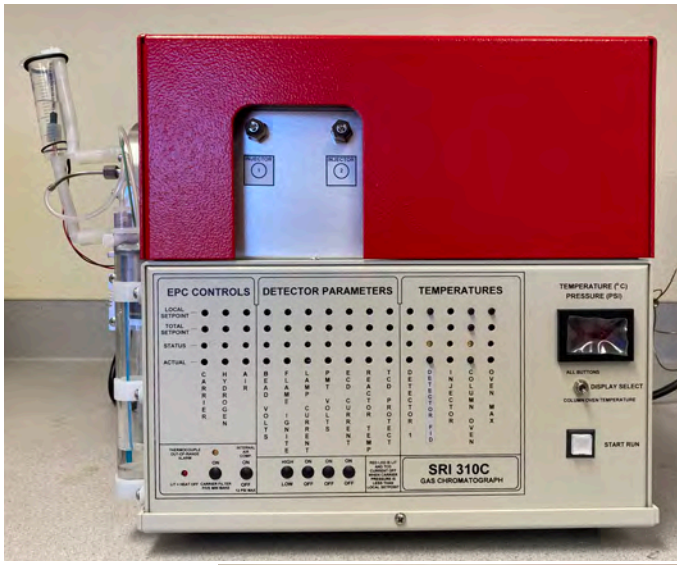
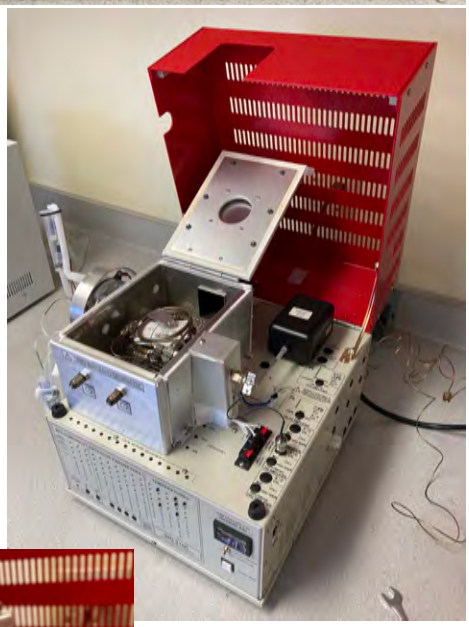
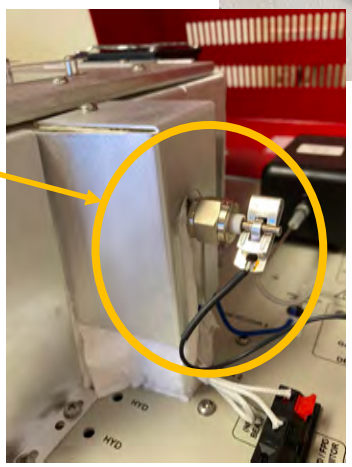
Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

SRI offers two **310MM Cannabis and Hemp GC systems**, the **standard configuration** (part# 0310-0091, $11,134.00, and the **310MM Edibles Cannabis and Hemp GC system** (part# 0310-0095, $12,896.00, 2022 pricing, prices subject to change, consult most recent price list. Both systems come with a flame ionization detector (FID, built-in hydrogen generator, built-in air compressor, MXT 502.2 capillary columns, Hayesep D packed column, and dual on-column injection ports.





While the 310MM models are also capable of analyzing terpenes, residual solvents in concentrates, and for the 310MM Edibles GC, edible cannabis products, this document will focus on basic potency testing in cannabis flower for the main cannabinoids of interest: CBD, delta-9-THC, and CBN, which is the most common and popular type of cannabis and hemp testing. (See additional cannabis and hemp testing documents on our website at *https://www.srigc.com/pages_document_downloads/*



Both versions of the 310MM GC include 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.

Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

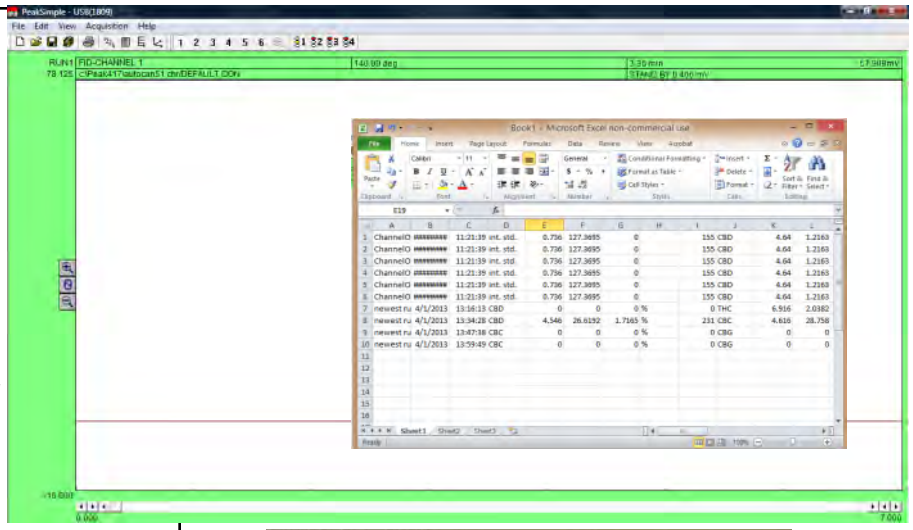
The cannabinoid molecules, Δ9-THC, CBD, and CBN (and for more advanced operators, CBC, Δ8-THC, and CBG) are separated by 5- and 30-meter MXT 502.2 metal capillary columns which are heated in the column oven. There is also a Hayesep D packed column, but that is only utilized for the residual solvents analysis, and not for potency testing, and therefore will not be discussed in this document.

