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Cannabis on Collision Course with Science

eXtraction Magazine

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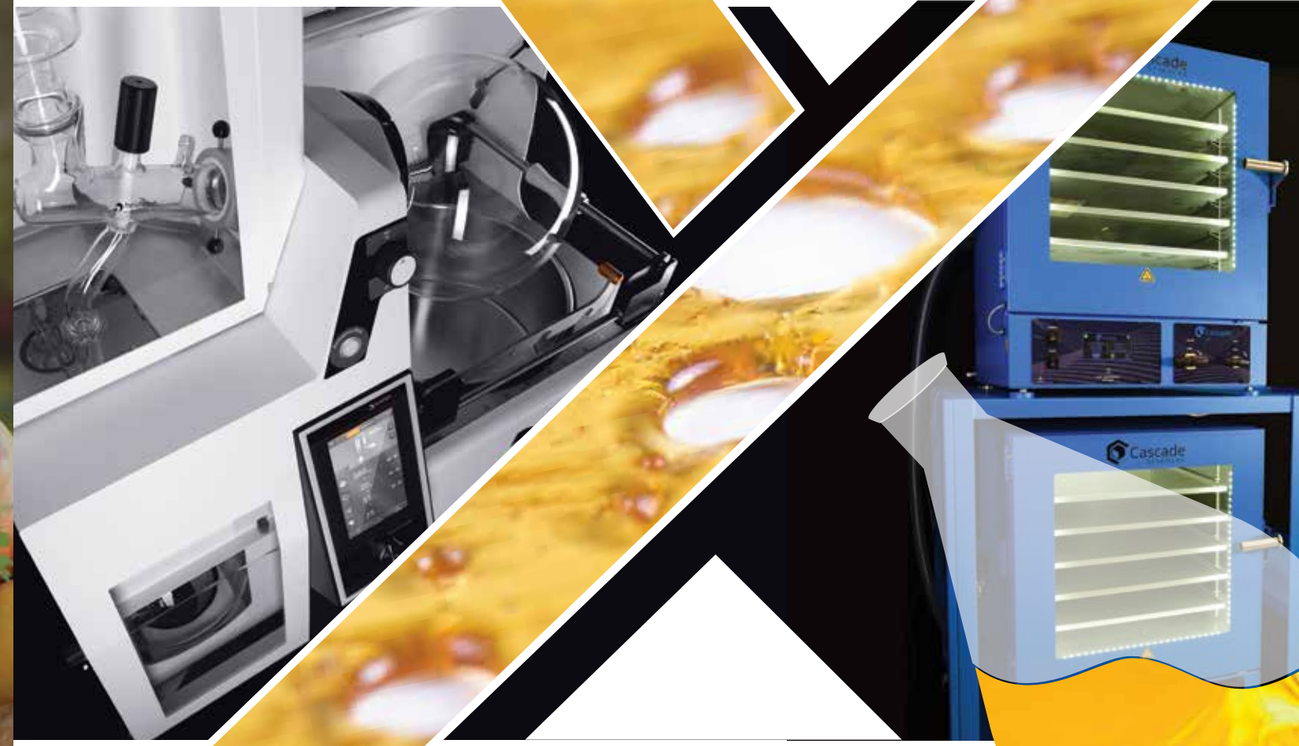

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Letter from the Editor

April 15th, 2019

If you experienced the T&T side of this issue first, you'll recall that I mentioned that this issue serves as a complement to our upcoming conference, Concentration 2019. Given the surge in concentrate sales, which are projected to reach somewhere near $8,500,000,000 by 2022, is there really any other topic in cannabis that warrants its own symposium?

One concept consistently mentioned when discussing cannabis concentration is that determining suitable extraction equipment first requires identifying what products you'll create. Oh, there's a macabre idiom that relates all of the different ways to perform tasks, but you likely don't need gory embellishments to grasp the notion of the myriad of products lining dispensary shelves. And you also likely don't need me to tell you that you're apt hear as many anecdotal opinions regarding what aspects *define* specific concentrates, extracts, and techniques as there are new cultivar names.

But who cares about all of the marketing names showered upon us for the same basic chemistry? As the recent paper published by Ulrich Reimann-Philipp and co-authors reported, 396 different cultivars reduced to 3 phytochemical classes, or chemovars, as distinguished by terpene content. (Reimann-Philipp, U. et al. "Cannabis Chemovar Nomenclature Misrepresents Chemical and Genetic Diversity; Survey of Variations in Chemical Profiles and Genetic Markers in Nevada Medical Cannabis Samples", Cannabis and Cannabinoid Research, 2019). To my knowledge, a study like this has yet to be done on concentrates, but what might the results reveal? That irrespective of chic names, concentrates, unless designed to mirror native plant chemistry, also cluster into a limited number of chemical classes?

What's integral for product manufacturers to reach triumph is having intimate knowledge on what *defines* the principles of cannabis extraction, method limitations and benefits, overhead costs, post-processing, compliance, and overall best practices for creating potentially award-winning products. What can an extraction method do, and perhaps, more importantly what *can't* it do? And what's vital to cannabis consumers when selecting a product is understanding how a product was made and who made it, discerning intrinsic health risks, such as ingestion of residual butane, and, of course, soliciting what molecules beyond THC and CBD make up the product's chemistry.

This issue of *EM* seeks to establish a point of reference on the fundamentals of cannabis/hemp extraction, an Extraction 101 of sorts, in our efforts to continue plucking treasure from chaff, given the notorious circulation of unsubstantiated information within the cannabis industry. And if we didn't cover an aspect of what you feel should be included in every extraction 101 discussion, shoot me an email, and we'll be sure to add it in next time around.

Until next time,
Cheers.

extraction magazine

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Cannabis Concentrates: The Origins

By Frenchy Cannoli

The origins of cannabis concentrates are lost to us; we only have various myths and legends from different producing countries to study, most of them quite recent, considering the long history of humanity and the cannabis plant, and none reliable.

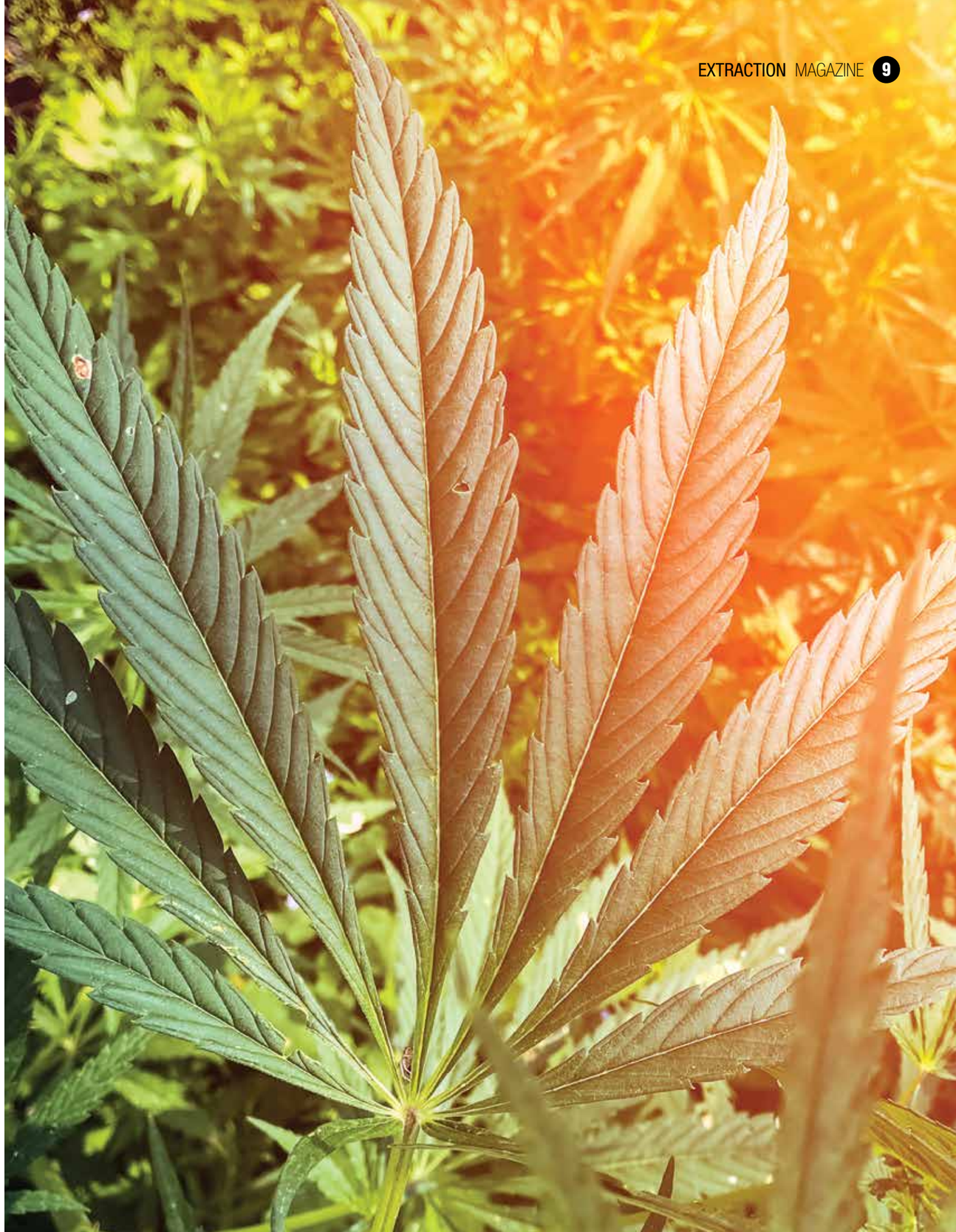
There is no archeological evidence or references specific to cannabis resin until the 9th century at the peak of the Muslim empire, whose advances in medical science was the source of modern medicine. Hashish is mentioned for the first time in two medical treatises. The great Islamic physician Rhazes [1], the recognized father of pediatrics, prescribed Hashish for pain relief in one, while his contemporary, the Arab physician Ibn Wahshiyah [2], warned of the potentially lethal effects of it, which he labeled a poison, in the other.

It is incredible to have no record whatsoever on Cannabis resin or Hashish until the Muslim empire while Cannabis was mentioned thousands of years earlier in Chinese medical texts and Hindu religious scriptures. The fact that the earliest references on Hashish are related to the medical aspect of the resin is fascinating but not unexpected, considering that the enzyme CBDA synthase is older than THCA synthase [3].
To find the origins of cannabis concentrates, we first need to find the birthplace of Cannabis, which is also unknown due to an early prehistoric dispersion and to the plant's ability to adapt and thrive at most latitudes and in most climates. A majority of experts believe the birthplace to be at the feet of the Himalayas from Bhutan to the Hindu Kush [4] or Central Asia [5].

The Asian continent is the birthplace of agriculture, of sedentary life and the rise of civilization. Agriculture in China is one of the oldest, with evidence of Cannabis, peas and rice farming over 10,000 years ago. The Fertile Crescent in Central Asia is where agriculture was born 15,000 years ago [6]. Northern India and Southern Afghanistan are the main centers of the origins of cultivated plants [7]. Excavations of prehistoric sites by Louis Dupree [8] and other archeologists indicate that early humans were living in the region known today as Afghanistan at least 52,000 years ago. Farming communities in Afghanistan were among the earliest in the world [9].

Around two and half million years ago *Homo erectus* migrated out of Africa and dispersed throughout Europe and Asia. It took our distant ancestors roughly 800,000 years to move from the African continent to Asia as confirmed by stone tools found in Malaysia that have been dated to be 1.8 million years old. Generation after generation of discovery and adaptation, every plant, animal, and event analyzed and memorized and passed down for the survival of the species. By the time *Homo erectus* was discovering the Asian continent, we can assume that a vast amount of knowledge was available in their continual search for food, medicine, fiber and the means to create tools. How long would it have taken a master forager to find a plant that offers the three most essential necessities to survival - food, medicine, and fiber?

If cannabis is so hard to hide in the twenty-first century, its discovery could not have been much of a challenge to our ancestors. It is pretty much impossible to access the seeds, the fiber, or any part of a Cannabis plant for that matter, without building up a layer of resin on the hands and fingers rapidly; this first accumulation of resin on our ancestor's hands was the first concentrate ever made, what would come to be called Charas [10] hundreds of thousands of years later. Charas is the oldest form of cannabis concentrate; it is the simplest and most effective method to collect live resin from the wild Cannabis plants at the peak of their flowering cycle. This method of production is hardly practiced anymore in Hashish producing countries but remains the unique collecting process in those countries at the feet of the Himalayas - Bhutan, Nepal and Northern India, all tropical regions with humid climates.



The principle of rubbing live resin from a cannabis plant is quite simple. Your hands are your tools and since water and oil are not conducive to collecting resin, absolutely dry hands are mandatory for the task. Remove the fan leaves from the plant. Caress the flowers with a gentle back-and-forth movement between your hands making sure not to bruise the plant material to avoid incorporating chlorophyll and plant water content in the resin gathering on your palms. Clean your hands of all leaf matter and start again until a thick layer of resin builds up by small increments on the palms of your hands.

Press and turn your thumb on the most resinous part of your other hand, snap the resin off and repeat the process until your hand is clean and your thumb holds all the resin mass. Transfer the collected resin from one thumb to the other and repeat the process on the other hand. It is a very basic technique adapted to the climatic conditions.

Beyond the technical aspect of collecting resin on the palms of one's hands, there is the experience of living for months at a time in remote valleys of the Himalayan mountains range. There is the magical and extraordinary feeling of caressing a Cannabis flower gently, feeling the resin layering on your hands and seeping into your body through the pores of your skin. There is the constant overload of terpenes and there is the high of Himalayan Charas, clean, cerebral, vibrant, energizing, with no plateau but levels of consciousness. Frenchy Cannoli is a consultant, educator, and writer in the Cannabis industry with special focus on hash making using traditional methods. Frenchy can be reached through his website at: www.frenchycannoli.com or seen on Instagram @frenchycannoli.

Fundamentals of Supercritical Carbon Dioxide Extraction

By Mike Padgett and John Van Antwerp, THAR Process

There are a number of different extraction techniques suitable in removing compounds of interest from cannabis plant material. Currently, the most popular techniques include those that utilize non-polar liquid solvents such as propane, butane or supercritical carbon dioxide (CO_2), or a more polar solvent such as ethanol. The challenge in using hydrocarbons such as propane and butane, is that they are flammable and, in some jurisdictions, banned from use. Where they are allowed, the use of hydrocarbon solvents generally requires a Class 1 Division 1 (C1D1) explosion-proof room to carry out the extractions. Other obstacles to consider are the purity of propane and butane. They are both petroleum distillates and could potentially contain toxic, poly-nuclear aromatic hydrocarbons (PAHs). These molecules would be difficult to detect in a standard residual solvent test and may require mass spectrometry for detection.

Ethanol extraction shares the same issue of flammability, and when the system is of sufficient scale, will also require an explosion-proof room for extraction. What makes supercritical CO_2 extraction different from other techniques is the ability to change the solvating properties of the CO_2. It's possible to manipulate the density of CO_2 by changing the pressure and temperature of the solvent. In addition, CO_2 is non-toxic and non-flammable, and is easily recovered, making it more environmentally friendly.

Let's cover a little background on the different physical states that CO_2 can exhibit. At room temperature and atmospheric pressure, CO_2 is a gas. If we put it in a cylinder under sufficient pressure, it is a liquid, even at room temperature. If we get it cold enough, CO_2 solidifies. Think of dry ice. Have you ever noticed what looks like steam coming off the solid dry ice? That is actually the solid changing into a gas! That ice is just solid CO_2. If we get above a critical temperature and pressure (1073 psi and 31°C), it becomes a supercritical fluid that exhibits the properties of liquids *and* gases. Having the properties of a liquid means that supercritical CO_2 has the ability to solvate compounds. Solvation simply refers to the interactions that occur between a solvent and dissolved moleculessolvent and dissolved molecule, which cause the formation of molecular aggregates between solute (species dissolved) and solvent. And having the properties of a gas means that the CO_2 practically has no surface tension, and thus will penetrate the matrix more easily than a liquid, increasing the speed of extraction. Changing the density of CO_2 changes its solvating properties, allowing us to "tune" the solvent to remove the compounds of interest (cannabinoids and terpenes) while leaving undesirable compounds, such as chlorophyll, behind. This unique property gives rise to a variety of extraction scenarios, including, in-line de-fat/de-waxing, collection of light fractions (terpenes and fragrances), and collection of heavy fractions (cannabinoids like CBDA, THCA).

Manipulation of CO_2 from gas, liquid, and supercritical states can provide many different outcomes on cannabis extraction. Examples include:

- Operate at low pressure to first partition and collect the terpenes and then increase the pressure in the extraction vessel to remove the cannabinoids.
- Run at higher pressure initially, extract all compounds of interest and have the ability to sequentially depressurize the supercritical CO_2 post-extraction to fractionate the extracts allowing for easier formulations.

- Depressurize the CO_2 where the acidic cannabinoids are no longer soluble, and they will drop out in the medium pressure collector. The neutral cannabinoids would still be soluble and would be collected in the low-pressure collector as CO_2 is depressurized back to a gas to be recovered. Material from the medium pressure collector could be winterized and made into shatter, while the decarboxylated, neutral cannabinoids from the low-pressure collector could be used for other products.
- Alternatively, if we wanted to combine acids and neutrals, we would set the pressure on the medium pressure collector low enough to drop both neutrals and acids out together.

One thing to remember is the myth that extractions with the "most weight" offer the best efficiency. Increasing the pressure of the extraction to 375-400 bar will decrease extraction time and permit the collection of a considerably larger amount of extract. This may not be the desired outcome, however. Using these parameters will allow us to collect twice as much extract by weight, but the purity of that extract could be as low as 30% cannabinoids by weight. By decreasing the pressure down to 275-300 bar and removing less waxes and chlorophyll from the starting biomass, the crude extract is generally near 60% cannabinoids by weight, with minimal increase in extraction time. This will help with post-processing steps, such as, winterization, decarboxylation, and short-path or wiped-film distillation.

Instrumental design considerations enable the measurement of CO_2 density during the extraction process and therefore offer the ability to precisely control the solvent-to-feedstock ratio. One must consider pumping CO_2 by weight, and not by volume, to understand the ratio. This ensures that despite changes in density, the correct solvent-to-feed ratio is achieved run-to-run and day-to-day, making a more robust process. If we were pumping by volume and the density, and therefore the solvating properties, of CO_2 changed, we could have an incomplete extraction resulting in poor recovery. Or if we ran out of CO_2, there could be no extraction at all.

Another benefit of CO_2 extraction is that it's a scalable process. By scaling the pumps, heat exchanges, process piping and the fraction collectors appropriately, whether we have an 18-liter extraction vessel or a 1,650-liter extraction vessel, we can keep the solvent-to-feed ratio the same and achieve 90+% extraction efficiency in as little as 2 hours.

Some individuals may have negative impressions of CO_2 extraction, because CO_2 infrastructure is more expensive

compared to other technologies such as hydrocarbon or ethanol. While it is true that the hardware for CO_2 extraction is more expensive, one needs to consider the rest of the infrastructure required for the competing technologies. For hydrocarbon extraction, this would be the cost of C1D1 explosion-proof room. For extractions employing ethanol, the most selective extraction requires the process to be performed at very low temperatures (-30°C or colder). This has an impact on heat load and therefore heating, ventilation, and air-conditioning (HVAC) requirements, explosion-proof rooms, and a means for removal and recovery of ethanol. This might be feasible for small systemssystems but becomes a much bigger challenge when scaling to very large systems. While the competing technologies can be scaled as well, one needs to consider the total cost of equipment, infrastructure, labor, and day-to-day operation. What is the price for health, safety, and GMP at industrial scale?

Some may think that CO_2 negatively effects the flavor of cannabis products. To that I say tune the conditions of CO_2 extraction. The ability to control these parameters at particular points in the extraction process can be achieved with proper engineering in addition to measurable quality with reproducible results. CO_2 can be dynamically altered to these quality parameters that won't elucidate those negative stereotypical flavors that may resemble freezer burn.

The same advantages that supercritical CO_2 exhibits for extraction can be used for chromatography as well. This can be used to purify individual cannabinoids for subsequent blending with plant extracts to design specific cannabinoid formulations, and ensure that batch-to-batch reproducibility is achieved. Chromatography can also be used to remediate tetrahydrocannabinol (THC) from hemp extracts. The extraction process not only concentrates cannabidiol (CBD) from hemp, but THC as well. The THC can be removed, and the rest of the cannabinoids can be collected to maintain the original profile minus the THC. The other potential use of chromatography is to remediate pesticides from contaminated feedstocks. One of the most commonly used pesticides, myclobutanil, can be easily removed using supercritical CO_2 chromatography. The CO_2 process-scale chromatography flashes CO_2 back to a gas and leaves the extracted fractions in a much smaller volume of ethanol (typically 2-4%) which can easily be removed to recover the cannabinoids.

In looking to the future, green methods of cannabis/hemp extraction must be at the forefront of our understanding and implementation. Innovation is happening at such a pace where we'll have a chance to witness the results in our current generation and generations to come; reducing, reusing, and recycling CO_2 that we have put into the environment.

Let Your Spirits Soar!
The Fundamentals of Ethanol Extraction

By Tamir Bresler

The cannabis flower has been used for religious and medicinal purposes for almost as long as human civilization itself. [1] Among its first recorded uses in medicine was as an extract into ethanol, with the tincture's curative powers being boasted about in the scientific literature as early as the 19th century. [2] In today's landscape, where many solvent and solventless extraction processes abound, is there still a place for this ancient technique?

To understand both the advantages and challenges of using an ethanol-based extraction system, it's important to review the chemical rules of solubility. Any molecule, from water to THC, can be described in terms of its polarity. When electrons are shared equally in a chemical bond, such as in the hydrocarbons propane and butane, no polarity is observed. If, however, electrons in a molecule are distributed unevenly—because some atoms are greedier for electrons than others—the molecule will become *polar* and behave like a tiny magnet. And just like a magnet, polar molecules are attracted to other polar molecules, and repelled from non-polar ones. This is from where we get the well-known adage, "like dissolves like."

