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features

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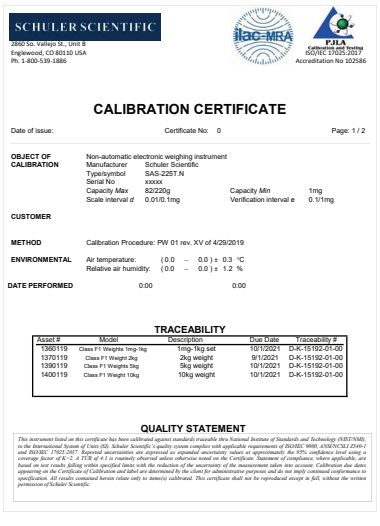

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Date of issue: Certificate No: 0 Page: 1 / 2

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Non-automatic electronic weighing instrument
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Serial No: xxxxx
Capacity *Max*: 82/220g — Capacity *Min*: 1mg
Scale interval *d*: 0.01/0.1mg — Verification interval *e*: 0.1/1mg

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METHOD Calibration Procedure: PW 01 rev. XV of 4/29/2019

ENVIRONMENTAL Air temperature: (0.0 – 0.0) ± 0.3 °C
Relative air humidity: (0.0 – 0.0) ± 1.2 %

DATE PERFORMED 0.00 0.00

TRACEABILITY

Asset #	Model	Description	Due Date	Traceability #
1360119	Class F1 Weights 1mg-1kg	1mg-1kg set	10/1/2021	D-K-15192-01-00
1370119	Class F1 Weight 2kg	2kg weight	9/1/2021	D-K-15192-01-00
1390119	Class F1 Weights 5kg	5kg weight	10/1/2021	D-K-15192-01-00
1400119	Class F1 Weight 10kg	10kg weight	10/1/2021	D-K-15192-01-00

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Cannabis Science and Technology® to Host Hemp Science & Technology Virtual Symposium

By: Megan L'Heureux

CANNABIS SCIENCE AND TECHNOLOGY® will host its first Hemp Science & Technology Virtual Symposium. Brian C. Smith, PhD, founder, CEO, and chief technical officer of Big Sur Scientific, will serve as the two-day event's program chair.

The two-day virtual symposium will take place October 28–29, 2020 and is free to attend. The symposium will feature talks from hemp scientists, researchers, and industry experts on a wide array of topics, including hemp analysis, cultivation, and extraction.

"During this health crisis, it's more important than ever to offer resources and virtual events to our community that share knowledge and help the hemp industry continue to push forward," said Mike Hennessy Jr., president and CEO of MJH Life Sciences™, parent company of *Cannabis Science and Technology*®. "We are excited to offer a new dedicated event for hemp professionals to learn more about the science and technology that can improve their work or lead to better products for consumers."

"I am excited to be working with *Cannabis Science and Technology*® to offer the Hemp Science & Technology Symposium to our industry," Smith said. "I have three state-of-the art symposia planned: New Developments in Hemp Testing, Optimizing Hemp Extractions, and Overcoming Hemp Growing Challenges. Each session will feature talks by industry experts, and a roundtable discussion where attendees can ask the experts questions. Don't miss this opportunity to learn how to improve your products and operations and advance your career."

Details on speakers and presentation titles will be announced in the coming weeks.

To register, please visit ***https://www.cannabissciencetech.com/view/cannabis-science-and-technology-r-to-host-hemp-science-and-technology-virtual-symposium.***

FDA Releases Cannabis Research Guidelines

By: Madeline Colli

THE US FOOD AND DRUG Administration (FDA) recently released a draft guidance on developing cannabis-based drugs. These guidelines come weeks after the White House announced it had completed reviewing the draft guidance.

In December 2018, Congress passed the 2018 Farm Bill (1), which was a groundbreaking piece of legislation that legalized the sale and cultivation of industrial hemp and its derivatives after decades of prohibition. For quite some time, lawmakers, advocates, and stakeholders have pressed the FDA to create a regulation structure to assist the industry. Recently, the FDA did just that by releasing a draft guidance on developing cannabis-based drugs (2).

These FDA guidelines come weeks after the White House announced it had completed reviewing the draft guidance (3). The new document (2) mentions that the guidance is "limited to the development of human drugs and does not cover other FDA regulated products." The draft documentation is meant to provide an outline for drug manufacturing. The agency is currently still working on developing guidelines that will allow cannabidiol (CBD) to be marketed and sold as a food item or supplement. CBD has increased in popularity in recent years because of its potential for treating medical conditions.

"A range of stakeholders have expressed interest in the development of drugs that contain cannabis and other compounds found in cannabis. Recent legislative changes have also opened new opportunities for cannabis clinical research. As that body of research progresses and grows, the FDA is working to support drug development in this area," said FDA Principal Deputy Commissioner Amy Abernethy (4).

Some of the legislative changes Abernathy is referring to is the 2018 Farm Bill, legalizing industrial hemp with less than 0.3% tetrahydrocannabinol (THC). Researchers are now able to use industrial hemp from any source as long as it doesn't exceed the allowed THC limit and not limiting them to use from the University of Mississippi, which is the country's only federally authorized cannabis manufacturer.

In the new draft guidance, there is an emphasis on THC testing, mentioning that investigators and sponsors may find it beneficial to calculate the level of delta-9 THC early in the development process of their proposed investigational drug product so that they can gain better insight into the potential control status. The document also provides step-by-step guidance on how to test for THC on a dry weight basis according to the FDA's standards (4).

The FDA is receptive to the public and has opened a 60-day comment period.

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Micro-Dosing & Vaporization

The Next Innovation in Cannabis Consumption

Most cannabis users have first-hand experience or have heard anecdotes of the negative effects—such as nausea and paranoia—associated with overconsuming cannabis. For both medical and recreational cannabis consumers, having the ability to monitor their therapeutic window, which is the range between the lowest effective dose and the dose that produces unwanted side effects, can be the difference between being a one-time buyer or a loyal consumer of cannabis products. Edibles and tinctures were some of the first product lines to provide reliable dose monitoring; now, vaporizer products are joining this new frontier through innovative hardware and technology companies.

Customized Inhalation Technology

Jupiter Research, a provider of inhalation technology for natural plant-derived extracts, created a technology that offers customized metered dosage delivery, Dose-CTI™, for cannabis vaporization. Previously, this technology was limited to a small number of custom hardware orders, but now it is available as a feature on most Jupiter devices, allowing extractors and licensed processors the ability to give their customers more control over their cannabis vaping experience. Jupiter metered dosage vaporizers are engineered with award-winning CCELL® heating technology and tailored to the viscosity and terpene profile of extract formulations to deliver a consistent dose with every activation.

Jupiter's Dose-CTI provides a unique micro-dose control customization feature for power supplies and all-in-one vaporizer products, offering brands the option to choose from one of three time-based metered dosage levels as a feature in their custom device. Using custom-built power supplies with time-based control circuit technology, users can select a vaporizer device with the cut-off time they prefer. Dose CTI technology can go beyond three, five, or 10-second activation time limits for customers looking for higher dosage time intervals. Precise dose delivery is available throughout Jupiter's diverse product portfolio—from 510 thread power supplies and 510 thread variable voltage power supplies to all-in-one vaporizers.

By upholding the highest standards for product quality and safety, Jupiter continues to be a market leader with a reputation for providing customers in the cannabis industry with the latest innovative technologies. This new customizable feature of first-level dose control attracts a broader base of customers, which provides them with the ability to regulate their intake. Dose control is core to Jupiter's commitment to technology development to meet the vaporization industry's most critical needs.

Multi-session ease-of-use metered dosage vaporizers allow consumers to control their cannabis intake by understanding how much and how often to dose to achieve the desired therapeutic effect. Dose CTI devices are inhale-activated, making them intuitive for experienced and novice users alike. For a single dose, consumers inhale slowly and steadily until the device shuts off automatically. Consumers can then inhale again, and the device will activate automatically and deliver another precise dose.

By providing a basic understanding of the key characteristics of cannabis dosing, consumers can feel empowered to make the most of this versatile product and explore the vast potential of inhalation.

For more information: JupiterResearch.com

The Pet Lab Syndrome

By ***Brian C. Smith***

The well documented inter-laboratory error problem in cannabis analysis means results from different laboratories cannot be compared. As a result, there arise disputes based on these varying results where both sides stubbornly defend their laboratory's results, the Pet Lab Syndrome. The problem can make it difficult to set a fair price for biomass or oil transactions. Suggested solutions will be offered.

Here is a scenario I have heard about far too often in this industry. Imagine a hemp extractor wants to buy biomass from a hemp grower. The farmer shows up with a certificate of analysis (COA) from his laboratory claiming 14% total cannabidiol (CBD) content. The extractor sends a sample of this hemp to his laboratory to be tested, and it comes back 9% total CBD. The two then argue about whose laboratory is right. Claims of a laboratory's quality, accreditations, previous experience, the brilliance of the lab director, and so forth are cited. This insistence that "my laboratory is always right" and "your laboratory is always wrong" is what I call the *Pet Lab Syndrome*—an overreliance or dogmatic insistence on the quality of one laboratory's results over another.

The inter-laboratory error problem, where different cannabis testing laboratories report radically different results on the same samples, has been well documented (1–7). It is the cause of the Pet Lab Syndrome. The causes of inter-laboratory error include variations in sample preparation, analytical methods, lack of standard reference materials, and poor training (5). Inter-laboratory error means that results from different cannabis laboratories cannot be compared, and that accuracy in cannabis analysis is a myth. The inter-laboratory error problem means that there are times, such as the purchase situation above, where different parties will have COAs with different results on the same sample. Who do we trust in these situations? I have offered my thoughts on solutions to the inter-laboratory error problem (6).

The inter-laboratory error problem has a corrosive effect on our industry. It sows seeds of doubt in the public's mind about the safety and efficacy of cannabis medicines (3,4), it makes it difficult for cannabis businesses to have the data they need to manage their businesses properly to make safe and effective medicines, and makes it difficult for transactions to take place if no one can agree on the composition of the thing being sold. Despite exposure of the problem and pleas for improvement (1–7), the inter-laboratory error problem is not going to be solved until sufficient regulation and oversight comes to the cannabis industry. In the meantime, I offer some thoughts on how we can deal with this problem.

How Do You Find a Laboratory You Can Trust?

Every cannabis business needs analytical chemical data to run properly. In my own business, where we make and sell cannabis analyzers (1,8–12), we need reliable reference data to calibrate and validate our instruments. Any analyzer's results are only as good as the reference data with which it is calibrated.

However, the inter-laboratory error problem is so bad that when we build calibration models using data from multiple laboratories we get poor results. However, when we build models from a single laboratory results improve. The variance here then is in the 3rd party laboratory's data, not within the instrument itself. In another example of the Pet Lab Syndrome, I have had customers insist there unit be re-calibrated to match their Pet Lab's numbers, despite overwhelming evidence that their unit matches the results from our ISO certified, state licensed laboratory just fine.

To find a reliable laboratory, I have literally roamed the country visiting dozens of cannabis analysis laboratories. I meet the lab director, observe analyses, and ask to see written standard operating procedures. In some cases, I will analyze the potency on a set of samples by mid-infrared spectroscopy (13), and have the laboratory run the same samples using their chromatographic method. Here the spectroscopy is used as an independent, orthogonal test of a specific laboratory's chromatography. Assuming the mid-infrared method is unbiased, the correlation of the chromatographic results and spectroscopy results is a measure of the quality of the chromatography. The results have been that some laboratories match the spectrometer's numbers well, some match poorly, and some not at all. Since the variable here is the laboratories, we must conclude that some laboratories are better than others. Only a small fraction of the laboratories I have visited have passed muster.

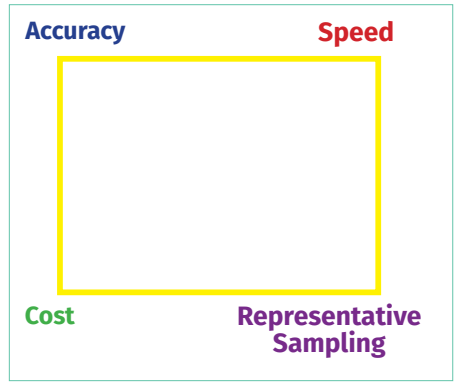
Does every cannabis business have to go to such lengths to find a laboratory they trust? No, but you need to do your homework before picking a laboratory. Certainly, you should visit a laboratory's facility, interview the lab director, and find out what degree level and relevant experience they possess. Ask how the technicians performing the analyses are trained. Watch analyses being performed. And most importantly, ask for a copy of

their method. If the method does not exist on paper it should be a red flag. If they refuse to give you a copy claiming it is "confidential information" that is another red flag. Slowly but surely standard methods are being promulgated in this industry. If your laboratory's method is not in conformance with any of the standard methods out there, that is a red flag too.

What about state licensing and International Standards Organization (ISO) certifications? These are necessary but not sufficient conditions to trust a laboratory. Certainly, if a laboratory is not state licensed and ISO certified it should be avoided. However, laboratories with these accreditations still suffer from the inter-laboratory error problem. One paper I coauthored in this journal (1), where the same cannabis distillate samples were sent to five different state licensed, ISO certified laboratories for potency and pesticide analysis, found troubling differences in results across the five laboratories. Thus, these certifications are no guarantee that a specific laboratory will do a good job on a specific sample.

Given all this uncertainty, what is a cannabis business to do? Accuracy may be a myth in this industry, but precision is not. Precision measures how reproducible results are for a given set of samples (14). Our work has clearly shown that different cannabis laboratories have different precisions (1). The precision of different laboratories is easily quantified. To determine this, put together a set of samples that you are confident are homogeneous. Let's say five samples. Split each sample into three aliquots, and ask your laboratory to analyze each sample. When the results come back you will have a set of three readings on each of the five samples. Calculate the average and standard deviation for each sample and analyte. Do this for multiple laboratories. The laboratory that has the best overall precision is who you should do business with. The point here is that in the absence of true accuracy, a laboratory that provides consistent results means you can compare samples to each other, compare results over time, and use this laboratory's data to run your business going forward.

Figure 1: The "Golden Rectangle of Chemical Analysis."



What About In-House Analyzers?

In practically every manufacturing industry, in-house testing is a common feature. In-house testing gives data on-the-spot about manufacturing processes and products, insuring that safe and effective medicines are made with high quality. Amongst the things that cannabis products may be required to be analyzed for are potency, pesticides, and heavy metals (various tests for mold and pathogens may be required as well, but since this type of testing is not my area of expertise, I won't comment further). Performing in-house testing for pesticides is impractical because the required instrumentation can cost hundreds of thousands of dollars. The expense of heavy metals testers are probably beyond the financial means of most cannabis businesses as well. However, there exist several types of potency analyzers that are accurate, easy to use, and cost a fraction of what a pesticide or metals analyzer may cost. Potency is the one thing that is practical for cannabis businesses to test for in-house.

How Do I Pick an In-House Potency Analyzer?

First, you should choose technology that best suits your needs. In previous columns I have discussed that to choose a testing method, four criteria should be used: accuracy, speed, cost, and the ability to perform representative sampling (15). These comprise what I call the "Golden Rectangle of Chemical Analysis" as seen in **Figure 1**.

In an ideal world a given analytical technology would be accurate, fast, low cost, and allow for representative sampling. The latter is particularly important for testing cannabis plant material because these samples are inevitably inhomogeneous and require significant amounts of testing to overcome sampling error (16). Because of this, any chosen method needs to be inexpensive and fast to allow the sample load required by representative sampling to be accomplished in a practical fashion. In a previous column I compared and contrasted chromatography and spectroscopy for potency analysis (15). Using the Golden Rectangle of Chemical Analysis, I concluded that chromatography was accurate, but suffered from being slow and expensive and hence did not lend itself well to representative sampling. On the other hand I concluded that infrared spectroscopy, while not necessarily as accurate as chromatography because it is a secondary method, enjoys advantages of speed, low cost, and the ability to handle a large sample load—meaning it is well suited to representative sampling programs.

How Do I Pick an In-House Analyzer Company?

After choosing an appropriate potency testing technology, you need to choose a vendor to do business with. You should ask the following two questions of your potential vendor.

1. Has this technology ever been used before in any application besides cannabis?

When I went to publish my first peer-reviewed paper on the use of mid-infrared spectroscopy for cannabis analysis, the editor, a very smart person, asked me the

above question. The reason she had to do this is that there exist analyzers claiming they can analyze cannabis for potency, but use technologies that have never been used before in the history of science to determine concentrations of analytes in samples. I am particularly alarmed by analyzers that use cell phones and the "cloud" to determine potency measurements. The need for an internet connection means your company's proprietary data is being sent over insecure links, to a mysterious location, to a server that may be easy to hack into. The details of how the results are determined on the cloud are typically kept secret, and the results magically appear on your phone's screen with no information whatsoever on how the device was calibrated or validated. Analyzers like this are to be avoided.

2. Where is the peer-reviewed data supporting your accuracy claims?
Peer review is how science knows what it knows. For example, when I want to publish a paper in this journal, a copy of the manuscript is sent to my peers. They make anonymous comments on the quality of my work and my paper. The verdict may be to publish the paper, re-write the paper, go back to the laboratory and do more work, or reject the paper. For any potency analyzer company to claim they have scientific proof of their accuracy data, that data must be published in a peer-reviewed journal like this one. Here are some examples of cannabis analyzers that have published peer reviewed accuracy data (1,8–12). Any claims made without peer-review are just that, claims. Application notes and white papers put out by instrument companies that are not peer-reviewed are not necessarily scientifically correct either. You should only consider doing business with a company that can back up their claims with peer-reviewed science.

What's a Cannabis Business to Do?

How do you run a cannabis business given the inter-laboratory error problem and Pet Lab Syndrome? In any situation where cannabis products are being bought or sold based on COAs, the buyer and seller must agree ahead of time on which laboratory's results will be used to set the purchase price. This is the only way to avoid the Pet Lab Syndrome. For potency analyses, there exist portable analyzers that can provide readings on the spot. The advantage of this is that data is obtained in minutes rather than days so transactions can take place quickly and easily. Also, the low cost per analysis of these tests means that multiple aliquots of the material being purchased can be analyzed to minimize sampling error. Of course, the buyer and seller need to agree ahead of time to abide by the results of whatever testing instrument is being used.

Conclusions

The inter-laboratory error problem continues to plague the cannabis industry. This has led to the Pet Lab Syndrome, where arguments of which laboratory is right inevitably occur. Cannabis businesses need to vet any laboratory they intend to do business with, paying particular attention to precision. Another solution to this problem is for cannabis businesses to do their own testing. Potency testing is the only in-house testing that is practical. How to choose an in-house potency tester was discussed. To alleviate the Pet Lab Syndrome problem, whenever cannabis products are bought and sold, the buyer and seller must agree on a laboratory or analyzer whose results will be used to set a purchase price.

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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 lab 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.

To Grind or Not To Grind

By **Lo Friesen**

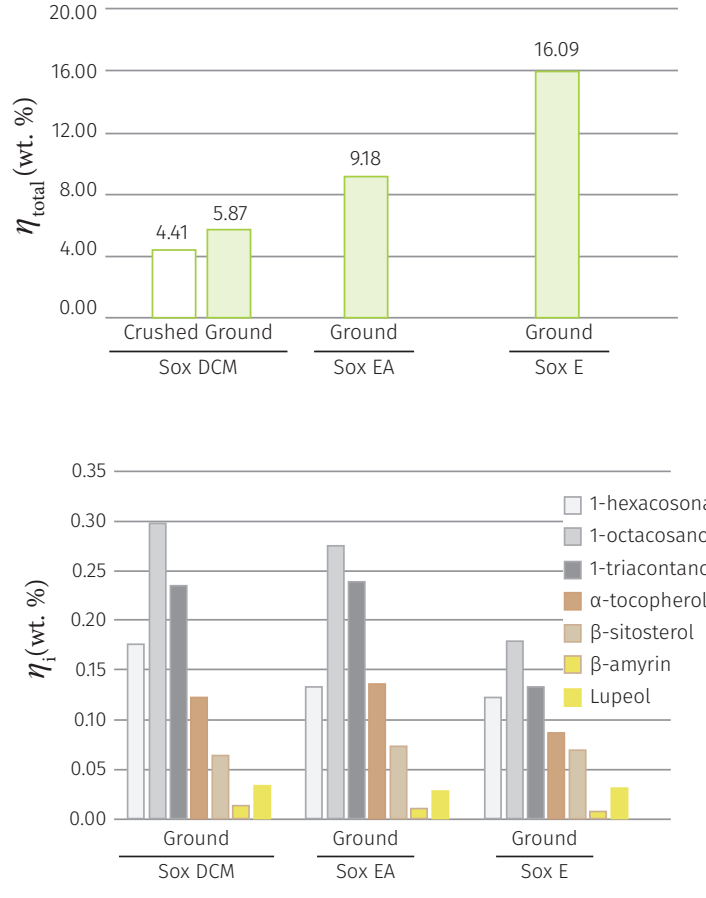
The debate is ongoing as to whether or not cannabis biomass should be ground prior to extraction. It seems there is enough evidence to demonstrate that the choice is dependent on the solvent and desired end product. This article explores the pros and cons of grinding cannabis prior to extraction along with the effects of particle size.