No gas tanks are needed to operate the 310MM GC. The built-in hydrogen generator and air compressor provide all the carrier gas and FID operation gases needed to conduct the most important cannabis and hemp analyses, including basic potency of cannabinoids. However, the 310MM GC can always be configured to run off of tank hydrogen, if the customer prefers.

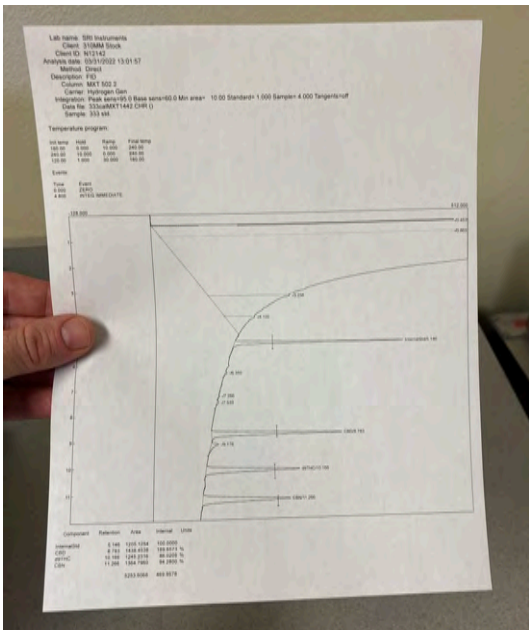
The entire GC plugs into any Windows computer using a USB cable.

Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

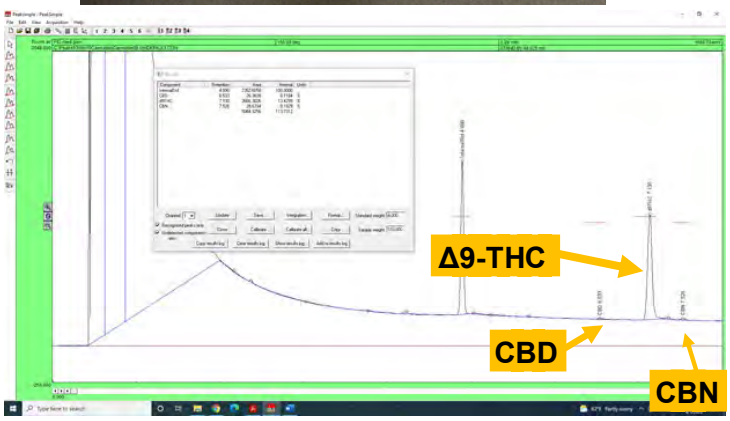
SRI's PeakSimple software is included with the GC. PeakSimple software collects the GC data and generates a calibrated result which can be printed or transferred to other programs such as Excel or Word. The latest version of the software can always be downloaded for free at *www.srigc.com.*



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

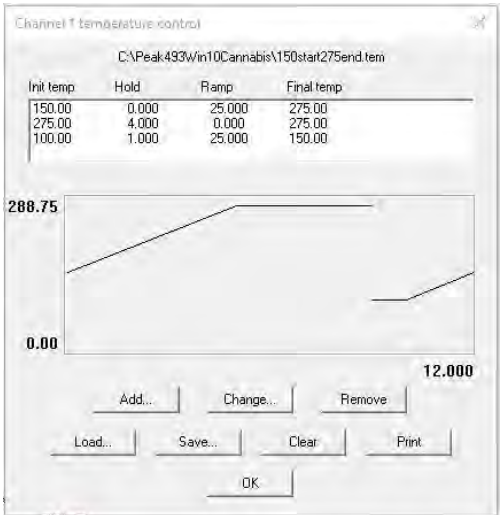


An actual cannabis sample is shown at right. Note that only the Δ9-THC peak is large, the other cannabinoids are much smaller. This is what you would expect for the majority of actual cannabis samples. On the other hand, when testing hemp samples, the CBD peak should be larger than the Δ9-THC peak.

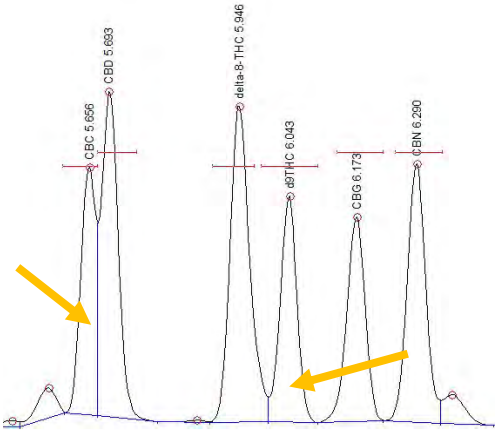


Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

For a quick 12-minute analysis that optimizes speed and peak separation for the MXT 502.2 column that comes standard with the 310MM GC, set the column oven temperature as shown to the right.

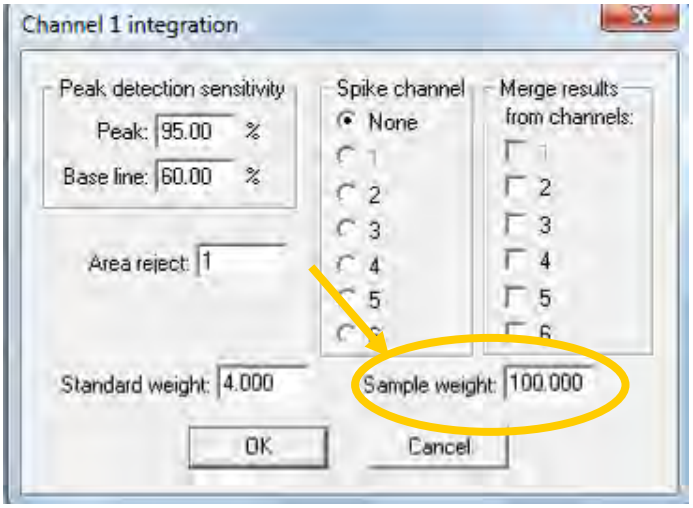


Certain samples and operators may wish to adjust the temperature program to achieve better separation or longer or shorter analysis times. As a general rule, a better separation comes at the expense of speed. The temperature program above should be sufficient for most users as it seeks to balance analysis time and separation.



