APPLICATIONS

Update . . . >Jan 2016

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Medical Cannabis / Marijuana

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Some prelim work . . . By Restek

- RESIDUAL SOLVENTS / PESTICIDES In Cannabis Extracts
- Detailed Quechers Extracts PLUS GCxGC TOF-MS - but MS is a bit limited for canabinoids terpenses etc
- Deribatisation of Cannabinoids

Cannabinoid Standards / Terpenes from Restek

eome GC Configurations (soecialised)

Some practical HINTS re GC set up / accessories

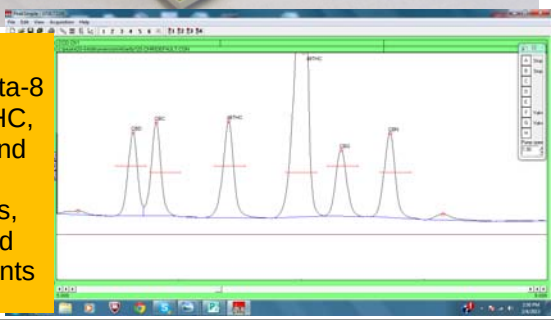
DISCLAIMER . . . In Australia use of Cannabinoids and even R&D on such STILL seems highly restricted) on a State by State basis and potential customers require full ID and possible registration/certification for ANY purchases / work being done in this field

FULLY at customers responsibility - No authenticity . . . No Sale !

NEW 2015+ WebSITE / SHOPPE

www.chromalytic.net.au

8610C Gas Chromatograph for Medical Cannabis Analysis



Measure CBD,CBC, delta-8 and delta-9THC, CBG, CBN and other cannabinoids, terpenes and residual solvents

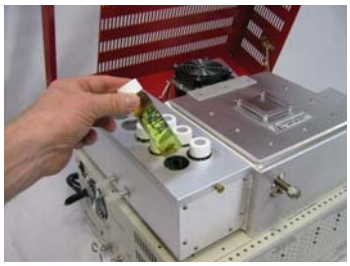
Flame Ionization Detector (FID)
Heated Flash Injector
400°C column oven
Built-in Incubator for heated extractions
15 meter Capillary column
PeakSimple Data System built-in
Hydrogen regulator/tubing kit
Field portable system
Heavy Duty shipping container
Low power consumption (< 800 watts)
Ships via FedEx/UPS or airline baggage
Small footprint for crowded lab benches
Friendly, easy to reach US tech support
Free training
Two Year warranty
Made in USA

Complete system
US$ 12,015.00 plus shipping

The SRI 8610C is the perfect size GC (gas chromatograph) for measuring CBD, THC and CBN levels in medical cannabis. It can also be used to test for synthetic cannabinoids like SPICE, butane residuals, terpenes, aromas and most edibles. The SRI 8610C is rugged enough for mobile applications and light enough to carry around. Simple operation makes training new operators easy. The built-in 50°C incubator speeds up the extraction process and is helpful in getting concentrates and/or butters to dissolve. A small cylinder of hydrogen (customer supplied) is used for carrier gas and lasts for months. The regulator and tubing for the cylinder is provided. Analysis time is about 8 minutes so up to 7 samples an hour can be analyzed. The included PeakSimple software (Windows XP/Vista/Win7/8) controls the GC as well as acquiring and calibrating the data. Simple one click export of the data to Excel or Word makes your final report look professional. Get half a day of free training with your GC at our tech support center near LAX (Los Angeles) airport.

System consists of two part numbers:

8610-0091 Cannabis Potency Testing GC complete	**$11,585.00**
8600-C350 Hydrogen Gas line kit	**430.00**
Total	**$12,015.00**



Built-in 50°C incubator

8610CannabisFlyer
Oct2013

Medical Cannabis Gas Chromatograph (GC)
Configuration choices February 2011

SRI can configure a gas chromatograph (GC) in hundreds of ways to perform almost any analysis. Two chassis sizes are available. The smaller 310C chassis is very portable while the larger 8610C chassis allows for more complex hard-ware. All SRI GCs are portable and easily shipped by UPS, FedEx and even as airline baggage.

Medical Cannabis contains many active cannabinoid compounds, but three are considered important, cannabidiol (CBD), THC, and cannabinol (CBN). A GC is the perfect tool for measuring the amount of these three compounds in plant material, resin , tinctures and edi-bles. Other analytical techniques such as HPLC and GC/Mass Spec can also be used, but are much more expensive to buy, and vastly more complicated to operate yet they do **NOT** provide supe-rior data. For this analysis, GC is the best solution. Unlike a HPLC, the GC naturally de-carboxylates the THCA (the original molecule produced by the plant) into Delta-9THC saving a processing and reporting step. Total cost to perform a GC analysis is less than one dollar, requires only .1 gram of sample and usually takes less than 5 minutes.

Four common configurations have become popular for measuring medi-cal cannabis.

1) Gasless, ultra portable, simple
2) Industry standard FID
3) Automated, hi-volume
4) Pesticides and potency both

Comes with a heavy duty shipping case.

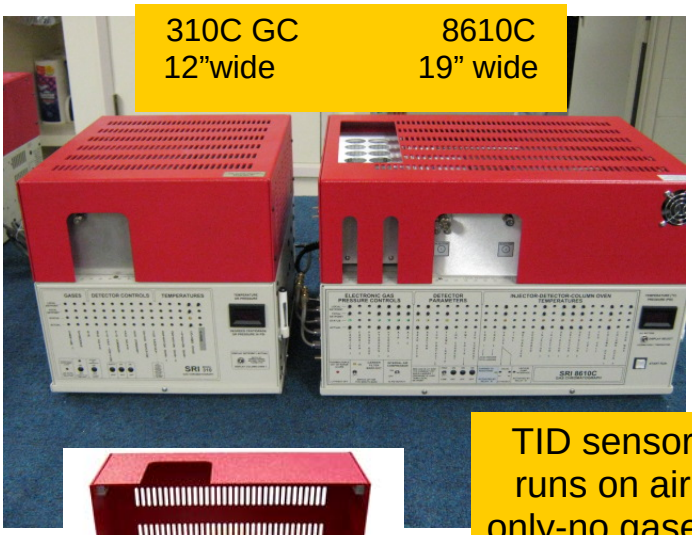
Weighs about 35 pounds (16 kilo-grams)

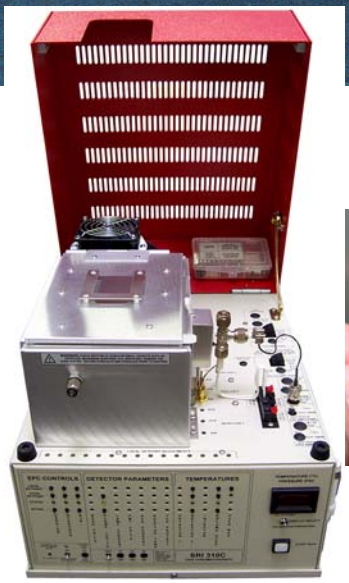
Medical Cannabis Gas Chromatograph (GC)
Gasless and Simple Configuration

Configuration #1
"Gasless" TID Detector based Potency Configuration
Part# 8610-0094 $9999.00

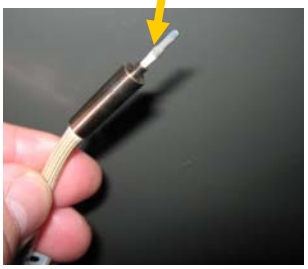
This GC is configured on the ultra-compact 310C chassis (only 12 inches wide) and includes an TID (thermionic ionization detector) which requires no gas cylinders to operate. All required gas is provided by the built-in "whisper quiet" air compressor and dryer. This GC configuration is appropriate for users with no prior GC experience, and/or for those who want maximum portability. You can literally carry the GC around under your arm, it's that portable.
Just add a Windows PC (XP, Vista, or Windows 7) desktop or laptop. SRI's easy to learn PeakSimple software is included.
The GC comes complete with syringes, and a starter pack of vials; everything you need except the standards and a balance.

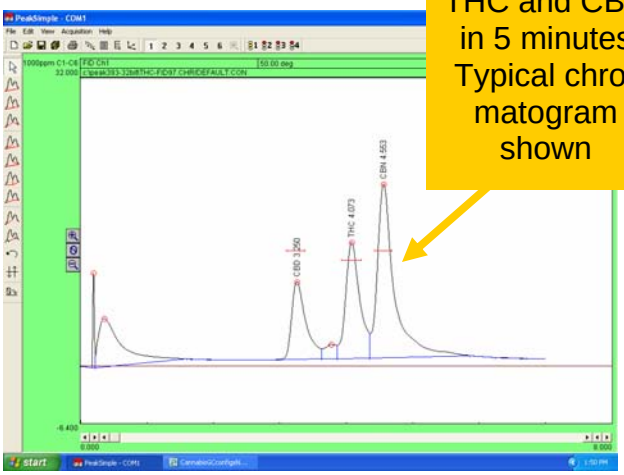
Run times can be as short as 3-4 minutes. A typical calibration chromatogram is shown at right.





TID sensor runs on air only-no gases required





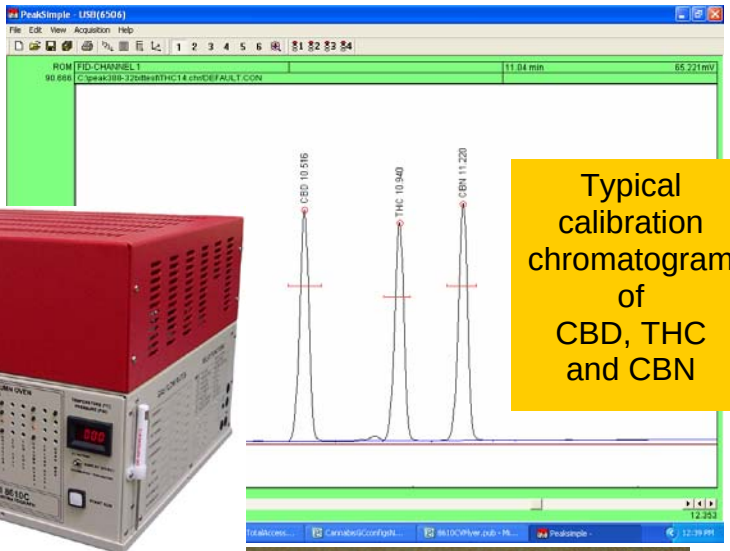
Measure CBD, THC and CBN in 5 minutes Typical chromatogram shown

Medical Cannabis Gas Chromatograph
Industry Standard FID Configuration

Configuration #2
FID Detector based
Potency Configuration
Part# 8610-0091 $10,210.00

This GC configuration includes an FID (flame ionization detector) which requires hydrogen gas to operate. Because hydrogen is used as a carrier gas, higher resolution is possible when measuring the CBD, THC and CBN molecules in cannabis. A photo of a typical hydrogen gas cylinder is shown at right. This GC configuration is appropriate for users with prior GC experience, for those who want to be equipped with industry standard hardware, or for those who may later wish to add the extra hardware required to measure the pesticide content of cannabis.
Run times can be as short as 3-4 minutes.

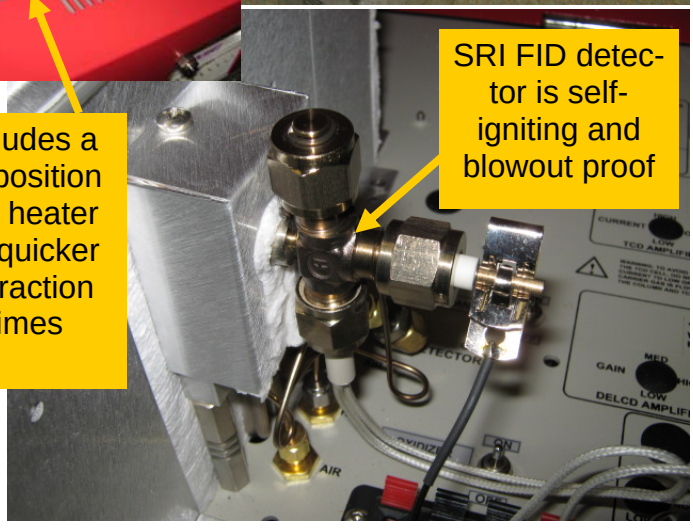
User's will need a hydrogen cylinder, Windows computer and AC power. Syringes and a starter pack of vials is included.



Typical calibration chromatogram of CBD, THC and CBN

Hydrogen Gas cylinder

Includes a 12 position vial heater for quicker extraction times



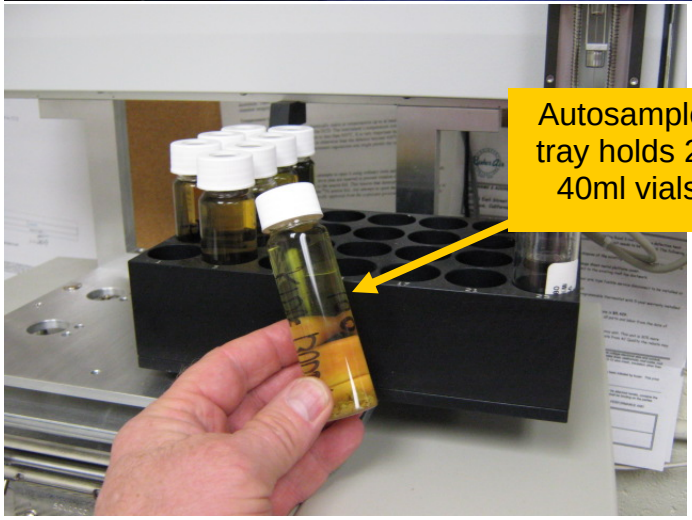
SRI FID detector is self-igniting and blowout proof

Medical Cannabis Gas Chromatograph Automated Hi-volume Configuration

Configuration #3
Potency test with FID detector and Autosampler
Part#8610-0093 $25,530.00

This GC configuration is appropriate for user's who have higher numbers of samples per day to analyze for CBD, THC and CBN. The autosampler accommodates 28 of the 40milliliter extraction vials so users do not have to transfer the THC extract from the extraction vial to a smaller autosampler vial thus saving an expensive and time consuming step. The autosampler makes it practical to take 2-3 samples from the same vial and average the results, leading to increased accuracy. The autosampler lets the user walk away or operate overnight. This configuration is appropriate for users with prior GC experience and who have or anticipate a high sample volume.

This configuration is not as portable as Configurations #1 or #2 since it is physically larger and the autosampler must be removed from the GC prior to transport.



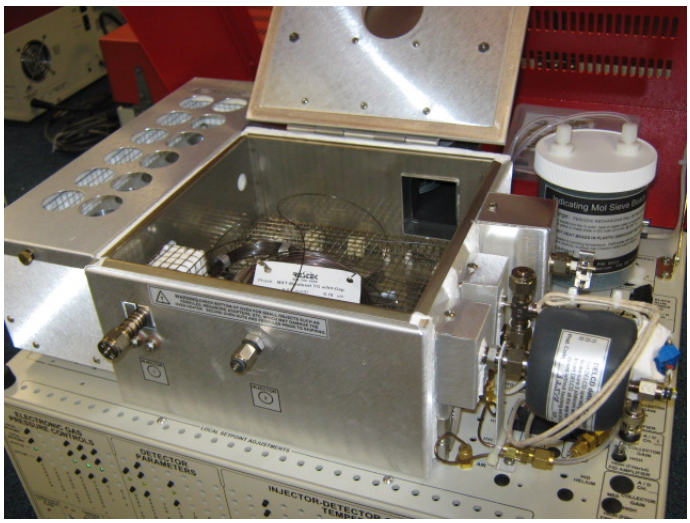
Medical Cannabis Gas Chromatograph Pesticides and Potency Configuration

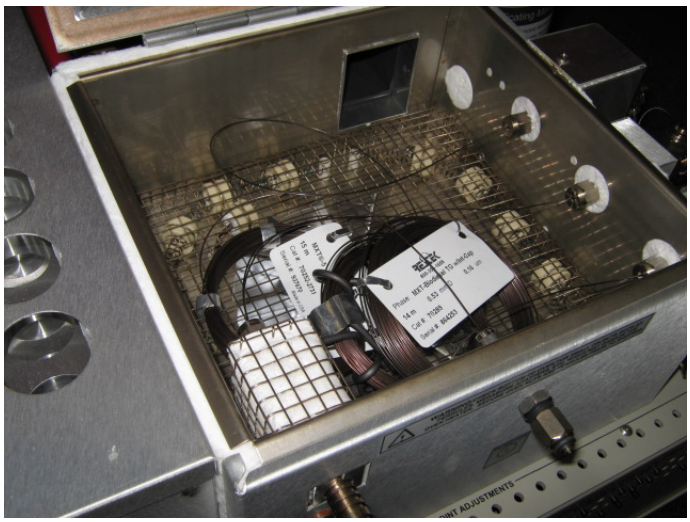
Configuration #4
Potency plus Pesticides
GC configuration
Part# 8610-0092 $21,889.00

This GC configuration permits two separate analyses which can be run simultaneously. The first analysis is for potency (CBD, THC and CBN) using a FID detector. The second analysis is for pesticides in cannabis using dual detectors. The NPD (nitrogen phosphorus detector) measures organo-phosphorus pesticides (Malathion) and many of the carbamate pesticides (Sevin).
The DELCD (dry electrolytic conductivity detector) measures organo-chlorine pesticides like Dursban, DDT, and Endrin.

The photos at right show the three columns, three detectors and dual injectors which make this possible.

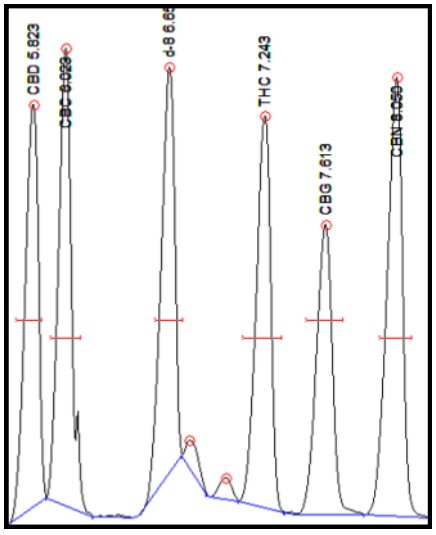
This GC configuration is appropriate for users with prior GC experience since the pesticide screen is more complex than the potency test. It should be understood that while 90% of all pesticides can be detected with this GC configuration, it is not possible to measure every possible pesticide since there are hundreds of pesticide molecules in a variety of chemical classes. It does allow the user to screen for most common pesticides in a very cost effective (less than 25 cents per analysis) manner using only .1 grams of sample.





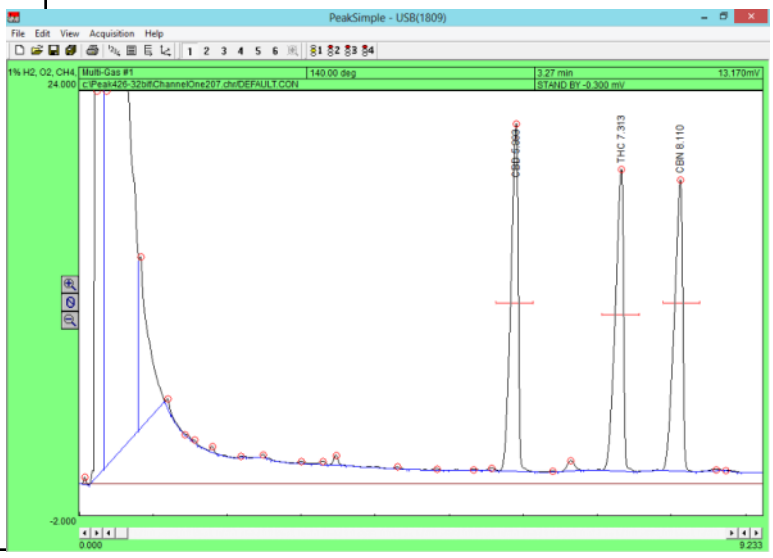
Calibrating Six Cannabinoids with CBD Reference Standard

There are six major cannabinoids that can be detected by the SRI 8610 FID Potency GC: Cannabidiol (CBD), Cannabichromene (CBC), D8-THC, D9-THC, Cannabigerol (CBG), and Cannabinol (CBN). Individual reference standards to calibrate all six cannabinoids can be obtained from chemical standard suppliers like Restek.



Sometimes all six cannabinoid standards are not available for calibration due to their cost or the time involved in individually injecting and calibrating. When this is the case, you can calibrate all six cannabinoids off of a CBD (or CBN) reference standard (obtained singly or in the Restek 3 Cannabinoid standard).

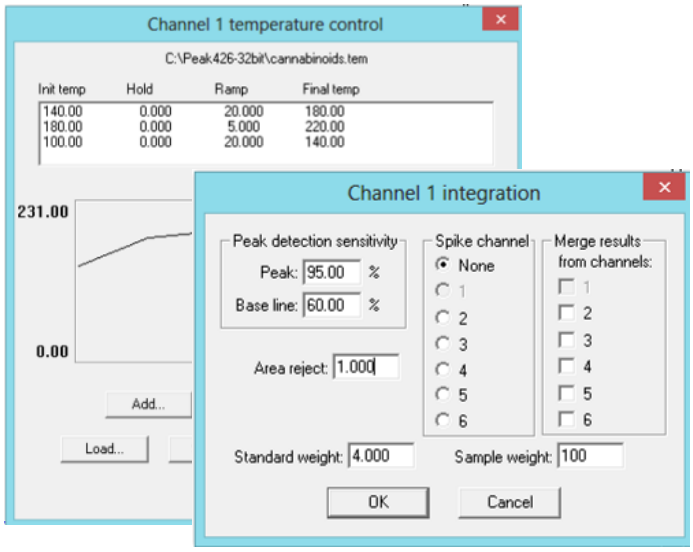
The six cannabinoid molecules are so chemically similar to each other that they will have near equal responses on the FID detector. 40% CBD will have the same area counts (and thus the same calibrated percent) as 40% THC (and 40% CBC, 40% CBG, etc.)* This principle can be confirmed by running a typical Restek cannabinoids standard and noticing that the three peaks are nearly identical in area counts.



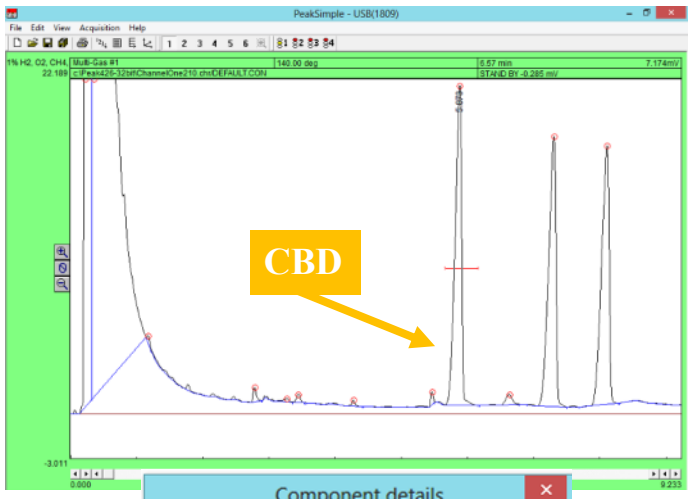
*For more information about this phenomena, read *A Contribution to the Improvement of Accuracy in the Quantification of THC by* A.J. Poortman-van der Meer and H. Huizer, *Forensic Science International 101*, 1999.

Calibrating Six Cannabinoids with CBD Reference Standard

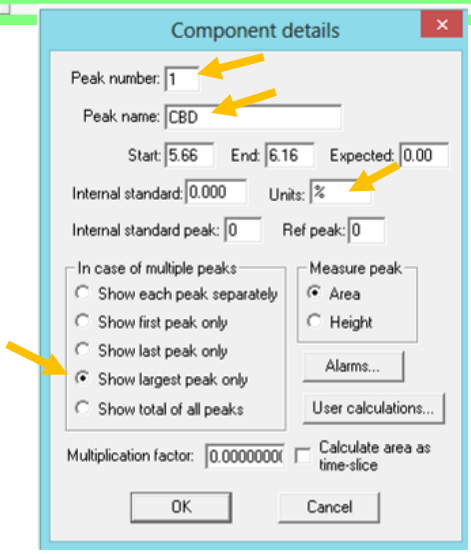
To calibrate all six cannabinoids off of a CBD reference standard inject either the Restek Cannabinoids Standard (containing CBD, THC, and CBN) or an individual CBD standard into the GC under your usual cannabinoid analysis temperature program and parameters. For more on this, see our document *Medical Herb Potency Testing* on our website at www.srigc.com.



When the run is complete you will see either the lone CBD peak or three cannabinoid peaks, depending on which standard you injected. Either way, the first peak is CBD. Right-click over the CBD peak and select "Add component". A red retention window should now be over the peak.

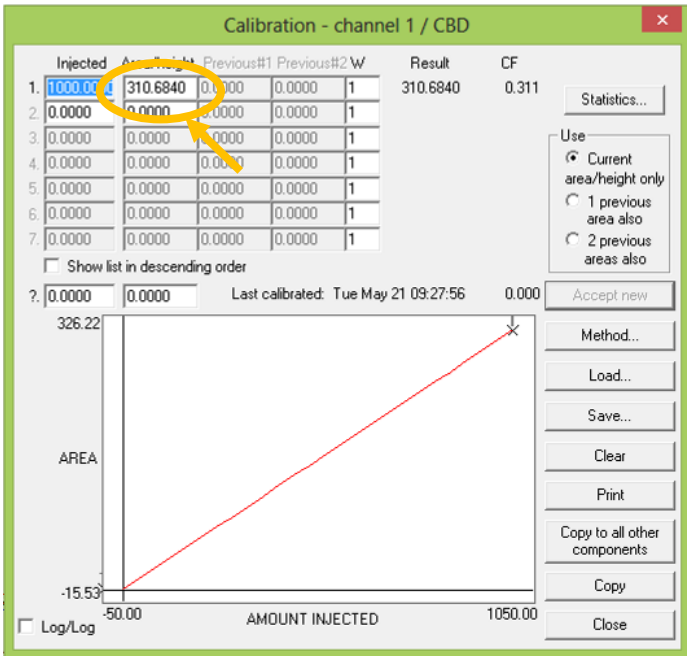


Right-click over the peak again and select "Edit component". In the "Component details" screen assign the peak a number, the name "CBD", enter "%" into the Units box, and select the "Show largest peak only" radio button before selecting the OK button.

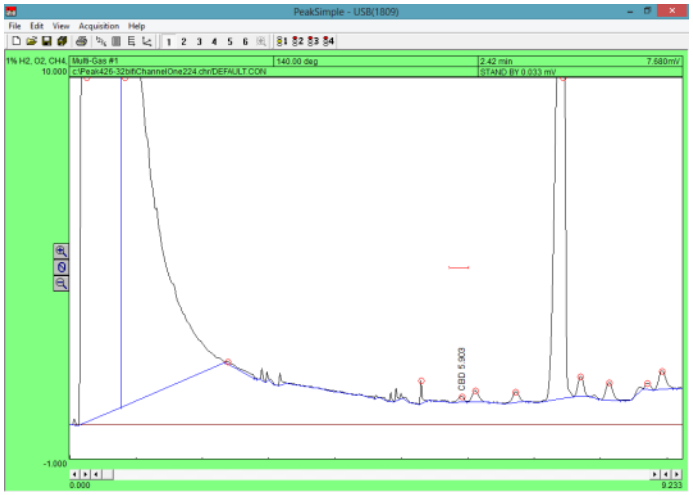


Calibrating Six Cannabinoids with CBD Reference Standard

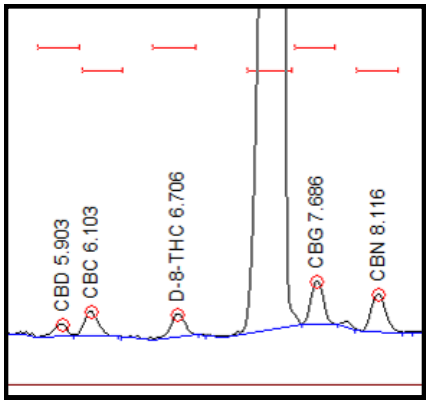
Right click on the CBD peak and select “Calibrate CBD”. Select “No” in the Load template calibration file screen and make sure “1” is selected on the “Recalibration level” screen. This will open the Calibration Screen. Enter “1000” under “Injected” (if you are using a 40% concentration calibration standard) and select the “Accept new” button. This will place the area of the CBD peak under the “Area/height” field. **Note this number**, as it will be used later to calibrate the other five cannabinoids.



Extract and inject an actual cannabis strain into the GC, preferably one in which you know there are all six cannabinoids present. Usually, (but not always) high potency strains and concentrates will have *some* amounts of all six cannabinoids. The chromatogram may look something like the one to the right.

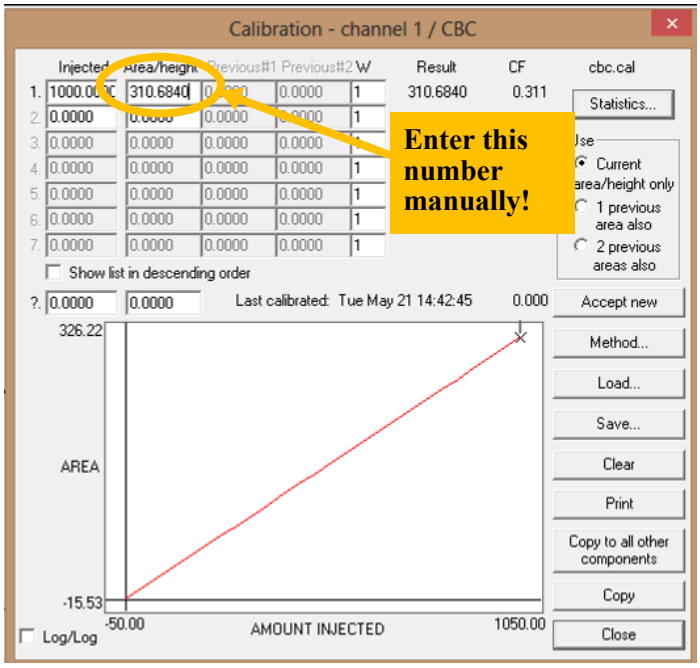


Identify the elution order on the stock MXT-500 column (other columns may have different elution orders). First out is (1) CBD, followed closely by (2) CBC. (3) D-8 THC comes out midway between CBC and (4) D-9 THC. (5) CBG comes out right after D-9 THC and just before (6) CBN. Add component windows and identify all the cannabinoids as shown to the right.

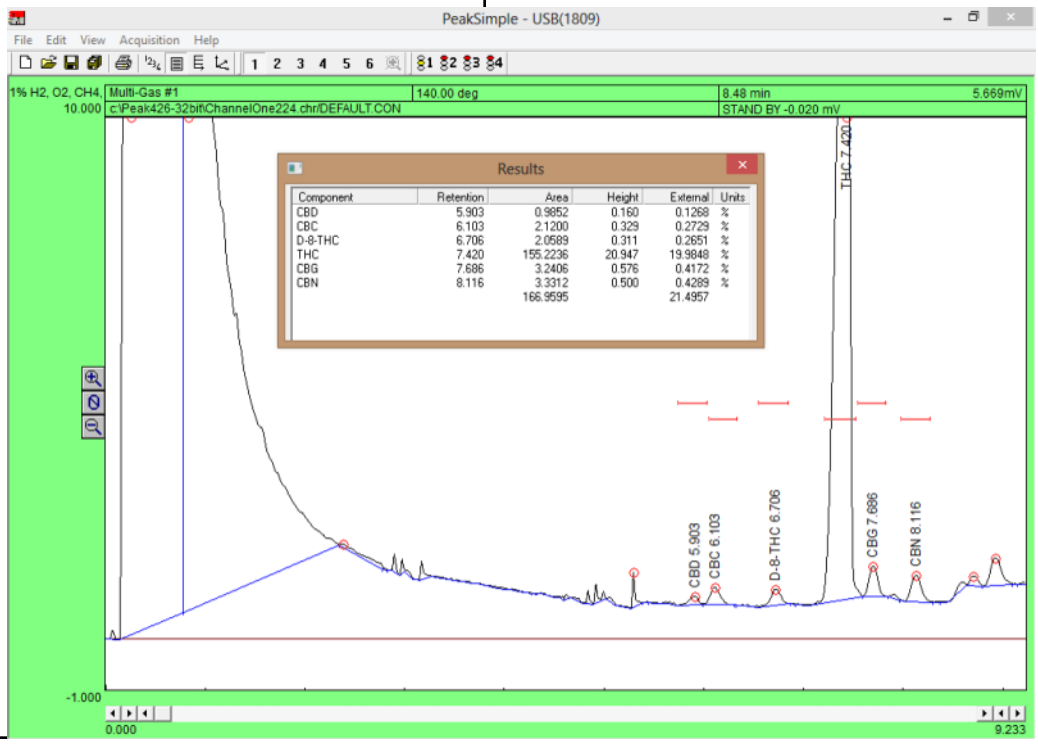


Calibrating Six Cannabinoids with CBD Reference Standard

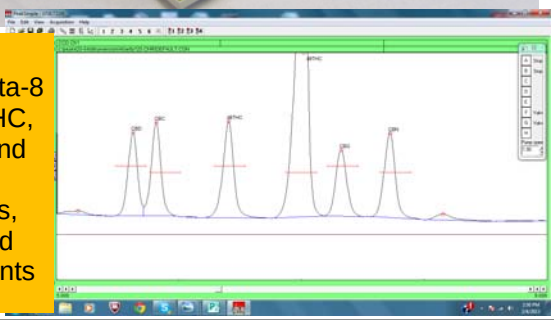
Calibrate each cannabinoid individually using the value obtained from the CBD calibration standard. Do not hit "Accept new", use the same area count that you obtained with the CBD Calibration Standard. For CBC, right click on the CBC peak and select "Calibrate CBC". In the "Calibration Channel 1/CBC" screen enter "1000" under "Injected" and the value you obtained from the CBD calibration standard under "Area/height". In our example, this number is "310.684". Save the calibration file and select "OK". Do this same process for the other cannabinoids.



Below is an actual strain with all six cannabinoids calibrated off of a CBD calibration standard.



8610C Gas Chromatograph for Medical Cannabis Analysis



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Flame Ionization Detector (FID)
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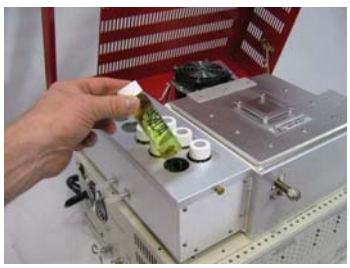
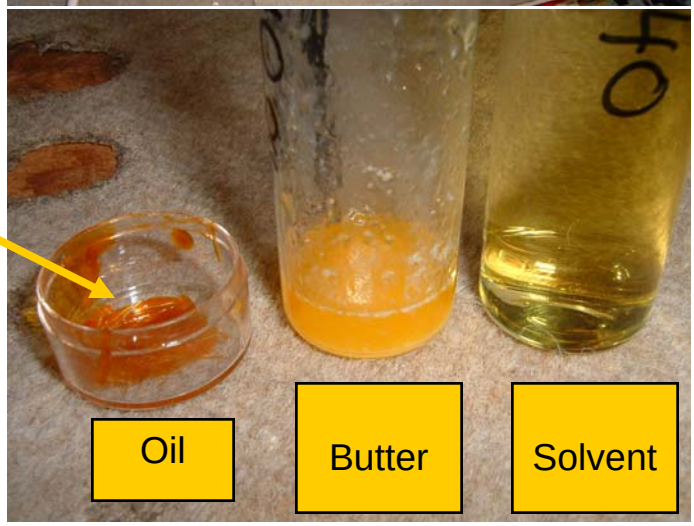


Built-in 50°C incubator

Measuring THC in Butter using the SRI 8610C GC

The THC in butter analyses were performed using an SRI 8610C GC configured for cannabinoid analysis.

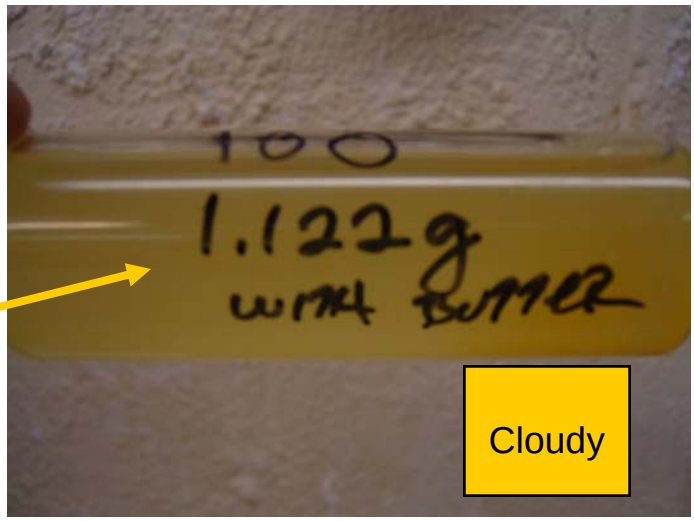
100 milligrams of a cannabis oil was weighed into two identical 40ml vials. The oil was a CO2 extract with an orange color. We used the oil for this test because it was very uniform in consistency.



The first vial was filled with methanol and placed in the built-in sample incubator which is part of this GC configuration. To the second vial was added 1 gram of butter. The butter vial was placed in the incubator WITHOUT solvent until the butter melted and dissolved the cannabis oil. The cannabis oil could clearly be seen to dissolve in the butter. The incubator was set to 50C. A third vial with no oil was loaded with 1 gram of butter for comparison.

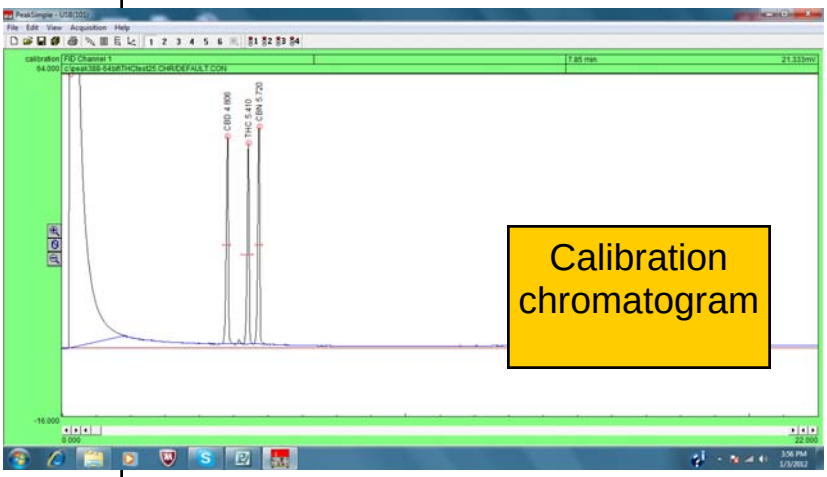
Measuring THC in Butter using the SRI 8610C GC

After 30 minutes in the incubator the two butter vials were filled with methanol and placed back into the incubator. Once the methanol warmed to 50C the butter vials were shaken for 30 seconds to disperse the butter into very fine droplets. This made a cloudy looking suspension The butter vials were again placed into the incubator for 30 minutes.



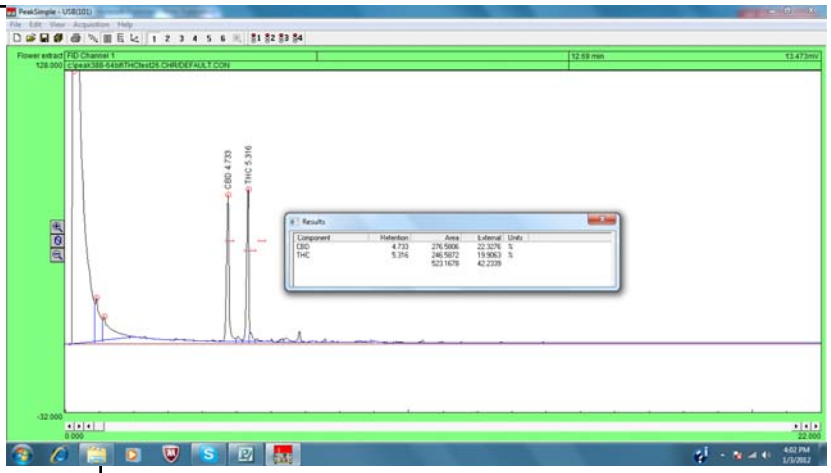
After another 30 minutes the butter solids dropped to the bottom of the vial leaving clear liquid in the top of the vial. Interestingly, the suspension did not clear at room temperature, only when heated in the incubator.

Meanwhile the GC was calibrated with a mixture of CBD, delta9THC and CBN each at a concentration of 333ng/ul. 1ul was injected on-column into a 15 meter MXT500 capillary column with .53mm id and a film thickness of .15 micron. The temperature program was set to start at 140C hold for 0.00 minutes , then ramp at 20 degrees per minute to 380 C then hold. The FID was set to 380C. Hydrogen carrier was used at 5psi or 10ml/min.

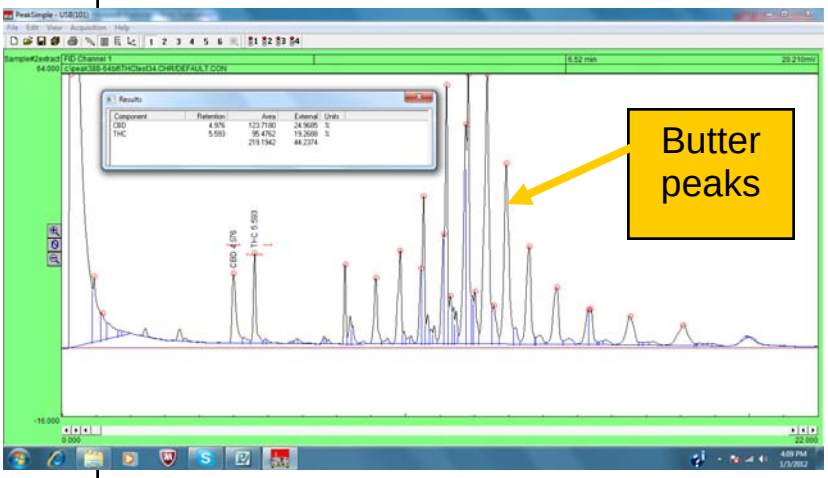


Measuring THC in Butter using the SRI 8610C GC

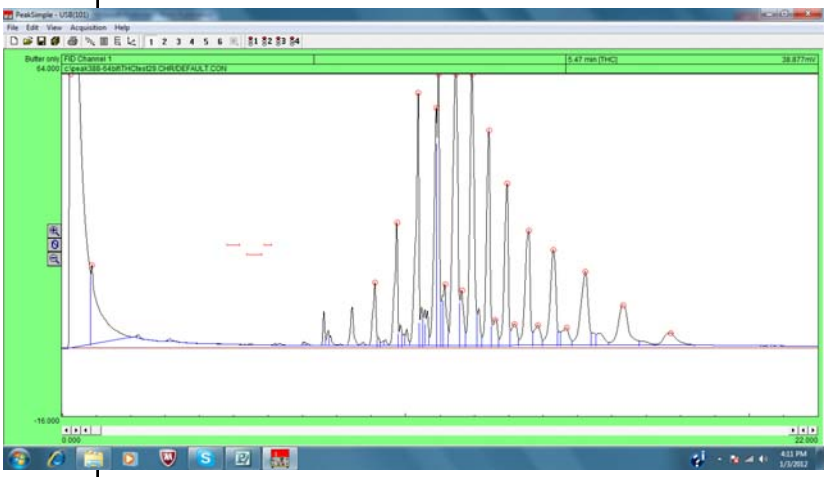
The oil only extract was injected and the results showed 22.3% for CBD and 19.9% for d9THC. Presumably this particular oil was prepared from industrial hemp since the CBD was so high.



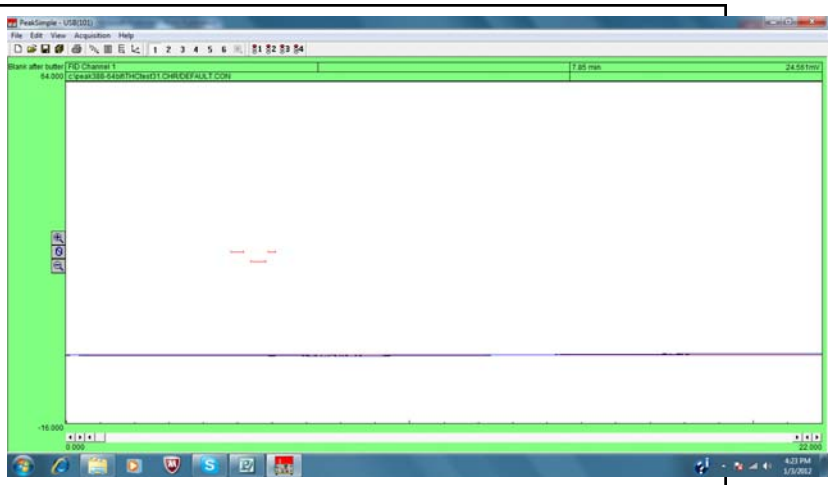
The vial with butter and oil was injected and the results showed 24.9% for CBD and 19.3% d9THC. Some thickening of the CBD is apparent while the THC peak looks much the same as the non-butter vial.



The vial with butter only (no oil) was injected for comparison. No interfering peaks were observed at the CBD or THC times but the butter peaks appear identical.



Measuring THC in Butter using the SRI 8610C GC



A blank run was made after the butter chromatograms. No carryover peaks or residue from the butter was observed.

We did notice that the retention times of the CBD and THC were shifted about 3% earlier with the 1 gram butter injections, but returned to the normal time in subsequent injections of non-butter samples.

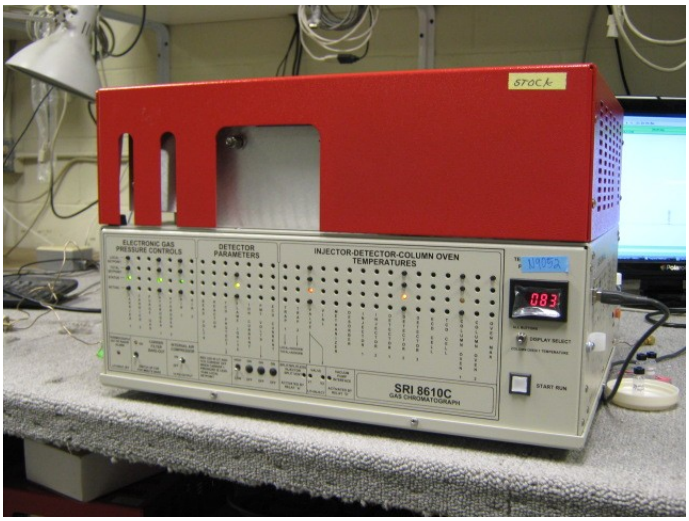
We made a more concentrated butter extract (3 grams butter in 40 ml methanol) and saw the retention times move even earlier.
We suspect the butter temporarily covers the stationary phase of the column resulting in less retention.

Conclusion:
This experiment shows that a simple methanol extraction completely transfers THC and CBD from butter into the methanol and avoids problems with the butter fats on the GC so long as the column is taken high enough in temperature during each analysis to elute the butter fats completely. The MXT500 column which was used is rated to over 400C which allows this high temperature operation. In addition the thin film promotes fast elution of the high boiling molecules. Even so, the analysis took 22minutes.

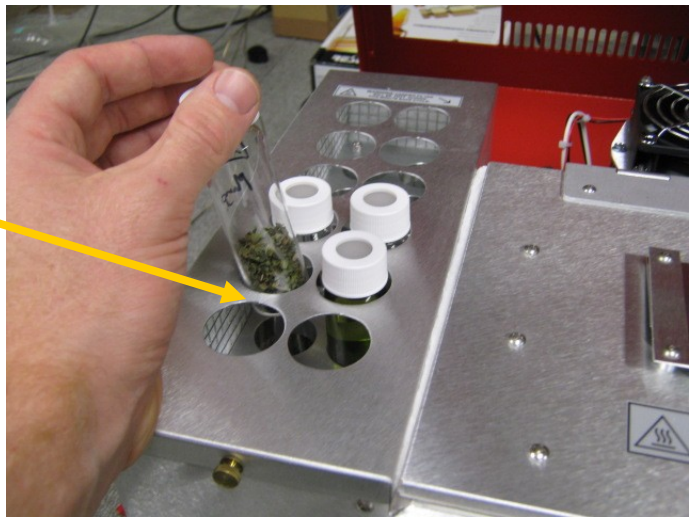
The peculiar shape of the CBD peak and the evidence that the butter increases the CBD number but not the d9THC is not explained and requires further investigation.

Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

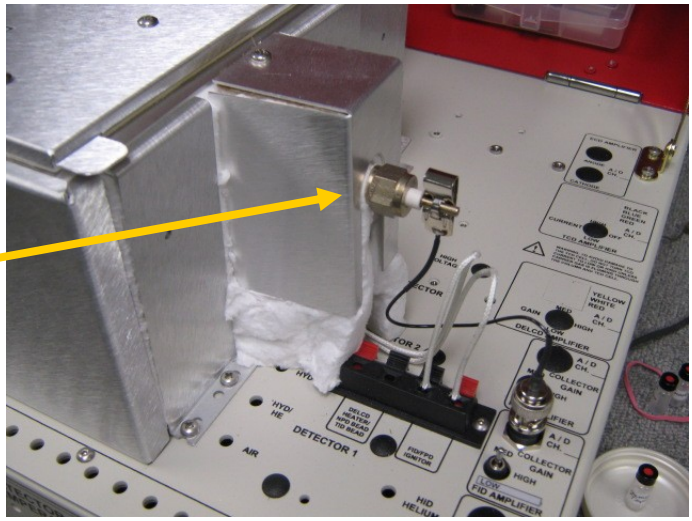
The SRI Medical Herb Potency 8610C GC is shown at right. This GC can also be used to test for the presence of terpenes in cannabis. The word terpene is usually taken to mean the non-psychoactive volatile molecules which make up the characteristic odor of cannabis even though delta-9-THC, CBD and other cannabinoids which are psychoactive, are also terpenes.



