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Note from the CEO

Expanding Cannabis Knowledge

WELCOME TO THE LATEST ISSUE of *Cannabis Science and Technology*! In May we held the 2022 Cannabis Science Conference West in Long Beach, California where we heard the latest developments in the cannabis industry as well as various insights from our new psychedelics track. Though each presentation and speaker brought their own perspectives, at the common core of the conference was the message that there is still so much we can and must do in the world of plant science and medicine.

In this issue, we conclude with the final installments of several multipart columns begun in prior issues. In the concluding part of her series on gas chromatography, Patricia Atkins looks at the final critical component to achieving successful gas chromatograms: the detector. She explains that, similar to instruments in an orchestra, the various components of gas chromatography systems each play a particular role and carry certain importance. The detector is like the filter that produces the final product in the best way possible, similar to producing the best orchestral auditory experience possible.

Previously, Brian Smith established the importance of Beer's Law in cannabis analysis, and now, starting with this issue, he expands this with the first installment of a new three-part column on quantitative spectroscopy. He provides practical and detailed tips on how to develop accurate spectroscopic calibrations while avoiding common pitfalls. Similarly, Lo Friesen wraps up her two-part exploration of vape products. Here she explores the history of vape products, the many extract types, how temperature and hardware impact the user experience, and more. In a peer-reviewed article, Dr. Weston Umstead from Chiral Technologies explores one alternative to the extraction of plant-based cannabinoids, namely the chiral method development screening for the enantiomeric separation of synthetic cannabidiol. Due to the continued increasing demand for cannabinoids for consumer and research purposes, Dr. Umstead proposed this method as a way to alleviate supply issues. We hear from Audrey Shor of Decarb Factor in part two of a guided tour through the protein structure and function influence of cannabinoid receptor 2 on cannabinoid signaling. Finally, Otha Smith III, creator of Tetragram, shares how his mobile app helps both the cannabis industry and cannabis users through real-world data.

We hope that you enjoy our June 2022 *Cannabis Science and Technology issue*!

Mike Hennessy Jr
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US Appeals Court Rules Hemp-Derived Delta-8 THC Products Legal

By Erin McEvoy

ON MAY 19, 2022 a federal appeals court ruled in a California vaping dispute that delta-8 tetrahydrocannabinol (THC) derived from hemp is "lawful" and eligible for trademark protection (1). The three-judge panel wrote that products containing delta-8 THC are generally legal because federal law defines hemp as "any part of" the cannabis plant, including "all derivatives, extracts, [and] cannabinoids," that contains less than 0.3% delta-9 THC by weight (2).

Delta-8 THC is an isomer of the psychoactive cannabinoid that occurs naturally in the cannabis plant, delta-9 THC. Delta-8 THC can be synthesized from cannabidiol (CBD), giving rise to products that offer intoxicating effects without needing to be sourced from cannabis, which remains illegal under federal law (1).

The ruling does not address the legality of selling consumable delta-8 THC product, however (1). The confusion over delta-8 THC's legality under federal law has prompted many states to limit or restrict how it can be sold. Though Congress legalized hemp derivatives such as delta-8 THC, it also directed the US Food and Drug Administration (FDA) to oversee how those products could be sold. The FDA has declined to authorize any cannabinoid products without a prescription, and the agency issued a consumer alert last September warning that delta-8 THC "may have potentially harmful by-products (contaminants) due to the chemicals used in the process." The Drug Enforcement Administration, meanwhile, signaled late last year that delta-8 THC was legal (2).

For more on this topic, please see our upcoming June installment of the "Stuck on Compliance" blog by Kim Stuck at: https://www.cannabissciencetech.com/blogs/stuck-on-compliance.

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Quantitative Spectroscopy:
Practicalities and Pitfalls, Part I

By ***Brian C. Smith***

The last three installments of this column (1–3) have been devoted to a discussion of Beer's Law, which is the mathematical relationship between the amount of light absorbed by a sample and concentration, which makes spectroscopy quantitative (4). In the first installment (1), I showed how to use Beer's Law to develop a calibration and then use it to measure concentrations in unknown samples. In the second column (2), Beer's Law was derived from first principles, and in the most recent installment (3) I demystified the absorptivity for you.

Beer's Law is important in cannabis analysis because most high performance liquid chromatography (HPLC) cannabis potency methods use an ultraviolet-visible (UV-vis) quantitative spectroscopy detection technique (5). Additionally, there exist cannabis potency analyzers that use quantitative infrared spectroscopy (6). Hence my statement in the first installment of this series (1), that Beer's Law is the most important equation in cannabis potency analysis.

Now that I have introduced Beer's Law and shown how to use it to perform quantitative spectroscopy, I will begin a series of columns containing practical tips and pitfalls to avoid to help you develop accurate spectroscopic calibrations. In this installment, we will discuss the importance of using standards that bracket the expected concentration range of standards in the unknowns, using the same chemical components in standards and unknowns, and making sure to minimize the error in concentration measurements of standards.

A Brief Review

Beer's Law is the equation that relates the amount of light absorbed by a sample to its concentration and has the form seen in **Equation 1** (1–4):

$$A = \varepsilon LC \quad [1]$$

where A is the absorbance, the amount light absorbed by a sample; ε is the absorptivity, a fundamental physical constant of a molecule; L is the pathlength or sample thickness; and C is the concentration.

The absorbance spectrum of a series of samples of known concentration called *standards* is measured. The peak height or peak area (the absorbance) of the molecule of interest (the *analyte*) is determined. A plot of absorbance versus concentration called a *calibration line* is then made (1–4). The slope of this line gives εL which is used in **Equation 2** to predict the concentration of analyte in unknown samples.

$$C_{unk} = A_{unk}/\varepsilon L \quad [2]$$

where C_{unk} is the concentration of analyte in an unknown sample, and A_{unk} is its absorbance.

Advice to Improve and Pitfalls to Avoid in Calibration

The rest of this and some upcoming columns will contain a list of practical tips that you should know that will help you do a better job of obtaining quality spectroscopic calibrations. The list will also contain pitfalls to avoid, which, if you fall into them, will prevent you from achieving your quantitative spectroscopy goals.

Use Standards that Bracket the Expected Concentration Range in Your Unknowns

My definition of a standard is a sample containing a known concentration of analyte whose matrix is the same as that of the anticipated unknown samples. A Beer's Law calibration line of peak areas versus concentration, built with five standard samples from the data seen in **Table I**, is shown in **Figure 1**.

This plot is for isopropyl alcohol (IPA) dissolved in water, and we have seen it before (1). Note that the plot extends from about 9% to 70% IPA. This means that this calibration can only be used on unknown samples whose IPA concentrations fall between 9% to 70%. This calibration cannot be used on IPA in water samples whose concentrations are above 70% or below 9% because the calibration line has no data points in these regions. We know nothing about the behavior of the calibration line outside the calibration range, so we cannot legitimately

use it there. To use this calibration outside its calibration range would be extrapolating the calibration, which is always tempting and is always wrong.

The maxim here is: "Use standards that bracket the expected concentration range in your unknowns" and is meant to solve this problem. By choosing standard samples whose highest concentration are above any anticipated unknown sample, and lower than in any anticipated unknown sample, your analyses will always be in the range where the calibration line actually has data and can be legitimately used. This means when developing your calibration method you will have to plan ahead, try to predict as best as possible what the highest and lowest unknown concentrations will be, and then make up your standards to bracket those concentrations accordingly.

Use Actual Components When Preparing the Standards

One of the big take aways from the last column (3) is that the absorptivity is matrix sensitive. That is, it depends upon a sample's chemical environment including temperature, pressure, pH, concentration, and chemical composition. In that column, I shared an anecdote about a customer of mine who tried to use a mid-infrared spectrometer calibrated to analyze cannabis buds to look for tetrahydrocannabinol (THC) in pizza. This analysis of course gave nonsensical numbers because there was a calibration applicability problem. No analytical instrument, be it a spectrometer or a chromatograph, can quantitate sample types it hasn't been calibrated to analyze.

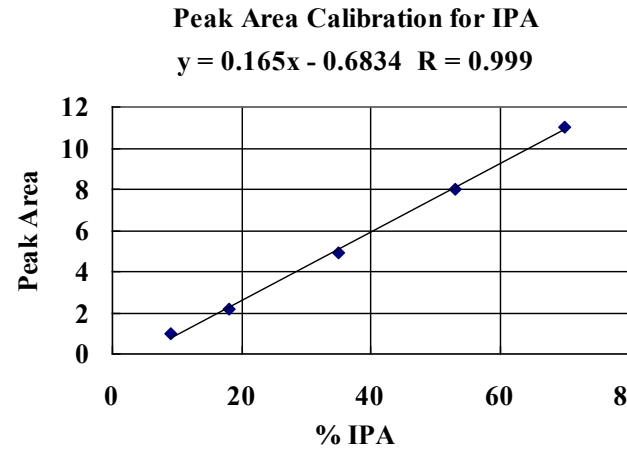
In quantitative spectroscopy the calibration applicability problem rears its ugly head when the analyte's absorptivity in the standards does not match that of the unknowns. This can happen when the chemical components used to make up standard samples do not match those in the unknowns. For example, the calibration line seen in Figure 1 is for IPA dissolved in water. This calibration will not work for IPA dissolved in acetone because IPA's absorptivity in these two sample matrices will be different.

Another problem with not using the same chemical components in standards and unknowns is illustrated by a story a colleague once shared with me. An analytical chemist was tasked with developing a spectroscopic method for measuring the amount of water in acetone samples obtained from a factory floor. The chemist, thinking that purer is always better, made water in acetone samples using 99% pure chromatography grade acetone that was available in the laboratory, and developed a lovely calibration with a great correlation coefficient (see reference 1 for the definition of this term). However, when the method was implemented, independent testing showed it gave inaccurate results. What happened?

Table I: Volume percent isopropyl alcohol (IPA) and peak area data used to plot the calibration line in Figure 1

%IPA	Area
9	1
18	2.2
35	4.9
53	8
70	11

Figure 1: A Beer's Law calibration line: a plot of peak area versus volume percent for isopropyl alcohol (IPA) dissolved in water.



The problem was although a good calibration was developed, it was the wrong calibration. Out in the factory the material being analyzed was made up using technical grade 95% pure acetone. This meant that the unknown samples contained 5% impurities, whereas the standard samples contained 1% impurities. This presents three problems. First, those impurities could affect the absorptivity of water meaning it might be different in the samples and unknowns, presenting us with a calibration applicability problem. Second, if the impurities in the technical grade acetone absorb light at the same wavelengths as water, they will skew the water concentration measurements. Lastly, all concentration determinations will be off because for a given absorbance measurement the unknowns will have 4% more water than the standards.

One of the biggest challenges in quantitative spectroscopy is making sure that the matrix of the standards matches the matrix of the unknown samples so that the absorptivity of your analyte calculated from the slope of your calibration line

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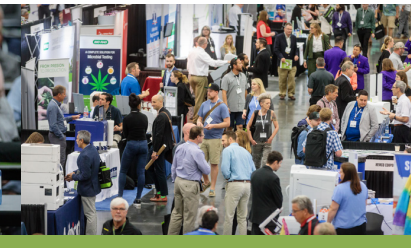

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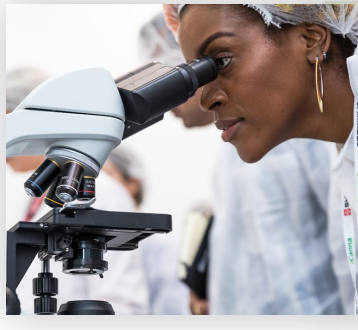

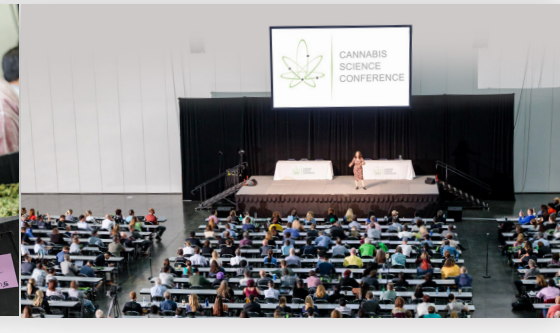

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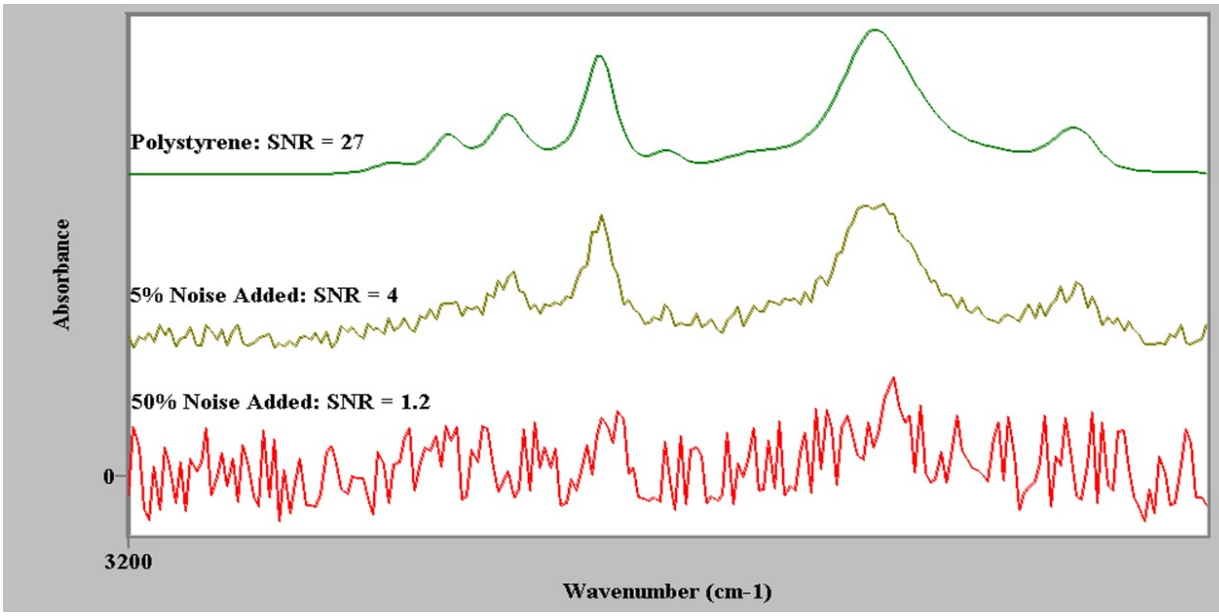
Figure 2: Three mid-infrared spectra of polystyrene measured with different SNRs.



will be the same as that for the analyte in the unknowns. This is why it is so important when making up standards to use the same chemicals for the standards and the unknowns.

Make Standards with Concentrations as Accurate as Possible

I have been talking a lot about making up standards, but haven't yet really shared with you how to make them. For simple single analyte quantitative spectroscopic analyses, like we have been discussing, it can be easy. For the IPA in water data shown in Figure 1, I started with a known solution of 70% IPA in water and diluted it with the appropriate amount of water to obtain standards containing 9%, 18%, 35%, and 53% IPA. Calibrating a spectrometer for more complex matrices, such as cannabis plant material, is complicated. In work I have discussed previously in this esteemed journal (7) for complex sample matrices one must calibrate using *matrix reference materials*. These are samples of the matrix of interest that have had analyte concentrations determined by an independent method. In my case, the standards were analyzed for cannabinoid concentrations using an HPLC method that used, wait for it, a quantitative UV-vis spectroscopy detection method making use of Beer's Law (5). Calibrating a spectrometer to analyze a complex matrix such as cannabis is more complicated than what I have been discussing in this column series (1–4), but all the information I will be sharing with you here is still relevant.

In a previous column I have talked about error, precision, accuracy, and a thing called the signal-to-noise ratio (SNR) (8). For any data set, the SNR can be thought of as the ratio of the measured value to the error in its measurement as seen in **Equation 3**.

$$\text{SNR} = (\text{Measured Value}) / (\text{Error in that value}) \quad [3]$$

A practical example of SNR is when you are talking on the phone. The volume of the caller's voice is the signal, and the static in the call is the noise. The greater the volume of the caller compared to the static the higher the SNR, the easier it is to understand the caller, and the higher the quality of the information received. On the other hand, when you have a bad connection the volume of the caller is low compared to the static, the SNR is low, the other person is hard to understand, and the quality of the information received is low. Hence, high SNRs in analytical data are always preferred over low SNRs.

In quantitative spectroscopy we use absorbance values in our calibrations, and since absorbance is usually plotted on the y-axis, it is the size of this error we are concerned about. **Figure 2** shows several mid-infrared spectra of polystyrene containing different SNRs.

