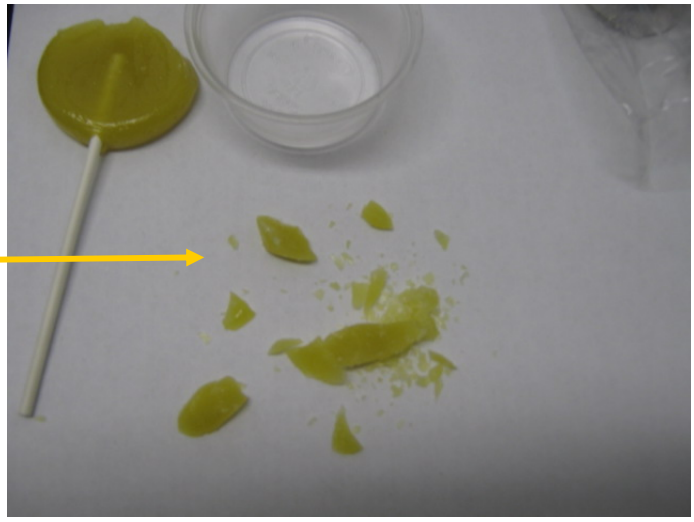
Medible Testing Using the SRI 8610C GC
Part 1 (Basic Sample Preparation)

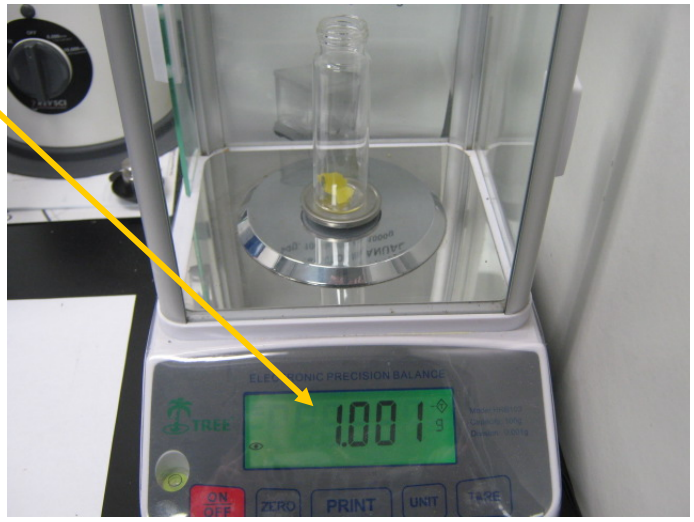
The SRI medical herb potency testing GC can be used to test most cannabis infused products. In the first part of this document, the basic sample preparation for medibles will be shown.

Most medibles have a relatively low concentration of cannabinoids. So in order to detect the cannabinoids, use 1 gram of sample.

Depending on the product, you may need to crush it into smaller pieces to get 1 gram.



1. Weigh out ~1 gram in a 40mL sample vial. It doesn't have to be exactly 1.000g. Just be sure to label the vial with the weight, so the appropriate weight can be entered into the sample weight box in peaksimple.



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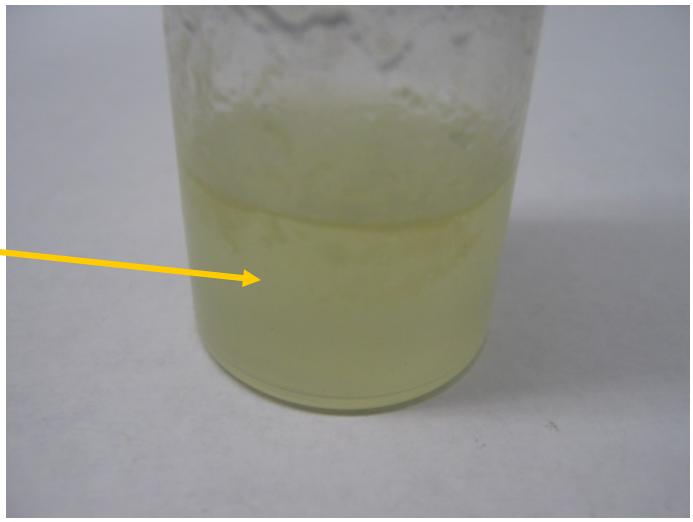
Part 1 (Basic Sample Preparation)

2. Add solvent into the vial. If the medible doesn't dissolve in methanol, weigh out another sample, and add 10 mL of water first to dissolve it.

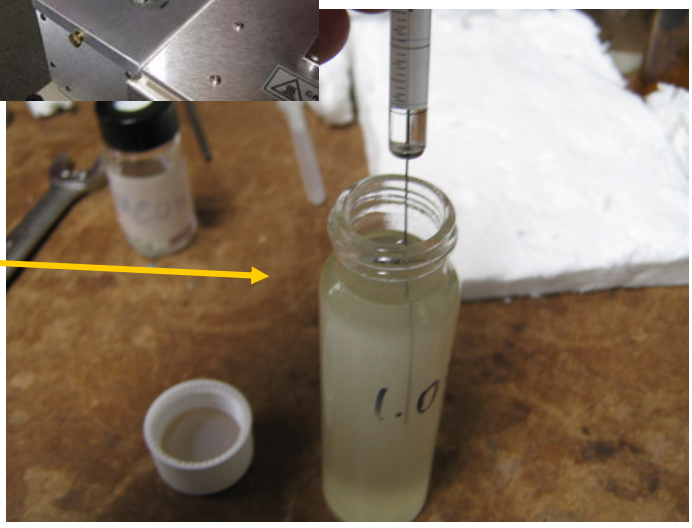


This particular medible was put in 10 mL of water for ~20 minutes, and dissolved very well. (Just methanol will work for most baked goods)

