THCA vs d9THC testing using the SRI 8610C GC

SRI gas chromatographs (GCs) that are configured for cannabis testing come in different sized chassis, with and without auto-samplers.

GC is the least expensive method (about 15 cents per sample) for testing cannabis potency and has long been the choice of many labs including the US Government's own lab.

HPLC systems can also be used to test cannabis although the equipment is more expensive to purchase and also more expensive to operate (about $5 per sample). HPLC has until now had one advantage over GC in that it can test for THCA as well as d9THC. THCA is the precursor molecule which the cannabis plant produces. With time and heat the THCA molecule loses one carbon and two oxygen molecules (de-carboxylates) to become d9THC. If the cannabis is smoked, the heat of the flame instantly decarboxylates the THCA into d9THC.

Edible forms of cannabis (medibles) are normally prepared with cannabis which has been deliberately decarboxylated prior to its addition to the flour or sugar "medible" ingredients. To verify that all the THCA in the cannabis leaves and flowers has been 100% decarboxylated (usually by simmering with butter, or otherwise heating above 100C) it is useful to be able to measure the THCA and also the d9THC in the same analysis.

Previously this was not possible with GC because the GC vaporizes the sample which is injected and due to the high heat, instantly decarboxylates any THCA in the sample, converting it into d9THC.

Recently we have learned how to stabilize the THCA molecule (derivatize) so it does not decarboxylate in the GC so we can measure THCA and d9THC separately in the same analysis just like using a HPLC but at much lower cost than buying and operating an HPLC system.

SRI 8610C GC

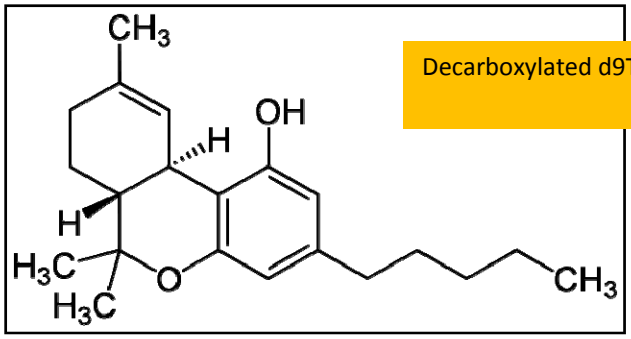
SRI 8610CV GC with Cobra auto-sampler

SRI Tech Support: 310-214-5092

THCA vs d9THC testing using the SRI 8610C GC

The decarboxylated d9THC molecule is shown at right.

d9THC's molar mass is 314.469g/mol

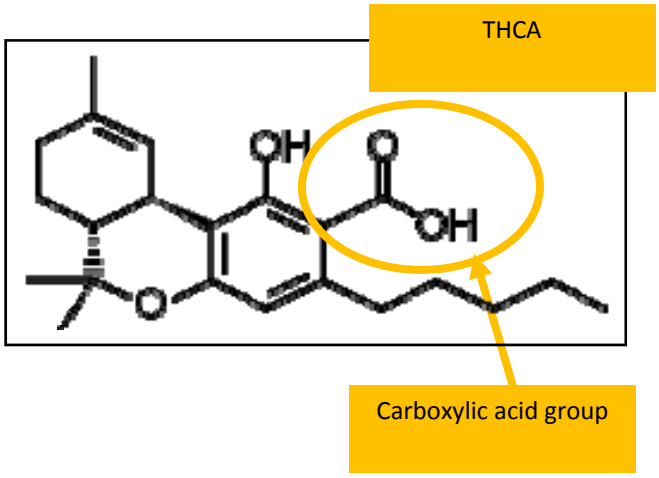


The precursor THCA molecule looks identical to d9THC except there is a carboxylic acid group attached.