12-min Run of Six Cannabinoids: Note the closeness of the CBD and CBC peaks, as well as Δ8 and Δ9-THC

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. (For example: If the sample weighed 0.104 grams, then "104" should be entered).



Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

Obtain the cannabinoid calibration standard from a chromatography supplier like Restek (restek.com) or one of the many other suppliers. The standards can be acquired individually or in 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 for purchase.

Whether you get three individual standards or the three-way, break the glass ampoule(s) and transfer the contents into 2mL septum vial(s). Restek provides one free vial with each standard. Use the pipet provided with the 310MM starter kit.

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.

Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

For most users, 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 (about 13%).

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 (for a total of 300uL). If you have the three-way standard, use the 100uL syringe to transfer 100uL of the standard into another 2mL (or smaller) vial and then add 200 uL of Clean extraction solvent (acetone, methanol, denatured alcohol, etc.)

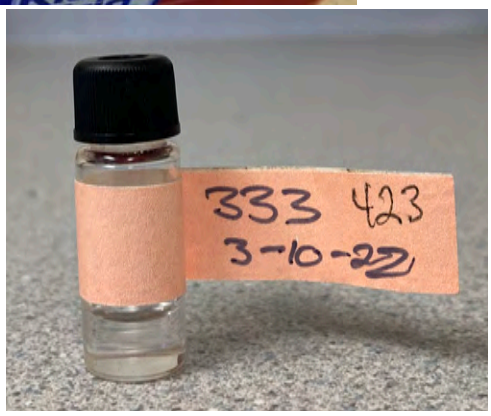
At the end of this step, you should have 300 uL of liquid in your 2mL vial.

Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

Prepare your "**dirty solvent**" extraction solvent by adding 1 gram of methyl stearate ("MS") to a gallon of acetone, denatured alcohol, or other solvent. Alternatively, for a smaller amount, add 264 milligrams of MS per liter of solvent. Label the bottle of solvent with the date you made it because each calibration standard will correspond with a particular bottle of solvent. When you run out of extraction solvent, you need to make a new batch of dirty solvent and new working calibration standard.

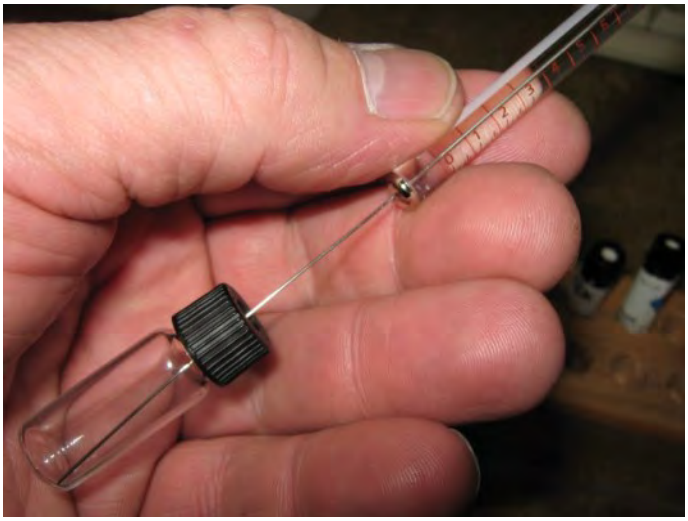
With your 100uL syringe, add an equal amount, or 300 uL, of dirty solvent to your 2mL working calibration vial. The important thing is that there is an equal amount of dirty solvent to whatever volume of liquid is in the vial.

At the end of this step, you should have 600 uL of working standard in the 2mL vial.

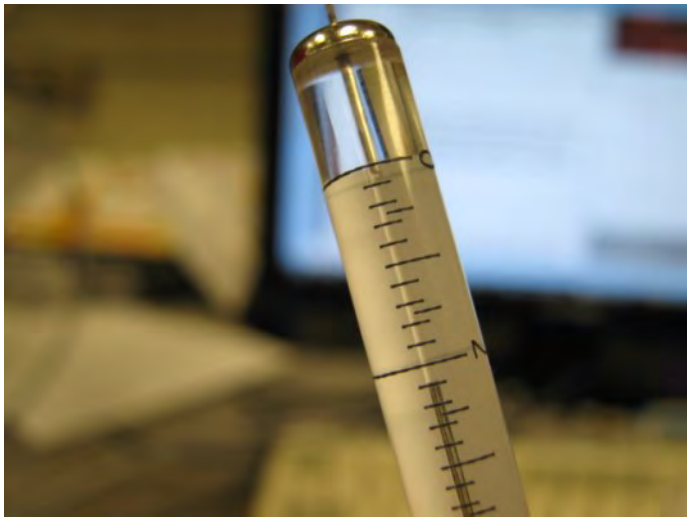


Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

With the working standard, rinse the syringe first then use the 10ul syringe delivered with the GC (SRI #8670-9550) to withdraw 2-3uL of liquid. Puncture the septum rather than open the vial to avoid letting the extraction 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 slant-ed upwards so any air bubbles will rise towards the needle.