The bottom spectrum, with an SNR of 1.2, is what true noise looks like in a spectrum: a collection of random, jagged, up and down features with no meaning. The middle spectrum, with

an SNR of 4, has peaks that are clearly due to polystyrene, but they are so small compared to the noise that they are not usable for quantitative work. The top spectrum, with SNR = 27, is much better with clearly seen peaks and very little noise. The peaks in this spectrum are of high enough quality to be used for quantitative analysis. In general, spectrometers are capable of SNRs of 100 or better on many sample types, which means the error level is often times less than 1%.

The sets of information we use to make Beer's Law plots, as seen in Figure 1, are absorbances and concentrations. Concentration information can be obtained by many different types of methods, including weighing out the amounts of analyte to be used in a standard ("gravimetric methods"), measuring the volume of analyte to put into standards as I discussed above, and from HPLC and gas chromatography (GC). For cannabis matrix reference materials, cannabinoid concentrations are often determined by HPLC and terpene concentrations by GC. In my own experience, discussions with other analytical chemists, and reading of the literature (9), concentration measurements often times only have SNRs of 20 at best, meaning the error level is often times 5% or greater. In general, concentration data has more error in it than absorbance data, which is why it is so important to make up and analyze standards as accurately as possible. Also, if you want to improve a spectroscopic calibration, your effort is best invested in correcting error in the concentration measurements because that is the surest and easiest way to increase accuracy.

Conclusions

In this column we have begun our discussion of practical tips to improve and pitfalls to avoid when developing quantitative spectroscopic methods. Using standards that bracket the expected concentration range of the analyte in the unknowns is required because it is always tempting, and always wrong, to extrapolate a calibration line to concentration ranges where you have no data. Using the same chemical components in standards and unknowns is important to ensure the analyte's absorptivity is the same in standards and unknown and to ensure quantitative accuracy. Making up standards carefully and minimizing the error in concentration measurements used in a Beer's Law plot is a sure way to increase the accuracy of your quantitative spectroscopic measurements.

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ABOUT THE COLUMNIST

BRIAN C. SMITH, PhD, is Founder, CEO, and Chief Technical Officer of Big Sur Scientific. He is the inventor of the BSS series of patented mid-infrared based cannabis analyzers. Dr. Smith has done pioneering research and published numerous peer-reviewed papers on the application of mid-infrared spectroscopy to cannabis analysis, and sits on the editorial board of *Cannabis Science and Technology*®. He has worked as a laboratory director for a cannabis extractor, as an analytical chemist for Waters Associates and PerkinElmer, and as an analytical instrument salesperson. He has more than 30 years of experience in chemical analysis and has written three books on the subject. Dr. Smith earned his PhD on physical chemistry from Dartmouth College.

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What's in a Vape? Part II

By ***Lo Friesen***

Since 2019's "vape crisis," cannabis and nicotine vapes have been under a roller-coaster of scrutiny—with good reason. In the second part of this two part series, we define the various categories of vapor products and how they are made, review the importance of temperature control, and predictions for the future of vapor products and technology.

Since 2019's "vape crisis," cannabis and nicotine vapes have been under a roller-coaster of scrutiny—with good reason. This is part II of a two part series, where we review the history of vape products, producing cannabis vapes, the myriad of extract types, and how temperature and hardware impact the user experience.

The long and unusual history of vapes began in the 1930s and has continued to this day. Innovation in the inhalables space has provided global consumers with a cornucopia of devices, products, and flavors in a number of different herbal and synthetic categories. A majority of liquids that are being vaped are nicotine and cannabis. It wasn't until the early 2000s that e-cigarettes and vaporizers became regulated under the US Food and Drug Administration (FDA) and equivalent agencies throughout the world. Because of this, the e-cigarette and vaping industry has been on a roller-coaster ride. At present day, the e-cigarette or vapor category is continuing to undergo great scrutiny as a result of unsafe products being sold to adults and minors. These unsafe products have caused serious illness and death among consumers. It is more important than ever to ensure that manufacturers are held accountable for the production of safe consumer goods. In this article, production methods, cannabis extract nomenclature, vaping temperature, and vape hardware will be reviewed as integral components of safe and effective vape manufacturing.

There are four main extraction methods that are utilized in the cannabis industry. Each extraction method has its strengths and weaknesses depending on cost, regulations, safety, versatility, and desired end product. In the US, 20% of cannabis products sold at retail are in the vape category (1).

Within the vape market, a majority of vapes are produced using distillate. CO_2 extracts and live resin extracts make up most of the remaining market share. Distillate is mainly produced using thin-film or wiped film evaporation methods, which result in cannabinoid concentrations of well over 80-90%. The goal of distillation and evaporation is to concentrate and purify an extract to produce these highly refined and purified distillates. Because of this, the ideal extraction method is ethanol extraction to produce high yield, low cost volume of cannabis extract to then run through distillate production at scale. Distillate is incredibly economical to produce at scale, which offers manufacturers a low-cost, high-tetrahydrocannainol (THC) base ingredient for vapor products. These affordable vapes, produced with low-cost distillate and often with non-cannabis derived flavorings are popular among consumers because of the affordability. These products are also perceived as "bang for your buck" because of the THC concentration, often exceeding 80% THC. Hydrocarbon extraction is often utilized for the extraction of cannabis derived terpenes, which are then used with distillate to produce a more flavorful end product. While many industry members know that distillate is not a good source for an optimal cannabis experience, it is cheap and has perceived value by consumers so it continues to maintain its position as the top cannabis vape product on the market.

Despite its market position, distillate products are being challenged as consumers become more educated and empowered in their search for a favorite cannabis product. Extracts with full-spectrum cannabinoid profiles offer a richer, more effective, and balanced experience along with the flavor profile of the cannabis it came from. These extraction methods are the best options for producing highly flavorful and full-spectrum vapor products. CO_2 extraction offers the most versatility and selectivity among the extraction methods because the temperature and pressure can be manipulated to more efficiently extract different fractions of desired

Figure 1: Headset Market Report: Cannabis Concentrates: A look at category data and performance (1).

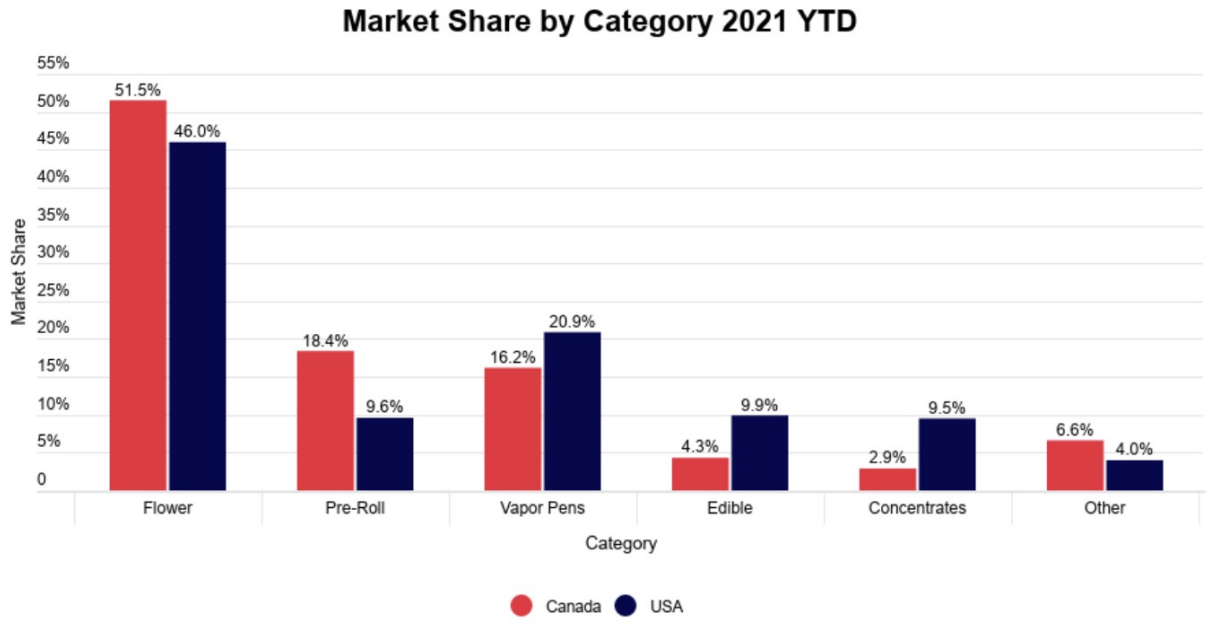
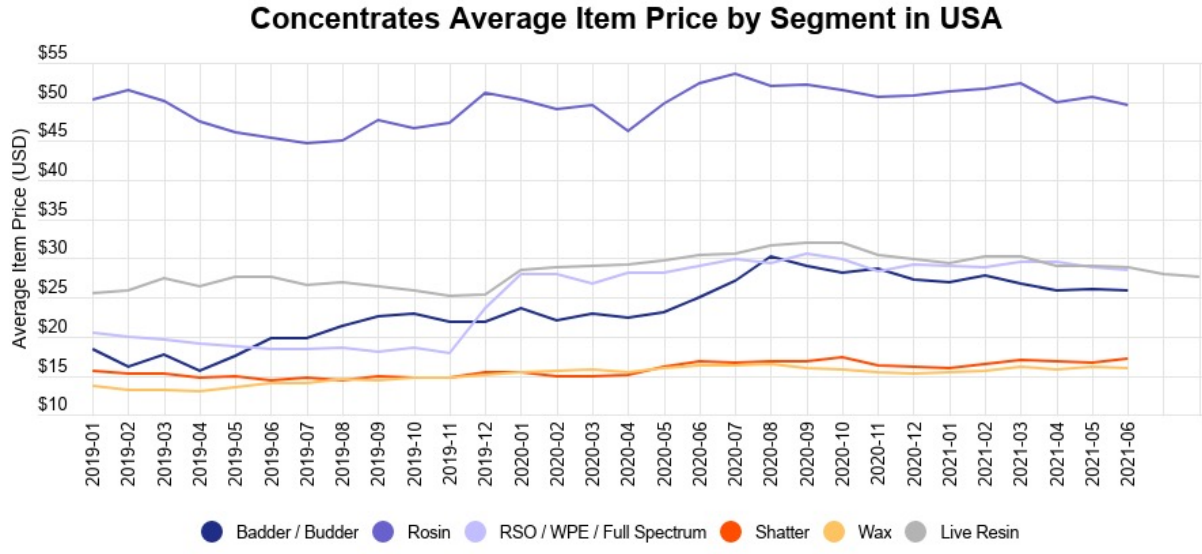


Figure 2: Headset Market Report: Cannabis Concentrates: A look at category data and performance (1).



compounds. For example, one can extract a rich and consistent terpene profile and cannabinoid profile simultaneously or separately using different parameters.

At one time, CO_2 extraction was the main method of extraction for the production of vapor cartridges. However, CO_2 extraction is much higher in cost and lower in efficiency than ethanol extraction. This results in higher cost vapor products, which has become a consistent challenge in a market that is continuing to bottom

out in prices (2). "Solventless" extraction is a category that has grown exponentially over the past two years. Methods in this category include rosin pressing and ice water hash. These physical separation methods are low in efficiency, but yield a highly desirable end product and consumers are willing to buy for the premium price as demonstrated in **Figure 2**.

Due to the nature of the method, rosin pressing must be done with a fine tuned method and high grade starting material to be able to produce an extract that will be flavorful, potent, and function in a vapor cartridge. If the extract is too thick or thin, the cartridge will not function or will leak. Temperature control and vape cartridge hardware have evolved to be able to support a wider range of extract types or to best function with very specific extract types, including rosin.

Vapor cartridges have a number of customizable features including:

- Material of the cartridge body
- Material of the cartridge wick
- Electrical current tolerance
- Aperture
- Volume

Each of these features affects the user experience and performance of the product. The most notable components are the materials used in the hardware, the electrical current range, and the hole size. The materials used to produce the hardware dramatically impacts the experience of the flavor as well as the safety of the product. It is increasingly important for consumers to be looking for hardware that will not leach heavy metals or toxic materials into the vapor stream, which will end up in the consumer's lungs. Findings have been published demonstrating that vapor from cannabis cartridges contained small amounts of chromium, copper, nickel, lead, manganese, and tin (3). Vapor hardware manufacturers have transitioned to more chemical resistant materials including ceramic to mitigate these risks.

Electrical potential range, usually measured in Volts, directly affects the temperature at which the extract is vaporized. The temperature of vaporization is important to ensure the vapor isn't too hot when entering the lungs. If the vapor is too hot, this can cause lung irritation. The temperature will also affect the compounds that are being vaporized. At too high a temperature, the compounds can convert into derivatives and result in different compounds entering the bloodstream than desired. There are also potential risks to consuming these derivatives as some compounds, such as terpenes, will convert into harmful toxicants like Benzene when heated over 400 °C. Some hardware manufacturers of vapor cartridges and devices have lowered the operating temperature and have also given the consumer more control over temperature settings with variable temperature controls. Lastly, temperature will affect the user's experience of the flavor. At too high of a temperature, the delicate flavor components will burn and leave the consumer with a harsh and unpleasant taste. While manufacturers and consumers are transitioning to lower temperature vaping, the vapor cloud naturally decreases in volume with lower temperatures. So, manufacturers are combating this by increasing the size of aperture within the cartridges. This will allow for more extract to be exposed to the wicking system and ultimately create more vapor per draw even at lower temperatures, which is what consumers are asking for.

In summary, the human lungs are extremely vulnerable, so pay attention to where your product is coming from. Inhalation is the fastest way to experience cannabis and often the most enjoyable because of the incredible benefits of the entourage effect. However, do the due diligence to vet and only consume products from trusted companies. Extraction method, cartridge hardware, and product testing should all play a factor in selecting the ideal cannabis product for you.

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ABOUT THE COLUMNIST

LO FRIESEN is the founder, CEO, and Chief Extractor of Heylo. With a background in chemistry and clinical research, Lo was inspired to explore cannabis as a medicine and to enter the emerging industry. She joined Eden Labs, a leading CO_2 extraction equipment manufacturer to support and expand a Research and Development department. There she managed the development of their latest and greatest CO_2 extraction system. In 2017, after working with Eden Labs and another cannabis processor, Lo launched Heylo with a mission to help people get more out of life with cannabis.

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Looking with Light:
Understanding Gas Chromatography, Part IV: Detectors

By ***Patricia Atkins***

In previous columns, we have looked at all the components necessary to achieve chromatographic separation in gas chromatography (GC) systems. Now, we will look at the final critical component to achieving successful chromatograms: the detector. The first components of the GC system are most critical for the separation of individual sample components, but it is the detector that actually specifies which types of components will be measured to create the final chromatographic results. Detectors range in functionality from simple general-purpose detectors (nonselective) to more advanced and sensitive (selective) detectors. In this column, we will investigate the differences between the commercially available detector in terms of sample type, size, and selectivity to understand which detectors fit into the most common types of cannabis GC analyses.

The components of a chromatographic system can be likened to instruments in an orchestra and the method the piece of music. Some instruments are almost truly essential like percussion while others expand the music or shape the direction of the sound. Like an orchestra, if the system is not tuned even the best method can produce jarring results. If the method is not well written, the composition can fall flat even with the best orchestra.

The final piece or the detector is the place where all this music or data comes to life. Imagine the difference between listening to a piece of music played in a small concert space versus an open area venue. Even though the instruments, musicians, and music are the same, the notes and flavors of the music will be different; losing some parts and being overwhelmed by others. The detector in a chromatographic system is that final filter which produces that final product of all the work in selecting mobile phases, stationary phases, instruments, columns, and settings. The detector, if chosen poorly is like listening to a concert on a poorly tuned radio compared to being at the event live.

Classifications of Detectors

The choice of detector is determined by many factors including the types of analytes, the concentration of the targets, and the fate of the sample being tested. Gas chromatography detectors are grouped under several categories. First, detectors can be either selective or nonselective (universal). *Selective detectors* are by their name, selective as to the types of analytes that can be detected and appear in the chromatogram. Selective detectors include detectors that only react with halogens, specific elements (S, N, C, P), or compounds with specific functionality (electronegativity). *Nonselective* or *universal detectors* work with a larger range of detectable compounds and are not limited to specific types of compounds or groups of elements (**Table I**).

Another way to look at detectors is by the fate of the sample after detection. Systems that consume or destroy the sample by evaporation, combustion, or mixing with other reagents to then measure the resulting materials are *destructive detectors*. Detectors that allow for recovery of the effluent or isolation of analytes are *non-destructive detectors* (Table I).