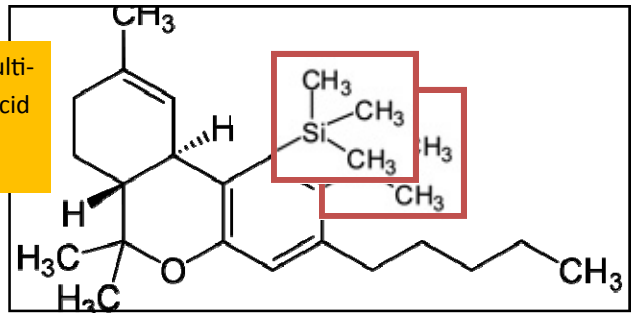
THCA's molar mass is 358.4733

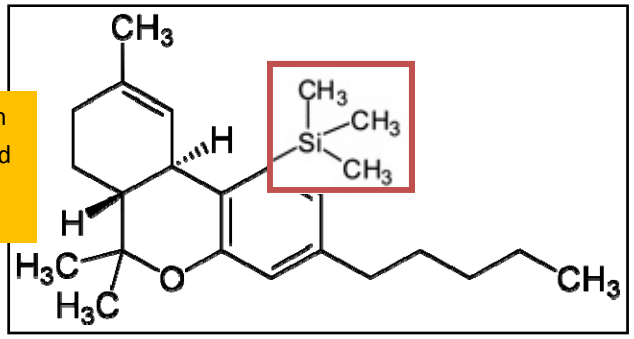
When heated above 100C the carboxylic acid group converts to CO2 and is removed from the molecule leaving d9THC.

So 1000 nanograms of THCA, injected into a hot GC injector loses 12.275% of its weight and transforms into 877.24 nanograms of d9THC.



Derivatizing the THCA (or the d9THC) substitutes a more stable silicon based group for the carboxylic acid or hydroxyl group or both. This new molecule has a higher response on the FID detector because each molecule now has more carbon-hydrogen bonds than the original THCA or d9THC.

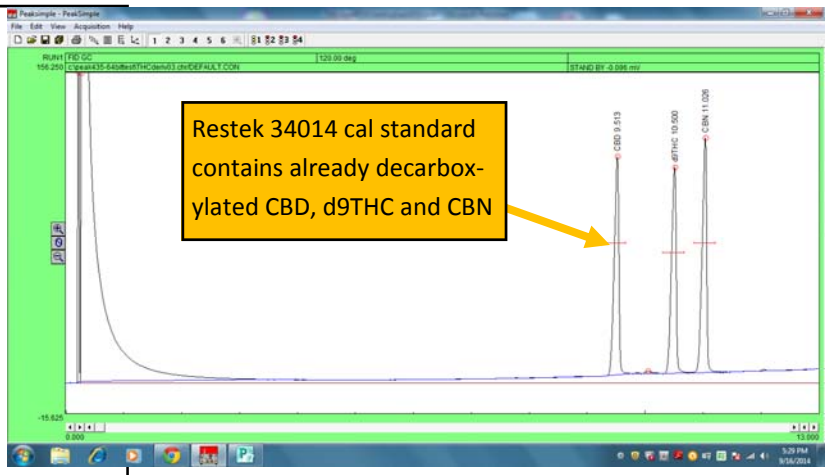




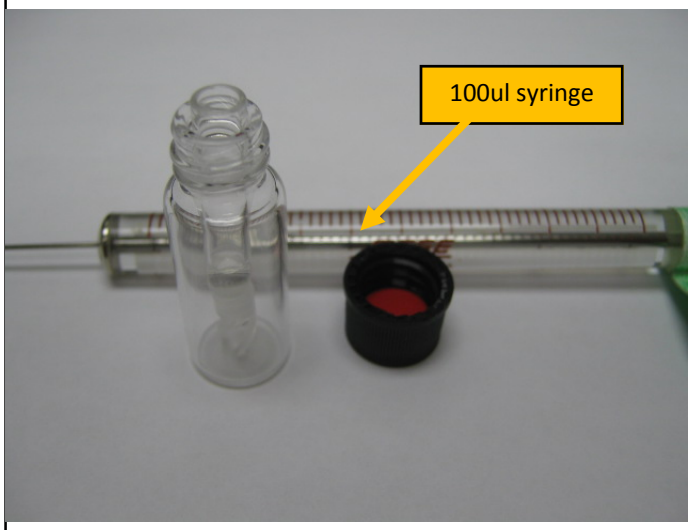
THCA vs d9THC testing using the SRI 8610C GC