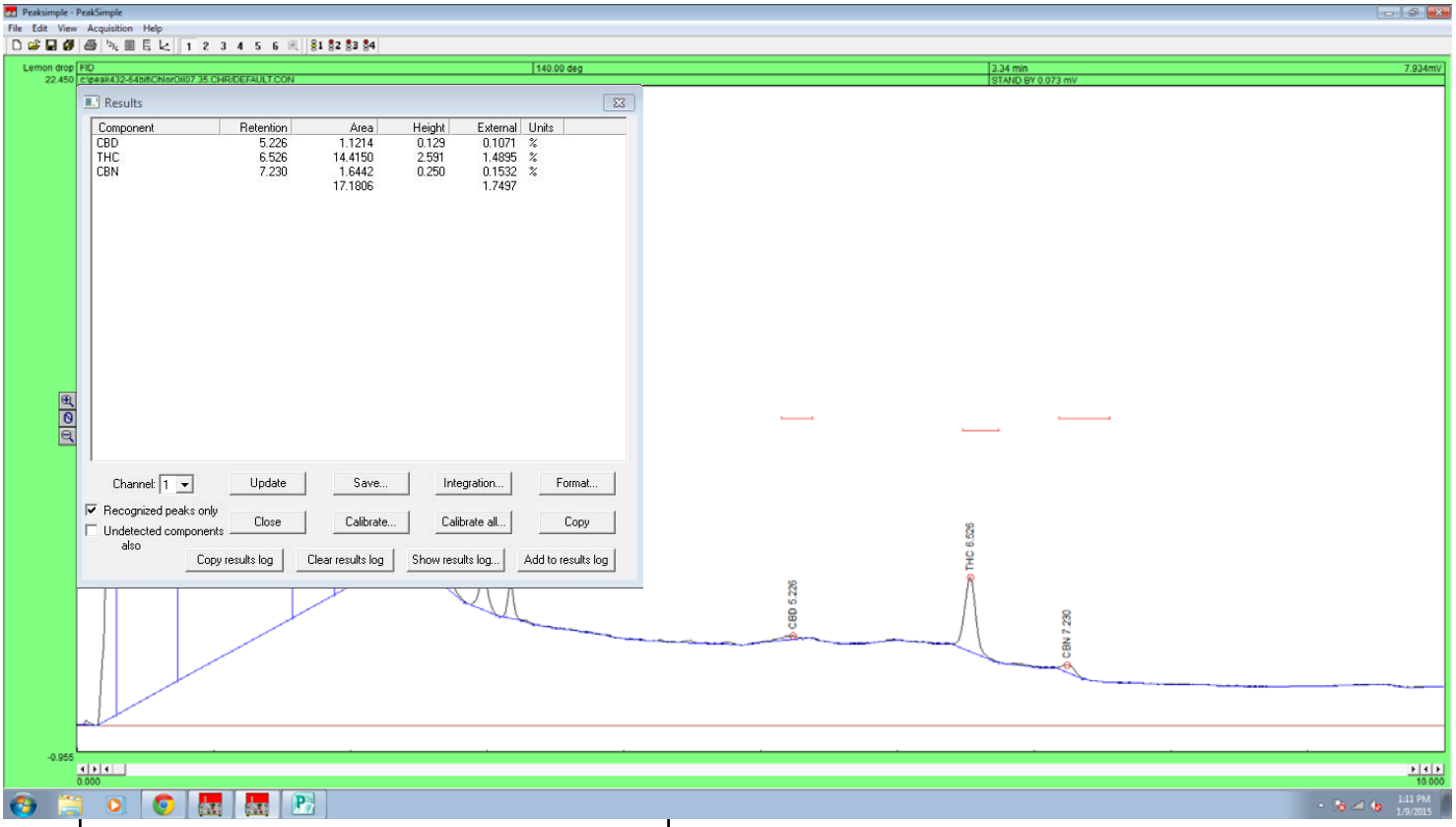
3. After the sample has been fully dissolved, add methanol into the vial until it is filled to the neck. Shake once, then let sit for ~5 minutes.

(To speed up the process of both the water extracting, and the methanol/water equilibration, the sample can be placed in the incubator on the GC.)

4. After ~30 minutes, when the extract is ready, pull 1 uL into a syringe, and inject into the GC.



Medible Testing Using the SRI 8610C GC
Part 1 (Basic Sample Preparation)



The chromatogram above shows the results for the sample.

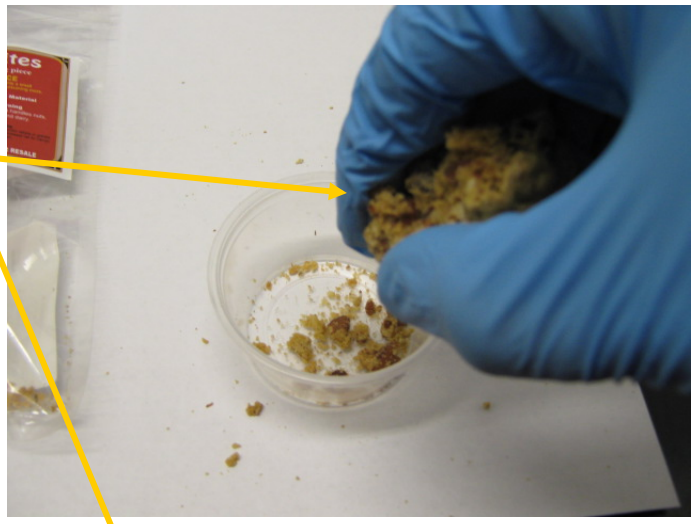
CBD = 0.1071%
THC = 1.4895%
CBN = 0.1532

This method isn't sufficient for certain medibles. See the second part of this document for advanced sample prep.

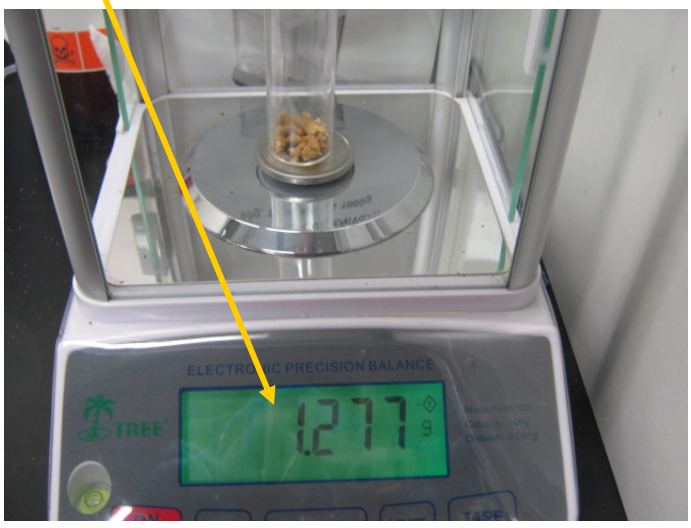
Medible Testing Using the SRI 8610C GC
Part 2 (Advanced Sample Preparation)

This sample preparation method should be used for all medibles, to prolong the life of your column. But it is mainly intended for medibles that do not dissolve easily or at all (cookies, brownies, cakes, etc.,).

1. Crush up the sample and weigh out ~1 gram.

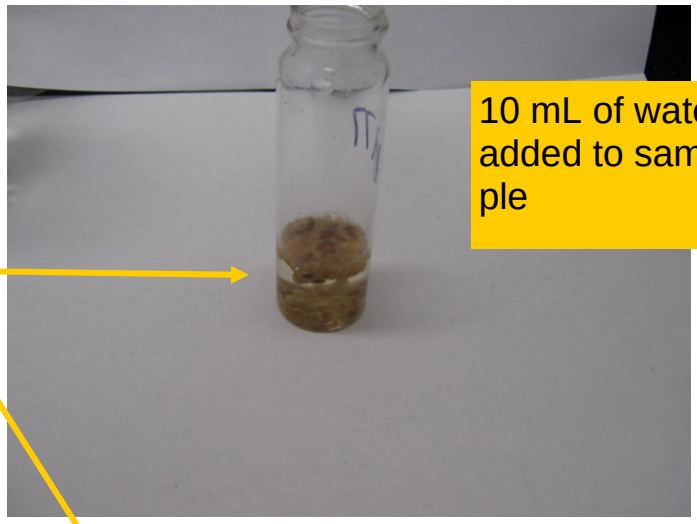


For samples with extra additives like; raisins, pecans, chocolate chips, etc.,. It is a judgment call, for the operator, on whether or not to include those additives into the sample. More likely than not, they will not contain any cannabinoids, but including them into the sample might be a more accurate repre-sentation of the sample.

