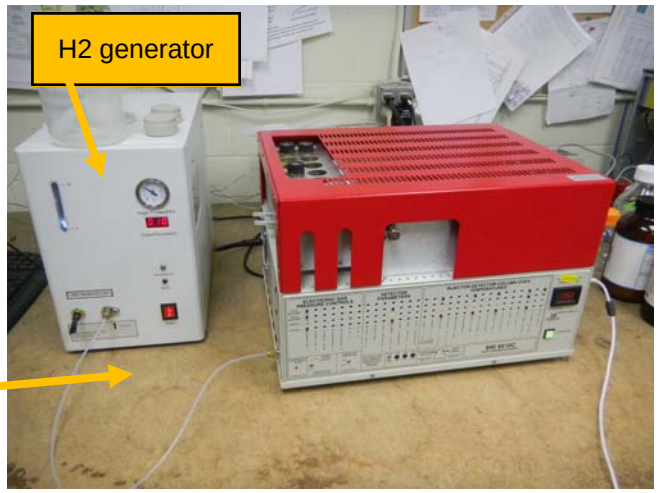
Cannabinoid Internal Standard Calibration

December 2016

SRI makes several models of gas chromatographs (GC) for cannabis testing. All models require the use of hydrogen carrier gas because they are all equipped with the same Flame Ionization Detector (FID), the “gold standard” method of detecting cannabinoids like CBD, THC, CBN and others.

This is the 8610C “professional model” (about $12K in Dec 2016) shown with the H2-100 hydrogen generator. ($3180 in December 2016) The hydrogen generator is optional, you could use a cylinder of hydrogen instead.



This is the 310C model (about $10K in December 2016) equipped with a built-in hydrogen generator. It generates the same quality of data as the 8610C above but does not have some of the “professional” features. The built-in hydrogen generator requires some human interaction every 8 hours, so it is best suited to intermittent testing operations, not 24/7 operation.

This is the Model 420 ($4995 in Dec 2016). It has the same built-in hydrogen generator as the 310C above, but is not temperature programmable. It does not provide as much detail (can’t distinguish CBD from CBC, for example) as the other two models above but is perfectly fine for routine CBD or THC level testing.

All models use the same software and are calibrated in the same way.

Cannabinoid Internal Standard Calibration

December 2016

The are two calibration methods that are used to measure cannabis, " external standard" (ES) and "internal standard" (IS).

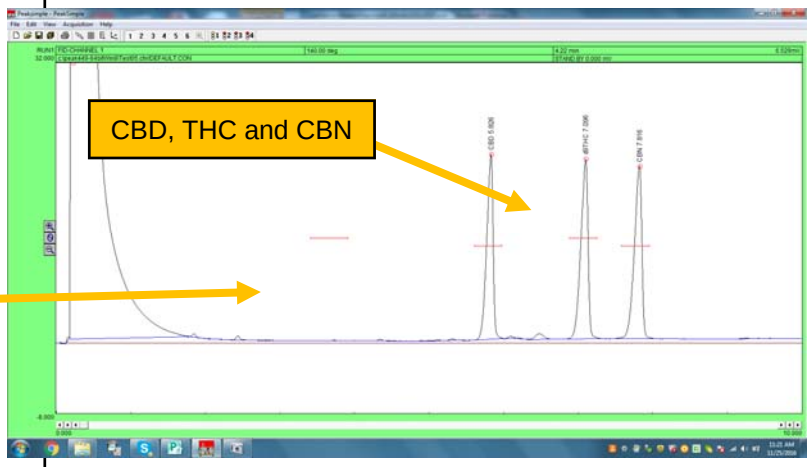
Both methods require that you purchase the certified calibration standard from Restek, Cerilliant or Lipomed which as of Dec 2016 are the only companies legally allowed to sell this.

The calibration standards are delivered in sealed ampules as shown at right. As of Dec 2016 the cost of an ampule ranges from $25 to $75.

You can buy the "Primary standards" as individual molecules (just CBD, just THC, etc) or you can buy a mixture of CBD, THC and CBN with each molecule dissolved in methanol solvent at a concentration of 1000 nanograms per microliter (ng/ul).

On the ampule it may say 1mg/ml or 1000ug/ml, but this is the same as 1000ng/ul.

If you inject the 3 way mix directly out of the ampule you get a chromatogram like this.



Notice there are just the 3 cannabinoid peaks and the large solvent peak at the beginning.

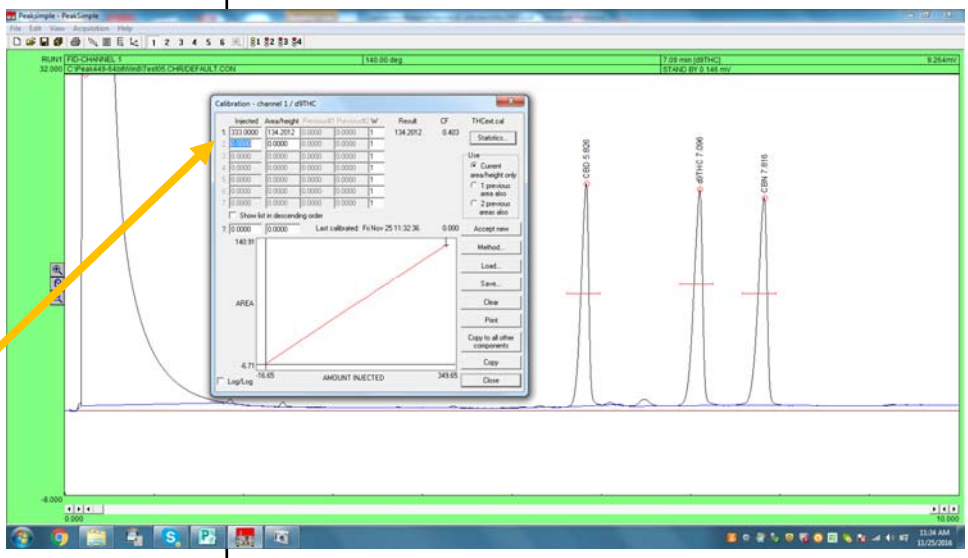
If you buy the standards as individual molecules, you can make a "working standard" by using the 100ul syringe supplied with the GC to mix the "primary standard" in any concentration in a separate vial. Most people mix the CBD, THC and CBN in equal amounts to make a "working standard with a concentration of 333ng/ul each, but there are no rules about this and it may make sense to adjust the relative amounts to more closely match the type of sample being measured. For example, hemp testers want the THC to be less than .3%, so it make sense for them to calibrate the GC at a lower level closer to what they would see in a real sample.

Cannabinoid Internal Standard Calibration

December 2016

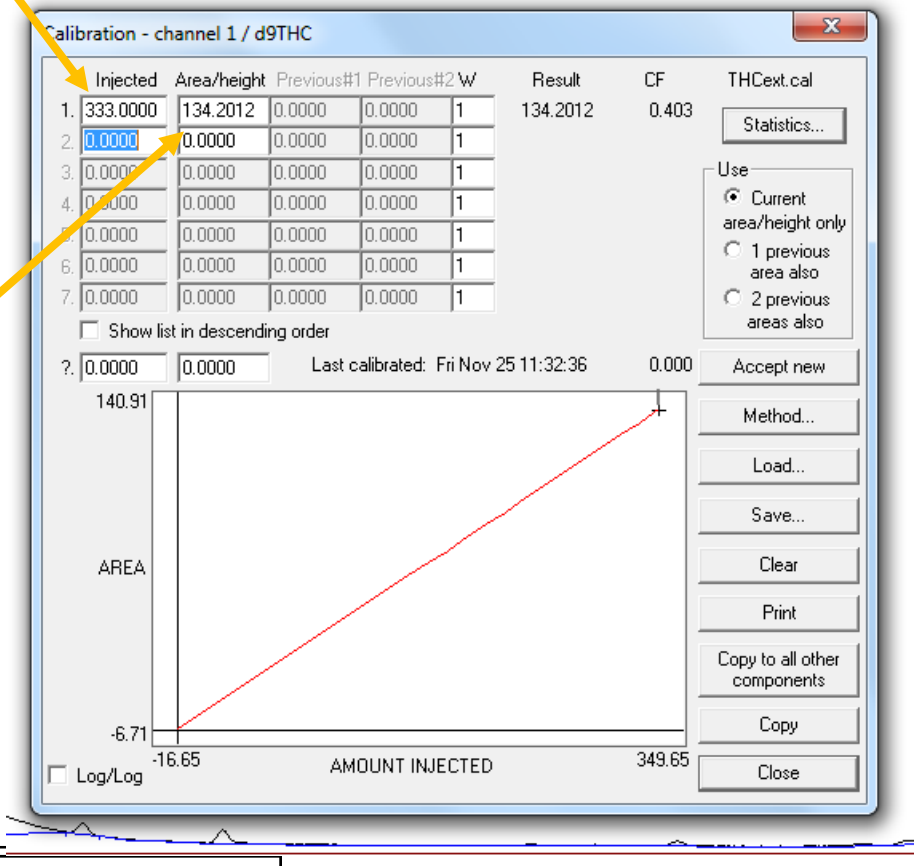
The “External Standard” calibration process starts by creating a calibration curve for each of the cannabinoids (CBD, THC and CBN).