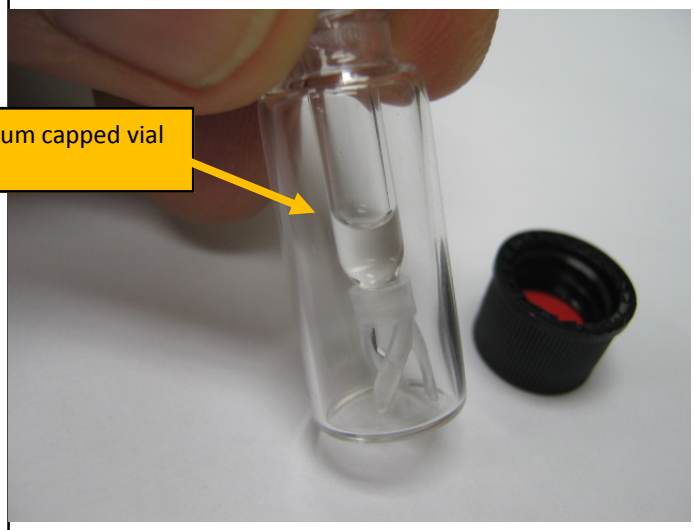
The chromatogram to the right shows the three peaks from Restek's #34014 calibration standard (www.restek.com). The CBD, d9THC and CBN are all completely decarboxylated in this mix.

1 ul of the Restek 34014 standard was injected into the GC with no sample prep at all.



50ul of the Restek 34014 mix was transferred into a 100ul vial using the 100ul syringe SRI provides with the GC. Vials and inserts like this are widely available.





A small, 50ul volume is all that is required. The Restek standard is in methanol.

THCA vs d9THC testing using the SRI 8610C GC

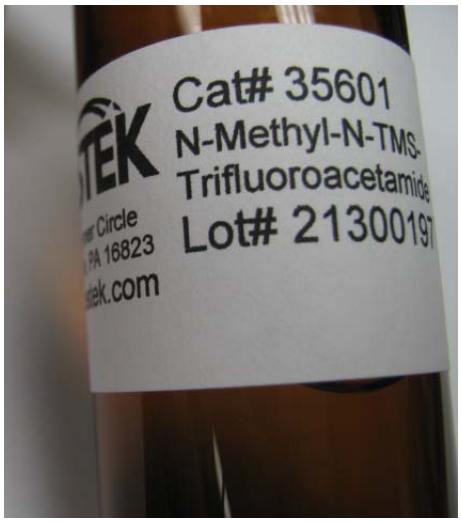
A small air compressor such as a fish aquarium pump is connected to a bent syringe needle (27gage 1.25" long) and placed in the vial containing the Restek mix to speed up the evaporation of the methanol solvent. It takes about 10 minutes to evaporate.

Its important that the end of the needle be above the liquid level so the liquid does not splash from the air bubbles.

Once the methanol solvent is completely dry, you will see some residue. This is the CBD, d9THC and CBN which have high boiling points and do not quickly evaporate.

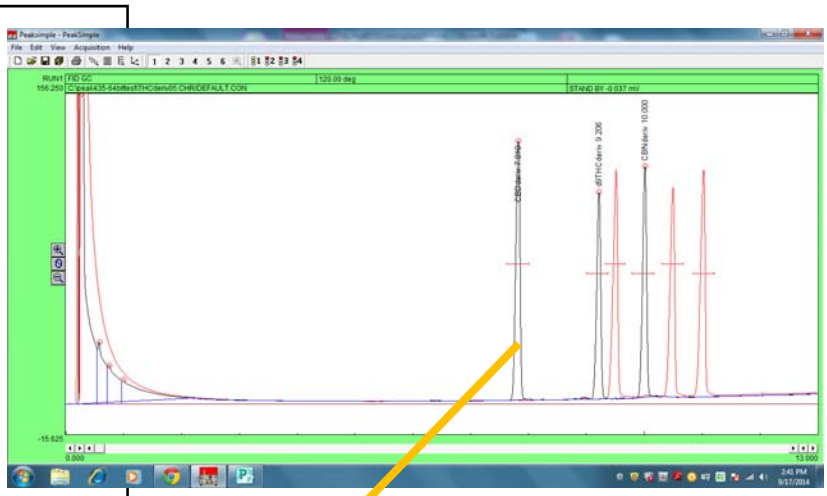
Add 50ul of the MSTFA derivatizing reagent. The CBD, d9THC and CBN will re-dissolve in the MSTFA. You may have to swirl the MSTFA a little to make sure the residues dissolve especially the ones at the very bottom of the 100ul insert.

It's important that the methanol is evaporated completely, as the derivatizing process will not work if methanol is present.