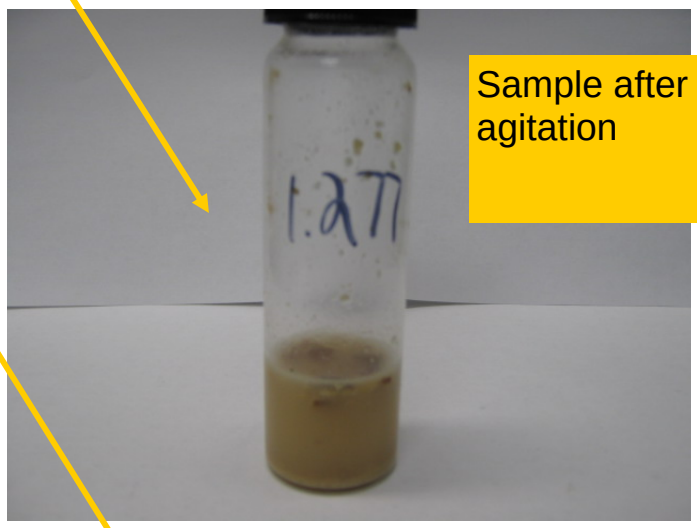


Medible Testing Using the SRI 8610C GC
Part 2 (Advanced Sample Preparation)

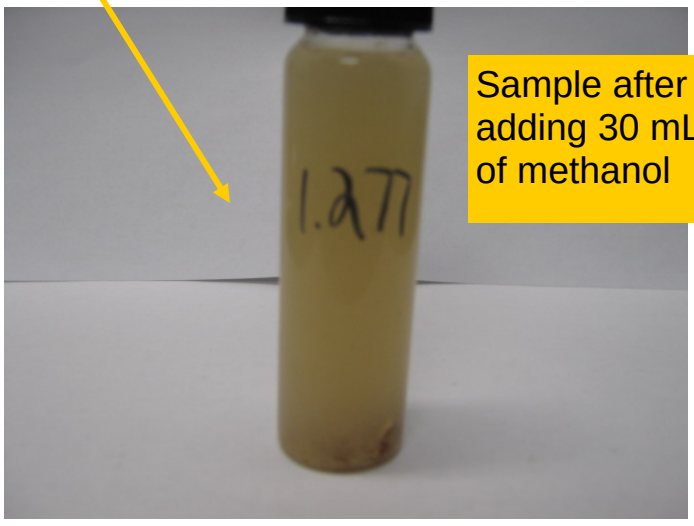
2. Add 40 mL of methanol to the vial. If the sample doesn't break up in methanol, weigh out another sample, add 10 mL of water, agitate vigorously to break up the sample, then fill the vial up to the lip with methanol, and place it into the incubator to extract.



10 mL of water added to sample



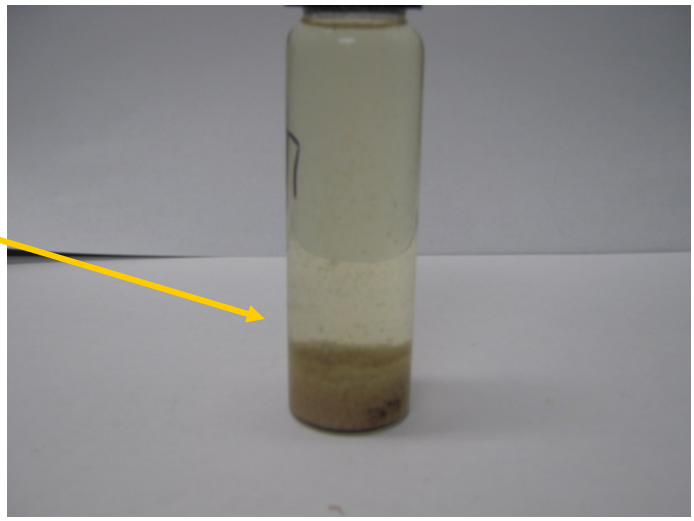
Sample after agitation



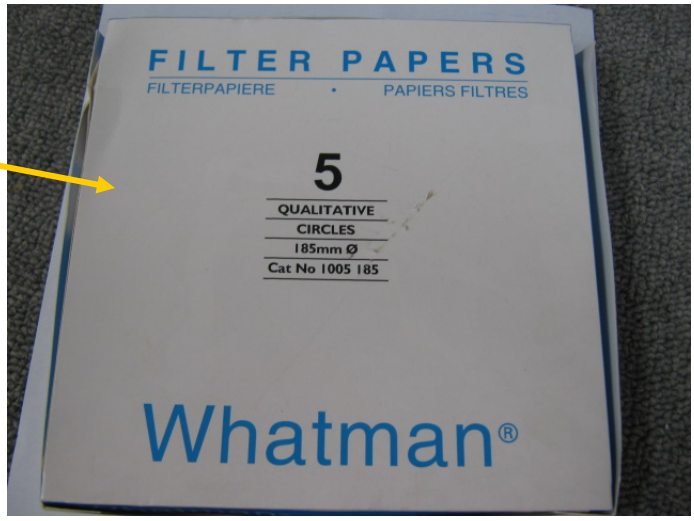
Sample after adding 30 mL of methanol

Medible Testing Using the SRI 8610C GC
Part 2 (Advanced Sample Preparation)

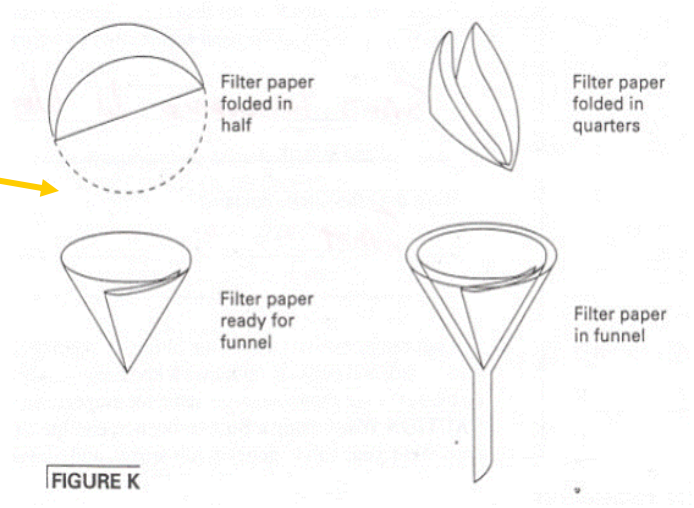
3. After letting the sample extract in the incubator for ~20 minutes, the majority of the undissolved particulates should sink to the bottom. There will still be some particulates floating in the solvent; these can clog syringes and columns. Although it is not absolutely necessary to remove the particulates, removing them will minimize risk of damage to syringes and columns.