The 12 vial sample heater (incubator) aids in extraction for potency testing, but can also be helpful in terpene analysis since the added heat makes the terpenes more concentrated in the gas headspace in the vial.



The GC includes SRI's Flame-Ionization Detector (FID) which is sensitive to all the terpene and cannabinoid molecules.



Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

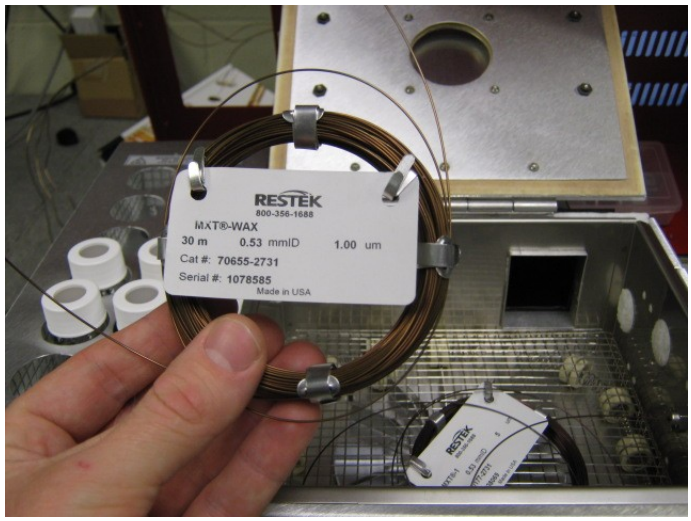
The terpene molecules commonly found in cannabis include:

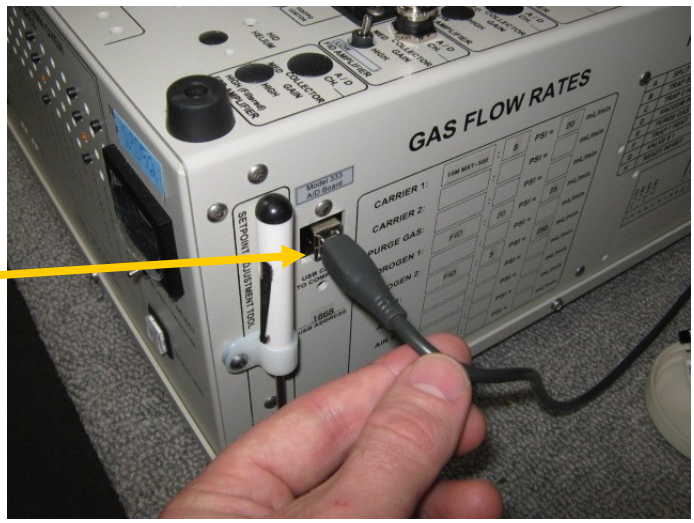
α-Pinene
β-Pinene
Camphene
Cineole (Eucalyptol)
γ-terpinene
β-Caryophyllene

But there are many more.

Many types of columns could be used to separate these molecules, but SRI currently suggests a 30meter MXT-WAX with 1 micron film thickness and .53mm id. The terpene analysis can be performed on other columns but the MXT-WAX provides the best separation.

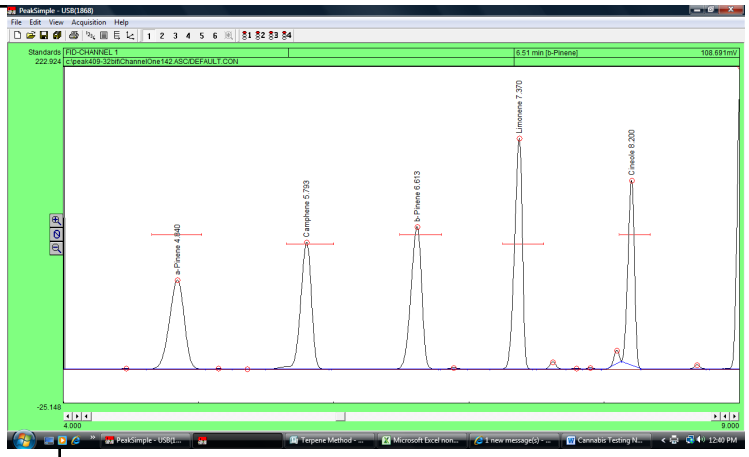
The entire GC plugs into any Windows computer using a USB cable.



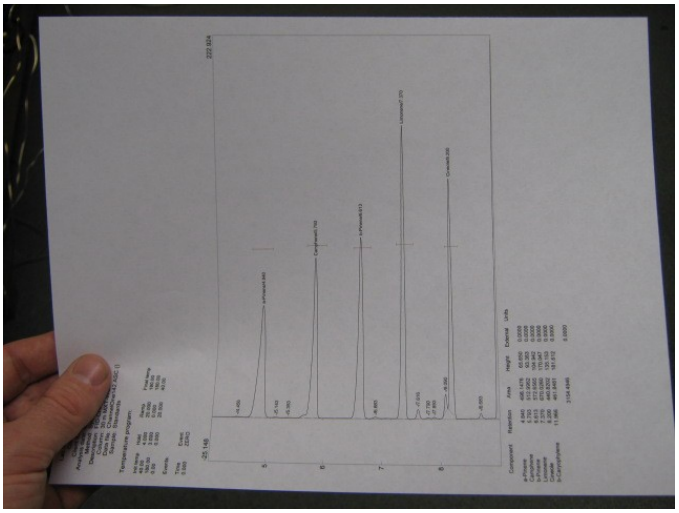


Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

SRI's PeakSimple software is included with the GC. PeakSimple software collects the GC data and generates a qualitative result which can be printed or transferred to other programs such as Excel or Word.

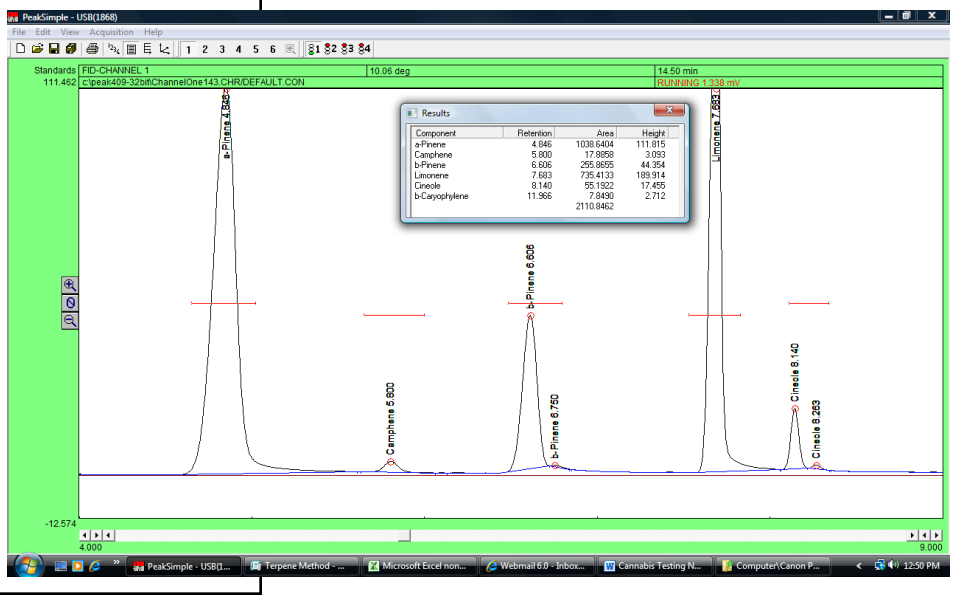


The chromatogram hardcopy printout at right shows a five terpene standard which was injected to identify these volatile odor compounds.



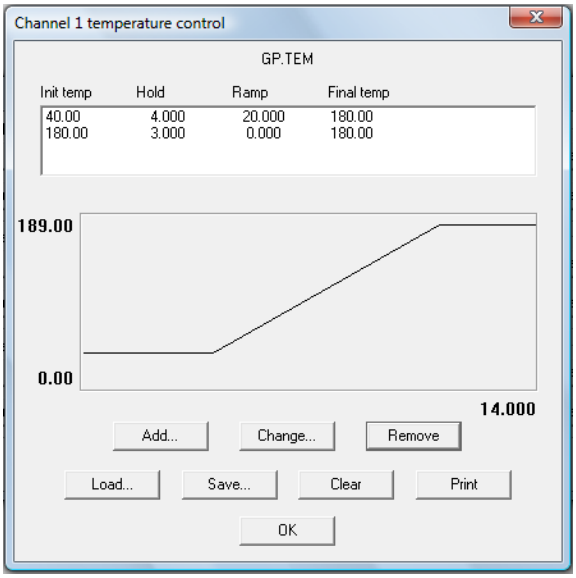
An actual cannabis sample run on the MXT-Wax column is shown at right.

The terpenes a-Pinene, Camphene, b-Pinene, Limonene, and Cineole are identified on the chromatogram.

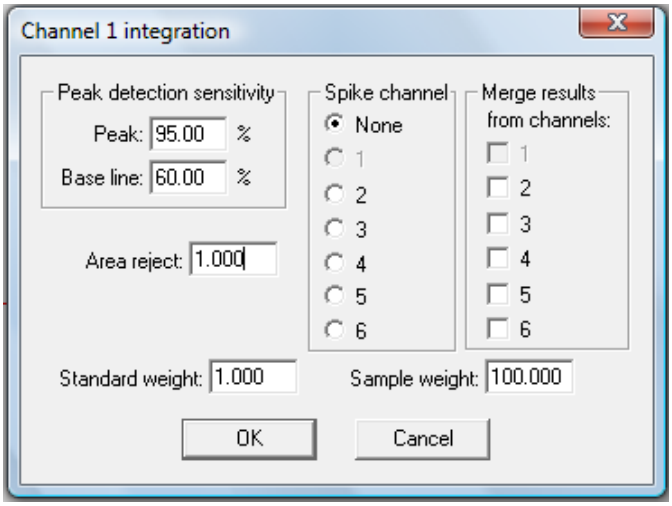


Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

Set the column oven temperature as shown at right. It is best not to exceed 180C or the MXT-WAX column may be damaged.

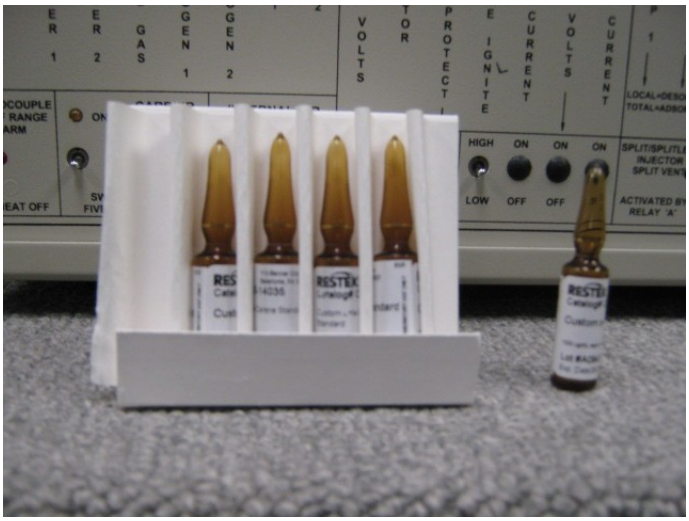


Set the Integration parameters as shown.

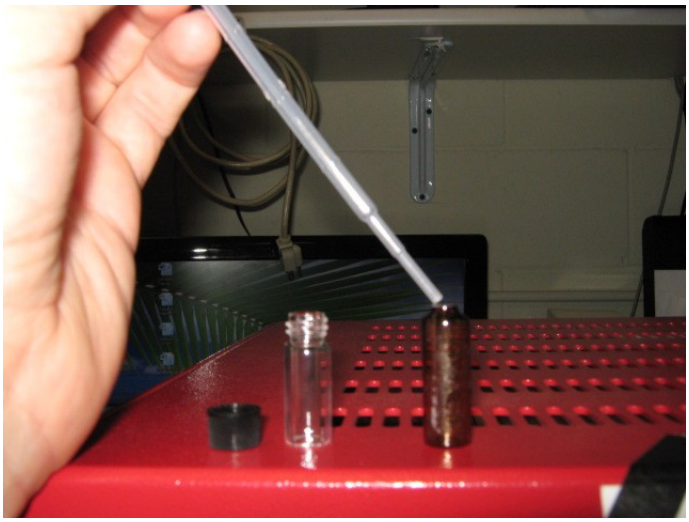


Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

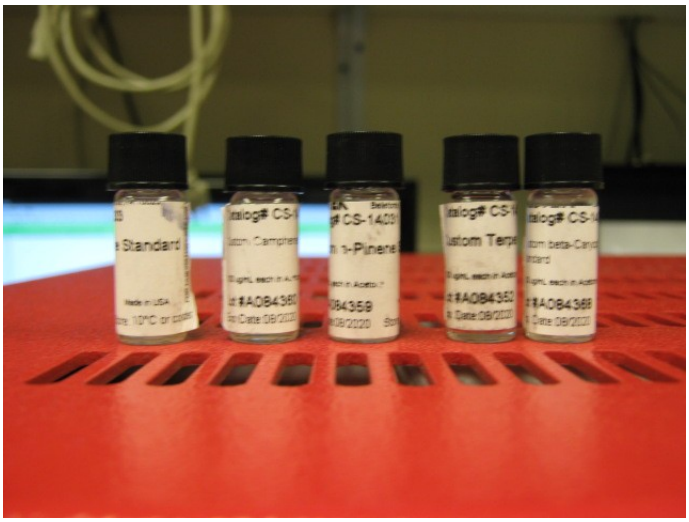
In order to identify terpenes in cannabis obtain the standards from a chromatography supplier like Restek (restek.com) (800) 356-1688.



Break the glass ampoule and transfer the contents into a 2ml septum vial (Restek #21154 and #24495). Restek provides one free vial with each standard.

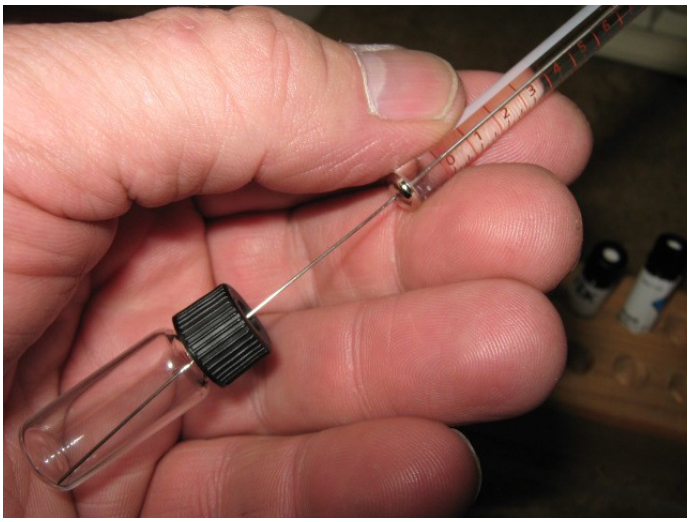


You will end up with one vial per terpene standard. There are 5-10 main terpenes in cannabis.

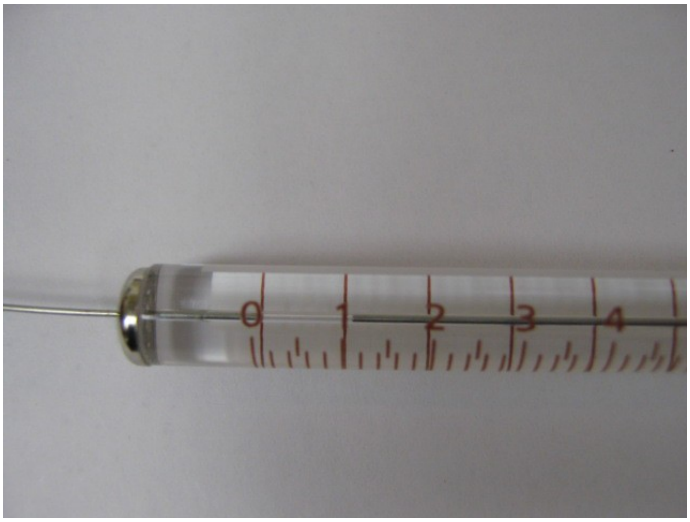


Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

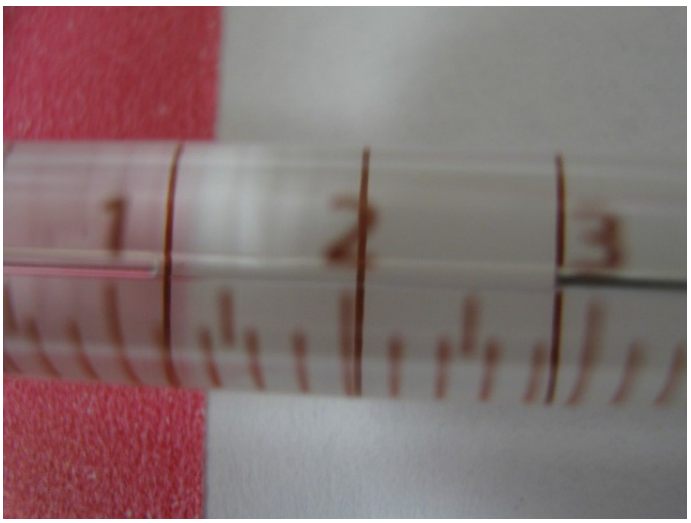
To qualitatively identify each terpene, the standard must be injected into the GC. Rinse the syringe first, then: use the 10uL syringe delivered with the GC (SRI #8670-9550) to withdraw 3-4uL of the standard. Puncture the septum rather than open the vial to avoid letting the methanol solvent evaporate each time the vial is opened. Pump the plunger several times to get rid of air bubbles.



With 3-4uL of liquid in the syringe, hold the needle vertically or at least slanted upwards so any air bubbles will rise toward the needle. With air bubbles removed, push the plunger to the 1uL mark. It is important to be as precise as possible. Wipe the needle with your fingers or a tissue to remove any liquid from the outside of the needle.



Pull the plunger back to the 3uL mark and note the amount of liquid. It should be 1.6-1.8 uL because the needle also contains .6-.8uL and this adds to the 1uL you measured with the plunger.



Leave the plunger at the 3uL mark.

Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

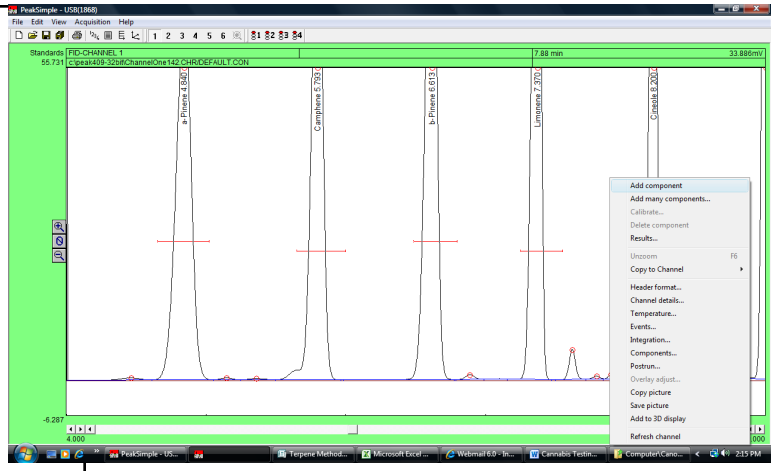
With the plunger still at the 3uL mark, place the needle up against the septum of the injection port (but not poking through it yet).

Press the Start Run button or press the spacebar on the key-board.

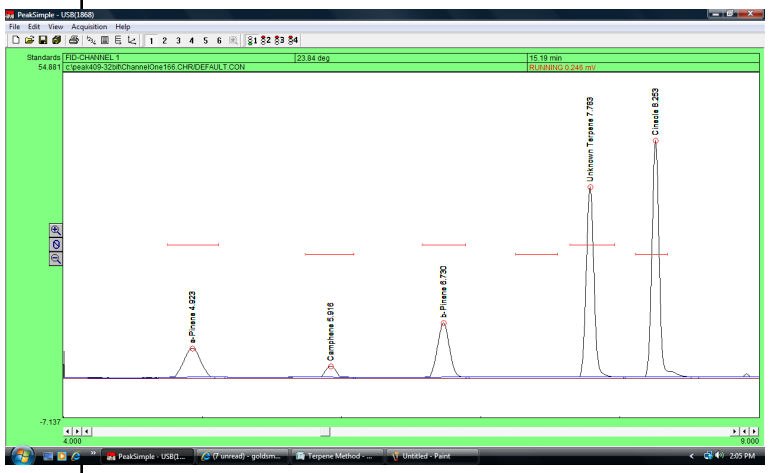
Insert the syringe all the way through the septum as far as it will go. Immediately depress the plunger. Twist the syringe one half turn (to wipe off any liquid on the tip of the needle) and then with-draw the syringe.

Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

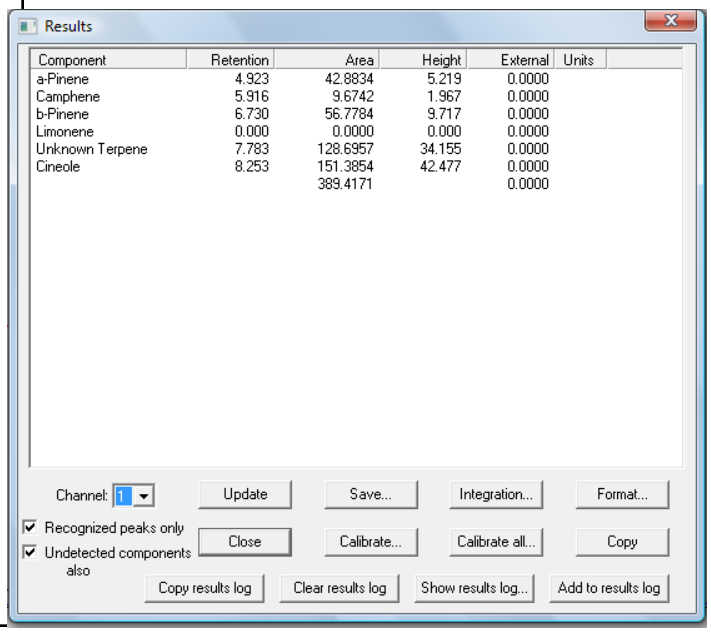
For this terpene standard we have five peaks. Identify the peaks so that each peak is defined by a “retention window”. See the PeakSimple tutorial describing the process of creating retention windows.



After qualitatively identifying the five terpene standards we can identify the same terpenes on subsequent sample runs of actual cannabis.



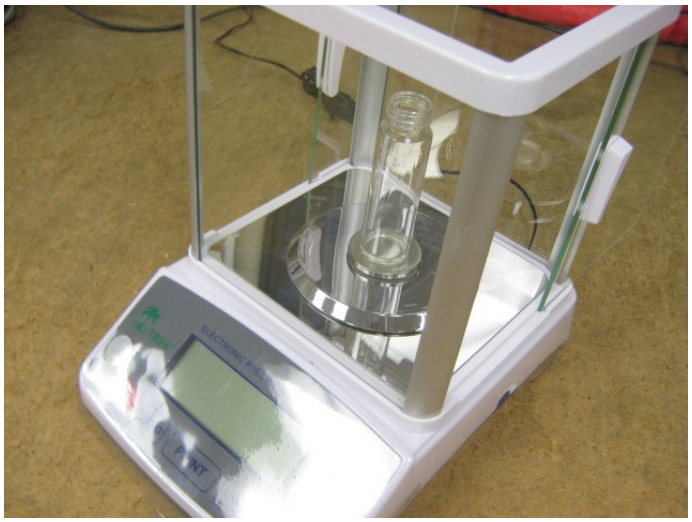
Navigate to the View/Results screen to see the report.



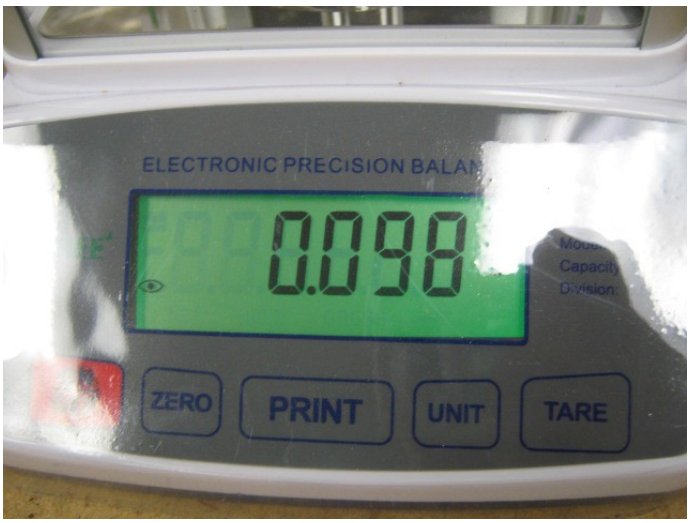
Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

Remove the cap from a 40mL vial and place it on a balance capable of reading 1 milligram (.001 gram). A balance like this can be purchased brand new for less than $300 on eBay.

With the 40mL vial on the balance, tare the reading (make the reading 0.000). Carefully add 100 milligrams of manicured cannabis to the vial. Drop the bits of cannabis into the vial slowly until the reading is close to 100 milligrams.



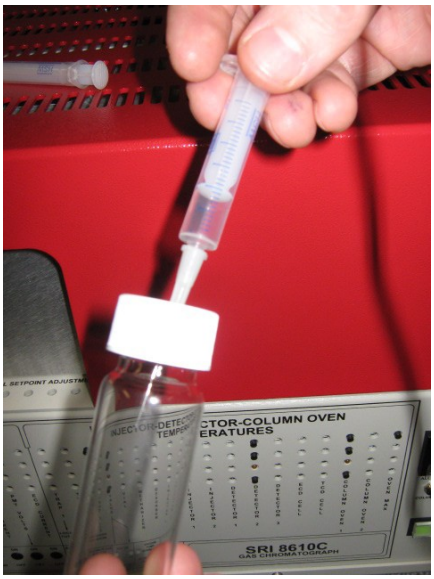
Don't worry if you are slightly under or above 100. In the photo at right, the reading is 98 milligrams which is close enough. Qualitative terpene analysis does not depend on an exact measurement of sample, but the operator may find it advantageous to use the same sample for a subsequent potency analysis. In this case, the reading on the scale will be important in properly measuring the cannabis sample. See the PeakSimple tutorial describing Medical Cannabis Potency.



Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

Seal the cap of the 40mL vial and let it sit for 30 minutes in the incubator.

Use a 3mL gas syringe to extract 1mL of gas from the “headspace” of the sample vial.

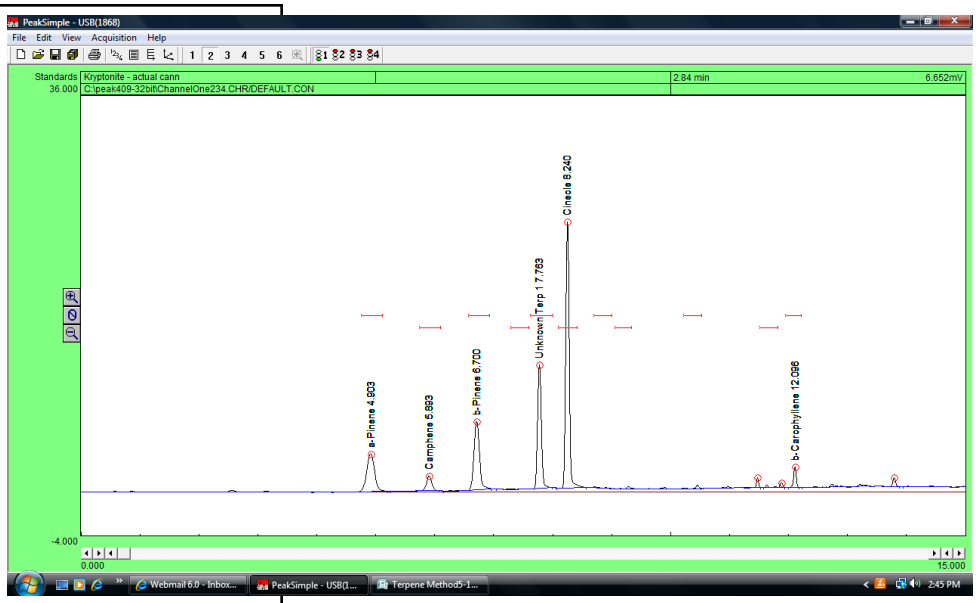


Inject the contents of the syringe into the injection port and start the run as shown previously.

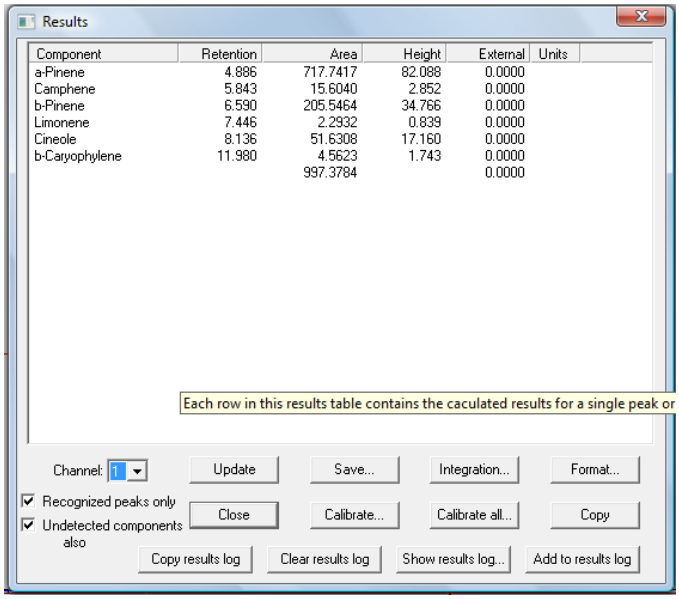
The picture at right shows a terpene sample vial filled to the neck with extraction solvent and ready to be injected for cannabis potency analysis. See the PeakSimple tutorial describing the process for Medical Cannabis Potency Testing.

Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

A real cannabis sample will look something like the chromatogram at right.



There may be several peaks under your known standard retention windows, there may be several unidentified terpenes without retention times.

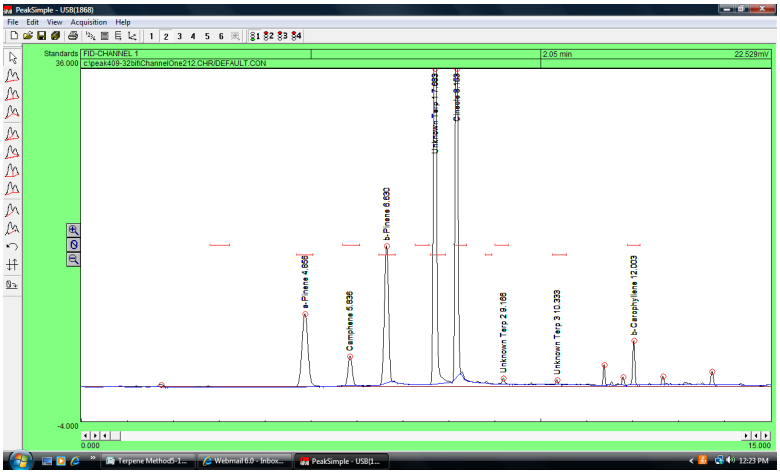


The Results screen will display the area counts of all peaks detected and identified with retention windows.

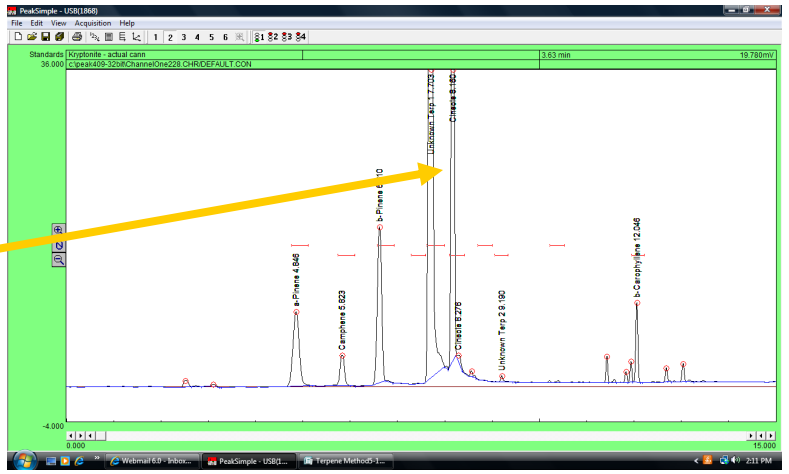
Print the chromatogram and results for a hardcopy record of the analysis.

Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

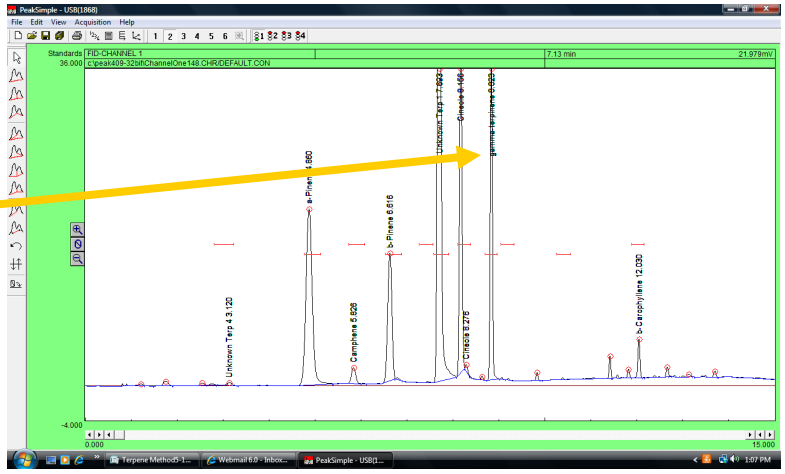
Here is what terpene analysis on the strain King Louie 13 OG looks like. Notice the presence of at least six terpenes: α– and β-Pinene, Camphene, Myrcene, Cineole, and β-Carophyllene.



This is a strain called Gush. Notice how, like many strains, it is highly concentrated in both myrcene and cineole. Also known as eucalyptol, cineole smells spicy, camphor-like, refreshing, and minty.

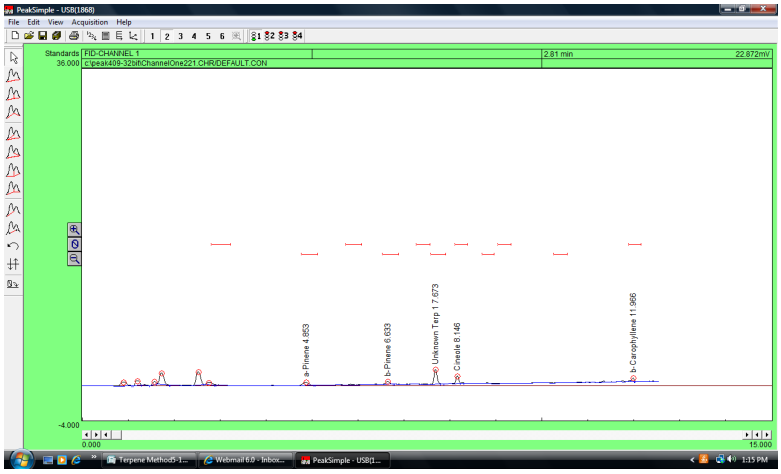


This sample of Green Crack has high concentrations of γ-terpinene. This terpene has a characteristic low-intensity lemon smell and is commonly used as an aromatic in foods, soaps, perfumes, and flavors.

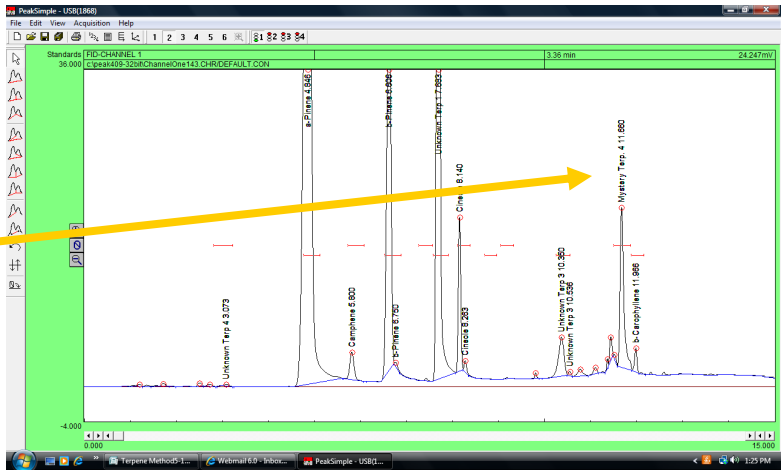


Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

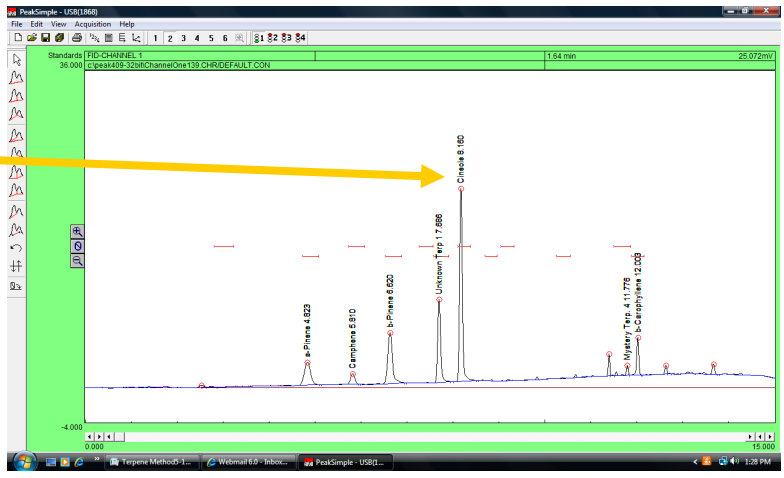
This sample was from visibly low-quality medical cannabis called Mango. Notice its overall low terpene concentrations.



This sample of Blue Dream was very high in overall terpene levels. Notice its high concentrations of an unknown terpene.

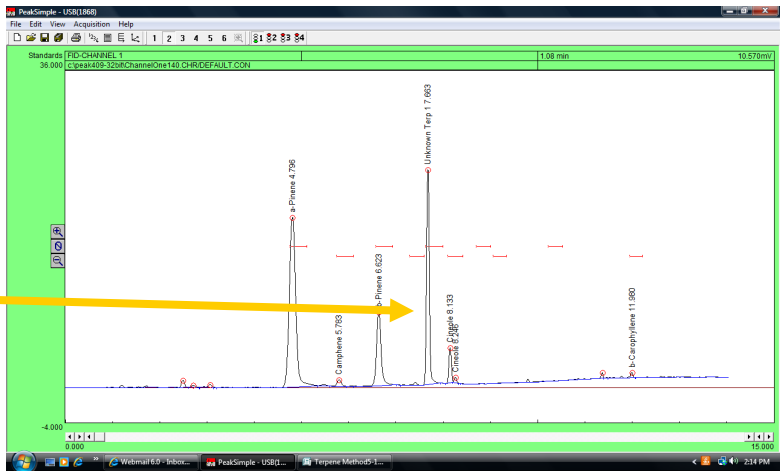


This is a strain called Super Sour Diesel. This chromatogram shows that it has the highest concentrations of the terpene cineole.

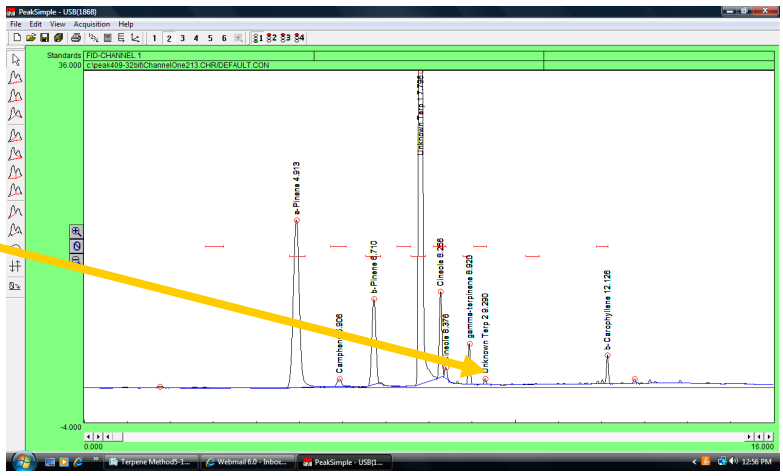


Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

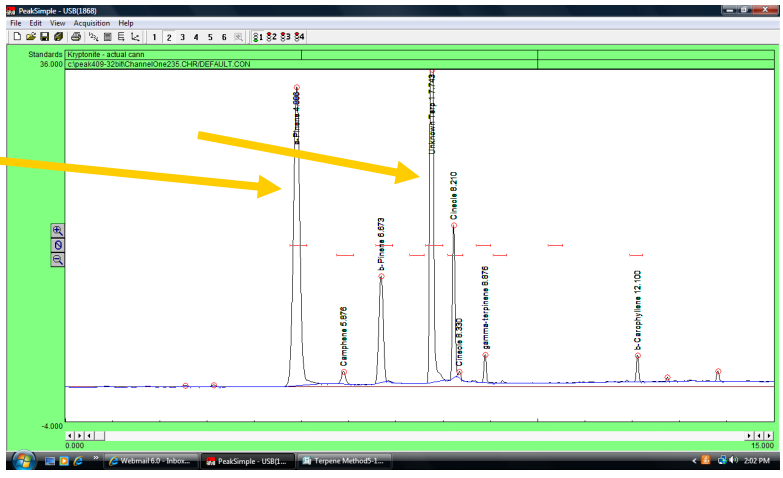
This is from a sample named Allen Wrench. Notice the high concentration of myrcene. This is typical of most strains as myrcene is the most common terpene in cannabis. Myrcene has a clove-like, earthy, vegetative, citrusy-mango smell.



This strain, AK-47, has a small concentration of an as yet unidentified terpene.

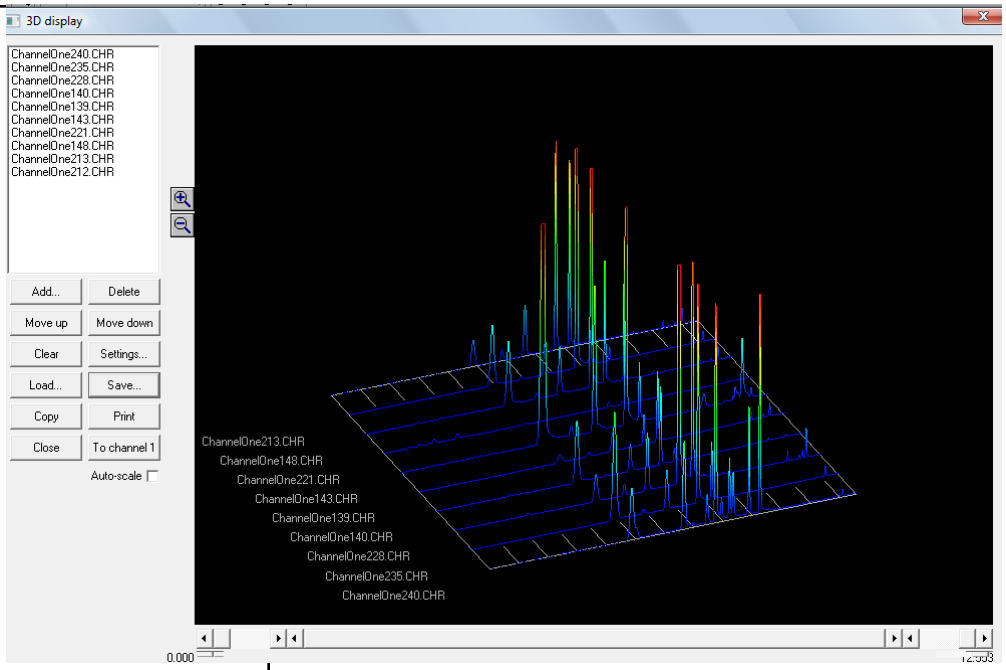


This is an outdoor variety of the strain Strawberry. Notice the nearly similar levels of α-pinene and myrcene. The terpene α-pinene has the characteristic odor of pine trees and is used in cleaning products like Pine-sol.



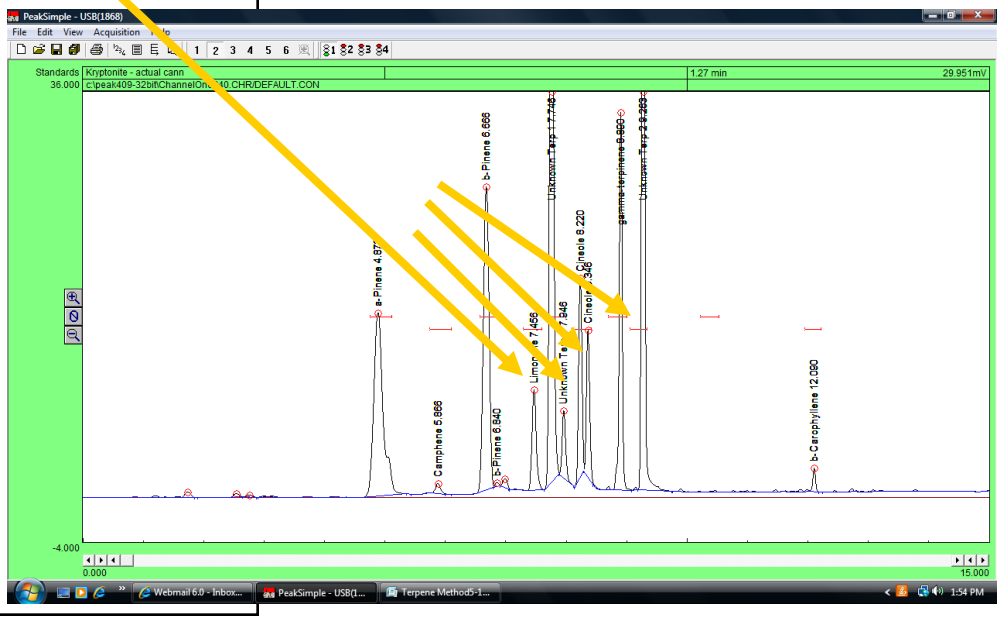
Medical Cannabis Terpene Measurement using the SRI 8610C FID GC

The user can display multiple terpene analysis runs on PeakSimple's 3D display. This feature makes it easy to compare multiple cannabis strains and to look for patterns.



This last terpene analysis is from a strain called Blueberry Jack. Notice the number of significant peaks (well over ten) compared to the usual cannabis sample.

SRI Instruments welcomes your feedback, knowledge and experience with terpene analysis. Please contact us if you have any questions or information to provide.



Calibration of Residual Solvents (Butane)

November 2013

To make a calibration for residual butane, inject a known amount of butane into an empty 40ml VOA vial.

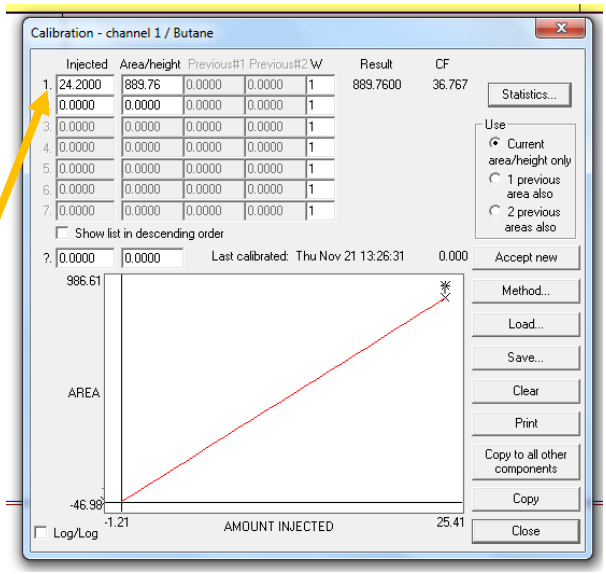
In the photo at right, we have injected 1ml of 1000ppm calibration gas in the VOA vial, so there is now 2.42ug of butane in the vial.

The calculation goes this this:

1mole of butane weighs 58.1 grams, and occupies 24000ml at room temp. One ml of butane (in a syringe, not under pressure) therefore weighs 2.42mg. The standard is .1% butane (1000ppm) so the weight of butane in the 1ml syringe (and also now the 40ml vial) is 2.42ug.

Inject 1ml of the vial headspace gas into the GC and record the area of the butane peak. In another vial place 100mg of concentrate and let it equilibrate. Inject 1ml of the concentrate headspace. If the peaks are the same size (area) then the amount of butane given off by the concentrate must also be 2.42ug.

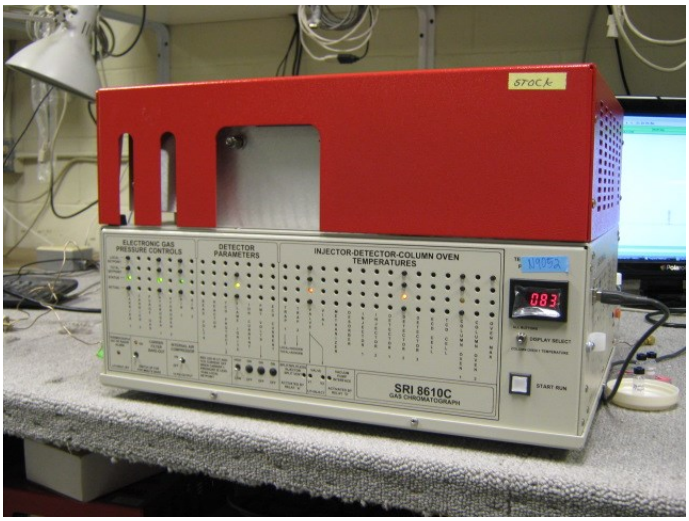
2.42ug (2420 nanograms) divided by 100mg (100,000,000 nanograms) equals .0000242 (24.2ppm). So a peak of this size is equivalent to a butane concentration (in the concentrate) of 24.2ppm.