Cannabis can be used in many different ways. In most cases, cannabis is ground before use. For example, the "perfect" grind or particle size is integral to the "perfect" joint. Similarly, the particle size is integral to the success of an extraction. This article explores the results of grinding cannabis prior to extraction along with the effects of particle size on extraction efficiency. The debate is ongoing as to whether or not cannabis biomass should be ground prior to extraction. It seems there is enough evidence to demonstrate that the choice surrounding grinding is dependent on the solvent and desired end product.

What Is the Ideal Particle Size?

For all of the extraction solvents, the right particle size can result in an extraction that simultaneously reaches optimal efficiency while minimizing undesirable compounds. Naturally, it seems as though the smaller the particle size the higher the yield. This may be true for total yield (n_{total}) by weight of extracted material, but yield specific to desired compounds is important to understanding the ideal particle size for extraction. A number of studies have demonstrated that exact theory—smaller particles size equates to higher total yield (1). Further examination of the data reveals that yield of individual compounds (n_i), or selectivity, often decreases with smaller particle size (**Figure 1**). This indicates that further processing will be required to refine the extract to a desired end product, specific compound, or specific blend.

Figure 1: Total yield (top) and individual yields (bottom) for the extracts produced from vine leaves by Soxhlet extraction with dichloromethane (DCM), ethyl acetate (EA), and ethanol (E).



How Does Particle Size Affect the Yield Based on Extraction solvent?

Ethanol Extraction

As we learned in my last column(2), ethanol is an extremely efficient

solvent. Ethanol extractions can be completed in as low as 15 min with a cannabinoid extraction efficiency of about 95%. Due to its efficiency, ethanol extraction can result in the extraction of undesirable compounds such as chlorophyll. By using large particle sizes for extraction, whole flower for example, the material has most of its cellular structure intact. This, along with utilizing cold ethanol, has dramatically reduced the extraction of chlorophyll. This in turn optimizes the extraction of cannabinoids and reduces the post processing steps.
CONCLUSION: Not To Grind

Hydrocarbon Extraction

Hydrocarbon solvents also have the potential to extract at high efficiency. So, grinding cannabis biomass prior to extraction is not necessary but would increase the efficiency of the extraction. Opening the surface area for extraction using hydrocarbon solvents is also coupled with undesirable by-products such as chlorophyll and plant waxes. These components require further processing to remove them from the extract. Obviously, this results in lower overall efficiency.
CONCLUSION: Not To Grind

CO_2 Extraction

CO_2 is a less efficient solvent than ethanol and hydrocarbon, therefore having room for improved yields via grinding input material. Increasing the surface area of the input material clearly leads to a more even pack of the extraction vessel achieving a more even extraction throughout the plant material and increased weight capacity within the vessel.
CONCLUSION: To Grind

What Equipment Can Be Used to Grind Cannabis?

The cannabis industry has created ample opportunity for other industries to serve the cannabis space with established solutions. The process of grinding or milling is common practice for hops, wheat, coffee, and beyond. On a small scale, standard blenders and coffee grinders have been utilized for reducing dried cannabis particle size prior to extraction or packing joints. On a large scale, cannabis processing operations are leaning on industrial scale grain mills, leaf shredders, and scaled-up laboratory sample preparation technology.

It is important to select equipment

that will offer precision and control with respect to particle size. This feature will support a more consistent extraction. In addition, ease of maintenance and sanitation is important as dried cannabis is highly resinous, which can cause resin build-up within the mill. This can translate to reduced yields if not properly managed. Frequent sanitation and maintenance of grinding equipment is integral to minimizing risk of loss or cross contamination.

Closing Thoughts

Ultimately, grinding, also known as milling, cannabis biomass increases efficiency by weight of extraction through increased input capacity, increased surface area of the plant material, increased packing uniformity, and reduced extraction time. However, dependent on the extraction solvent and desired end product, the particle size will affect the overall outcome of the end product.

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(2) L. Friesen, *Cannabis Science and Technology* **3**(6), 14–20 (2020).

Erratum

In the July/August installment of "Extraction Science" there were several typographical errors in Figures 3 and 5. We apologize for these mistakes. The corrected figures appear online at: https://www.cannabissciencetech.com/view/exploring-the-chemical-make-up-of-cannabis-extract-by-method.

ABOUT THE GUEST 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 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.

Treating Immunocompromised Patients During the COVID-19 Pandemic:

A Conversation with Dr. Bonni Goldstein

By ***Joshua Crossney***

Everyone has been affected by the COVID-19 pandemic and most of us have had to adjust our work schedules in creative ways. I had the opportunity to discuss what it is like to treat immunocompromised patients in these challenging times with Dr. Bonni Goldstein. Many of you know Dr. Goldstein from the critically acclaimed documentary, *Weed the People* (1), or from her amazing keynote presentations at past Cannabis Science Conferences by CSC Events, LLC (2).

Q: Can you tell our readers a little about your practice and the type of patients you treat?

A: My practice opened in 2008 and I was hired on as Medical Director. I purchased the practice in 2011. Since I trained as a pediatrician, I am focused on treating pediatric patients with severe epilepsy, autism, cancer, autoimmune disorders, and other serious conditions. I still follow many of our adult patients who have been coming to my practice for many years. Most suffer with chronic pain, mood or sleep disorders as well as cancer, post-traumatic stress disorder (PTSD), and some other serious illnesses.

Q: How have you adapted to treating patients during the COVID-19 pandemic?

A: Since we have so many vulnerable patients, I am now seeing everyone through telemedicine only. Everyone seems to be accepting of this "new normal," although I miss seeing my pediatric patients in person.

Q: How is telemedicine different from seeing patients in person? Have you seen an improvement in telemedicine technologies?

A: I was doing some telemedicine prior to COVID-19 as some patients who have serious conditions found it difficult to travel to my office. So not much has changed. There are so many telemedicine platforms available to physicians that it is quite easy to continue to take care of patients and connect with them when they need advice.

Q: Are you aware of or involved with any research studies or clinical trials that involve cannabinoids as treatments for COVID-19? If not, do you have any thoughts on potential opportunity here?

A: It appears that COVID-19 causes a severe "cytokine storm" in some patients, where an over-abundance of cytokines—a group of inflammatory chemicals released from our immune cells—are released and create havoc. It is this "hyper-immune inflammatory" response that leads to severe and often fatal respiratory symptoms, causing acute respiratory distress syndrome (ARDS). Although COVID-19 is new to us, cytokine storm and ARDS are well known to physicians and researchers as numerous other infections cause the same response. In 2015, a group of researchers at the University of South Carolina School of Medicine demonstrated that tetrahydrocannabinol (THC), with its well-known anti-inflammatory properties, can improve survival in animals with cytokine storm and ARDS. They used a mouse model of enterotoxin (a toxin that affects intestines, such as one might experience with food poisoning) to trigger cytokine storms and ARDS. The mice that received the toxin and no treatment all developed cytokine storm and ARDS, resulting in the death of 100% of mice by day 5. The mice that received the toxin and then received four daily doses of THC all survived and were found to have decreased cytokines and lung inflammation. These types of results—100% mortality without THC versus 100% survival with THC—is certainly worth pursuing further, especially since we are struggling to find effective and safe treatment for COVID-19. In another mouse study, just published in July 2020, researchers found "a potential protective role for CBD [cannabidiol] during ARDS that may extend CBD as part of the treatment of COVID-19 by reducing the cytokine storm, protecting pulmonary tissues, and re-establishing inflammatory homeostasis." Another study from Israel reported CBD plus a specific terpene combination treated life-threatening cytokine storm and was more effective than CBD isolate and also more effective than a corticosteroid commonly used for this condition. So, there is no question in my mind that human clinical trials of CBD and THC are indicated to help prevent death and potentially all or some of the complications associated with this terrible virus (3-5).

Q: Aren't "cytokine storms" also associated with the use of ibuprofen and other nonsteroidal anti-inflammatory drugs (NSAIDs) during COVID-19?

A: At the beginning of this pandemic, there was some question as to whether or not NSAIDs (such as ibuprofen and naproxen) worsened the outcome for those with COVID-19. As of the latest findings, there is not enough evidence to say whether NSAIDs are dangerous or beneficial for those with COVID symptoms. French researchers are recommending those with COVID-19 avoid NSAIDs until more is known (6), however the World Health Organization (WHO) recognizes that there is no evidence so far to prove that NSAIDs make coronavirus worse. They are no longer recommending against the use of NSAIDs as they did a number of months ago. It is very important that people understand that we do not know the complete story with COVID-19 and that it is going to take more time to fully comprehend the critical aspects of this virus that will lead the medical community to making "correct" recommendations. We should all expect that COVID-19 recommendations will change over time as we learn and understand more about this virus.

Q: I read your article entitled, "Trade-in Your Ibuprofen for Cannabis" on your website bonnigoldsteinmd.com (7). I had no idea so many NSAIDs users were hospitalized every year for gastrointestinal disorders.

A: Yes! It is crazy to think about how we consider NSAIDs to be "very safe" because they are sold over the counter. I meet people in my practice who go through a big bottle of ibuprofen every month! The side effects are real and can be quite dangerous, but cannabis is a great alternative and since we have numerous nonintoxicating cannabinoids on the market, those that are not interested in experiencing this effect can still use cannabis to help with inflammatory pain.

Q: How do patients suffering with inflammation know which cannabis is best for anti-inflammatory effects?

A: This is a great question. THC, CBD, and many other cannabinoids are anti-inflammatory. Since we have been unable to do human clinical trials to see which one might work best for certain inflammatory conditions, patients are left to figure it out for themselves. For those who struggle with inflammation as part of their illness (which is almost everyone as inflammation is the common denominator for so many conditions), I encourage the use of both CBD and THC in various ratios depending on a person's response and preference. Some patients prefer to use high ratio CBD:THC products and others find lower ratios to be beneficial. Additionally, the raw phytocannabinoids THCA and CBDA both have anti-inflammatory properties and can be taken as well and I often recommend them in conjunction with CBD or THC as the synergies between these compounds can often give great results. Furthermore, beta-caryophyllene, a terpene that is also considered a cannabinoid since it binds to the Type 2 cannabinoid receptor, has potent anti-inflammatory properties as well and should be included in an anti-inflammatory cannabinoid medicine regimen.

About the Interviewee

A native of New Jersey, **Dr. Bonni Goldstein** received her undergraduate education at Rutgers College. She pursued her medical degree at Robert Wood Johnson Medical School at the University of Medicine and Dentistry of New Jersey. Her post-doctoral education included internship and residency at Childrens Hospital Los Angeles. Dr. Goldstein also served as Chief Resident at Childrens Hospital Los Angeles. She was a Clinical Instructor in Pediatrics at USC School of Medicine in Los Angeles, Emergency Transport Attending Physician at Childrens Hospital Los Angeles, and Emergency Medicine Attending Physician in the Pediatric Emergency Department at Los Angeles County-USC Medical Center. In 2008, Dr. Goldstein developed an interest in the science of medical cannabis after witnessing its beneficial effects in an ill friend. Since then she has been evaluating both adult and pediatric patients for use of medical cannabis. Dr. Goldstein has given numerous lectures to many patient support programs, including the Cancer Support Community, Southern California Prosthetics, and Pediatric Epilepsy. She has also lectured at CannMed 2016 at Harvard University, United in Compassion Medical Cannabis Symposium in Sydney, Australia, Patients Out of Time 2015, and United Patients Group Conference 2016. She is currently the Medical Director of Canna-Centers, a California-based medical practice devoted to educating patients about the use of cannabis for serious and chronic medical conditions. She is a Medical Consultant to WeedMaps.com. She is a Member of the International Association of Cannabis as Medicine, the International Cannabinoid Research Society, and the Society of Cannabis Clinicians.

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ABOUT THE COLUMNIST

JOSHUA CROSSNEY is the columnist and editor of "Cannabis Crossroads" and a contributing editor to *Cannabis Science and Technology* magazine. Crossney is also the president and CEO of CSC Events. Direct correspondence to: **Josh@CannabisScienceConference.com**

As submitted to Cannabis Science and Technology

Rapid Analysis of 16 Major and Minor Cannabinoids in Hemp Using LC–MS/MS with a Single Sample Dilution and Injection

BY AVINASH DALMIA, SABA HARIRI, ERASMUS CUDJOE, JACOB JALALI, AND FENG QIN

With the legalization of hemp in the US, there has been an influx of new hemp-based consumer products to market including dried hemp plants, drinks, vitamins, protein powders, and beauty products. In turn, innovative testing and analysis is required to ensure safety, quality, and labeling accuracy. A rapid liquid chromatography–tandem mass spectrometry (LC–MS/MS) method has been developed for the quantitative analysis of 16 cannabinoids in hemp samples with a single dilution protocol. Unlike traditional methods for cannabinoid analysis, this study did not require multiple detectors or multiple injections. The method is highly sensitive, and the limit of quantification (LOQs) of all cannabinoids were well below US limits for cannabinoids in hemp products. In addition, the method achieved a wide linear dynamic range with a good regression correlation fit ($R^2 > 0.99$) for all 16 cannabinoids. The method therefore enabled quantitation of the cannabinoids over a range of 0.03–100% in hemp samples.

WITH THE PASSING of the landmark Farm Bill by the United States congress in 2018, the growth and sale of hemp by farmers was legalized (1). Hemp, which can be used in consumable and beauty products ranging from beverages, vitamins, and protein powders to sunscreen and moisturizing lotions, is a strain of the *Cannabis* species and contains high concentrations of pharmaceutically active cannabinoids. Hemp naturally contains high concentrations of the cannabinoid cannabidiol (CBD), which is purported to have multiple medicinal uses for patients with epilepsy, pain, and nausea (2). Hemp also contains relatively smaller amounts of other cannabinoids such as tetrahydrocannabinol (THC). To ensure compliance with federal government regulatory requirements for hemp and help support quality control and labeling accuracy, hemp should contain less than 0.3% total THC. This is defined as the sum of THC and its acid form, tetrahydrocannabinolic acid A (THCA-A). According to United States Department of Agriculture (USDA) rules for hemp production, any hemp plant with a total THC level exceeding 0.3% (wt/wt) is considered marijuana, which remains classified as a Schedule I controlled substance regulated by the Drug Enforcement Administration (DEA) under the Controlled Substances Act. If a hemp farmer's product exceeds the THC levels enforced by USDA rules, the product must be disposed of, resulting in an economic strain on the grower (3). Different strains of hemp and cannabis vary in their composition of cannabinoids depending on the plant's tissue type, age, variety, growth conditions, harvest time, and storage conditions. The amount of different cannabinoids and their interaction may determine different effects and adverse side effects (4). It is therefore important to devise methods that can quickly and accurately determine different cannabinoids to distinguish different strains of hemp and cannabis products.

Traditionally, the most commonly used method for cannabinoid analysis in cannabis and hemp is gas chromatography (GC) coupled to a flame ionization detector (FID) or a mass spectrometry (MS) detector. This approach has limitations in correct quantitation of cannabinoids since they may thermally degrade in the GC injection port (5,6). Liquid chromatography (LC) with ultraviolet (UV) detection does resolve the limitations of GC methods for cannabinoid analysis due to decomposition (6–10), but the lack of selectivity with LC and the method's narrow linear dynamic range can result in inaccurate quantification of cannabinoids in hemp and cannabis samples (11). Liquid chromatography tandem mass spectrometry (LC–MS/MS) offers much higher linear dynamic range, selectivity, and sensitivity compared to LC-UV and it can therefore be utilized for more accurate quantification of cannabinoids in the range of 0.1–100% with much higher selectivity than LC-UV (12–15).

This article describes the sample preparation and analytical method for the chromatographic separation and quantitative monitoring of 16 primary cannabinoids (covering seven different subclasses) in the hemp matrix by LC–MS/MS.

Experimental

Hardware and Software

Chromatographic separation was

conducted on a PerkinElmer LX50 ultrahigh-pressure liquid chromatography (UHPLC) system, while detection was achieved using a PerkinElmer QSight 420 LC–MS/MS detector with an electrospray ionization (ESI) source. All instrument control, data acquisition, and data processing was performed using the Simplicity 3Q software platform.

Sample Preparation Method

The step-by-step sample preparation procedure is described with an overall dilution factor of 100,000. For each sample, approximately 5 g of ground hemp was used as a representative of each sample batch. In our method, hemp was already received after grinding and therefore there was no need for further grinding. Note that hemp plant material would need to be ground to smaller particle size for efficient extraction of cannabinoids if it was present in its native form. Next, 1 g of this ground sample was then weighed out into a 50 mL centrifuge tube on an analytical balance (±0.001 g). The weight of the sample aliquot was recorded and 3–4 replicates were produced from the representative sample of each batch. Then 30 mL of 80:20 methanol–water was added to the sample aliquot in the centrifuge tube, which was then capped. This extraction solvent composition comprising mainly methanol with a small amount of water has previously demonstrated good recovery of cannabinoids from hemp products (9,13). The centrifuge tube was placed on a vortex mixer and agitated for a total of 15 min, before being spun for 5 min at 3000 rpm.

Following centrifugation, the plunger of a 3 mL polypropylene disposable syringe was removed and a 0.22 µm nylon syringe filter was then secured onto the tip of the syringe. Next 1.5 mL of the supernatant from the centrifuge tube was removed with a disposable transfer pipette and transferred into the 3 mL disposable syringe. The plunger was then inserted into the syringe barrel and the supernatant was filtered through the 0.22 µm nylon filter into a 1.5 mL centrifuge tube. The filtered supernatant was diluted 1000-fold by adding 30 µL of filtered extract into 970 µL of solvent containing 80:20 methanol–water in a new 1.5-mL centrifuge tube, before being mixed thoroughly on a vortex mixer for ~10 s. The mixed extract was diluted 100-fold further by pipetting 10 µL of the filtered supernatant into 990 µL of 80:20 methanol–water in a 2 mL sample vial, giving an overall dilution of 100,000. This was mixed thoroughly on a vortex mixer for ~10 s and loaded into the LX-50 autosampler for analysis using the LC–MS/MS method described herein.

Table I: HPLC–MS/MS method parameters

LC Conditions	
Column	Restek Raptor ARC-C18, 2.7 µm, 4.6 x 150 mm
Mobile phase	Solvent A: Water with 0.1% formic acid and 5 mM ammonium formate Solvent B: Acetonitrile with 0.1% formic acid
Run time	10 min (includes column equilibration time)
Flow rate:	1.5 mL/min
Autosampler temp.	10 °C
Oven temp.	30 °C
Injection volume	3 µL
MS Source Parameters	
ESI Voltage	5100 V in positive ion mode (neutral cannabinoids) and -4200 V in negative ion mode (cannabinoid acids)
Nebulizer gas	450 arbitrary units
Drying gas	150 arbitrary units
Source temperature	425 °C
HSID temperature:	275 °C
Detection mode	Time-managed MRM

LC Method and MS Source Conditions

The HPLC–MS/MS method, LC gradient, MS source, and multiple reaction monitoring (MRM) parameters are shown in **Tables I**, **II,** and **III,** respectively.