4. To remove the particulates, use filter paper.

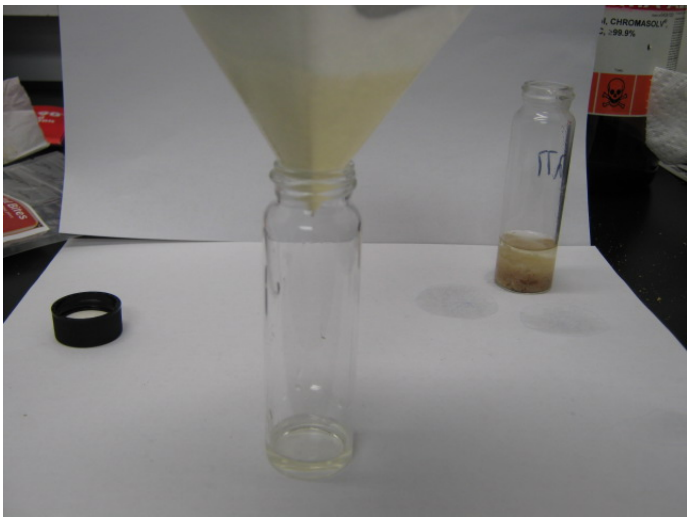


5. Fold the filter paper into a cone. Optionally, place cone into a funnel.

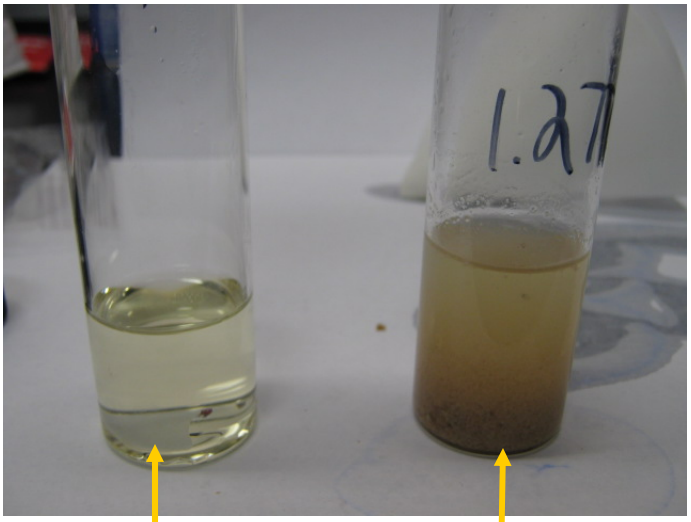


Medible Testing Using the SRI 8610C GC
Part 2 (Advanced Sample Preparation)

6. Let the extract drip through the filter paper. It is not necessary to get all the extract filtered. All that is needed, is enough to run through the GC.



7. Run the clean extract through the GC.



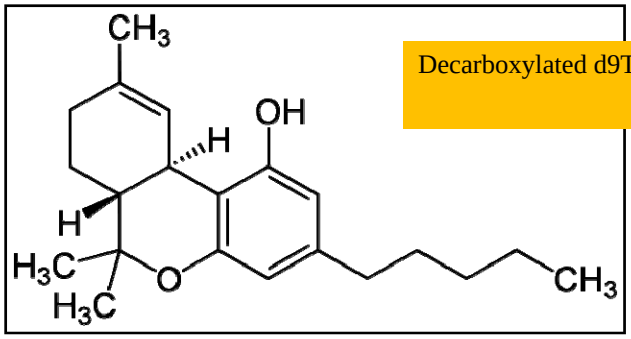
*This document only contains information concerning sample preparation for medibles, and other cannabis infused products. For information on testing THCA in similar samples, please continue reading for a simplified version of our document called, “THCA vs. d9-THC measurement”. To see the full document, visit http://srigc.com/THCAvsd9THCtestingDec2014.pdf

THCA vs d9THC testing using the SRI 8610C GC (Simplified)

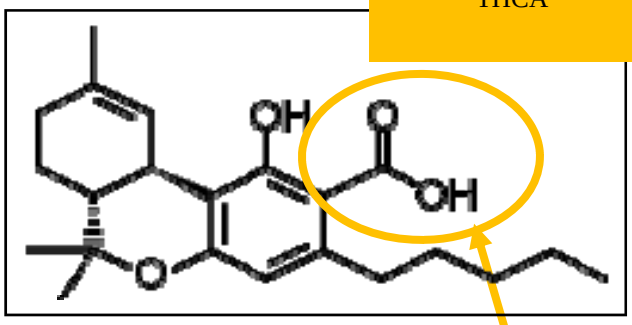
Edible forms of cannabis (medibles) are normally prepared with cannabis which has been deliberately de-carboxylated prior to its addition to the flour, sugar, or other “medible” ingredients. To verify that all the THCA in the cannabis leaves and flowers has been 100% decarboxylated (usually by simmering 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 to easily do with the GC, because the GC vaporizes the sample during the injection, due to the high heat, instantly decarboxylates any THCA in the sample, and converting it into d9THC.

Recently we have learned how to stabilize the THCA molecule (derivatize) with relative ease, so it does not decarboxylate in the GC; allowing the measurement of 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.