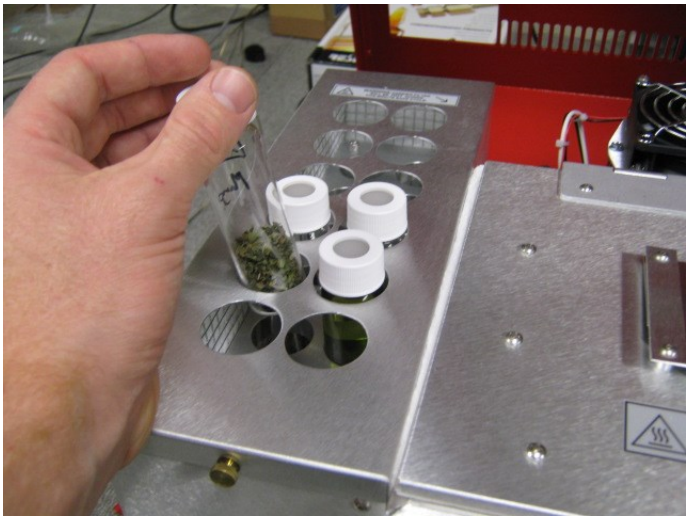
So the calibration curve would like the one at right.

Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

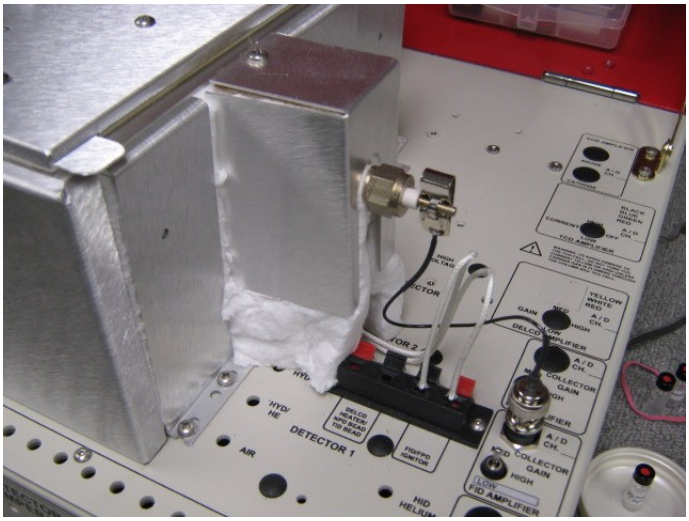
The SRI Medical Herb Potency 8610C GC is shown at right. This GC can also be used to test for residual solvents (i.e. butane, acetone, gasoline residue, etc.) in medical cannabis. These solvents are used in the extraction process to create medical cannabis hash oils and concentrates.



The 12 vial sample heater (incubator) aids in extraction of samples for potency testing, but can also be helpful in residual solvent analysis since the added heat makes any solvents more concentrated in the gas headspace in the vial.



The GC includes SRI's Flame-Ionization Detector (FID) which is sensitive to hydrocarbons (solvents, terpenes, and cannabinoid molecules).



Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

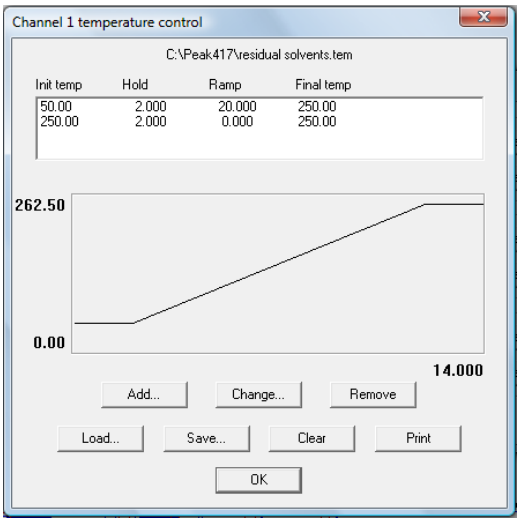
Solvents used to make cannabis extractions commonly include:
Butane
Isopropanal Alcohol
Acetone
Ethyl Alcohol (Ethanol)
Methyl Alcohol (Methanol)
Petroleum Ether
And in some cases Naphtha or even Gasoline (which contains hazardous chemicals like Benzene, Toluene, and Xylene, also known as BTEX).

Many types of columns could be used to separate these molecules, but SRI suggests a 15 meter MXT-1 with a 5 micron film thickness and .53mm id. This column can distinguish between solvents like pentane and hexane and does a good job of separating terpene molecules.

The residual solvent analysis can also be performed on the MXT-500 column that comes standard with the Potency GC, but the separation of volatile hydrocarbons will not be as good. For the best separation of terpene molecules, a 30 meter MXT-Wax is recommended but solvent separation will not be as good, and buying the column will be more expensive. As with all GC analysis, the operator must decide what compounds are most important to detect and select the proper column accordingly.

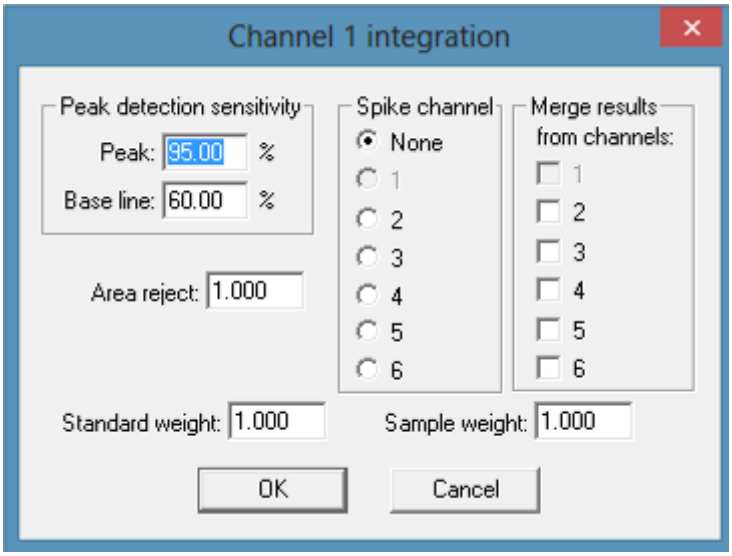
Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

Set the column oven temperature as shown at right. Although we are only interested in the early eluting solvents and adulterants, the "heavier" terpene molecules are also injected onto the column, and these must be allowed time to come out. The light hydrocarbons come out during the two minute hold, BTEX between 50 and 130 degrees, and the terpenes after that. The final temperature hold at 250 ensures that the heaviest molecules are "baked-out" of the column.



Thus, it can be convenient to perform butane and residual solvent ***and*** terpene analysis in one run. For more information on terpene testing, please see the tutorial describing medical cannabis terpene analysis.

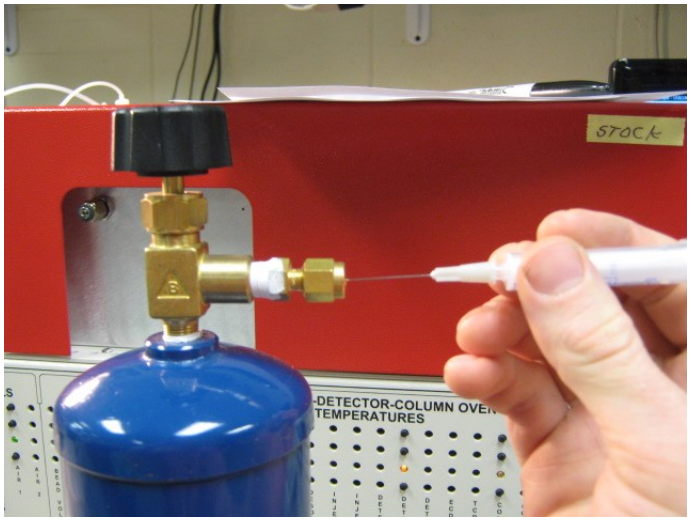
Set the Integration parameters as shown.



Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

In order to identify residual solvents in cannabis samples known standards must be injected. There are many ways to do this, but SRI recommends using a C1 to C6 gas standard at 0.1% concentration (1000 ppm for each gas). You can pick a gas standard from Grace Davison (part # M7017).

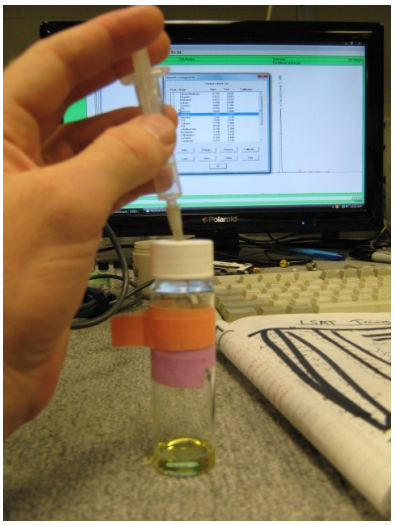
Pressurize the gas cylinder by turning the release valve slightly counterclockwise. Pierce the septum with a 3 mL gas syringe and withdraw 1 mL of gas. Remove the syringe from the gas sample bottle.



Or, alternatively, place the 3 mL syringe needle into a standard disposable lighter and suck out 1 mL of butane.

Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

To identify gasoline and its constituents that remain after evaporation (BTEX) obtain some gasoline and place it into an airtight vial. Using the 3 mL syringe, suck out 1 mL of headspace gas from the top of the vial.

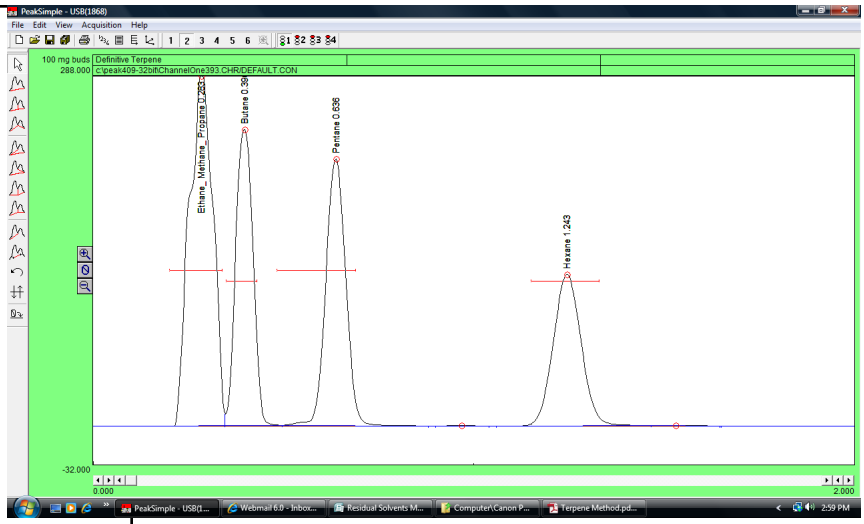


With the syringe plunger still at the 1mL mark, place the needle up against the septum of the injection port (but not poking through it yet). Press the Start Run button or press the spacebar on the keyboard.

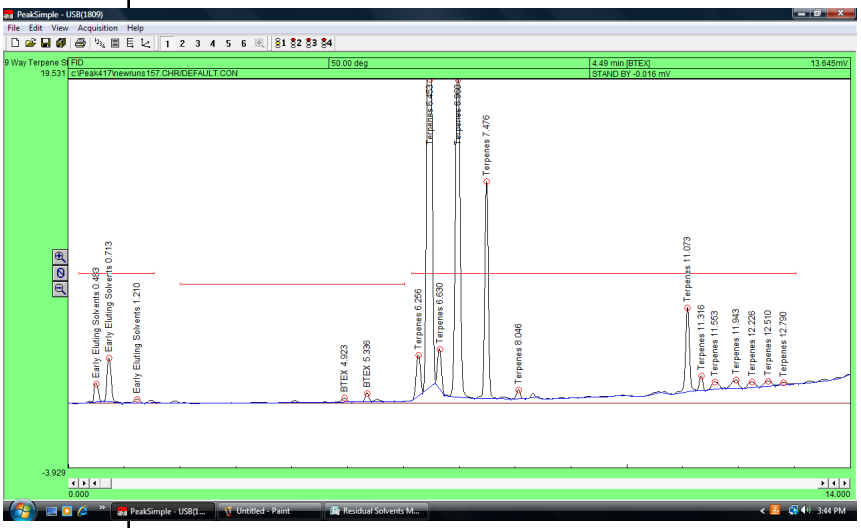
Insert the syringe all the way through the septum as far as it will go. Immediately depress the plunger and quickly withdraw the syringe.

Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

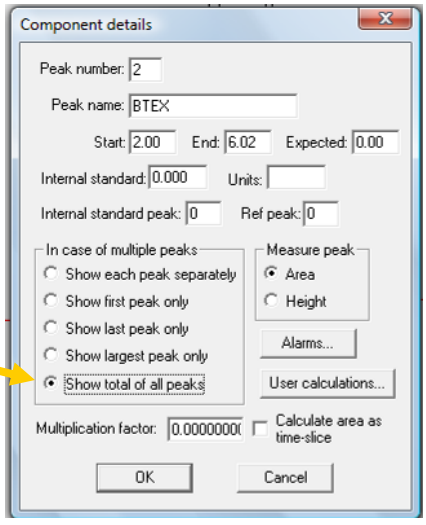
After injecting the C1—C6 standard we see four peaks: ethane, methane, and propane (which all elute together); butane; pentane; and hexane. Identify the peaks so that each peak is defined by a "retention window". See the PeakSimple tutorial describing the process of creating retention windows.



Since it may be difficult, if not impossible, to obtain reference standards for all the various residual solvents in cannabis it may be more practical to place blanket retention windows over categories of residual solvents. In the chromatogram to the right, one retention window covers the organic solvents, the second covers BTEX, and the third encompasses all the terpenes.



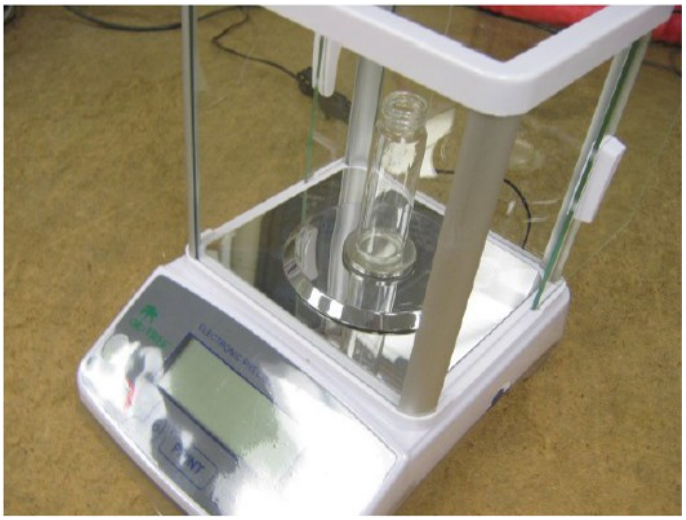
In this case, all the peaks under the retention window need to be quantified. In the Edit Component screen select "Show total of all peaks".



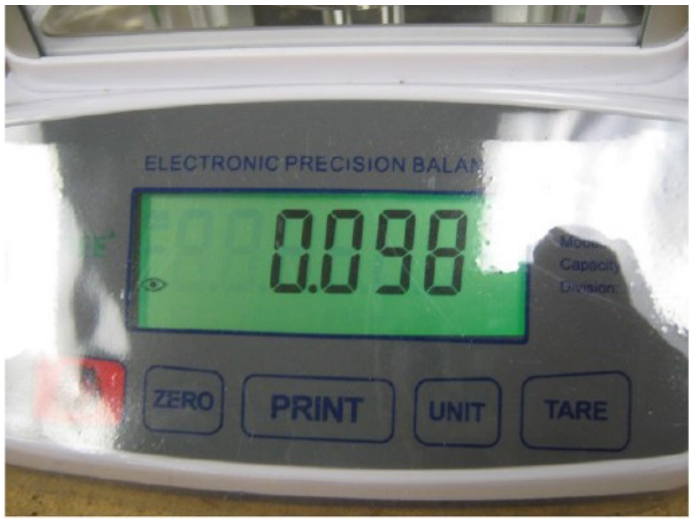
Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

Remove the cap from a 40mL vial and place it on a balance capable of reading 1 milligram (.001 gram). A balance like this can be purchased brand new for less than $300 on eBay.

With the 40mL vial on the balance, tare the reading (make the reading 0.000). Carefully add 100 milligrams of manicured cannabis to the vial. Drop the bits of cannabis into the vial slowly until the reading is close to 100 milligrams.

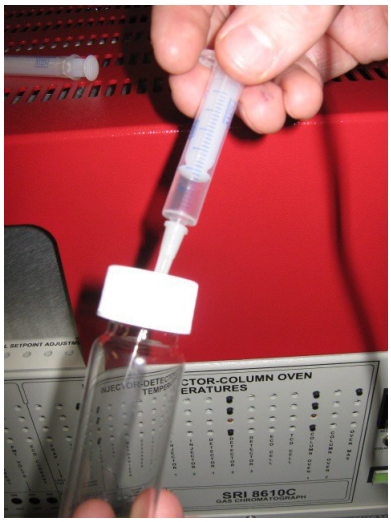


Don't worry if you are slightly under or above 100. In the photo at right, the reading is 98 milligrams which is close enough. Qualitative butane and residual solvent analysis does not depend on an exact measurement of sample, but the operator may find it advantageous to use the same sample for a subsequent potency analysis. In this case, the reading on the scale will be important in properly measuring the cannabis sample. See the PeakSimple tutorial describing Medical Cannabis Potency.



Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

Seal the cap of the 40mL vial and let it sit for at least 15 minutes in the incubator. Use a 3mL gas syringe to extract 1mL of gas from the “headspace” of the sample vial.

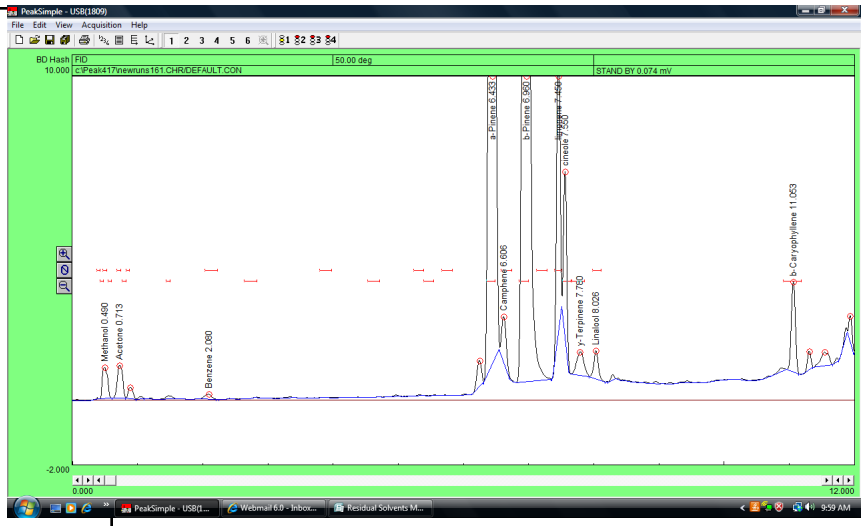


Inject the contents of the syringe into the injection port and start the run as shown previously.

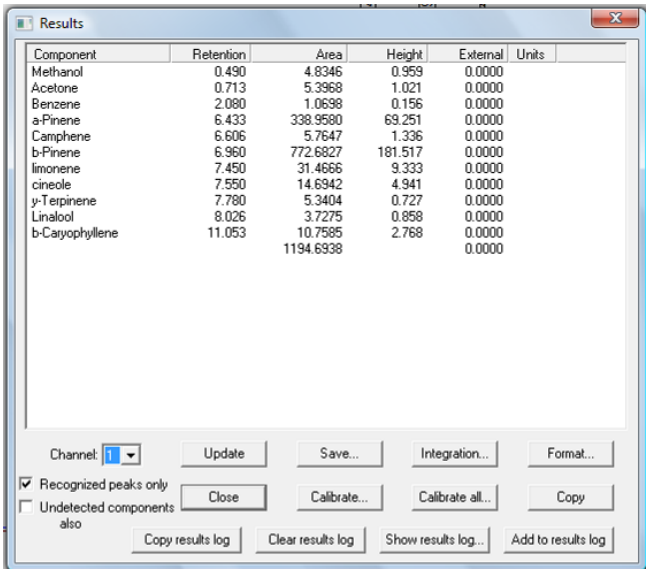
The picture at right shows a butane and residual solvent sample vial filled with 40 mL of extraction solvent and ready to be injected for cannabis potency analysis. See the PeakSimple tutorial describing the process for Medical Cannabis Potency testing.

Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

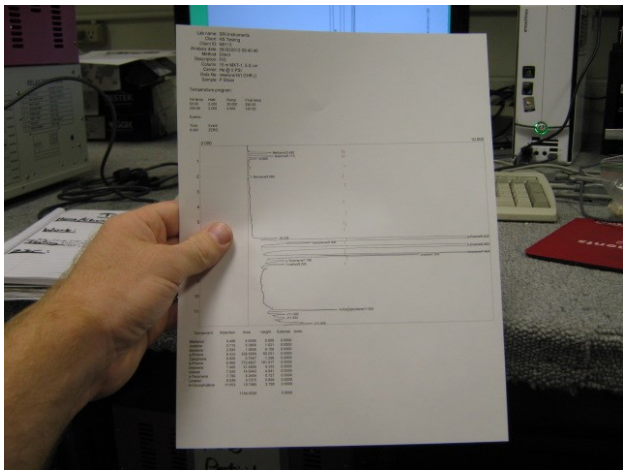
A real cannabis flower sample will look something like the chromatogram at right. This particular sample has standard levels of organic solvents (which are present in low levels naturally in plant matter) and multiple terpenes.



The Results screen will display the area counts of all peaks detected and identified with retention windows.

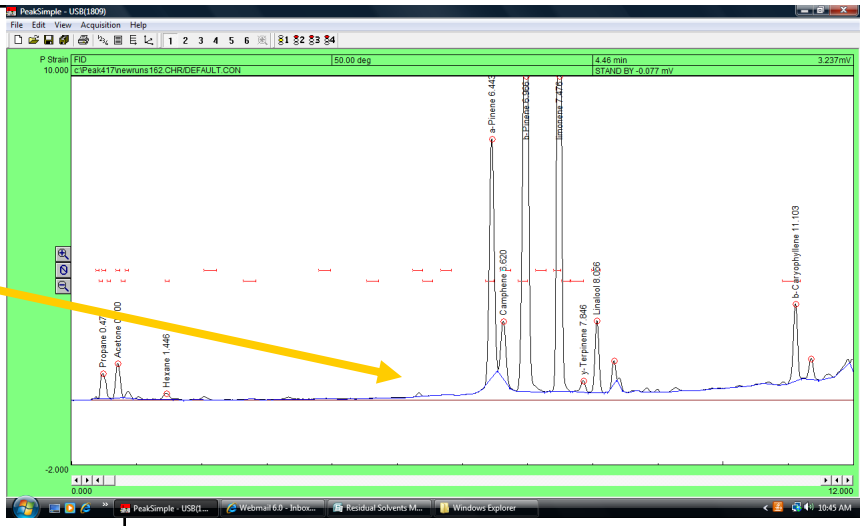


Print the chromatogram and results for a hardcopy record of the analysis.

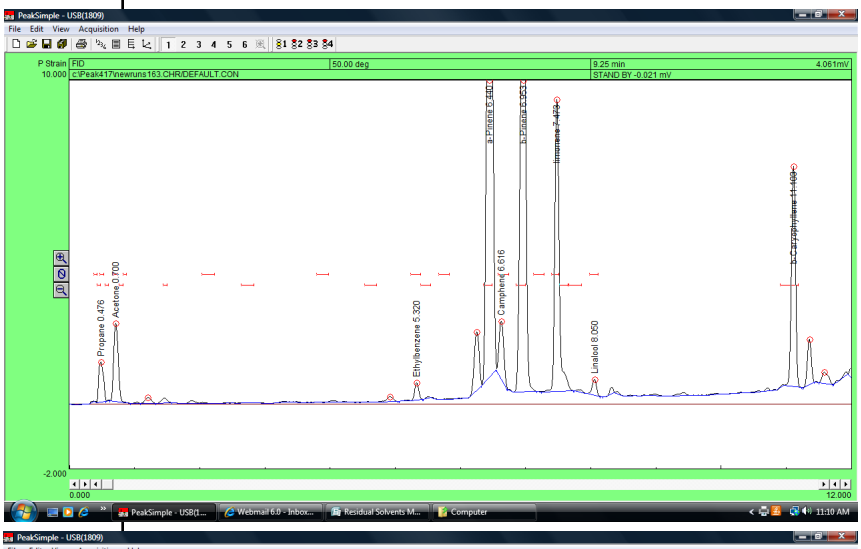


Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

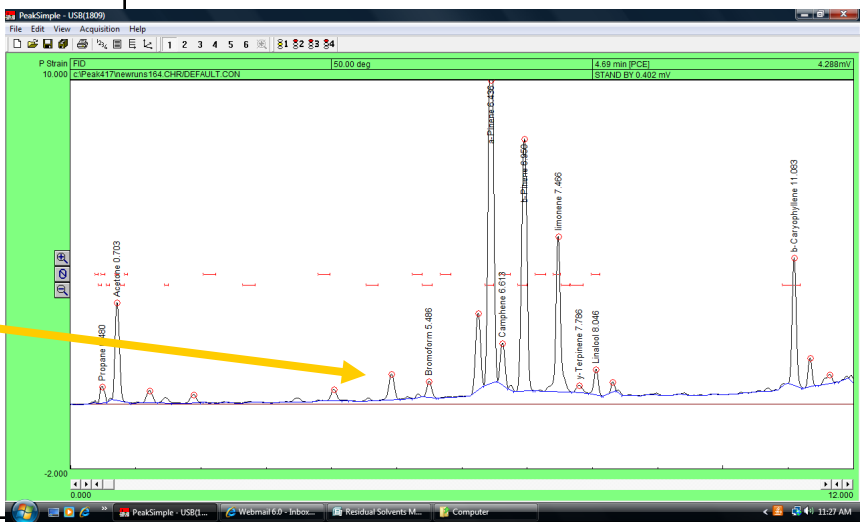
Super Sour Diesel flowers. Notice the lack of any BTEX-like adulterants.



Blue Dream concentrate.

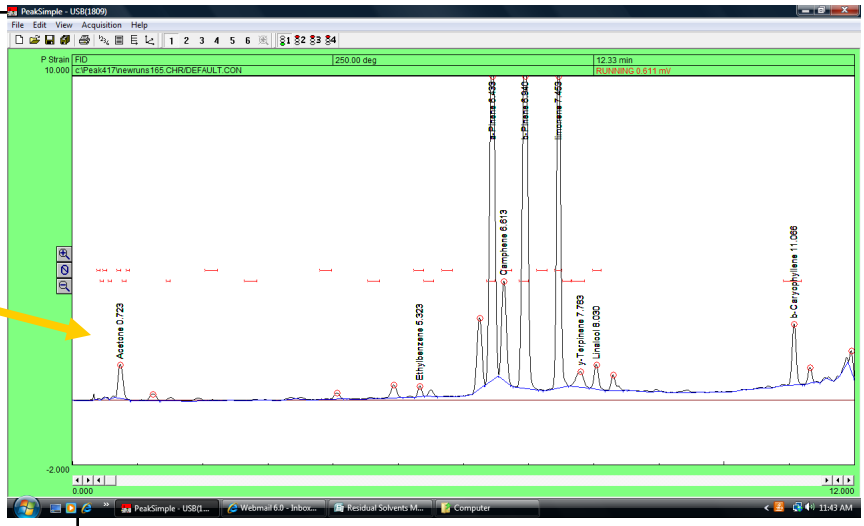


Mr. Nice concentrate. Notice the three small peaks in the BTEX area.

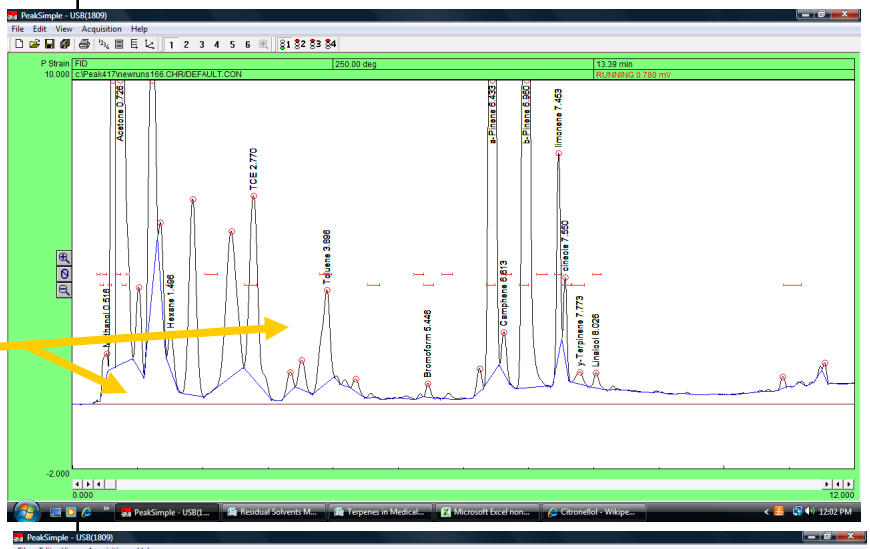


Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

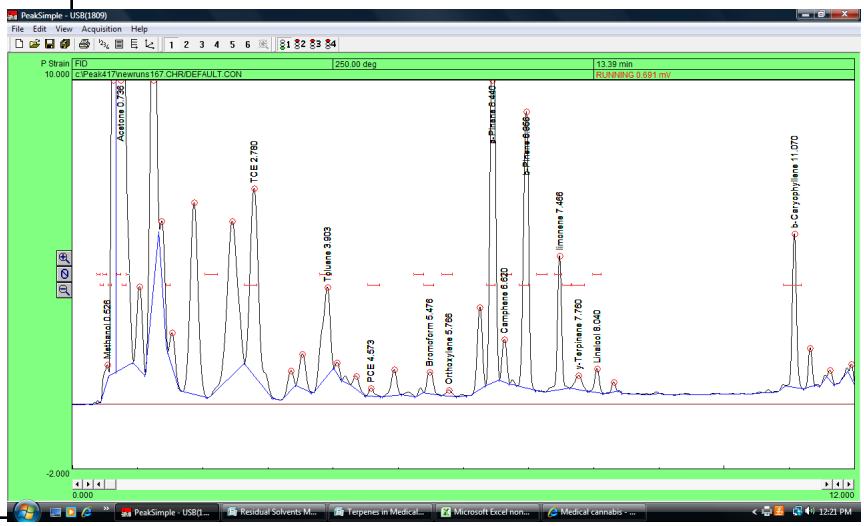
Sour OG Concentrate. Notice the low levels of organic solvents.



Outdoor-grown flower spiked with gasoline fumes. Notice the high concentrations of organic solvent and BTEX adulterants.

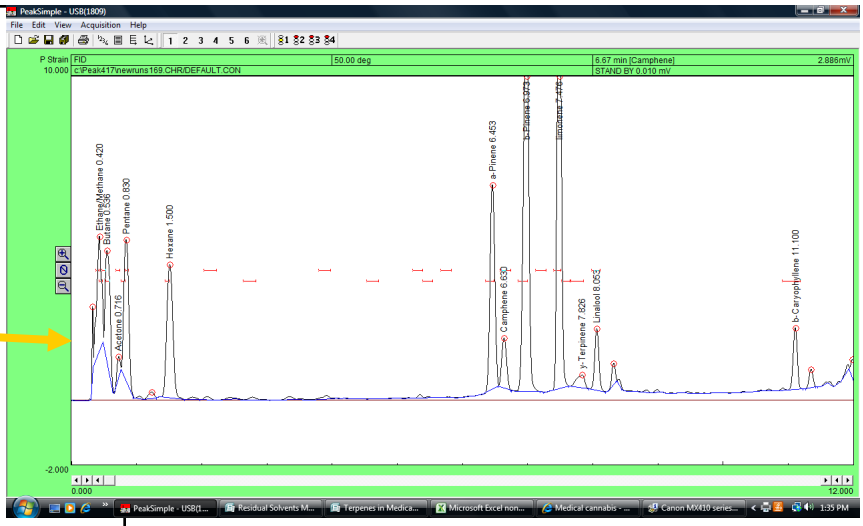


Mr. Nice Concentrate spiked with gasoline fumes.

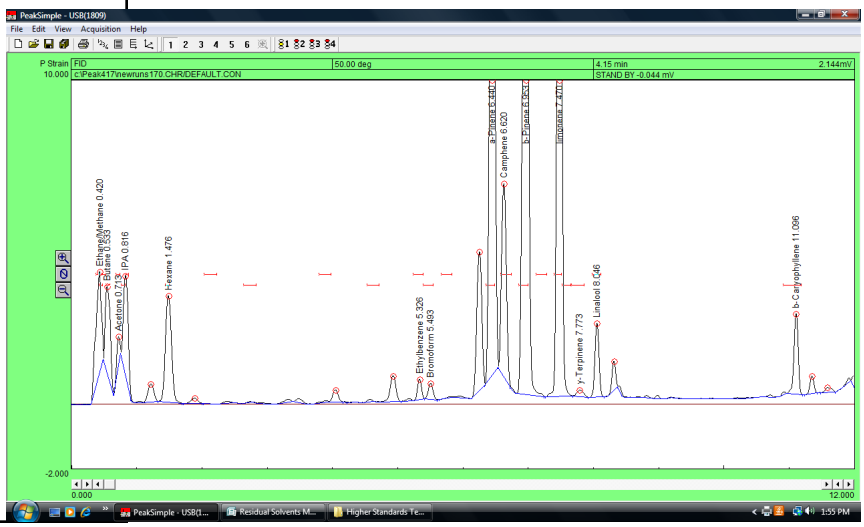


Butane and Residual Solvents in Medical Cannabis Flowers and Concentrates using the SRI 8610C FID GC

Super Sour Diesel Flowers spiked with organic solvents (C_1-C_6). Notice the elevated concentrations of organic solvents.



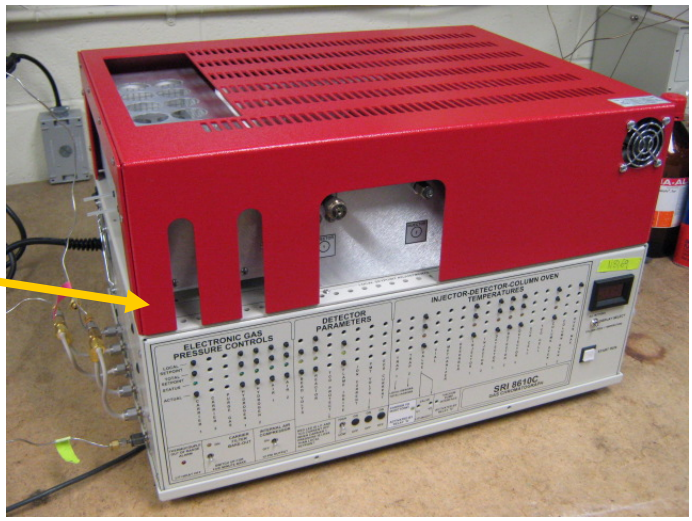
Sour OG concentrate spiked with organic solvents (C_1-C_6).



Real world medical cannabis samples will always contain some concentration of organic solvents (plant matter gives off trace amounts of ethane, methane and other gases as it slowly decays), so the presence of minute quantities of these gases should not be alarming. As the operator gains experience running samples they will be more qualified to determine what acceptable and unacceptable levels of these compounds are.

Medical Cannabis Pesticide Screening using the SRI 8610C GC

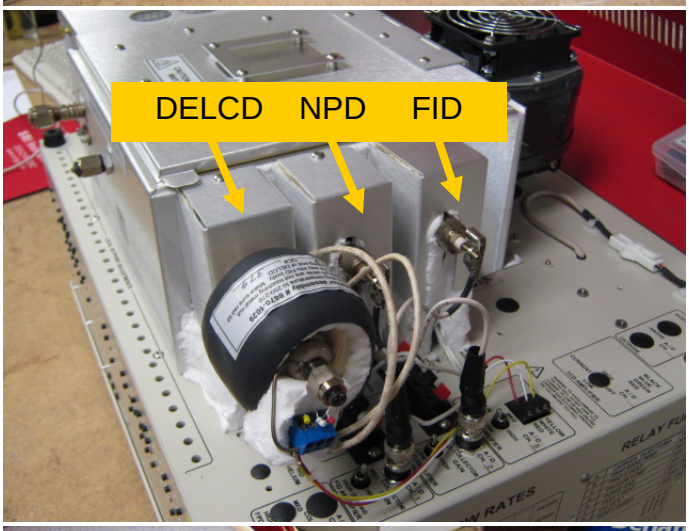
The SRI 8610C Gas Chromato-graph (GC) configured for Medical Cannabis Potency and Pesticide testing is shown at right.



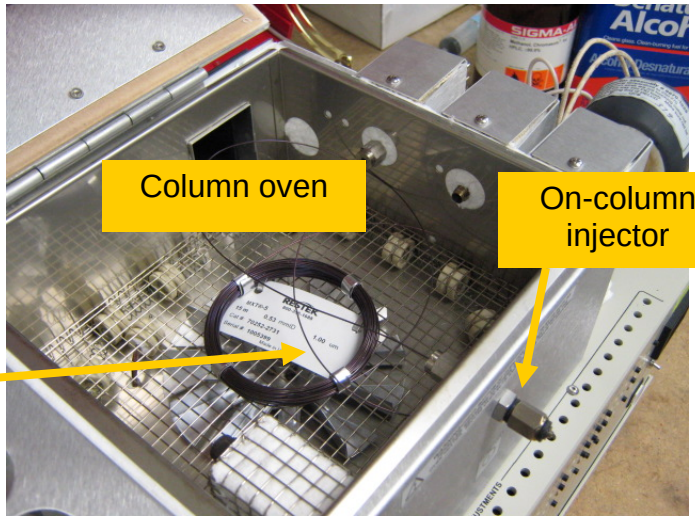
The GC is equipped with three detectors:

FID (flame ionization detector)
NPD (nitrogen/phosphorus)
DELCD (dry electrolytic conductivity)

Refer to the GC manual or pdf documents on the SRI website www.srigc.com for specific instructions on the detectors.



This GC can be used for potency testing only by using the on-column injector and the FID detector. In this case only a single column is re-quired in the column oven.

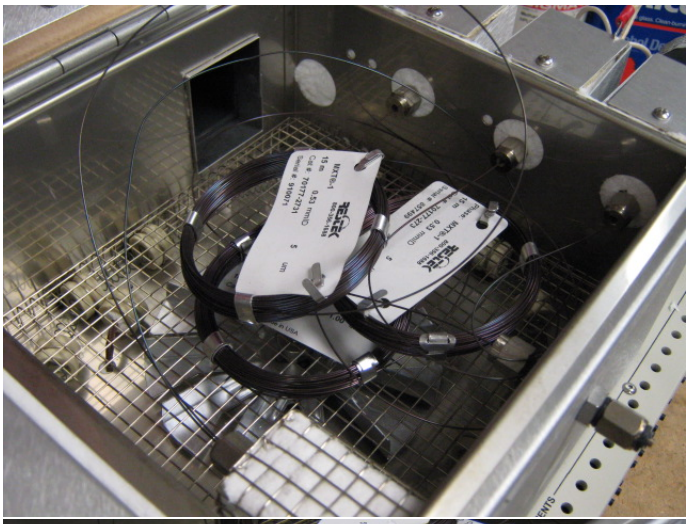


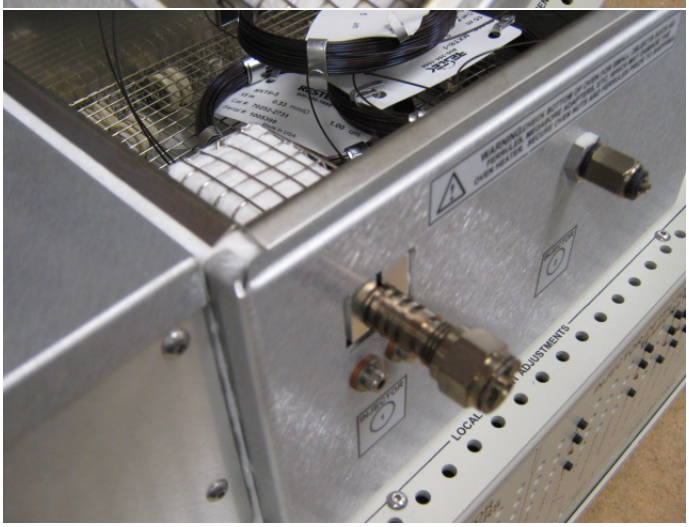
Medical Cannabis Pesticide Screening using the SRI 8610C GC

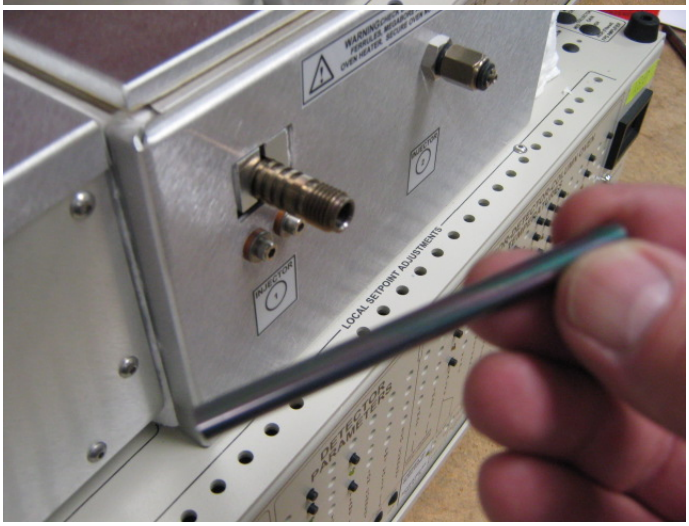
Two additional columns can be connected to the Heated Injector. One of these columns goes to the NPD detector and the other to the DELCD detector. The Heated Injector splits the sample onto the two columns using a two hole ferrule Restek part# 20246

The Heated Injector and on column injector are side by side on the front of the GC's column oven.

The Heated Injector includes a removeable quartz lined stainless steel tube. Cannabis samples (100 milligrams) are inserted into the tube and then into the Heated Injector which at 200C thermally desorbs pesticides off the cannabis and onto the two columns.



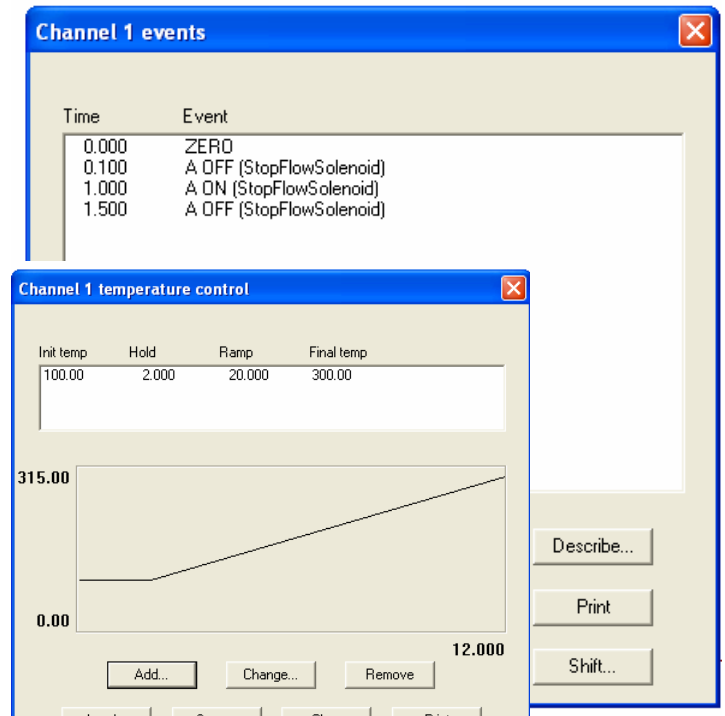


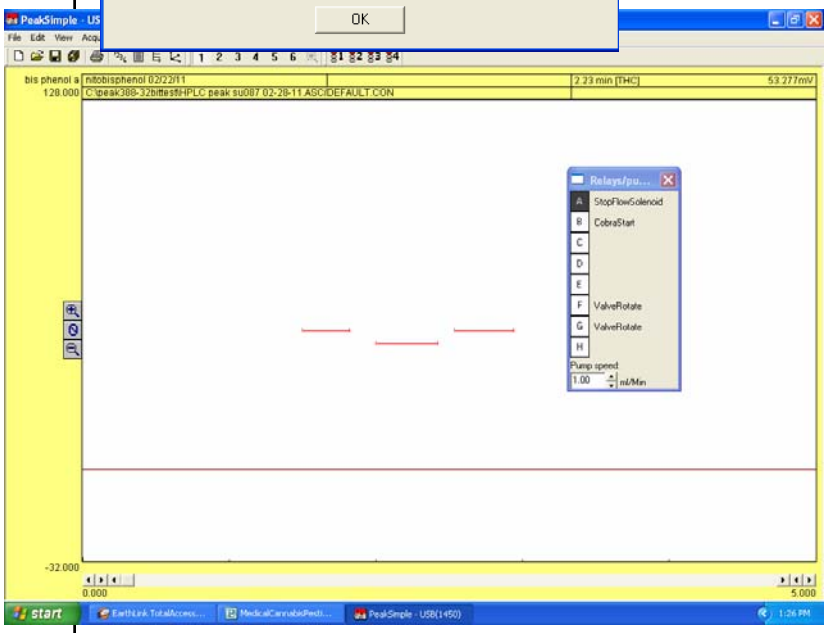


Medical Cannabis Pesticide Screening using the SRI 8610C GC

Edit the Event table in Channel 1 of the PeakSimple software to turn the carrier gas to the Heated Injector on and off at the times shown.

Enter the temperature program shown at right. The column oven starts at 100C for two minutes, then ramps at 20 degrees per minute to 300C.





Manually actuate Relay A prior to the start of the analysis. Display the Pump/Relay window and click the A button to actuate Relay A. When it is actuated, Relay A turns the carrier gas flow to the heated injector off.

Medical Cannabis Pesticide Screening using the SRI 8610C GC

Take a common cotton ball and make a small wad about the size shown.

Use a screwdriver or other tool to push the cotton wad about halfway down the tube.

Place the tube on the balance and then ‘tare” the balance to make it read 0.000 grams

Medical Cannabis Pesticide Screening using the SRI 8610C GC

Manicure the cannabis sample and scoop 100milligrams (.1gram) into the tube.

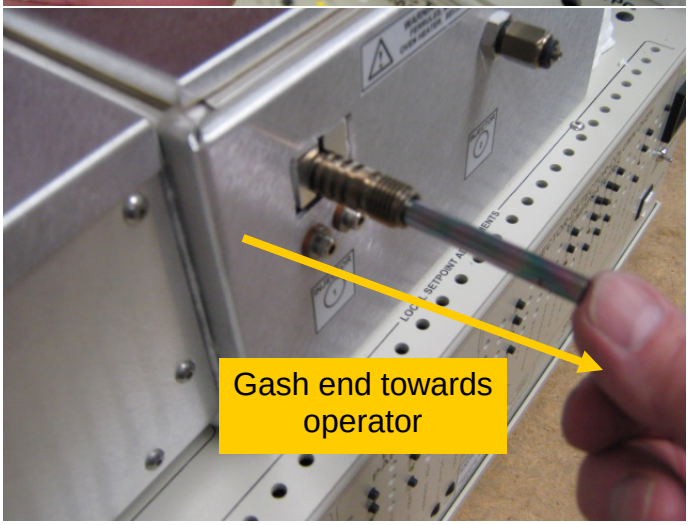
Weigh the tube until you get approximately 100 milligrams. You do not have to get exactly 100 so long as you are close (95-105 mg). The photo are right shows the weight at 99 milligrams. You can correct for the actual sample weight in the PeakSimple software after the analysis.

Stuff a little more cotton into the tube to hold the cannabis sample in place. Do not pack the cotton and cannabis tightly. The cotton should just be tight enough to prevent the cannabis from escaping the tube. The cannabis should be loose, NOT packed down.

Medical Cannabis Pesticide Screening using the SRI 8610C GC

Since the injector is HOT, use a tool like a 9/16” socket to remove the septum nut.

Insert the tube filled with cannabis into the injector. At this time the carrier gas is off so no gas will escape while you are inserting the tube.



The tube has a gash at one end.

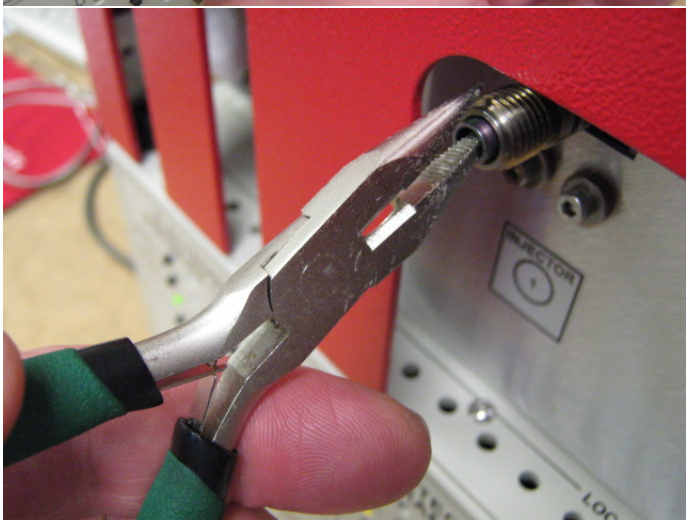
<u>The gash end MUST be towards the operator.</u>

Medical Cannabis Pesticide Screening using the SRI 8610C GC

Start the analysis by pushing the start button on the GC. You can also push the spacebar on the computer keyboard. The Event table in PeakSimple will de-actuate Relay A at .1 minutes into the analysis which will cause the carrier gas to strip the pesticides from the now HOT cannabis and deposit the pesticide molecules on the two columns.

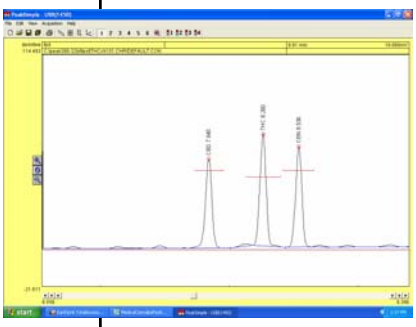
If your GC is equipped with a second injector and FID detector for potency measurement, you can inject the potency extract in the other injector anytime in the first 1 minute of the analysis.