Figure 1. These "heat maps" are topographical representations of electron density in a molecule, a standard method of displaying bond polarity. Red regions represent areas of high electron density, green of intermediate, and blue of low density. In the ball-and-stick figure, atoms are colored according to standard convention: Black = carbon, white = hydrogen, and red = oxygen

Figure 1

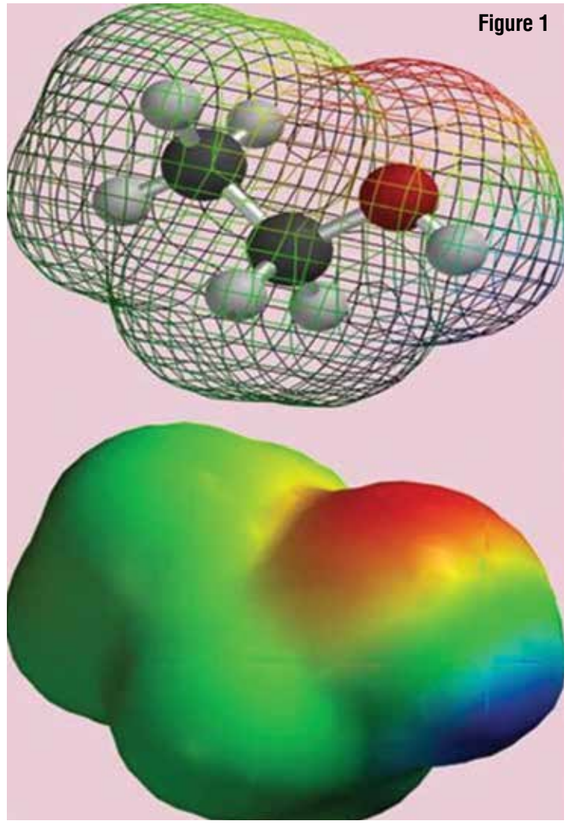
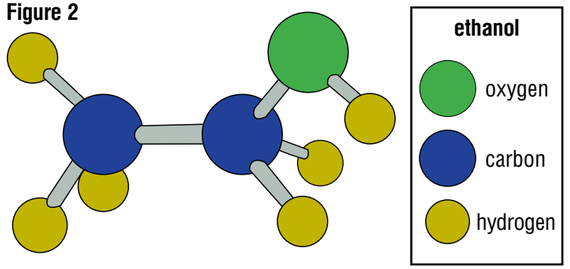


Figure 2. Ethanol is an amphipathic molecule, containing both a polar and non-polar component. The alcohol of a two-carbon hydrocarbon, ethanol's 'tail' is made up of the electrically neutral ethyl group, while the 'head' is composed of the electrically charged oxide group.

Figure 2



Ethanol is a predominantly polar molecule, due to its small size and alcohol moiety, with a partition coefficient of 0.661. [3] Because of this polar head, ethanol is notoriously capable of dragging water-soluble particles out of the plant during extraction, such as the cell's chlorophyll and plant waxes. However, ethanol is also an amphipathic molecule, containing both polar *and* non-polar components. Its hydrocarbon ethyl group, made up of two carbons and five hydrogens (C_2H_5), makes it hydrophobic enough to successfully solvate a wide range of non-polar compounds, including cannabinoids and terpenes.

Another important detail to note is that hotter temperatures prime polar molecules to dissolve more readily than in the cold. In molecular terms, temperature is a direct measurement of the rate at which a molecule vibrates, rotates, and generally moves around. At higher temperatures, a molecule of, say, THC, is going to collide with a molecule of ethanol faster than at colder temperatures, leading to a quicker solvation and extraction. Also, on the inside of the plant matrix, each cannabinoid and terpene that we want to get our hands on is surrounded by an intricate, interconnected lattice of water molecules. Turning up the heat makes it easier to break this framework apart, at which point our solvent swoops up the groovy treasure that awaits. So ethanol extractions generally favor colder temperatures than the other popular techniques.

Given this information, what does it all mean? First of all, the cold temperatures that are common in ethanol extractions go a long way towards preserving the many delicate and volatile cannabinoids and terpenes, which don't always survive the punishing treatment of hydrocarbon or supercritical CO_2. [4] It's these unique profiles of varying bioactive molecules that give each cultivar the special properties it takes to tackle different problems! Probing the exact array of what works best at treating a given condition is of keen interest to researchers at the moment, and at its heart is the ability to successfully preserve, capture, and retain the entire spectrum of cannabinoids and terpenes in any given cultivar. [5]

One of the mainstay challenges in modern cannabis extraction has been the maintenance of terpene consistency across

separate batches, and retaining terpene content in these products post-extraction. Of the multitudes of cannabis-derived terpenes that have been identified so far, over 15% of them are considered “light” and can evaporate even at room temperature. [6] Even mild amounts of heat, as well as high-pressure and mechanical agitation, can lead to their degradation. The elevated temperatures applied during many standard extraction techniques can therefore result in a significant loss of terpene content. [4]

One solution has been to capture terpenes by condensation as they escape and reintroduce them into the final distillate later on. [7] Another has been based on cultivar analysis, whereby laboratory services compare the terpene composition of the starting material and the finished product, and create a master mix that quantitatively reintroduces the missing components. [8]

Quick Wash Ethanol (QWET) provides a more elegant and simple solution than all of the above. The entire process is done at -20°C, with both the ethanol and cannabis cooled with dry ice prior to extraction. The cold temperatures tamper down the hydrophilic properties of the solvent, reducing the amount of chlorophyll that’s pulled out and definitively toning down the distinctive earthy, bitter flavor which has been associated with ethanol extractions for so long. The low temperatures also prevent the solvation of plant waxes and cuticle, which, in other extraction methods, require a winterization and filtration step to remove. [9]

California-based Capna Labs specializes in manufacturing QWET extraction systems. Their method works on the principle that long incubation times of cannabis in ethanol are unnecessary and even counter-productive, pulling out excess chlorophyll without a reciprocating increase in extraction efficiency. Cannabinoids and terpenes will readily dissolve into ethanol when placed in contact with the plant matrix, and therefore a short incubation period of 3-10 minutes is all that’s needed. From there, the crude extract can be distilled and concentrated as desired using usual laboratory methods.

While regulatory challenges remain when it comes to obtaining the large quantities of pure ethanol required for cannabis extraction, QWET and other ethanol-based extraction models have remained a mainstay in the preparation of both at-home and industrial cannabis extracts.

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Hydrocarbon Extraction: Look Past the Open Blast!

By Dustin Mahon, EC Labs LLC, Prescott Logic Technologies LTD

Hydrocarbon (butane/propane) extraction and B.H.O. (Butane Hash Oil) have always gotten a bad rap. The ease of production and regular availability of canned butane, coupled with its low vapor point and flammability, has made for some explosive results. The news stories that keep popping up have bestowed the bad name to this technique. These stories were almost always caused by someone that was uneducated and "open blasting".

Open blasting is a technique where plant material is packed into a vessel that has a very small opening at the top, and a large opening at the bottom that is covered with a filter. A can of liquid butane is then deployed into the vessel through the small opening and captured in a collection vessel. The collection vessel is then heated, causing the hydrocarbons to boil off into the atmosphere. What is leftover is a raw hash oil. Butane and propane are heavier than the atmosphere, therefore they sink to the ground. This is where most people get into trouble.

Since most of these extractions are occurring illegally, the person doing the act tends to try and hide the action, and typically (especially in Colorado) that means using a basement. Once the atmosphere reaches a 1.8% saturation of butane, it becomes explosive. This is known as the Lower Explosive Limit (LEL). The only thing missing is an ignition source. This can be a pilot light on a water heater, a refrigerator fan kicking on or anything that generates a spark, even static electricity. Hazardous solvent extractions should only be performed by trained professionals, with professional, peer-reviewed equipment in a professional, peer-reviewed facility.

This means the application of "closed loop" extraction systems, the same concept applies from open blasting, in that a vessel is filled with plant material and saturated with liquid hydrocarbons. In open blasting, there are openings in the vessel. In closed loop, all of the solvent is maintained in a closed vessel and never sees the light of day. In a closed loop system, the extraction vessel is filled with plant material. The vessel will have a bottom cap that contains a filter and is connected to a collection vessel. The top of the vessel will be caped after filling with material, thus creating a closed vessel. The vessel and the collection chamber are both vacuumed free of atmosphere. This is a crucial step, because neither butane nor propane exist as a liquid in our atmosphere. Under vacuum they are still liquids. The vacuum helps pull the liquid solvent out of an operating tank and runs it through the top cap. Once the solvent runs over the plant material, through the filter and into the collection vessel, the collection vessel will now be filled with a solution of hash oil and liquid hydrocarbons. By adding heat to the collection vessel, the hydrocarbons are converted back into a gas. The gas can then be distilled out of the collection vessel and re-condensed back into the starting operating tank, thus completing the "loop". The hydrocarbons can then be reused.

This process must be performed inside of a room specifically engineered for the process. The relatively new requirement of Class 1 Division 1 extraction rooms with hazardous exhaust systems has really stepped up the level of safety in these facilities. C1D1 is an electrical classification given to electronics that are designed to be intrinsically safe and "explosion proof" by the International Electrical Code (IEC). This electrical classification coupled with a hazardous exhaust system that meets the specifications of the International Mechanical Code (IMC), provides for a nearly impossible environment for explosion. Additionally, flammable gas detection units are implemented in these facilities. Gas sniffers are mounted at 12" or lower from the ground to accurately detect the amount of flammable vapor in the area. Visual strobes and audio horns are set off to alarm operators of leaks. These are set to go off well before the vapors reach LELs.

As far as end user product safety goes, most legal states have adopted a level of analytical testing that can measure the amount of residual solvent left in a product. Some states have adopted limits in the single digits of PPM (parts per million). PPM is a term that gets used a lot but I don't think that many people understand how little of a number we are talking. Percent is a measurement all of us are familiar with. This is a scale of 0-100, or parts per one hundred. So, 1% (1 part per 100) would be 10 parts per 1,000, or 100 parts per 10,000 or 1,000 parts per 100,000 or 10,000 parts per million. So, 100 parts per million would equate to 10 per 100,000 or 1 per 10,000 or 0.1 per 1,000 or 0.01 per 100 (0.01%). The United States Pharmacopeia allows for 5000 ppm of residual hydrocarbons in pharmaceutical products (0.5%). [1] In comparison, pharmaceutical products are 99.5% solvent free, while cannabis products are 99.99% solvent free at 100 ppm. While these tests are the limits for what is contained in the final product, obtaining Non-Detectable (ND) levels of hydrocarbons is very achievable, and frequently accomplished.

Butane and propane are both non-polar hydrocarbons. Non-polar is easier explained as non-water soluble. The compounds that we quest after in the cannabis plant are mostly oils, therefore a non-polar solvent would make sense. Terpenes are complex hydrocarbons and would make sense that they are extracted and retained in hydrocarbon extractions. While both butane and propane are considered non-polar solvents, they both have some pretty different abilities that make them desirable. It's like drawing a line in the sand, and anything that falls to the left is considered non-polar, and anything that falls to the right is polar (i.e. alcohol). Propane exists on the far left of the spectrum and it has virtually no affinity for water.

Butane, on the other hand falls pretty close to the middle. So why use both, and not just one? Certain terpenes will have some polar properties, and the propane will miss them, losing out on flavor. On the inverse, pure butane, because of its slightly polar properties and pressures, tends to produce darker colored extracts. Butane only has a pressure of 40 psi, making it relatively slow moving and allowing it to pick up more impurities during extraction. Propane is almost 4x more pressure at 150 psi and can be too fast and miss some of the compounds. A good mix of the two seems to be the magic ticket of major flavor, color and yield.

The plant grows most of its desired compounds in its trichomes predominantly on the outside of the flower. As the solvent contacts the plant material, it starts to dissolve the trichomes into solution. The longer it touches the trichome, the more it will extract. Nearly 85-90% of the compounds exist in the large bulbous head of the trichome. For high potency

Reference

▲ [1] "<467> RESIDUAL SOLVENTS", USP 40, *Chemical Tests*, https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c467.pdf

concentrates, we really only want to decapitate the trichome heads. Dissolving more will start to lead to larger proportions of plant impurities like waxes, fats, lipids, or chlorophyll getting into the concentrate. For edible products, lower potencies, but higher efficiencies are desirable.

Heat plays a major role in the chemistry of cannabis. The low boiling points of both butane (34°F) and propane (-40°F) allows for very low heat to remove any residual solvents. It also makes them ideal for the retention of terpene content as well as the preservation of the acidic cannabinoids. Heat causes the terpenes to boil off and decarboxylation of cannabinoids. To achieve an optimal, high end dabable extraction, the plant material should be fresh and still full of water. This technique is known as “Fresh Frozen” or “Live Resin”. Fresh plant material has been shown to have significantly more terpenes than its dried counterpart.

The freezing temperatures cause the waxes to fall out of solution and stay behind in the plant material. In turn the technique produces a much smoother dab with a superior flavor. It is a much more labor-intensive process and the end yields are greatly lower, but the end quality is significantly better. Many extraction artists have started to introduce a technique that actually grows THCA crystals, causing a separation of the terpene layer. The crystals are testing over 99% pure.

The vast majority of hydrocarbon extractions will be performed on dried plant material. Over 70% of the plants weight is water. By drying the plant material out, more material can fit into each run. The end result is much higher yields, and much lower labor costs. The process is simpler and requires less specialized equipment.

The equipment, the compliance and safety measures required, and the stigma of hydrocarbon extraction are its biggest downfalls. Being a non-polar solvent, it will only extract 75-85% of the cannabinoid content, so it leaves some money on the table. C1D1 equipment seems to add another digit to the price tag and can be difficult to source. Most of the regulators err on the side of caution and can create “interesting” issues for operators. Some people are turned off by the word “butane” or “propane” and will simply not use it. They have seen too many headlines and have a negative association.

I truly love aspects of all forms of extraction. I think there is a place for everything. When it comes to producing and preserving the highest cannabinoid and terpene content extractions, keeping in mind the overall cost, hydrocarbon extraction is one of the superior ways. It is also one of the fastest growing segments of the industry and should not be overlooked. Look past the open blast and realize the awesome power that is hydrocarbon extraction.

Life After Extraction: Standardization Begins with Distillation

By Steven P. Bennett, PhD.,
Prescott Logic Technologies, Ltd.

A successful cannabis producer needs to weigh many variables when delivering products that are properly differentiated and branded to compete in the market(s) selected. Often, the markets are so nascent that it might be difficult to ascertain, let alone execute with a competitive advantage. As such, producers are depending more on the processing of cannabis into various derivatives, rather than focusing on cultivating the drifting genetics of esoteric cultivar names. Consistency of process, formulation, and consumer experience each rely on the "standardization" of procedures and that begins in the world of "post-processing" of cannabis.

There are two pathways to consider with cannabis extraction that I will term "Niche" and "Classical". Niche processing refers to techniques where the concentrated output material is primarily used directly by those consumers that make their decisions based mainly on the combined taste and effect of products, as determined by dab. The utilization of water for separation of trichomes, mechanically pressed live rosin, and extraction of cannabis with butane and propane all yield what are typically regarded as "connoisseur" products, as they all produce vibrant, fruity, and heady experiences to the seasoned users, but often render the novice incapacitated. Although there is a business case for each of the methodologies listed, the extraction efficiency for each method is marginal to adequate in the "Niche" laboratories and efficiencies are supplemented with more traditional or "classical" techniques to maximize returns from the starting material.

Classical processing methods have been adopted from the botanical, pharmaceutical, and even petroleum industries. These well-established businesses seek the highest-throughput methods with the fewest steps possible and minimal dependence on human intervention and, consequently, error. Often, these are methods that are considered the most scalable, implying that they're the most efficient and of the highest-throughput when considering operations in *acres* rather than square feet. To be clear, however, it is the responsibility of each producer to determine the process that works best for the product assortment they wish to make. The market can bear all of the cannabis products that can be delivered to them, as long as their quality is ensured.

The classical cannabis processing pathway is: 1) extraction, usually in the form of CO_2 or ethanol; 2) fractional distillation of the resultant crude oil; and 3) chemical separation/isolation of pure cannabinoids, generally through the use of preparative chromatography. The techniques of extraction, distillation, and isolation have been used in series for decades to purify many compounds we use today. It is the process of oil refinement, whether peppermint or kerosene, and with some adaptation, these same principles apply to efficiently delivering consistent products from starting material as inconsistent as cannabis biomass. Despite the diverse number of interesting molecules in a cannabis extract that may confer therapeutic benefit, they can only be processed in high-efficiency when they are prevalent at concentrations above 1%.

To summarize, the classical processing paradigm describes groups that want to "harvest molecules" from the plant in order to reassemble them in reproducible ways for a consistent consumer experience, while the niche markets are focused more on "craft" consumer experiences. The extraction efficiencies of the niche methods, however, pose the question of "when" to adopt classical methodologies in their process and not "if."

When will you chose to use ethanol in your process? Some may wish to steer away from it completely, but even with material that has 85% of the cannabinoids blasted away from butane, there is a loss that cannot be reconciled, particularly at scale. Eventually, every processor will seek ways to salvage the last molecules of THC and CBD from their process and turn to a polar solvent, namely ethanol, to dissolve these molecules for subsequent recovery. Food-grade ethanol has become the solvent of choice in the cannabis industry due mainly to its availability and safety profile. Ethanol is notorious for penetrating cell membranes indiscriminately and, in the case of biomass, non-selectively dissolving (extracting) the components therein. Given the low boiling point (78°C) of ethanol and its relatively low molecular weight (46.07 g/mol), the solvent can be easily recovered from any oil with the simple application of heat. So, the case can be made to extract first with one of the abovementioned methods, and then utilize less volumes of ethanol to recover the rest. Alternatively, a processor could decide to use solely ethanol for extraction, thereby skipping a secondary extraction step altogether.

Before everyone jumps ahead and relies solely on ethanol for their processing needs, it's important to first understand the desired product mix and how each might be impacted by the chosen extraction process. The reality is that the oil extracted from cannabis can only be as good as the starting material. Whereas it's true that quality products only come from quality starting materials, it is also true that quality gives rise to quantity when harnessing efficient processing.

"Good" cannabis, for example, is typified by potency in a cannabis plant. If a cannabis flower tests at 30% THCA, more cannabinoids can be harvested during processing. The resulting refined, distilled THC oil is theoretically no different than THC oil that has been refined from starting material at 10%, except that there is three times the amount of THC.

Post-processing of oil is, by virtue, a standardization step that is absolutely required to deliver consistent products to market. Medical product formulation will never be possible with plant matter or crude extracted oil. Even if it is accepted as therapeutic, the variation in plant matter is carried over to the crude oil derived from it. So, whereas the efficacy of Rick Simpson Oil (RSO) is not being debated, its lack of consistency cannot be defended. The art of post-processing may, therefore, not appeal to those that support only "full spectrum" oils, but will certainly appeal to the business that wants to normalize batch-to-batch variations within their products, deliver only consistent experiences to their customers, and maximize earnings.

Winterization

Following extraction of any sort, the next step of classical cannabis processing is "winterization." As the name suggests, it's a cold process that is the most commonly employed dewaxing method for cannabis oil. Although there are procedural nuances, it can simply be described as the addition of a polar solvent (ethanol) at 5-10 times the volume of crude oil, homogeneous blending, and exposure to a deep freeze (-40°C) for 24-48 hours. At these cold temperatures, the waxes and lipids from the extract are forced out of solution (precipitated) as their solvency in ethanol is temperature-dependent.

These fats need to be quickly removed while maintaining a temperature of -40°C through filtration. Whether fed by gravity through a filtered Buchner funnel or by force through a series of ventricular filters, any slight warming of the ethanol-oil mixture will result in re-dissolved waxes that can wreak havoc on processing efficiencies and increase machine downtime. The filtration is also paramount to remove any particulate matter in the oil. Proper winterization and filtration of cannabis oil can make the difference between smooth processing and firefighting during fractional distillation.

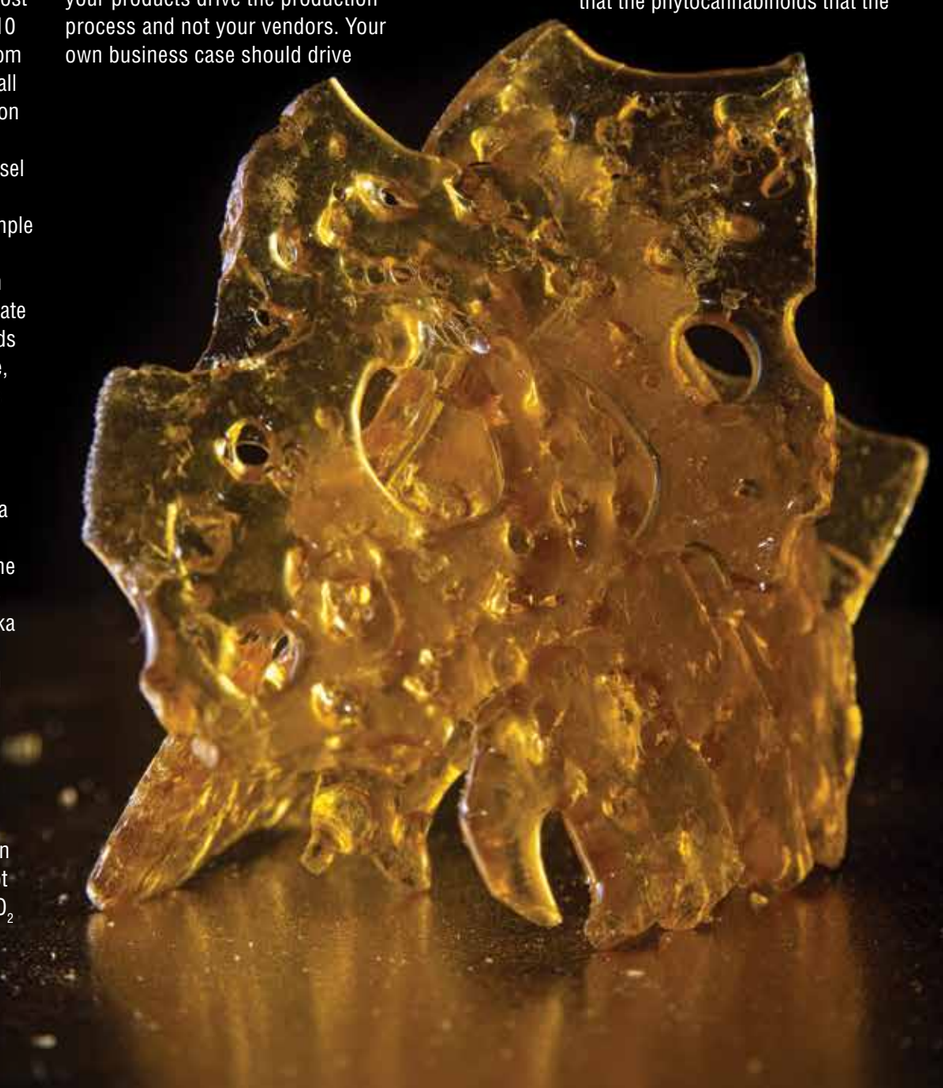
Solvent Recovery

The next step of processing, solvent recovery, is more straightforward than it is simple. If you have followed the bouncing ball through winterization and filtration, a cannabis producer is now working with crude oil deprived of most fats and waxes and suspended in 5-10 volumes of ethanol. To separate it from the now refined, crude cannabis oil, all that's required is simple pot distillation and no, not the pot we are typically referring to, but, rather, a heated vessel (with or without agitation) and an external condenser. The classic example of this is a rotary evaporator, where large round bottom flasks are held in warm baths under vacuum to evaporate and condense relatively volatile liquids from less volatile liquids, in this case, a refined cannabis oil that resembles amber, translucent motor oil.

