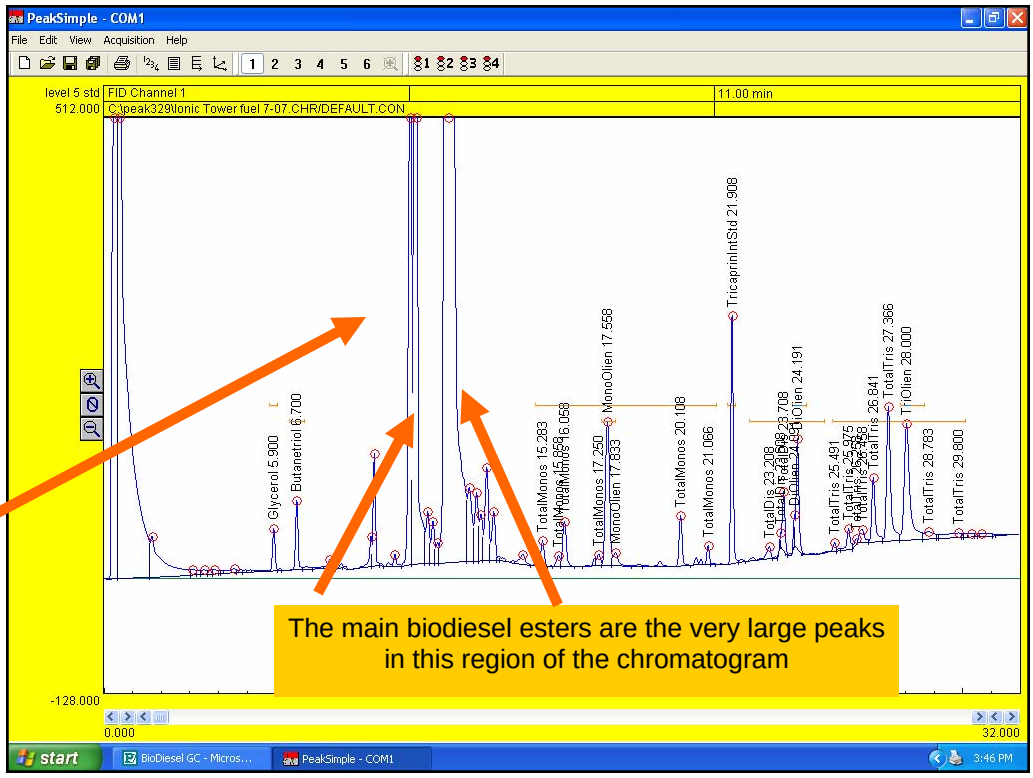
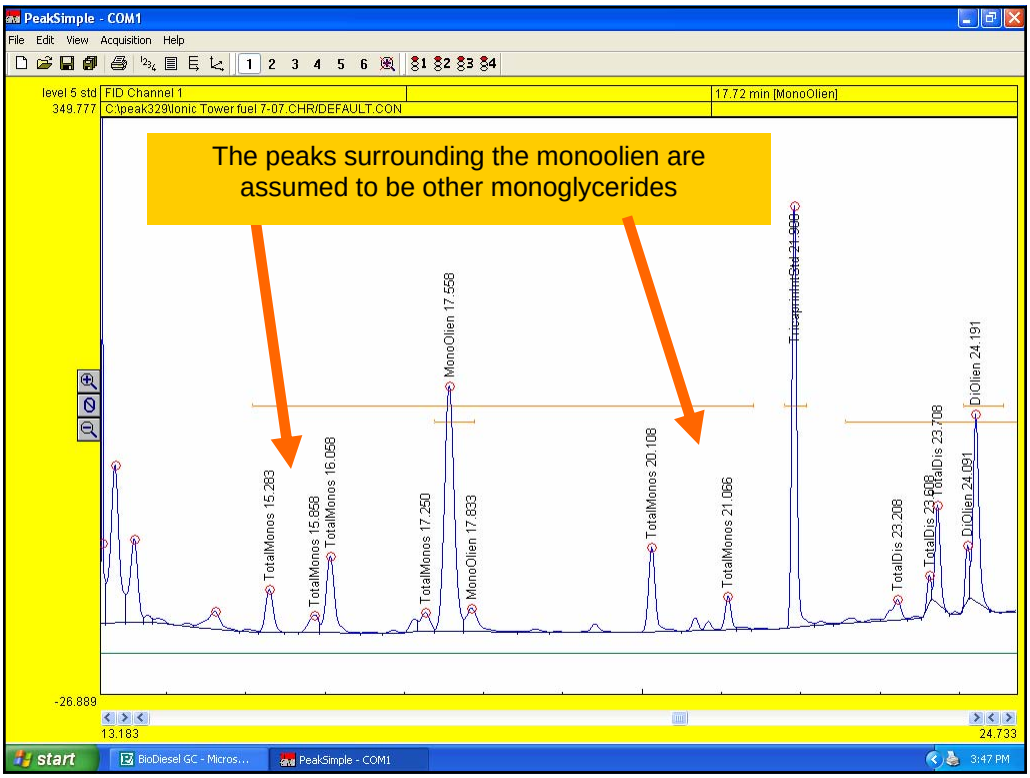
Now that the system is calibrated, prepare an actual BioDiesel sample by placing 100ul of BioDiesel, 100ul of Tricaprin in pyridine, 100ul of Butanetriol in pyridine, and 100ul of MSTFA in a vial. Allow 20 minutes for the reaction then dilute to 8ml with heptane.