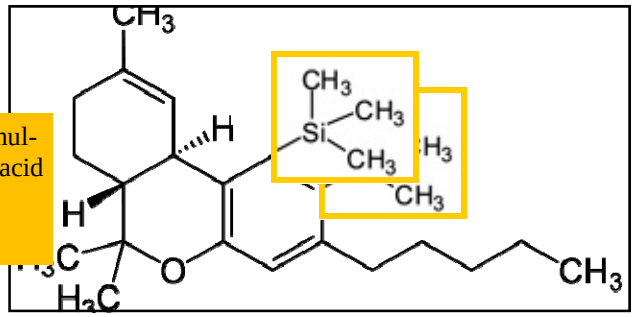


Decarboxylated d9THC

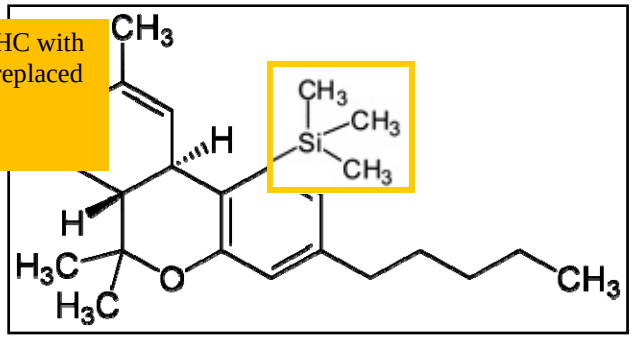


THCA

Carboxylic acid group



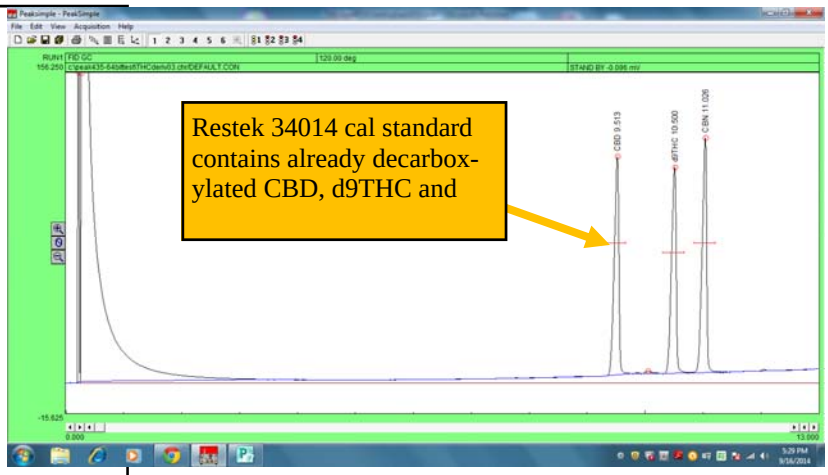
Derivatized THCA with multiple Carboxylic/hydroxyl acid groups replaced



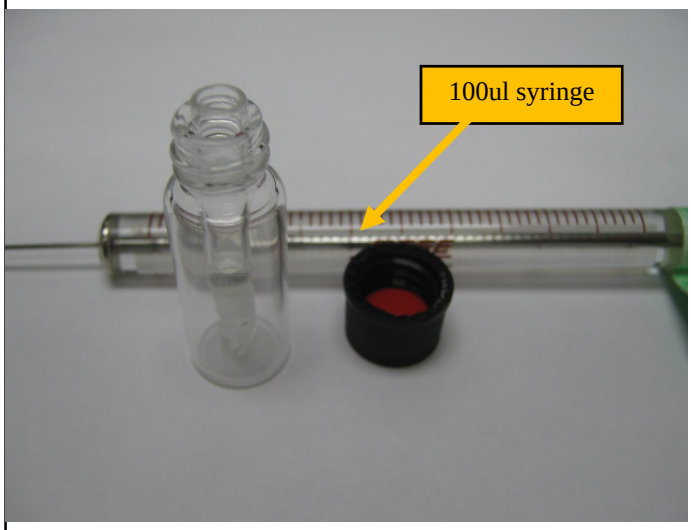
Derivatized d9THC with hydroxyl group replaced

THCA vs d9THC testing using the SRI 8610C GC

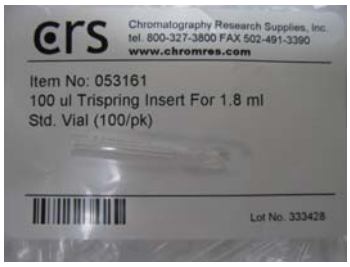
The chromatogram to the right shows the three peaks from Restek's #34014 calibration standard (www.restek.com). The CBD, d9THC and CBN are all completely decarboxylated in this mix.
1 ul of the Restek 34014 standard was injected into the GC with no sample prep at all.

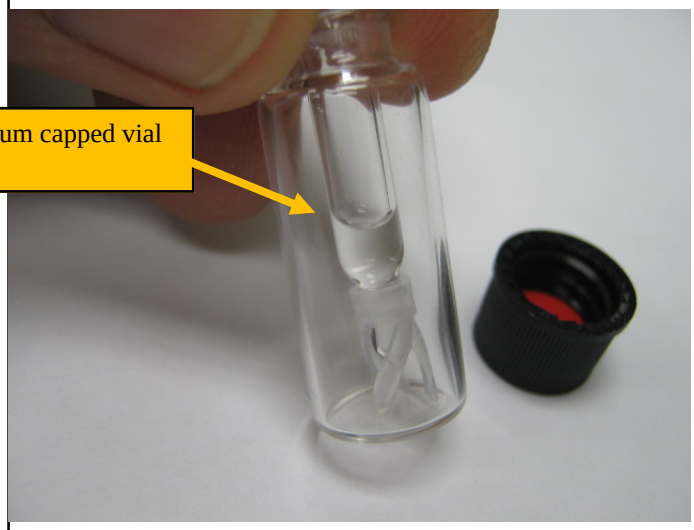


50ul of the Restek 34014 mix was transferred into a 100ul vial using the 100ul syringe SRI provides with the GC. Vials and inserts like this are widely available.



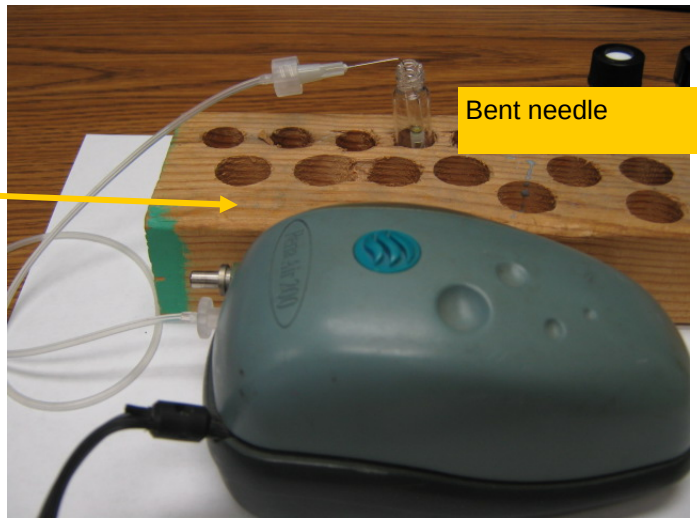
A small, 50ul volume is all that is required. The Restek standard is in methanol.