A final classification of for gas chromatography detectors is their type of response as a function of either their measurement of concentration or mass. *Concentration detectors* measure an analyte's concentration in the mobile phase whereas *mass flow* or *mass detectors* measure the absolute amount of the analytes in the carrier gas (Table I).

Understanding Universal Detectors

The choice of a detector is often heavily driven by the types of samples and analytes that the laboratory will be analyzing. Many laboratories depend

Table I: Common GC detectors: type and selectivity

Detector	Acronym	Type	Selectivity	Response Type	Destructive
Flame ionization detector	FID	Non-selective / universal	Carbon compounds	Mass	Yes
Thermal conductivity detector	TCD	Non-selective / universal	Thermal conductivity	Concentration	No
Mass spectrometer	MS	Non-selective / universal	Ionized molecular fragments	Mass	Yes
Electron capture detector	ECD	Selective	Electronegative groups	Concentration	No
Nitrogen-phosphorus Detector	NPD	Selective	Nitrogen, phosphorus, halogenated compounds	Mass	Yes
Flame photometric detector	FPD	Selective	Sulfur, phosphorus, halogenated compounds	Mass	Yes

heavily on universal or nonselective detectors to be the workhorse for the laboratory and hope that the wide range of functionality will suit most applications. The three most common universal detectors are the flame ionization detector (FID), the thermal conductivity detector (TCD), and the mass spectrometer (MS).

The most popularly used universal detector is the flame ionization detector or FID. It is sensitive to a wide variety of compounds with the exception of most inorganic gases, noble gases, halogenated compounds, and a few others. The FID is selective to hydrocarbons. It does not detect common contamination or background chemicals like carbon dioxide and water, which increases sensitivity to hydrocarbons. The FID is a destructive mass detector that measures the ions formed during combustion of the analytes in an air-hydrogen flame. In the flame, the sample undergoes pyrolysis to produce ions.

The ions produced are proportional to the concentration of the analytes in the gas phase. The ions are detected by the ion potential difference from two oppositely charged electrodes. The positive electrode is the nozzle where the flame is produced and where the negative electrode is a collector plate where the ions collect and produce a current upon colliding with the plate, which is measured as number of carbon atoms per unit of time and interpreted by an integrator into the chromatogram. Most responses are measured as time (*x*-axis) versus ion response (*y*-axis). This measurement is not affected by gas flow rate changes and is dependent on the mass of carbon atoms detected in a unit of time making it a mass detector (**Figure 1**). The advantages of the FID are its ease of use, sensitivity, wide range of analytes, resistance to interference from gases and water, and since it is a commonly used detector, the costs are less

Figure 1: Flame ionization detector (FID) general diagram.

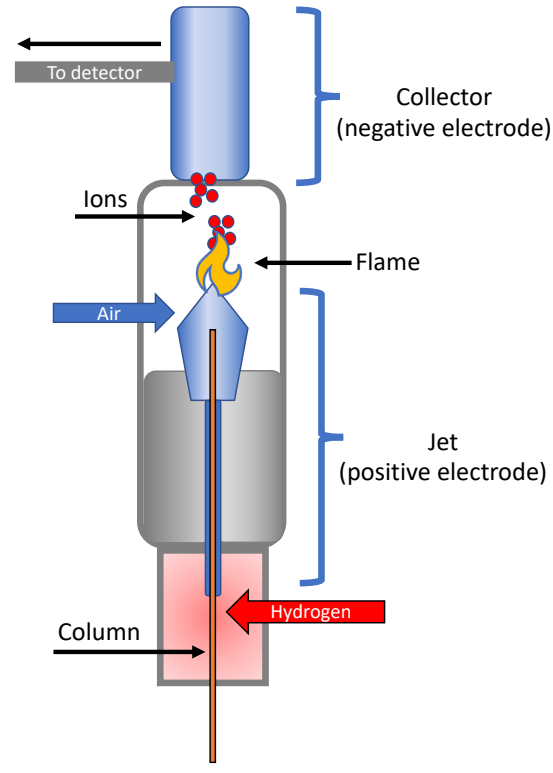


Figure 2: Thermal conductivity detector (TCD) general diagram.

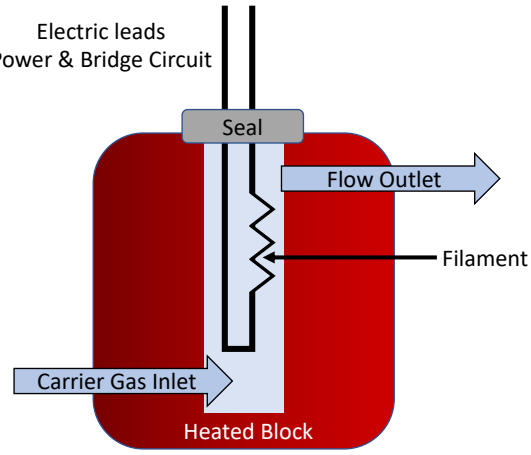
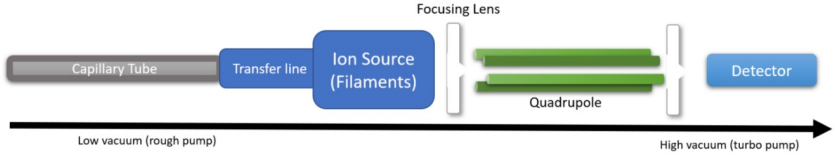


Figure 3: General diagram of simple quadrupole MS.



expensive than some other types of detectors. The disadvantage of using FID is that some compounds without a carbon-hydrogen bond are difficult to detect and the samples are destroyed during combustion.

The second type of universal detector is the thermal conductivity detector (TCD) or a katharometer. The TCD is a nondestructive concentration detector that measures the change in the thermal conductivity of the carrier gas by the presence of an analyte. The TCD contains a heated filament in a source made from a thin platinum, gold, or tungsten-rhenium wire whose power is kept constant by an applied current. The temperature of the source is dependent on the thermal conductivity of the gases. The resistance in the wire is dependent on temperature, which in turn is dependent on the thermal conductivity of the gas. As compounds elute from the column, they mix with the carrier gas and the conductivity decreases, which increases the filament temperature and resistance which then changes the current causing a response in the detector. The sensitivity is proportional to the filament current and inversely proportion of the incurred temperature and the flow rate of the gas. This makes the detection a measurement of concentration.

TCDs usually contain two detectors: one is the reference for the carrier gas and the other measures the conductivity of the sample and carrier gas mixture. Gases, such as helium or nitrogen, typically used in this application have a high thermal conductivity which increases the sensitivity of the sample detection. The advantages for a TCD are ease of use and a wide range of analytes, but the disadvantages include lower sensitivity than an FID which is two to three times more sensitive than TCD. TCD is able to see gases not visible to an FID detector, but it is also subject to more background noise from common contamination like water and carbon dioxide.

The third most commonly used type of universal detector is the mass spectrometer (MS), which we discussed in a previous column (1). As the name suggests, the MS is a mass detector rather than a concentration detector where compounds are subjected to ionization and the mass-to-charge ratio of charged particles results in a mass spectrum (intensity versus mass-to-charge plot). The ions can also be measured and plotted by abundance (*y*-axis) versus time (*x*-axis) to create a *total ion chromatogram* (TIC). The TIC is necessary to calculate quantitative results where the spectrum provides information on compound identity. Spectra for gas chromatography–mass spectrometry (GC–MS) are well documented and characterized by many organizations including National Institute of Standards and Technology (NIST) and these spectrum are widely available as either free or paid databases often incorporated into a manufacturer's MS system.

The main parts of a mass spectrometer are an inlet or transfer line, an ion source, a mass analyzer, and a detector (**Figure 3**). Sample inlets allow for the controlled introduction of a gaseous or vaporized liquid sample (or solid via a heated probe) through an aperture where the sample passes to an ionization source that generates ions. Most ionization techniques fall into either "hard" ionization or "soft" ionization depending on the ionization energy involved and the degree of fragmentation that results.

Hard ionization uses high quantities of energy in fragmenting the target molecules and result in a large number

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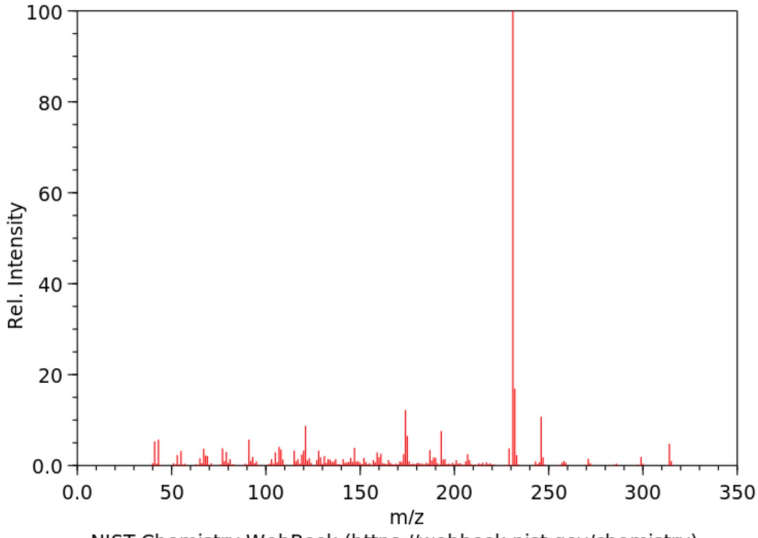
Figure 4: Example of GC–MS EI mass spectrum for cannabidiol.



of fragments from the rending of bonds in the original molecule. The fragments tend to have lower mass-to-charge ratios (*m*/*z*) than the parent molecule. The most common hard ionization technique for organic molecules is electron impact ionization (EI) that uses a high-energy electron beam (~70 eV) to form radical cations which then decompose to smaller fragments. These fragments are the basis for the mass spectrum (sometimes referred to as the molecular fingerprint) and its use in identifying compounds (**Figure 4**). EI requires a system that can be kept under high vacuum.

Soft ionization uses small amounts of energy to ionize molecules and result in only a small number of fragments. The most commonly used soft ionization for GC–MS is chemical ionization (CI). In chemical ionization techniques, ion fragments are produced by the collision between sample molecules and a collision gas. This type of ionization requires lower energy than other types of ionization depending on the type of sample and collision gas.

CI often provides simpler spectrum with little to no fragmentation. In CI, the molecular ion peak $[M+1]^+$ is present and is helpful to determine molecular mass. This simpler spectrum can limit the amount of structural information for a particular sample or element but can be useful when other stronger ionization techniques—such as EI—that can make molecular ion peaks undetectable. CI techniques, similar to EI techniques, tend to be used in conjunction with systems under high vacuum.

The benefit of MS detectors is that they are very sensitive and provide information regarding compound identity in addition to quantitative data. The chromatographic separation is not as important to accurate quantitation since coeluting peaks can be separated by mass in the MS. There are many types of MS systems including some MS-MS tandem systems. Some of the disadvantages to mass spectrometry systems include cost, expertise, and operation. These systems do cost more than other universal detectors and require extra components such as vacuum pumps. There is more data produced for interpretation and the learning curve for an MS system can sometimes be more difficult than the other detectors.

Staying Selective with Detectors

Selective detectors, at first glance, seem to be almost like unitaskers in a laboratory needing multitaskers. These detectors are specialists at the compounds they detect. They are not meant for every type of analysis. Reverting back to our music metaphor, they are the conga drums when a Latin beat is required or the trill of the piccolo in a rousing Sousa march to get everyone clapping. These instruments are not the go-to instruments of the laboratory (or orchestra), but when you need their skills, they are often irreplaceable.

The first detector is commonly used in environmental laboratories where there are a lot of compounds with electronegative groups that are sometimes not amenable to other detectors, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides. The electron capture detector (ECD) is a selective, nondestructive concentration detector best used for trace level detection of compounds with functional groups such as halogenated compounds, conjugated double bonds, peroxides, quinones, nitriles, nitrates, and other electronegative groups.

An ECD uses electrons emitted from a radioactive nickel-63 or tritium emitter. The emitter ionizes the carrier gas (commonly nitrogen) to release electrons. A constant current passes between two electrodes. Analytes pass over the emitter upon leaving the column and decrease the current between the electrodes by capturing of electrons with the analyte's electro-negative functional groups. The detector is unable to detect changes in current

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Figure 5: Simple electron capture detector diagram.

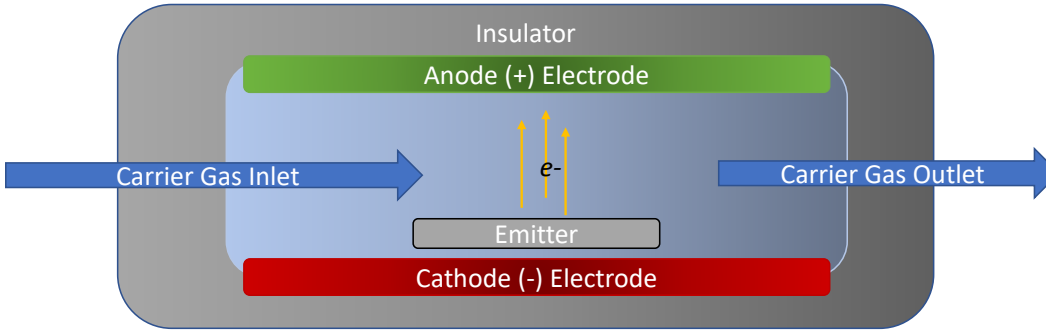


Figure 6: Basic nitrogen-phosphorus detector diagram.

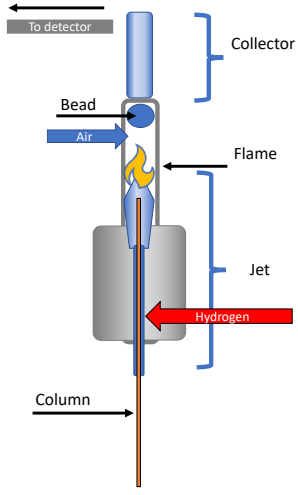
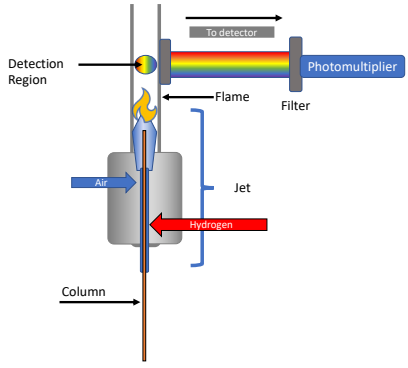


Figure 7: Basic Flame Photometric Detector (FPD) Diagram



for analytes that lack electro-negative groups. The loss of charge is measured, and signal is produced.

An ECD is highly selective and sensitive to analytes with electronegative functionality (up to 1000x more than FID and 10^6 more than TCD); however, it has issues with limited signal range and dangerous components because of radioactivity. These detectors are best suited for specific applications such as pesticide, herbicide, or PCB analysis.

Nitrogen-phosphorus detectors (NPD) are selective, destructive mass detectors that can be used to detect analytes with nitrogen, phosphorus, or halogen substituents. NPD are often found in laboratories that test for low levels of drugs or pesticides. This detector burns compounds in a plasma around a rubidium bead (collector) with hydrogen and air. Compounds with nitrogen, phosphorus, or halogens produce ions that are attracted to the bead. The number of ions colliding with the collector is measured (**Figure 6**).

The final commonly used selective detector we will look at is the flame photometric detector (FPD). This destructive mass detector can detect halogenated and sulfur compounds like the NPD but instead of nitrogen, the FPD can also detect tin or sulfur compounds. These compound can include important pollutants such as mercaptans, sulfides, and alkyl tins found as by-products of petroleum, paper processing, and marine antifouling paints that prevent barnacle and mussel growth on oil rigs, boats, and barges.

The FPD uses a hydrogen-air flame to burn compounds to produce light. A monochromatic filter allows the selected wavelengths of light to pass to a photomultiplier to generate a signal. Each wavelength of light corresponding to a particular light producing species (that is, sulfur 394 nm or phosphorus 526 nm) requires a different filter to be detected; so, species can only be measured one at a time.

Final Thoughts

The decision of which gas chromatography detector to choose comes down to the type of analyses that you will be performing, the chemistry of the analytes, and the degree of sensitivity needed for the analysis. Universal detectors are often average or pretty good for a lot of targets but not particularly great at detecting any one target or getting down to very low detection limits. Selective detectors are great only for a small number of targets making their range of use very narrow; but for the compounds they do detect, they can measure with great sensitivity (**Table II**).