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

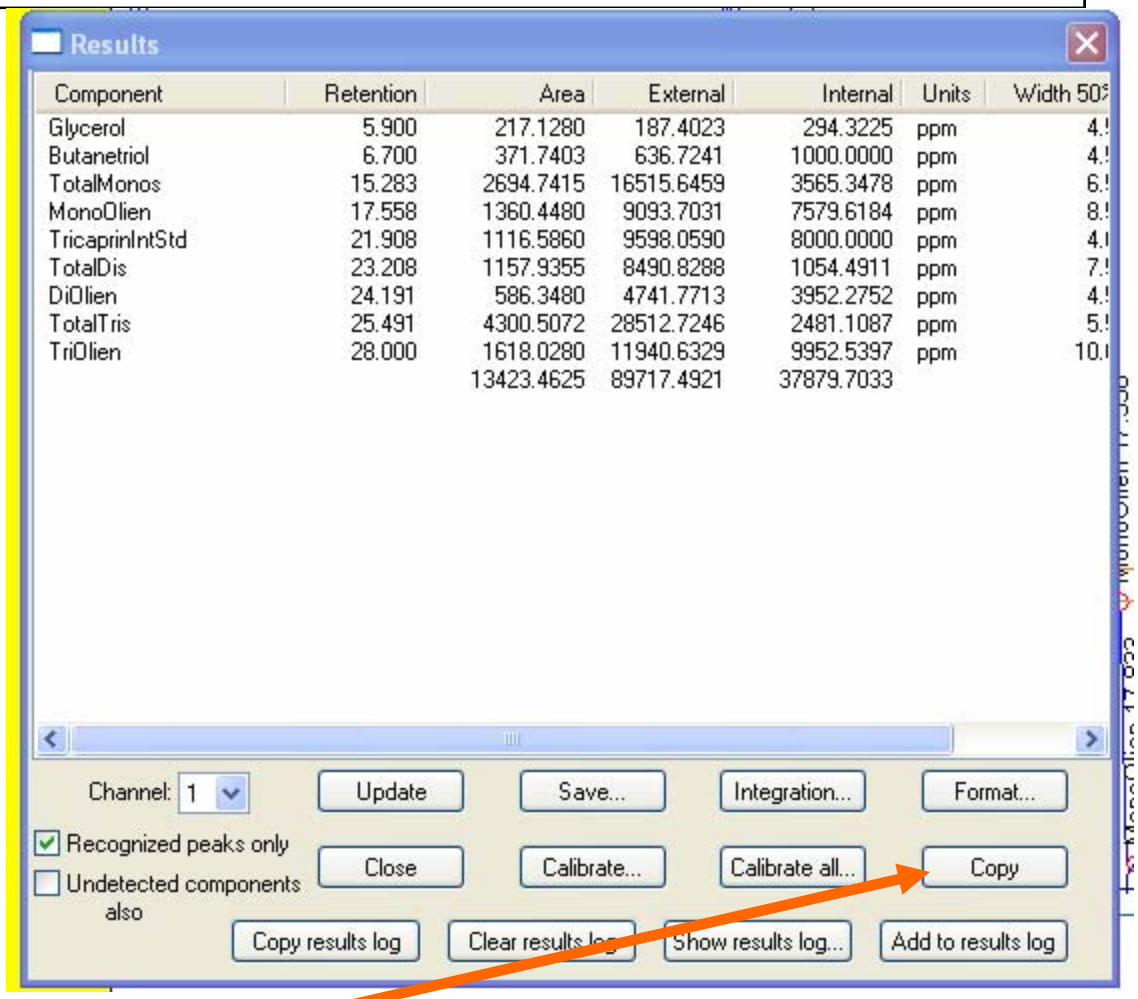


Zoom in to the region surrounding the mono, di and triolien. Stretch the total mono, di and tri component window so it includes the small peaks surrounding the monoolien, diolien and triolien. The ASTM 6584 method does not precisely define which peaks to include or exclude, so there is some judgement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



Performing ASTM 6584 free and total glycerin in BioDiesel using an SRI Gas Chromatograph and PeakSimple software version 3.67

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