Table II: LC gradient method

Step	Step Time (min)	Flow Rate (mL/min)	%A	%B
1	0	1.5	25	75
2	6	1.5	25	75
3	7.5	1.5	15	85
4	7.6	1.5	0	100
5	9.0	1.5	0	100
6	9.1	1.5	25	75
7	10.0	1.5	25	75

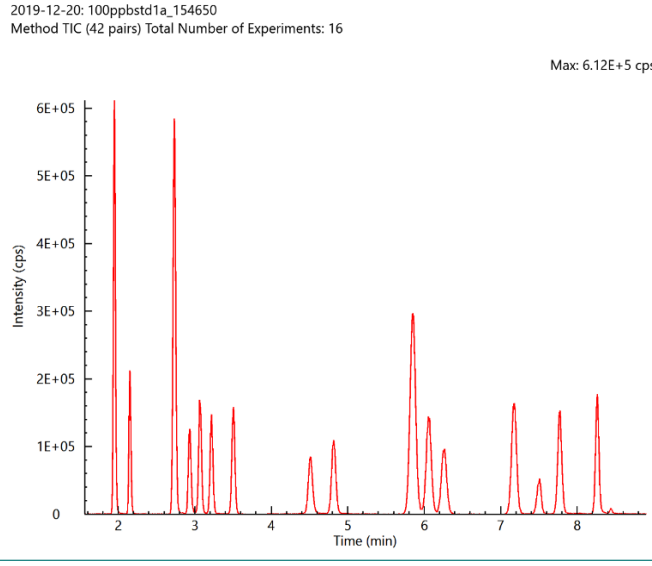
Solvents, Standards, and Samples

All solvents and diluents used were LC–MS grade and filtered via 0.45 µm filters. Upon arrival in the analytical laboratory, all standards were further diluted using acetonitrile. The standards of 16 cannabinoids at a concentration level of 1000 ppm were obtained from Cerilliant. A stock standard containing 20 ppm of each of the 16 cannabinoids was prepared by adding 100 µL of each individual standard to

Table III: MRM mass parameters for 16 cannabinoids. The * annotation indicates that these MRM masses were based on an M+1 isotope of the monoisotopic peak as precursor ion.

Cannabinoid	Precursor Ion Mass	Product Ion 1 Mass	Product Ion 2 Mass
Cannabidivarinic acid (CBDVA)	329.2	107	217.1
Cannabidivarin (CBDV)	287.2	165.1	135
Cannabidiolic acid (CBDA)	357.2	245.1	107
Cannabidiolic acid (CBDA)*	358.2	246.1	107
Cannabigerolic acid (CBGA)	359.2	136	191.1
Cannabigerol (CBG)	317.2	193.1	123
Cannabidiol (CBD)	315.2	193.1	259.1
Cannabidiol (CBD)*	316.2	194.1	260.1
Tetrahydrocannabivarin (THCV)	287.2	165.1	135
Tetrahydrocannabivarinic acid (THCVA)	329.2	107	217.1
Cannabinol (CBN)	311.2	223.1	241.1
Cannabinolic acid (CBNA)	353.2	279.1	222.1
Δ^9-Tetrahydrocannabinol (d9-THC)	315.2	193.1	259.1
Δ^9-Tetrahydrocannabinol (d9-THC)*	316.2	194.1	260.1
Δ^8-Tetrahydrocannabinol (d8-THC)	315.2	193.1	259.1
Cannabicyclol (CBL)	315.2	235.1	81
Cannabichromene (CBC)	315.2	193.1	259.1
Δ^9-Tetrahydrocannabinolic acid (THC-A)	357.2	245.1	107
Δ^9-Tetrahydrocannabinolic acid (THC-A)*	358.2	246.1	107
Cannabichromenic acid (CBCA)	359.2	191.1	136

Figure 1: LC–MS/MS chromatogram showing the separation of the 100 ppb standard of 16 cannabinoids.



3.4 mL of acetonitrile solvent, bringing the total volume of standard to 5 mL. For calibrants, the mixture was serially diluted to give concentration levels of 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 µg/mL (ppm). The results reflect the averaged triplicate injections for all calibrants and samples.

Results and Discussion

Separation and Detection Limits of 16 Cannabinoids

Figure 1 shows the LC–MS/MS chromatogram of a standard containing 100 ppb of 16 cannabinoids, all well resolved in less than 9 min. The LC method was able to obtain baseline separation of all the analyzed cannabinoids including the resolution of critical pairs containing Δ^9-THC–Δ^8-THC and CBD–CBG. **Table IV** lists the retention time of the 16 cannabinoids in solvent standard. To check for possible analyte carryover or background interference, an acetonitrile blank was run twice, both after the calibration set and the samples. In all cases, no carryover was observed for any of the analytes. LC–MS/MS methods show good specificity for analysis of cannabinoids in the presence of other interfering compounds in hemp matrix such as terpenes, because they have very unique precursor and product ion masses. However, LC–MS/MS shows poor specificity for cannabinoid isomers unless they are separated in time, either by LC or ion mobility. Therefore, it was important to achieve the baseline resolution of isomers of neutral cannabinoids and acidic cannabinoids with LC conditions. Table III demonstrates clearly that the mass spectrometer cannot distinguish between isomers of neutral and acidic cannabinoids. For example, the MRM table shows that isomers of neutral cannabinoids such as THC-8, THC-9, CBC, and CBD have the same major product ions at nominal masses of 259 and 193 Da, with the same precursor ion mass of 315 Da. The MRM table shows that another isomer of these cannabinoids, namely CBL, has

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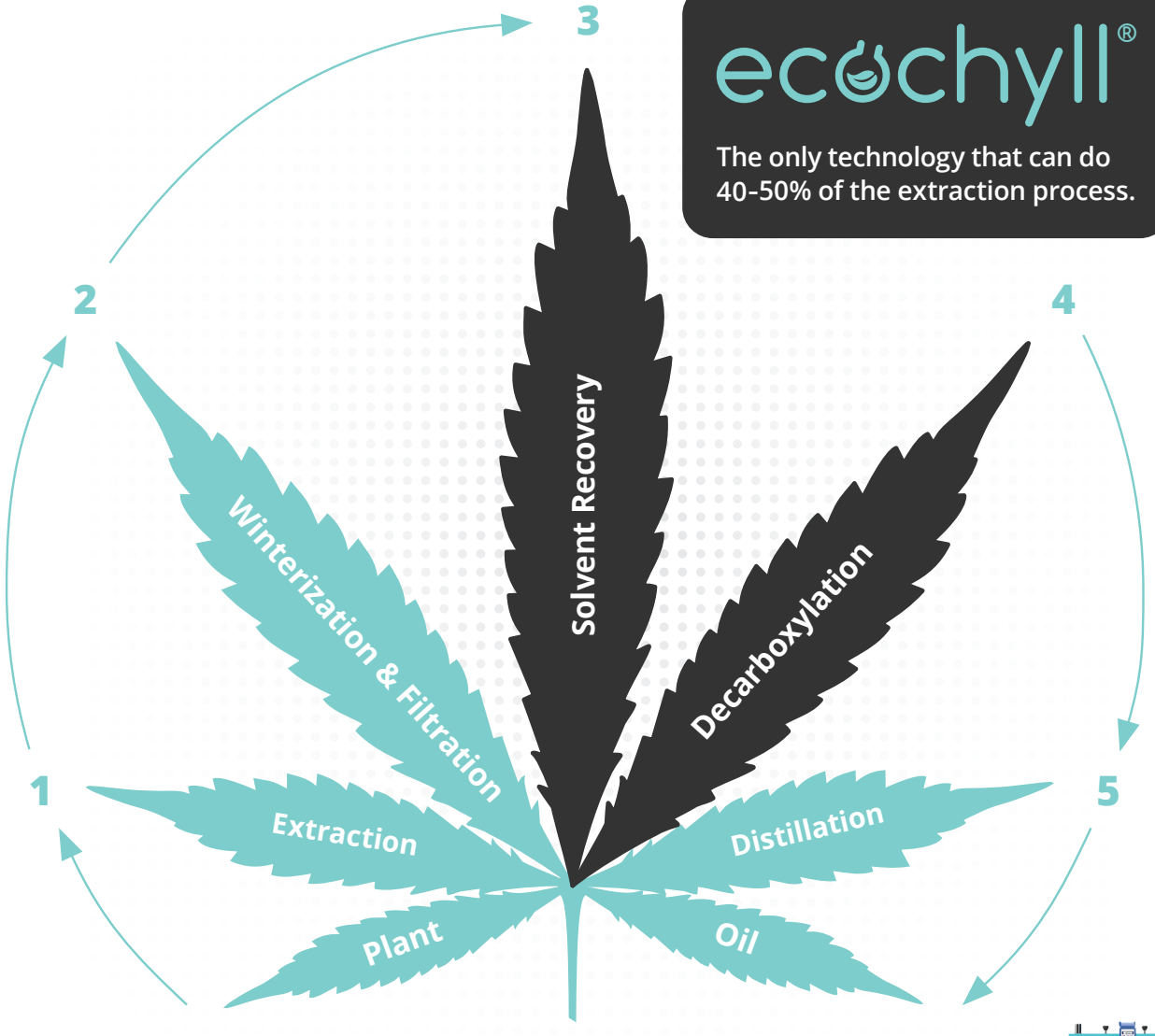

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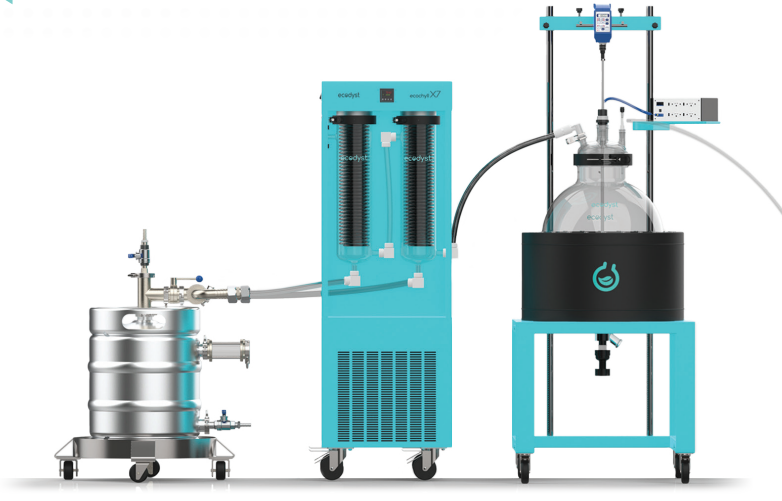
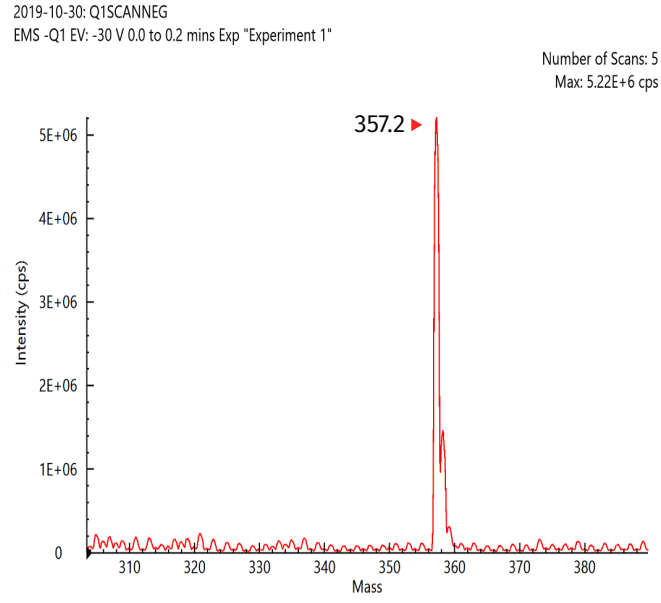


Table IV: Retention times for the 16 cannabinoids in solvent standard

S. No.	Cannabinoid	Retention Time/min
1	Cannabidivarinic acid (CBDVA)	1.94
2	Cannabidivarin (CBDV)	2.14
3	Cannabidiolic acid (CBDA)	2.71
4	Cannabigerolic acid (CBGA)	2.91
5	Cannabigerol (CBG)	3.05
6	Cannabidiol (CBD)	3.20
7	Tetrahydrocannabivarin (THCV)	3.49
8	Tetrahydrocannabivarinic acid	4.48
9	Cannabinol (CBN)	4.79
10	Cannabinolic acid (CBNA)	5.79
11	Δ^9-Tetrahydrocannabinol (d9-THC)	6.01
12	Δ^8-Tetrahydrocannabinol (d8-THC)	6.21
13	Cannabicyclol (CBL)	7.13
14	Cannabichromene (CBC)	7.46
15	Δ^9-Tetrahydrocannabinolic acid	7.74
16	Cannabichromenic acid (CBCA)	8.23

Figure 2: Mass spectra of an acidic cannabinoid (THCA) in negative ion mode using an ESI source.



different major product ions in comparison to the major product ions for the other four isomers (THC-8, THC-9, CBD, and CBC). In this case, we would need to obtain baseline resolution of CBL with the other four isomers with LC for higher selectivity, since CBL's minor product ions have the same mass as the major product ions of the other four isomers.

The optimization of the MS signal for cannabinoids showed that neutral cannabinoids and acid cannabinoids ionize better in positive and negative ion mode, respectively. Recent work published in literature has claimed that negative mode ESI can cause decarboxylation of acidic cannabinoids. The author of this article further postulated that this might result in a concern for quantitation of acidic cannabinoids in negative ion mode using LC–MS with no calibration or quantitation data (16). We did not observe decarboxylation of acidic cannabinoids in negative ion mode using ESI source in our instrument.

Figure 2 shows MS spectra of an acidic cannabinoid (THCA) in negative ion mode using LC–MS conditions published in our work with very good signal for $[M\text{-}H]^-$ ion at mass of 357.2 Dalton only without any decarboxylation. We think that decarboxylation of acidic cannabinoids was observed in the earlier paper by inducing either in-source fragmentation using high voltage to accelerate ions from ion source to a mass spectrometry analyzer, or excess heat in the author's source design. It is very common to induce in-source fragmentation of compounds using high voltage in an ESI source (17,18). In different ESI source designs on the market, these voltages are called either fragmentor, cone, or entrance voltages. In addition, another recent paper showed the possibility of measuring acidic cannabinoids in negative ion mode using an LC–MS/MS method with no calibration and quantitation issues and better detection limits for these compounds in negative ion mode as compared to positive ion mode (13).

Even in the case that decarboxylation results for acidic cannabinoids in negative ion mode were correct in the earlier reference with their ESI ion source parameters, as long as the LC method separates both neutral and acidic cannabinoids in time, there is no concern about quantification of acidic cannabinoids. Since we do not observe decarboxylation of acidic cannabinoids in negative ion mode and we are separating both acidic and neutral cannabinoids in time using our LC method, the concerns raised by the earlier paper (16) for quantification of acidic cannabinoids in negative ion mode are not valid for our LC–MS/MS method.

Since MS instruments are much more sensitive than UV detectors, it was possible to detect all cannabinoids with a limit of quantitation (LOQ) of

Table V: Amount of 16 cannabinoids, total THC and CBD, and their LOQ in ground hemp sample

S. No.	Cannabinoid	Amount/%	Calculated LOQ %
1	Cannabidivarinic acid (CBDVA)	0.057	0.03
2	Cannabidivarin (CBDV)	< LOQ	0.03
3	Cannabidiolic acid (CBDA)	5.73	0.03
4	Cannabigerolic acid (CBGA)	0.199	0.03
5	Cannabigerol (CBG)	0.072	0.03
6	Cannabidiol (CBD)	0.153	0.03
7	Tetrahydrocannabivarin (THCV)	< LOQ	0.03
8	Tetrahydrocannabivarinic acid (THCVA)	< LOQ	0.03
9	Cannabinol (CBN)	< LOQ	0.03
10	Cannabinolic acid (CBNA)	< LOQ	0.03
11	Δ^9-Tetrahydrocannabinol (d9-THC)	0.066	0.03
12	Δ^8-Tetrahydrocannabinol (d8-THC)	< LOQ	0.03
13	Cannabicyclol (CBL)	< LOQ	0.03
14	Cannabichromene (CBC)	0.163	0.03
15	Δ^9-Tetrahydrocannabinolic acid (THC-A)	0.125	0.03
16	Cannabichromenic acid (CBCA)	0.601	0.03
17	Total CBD (CBD + 0.877*CBDA)	5.18	0.06
18	Total THC (THC + 0.877*THC-A)	0.176	0.06

3 ppb or ng/mL. LOQ was determined based on a signal to noise ratio of 10 or more for quantifier transitions, as well as an ion ratio matching within 30% relative of the average expected ion ratio of qualifier to quantifier transitions of all the standards. Due to the very high dilution factor of 100,000 for hemp extract, we did not see any difference in noise level for all of the cannabinoids in both solvent standard and hemp extract. In addition, a higher dilution factor of hemp extract will result in minimal or no signal suppression for cannabinoids from hemp matrix. For the above reasons, we are justified in concluding that the LOQ for all of 16 cannabinoids would be quite similar in both solvent standard and hemp extract with a dilution factor of 100,000. Based on an overall dilution factor of 100,000, the detection limits of all the cannabinoids was 0.03% in hemp matrix.

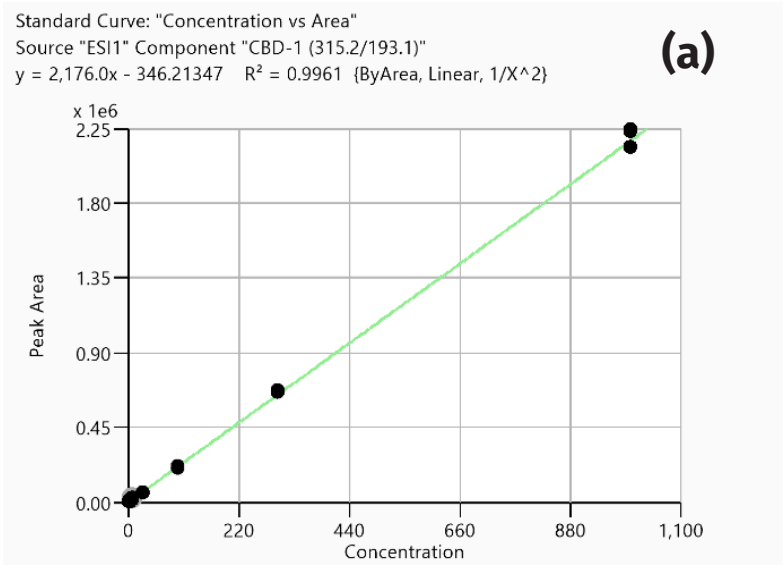
The Linear Dynamic Range

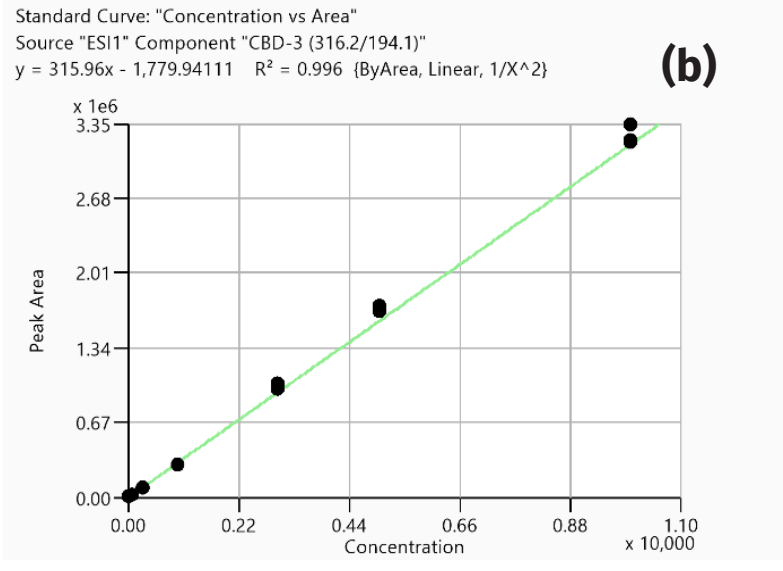
The calibration curves for 16 cannabinoid standards were generated in a concentration range of 3–1000 ppb with MRM transitions based on monoisotopic ions as precursor ions. This enabled quantitation of the cannabinoids over a range of 0.03–10% in hemp samples diluted by factor of 100,000. There is the possibility that concentrations of major cannabinoids such as Δ^9-THC, THCA, CBDA, and CBD could be higher than 10% in particular strains of cannabis flower, hemp, and their concentrated extracts. Therefore, for these samples, the calibration curves were extended to 10 ppm, which corresponds to 100% cannabinoid in samples based on an overall dilution factor of 100,000. Using 10 ppm is a relatively high level of cannabinoid for MS detectors. To overcome saturation effects, MS transitions of these compounds were also measured based on a first isotope as a precursor ion, which has 5–10 times lower signal than MS transitions based on a monoisotopic ion as a precursor ion.