Other considerations in choosing detectors could be ease of use, amount of

Table II: GC detector parameters: sensitivity, range, gases, and applications

Detector	Acronym	Sensitivity	Linear Range	Carrier Gas	Support Gases	Applications	Species Detected
Flame ionization detector	FID	pg–ng	10^5–10^7	Hydrogen, helium, or nitrogen	Hydrogen and air	General	Most organic compounds; especially with C-H bond
Thermal conductivity detector	TCD	low ng	10^4– 10^6	Hydrogen, helium, or nitrogen	Reference gas same as carrier gas	General	Most thermally conductive compounds
Mass selective detector	MSD	ng scan; pg SIM	10^5–10^6	Hydrogen, helium, or nitrogen	Vacuum (EI or CI) or collison gas (CI)	General	Wide range
Electron capture detector	ECD	ng–pg	10^4–10^6	Hydrogen, helium, nitrogen, or argon and methane	Nitrogen or argon and methane	Pesticides, PCBs	Halides, nitrates, nitriles, oxygen containing compounds, anhydrides, organometallics
Nitrogen-phosphorus detector	NPD	low pg	10^5	Helium or nitrogen	Hydrogen and air	Drugs, pesticides	Nitrogen and phosphorus
Flame photometric detector	FPD	pg	10^3–10^5	Hydrogen, helium, nitrogen, argon	Hydrogen, air, and oxygen	Environmental pollutants such as alkyl tins, petroleum, and paper processing by-products	Sulfur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium—light producing elements

training required, cost of systems, and cost of additional components such as makeup gases, vacuum pumps, and so forth. The workhorses for the cannabis GC laboratory should always start with an FID and an MS system to cover both the range of concentration and compounds. If a choice has to be made between the FID system and an MS system, often the MS is a better choice since most samples can be diluted down to an analytical range on an MS, but there are many trace analyses out of the range of many FID systems.

As for the more selective GC detectors, they can have their place in the cannabis laboratory if some targets are not well detected using an FID or MS system. These detectors can be utilized to fully investigate some of the more problematic pesticides that are not well suited for FID or MS. Selective and specialized GC detectors such as an ECD or NPD can be considered to increase accuracy of selected pesticide screenings.

Hopefully this look into the different types of GC detectors has given you a deeper understanding of gas chromatography systems so that you can make knowledgeable choices that work with your samples and chemistries to produce better chromatography and accurate results.

Further Reading

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ABOUT THE COLUMNIST

PATRICIA ATKINS is a Senior Applications Scientist with Spex, an Antylia Scientific company and has been a member of many cannabis advisory committees and working groups for cannabis including NACRW, AOAC and ASTM.

Passing the Test: Understanding Proficiency Testing

Testing laboratories in the analytical world use proficiency tests (PTs) to comply with their accreditation requirements and evaluate analysts' performance. PTs are an integral part of a quality management system (QMS) under quality assurance and control (QA/AC). Understanding the core components of the QMS is an important part of passing any PT test. Unacceptable PT results may have little to do with the result itself but reflect the use and application of statistics, standards, and methods. As more cannabis and hemp laboratories join implement quality management systems and become accredited under standards organizations, they also become subject to the need for proficiency testing. It can be argued that many PTs directed toward the general laboratory community are not a good fit for purpose in the cannabis community. In this article we discuss the purpose and best practices for PTs and how they can be better adopted to the cannabis market.

UNDERSTANDING PTS

PTs are characterized materials created to represent the types of samples, matrices, and analyte targets being evaluated in laboratories. PTs are assigned reference values which are not disclosed to participants. PT samples are treated like blind samples; in which the nature and quantities are unknown. PT tests may provide basic information regarding the target identity, quantitative range and other information which directs the analyst in how to perform sample preparation or analyses. PT samples can come in many different forms from solid or liquid matrices to extracted oils, or solid dosage formulations like capsules and tablets. Analysts are expected to prepare and treat PTs in the same way similar types of samples would be routinely processed.

PT participants confidentially share their results with the PT provider for final evaluation and grading. In this way, PTs serve as an indicator for the competency of a laboratories staff and analytical performance. Results reported to the PT provider are compared to the established reference values for that PT. A reference laboratory obtains established reference values or averages the values reported by the PT participants (consensus value).

Results can be reported individually, meaning each analyst reports a result, or by calculating an average from all analysts performing the PTs. The individual result entry method is often preferred because it serves to document passing or not passing results at an individual level. This is most helpful for assessing and attributing competency of each lab analyst performing the PT. Participants who achieve passing PT results are ensuring the validity and reliability of their lab's test results.

PTs should be an integral part of all testing labs' quality system. When evaluating PT programs, laboratories must consider some critical points such as: the qualifications of the PT administrator, the quality and qualifications of the PT provider, and the accessibility of data. Within QMS systems based on ISO guidelines, laboratories are required to use certified providers for their analytical standards, methods, and PTs. ISO 17025 laboratories must use PT providers accredited to ISO 17043 and CRM providers to ISO 17034.

Methods used for PTs should have previously been validated by the laboratories or standards organizations which have issued those methodologies (i.e., AOAC, ASTM). In cases that standardized methods are not available; the laboratory should validate or verify their methods that will be used for the PT before the study begins. PTs are not a means of methods validation. A suitable QC-known, certified reference material or primary reference standard is employed for validating an analytical method. If a certified reference material is not available; a suitably validated and characterized material (usually by consensus or round-robin testing) can be validated for use as standard in lieu of a CRM.

Acceptable PT results can serve as method verification when PTs are performed by the lab's validated method. After the in-house methods have been validated, the methods can be verified by successfully passing a PT from an accredited third-party provider. In this example, PT is integral to the QMS of the participant lab.

PTs serves to measure the ongoing proficiency of independent laboratories through interlaboratory comparison of test results for the same sample. Some regulatory bodies stipulate the frequency of PT participation. Testing laboratories participate in accordance with their accreditation audit schedule. Some accreditation audits only occur once every other year. Thus, the labs would participate in PTs once every two years.

When determining the schedule and budget for PT participation, it is best to consider the accreditation goals and regulatory requirements for the laboratory when determining the schedule and budget for PT participation. It is best for each analyst who is reporting data to perform PT at least annually to monitor performance during a twelve-month period. Longer stretches without PT participation could negatively impact timely identification of non-conformance and delay implementation of effective corrective action. Laboratories that do not achieve acceptable results on their PT should examine analyst training and method procedures to check for deficiencies.

FIGURE 1: Unit conversions

Concentrations and Conversions

Weight to Weight Concentrations

Name	Symbol	Equivalence			
Parts per thousand*	ppt*	g/kg	mg/g	µg/mg	ng/µg
Parts per million	ppm	mg/kg	µg/g	ng/mg	pg/µg
Parts per billion	ppb	µg/kg	ng/g	pg/mg	fg/µg
Parts per trillion**	ppt**	ng/kg	pg/g	fg/mg	ag/µg

Weight to Volume Concentrations

Name	Symbol	Equivalence			
Parts per thousand*	ppt*	g/L	mg/mL	µg/µL	ng/nL
Parts per million	ppm	mg/L	µg/mL	ng/µL	pg/nL
Parts per billion	ppb	µg/L	ng/mL	pg/µL	fg/nL
Parts per trillion**	ppt**	ng/L	pg/mL	fg/µL	ag/nL

Concentration Conversions

Name	Symbol	ppt*	ppm	ppb	ppt**
Parts per thousand*	ppt*	—	1×10^{3}	1×10^{6}	1×10^{9}
Parts per million	ppm	1×10^{-3}	—	1×10^{3}	1×10^{6}
Parts per billion	ppb	1×10^{-6}	1×10^{-3}	—	1×10^{3}
Parts per trillion**	ppt**	1×10^{-9}	1×10^{-6}	1×10^{-3}	—

PTS CALCULATIONS AND RESULTS

Once participants analyze the PT, the participants report their results to the PT provider for review and evaluation. PT providers use customized software programs to evaluate participants' results using programmed algorithms and statistics that meet the accrediting bodies guidelines. ISO guidelines (ISO 13528) have two basic methods for statistical evaluation of proficiency tests: the En-value and the z-score. An En-value is most commonly used in interlaboratory comparisons where the laboratories report their uncertainty calculations. (**Equation 1**).

$$E_n = \frac{X_i - X_{ref}}{\sqrt{u^2_X + u^2_{ref}}}$$

Equation. 1 En-Value Equation: Xi is lab reported value versus the Xref value reported by PT provider divided by the square root of the combination of uncertainties from the lab value and the reference value expressed using k=2 with a 95% coverage factor. Results with En-value between 1 and -1 are acceptable and agree with the reference values. Results with En-values greater than one or less than -1 are not acceptable.

The most practical of the statistical functions for chemical and biological analyses is the z-score. (**Equation 2**).

$$\text{Z-score} = \frac{X_i - \mu}{\sigma}$$

Equation 2. Z-score equation where µ is the mean subtracted by the Xi lab reported value; divided by the standard deviation, s.

The z-score can be used within interlaboratory comparisons without an uncertainty calculation; The z-score assumes all reported samples are from the same population, batch, or sample set and have the same uncertainty.

The results are calculated after the outliers are removed or are obtained from robust statistics. Z-scores less than two are considered acceptable. Scores between two and three are considered suspect while scores greater than three are unacceptable.

Once participant results are compared to reference values and assigned acceptable or unacceptable scores, those scores are provided to the laboratories. Results of a particular PT scheme may be deemed suspect if a clear bias or trend is apparent. Even if the results themselves are passing, the PT results can be considered unsatisfactory if there is a clear and measured bias. All failures or unsatisfactory results should be directed towards root cause analysis and corrective actions indicated by the lab's QMS.

FIGURE 2: Dilution tips

Dilution Tips

- Make sure you keep track of units.
- Unify units when possible (i.e. convert to grams, µg uniformly).
- Don't forget purity when making a stock solution.
- Don't forget to account for the weight or volume of internal standards or spiking solutions.
- Make sure your calibration points are within range of your analytical targets and within the dynamic range of the instrument, if not either the samples or standards will need to be diluted.
- Start calculations with the stock solution or raw material dilution.

Starting Material or Stock Starting Calculation

X **(mass or volume units of Target) / Final (mass or volume) * Purity (or Concentration) of material * 10^6**

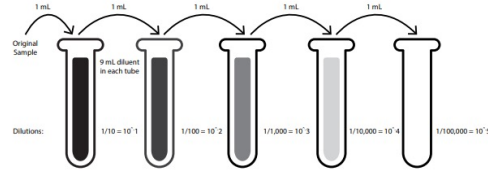
- Another type of dilution is a simple dilution using a dilution factor as seen in the equations below.

Simple Dilution Equations

Dilution = Volume of Mass of Sample / Total Volume or Mass of (Sample + Diluent)

Dilution Factor = Total V of (Sample + Diluent) / V of Sample
** or we can simply say the reciprocal of dilution

- In order to make calibration curves, most people employ a mixture of simple dilutions separate from each other or use a serial dilution which is a series of dilutions, where each dilution is cumulative. Typical serial dilution scheme:



CORRECTIVE ACTIONS

In the event that analysts or laboratories fails PTs, there should be a procedure for review along with a plan of action to correct the problems found. First, a root cause analysis is conducted to identify and document the problems. If a root cause analysis finds deficiencies within a system, then corrective action is devised to remedy issues. A retesting of a corrected system must be conducted to determine if the correction was successful.

Points of review should include laboratory processes and all internal quality control data. Points to reexamine during a system and procedure review for a PT failure include:

- ✓ Preparation – Did the preparation process differ between routine samples and the PT samples?
- ✓ Instrumentation and equipment - Does the deficiency lie within the system, and can it be corrected with replacement, repair, or recalibration?
- ✓ Environment – Where are all materials and equipment maintained at the correct temperature?
- ✓ Examine all processes, standards, and calculations:
  - Were all the calculations correct and documented including unit conversions? (**Figure 1**)
  - Are all the dilutions correct and within the correct dynamic range for the system? (**Figure 2**)
  - Were the standards, reagents, QC samples and controls within expiration? Were samples and standards prepared fresh or new standards opened?

BEST PRACTICES FOR PTS PREPARATION

The proper handing and processing of PT samples can be critical in passing PTs. There are other additional areas which play key roles in passing these tests including instrument calibration, maintenance, and updating procedures and methodologies. Here is a list of helpful points to assist laboratories and analysts in passing their PTs. (Figure 2)

PTS LIMITATIONS

PTs are a necessary and helpful tool in the QMS arsenal of a laboratory. But they do have some limitations and misuses. In PT schemes where interlaboratory tests are used to establish values, there is often a

retrospective aspect to the tests. There are often routine samples or analyses not reflected by the PT schemes either because the PT is offered in a different sample format or matrix than what the lab routinely tests.

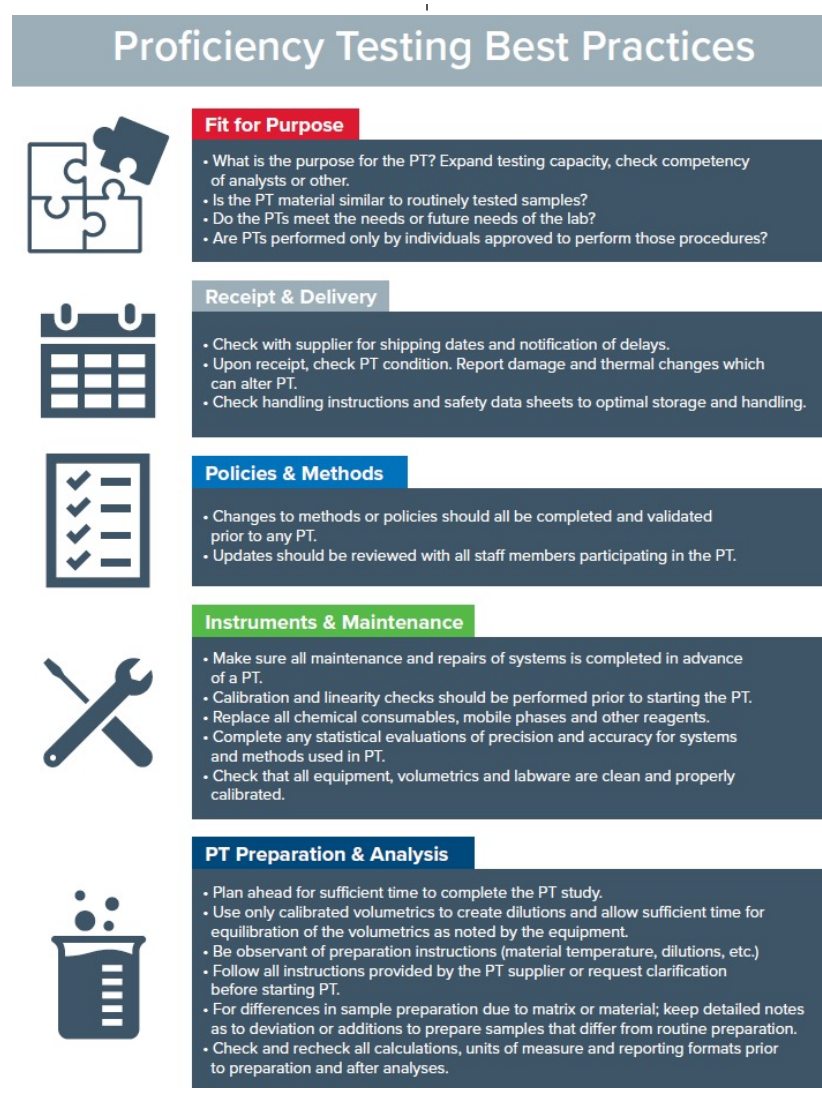
For example, if a laboratory tests pesticides in cannabis extracts in ethanol but the PT is pesticides in plant material, the PT doesn't match the preparation routine of that laboratory. In this case, the laboratory must document all the steps taken to prepare the PT sample and the ways in which it could differ from the preparation of actual samples. The best practice is to try and match the PT to the types of routine samples run by the laboratory. If a commercial PT program does not offer an obvious match in an established PT program; the laboratory can work with the PT provider to create the matrix-matched PT suitable for that laboratory's needs.

An unacceptable PT only indicates a problem exists. It does not identify the root cause of the problem. Conversely, an acceptable PT result does not indicate competence in all areas of QMS. PTs are not substitutes for using quality controls and reference standards. They all work collectively within the QMS.

CONCLUSIONS - TYING IT ALL TOGETHER

PTs serve to measure the ongoing proficiency of independent laboratories through interlaboratory comparison of test results for the same characterized material. PTs participation is often stipulated by the regulatory authority overseeing laboratories operations or an accrediting body to which laboratories comply, or often both authorities concurrently. It is best to consider the laboratories accreditation goals and regulatory requirements when determining the schedule and budget for PTs participation. It is best to perform PTs at least annually to monitor lab performance during a twelve-month period. Longer stretches without PTs participation could negatively impact timely identification of non-conformance and delay implementation of effective corrective action. PTs are useful as tools to verify continuous improvement, resolve or monitor effective corrective actions, and demonstrate training and competency for lab analysts. PTs are a multi-purpose external quality assessment tool and should be an integral part of the lab's QMS.