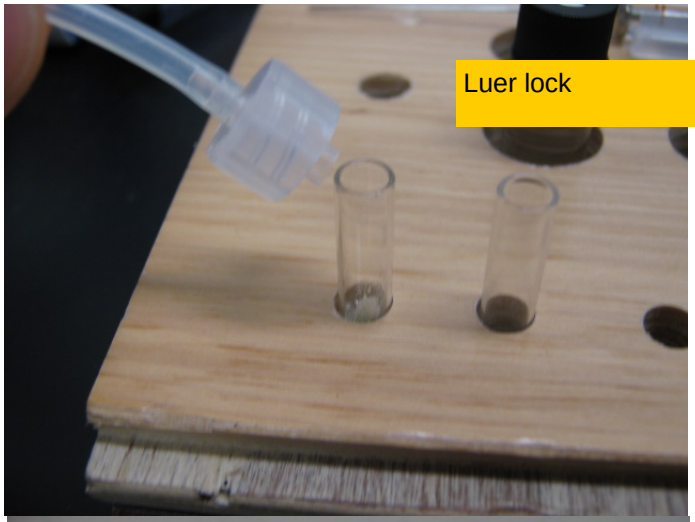
THCA vs d9THC testing using the SRI 8610C GC

A small air compressor such as a fish aquarium pump is connected to a bent syringe needle (27gage 1.25” long), or preferably a 1/16th barb to a male or female luer lock, 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.



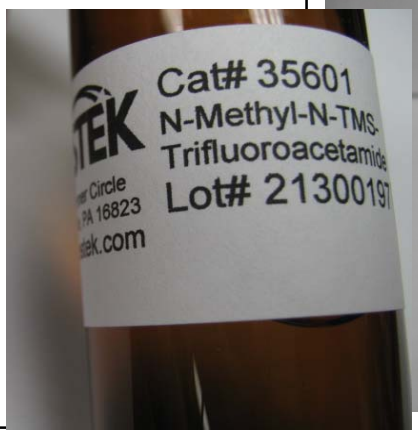
Its important that the end of the needle be above the liquid level so the liquid does not splash from the air bubbles.

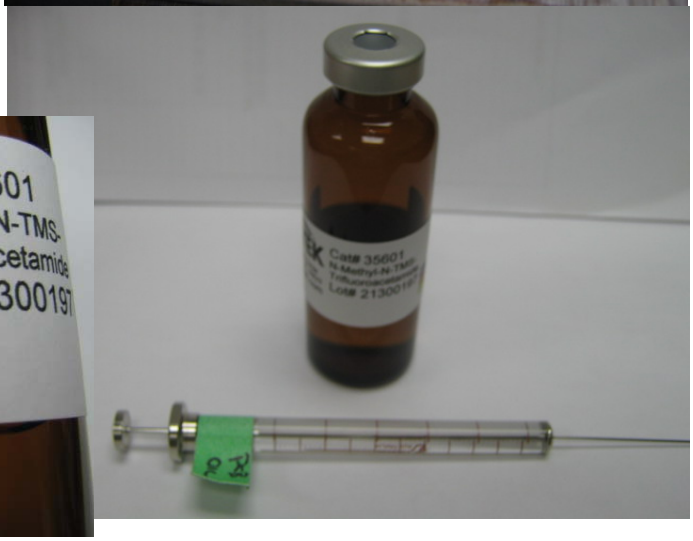
Once the methanol solvent is completely dry, you will see some residue. This is the CBD, d9THC and CBN which have high boiling points and do not quickly evaporate.



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.

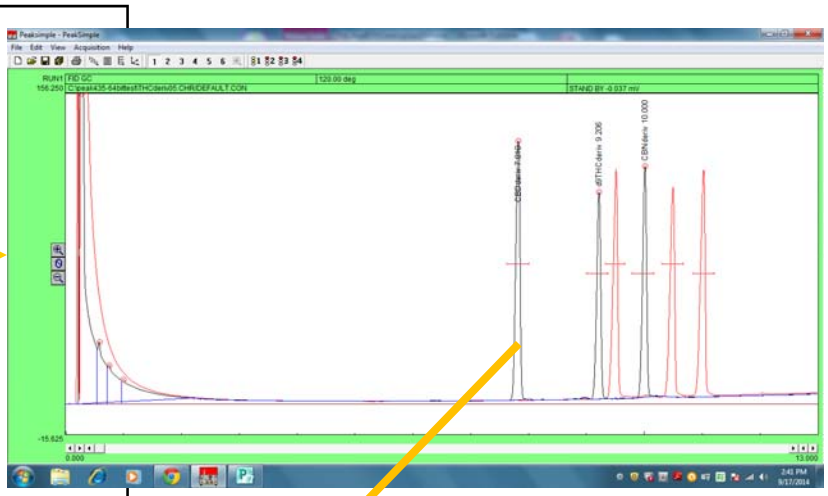
It’s important that the methanol is evaporated completely, as the derivatizing process will not work if methanol is present.



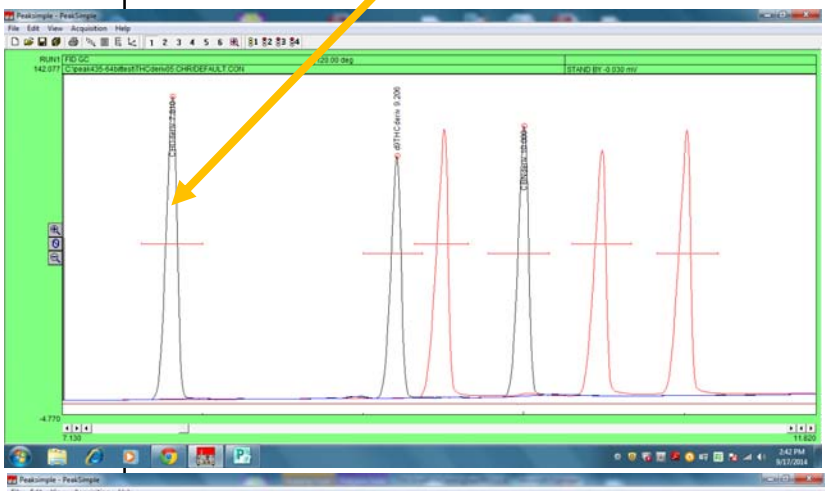


THCA vs d9THC testing using the SRI 8610C GC

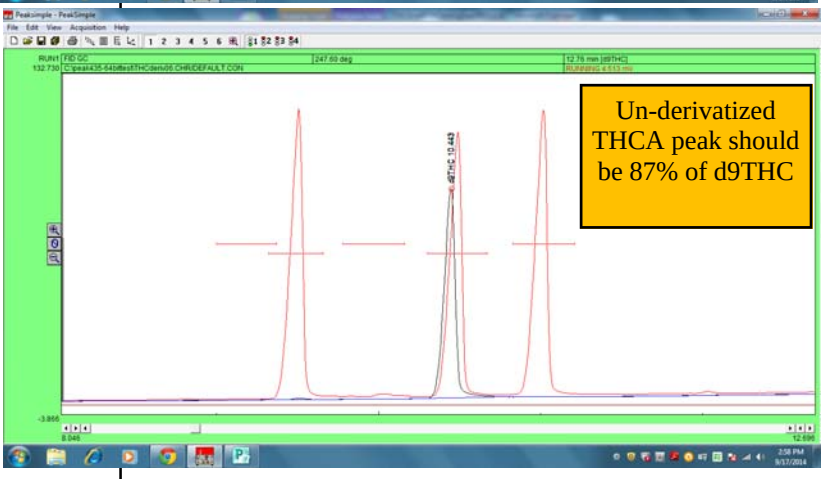
The derivatized Restek standard looks like the chromatogram to the right. The peaks in red are the original un-derivatized, already decarboxylated CBD, d9THC and CBN peaks.