By utilizing the calibration curves described above, it was possible to analyze major cannabinoids (CBD, CBDA, THC, and THCA) in a range of 0.03–100% without changing the dilution factor of samples. In previous studies, different groups have been able to measure these compounds in the range of 0.05–100% by using either two different injections with different sample dilution factors and UV detectors (9,10), or by using two different detectors (UV and MS) with different linear dynamic ranges (14). This new concept of measuring cannabinoid concentrations in samples over a wide dynamic range (0.03–100%) is significantly more cost effective because it does not require multiple injections or the use of two different detectors. This also results in a higher sample throughput compared to previously reported methods.

Approximately, 5-level or higher calibration fits were determined for all 16 cannabinoids. Representative linearity plots for CBD are shown in **Figure 3** over a concentration range of 3 ppb to 1 ppm and 30 ppb to 10 ppm using MS transitions based on monoisotopic mass and its first isotope as a precursor ion, respectively. The R^2 values for calibration curves of all of 16 cannabinoids were above 0.99. The accuracy of the calibration curve was checked by comparing back-calculated concentrations from a calibration curve with known concentrations of each cannabinoid and the criterion of maximum deviation of 15%. The precision studies showed that the relative standard deviation (RSD) of response of calibration standards was less than 10%.

Figure 3: Linearity plots for CBD over concentration range of 0.003–1 ppm (a) and 0.030–10 ppm (b) using signal from MS transitions based on monoisotopic mass and its first isotope as precursor ion, respectively.





Hemp Sample Analysis Using the LC–MS/MS Method

A sample of ground hemp, obtained from Emerald Scientific, Inc., was extracted with the described solvent extraction sample preparation method and present cannabinoids were quantified using the LC–MS/MS parameters described in Table I. Software was used to calculate the percentage of cannabinoids in the sample by considering the dilution factor and mass of the extracted hemp sample. To determine the amount of cannabinoids in the original hemp sample in wt/wt, we had to multiply the amount of cannabinoids determined in the hemp sample in g/mL of extract with a dilution factor of 100,000, since 1 mL of extract would be the equivalent of 10^{-5} gm of hemp sample. To further convert this wt/wt amount into % wt/wt, we had to multiply the above number by 100. The amounts of different cannabinoids measured in this hemp sample are listed in Table IV. Table IV also shows the calculated percentage of total CBD and THC by summing the acid and neutral forms of each (CBD + CBDA and THC + THCA). For this calculation, a correction factor of 0.877 was applied to the acid forms because of the extra molecular weight of the acid. The total amount of THC in this hemp sample was experimentally derived to be 0.176% (wt/wt), well below the USDA limit of 0.3% (wt/wt) for legal hemp product. Table IV also includes the calculated LOQs in hemp matrix for each cannabinoid, which were established based upon the dilution factor of 100,000, a signal to noise ratio over 10 for quantifier transitions, and an ion ratio matching within 30% relative of the average expected ion ratio of a standard.

Advantages of an LC–MS/MS Method for Cannabinoid Analysis in Hemp and Cannabis-Related Matrices Compared to Traditional Analytical Methods

LC–MS/MS provides higher selectivity and specificity for cannabinoid analysis compared to previously reported methods because it measures the unique fragments of each compound's molecular ion. In comparison, LC-UV has much lower selectivity because it measures the signal of cannabinoids at a fixed wavelength of light, which can result in matrix interference caused by compounds found in cannabis such as terpenes. These compounds can give a

signal at the same wavelength as cannabinoids and cause matrix interference if they coelute with cannabinoids in cannabis samples (11). A further advantage of this LC–MS/MS method for cannabinoid analysis is that the high dilution factor results in little contamination of chromatography columns by the sample matrix. This increases the methods cost effectiveness because the columns used will have an extended lifetime.

LC–MS/MS methods also provide high sensitivity due to advanced MS detectors, and minor cannabinoids can therefore be easily detected at levels of 0.03–0.1% or lower. This is achieved by utilizing a much higher dilution factor of 100,000 in comparison to the factor of 1000–6000 used for analysis of cannabinoids with LC-UV. The higher sensitivity of this method also means that calibration curves must be generated up to concentration levels of 1–10 ppm, compared to the much higher concentrations (50–250 ppm) required for previous LC-UV methods. The cannabinoid standards used to generate these curves are very expensive, and a 5–10-fold decrease in their consumption with the new LC–MS/MS method can represent an impactful cost-saving measure for laboratories. In addition, the use of a 10 times higher concentration of standards in LC-UV methods could exacerbate the LC autosampler carryover issues in LC-UV methods and this might lead to more inaccurate cannabinoid quantitation in cannabis and hemp samples compared to the results seen in the new LC–MS/MS method. A multiple sample dilution method with many injections would be needed to analyze both major and minor cannabinoids over a wide concentration range (0.1–100%) with UV detectors (9,10). This unique LC–MS/MS method can measure the cannabinoids over a wide range of 0.03–100% with a single sample dilution and injection.

Conclusion

This work has demonstrated the effective baseline chromatographic separation of 16 cannabinoids, including critical pairs of Δ^9-THC–Δ^8-THC and CBD–CBG. In a rapid 10 min LC–MS/MS method, 16 cannabinoids were quantitated in the range of 0.03–100% in hemp samples with a single dilution protocol and no carryover. The ground hemp sample tested in this work contained less than 0.3% total THC, as expected from legal hemp products. This LC–MS/MS method does not require use of multiple detectors (MS and UV) or multiple injections of samples with different dilutions to monitor both the low and high abundant amounts of cannabinoids in different hemp and cannabis related samples. In addition, the method can be easily extended to monitor cannabinoids in other cannabis-related samples including cannabis flower, concentrates, and edibles using a single dilution method with a single sample injection.

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Back to the Root

The Role of Botany and Plant Physiology in Cannabis Testing, Part III:

Genetic and Environmental Factors Associated with Terpene Synthesis in Plants

BY GWEN BODE

Terpenes are a class of compounds that have become of increasing interest to a broad swath of the cannabis industry because of their desirable consumer appeal and undesirable potential to act as interfering compounds in certain analytical assays. In Part III of "Back to the Root" we explore the role that terpenes play in plant immunity, defense, and signaling. A discussion of synthesis pathways will broaden our understanding of how and where these molecules are produced within plant tissues, as well as how they are expressed in plant defense and immunity. Finally, this article investigates environmental and genetic factors that influence terpene production in plants and the implications for cannabis.

AS THE BODY of research on cannabis continues to grow, terpenes have become of increasing interest to the cannabis industry as a whole from grower to scientist. *Terpenes* are a broad class of organic compounds classified by the number of 5-carbon isoprene units composing the molecule. *Monoterpenes* are composed of two isoprene units, sesquiterpenes have three, and diterpenes have four. Terpenes are secondary metabolites, which is just a way of saying that they are not involved in primary metabolic functions related to growth, development, or reproduction. This does not mean, however, that secondary metabolites are any less important to plants simply because their role is outside of primary metabolism. Terpenes in particular play an important role in plant immuno-defense systems, acting both directly as a primary defense and indirectly as communication molecules as we will see later.

Plant Defense Systems

To properly begin our discussion of terpenes, we need to consider their botanical importance, which means we need to understand a little bit about plant defense. In part II of "Back to the Root," we began to explore plant defense systems as they relate to the movement of systemic pesticides in plants (1). As a reminder, plant defenses can fall into two primary categories; *induced* defenses, which occur in response to stimuli that indicates a threat, and *constitutive* defenses, which are ever present regardless of attack (2). However, distinguishing between these categories can be a little murky; hypothetically plants that experience a consistent level of threat are thought to develop constitutive defenses while plants that experience varying levels of threat are more likely to rely on induced defenses (2). For example, plants in environments with a consistent level of herbivory, high or low, should develop constitutive defenses to defend them from herbivores. On the other hand, plants living in environments where levels of herbivory vary—say due to seasonal variations in the activity of herbivores—hypothetically will develop induced responses to conserve resources during times of lower threat. Keep in mind however, that these two strategies are not mutually exclusive and most plants tend to have both induced and constitutive defenses.

In cases of induced defenses, the plant needs to first perceive an imminent threat to trigger a defense response. Some examples of threatening stimuli include detection of insect footsteps or eggs, mechanical damage, introduction of hydrogen peroxide (H_2O_2) from insect feeding secretions, or fragments of cell walls from pathogens (2). We will begin by exploring the detection of pathogenic microorganisms. Plants detect pathogens at the cellular level; embedded in the plasma membrane of plant cells are *receptors*, proteins that recognize microbe-associated molecular patterns (MAMPs) (2). Some examples of MAMPs include oligosaccharides, peptides, and enzymes (2). These patterns tend to be highly conserved in the microbes' genome and allow for the detection of entire groups of microbes that share a recognized molecular pattern (2). These patterns are highly conserved because they are usually critical to a microbe's ability to survive, reproduce, or form colonies making them unlikely to be easily changed without serious consequences. Once a specific molecular pattern is recognized by its corresponding receptor, a signal pathway is triggered that induces a defense response.

As with all immuno-defense responses, this amounts to an arms race driven by selection and evolution. Over time, selection will favor those pathogens that possess a trait that improves their chances to avoid detection by the host, survive, and reproduce, resulting in the evolution of resistant microbe populations. We refer to these traits as *effectors*, some examples of which include changes to the identifying pattern that are just enough to make it unrecognizable while remaining functional or behaviors such as shedding an identifiable structure upon entering a host (2). In turn the hosts have evolved a way to still detect pathogens that have avoided detection by the more generalist receptors. In plants this amounts to a second, more specific level of defense involving specialized plant receptors called *R proteins* (R for resistance) that detect specific effectors and trigger a defense response (2). By combining broad detection of large groups of microbes with recognition of more specific effectors, plants have what amounts to a multilayered pathogen detection system.

> "Plants have been shown to be highly sensitive **when it comes to detecting the pitter patter of insect feet;** very slight disruptions of the epidermis by **the claws of caterpillar feet are enough to trigger** a defense response in plants (2)."

The mechanism of herbivore detection is thought to be similar to that of pathogen detection; plants possess receptor proteins able to recognize herbivore-associated molecular patterns (HAMPs) (2). Some molecular patterns identified by research include chemicals released when an insect walks across a plant surface, as well as insect secretions from both feeding and egg-laying (2). While many of the receptors involved in microbial detection are well understood, less is known about the receptor proteins that detect herbivore-associated molecular patterns. Molecular and chemical cues are not the only way that plants detect insect threats. Plants have been shown to be highly sensitive when it comes to detecting the pitter patter of insect feet; very slight disruptions of the epidermis by the claws of caterpillar feet are enough to trigger a defense response in plants (2). This shows that in the case of footsteps at least, plants are sometimes responding to mechanical damage, rather than chemical cues released by the insects as they walk.

In the case of feeding insects, detection cues that trigger a defense response can be either chemical, through molecular recognition by a receptor, or mechanical and seem to vary amongst

plants. Some plants respond similarly to wounding by feeding insects as they do to simulations such as leaf clipping, suggesting that mechanical cues are triggering a defense response (2). Other plants treat these as two different threats that elicit different responses, indicating that they are using chemical cues such as feeding secretions to distinguish between threats (2). To the best of my knowledge at this time, little has been done regarding insect wounding studies on *Cannabis* subspecies (ssp.) (see footnote below) though there has been some indication that insect wounding increases resin production (3). This is an important knowledge gap because as any grower knows, pests are a major source of crop damage and loss for both indoor and outdoor cannabis grow facilities. A better understanding of how cannabis plants detect insect damage might allow growers to better tap into native plant defense systems.

Broadening our understanding of the cues that trigger different defense responses, the receptors that detect those cues, and how cannabis responds to threats would enhance our knowledge of the chemical ecology of cannabis. Discovering both microbe-associated and herbivore-associated molecular patterns that cannabis plants use to detect threats might allow for a sort of immunization of plants, called *systemic acquired resistance* (*SAR*). Additionally, plants can inherit immunity and resistance to pathogens and pests, making selective breeding another highly viable option for improving crop health as genetic research on cannabis advances. As I have advocated previously, until we have a better understanding of the relationships of the varied chemotypes in the genus *Cannabis* (high-cannabidiol [CBD], high-tetrahydrocannabinol [THC], fiber producing, and so on) each should be investigated individually since so much variation among plant responses can and does exist.

The Role of Terpenes in Plant Defense

Now that we have an idea of how plants identify threats through molecular and physical cues, we're ready to move on to a discussion of the role that terpenes play in plant defense. So far we have predominantly talked about the detection of cues that result in induced defenses, wherein the plant is responding to a threat in response to some cue. Another way to think about plant defense is to consider whether the defense is direct, attacking the threat organism outright, or indirect. Terpenes can act in both indirect and direct defense. As direct defense, many terpenes have antifungal or antibacterial action while others are repellent or outright toxic to insects (2,4). Terpenes can also act as a direct defense by deterring insect egg-laying (2,5). If the eggs are not laid, the caterpillars won't hatch and consume the plant; really a rather proactive approach.

Indirect defenses are perhaps the more interesting, from a behavioral standpoint, because they require communication: intraplant (from one part of the plant to another), plant to plant, or plant to insect. Keep in mind that while it's common to think of communication generally as sound or movement, plant communication is done through cell-signaling and the emission of volatile organic compounds (VOCs). When a plant is attacked, communicating the need to ramp up defenses to all tissues at risk is crucial. Plants generally communicate this need using salicylic acid in the case of microbial attack and jasmonic acid in the case of insect attack. These acids are transported through the plant via the phloem. Translocation of these compounds is relatively slow and is further compounded by the mosaic of connectivity of plant vascular systems (2). This means that the leaves immediately next to a leaf under attack might not have a direct vascular connection, but would surely benefit from a ramped up defense response.

What is a plant to do? Both salicylic and jasmonic acid can be converted to volatile methyl esters, which when released can communicate a threat to other parts of the plant that have limited vascular connectivity (2). Usually release is precipitated by wounding in the case of *jasmonates*, the group of compounds that include jasmonic acid and methyl jasmonate (2). Methyl jasmonate has even been shown to induce the synthesis of defense compounds in neighboring plants in leaf wounding studies wherein wounded plants are grown in the vicinity of nonwounded plants and both are measured for defense responses (2). Less is known about the ability of methyl salicylate to confer systemic microbial resistance to neighboring plants (2). Jasmonates can also regulate the emission of other stress induced volatile organic compounds including terpenes (4). It is yet unknown how exactly plants perceive these volatile signaling molecules. Interestingly, terpenes themselves can stimulate the biosynthesis of jasmonic acid indicating that there is a very close molecular interplay between

Footnote: In trying to find a concise but accurate way of expressing the uncertainty surrounding the speciation of cannabis, I have chosen to refer to the genus (*Cannabis*) followed with the abbreviation for subspecies, because this best reflects the formal taxonomy of cannabis, which recognizes only one species (*Cannabis sativa L.*), while encompassing all varieties and or chemotypes.

these compounds (2). It makes sense for defense signaling molecules to have a reciprocal response to each other, since that amounts to what is effectively a two-way communication channel.

Once released, terpenes can induce the expression of a multitude of herbivore- and pathogen-defense genes (2,4). Riedlmeier and colleagues showed in the model organism *Arabidopsis thaliana* that α- and β-pinene induced defense responses, as well as stimulating the expression of several genes related to salicylic acid production and SAR (6). Further, both pinenes and camphene elicited a defense response in neighboring plants, showing that these terpenes can act as communication molecules between plants (6). Evidently, monoterpenes in particular play an important role in intra- and interplant communication.

Another interesting way that terpenes and jasmonates can function in indirect defense is by attracting herbivore predators and parasites (2,4). When a plant has been wounded, the released terpenes and other volatiles act as a distress signal that has wasps and other insect predators buzzing in. The mixture of volatiles emitted by a plant in response to herbivory can be specific to the plant and the attacking herbivore, meaning that insects, allies, and threats alike can tell who is being attacked and by whom (2). Increasingly, some cannabis growers have opted to use biological controls such as beneficial insects over traditional chemical pesticides. Understanding more about the specific volatile signature of cannabis plants under various insect stressors could prove useful by enhancing the effectiveness of biological controls.

It is not well understood how exactly insects, both herbivores and predators of herbivores alike, are distinguishing between these volatile molecular cocktails, which can sometimes contain hundreds of compounds. Much debate surrounds the topic, though it seems most likely that insects are relying on different ratios of compounds for identification rather than identifying species-specific volatiles (2). It is worth mentioning briefly that terpenes can also play a role in attracting pollinators, though this doesn't apply in the case of cannabis which is a wind pollinated species.

Terpenes and Abiotic Stress

Cannabis ssp. can produce an extensive assortment of terpenes, with more than 150 unique terpenes identified in the resin of various cannabis chemotypes (7). We now have a good idea of the role terpenes play in plant defense, so let's talk about abiotic stress. *Abiotic stress* is any nonliving factor that negatively impacts a plant's ability to survive, grow, or reproduce. Some examples of abiotic stress include nutrient deficiencies, drought, salinity, temperature, and damage from reactive oxygen species. Because plants are sessile (they can't run away), being able to adapt to abiotic stress is crucial to their survival. It has been demonstrated that plants emit terpenes at higher levels when they experience heat stress (8). Terpene emissions should be expected to increase as temperature increases because heat makes them more volatile, resulting in a higher vapor pressure. However, more terpenes are emitted under temperature stress than can be accounted for by vapor pressure changes alone, making the case that biosynthesis has increased as well. Further, research has shown that plants fumigated with certain monoterpenes recover more rapidly from high temperature exposure, as measured by photosynthesis rates, than untreated controls (8). This indicates that terpene exposure beneficially impacts recovery time for plants exposed to heat stress.

Another abiotic stress that terpenes can alleviate is oxidative stress. Oxidative stress can result in damage to cells and biomolecules such as DNA, making the control of reactive oxygen species critical. Further, oxidative stress in plants is an additional consequence of many other biotic and abiotic stresses including herbivory, temperature, and light stress. Monoterpenes and sesquiterpenes both have been shown to decrease reactive oxygen species in fumigation and genetic modification studies (8). It has also been shown that plants produce high levels of sesquiterpenes when exposed to ozone (O_3), a common reactive oxygen species (4). Alternatively, when monoterpene biosynthesis was inhibited, plants showed higher levels of oxidative damage and reduced photosynthesis (8). The evidence that terpene presence improves recovery from oxidative stress while terpene suppression results in greater damage suggests that terpenes play an important role in oxidative stress responses.

Drought stress and salt stress are also abiotic stressors that have been shown to increase terpene emission by plants, but inconsistently (8). This suggests that a terpene response to these stressors may be species specific, indicating that they might induce terpene release in *Cannabis* ssp. or they might not. More research is needed to determine what effects drought and salt stress might have on terpene emissions in cannabis, if any. Low nitrogen availability appears to have an impact on isoprene emissions in plants in general, though whether or not it impacts terpene emission is not well understood. Because isoprene is the building block of terpenes this relationship bears further investigation. In hemp, there has been some evidence that

"As the body of molecular and genetic knowledge accumulates, **we will be better equipped to understand the ecological role of terpenes in cannabis;** the study of which will provide benefits beyond knowledge alone."

nitrogen metabolism-related genes and genes involved in secondary metabolism are coregulated (9). However, more research is needed to determine if terpene genes specifically are coregulated with genes related to nitrogen-metabolism.