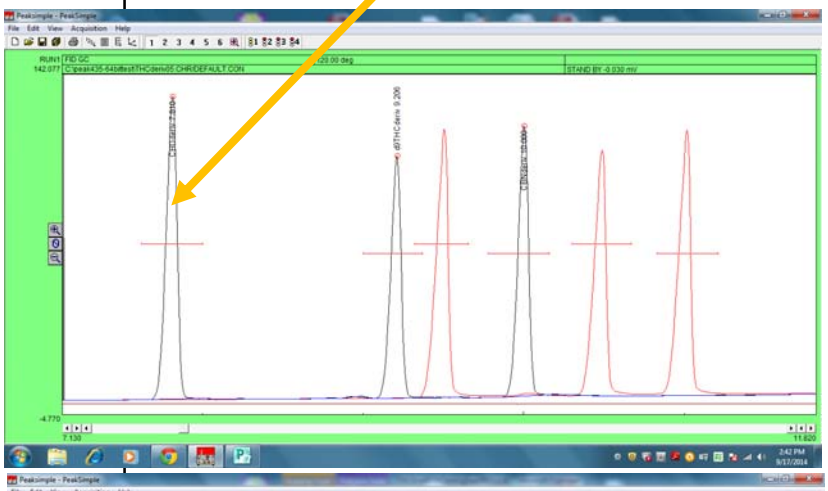


THCA vs d9THC testing using the SRI 8610C GC

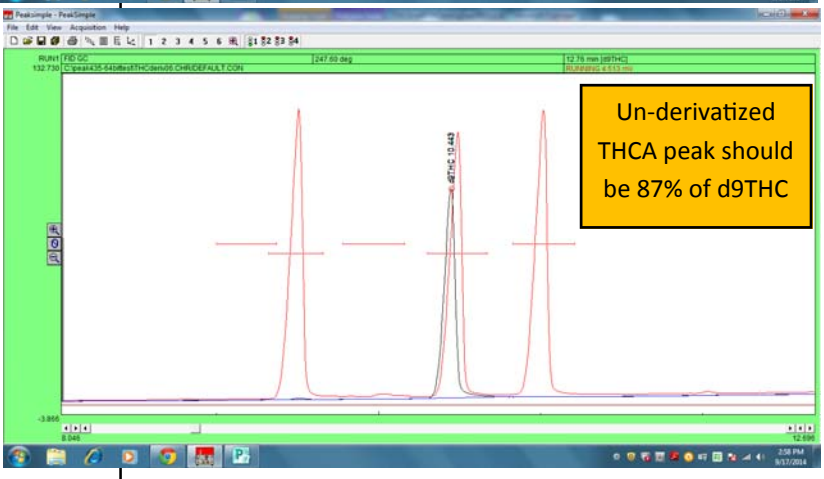
The derivatized Restek standard looks like the chromatogram to the right. The peaks in red are the original un-derivatized, already decarboxylated CBD, d9THC and CBN peaks.



The peaks in black are the derivatized CBD, d9THC and CBN. You can see that the retention time of the derivatized peaks has shifted earlier and the peaks are 10-20% larger.

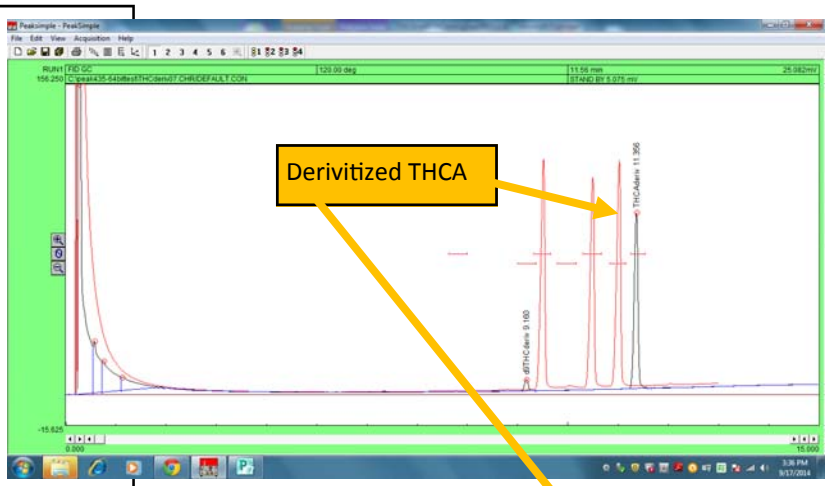


Here is the chromatogram of un-derivatized THCA (Restek# 34093) in black. Notice that the peak comes out at the same time as the d9THC. The un-derivatized THCA decarboxylates in the GC and becomes d9THC, so it makes sense that it elutes at the same time as d9THC. The size of the un-derivatized THCA peak should be 87% of the d9THC peak if the THCA standard completely de-carboxylates in the GC injector and both the 34014 and 34093 standard each contain 1000ng/ul as stated on the label.