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.

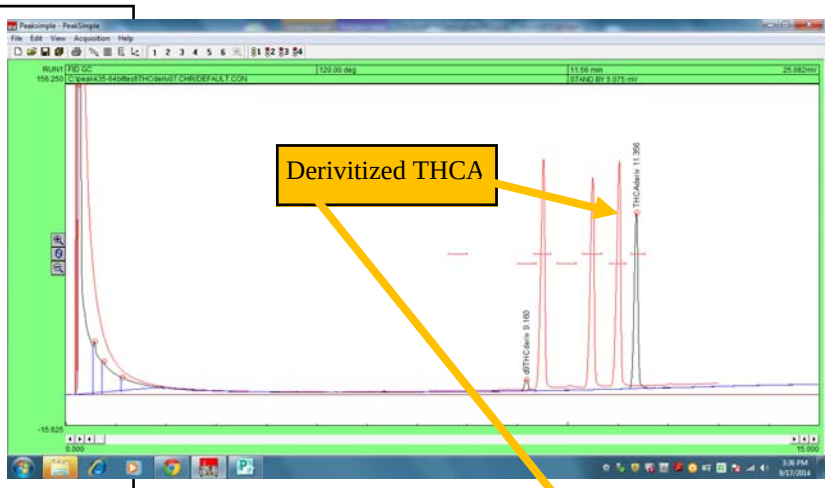


Here is the chromatogram of un-derivatized THCA (Restek# 34093) in black. Notice that the peak comes out at the same time as the d9THC. The un-derivatized THCA decarboxylates in the GC and becomes d9THC, so it makes sense that it elutes at the same time as d9THC. The size of the un-derivatized THCA peak 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.

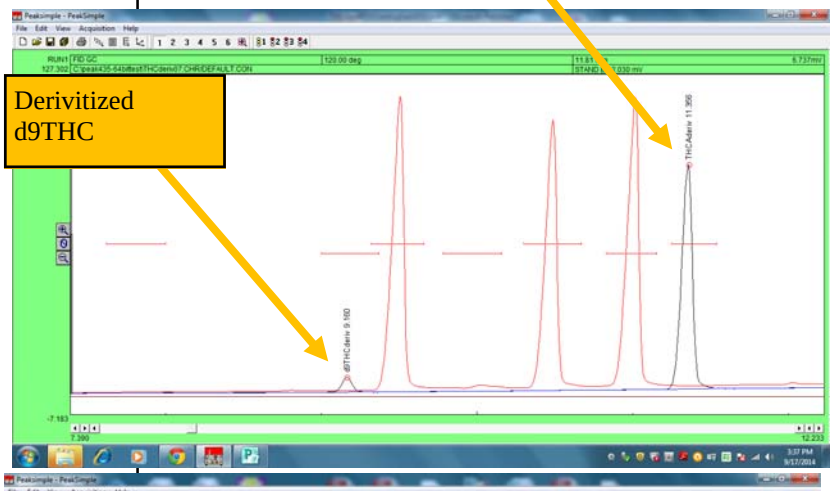


THCA vs d9THC testing using the SRI 8610C GC

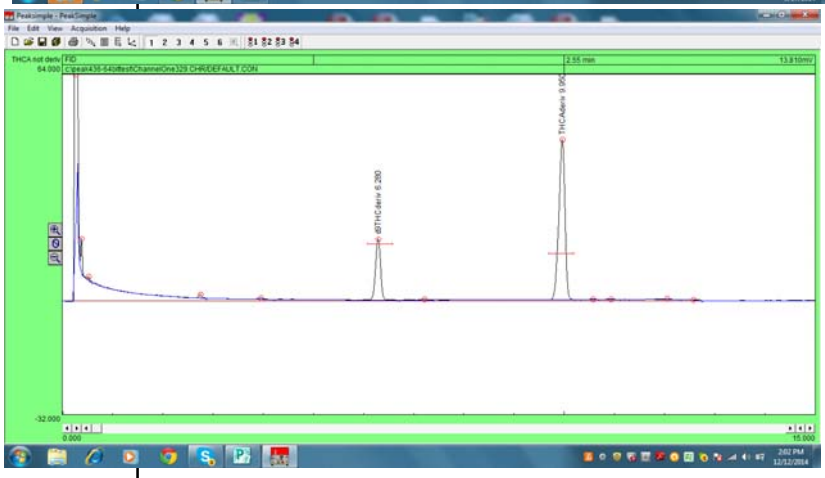
Here is what the derivatized THCA looks like in black.



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.

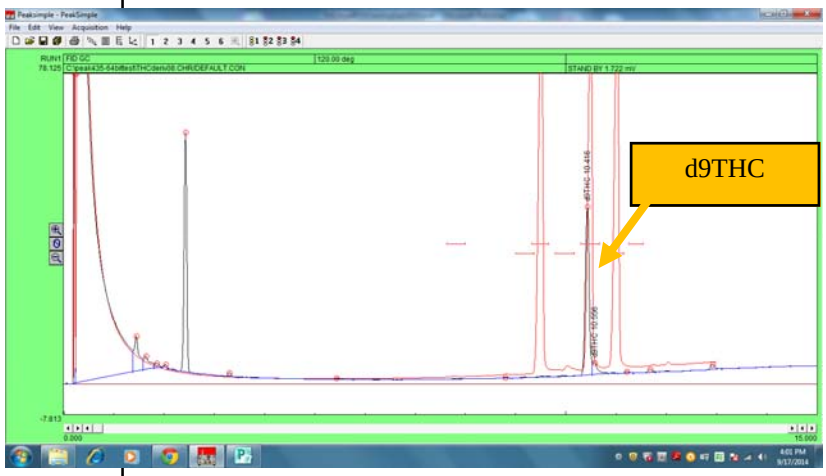


Here is another bottle of THCA standard. You can see in this chromatogram that about 25% of the THCA has decarboxylated into d9THC. You might think this makes the THCA standard useless, but because we already have a good calibration for the d9THCderiv peak (from the 34014 sample) , we can calibrate the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