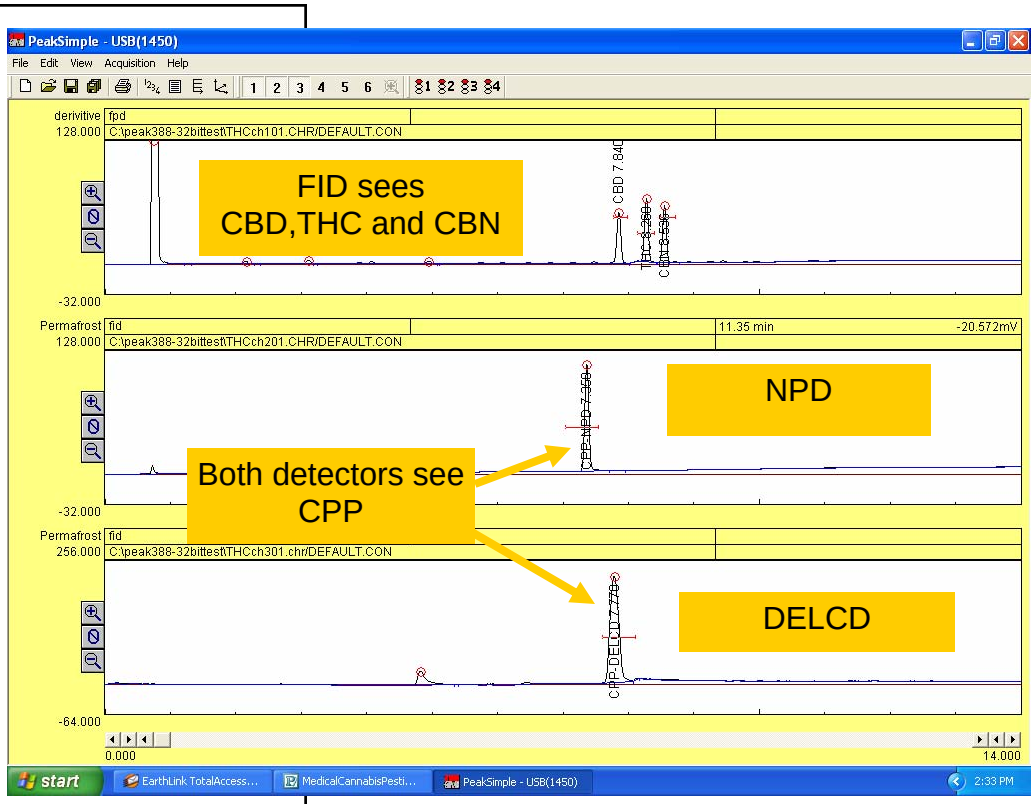
At 1 minute into the analysis, the carrier gas is turned off for 30 seconds. During that 30 second period remove the tube from the HOT injector using a tool to avoid burning your fingers. Place the HOT tube in a beaker to cool off. You must replace the septum nut within the 30 second window.



Medical Cannabis Pesticide Screening using the SRI 8610C GC

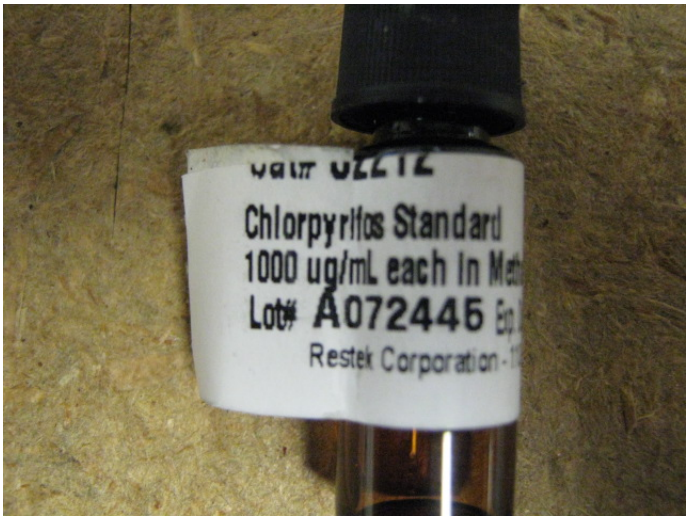
To calibrate the Potency channel (channel 1), inject 1ul of the 333ng/ul calibration mixture into the on-column injector. You should see three equal size peaks.





Preparation of the 333ng/ul working standard is described in another publication.

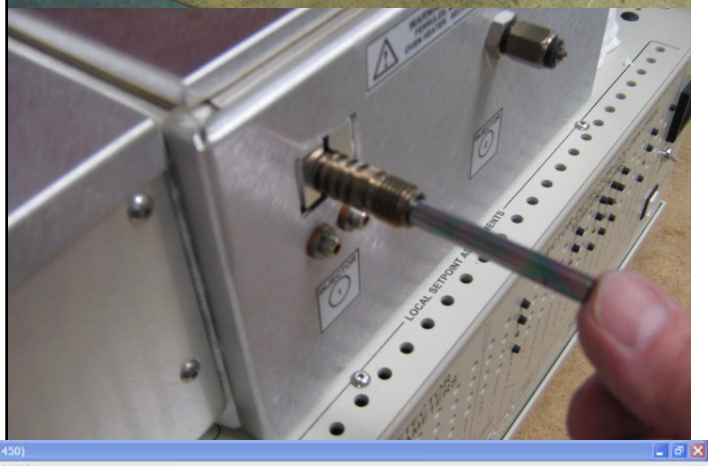
The two pesticide detectors (NPD and DELCD) are calibrated with a pesticide standard such as Chlorpyrifos. Restek part# 32212 is 1000ug/ml (1000ppm) of chlorpyrifos (CPP) in methanol. CPP was chosen as the calibration pesticide because it has both phosphorus (which the NPD detects) and chlorine (which the DELCD detects). So the one pesticide can be used to calibrate both detectors.



Medical Cannabis Pesticide Screening using the SRI 8610C GC

Deposit 1ul of the CPP standard on a clean cotton wad in the tube.

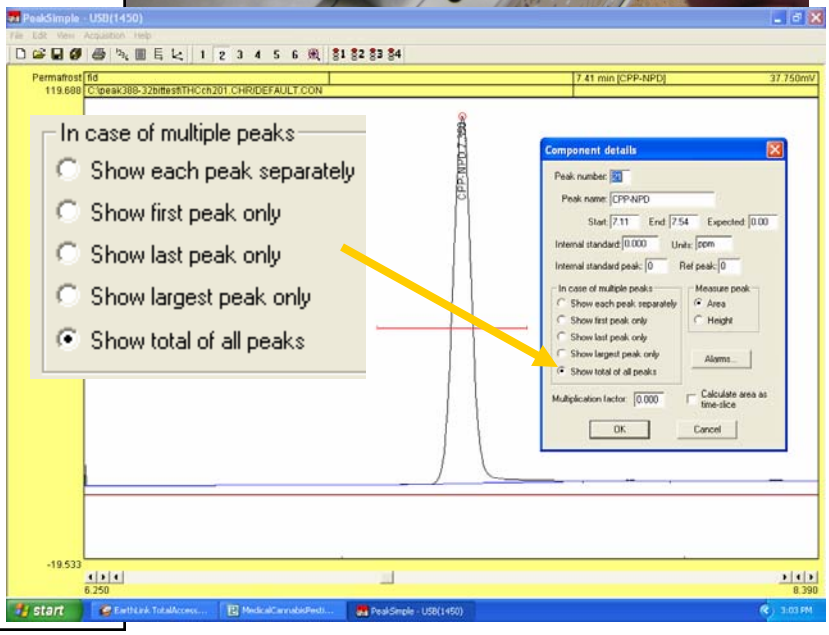
Then desorb using the standard program and events.



There should be a single peak on the NPD and DELCD channels.

Create a retention window for the CPP peak in the NPD channel and another similar retention window in the DELCD channel.

Notice that the retention window has
“Show total of all peaks” selected



Medical Cannabis Pesticide Screening using the SRI 8610C GC

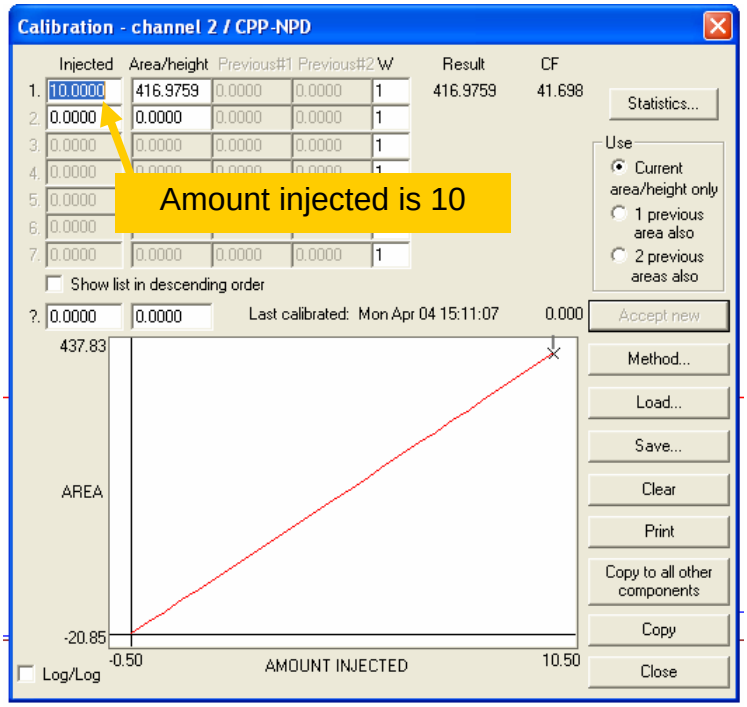
Create a calibration curve for the CPP in both NPD and DELCD channels. Note that the amount injected is set to 10.

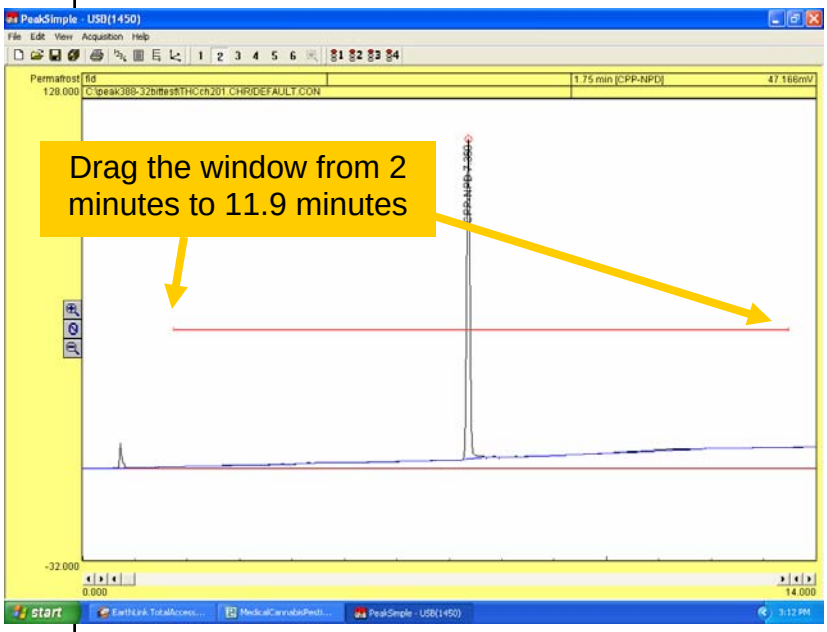
We injected 1ul of CPP standard which contains 1000 nanograms of CPP. Since we will be desorbing 100milligrams of cannabis, 1000 nanograms is 10ppm, hence the number 10 in the amount injected column.

Drag the retention window across the entire screen except for the first 2 minutes. This will have the effect of adding up all the peaks detected during the analysis and applying the CPP calibration to the total of the peaks, regardless of whether a particular peak is CPP or another pesticide.

Unlike the potency analysis where the results are reported in Percent, the pesticide results are reported in ppm (parts per million) because the concentration should be very low.

1,000,000 ppm =100%
100,000ppm=10%
10,000ppm=1%
1000ppm=.1%
100ppm=.01%
10ppm=.001%
1ppm=.0001%

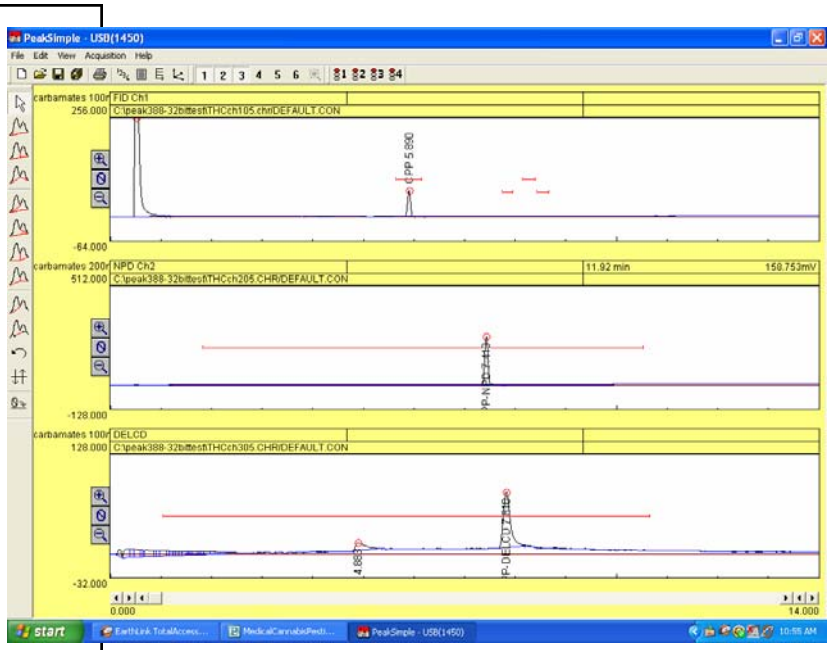




Medical Cannabis Pesticide Screening using the SRI 8610C GC

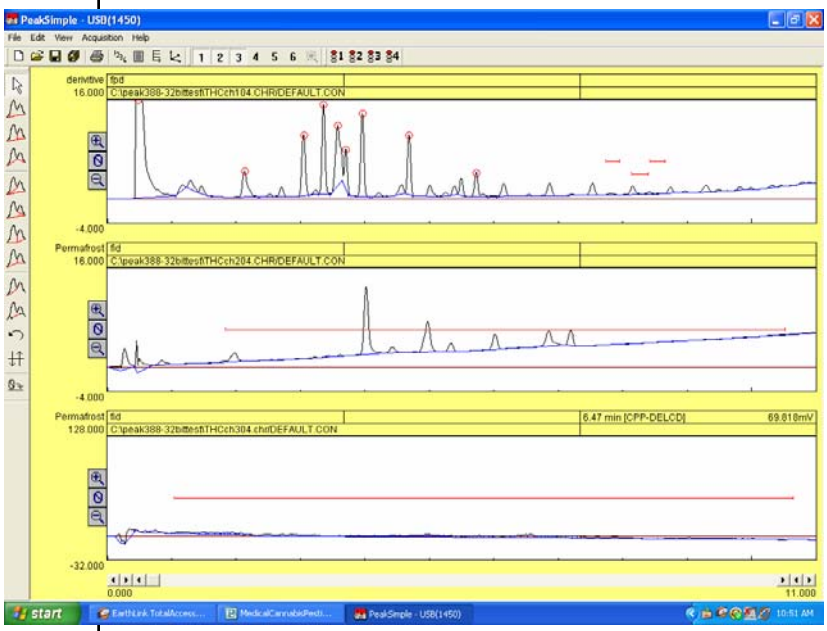
The chromatograms at right show the CPP peak on all three channels. The top channel (FID) was injected with the CPP standard just for comparison. Normally the FID channel is used for potency (CBD, THC, CBN).

The NPD (channel 2) and the DELCD (channel 3) show the CPP standard desorbed from the desorber tube.



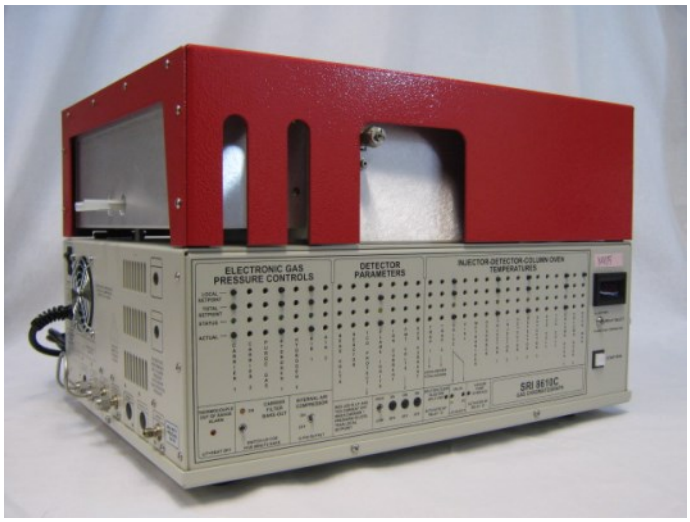
The chromatograms to the right show carbamate pesticides.

You can see the NPD responds but the DELCD does not. Since the carbamates do not have chlorine this makes sense.

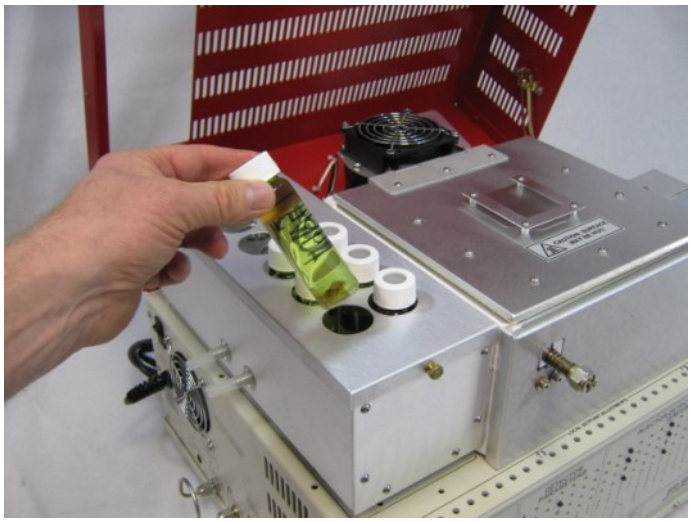


Medical Cannabis Potency Testing using the SRI 8610C FID GC

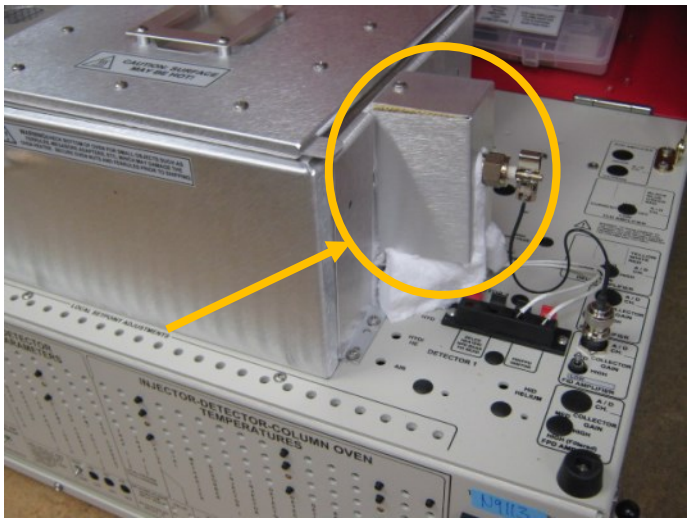
The SRI 8610C FID GC with a 12-vial sample heater is designed for testing the **potency of medical cannabis (cannabinoids)**. With minor configuration and procedural changes the GC can also test for **terpenes** and **residual solvents** in concentrates (see our documents on our website at www.srigc.com/documents.htm).



The 12-vial sample heater aids in a quicker extraction of the cannabinoids in solvent and maintains the extracted samples at 50° C for better reproducibility.

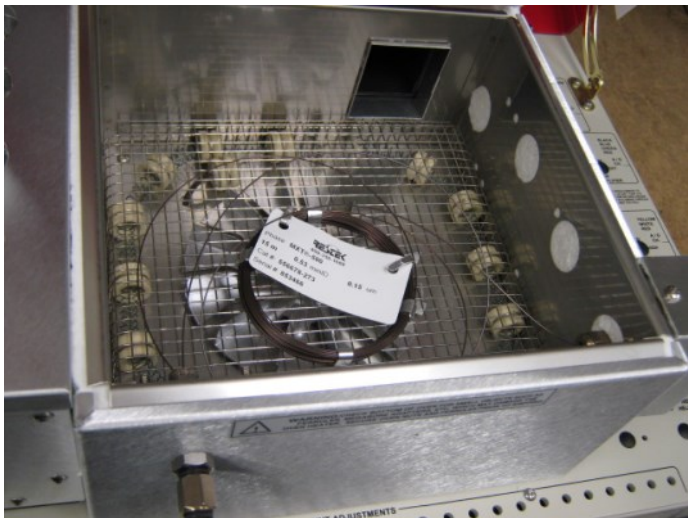


The GC includes SRI's Flame Ionization Detector (FID) which is able to measure the cannabinoid molecules based on its ability to detect the combustion of hydrocarbon molecules.

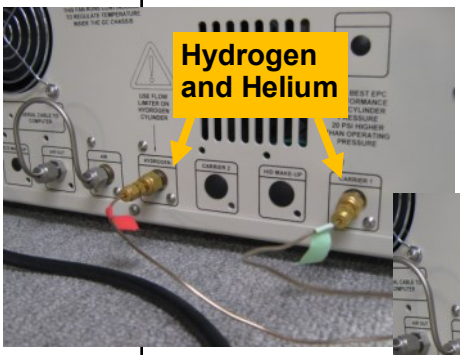


Medical Cannabis Potency Testing using the SRI 8610C FID GC

The cannabinoid molecules, Δ9-THC, CBD, and CBN (and for more advanced operators, CBC, Δ8-THC, and CBG) are separated by a 15-meter metal capillary column which is heated in the column oven.



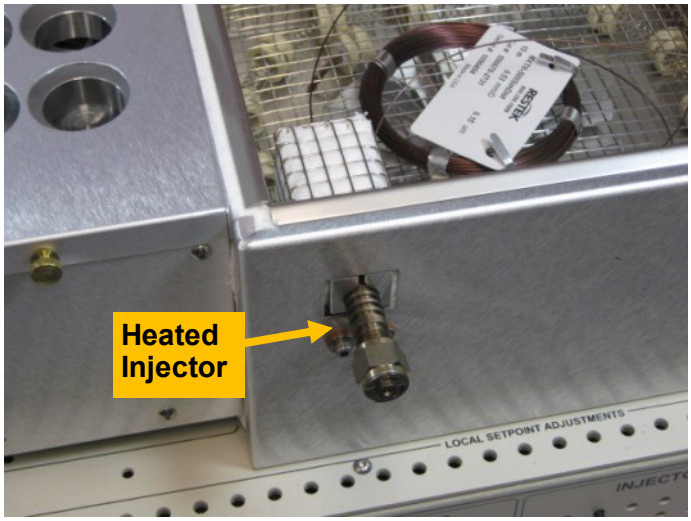
Hook up the gas lines to the left side of the GC. The GC can be operated with hydrogen or helium as a carrier gas. When using hydrogen as a carrier gas, cap off the hydrogen gas inlet and connect the hydrogen to the "Carrier 1" inlet.



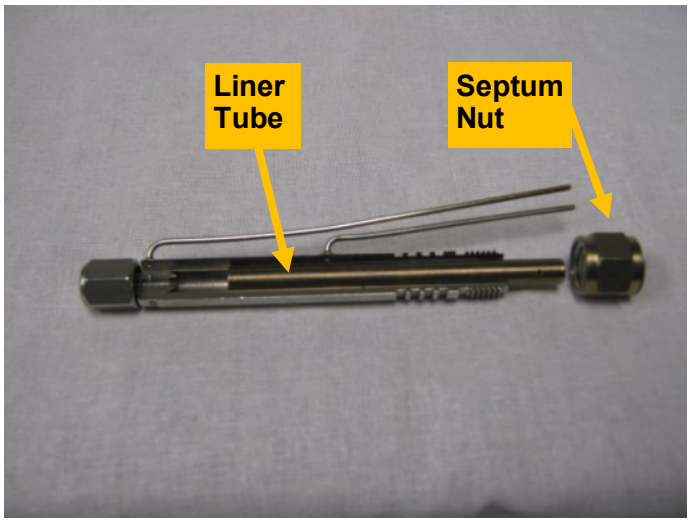
The entire GC plugs into any Windows computer using a USB cable.

Medical Cannabis Potency Testing using the SRI 8610C FID GC

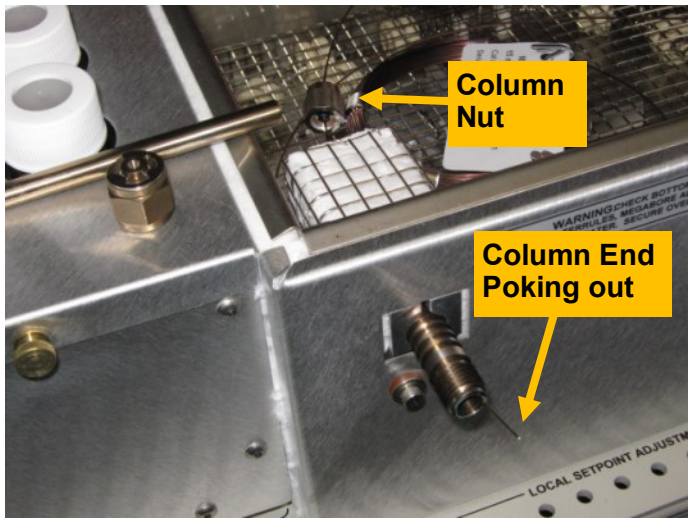
The Heated Injector completely de-carboxylates the cannabinoid acids before they enter the GC column. This provides for sharper peaks and more accurate results. The Heated Injector temperature should be **set to 250 C** using the front panel controls of the GC under “Injector 1”.



Here is a cut-away view of the inside of the heated injector with the septum nut unscrewed and the liner tube slid part-way out.

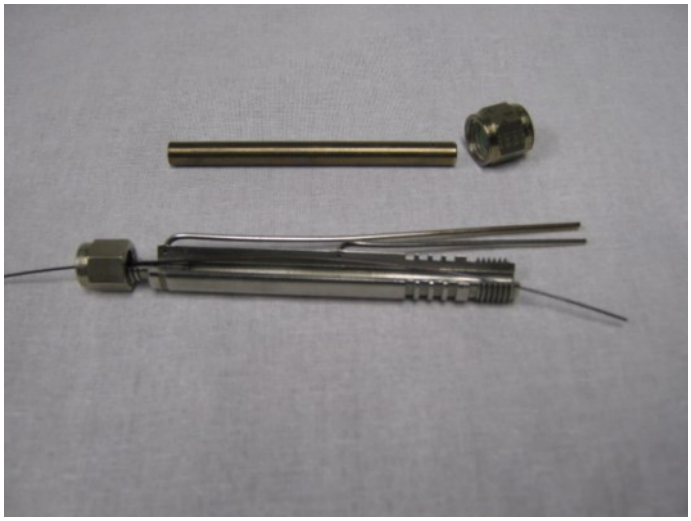


Unscrew the septum nut on the front end of the heated injector and then remove the liner. Loosen the 1/8” column nut securing the capillary column to the heated injector so that the end of the column can be slid all the way out through the end of the column. You may want to take this opportunity to trim 1/2” off the end of the column.



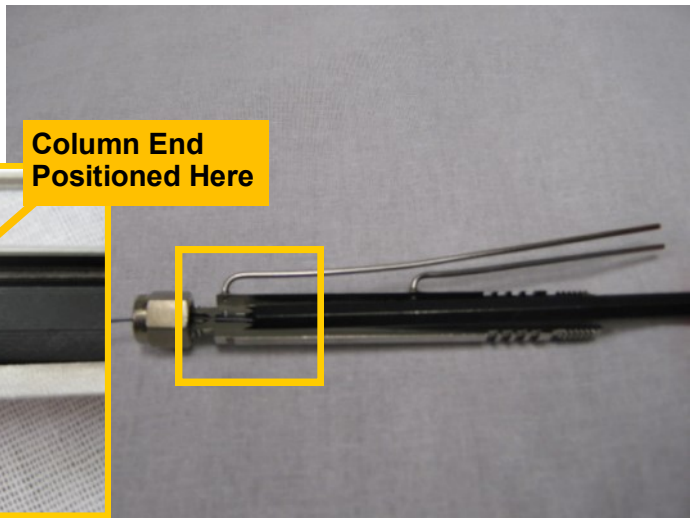
Medical Cannabis Potency Testing using the SRI 8610C FID GC

Here is a cut-away view of the column slid all the way through the heated injector. Notice how the liner tube has been taken out.



For best results, the column should be placed just at the back of the heated injector. Use something long and skinny enough to *just* fit into the heated injector (a 7/32" hex wrench works well) in order to gently push the end of the column to the back of the heated injector. Make sure that the tool pushes the column all the way into the proper position, as it is easy for the column to slip past the tool and not be positioned properly.

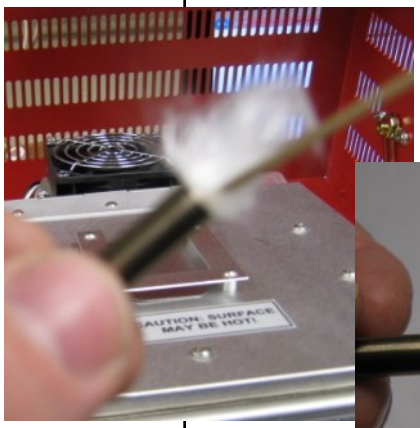
Once the end of the column is placed properly, tighten the 1/8" nut securing the column to the heated injector. Make sure to tighten the nut securely, but not so much that the soft graphite ferrule is smashed.



Medical Cannabis Potency Testing using the SRI 8610C FID GC

Peak shape and results are best when deactivated wool is inserted into the liner. Inside the injector, the sample is injected into the wool, where it is then thermally desorbed (heated off) before it enters the column. The wool should be re-packed if you suspect contamination and/or when you replace the septum. Under standard operating conditions, the wool could last indefinitely. Deactivated wool can be obtained from Restek (P/N 24324).

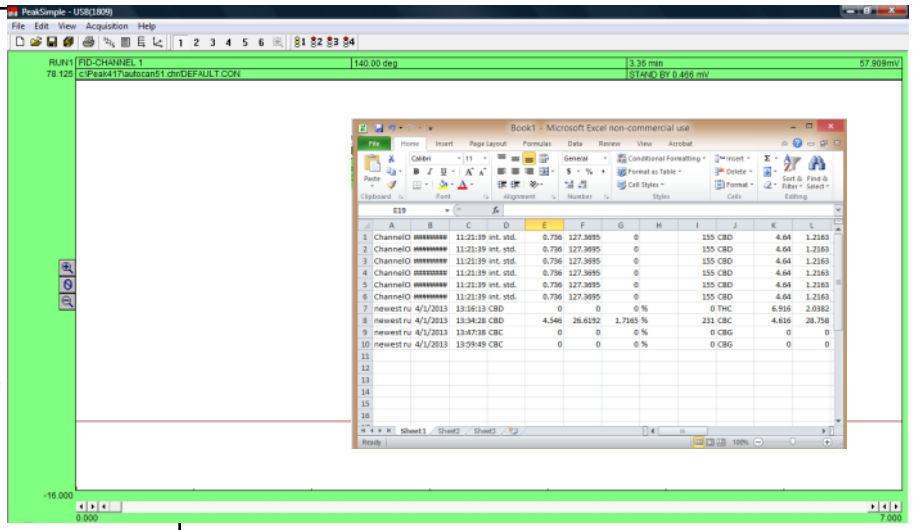
Take a finger-sized pinch and twist it several times to make it easier to stuff into the liner. Use something skinny like a small file or a piece of tubing to insert the plug of wool into the tube. Ideally, the center of the plug is positioned where the syringe will deposit the sample in the heated injector. To see if your plug is positioned properly insert the syringe into the tube (**into the end with the hole, this is very important)** until it is about 1/4" away from being completely in. You should feel the syringe tip enter and remain in the plug of wool.



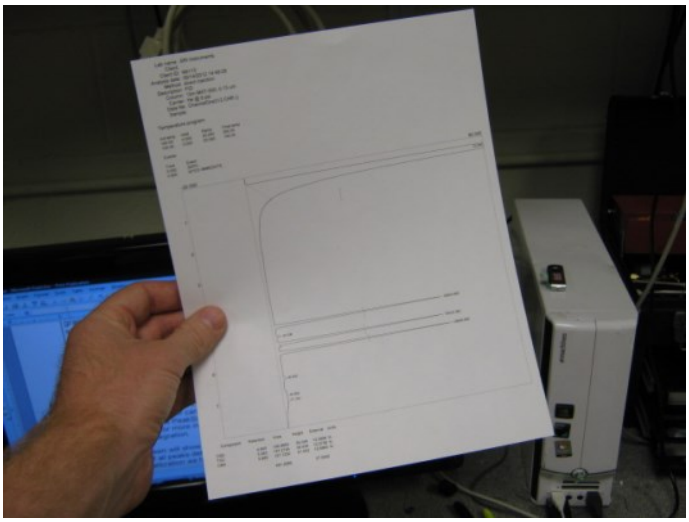
Once the column is positioned properly and the wool is inserted in the liner, replace the tube in the injector (**with the hole end facing out**) and screw on the septum nut. Don't screw on the septum nut too tight, as it will tear up the septum. Finger tight is best.

Medical Cannabis Potency Testing using the SRI 8610C FID GC

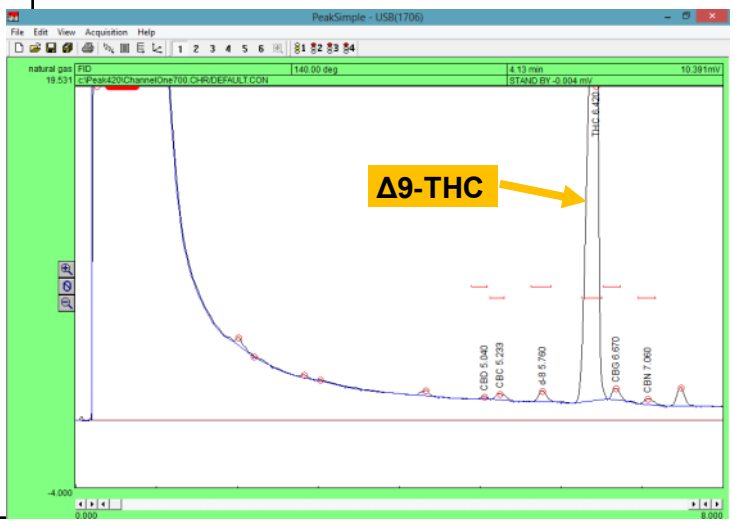
SRI's PeakSimple software is included with the GC. PeakSimple software collects the GC data and generates a calibrated result which can be printed or transferred to other programs such as Excel or Word. The latest version of the software can always be downloaded for free at www.srigc.com.



The chromatogram hardcopy printout at right shows the three peaks, CBD, Δ9-THC and CBN, which were injected to calibrate the GC.

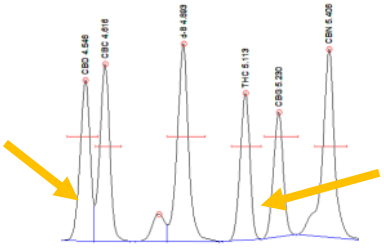


An actual cannabis sample is shown at right. Note that only the Δ9-THC peak is large, the other cannabinoids are much smaller. This is what you would expect for the majority of actual cannabis samples.



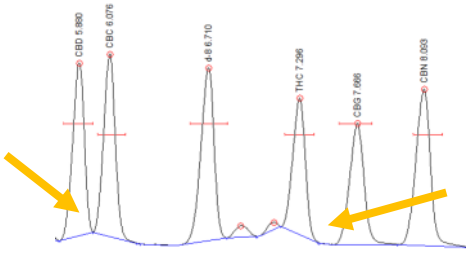
Medical Cannabis Potency Testing using the SRI 8610C FID GC

For a quick 8-minute analysis that optimizes speed *and* peak separation, set the column oven temperature as shown to the right.



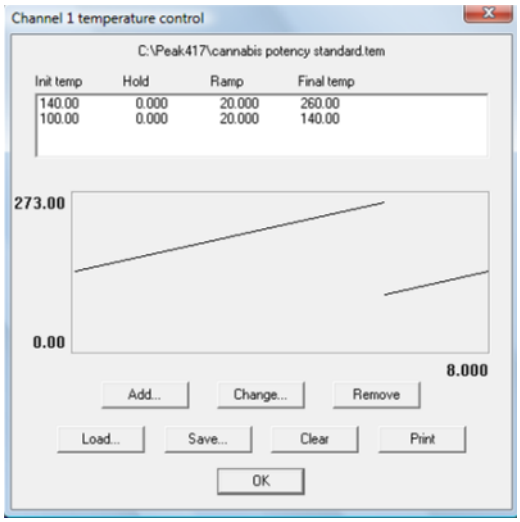
8-min Run: Note the closeness of the CBD and CBC peaks, as well as THC and CBG.

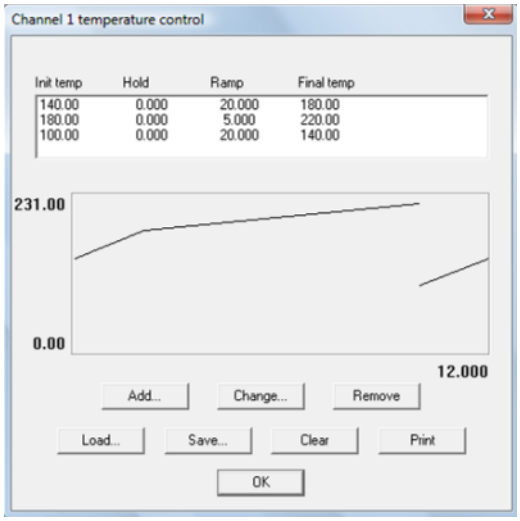
Sometimes, a better separation is preferred at the expense of speed. For a longer 12-minute analysis, set the column oven temperature as shown to the right.

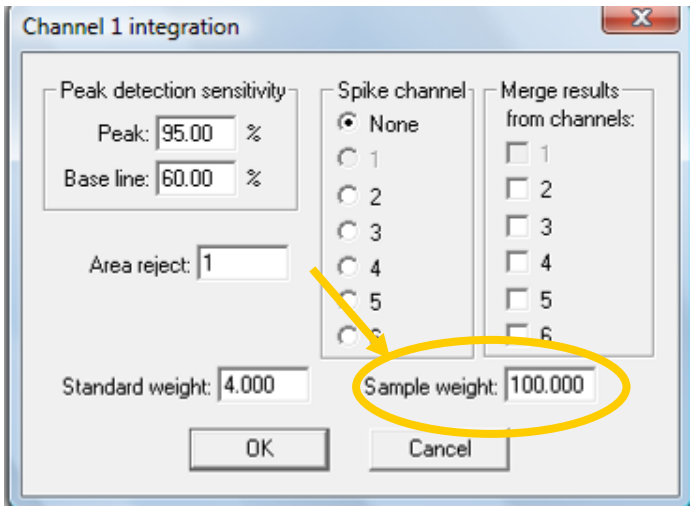


12-min Run: Note the baseline separation of CBD and CBC and the distance between THC and CBG.

Set the integration parameters as shown. Note the "Sample weight" box. When you calibrate the GC it will be set at 100. When you run actual cannabis samples, the weight of the sample will be entered. (ex. If the sample weighed 0.104 grams, then "104" should be entered).





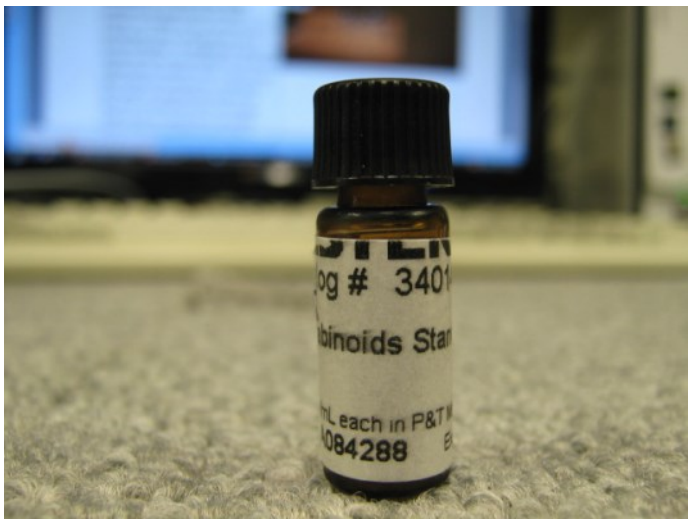


Medical Cannabis Potency Testing using the SRI 8610C FID GC

Obtain the cannabinoid calibration standard from a chromatography supplier like Restek (restek.com). The standards can be acquired individually, but SRI recommends a more convenient three-way (THC, CBD, CBN) cannabinoid standard. The standards are available at a concentration of 1000 ng/ul in Methanol. No license is required to purchase.

Break the glass ampoule and transfer the contents into a 2mL septum vial. Restek provides one free vial with each standard.

Whether you have three vials (individual standards of THC, CBD, or CBN) or one vial of 3-way standard, they will each be at a concentration of 1000ng/ul. We will refer to these standards as primary standards. Ideally, when not in use they should be kept in a refrigerator with an un-pierced septum so that the methanol will not evaporate and increase the concentration of the cannabinoids in the standard. When calibrating with the primary standards the percent concentration of the cannabinoids will be 40%.

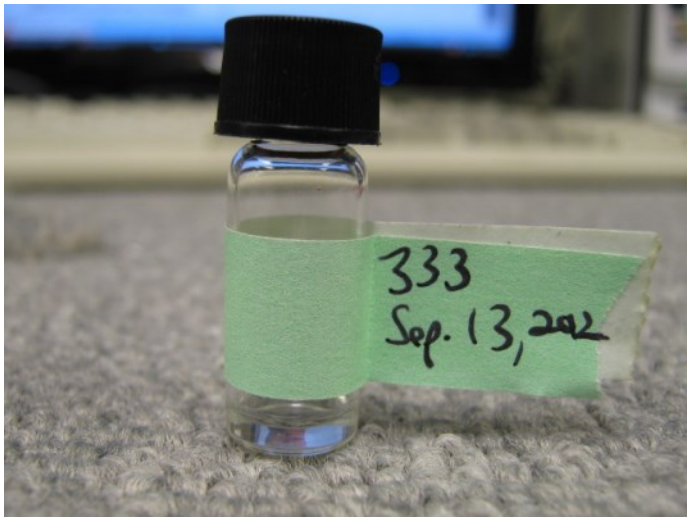


Medical Cannabis Potency Testing using the SRI 8610C FID GC

SRI recommends preparing a “333 working standard” rather than using a primary standard to calibrate. Not only will this help to preserve the purity of your primary standard and get more mileage out of it, but it will also calibrate the GC at percent concentrations that more closely resemble cannabis flowers (13.32% instead of 40%).

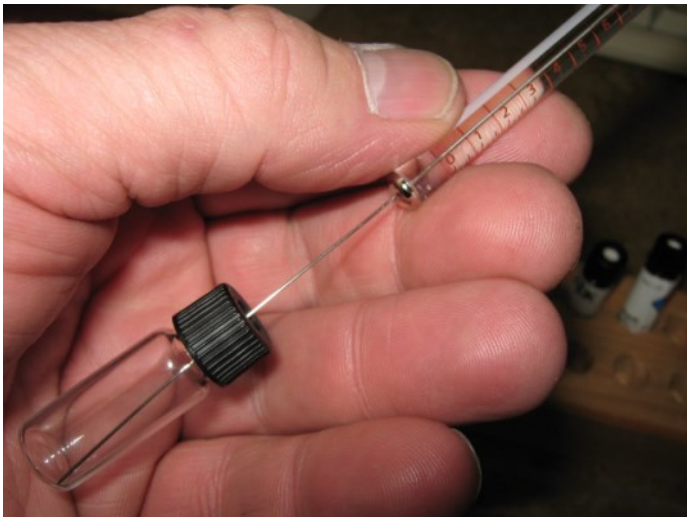
If you have separate cannabinoid standards, use the 100uL syringe, which is included with the SRI GC, (Restek#24863) to transfer 100uL of each 1000ng/ul (primary) standard into another 2mL vial. If you have the 3-way standard, use the 100uL syringe to transfer 100uL of the standard into another 2mL vial (or smaller) and then add 200uL of methanol.

After either method, you will end up with 300uL of working standard containing 333ng/ul each of the three compounds (CBD,THC, and CBN). Label the primary and working standards with both a name and a date.

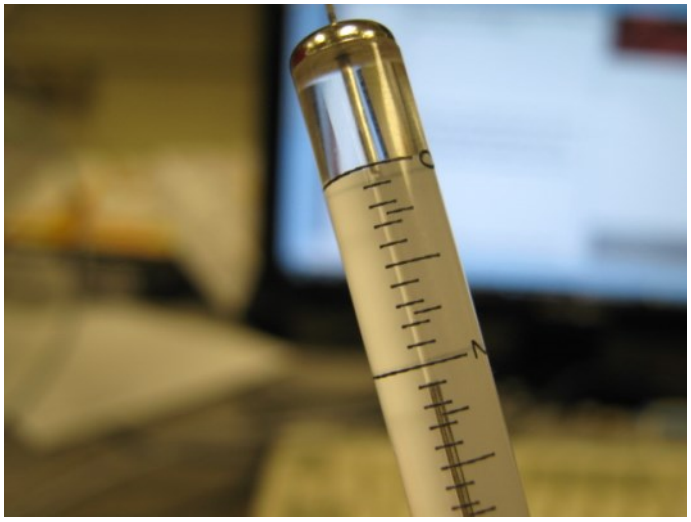


Medical Cannabis Potency Testing using the SRI 8610C FID GC

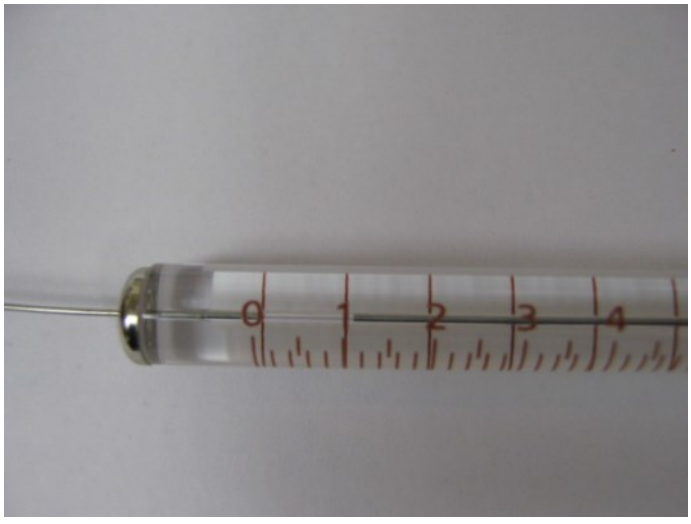
Rinse the syringe first then use the 10ul syringe delivered with the GC (SRI #8670-9550) to withdraw 2-3ul of the working standard. Puncture the septum rather than open the vial to avoid letting the methanol solvent evaporate each time the vial is opened. Pump the plunger several times to get rid of air bubbles.



With 2-3ul of liquid in the syringe, hold the needle vertically or at least slanted upwards so any air bubbles will rise towards the needle.



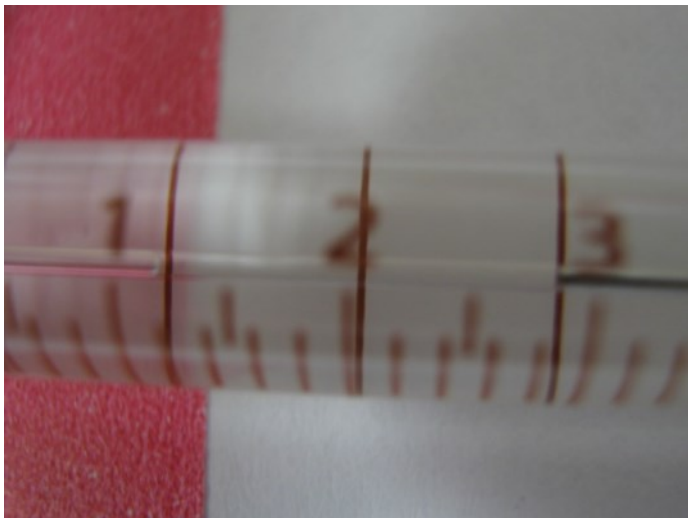
With air bubbles removed, push the plunger to the 1ul mark. It is important to be as precise as possible. Wipe the needle with your fingers or a tissue to remove any liquid from the outside of the needle.



Medical Cannabis Potency Testing using the SRI 8610C FID GC

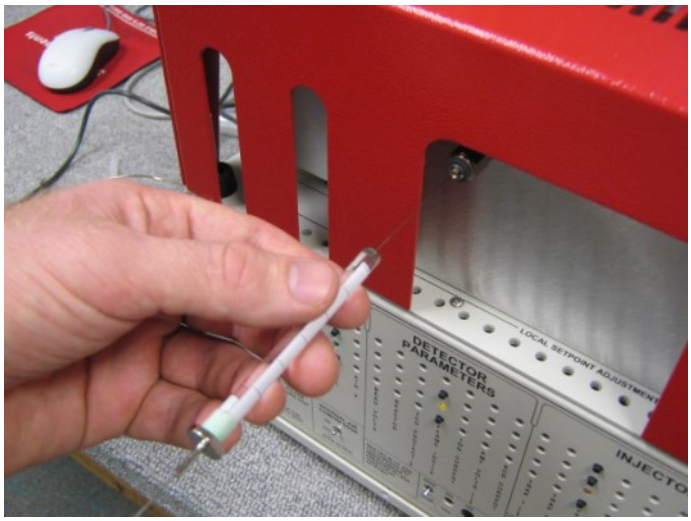
Pull the plunger back to the 3ul mark and note the amount of liquid. It should be 1.6-1.8ul because the needle also contains 0.6 - 0.8ul and this adds to the 1ul you measured with the plunger.