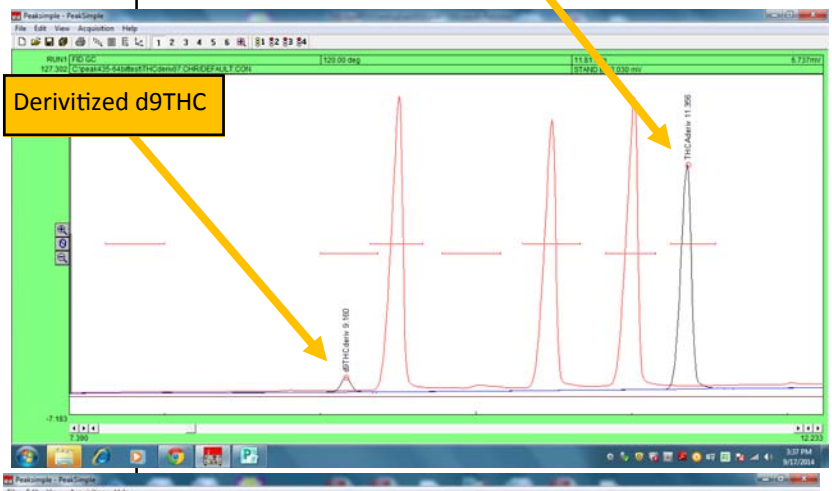


THCA vs d9THC testing using the SRI 8610C GC

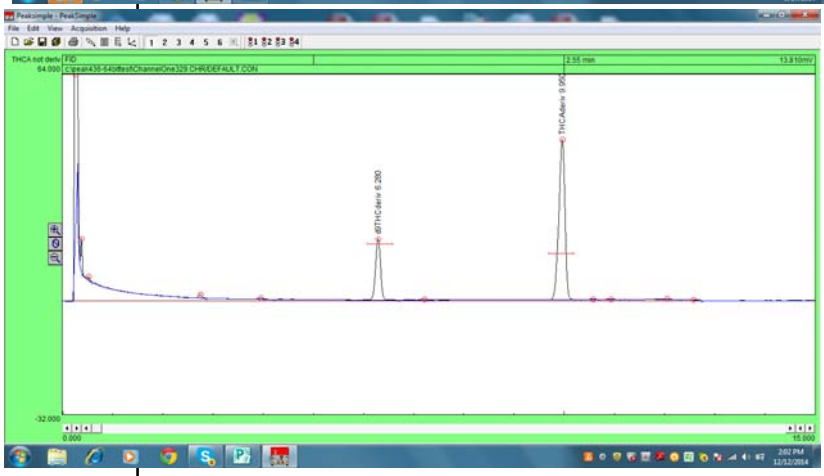
Here is what the derivatized THCA looks like in black.



This is the same chromatogram zoomed in for more detail. Notice that a small THCderiv peak was also detected because some of the THCA standard had spontaneously decarboxylated in storage to d9THC. This may be why the standards are shipped in dry ice.