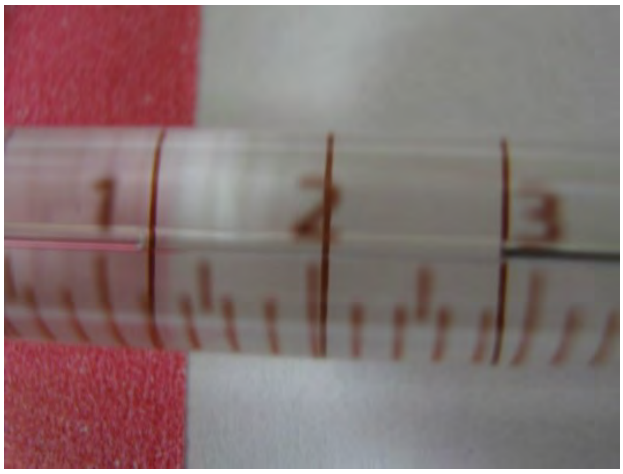


With any air bubbles removed, push the plunger to the 1uL mark. It is important to be as precise as possible, especially if using an external standard method of calibration. While the internal standard calibration standard effectively removes operator syringe error, it is still good practice to be as precise and consistent with your injection volumes as possible. Wipe the needle with your fingers or a tissue to remove any liquid from the outside of the needle.

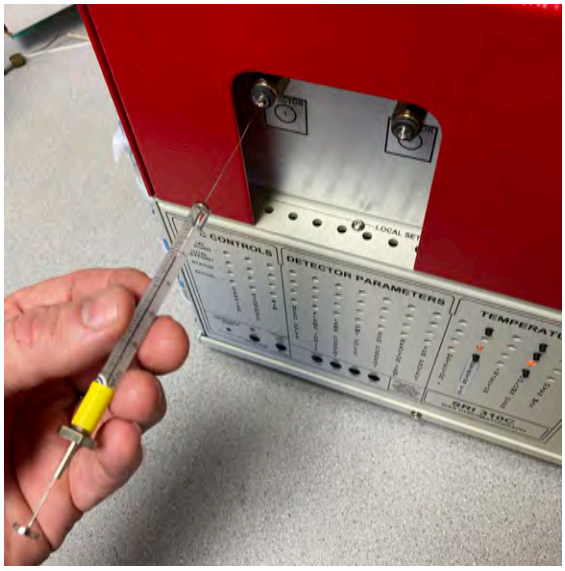
Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

Pull the plunger back to the 4ul mark and note the amount of liquid. It should be 1.6 - 1.8 uL because the needle also contains 0.6 - 0.8 uL and this adds to the 1uL you measured with the plunger.

Leave the plunger at the 4uL mark.



With the plunger still at the 4uL mark, place the needle up against the septum of Injector 1, not Injector 2 (which is only for residual solvents), but not poking through it yet.



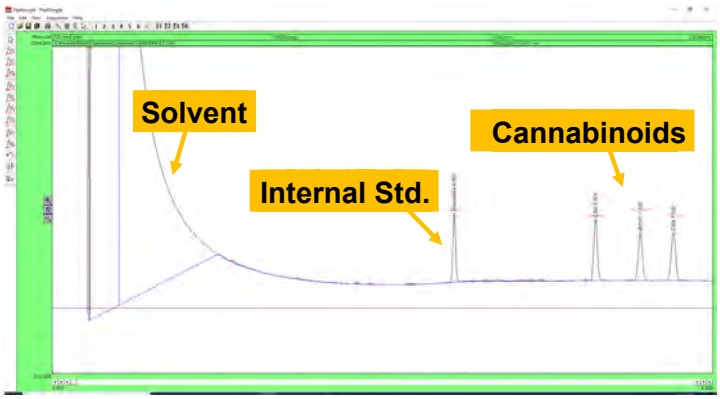
Press the Start Run button or hit the Spacebar on the keyboard with PeakSimple open to start the run.

After the run is started, insert the syringe all the way through the septum as far as it will go.

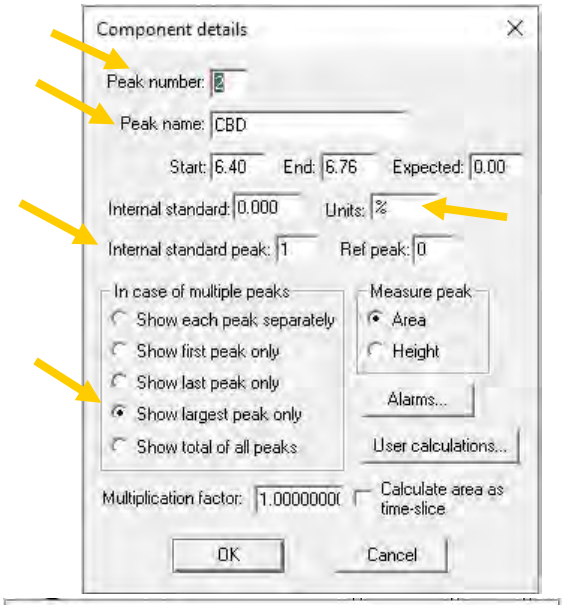
Immediately depress the plunger. Twist the syringe one half turn (to wipe off any liquid on the tip of the needle) and then withdraw the syringe.

Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

Once the run is completed you should see a large solvent peak near the beginning, the internal solvent peak somewhat towards the middle, then closer to the end, three cannabinoid peaks of roughly equal size (there will also probably be a small Delta-8 THC peak between the 1st and 2nd peak). If the software has not already provided them by default, add retention windows to the three peaks by right clicking on the peak and selecting "Add component."



Identify all four peaks (from left to right: Int. Std., CBD, THC, CBN) by right-clicking on each peak and selecting “Edit component”. Assign each peak a unique number and name (for example: IntStd, CBD, THC, or CBN), select “show largest peak only”, and add a “%” sign to the “Units” box.