Leave the plunger at the 3ul mark.

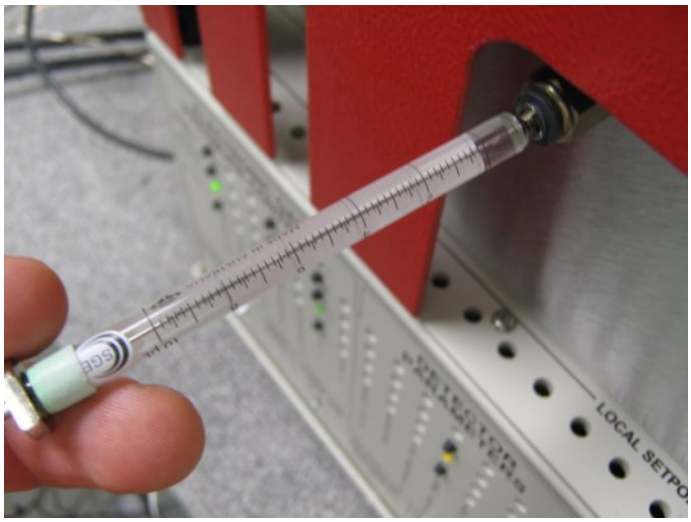


With the plunger still at the 3ul mark, place the needle up against the septum (but not poking through it yet).

Press the Start Run button or hit the Spacebar on the keyboard to start the run.

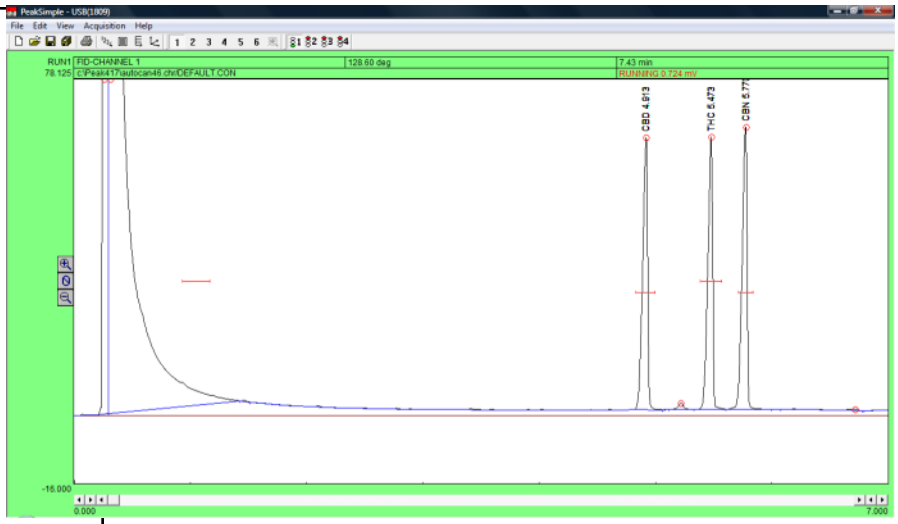


Insert the syringe all the way through the septum as far as it will go. Immediately depress the plunger. Twist the syringe one half turn (to wipe off any liquid on the tip of the needle) and then withdraw the syringe.

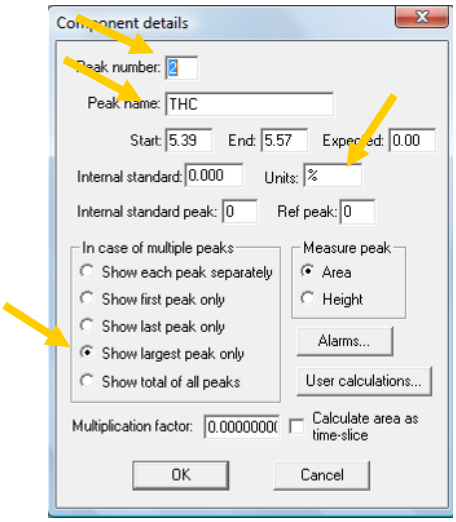


Medical Cannabis Potency Testing using the SRI 8610C FID GC

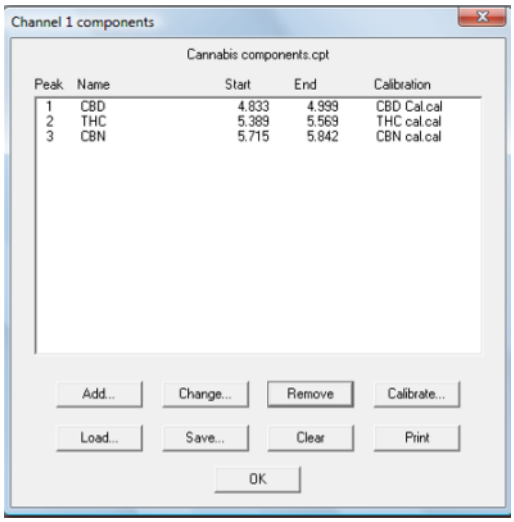
Once the run is completed you should see a large solvent peak near the beginning, then closer to the end, three peaks of roughly equal size (there will also probably be a small Delta-8 THC peak between the 1st and 2nd peak). Add retention windows to the three peaks by right clicking on the peak and selecting "Add component". See the PeakSimple tutorial describing the process of creating retention windows.



Identify the three peaks (from left to right: CBD, THC, CBN) by right-clicking on each peak and selecting "Edit component". Assign each peak a unique number and name (CBD, THC, or CBN), select "show largest peak only", and add a "%" sign to the "Units" box. Press the "OK" button to exit back to the main chromatogram screen.



Right click on the chromatogram and select "Components" to open the "Channel 1 Components" Screen. Here will be displayed a list of all the components with named retention windows and unique peak numbers. Select "Save" and name the component file so that if you exit PeakSimple your component and calibration files will not be lost.

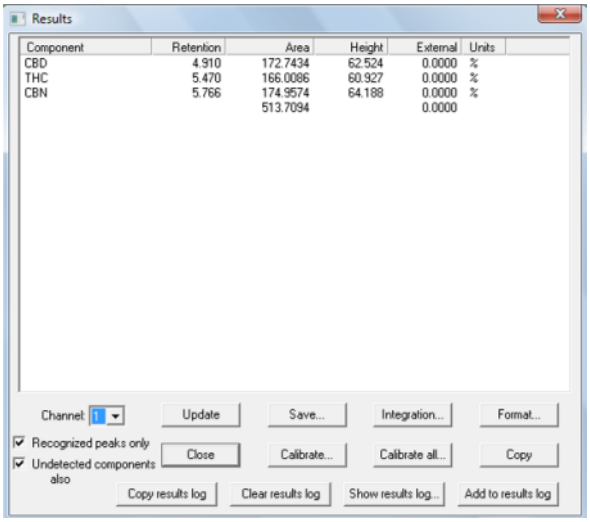


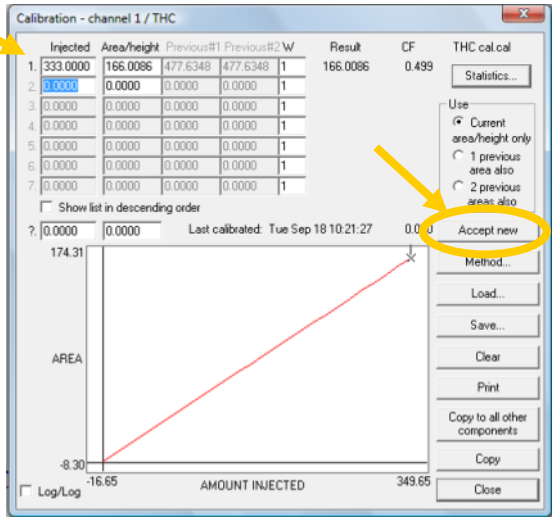
Medical Cannabis Potency Testing using the SRI 8610C FID GC

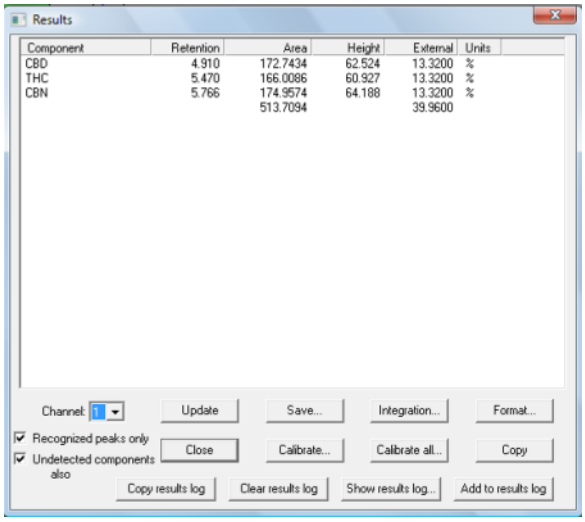
Check the Results screen. With the FID Gain switch on “MED”, when you inject a primary standard the area counts for the cannabinoids should be between 420 and 540 and roughly equal to each other (+/- 30). If you inject the working standard the area counts should be between 140 and 180 and roughly equal (+/- 10). If you operate the FID Gain switch on “HIGH” (not recommended because with concentrates cannabinoid levels will go off scale) the area counts will be about 20 times greater.

Calibrate each peak by creating a calibration curve. See the PeakSimple tutorial describing this process. In the calibration curve enter the amount of standard you just injected. This will be 333 (for 333ng/ul) or 1000 (for 1000ng/ul). Type this number in the top left cell of the spreadsheet in the calibration curve. Then click the Accept New button to transfer the peak’s area into the top row 2nd column. Save the curve under some name. Do this for all the peaks.

Navigate to the View/Results screen to see the report. With the integration screen and components setup as discussed earlier in the document the percent concentrations of CBD, THC, and CBN will each be displayed as 13.32% (or 40% if primary standards were injected). You are now calibrated and ready to inject real cannabis samples.



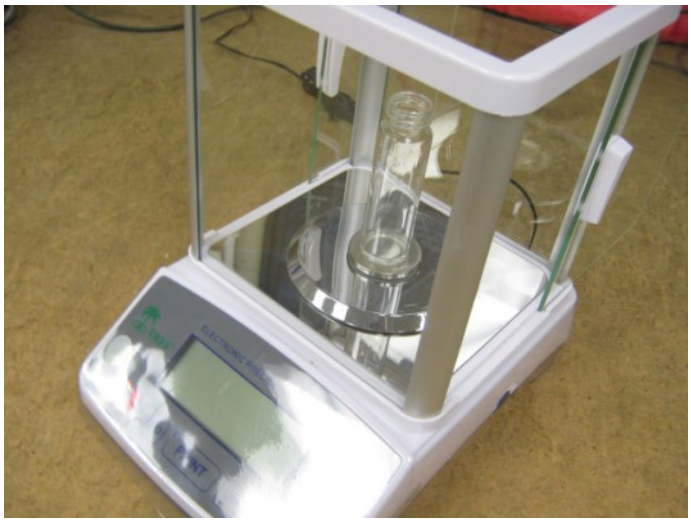




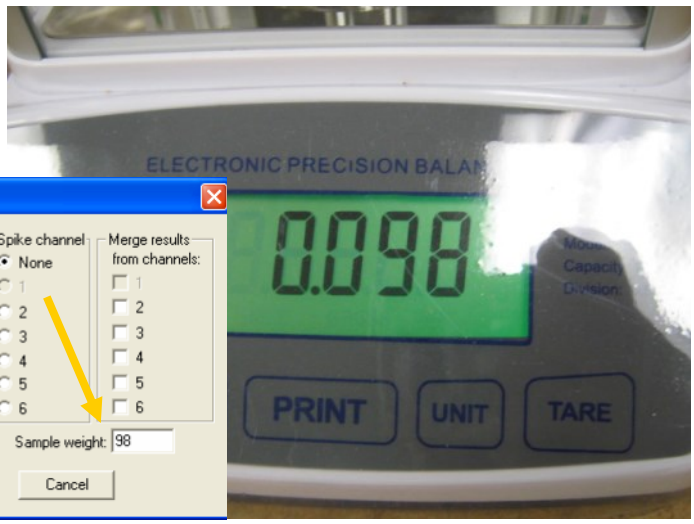
Medical Cannabis Potency Testing using the SRI 8610C FID GC

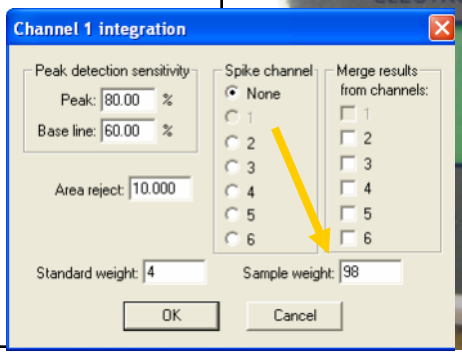
Remove the cap from a 40ml vial and place it on the balance. The balance should be capable of reading 1 milligram (0.001 gram). A balance like this can be purchased brand new for less than $300 on E-bay.

With the 40 mL vial on the balance tare the reading (make the reading 0.000). Then carefully add 100 milligrams of manicured cannabis. Drop the bits of cannabis into the vial slowly until the reading is close to 100 milligrams. Make sure to write down the exact weight of the sample somewhere, preferably on the vial itself.



Don't worry if you are slightly under or above 100. In the photo at right, the reading is 98 milligrams which is close enough. You will enter the reading in the sample weight field in PeakSimple software which will mathematically correct the calculated answer to compensate for weights slightly above or below 100.



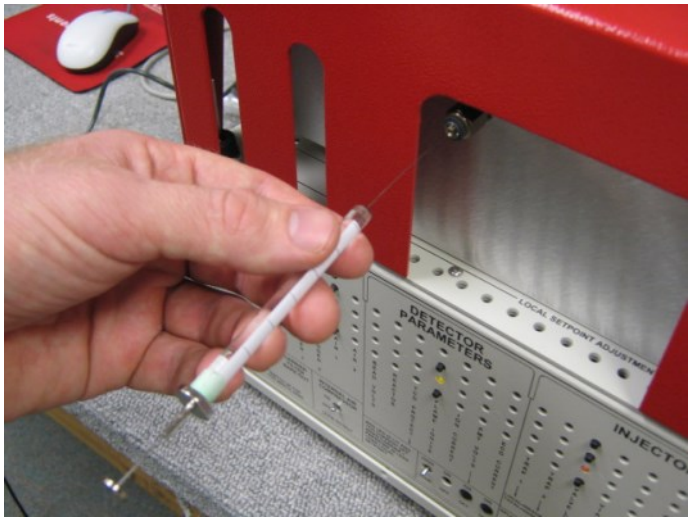


Medical Cannabis Potency Testing using the SRI 8610C FID GC

Remove the 40ml vial from the balance and fill it with 40ml of extraction solvent. You can use 70% or 91% IPA (isopropyl alcohol), methanol (methyl alcohol), ethanol, acetone, chloroform or other solvents. We recommend using either methanol, or for a cheap and efficient solvent, **denatured alcohol (a mixture of ethanol and methanol) that can be obtained at most hardware stores for less than $20 a gallon**. Non-polar solvents like hexane are not recommended because they do not extract the cannabinoids as well as polar solvents. IPA can also be a difficult solvent to work with.

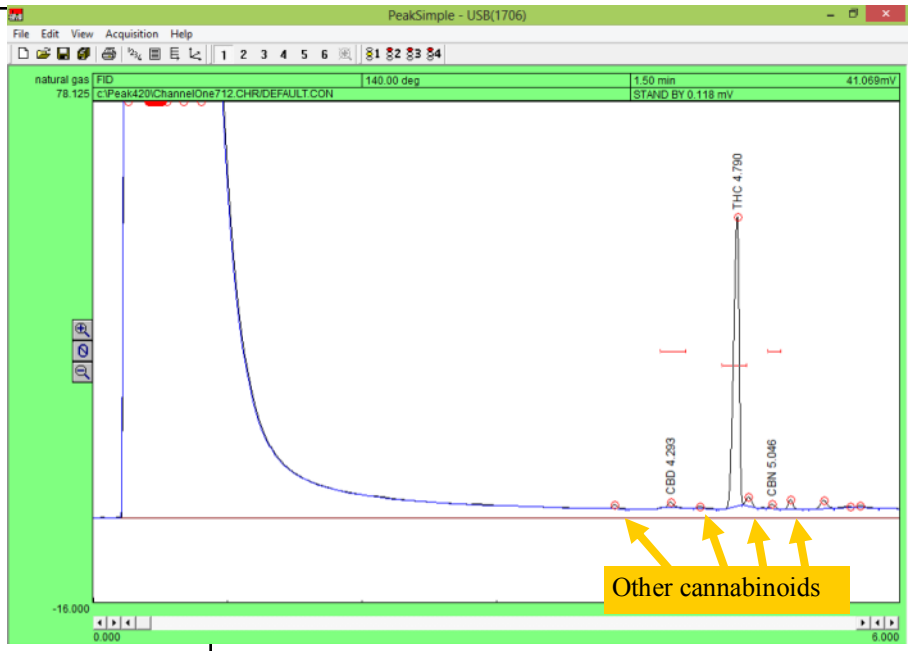
Shake the vial for a few seconds and then let it sit for about 20 minutes in the incubator (longer without heat).

Use the 10ul syringe which comes with the GC to inject 1ul of the extract as shown previously with the calibration standard. It is important to be very precise with the syringe since the overall accuracy of the test depends on this. Don't forget to enter the exact Sample weight in the proper field on the integration screen.



Medical Cannabis Potency Testing using the SRI 8610C FID GC

A real cannabis sample will look something like the chromatogram at right. There will be one big peak (THC) and much smaller ones for CBD and CBN.



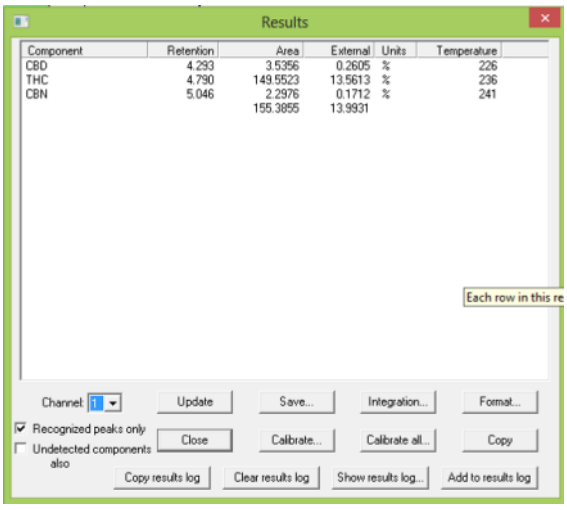
CBN may or may not be detected or it may blend into the much larger THC peak. When this happens you can use the slower temperature program and/or lower the carrier pressure to get better separation of the peaks.

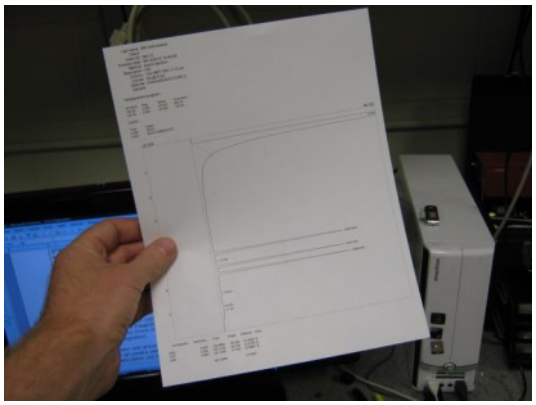
There may be other peaks which are not CBD, THC or CBN. These other peaks are cannabinoids (CBC, Delta-8 THC, CBG, and others) for which there may or may not be calibration standards available at this time.

It may be necessary to manually integrate some of the peaks for the most accurate quantification of cannabinoid potency. See the PeakSimple Advanced Tutorial for more information on manual Integration.

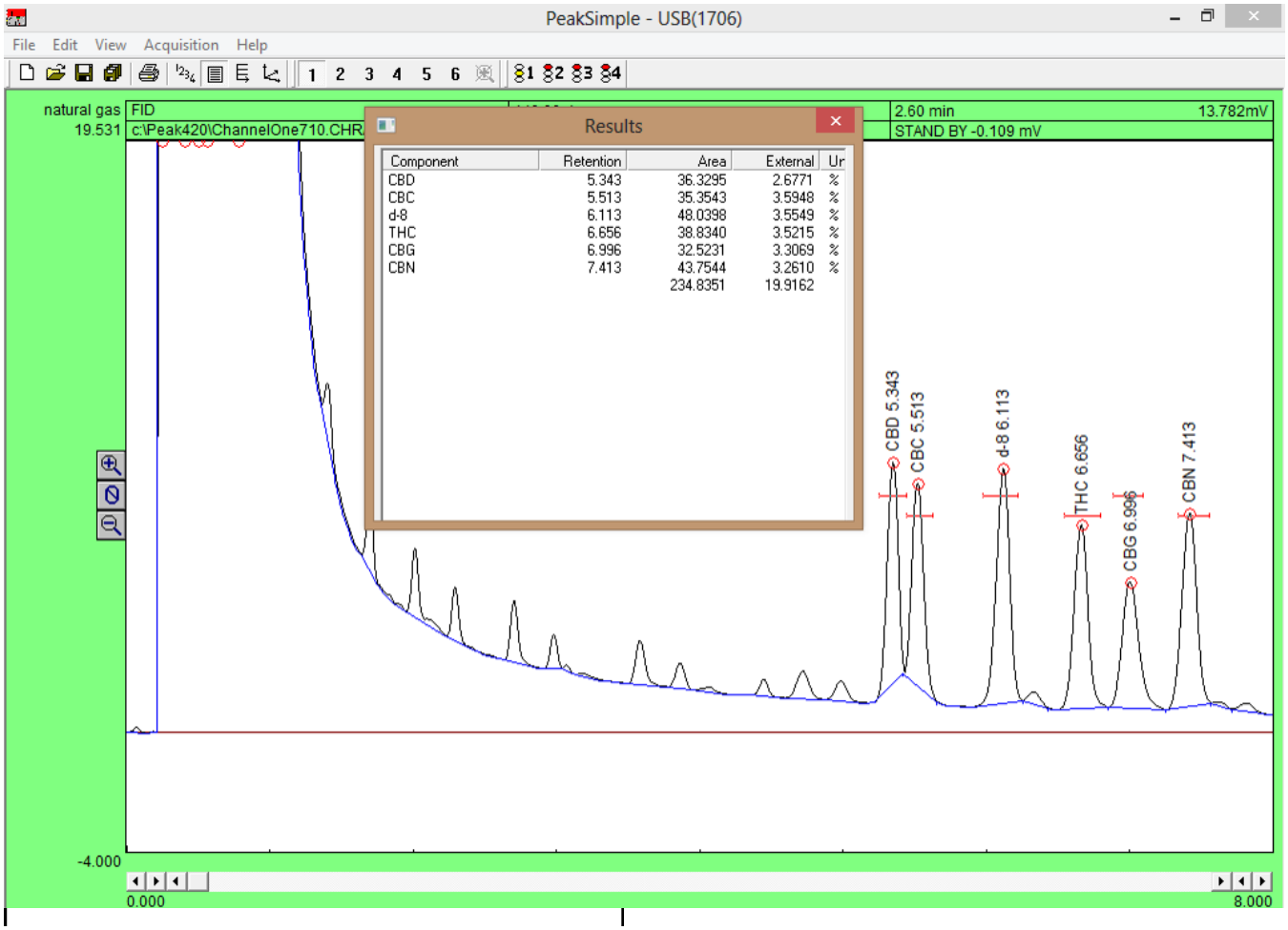
The Results screen will show the concentration of all peaks detected based on the calibration we have previously done.

Print the chromatogram and results for a hardcopy record of the analysis.





Medical Cannabis Potency Testing using the SRI 8610C FID GC



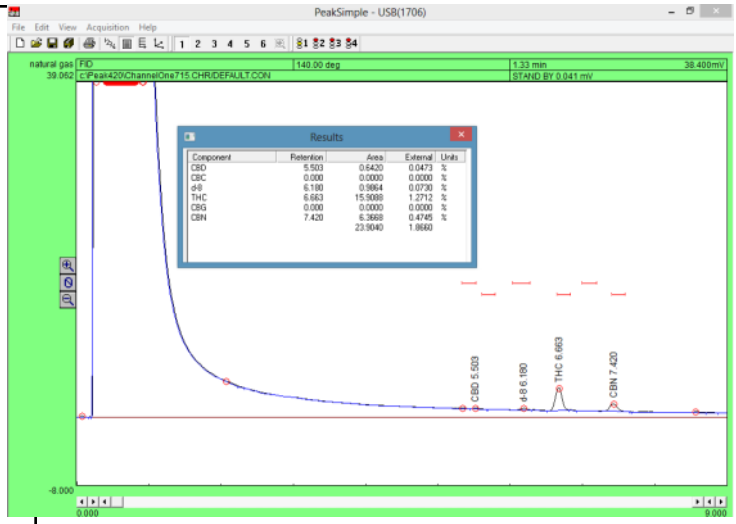
The chromatogram above shows a six-cannabinoid calibration standard. In addition to CBD, Δ9-THC, and CBN; Cannabichromene (**CBC**), **Δ8-THC**, and Cannabigerol (**CBG**) are now identifiable and quantifiable.

Notice how even in the calibration standard, CBD and CBC, and, THC and CBG, elute very close to each other. In real world samples this effect can be even more pronounced. The goal is *always to achieve the best separation*.

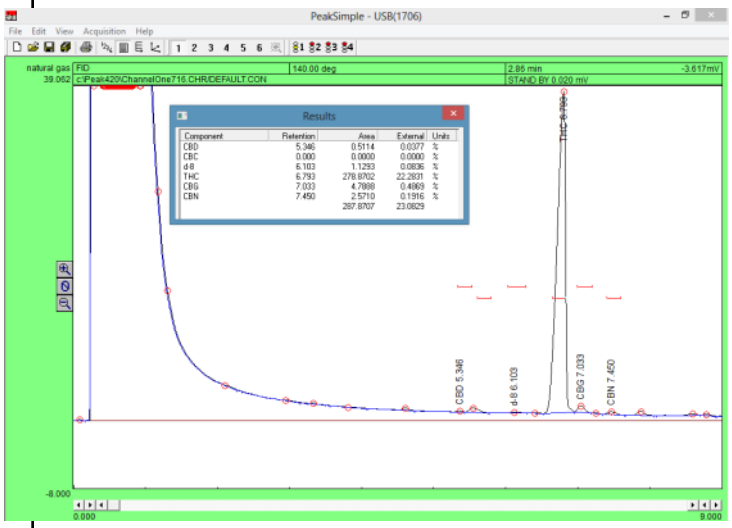
Most cannabis samples contain at least *some* amount of all six cannabinoids (as well as a few others). Because the chemical structure of all six cannabinoids are so similar, a calibration value for one cannabinoid (i.e. CBD or THC) can be used to accurately calibrate another cannabinoid (i.e. CBC, CBG, or Δ8-THC) for which a standard is unavailable. For further information, consult a future paper entitled "*Calibrating Six Cannabinoids with CBD Calibration Standard*".

Medical Cannabis Potency Testing using the SRI 8610C FID GC

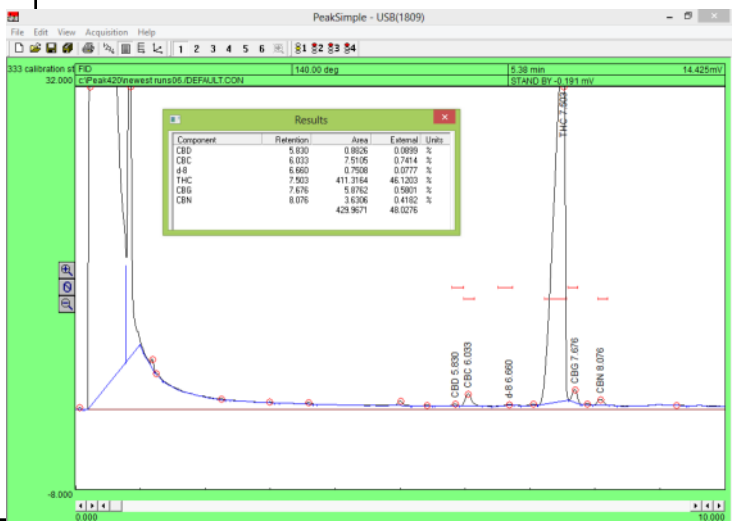
This is a chromatogram of a low-potency cannabis flower with 1.2% THC.



This is a chromatogram of a high-potency cannabis flower with 22.3% THC.



This is a chromatogram of a typical cannabis concentrate with 46.1% THC.



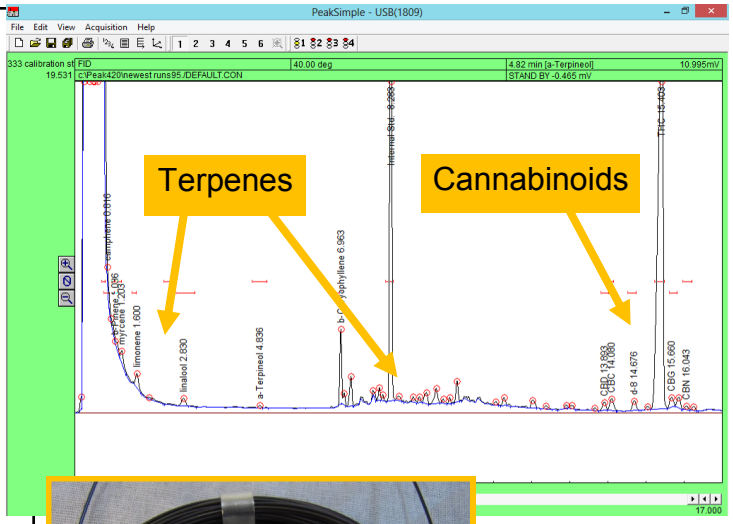
This concludes the *Medical Cannabis Potency Testing* Document. See www.srigc.com for more documents, resources, and sales information.

Medical Cannabis Potency *and* Terpenes Analysis
Using the SRI 8610C FID GC

The cannabinoids (psychoactive components) and terpenes (aroma compounds) can be measured quantitatively on one 20-minute run using the stock medical cannabis potency column (15-meter MXT-500 with a .15 um film) on an 8610C FID Medical Cannabis Potency GC.

Refer to the "Medical Cannabis Potency" Document found on our website www.srigc.com in order to setup the software, extract and inject the sample, and interpret the results. The only difference between the potency analysis and running potency *and* terpenes is the **gain setting** and the **temperature program**.

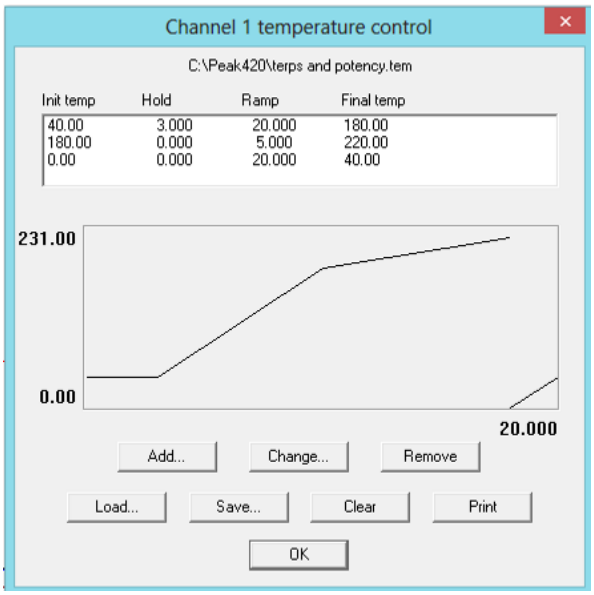
With the red lid open and staring down, the FID gain switch is located next to the FID detector on the far right of the GC. Usually the gain switch for the FID detector is set up to "MED". For this analysis, put the switch in the middle position on "**HIGH**". This will make the FID sensitivity about 20 times greater. This is important because the terpenes are present at much smaller concentrations in cannabis samples than the cannabinoids. This may present a problem with concentrates exceeding 40% THC (the peak will go off-scale) but typical cannabis flowers will present no problems. **Remember to recalibrate after changing the gain setting.**

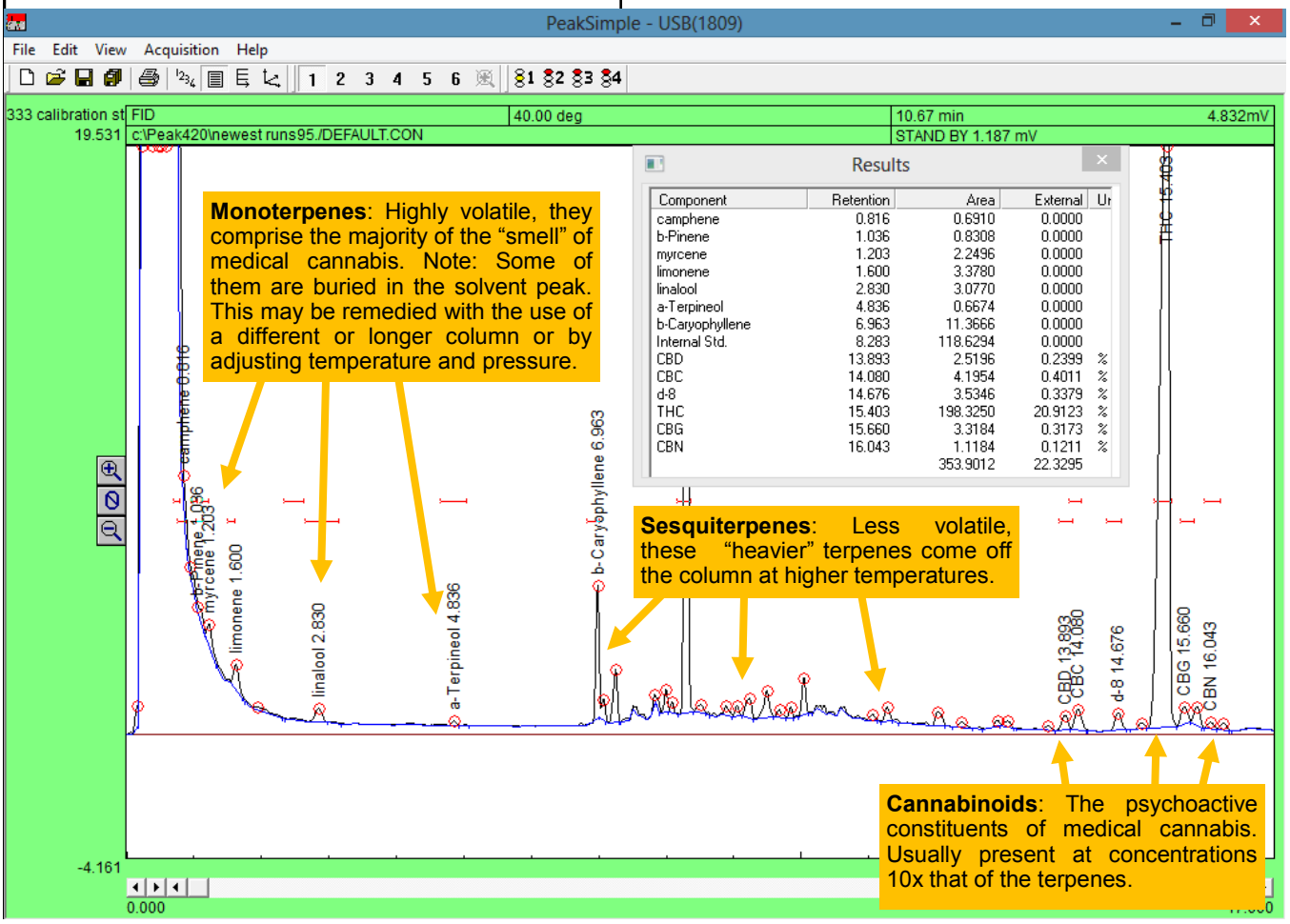


Medical Cannabis Potency *and* Terpenes Analysis
Using the SRI 8610C FID GC

The temperature program for the potency and terpenes run is shown to the right. The temperature begins and holds for 3 minutes at 40 C to allow the most volatile terpenes to separate. Between 180 C and 220C a slower temperature ramp achieves optimum separation of the cannabinoids.

A terpenes and potency chromatogram will look something like the one below. The cannabinoids have been calibrated, the terpenes have not.





Heated vs. On-Column Injection for Medical Cannabis Potency Testing

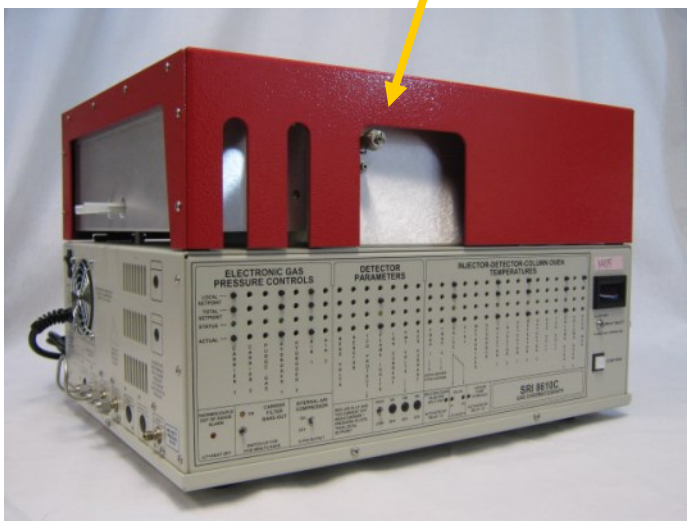
Older models of the SRI 8610C FID Medical Cannabis Potency GC were equipped with an **on-column injection** system while current versions are equipped with a **heated injector**. Either injection system can be used to accurately quantify cannabinoids in medical cannabis.

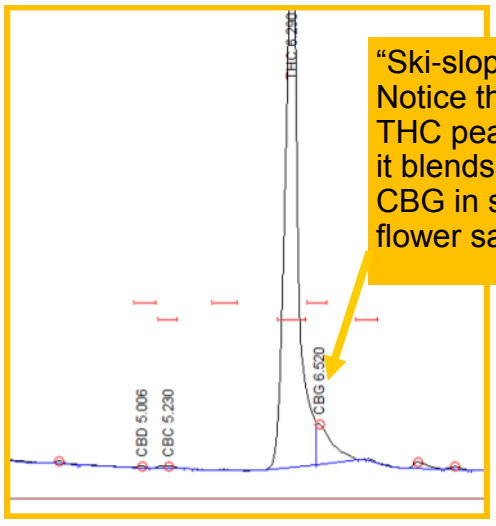
SRI prefers the heated injection because it improves separation and leads to more accurate quantification of the cannabinoids. It comes standard with the current Medical Cannabis Potency GC. As a service to our Medical Cannabis FID GC owners, we will upgrade on-column injectors to heated injectors for the special low-price of $500.00.

The on-column injector takes several seconds to decarboxylate the cannabinoids in the first meter of the column. Because of this, a smear or "ski-slope" effect can be seen in some samples where THC tails off into CBG and sometimes CBN. This can make it harder to integrate and quantify these cannabinoids. This problem can be remedied with the installation of a heated injector, which instantly decarboxylates the cannabinoids at 250° C before they enter the column.

On-column Injector

Heated Injector

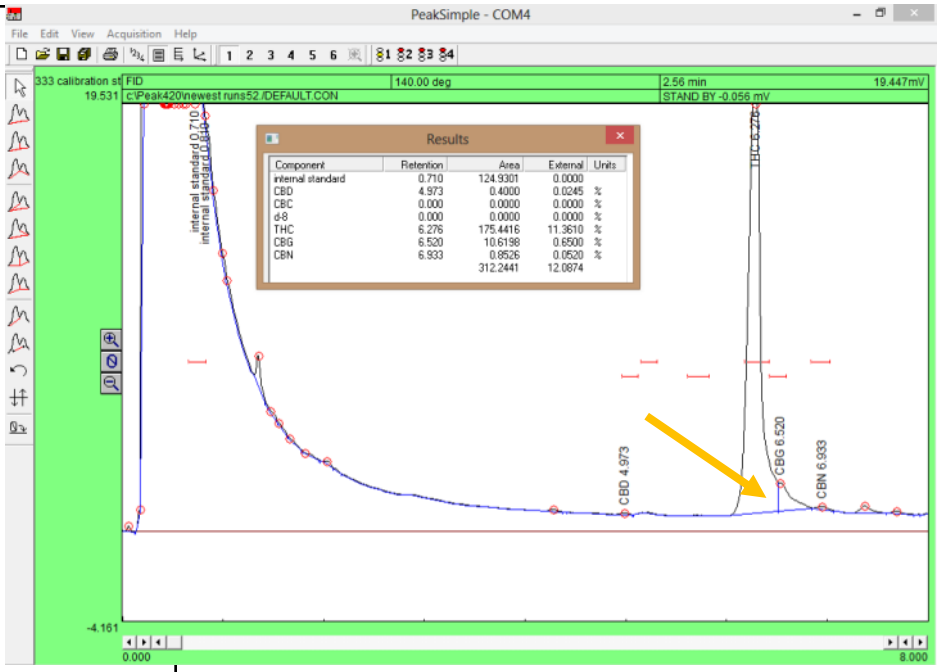




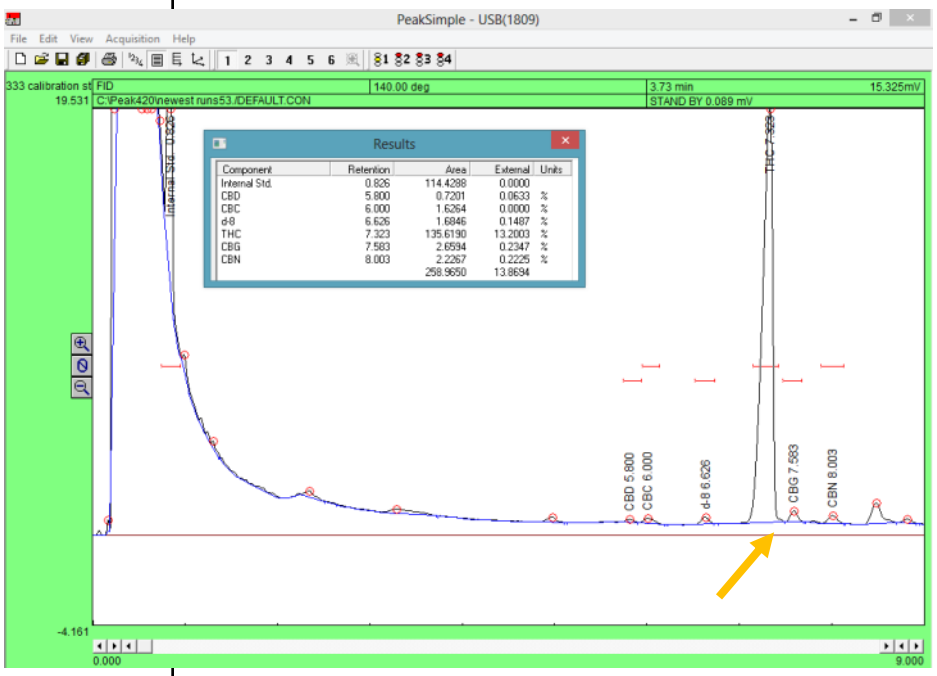
"Ski-slope" effect: Notice the tail on the THC peak and how it blends into the CBG in some actual flower samples.

Heated vs. On-Column Injection for Medical Cannabis Potency Testing

To the right is a chromatogram of a typical medical cannabis flower on an on-column injector. Notice the ski-slope effect with the large THC peak tailing into CBG.



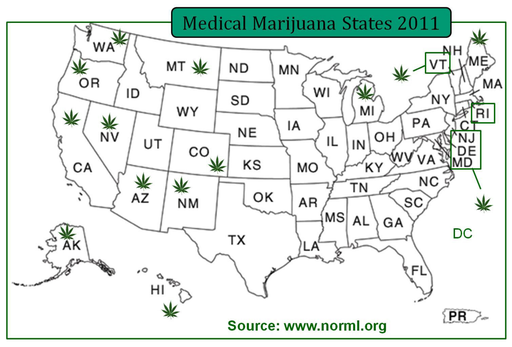
The same medical cannabis extraction run on a heated injector. Notice the crisp THC peak with baseline separation between it and CBG. Also, notice how cannabinoids present in trace amounts (CBC, Δ8-THC, CBN) have more pronounced peaks.



High Quality Analysis of Pesticides in Marijuana for Food and Medicine using QuEChERS, Cartridge SPE, GCxGC-TOFMS, and LC-MS/MS

Jack Cochran, Julie Kowalski, Sharon Lupo, Michelle Misselwitz, Amanda Rigdon, Jason Thomas, Restek Corporation
Frank Dorman, Jessica Westland, Amanda Leffler, The Pennsylvania State University

- Over 15 states in the USA have medical marijuana laws.
  - Therapeutic benefits include pain relief, nausea control, appetite stimulation, and muscle relaxation.
  - Marijuana is illegal on the federal level so patients have no assurances on medicine safety, including for pesticide residues.
- We used the QuEChERS sample preparation approach for extracting pesticides from marijuana.
  - But dispersive SPE did not have the cleanup capacity for GCxGC work.
  - Instead, we employed cartridge SPE for cleanup for GCxGC.
- GCxGC-TOFMS and LC-MS/MS were used for pesticide determinations in cleaned up QuEChERS extracts.
  - The selectivity of advanced techniques was needed due to sample extract complexity, even after dilution/cleanup.
  - LC-MS/MS was necessary for abamectin because it does not gas chromatograph.

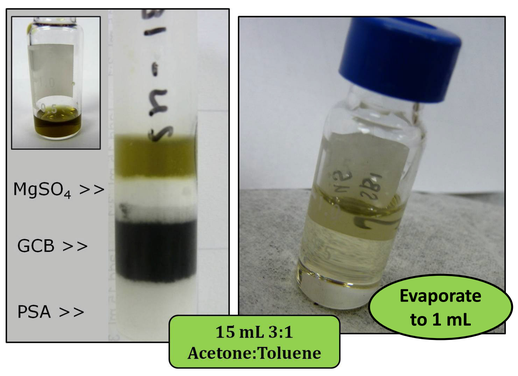


Preparing Marijuana Samples at PSU

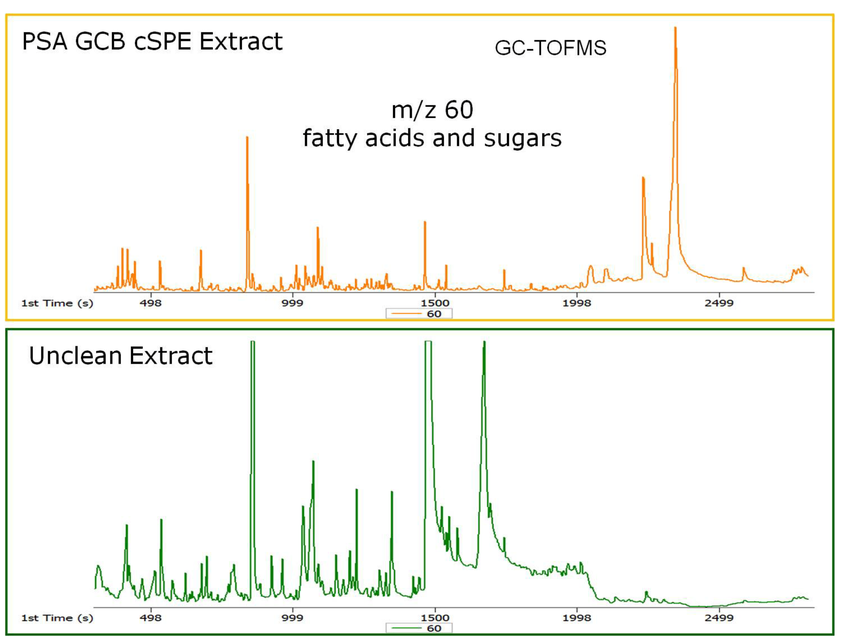
QuEChERS Procedure for Marijuana

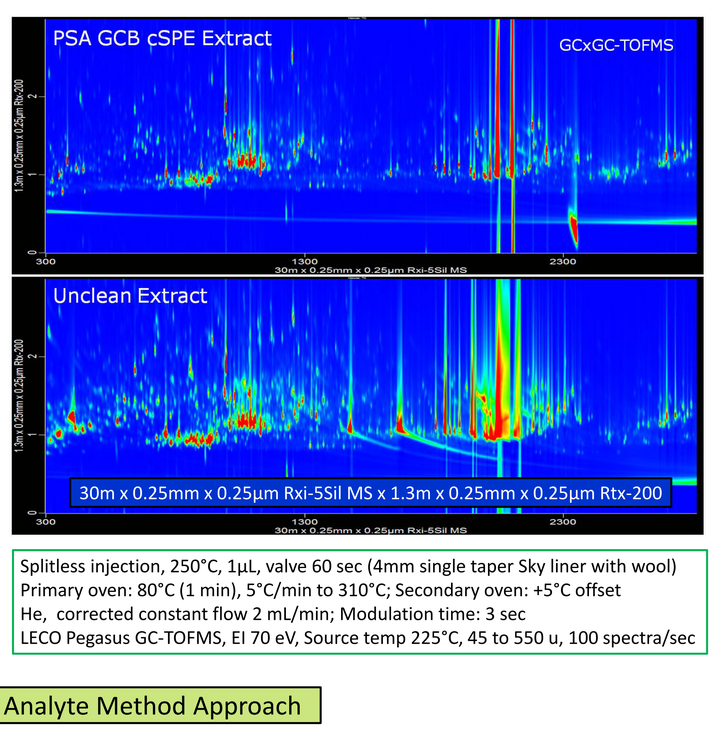
Up to 2 g ground marijuana – 50 mL centrifuge tube
10 mL MeCN
10 mL H_2O
Shake to wet
Soak one hour
Add spikes and internal standards
Vortex 30 min
Add QuEChERS EN salts
Shake 1 min
Centrifuge 5 min at 3000g
Remove extract for cleanup and analysis
GCxGC-TOFMS and LC-MS/MS

Cartridge SPE Clean-up Prior to GCxGC-TOFMS



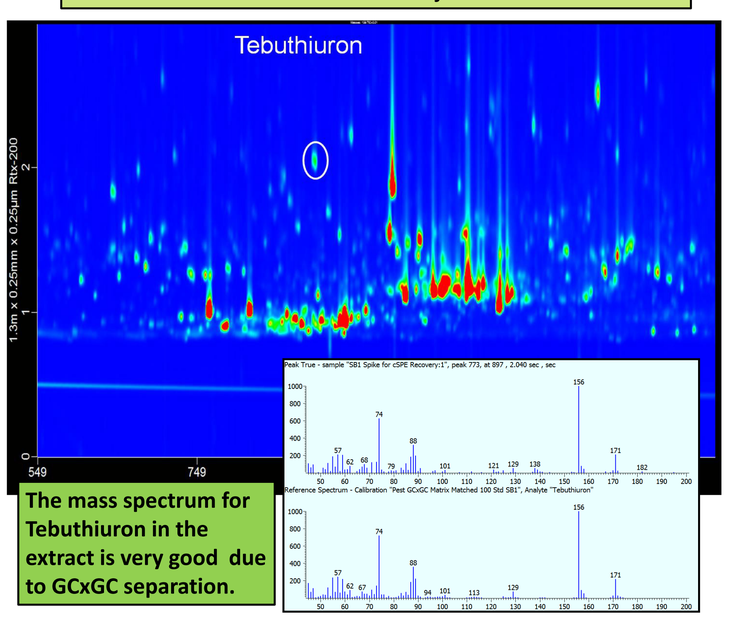
High Capacity Cartridge SPE Produces a Cleaner Extract by Removing Interfering Fatty Acids and other Matrix Co-extractives





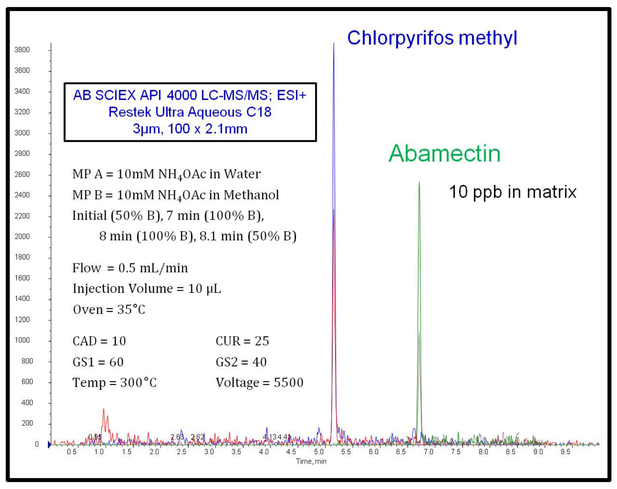
Splitless injection, 250°C, 1µL, valve 60 sec (4mm single taper Sky liner with wool)
Primary oven: 80°C (1 min), 5°C/min to 310°C; Secondary oven: +5°C offset
He, corrected constant flow 2 mL/min; Modulation time: 3 sec
LECO Pegasus GC-TOFMS, EI 70 eV, Source temp 225°C, 45 to 550 u, 100 spectra/sec

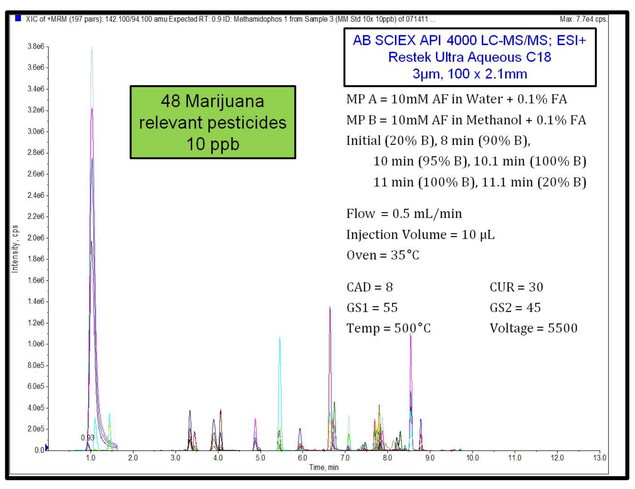
GCxGC Separates Pesticides from Remaining Matrix Co-extractives in a Marijuana Extract



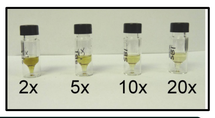
The mass spectrum for Tebuthiuron in the extract is very good due to GCxGC separation.