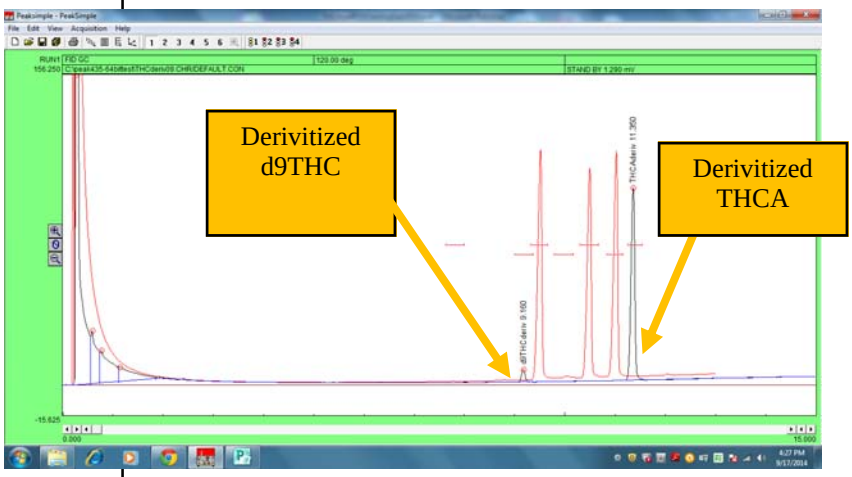


THCA vs d9THC testing using the SRI 8610C GC

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.

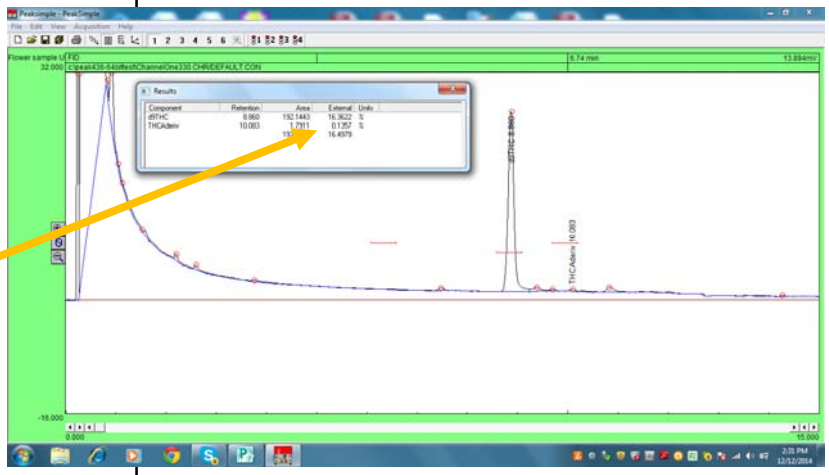


This shows the same extract after derivitization. Apparently there was a ratio of about 10:90 of d9THC to THCA in the extract. The cannabis used for this sample was very fresh and un-cured, so it might be expected that the extract would contain mostly THCA.

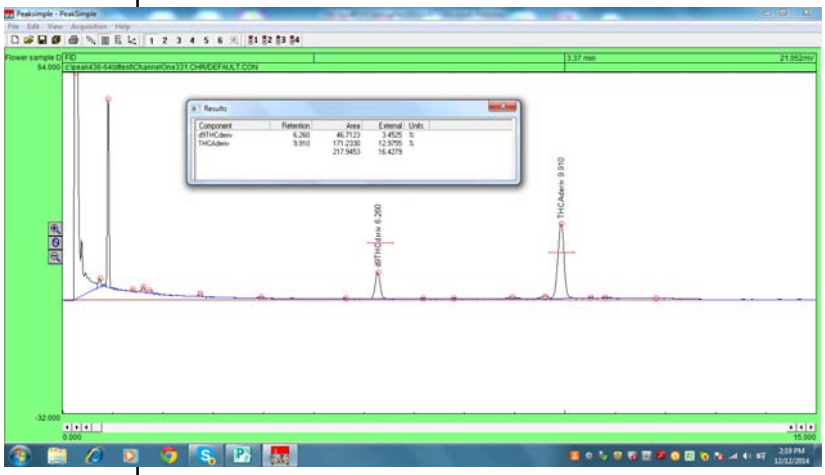


THCA vs d9THC testing using the SRI 8610C GC

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

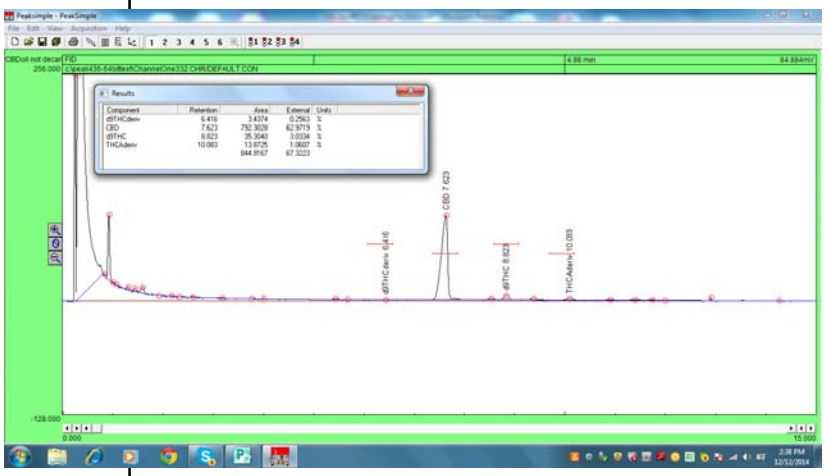


Here is the sample sample after derivatization. Notice that the sum of the d9THCderiv and THCAderiv peaks equal 16.42% which is almost exactly the same as the underivatized result.

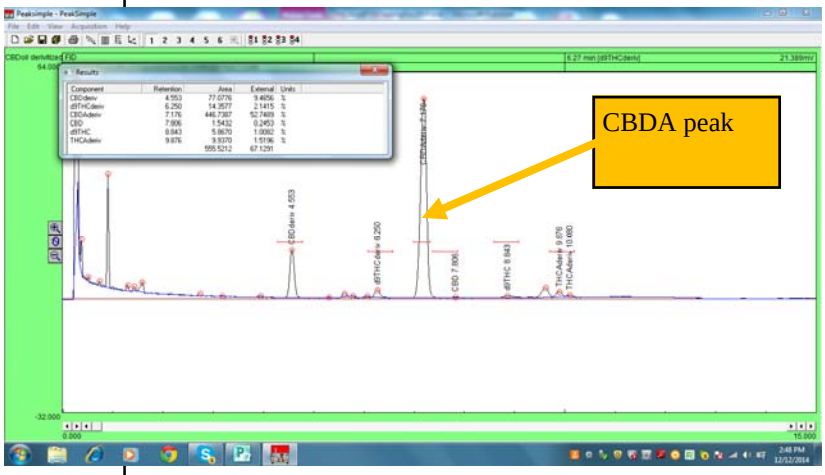


THCA vs d9THC testing using the SRI 8610C GC

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.