In the top left cell of each calibration curve you enter the number of ng/ul you injected. So if you injected 1ul of the 3way standard (which is supplied at 1000ng/ul) you would enter the number 1000 into the top left cell.



If you had made a “working standard” consisting of equal amounts of CBD, THC and CBN, then you would enter the number 333 into the top left cell.

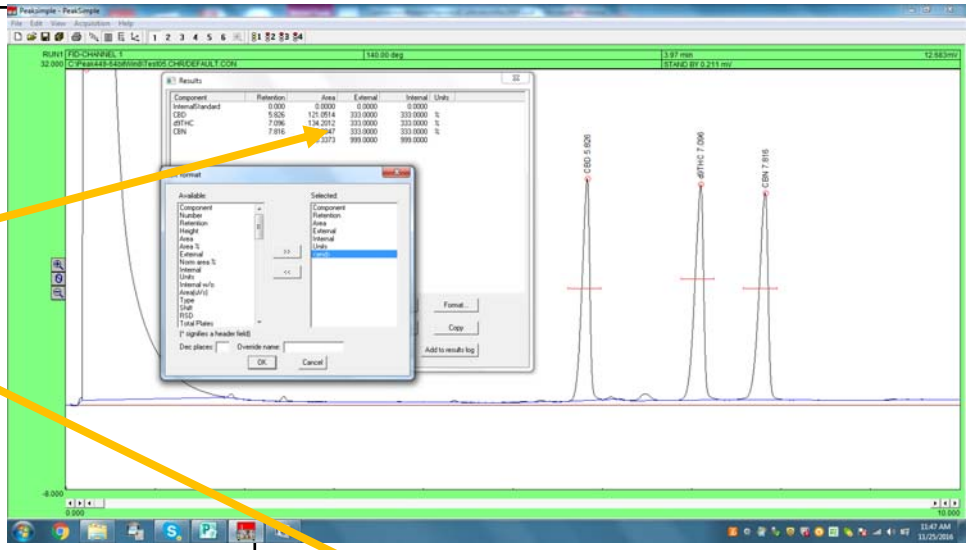
In the next cell you enter the area of the peak. You can type this in, or you can click the “Accept New” button. In the example at right, the peak area is 134.2012. This creates a single level calibration curve which describes the relationship between the peak area and the number of nanograms injected into the GC. If the GC system is linear in its response, then presumably if you inject 2ul instead of 1ul the peak area would be about 268 area counts instead of 134.



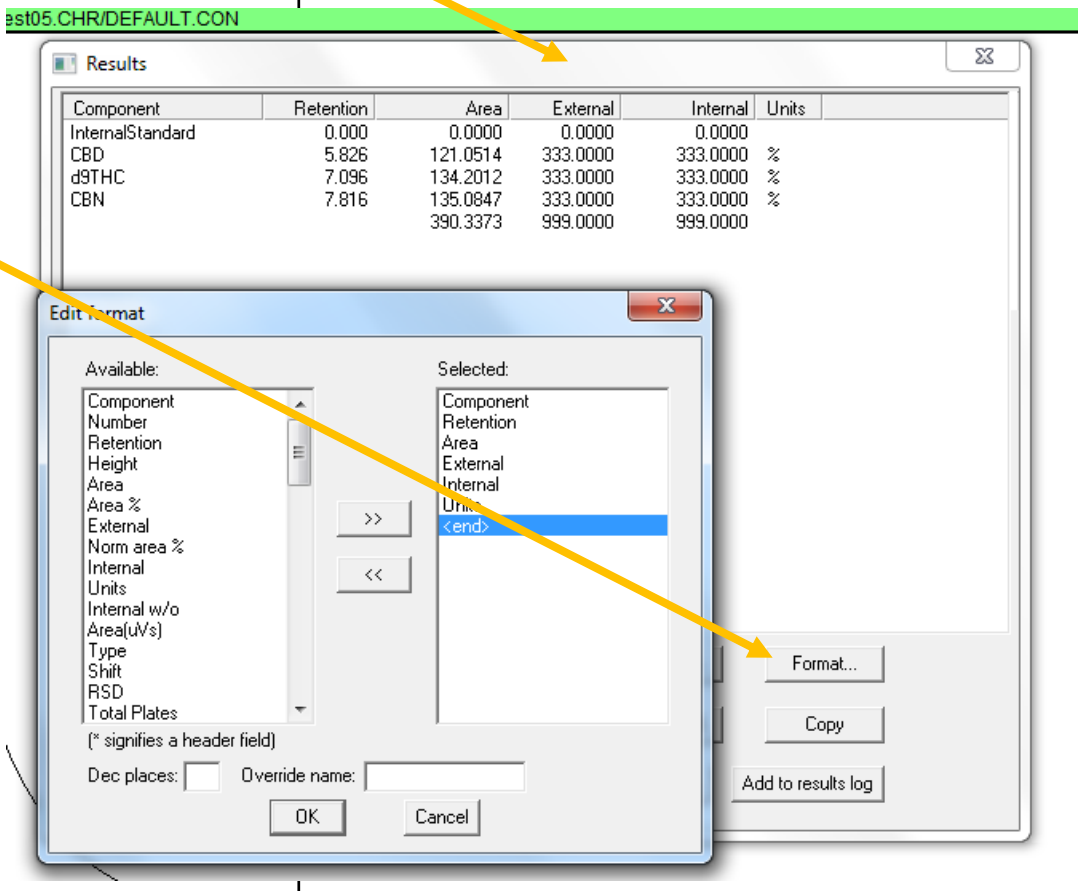
Cannabinoid Internal Standard Calibration

December 2016

When you navigate to the View/ Results screen, you see the calculated answer shows the number 333 in both the External and Internal columns.



You can click the Format button to select whether the External, Internal or both results appear in the report.



Cannabinoid Internal Standard Calibration

December 2016

Sometimes you might want the answer in ng/ul. If you were measuring a beverage for instance, you could inject 1ul of the THC infused beverage directly into the GC and get the answer in ng/ul or mg/ml directly with no further math required.

For flower or concentrate samples, most people prefer the answer in the percent of THC in the flower or sample.

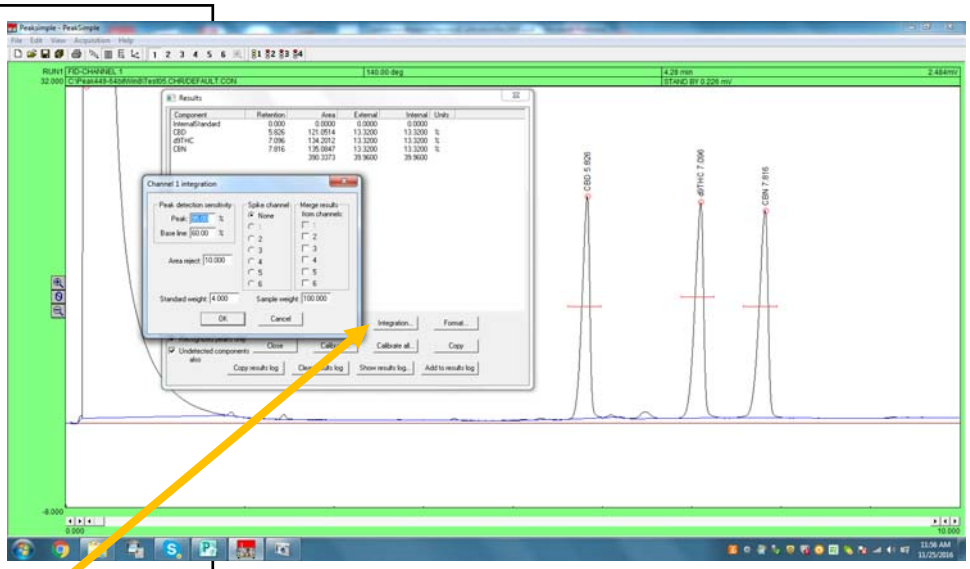
To convert the calibrated result from ng/ul to percent in the flower or concentrate:

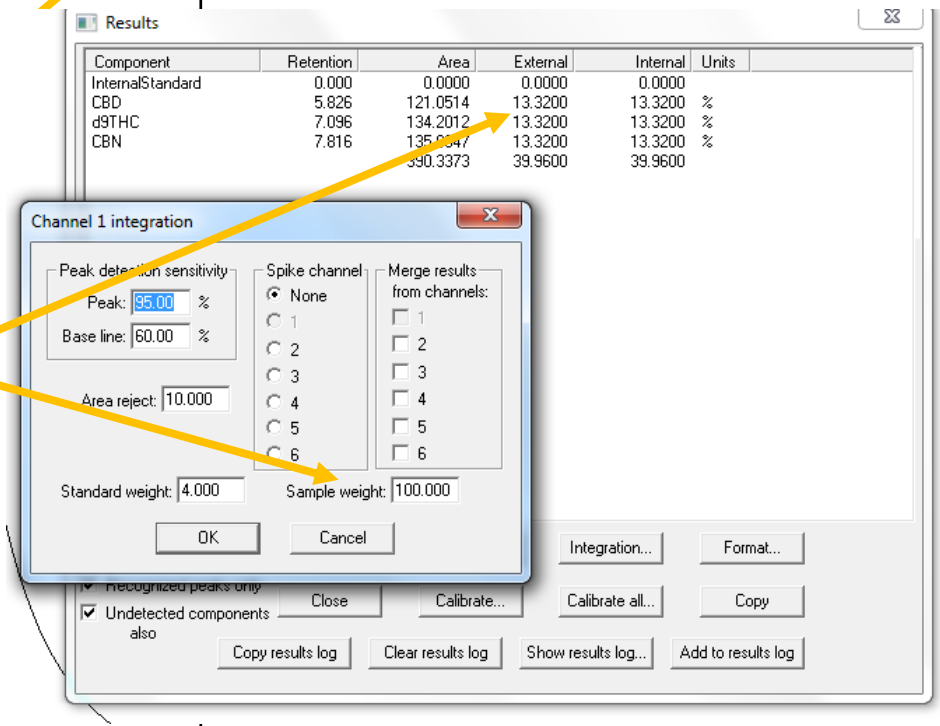
Click the Integration button to enter the “Standard Weight” and “Sample Weight”.

Enter the number 4 into the “ Standard Weight” box and the number 100 into the “Sample Weight” box. This multiplies the result from the calibration curve (333) by 4 and then divides by 100 to equal 13.32.

This is the concentration of the CBD, THC or CBN which would be in the starting sample if the starting sample weighed 100 milligrams.

If the sample weighed either more or less (its tedious and time consuming to try to weigh out exactly 100 milligrams), you enter the actual weight of the sample into the sample weight box and the answer is corrected for the actual weight of sample you used.



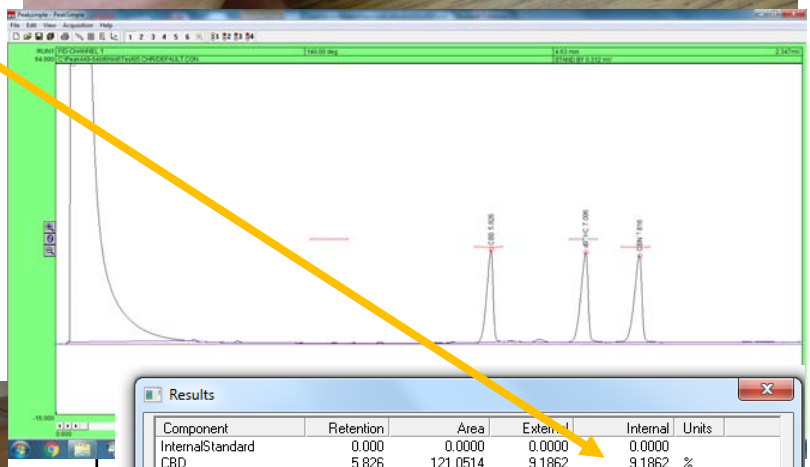


Cannabinoid Internal Standard Calibration

December 2016

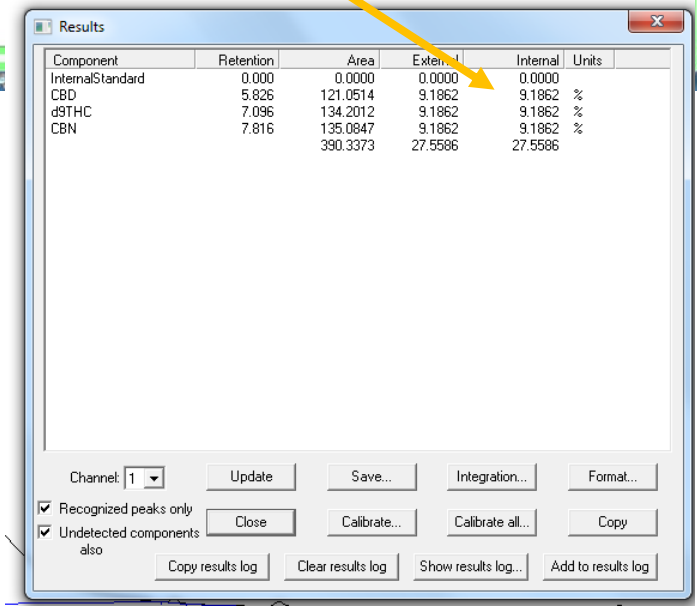
While the “External Standard” method of calibration is simple and easy, it is very dependent on injecting the same amount of sample each time.

If you inject less the answer comes out less.



Even the most experienced GC operator can only keep the amount injected within a few percent variation, and it takes extra time and patience to fuss with the syringe to get the amount injected the same each time. Even a robot type auto-sampler (which SRI also sells) has trouble injecting exactly the same amount every time.

every

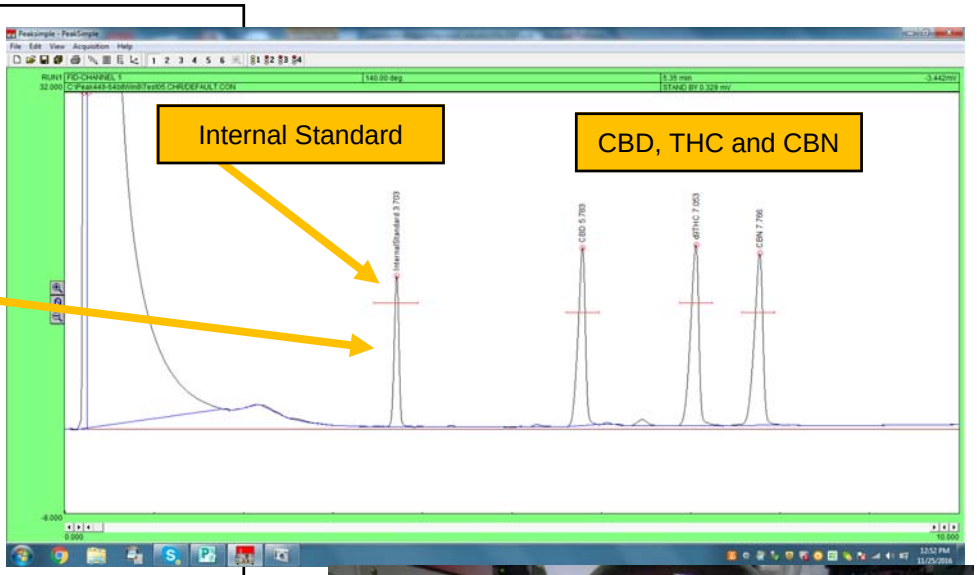


The “Internal Standard” method of calibration eliminates most of this problem.

Cannabinoid Internal Standard Calibration

December 2016

A calibration chromatogram for “Internal Standard” has an additional peak that was not present in the “External Standard” calibration chromatogram.



There are many possible molecules which could be chosen as the “Internal Standard” peak.

SRI suggests the Methyl stearate molecule (CAS# 112-61-8) because its cheap, non-toxic and elutes in a convenient place in the chromatogram just before CBD. Its oil from the palm tree and used widely in lipstick, cosmetics and hand lotion.

You can buy 95% pure (or better) Methyl stearate in 500 gram jars for about $30 (16 cents/gram) from TCI or 99% pure from Sigma-Aldrich for $10 per gram.

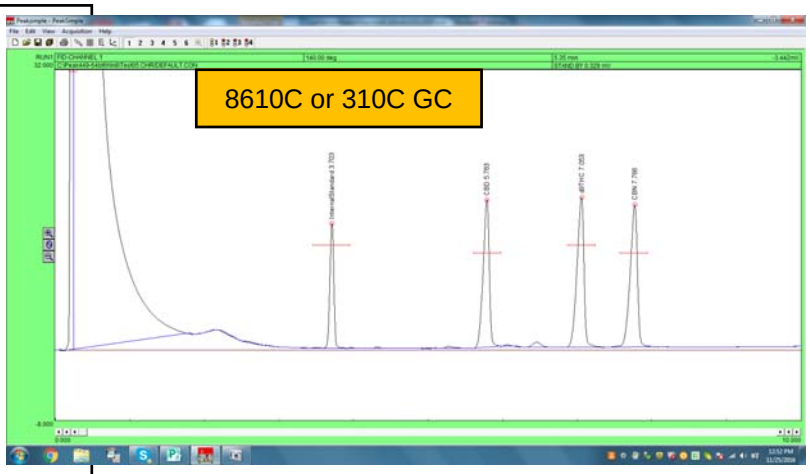
The more expensive MS is fine but does not improve the results.