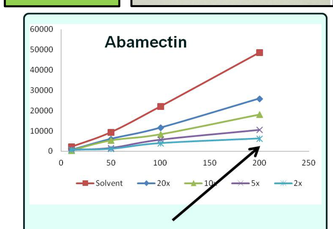
LC-MS/MS of Marijuana Pesticides – Abamectin Required a Single Analyte Method Approach

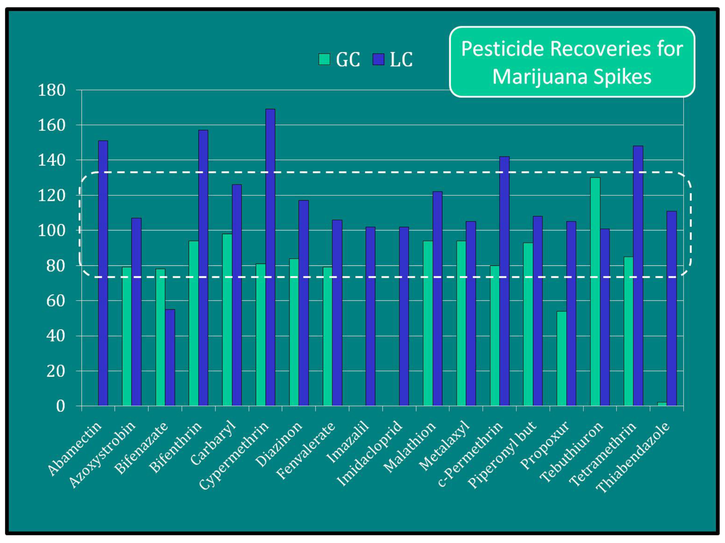




Dilute and shoot?







Incurred Pesticides in Marijuana Sample 2Q2

ppb

Pesticide	LC	GC
Imazalil	410	NA
Bifenazate	1100	2180
Piperonyl butoxide	37	41
trans-Permethrin	660	1100
cis-Permethrin	1200	690
o-Phenylphenol	NA	280
4,4'-DDE	NA	30

NA = not analyzed by this method

"Effervescent Health Formula"

Technical Article

High-Quality Analysis of Pesticides in Cannabis

Using QuEChERS, Cartridge SPE Cleanup, and GCxGC-TOFMS

By Jack Cochran, Julie Kowalski, Sharon Lupo, Michelle Misselwitz, and Amanda Rigdon

- Quickly and effectively extract medical marijuana samples for pesticide analysis.
- Cartridge SPE cleanup of dirty extracts improves GC inlet and column lifetimes.
- Selective GC columns increase accuracy of pesticide determinations for complex samples.

Over 20 states in the U.S. have legalized the use of recreational or medical cannabis because of therapeutic benefits for ailments such as cancer, multiple sclerosis, and ALS. Dosing methods include smoking or vaporizing and baked goods. Unlike other prescribed medicines regulated by U.S. FDA, marijuana is a Schedule 1 drug and is illegal on the federal level. As a result, medical cannabis patients have no safety assurances for their medication, which could contain harmful levels of pesticide residues. Currently, medical marijuana pesticide residue analysis methods are poorly defined and challenging to develop due to matrix complexity and a long list of potential target analytes.

In order to address matrix complexity, we combined a simple QuEChERS extraction approach with cartridge SPE (cSPE) cleanup, followed by GCxGC-TOFMS. Acceptable recoveries were obtained for most pesticides, and incurred pesticide residues were detected in some of the illicit marijuana samples used for method development.

QuEChERS Extraction Saves Time and Reduces Hazardous Solvent Use

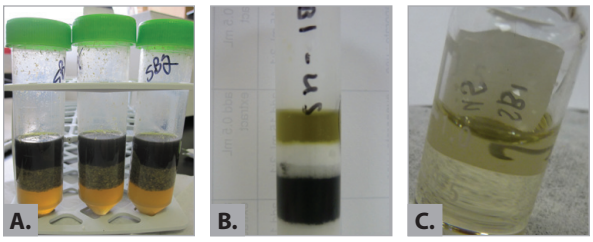
Trace residue extraction procedures from dry materials like medical cannabis typically involve large amounts of solvent, long extraction times, and tedious concentration steps similar to the Soxhlet procedure or multiresidue methods from the Pesticide Analytical Manual. QuEChERS, with its simple 10 mL acetonitrile shake extraction and extract partitioning with salts and centrifugation, offers time savings, glassware use reduction, and lower solvent consumption.

Water was added to finely ground, dry cannabis samples to increase QuEChERS extraction efficiency, especially for more polar pesticides. A vortex mixer was used to shake the solvent and sample for at least 30 minutes prior to extract partitioning. When finished, it was easy to transfer the supernatant from the QuEChERS extraction tube for subsequent cSPE cleanup prior to analysis with GC or LC (Figure 1).

Cartridge SPE Cleanup Improves GC Inlet Uptime

Injecting chlorophyll-laden extracts into a GC gives reduced recoveries for less volatile pesticides, and results in degradation of sensitive pesticides like DDT and Dicofol (Table I). SPE cleanup with a 500 mg graphitized carbon black/500 mg PSA cartridge removes chlorophyll and traps fatty acids that interfere with qualitative pesticide identification and bias quantification. cSPE has increased sorbent capacity over dispersive SPE for thorough cleanup of complex extracts.

Figure 1: A quick and easy QuEChERS extraction, combined with cSPE, effectively prepared extracts for pesticide residue analysis from highly complex marijuana samples.



Post-centrifugation QuEChERS extracts | QuEChERS extracts loaded on SPE cartridge | Final extract

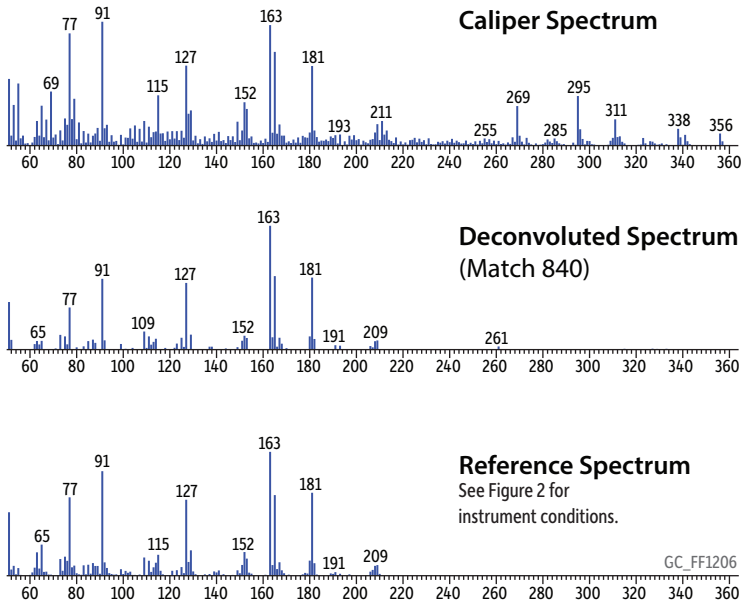
Orthogonal GC Columns Increase Separation Power for More Accurate Pesticide Results

GCxGC is a powerful multidimensional approach that gives two independent separations in one instrumental analysis. An Rxi®-5Sil MS and Rtx®-200 column combination distributes pesticides broadly in both dimensions, providing a highly orthogonal GCxGC system. More important though is separating pesticides from potential isobaric matrix interferences, as seen in the surface plot for the insecticide cypermethrin (Figure 2). Cypermethrin gas chromatographs as four isomers, and all would have experienced qualitative interference and quantitative bias from peaks in the foreground of the surface plot had only 1-dimensional GC been used. With GCxGC-TOFMS, cypermethrin was unequivocally identified in a marijuana sample at a low ppm level (Figure 3).

Summary

QuEChERS and cSPE produced usable extracts from highly complex cannabis samples for high-quality pesticide residue analysis. The multidimensional separation power of GCxGC-TOFMS was then used to correctly identify and quantify pesticides in these complex extracts.

Figure 3: Positive mass spectral identification of incurred cypermethrin in illicit marijuana.



Acknowledgment: Randy Hoffman, a Police Evidence Technician at The Pennsylvania State University (PSU), supplied the seized marijuana samples while overseeing their handling. Frank Dorman at PSU assisted with QuEChERS extractions.

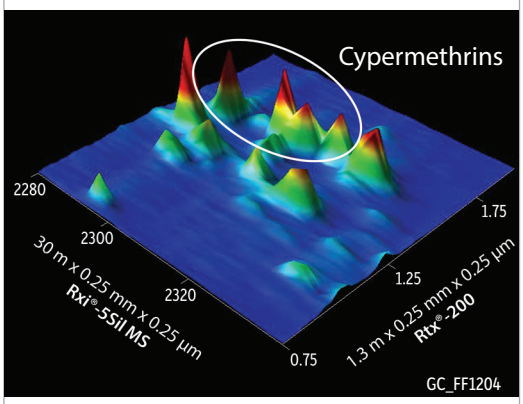
Initially published in *Restek® Advantage*.

Table I: Pesticide recoveries for a QuEChERS extract of cannabis give higher results when cSPE is used for cleanup. Dicofol and DDT are degraded in the inlet for the dirtier extract, yielding high DDD results.

Pesticide	Classification	With cSPE Cleanup (%)	Without cSPE Cleanup (%)
4,4'-DDD	Organochlorine	83	230
4,4'-DDT	Organochlorine	77	9
Bifenthrin	Pyrethroid	86	89
Dicofol	Organochlorine	84	ND
Azinphos methyl	Organophosphorus	79	53
trans-Permethrin	Organochlorine	68	17
Pyraclostrobin	Strobilurin	73	19
Fluvalinate	Pyrethroid	72	23
Difenoconazole	Triazole	67	21
Deltamethrin	Pyrethroid	68	20
Azoxystrobin	Strobilurin	72	27

ND = no peak detected

Figure 2: GCxGC-TOFMS and orthogonal Rxi®-5Sil MS and Rtx®-200 columns allow incurred cypermethrins in a marijuana extract to be separated from interferences (m/z 163 quantification ion).

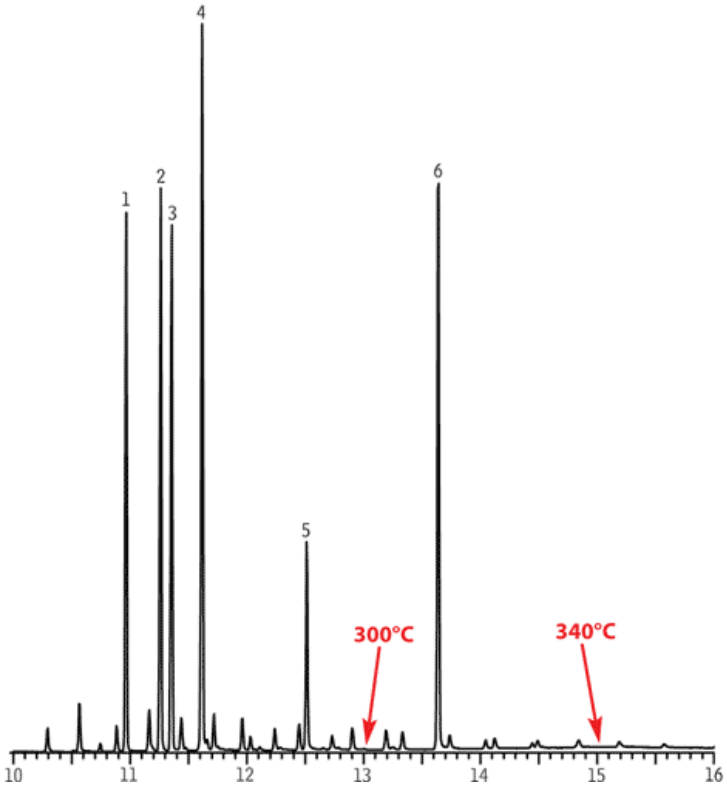


Peaks	RT 1 (sec.)	RT 2 (sec.)
1. Cypermethrin 1	2292	1.50
2. Cypermethrin 2	2304	1.54
3. Cypermethrin 3	2310	1.53
4. Cypermethrin 4	2313	1.58

Column: Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623), Rtx®-200 1.3 m, 0.25 mm ID, 0.25 µm (cat.# 15124); **Sample:** Diluent: Toluene; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: Sky® 4mm single taper w/wool (cat.# 23303.1); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min; **Oven:** Oven Temp: Rxi®-5Sil MS: 80 °C (hold 1 min) to 310 °C at 5 °C/min, Rtx®-200: 85 °C (hold 1 min) to 315 °C at 5 °C/min; **Carrier Gas:** He, corrected constant flow (2 mL/min); **Modulation**: Modulator Temp. Offset: 20 °C; Second Dimension Separation Time: 3 sec.; Hot Pulse Time: 0.9 sec.; Cool Time between Stages: 0.6 sec.; **Instrument**: LECO Pegasus 4D GCxGC-TOFMS; For complete conditions, visit www.restek.com and enter GC_FF1204 in the search.

Figure 2 A 12m x 0.20mm ID x 0.33µm Rxi™-5ms column provides baseline resolution and short analysis time for cannabinoids.

1. cannabidiol
2. Δ^8-tetrahydrocannabinol
3. Δ^9-tetrahydrocannabinol
4. cannabinol
5. 11-hydroxy-Δ^9-tetrahydrocannabinol
6. 11-nor-Δ^9-tetrahydrocannabinol carboxylic acid



GC_PH00891

Column:	Rxi™-5ms 12m, 0.20mm ID, 0.33µm (cat.# 13497)
Sample:	1000µg/mL each component in methanol
	1.0µL, split, split ratio 25:1, 4mm ID base-deactivated single gooseneck inlet liner w/wool
Inj.:	(cat.# 20798-211.1)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	40°C to 340°C @ 20°C/min. (hold 5 min.)
Det:	MS
Transfer line temp.:	280°C
Scan range:	100-550 amu
Ionization:	EI
Mode:	scan

References

1. Smith, F. and J. Siegel *Handbook of Forensic Drug Analysis* Elsevier Academic Press, 2005, pp. 98-151.
2. Clouette, R., M. Jacob, P. Koteel, and M. Spain *Journal of Analytical Toxicology* 17 (1): 1-4 (Jan./Feb. 1993).

RELATED SEARCHES

marijuana, *cannabinoid metabolites*, *Rxi-5ms*, *THC*

Technical Article

High-Quality Analysis of Pesticides in Cannabis

Using QuEChERS, Cartridge SPE Cleanup, and GCxGC-TOFMS

By Jack Cochran, Julie Kowalski, Sharon Lupo, Michelle Misselwitz, and Amanda Rigdon

- Quickly and effectively extract medical marijuana samples for pesticide analysis.
- Cartridge SPE cleanup of dirty extracts improves GC inlet and column lifetimes.
- Selective GC columns increase accuracy of pesticide determinations for complex samples.

Over 20 states in the U.S. have legalized the use of recreational or medical cannabis because of therapeutic benefits for ailments such as cancer, multiple sclerosis, and ALS. Dosing methods include smoking or vaporizing and baked goods. Unlike other prescribed medicines regulated by U.S. FDA, marijuana is a Schedule 1 drug and is illegal on the federal level. As a result, medical cannabis patients have no safety assurances for their medication, which could contain harmful levels of pesticide residues. Currently, medical marijuana pesticide residue analysis methods are poorly defined and challenging to develop due to matrix complexity and a long list of potential target analytes.

In order to address matrix complexity, we combined a simple QuEChERS extraction approach with cartridge SPE (cSPE) cleanup, followed by GCxGC-TOFMS. Acceptable recoveries were obtained for most pesticides, and incurred pesticide residues were detected in some of the illicit marijuana samples used for method development.

QuEChERS Extraction Saves Time and Reduces Hazardous Solvent Use

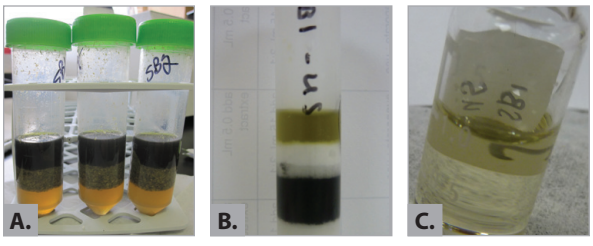
Trace residue extraction procedures from dry materials like medical cannabis typically involve large amounts of solvent, long extraction times, and tedious concentration steps similar to the Soxhlet procedure or multiresidue methods from the Pesticide Analytical Manual. QuEChERS, with its simple 10 mL acetonitrile shake extraction and extract partitioning with salts and centrifugation, offers time savings, glassware use reduction, and lower solvent consumption.

Water was added to finely ground, dry cannabis samples to increase QuEChERS extraction efficiency, especially for more polar pesticides. A vortex mixer was used to shake the solvent and sample for at least 30 minutes prior to extract partitioning. When finished, it was easy to transfer the supernatant from the QuEChERS extraction tube for subsequent cSPE cleanup prior to analysis with GC or LC (Figure 1).

Cartridge SPE Cleanup Improves GC Inlet Uptime

Injecting chlorophyll-laden extracts into a GC gives reduced recoveries for less volatile pesticides, and results in degradation of sensitive pesticides like DDT and Dicofol (Table I). SPE cleanup with a 500 mg graphitized carbon black/500 mg PSA cartridge removes chlorophyll and traps fatty acids that interfere with qualitative pesticide identification and bias quantification. cSPE has increased sorbent capacity over dispersive SPE for thorough cleanup of complex extracts.

Figure 1: A quick and easy QuEChERS extraction, combined with cSPE, effectively prepared extracts for pesticide residue analysis from highly complex marijuana samples.



Post-centrifugation QuEChERS extracts | QuEChERS extracts loaded on SPE cartridge | Final extract

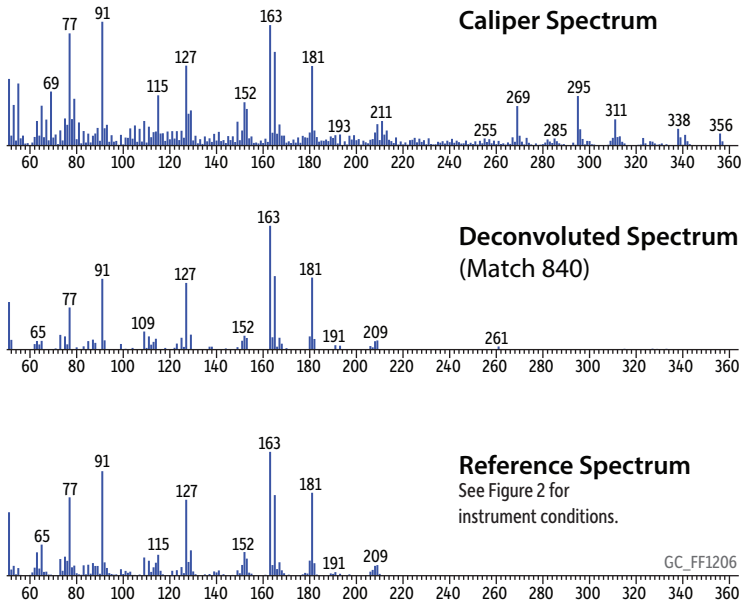
Orthogonal GC Columns Increase Separation Power for More Accurate Pesticide Results

GCxGC is a powerful multidimensional approach that gives two independent separations in one instrumental analysis. An Rxi®-5Sil MS and Rtx®-200 column combination distributes pesticides broadly in both dimensions, providing a highly orthogonal GCxGC system. More important though is separating pesticides from potential isobaric matrix interferences, as seen in the surface plot for the insecticide cypermethrin (Figure 2). Cypermethrin gas chromatographs as four isomers, and all would have experienced qualitative interference and quantitative bias from peaks in the foreground of the surface plot had only 1-dimensional GC been used. With GCxGC-TOFMS, cypermethrin was unequivocally identified in a marijuana sample at a low ppm level (Figure 3).

Summary

QuEChERS and cSPE produced usable extracts from highly complex cannabis samples for high-quality pesticide residue analysis. The multidimensional separation power of GCxGC-TOFMS was then used to correctly identify and quantify pesticides in these complex extracts.

Figure 3: Positive mass spectral identification of incurred cypermethrin in illicit marijuana.



Acknowledgment: Randy Hoffman, a Police Evidence Technician at The Pennsylvania State University (PSU), supplied the seized marijuana samples while overseeing their handling. Frank Dorman at PSU assisted with QuEChERS extractions.

Initially published in *Restek® Advantage*.

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Difenoconazole	Triazole	67	21
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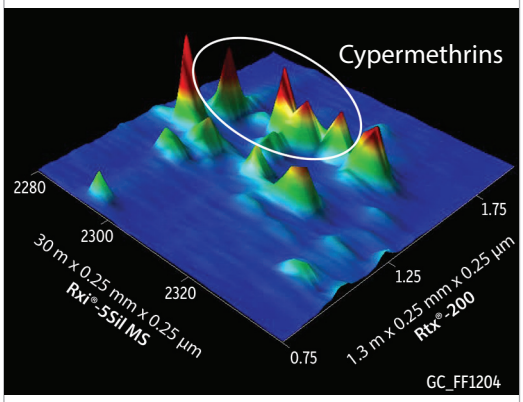
Figure 2: GCxGC-TOFMS and orthogonal Rxi®-5Sil MS and Rtx®-200 columns allow incurred cypermethrins in a marijuana extract to be separated from interferences (m/z 163 quantification ion).



Peaks	RT 1 (sec.)	RT 2 (sec.)
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2. Cypermethrin 2	2304	1.54
3. Cypermethrin 3	2310	1.53
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Column: Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623), Rtx®-200 1.3 m, 0.25 mm ID, 0.25 µm (cat.# 15124); **Sample:** Diluent: Toluene; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: Sky® 4mm single taper w/wool (cat.# 23303.1); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min; **Oven:** Oven Temp: Rxi®-5Sil MS: 80 °C (hold 1 min) to 310 °C at 5 °C/min, Rtx®-200: 85 °C (hold 1 min) to 315 °C at 5 °C/min; **Carrier Gas:** He, corrected constant flow (2 mL/min); **Modulation**: Modulator Temp. Offset: 20 °C; Second Dimension Separation Time: 3 sec.; Hot Pulse Time: 0.9 sec.; Cool Time between Stages: 0.6 sec.; **Instrument**: LECO Pegasus 4D GCxGC-TOFMS; For complete conditions, visit www.restek.com and enter GC_FF1204 in the search.

Technical Article

Don't Overestimate Cannabidiol During Medical Cannabis Potency Testing by Gas Chromatography

By Jack Cochran

Accurate potency testing of medical cannabis with gas chromatography (GC) depends principally on choosing a column with the right selectivity; otherwise, coelutions between cannabinoids of interest may cause error in potency measurements. Cannabidiol is one of the chief cannabinoids with pharmacological value and provides relief against nausea, anxiety, and inflammation. Potency testing for medical marijuana is often done using "5-type" GC columns since they are commonly available in most labs. However, on 5-type columns cannabidiol can coelute with cannabichromene, a compound that likely also has medical value and is increasingly becoming part of potency testing. To identify and report both of these compounds accurately, a GC column with a different stationary phase is needed.

Proper Column Choice Results in More Accurate Potency Data

As shown in Figure 1, cannabinoids are aromatic compounds, meaning they will likely be better separated on a column that contains aromatics in the stationary phase because these stationary phases are more selective for aromatic-containing analytes. A fully non-aromatic stationary phase, like a "1-type" (100% dimethyl polysiloxane) column is not appropriate for this analysis since cannabichromene (CBC) and cannabidiol (CBD) will coelute completely. While 5-type columns (5% phenyl) contain some aromatic component, they generally also produce coelutions for cannabichromene and cannabidiol, depending on the conditions used. At best, CBC and CBD can be only partially resolved on 15 m 5% phenyl columns. Much better separations are obtained on higher phenyl-content phases, such as Rxi®-35Sil MS (35% phenyl type) and Rxi®-17Sil MS (50% phenyl type) columns, as they offer excellent selectivity for aromatic cannabinoids. Not only do both columns resolve cannabichromene and cannabidiol, the chromatograms in Figures 2 and 3 demonstrate that they also separate delta-8-tetrahydrocannabinol (d8-THC), delta-9-tetrahydrocannabinol (d9-THC), cannabigerol (CBG), and cannabinol (CBN). Although both columns perform well, the Rxi®-35Sil MS column is recommended because of the slightly faster analysis time and greater space overall between the peaks of interest.

While stationary phase selectivity is the most important factor in choosing a GC column for cannabinoid analysis, there are some additional aspects of this work that will benefit labs doing medical marijuana potency testing. First, cost savings were achieved by using a 15 m column. When a column with the proper selectivity is used, a 15 m column easily provides the separating power needed for this analysis at about half the cost of a 30 m column. Also, the 0.25 mm x 0.25 µm format has good sample loading capacity and is robust, especially when a proper split injection is used with a Sky® Precision® split liner with wool. Finally, hydrogen carrier gas was used here instead of helium. Using hydrogen provides a faster analysis, increasing sample throughput. Hydrogen carrier gas is a convenient way to speed up run times, increase productivity, and reduce the cost and availability concerns associated with using helium carrier gas.

Figure 1: Since cannabinoids are aromatic compounds, a GC column that contains aromatics in the stationary phase will provide much better separations than a column with a non-aromatic phase.

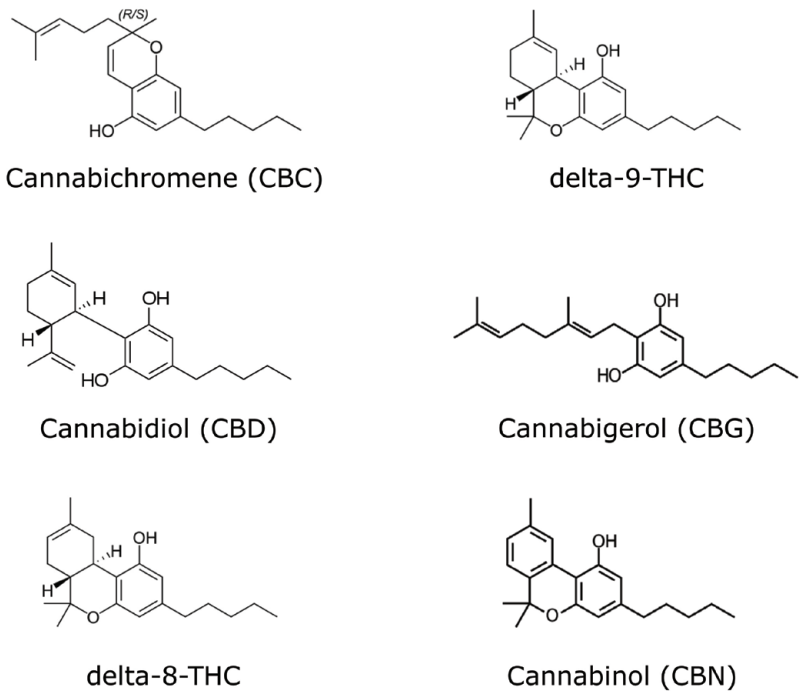


Figure 2: The Rxi®-35Sil MS column provides both the best separation and the fastest analysis time, making it the ideal GC column choice for medical cannabis potency testing.

Peaks
1. Cannabichromene
2. Cannabidiol
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

Column: Rxi®-35Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13820); **Sample:** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 225 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.9 min); **Carrier Gas;** H_2, constant flow; Flow Rate: 2.5 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument:** Agilent/HP6890 GC

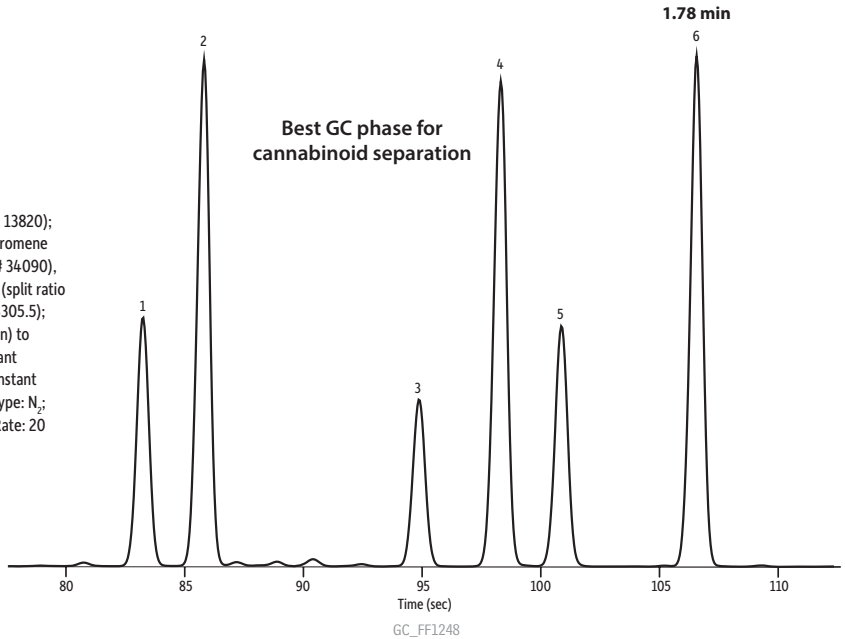
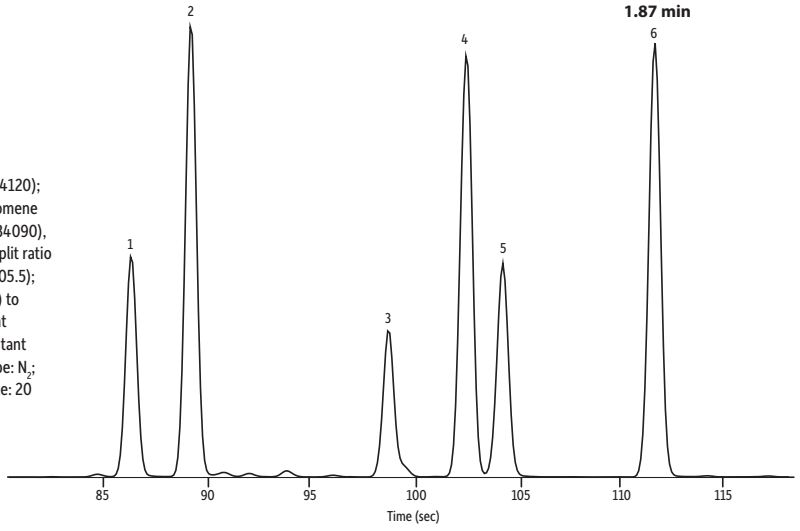


Figure 3: Cannabinoids can be effectively separated on an Rxi® 17Sil MS column, but with slightly less resolution than that obtained with the optimal selectivity of the Rxi®-35Sil MS column.

Peaks
1. Cannabichromene
2. Cannabidiol
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

Column: Rxi®-17Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 14120); **Sample;** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 225 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.9 min); **Carrier Gas:** H_2, constant flow; Flow Rate: 2.5 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument:** Agilent/HP6890 GC



Adjusting Conditions for 5-Type Columns

While using an Rxi®-35Sil MS column provides the best selectivity and speed for cannabinoid analysis, cannabidiol potency can be determined in medical cannabis using a 5-type column under certain conditions. If you already have a 5-type column for this work, you can vary the GC conditions, especially carrier flow and oven temperature program, and still separate cannabichromene and cannabidiol, just not as quickly or easily as with the Rxi®-35Sil MS column. Figures 4 and 5 show this analysis on Rxi®-5ms and Rxi®-5Sil MS columns, respectively. Again, the 0.25 mm x 0.25 µm format was used here because it offers better efficiency than wider bore columns (e.g., 0.32 mm and 0.53 mm IDs), which may not separate cannabichromene and cannabidiol under any operational conditions.

Figure 4: The selectivity of a 5-type column is not sufficient to fully separate cannabichromene and cannabidiol, resulting in less accurate medical marijuana potency testing.

Peaks
1. Cannabichromene
2. Cannabidiol
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

Column: Rxi®-5ms, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13420); **Sample;** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 250 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.6 min); **Carrier Gas:** H_2, constant flow; Flow Rate: 1.6 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument:** Agilent/HP6890 GC

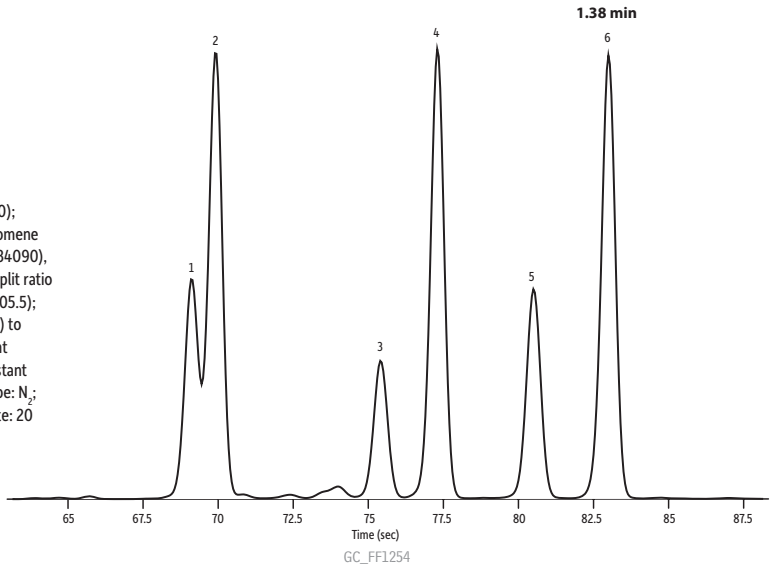
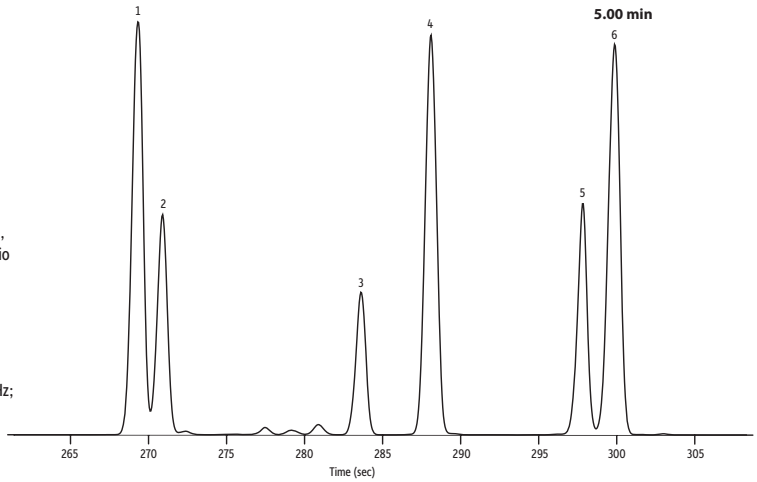


Figure 5: Rxi®-5Sil MS columns offer better resolution of key cannabinoids than standard 5-type columns, but the incomplete separation and longer analysis time mean further optimization is needed for accurate reporting.

Peaks
1. Cannabidiol
2. Cannabichromene
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

Column: Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620); **Sample;** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 150 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.7 min); **Carrier Gas:** H_2, constant flow; Flow Rate: 1.6 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument:** Agilent/HP6890 GC



Note that even though these are both 5-type columns, the elution order of cannabichromene and cannabidiol changed. This is due to two things. The first is that Rxi®-5ms and Rxi®-5Sil MS columns differ slightly in selectivity for certain compounds; even though they are both considered 5-type columns, they contain different stationary phases that retain some compounds differently. The second reason is that the GC oven programs are different for the columns, which means that the compounds are eluting at different temperatures. You may be able to further optimize the separation of cannabichromene and cannabidiol on a 5-type column, but the selectivity and faster analysis that can be obtained using a high-phenyl content Rxi®-35Sil MS column make it ideal for potency determinations in medical cannabis.

To sum things up, proper column choice is essential for accurate and robust cannabis potency testing. Using the right column not only gives you more confidence in your potency values, but it also saves you time and money. Switching to hydrogen carrier gas can reduce your costs even further, while increasing sample throughput.

Visit www.restek.com/medical-cannabis for Restek® GC and LC columns, accessories, reference standards, and other products and resources for medical marijuana analysis.

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Terpenes in Impinger Extracts of Kryptonite and Blueberry Strains of Medical Cannabis

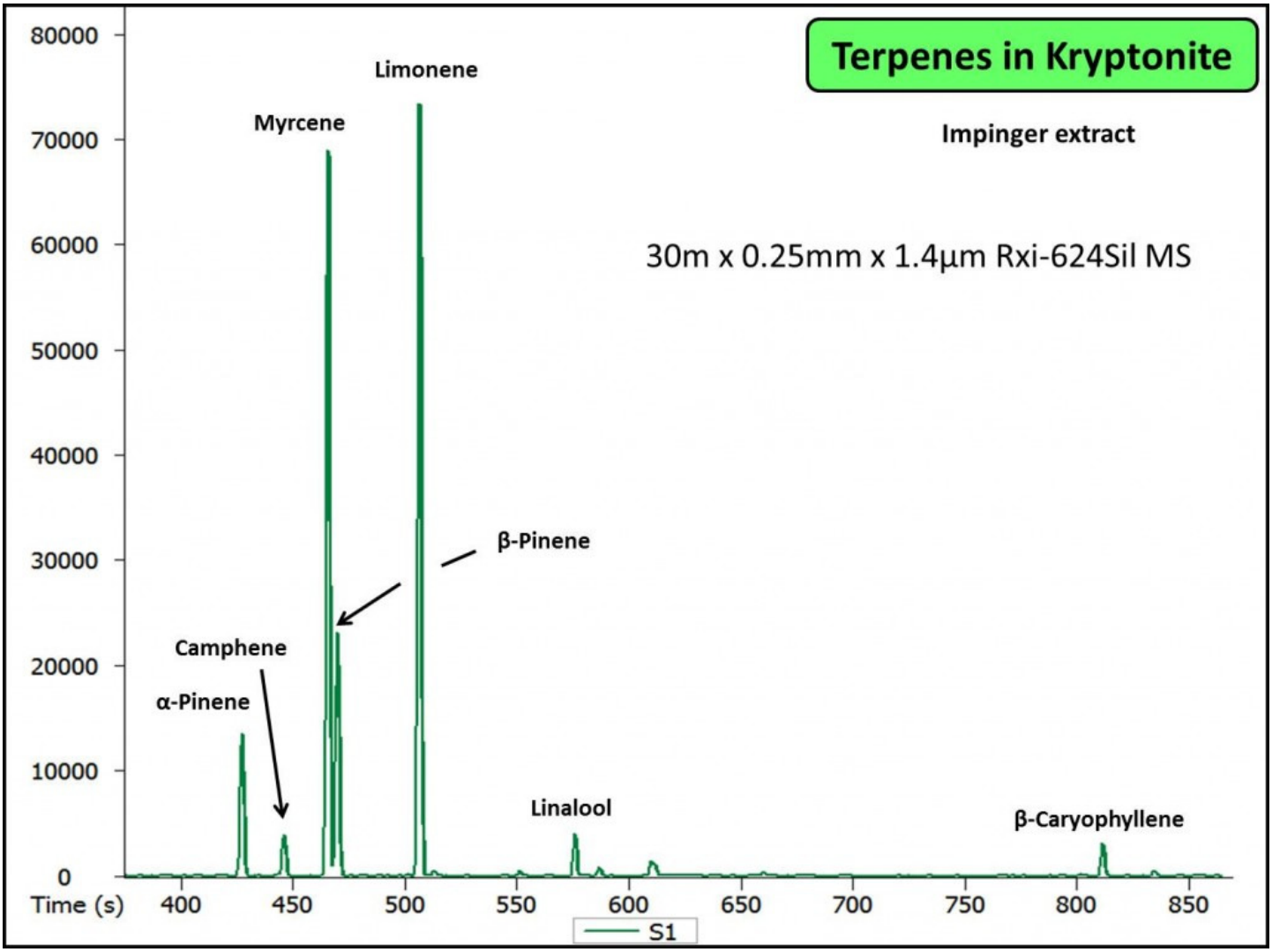
March 17th, 2014 by Jack Cochran

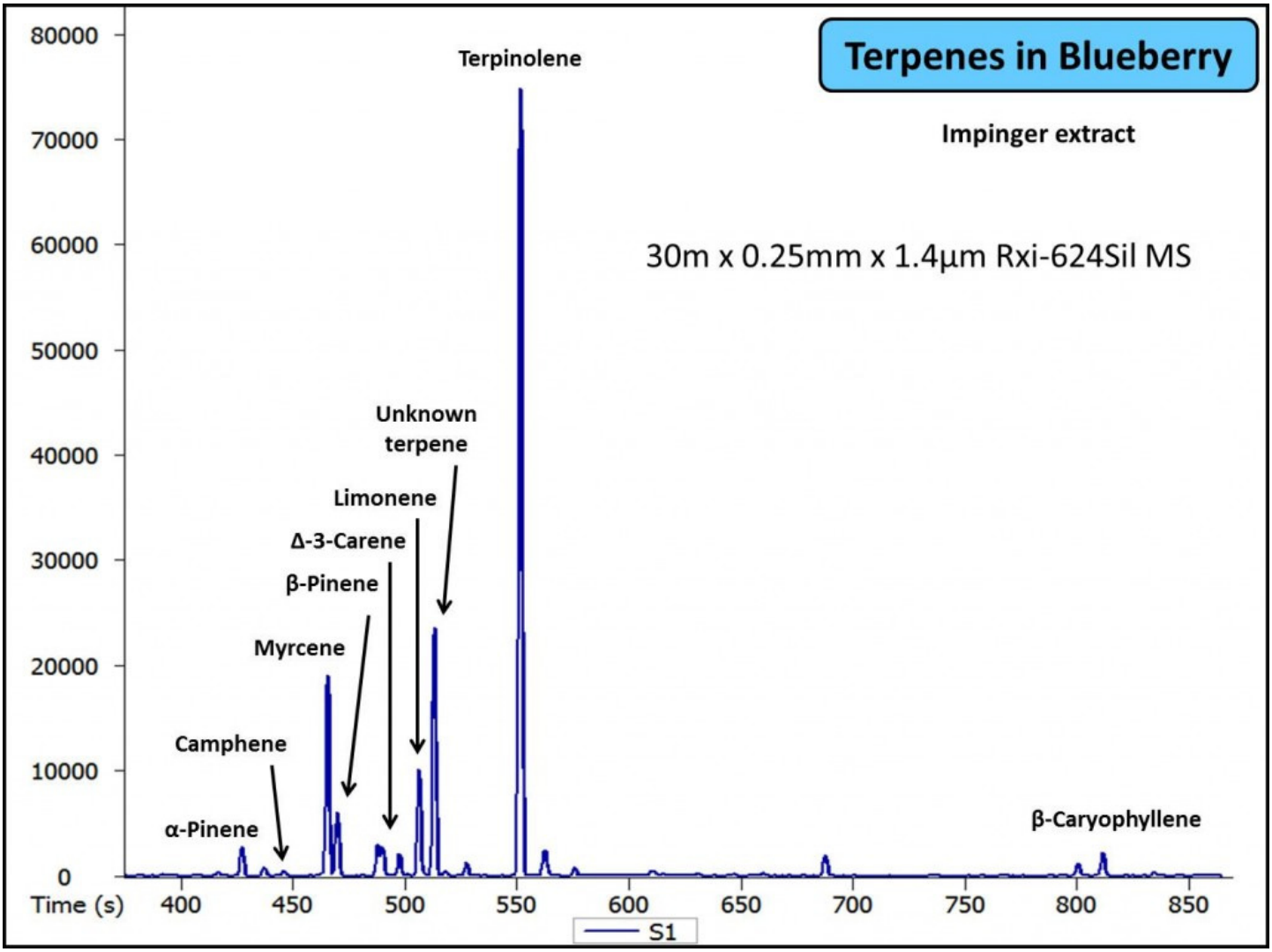
As noted in my earlier post, Terpenes in Medical Cannabis, terpenes are an important class of aroma compounds that may contribute to the medicinal benefits of cannabis, via the so-called "entourage effect". I profiled some of the terpenes listed as important for medical cannabis using our 30m x 0.25mm x 1.40µm Rxi-624Sil MS, achieving a promising separation on a standard I put together. Shown below are some impinger extracts provided by SRI Instruments for Kryptonite and Blueberry strains of medical cannabis. Importantly, these extracts do NOT contain any cannabinoids, which would elute late, if at all, from the thick-film 624Sil MS column, nor do they contain chlorophyll, another compound that plays havoc with GC inlet liners and stationary phases. Part of the beauty of headspace extraction techniques for terpenes is leaving the involatile material behind, and in this case, compressed air was used to sweep the terpenes from the cannabis to a vial containing methanol for trapping the terpenes.