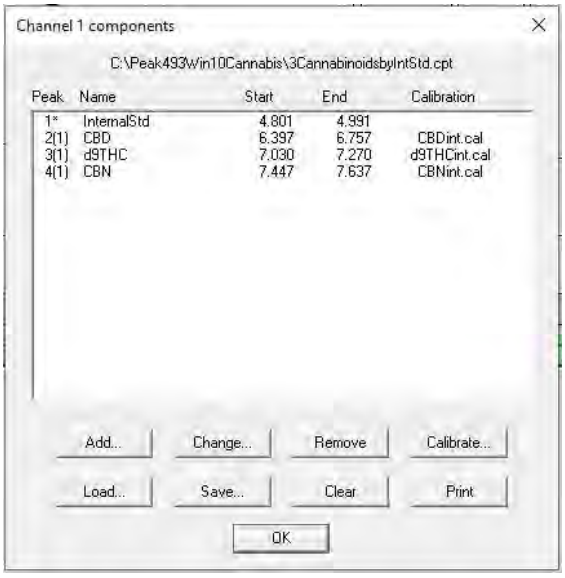


For the Internal Standard Peak only, assign it Peak number "1".

For the three cannabinoids only, insert the number "1" into the Internal standard peak box.

Press the “OK” button to exit back to the main chromatogram screen.

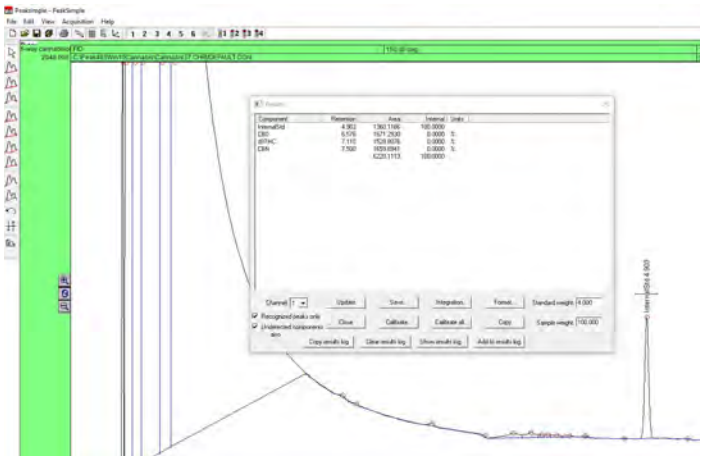
Right click on the chromatogram and select “Components” to open the “Channel 1 Components” Screen. Here will be displayed a list of all the components with named retention windows and unique peak numbers.



Note the parenthesis and the number "1" in front of CBD, THC, and CBN, indicating that the calibration of these peaks are based on the internal standard peak. Select “Save” and name the component file so that if you exit Peak-Simple your component and calibration files will not be lost.

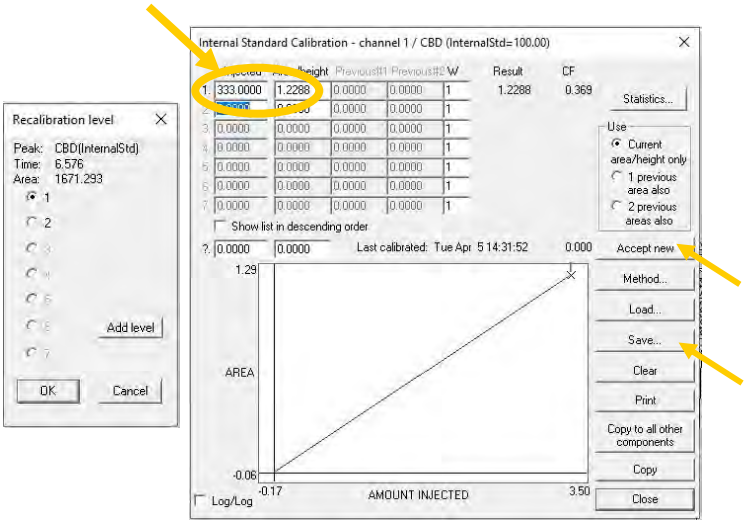
Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

Check the results screen, when you inject a working standard, the area counts for the cannabinoids should be approximately 1500 area counts and roughly equal to each other (+/- 200).

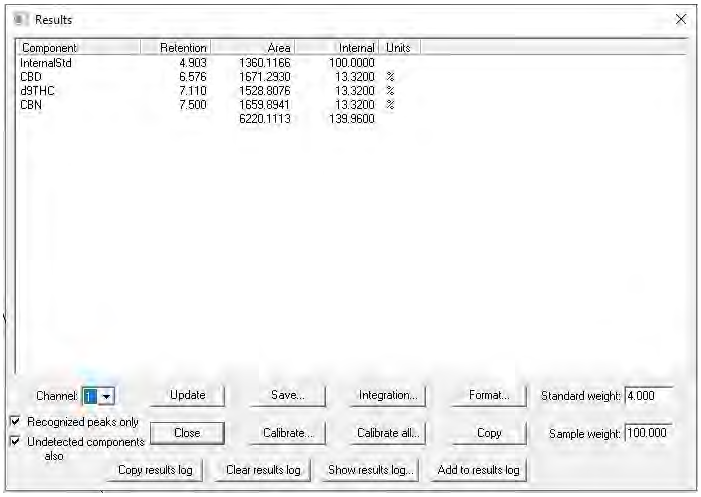


Now, calibrate each cannabinoid peak by right clicking over the peak and selecting "Calibrate CBD/THC/CBN." Hit the "OK" button on the Recalibration level screen.