Terpenes in Cannabis

In *Cannabis* ssp. terpenes are both produced and stored in glandular trichomes found on all aerial parts of the plant, but female flowers possess the greatest quantity of trichomes. Cannabis flowers have three morphologically different types of trichomes: *bulbous*, which are the smallest and produce few secondary metabolites; *sessile*, which sit on a short stalk, topped with a round disk of secretory cells and have a storage compartment that extends below the surface; and *stalked*, which are structurally similar to sessile trichomes but with a larger head and a longer stalk (10). Vegetative leaves and anthers do not possess stalked trichomes, but do have sessile trichomes (10). Trichome morphology alone cannot tell us about any chemical differences or the developmental relationship between these different trichome types.

Livingston and colleagues sought to greatly broaden our understanding of cannabis trichome structure and chemical composition, finding evidence that on female flowers, stalked glandular trichomes develop from immature, "sessile-like" trichomes (10). These immature trichomes differ from "true" sessile trichomes found on leaves and anthers in several key ways. True sessile trichomes were shown to have a greater ratio of sesquiterpenes to monoterpenes and to sit directly on the surface of the epidermis (10). In contrast, stalked glandular trichomes and their immature, sessile-like precursors have a greater ratio of monoterpenes to sesquiterpenes and sit on a stalk that lengthens as the trichome develops (10). The two can also be distinguished by the number of cells they have and their fluorescence (10). Which cannabinoids the trichomes produced was not found to vary across trichome types but the quantity of cannabinoids did, with stalked trichomes having the highest cannabinoid content (10). This study is especially noteworthy because Livingston and colleagues tested both the model hemp variety "Finola" and two "marijuana-type" (high THC) varieties: "Purple Kush" and "Hindu Kush." Because they were so thorough they were able to show that their findings on trichome structure were consistent across these chemotypes.

It is well known that terpenes and cannabinoids alike are produced and stored in various glandular trichomes; more recently researchers have begun to examine terpene synthesis pathways and their related genetic components in *Cannabis* ssp. As mentioned earlier, terpenes are composed of isoprene units, the number of which determine the type of terpene (mono, sesqui, di, and so on). Terpenes are synthesized via two primary metabolic pathways: via the mevalonic pathway from acetyl-CoA or via the methylerythritol phosphate pathway from pyruvate (2). Special enzymes called *terpene synthases* catalyze the chemical reactions that convert precursors into terpenes; the diversity of terpenes found in cannabis is reflective of the diversity of genes related to terpene synthase enzymes in the cannabis genome (7). Many of these terpene synthases can produce more than one type of terpene, possibly explaining why certain terpenes in cannabis tend to co-occur (7). Monoterpenes and cannabinoids share a precursor molecule, 10-carbon geranyl diphosphate (GPP C_{10}), though sesquiterpenes have a different 15-carbon precursor (7). The fact that monoterpenes and cannabinoids share a precursor is logical because, as we have learned, cannabinoids are produced in greater quantities within stalked trichomes that have a greater proportion of monoterpenes.

An increasingly popular way to clarify the relationships surrounding terpene synthesis, terpene synthases, and various terpene synthesis-related genes is through transcriptomic analysis. Transcriptomic analysis looks at the *transcriptome*, or total set of RNA transcripts produced by the genome in a specific cell or under specific conditions. It has become increasingly popular with researchers of secondary metabolites because it reflects the genes that are actively being expressed at any given time, allowing for the identification of comprehensive sets of genes involved in the synthesis of compounds of interest. Several research groups have recently applied this methodology to illuminating terpene synthesis in cannabis. Booth and colleagues used transcriptomic analysis to identify nine major cannabis terpene synthases in the "Finola" hemp variety (11). The products of

these synthases are responsible for most of the terpenes present in "Finola" resin including β-myrcene, (*E*)-β-ocimene, (-)-limonene, (+)-α-pinene, β-caryophyllene, and α-humulene (11). This was a significant contribution to understanding terpene synthesis pathways in cannabis because these terpenes are frequently detected at significant levels in cannabis and hemp, in my experience.

The researchers were also able to compare their discovered genes with the recently sequenced genome of "Purple Kush," a "marijuana-type" (high-THC chemotype) cannabis strain. Some terpene synthase-related genetic overlap between the varieties was observed, but not all of the genes identified were present in both chemotypes. The researchers speculated that some genes might have evolved to have different functions over time in different cannabis varieties (11). For example, they found a gene involved in α-pinene synthesis in "Finola" but that gene was not present in the "Purple Kush" genome, a strain that tends to have high levels of α-pinene (11). This implies that some other gene, or combination of genes, might be responsible for α-pinene synthesis in "Purple Kush." This highlights the need to research as many chemotypes as is practical because genes can evolve over time, resulting in multiple synthesis pathways to the same product.

Building off the work of Booth and colleagues, Zager and colleagues did a transcriptomic analysis of nine recreational cannabis strains, including the high-CBD recreational strain "Canna-tsu." They found that the genes identified by Booth and colleagues for β-myrcene, (2)-limonene, α -pinene, β-caryophyllene, and α-humulene, were expressed at high levels across all strains (12). They also identified a gene that codes for a terpene synthase responsible for producing linalool and nerolidol in these strains (12). This makes a strong argument for the close relationship of recreational cannabis chemotypes and also identifies another terpene (linalool) commonly found at significant levels in cannabis. Nerolidol on the other hand, is relatively rare in my experience, making the fact that it shares a synthase with linalool interesting.

In addition to characterizing chemical differences in different trichome types across different cannabis varieties, Livingston and colleagues also did a transcriptomic analysis of different flower trichome types from the model hemp variety "Finola." They found that there was no significant difference in gene expression between stalked trichomes and their immature prestalk precursors (10). Livingston and colleagues also identified two previously uncharacterized terpene synthases in "Finola," which produced terpinolene and β-ocimene in recombinant studies (10). Prior to this study, no terpene synthase producing terpinolene had been identified.

Conclusion

It's exciting to see so much novel, molecular research exploring terpene synthesis pathways in both hemp and recreational cannabis chemotypes. As the body of molecular and genetic knowledge accumulates, we will be better equipped to understand the ecological role of terpenes in cannabis; the study of which will provide benefits beyond knowledge alone. We will also be able to move closer to clarifying the relationship between *Cannabis* ssp. and the genetic factors that characterize strains of recreational cannabis. In the next and final article of this series we will explore cannabinoids, specifically what we know about them botanically and what we don't.

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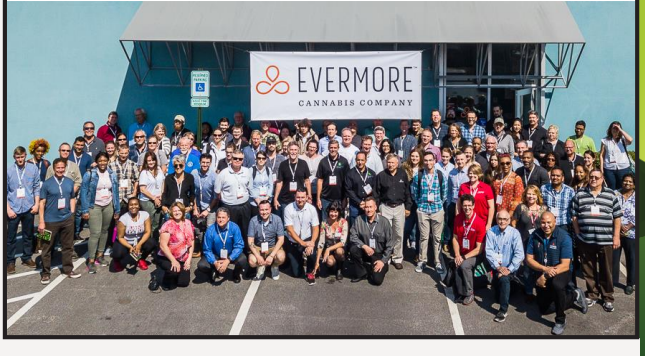

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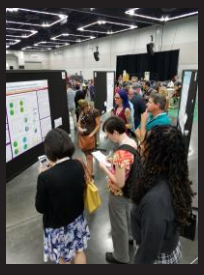



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Budtender:
Is That a Harmful Mold in My Bud?

BY RYAN KOBYLARZ

As the novel COVID-19 pandemic spreads through our country, it is a somber reminder that our public health regulations and methods of analysis should be constantly evaluated. As we learn more about diseases on a genetic level, we become more reliant on DNA based technologies. As we advance our understanding of DNA based technologies, application toward public safety regulations should be a natural conclusion. This article presents an argument for moving away from traditional culture-based methods and towards employing methods with increased specificity and accuracy.

IN THE UNCERTAIN times brought about by COVID-19 and the cannabis industry being designated as an essential business, it creates an opportunity to evaluate how the microbial rules are determined in the cannabis industry and how testing laboratories across the country analyze these samples. There is no doubt that cannabis testing laboratories and regulators across this country work together to create a program that ensures all people are consuming a quality and, most importantly, a safe product. It is also important to regularly ask whether the testing rules are stringent enough and in line with the knowledge and technologies that are available today. Is there a good reason for medical and adult-use products to have different action limits? Are our testing methods specific enough? Are we employing methods that mitigate subjectivity in analysis?

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Current Regulations

Action limits are defined thresholds that determine whether a product is safe for consumption. What thresholds are in place now and how were they determined? Some action limits are qualitative, which is essentially an absence or presence of analysis for Shiga-Toxin producing *Escherichia Coli* (STEC), *Salmonella* spp., and Aspergillus (*flavus*, *niger*, *terreus,* and *fumigatus*), these are determined on a state by state basis by state regulators. Many of the existing action limits come from the recommended standards set forth by the American Herbal Pharmacopoeia in its 2014 publication, *Cannabis Inflorescence: Standards of Identity, Analysis, and Quality Control* (1). This seminal publication has put forth recommended quantitative tolerance limits at levels that have been determined acceptable and safe for human consumption.

Quantitative action limits for microbial load can appear to be subjective at first glance. The quantitative recommended limits were put forth in 2014, after the authors had collected and compiled data and resources in the years prior. How much of these determinations were based on the existing technologies of the time? How much of the determinations were due to the legality surrounding cannabis testing at the time? What rationale is there for different action limits for medical cannabis and adult-use cannabis?

When we compare the technologies of the time to today, we are comparing culture-based plating testing to quantitative polymerase chain reaction (qPCR). Both methods identify colonies but in very different ways; culture-based plates inclusively count all yeast and mold species that grow colony forming units on an agar plate and qPCR exclusively identifies DNA of specific yeasts and mold species.

As the accepted legality of cannabis increases, so does the cultivation and testing of cannabis. This has presented us with greater resources and opportunities to pool all of our collective data and experiences. An example of such new knowledge are biological controls. Cultivators, many of whom strive to create clean and organic cannabis products, aim to move away from harmful pesticides and fungicides, and are turning to biocontrols to help ensure the naturalness of the product intended for medical patients. We know that these biocontrols include certain types of fungi that are harmless to humans, but they are also included in the quantitative count on culture-based plates. It becomes important that we have testing methods that do not unnecessarily punish cultivators for trying to create their vision of a quality product.

Advanced Technologies

Understanding the technologies available to us can help us understand where we are and where we can go from here. Culture-based plating is the traditional method for enumerating total yeast and mold. With this method, samples are "plated" on nutrient-rich media that will support the growth of general yeast and mold fungi and incubated for a predetermined amount of time. After which, yeast/mold colony forming units (CFUs) are tallied and the cumulative result is the total fungal bioburden present in the sample. Knowing the quantified bioburden present in the sample is an excellent indicator test, however, it misses the mark when it comes to distinguishing between those that are benign and those that are pathogenic to humans. Aspergillus, a ubiquitous mold that has several species—for example, *A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*—that are known to be harmful to humans and cannot be visually identified by commercial culture-based plating methods. There are noncommercial plate-culture methods, but they require

a trained mycologist to visually identify the species in the presence of other yeasts and molds.

PCR is a molecular detection technology that is rapidly becoming a tool in every microbiology laboratory. The principle of a PCR assay, simplified, has four steps:

1) All DNA is extracted from the sample;
2) Three DNA oligomers—two "primers" that contain very specific DNA sequences and one fluorophore marker "probe"—are added to the solution containing all DNA from the sample;
3) The mixed solution, in a thermocycler, undergoes iterative cycles of heating, where the primers will bind to unique DNA sequences of the target organism, if present in the solution, and create a copy of the DNA sequence;
4) The probe is bound to this DNA copy at the end of each cycle and then emits light that is captured and measured by a camera.

The amount of DNA copies and light emitted is amplified with each cycle. By measuring the amount of light emitted, it is possible to reverse calculate the initial concentration of the target organism—a PCR assay known as qPCR.

PCR assays have several key advantages compared to culture-based plating methods. PCR assays have high specificity and species level resolution by identifying specific DNA sequences that are unique to the target organism or species. Most PCR assay kits come with 96-well plates, making it a high throughput assay. The exponential amplification of the PCR assay confers greater sensitivity that enables detection of very low amounts of target DNA, which may be out competed by nontarget microorganisms on culture-based plates. Speed is another advantage; most PCR assays only require an overnight incubation in an enrichment broth and the PCR analysis can be conducted the next day.

Despite the evident advantages, the widespread implementation of PCR is limited by two valid arguments: cost and the DNA of dead cells. All PCR equipment, kits, and reagents necessary for PCR assays can incur greater upfront cost relative to the culture-based plating method. High throughput efficiencies can help offset some of the costs. Secondly, the sensitivity of PCR assays allows for the detection of DNA from dead and nonviable cells. The purpose of cannabis microbial screening is to screen for the living microorganisms that may have a negative effect on human health and dead or nonviable cells do not pose the same threat to human health. However, more PCR assays are now incorporating a pretreatment step to deactivate or neutralize the DNA from dead or nonviable cells, eliminating the possibility of a false positive.

There might be some unintended consequences by relying on culture-based plating testing with its limitations. Some regulators are considering or have allowed increased total yeast and mold action limits for cannabis intended for adult-use. Two questions immediately come to the forefront: Are adult-use cannabis products inherently less safe or less clean than medical products now that they have higher action limits? Are medical cultivators now turning to potentially harmful chemical-based pesticide and fungicides to eliminate microbial contamination in their products? In light of public safety, and especially with the stay at home orders placed, perhaps the question we should be asking regulators is why is it okay for the state to say "here you go pal, this product would not be acceptable for medical consumers but since you are consuming this for fun, you can handle a wee bit of a cough"?

There is no real reason to allow different action limits for cannabis based on the distinction between medical use versus adult-use. Some of the latest qPCR assays for cannabis testing have the capability to quantify total yeast and molds counts, a potential replacement for the traditional culture-based plating method. Even with the additional complexity that comes with quantitation of many different yeasts and mold, a successful qPCR method can be more accurate, reliable, and truer to the spirit of the regulations than plating methods.

Conclusion

The cannabis industry has changed significantly from prelegalization to today. Long gone are the days of cannabis being grown, cured, and stored in poor, unregulated environmental conditions. In today's regulated cannabis industry, the occurrence of pathogenic microbial contaminants is very rare. The cannabis industry is in its infancy and is still evolving. We, as stewards of public health, should constantly strive to evaluate the changing regulations and new advances in testing technologies. Testing laboratories serve to minimize hazardous risk to the public, particularly the immune-compromised population. The recent vaping crisis and the COVID-19 pandemic are all too much a reminder that the field of public health and safety must be a constantly evolving one.

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Cannabis Extracts:

The Need for Standardization and Accreditation in Emerging Industries

BY JEREMY E. MELANSON AND ANNA WILLIAMS

As the market for legalized cannabis extract products, such as edibles and vape liquids, continues to grow, laboratory testing to ensure the quality of these products has not kept pace with the range of new products available. The wide range of products will require a suite of different methods that may need to be validated for each sample type. For example, some testing methods for tetrahydrocannabinol (THC) in dried cannabis material are not appropriate for measuring THC in foods and beverages. This article highlights challenges facing the cannabis extracts industry and emphasizes the need for standardized methods and the role of laboratory accreditation.

Challenges with Method Validation

RELATIVE TO DRIED cannabis material, cannabis extracts and edibles pose a significant measurement challenge because of their high complexity and diversity. For instance, food products such as chocolate create difficulties with cannabinoid extraction due to the high fat content and also yield a multitude of interference peaks that can hinder accurate quantitation. In some cases, more complex samples will require the added specificity offered by techniques such as liquid chromatography–tandem mass spectrometry (LC–MS/MS) (1). While LC–MS/MS offers superior specificity relative to conventional liquid chromatography with optical detection, it still requires rigorous validation to ensure accuracy because of its susceptibility to matrix effects that suppress signal.

Validated test methods that are accurate, precise, and robust are required to ensure reliability of cannabis testing results. In addition, the use of validated methods is a requirement of ISO/IEC 17025 and has been specified in cannabis regulations of many jurisdictions. Rigorous method validation is labor intensive, and without suitable reference materials available for all sample types, it often requires relatively large amounts of the target analytes for spiking and recovery experiments to demonstrate accuracy. This can be particularly challenging for assessing accuracy for cannabinoids such as Δ^9-tetrahydrocannabinol (Δ^9-THC), with

most laboratories limited to using the 1 mg/mL commercial Δ^9-THC standard solutions for legal reasons, which are too dilute for effective spike and recovery studies.

Therefore, validation of cannabinoid methods in extracts and edibles is costly, time consuming, and typically impractical for many testing laboratories that could lack the resources or expertise. For similar matrices, such as different chocolate varieties, there is considerable debate about whether a complete method revalidation is required, or a more streamlined method verification is sufficient. Regulations in some jurisdictions do not provide clear guidance on this issue, so many laboratories will likely choose the most cost-effective option.

The Need for Standard Methods

To help reduce the need for individual laboratories to validate their own methods for cannabis testing, there is a growing need for standardized testing methods in the cannabis industry (2), and the edibles and extracts industry in particular. Already prevalent in the food testing industry and other established markets, standard test methods help ensure harmonization of testing results across different laboratories, even across international borders. While standard methods take considerable time to develop and generally involve some form of interlaboratory study, the burden of the full method validation is shouldered by only a small number or even a single laboratory. The end result is not only greater harmonization and reliability of results, but also a significant cost savings across the entire industry as users of the standard method can avoid costly validation studies. This cost savings could be vital to the long-term viability of licensed cannabis producers, which continue to be undercut by the illicit market.

> "Ultimately, **the process of accreditation through a reliable accreditation body** gives the public confidence that a testing laboratory is competent, **ensuring consumers have access to a quality product**."

Fortunately, there are considerable efforts underway by several organizations dedicated to the development of standard test methods for cannabis products. ASTM International formed Committee D37 on Cannabis to develop standards for cannabis, its products, and processes (3). With nine subcommittees ranging in scope from "Quality Management Systems" to "Processing and Handling," the Laboratory subcommittee is dedicated to the development of standard test methods for cannabis products. A "Standard Practice for Laboratory Test Method Validation and Method Development" specific to the cannabis industry has been published (4) and several test method standards are in development. AOAC International has developed its Cannabis Analytical Science Program (CASP), which is a forum where the science of hemp and cannabis analysis can be discussed, and cannabis standards and methods developed (5). Several AOAC standard method performance requirements (SMPR) have been published, and an official method for cannabinoids in cannabis dried plant materials, concentrates, and oils using liquid chromatography-ultraviolet detection (LC-UV) and LC–MS/MS has been published (6). Finally, the US Pharmacopeia (USP) has established an Expert Panel on cannabis, tasked initially with the development of the equivalent of a monograph for cannabis for medical purposes (7).

While the majority of standard test methods in development are dedicated to dried cannabis plant material, these organizations are prioritizing standards development for extracts and edibles. For instance, AOAC's CASP has developed a new working group dedicated to "Cannabis and Consumables." In addition, ASTM D37's Laboratory subcommittee has initiated a work item on the characterization of vape liquid components by gas chromatography–mass spectrometry (GC–MS). The complementary resources from these organizations and others will undoubtedly lead to greater standardization in the cannabis extracts industry.

The Role of Laboratory Accreditation

Accreditation is defined as "a third-party attestation related to a conformity assessment body (the laboratory) conveying formal demonstration of its competence to carry out specific conformity assessment tasks (the tests)." In other words, it is a formal process by which an independent party, an accreditation body (AB), evaluates and acknowledges a laboratory's technical competence to perform specific tests, which usually are listed on a scope of accreditation. The AB accomplishes this by using trained and qualified technical experts to evaluate the laboratory against the appropriate international standard, in this case ISO/IEC 17025. The AB may also use additional program-specific and regulatory requirements in assessing the competence of the laboratory.

In an industry where there are few standard methods, where one hears that you can "pay to play," and where

there are laboratories popping up run by staff with little experience in operating a testing laboratory, it is extremely important to have an experienced, independent, third party accrediting body such as A2LA evaluating the laboratory. An AB confirms a laboratory's adherence to appropriate quality management system standards and standard methods or their own internally developed methods when assessing a laboratory to ISO/EIC 17025. Through this assessment, the AB can verify that those methods produce valid results. Ultimately, the process of accreditation through a reliable AB gives the public confidence that a testing laboratory is competent, ensuring consumers have access to a quality product.