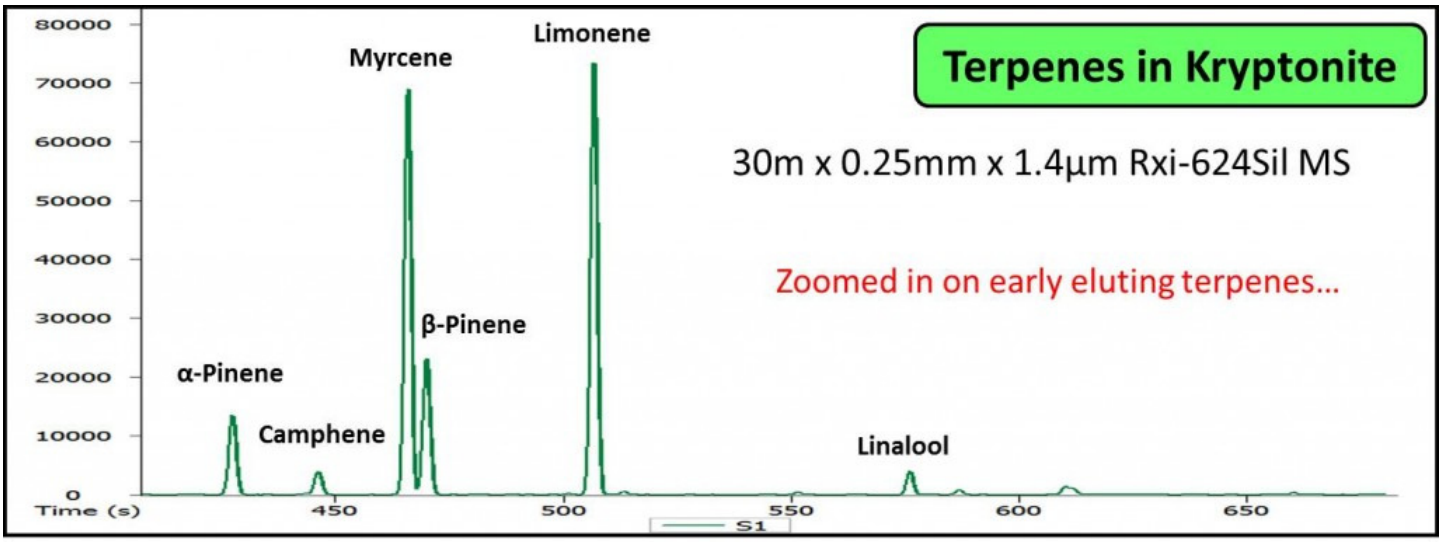
As you look at the chromatograms below, it is important to note that this is ONLY qualitative work at this point and that different headspace methods (e.g. purge-and-trap, static headspace, SPME, etc.) could yield much different chromatograms. Solvent extraction or steam distillation, would likely be even more different, including resulting in more intense peaks for later eluting (less volatile) terpenes. The point of this work is to show initial efforts to characterize chromatographic elution order for some medical marijuana terpenes and analyze the first "real world" samples to show how terpene profiles for different medicines can be dissimilar.

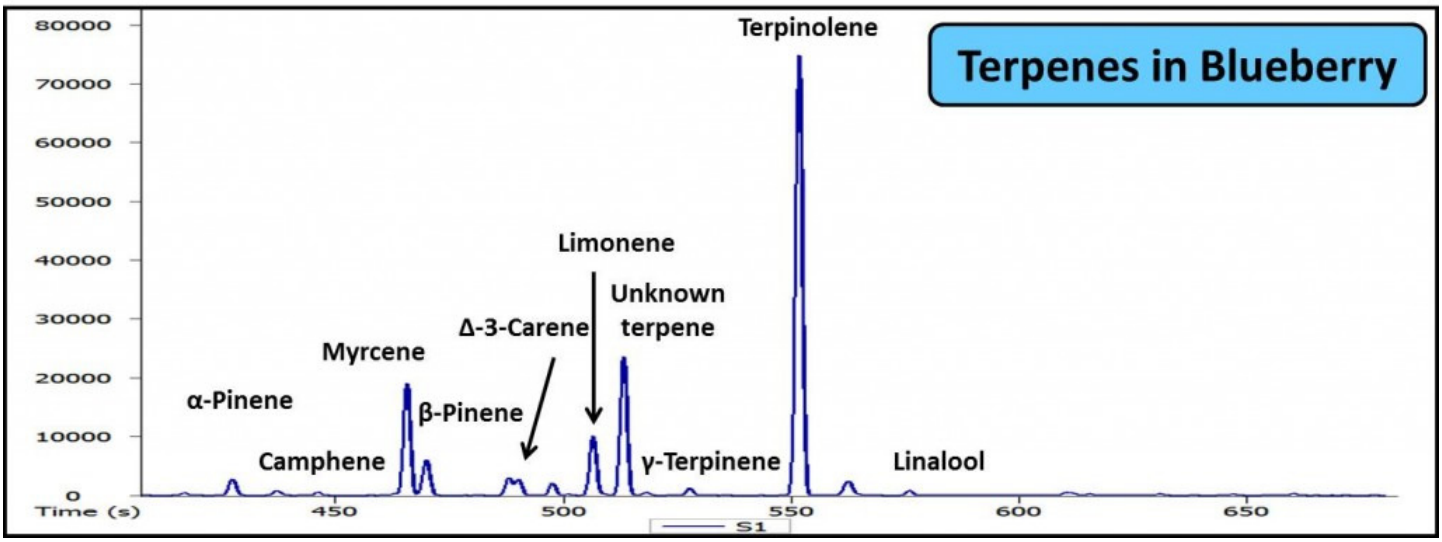
Take a look back on the GCxGC-TOFMS work that shows very nice multidimensional separations of terpenes, sesquiterpenes, and oxygenated terpenes in a solvent extract for cannabis.

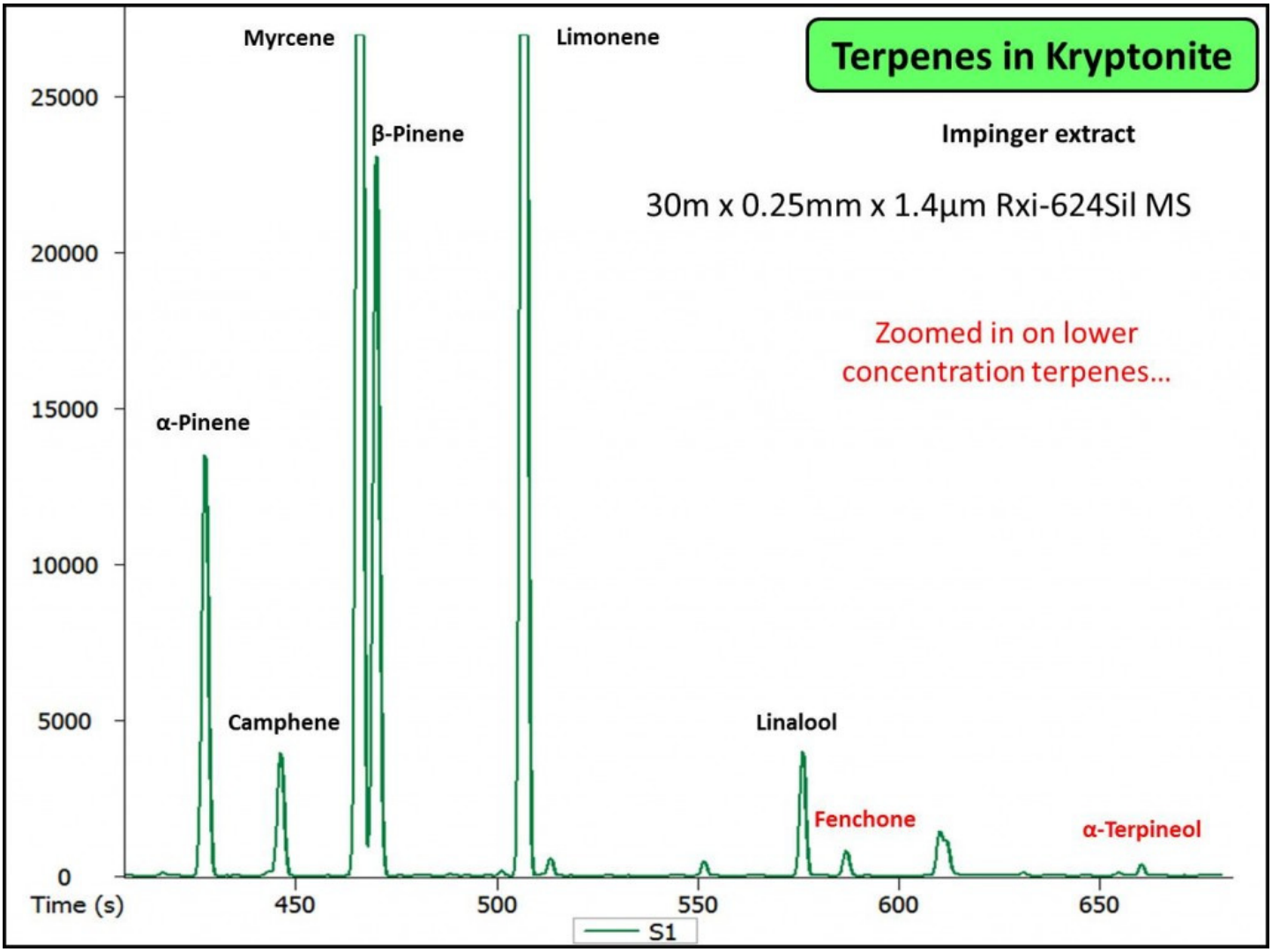
Restek continues to support the medical cannabis analysis community with GC and LC columns, accessories, and reference materials. Check out our Medical Marijuana web page.

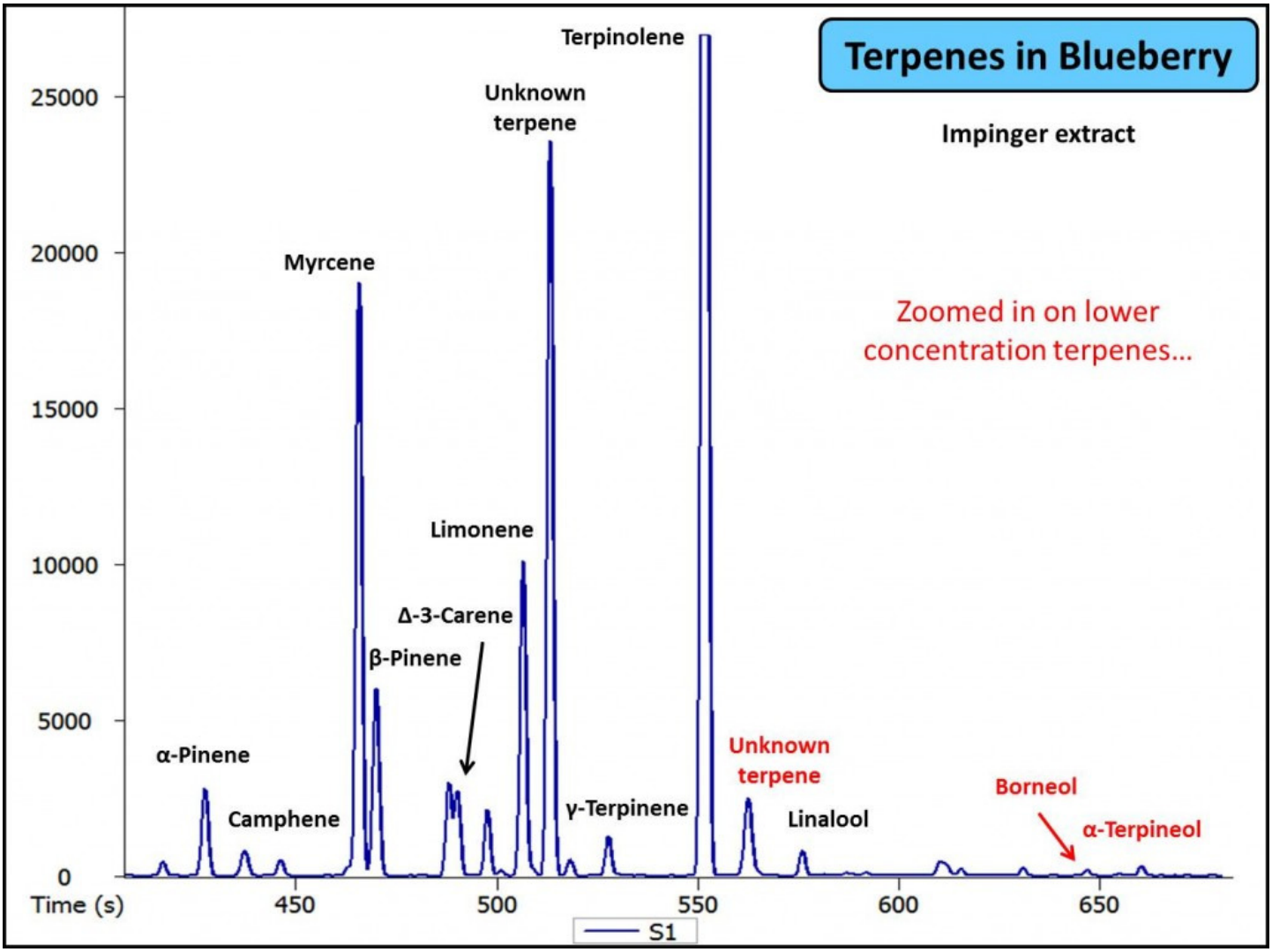












This entry was posted on Monday, March 17th, 2014 at 6:32 pm and is filed under QuEChERS, GCxGC, Medical Marijuana. You can follow any responses to this entry through the RSS 2.0 feed. You can leave a response, or trackback from your own site.

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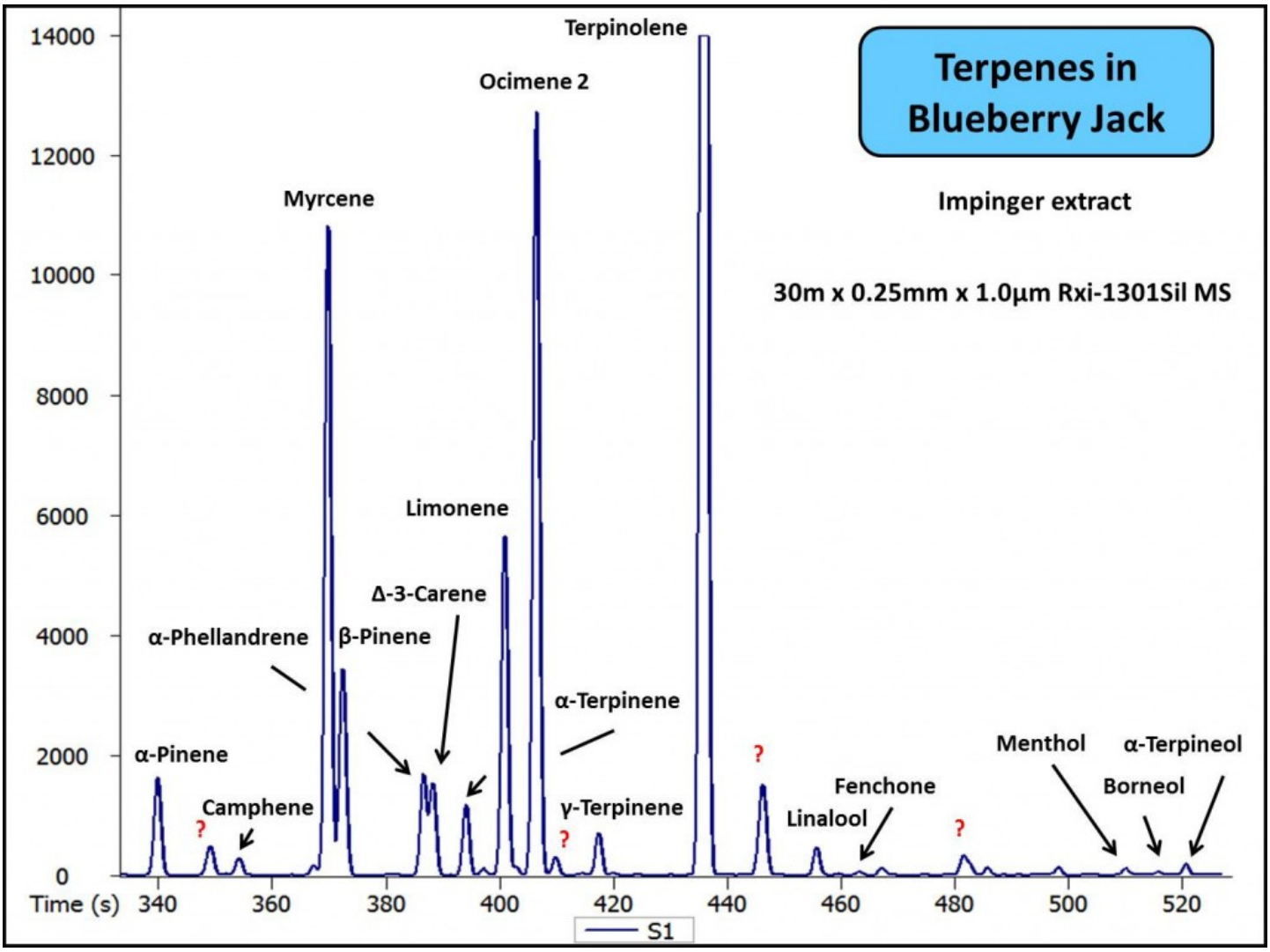
« Early Eluting Terpenes – GC – Medical Cannabis
Keep up with ChromaBLOGraphy — new subscription option added »

Terpenes in Blueberry Jack Medical Cannabis – GC – More Identified

March 26th, 2014 by Jack Cochran

Based on acquisition of new terpene standards I was able to better profile the Blueberry Jack medical cannabis impinger sample on the beta-version 30m x 0.25mm x 1.0µm Rxi-1301Sil MS GC column. Check it out…

I'm looking for suggestions on terpene identification for the ones marked by "?" in the chromatogram below. Help, please!



This entry was posted on Wednesday, March 26th, 2014 at 10:37 pm and is filed under New GC Columns, Medical Marijuana. You can follow any responses to this entry through the RSS 2.0 feed. You can leave a response, or trackback from your own site.

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« Some answers on the Chromatography challenge posted before: Does a GC-capillary column produce different retention times when installed in the opposite direction?
LC/MS/MS Analysis of Synthetic Cannabinoid Metabolites in Urine – The Saga Begins »

First QuEChERS Extraction of Marijuana with GCxGC-TOFMS Analysis, dudes…

April 20th, 2011 by Jack Cochran

My Restek colleagues Julie Kowalski, Michelle Misselwitz, and Amanda Rigdon, along with Professor Frank Dorman from The Pennsylvania State University (PSU), report here what we believe is the first QuEChERS extraction of marijuana, with subsequent analysis using GCxGC-TOFMS. We were assisted in this task by Randy Hoffman, a Police Officer Specialist/Evidence Technician at PSU, who very kindly donated the samples confiscated from some students who probably should have had their minds on class, not grass.

Our interest in this topic is mainly about medicine, since at least 15 states (Pennsylvania is not one of them) and Washington DC have enacted laws to legalize medical marijuana. When you fill your prescription, how do you know your remedy is active (potency, or cannabinoid content), pesticide-free, and without bacteria or mold or fungus? Well, you probably don't, but eventually FDA might get involved and we'll need good, robust analytical methods, especially for pesticide analysis. We think that you might be able to do one extraction for both potency and pesticide determinations and we're high on QuEChERS, so we went for it.

First, the potency work, or cannabinoids determination. Although you don't need GCxGC for the BIG THREE (cannabidiol, Δ^9-THC, cannabinol; by the way, Restek has a reference material containing these compounds…), we used it to illustrate one of the benefits of that technique, the structured chromatogram. In the first figure below, the GCxGC contour plot (or chromatogram), you can see that compound classes position themselves in certain areas. This helps identification, and makes discovery of new compounds within classes a bit easier (e.g. perhaps there are undiscovered cannabinoids out there with medicinal benefits). Zooming in, we can see the terpenoid classes, which are thought to have therapeutic effects. Finally, you can see the cannabinoids, including cannabidiol, one of much interest given that "it has been shown to relieve convulsion, inflammation, anxiety, and nausea, as well as inhibit cancer cell growth" (http://en.wikipedia.org/wiki/Cannabidiol).

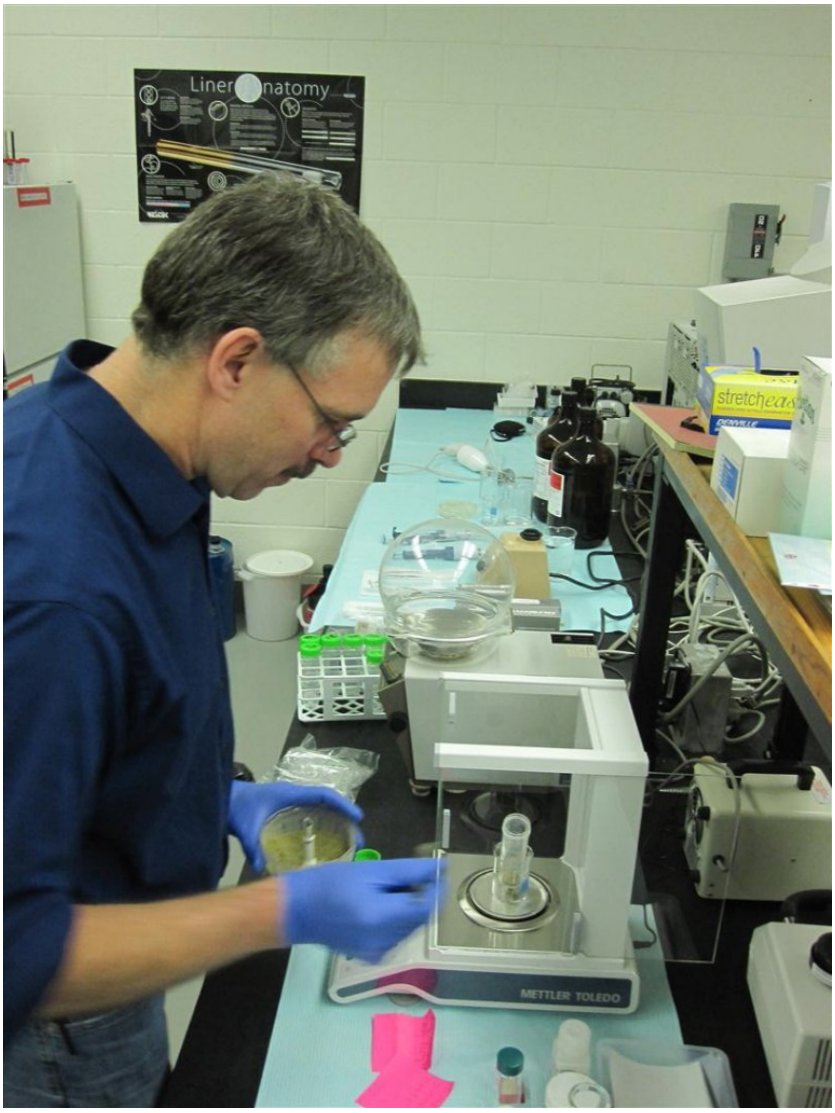
We quantified cannabidiol (CBD), Δ^9-THC (THC), and cannabinol (CBN) for 4 marijuana samples using QuEChERS and GCxGC-TOFMS and the results are presented in the table below. Since the samples had been stored in an evidence locker for over a year in some cases, the CBN content is relatively high versus fresh marijuana. CBN increases as THC degrades. The THC content is in line with what is typically reported for higher grade illicit marijuana.

Stay tuned for a report on pesticide analysis of marijuana using QuEChERS and GCxGC-TOFMS. As you might imagine, the extracts are extremely complex, similar to what we saw in our dietary supplements work.

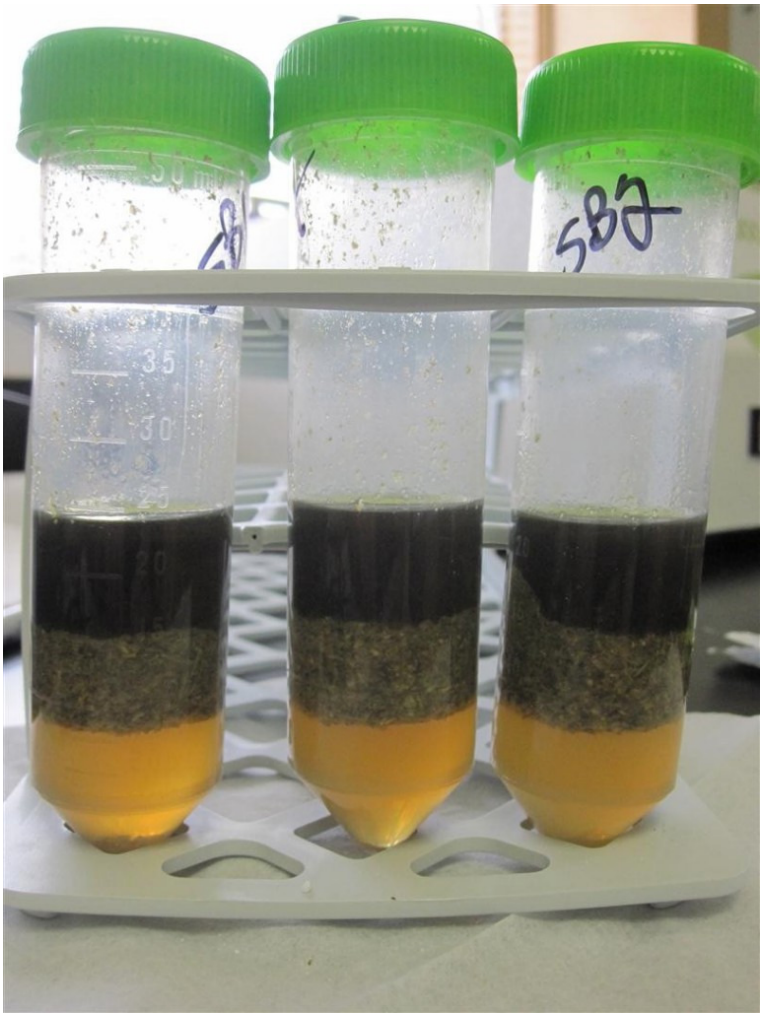
Siezed marijuana for QuEChERS extractions at PSU.

Marijuana for grinding prior to QuEChERS extractions.

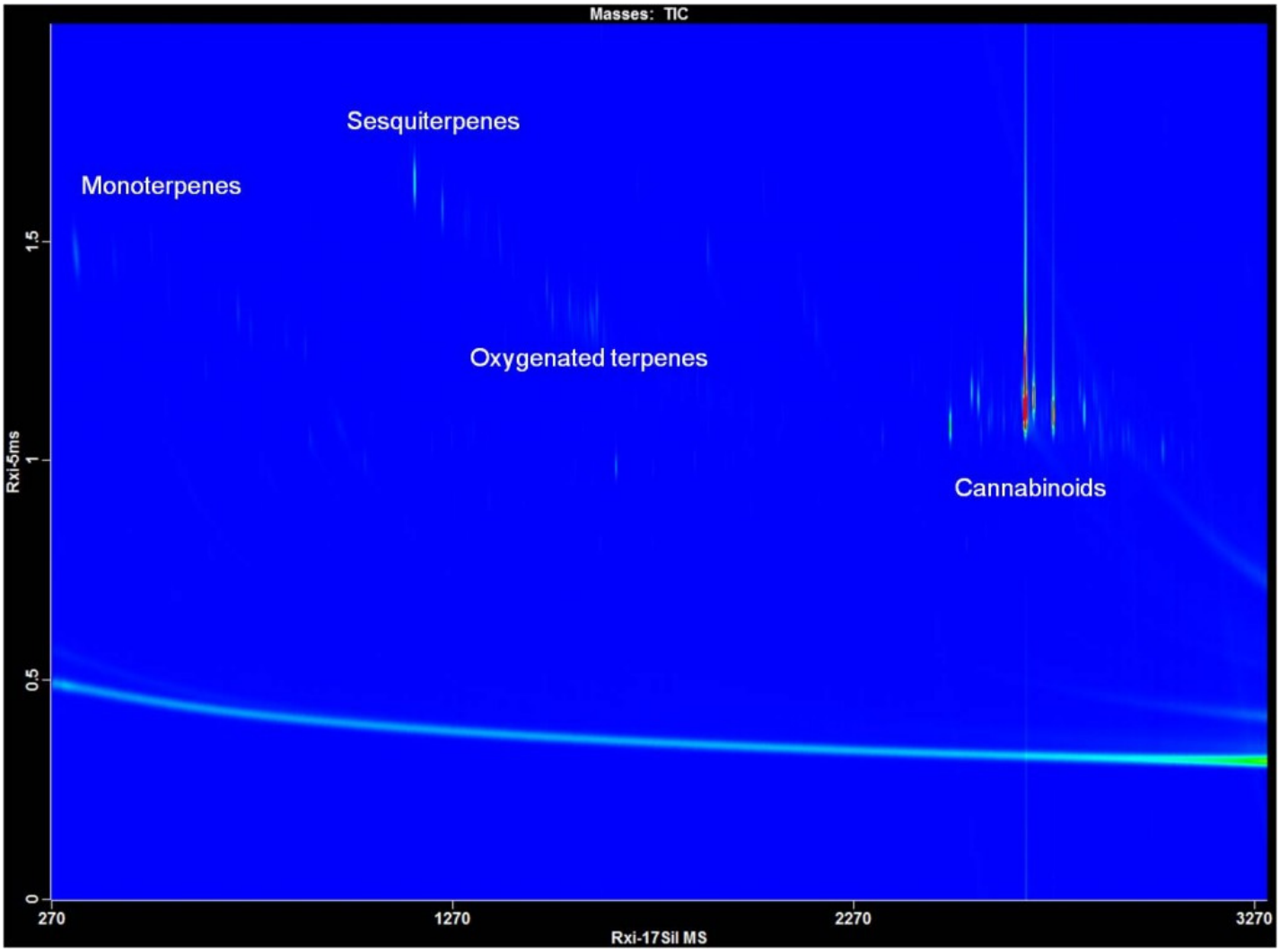
Professor Frank Dorman at Penn State University grinds the goods.



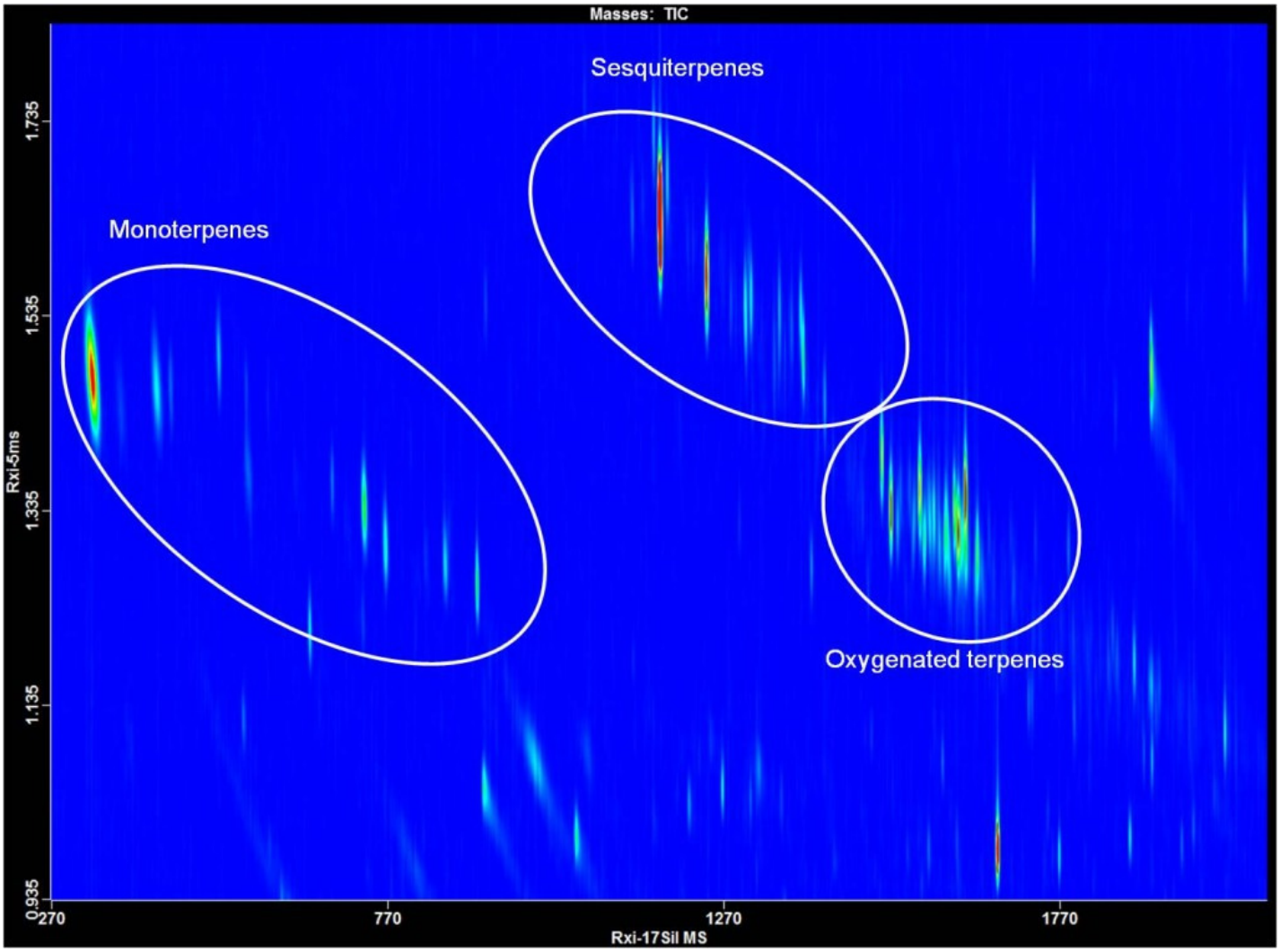
Weighing the marijuana into the QuEChERS extraction tubes. It is full of static!



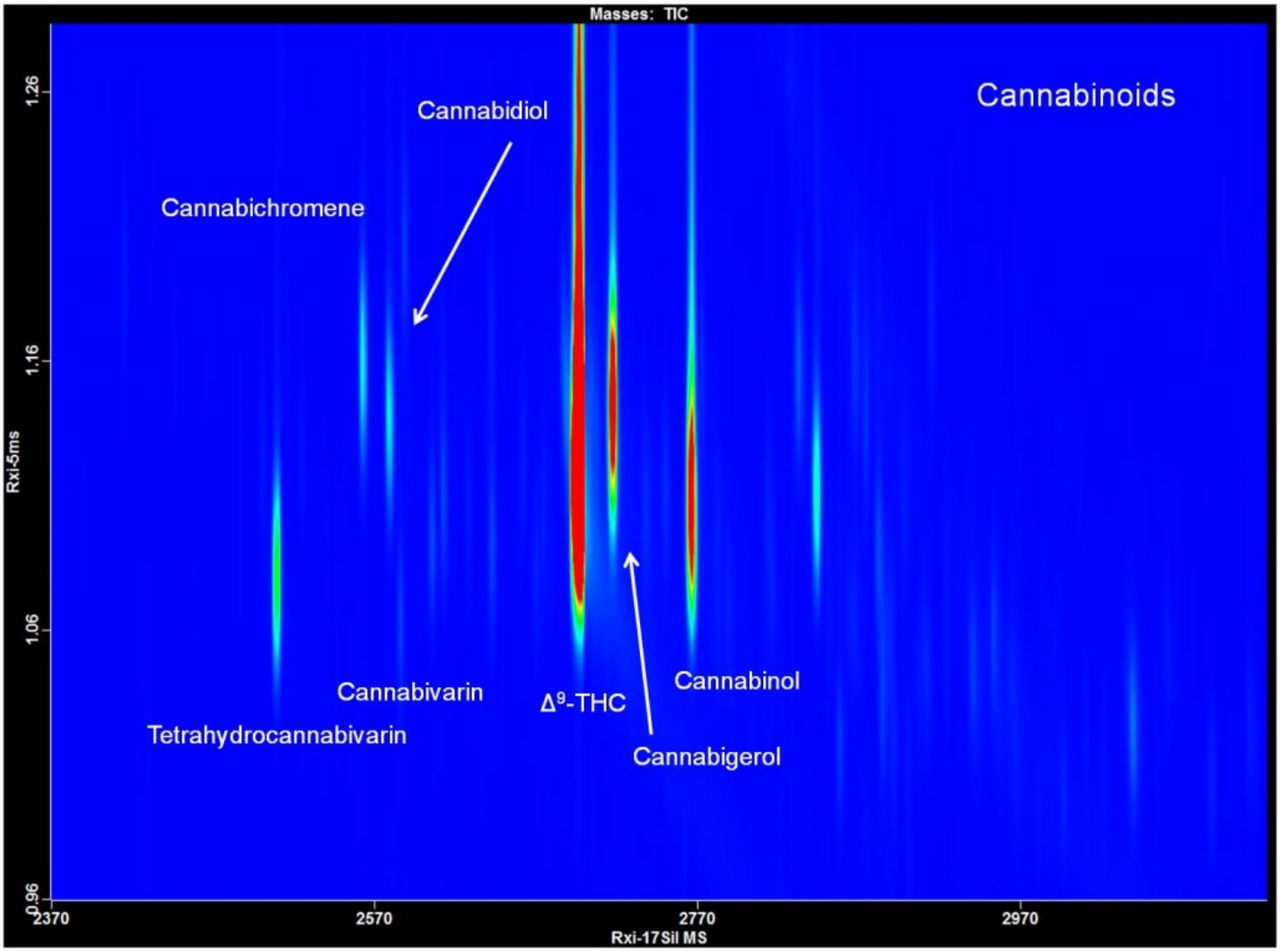
The first QuEChERS extracts of marijuana. They are almost black, and are very complex.



GCxGC-TOFMS contour plot of QuEChERS marijuana extract showing "structured chromatogram", where compound classes elute in certain regions. Rxi-17Sil MS x Rxi-5ms column combination.



Zooming in on the GCxGC terpenoid region for QuEChERS extracts of marijuana.



The cannabinoid region of the GCxGC chromatogram of a QuEChERS extract of marijuana.

Sample	CBD	THC	CBN
S1	0.029	12	1.1
S2	0.016	4.3	1.3
S3	0.034	9.0	1.3
SB9	0.15	10	1.7

Cannibinoid results in percent for samples of marijuana analyzed by QuEChERS and GCxGC-TOFMS.

This entry was posted on Wednesday, April 20th, 2011 at 11:14 pm and is filed under GC/MS, QuEChERS, GCxGC, Medical Marijuana. You can follow any responses to this entry through the RSS 2.0 feed. You can leave a response, or trackback from your own site.

6 Responses to "First QuEChERS Extraction of Marijuana with GCxGC-TOFMS Analysis, dudes…"

1. May 4

Hi,

Great work! I have been doing a Quechers extraction of cannabinoids for edible food products as well as a Quechers extraction of cannabis flowers for pesticide residue testing for over a year. Our laboratory tests medical cannabis samples in California. We would love to see restek offer standards for CBG, THCV, CBC, as well as THCA and CBDA (THCA and CBDA are the major cannabinoid constituents of raw cannabis flowers but are degraded into THC and CBD on a GC column).

I would love to share some data with your group if you are interested.

Thanks,
Josh Wurzer
Laboratory Director
SC Laboratories Inc.

2.  *Jack Cochran* says:
May 12, 2011 at 1:55 am

Greetings Josh!

Thanks so much for your kind comments and for letting me know of your use of QuEChERS. I'll forward your reference materials request to our standards group. I do know they are interested in expanding the line, but some of those neat compounds are SO expensive. I'm just getting ready to post on our pesticide results for the illicit marijuana we extracted. We found numerous pesticides, so the work turned out to be quite interesting. The samples are unbelievably complex, and needed a multidimensional technique, in this case, GCxGC, for the quantitative effort.

Regards,

Jack

3.  *Blake Meinert* says:
May 19, 2011 at 8:45 pm

Hello Jack,

I have had substantial experience analyzing for chlorinated pesticides in soil and water, but I had not considered their use in marijuana crops. What pesticides did you primarily see? Is there one primary pesticide of choice with growers that you know of? This is interesting.

Thanks,
Blake

4.  *Jack Cochran* says:
May 23, 2011 at 2:47 pm

Hi Blake:

The pesticides we saw on our small sample size were o-phenylphenol, hexachlorobenzene, metalaxyl, chlorothalonil, imazalil, and cypermethrin. Interestingly, all are fungicides except for the insecticide, cypermethrin. Mold/fungus apparently is a big problem for marijuana that is being dried/stored, so maybe this finding isn't surprising.

With the small sample size we had, and the fact that all of our samples were illicit marijuana, I'm not sure if there is a "primary pesticide of choice". At least with the medical marijuana, it may be that bifenazate (Floramite) and abamectin (Avid) are the "pesticides of choice" to control spider mites in indoor grow operations. But since this doesn't seem to be a well regulated area yet, I'm not sure if we know what to expect as regards pesticide use.

Jack

5. *High Quality Analysis of Pesticides in Marijuana using QuEChERS, cartridge SPE cleanup, and GCxGC-TOFMS « ChromaBLOGraphy* says:
May 22, 2011 at 3:28 am

[…] we reported on what we believe is the first application of QuEChERS for marijuana, using it for potency analysis with GCxGC-TOFMS. Ultimately, the plan was to determine pesticides […]

6. *The Bard Hits the Bong? « ChromaBLOGraphy* says:
July 2, 2011 at 9:47 pm

[…] cannabis to (1) develop methods for possibly fingerprinting marijuana types, (2) characterize marijuana potency, and (3) analyze for pesticides in marijuana with GCxGC-TOFMS and […]

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« Difficult QuEChERS extracts: Removing fatty acid interferences
Even More Technical Service "Red Flags" – GC »

Accurate Quantification of Cannabinoid Acids and Neutrals by GC – Derivatives without Calculus

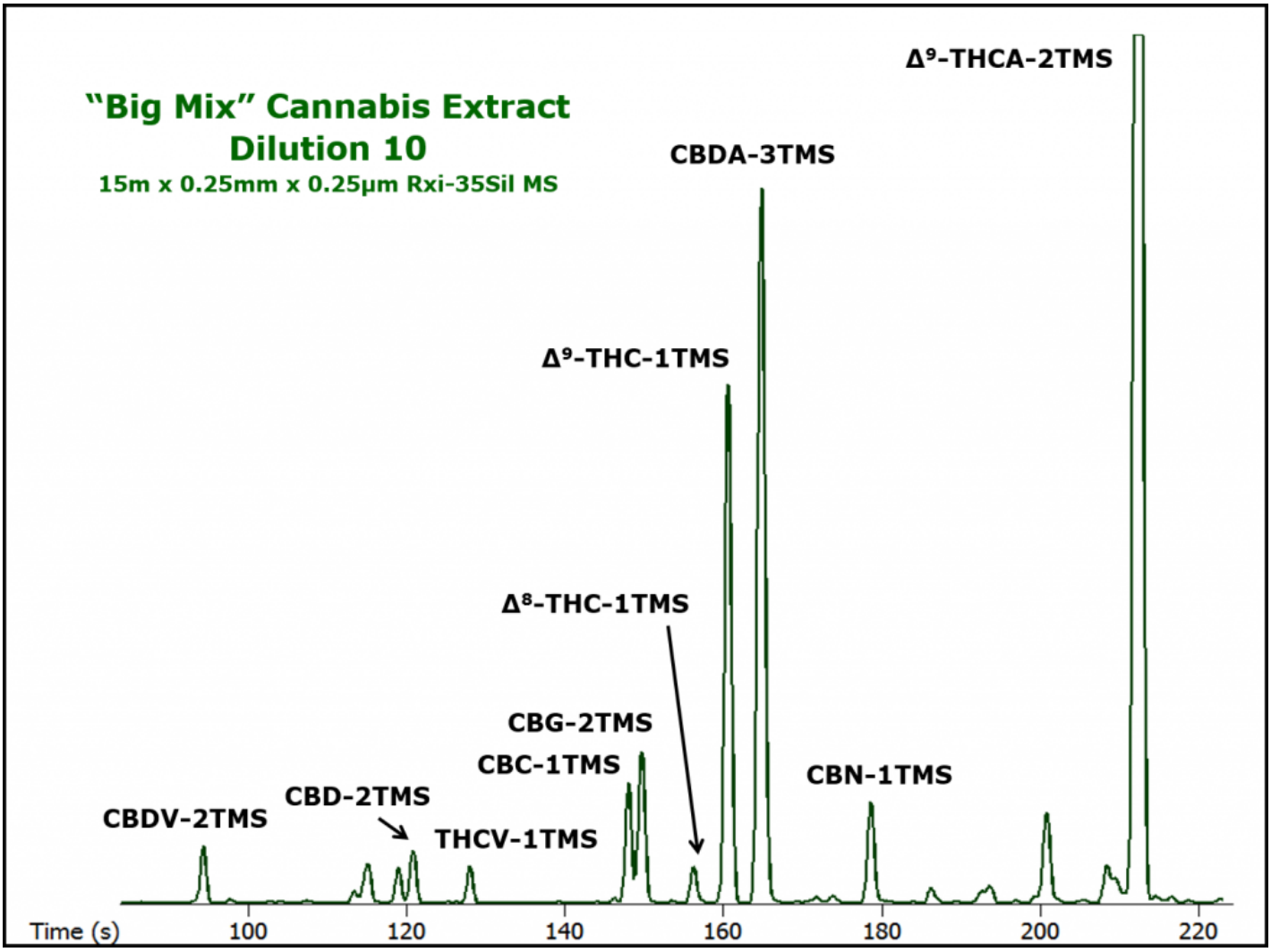
September 9th, 2015 by Amanda Rigdon

Derivatization is a widely-used technique for GC sample preparation across many industries and in widely varied matrices from soil to plastics to blood that is used to make polar and active compounds more amenable to good GC analysis. If you're careful about testing your derivatization procedure during method development, you can be confident that you'll have a reproducible method that can vastly improve the quality of your GC results. While derivatization does require some extra sample handling, the procedure I developed for cannabis plant matrix is very straightforward and easy to perform:

Derivatization Procedure for Cannabis and Hemp Plant Matrices:

- Place 100µL of plant extract into a 1mL Micro-Vial
- Evaporate to dryness at 50°C under a gentle stream of nitrogen
- Add 50µL ethyl acetate and 50µL BSTFA + 1% TMCS
- Incubate at 70°C for 30 minutes
- Cool and dilute with ethyl acetate if desired

In my last blog, I introduced the concept of derivatization for use in cannabis or hemp cannabinoid testing. Once acidic cannabinoids are derivatized, they no longer break down in the GC inlet and can be quantified separately from the neutral cannabinoids. I demonstrated this through derivatization of high-level solvent standards, but work with solvent standards is a far cry from matrix work, which means the procedure needed to be tested in matrix. To kick off the matrix test, I spiked an extract with the most common cannabinoids of interest, derivatized it using the procedure listed above, and my colleague, Jack Cochran, analyzed it via GC-FID with our Rxi-35Sil MS GC column. We can see that we have a beautiful chromatogram with all of the derivatized cannabinoids separated, and very little matrix interference.



In addition to confirming that all derivatization sites are indeed derivatized by analyzing the standards with GC-MS (this is shown in my last blog), we also tested derivatization efficiency using a cannabis extract previously generated at Penn State University with the help of Professor Frank Dorman and a Police Officer Specialist. Because derivatization is a chemical reaction, the derivatization reagent gets used up during the derivatization reaction. Because plant matrix contains many other derivatizable compounds like sugars and sterols, these other compounds may compete for the derivatizing reagent, possibly resulting in the reagent getting used up before all of our analytes of interest can be derivatized.

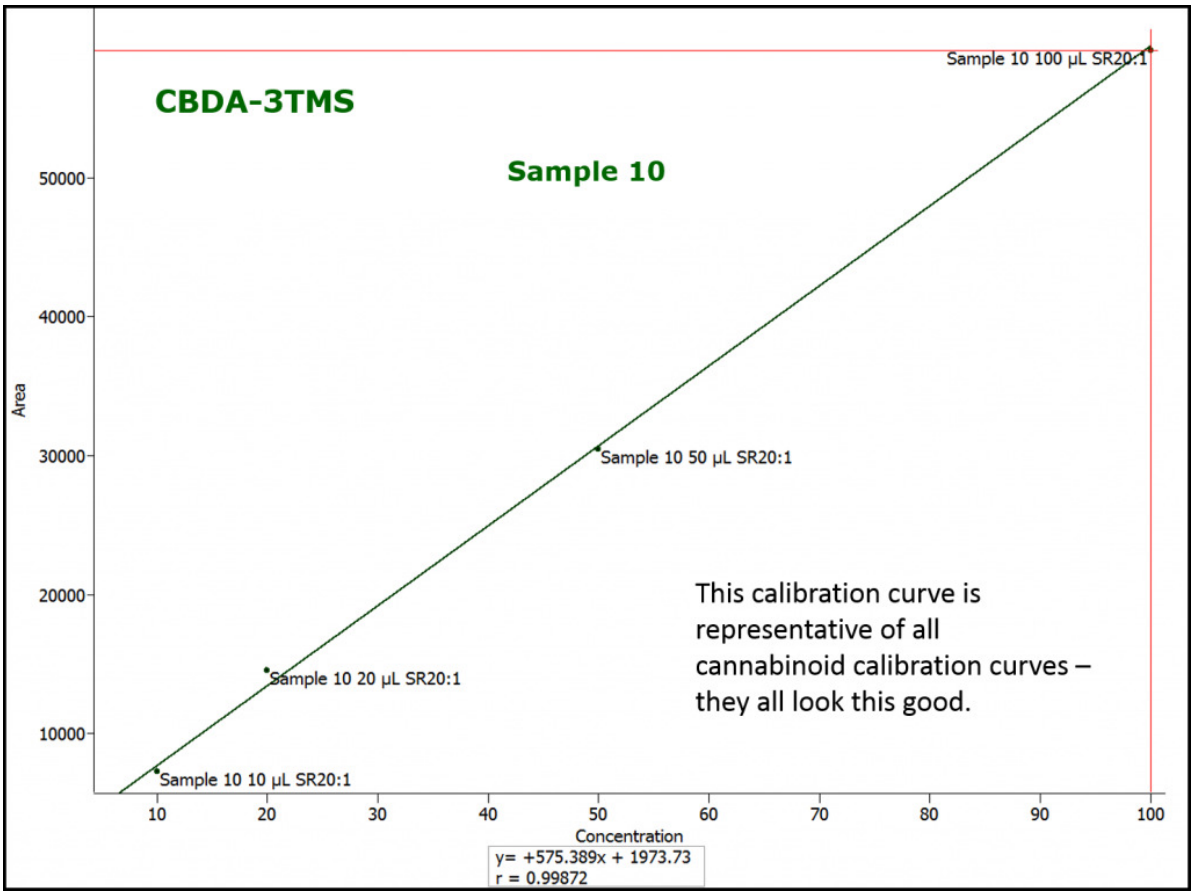
So how can we be sure our derivatization is going to completion in the presence of matrix? There are a couple things we can do, the first of which is really simple. We can see in our procedure that we use a hefty 50µL of derivatizing reagent per 100µL of cannabis extract. We know that our extract contains a lot less than 50mg of plant matrix, not all of which is derivatizable. This means that by adding 50mg of BSTFA per 100µL of sample, we can be confident that we have a significant excess of derivatizing reagent as compared to derivatizable groups in our sample. Excess derivatizing reagent means that it will never be completely used up, ensuring the reaction will go to completion no matter what.