FIGURE 2: Best practices for PTs



FURTHER READING

- ISO/IEC: 17043:2010 – Conformity assessment – general requirements for proficiency testing
- ISO/IEC: 17025- General requirements for the competence of testing and calibration laboratories
- USP Proficiency Testing Program: www.usp.org/proficiency-testing
- NSI Laboratory Solutions PT Program: www.nsilabsolutions.com/proficiency-testing/
- Spex Knowledge Base for white papers on dilutions, calibrations, and standards: www.spex.com/KnowledgeBase/AppNotesWhitepaper

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The Chiral Separation of the (+) and (-) Enantiomers of Cannabidiol

BY WESTON J. UMSTEAD

With the continued increase in the demand for cannabis-based products, extraction of cannabinoids from plant-derived materials might not remain a feasible route into the future. Synthetic routes for cannabinoid production have been developed to assist with specific needs that arise based on demand, and will certainly be developed into the future as new needs arrive. Given the specificity by which living organisms synthesize compounds, special care needs to be given when developing these synthetic processes to ensure proper stereochemical control. This ensures the formation of undesired or unexpected isomers of the intended target is avoided. This publication investigates the chiral method development screening for the enantiomeric separation of synthetic cannabidiol (CBD) on polysaccharide-based chiral stationary phases with both normal-phase and reversed-phase mobile phases, using high performance liquid chromatography (HPLC) and ultrahigh pressure liquid chromatography (UHPLC). Several new chiral separations of CBD enantiomers on CHIRALPAK IA, ID, IE, and IG are reported, with additional focus given to the use of longer columns, or the conversion from gradient to isocratic methods, to achieve complete baseline resolution of the enantiomers.

Plants and other living organisms are capable of synthesizing countless numbers of compounds required for said organism's biological pathways. Because of downstream requirements for transformation or uptake, these compounds are often produced as a single enantiomer. That is a compound that has two configurations that are nonsuperimposable mirror images, resulting from an atom with a tetrahedral geometry (a chiral center) that has four different chemical entities bonded to it. Cannabis is such an example—it contains a complex mixture of major and minor cannabinoids, as well as terpenes and other plant-based compounds. Most of these compounds contain a chiral center, but are only found as a single enantiomer. A notable exception is cannabichromene (CBC), which has been documented as a mixture of both (+) and (-) enantiomers (1).

With continuing increase in the demand for cannabis-based products, lab-based synthetic routes can be developed to help alleviate potential supply bottlenecks, was well as provide access to the non-naturally occurring enantiomers and derivatives for clinical study or commercialization. For the synthesis of naturally occurring cannabinoids, or any cannabis-based product intended for human consumption, it is not a stretch to assume that at some point chiral testing could be required by a governing body, much like the US Food and Drug Administration's (FDA) current requirements for pharmaceuticals testing (2).

The example presented in this publication is CBD. While cannabis synthesizes only the (-)-CBD enantiomer (3), synthetic routes have been reported to generate the non-naturally occurring (+)-CBD enantiomer (4). At present, the function of (+)-CBD is not fully established; however, it has been shown that it binds with a higher affinity to the CB_1 and CB_2 cannabinoid receptors than the naturally occurring (-)-CBD (4).

This publication explores the chiral separation of (+) and (-) enantiomers of CBD on polysaccharide-based chiral stationary phases, with normal-phase and reversed-phase mobile phases with high performance liquid chromatography (HPLC) and ultrahigh pressure liquid chromatography (UHPLC), with the goal of identifying baseline separations for the accurate quantification. Similar separations under reversed-phase conditions were reported previously (5), however this work improves on those methods by simplification of the mobile phase, and the use of a smaller particle size to significantly improve the analysis time.

Experimental

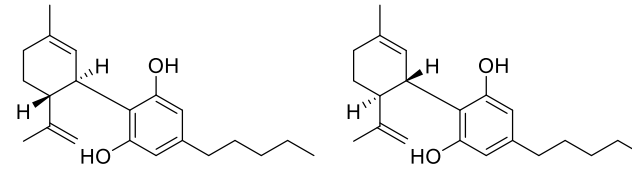
Samples of synthetic (+) CBD and (-) CBD were generously provided by KinetoChem, LLC. For normal-phase screening, each enantiomer was prepared separately as 2 mg/mL solutions in ethanol, and mixed in a 1:1 ratio. The columns screened included CHIRALPAK® IA-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, IJ-3, and IK-5 (150 mm L x 4.6 mm i.d.). For reversed-phase screening, each enantiomer was prepared separately as either a 1.2 mg/mL solution in MeOH (+ CBD) or 2.2 mg/mL in MeOH (-CBD), and mixed in a 1:1 ratio. The columns screened included CHIRALPAK® IA-5, IB N-5, IC-5, ID-5, IE-5, IF-5, IG-5, IH-5, IJ-5, and IK-5 (150 mm L x 4.6 mm i.d.). Solvents were purchased from Pharmco, were HPLC-grade or higher, and were used as-is. The hexanes (Hex) used contained 95% n-hexane, and the ethanol (EtOH) was reagent alcohol (90% EtOH denatured with 5% methanol [MeOH] and 5% 2-propanol [IPA] v/v/v). HPLC screening and optimization was performed on an Agilent 1200 equipped with a quaternary mixing pump utilizing a diode array detector (DAD). UHPLC optimization was performed on an Agilent 1290 equipped with a quaternary mixing pump, and a DAD.

Results and Discussion

Normal-Phase Chromatography

The individual enantiomers of CBD were mixed in a 1:1 ratio (as described earlier) and screened for chiral separation under normal-phase conditions using solvent mixtures Hex-EtOH = 95:5 (v/v) and Hex-IPA = 95:5 (v/v), on all available immobilized polysaccharide columns offered by Daicel. These starting conditions (95:5) provided for good retention on most columns. The initial screening resulted in a number of baseline or near baseline separations on most columns in the screening. Four columns, IA-3, ID-3, IE-3, and IG-3, all of a 3 µm particle size, yielded baseline or greater than baseline resolution. In all cases, the non-naturally occurring (+) CBD eluted first, with the naturally occurring (-) CBD eluting second.

Figure 1: Enantiomers of cannabidiol (- CBD, left; + CBD, right).



Because applications can vary widely from user-to-user, the conditions provided in this work should be considered a starting point for further application-specific optimization. For this reason, performance specifications (retention factor [k'], selectivity [α], or resolution [R_s] for instance) are not provided, as they will be different after end-user optimization. The goal therefore was to establish conditions that provided for a complete baseline resolution. The chromatographic conditions used to generate **Figures 2–7** are listed in **Table I** for reference, and are discussed further below.

The enantiomers of CBD were well resolved on IA-3 with both Hex-EtOH and Hex-IPA mobile phases (**Figures 2 and 3**). The separation was further improved from the initial screening by increasing the column length from 150 mm to 250 mm. This resulted in a greater than baseline separation of the enatiomers. Baseline resolution on ID-3 with Hex-IPA (**Figure 4**) was achieved on a shorter 150 mm length column by increasing the retention of the CBD enantiomers. This was accomplished by decreasing the elution strength of the mobile phase from Hex-IPA = 95:5 (v/v) to Hex-IPA = 97:3 (v/v). A longer column could likely also have been used with the original mobile phase conditions to achieve a similar result. The separation on IE-3 (**Figure 5**) was achieved using Hex-IPA = 95:5 (v/v) and a longer 250 mm length column. Finally, IG-3 (**Figures 6 and 7**) also provided good separation using both Hex-EtOH and Hex-IPA = 95:5 (v/v) mobile phases, on a longer 250 mm length column.

Given the large degree of separation that was achieved on the 3 µm particle size of IG (IG-3), the separation was also checked on a UHPLC instrument utilizing the sub-2-µm (1.6 µm) particle size equivalent, CHIRALPAK® IG-U. Smaller particle sized columns can be utilized at higher equivalent flow rates compared to their larger particle size analogs, without as noticeable of a drop in column efficiency. This arises from the reduced influence of the resistance to mass transfer, or C-term, of the Van Deemter equation. Because analytes are capable of interacting and departing from a small particle more efficiently than a large particle, the linear velocity (flow rate) of the mobile phase can be increased with less of a deleterious effect on resolution or selectivity

Figure 2: Separation of (+) and (-) CBD on IA-3 with Hex-EtOH = 95:5 (v/v).

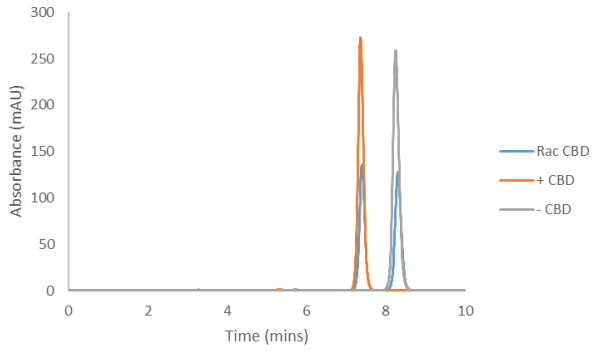


Figure 3: Separation of (+) and (-) CBD on IA-3 with Hex-IPA = 95:5 (v/v).

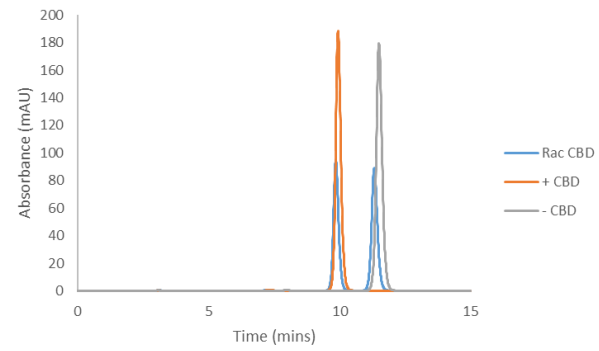


Figure 4: Separation of (+) and (-) CBD on ID-3 with Hex-IPA = 97-3 (v/v).

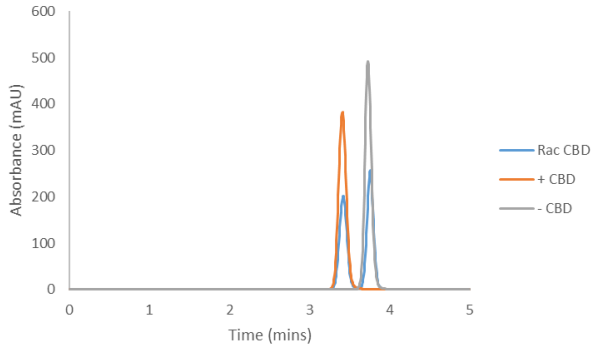


Figure 5: Separation of (+) and (-) CBD on IE-3 with Hex-IPA = 95:5 (v/v).

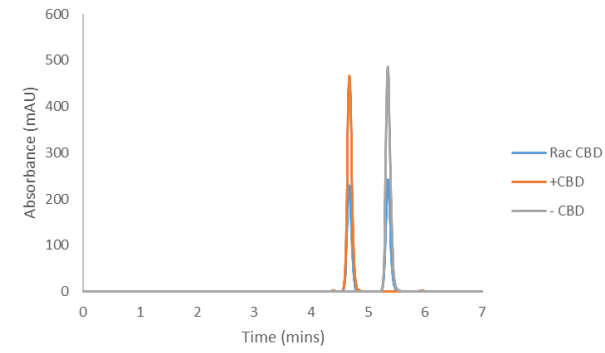


Figure 6: Separation of (+) and (-) CBD on IG-3 with Hex-EtOH = 95:5 (v/v).

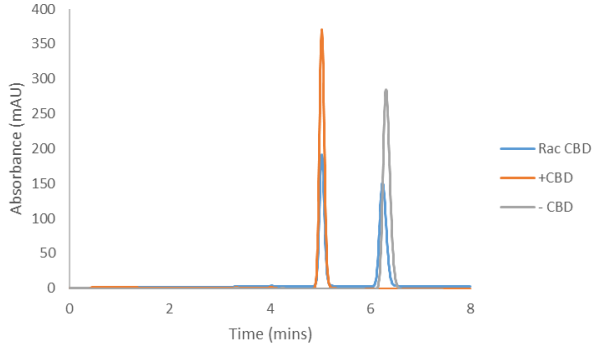
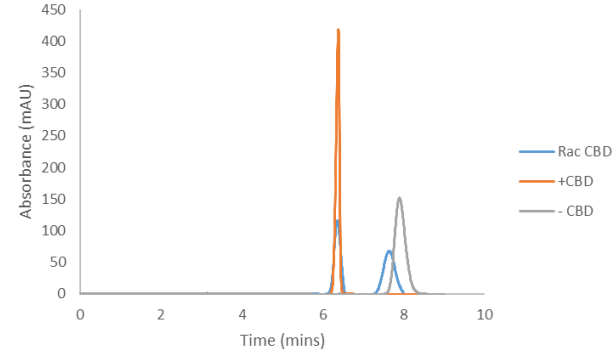


Figure 7: Separation of (+) and (-) CBD on IG-3 with Hex-IPA = 95:5 (v/v).



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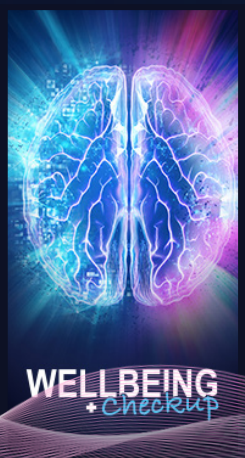

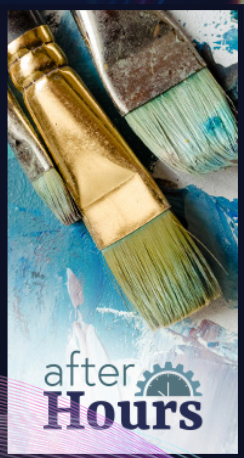

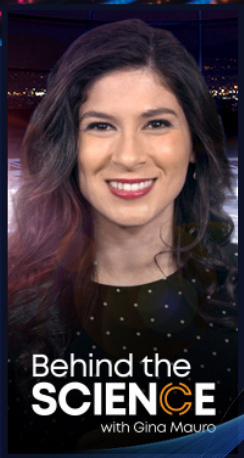

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Table I: Summary of the normal phase chromatographic conditions for the separation of (+) and (-)-CBD

Column	CHIRALPAK® IA-3 (250 mm x 4.6 mm i.d.)		CHIRALPAK® ID-3 (150 mm x 4.6 mm i.d.)	CHIRALPAK® IE-3 (250 mm x 4.6 mm i.d.)	CHIRALPAK® IG-3 (250 mm x 4.6mm i.d.)	
Moblie Phase	95:5 = Hex-EtOH	95:5 = Hex-IPA	97:3 = Hex-IPA	95:5 = Hex-IPA	95:5 = Hex-EtOH	95:5 = Hex-IPA
Flow Rate	1.0 mL/min					
Detection	UV 230 nm ref. 450 nm					
Temperature	25 °C					
Sample	(+) and (-) CBD – 2.0 mg/mL in EtOH					
Injection Volume	2 µL					

Table II: Summary of the reversed-phase chromatographic conditions for the separation of (+) and (-)-CBD

Column	CHIRALPAK® IA (5 µm)	CHIRALPAK® IG (5 µm) (150 mm x 4.6mm i.d.)
Moblie Phase	45:55 = Water-ACN	30:70 = Water-ACN
Flow Rate	1.0 mL/min	
Detection	UV 230 nm ref. 450 nm	
Temperature	25 °C	
Sample	(+) CBD – 1.24 mg/mL in MeOH	
Injection Volume	(-) CBD – 2.22 mg/mL in MeOH	

Figure 8: Van Deemter plot for varying particle sizes of CHIRALPAK IA immobilized polysaccharide CSP and the separation of trans-stilbene oxide.