Decarboxylation

This next step of post-processing is a bit controversial. In some labs, once you follow everything listed above, the oil is ready for the last stage of post-processing, short path distillation (aka wiped film or thin film distillation). Prior to short path distillation, the oil must be decarboxylated to release the carboxylic acid (COOH) moieties from the cannabinoid molecules, thereby "activating" them. Whereas decarboxylation before distillation cannot be debated, precisely where in the process it is employed should not be a decision made solely by your CO_2 manufacturer.

THC is more soluble in CO_2 than its inactivated cousin THCA. Many CO_2 equipment manufacturers suggest decarboxylation of dried plant material in vacuum ovens prior to CO_2 extraction. As efficient as this may be for some, others may prefer terpene profiles intact for use in marketing the best possible vaporizer pens. The terpenes a producer can take from flower that has not been first heated for decarboxylation are far superior to those caught from flower that's been heated. It is therefore critical that your products drive the production process and not your vendors. Your own business case should drive your company's decisions, not your equipment distributors. Knowing which products (cough…vape pens!!) can be compromised by following protocols that are not your own is an imperative part of a successful business strategy.



If you did not address the issue prior to extraction, decarboxylation is the next post-processing step before the crude material is ready for fractional distillation. It has long been proposed that the phytocannabinoids that the plant biosynthesizes are in the acid ("a" form or COOH), which are termed "inactivated" to human physiology, which likely is inaccurate at best. Notwithstanding, these COOH moieties need to be removed prior to fractional distillation or they will impede the process by "foaming" through the system. That is, rather than distilling liquids from liquids, the crude oil that enters the apparatus releases the CO_2 from the decarboxylation, and creates a foamy material that makes its way through the distiller. For proponents of RSO, we are not making claims that inactivated cannabinoids are not of value, but they do hinder classical cannabis processing efficiencies.

Fractional Distillation

Once the oil is winterized and filtered to remove waxes, then heated to eradicate residual ethanol and carboxylic acids, the oil is ready for the final stage of post-processing: fractional distillation. Fractional distillation describes the separation of molecules based on the differential thermal energy required to "boil" them off from each other. In the presence of oxygen, the introduction of heat leads to the oxidation of thermally labile (i.e. sensitive) molecules, including cannabinoids. As such, an increase in vacuum (or lack of oxygen) is inversely proportional to the evaporative capacity of a given matrix. The more vacuum, the less heat required to thermally separate molecules from each other with less (or without) oxidation.

Fractional distillation describes the separation of molecules based on boiling point and molecular weight. This concept is relatively simple, since different molecules (fractions) evaporate at different temperatures and can be condensed and collected as a function of that temperature, which can be reduced as a function of vacuum pressure.

Consider the mixture of alcohol (food grade ethanol, non-denatured) and water. Water has a boiling point of 100°C and ethanol has a boiling point of 78°C. Consider a liter of water, mixed with ethanol in a kettle, and placed on a stovetop set at 85°C. The ethanol (with a BP more than 78°C because of its "matrix" within water) will slowly dissipate into the atmosphere if the temperature holds between 80 and 100°C. Theoretically, as the temperature approaches 100°C, the last molecules of ethanol have departed and the concentration of water increases.

Wiped-film distillation, often called thin film, is nothing more than the mixture of ethanol and water at its core. The application of heat at a specific temperature "induces" certain molecules to convert from a liquid to a gaseous state. Just like every other aspect of cannabis processing, change the phase and reap the rewards. Wiped-film distillation, i.e. Prescott Distillation technology, utilizes heat combined with the evaporative potential of molecules of interest.

Taking this together with the knowledge of "pot" distillation, any person can pour tomato soup into a vessel on the stove and boil the contents. "Distillation" does not describe the simple application of heat, but also the mindful trapping of these molecules in thermodynamically appropriate ways. Heating a sample in order to release certain components can only be married to the mindful "trapping" of their release by the absence of that same heat.

The decreased amount of thermal energy from the evaporative to the condensing surface corresponds with the efficiency of mass transfer. A hot pot of tomato soup will burn on the bottom and evaporate on the surface. Stirring the

soup increases the surface area for evaporation but prevents it from burning. The wiped-film evaporator functions on the same premise: maximize the surface area that is exposed to heat to most efficiently evaporate (and condense) the molecule(s) of interest. The take-home is that if someone doesn't stir the tomato soup on the stove, it will burn on the bottom and boil at the top. In order to efficiently separate the water from the tomatoes, stir fast and enter, the wiped-film distillation system.

The distillation of cannabis follows the same process, with some exceptions. The bottom of the pot is the inside surface of the molecular still, which still requires constant stirring to prevent burning. The cannabis oil, now propelled by gravity, passes down the central still, analogous to the bottom of a hot pot turned sideways. As the source of heat at the base of the pot remains constant, the tilting of the pot exposes more tomato soup to the surface and promotes further evaporation of water, thereby further condensing the soup. Taken further, imagine the hot soup being passed from one hot pot to another, increasing the evaporation of water from one surface to the next. Wiped-film distillation is merely a succession of hot pots turned sideways under constant stirring to maximize evaporation. All that needs to be understood further is that water and ethanol have low molecular weights, relative to cannabinoids. The distance between the evaporative surface in a rotovap and the condensing surface can be measured in meters (consider the external condenser of a rotary evaporator), whereas THC, with a molecular weight near 314 g/mol, requires an internal condensing surface that is only centimeters from where it is evaporated to efficiently separate the wanted fractions from the unwanted fractions.

It cannot be overstated: there is not a "right" way to process cannabis. There are, however, some business decisions that need to be made to determine the most optimal products for a given market and the processing equipment required to get there at the anticipated scale. Gone are the days of the equipment vendors telling customers what they need. The success of producers will be determined by the purposefulness of their decisions, rather than the throughput of their equipment.

If simply pressing "start" on a machine has made you rich, why did you even read this article? The "best" way to process cannabis is a result of *your* business plan, not of the vendors'. Equipment vendors provide tools to execute *your* business strategy. If any licensed producer is counting on equipment purchases to take them to the promised land, they have merely underestimated the importance of their own branding and marketing along the way. As such, cannabis producers with either classical or niche approaches should seek solutions to their specific business challenges rather than simply increase equipment budgets.

What Makes an Award-Winning Concentrate/Oil?

By Cobra Extracts

The answer to this question is the same across all varieties of concentrates, particularly those that require somewhat sophisticated laboratory processing – exceptional starting material and a highly experienced extraction artisan. For the sake of this article, the focus will be more on the aesthetic aspects of the oils as those are what generally determines a winner.

The similarities between wine and cannabis are many. There are well-established means of assessing wines so applying a similar method to cannabis seems natural with one key exception. Consumers know with relative certainty what affects wine will have – they get drunk. Most don't differentiate between different gradients of being drunk, just the inevitable quantity driven consequences. Cannabis produces a vast and mysterious range of psychoactive, emotional, and physical effects that are generally the biggest draws to the product but also what makes it so difficult to assess.

Understanding what people desire and value in a connoisseur-level cannabis product is critical to making an award-winning product. With so few industry-accepted grading frameworks and highly individualized preferences, making educated and generally accepted assessments across regions, brands, and types of product is incredibly difficult. Here is one view of how those assessments should be graded and assessed.

Types of products

Flower offers the consumer a variety of different evaluation opportunities that are different from a vape pen, shatter, crumble, or even pre-rolled flower. Edibles are an entirely different beast as the metabolic chemistry associated with cannabinoids processed by the liver are quite different than those associated with an alveoli-based absorption. Given these differences, homing in on the type and associated attributes of the product being evaluated is the first step.

The ceremony of selecting the buds includes smelling (arguably the most compelling component), observing the nuanced colors, seeing the level of trichrome coverage ('sugar'), touching it to feel how sticky/soft/fresh it is, and (often to a lesser extent) a reaction to its cost.

Without a standardized ranking of these things, all of which are highly relative to the person making the assessment, how does one differentiate something with an amazing sour smell but relatively no sugar, versus something that looks like it was dipped in sugar and is super sticky but has no nose?

The snobbery associated with concentrates has evolved to the point of prejudice, even if subconsciously, with color topping most people's list of desirable attributes. Yes, bright yellow or even completely translucent concentrates are beautiful; however, they can fall well short of the full sensory compliment offered by darker products. Take raw THC distillate as an example. Done properly, it's a light golden yellow and can produce a narrow and underwhelming effect. Add in some terpenes and a bit of CBD, and the feeling we all know, and love is unleashed.

To limit further tangents, the focus of our discussion will be on producing what may well be the most nuanced and misunderstood concentrate of them all…Shatter (specifically Cobra Extracts Pie Hole Shatter). Even within this relatively narrow product category, there are variants: Shatter, Pull Snap, Sap, Crumble, Live Resin, Sauce, Sugar, etc. To keep things relatively simple, good old-fashioned shatter will be examined.

So, what makes a great, award-winning shatter? From a purist's perspective, it is, quite simply, a product that brings out the absolute best of the flower from which it was extracted. It should be crystal clear to emulate the purity of the master grower's biomass. When exposed to air, its presence should

be immediately known via a full and heady aroma. Like a snowflake, the individual characteristics of each shard captivates and fascinates the onlooker.

With zero need to filter adulterants through a water piece, a nectar collector affords the eager aficionado an opportunity to experience natures greatest gift in its purest form. Upon inhalation, the flavors and aromas play quickly with sensationally progressive head and body changes. Held captive briefly then set free, the cloud should advertise and impress sesh-mates.

Color, clarity, nose, smoothness, effects, and emotional reaction are core to assessing the quality of a product. Guiding and directing the mechanics and chemistry required to transform biomass to award-winning shatter is akin to blending a sophisticated Meritage.

Color: From a molecular perspective, pure THC is light yellow. Pure THC-A is an opaque crystalline white. Terpenes tend to be clear to light yellow/green. Vitamins, flavonoids, other cannabinoids, chlorophyll, and other plant materials all tend to have a darkening effect. Some darker oils have far more effects than 'pure' THC distillate. The combination of non-THC cannabinoids and organic matter produce what is commonly referred to as the 'entourage effect'. This means they pair well with THC regarding how the body metabolizes and interacts with those compounds. So lighter tends to be more highly sought after, but caution has to be taken to ensure that enough of the good stuff is left with the shatter to make it, well, great shatter.

Clarity: A great shatter should be very translucent and have no cloudiness. Cloudiness is indicative of over-processing, unintentional sugaring, inadequate purification processing, or poor laboratory best practices.

Nose: Several players in the entourage effect make their fragrance known. Super gasoline-soaked Girl Scout Cookies? Thank the terpenes *beta*-caryophyllene and linalool. Old School OG Kush, on the other hand, gets its distinctive aroma from limonene and *beta*-pinene. And since terpenes have been reported to have medicinal properties of their own, their inclusion in cannabinoid concentrates not only lends to the synergies described by the entourage effect; their presence also augments the wealth of beneficial molecules the concentrate contains.

Smoothness: Counter to what pop culture portrays, lung-splitting, eye-watering, drool-inducing coughing is not necessarily a sign of a great product. In its purest form (diamonds, or THC-A powder) little-to-no coughing is common. Coughing is generally induced by irritants. Natural cannabis is not an irritant. Temperature can play a big role in smoothness but a properly taken hit of an award-winning shatter should go down like 50-year-old scotch (but through the other pipe obviously).

Effects: This is the most wildly variable component of any cannabis product. Due to the extreme level of variety in how identical products affect different people, this aspect should be measured more by intensity than by nuance. Great shatter will announce itself whether the effects are individually enjoyable or not. Sitting and staring at the back of your hand is a planned evening for some, but the Type-A folks would likely want something gigglier or attention focusing. Regardless, it's the degree to which the effects are produced that is the measure.

Emotional Response: Again, a highly individualized component but one that is incredibly important. Cannabis has always been a social and ceremonial indulgence. While yesterday's picked at, dab-tool assaulted, glob of goo is likely still a great smoke, it doesn't induce that coveted wow-factor.

While we've established a baseline for categorizing the what's of an award-winning extract, we haven't delved much into the how's. Creating commercial cannabis concentrates requires detailed process engineering, compliant lab configuration, pharmaceutical-grade analytical and processing equipment, and an immunity to a pandemic-level assault on green capitalism. Extracting cannabis oil is a fairly straightforward process that can be broken into several phases: Biomass Curation, Primary Extraction, Filtration, Separation, and Polishing.

Biomass Curation: Great shatter starts with great biomass. The artisan extractor selects trim like a chef at a farmer's market. Experience and skill guide the seasoned extractor toward fresh, high potency, lab tested starting material. Relationships with farmers and others in the supply chain are critical as environmental impacts can have deleterious effects on quality. Lab testing helps alleviate some of this uncertainty, but there is no substitute for a trained eye and nose.

All organic matter has the ability to decay and transform into an undesirable state. Think of a fresh banana. Cut from the tree it has a green peel and a bitter taste. Over several days the peel will yellow and the banana will sweeten. Several more days uneaten, the peel will spot and turn brown and the banana will turn to mush. The same concept holds true for cannabis and

getting it into the lab at the peak of freshness is important. The onus falls on whoever is purchasing the material to inspect and make sure the master grower harvested within the timeframe that nets maximum trichrome production before degradation begins.

The transportation of the biomass from farm to lab is also important. Leave that un-ripened banana on the dashboard of a delivery truck all day with no air-conditioning? How appetizing is it going to be?

From the moment the farmer's scythe severs the stalk, the fruits the plant has presented begin a slow and deliberate decay. Care must be taken to keep things as fresh as possible. Low temperatures, shielding from ultraviolet rays, limited exposure to air, and speed to process all set the stage for a successful, and potentially award-winning, extraction.

Once the biomass has been white-gloved into the lab, preparation begins. Take care to reclaim any kief that abandoned leaf in transport. Sugar leaf, shake, and the calyx itself need to be separated from waterleaf, stem, trellis, and any other foreign matter. Breaking large masses by hand is preferable to a grinder, mill, or blender, as those create friction and broken cell structures that encumber the filtration process later.

Primary Extraction: Once the biomass has been selected, attention must be directed toward selecting the extraction method. Solvent-based extractions (i.e. hydrocarbon, CO_2, ethanol) and mechanical separation of trichomes (i.e. bubble hash, rosin) are all tools of the trade. Hydrocarbon extractions are fast, have little operator control, and utilize chemicals that could cause bodily harm if not properly removed before consumption. CO_2 is precise and clean but tortoise-like in its efficiency. Mechanical processes are less practical at a commercial processing level.

In our opinion, the leader of the pack when it comes to efficient and delicate processing is food-grade ethanol. More advanced techniques involve combinations of CO_2 and ethanol processing, but that treads dangerously close to proprietary processes not a part of this article.

With the biomass properly curated, it's time to begin the primary extraction. It should go without saying, but lab conditions should be pristine. All beakers, flasks, tanks, and utensils should be sterilized. Aside from just looking super cool, lab coats, protective eyewear, and nitrile or latex gloves are mandatory. Ethanol and CO_2 should be food grade or better.

Ethanol should be well below ambient temperature.

Each step of the process should be ready to go before starting, including downstream processes. A timer should be utilized as well as a log that captures key data points (inclusive of those required by regulators). There is nothing more frustrating than getting an amazing result and look to reproduce the steps only to find some data wasn't captured.

With the cannabis safely in the extraction vessel, proceed to add solvents and observe for desired results. Over time, the extraction artist will come to understand the peculiarities of each ingredient and study the causal relationships. There really isn't one specific recipe that will produce the exact same result each time and there is no substitute for experience.

Filtration: Once the initial tincture/crude is produced and the raffinate disposed of properly, it is necessary to start separating the desired from undesirable. The primary offending compounds from CO_2 extractions are fats and waxes, residual CO_2, and (parameter dependent) chlorophyll. For CO_2, it's important to embrace the spirit of what is trying to be accomplished. When banging out crude for distillate, high pressures and higher temperatures will produce an efficient, albeit rather nasty, crude in a relatively short period of time. On the other end of the spectrum is where delicate terpenes can be coaxed from their chemical brethren. Don't take a sledgehammer to an archaeological dig. Most closed-loop professional systems allow enough operator control to selectively extract desired compounds.

The primary undesirables from an ethanol extraction are chlorophyll and ethanol. CO_2 crude can be winterized by mixing in ethanol, freezing the ethanol, then filtering the tincture. There are many different methods of filtering the primary crude or tincture. Smaller scale operations can use the tried and true Buchner funnel with a vacuum pump assist. Those looking to scale into a commercial operation can utilize exceptionally large Buchner funnels (Drain Droid type) or systems that can provide in-line filtration (plate and frame or lenticular/depth filtration).

Choice of filtration medium is fairly intricate as are the adsorbents and filtration aids that commercial producers employ. Your local lab supply should be able to make some recommendations specific to your needs.

Separation: With ethanol being a core component to both types of the extraction process, it's important to separate the ethanol from the cannabis. Keeping in mind that we are dealing with delicate phytomolecules that we want to take us on a

majestic cerebral journey, we look to chemistry and physics for guidance.

Ethanol will boil at sea level at 78°C. Cannabis oils start to break down and decarboxylate at these temperatures (for clarity, at 78°C and sea level, it would take a relatively long time for the decarboxylation process to occur). It would be best to keep the tincture at as low a temperature as possible but still be able to evaporate the ethanol from the oil. Liquids boil off as vapors. Since ethanol is also flammable, it is best to have a means of getting the vapor back into a liquid form. Here's where physics come in.

Just like it takes less energy to boil water on a mountain top due to the decrease in atmospheric pressure, ethanol can be made to boil at less than half the sea level temperature by subjecting it to vacuum. There are charts illustrating the relationship between the boiling point (temperature) of a liquid and its relative pressure (vacuum). With the tincture in a closed environment, lowering the pressure by using a vacuum pump allows the evaporation to happen in the 30-35°C range. A condensing coil or cold-finger allows reduced temperatures to force the vapor back to its liquid form. Rotary evaporators (rotovaps) are excellent for this process and come in sizes from 1-50 liters.

For people looking to produce vast volumes of oil and have access to a sizeable mint, falling-film and steam-distillation systems are available. With the bulk, but not all, of the ethanol safely separated from the oil, it is time for the final polishing.

Polishing: Even in very large rotovaps, it's impractical to recover all of the ethanol. Even taking the residual levels down too far increases the viscosity of the oil to the point where it needs to be scraped off of the evaporation flask. Scraping is bad; remember this is about to be an award-winning shatter.

After all of the hours and processes that led up to this, over-distilling is heartbreaking. Don't do it. Final polishing can take several forms from re-filtering, running it through a flash chromatography system, or the simpler (and most desirable) purge in a vacuum oven. The same concepts that applied to the distillation discussion above apply here, except instead of a rotovap, a vacuum oven will be used.

Keeping the ethanol boiling point curve in mind, the vacuum oven(s) should be set to somewhere between 28°C and 33°C. This would need to be adjusted based on altitude and the depth of vacuum produced by the pump. While the oven is getting to temp, prepare a baking sheet (standard sized) and line it with quality parchment paper. Teflon or silicone sheets can be used but there is something nostalgic and simple about parchment. Fold the edges so it forms a 'boat' such that all edges are covered, and oil can't seep through the corners to the underlying tray. The oil should be poured such that it just touches the edges of the pan. Here again, we employ physics.

The residual ethanol will boil off, evacuate the oven, and re-condense in a cold trap. The less time the oil can be in the oven the better. The rate of ethanol evaporation is directly tied to the amount of surface tension differential that exists between the oil and the air inside the oven. Keeping the tincture thin ensures maximum surface area and low surface tension. Watching the evaporation is nearly hypnotic.

As the vacuum pump pulls air out of the chamber, large elegant bubbles of departing ethanol create a sea of roiling froth that can be as messy as it is beautiful. Care needs to be taken to ensure that the temperature relative to the level of vacuum is monitored to avoid 'muffining' the tincture. Muffining is when gases (ethanol or hydrocarbons) come out of the oil in very large bubbles. The bubbles can expand to the edges of the confined space (in this case an oven), leaving it sticky and wasting valuable product. It's called muffining as it is akin to how the tops of muffins expand in an oven. Cannabis oil isn't quite Super Glue but its close. The fewer surfaces it touches, the better.

The purging process is slow, and it should be. It's not uncommon for a full purge to take several days, although it can happen much faster. At 12-hour intervals or when the bubbling activity slows and the tincture has started to solidify into a slab, remove it from the oven and 'flip it over'. Place the slab back in the oven and allow it to reach vacuum again. This allows the other side of the slab to be exposed to the air and for remaining ethanol to have another path out. The slab is done once it can be held by fingertips without sticking and cracks in a clean line like stained glass.

If all has gone well, and all of the many opportunities for failure have been avoided, what should result is a beautifully clear, aromatic, mouth-watering, anticipation-inducing and potentially even award-winning shatter.