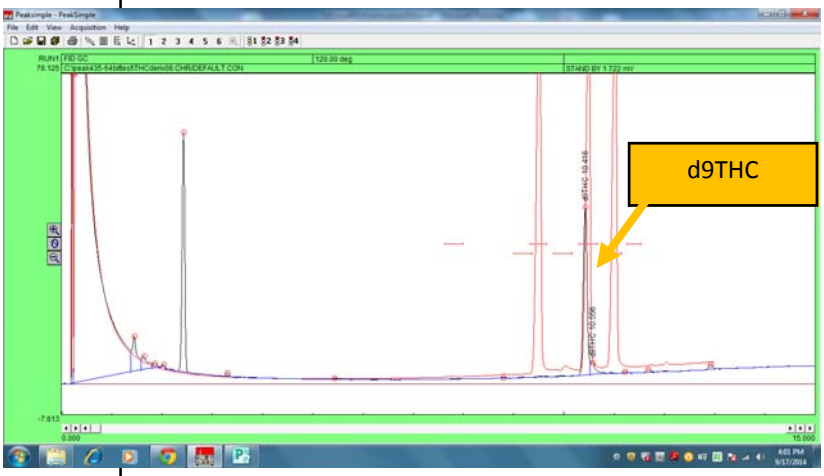


Here is another bottle of THCA standard. You can see in this chromatogram that about 25% of the THCA has decarboxylated into d9THC. You might think this makes the THCA standard useless, but because we already have a good calibration for the d9THCderiv peak (from the 34014 sample) , we can calibrate the THCA derivative peak by subtraction.



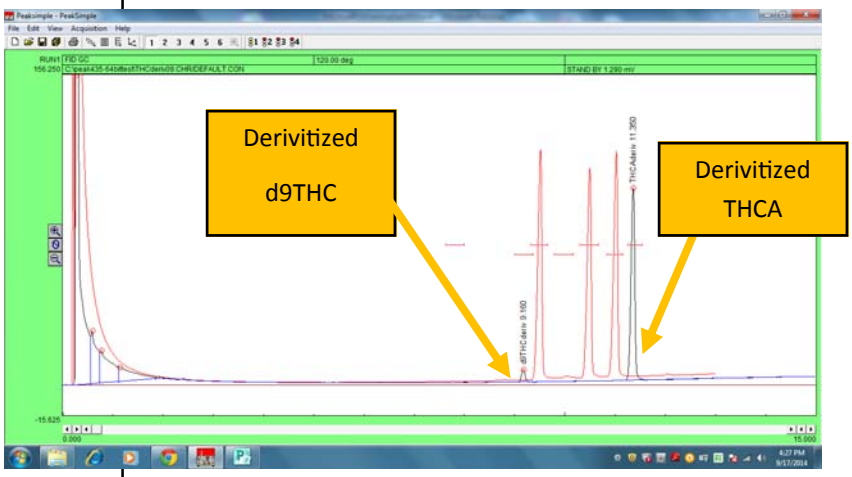
THCA vs d9THC testing using the SRI 8610C GC

This is a chromatogram of some un-derivitized cannabis. The early peak is the nC16 internal standard peak which we typically add to the extraction solvent. The benefits of the internal standard are discussed in another publication.



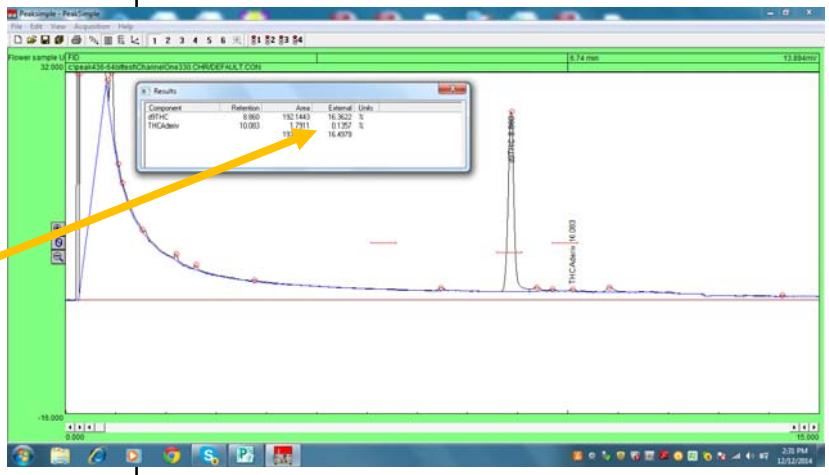
This shows the same extract after derivitization. Apparently there was a ratio of about 10:90 of d9THC to THCA in the extract.

The cannabis used for this sample was very fresh and un-cured, so it might be expected that the extract would contain mostly THCA.