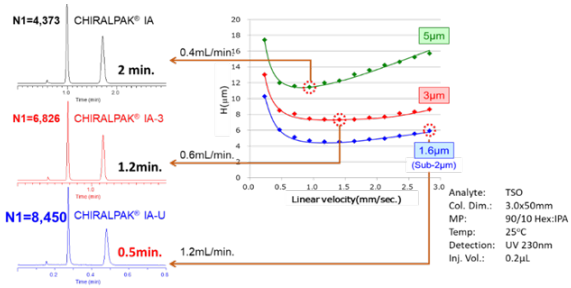
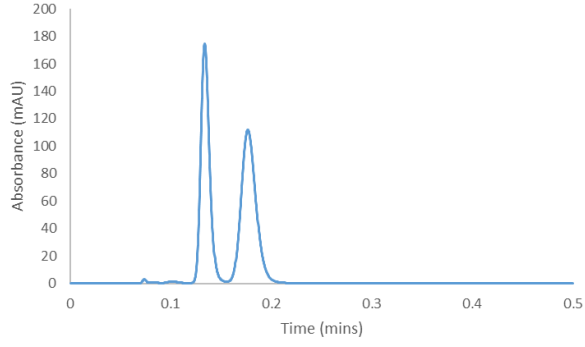


Figure 9: Separation of (+) and (-) CBD on IG-U with Hex-EtOH = 95:5 (v/v).



(6). Plotting the Van Deemter equation visually demonstrates the relationship between theoretical plate height and linear velocity, where the relative minimum of the curve is the theoretical optimal flow rate to achieve the greatest column efficiency (**Figure 8**). There are a few factors that affect the overall appearance of the curve, one being the packing efficiency of smaller particles. Because sub-2-µm particles pack more efficiently compared to 3- and 5-µm particles, the overall theoretical plate height (H) is intrinsically lower. As shown in Figure 8, an increase in the linear velocity moving left to right produces an upward sloping curve, indicating a loss of efficiency as the theoretical plate height increases. However for the blue trace (sub-2-µm), the slope is not as steep as the red and green traces (3 µm and 5 µm, respectively), indicating less of loss of column efficiency (for sub-2-µm). It should be noted that to maximize the benefits of a sub-2-µm particle size, a UHPLC should be used. UHPLC systems are optimized to reduce extra-column dead volume from excess tubing, unnecessarily large inner diameter tubing, and large flow cell volume, which will reduce the efficiency of any column, put specifically columns with a smaller particle size. UHPLCs are also capable of achieving higher operating pressures compared to standard HPLCs, meaning faster flow rates for increased analysis speed. Under the mobile phase conditions of Hex-EtOH = 95:5 (v/v), the flow rate on an IG-U 50 mm length by 3.0 mm inner diameter column was increased from 1.0 mL/min to 3.5 mL/min, which resulted in the separation of (+) and (-) CBD in under 15 s (**Figure 9**). As the selector for IG-U is the same as IG-3 (only the particle size is different), the elution order is the same as in Figure 6. This ultra-fast analysis would allow for a rapid check of multiple batches of material in only a few minutes, saving significant time for quality control (QC) and quality assurance (QA) release testing.

Reversed-Phase Chromatography

Sample preparation for reversed-phase screening was slightly different from normal phase. The enantiomers were prepared separately in MeOH (as described above), and mixed in a 2:1

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Figure 10: Separation of (+) and (-) CBD on IA with water-ACN = 45:55 (v/v).

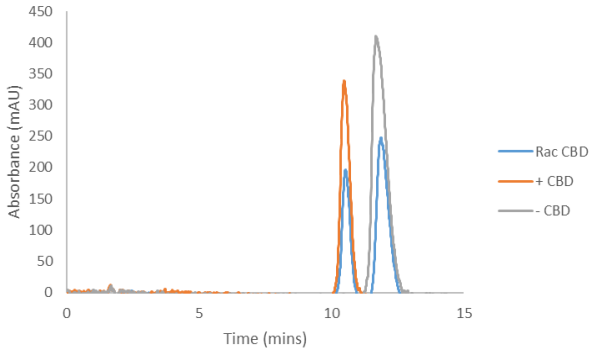


Figure 11: Separation of (+) and (-) CBD on IG with water-ACN = 30:70 (v/v).

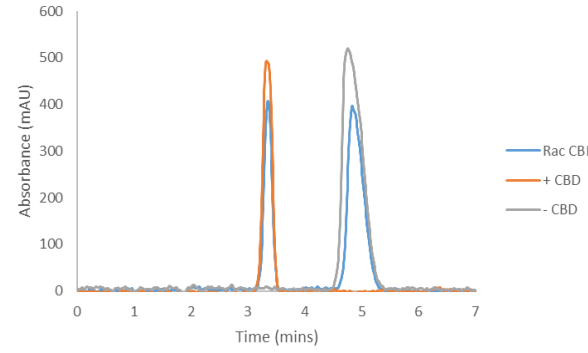
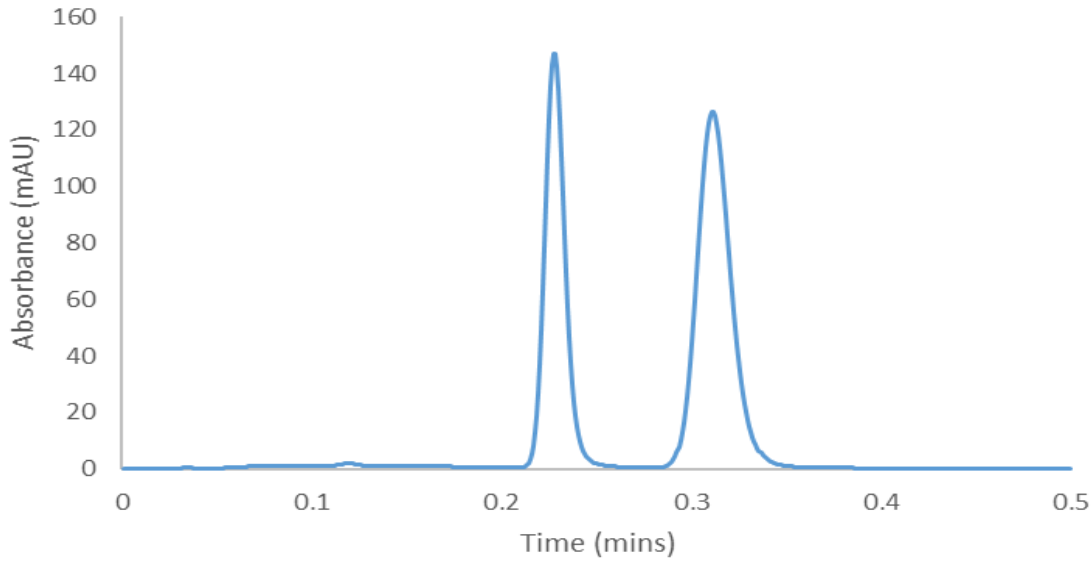


Figure 12: Separation of (+) and (-) CBD on IG-U with water-ACN = 30:70 (v/v).



ratio. The 2:1 ratio was chosen simply to better visualize any elution order reversal from what was seen under normal phase conditions. A reversal of elution order can be an important handle to optimize a separation. For analytical applications, it is often preferred to have a low-level impurity elute in front of the main peak to achieve a better limit-of-detection (LOD) or limit-of-quantification (LOQ), as this avoids the possibility that the main peak will tail into the impurity, negatively affecting the ability to detect, and integrate it. However, for a preparative application, the main peak or enantiomer of interest should elute first to achieve a higher chiral purity.

The 2:1 mixture was screened under reversed-phase conditions using a gradient of 34 min, rather than performing a retention check as was done for normal phase. The gradient started at 90% water and decreased to 10% water over 20 min, followed by a 6 min hold at 10% water, and a final 8 min re-equilibration at 90% water. Given the higher viscosity of aqueous mobile phases relative to normal phase alkane–alcohol mobile phases, 5 µm particle size columns were used for this screening (rather than 3 µm particle size). From this screening, baseline separations were observed on CHIRALPAK® IA and IG (5 µm), as well as several partial separations on other columns.

Similar to the normal phase applications, the methods described for reversed-phase should not be considered optimized. The goal was again to achieve a complete baseline resolution. To improve the rate of sample analysis (34 min might be considered too long for some applications), the initial gradient method was converted to as isocratic method. This resulted in a significant time savings, nearly 30 min for the separation on IG. Although polysaccharide-based columns can be used under gradient mode, for repeated analyses, an isocratic method can be preferred as it does not require column re-equilibration after each run. The final chromatographic conditions used to generate Figures 10 and 11 are listed in **Table II** for reference, and are discussed further below.

From the initial gradient, isocratic conditions of Water-ACN = 45:55 (v/v) resulted in a baseline resolution of the CBD enantiomers on IA (**Figure 10**). Although IG had a shorter retention compared to IA, it showed a greater selectivity. Because of this, a higher percentage of organic solvent (ACN) was used to elute the enantiomers from the column faster, decreasing the analysis time. After conversion from gradient to isocratic conditions, Water-ACN = 30:70 (v/v) was found to maintain a greater than baseline resolution (**Figure 11**). In both cases, no reversal of elution order was observed from the respective normal phase conditions.

Similar to the normal phase separation in IG-3, the resolution on IG under reversed phase conditions was also sufficiently resolved to merit checking under UHPLC conditions. With a mobile phase of water-ACN = 30:70 (v/v), the flow rate on an IG-U 50 mm length by 3.0 mm inner diameter column was increased to 2.5 mL/min., resulting in a sub-20 second separation (**Figure 12**). Again since the selector of IG-U is the same as IG (5 µm), the elution order is the same as in Figure 11.

Conclusions

This publication presents several new normal-phase and reversed-phase HPLC and UHPLC methods for the chiral separation of (+) and (-) CBD. Multiple columns and mobile phase conditions provide flexibility for users to choose conditions that best suit their laboratory or company needs. The reversed-phase methods have the added benefit of being mass spectrometer (MS) compatible should this be a requirement or need for a particular application. The added separations on Chiralpak IG-U provide conditions for rapid sample analysis, which would help conserve a considerable amount of time should these methods be implemented for high-volume testing.

Disclaimer

As a responsible provider of quality products and services, Daicel Chiral Technologies makes available analytical techniques that may be of use to a broad range of customers and applications. It does not however support or promote the use of its products or services in connection with any contraband activities or products related to cannabis, including but not limited to illegal or illicit drug manufacturing, testing, or consumption.

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Cannalingo Part II: Connecting Molecular Conversations of CB2R with the Cannabis Experience

BY AUDREY SHOR

Part II of a guided tour through the protein structure and function influence of cannabinoid receptor 2 (CB2R) on cannabinoid signaling.

OUR JOURNEY THROUGH the molecular maze of cannabinoid receptor signaling continues with an in-depth exploration of the structure, function, and variants of cannabinoid receptor 2 (CB2R). The second of two characterized cannabinoid receptors, CB2R, plays a significant, albeit lesser, role in cannabinoid signaling compared to CB1R. Of the five variants of CB2R that have been characterized in humans, two are silent, meaning that there is no change to the protein. Three of the variants are missense mutations, resulting in changes to the protein sequence, and potentially function. It is hypothesized that slight changes in the chemistry of these three amino acids may impact the cytoplasmic function and the internal signaling of the protein. The impact may influence the functional response to cannabinoids for an individual who expresses a CB2R variant. The structural and functional impact of these three variants will be considered as the role of CB2R in cannabinoid signaling is explored.

CBR2 was the second cannabinoid binding receptor identified in humans. It is a 360 amino acid protein that shares 44% of the same amino acid sequence as CB1R. Like CB1R, CB2R is also expressed in the cell membrane, where it awaits binding to ligands and becomes activated to initiate a series of signals that ultimately induces the effects of cannabinoids both foreign and endogenously synthesized. CB2R is more conserved across species, generally meaning the sequence of this protein has not changed or mutated much since the species who express this protein parted ways on the evolutionary tree.

We can find CB2R expressed in neuronal, glial, and endothelial cells in the brain where it contributes to the regulation of neuronal activity (1). It is most abundant in immune tissue such as the spleen, tonsils and thymus, cardiovascular and respiratory system, and reproductive tissue including the testis (**Figure 1**) (2,3). While CB2R is not expressed as abundantly as CB1R, the expression of CB2R is inducible, meaning that certain stimulation can cause this receptor to increase its expression within the membrane. While this might sound exciting, this type of stimulation is not usually like the ones we seek out for fun. Addiction, inflammation, anxiety, and epilepsy serve to stimulate CB2R expression (4). This explains why CB2R is such a popular target of emerging drug discovery efforts. CB2R serves as a possible disease or condition-associated target for potential therapeutic treatments to prevent inducing its expression with over stimulation caused by conditions such as anxiety and epilepsy.

The endocannabinoid 2-arachidonoylglycerol (2-AG) binds to and activates CB2R, while anandamide (AEA) does not. What's interesting is, 2-AG basal expression is 1000x higher than AEA in the brain. This abundance in expression contributes to the inducible nature of CB2R. Delta-9-tetrahydrocannabinol (Δ^9-THC) binds readily to CB2R, while cannabidiol (CBD) does not. Signaling by

CB2R is impacted by THC products, rather than CBD products.

CB2R is encoded by the *CNR2* gene located on chromosome 1. When expressed, it serves as a membrane embedded, G-coupled protein receptor (GCPR). Once activated by binding to a ligand, such as THC, the intracellular components of the receptor move to seemingly kick an intracellular G-protein into activation. This results in a domino effect, amplifying cell signaling that changes the cell's behavior thereby inducing the analgesic and other effects of cannabinoid signaling. CB2R's structure is typical of G-coupled protein receptors, including seven transmembrane domains that serpentine through the cell membrane, with four extracellular domains and four cytoplasmic, intracellular loops (**Figure 2**). The extracellular domains interact with ligands outside of the cell, such as THC, while the intracellular loops communicate the cellular response from the receptor being activated by the ligand binding to it (**Figure 3**).

There are two versions of CB2R expressed in humans: CB2A and CB2B. CB2A is a slightly longer version of the two, having a longer sequence at the start of the receptor. CB2A is predominantly expressed in the testis and to a lesser extent other tissues including the central nervous system and immune system. Whereas CB2B is predominantly expressed in the spleen and immune cells, followed by other peripheral tissues. How the CB2A structure and function changes because of the increase in length is yet to be determined. Some evidence suggests that the longer isoform may demonstrate more sensitivity to increased expression when induced by receptor agonists.

Similar to chromosome 6 in the CB1R story, chromosome 1 has also

Figure 1: Relative CB2R expression by tissue type.

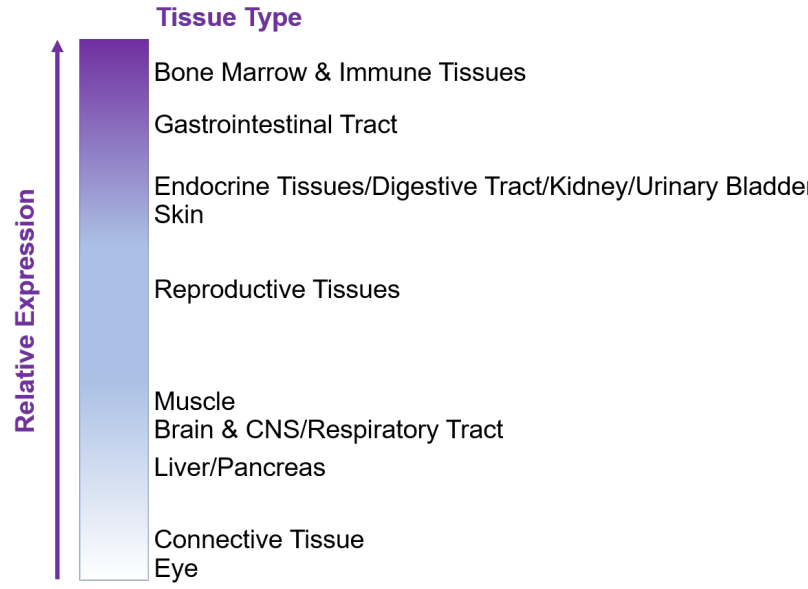


Figure 2: Linear cartoon of CB2R.

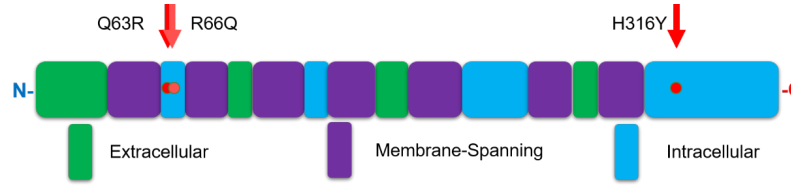


Figure 3: Membrane embedded cartoon of CB2R, a GCPR.