Achieving Acceptance Through Experience-Based, Custom Formulations

By Tristan Watkins, PhD., LucidMood

Cannabis cultivar names are meaningless to most consumers, and especially meaningless to the novice mainstream consumer. A rising number of consumers expect predictable products that are labeled by intended mood or effect. Nearly half of current consumers wish products were labeled by intended mood or effect, and over one third of former users quit because they did not like the unexpected effects of cannabis. Cultivar names do not convey intended benefits or unwanted effects, forcing the user to guess and hope that the product they select is the product they actually want. Rethinking the way cannabis products are created, validated, and marketed will appeal to the mainstream, and subsequently increase overall acceptance of cannabis consumption.

The new mainstream consumer expects the same thing out of cannabis products as they do with any other packaged commodity. BDS Analytics reports that 37% of former cannabis consumers stopped using cannabis because of experiencing unwanted effects (BDS Analytics). This means that more than one third of new cannabis consumers had such a negative experience that they never went back. This is horrible for customer retention and likely worse for public acceptance. Even current consumers are unhappy with current product offerings, with 41% reporting they wish more products were clearly labeled by intended mood or effect BDS Analytics. [1] With so many members of the cannabis community calling for change, isn't it time we start to listen?

Current cannabis products are built on a weak foundation -- the cultivar name plant name. Not only do plant names fail to convey meaningful information, their composition is notoriously inconsistent. The Blue Dream from one grow is seldom the same Blue Dream in another. Even when the same genetics are used, it is virtually impossible to maintain identical growth environments, meaning that cannabinoid, terpene, and flavonoid profiles differ with each growth cycle. Inconsistent cultivar profiles mean unpredictable effects. Would you buy a sports supplement that had a significant chance of making you tired and lethargic? What about a relaxing tea blend that sometimes shot your anxiety through the roof? If this isn't acceptable in other consumer packaged goods, then why should cannabis be exempt?

What happens when you extract from a cultivar? Different extraction processes yield dramatically different compositions. Generally speaking, true solventless extracts (e.g. ice water hash, rosin press) yield a phytochemical profile similar to the biomass used, whereas a CO_2 process yields an incomplete composition profile with minimal terpene content. [1] An untrained cannabis consumer is unlikely to understand this nuance, adding to the guesswork in decision-making and increasing the likelihood of an unwanted or unpredictable effect. In the end, extraction techniques suffer from similar issues as cured flower.

So how do we overcome the issue of predictability? Since cultivars are inherently unpredictable, we should altogether stop using them. Companies like LucidMood are doing just that. Instead of building a product line on the weak foundation of cultivars, companies should work towards formulating their products from the ground up. Isolate each compound of interest, study the effects of each compound, formulate products based on those findings, validate that your proprietary composition delivers a predictable effect, and then name it as such. Although this process takes considerable expertise, it dramatically improves predictability and strongly appeals to the mainstream consumer.

Let's dig into this idea a bit more by examining the utility of terpenes. Terpenes are a major factor in creating wanted and unwanted effects. Some terpenes, like linalool and terpinolene, can make people feel sleepy while other terpenes, like limonene or borneol, can make people feel more alert or refreshed. With this information, why not create one product high in linalool and terpinolene for evening use and another product high in limonene and borneol for daytime use? With formulated cannabis, you can create products with consistent composition profiles that deliver discrete effects. Not only does formulating your products allow you to add in exactly what you want, it allows you to deliberately leave out any compounds that may interfere with your intended effect. Would you drink a caffeinated tea right before bedtime just because it also had chamomile? Of course not! Then why would you choose a cannabis product with compounds that deliver inconsistent or competing effects?

The current cannabis market may not be demanding formulated cannabis products, but the market is still in its infancy. It pays to plan for the future, and the future will undoubtedly demand predictable products. With more predictable products, the novice mainstream user feels more comfortable making purchases. As the novice mainstream market grows, so does acceptance. And with greater acceptance comes more rapid changes in legislation, hopefully at the federal level. The goal of everyone in the industry should be to grow the industry as a whole, expand the consumer base, and increase profits. Right now, catering to the early adopters may be the answer, but that strategy is not future-proof. It may be wise to invest in your R&D department, learn more about the myriad of compounds naturally expressed in cannabis, and begin designing your own formulated products.

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Defining "Full-Spectrum": Understanding the Complex Chemistry of Cannabis Products

By Jacqueline L. von Salm, PhD., Plants of Ruskin and AltMed FL, LLC

The buzz words "full-spectrum" and "entourage effect" have taken the cannabis industry by storm. For example, cannabinoid analysis of Rick Simpson Oil (RSO) often shows no significant potency difference of Δ9-tetrahydrocannabinol (THC) compared to concentrates or vape pens, but patients consistently claim more powerful effects. The answer is almost always one of these two phrases without much description beyond those four words. Are terpenes more abundant? Are there other natural products playing a role? And what are these products "full" of exactly?

Rick Simpson claims that true RSO contains little to no terpenes and flavonoids, and the chemistry responsible for activity against cancer and other ailments are chlorophylls and various plant components. [1-2] Chlorophylls cannot be the only molecules responsible, since chlorophyll is the green color of all plants and is required for photosynthesis. Their potential in cancer prevention should not be completely ignored [3], but if they were the only active components, it would imply that any photosynthesizing plants, algae or cyanobacteria could potentially cure cancer.

As for a lack of terpenes and flavonoids, it is nearly impossible to completely destroy all cannabis terpenes in an extract when you consider larger terpenes like sesquiterpenes and diterpenes. Distillate with cannabinoid purity close to 95% might be devoid of terpenes, but this obviously does not have the same effects as RSO, so, what is RSO?

If cannabinoid content is similar or possibly less than other oils on the market, the next logical metabolites to analyze are terpenes. Terpenes are often documented as comprising anywhere from 0.5-3.5% of the total weight of dried cannabis plants [4], but some products on the current market can exhibit higher levels, such as the MiΔv Lemon Bubba chemovar, which revealed 5.7% terpenes at 9.1% moisture content. That number does not include unidentifiable sesquiterpenes that can often be seen as abundant as *beta*-caryophyllene, and with percentages that high, it would make sense that these play a more significant role than just a byproduct.

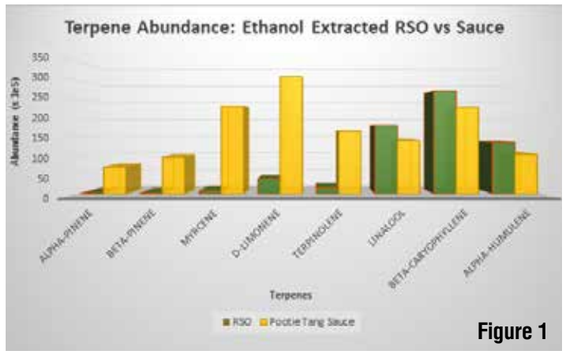
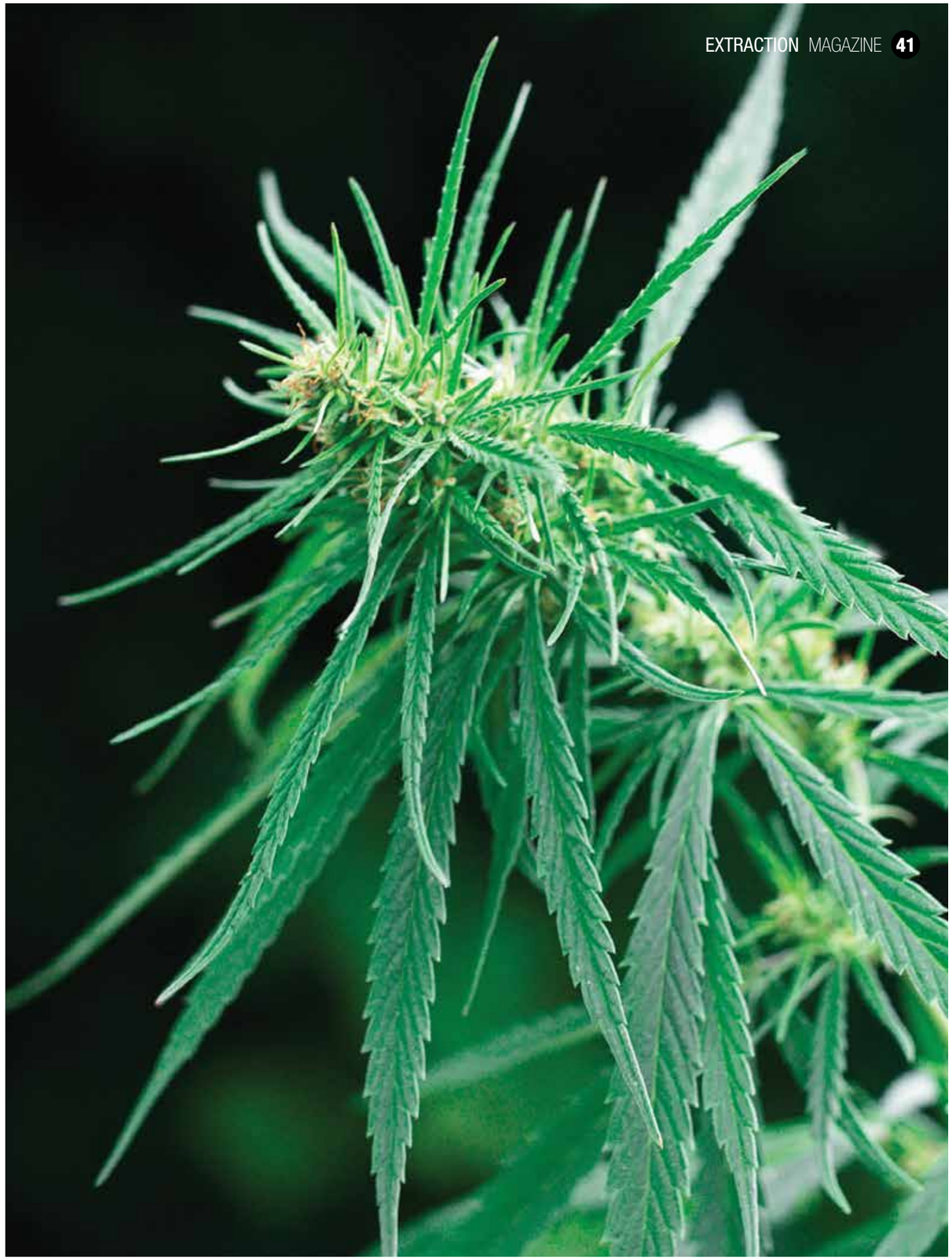


Figure 1: Comparison of terpenes present in RSO versus Pootie Tang Sauce concentrate. A 20% increase in the total abundance of linalool, *beta*-caryophyllene, and *alpha*-humulene is observed in RSO. Stereotypical monoterpenes are nearly absent.



Other biologically active compounds have also been documented [5], but which ones are therapeutically relevant? All of these questions and more led to the analysis of a dozen separate batches of ethanol- extracted RSO from MΔ The analysis was done via GC/MS headspace to assess the terpene content of separate, random batches of mixed cultivars and compared to a minimally processed concentrate (sauce) from Pootie Tang (Figure 1). The potency of all RSO products analyzed was approximately 55-70% total cannabinoids, and concentrates were 70-85% total cannabinoids, all of which were high THC extractions.

The stereotypical cannabis monoterpenes were less abundant in RSO than sauce, as predicted by Rick Simpson, but this already complicates any descriptions of "full-spectrum" extracts. This term is also often used for full terpene profiles in products. Stereotypical terpenes in this case include *alpha*-pinene, *beta*-pinene, myrcene, D-limonene and terpinolene. However, it gets interesting when you look at the relative abundance of slightly less volatile terpenes such as linalool, *beta*-caryophyllene, and *alpha*-humulene.

It should be noted that the RSO was created as a second extraction of plant material with room temperature ethanol, so the absence of volatile terpenes is somewhat expected. The surprising part is that patients continue to claim greater sleep aid and pain relief despite negligible myrcene content. Linalool, *beta*-caryophyllene, and *alpha*-humulene have all been documented to help insomnia and analgesia, [6-10] but it still seems likely that other compounds are also playing a role for cancer therapies.
Additionally, sesquiterpenes like santalene derivatives (common in sandalwood) and azulene derivatives were more abundant in RSO, which are not often seen in processed cannabis oils like concentrates. These are well-known for their effects as analgesics and possibly anti-cancer properties. [11-13]

The next biologically active compounds in enough abundance to be considered are flavonoids and phytosterols. Flavonoids include polyphenols like quercetin or anthocyanins in wine, berries and green tea. Purple cannabis phenotypes are often higher in pigments like anthocyanins giving them their iconic purple color.

Quercetin and other flavonoids are known to have anti-cancer properties with quercetin specifically acting on cannabinoid receptors throughout the human body. [14-15] Cannabis produces similar flavonoids to other plant species as well as its own appropriately named cannflavins. Research on these compounds documented 30 times the potency of aspirin as anti-inflammatories by inhibiting the prostaglandin E_2 release *in vitro*. [16]

Based on these results, analysis of the cannflavins and quercetin via LC/MS/MS shows RSO having an increased number of cannflavins A, B, C and quercetin as compared to concentrates from the same Pootie Tang concentrate from Figure 1. A purple phenotype was also included for comparison, Purple Linda, which was predicted to have a higher flavonoid content (Figure 2). Quercetin concentrations (ug/g) were 26% higher in RSO than Purple Linda sauce and 44% higher than Pootie Tang sauce.

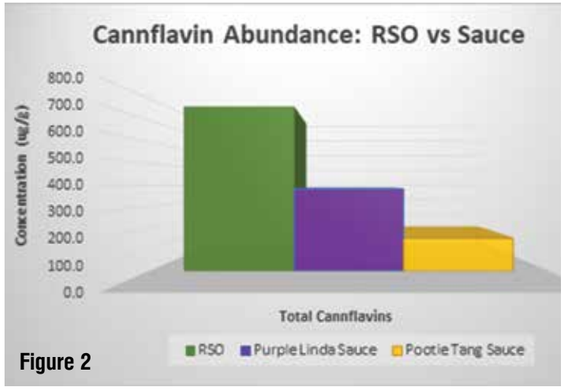


Figure 2

Figure 2: Comparison of cannabis flavonoids between RSO and sauce concentrates. Purple Linda (a purple cultivar with inherently higher flavonoid content due to its pigmentation) contained 50% of the three flavonoids as compared to RSO. Pootie Tang (representative of typical non-purple cultivars) contained 20% of the flavonoids present in RSO.

This data provides solid evidence that the chemical composition of flower and "full-spectrum" derivatives needs to be better defined. We must also always remember that certain molecules can be promiscuous, meaning they act on various receptors in the human body without specificity. This can have both beneficial (combination therapy) and detrimental effects (PAINS) [17], but could also explain how mixtures of these various biologically active compounds are more effective than target specific drugs. [18]
Rather than focusing on the daunting task of recreating the nature of flower for now, efforts can start with finding the differences between current products and methods. Understanding these chemical differences will help explain the wide range of properties shown by product made from *Cannabis* spp. We can then work our way up to comprehending the complexity of biological effects these compounds have on the human body from flower, concentrates, to purified cannabinoids.

Other questions worth asking: is RSO more bioavailable because it contains a more complex mixture of compounds (maybe slightly more water-soluble)? Are specific compounds more bioavailable when consumed orally versus inhalation, since larger compounds are not volatile? With so many questions, it is hard to know where to start, but much of the research on these compounds individually shows the right properties to act as anti-cancer, analgesic, and anti-insomnia agents. [19] Describing nature is always going to be a complicated endeavor, but it seems like the industry is finally headed in the right direction for medical patients and consumers alike.

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Author Bio: Jackie is the Director of R&D and Laboratory for Alternative Medical Enterprises, LLC (MΔ). She holds a PhD in Natural Products and Organic Chemistry from the University of South Florida with multiple publications in chemical ecology and drug discovery from natural extracts.

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Reactors' Capabilities for Post-Extraction

By Dashiell Davis, Ace Glass

When looking at post-extraction equipment, glass reactors are sometimes forgotten in the dizziness caused when choosing what's right for one's lab. One of the first points Ace Glass's Dr. Jim Carey likes to introduce when discussing glass as opposed to stainless steel reactors is visibility. "The beautiful thing about glass is that you can see through it." Glass reactors can be a versatile and vital part of the process, especially in relation to recrystallization, decarboxylation, mixing, and solvent recovery. There are three main glass reactors with applications in the cannabis industry: large-scale, bench-scale, and filter reactors; however, filter reactors have the most versatility.

Recrystallization

Recrystallization is one of the best and, per Dr. Carey, most cost-effective ways to increase purity in your product to 99.5% or better, especially in relation to the costs of chromatography. It also provides a solid instead of a liquid form. It is also a method in which filter reactors really reign.

Recrystallization involves taking your relatively pure distillate and combining it with a non-polar solvent, such as hexane or pentane, into a solution, and applying heat. Heating it to the boiling point of the solvent will super-saturate the solution. The entire solution can then be cooled and agitated. This will allow crystals to grow in different orientations. Agitation is important because along with temperature, this will yield larger and more crystals.

Changing the temperatures via a recirculator, and agitation, are critical in filter reactors. Somehow though, the solvent needs to be removed.

This is where filter reactors really shine. Not only can they do everything the large-scale and bench-scale reactors can, they can also easily:

- Remove the solvent.
- Allow the addition of other solvents.
- Drain the solvents without removing the crystals.
- Dry the crystals out by pulling vacuum.

There are other ways to perform recrystallization, such as using a -80°C refrigeration unit or manually with jars, hot plates, a freezer, and filtering. However, the freezers tend to break down over time. Both of these processes can slow your supply chain considerably and result in losses of time and efficiency. So, the question then becomes, which option is right for you?

Here are four factors that matter:How much are you willing to spend?

- How much space do you have in your laboratory?
- How much efficiency are you looking for?
- Where does the equipment fit in your supply chain (more important for larger processors)?

The first two points are based on your budget and your available room. On the third point, the glass reactors allow for processing everything in one reactor system and seeing it as it happens. The fourth point highlights the importance of versatility in that one piece of equipment can be used for multiple things, one of those being decarboxylation.

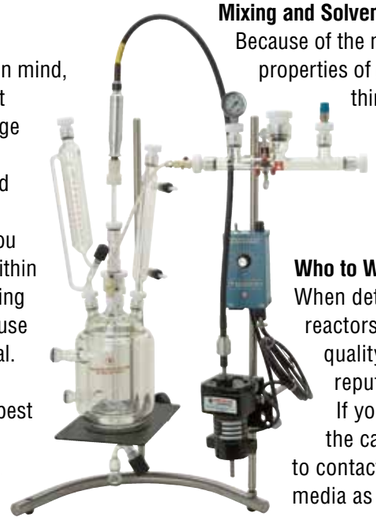
Decarboxylation

Decarboxylation is another process for which the filter reactors are ideal. For processers that are only focused on creating cannabis for inhalation, this is not as important. The user will decarb the cannabis themselves when they light it. However, for all other cannabis products, decarboxylation is essential. It is the process of heating to get your cannabis into the active form and ready for consumption.

Beyond filter reactors, bench-scale or large-scale reactors can handle decarboxylation too. However, there are other equipment options such as rotary evaporators (rotavaps), heating mantles with round bottom flasks, or short paths. The question of how to decide, again, comes down to the same four factors from before.

With the previously mentioned four factors in mind, let's examine the aforementioned equipment options. Rotavaps have the temperature range to decarb since they have the ability to heat to around 100°C. They are not normally used this way; they are made ideally for solvent recovery. If the temperature gets too high you run the risk of distilling other compounds within the solution. Round bottom flasks with heating mantles can work as well, but if you do not use agitation, this method can burn your material.

Short Paths work well but may not be your best choice because of your supply chain. Many processers know when they need to decarb. That time is generally before distilling to prevent CO_2 bubbles forming in their distillation. So, if you are using them for decarbing, they may not be ready to be used for distillation, and that may cause a bottleneck. "Everything comes down to time," said Dr. Carey.



Mixing and Solvent Recovery

Because of the motors, agitators, and temperature properties of glass reactors, they can do many other things. A significant percentage of people who have reactors use them for mixing. If you only have the space or funds for one or two pieces of equipment, reactors can also perform solvent recovery.

Who to Work With

When determining who you want providing your reactors, make sure to consider the company's quality, longevity, customer service, reputation, and if they are made in America. If you want to learn more about glassware for the cannabis processing community, be sure to contact Ace Glass. You can find them on social media as well as at aceglass.com.