ISO/IEC 17025:2017 for Emerging Industries

ISO/IEC 17025:2017 takes a risk-based approach and allows for flexibility for laboratories in emerging industries. One of the biggest issues facing laboratories in new industries is inconsistent test results, and cannabis is not an exception to this. Several factors validate this reality, including the lack of standard methods, sampling plans that are not statistically valid, and a lack of accredited proficiency testing providers and reference material producers.

When an AB assesses a laboratory to ISO/IEC 172025, the assessor utilized will be a technical expert in the field that the laboratory is being accredited to. This means that an analytical chemist with experience with biological techniques will be assessing the ins-and-outs of the laboratory as a peer. The writers of ISO/IEC 17025:2017 took into account that not all industries are equal. This is why they created it with a risk-based mindset, allowing different laboratories to meet the intent of the standard in several different ways. Smaller laboratories in emerging industries are able to take an approach to meeting ISO/IEC 17025:2017 that may not work for larger, more established laboratories. There are several clauses that take into account areas that can vary widely from industry-to-industry. ISO/IEC 17025:2017 includes a section applicable to laboratories not able to use standard methods, as well as laboratories performing their own sampling. This allows for the assessor to take a deep dive into the method validation and sampling plans developed, ensuring that the laboratory is able to get consistent and reliable results. ISO/IEC 17025:2017 also includes several alternates to commercial proficiency testing and use of reference materials in emerging industries where these may not be available. The laboratory is able to ensure the validity of their results in the ways that best meet their industry needs, and it is up to the AB to determine if they are using the resources available to them when possible.

In addition to ISO/IEC 17025:2017, laboratories have the ability to be assessed to additional requirements by their AB. Some examples of programs that A2LA's cannabis laboratories have opted to be assessed to include an assessment to the American's for Safe Access (ASA) Requirements adopted from the American Herbal Products Association or the AOAC Food Testing Requirements. The ASA program requirements incorporate key issues in the cannabis industry, including security, competency of personnel, and interactions with regulators. The AOAC program requirements offer additional requirements that can be beneficial when testing edibles, such as handling and confirming the validity of reagents and equipment, competency of staff, and quality control. Although ISO/IEC 17025:2017 is a great framework for cannabis laboratories, seeking accreditation to additional program requirements is one way to ensure that their customer base and the public has additional reassurance that their results are going to be consistent and reliable.

Conclusion

The countless possibilities for cannabis extract products will undoubtedly create difficulties for testing laboratories to achieve accurate and reliable results. Standardized testing methods combined with appropriate laboratory accreditation will be effective tools in meeting these challenges, and will help ensure the long term viability of regulated cannabis markets.

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Size Matters: Optimizing Cannabis Milling

Blake Grauerholz, Dr. Markus Roggen

ABSTRACT

Does Milling improve extraction or destroy the cannabis? We postulated that the surface area to volume ratio of cannabis would affect CO2 extraction efficiency and speed. Therefore, we wanted to investigate the changes to terpene and cannabinoid composition in both the raw plant material and extract as a function of particle size and shape. For this study cannabis flower was ground to varying particle sizes using a milling system, along with other, less sophisticated, milling methods to study the effects on our extraction method. This milling study is a further piece of the puzzle of our extraction optimization project. By testing the extract oil and spent material, an ideal balance of efficiency and terpene preservation, while preventing degradation to the starting material, can be achieved.

METHODOLOGY

To study the effect milling has on freshly dried cannabis, a homogeneous mixture of whole plant material was ground from 1mm<10mm using a Fritsch P19 milling system. A mixed particle size produced with a food processor, and an analysis of un-ground material was also studied. Additionaly, the RPM of the milling system was also varied in order to investigate the potential of thermal degradation caused by the increased rotor speed. Lab testing was performed on pre & post milled material. A selected range of particle sizes was used for CO2 extraction at normal production parameters to study the effect milling has on cannabis oil yield and composition in SFE.

Once a ideal particle size is selected for extraction, the packing density of the cannabis within the extractor vessel can be studied to further improve recovery of THC.

Every run of particle sizing and extract fractions were analyzed for total cannabinoid and terpene content.

RESULTS

Non-ground offers no precision and greatly reduces efficiencies in SFE.
Smaller particles give higher cannabinoid and terpene yields.
Milling has little affect on terpenes and molecular makeup of cannabis.

DISCUSSION/CONCLUSION

- Insignificant effect on decarboxylation at higher RPM or smaller particle sizing
- Recovery of both terpenes and cannabinoids is increased with decreased particle size (Fig. 1-2)
- Finer, more uniform, particle sizing provides better precision in extraction
- The cannabinoid fraction is of higher quality with smaller particle size (Fig. 6)
- The terpene fraction appears to improve in quality with larger or irregular (Blender) particle size.
- Further optimization on CO2 extraction effeciencies can be achieved by milling.
- Packing density has an effect on total recovery, gravity fed being best for our machine (Fig. 3)

Fig. 1 Cannabinoid Recovery by Size

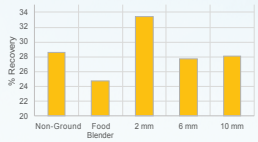


Fig. 2 Terpene Recovery by Size

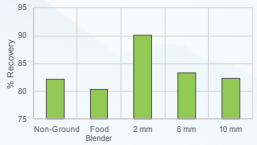


Fig. 3

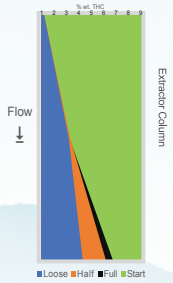


Fig. 4 % wt. Terpenes by Size

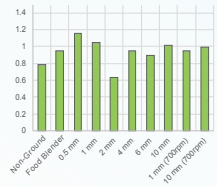


Fig. 5 Terpene Concentration F1 (Food Blender*)

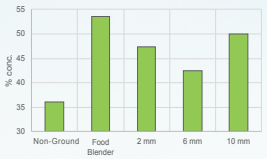
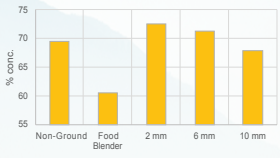


Fig. 6 Cannabinoid Concentration F2 (2 mm*)



INTRODUCTION

A) Particle Study (200rpm):
1 Food Blender
2 0.5 mm
3 1 mm
4 2 mm
5 4 mm
6 6 mm
7 10 mm
8 1 mm (700rpm)
9 10 mm (700rpm)
10 non-ground (baseline)

B) Extraction Study (3kg)(580rpm):
1 Food Blender
2 2 mm
3 6 mm
4 10 mm
5 non-ground (baseline)

C) Column Behavior Study (4kg):
1 Gravity Feed
2 Light packing middle & top
3 Tightly pack entire column

Cannabinoid profile on post-extracted core samples taken from column:
Top (1)
Middle (2)
Bottom (2)

For more information, please visit: www.OutCo.com, & www.CBDVL.com

A Brief Review
of Derivatization Chemistries for the Analysis of Cannabinoids Using GC–MS

BY ANTHONY MACHERONE

Determination of cannabinoid content in cannabis and cannabinoid products derived from cannabis and hemp is regulated in every jurisdiction where medicinal or adult use recreational programs have been legalized. A primary purpose of this testing is to quantify the total amount of psychoactive Δ^9-tetrahydrocannabinol and other pertinent cannabinoids such as total cannabidiol, cannabinol, and cannabigerol content. High performance liquid chromatography with ultra-violet detection is the most common choice for the analysis. Gas-phase applications can also be used but the laboratory must determine if they will chemically modify (derivatize) the target cannabinoids or analyze them in their natural state. This choice affects sample preparation procedures and the analytical conditions of the gas chromatography (GC) system. This article focused on the use of GC–mass spectrometry (MS) for the analysis of derivatized cannabinoids in hempseed oil matrix. Sample preparation and synthetic conditions for silylation are discussed. Analytical results and observations of artifacts resulting from the derivatization processes and the analytical system are presented.

AT LEAST 70 known cannabinoids have been identified in *Cannabis* spp. (1,2). Nonetheless, state and country regulatory entities have focused on only a handful where medicinal or adult recreational use of cannabis or cannabinoid products has been legalized. These include Δ^9-tetrahydrocannabinolic acid (THCA), Δ^9-tetrahydrocannabinol (THC), cannabidiolic acid (CBDA), cannabidiol (CBD), cannabinol (CBN), and cannabigerol (CBG). The most common analytical procedure to identify and quantify cannabinoids in these products is high performance liquid chromatography (HPLC) with ultraviolet (UV) detectors. Typical HPLC-UV methods measure the cannabinoids listed above and 5–13 more that are commercially available as certified reference materials (CRM). Sample preparation for cannabis inflorescence is generally liquid extraction with methanol or ethanol followed by dilution. The sample preparation procedure for oils, concentrates, resins, and tinctures is simply dissolving the sample in methanol or ethanol which is again, followed by dilution. The purpose of dilution is two-fold: 1) contemporary cannabis, hemp, or cannabinoid products may contain 20–30% (wt./wt.) THCA, THC, CBDA, or CBD and dilution brings these concentrations into a µg/mL calibration range; and 2) dilution mitigates interferences from other endogenous chemicals, such as terpenes, that are commonly present in the 1–3% (wt./wt.) range.

Another common analytical methodology for the determination of cannabinoid content is gas chromatography–mass spectrometry (GC–MS). These analyses typically begin with a liquid extract of a sample that is injected into a hot inlet on the GC (commonly ≥250 °C). The liquid is volatilized, rapidly transferred through the inlet, and condensed onto the head of a chromatographic column. If this is performed properly, the condensed sample will form a tight band at the column head prior to traversing the column to perform the separation of the sample components (3). With respect to cannabinoid analyses using GC–MS there are caveats that should be addressed. This work reviews GC–MS methodologies, the problems that can be encountered in various procedures, discusses derivatization chemistries with a focus on silylation of common cannabinoids, presents results, and examines artifacts in the data.

The Purpose for Cannabinoid Derivatization

Cannabis spp. which includes hemp, do not directly synthesize THC, CBD, CBN, or other neutral cannabinoids (4). In the living plant, acid phytocannabinoids are biosynthesized. Post-harvest,

these acids naturally decarboxylate to their neutral analogues upon exposure to light and heat. Acid phytocannabinoid decarboxylation also occurs in the hot inlet of a GC and in liquid chromatography–mass spectrometry (LC–MS) with electrospray ionization (ESI) in the negative ionization mode (5). Furthermore, in the hot inlet of a GC, cannabinoid degradation products are formed. For example, under typical GC conditions about 70% of THCA converts to THC with the remaining 30% comprised of degradation products CBN and dihydrocannabinol (6).

To avoid cannabinoid decarboxylation and degradation in GC methodologies, chemical derivatization is used. Chemical derivatization is a common procedure in GC analyses and serves several purposes. Firstly, derivatization caps polar or reactive moieties in a target analyte which improves chromatography and repeatability. Secondly, derivatization increases the volatility of the analyte rendering it more amenable to chromatography at lower temperatures (7). Lastly, derivatization chemistries can be used to functionalize the analyte and make it more amenable to GC–MS using other ionization mechanisms such as negative chemical ionization (NCI). For example, estrogens and androgens were derivatized with pentafluorobenzoyl chloride and pentafluorobenzyl hydroxylamine for analysis with GC–tandem mass spectrometry (GC–MS/MS) using NCI. The formation of pentafluorobenzoyl esters and pentafluorobenzyl oximes afforded sub-pg/mL limits of quantitation (LOQ) for these analytes in serum (8–11). Cannabinoids have also been functionalized for analysis by GC–MS using NCI, however, the derivatizing reagents and synthetic conditions must be carefully chosen to avoid undesired side-reactions and artifacts. The use of trifluoracetic acid anhydride (TFAA) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) for the derivatization of THC and CBD was shown to convert CBD to THC and Δ^8-THC (9). This phenomenon was also reported when derivatizing with pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH) (10). The conversion of CBD to THC has been determined to occur through an acid catalyzed stable carbocation intermediate (11). It is therefore suggested that acidic reagents or reagents that become acidic upon storage like dichloromethane (DCM) are avoided when extracting and derivatizing cannabis and cannabinoid products.

Figure 1: tPSA for common cannabinoid fragment moieties ranging from nonpolar on the left to moderately polar on the right.

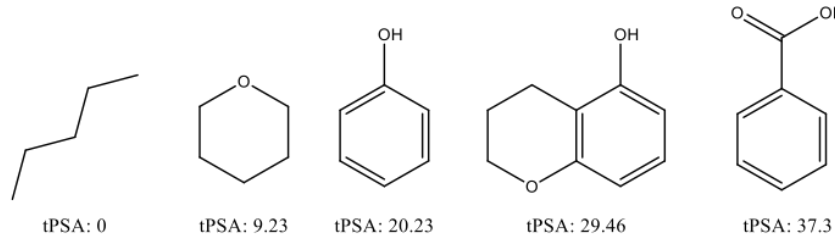
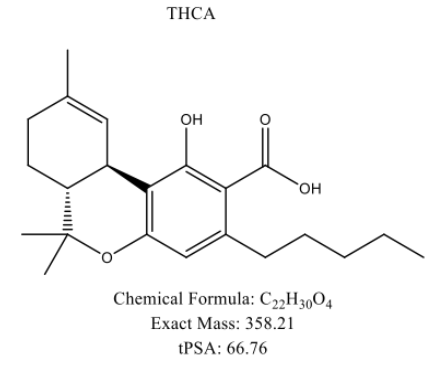


Figure 2: The tPSA of THCA is the sum of the fragment moieties.



Polarity

Functional moieties such as hydroxyls (–OH), carboxyls (–COOH), and amines ($–NH_2$) are polar and reactive. Their presence in a molecule adversely effects chromatographic peak shape on nonpolar or low-polarity column stationary phases. A common measure of polarity is total polar surface area (tPSA) and is measured in Å^2. Total polar surface area is the surface area of oxygen and nitrogen atoms and their attached hydrogen atoms in a molecule and is an indicator of hydrogen bonding capacity (12). Cannabinoids typically analyzed in testing laboratories are comprised of carbon, hydrogen, and oxygen and the functional moieties include alkanes, tetrahydropyrans, phenols, resorcinols, chromenes, and carboxylic acids. **Figure 1** illustrates common fragments in cannabinoid molecules and their tPSA. **Figure 2** is the chemical structure of THCA and some physicochemical properties including tPSA. The sum of the chromen-5-ol and benzoic acid tPSA equals the tPSA of THCA. This text will discuss how these are affected by derivatization.

Volatility

There is an observable and proportional relationship between boiling point and chromatographic elution times and an inverse relationship between boiling point and vapor pressure or volatility. In general, cannabinoids are high boilers, less volatile, and retained on a GC column until the oven reaches temperatures well over 200 °C.

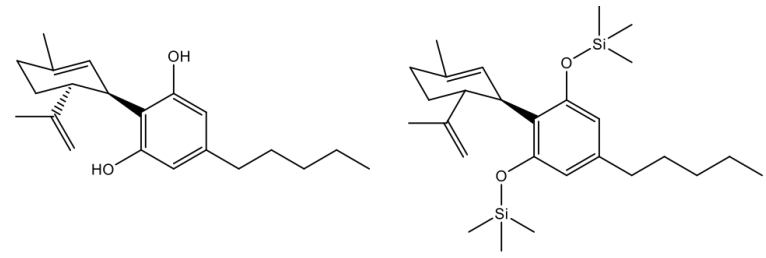
The polarity of a molecule also affects its volatility because hydroxyls (–OH), carboxyls (–COOH), and amines ($–NH_2$) are not sufficiently volatile. Derivatization addresses these issues by improving molecular volatility and decreasing hydrogen bonding.

Example: Derivatized Versus Underivatized Cannabinoids

A data-driven, untargeted analysis of six CBD oil pet supplements was performed using a GC-quadrupole time-of-flight (GC-QTOF) instrument (13). The samples were analyzed without derivatization on a moderately polar DB-35MS UI column (35%-phenyl-methylpolysiloxane). Under the experimental conditions, the retention time for underivatized CBD was 13.47 min eluting at an oven temperature of approximately 295 °C. In another experiment, cannabinoids, including CBD, were derivatized with N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) capping both –OH moieties with trimethylsilyl groups and analyzed on a HP-5MS UI column (14). In that work, the retention time of CBD was 12.55 min eluting at an oven temperature of approximately 295 °C.

In addition to the fact that in the underivatized form, cannabinoids should be analyzed with more polar columns, there are several points to note in this example. First, the polarity of CBD is reduced by derivatization making the molecule more volatile. Second, derivatized CBD has a calculated boiling point approximately 55 °C higher than underivatized CBD. And, finally, derivatized CBD elutes at an oven temperature equal to its underivatized counterpart yet its retention time was approximately 0.92 min earlier. This latter point is most interesting because the reduction in polarity of the derivatized CBD is expected to result in a longer retention time on the low polarity HP-5MS UI column (5%-phenyl-methylpolysiloxane) due to improved interaction with the stationary phase. However, this was not observed experimentally. **Figure 3** illustrates CBD, its derivatized counterpart, and physicochemical properties for each including tPSA and boiling point in Kelvin.

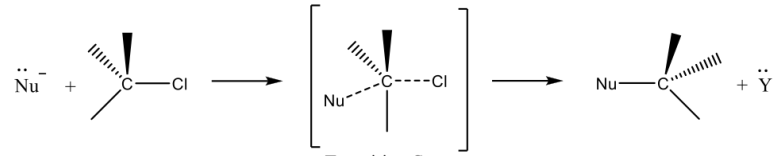
Figure 3: CBD and bis-trimethylsilyl CBD. The boiling point increases after derivatization but tPSA is reduced by more than 50%



Cannabidiol
Chemical Formula: $C_{21}H_{30}O_2$
Exact Mass: 314.22
Boiling Point: 888.56 [K]
tPSA: 40.46

bis-trimethylsilyl Cannabidiol
Chemical Formula: $C_{27}H_{46}O_2Si_2$
Exact Mass: 458.30
Boiling Point: 942.3 [K]
tPSA: 18.46

Figure 4: . Generic S_N2 reaction. Note complete stereochemical inversion (18).



Silylation of Cannabinoids

The mechanism of silylation is S_N2 (bimolecular nucleophilic substitution) alkylation in which a nucleophile attacks the substrate opposite to the leaving group. This is shown for a generic reaction in **Figure 4**. Cannabinoids commonly analyzed in testing laboratories contain alcohols, phenols, resorcinols, and carboxylic acids. These react with silylation reagents to form chemical derivatives.

Sample Preparation

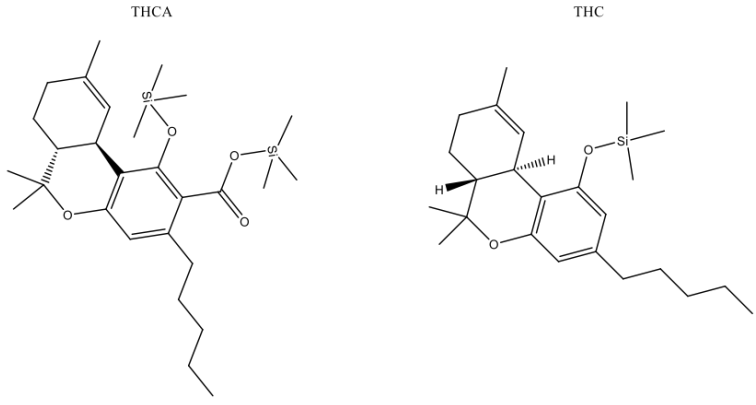
Cannabis inflorescence and hemp plant material are commonly extracted with solvents such as methanol and ethanol then further diluted prior to analysis. It is important to use high-purity commercial solvents and filter the extracts to remove particulate matter that may foul the analytical system. Solvents such as methanol and ethanol are protic, polar, and hydrophilic. S_N2 derivatization chemistries are negatively impacted by these factors. Polar, protic solvents hydrogen bond with nucleophiles and slow reaction rates and the presence of water can hydrolyze silylation reagents leading to incomplete derivatization or no reaction at all (15). The presence of water can also hydrolyze the derivative. Therefore, if derivatization will be performed, the extract will have to be dried down to remove problematic solvents and water prior to synthesis. Proper sol-

vents for silylation are polar and aprotic, for example, ethyl acetate, acetonitrile, pyridine, or N, N-dimethylformamide (DMF) but care should be taken to ensure that these reagents are dry.