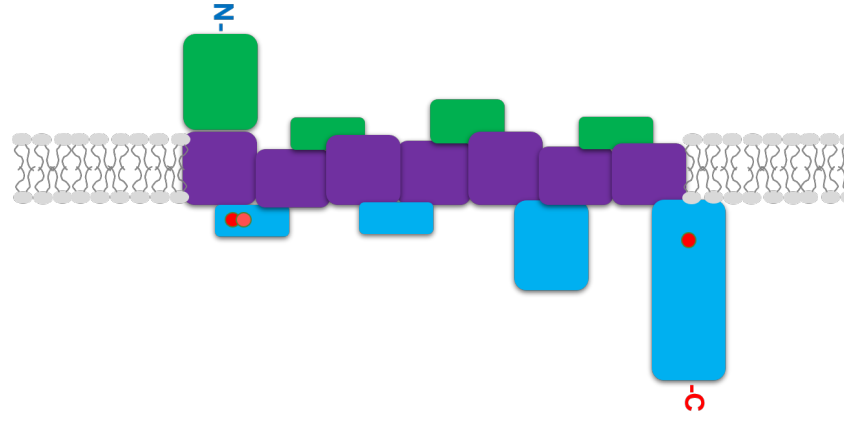
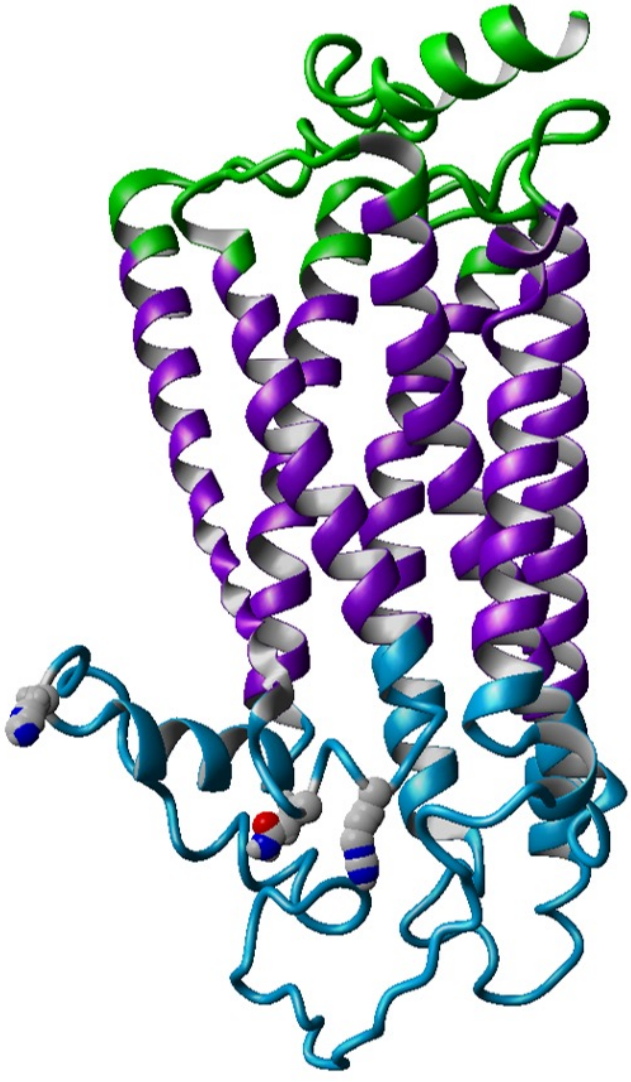


Figure 4: Structure of wild type CB2R with amino acid side chains mutated in variants displayed.



been demonstrated to have duplication events; extra copies of gene sequences may be present on the chromosome of certain individuals. However, these duplication events have not been observed in the portion of the chromosome that encodes for CB2R. Rather, the variant story associated with CB2R is far more exciting. Five variants have been characterized among people. To appreciate the intricacies of these variants, a short explanation about proteins is in store.

Proteins, the working molecules of life, are polymers assembled by building blocks of amino acids. There are 20 different amino acids found in proteins that support life. These amino acids differ in their chemistry; some are polar (hydrophilic, water-loving) and readily interact with the aqueous external and internal cellular environment. Hydrophilic dominant protein sequences will take on a three-dimensional (3D) shape that maximizes their interaction with water and other polar molecules. Other amino acids are nonpolar (hydrophobic or water-fearing), and exclude themselves from aqueous environments; hydrophobic dominating sequences of proteins often found in lipid filled membranes. Then, there are amino acids that can acquire a charge at physiological and more extreme pH. These amino acids can be negative or positively charged and often confer support for building the 3D shape or interacting with other molecules through ionic binding (the formation of salt bridges).

Proteins evolved to perform specific reactions to support the biochemistry of life. While proteins can be relatively short—a few 100 amino acids—to incredibly large—tens of thousands of amino acids—not all possible sequences of amino acids are observed in life. The energetic stability, as well as the ability of the resulting structure to support the given function of the protein dictate whether a protein is expressed and utilized to support life. Since life first began as single-celled organisms, life has been bombarded with exposures that mutate or change the DNA sequence that encodes for proteins. Some of the mutations resulted in variants of the protein that acquired a more efficient function, while others were removed from the population because the mutation did not support life. Over time, the mutations accumulated, duplication or removal of genetic material occurred, which ultimately lead to the production of new genes and proteins as the amino acid sequence changed, substituting, inserting, duplicating, or deleting amino acids along the way. As these new working molecules were introduced,

new functions and interactions contributed to more sophisticated cellular functions and life forms. Fortunately, the atmosphere on Earth has become more hospitable since life first evolved and we have acquired some molecular machinery that can help life overcome certain mutations. However, we still find variation among the population, imposed by natural errors in cellular replication or induced by exposures to things such as ultraviolet (UV) light damage or carcinogens. Regardless, protein variants persist.

Two of the CB2R variants identified, F97F and C313C are silent mutations. Meaning that while the DNA sequence of the gene has changed, the amino acid encoded for by that part of the gene has remained the same. Position 97 of the amino acid sequence is still phenylalanine and 313 is still cysteine. There is no change in the amino acid sequence or shape of the protein, but this also goes to show that not all mutations are bad.

The other three variants—Q63R, R66Q, and H316Y—are a bit more interesting (**Figure 4**). These are missense mutations because the amino acid in the protein sequence changes, but these have the added intrigue of changing the chemistry of that position along the protein too. We can begin to explore the impact variants may have on CB2R structure and function by running molecular dynamic simulations (MDS). In other words, running modeling calculations to determine the changes in stability of the overall protein, and even pinpoint where in the sequence the structure may change. These are simulations, predictions of what may be occurring under ideal physiological conditions. While not grounded truth, MDS provide us with a preview of what may be. To run an MDS, we start with the amino acid sequence of the protein under study. Working with a protein whose structure has been determined always provides more probability in your comparisons. Fortunately, the structure for CB2R has been

Figure 5: MDS demonstrating the overall protein stability (A) and by amino acid residue (B) of CB2R. The overall protein movement is significantly less stable among the variants compared to wild type CB2R (A). The variants demonstrated altered stability in ligand binding regions compared to wild type (B).

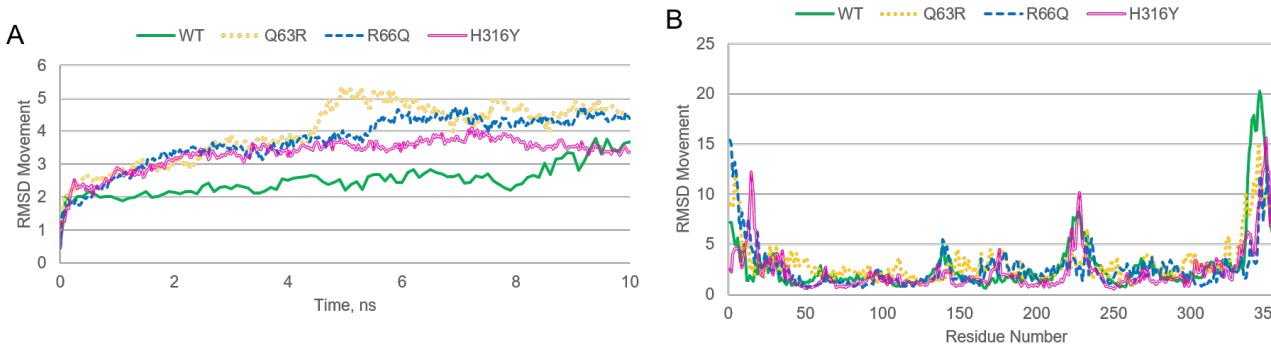
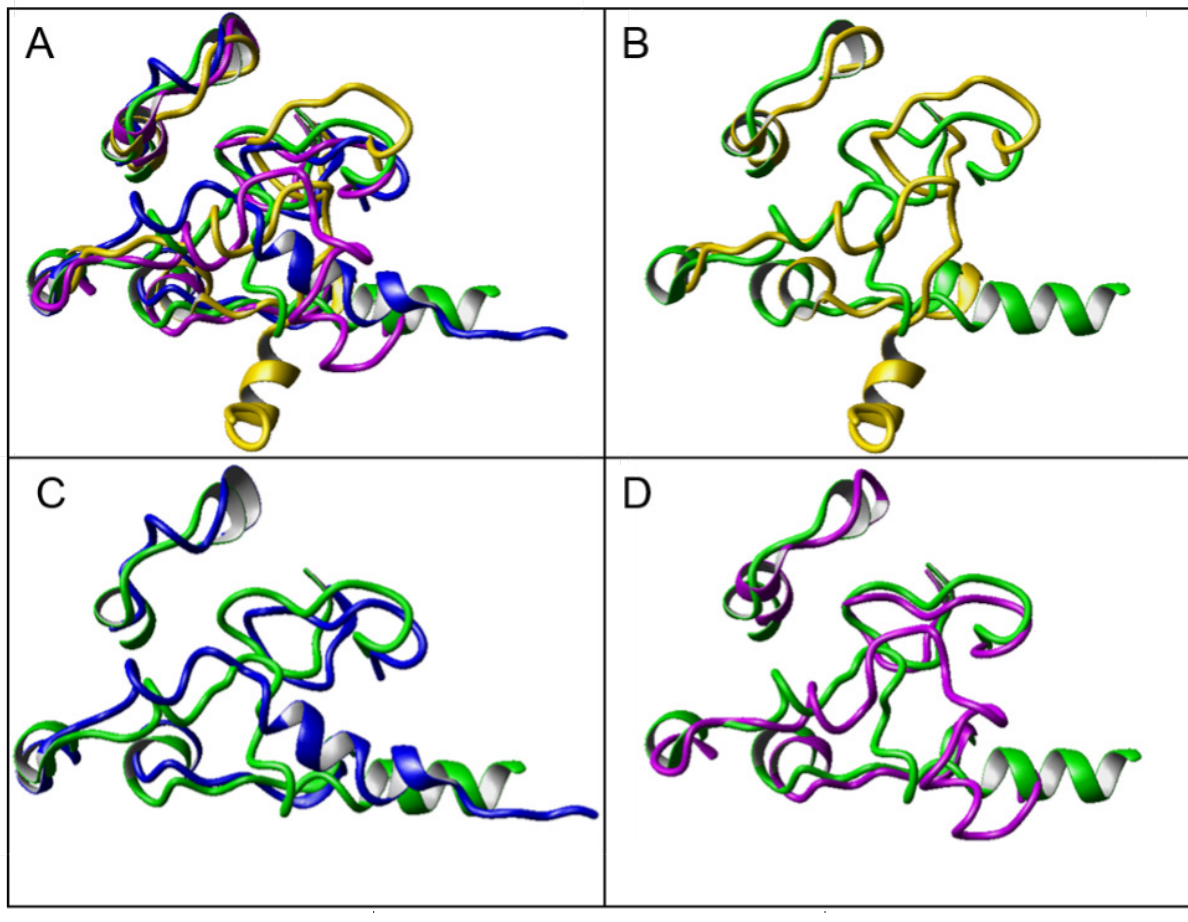


Figure 6: Superimposed images of looking down the extracellular structure of wild type CB2R (green) and; all three variants (A), Q63R (yellow, B), R66Q (blue, C), and H316Y (pink, D).



resolved and published. Next, we run some threading-based programs to predict the structure of the variants associated with CB2R. After scoring the possible structures, we can begin the MDS process. I did this on CB2R and these three variants, and found some compelling evidence to justify subsequent investigations into learning more about just how these variants contribute to the multiverse of CB2R signaling among people.

While all three variants are tolerable, an individual can survive these mutations, all three are significantly less stable than the wild type (original) protein (**Figure 5A**). The more movement observed as the protein takes on its shape during the first 10 ns of the simulation, the less energetically stable the protein. The first two variants at positions 63 and 66 of the sequence are both located in the first intracellular loop, while the variant at 316 is in the last intracellular portion of the protein (Figures 2 and 3). The first, Q63R, shorthand for glutamine, a polar amino acid, at position 63 is mutated to an arginine, a basic amino acid that is usually positively charged at physiological pH. This position of the protein is not super conserved among species, however the amino acid that is usually found here is either glutamine or arginine. This may seem like no big deal, however some hot off the press findings suggest that this variant may impact the severity of COVID-19, impacting the regulatory activity of endocannabinoids in immune cells, increasing the risk of inflammation. The Q63R variant may disrupt the G-protein's ability to dock and be activated by the receptor (5). This does not unlock mysteries about why one individual may be more sensitive to cannabis exposure, but it sure does open the door for more research into the role this variant may play during our current pandemic life. The impact on structure is not incredibly extreme, especially right where

Enzymes target specific **amino acids** that can be phosphorylated (namely serine, threonine and tyrosines), **adding** a phospho group to an amino acid **drastically changes** its **chemistry** and often contributes to the interactions between other proteins; opening the docking site to support these **interactions**.

the mutation is located. However, this change in chemistry may impact interactions the protein has with ligands in the extracellular domain (**Figure 6B**). More research is needed to sift through the impact of the variant further.

The R66Q variant is the opposite: an arginine at position 66 is mutated to a glutamine. Similarly, R66Q does not drastically change the structure of the protein at this intracellular loop, however the simulation suggests that ligand binding may be altered as well (**Figure 6C**). Unlike position 63, 66 is highly conserved across species. When it is not, the options for this position are generally arginine or glutamine. In fact, this arginine is part of a highly conserved sequence in CB2R, RSSVT (arginine, serine, serine, valine, threonine) that serves as a docking site for 14-3-3. When CB2R is bound to ligand and activated, 14-3-3 will bind to this region. This interaction may induce delays in the G_2 to M cell cycle progression and slow down cellular replication. Disrupting this interaction could result in problems for the cell and subsequently derived cells. The progression from G_2 to M serves as a checkpoint for ensuring that the cell is ready for mitosis (or meiosis depending upon the cell type). The cell must have enough energy, materials, and cellular machinery ready to support the process of dividing the cell into two daughter cells. Rushing through this phase may result in problems in the resulting daughter cells, which may be perpetuated in subsequent progeny from this faulty cell. Replacing the arginine in the RSSVT sequence may prevent the serines and threonine from becoming modified by phosphorylation. Enzymes target specific amino acids that can be phosphorylated (namely serine, threonine and tyrosines), adding a phospho group to an amino acid drastically changes its chemistry and often contributes to the interactions between other proteins, opening the docking site to support these interactions. The shape of the RSSVT sequence must complement the active site of the enzyme that adds the phospho group to the amino acid. The positive charge on arginine is significant to maintaining the complementary interactions with the enzyme. If the positively charged arginine is not in the sequence, QSSVT instead, the enzyme will not phosphorylate the serines or threonines as efficiently, 14-3-3 may not dock, and the cell can transition to M phase without conducting the appropriate quality control checks to make sure it is ready to do so. The impact this has on the cannabis experience is not quite elucidated yet, however other health consequences might be considered before teasing out this role.

The H316Y variant is characterized as a cyclic, polar, sometimes positive at fluctuations of pH common to the biochemistry of life amino acid to a cyclic, polar, and often phosphorylated amino acid. Histidine (H) and tyrosine (Y) share some characteristics yet are quite different amino acids. Different enzymes recognize, and physiological conditions influence, the phosphorylation of histidine versus tyrosine. This position along CB2R is found in the final intracellular domain and is significant in kickstarting the activation of the G-protein that initiates the resulting cellular signaling upon receptor activation. Activating the G-protein is an important part of this signaling pathway, so it is not surprising to learn that position 316 is in a highly conserved region of the protein. Fortunately, this variant appears to be well tolerated and must not impact the initiation of pathway too much. The MDS suggests that while the intracellular domain may not alter its shape too drastically, several potential ligand binding sites located on the extracellular domains of the receptor may be positioned differently (**Figure 6D**). Altering the shape of the ligand binding region of the receptor may impact cannabinoid binding efficiencies.