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Top 5 Products for ANXIETY

1. **Cinderella's Dream**
   Smokey Point Productions (Spp)
   Type: Edible # Sessions: 11
   Average Rating: 4.9 ★★★★★
2. **Pink Cookies**
   Nw Cannabis Solutions
   Type: Concentrate # Sessions: 13
   Average Rating: 4.6 ★★★★½
3. **Blue Dream CO_2 Oil**
   Happy Cat
   Type: Concentrate # Sessions: 23
   Average Rating: 4.4 ★★★★½
4. **Ostara CO_2 Oil**
   Happy Cat
   Type: Concentrate # Sessions: 18
   Average Rating: 4.4 ★★★★½
5. **Juicy Fruit CO_2 Distilalte**
   Leafwerx
   Type: Concentrate # Sessions: 17
   Average Rating: 4.3 ★★★★½

Top 5 Products for DEPRESSION

1. **Candyland CO_2 Oil**
   Avitas
   Type: Concentrate # Sessions: 10
   Average Rating: 4.9 ★★★★★
2. **Shangri-La CO_2 Oil**
   Happy Cat
   Type: Concentrate # Sessions: 23
   Average Rating: 4.9 ★★★★★
3. **Sugar Babe CO_2 Oil**
   Avitas
   Type: Concentrate # Sessions: 12
   Average Rating: 4.8 ★★★★½
4. **Pink Cookies**
   Nw Cannabis Solutions
   Type: Concentrate # Sessions: 21
   Average Rating: 4.6 ★★★★½
5. **Shangri-La Bho**
   Sticky Budz
   Type: Concentrate # Sessions: 14
   Average Rating: 4.5 ★★★★½

Top 5 Products for BIPOLAR DISORDER

1. **Candyland CO_2 Oil**
   Avitas
   Type: Concentrate # Sessions: 32
   Average Rating: 4.9 ★★★★★
2. **Sugar Babe CO_2 Oil**
   Avitas
   Type: Concentrate # Sessions: 25
   Average Rating: 4.8 ★★★★★
3. **Shangri-La CO_2 Oil**
   Happy Cat
   Type: Concentrate # Sessions: 39
   Average Rating: 4.8 ★★★★½
4. **Pink Cookies**
   Nw Cannabis Solutions
   Type: Concentrate # Sessions: 63
   Average Rating: 4.7 ★★★★½
5. **Soul Assassin CO_2 Extract**
   Leafwerx
   Type: Concentrate # Sessions: 60
   Average Rating: 4.6 ★★★★½

Top 5 Products for PTSD

1. **Cinderella's Dream**
   Smokey Point Productions (Spp)
   Type: Concentrate # Sessions: 30
   Average Rating: 4.8 ★★★★½
2. **Soul Assassin CO_2 Extract**
   Leafwerx
   Type: Concentrate # Sessions: 30
   Average Rating: 4.7 ★★★★½
3. **Zkittlez Honey Oil**
   Skagit Organics
   Type: Concentrate # Sessions: 31
   Average Rating: 4.7 ★★★★½
4. **Blue Dream CO_2 Oil**
   Happy Cat
   Type: Concentrate # Sessions: 70
   Average Rating: 4.6 ★★★★½
5. **Pink Cookies**
   Nw Cannabis Solutions
   Type: Concentrate # Sessions: 35
   Average Rating: 4.6 ★★★★½

Top 5 Products for PMDD

1. **Girl Scout Cookies CO_2 Oil**
   Phat Panda
   Type: Concentrate # Sessions: 10
   Average Rating: 5.0 ★★★★★
2. **Candyland CO_2 Oil**
   Avitas
   Type: Concentrate # Sessions: 24
   Average Rating: 4.9 ★★★★★
3. **Green Crack CO_2 Oil**
   Happy Cat
   Type: Concentrate # Sessions: 16
   Average Rating: 4.8 ★★★★½
4. **Girl Scout Cookies BHO**
   Phat Panda
   Type: Concentrate # Sessions: 14
   Average Rating: 4.8 ★★★★½
5. **Sugar Babe CO_2 Oil**
   Avitas
   Type: Concentrate # Sessions: 24
   Average Rating: 4.7 ★★★★½

Terpenes and Testing Magazine
Terpenes, Horticulture, Chemistry & Analytics

Under Pressure: Rosin Separation Variables and Industry Advancements

By Blake Grauerholz, OutCo

Rosin manufacturing, while simple in its design, requires great attention to detail in order to be optimized and achieve the best quality end product. Throughput, yields, and quality are all extremely variable and dependent on the starting material quality, freshness, the type of pressing screen used, temperature, and the size of press plates. Assuming the starting material is hash, the oil content of the trichome heads will play a heavy role into the amount of material that can physically fit into a pressing screen, as well as the return on rosin oil. Material that is dry, old, or otherwise of low quality will not contain the high concentrations of oils and resins that are desired. This lower grade material weighs less and will therefore take up more volume in your pressing screen as it is composed of more plant matter than it is of oil, which results in lower yields during post processing.

To elaborate more on batch sizing compared to starting material quality, better quality material will yield more rosin. From personal experience, as much as a half-pound (222g) of fresh frozen water hash has been processed in a single press and yielded 76%. Higher yield percentages working with premium quality starting material are achievable and are well documented within the industry. Comparatively, working with low grade cooking hash or kief, even when using the same screen size and method, results in a dramatically lower yields. Lower quality hash, being less rich in the oils attempted to be expelled, fails to compress to the same degree during pre-press preparation, lowering packing density, and compounding the reduction in yields due to oils failing to exit the “brick”. For example, only 180g of low quality hash could be compressed to fit the plate dimensions, and an 18% yield was observed, exemplifying reductions in throughput and yield.

A quality pressing screen is vital to an efficient rosin separation. The pressing screen acts as a filter to keep particulates and non-oleo compounds from mixing with the rosin fraction. The screen is filled with starting material (i.e. hash, kief, flower) and pressed using moderate pressure on the heating plates where the rosin oil is squeezed from the starting material. Input weights are dependent on the aforementioned quality, but also pressing screen size and the quality of the screens themselves. Outco now exclusively works with stainless steel mesh screens from *Pure Pressure* as opposed to more traditional nylon mesh. These stainless-steel screens are the new standard primarily due to their resistance to blowouts, which is when the screen ruptures and leaks the starting material into the rosin oil. In addition to overall durability, stainless steel screens possess increased throughput potential. The large mesh sheets allow the user to freely adjust the press size and corresponding input weight to suit their batch size, rather than having to conform to pre-sewn fabric bags. Another added benefit of stainless steel versus fabric bags is the elimination of contamination due to nylon fibers coming loose and getting stuck in the extract, or from the actual manufacturing facilities that produce the nylon bags.

Filling open stainless steel screen sheets with larger quantities of material requires special handling and experience compared to nylon mesh. With preformed, sewn nylon mesh bags, operators can pour granulated hash or kief into the bag using a funnel. This results in a loose packing density that limits production throughput and can also create an inconsistent packing density that can cause a rupture in the bag once under pressure. Very little success has been seen when pressing with a single nylon screen and leading

Step 1

Step 2

Step 3

Step 4

Step 5

Step 6

to often double, or triple bagging the hash when using nylon bags. This helps reduce blow outs but at the cost of aerating and agitating the rosin oil as it has to flow past multiple layers of screens. This agitation causes a multiphasic, amorphous reaction in the finished product causing premature “buddering” texture. This buddered product loses its aroma and flavor faster.

To fill the large volume capacity of stainless steel bags, pre-forming the hash is necessary. The hash must be pressed and shaped into a small brick on parchment paper so that the stainless-steel screen can be folded around the hash mass. Pre-press molds make this task simple, although processors that are comfortable with handling high-quality resin can form the material free-hand, without the aid of tools. This also allows a perfectly shaped brick to be made that fits your pressing plates precisely. Once formed, the screen can be wrapped and folded around the hash like a burrito. While more durable, stainless screens can still blowout under too much pressure and/or if the hash brick is too thick. Methods can be developed for cultivars by testing packing density, pressure ramp up times, and temperature. This will take some experimenting for every batch processed but will cut down on production time and costs thanks to the increased capacity stainless steel screens offer.

*In collaboration with *Pure Pressure*

The Perspective from Italy: Cannabis, Hemp, Extractions, and Analysis

By Giuseppe Desando & Giovanni Isoldi, Materia Medica processing, & Stefano Zaccherotti, Società Italiana Canapa Medica (Sicam)

MEDICAL CANNABIS

Since 2013 medical cannabis can legally be prescribed by physicians to treat an array of diseases showing evidentiary data in the scientific literature. Between 2014 and 2015, the first local cultivation began in Florence in the military chemical-pharmaceutical plant (SCFM). By 2018, the SCFM produced 150 kg of dried flowers. Notwithstanding additional imports from Bedrocan of 450 kg (the Netherlands) and Aurora of 100 kg (Pedanios, Germany), the 600-700 kg state production is unable to meet the national demand for medical cannabis (estimated at around one ton). Although the SCFM remains the only authorized institution cultivating medical cannabis in Italy, there is a growing possibility for private companies to begin pharmaceutical operations (EuGMP) under stringent regulations after government authorization (tender expected in 2019). Table 1 highlights the cultivars available in Italy.

COMMERCIAL Name	THC (%)	CBD (%)	Manufacturer
BEDROCAN	22	<1	BEDROCAN(NL)
BEDICA	14	<1	BEDROCAN(NL)
BEDROBINOL	13	<1	BEDROCAN(NL)
BEDIOL	6.5	8	BEDROCAN(NL)
BEDROLITE	<1	9	BEDROCAN(NL)
FM1	14-15	<1	SCFM(IT)
FM2	5-8	8-12	SCFM(IT)
PEDANIOS 22:1	17-26	<1	AURORA (CA)
PEDANIOS 8:8	5-8	6-12	AURORA (CA)
PEDANIOS 1:9	<1	8-10	AURORA (CA)

Table 1: Cultivars available in Italy

Currently, the Italian cannabis varieties for therapeutic use include FM1 (THC 13-20%, CBD <1%), and FM2 (THC 5-8%, CBD 6.5-12%), selected to act as a counterpart to the two Dutch cultivars more commonly used, i.e., Bedrocan and Bediol. Medicinal cannabis is only sold in pharmacies as a ground flower or galenic preparation made by pharmacists in forms like oils and capsules. The main difficultly is that the production of galenic preparations must be accompanied by a quantitative analysis of the content of the main cannabinoids. This create difficulties for pharmacies with inadequate infrastructure and know-how to prepare such formulations. Additionally, this results in great product variability between individual pharmacies. Many pharmacies have updated and purchased analytical tools like HPLC (high pressure liquid chromatography) to perform internal analyses.

Since June 2017, pharmacists have sold cannabis relatively below cost. With the update of the national medicine tariff, a fixed price of 9 euros (just over $10) was imposed on cannabis. Pharmacies spend 6.88 to 10 euro per gram ($7.73-11.23) before shipping costs (which average 20 euros, or $22.46) in addition to the 22% tax rate. Supply difficulties, galenic analysis requirements, profit limitations for pharmacies and a lack of standardization are the mains hurdles restricting the growth of the Italian medical cannabis sector.

HEMP: THE ITALIAN CANNABIS LIGHT PHENOMENON

A December 2016 law made the cultivation of industrial hemp legal without specific authorization in Italy. While limited to EU certified cultivars [1], farmers are now permitted to grow hemp. These varieties, used for fibers and seeds, have been cultivated for centuries in Italy. In 2017, Easyjoint made CBD flowers readily available on the Italian market with the introduction of 'Cannabis Light'.

Currently, the only regulated aspects of the Italian hemp industry concern how the product is labeled and the quantification of THC content. The regulatory framework has yet to be developed, and cannabis currently occupies an ambiguous legal status where the flowers are commercially referred to as "collectors' items, decorative plants, or for technical use only".

These cultivars are listed and certified by the European community as industrial, i.e., capable of developing THC concentrations not exceeding 0.2%. Ironically, some of these varieties, while grown for decades in Italy, are more likely to exceed the THC limit than other varieties on the market in the rest of the world. For example, the Carmagnola cultivar, that takes its name from the municipality where it was historically cultivated, was used centuries ago to create rope and fabric, yet tends to reach THC levels well above 0.6% (the THC limit imposed on farmers in Italy).

While the 2016 law was intended for the industrial use of hemp, it has inadvertently resulted in the explosion of the "cannabis light" market with proliferation of over 600 businesses and retailers, so-called "hemp shops", that produce an annual turnover of more than 40 million euros (nearly $45 million). In Europe last year, agricultural land dedicated to hemp cultivation has increased from 8,000 to 33,000 hectares. After the Netherlands and ahead of Lithuania, Italy is the fourth largest cultivator of hemp with 2,300 hectares of agricultural land. Since 2016, cannabis trade and research expos have become widespread and numerous, with around 20 significant fairs throughout Italy. Many growers have quickly evolved from cultivating certified varieties to more interesting genetic profiles based on terpenes. Although these varieties are not present in the official European registry, it is virtually impossible for authorities to properly evaluate the plant's origin. In fact, the only document growers require to place their products on the market is the original label from the seed bag demonstrating the plant is a EU certified cultivar. Regulatory controls are limited to THC analysis of inflorescences from random crop testing, without involving any detailed genetic analysis that would be extremely expensive. The Italian market is flush with low quality products, depending upon analysis derived from unproven laboratories that focus on THC content rather than dangerous contaminants. Nevertheless, the sale of such dubious products is unrelenting.

TESTING

Aside from internal analysis in local pharmacies, laboratories offering analytical services to hemp and hemp derivatives rely on non-standardized testing methods. The only possible accreditation is for public laboratories or research institutes, while facilities offering cannabis testing are largely non-specialized labs. In order to make label claims and demonstrate legality, growers only test for CBD and THC content. At the same time, cannabis-testing labs remain in their infancy and lack standardization. Materia Labs was founded with the motivation to offer a dedicated analytical platform for the analysis of hemp. While we

ANALYTICAL PARAMETERS	SPECIFICATIONS	REFERENCES (methods)
Appearance	Brownish-green milled or whole dried flowers with a characteristic smell	Visual
Identity (Chromatography) • a) THC, CBD, other cannabinoids • b) terpenes: myrcene, linalool, limonene, bisabolol, *beta*-caryophyllene, caryophyllene oxide	a) compliant b) compliant	Validated method
Assay	THC: 17.0-26.0% (*) CBD (Cannabidiol): < 1% (*) (*) expressed as the sum of the acidic form and the decarboxylated form	Validated GC/FID method or other validated chromatographic methods
Other cannabinoids:	Cannabigerol (CBG): < 1.0% Cannabichromene (CBC): < 1.0% Tetrahydrocannabivarin (THCV): < 1.0% Cannabinol (CBN) < 0.5% (*) (*) not more than 1.0% at the end of expiration	Validated GC/FID method or other validated chromatographic methods
Microbiological purity (For preparation of infusion or decoctions using boiling water and inhalation of vaporized products)	Requested method to reduce the microbial contamination: gamma irradiation at the dose of 10 Kilogray, or equivalent method TAMC < 102 CFU/g TYMC < 101 CFU/g Absence of *Salmonella* (in 25g) Absence of *Escherichia coli* (in 1g) Absence of Bile tolerant gram-negative bacteria Absence of *Staphylococcus aureus* (in 1g) Absence of *Pseudomonas aeruginosa* (in 1g)	Ph.Eur. 5.1.4-1, 2.6.12, 2.6.31
Aflatoxins	Total (B1:B2; G1 & G2) < 4µg/kg B1 < 2µg/kg	Ph. Eur 2.8.18
Heavy metals	Cd: < 1.0 ppm Hg: < 0.5 ppm Pb: < 5 ppm As: < 1 ppm	Ph. Eur 2.4.27
Foreign materials	Absence of stalks, fibers, insects or other pests	Ph. Eur 2.8.2
Pesticides and fumigants	Statement of non-use (mandatory)	
Loss on drying	< 10% moisture	Ph.Eur. 2.2.32 Method C

FOR ACCEPTANCE OF THE ABOVE SPECIFICATIONS AND PRODUCTION ACCORDING TO GOOD MANUFACTURING PRACTICE (GMP) GUIDELINES AND GOOD AGRICULTURAL AND COLLECTION PRACTICE (EU COMMISSION DIRECTIVE 91/356/EEC, AS AMENDED BY DIRECTIVE 2003/94/EC)

Table 2: Quality requirements for GMP Italian Medical Cannabis

PENNSYLVANIA

releaf.

Data collected by Releaf App
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Top Products for ANXIETY

1. **Reserve-Northern Lights #5**
   Cresco
   Type: Concentrate # Sessions: 11
   Average Rating: 5.0 ★★★★★
2. **Granddaddy Purple**
   Vireo
   Type: Concentrate # Sessions: 6
   Average Rating: 4.8 ★★★★★
3. **Orange Cookies**
   Dank
   Type: Concentrate # Sessions: 5
   Average Rating: 4.6 ★★★★½
4. **Blue Dream**
   Ilera
   Type: Concentrate # Sessions: 12
   Average Rating: 4.5 ★★★★½
5. **Golden Goat**
   Ilera
   Type: Concentrate # Sessions: 15
   Average Rating: 4.4 ★★★★½

Top Products for DEPRESSION

1. **Golden Goat**
   Ilera
   Type: Concentrate # Sessions: 8
   Average Rating: 4.4 ★★★★½
2. **Blueberry Skunk**
   Liberty Bell Blend
   Type: Flower # Sessions: 3
   Average Rating: 4.3 ★★★★½
3. **Rhythm for a Cause**
   Rhythm
   Type: Concentrate # Sessions: 3
   Average Rating: 4.3 ★★★★½
4. **Sunset Sherbert**
   Dank
   Type: Concentrate # Sessions: 3
   Average Rating: 4.3 ★★★★½

Top Products for MIGRAINES

1. **Lemonhead**
   Dank
   Type: Concentrate # Sessions: 6
   Average Rating: 4.5 ★★★★½
2. **Rhythm Energize**
   Dank
   Type: Concentrate # Sessions: 12
   Average Rating: 4.1 ★★★★☆

Top Products for PTSD

1. **Northern Lights**
   Standard Farms
   Type: Concentrate # Sessions: 5
   Average Rating: 4.8 ★★★★½
2. **Mendo Breath**
   Standard Farms
   Type: Concentrate # Sessions: 6
   Average Rating: 4.7 ★★★★½
3. **Reserve-Northern Lights #5**
   Cresco
   Type: Concentrate # Sessions: 19
   Average Rating: 4.6 ★★★★½
4. **Granddaddy Purple**
   Vireo
   Type: Concentrate # Sessions: 7
   Average Rating: 4.6 ★★★★½
5. **Orange Cookies**
   Dank
   Type: Concentrate # Sessions: 8
   Average Rating: 4.5 ★★★★½

Top Products for CHRONIC PAIN

1. **Ican Capsules**
   Terrapin Pennsylvania
   Type: Pill # Sessions: 10
   Average Rating: 5.0 ★★★★★
2. **Long's Peak Blue**
   Terrapin Pennsylvania
   Type: Flower # Sessions: 18
   Average Rating: 4.8 ★★★★½
3. **Moonshine Haze**
   Terrapin Pennsylvania
   Type: Flower # Sessions: 19
   Average Rating: 4.5 ★★★★½
4. **Cookies N Cream**
   Ilera Healthcare - Stick Vape
   Type: Flower # Sessions: 10
   Average Rating: 4.4 ★★★★½

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have yet to perform proficiency tests, as we await more international industry standardization, Materia Labs is making strides in the professionalization of hemp analysis. In February at the ASTM conference in Rome, an institution for international standards, hardly any Italian organizations were in attendance. In this landscape, with the hemp bubble about to burst, the market focus will shift to extracts and CBD oils. With extracts and oils, quality is far more critical because of the possibility of contaminants to become more concentrated. Studies on the quality of CBD oils in Italy and Europe have demonstrated, irrespective of label claims, the presence of contaminants and THC. [2]

MEDICAL CANNABIS AND HEMP EXTRACTION INDUSTRY

Due to the unavailability of pharmaceutical extracts from medicinal cannabis on the national market, pharmacists retain the only possibility of administering THC in the form of oil or other preparations. Many pharmacists have begun to perform small maceration or ultrasound extractions, suspending the extract in olive or MCT oil in a 1:10 solvent-drug ratio [3]. Only a few pharmacies have begun to prepare extracts in the form of resins with the use of ethanol and rotary evaporation.

Today, the use of more advanced technologies is not possible, as pharmacists prepare the product only after the prescription (extemporary preparation) is received and are thus unable to keep the product in stock in advance. For the pharmaceutical sector there is growing speculation about a SCFM project to produce a standardized, pharmaceutical-grade GMP (Good Manufacturing Practice) oil, but this can only be achieved after crop yields are significantly increased.

For the hemp extraction industry, the 0.2% THC legal limit in Italy and Europe has limited extracts to states with licenses (*ex*. Germany and the Czech Republic). As a result, nearly all of the extraction of legal hemp is 'homemade' and non-professionalized, limiting both quality and safety. The demand for third-party extraction by farmers with unsold hemp is very high and absorbed by non-professionalized home operators. While some companies have developed processes that avoid the concentration of THC and produce good quality products, the productivity is not enough to meet the demand. Additionally, European authorities do not consider CBD products food, leading to the presence of products on the market with questionable content and safety.

NEW MEXICO

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Top 5 Products for PTSD

1. **Chocolate Bar**
   Cannaceutics
   Type: Edible # Sessions: 29
   Average Rating: 4.9 ★★★★★
2. **Elevated Indica Milk Chocolate Bar**
   Elevated
   Type: Edible # Sessions: 93
   Average Rating: 4.7 ★★★★½
3. **Lavender**
   Me
   Type: Flower # Sessions: 27
   Average Rating: 4.7 ★★★★½
4. **Warlock**
   Ultra Health
   Type: Concentrate # Sessions: 67
   Average Rating: 4.4 ★★★★½
5. **ACDC**
   Curio Wellness
   Type: Concentrate # Sessions: 58
   Average Rating: 4.3 ★★★★½

Top 5 Products for MIGRAINES

1. **Elevated Indica Milk Chocolate Bar**
   Elevated
   Type: Edible # Sessions: 12
   Average Rating: 5.0 ★★★★★
2. **Cannaceutics Fudge**
   Cannaceutics
   Type: Edible # Sessions: 5
   Average Rating: 5.0 ★★★★★
3. **Cherry Chocolate**
   Ultra Health
   Type: Edible # Sessions: 5
   Average Rating: 4.6 ★★★★½
4. **Marqaha**
   Marqaha 50:50
   Type: Tincture # Sessions: 5
   Average Rating: 3.4 ★★★½☆
5. **Girl Scout Cookies**
   Verdes
   Type: Flower # Sessions: 13
   Average Rating: 3.3 ★★★½☆

Top 5 Products for FIBROMYALGIA

1. **Peppermint Patty**
   Cannaceutics
   Type: Edible # Sessions: 26
   Average Rating: 4.9 ★★★★★
2. **Chocolate Bar**
   Cannaceutics
   Type: Edible # Sessions: 40
   Average Rating: 4.9 ★★★★★
3. **Elevated Indica Milk Chocolate Bar**
   Elevated
   Type: Edible # Sessions: 128
   Average Rating: 4.8 ★★★★½
4. **Granddaddy Purple**
   Terpenated Cartridge
   Type: Concentrate # Sessions: 37
   Average Rating: 4.3 ★★★★½
5. **Ghost Haze**
   Ultra Health
   Type: Concentrate # Sessions: 43
   Average Rating: 4.1 ★★★★☆

Top 5 Products for ANXIETY

1. **Cannatonic-Verdes**
   Verdes Foundation
   Type: Flower # Sessions: 14
   Average Rating: 5.0 ★★★★★
2. **Elevated Indica Milk Chocolate Bar**
   Elevated
   Type: Edible # Sessions: 14
   Average Rating: 5.0 ★★★★★
3. **Cherry Chocolate**
   Ultra Health
   Type: Edible # Sessions: 14
   Average Rating: 4.9 ★★★★★
4. **Pull N Snap**
   Infusiasm
   Type: Concentrate # Sessions: 13
   Average Rating: 4.7 ★★★★½
5. **Warlock**
   Ultra Health
   Type: Concentrate # Sessions: 50
   Average Rating: 4.3 ★★★★½

Top 5 Products for DEPRESSION

1. **Kobe**
   Urban Wellness
   Type: Flower # Sessions: 7
   Average Rating: 5.0 ★★★★★
2. **Micro-Dose Ice Milk Chocolate**
   Bhang
   Type: Edible # Sessions: 10
   Average Rating: 4.9 ★★★★★
3. **Querkle**
   Urban Wellness
   Type: Flower # Sessions: 6
   Average Rating: 4.8 ★★★★½
4. **Shark Shock**
   Minerva
   Type: Flower # Sessions: 233
   Average Rating: 4.8 ★★★★½
5. **HDark Chocolate Bar**
   Elevated
   Type: Edible # Sessions: 7
   Average Rating: 4.7 ★★★★½

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With these factors in mind, Materia Medica Processing was created, utilizing methods that allow us to obtain a THC-free (under detection limits) product, with a CBD concentration that exceeds 70%. This allows us to avoid product contamination problems, vary the concentration of final products, and guarantee maximum safety through a series of analyses that range from cannabinoid content, to pesticides, heavy metals and microbiological contaminants.