As an example of sample preparation and derivatization, consider an accurately weighed 0.2 g homogeneous sample of cannabis inflorescence (16). To this, 20 mL high-purity methanol (P/N 5190-6896, Agilent Technologies) was added. The suspension was vortexed or shaken for 10 min to extract the cannabinoids, then centrifuged at 5000 rpm for 5 min. A 1.0 mL aliquot of the supernatant was filtered into a new vial with a 0.45 µm regenerated cellulose syringe filter (P/N 5190-5107, Agilent Technologies). To 10 µL of the filtered extract, 190 µL of high-purity methanol was added. The solution was briefly vortexed to mix and heated gently under nitrogen to dry. To the vial, 200 µL 10% (v/v) MSTFA in ethyl acetate was added. The vial was capped and heated at 60 °C for 30 min to derivatize. After cooling, the analysis was made directly from the vial. The chemical structures of silylated THCA and THC are shown in **Figure 5**.

Oils, resins, and concentrates pose another problem. Although these sample types are commonly dissolved in solvents such as methanol and ethanol and diluted before analysis, drying these solvents down prior to derivatization results in the matrix being reconstituted in the sample container. In this case, polar, aprotic solvents that are miscible with the matrix should be used. The sample should be dissolved and diluted to the appropriate factor for analysis. Then, an aliquot of the sample is taken for derivatization. For example, a nominal 0.5 mL aliquot of a CBD oil was added to a 50 mL Class A volumetric flask. The weight of the sample was accurately determined to be 0.45 g. The flask was brought to volume with high-purity ethyl acetate and was not diluted further. A 1.0 mL aliquot of the solution was filtered into a new vial with a 0.45 µm regenerated cellulose syringe filter. A 200 µL aliquot of the filtered solution was transferred to a fresh auto-sampler vial and 200 µL 10% (v/v) MSTFA in ethyl acetate was added (this resulted in a 2-fold dilution factor). The vial was capped, vortexed briefly to mix, and heated at 60 °C for 30 min to derivatize. Upon analysis of the derivatized sample, the CBD content was determined from linear regression of a known calibration curve to be 58.0 µg/mL. To determine the percent by weight of CBD in the sample, **Equation 1** was used.

Figure 5: Derivatized THCA (left) and THC (right) after reaction with MSTFA.



Equation 1. $$\%\ (^{wt.}/_{wt.}) = \left(\frac{concentration\ (\mu g/mL)\ *V\ (mL)*DF}{m\ (g)}\right)\left(\frac{g}{1\times10^{6}\mu g}\right)*100$$

where, Concentration = concentration of analyte from linear regression analysis, V is volume of solvent, DF is the dilution factor (unitless), and m is the mass of sample.

Subsituting from above

$$\%\ (^{wt.}/_{wt.}) = \left(\frac{58.0\mu g/mL)\ *50mL*2}{0.45\ g}\right)\left(\frac{g}{1\times10^{6}\mu g}\right)*100 = 1.29\ \%\ (^{wt.}/_{wt.})$$

GC–MS Spectra of Derivatized Cannabinoids

Organic hempseed oil was purchased from an online retailer. The material was screened using GC–MS in electron ionization (EI) mode and determined to be free of cannabinoids, terpenes, and residual solvents. Δ^8-THC, Δ^9-THC, THCA, CBD, and CBDA were spiked into the matrix and the matrix was dissolved in ethyl acetate and derivatized as described above. The derivatized samples were analyzed on an Agilent Intuvo 9000 GC – 5977B GC/MS system in EI mode using two low-polarity HP-5MS UI columns connected with a mid-column backflush flow chip. The oven was held at 70 °C for 1 min then ramped at 20 °C/min to 300 °C and held for 4 min. The MS was operated in scan mode over the mass range of 35 *m/z* to 600 *m/z*. The retention times for the compounds are given in **Table I**.

Table I: Cannabinoid retention times.

Cannabinoid	Retention Time (min)
CBD	12.545
Δ^8-THC	13.021
Δ^9-THC	13.090
CBDA	13.803
THCA	14.597

The extremely narrow peak widths of approximated 0.04 min were enough to chromatographically resolve Δ^8-THC and Δ^9-THC with baseline resolution. This is shown in **Figure 6**. The mass spectra for each are shown in **Figure 7**. The molecular ion at 386.2 *m/z* is observed for both isomers but the ion ratios of the fragments differ. Also observed is the M-15 ion (371.2 *m/z*), which is very common with trimethylsilyl (TMS) derivatives. Other common ions for TMS derivatives include 73 *m/z* (trimethylsilane) and 147 *m/z* (hexamethyl disiloxane).

GC–MS Artifacts

In this work, derivatization of cannabinoids with MSTFA as the silylation reagent yielded the desired products with no observation of partially derivatized compounds. The success of the derivatization is closely related to the synthetic conditions. The use of 10% silylation reagent in a GC "friendly" solvent such as ethyl acetate worked very well. Ethyl acetate is a hydrophobic, moderately polar, aprotic solvent commonly used in GC-based analyses. In conjunction with the derivatizing reagent, dilute solutions (0.5–1.0% v/v) of pyridine in ethyl acetate have been employed to both activate acidic protons and scavenge leaving groups like halogen ions. Other choices for silylation reagents would be N-O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) which is more reactive than MSTFA, especially if catalyzed with trimethylchlorosilane (TMCS). The same conditions used in this work with MSTFA can be employed with BSTFA.

It is important to use fresh solvents and to ensure that they are dry. Solvents to avoid include acetone, methanol, and ethanol. Dichloromethane (DCM) is often used in gas-phase analyses. It is hydrophobic, heavier than water with a polarity slightly higher than ethyl acetate. However, as DCM ages it becomes acidic as shown in **Equation 2**.

Equation 2: $CH_2Cl_2 + H_20 \rightarrow CH_20 + 2HCl$

As noted above, the presence of mineral acids in solvents can cause CBD

Figure 6: Overlaid TIC chromatograms for derivatized cannabinoids. Δ^8-THC elutes approximately 0.07 min earlier (blue). Red is Δ^9-THC at 13.090 min

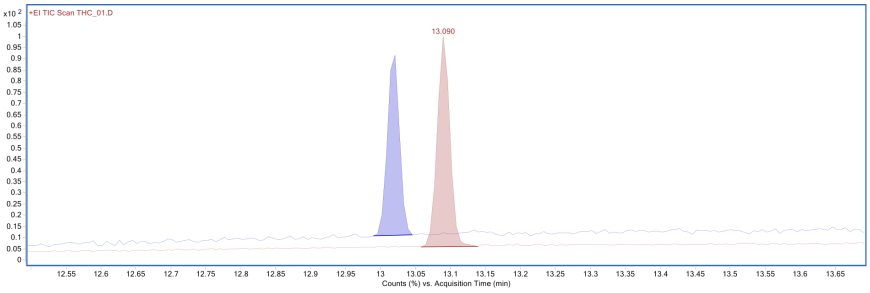
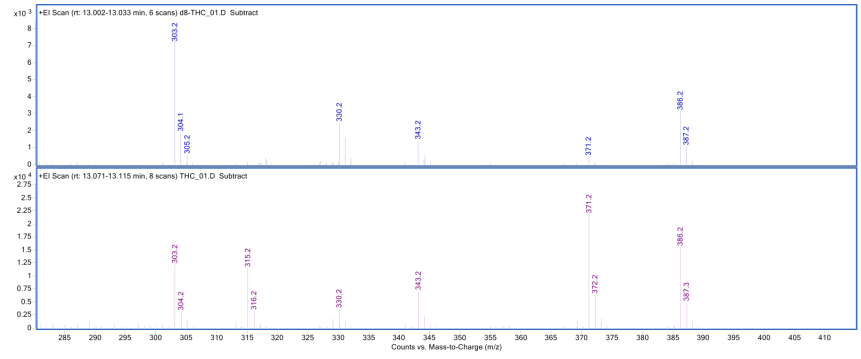


Figure 7: Mass spectra of Δ^8-THC (top) and Δ^9-THC (bottom). Similar ions are observed but the ion ratios differ.



(and CBDA) to transform into THC (and THCA), respectively. Furthermore, DCM degrades over time. Therefore, stabilizers like amylene (2-methyl-2-butene) are added to DCM to scavenge free radicals created by degradation processes. These additives produce by-products (artifacts) that can be observed in the analysis and may interfere with the results (17).

Silylation of carboxylic acids with MSTFA generally yields the desired derivative with few artifacts, but hindered phenols can yield mixtures of derivatized and underivatized products if not heated long enough or in the absence of a catalyst (18). However, small artifacts are commonly observed in the mass spectra. Some of the artifacts are derived from the derivatizing reagent itself and others from the system or from handling system components such as septa or liners with bare hands. The chemical structure of MSTFA is given in **Figure 8**. **Figure 9** shows the mass spectra of minor artifacts observed in the analysis.

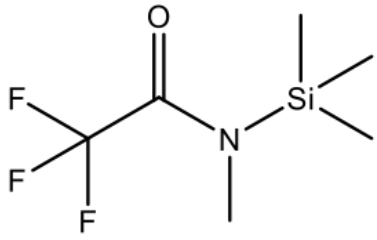
Bis-trimethylsilyl trifluoroacetamide, octamethyl trisiloxane, methylamine, and hexamethyl disiloxane are by-products of MSTFA. Other common artifacts include the observation of 207 *m/z*, and 281 *m/z* (hexamethyl-cyclotrisiloxane and octamethyl-cyclotetrasiloxane, respectively) which are common column bleed and septum bleed ions. The latter often manifests as discreet, evenly spaced peaks in the chromatogram and the former manifests as a rising baseline as the oven temperature is increased. Trimethylsilyl palmitic acid and trimethylsilyl steric acid were also observed in this analysis. Their presence was most likely because of handling the septum without gloves or possibly from the hempseed oil matrix.

Conclusions

At the federal level in the United States, marijuana is still a Schedule I controlled substance and legalization occurs at the state level only. In Canada, medicinal and adult use recreational cannabis programs are legal and regulated by the federal government. Since the passage of the Farm Bill in the US, hemp has been legalized as an industrial crop and the United States Department of Agriculture (USDA) has been tasked with its regulation. As of June 2020, the USDA has only designated potency testing of hemp and require the analysis to be performed by HPLC or GC methodologies. Cannabis is differentiated from hemp based on the total psychoactive THC content with any material greater than 0.3 % (wt./wt.) being identified as marijuana (cannabis). At least one US state has stipulated potency testing to be performed by GC–MS. Elsewhere, it is at the laboratory's discretion.

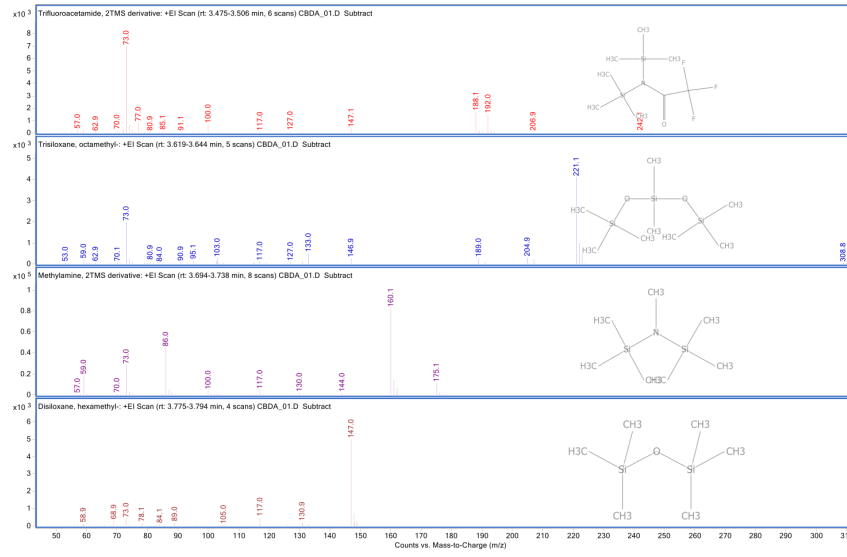
The analysis of cannabinoids in the myriad sample types available in the cannabis industry is of critical importance. From a safety point of view, THC or CBD content described on a product label must be quantitatively accurate. Where GC–MS is chosen as the analytical system, the laboratory must determine if they will perform the analysis with or without derivatization. This choice in turn affects the sample preparation procedures and the choice of analytical column stationary phase and conditions. If the laboratory choses to derivatize the cannabinoids, proper synthetic conditions need to be empirically determined to maximize derivatization efficiency and minimize

Figure 8: Chemical structure of MSTFA.



artifacts that may interfere with the analysis. This work discussed the purpose and outcomes of derivatization, the synthetic procedures for silylation of cannabinoids including suggested solvents and ones to avoid, a proper column choice for derivatized cannabinoids, analytical conditions for the analysis, and examined the results and artifacts that may be observed.

Figure 9: Mass spectra of common GC–MS artifacts observed with silylation reagents. From top to bottom: bis-trimethylsilyl trifluoroacetamide, octamethyl trisiloxane, methylamine, and hexamethyl disiloxane.



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The author does not advocate the use of marijuana or cannabinoid products.

Separation of Eight Cannabinoids

With the recent legalization of both medicinal and recreational marijuana in the United States, analysis of individual cannabinoids has captured the public's interest at a new level. As such, many new cannabis products are now available, i.e., edibles, vaporizers, and extracts to name a few. The increased marketability of the product has incited consumers to take a greater interest in the quality and craft ability of the products being sold. Through the quantification of individual cannabinoids, the consumer can make an informed decision about the possible effects they could expect from the products they purchase. Therefore, the need for accurate, robust, and affordable analysis tools are of the upmost importance.

With health, safety, and edibles dosing as the primary motivation, Hamilton Company developed an HPLC method that isolates eight major cannabinoids. The HxSil C18 (3 µm) column provides an accurate, cost effective, and robust solution that can be used in any HPLC system.

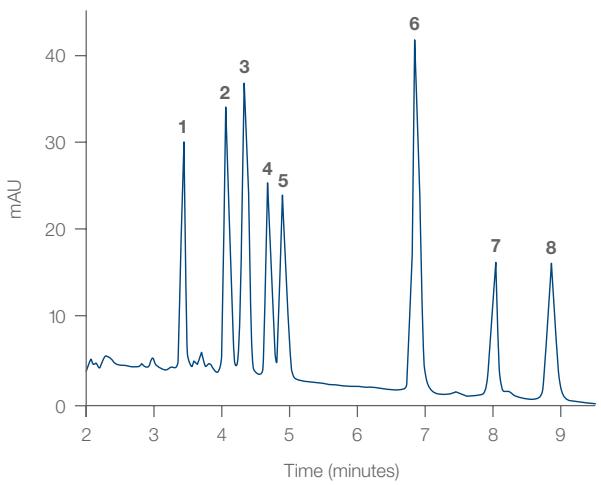
Column Information

Packing Material	HxSil, 3 µm
Part Number	79641

Chromatographic Conditions

Gradient	0–10 min, 78–92% B 10–15 min, 78% B
Temperature	Ambient
Injection Volume	5 µL
Detection	UV at 230
Dimensions	150 x 4.6 mm
Eluent A	20 mM NH_4COOH pH 3.5
Eluent B	Acetonitrile
Flow Rate	1.0 mL/min

Separation of Eight Cannabinoids



Compounds:

1: Cannabidivarin (CBDV)
2: Cannabidiol (CBD)
3: Cannabidiolic Acid (CBDA)
4: Cannabigerolic Acid (CBGA)
5: Cannabigerol (CBG)
6: Cannabinol (CBN)
7: Δ-9-Tetrahydrocannabinol (Δ-9-THC)
8: Δ-9-Tetrahydrocannabinolic Acid (Δ-9-THCA)

Author: *Adam L. Moore, PhD, Hamilton Company*

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Invasion of the Drones:

How Unmanned Aerial Vehicles Can Help Cannabis Growers Improve Crop Quality, Lower Costs, and Save the Planet

BY RYAN DOUGLAS

Drones will soon become an integral part of commercial cannabis cultivation. From open-air farms to indoor grow ops, drones can help cultivators operate a more efficient business, produce healthier plants, reduce their carbon footprint, and decrease their overall cost of production. Although drone use is not yet commonplace among cannabis growers, this is certain to change as cultivators learn more about the benefits of incorporating this technology into their commercial operations.

photo provided by Kevin Hill Parabug

A **DRONE IS** an unpiloted aircraft. Officially referred to as an unmanned aerial vehicle (UAVs), a drone is essentially a flying robot that can be controlled remotely or flown autonomously. Originally developed for military and aerospace applications, drones are becoming increasingly popular among hobbyists and photographers alike. In agriculture, they are playing a more integral role in the crop production process, helping farmers to gather data and perform tasks more quickly and more consistently than ever before.

This article explores the benefits of integrating drones into commercial cannabis operations and helps the reader decide which technology is appropriate for their business.

Indoor

Researchers are developing innovative applications for the use of drones in indoor vertical grow environments. Indoor vertical farms typically have high ceilings and multiple levels of production that can present scouting challenges for the cultivation team. Plant inspections allow growers to catch plant problems before they get out of hand, and in vertical grow operations this involves pushing around rolling ladders and spending most of the work day climbing up and down them. Accessing each level of plant production can be difficult and time-consuming, and this process is neither consistent or efficient.

Enter the drones. In Denmark, a partnership between Arhus University Engineering College and Nextfood, a technology company that develops vertical farming systems, has resulted in a prototype Indoor Image Acquisition Drone, or IIAD, to help growers monitor plant health and prevent crop damage in vertical growing situations. Made with a compact frame for maneuvering in tight spaces, the IIAD uses three onboard cameras that allow it to navigate inside of a warehouse without relying on global positioning systems (GPS) or indoor positioning systems (IPS). One downward facing camera is used for following lines that lead the drone to each growing station, while a front facing camera is used for positioning, imaging, and reading QR codes. Using ultrasonic sensors, the drone is designed to avoid obstacles along its route, and can fly between different levels of plant production with ease. The IIAD can recognize individual plants by reading their QR codes and through subsequent image analysis can provide insight into plant maturity, nutrient deficiencies, and potential disease problems.

Greenhouse

Cannabis growers can benefit from incorporating drone technology in their greenhouse operations to help control flying insects like moths. Although moths don't eat cannabis plants, their offspring do. Given their light green color, many caterpillars seamlessly blend into the crop and can feed unnoticed until severe damage becomes visible to the naked eye. In addition to damage from feeding, caterpillar feces can render cannabis flowers unsaleable. Until now, growers could only screen moths from entering their facilities, since most pesticides used for controlling caterpillars are prohibited for use on cannabis crops.

PATS Indoor Drone Solutions in the Netherlands is developing a drone that eradicates flying pests without the use of manual labor or pesticides. Their solution involves the use of base stations mounted throughout the greenhouse that continuously scan the cultivation environment for harmful flying insects. Once an insect is detected, the base station launches a mini-drone to seek and destroy the flying insect by sucking the insect into their propellers. Once the insect is eliminated, the drone returns to the base station.

Although still in development, the use of these mini drones has the potential to provide growers a completely autonomous pest control solution while helping to eliminate insecticide use and the labor required to apply them. "Making a plan, scouting, and doing treatments is very laborious," says Bram Tijmons, CEO and cofounder of PATS Indoor Drone Solutions. "We want to be better than the alternatives that exist right now, and our technology is a better fit with integrated pest management programs."

Farms

Outdoor cannabis cultivation holds the greatest number of potential uses for drones. This is because outdoor farms are usually several acres in size, with some hemp plantations exceeding 100 acres. Drones allow farmers to decrease money spent on manual field labor, while increasing the consistency of the task performed. This results in a lower cost of production, while increasing both the volume and quality of plants cultivated. Now let's discuss six of the most promising uses of drones for outdoor cultivators of cannabis: soil analysis, crop mapping and surveying, seed planting, irrigation management, flowering cycle interruption, and pest and disease management.

Soil Analysis

Drones can help farmers analyze their land prior to planting large crops. By equipping drones with specialized sensors and cameras, farmers can accurately assess the quality of a field prior to planting. Armed with data compressed into an easy-to-read format, a farmer can choose to avoid planting in consistently wet areas of a farm or where the soil is of poor quality. If the farmer choses to amend the soil to make it more conducive for cultivation, they only need to amend the affected areas identified by the drone's analysis, instead of broadly amending the entire field.