These are viable variants of CB2R. Albeit, there is some evidence to suggest that the 63 and 316 variants have been associated with autoimmune

disorders. Laboratory-based studies of the Q63R and H316Y variants expressed in cell lines suggest that the variants can bind to cannabinoids and induce signal transduction. However, cannabinoid agonists, such as AG-2 and WIN55212-2 (a synthetic agonist), had reduced efficacy on the variants. Further, these CB2R variants demonstrated greater constitutive activity compared to the wild type receptor (6). All three variants share potential modifications to the ligand binding regions of the extracellular domains of the receptor (Figures 5B and 6). Superimposing the structures suggest that ligand binding ability of the variants may be altered, with H316Y having the most significant changes in shape (notice the alpha helix to the lower right of the wild type has become disordered in the H316Y variant).

Interest in studying the role CB2R and its variants has increased since expression of this receptor has been observed in brain and CNS tissues. Evidence suggests that CB2R may play a role in neuropsychiatric disorders such as depression, schizophrenia, and substance abuse. Clearly, more research is needed to better understand how variants of this receptor impact an individual's response to cannabinoids.

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A Focus on Safety, Integrity, and Innovation:

One Cannabis Laboratory Strives to Exceed Standard Testing Practices and Client Relations

By ***Erin McEvoy***

Cannabis testing laboratories play an important role in ensuring consumer safety and quality throughout the industry. These laboratories face many challenges—from the variety of samples in the industry to changing regulations and testing requirements and everything in between. In this interview excerpt, Sarah Ahrens, Founder and CEO of True Labs for Cannabis LLC, explains her philosophy and approach behind building her cannabis analytical testing laboratory in New Jersey. She also covers current developments in the cannabis testing industry and what needs to be done to ensure it has a transparent and productive future that benefits producers and consumers alike.

Q: Can you tell us about your background? What are the main reasons you chose to start True Labs for Cannabis LLC (TLC)?

A: Sarah Ahrens: Before entering the cannabis industry, I had a successful career in sales, but if I were to start my career over again, I think I would have become a research scientist. My interest in cannabis and being a part of a new market, combined with my interest in science, led me to land on the idea of starting a cannabis analytical testing laboratory.

Beyond my professional background and interests, though, lies a personal passion for quality. Transparency and integrity are rooted in my DNA. I want to know what goes into the products that I buy for myself and my family. I'm health-conscious—I read all the ingredient labels and buy organic as much as possible. So, it was easy for me to see how I could help bring safety and transparency to the New Jersey cannabis market. It's not a coincidence that TLC also stands for "tender loving care."

Q: Did you face any unique challenges as the first woman-owned testing laboratory on the East Coast?

A: Ahrens: There are challenges associated with starting any business, but it has been a big benefit and a differentiator to be the first certified woman-owned cannabis testing laboratory on the East Coast. It feels good to be the first, but I certainly hope I'm not the only woman-owned laboratory for long. We need more women leaders in cannabis and in testing.

There have been challenges I've faced as a female entrepreneur, though, and particularly when it comes to access to capital. Being in the cannabis industry has its own banking and capital restrictions but combine that with the fact that less than 3% of investor capital goes to women-owned businesses, securing funding becomes a really steep hill to climb. It has certainly helped that I have a lifetime career in sales, but most female entrepreneurs are not so lucky. There's a lot more the industry could do to break down gender barriers when it comes to financially supporting women business enterprises (WBEs).

Q: What has been the most unexpected or rewarding experience you've had since starting TLC?

A: Ahrens: There have been several already. It feels special to blaze a trail by being the first certified woman-owned cannabis testing laboratory on the East Coast. I also never expected to play a big advocacy role, but it is so important in a market that is just getting started. It has been a great experience to develop a louder voice to stand up for consumer safety and robust testing standards. It is also amazing how small this industry can get so quickly, and how there are very few degrees of separation between people in cannabis—it starts to become a very large family.

But hands-down, the most rewarding experience has been to see my dream of starting this business become a reality. On that note, I must give a shout out to our Chief Science Officer, Dr. Carl Christianson. He has been the best partner and scientific leader I could have ever imagined, and True Labs would not be where we are today without him and his guidance.

Q: Why is it important to note that your laboratory has been "built from the ground up?" What sorts of challenges did your team face while building this laboratory?

A: **Ahrens:** At True Labs, we are dedicated to our craft: cannabis testing. Being 100% focused on servicing one industry allows us to continuously innovate, offer a wider array of industry-specific testing, and increase efficiencies. Our scientific leadership team has more than 18 years of cannabis testing experience! That is very hard to find in a new market.

We've built our lab in a way that fits our values and ideals, with integrity threaded throughout our processes. We are using advanced technology and instrumentation as the cornerstone of the state-of-the-art facility we set up. So, we are not just taking someone's cookie cutter approach to turning around a profit, but we are purpose-driven and truly focused on being trusted partners for our customers. We also want to be a reputable source of knowledge and insight for consumers.

As far as challenges go, we've experienced what you would typically see for any startup business, namely timeline delays for various reasons and added and unexpected costs, although we've had some pleasant surprises with cost reductions and savings as well.

Q: **How important is the certificate of analysis (CoA) you provide for cannabis products tested in your laboratory?**

A: **Ahrens:** Extremely important. Without a CoA, there is no proof that products were tested and no insight into what substances and contaminants were identified and quantified. A CoA is like a report card, in that it details whether you are passing or failing, and for the latter, it details how you're failing. However, the CoA is just a representative document meant to summarize all the work that went into generating that data. The most important thing we can do as a lab is make sure this is presented clearly and concisely, making the information accessible and usable for every consumer.

This interview excerpt has been edited for length and clarity. To read the full interview with Sarah Ahrens, please visit: www.cannabissciencetech.com/view/a-focus-on-safety-integrity-and-innovation-one-cannabis-laboratory-strives-to-exceed-standard-testing-practices-and-client-relations/.

Tetragram Provides Cannabis Users a Safe Environment to Explore the Medicinal Benefits of the Plant

BY MADELINE COLLI

SUFFERING FROM A tragic accident, Otha Smith III, sought out medical cannabis to treat his underlying health conditions. A main issue Smith encountered was that there were no instructions or guides on how to use medical cannabis. While a growing audience wants to see legalization and decriminalization, the US federal government labels cannabis as a Schedule I drug along the likenesses of heroin, lysergic acid diethylamide (LSD), methaqualone (meth), and so on. Because of this scheduling, it is difficult to obtain research on cannabis so that more can be known about the plant. Smith wanted a platform where novices and more experienced users could anonymously share their experiences as well as what cannabis products worked and did not work for them. With this idea, Tetragram was born. Smith created Tetragram as a mobile application that can be easily downloaded onto your mobile device, tablet, and so forth. By using this app, users can anonymously track cannabis products and tailor a cannabis regimen with products best suited for their needs. Here, Smith shares his personal journey and how it led to an app to help advance the cannabis industry through real-world data.

A Patient First

In 2006, Smith was involved in a serious car accident where he almost lost his life. From the accident, he had a six-inch, 34-plus staple scar on the top of his head. Once he was discharged from the hospital, Smith was prescribed several different kinds of opioids for his neck and back pain. "After three years of using prescription pills, I decided I wasn't going to use pills every day for the rest of my life and completely stopped use," Smith said.

Never shy around cannabis, as he had been a fan of the plant during high school and college, Smith decided to take a chance on cannabis to self-medicate for his health ailments rather than use pills. "Once Maryland legalized cannabis for medical use, I hurried up and got my medical card, but I will never forget how overwhelmed I felt during my initial visit to a dispensary," he explained.

In Maryland, each dispensary is required to have a clinical director on staff. Smith found this very helpful and was able to learn more about cannabis. "I became good friends with the clinical director at my local dispensary, Barbara Orchester. She was instrumental in educating me on the medical benefits of cannabis," he said. "Barbara was the one responsible for making me aware of terpenes and cannabinoids and the effects associated with each. But consequently, since cannabis affects everyone differently, I didn't feel comfortable with other people's recommendations."

Smith decided to venture out on his own through a lot of trial and error to find the right product to address his medical needs. Figuring out the right product, though, didn't come quickly and was, at times, frustrating due to the huge selection of products to choose from.

Entrepreneurial Drive

Inspired by his own medical cannabis journey, Tetragram was born. "As a patient myself, I can remember how frustrating it was to find the right cannabis product to address my medical needs. More importantly, I've worked in dispensaries and understand how challenging it is for doctors and dispensary staff to make accurate product recommendations to customers," said Smith. Every cannabis user goes through their own trial and error journey to find the best product or products for them. "I vividly recall everyone I spoke with, whether they were a patient, worked at a dispensary, or a doctor; one thing that was constant in all my conversations was people recommending that I keep a journal. I noticed dispensaries would sell paperback journals and I met a few people that went as far as creating detailed excel spreadsheets to keep track of products they purchased and how it made them feel. That's when I said to myself, there needs to be an app that makes the process of recording my experience

Analysis of Natural Cannabinoids and Metabolites from Urine Using Styre Screen® HLB and SelectraCore® C18 Column on LC-MS/MS

AS MORE STATES in the US legalize marijuana for recreational and medical use, it is important to be able to accurately and precisely quantitate cannabinoids from biological matrices. This application note outlines a solid phase extraction (SPE) procedure for cannabinoids in urine and a 12-minute liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The panel includes isomers Δ^9-THC and Δ^8-THC, which were successfully separated using C18 core-shell column.

INSTRUMENTATION:

Samples were extracted on Styre Screen® HLB SPE column (SSHLB063, 3mL 60mg sorbent). SelectraCore® C18 core-shell Column (SCS27-C181021, 100 mm x 2.1 mm, 2.7 µm) with a guard cartridge was used to analyze the extracts on Shimadzu Nexara LC-30AD w/MS-8050 LC-MS/MS system. Water & methanol containing 0.1% formic acid were used as mobile phase A and B respectively.

CONCLUSION:

A LC-MS/MS and SPE extraction method was developed for the analysis of four natural cannabinoids and the two major Δ^9-THC metabolites in urine. The addition of 1 mL of acetonitrile in the sample preparation helps prevent analytes from sticking to the test tube. The relative recovery of all analytes at low, medium & high concentrations was greater than 90% with a relative standard deviation of less than 8%. Apart from COOH-THC and OH-THC, all other analytes had matrix effects between +10% and -10%.

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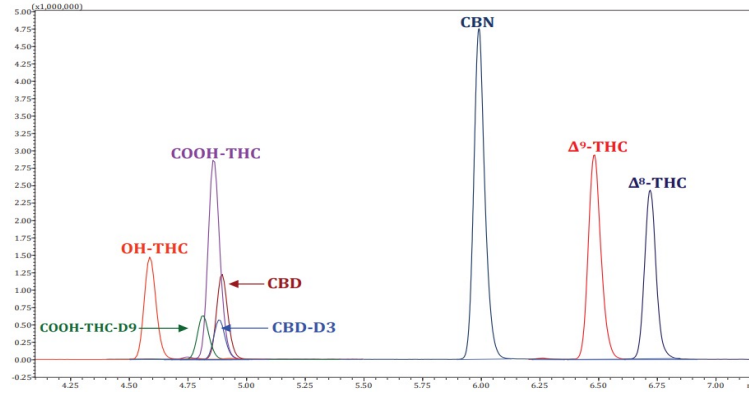
Relative recovery of target analytes (N=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
Δ^9-THC	98%	4%	97%	2%	99%	2%
Δ^8-THC	93%	4%	94%	2%	95%	1%
OH-THC	103%	3%	99%	1%	105%	3%
COOH-THC	94%	7%	95%	3%	100%	2%
CBD	96%	4%	98%	1%	100%	1%
CBN	99%	4%	93%	2%	96%	1%

FIGURE 1: Chromatogram of an extracted 50 ng/mL urine sample .



Extraction Procedure:	
Sample pre-treatment	1 mL urine + ISTDs + 1mL Acetonitrile + 1 mL pH 7.0 phosphate buffer Vortex and centrifuge samples for 10 minutes at 3000 rpm
Condition	2 mL methanol 2 mL pH 7.0 phosphate buffer
Load	Load the samples at 1 to 2 mL/min
Wash	3 mL DI water 3 mL 50% methanol
Elute	3 mL 60:40 methanol: hexane *Note: shake or vortex elution solvent well before use*
Post elution	Evaporate and reconstitute in mobile phase or methanol

with cannabis and cannabidiol (CBD) fun and engaging," Smith said.

With cannabis still federally illegal, there isn't a lot of sufficient data or published studies for people to rely upon for guidance. The medicinal plant also affects every user differently, which can make the process more challenging. "Tetragram is a smart, digital journal that empowers medical cannabis patients and recreational users with the ability to track, rate, and share their personal experiences with cannabis through our mobile app," Smith explained. "Tetragram is free and will always be free for consumers to download from the Google Play Store or the Apple App Store." Smith further explained that they are currently developing a V2 of Tetragram which will provide new features and functionality not just specifically for consumers, but for dispensaries, cultivators, and consumer packaged goods (CPG) companies, as well as the medical community.

Tetragram's growth and rise in popularity has been solely organic. "Myself, along with my two business partners, Julius Moore (CTO) and Lucas Roe (CDO), are each medical cannabis patients. Therefore, we take privacy extremely seriously, which is why Tetragram is HIPAA-compliant as well. Though users can share their reviews with others on Tetragram, the user's personal identity remains anonymous," Smith added.

With the COVID-19 pandemic, it was both good and bad for the app. During the pandemic, the public was placing stronger focus and attention on personal mental and physical health. Cannabis consumption dramatically rose during this period. More products were being purchased and people were paying closer attention on the effects associated with each product they were purchasing. "The only downside to launching Tetragram during the COVID-19 pandemic was that there were very limited options to make consumers aware of Tetragram. Cannabis companies have very limited options for marketing their products and services to consumers due to cannabis being federally illegal," he said. Cannabis companies mainly rely on tradeshows and conventions to reach consumers. With everything shutdown, it was very difficult to introduce new or current products.

In the cannabis market, there are so many products to choose from. This can make it daunting to novices interested in becoming cannabis users or even be challenging to the most experienced of consumers. "With Tetragram, we empower the consumer to take control of their personal journey with cannabis by providing them with a platform that makes it fun to keep track of each product purchased and its associated effect. By doing so, consumers will be able to draw conclusions more effectively on not only which product but understand which terpenes and cannabinoids and consumption methods are providing them with the most relief," Smith said. The app is also available nationwide which allows users to access it and explore products no matter what state they are located in.

To access Tetragram, you can download it from the App Store or Google Play store. Upon downloading and opening the application, you will be prompted to create an account and verify your email address. Once these steps are completed, users will be able to sign in and begin logging product information. Smith further added, "We placed a lot of time on the user experience to make sure Tetragram would be easy to navigate. Once you open Tetragram, simply click on our logo and you can start logging away by first entering in the product information, then where you made the purchase, and how you consumed the product." Users are also able to select a medical condition from a list of default options or create their own symptom, rate that symptom before and after use, and add any detailed comments associated with the product. Another aspect of the app that gives users more flexibility is that they are able to choose what method of consumption they are using. For example, if the user is consuming orally, topically, or inhaling and will then be able to select the device being used (bong, joint, vape pen) or form of the product (flower, hash, shatter).

A major issue in the cannabis industry Smith sees is the need for standardization. Each state across the country that has medical or adult-use programs require products to be tested by a laboratory, but testing requirements vary from state-to-state. "This is the reason why we aim to get the certificate of analysis (COA) directly from the cultivator to make sure the information presented on the label matches the COA. But the problem is much bigger than just blaming the manufacturer: for example, testing facilities have been caught taking more money from companies to increase the percentage of tetrahydrocannabinol (THC) on the COA," Smith said. Having the same standardization across all states would ensure that every laboratory is following the same rigorous testing requirements to provide safe and accurate products to consumers.

Smith is proud of what he and his partners have created with Tetragram. The app provides a platform and haven for users to track the products that work best for them and see what works for others anonymously, which helps improve their quality of life. It is why Tetragram's slogan is, "Built for patients, by patients." If you are interested in using Tetragram or learning more about it, they can be found on social media (for Instagram and Facebook, they can be found @ **thetetragramapp**) or you can visit their website at tetragramapp.com. Smith is also involved in several exciting research projects. Listen to our recent podcast episode to learn more:

https://www.cannabissciencetech.com/view/ep-6-tetragram-how-patient-data-is-powering-research.

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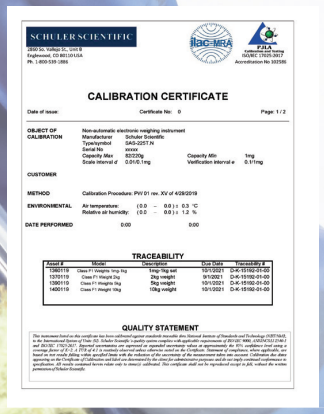
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