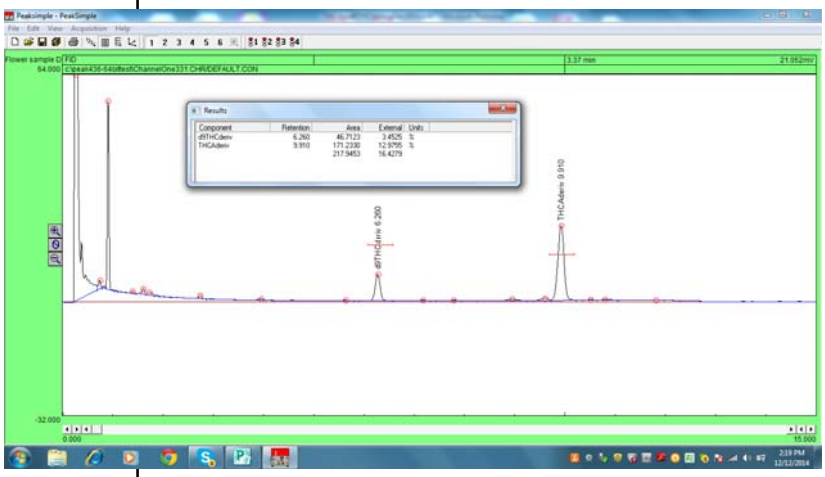


THCA vs d9THC testing using the SRI 8610C GC

Here is another un-derivitized cannabis extract. The d9THC peak (really the d9THC plus the decarboxylated THCA) calculates out to 16.36%

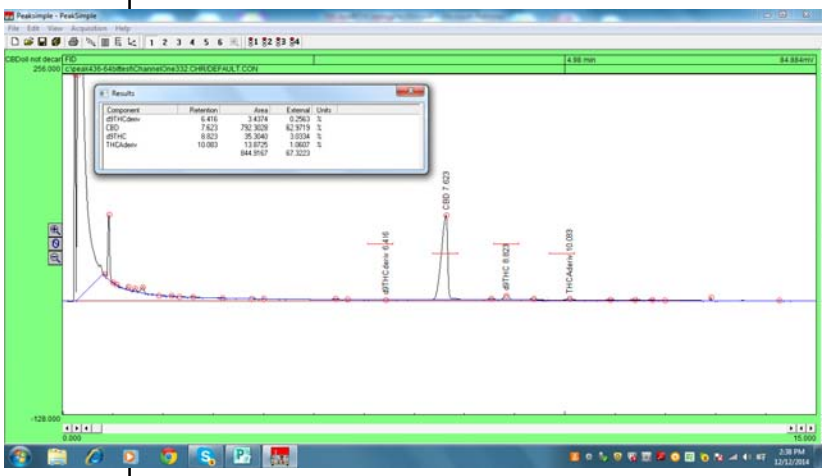


Here is the sample sample after derivatization. Notice that the sum of the d9THCderiv and THCAderiv peaks equal 16.42% which is almost exactly the same as the underivatized result.

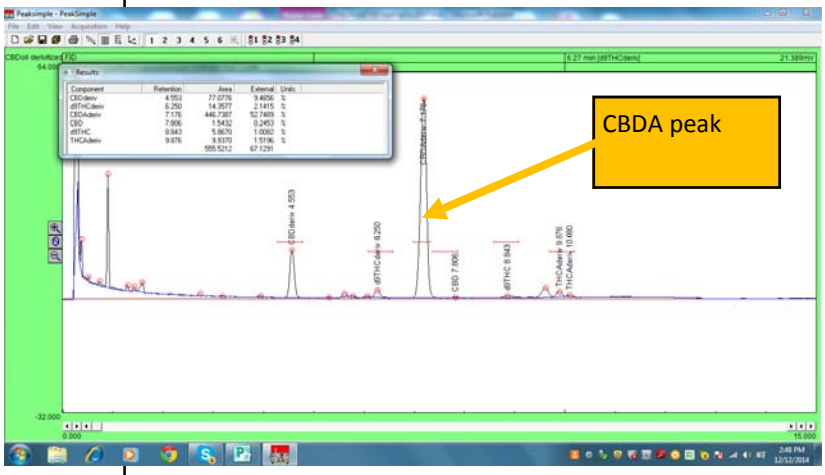


THCA vs d9THC testing using the SRI 8610C GC

Here is a un-derivatized CBD oil measuring 63% CBD (really CBD plus CBDA) but the CBDA decarboxylates in the GC's hot injector.



Here is the sample sample after derivatization.



Summary:

This shows that with a simple and low cost derivatization step in the sample preparation, GC can be used instead of HPLC to measure the acid forms as well as the decarboxylated form of most cannabinoids.