At room temperature it’s a white powder.

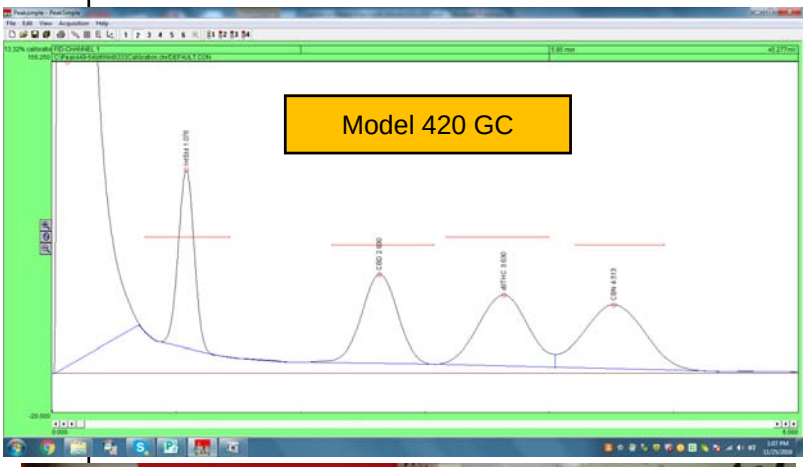
Cannabinoid Internal Standard Calibration

December 2016

This is what a 333ng/ul CBD, THC and CBN chromatogram looks like on the 8610C or 310C GC. The Methyl stearate Internal standard is mixed in with the " Working Standard" so there are four peaks.



This is the same calibration mix on the Model 420 GC. The peaks are a little less sharp, but the analysis time is shorter.



The "Internal Standard" analysis process starts by preparing the IS extraction solvent.

Either Denatured Alcohol or Acetone can be used. Both cost about $15 per gallon at Home Depot or any hardware store. You can transfer the alcohol or acetone into smaller bottles if you want, or you can work with the gallon.

Cannabinoid Internal Standard Calibration

December 2016

Weigh out 1 gram of the Methyl stearate (MS). It doesn't matter if you are a little off in the measurement.

Put the 1 gram of MS in the gallon of alcohol or acetone.

You can use a smaller bottle if you like. Use 246 milligrams of MS per liter. Don't use a water bottle or anything someone (a child) might accidentally drink from.

Be sure to label the bottle (or gallon) with the date because each batch of extraction solvent you make will correspond to a different calibration standard. When you run out of extraction solvent you will have to make a new batch and a new calibration standard. A gallon gives you enough for 100 analyses. A liter enough for 24.

The MS dissolves quickly in the Acetone, but can take a while in the alcohol. It should be totally dissolved before you use it. Warming the solvent helps dissolve the MS, but don't put it in the oven or microwave since these are flammable solvents. The glass bottles help you be sure that the MS is totally dissolved.

Cannabinoid Internal Standard Calibration

December 2016

Make a “Working Internal Standard” calibration mixture from the Primary Standard and the Extraction solvent (which has had the MS added).

It doesn’t matter if you use a 1000ng/ul primary standard or a 333ng/ul primary standard or any concentration you prefer. The important thing is to put exactly the same amount (50/50) of the primary standard and the Extraction solvent in the small vial using the 100ul syringe. Typically you might use 500ul each.

This keeps the ratio of the CBD,THC,CBN to the MS the same as it will be when you do an actual extract, since both the THC and the MS are both diluted by 50%.

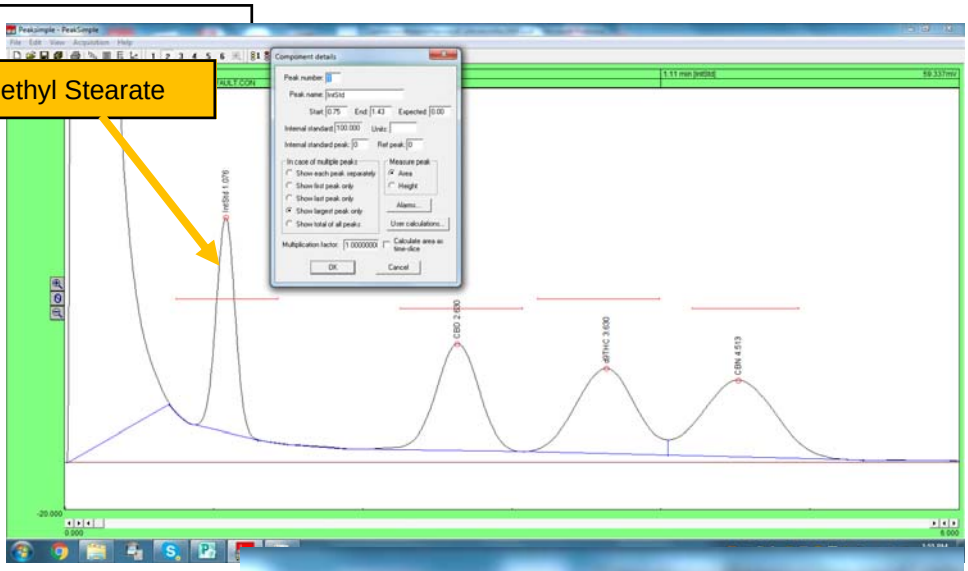
The actual extract of 100mg flower or concentrate is made from the same batch of Extraction Solvent (this is why the date or batch number is important).

As long as the calibration standard is made from the same batch of extraction solvent as you use for an actual extract, any errors in weighing out the MS cancel out.

Cannabinoid Internal Standard Calibration

December 2016

Inject 1 ul of the “Working Internal Standard” mix (has the MS added) to get a chromatogram. The one shown was run on a Model 420 GC.

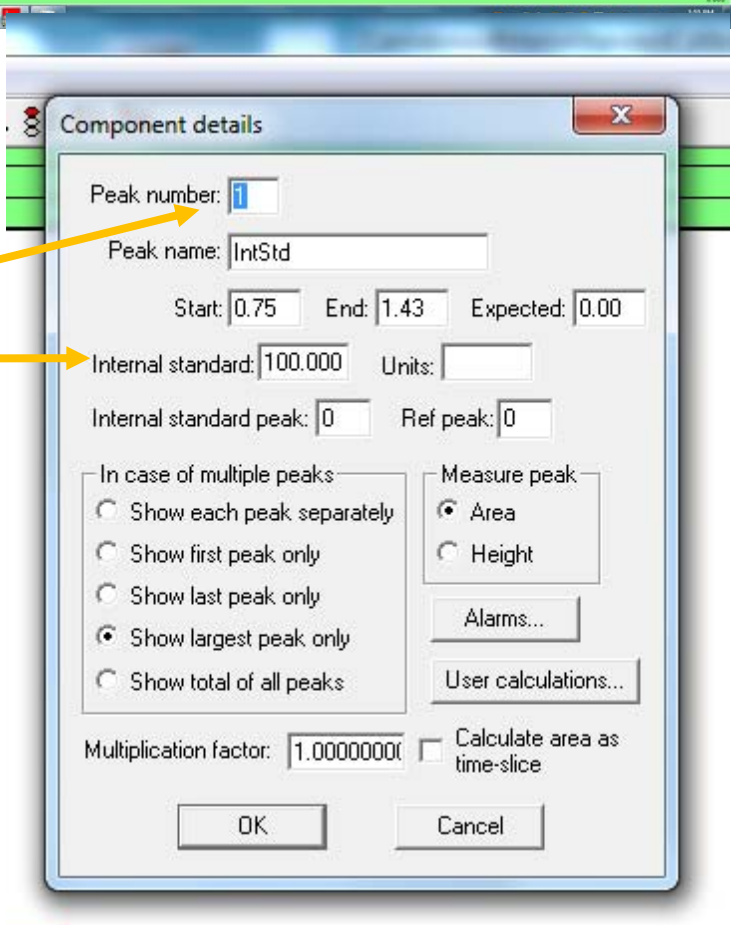


Create a Retention window for each of the 4 peaks. The retention window is the horizontal red bar in the middle of the screen which identifies the peak.

In the Component Details screen for the MS peak enter the number 1 for the Peak number and the number 100 in the Internal Standard box.