Italian extraction equipment solutions remain limited, with only CO_2 and fluorinate gas providers. Yet, our long tradition in medicinal plant extraction (Aboca, Indena, Epo and many more), and established specialized pharmaceutical sector both lend easily transferable processes and solutions to the world of cannabis. As a result, as soon as the regulatory framework is clear, the industry is poised for rapid growth. Additionally, we can also rely on the existing industry excellence of our neighbors (as demonstrated in the use of European products in the American market) like Germany for the production of distillation equipment and France in chromatography instrumentation.

In Italy, where artisanal and agricultural products excel, the rapid development of the cannabis sector can help promote a new and better understanding of the plant.

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TO CARRY THE TORCH
Welcoming the Next-Generation of Cannabis Scientists

Letter from **the Editor**

April 15th, 2019

This issue serves as a prelude to our upcoming conference, CONCENTRATION 2019. We wanted to divide and conquer each pathway that we explore in our publications with our conferences this year, and as such, Concentration 2019 will focus on the extraction of cannabis and hemp. Later in early August, CannaFarmCon 2019 will delve into cannabis farming and agronomy. The quantitative analysis of analytes and contaminants in botanical plants and products is ubiquitous, therefore, analytical chemistry will be integral to each symposium we do.

The analytical chemist knows that, blessing or curse, in any direct he or she looks, there is data to be studied. It's *all* data, isn't it? From the sampling of whatever, measurement by whichever device, to the processing of the results. These words could describe a sensory analysis of a smell, perhaps the deceivingly funky fragrance of Vic Secret hops; or the analysis of cannabis inflorescences for pesticide and mycotoxin contamination. Sample, measure, process…these three terms are at the core of analytical decision making. And whether you're a cannabis farmer, processor, scientist, medical doctor, regulator, caregiver, consumer, or even skeptic, there are analytical decisions to be made. Cannabis analytics define the legitimacy of the industry. They validate it.

Which brings us to this issue. Given that CONCENTRATION 2019 will be divvied up between extraction and analytical science and technology, we found it fitting to mirror those themes herein. One of the most prevalent topics in the media regards the failure of cannabis samples for containing contamination beyond permissible levels. Some of these samples made it into dispensaries, forging a scenario rife with finger-pointing, fines, decreased legitimacy within the industry, and increased levels of consumer mistrust. In short, a lose-lose situation for all those involved.

Therefore, it's integral for laboratories and cannabis farmers and product manufacturers to arm themselves with defensible data. To that end, instrument manufacturers are consistently venturing out onto the frontier, designing more sensitive and specific instrumentation, all the while aiming for lower costs. They are also increasingly developing turnkey solutions such that those with less experience with analytical instrumentation can still utilize the tool in their quest for wisdom.

You may find this issue to be heavy on mass spectrometry, and for good reason. While chromatographical separations have been well-discussed, increasing demands for ensuring product safety require more sensitive detectors. Consider pesticide analysis. Given the glut of analytes that need to be quantified when declaring a product pesticide-free (or at least below permissible concentrations), instrumentation that not only resolves the assortment of chemicals, but also pushes detection limit boundaries can better ensure success.

There's no reason or excuse to be without data. Or to gamble on product quality. While meticulous labs act as industry sentinels, protecting the populace from contaminated products, there's accessible knowledge to excavate, for any cannabis business to embrace.

Until next time,

Cheers.

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Contents

To Carry the Torch: The Next-Generation of Cannabis Scientists

By Jason S. Lupoi, PhD.

Have you ever pondered the thought of nearly 16,000 chemists descending upon an odd land where kids and adults alike frolic with mice, dogs, and ducks? I *lived* it in early April 2019. And while the complete scene was rather daunting, a specific happening beckoned like a revival. The occasion was the American Chemical Society (ACS) National Meeting. The city was none other than Orlando, Florida. And while hordes flocked to vacate as Mouseketeers, a sizeable slice of the scientific community came to hear the good word regarding the latest in chemical research and technology.

I was in attendance specifically to support the ACS-Cannabis Chemistry subdivision, called CANN. You may recall from your read of a previous *T&T* issue [1] that this year's meeting recognized several scientists who have added to the foundation of cannabis research and scientific inquiry, through a scholarship and award named after Mahmoud ElSohly. Before delving into the specific chapters of this story, it's worth introducing the cast. Julia Bramante is the current Chair of CANN, and also is employed as the Marijuana Reference Laboratory Lead Scientist at the Colorado Department of Public Health and Environment. Ezra Pryor is the Founder of CANN and is the Applied Market Specialist at Heidolph North America. Kyle Boyar is the Vice Chair, and Field Applications Scientist at Medicinal Genomics Corporation. Amber Wise, Ph.D. is the Scientific Director of Medicine Creek Analytics. Together, these four governed the CANN symposium, while the ElSohly award winners Stephen Goldman (PhytaTech), Bryant Jones (University of Minnesota), Monica Vialpando, Ph.D. (Vialpando, LLC), Michael Coffin (Bloom Farms), and a host of other scientists regaled the audience with wit and expertise.

The advance of cannabis science is paramount to greater legitimization, validation, and acceptance. The cannabis plant has bequeathed a generous glut of medicinal molecules for researchers to identify, quantify, and understand, as modern Earthlings resuscitate ancestral knowledge, this time, however, bolstering anecdote with scientific fact. Mahmoud ElSohly, Ph.D. has contributed a decent chunk of the available scientific literature on cannabis chemistry, and so, it was inspiring to see ElSohly pass the proverbial torch to the awardees, whose passion for cannabis is undeniable, and limitless. This article canvases some of the event's bullet points.

Several researchers came equipped with novel methods and data to share. Mass spectrometry was a clear focal point (as it is in this issue), given the analytical power it provides, and the well-founded emphasis on contaminant detection in cannabis. Zack Iszard, of Confidence Analytics, showcased some interesting molecular findings unearthed in commercial cannabis concentrates, which he hypothesized could be isomers of THC. He offered up his data to anyone with more time to data mine.

Michael Coffin presented data illustrating significant differences between the reported terpene profile from a commercial blend of terpenes meant to convey the fragrance of Granddaddy Purple (GDP), and what terpenes were *actually* measured in natural GDP. This kind of finding instructs as to why it's important to characterize what molecules specifically distinguish one cultivar from another, such as the work of Justin Fischedick, Ph.D. (a past CANN awardee) and Arno Hazekamp, Ph.D. [2, 3]

Jahan Marcu, Ph.D. carved through science fiction and hypocrisy, detailing how THC makes an appearance on three different drug schedules (I, II, III), and how the GW Pharmaceuticals product Sativex® has been in phase 3 clinical

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trials since 2004, while it has been licensed for use in 30 other countries. One of the more sobering images shown in Marcu's presentation depicted the amounts of heroin and fentanyl that constitute the LD_{50}, or the dose that killed 50% of the test population (22 and 11 mg per kilogram of body weight, respectively), and then juxtaposed these miniscule amounts with the dose representing the LD_{50} of THC, which, as Marcu noted, *could* kill you if dropped from above (It was on a pallet).

Monica Vialpando, Ph.D. provided insight into cannabis product formulation, sharing her eyewitness scientific discovery through both tribulation and triumph. Her discussion exemplified practicing the scientific method when performing any kind of R&D, and harkened to the thought that in research, the balance of diligence and patience is paramount for success. One of her recent formulations is a cannabis-based sunscreen, the idea for which stemmed from the concept that THC can protect plants from ultraviolet light during growth stages.

Mahmoud ElSohly also spoke on the program at Ole Miss, saying that even though his group has the government contract for working with cannabis, they are also probably the most restricted in what they can do. "I welcome the opportunity to work with other growers," ElSohly stated. ElSohly has spent a career analyzing cannabis chemistry. His recent tally of molecules in cannabis is now at 565, across 25 different chemical classes. And given ElSohly's role at the Research Institute of Pharmaceutical Sciences at the University of Mississippi, he naturally spoke on the medicinal aspects of cannabinoids, and the often-seen U-shaped activity curve, where a Goldilocks' zone at moderate concentrations provides enhanced benefits over low and high dosages.

One of the coolest statements of the event came from Kyle Boyar, who reported on having analyzed medicinal, cannabis-infused pho. Boyar's seminar delved into the world of microbial testing, and the limitations and successes of the various methods for quantifying bacterial contamination. "How can you determine the difference between *Aspergillus* species from plating when everything looks the same?", Boyar questioned. Boyar also discussed the interference of citric acid, a common ingredient in edibles, on the detection of coliform, and cautioned against using gamma irradiation on cannabis plants in efforts to rid the plant of bacterial growth, since (a) the radiation targets the surface of the cannabis flower, but does not necessarily eradicate endophytes *within* the flower, and (b) can result in some serious losses of monoterpenes.

Brad Douglass, of The Werc Shop, provided a wonderful discourse on the concept of adding flavoring agents into cannabis products. "Are we causing ourselves damage as a society?", he asked. "Are some products truly toxic?" There is no prohibited ingredient list, and the mushrooming world of nicotine-based vape liquids has caused a tizzy with regulators, as science tries to catch up with potential hazards. Even some terpenes could pose problems. Douglass mentioned that humulene is not on the Food and Drug Administration's Generally Recognized as Safe (GRAS) list, and is not allowed as a flavor additive in some states. If you happen to be drinking an IPA like I am, that fact is a bit unsettling, right?

In most states, consumers can stop and smell the flowers (sadly, no dice on this in Pennsylvania, yet). Some may purchase select cultivars based on smell. Enter sensory psychologist Avery Gilbert, Ph.D., who studied the selection of specific cannabis scents. Gilbert evaluated the data supplied by 61 panelists, who were surveyed on 48 odors used in describing the complex fragrances cannabis terpenes create. He found two main classes: woody, earthy, and herbal; and citrusy, lemony, pungent, and sweet. And amazingly, although the fragrance of cannabis has absolutely zero to do with potency, the panelists felt that the citrusy cultivars had higher potency. What's more, when posed the question as to how much they'd be willing to pay for the product, they proffered $2 more per gram.

These were the highlights of the ACS-CANN symposium, filtered through my eyes and ears. While I would bet on there being more than a few handfuls of mortified squares shocked that cannabis was on the intellectual menu for three straight days, what was very apparent was that the audience was eager to educate themselves, and enthralled at the relevance, importance, and necessity of cannabis science being showcased during an ACS National Conference. After all, can you think of anything more of a gorgeous topic, so rife with chemical, botanical, agronomical, psychological, and medicinal opportunities for scientific inquest, wrapped up in seemingly extraterrestrial inflorescences? I know I can't.

And my sincere apologies, Stephen Goldman, for not making it to your talk. Apparently the Pittsburgh International Airport had an airport-wide "computer glitch", which delayed my carefully crafted travel plans. For those of you who are reading this that missed out on the ACS-CANN symposium, you can visit with the ElSohly award winners at Concentration 2019, for a revisit and celebration of their offerings to cannabis science.

Everything is Turning Up Terpenes!

By Cindy S. Orser PhD., Digipath Labs NV

In this ever fast evolving field of cannabis testing, innovation and entrepreneurship, we've now turned our full attention to terpenes. While everyone else had been solely fixated on the amount of THC contained in everything cannabis, terpenes were not only being lost in the extraction and purification process but also in the discussion. As many now realize, terpene content now deserves equal label space to cannabinoids. In fact, terpenes are the distinguishing chemicals in the cannabis experience, what Ethan Russo dubbed the "Entourage Effect" years ago and now others the "Ensemble Effect."

Terpenes are widely produced by plants, insects and mammals. Yes, even you make some very important terpenes! In fact, both sex hormones, progesterone and testosterone are *endo*-terpenoids as well as cholesterol. Plants produce terpenes as volatile organic compounds (VOCs) to communicate with their environment, to attract pollinators, to repel insect and microbial pests. Terpenes are the dominant component of essential oils derived from plants. In cannabis, terpenes accumulate in glandular trichomes from 1 to 3% weight in the plant. [1-3] Terpenes, considered "generally regarded as safe" or GRAS compounds by the FDA, are widely used in the fragrance and flavoring industry and are just now coming into their own for their perceived physiological effects. In general, some terpenes are thought to be sedative based on animal studies showing their interaction with mammalian receptors, GABAA and GABA, the main inhibitory neurotransmitter in the brain that exerts its effects through reducing neuronal excitability and are the target of widely used anti-depressants.

Luckily, Nevada was the first and still only one of two states (Pennsylvania being the other) that require terpenoid analyses by third party independent testing labs. Because of that, Digipath Labs was amassing volumes of terpene data, that when analyzed by principal component analysis (PCA), demonstrated that terpene chemoprofiles define cultivar associations. In Southern Nevada, for example, there are three distinct terpene clusters found within Drug Type I cannabis which represents 98.3% of what is being grown under 404 different cultivar names. [1, 2, 3] Now that the rest of the country is catching up with Nevada, the actual analytical analysis of terpenes is a key topic.

Terpenes are hydrocarbons made up of isoprene subunits assembled into either monoterpenes (two isoprene units) or sesquiterpenes (three isoprene subunits). They can be linear or cyclized and if a terpene contains elements other than carbon and hydrogen, they are called terpenoids. There are more than 100 different cannabis terpenoids but with only a handful being prevalent including *beta*-myrcene, limonene, *beta*-caryophyllene, *alpha*-pinene, *alpha*-humulene, *beta*-pinene and terpinolene.

Terpenes have high vapor pressures, are extremely volatile, and thus are excellent candidates for static headspace gas chromatography (GC) analysis. There are analytical choices to be made, starting with instrumentation: Headspace-GC-mass spectrometry (MS), GC-MS/MS, GC-FID-MS, or HPLC-FID (HPLC = high performance liquid chromatography; FID = flame ionization detectors). The next decision is whether or not to extract the cannabis sample matrix with a solvent, such as methanol or simply place a small amount (10-50 mg) of the sample into a headspace vial, which is then capped, heated up and a sample of the gas phase is taken and sent to the detector. Unfortunately, this is not a simple decision, since cannabis product matrices are extremely varied and plant material will not dissolve in solvent. In general, extraction gives more reliable quantitative numbers.

The second decision is which detector to use: flame ionization detector (FID) or mass selective detection (MSD) or both. The operation of the FID is based on the detection of ions formed during combustion of organic compounds in a hydrogen flame. The generation of these ions is proportional to the concentration of organic species in the sample gas stream. One advantage of the FID detector is that it will not saturate like a MS detector. The FID also has an expanded linear range from 0.01 to 1.5% with a single injection which takes about 35 minutes to run.

If using intact samples, a GC-headspace-MS has the advantage of not requiring organic solvents (and their ultimate disposal) which can also potentially interfere with the chromatographic analysis or contaminate the GC system. The overriding advantage of GC-MS is that it provides a means of peak identification and purity, using spectral data matching against reference spectra in a library such as NIST MassHunter, with a run time of around 18 minutes for 22 terpenoids. The individual terpene concentrations in the unknown samples are determined by using a linear regression analysis of the linear calibration curve constructed from the known calibrator levels of individual terpene certified reference materials.

Innovative instrument purveyors have combined headspace-GC-MSD with FID using a capillary flow technology that

splits the column effluent in a precise manner to the two detectors. The use of both FID and MSD allows for more comprehensive data analysis as terpenes are often in high percent values, which would saturate the MS, whereas the FID will not saturate, while illustrating the advantage of the MSD's selectivity to rule out interfering species that may otherwise be misidentified as a target analyte when using FID detection alone. Agilent has published a technical note combining the dual detector approach for the analysis of 22 terpenes in less than 6 minutes using both FID detection for quantification and extended linear range and MSD for terpene speciation. [4]

A lack of standardization in testing methods is hampering the cross-comparability of terpene chemotype data as well as all other analytes between states and between testing labs. Nonetheless, terpene content contributes to potency and should be included in cannabis and cannabis-based product analyses. Terpene analysis also provides useful chemoprofiling discriminatory data for deconvolution of the current cultivar naming. And lastly but importantly, terpenes are the basis for consumer perception of cannabis aroma.

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HPLC Method Development for Baseline Resolution of Seventeen Cannabinoids

By Edward Franklin, PhD. and Melissa Wilcox, Regis Technologies

The global cannabis industry is growing rapidly, with many countries and US states adding regulatory frameworks for medical and recreational programs. Quality control is an essential component in protecting the health and safety of the consumer in this emerging market, and there is increasing demand upon cannabis testing laboratories for analytical determination of multiple cannabinoids. Current regulations surrounding potency vary by jurisdiction, but usually require testing for the active forms of THC and CBD. In addition, many require testing for the acid forms, THCA and CBDA, along with other cannabinoids like CBG, CBGA, THCV, CBC, CBL, and CBN. As regulations evolve, and as research interests in minor cannabinoids expand, it is important to have robust analytical methods in place that are capable of meeting those needs.

The preferred technique for quantifying cannabinoids is HPLC (High Pressure Liquid Chromatography) with detection by UV (Ultraviolet) or MS (Mass Spectrometry). In general, all approaches to HPLC method development look to balance several elements, among which are the ultimate goals of the analysis, resolution of target compounds and potential interferences, speed, and assay robustness. Upon evaluating the molecules of interest in terms of their charges, polarities, and other functionalities, chromatographic method developers turn their focus to column and solvent selection, pH conditions, buffer selection and concentration, temperature, etc. Specific approaches can differ depending upon the primary goals of a separation. For example, if comprehensive characterization of a complex sample is desired, approaches to maximizing overall separation at the expense of analysis time may be acceptable. If, on the other hand, resolution of only a particular critical pair is required, speed and selectivity (for the crucial pair) may be the primary focus.

With these concerns in mind, we set out to develop an HPLC method capable of fully resolving 17 cannabinoids in a minimal amount of time. Additionally, a second objective concerning the resolution of a specific critical pair of THC isomers (Δ8-THC and Δ9-THC) was explored.

Seventeen analytical reference cannabinoid standards (1 mg/mL) were acquired from Cerilliant (Round Rock, TX, USA) and combined to a final component concentration of approximately 59 µg/mL in 53:47 methanol:acetonitrile. The mixture was composed of Δ8-tetrahydrocannabinol (Δ8-THC), Δ9-tetrahydrocannabinol (Δ9-THC), cannabichromene (CBC), cannabichromenic acid (CBCA), cannabicyclol (CBL), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabidivarinic acid (CBDVA), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabinol (CBN), cannabinolic acid (CBNA), exo-tetrahydrocannabinol (exo-THC), tetrahydrocannabinolic acid A (THCA-A), tetrahydrocannabivarin (THCV), and tetrahydrocannabivarinic acid (THCVA).

Column:	Evoke C18; 15 cm x 4.6 mm; 3 µm	
Instrument:	Shimadzu Nexera	
Mobile phase A:	Water + 0.1% formic acid (+ ammonium formate concentration specified with chromatogram)	
Mobile phase B:	Acetonitrile + 0.1% formic acid	
Flow:	2.0 mL/min	
Gradient:	Time (min.)	%B
	0.00	75
	15.00	90
Oven Temp:	30° C	
Inj. Vol:	5 µL	
Detection:	228 nm	

Table 1 – Chromatographic conditions used in the development of the method to separate 17 cannabinoid analytical reference standards.

Chromatographic method development was performed on a Shimadzu Nexera (Kyoto, Japan) using an Evoke C18, 15 cm x 4.6 mm column, packed with 3 µm fully porous particles from Regis Technologies, Inc. (Morton Grove, IL, USA). Reversed-phase conditions were screened using different organic modifiers (methanol and acetonitrile) in both isocratic and gradient modes of operation. Acid additives (formic acid and trifluoroacetic acid) were also investigated and found important in achieving adequate retention and maintaining the peak shape of carboxylated species (e.g. CBCA, CBDA, etc.). The conditions that resulted in the most baseline resolved peaks and served as the foundation for further method development are listed in Table 1.

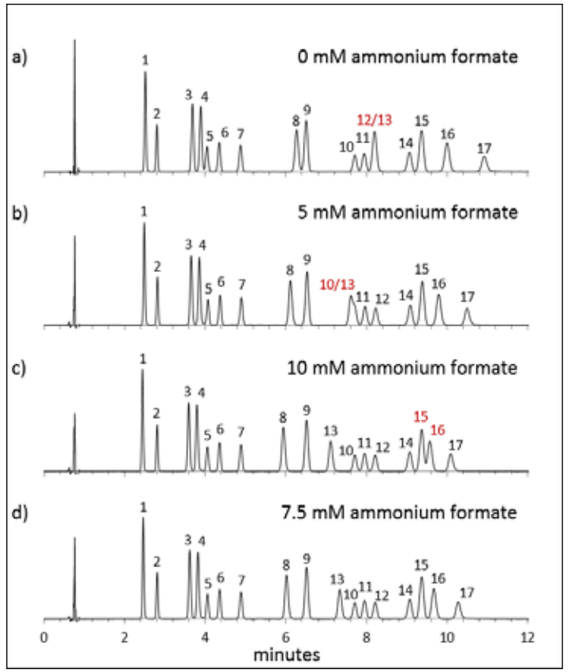


Figure 1 – Effect of the addition of ammonium formate to mobile phase A. a) No ammonium formate added. b) 5 mM ammonium formate added. c) 10 mM ammonium formate added. d) 7.5 mM ammonium formate added. Additional chromatographic conditions listed in Table 1.