In the Calibration Curve screen enter the amount of standard you just injected into the top left cell of the calibration spreadsheet. For the working standard this will be 333 (for 333ng/ul) or 1000 (for 1000ng/ul) for a primary standard. Then, click the Accept New button to transfer the ratio of the peak area to the internal standard peak area, which should be a number close to "1", into the top row second column. Save the curve under some name. Do this for all three 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.**

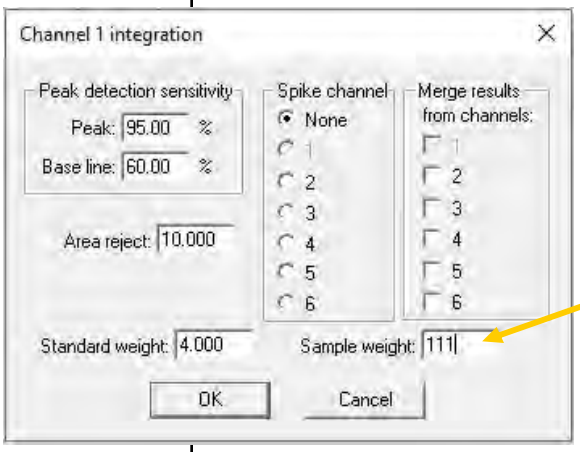


Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

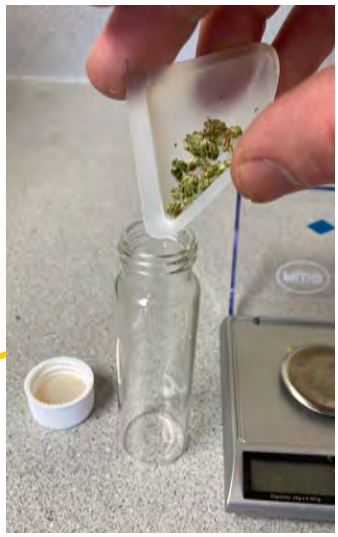
Use a balance capable of reading 1 milligram (0.001 gram). A balance like the one pictured comes with the 310MM Cannabis GCs. Turn on the balance, place a piece of paper or some kind of light tray on the scale, and hit the TARE button to zero the balance.

Carefully add approximately 100 milligrams (0.100 grams) of manicured cannabis (or non-sticky concentrates) onto the paper/tray. Drop the bits of cannabis onto the balance slowly until the reading is close to 100 milligrams. Do not worry if you are slightly under or over 100 milligrams. In the photo at right, the reading is 111. Just make sure to write down the exact weight of the sample somewhere, preferably on the 40-mL vial itself.

Later 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.



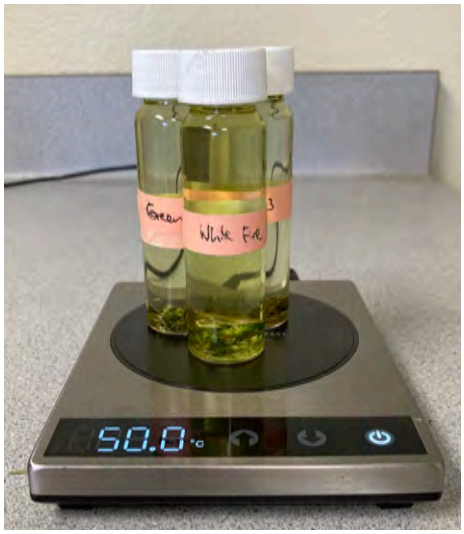
Carefully add <u>all</u> of the weighed-out cannabis to a 40-mL vial.



Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

Fill the 40-mL vial with 40-mL of your dirty extraction solvent. This can be done by measuring out 40mL precisely in a beaker or graduated cylinder or, more simply, by filling the 40-mL vial up to the neck with dirty solvent.

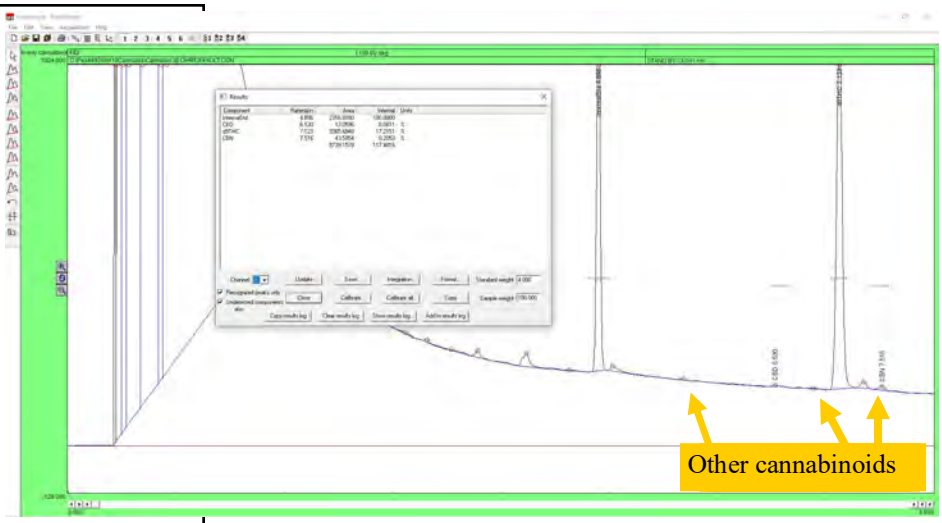
Place the cap onto the 40-mL vial. Shake the vial for a few seconds and then let it sit for at least an hour to allow the solvent to extract the cannabinoids. This extraction step can be sped up by utilizing a hot plate or other heating device to heat the sample(s) at **low heat**. ***FOR SAFETY PURPOSES: DO NOT HEAT SAMPLES MORE THAN 50 DEGREES CELSIUS.***



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 as precise as possible with the syringe, even if the internal standard calibration method is used. Don't forget to enter the exact Sample weight in the proper field on the Results screen during or after the sample has been run.

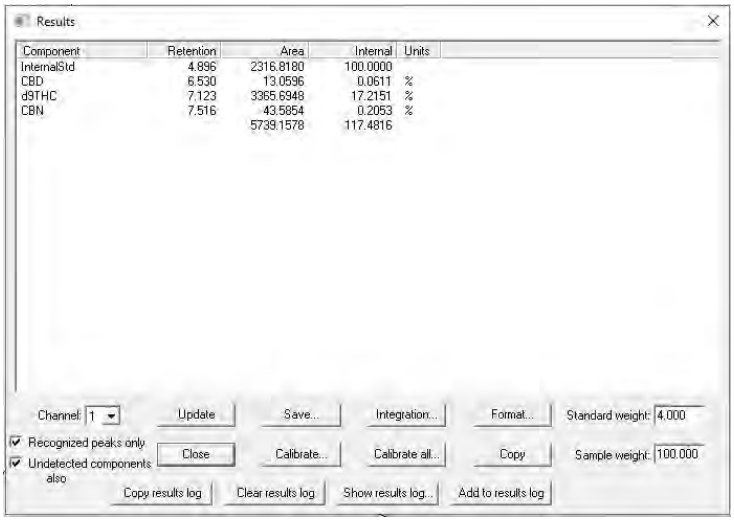
Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

A typical 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.