A more quantitative way to test derivatization efficiency in a matrix where you can't get blanks is to evaluate analyte linearity with differing amounts of matrix. For example, if you derivatize four THCA-containing samples prepared using 10, 20, 50, and 100µL of cannabis extract and plot the area of THCA versus sample amount, you should end up with a straight line if your derivatization is going to completion. If it's not, then you'll likely see THCA area fall off for the samples containing more matrix since the derivatization reagent is being used up before all the analyte in the higher matrix level sample is derivatized. To test our procedure, we did just that. We can see that our linearity looks beautiful for all of the ca completion.

Sample 10 Dilution Derivatization Linearity

Compound	Mol Wt		t_R sec	t_R min		r
CBDV-2TMS	430		94.6	1.58		0.9994
CBD-2TMS	458		121.0	2.02		0.9995
THCV-1TMS	358		128.2	2.14		0.9991
CBC-1TMS	386		148.2	2.47		0.9989
CBG-2TMS	460		149.9	2.50		0.9993
Δ^8-THC-1TMS	386		156.4	2.61		0.9993
Δ^9-THC-1TMS	386		160.7	2.68		0.9990
CBDA-3TMS	574		164.8	2.75		0.9987
CBN-1TMS	382		178.7	2.98		0.9988
Δ^9-THCA-2TMS	502		212.3	3.54		0.9992

15m x 0.25mm x 0.25µm Rxi-35Sil MS; 10, 20, 50, 100 µL samples



In addition to verifying that the derivatization reaction goes to completion in the presence of plant matrix, we also verified the procedure using several different samples which were generated at the same time as the sample shown in the figure above. Our preliminary work is still looking good, which is exciting, but what about all of the other matrices cannabis chemists have to work with? Well, we're planning on moving the work forward into edible matrices next, so stay tuned for an update!

This entry [illegible] led under Tips & Tricks, Medical Marijuana.

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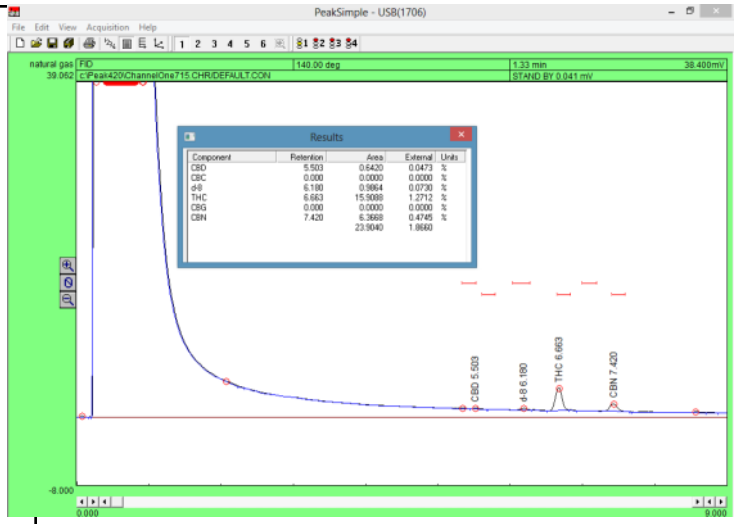
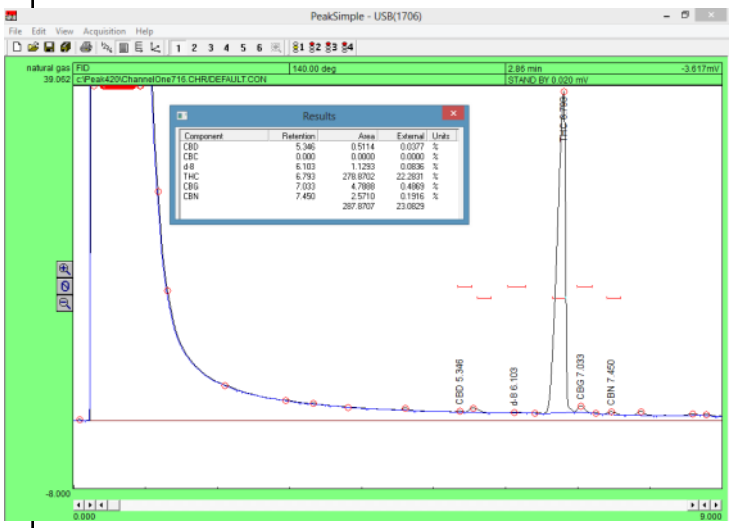
Medical Cannabis Potency Testing using the SRI 8610C FID GC

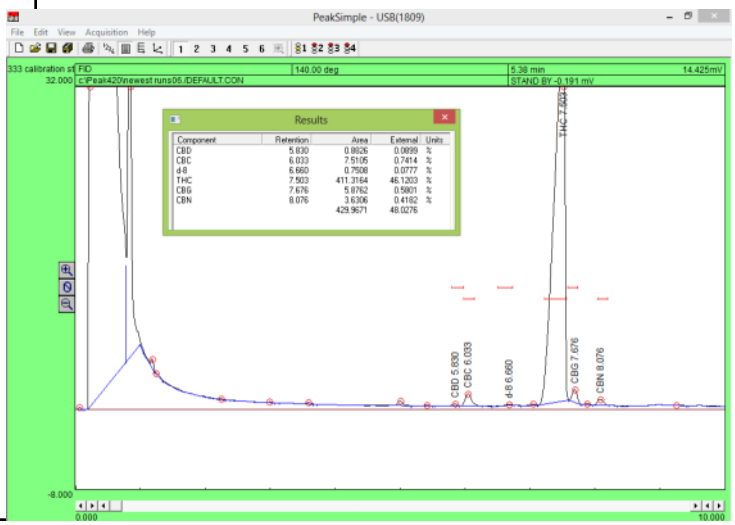
This is a chromatogram of a low-potency cannabis flower with 1.2% THC.



This is a chromatogram of a high-potency cannabis flower with 22.3% THC.



This is a chromatogram of a typical cannabis concentrate with 46.1% THC.



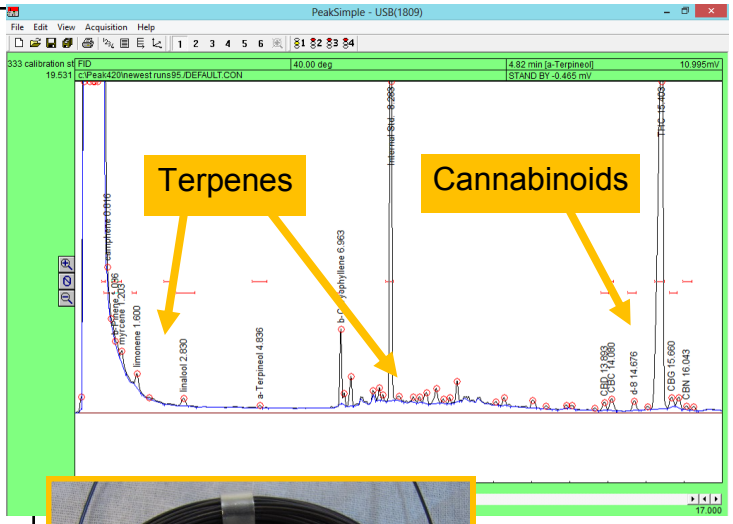
This concludes the *Medical Cannabis Potency Testing* Document. See www.srigc.com for more documents, resources, and sales information.

Medical Cannabis Potency *and* Terpenes Analysis
Using the SRI 8610C FID GC

The cannabinoids (psychoactive components) and terpenes (aroma compounds) can be measured quantitatively on one 20-minute run using the stock medical cannabis potency column (15-meter MXT-500 with a .15 um film) on an 8610C FID Medical Cannabis Potency GC.

Refer to the "Medical Cannabis Potency" Document found on our website www.srigc.com in order to setup the software, extract and inject the sample, and interpret the results. The only difference between the potency analysis and running potency *and* terpenes is the **gain setting** and the **temperature program**.

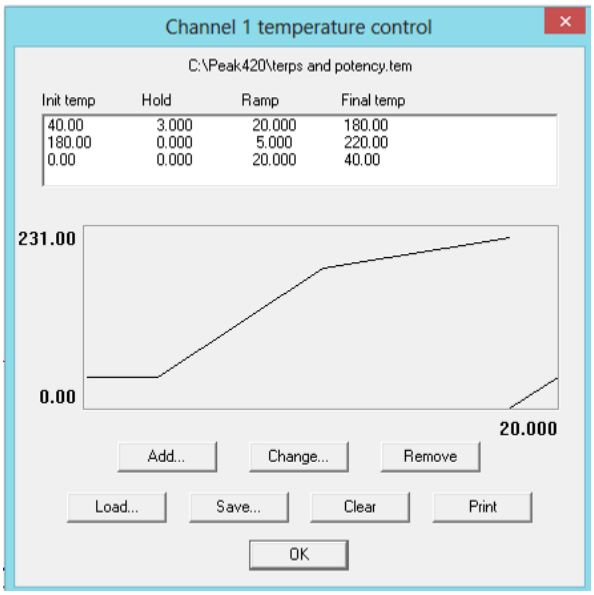
With the red lid open and staring down, the FID gain switch is located next to the FID detector on the far right of the GC. Usually the gain switch for the FID detector is set up to "MED". For this analysis, put the switch in the middle position on "**HIGH**". This will make the FID sensitivity about 20 times greater. This is important because the terpenes are present at much smaller concentrations in cannabis samples than the cannabinoids. This may present a problem with concentrates exceeding 40% THC (the peak will go off-scale) but typical cannabis flowers will present no problems. **Remember to recalibrate after changing the gain setting.**

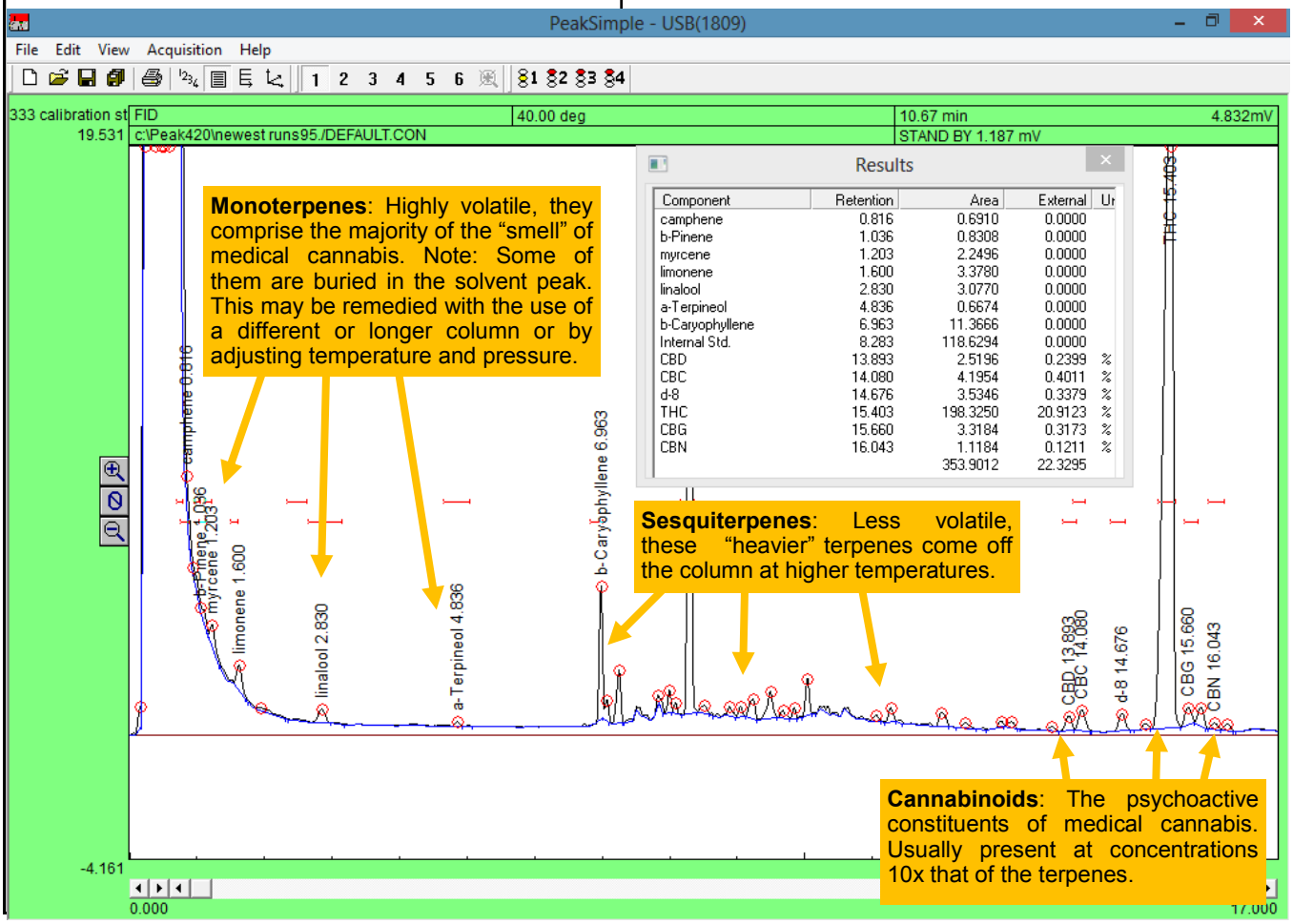


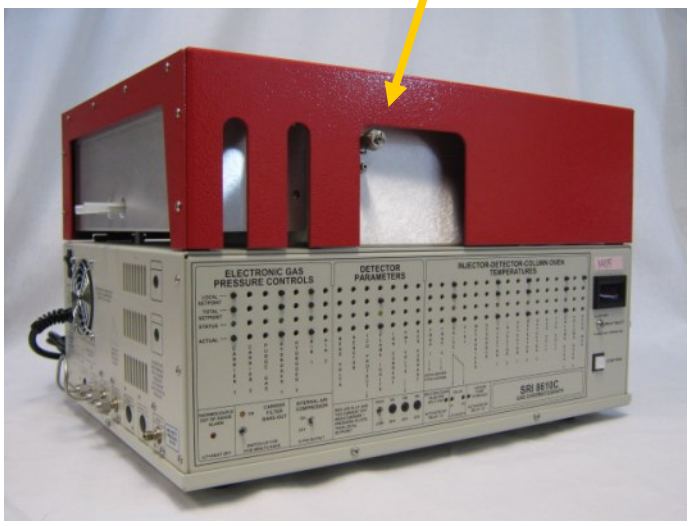
Medical Cannabis Potency *and* Terpenes Analysis
Using the SRI 8610C FID GC

The temperature program for the potency and terpenes run is shown to the right. The temperature begins and holds for 3 minutes at 40 C to allow the most volatile terpenes to separate. Between 180 C and 220C a slower temperature ramp achieves optimum separation of the cannabinoids.

A terpenes and potency chromatogram will look something like the one below. The cannabinoids have been calibrated, the terpenes have not.



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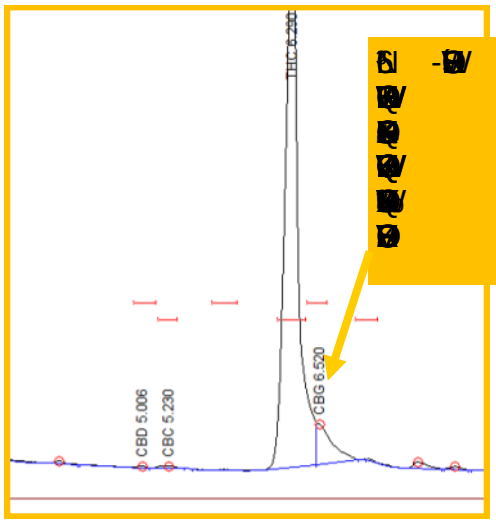
DETECTOR PARAMETERS
INJECTOR-DETECTOR-COLUMN OVEN TEMPERATURES
SRI 8610C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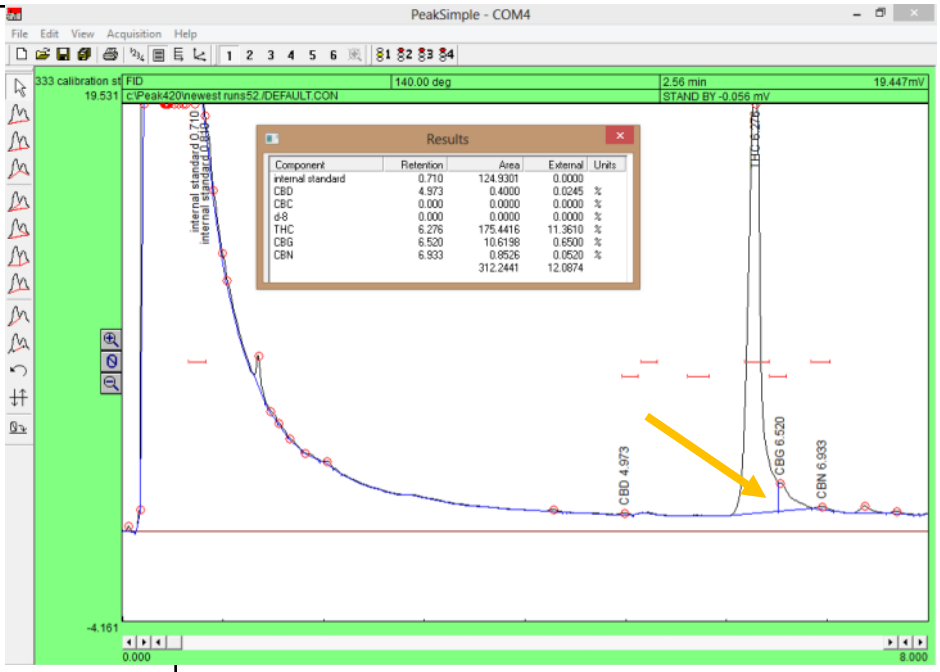
CBC 5.230
CBG 6.520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CBD 4.973 0.4000 0.0245 %
CBC 0.000 0.0000 0.0000 %
d-8 0.000 0.0000 0.0000 %
THC 6.276 175.4416 11.3610 %
CBG 6.520 10.6198 0.6500 %
CBN 6.933 0.8526 0.0520 %
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CBD 4.973
CBG 6.520
CBN 6.933
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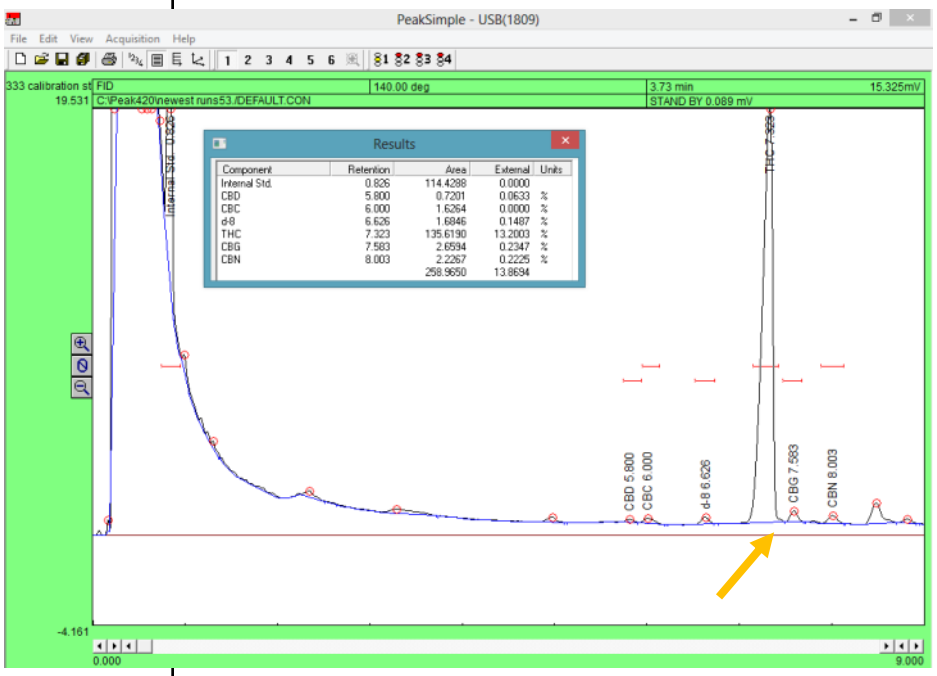
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Component Retention Area External Units
Internal Std. 0.826 114.4298 0.0000
CBD 5.800 0.7201 0.0633 %
CBC 6.000 1.6264 0.0000 %
d-8 6.626 1.6846 0.1487 %
THC 7.323 135.6190 13.2003 %
CBG 7.583 2.6594 0.2347 %
CBN 8.003 2.2267 0.2225 %
258.9650 13.8694
CBD 5.800
CBC 6.000
d-8 6.626
CBG 7.583
CBN 8.003
-4.161
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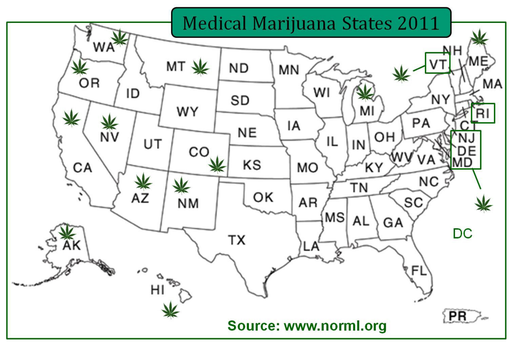
9.000

High Quality Analysis of Pesticides in Marijuana for Food and Medicine using QuEChERS, Cartridge SPE, GCxGC-TOFMS, and LC-MS/MS

Jack Cochran, Julie Kowalski, Sharon Lupo, Michelle Misselwitz, Amanda Rigdon, Jason Thomas, Restek Corporation
Frank Dorman, Jessica Westland, Amanda Leffler, The Pennsylvania State University

- Over 15 states in the USA have medical marijuana laws.
  - Therapeutic benefits include pain relief, nausea control, appetite stimulation, and muscle relaxation.
  - Marijuana is illegal on the federal level so patients have no assurances on medicine safety, including for pesticide residues.
- We used the QuEChERS sample preparation approach for extracting pesticides from marijuana.
  - But dispersive SPE did not have the cleanup capacity for GCxGC work.
  - Instead, we employed cartridge SPE for cleanup for GCxGC.
- GCxGC-TOFMS and LC-MS/MS were used for pesticide determinations in cleaned up QuEChERS extracts.
  - The selectivity of advanced techniques was needed due to sample extract complexity, even after dilution/cleanup.
  - LC-MS/MS was necessary for abamectin because it does not gas chromatograph.

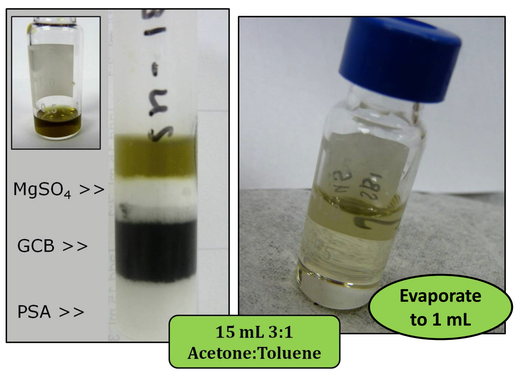


Preparing Marijuana Samples at PSU

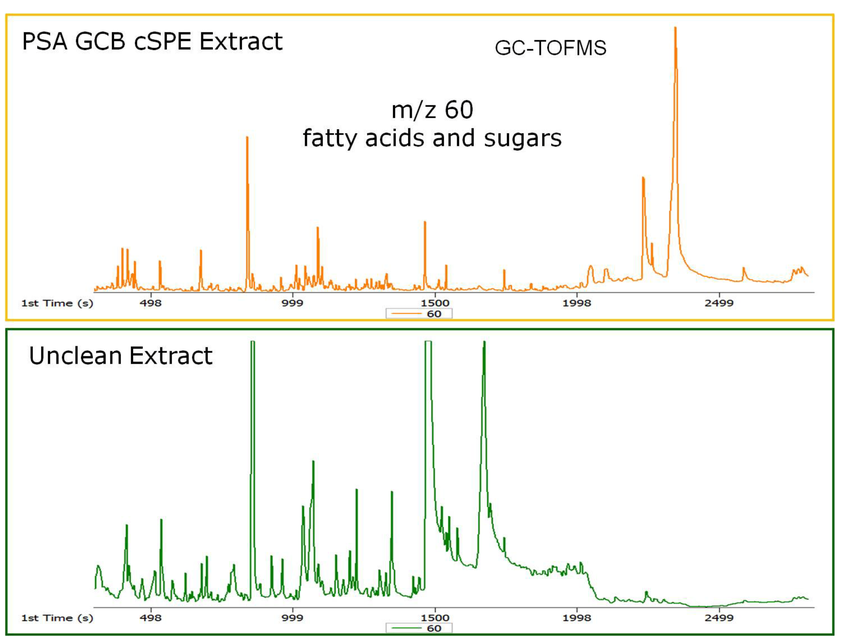
QuEChERS Procedure for Marijuana

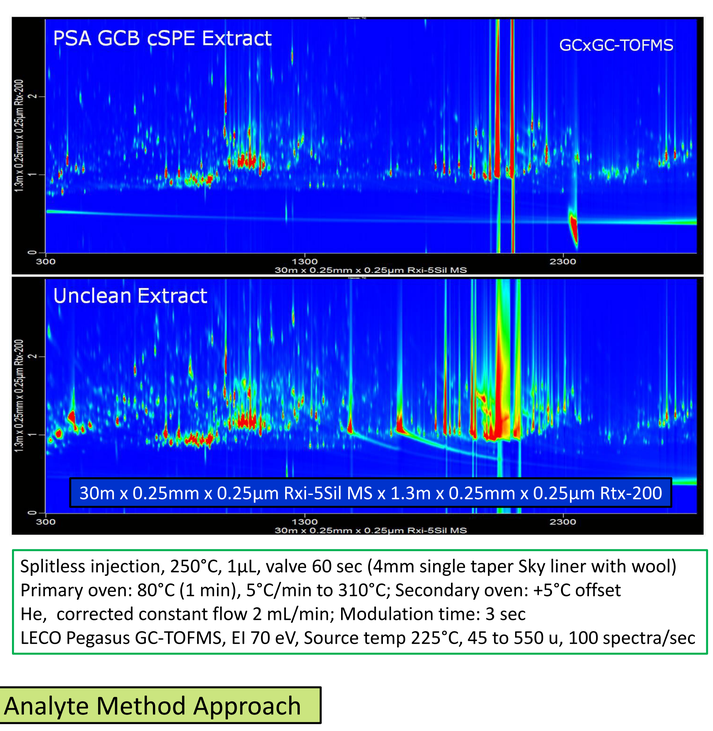
Up to 2 g ground marijuana – 50 mL centrifuge tube
10 mL MeCN
10 mL H_2O
Shake to wet
Soak one hour
Add spikes and internal standards
Vortex 30 min
Add QuEChERS EN salts
Shake 1 min
Centrifuge 5 min at 3000g
Remove extract for cleanup and analysis
GCxGC-TOFMS and LC-MS/MS

Cartridge SPE Clean-up Prior to GCxGC-TOFMS



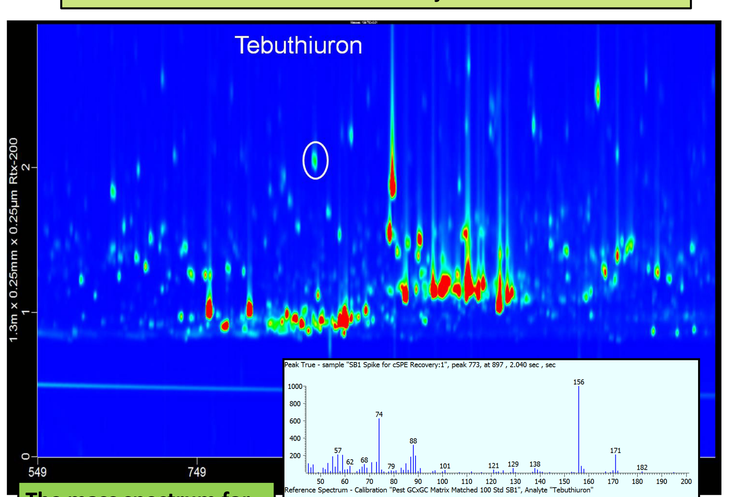
High Capacity Cartridge SPE Produces a Cleaner Extract by Removing Interfering Fatty Acids and other Matrix Co-extractives





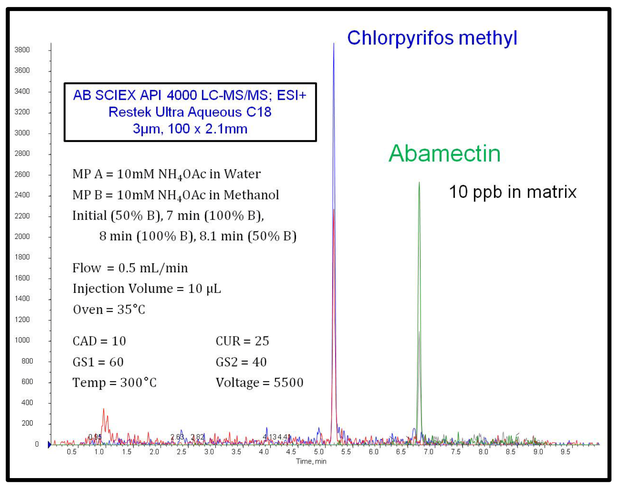
Splitless injection, 250°C, 1µL, valve 60 sec (4mm single taper Sky liner with wool)
Primary oven: 80°C (1 min), 5°C/min to 310°C; Secondary oven: +5°C offset
He, corrected constant flow 2 mL/min; Modulation time: 3 sec
LECO Pegasus GC-TOFMS, EI 70 eV, Source temp 225°C, 45 to 550 u, 100 spectra/sec

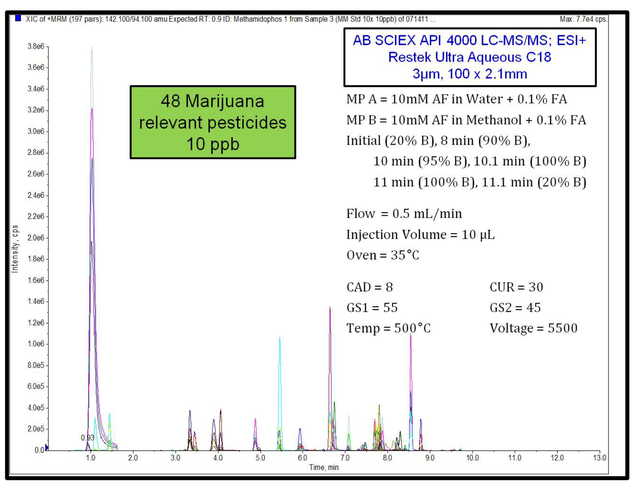
GCxGC Separates Pesticides from Remaining Matrix Co-extractives in a Marijuana Extract



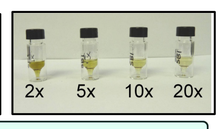
The mass spectrum for Tebuthiuron in the extract is very good due to GCxGC separation.

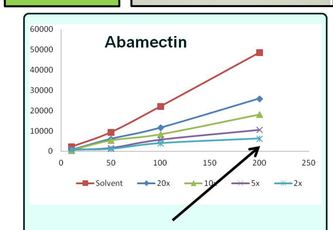
LC-MS/MS of Marijuana Pesticides – Abamectin Required a Single Analyte Method Approach

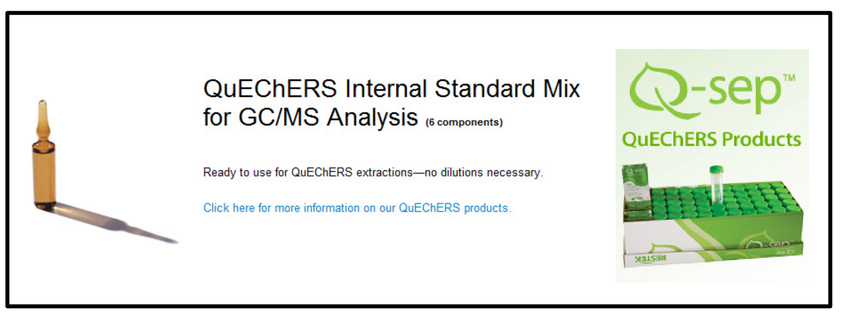


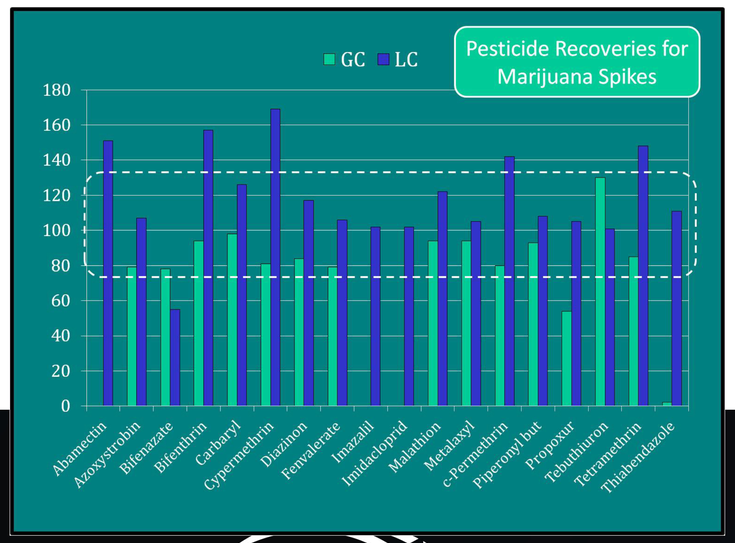


Dilute and shoot?







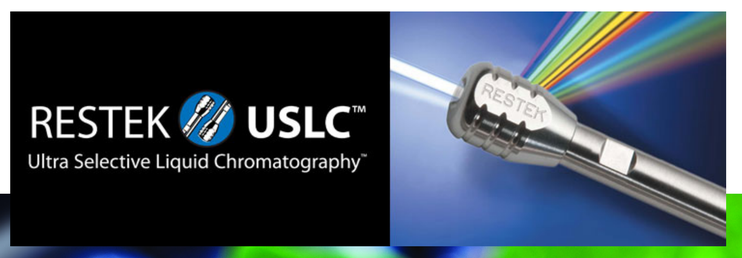


Incurred Pesticides in Marijuana Sample 2Q2

Pesticide	LC (ppb)	GC (ppb)
Imazalil	410	NA
Bifenazate	1100	2180
Piperonyl butoxide	37	41
trans-Permethrin	660	1100
cis-Permethrin	1200	690
o-Phenylphenol	NA	280
4,4'-DDE	NA	30

NA = not analyzed by this method

"Effervescent Health Formula"



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Technical Article

High-Quality Analysis of Pesticides in Cannabis

Using QuEChERS, Cartridge SPE Cleanup, and GCxGC-TOFMS

By Jack Cochran, Julie Kowalski, Sharon Lupo, Michelle Misselwitz, and Amanda Rigdon

- Quickly and effectively extract medical marijuana samples for pesticide analysis.
- Cartridge SPE cleanup of dirty extracts improves GC inlet and column lifetimes.
- Selective GC columns increase accuracy of pesticide determinations for complex samples.

Over 20 states in the U.S. have legalized the use of recreational or medical cannabis because of therapeutic benefits for ailments such as cancer, multiple sclerosis, and ALS. Dosing methods include smoking or vaporizing and baked goods. Unlike other prescribed medicines regulated by U.S. FDA, marijuana is a Schedule 1 drug and is illegal on the federal level. As a result, medical cannabis patients have no safety assurances for their medication, which could contain harmful levels of pesticide residues. Currently, medical marijuana pesticide residue analysis methods are poorly defined and challenging to develop due to matrix complexity and a long list of potential target analytes.

In order to address matrix complexity, we combined a simple QuEChERS extraction approach with cartridge SPE (cSPE) cleanup, followed by GCxGC-TOFMS. Acceptable recoveries were obtained for most pesticides, and incurred pesticide residues were detected in some of the illicit marijuana samples used for method development.

QuEChERS Extraction Saves Time and Reduces Hazardous Solvent Use

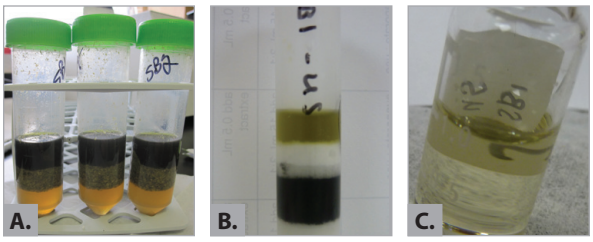
Trace residue extraction procedures from dry materials like medical cannabis typically involve large amounts of solvent, long extraction times, and tedious concentration steps similar to the Soxhlet procedure or multiresidue methods from the Pesticide Analytical Manual. QuEChERS, with its simple 10 mL acetonitrile shake extraction and extract partitioning with salts and centrifugation, offers time savings, glassware use reduction, and lower solvent consumption.

Water was added to finely ground, dry cannabis samples to increase QuEChERS extraction efficiency, especially for more polar pesticides. A vortex mixer was used to shake the solvent and sample for at least 30 minutes prior to extract partitioning. When finished, it was easy to transfer the supernatant from the QuEChERS extraction tube for subsequent cSPE cleanup prior to analysis with GC or LC (Figure 1).

Cartridge SPE Cleanup Improves GC Inlet Uptime

Injecting chlorophyll-laden extracts into a GC gives reduced recoveries for less volatile pesticides, and results in degradation of sensitive pesticides like DDT and Dicofol (Table I). SPE cleanup with a 500 mg graphitized carbon black/500 mg PSA cartridge removes chlorophyll and traps fatty acids that interfere with qualitative pesticide identification and bias quantification. cSPE has increased sorbent capacity over dispersive SPE for thorough cleanup of complex extracts.

Figure 1: A quick and easy QuEChERS extraction, combined with cSPE, effectively prepared extracts for pesticide residue analysis from highly complex marijuana samples.



A. Post-centrifugation QuEChERS extracts

B. QuEChERS extracts loaded on SPE cartridge

C. Final extract

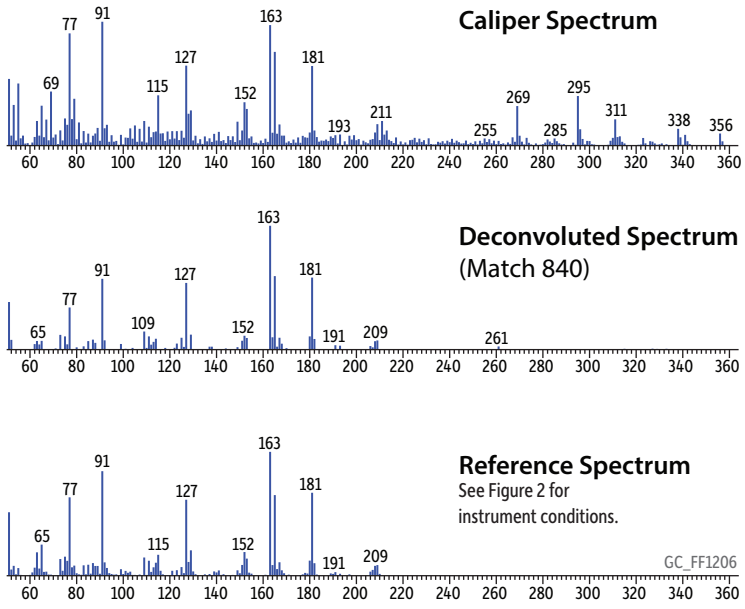
Orthogonal GC Columns Increase Separation Power for More Accurate Pesticide Results

GCxGC is a powerful multidimensional approach that gives two independent separations in one instrumental analysis. An Rxi®-5Sil MS and Rtx®-200 column combination distributes pesticides broadly in both dimensions, providing a highly orthogonal GCxGC system. More important though is separating pesticides from potential isobaric matrix interferences, as seen in the surface plot for the insecticide cypermethrin (Figure 2). Cypermethrin gas chromatographs as four isomers, and all would have experienced qualitative interference and quantitative bias from peaks in the foreground of the surface plot had only 1-dimensional GC been used. With GCxGC-TOFMS, cypermethrin was unequivocally identified in a marijuana sample at a low ppm level (Figure 3).

Summary

QuEChERS and cSPE produced usable extracts from highly complex cannabis samples for high-quality pesticide residue analysis. The multidimensional separation power of GCxGC-TOFMS was then used to correctly identify and quantify pesticides in these complex extracts.

Figure 3: Positive mass spectral identification of incurred cypermethrin in illicit marijuana.



Acknowledgment: Randy Hoffman, a Police Evidence Technician at The Pennsylvania State University (PSU), supplied the seized marijuana samples while overseeing their handling. Frank Dorman at PSU assisted with QuEChERS extractions.

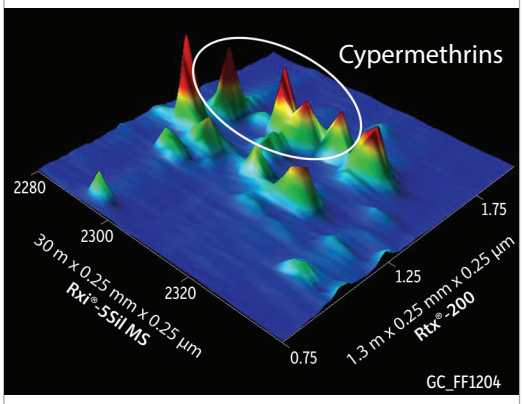
Initially published in *Restek® Advantage*.

Table I: Pesticide recoveries for a QuEChERS extract of cannabis give higher results when cSPE is used for cleanup. Dicofol and DDT are degraded in the inlet for the dirtier extract, yielding high DDD results.

Pesticide	Classification	With cSPE Cleanup (%)	Without cSPE Cleanup (%)
4,4'-DDD	Organochlorine	83	230
4,4'-DDT	Organochlorine	77	9
Bifenthrin	Pyrethroid	86	89
Dicofol	Organochlorine	84	ND
Azinphos methyl	Organophosphorus	79	53
trans-Permethrin	Organochlorine	68	17
Pyraclostrobin	Strobilurin	73	19
Fluvalinate	Pyrethroid	72	23
Difenoconazole	Triazole	67	21
Deltamethrin	Pyrethroid	68	20
Azoxystrobin	Strobilurin	72	27

ND = no peak detected

Figure 2: GCxGC-TOFMS and orthogonal Rxi®-5Sil MS and Rtx®-200 columns allow incurred cypermethrins in a marijuana extract to be separated from interferences (m/z 163 quantification ion).



Peaks	RT 1 (sec.)	RT 2 (sec.)
1. Cypermethrin 1	2292	1.50
2. Cypermethrin 2	2304	1.54
3. Cypermethrin 3	2310	1.53
4. Cypermethrin 4	2313	1.58

Column: Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623), Rtx®-200 1.3 m, 0.25 mm ID, 0.25 µm (cat.# 15124); **Sample:** Diluent: Toluene; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: Sky® 4mm single taper w/wool (cat.# 23303.1); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min; **Oven:** Oven Temp: Rxi®-5Sil MS: 80 °C (hold 1 min) to 310 °C at 5 °C/min, Rtx®-200: 85 °C (hold 1 min) to 315 °C at 5 °C/min; **Carrier Gas:** He, corrected constant flow (2 mL/min); **Modulation**: Modulator Temp. Offset: 20 °C; Second Dimension Separation Time: 3 sec.; Hot Pulse Time: 0.9 sec.; Cool Time between Stages: 0.6 sec.; **Instrument**: LECO Pegasus 4D GCxGC-TOFMS; For complete conditions, visit www.restek.com and enter GC_FF1204 in the search.

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Reliably Confirm Cannabinoids by GC-MS

Using a 12m x 0.20mm ID 0.33µm Rxi®-5ms Column

by Kristi Sellers, Clinical/Forensic Innovations Chemist

- Baseline resolution for all major metabolites.
- Ultra-low bleed at 300°C, for accurate data.
- Bake column at 340°C, to remove derivatization by-products and prolong column life.

Marijuana is one of the most abused substances in the United States. Its common abuse stems from its widespread availability and because it is inexpensive, compared to other abused substances such as cocaine and heroin. Marijuana use typically is determined by screening for its major metabolite in urine, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (Δ9-carboxy-THC), using an immunoassay. When screening results are positive, gas chromatography/mass spectrometry (GC/MS) is employed for confirmation. Marijuana use also can be determined by analyzing other sample matrices, such as blood, hair, oral fluid, or body tissues but, again, positive results require GC/MS confirmation.[1]

GC/MS confirmation methods require sample clean-up and derivatization of target analytes, and call for a capillary GC column that can produce reliable identification and quantification results. Δ9-carboxy-THC is the primary target in GC/MS confirmation analysis, but other marijuana metabolites present in samples include cannabinol, cannabidiol, 11-hydroxy-Δ9-tetrahydrocannabinol (Δ9-hydroxy-THC), Δ9-tetrahydrocannabinol (Δ9-THC), and Δ8-tetrahydrocannabinol (Δ8-THC). Further, a guard column typically is recommended for this analysis, to prevent non-volatile residue in the sample matrix from contaminating the analytical column. The guard column should have an internal diameter approximately equal to that of the analytical column, to minimize changes in flow rate.

For the analysis we show in this article, we used MTBSTFA (N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide) to derivatize the target compounds.[2] The analytical column we chose is our new 12m x 0.20mm ID x 0.33µm Rxi™-5ms column (5% diphenyl / 95% dimethylpolysiloxane stationary phase). The small internal diameter makes this column compatible for use with mass spectrometers, because the column can be operated using a 1.0mL/min. flow rate. The short length produces analysis times of less than 15 minutes for the major metabolite, Δ9-carboxy-THC, which elutes last. Because the target compounds have relatively high molecular weights (310-358 amu, underivatized — see Figure 1), the GC oven must be programmed to a relatively high temperature, 300°C, to keep analysis time short.

The column and conditions we used ensure baseline resolution for all of the metabolites in Figure 2. Figure 2 also shows that the ultra-low bleed exhibited by the Rxi™-5ms column does not interfere with the analysis. The GC oven must heated to an even higher temperature between samples, 340°C, to bake sample matrix interferences and derivatization by-products from the system. Derivatization by-products can be seen in the baseline in Figure 2.

The results of this analysis demonstrate that a 12m x 0.20mm ID x0.33µm Rxi™-5ms column has the selectivity and inertness needed to provide baseline resolution, suitably short analysis times, and no interference from bleed at high temperature. We highly recommend it for this analysis.

Figure 1 Cannabinoids have relatively high molecular weights, so high temperatures must be used in their analysis.

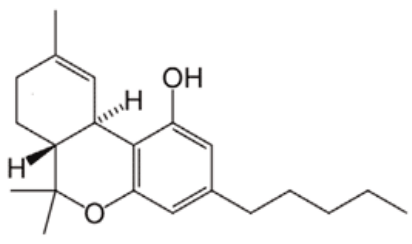
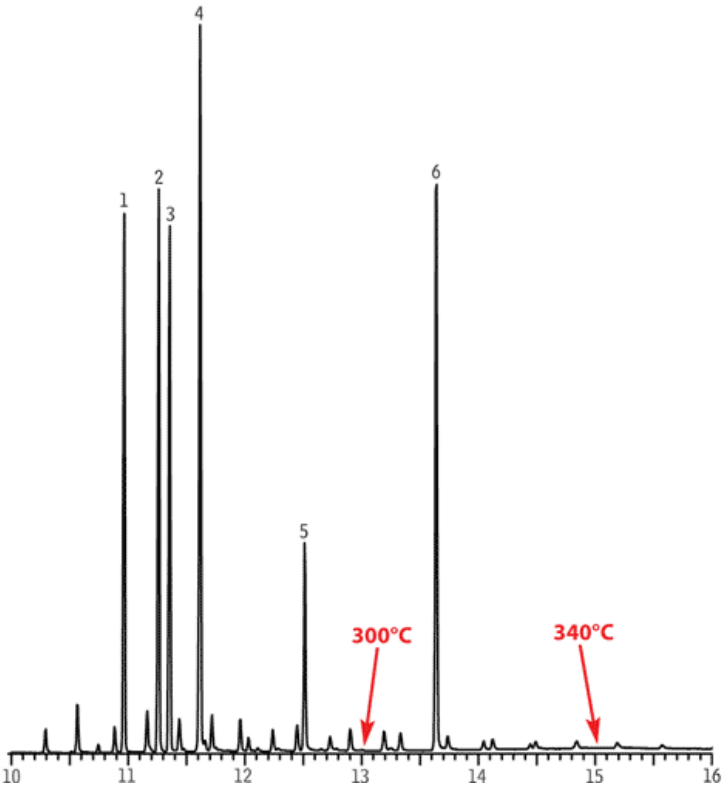


Figure 2 A 12m x 0.20mm ID x 0.33µm Rxi™-5ms column provides baseline resolution and short analysis time for cannabinoids.

1. cannabidiol
2. Δ^8-tetrahydrocannabinol
3. Δ^9-tetrahydrocannabinol
4. cannabinol
5. 11-hydroxy-Δ^9-tetrahydrocannabinol
6. 11-nor-Δ^9-tetrahydrocannabinol carboxylic acid



GC_PH00891

Column:	Rxi™-5ms 12m, 0.20mm ID, 0.33µm (cat.# 13497)
Sample:	1000µg/mL each component in methanol
Inj.:	1.0µL, split, split ratio 25:1, 4mm ID base-deactivated single gooseneck inlet liner w/wool (cat.# 20798-211.1)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	40°C to 340°C @ 20°C/min. (hold 5 min.)
Det:	MS
Transfer line temp.:	280°C
Scan range:	100-550 amu
Ionization:	EI
Mode:	scan

References

1. Smith, F. and J. Siegel *Handbook of Forensic Drug Analysis* Elsevier Academic Press, 2005, pp. 98-151.
2. Clouette, R., M. Jacob, P. Koteel, and M. Spain *Journal of Analytical Toxicology* 17 (1): 1-4 (Jan./Feb. 1993).

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marijuana, *cannabinoid metabolites*, *Rxi-5ms*, *THC*