Crop Mapping and Surveying

Once a crop is growing in the field, these same sensors and cameras can provide farmers with valuable data that is critical to maintaining plant health. Nutrient deficient plants or infestations from damaging insects will appear different than the rest of the crop. The cultivator can use this information to apply fertilizer in select areas of the farm or spot spray pesticides to affected areas. This saves the time and money from broadly applying these materials to the entire crop when it is not necessary. Drones can also be used to inventory the crop by plant count and even help to predict yields prior to harvest.

Seed Planting

When cannabis is seeded directly into the ground, not every seed germinates. This happens when seeds are of poor quality, the environmental conditions are not conducive to germination, or they get eaten by animals. Drones can help identify "dead spots" in the field shortly after the crop begins to emerge and then be dispatched to re-seed these areas. Some drones can handle a payload in excess of 20 lbs, so they can also carry water to irrigate the seeds once they have been dropped.

Irrigation Management

Armed with thermal and infrared cameras, drones can help farmers use less water to grow their crops. Overhead views of a field can help identify leaks and malfunctioning irrigation equipment, as well as dry areas or chronically waterlogged soil. Identifying and fixing these problems early can help improve plant health and prevent water waste.

Flowering Cycle Interruption

Cannabis crops are *photoperiodic*, meaning they initiate flowering once they are exposed to long, uninterrupted periods of darkness. This can present a problem for growers that seed directly into the ground, or transplant rooted clones outdoors when nighttime is still longer than daylength. Seedlings can begin flowering shortly after emerging from the ground, and rooted clones can begin flowering just after being transplanted. This can result in extremely short plants and an accompanying small yield. To prevent premature flower onset, outdoor growers must break up long nights by using interruption lighting, which can provide a logistical problem since most outdoor crops are several acres in size.

To solve this problem, drones carrying lights can be programmed to fly over crops at night, very slowly and just above the plant canopy. By making multiple passes, or by using multiple drones, enough light can fall on the crop to prevent it from prematurely going into flower. The cost of renting and operating the drones would be far less than the cost of mounting a comprehensive lighting infrastructure over several acres of farmland.

Drones equipped with specialty cameras and sensors can be used to gather the following data:

- *3D mapping*
- *Canopy and below canopy analysis*
- *Crop yield predictions*
- *Field uniformity*
- *Invasive species detection*
- *Irrigation problems*
- *Plant counts*
- *Plant height*
- *Soil conditions*
- *Soil moisture levels*
- *Soil temperatures*
- *Topographical analysis*
- *Weed and disease detection*

Pest and Disease Management

Pesticide and fungicide applications are much more consistent and precise using drones. Until now, crop dusting airplanes, helicopters, and tractors have been the go-to method for spraying large fields. However, these methods are not ideal, since coverage can be sporadic and subject to prevailing winds. For mountainous crops, it can be difficult for airplane pilots to maintain a consistent flight height, and tractors aren't much help over steep terrain.

In contrast, sensors on drones can automatically adjust height according to the terrain, ensuring spray accuracy and even application rates in the face of wind gusts and changing flight speeds. Drones can also fly much closer to the crop, ensuring consistent coverage across the field. For cultivators that battle damaging pests with beneficial insects, drones can be programmed to evenly distribute these insects over tall crops and challenging terrain that would otherwise be difficult for employees to access. Drone application of biologicals can result in reduced labor, faster applications, and more consistent distribution over large areas.

Laws and Permits

Operating a drone for commercial purposes in the United States requires certification by the Federal Aviation Administration (FAA). To receive a commercial license, drone pilots must go through much of the same training as a pilot seeking certification to fly a small airplane. This is because to the FAA, a drone operated for commercial purposes is an aircraft. If the drone is not used for hobby purposes, and it weighs more than .55 lbs, the operator must receive a Remote Pilot Certificate from the FAA.

Certification requires that the operator pass an initial aeronautical test, be at least 16 years old, and be able to read, speak, and write English, as well

photo provided by PATS Indoor Drone Solutions - Drone Gerbera

as pass a background security check by the Transportation Security Administration. Once licensed, the operator is given a unique registration number that must be visibly and legibly displayed on their aircraft, and they must pass a recurrent aeronautical knowledge test every 24 months.

The FAA has very specific rules that must be followed to fly a commercial drone in the national airspace. Such rules include maintaining minimum distances from buildings and clouds, and the operator must maintain a visual line of sight of their drone at all times. Commercial drones can only be flown during daylight hours, and during autonomous operations where the remote pilot inputs a flight plan that results in automated flight, the operator must have the ability to take control at any point during that flight. Commercial drones must be less than 55 lbs, and pilots cannot operate more than one drone at a time. Maximum airspeed cannot exceed 100 miles per hour, and maximum height cannot exceed 400 feet.

For activities that fall outside of the above parameters, operators must obtain an independent waiver. Flying at night requires a special waiver, as does flying multiple drones at the same time, called a "swarm waiver." Operating a drone that weighs more than 55 lbs is a different waiver, as well as flying in certain airspaces, such as close to an airport.

Types of Drones

There are basically three types of drones that are used for agricultural purposes: surveyors, sprayers, and broadcast spreaders.

Drones used for surveying crops utilize cameras to collect data from a bird's eye view. These aircrafts don't need to haul much weight besides a camera, so they are typically smaller and lighter than other drones used for more heavy-duty jobs. Surveillance drones can be either fixed wing models that resemble small airplanes, or multirotor drones that use various propellers. Fixed wing drones are best for covering a lot of ground in a short time, and they are capable of covering up to 10 times the acreage as a multirotor drone. Multirotor drones are best for surveillance projects that require low, slow hovering action where accuracy and high resolution images are desired. Fixed wing drones can run $10,000 to $25,000 while multirotor drones are a bit more economical, around $2,000 to $10,000.

Sprayer drones are used for spot irrigation, application of liquid fertilizer, pesticide and fungicide applications, and sanitation. The largest sprayer drones can hold up to 20 L, roughly the same volume as a standard backpack sprayer. A sprayer drone can cover approximately 25 acres per hour, and with multiple drones a farmer could cover additional acreage in the same amount of time. Even compared to tractor sprayers, drones still come out on top, allowing the farmer to cover more space much faster. Sprayer drones with a tank capacity of 10 L start around $10,000.

Broadcast spreaders are used for seeding, disbursement of pelletized fertilizer, and the distribution of beneficial insects. Like sprayer drones, broadcast spreaders must hold several pounds of weight, so they are typically larger than drones used only for surveying. Spreaders can have up

Agricultural drone service providers in the United States

AGERpoint
https://www.agerpoint.com/

AgEagle
https://www.ageagle.com/

Ceres
https://www.ceresimaging.net/

Empire Unmanned
https://empireunmanned.com/

Farm Shots
http://farmshots.com/

Parabug
https://www.parabug.solutions/

Precision Hawk
https://www.precisionhawk.com/

Taranis
https://taranis.ag/

to a 16 L capacity with an adjustable spreading range of up to 30 m. Growers should expect to spend around $10,000 for a drone with a payload capacity of 15 lbs.

Value

Large farms may utilize multiple drones to accomplish tasks more quickly, and they may require more than one type of drone throughout the crop cycle. In addition to the purchase price, there are also maintenance costs and licensing fees. Although these costs can collectively push six figures, it's best to keep the purchase price in perspective. A new tractor can cost several hundred thousand dollars, and not achieve anywhere near the same efficiency of a drone. They also carry a much higher operating cost in terms of manual labor, fuel, and maintenance.

The value of a drone goes beyond just the monetary benefits and extends to a grower's environmental impact as well. Farmers should anticipate more restrictive environmental regulations surrounding the cultivation of cannabis in the future, and the use of drones can help commercial growers set the standard for environmental stewardship. Many drones are solar-powered, so there are no emissions over the field. In greenhouses, they can be an effective method of insect control without the use of pesticides, creating a more ecologically-friendly grow operation. Outdoors, drones can maintain a spray height of just 2 feet above the plant canopy, eliminating pesticide drift and the subsequent contamination of neighboring plants and wildlife. Compared to alternatives such as crop dusters, helicopters, tractors, or manual backpack sprayers, drones win every time.

How to Start

Cannabis cultivators interested in incorporating drones into their cultivation program should consider contracting a licensed commercial drone operator. This can be a safer and less costly introduction to drones for the grower. In addition to the upfront cost of the aircraft, there are also strict permitting requirements and a considerable learning curve to successfully operate the drone. Most growers don't have the time required to learn how to operate a drone, or the free time to troubleshoot every issue that arises during regular operation. It is less costly, less time consuming, and less risky to hire a commercial drone operator. After a few seasons, if the grower is satisfied with the results and potential return on investment (ROI) of owning a fleet of agricultural drones, the purchase is much less risky.

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photo provided by Kevin Hill Parabug

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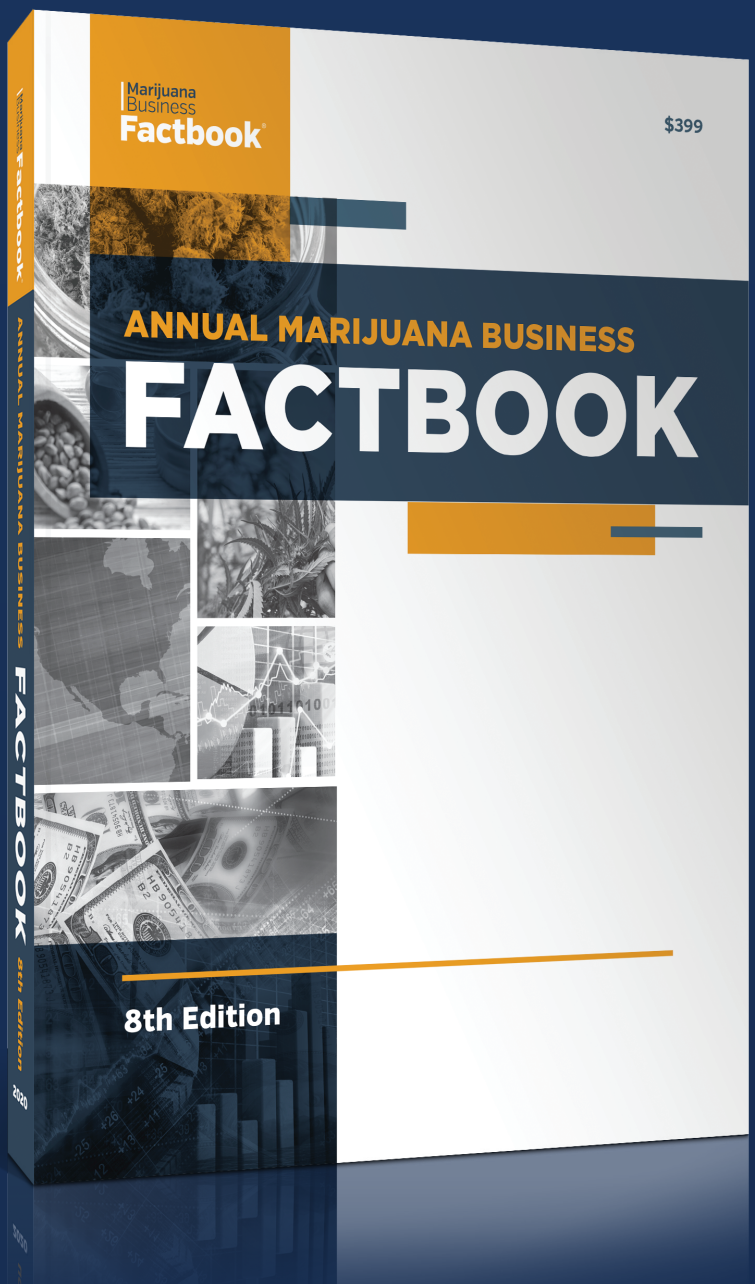

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Analytical Instrument Demand for Cannabis Laboratory Testing in North America

With the proliferation of cannabis products on the market and consumption in North America continuing to rise, the demand for analytical instruments for cannabis testing is expected to increase at a robust pace over the next few years, fueled by the growing concerns for safety. Laboratories are expanding their capabilities beyond potency testing for tetrahydrocannabinol (THC) and cannabidiol (CBD) content, to include the ability to test for pesticides and fungicides, solvent residues, heavy metals, microbes, foreign organic matter, and other substances of interest. The most common technologies used for these tests are high performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS), gas chromatography (GC) and GC–MS as well as inductively coupled plasma-mass spectrometry (ICP-MS).

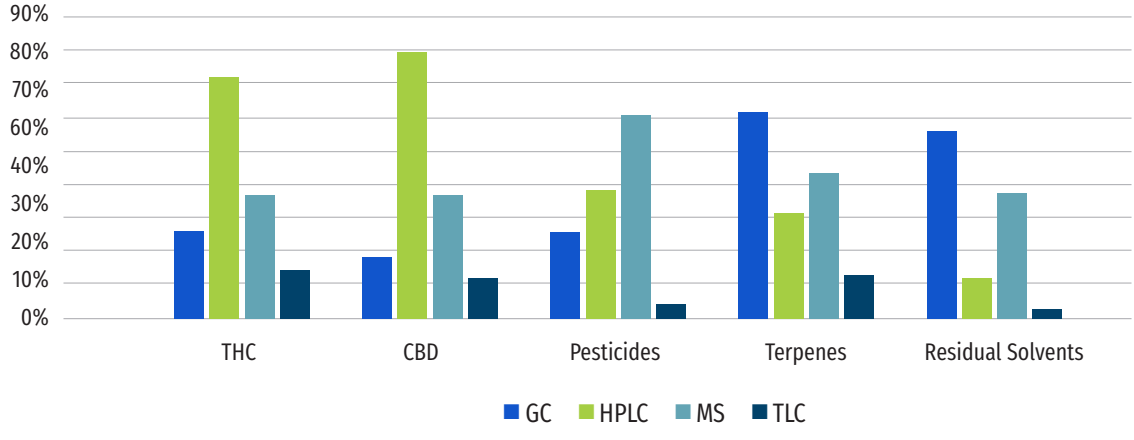
Tests for THC and CBD content are some of the most important in the cannabis testing market, particularly because the consumer uses these measurements to make purchase decisions on marijuana or hemp products. But consumers are becoming increasingly aware of the abundance of unsafe products available on the market and expecting assurances the items purchased (such as flowers, edibles, oils, concentrates, vapes) at their local dispensaries are safe and that the required tests have been performed properly.

GC is a key instrument in cannabis laboratories, but its use for certain applications has become less frequent. In a recent survey of cannabis laboratories, labs were asked which technologies they used to perform tests on cannabis products. For potency, the technology most laboratories used was HPLC. This is a significant shift from a few years ago, in which laboratories were mainly using a GC method. The availability of turnkey HPLC instruments has made it easy for less experienced chromatographers to adopt HPLC technology.

Laboratories are leveraging mass spectrometry technologies, including LC–MS, GC–MS, and ICP-MS, with the growing concern for safety and regulatory mandates. For pesticide testing, cannabis laboratories are using a combination of LC–MS and GC–MS methods, while for heavy metals, laboratories are using ICP-MS. Laboratories continue to use GC for measuring residual solvents and obtaining terpene profiles.

In addition to the survey, this article refers to data from the 2020 market report focused on the North American cannabis market for laboratory instrumentation from independent market research firm TDA. The report features industry benchmarks (such as market size and growth estimates) for cannabis testing laboratories in North America, including a survey of end users. For more information about this report, contact Glenn Cudiamat, president & CEO, at (310)-871-3768 or **glenn.cudiamat@tdaresearch.com** or visit **www.tdaresearch.com/cannabis**.

Figure 1: Survey results from the question: What technology (or technologies) are you using to test for THC, CBD, pesticides, terpenes, and residual solvents?



The Hidden Costs of Falling Films

BY NICK SHREWSBURY

Falling films are usually only a partial solvent recovery tool. They often carry hidden costs and time loss because they are designed to recover just 70–80% of the solvents from a tincture of full spectrum oil suspended in a solvent. This requires manufacturers to purchase additional rotary evaporators that significantly slow the solvent recovery process. An alternative option for extractors to evaluate are all-in-one industrial solvent recovery and decarboxylation systems.

The Facts About Falling Films

IF YOU HAVE been around the hemp or cannabis sectors, you have likely heard of falling films. They are widely considered to be one of the fastest solvent recovery systems on the market.

While they are a useful addition to the cannabis space, it is important to note that most falling films are not a one-stop-shop for solvent recovery. The vast majority can only evaporate 70–80% of solvents from a tincture, meaning extractors need to expend more capital on inefficient rotary evaporators that entirely erase the speed gain of a falling film.

How Do Falling Films Work?

Solvent recovery is one of the major bottlenecks in hemp and cannabis extraction processes. Whether manufacturers are using ethanol, CO_2, or hydrocarbons to extract, they often use solvents such as ethanol in the initial extraction, the winterization or dewaxing, or the chromatography remediation phases. Falling films are distillation machines that use evaporation and condensation to "recover" solvents and isolate desired products such as full spectrum oil.

Falling films operate on a basic principle: It is easier to evaporate a thin film of water than a large pool. Most falling films are large columns that contain multiple vertical heated tubes. Each tube channels a thin falling film of solution. In this way, solvents such as ethanol can be quickly vaporized while the remaining solution trickles down to be captured at the bottom of the machine.

Falling Film Challenges

Falling films work best with dilute solutions that are mostly ethanol. Once the majority (70–80%) of the solvents have been recovered, a tincture of full-spectrum oil and ethanol becomes viscous and does not move in a uniform film. At that stage, the small tubes within a falling film can become obstructed while the high temperatures burn the oil.

This kind of obstruction has to be avoided at all costs. Falling films are notoriously difficult and costly to repair

once the interior system has been compromised. For this reason, most falling films (except those that are hundreds of thousands of dollars) are only designed to reclaim 70–80% of solvents. They require extractors to make an additional purchase of a 20 L or 50 L rotary evaporator to "polish" off the residual ethanol.

Depending on the company you choose, that additional rotary evaporator will easily add $50,000 to $80,000 to your price tag (not including additional shipping, certification, and training costs). In addition, the high operating temperatures that falling films use tend to spoil terpenes, flavonoids, and full-spectrum oil.

Working with Real Numbers

Let's look at some numbers to understand the actual time loss involved.

If you are working with 100 gallons of tincture that is 90% ethanol and 10% cannabidiol (CBD) oil, you can expect a 25 gph falling film to recover 67.5 gallons of ethanol in 2.5 h. However, at that point you would be left with 22.5 gallons of concentrated tincture that needs to be loaded into a 50 L rotary evaporator.

A 50 L rotary evaporator can reach 4 gph for very dilute tincture solutions, but if you are working with concentrated tincture, you can expect to get half that rate, 2 gph. Your rate to evaporate 22.5 gallons would be at least 11 h of continuous operation, not including the time to load and empty the system.

The total run time for that 25 gph falling film and 50 L rotary evaporator was 13.5 h to evaporate 90 gallons of ethanol. In other words, it's really more of a 6.5 gph falling film.

That's not fast, especially considering the price you paid for two different systems and the work to load and empty the rotary evaporator every 2 h.

The All-In-One Solution

In conclusion, falling films are not fast solutions if they need to be paired with a rotary evaporator. They carry high hidden costs and labor expenditures.

This is a problem my company has been working hard to innovate. Our industrial systems are designed to replace all this equipment. Working with the same 100 gallons of tincture as above, our system (X9 200 liter EcoChyll) could evaporate all 90 gallons of ethanol in 6 h. That is more than double the speed of the 25 gph falling film and 50 L rotary evaporator.

In addition, our 200 L system could decarboxylate all 10 gallons of CBD oil immediately. It would take 2 h to decarboxylate. Normally, decarboxylation reactors that can handle 10 gallons of oil cost more than $60,000 and take 6 h to run.

All told, you could spend hundreds of thousands of dollars for a 25 gph falling film, a 50 L rotovap, and a 100 L decarboxylation reactor to process 100 gallons of tincture in 20 h (not including labor to load and empty systems). Or you could spend far less to buy one of our systems and do it all in 8 h. We are pretty proud of those numbers.

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