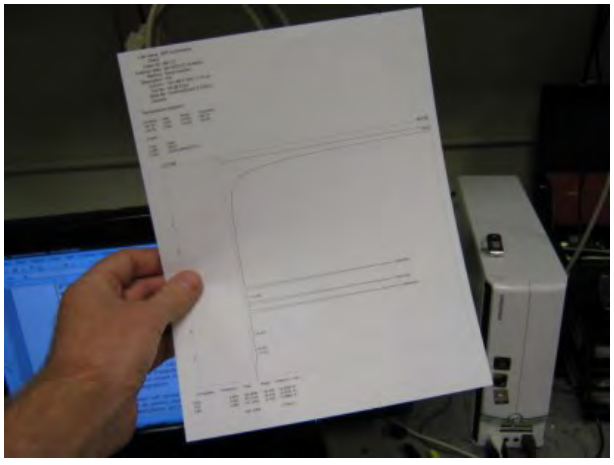
CBD and CBN may or may not be detected based upon the particular sample and the size of the "area reject" selected in the Integration screen.

There may be other peaks close to CBD, THC, and CBN which are not any of these three cannabinoids. These other peaks are also cannabinoids (CBC, Delta-8 THC, CBG, and others) for which there may or may not be calibration standards available.



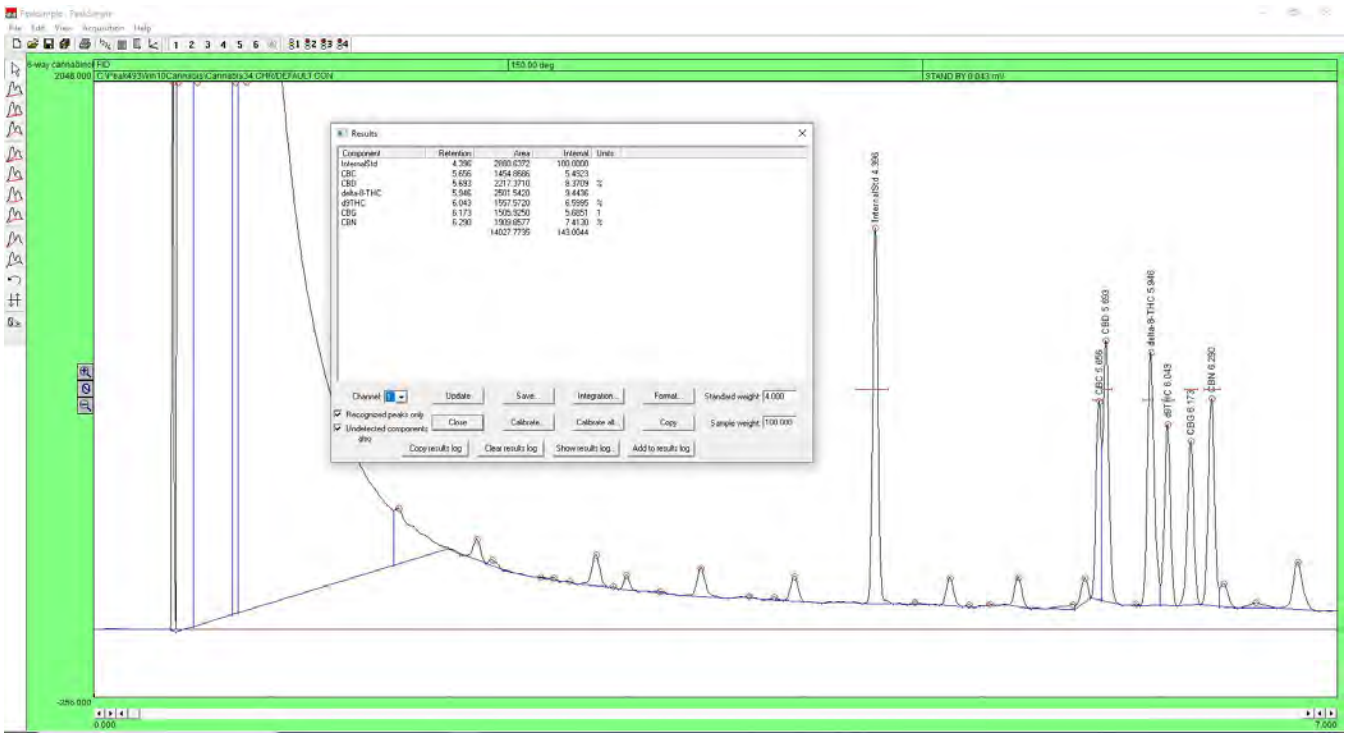
It may be necessary to manually integrate some of the peaks for the most accurate quantification of cannabinoid potency. See the "PeakSimple Advanced Tutorial" at https://www.srigc.com/cn/downloads/133/PeakSimple%20Advanced.pdf for more information on manual integration.

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 hard copy record of the analysis or save as a pdf for a digital copy.

Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems



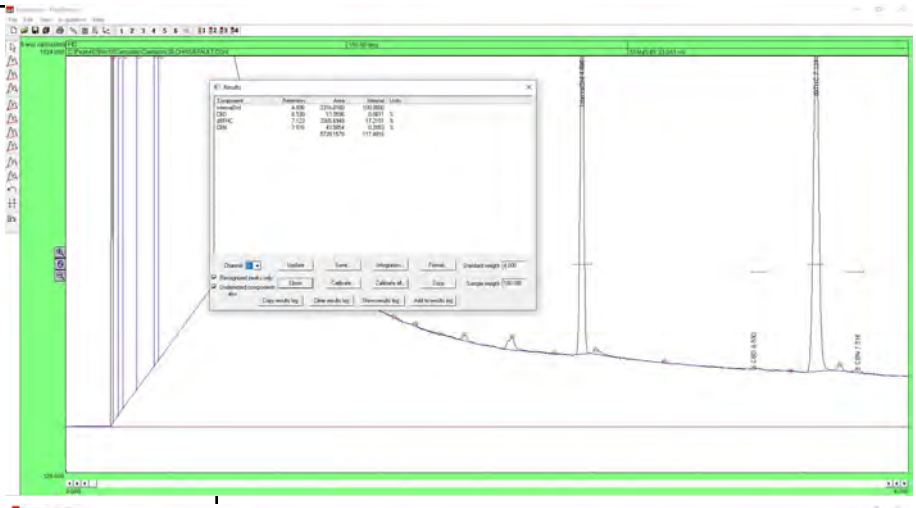
The chromatogram above shows a six-cannabinoid calibration standard. In addition to CBD, Δ9-THC, and CBN; Cannabichromene (**CBC**), **Δ8-THC**, and Cannabigerol (**CBG**) are now identifiable and quantifiable.

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*.

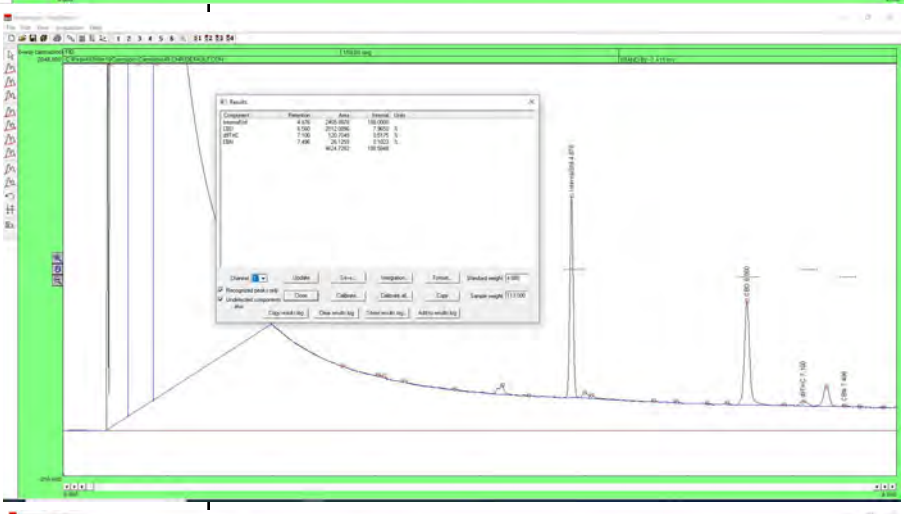
Most cannabis samples contain at least *some* amount of all six cannabinoids (as well as many others). However, because the chemical structure of all six cannabinoids are so similar, a calibration value for one cannabinoid (i.e. CBD or THC) can be used to accurately calibrate another cannabinoid (i.e. CBC, CBG, or Δ8-THC) for which a standard is unavailable or too expensive. **For further information, consult our document "*Calibrating Six Cannabinoids with CBD Calibration Standard*"** at *https://www.srigc.com/cn/downloads/90/Calibrating%20Six%20Cannabinoids%20with%20CBD%20Calibration%20Standard%20-%20August%202013.pdf*

Cannabis and Hemp Potency Testing using the SRI 310MM Cannabis and Hemp GC Systems

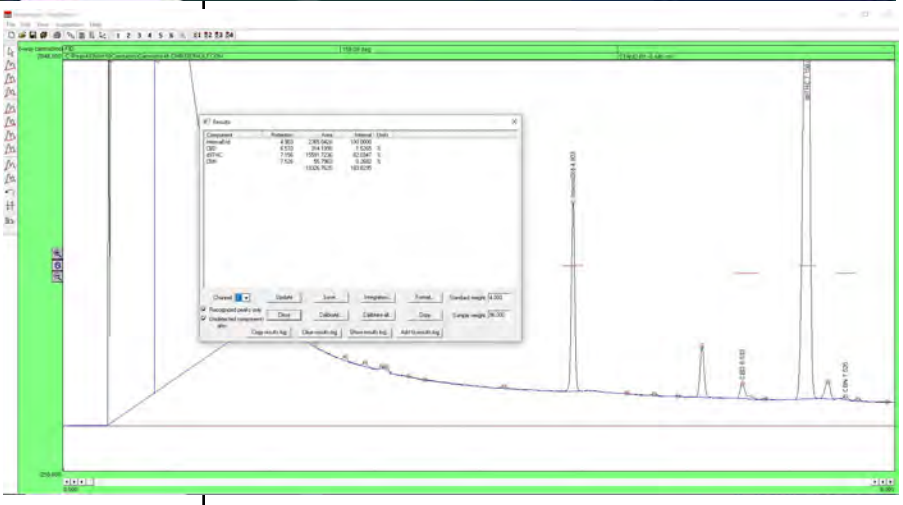
This first chromatogram is from a typical cannabis flower with 17.2% THC and less than 0.1% CBD.



This second chromatogram is from a typical hemp sample with 8.0% CBD and 0.5% THC.



This third chromatogram is from a high-purity cannabis concentrate with 82.0% THC and 1.5% CBD.



This concludes the Cannabis and Hemp Potency Testing document. See *www.srigc.com* for more documents, resources, and sales information or visit our YouTube channel at *www.youtube.com/channel/UCQHQDR6_nsDKDUboEaW1R6g* for helpful videos on cannabis analyses and other topics.