Figure 1a shows the baseline-subtracted chromatogram for the separation of the 17 cannabinoid test mixture using the conditions listed in Table 1. Baseline resolution is achieved for each of the component peaks with the exceptions of CBGA and CBG (Rs = 1.40), THCVA and CBN (Rs = 1.42), and the coelution of Δ8-THC and CBNA at 8.20 minutes. In efforts to improve the resolution of these pairs, the effect of adding ammonium formate to mobile phase A in concentrations

ranging between 5 and 10 mM was investigated. The addition of ammonium formate to formic acid mobile phases increases the ionic strength as well as slightly raises the pH.

As shown in Figure 1, the addition of ammonium formate to mobile phase A resulted in reduced retention of the carboxylated cannabinoids while the decarboxylated species remain unaffected, thus baseline-resolving CBGA/CBG and THCVA/CBN. With 5 mM ammonium formate, the retention time of CBNA is shifted to 7.63 minutes and co-elutes with exo-THC, an impurity formed in the synthesis of Δ9-THC (Fig. 1b). By increasing the concentration to 10 mM ammonium formate, the retention of CBNA is shifted, causing it to elute earlier than the THC isomers, but THCA-A is shifted into co-eluting with CBC (Fig. 1c). An intermediate concentration of 7.5 mM ammonium formate was found to provide baseline resolution of all 17 cannabinoids in the test mixture (Fig. 1d).

With typical re-equilibration time, run-to-run results were found to be reproducible. Nevertheless, it should be noted that since ammonium formate is added to only the aqueous component of the mobile phase, the total ionic strength changes throughout the gradient runtime. For example, when 7.5 mM ammonium formate in mobile phase A is used in the gradient listed in Table 1, the total concentration on the column changes from 1.875 mM to 0.75 mM over the course of the 15-minute run. Attempts to maintain a constant concentration by adding an intermediate concentration of salt to both mobile phases A and B resulted in unfavorable retention time shifts at either the early portion or the latter portion of the chromatographic run. Thus, the concurrent gradients in eluotropic strength and pH/ionic strength synergistically serve to provide the separation shown in Figure 1d.

In some assays, analysts are concerned with improving the resolution of certain critical pairs. This may be especially true in cases where one component is far more abundant than the other. In the gradient separations shown in Figure 1, the resolutions between Δ9-THC and Δ8-THC are approximately 1.50. These isomers are neutral, and their retentions are largely unaffected by changes in mobile phase pH or ionic strength. Often, it is possible to improve resolution by running an isocratic analysis and by reducing eluent strength. In the case of Δ9-THC and Δ8-THC, the greatest effect is observed by changing the composition of mobile phase B.

Figure 2 plots the effect of varying the percentage and composition of mobile phase B (MPB) on the isocratic

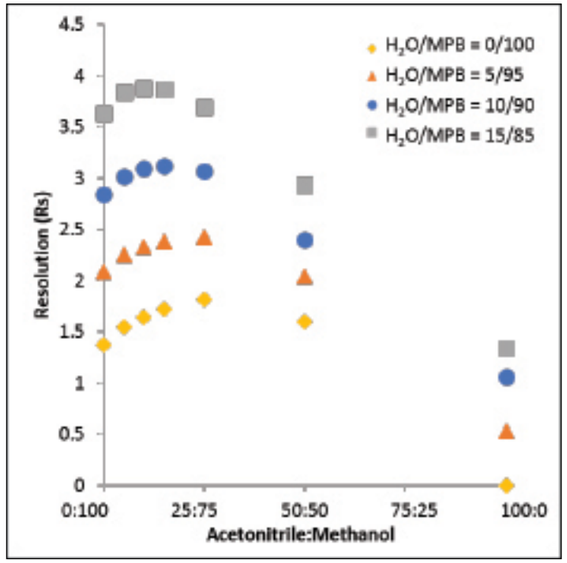
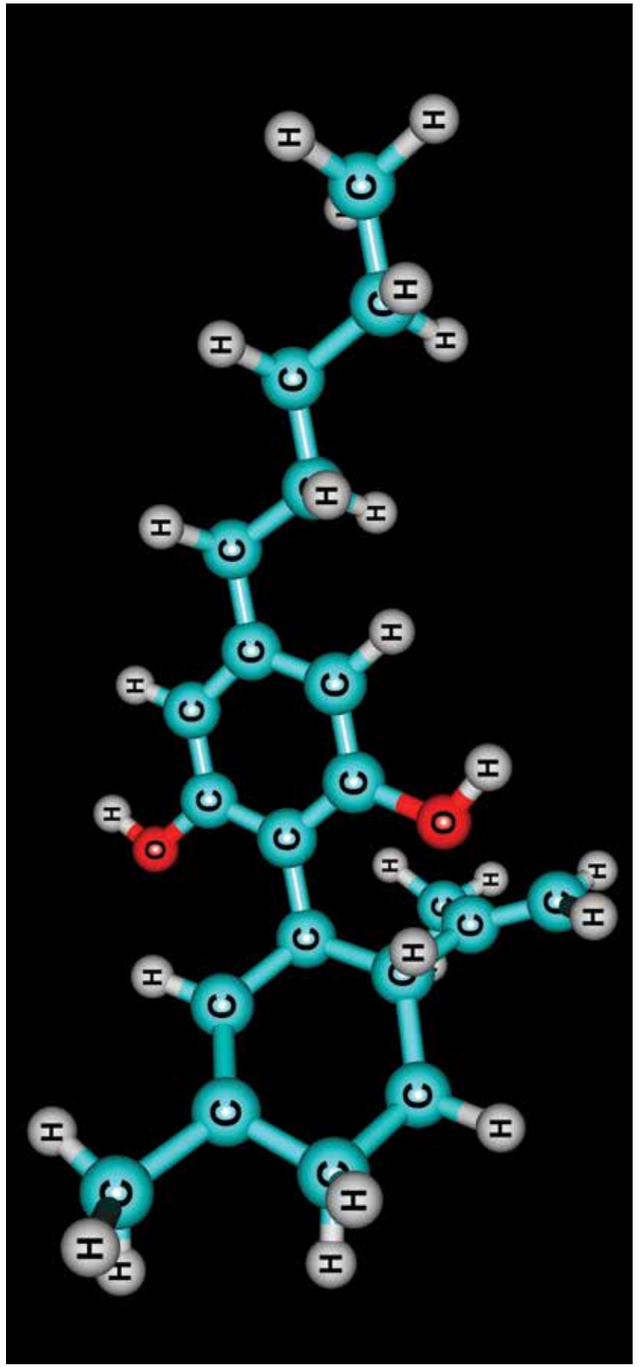


Figure 2 – The effect of the percentage and composition of mobile phase B (MPB) on the resolution of Δ9-THC and Δ8-THC. A blended organic modifier results in better resolution than pure methanol or pure acetonitrile. Evoke C18, 15 cm x 4.6 mm, 3 µm, 1.5 mL/min.

resolution of 1:2 Δ9-THC:Δ8-THC using the same Evoke C18, 15 cm x 4.6 mm column. Consider the analysis when performed with H_2O/MPB = 10/90. The resolution of Δ9-THC and Δ8-THC is 1.06 when MPB = 100% acetonitrile. When MPB = 100% methanol, the resolution is 2.84. Maximum resolution (Rs = 3.12) is observed when MPB = 15:85 acetonitrile:methanol. That relatively minor improvement in resolution afforded by the blended MPB might suggest pure methanol to be the preferred organic modifier for this analysis, especially given the convenience of using a single solvent over pre-mixing a blend of acetonitrile:methanol or investing in alternative pumping instrumentation (e.g. quaternary pumps). With complex samples, though, care must be taken to observe how a desired change in selectivity can affect other analytes in the separation.

A brief example serves to illustrate that several parameters should be considered when developing a chromatographic method for the resolution of complex samples involving key critical pairs. Consider again the separation of 1:2 Δ9-THC:Δ8-THC in the presence of CBL. In Figure 2, it can be seen that the resolution of the THC isomers is superior with pure methanol than with pure acetonitrile as the organic modifier.



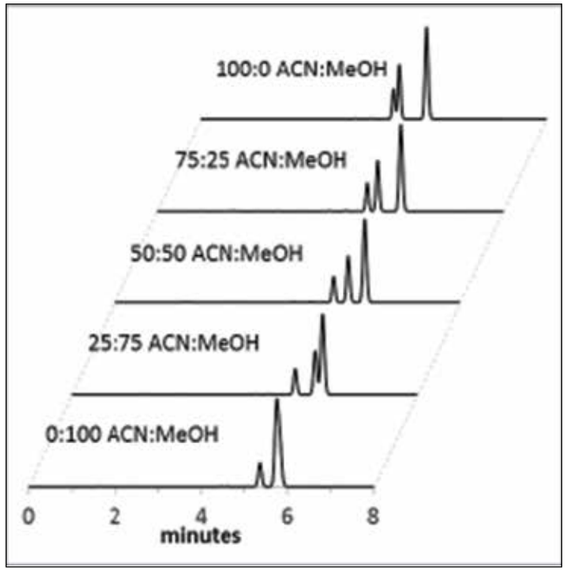


Figure 3 – Separation of 1:2:3 Δ9-THC:Δ8-THC:CBL. An organic modifier of pure methanol results in the co-elution of Δ8-THC and CBL while pure acetonitrile results in incomplete resolution of the THC isomers. A 50:50 blend of acetonitrile:methanol resolves all three analytes. Evoke C18, 15 cm x 4.6 mm, 3 µm, 1.5 mL/min, H2O/MPB = 10/90.

As shown in Figure 3, though, if CBL is present, it co-elutes with Δ8-THC in H_2O/methanol = 10/90. CBL elutes well away from the critical pair if pure acetonitrile is used, but the THC isomers are insufficiently resolved (Rs = 1.06). A 50:50 blend of acetonitrile:methanol provides good resolution, with Rs > 2.5 for both pairs. So, while binary mobile phase systems are very common in reversed-phase HPLC separations, ternary mobile phases can provide access to unique selectivity.

To recap, we developed an HPLC method that fully resolves 17 cannabinoids by using screening runs that altered concentrations of organic and acid modifiers and provided the foundation for further development. The addition of ammonium formate to mobile phase A gave a means to shift the retentions of the carboxylated species relative to the neutral ones, and an optimized concentration allowed for the baseline resolution of all cannabinoids in the test mixture. In addition, the use of a ternary mobile phase system (water, methanol, acetonitrile) was shown to improve the resolution of THC isomers while permitting the flexibility to avoid potential interferences.

Mycotoxins in Cannabis - Lessons From Other Stored and Packaged Goods

By Kimberly Ross, PhD., Peak Compliance, LLC

Mycotoxin detection for cannabis products is becoming increasingly common as more states implement testing programs for their new markets. While there's thousands of molecules classified as mycotoxins, the typical suite of analytes now mandated for cannabis include Aflatoxins A1, G1, A2, G2, and Ochratoxin A. These small-molecule secondary metabolites are produced by various species of fungi (colloquially termed 'mold') and exhibit notable toxicity or carcinogenic potential in humans. Quantitation of these molecules is approached differently than detection of the organisms that produce them (screening for *Aspergillus* species such as *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus* are also included in many state regulatory programs). To detect mold species, they are cultured with selective media, or their DNA is targeted and amplified. Typically, the small molecules such as aflatoxins or ochratoxin are measured using liquid chromatography, often coupled with mass spectrometry to detect very low levels in parts-per-billion.

Mycotoxins are known to contaminate cereal crops including wheat, walnut, corn, cotton, peanuts and tree nuts. [1,2] Dried fruit, red wine, milk, coffee, and spices [3] may also harbor the toxins. Molds and mold spores are ubiquitous in the outdoor environment, in quantities that vary seasonally and biogeographically. [4] They require moisture to proliferate and produce the small-molecule secondary metabolites that have the potential to cause toxicity in humans. In an indoor cultivation setting, to establish optimal conditions for plant growth, humidity is generally higher than in the typical indoor environment. Increased humidity, though, is also favorable for mold proliferation. Therefore, precautions should be taken to eliminate any sources of standing water (irrigation reservoirs, mop buckets, wet floors from watering or feeding applications).

Mold exposure is tricky to trace epidemiologically, no matter the source. In contrast to an infectious disease caused by pathogens, these organisms do not colonize or reproduce in the host like a virus or bacterial infection. Rather, they excrete compounds that can cause asthma or an allergic-type response in the short-term, and respiratory problems or persistent cough over time. [5] Symptoms of chronic exposure are difficult to identify and vary among individuals. They may often mistakenly be attributed to other causes such as hay fever or pet dander. Clinical diagnosis is fraught with skepticism because no specific test has been shown to distinguish the symptoms from other ailments. [6] Repeated exposure over time may contribute to more serious conditions, including inflammatory responses that can affect the brain [7] (sometimes described as 'brain fog'), and may potentially threaten liver and kidney health [8] (hepatoxicity and nephrotoxicity, respectively). Therefore, the presence of mold in cultivation settings also becomes an occupational concern for workers in these environments daily, in addition to jeopardizing the quality of the final products. [6,9]

Many people consume cannabis. Adverse reactions are rare. In terms of risk, immunocompromised or especially sensitive individuals, including infants and elderly, are more susceptible to consumption of contaminated coffee, cereals, milk, or cannabis, and are therefore more likely to suffer increased negative health effects. The source of symptoms, typified by overall malaise, may not be suspected to be connected with cannabis consumption by the individual or their health care provider. Furthermore, states' compliance regulations have included only five of the most common mycotoxins, but the diversity of these molecules is vast, as are the types of species which can produce them—most of which are outside the scope of state-mandated testing. For example, other species of *Aspergillus* such as A. *ochraceus*, A. *carbonarius*, A. *parasiticus*, A. *sulphureus*, A. *sclerotiorum*, and A. *nomius*, are known mycotoxin producers but are not included on most state lists. Additional genera, such as *Penicillium, Emericella, Fusarium, Mucor,* and others may also cause concern but are not specifically screened.

Several years ago, the popular press picked up on published research evaluating the prevalence of mycotoxins in coffee. [10-12] Suddenly, blog posts were everywhere attempting to sort fact from fiction. If many people drink coffee daily without perceptible symptoms, is there really a cause for concern? Where and when did these so-called mycotoxins creep into the coffee supply chain? Were some beans better than others, and if so, why? It turned out that coffee beans sourced from many different farms and aggregated tended to test positive more often than single farm-sourced batches. Organic and conventional practices were not statistically different in terms of mycotoxin prevalence. [12] It's suggested the reduction in quality could be traced to non-ideal harvesting, storage and packaging practices. Furthermore, if the processing plant becomes contaminated from sub-optimal batches, transmission can occur at that stage to cross-contaminate batches that would have been otherwise free from mycotoxin load. [11]

The lessons learned from other industries that store and package plant-based products, such as coffee, could certainly prove informative for cannabis. Molds can affect many crops during growth, harvest, storage, and processing. [2] For cannabis, appropriate curing processes are critically important, and environmental conditions must be tightly controlled in order to protect the quality of the batch until delivery to the end user. [9] Baseline data should be collected on cannabis samples of varying quality to establish the prevalence of these compounds, ideally beyond the five most common in state cannabis regulations. [13] Building a comprehensive dataset can inform regulators in making data-driven decisions, based on a better understanding of mycotoxin load and diversity in commercial cannabis and public health risk.

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The Fundamentals of LC/MS

By Sean Myers and Stephen Goldman, PhytaTech

Liquid Chromatography/Mass Spectrometry (LC/MS) is fast becoming the preferred tool of liquid chromatographers. It is a powerful analytical technique that combines the resolving power of liquid chromatography with the detection specificity of mass spectrometry. This specificity allows for decreasing Method Detection Limits (MDL) and Limits of Quantitation (LOQ); an LC/MS can be used as a highly selective and sensitive tunable detector. An MS chromatogram for a single mass often produces an interference-free signal that offers high precision and low minimum detection limits. LC/MS data may also be used to provide information about the molecular weight, structure, identity and quantity of specific sample components. Due to its superior sensitivity, high mass accuracy and robust performance, LC/MS plays a key role in analysis and is widely used in many industries such as clinical, pharmaceuticals, food safety and environmental. LC/MS systems facilitate the analysis of samples that traditionally have been difficult to analyze and significantly expands the effective analytical use of mass spectrometry to a much larger number of organic compounds. Sample types range from small compounds to large proteins.

Liquid chromatography (LC) is a separation technique. It separates the components of a sample based on the differences in their affinity or retention strength for the stationary and mobile phases. Upon separation by LC, the components can then be detected using the analytes' properties such as ultraviolet-visible (UV-VIS), fluorescence, refractive index, evaporative light scattering or electrical conductivity. Chromatograms obtained using optical detectors primarily identify or qualify substances based on retention time and quantitate substances based on the peak area and intensity. LC offers great quantitative accuracy for analytes that can be chromatographically resolved, however, achieving required resolution is challenging for complex samples where multiple components elute approximately at the same time.

In contrast, mass spectrometry (MS) acquires mass information by detecting ions. It offers molecular-weight and structural information using a highly sensitive detection technique that ionizes the sample components, separates the resulting ions based on their mass-to-charge ratios (m/z) and measures the intensity of each ion. A mass spectrum plots the relative ion intensities against the m/z values, and a series of mass spectra are generated at each time point. This information indicates the concentration level of ions that have a given mass, and is extremely valuable for the unique identification of molecules. Moreover, MS provides added specificity, sensitivity, and the convenience of simultaneous multicomponent analysis. A MS combined with a LC can selectively detect compounds of interest in a complex matrix, thus making it easy to find and identify suspected impurities at trace levels. LC/MS combines the outstanding separation resolution of LC with the excellent qualitative capabilities of MS. With high sensitivity and high detection selectivity, LC/MS provides the flexibility of simultaneous multi-component analysis and improved productivity and efficiency to HPLC analyses.

MS/MS

Mass spectrometry involves the control of ion movement by applying electrostatic fields. It is used to focus the ions generated at the ion source into a beam, and simultaneously removes non-ionic gas particles from the system by progressive pumping and partitioning. This is important for achieving high-sensitivity analysis as residual particles interfere with the ion beam. Ion transmission and focusing is achieved by applying electric fields or radiofrequency (RF) voltage to the quadrupole ion guide. This causes the flow of charged particles to bend in the magnetic field and separation of charged particles by their mass number. With the use of this electromagnetic interaction, ions can be separated and measured according to m/z.

A single quadrupole mass analyzer contains four parallel cylindrical metal rods (electrodes with a hyperboloidal interior surface) inside a vacuum chamber, positioned equidistant from the center axis. The continuous ion source generated in the ionization unit is first accelerated in the z-direction by a relatively weak voltage. These ions pass through a tiny orifice and enter the quadrupole. Both a direct current (DC) and high frequency alternating current (or RF) are applied to the quadrupole causing the ions passing through this electric field to oscillate in the x- and y- directions. When a given

set of parameters are applied to the poles, certain ions of a specific m/z range maintain a stable oscillation and pass through the quadrupole. On the contrary, the oscillations of ions with other m/z values become unstable, causing them to collide with the poles and not be detected, such that only the ions with the target m/z successfully pass through to the detector. The quantity of ions that reach the detector is converted to a signal and output to a computer.

There are limitations of a single mass spectrometer. A single MS may not provide reliable quantitative and qualitative information in cases where resolution is insufficient for both chromatography and m/z (e.g. isomers). This is particularly the case where the sample matrix is complex and the target analytes are in trace concentrations. Therefore, a technique that provides a higher selectivity, specificity and sensitivity, and gives additional unique mass and structural information of the target analytes is required.

MS/MS, also known as a tandem MS, serves as a solution for the challenges faced by a single MS analysis. MS/MS is accomplished by a process called collision-induced dissociation (CID) in which ions break apart as a result of collisions with other molecules. An MS/MS system consists of two quadrupole mass analyzers connected in series with a collision or fragmentation cell in between. The precursor ions (m/z) selected by the first mass analyzer (MS1) are separated and enter the collision cell filled with chemically inert gas (e.g. He, Ar, Xe or N_2). Collisions between the precursor ions and inert gas are induced by applying an oscillatory field. These collisions cause conversion of kinetic energy into molecular excitations that then cause chemical bond breakage and generate product ions. The degree of fragmentation and product ion species depends on the energy supplied. As collision energy increases, the abundance of the molecular precursor ion decreases and fragmentation occurs to generate a variety of product ions. These product ions are then separated by the second mass analyzer (MS2) and passed on to the detector.

Multiple Reaction Monitoring (MRM) is the selective monitoring of a specific few product ions. Prior to performing MRM, a product ion scan is usually conducted to determine the product ions of highest abundance. These product ions are then scanned at the specified retention times per the LC separation. This allows the MS/MS system to remove noise and interference and selectively target particular ions for quantitation to deliver a higher specificity and sensitivity.

Pesticides and Mycotoxins

More than half of the United States has legalized the use of medical cannabis. Like traditional agriculture crops, pesticides are sometimes used in cannabis cultivation to protect plants from pests and improve growth yield. But as is the case in traditional crops, chronic exposure to pesticides can pose serious health risks; therefore, pesticide analysis in cannabis is an important consumer safety topic. Moreover, many of today's cannabis products are inhaled after combustion, so there is growing concern among consumers and regulators because of the unknown effects of pesticide compounds when they are inhaled.

Furthermore, the growing conditions for cannabis are also conducive to the growth of molds and fungi, which can

produce carcinogenic mycotoxins including ochratoxin A and aflatoxins. As a result, testing for the levels of mycotoxins in cannabis is important to ensure consumer safety and quality control. Some states, including California, regulate or have proposed regulation of aflatoxin residues in cannabis. Action levels defined for aflatoxins are well below those outlined for most pesticides and quantitation in the parts per trillion range is necessary.

The chemical diversity of these compounds also presents a considerable challenge to chemists, who require a range of analytical techniques to extract them from various samples types, and then to accurately determine their identity and concentration at trace levels. In the environmental field, LC/MS is widely utilized for the qualitative and quantitative determination of known and trace-level emerging contaminants. High performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) has emerged as the method of choice for pesticide and mycotoxin analysis because it offers superior selectivity, sensitivity, ruggedness, and does not require extensive sample preparation before analysis.