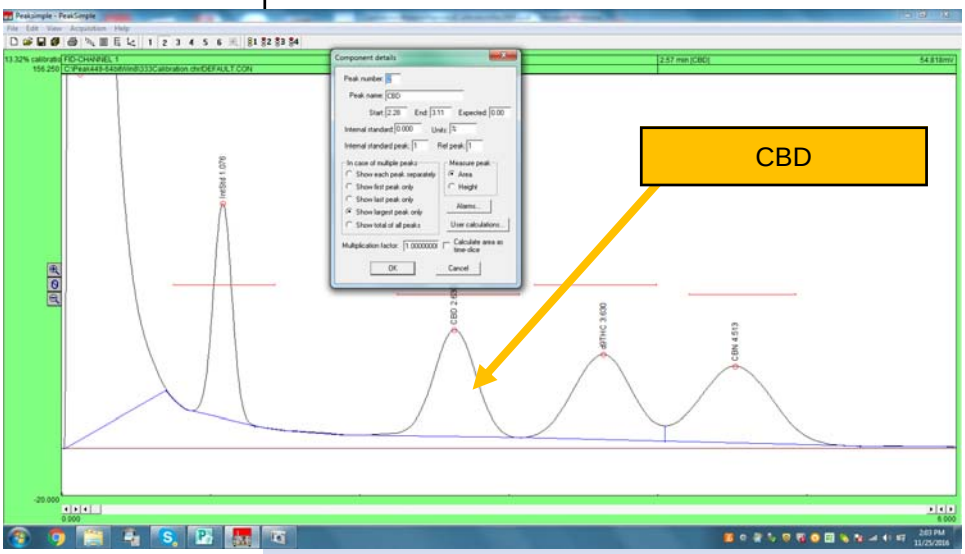
It does not matter if you pick a number other than 100. Any number except 0.00 will do. If there is any non-zero number in this box it tells PeakSimple (the software) that this is an Internal Standard peak.

Pick any name you like for the MS Internal Standard peak name. In the example, it is just labelled IntStd.



Cannabinoid Internal Standard Calibration

December 2016



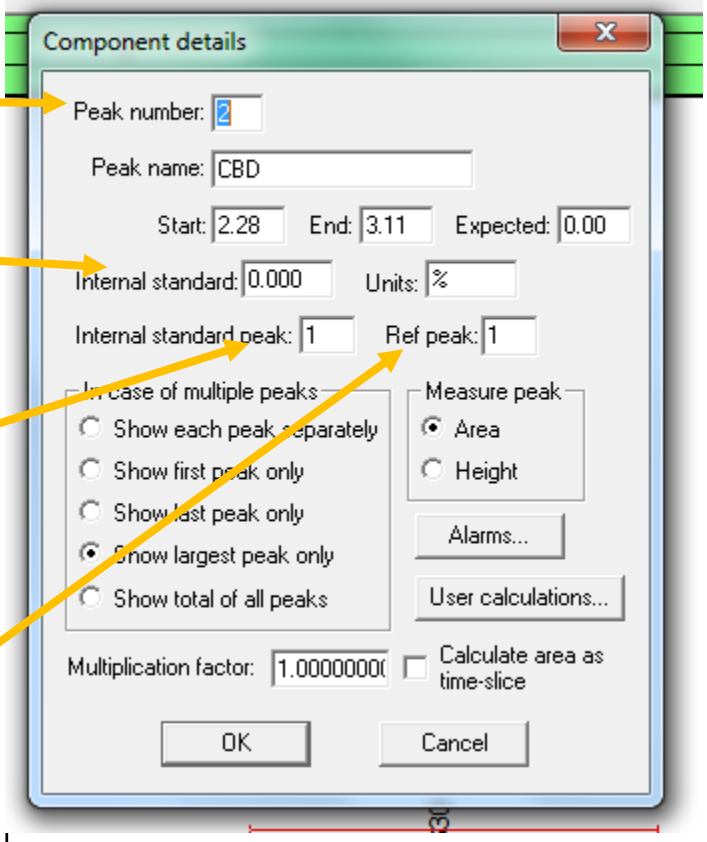
In the Component Details screen for CBD enter the number 2 for the peak number (no two peaks with the same number).

Make sure the Internal Standard box is 0.00 since the CBD is not itself an Internal Standard.

Make sure the Internal Standard Peak box shows the number 1. This tells PeakSimple that peak#1 (the MS) is the Internal Standard peak for CBD. Having an Internal Standard peak corrects for injection volume shifts.

Make sure the Ref peak box shows the number 1. This tells PeakSimple that peak#1 (the MS) is also the reference peak for CBD. Having a reference peak corrects for retention time shifts. Peak#1 can be both the Internal Standard peak and also the Reference peak.

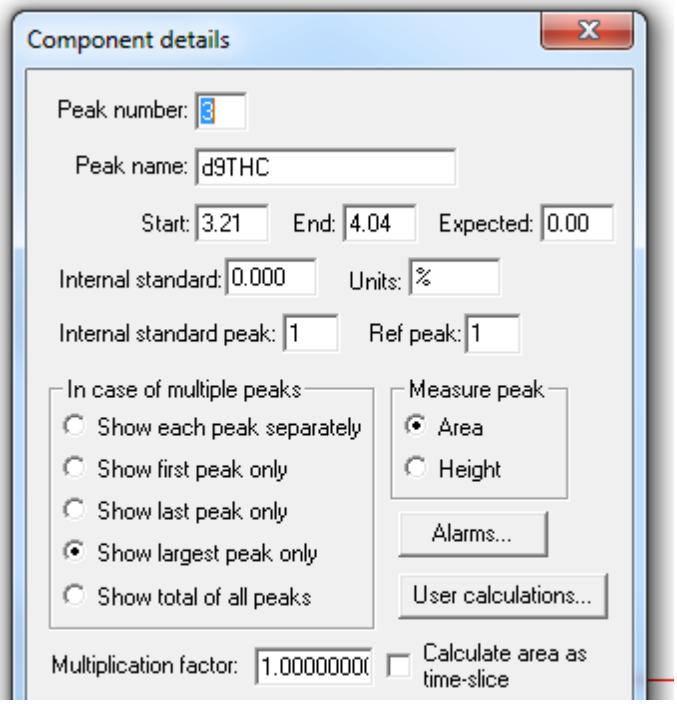
Enter the percent (%) sign in the Units box.



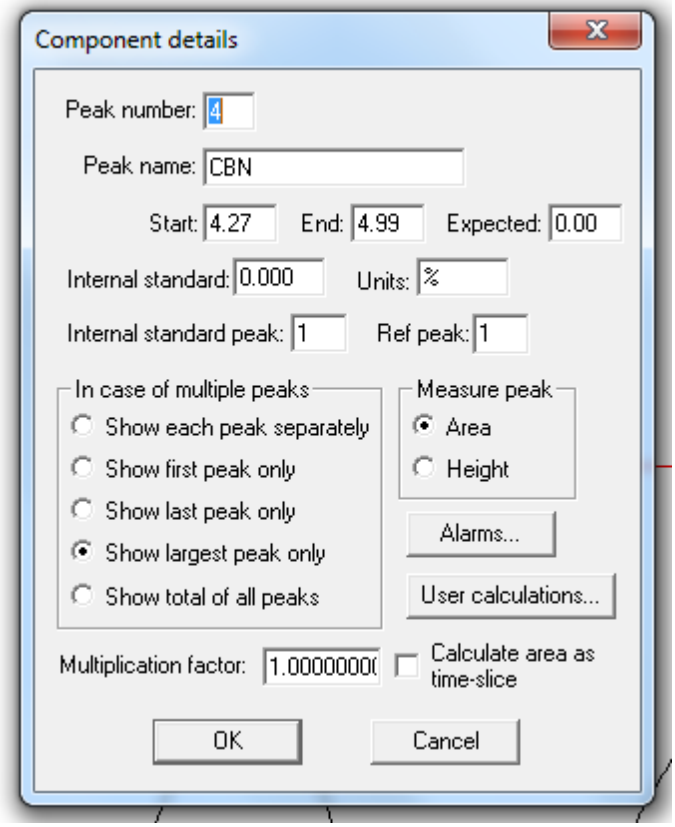
Cannabinoid Internal Standard Calibration

December 2016

Do the same for THC except make the peak number 3



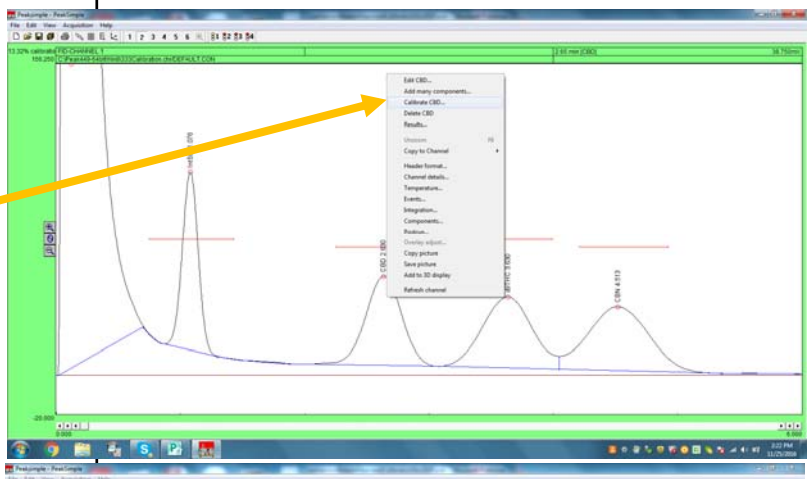
Do the same for CBN except make the peak number 4.



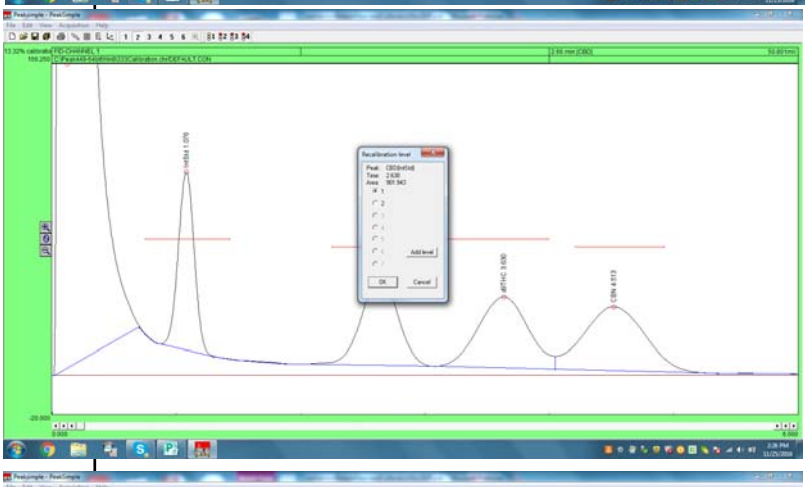
Cannabinoid Internal Standard Calibration

December 2016

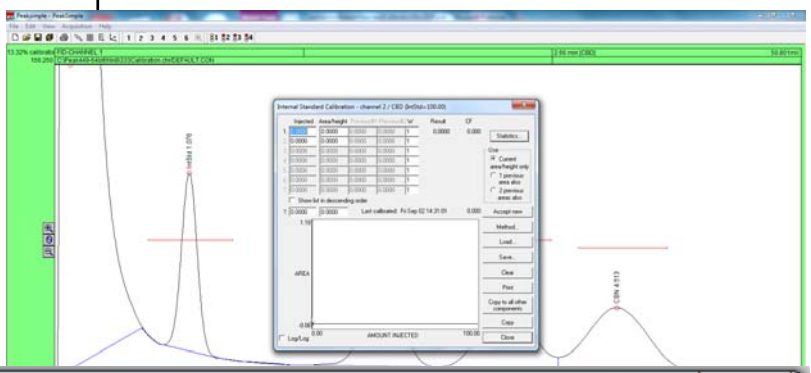
Point to the CBD peak with your mouse and right click. A menu will pop up. Left click on "Calibrate CBD".

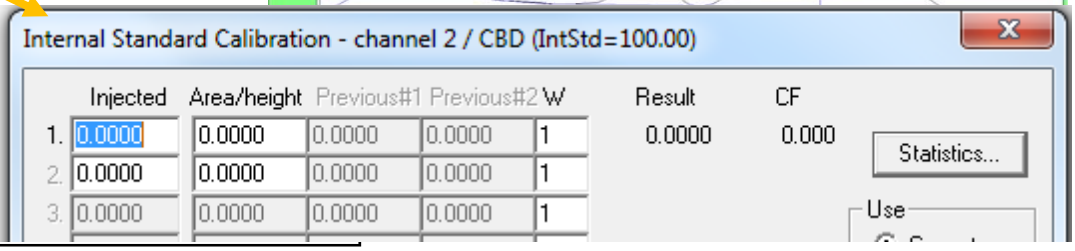


At the Recalibration Level screen select the Level 1 radio button if it is not already selected.



This takes you to the blank calibration curve screen. Notice that the screen is labelled Internal Standard Calibration which is different than the label when doing an External Standard Calibration.





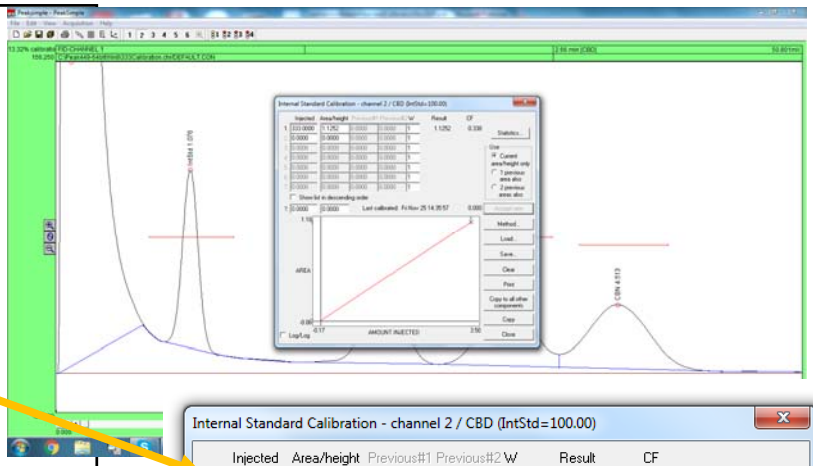
Cannabinoid Internal Standard Calibration

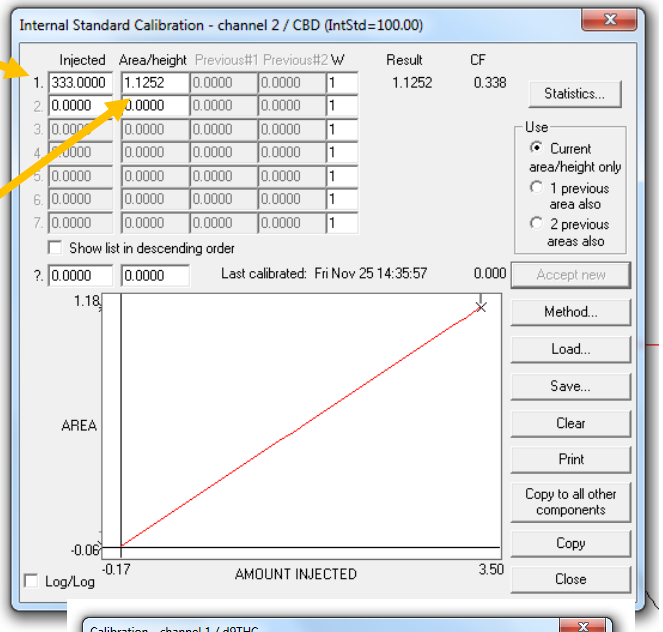
December 2016

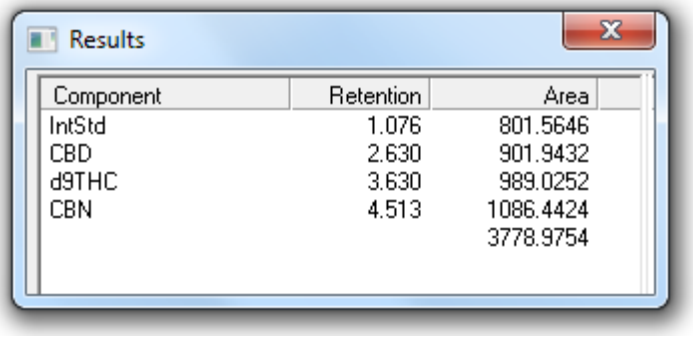
Enter the ng/ul of your “Primary Standard” in the Injected column, top row.

In this case the number is 333, but it should be whatever the concentration of the standard was that you used to prepare the Internal Standard Calibration mixture.

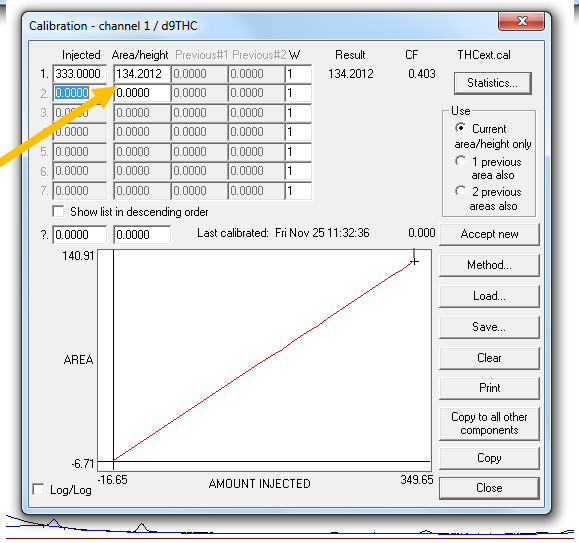
Click the “Accept new” button, and PeakSimple calculates the ratio of the CBD peak Area to the Internal Standard peak area and puts that ratio in the next cell to the right. In this case the number is 1.1252, which is 901.9432 divided by 801.5646 = 1.1252.







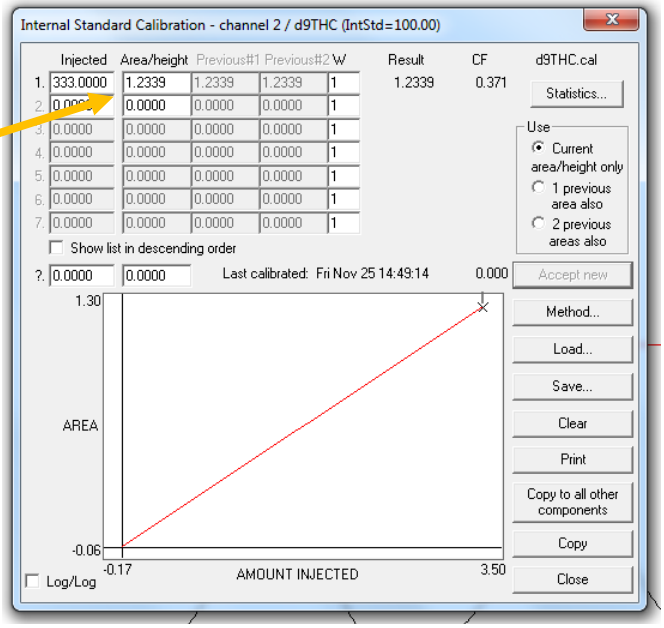
Notice how this is different than the External Standard Calibration curve where the number is the area of the CBD peak, not the ratio.

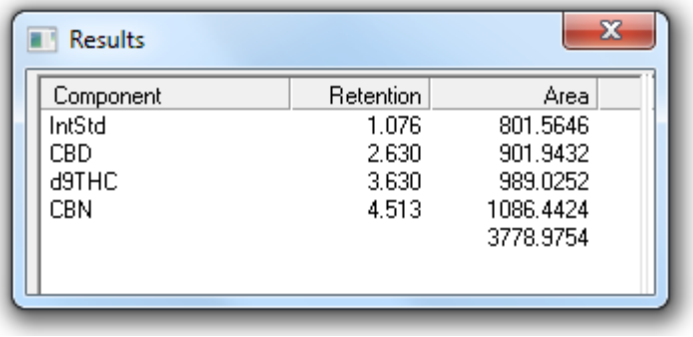


Cannabinoid Internal Standard Calibration

December 2016

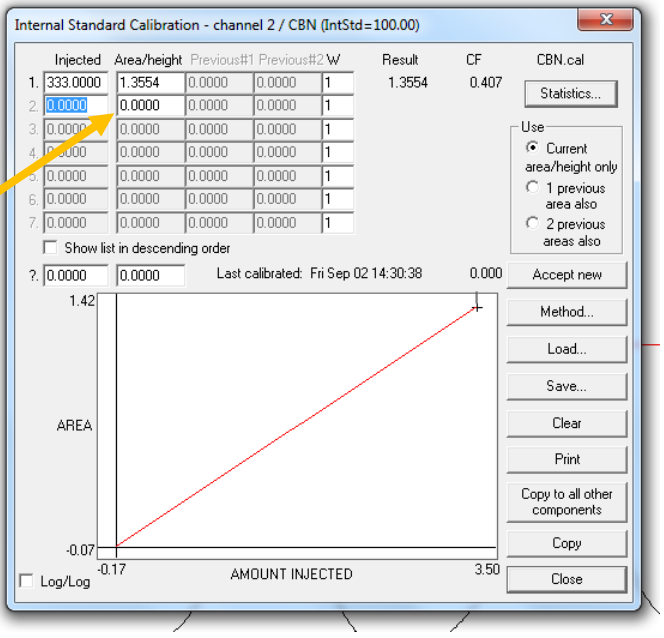
Do the same for the THC peak.





And the CBN peak.

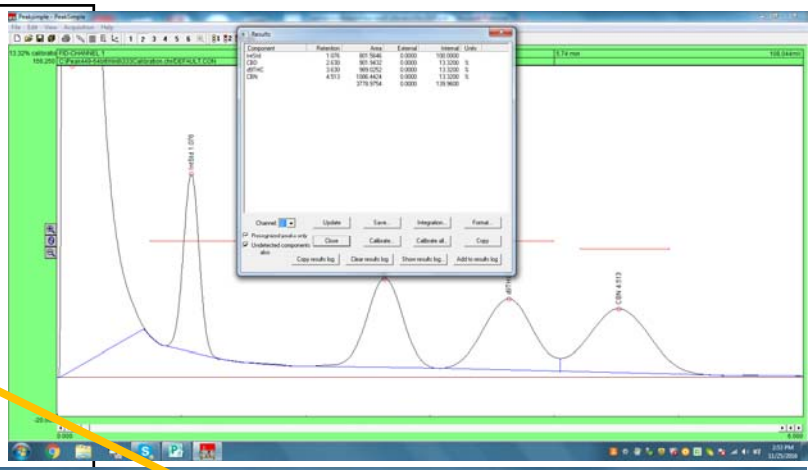
Notice that the ratios are slightly different for each peak.



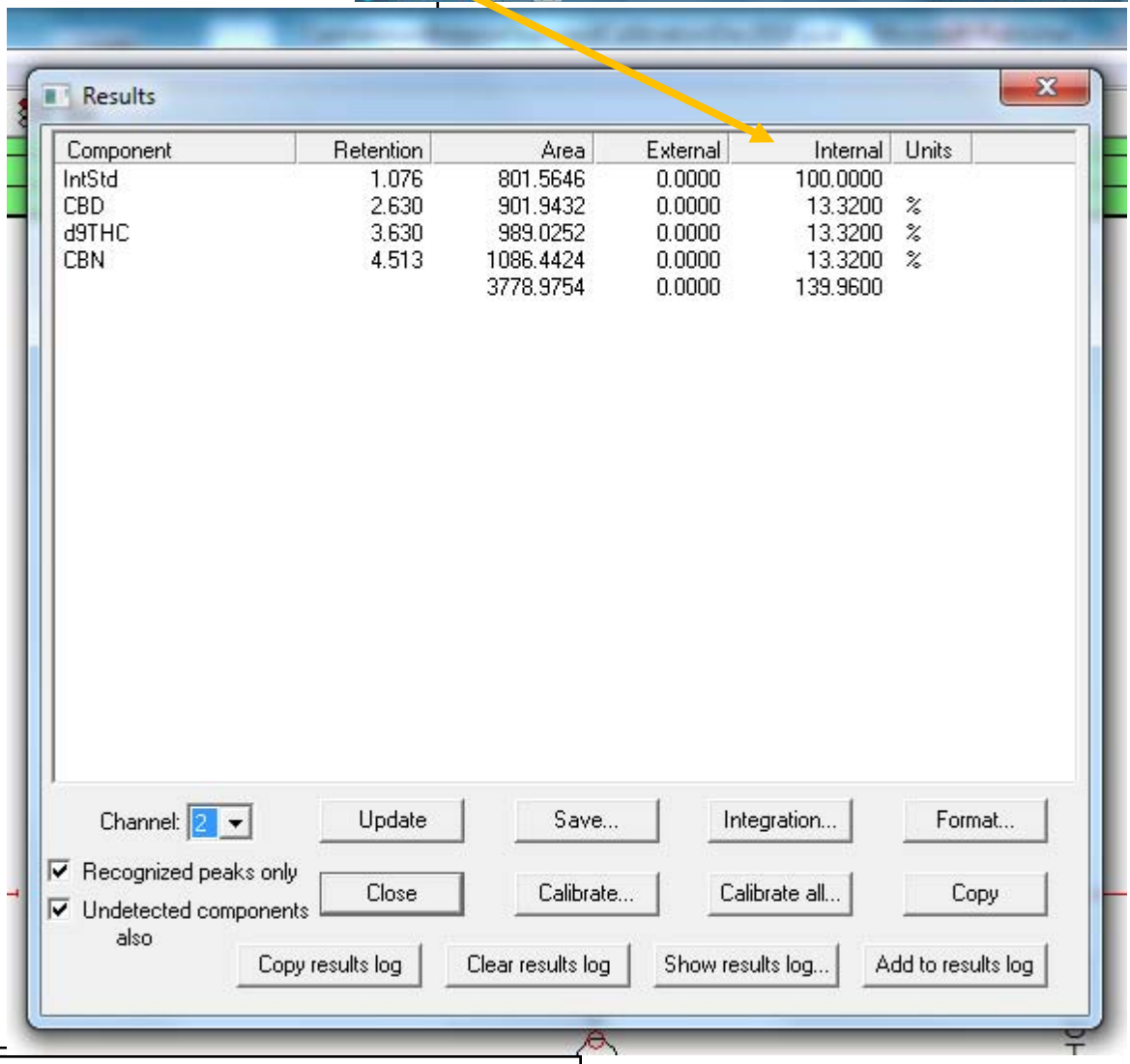
Cannabinoid Internal Standard Calibration

December 2016

Go to View/Results (the Results screen) and notice that the answers are the same as in the External Standard Calibration (13.32%) but the answers appear under the Internal column heading. All the answers under the External column heading are now 0.000.



Click the Format button to delete the External column if you like.

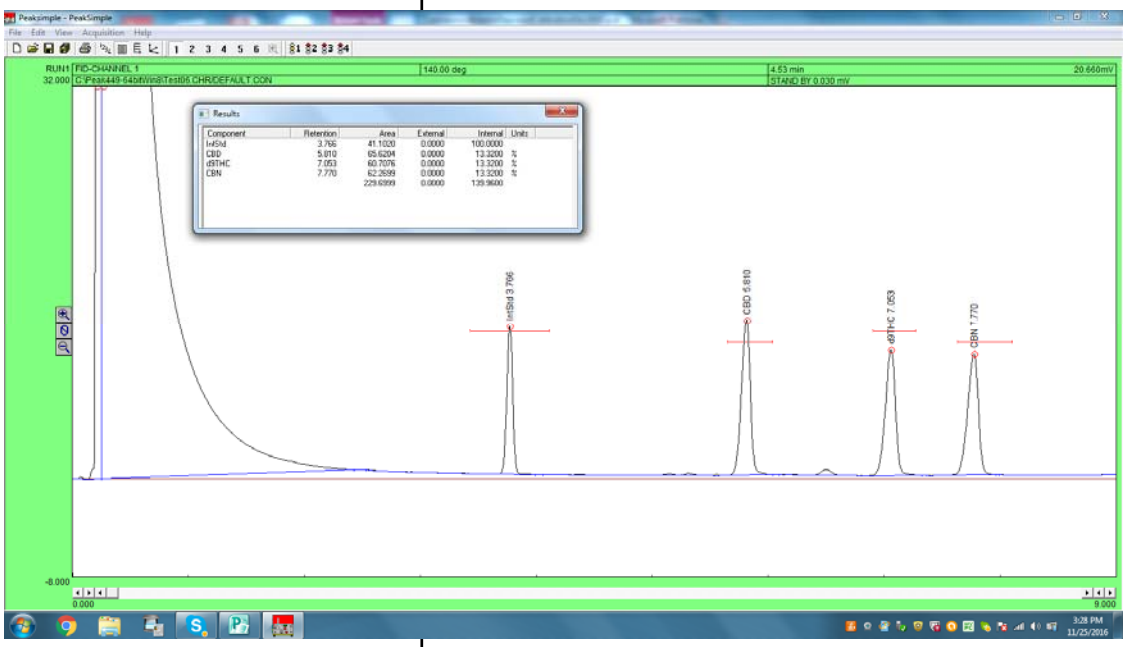


Cannabinoid Internal Standard Calibration

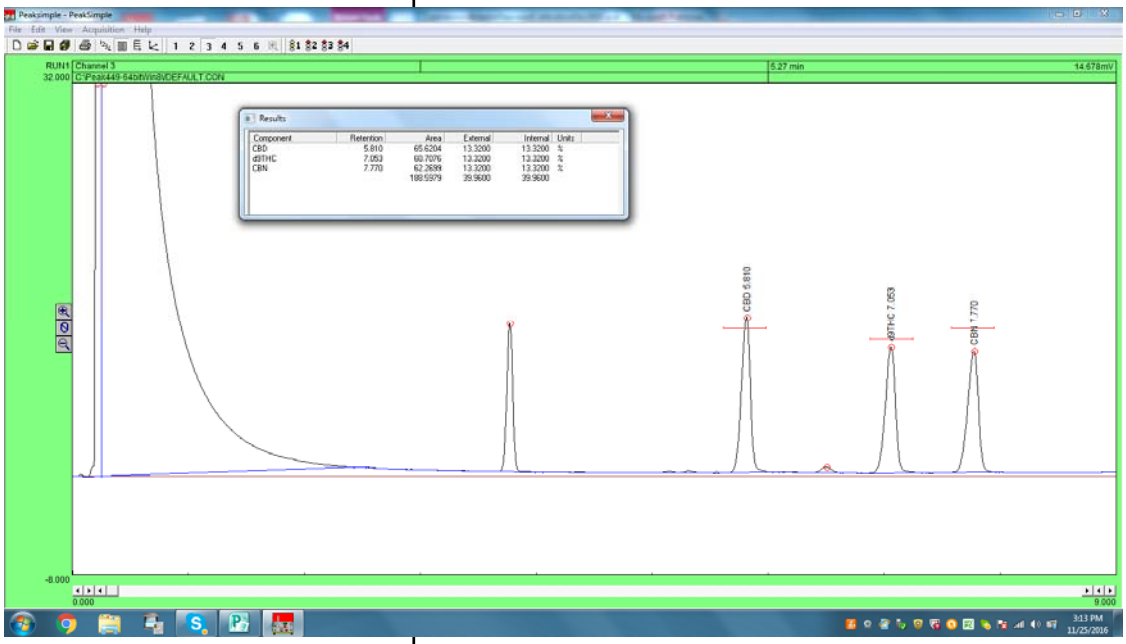
December 2016

Here is a 1ul injection of the 333ng/ul calibration mixture with Internal Standard calibrated using the Internal Standard method.

The Results all show exactly 13.32% for CBD, THC and CBN.



Here is the same exact data calibrated using the External Standard method. Both the peak areas and concentrations are identical.

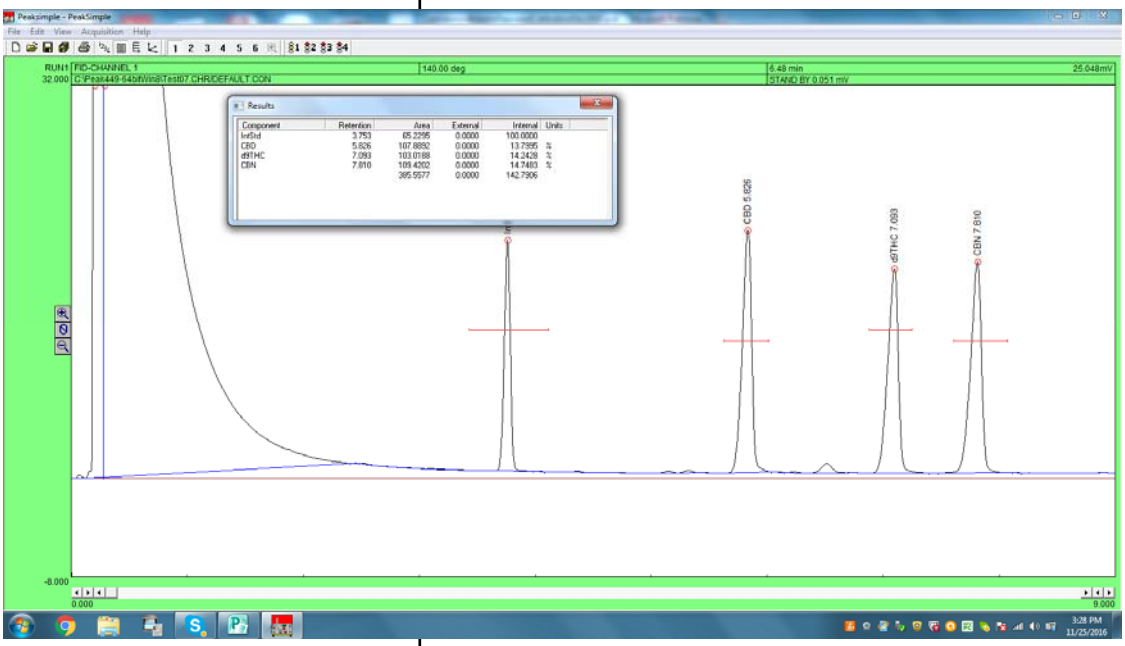


Cannabinoid Internal Standard Calibration

December 2016

Here is a 1.5ul injection of the 333ng/ul calibration mixture. The results are calculated using the Internal Standard calibration curves.

The Results for CBD, THC and CBN are within 7% even though the amount injected was 50% larger.



Here is the same exact data calibrated using the External Standard method. The answers are all 50% too high.