One of the biggest challenges for cannabis testing labs is obtaining a targeted compound list for which to perform the analysis. Because there is no federal guidance for the analysis of pesticides in cannabis samples, different states have developed their own testing guidelines. Oregon was the first state to determine comprehensive guidelines for pesticide residue analysis in cannabis and set regulatory limits for 59 pesticides. However, California has issued more stringent action limits for 66 pesticides and five mycotoxins residues in cannabis flower and edibles. As government agencies debate the legalities of cannabis consumption for recreation and medicinal purposes, cannabis testing labs are faced with inconsistent and even non-existent maximum residue limits. This is especially important to note since crop protection agents are commonly used to increase cannabis yields and appearance. In addition, proximity to other traditional agricultural productions, fields, and facilities, as well as environmental pest controls (such as mosquitos) has the potential to induce external contamination of cannabis crops, whether grown indoor or outdoor. Analysis must be sensitive and specific using an instrument capable of matching all chemical residues to the lowest possible limit.

Numerous methods for pesticide and mycotoxin analysis in cannabis have been published but these studies have certain deficiencies, such as not being able to achieve detection limits to meet state action limits, the use of time-consuming sample preparation methods (for example, QuEChERS with dispersive solid-phase extraction), and poor matrix recoveries for many of the compounds. These deficiencies substantially increase the cost, complexity, and turnaround time of analysis. Sample variation can also prove problematic since cannabis can be consumed by ingestion, topically, or via inhalation. With so many variables, cannabis labs are faced with quantifying many residues with dirty matrices and require rugged instrumentation and solid application support from the instrument vendor to manage this workflow. Using high-resolution mass spectrometry, today's cannabis lab can analyze pesticide residues, mycotoxin contaminants and trace cannabinoids in a variety of matrices for next-generation cannabis products.

Trace Cannabinoids and Terpenes

The cannabis plant produces a large variety of compounds known as cannabinoids, many of which have not been detected in any other plant. Most of them are present at very low levels, especially in commercial cannabis products, making it difficult to accurately detect them. The pharmacological uses of most of these trace cannabinoids have yet to be determined, and a host of novel products are, and will be, formulated from the isolation of these chemicals. Next-generation medicinal cannabis products will attempt to target these cannabinoids and to generate novel formulations and delivery methods. Accurate determination of not only the identity of these molecules, but also their potency will be an essential analysis requirement.

In addition, at least 200 terpenes have been identified in cannabis, with unique cultivars presenting varying terpene profiles, which contribute to distinct flavor and aroma. The ability to quantify relevant terpenes in cannabis products is highly desirable and increasingly demanded by both growers and consumers.

As market demand for these highly technical analyses becomes more prevalent, the use of high-resolution mass spectrometry coupled with HPLC will become paramount. Much like trace residue testing for pesticides and mycotoxins, the sensitivity and specificity of tandem MS will become a valuable tool in the creation of methods and techniques to discover, qualify, and quantify these trace cannabinoids and minute terpenoids. Comprehensive methods that attempt to assimilate all these analyses into a cost-effective, robust, and timely process will become standard procedure in cannabis testing.

Untangling Terpenes

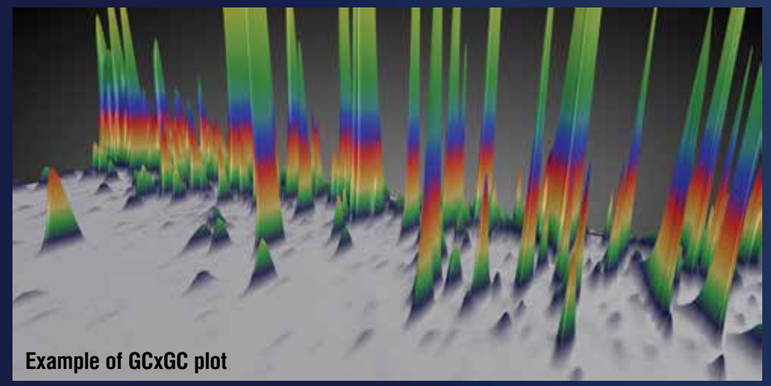
By Kevin Koby, Abstrax Labs

While the domino-effect of recreational legalization is a welcome development for everyone involved in the commercial cannabis industry, past years of criminalization have prevented a great deal of viable research. Now, held up to the light of public scrutiny and the gauntlet of verifiable, repeatable testing, cannabis is revealing itself as increasingly complex. While scientific advances in the field are occurring, particularly regarding cannabinoids, the full nature of terpenes remains largely unexplored.

Ultimately, a deeper understanding of these compounds is necessary to answer crucial, but industry-specific questions. For example, what are agreeable definitions for terms like 'tangie', 'skunk', and 'diesel' in regard to the flavor and terpene content of particular cultivars? Granted, subjects like these may seem esoteric or unimportant to people unfamiliar with cannabis. However, they are integral to the chemists, analysts, manufacturers, patients, and recreational customers seeking a particular flavor, sensorial experience, or effect in cannabis and/or terpene-related products.

Legwork

Abstrax' main focal point is the research behind these mysteries. So, it's our goal to continually push our own intellectual limitations regarding theoretical and analytical terpene testing. This pursuit means continually seeking improved equipment, updating operating protocols, and placing more intense scrutiny on the variable details of industry-related developments.



Example of GCxGC plot

As terpenes are radically affected by a wide range of factors, even small changes in a growing cycle, transportation method, storage methodology, and/or mixing procedure can be drastic. They're easily damaged, degrading through exposure to sunlight, oxygen, and even the unavoidable passage of time. When this happens, a terpenoid is often formed. Over 100 terpenes have been identified in various cannabis samples, each with unique characteristics like taste and effect that are vulnerable to this degradation. In addition to the other factors mentioned, the methods by which terpenes are studied must be carefully selected to unlock their mysteries.

Processing and Separation

The cannabis industry is still pretty new, so there's few established standardized procedures for analytical testing. These methods will usually entail some type of chromatography which separates molecules based on their polarity. The laboratory process for the separation of a mixture, chromatography consists of a liquid or gas (called the mobile phase) moving a mixed sample through a solid structure (the stationary phase). This usually happens within a column, a sealed, metal cylinder. In other words, sample material is forced through a tube full of filters so a detector can measure its individual parts. Here are two analytical methods common in cannabis testing labs:

1. **High-Performance Liquid Chromatography (HPLC):** Inert liquids are pumped through a short column at a specific pressure. The flow is standardized so that a sample can be injected into the otherwise stable system. As the material moves through the column, filtration processes separate the various parts of the sample. This data is measured by built-in detectors and sent to a computer.
2. **Gas Chromatography (GC):** An inert, carrier gas is released into tubes moving through a column within a specialized oven. This flow is sustained while a sample is introduced to the system via an injector. These small amounts pass through the filters of the column, which divide the material. Separated analytes are measured using a detector, such as a flame ionization detector (FID), which combusts the separated compounds, thereby producing ions.

The Right Tool for the Job

Like their names suggest, the primary difference between the two is the use of liquids relative to gases for separation. Either way, inert liquids or gases force a given sample through a separation medium, dividing that formerly mixed material into individual, measurable components. Also, GC is typically used for volatile species, like terpenes or residual solvents, whereas LC is often selected for the separation of non-volatile, or thermally labile compounds, such as distinguishing THCA and THC.

Another significant difference between the two is that liquids are more viscous than gases. This means that HPLC requires a significant amount of pressure to move those liquids through its column. That additional pressure necessitates a thicker, stronger column to withstand the pumping machinery. While this process is ideal for samples with higher molecular weights, the additional and beefier equipment required is expensive, slow to implement, and can require lengthier training.

GC, on the other hand, doesn't require pressure pumps, so the more delicate machinery can be utilized. GC has the option to use capillary columns which require less material. Gaseous samples interact with the functionalized walls of the column, compared to the HPLC column, which is filled with tightly packed material, often silica beads with octadecyl carbon sidechains attached. GC requires less sophisticated machinery and advanced training, and offers faster analyses than HPLC, again provided that the analytes are suitable for the method.

Doubling Down

These analytical methods are widely used and accepted in modern pharmaceutical and medicinal fields. Terpenes are so profoundly intricate, however, that even the traditional chromatographic methods aren't always up to the task. Terpenes sometimes appear as isomers: compounds with the same elemental formulas, but with different molecular structures. Some detectors may be fooled when isomeric terpenes appear within a sample. Isomers often act differently than their counterparts, so there can be different physiological effects within the scope of a single terpene. This information further complicates the academic study of the 'entourage effect'. That is, the process through which terpenes interact with each other and/or cannabinoids in various ways, depending on the mixture.

To get a better grasp on these topics, Abstrax utilizes Two-Dimensional Gas Chromatography (GCxGC). The process begins the same as GC - a sample is introduced to a column via injector. But, instead of passing through a detector at the end of that column, the material moves on to a second column with different selectivity before appearing in colored plot charts. This differentiation helps show the versatility within samples. One good example is that even though regular GC can distinguish some monoterpenes (10 carbon atoms) from sesquiterpenes (15 carbon atoms), GCxGC can distinguish in high resolution and look behind peaks that would otherwise be the sum of multiple peaks in a regular GC. Identifying the exact constituents of samples is integral to achieving better-structured characterizations of the various chemovars. The implementation of GCxGC has been massively beneficial in our efforts to improve our process and understanding.

Tips and Challenges for Defensible Analysis of Pesticides in Cannabis Products

By Marco Troiani and Savino Sguera, Digamma Consulting

As the cannabis industry becomes more regulated, analytical laboratories must generate data that results in their routinely passing regulatory audits. All analyses performed by the labs are challenging, but one of the biggest issues the industry faces is the analysis of pesticides.

Pesticide analysis can be more challenging than other analyses due to low action levels, in parts per billion (ppb) or nanograms (ng). Other contaminant evaluations, such as residual solvents, often have action levels in the parts per million (ppm) or microgram (µg) range, which is 1,000 times greater than ppb. Other organics, such as terpenes and cannabinoids, are present at higher levels such that percent (%) or milligrams-per-gram (mg/g) are used.

Heavy metal limits are also in the ppb range. The thorough digestion used in the analysis creates a more favorable signal-to-noise (S/N) environment in the inductively coupled plasma mass spectrometer (ICP-MS) than we see in LC-MSMS or GC-MSMS for pesticide analysis, where LC and GC are liquid and gas chromatography, respectively. Because there are only 92 naturally occurring elements, complete digestion of heavy metals allows for each to be measured accurately with little interference. In the analysis of pesticides, however, the number of interferences and similar compounds that may occur is extraordinarily high. This is why the technology used for pesticide analysis utilizes a series of mass filters to accurately detect analytes at trace levels.

ANALYTES

No singular ion source is sufficient to quantify all pesticides on most cannabis monitoring lists. Electrospray Ionization (ESI+/-) is typical for LC-MSMS, but is not effective for all analytes. A second source of ionization is currently necessary to detect remaining pesticides and varies between the following: Electron Impact (EI+) Ionization used for GC-MSMS and Atmospheric Pressure Chemical Ionization (APCI) for LC-MSMS or GC-MSMS.

ESI+/- is dependent on the following conditions: an acidified solvent, analytes that can receive positive charge from proton, and analytes that are stable as cations without counter-ions. The mass spectra often exhibit peaks of M+H, which is the molecular mass of the target analyte plus one hydrogen atom. Adducts are not uncommon and cation species typically replace H in the formula M+X, where X can be Na^+, K^+, NH^{4+}, or other monovalent cations substituted for the proton.

EI+ is dependent on the following conditions: an inert, gaseous mobile phase, and analytes that can receive stable positive charge from loss of an electron. The mass spectra often reveal peaks of masses less than or equal to the molar mass. The nature of the EI+ source assures that fragments are common but adducts are not observed.

Nearly all pesticides on the California monitoring list for cannabis analysis are amenable to ESI+/- ionization and perform well using LC-MSMS. Some compounds require either EI+ ionization or APCI+/- ionization to be detected. These analytes include azoxystrobin, captan, chlordane, chlorfenapyr, chlorpyrifos, cyfluthrin, cypermethrin, methyl parathion, pentachloronitrobenzene, and permethrin.

Many analytes begin to degrade in solution under two common conditions: after removal from a freezer when temperatures subsequently rise and degradation reactions occur at higher rates; and when combined with other analytes or matrix components, wherein the half-life of the analyte in solution sharply decreases. Captan hydrolyzes in the presence of mildly basic water, and many other pesticides have basic chemical properties. A combined standard of all pesticides on a monitoring list most likely will not be accurate for more than a 24-hour period at room temperature. Therefore, multi-part standard mixes and matrix-matched calibration standards must be prepared daily to assure accuracy.

MATRIX

Cannabis testing labs face a challenge in finding matrix blanks. Although toxicology and pharmaceutical labs have well-controlled blank matrices, cannabis labs face variation similar to that seen in environmental labs. The major categories of matrices analyzed in cannabis labs are the following: flower, concentrates, edibles, and topicals.

Across all matrices, there are typically four major interferences observed (Figure 1): cannabinoids and terpenes, waxes and lipids, carbohydrates and amino acids, and polymers. Because each matrix class has varying ratios of the interfering compounds, it is necessary to matrix-match the calibration to ensure consistent recoveries.

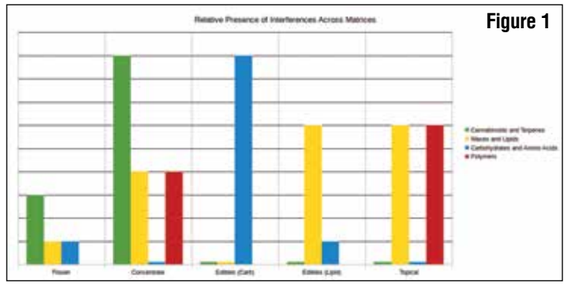


Figure 1

Matrix-blank material must be selected from a pesticide-free material that matches the composition of interference compounds present in a true matrix. Organic cannabis is recommended for flower, but in lieu of that, buds of hops can serve as a proxy. Organic hemp oil is recommended for concentrates and is readily available at retail stores. Organic dry cereal is recommended for carbohydrate edibles and organic chocolate and coconut oil are recommended for lipids in edibles and topicals. Using the same matrix blank material for calibration and quality control allows for method variations to be controlled enough to comply with regulatory standards (+30% recovery) of California and within the EPA standards (+20%). It is recommended to test all matrix blank materials for the presence of analytes before adoption as a standard blank material.

Matrix-matched calibration is necessary because clean-up steps invariably cause analyte loss from extraction solvents. The best way to have the quality control samples match the client and calibration samples is to matrix calibrate with a matrix blank and run your quality control tests on that same blank.

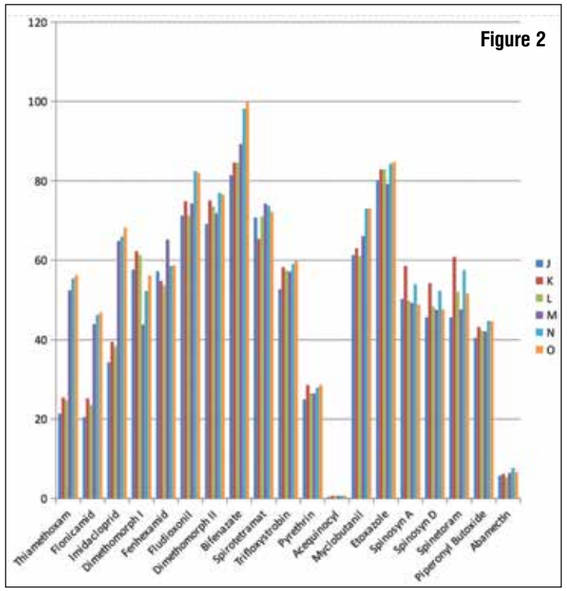


Figure 2

Figure 2 provides data from a study presented at the American Chemical Society 2016 conference in Philadelphia, where the extraction efficiency of each analyte on the Nevada cannabis pesticide monitoring list is illustrated. Each analyte has a different percent recovery, and to correct for this variation, matrix-matched calibration is necessary. The percent recoveries of some pesticides are as low as 20%, well below the 80% recovery minimum in most analytical industries and the 70% minimum in California's cannabis industry.

HOMOGENIZATION

Proper homogenization (Figure 3) of each sample tested is required for reproducibility of reported data. Pesticide distribution is often not uniform, so samples should be homogenized to fine particle sizes and well mixed. Fine particle mesh also allows less acetonitrile to be sequestered in the plant matrix, and for a greater volume of acetonitrile to be collected after sample extraction. This enables a larger ratio of sample mass to extraction volume, which gives lower limits of detection for a method.

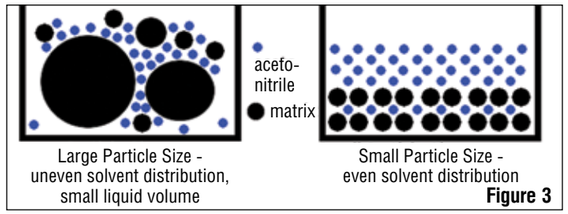


Figure 3

EXTRACTION

LC and GC systems have different vulnerabilities when it comes to matrix interferences, and require extraction clean-up approaches that protect each instrument. Interfering hydrophilic species (carbs, amino acids, proteins) are problematic for GC's hot and dry environment, which causes Maillard reactions. [1] The Maillard reaction (Figure 4) is a bond formation between carbohydrates and amino acids and is seen often in cooking when foods brown or caramelize. These reaction products are sticky and difficult to remove from the dry and hot environment in the GC system, making QuEChERS necessary for GC-MSMS maintenance. Because heat and exposure to oxygen is a necessary element of the Maillard reaction, the LC system's cooler and wetter environment prevents formation of Maillard products. [1]

Interfering hydrophilic compounds are effectively removed from sample extract with QuEChERS salts. Interfering hydrophobic species (waxes, hydrocarbons) are problematic for LC because some have higher affinity for the C18 column than a mobile phase like methanol [2], and subsequently may irreversibly bind to the column, clog the ESI probe, or dirty the quadrupole, altering retention times or chromatographic resolution. Thankfully hydrophobic analytes can be removed with a lipophilic purge.

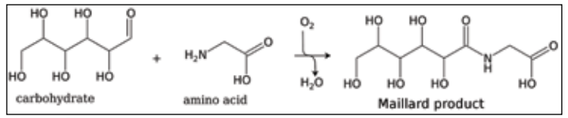
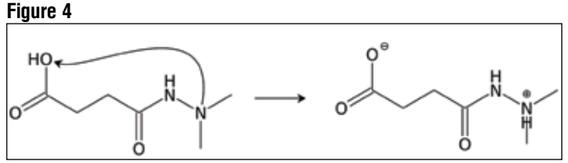


Figure 4



QuEChERS salts cause the loss of the ionic analyte daminozide because the pesticide's high polarity causes it to bind preferentially to the dehydrating salts over the acetonitrile solvent. This step is incompatible with LC pesticide analysis, where daminozide is present on the pesticide monitoring list. Using 10-50 mg of graphitized carbon black (GCB) dispersed in 1.0-mL of extraction solvent used as a non-specific extract clean-up is preferred to QuEChERS when daminozide is on pesticide monitoring list. GCB clean-up in lieu of QuEChERS is not as effective but it's compatible with LC systems. This method is not recommended on GC systems due to the inability to remove nearly all carbohydrates and proteins from extraction solvents, and the aforementioned Maillard reactions that can occur.

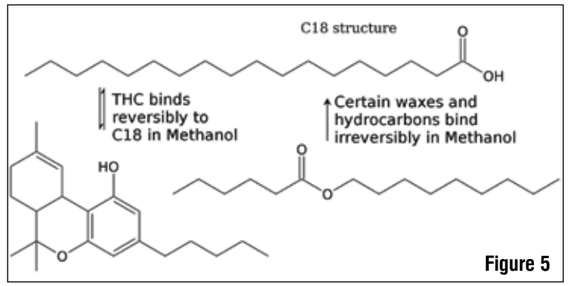


Figure 5

LC column packing material is typically functionalized with C18 moieties, which can cause issues with strongly hydrophobic components in a sample matrix (Figure 5). C18 is very aliphatic and binds strongly to certain compounds, such as aliphatic fatty acids, their triglycerides, and wax derivatives. Lipophilic clean-up steps, such as a hexane biphasic purge, may be necessary for proper LC-MSMS column maintenance. A GC system's high heat allows for the off-gassing of aliphatic hydrophobic compounds due to their thermal lability and relatively low boiling points. Therefore, it's important to purge and remove hydrophobic matrix interferences from extracted samples for LC analysis.

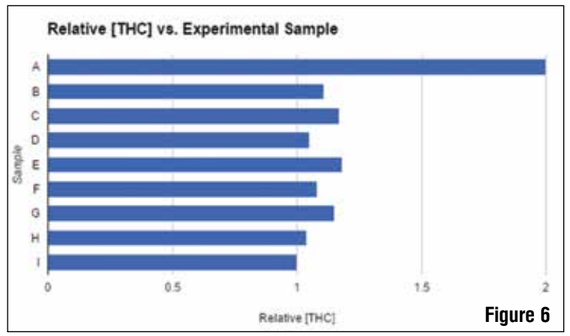


Figure 6

QuEChERS alone does not completely remove hydrophobic interferences, such as THC or waxes. In Figure 6, where relative THC concentration is compared across several extract preparations, sample A used QuEChERS only, while B-I involved various amounts of GCB as an additional step.

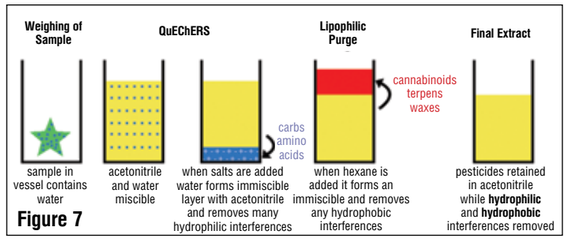


Figure 7

Both QuEChERS and lipophilic purges utilize liquid-liquid biphasic extraction to selectively remove compounds from an extract. When combined, as outlined in Figure 7, we can sequentially remove water and hydrophilic species, and then aliphatic hydrophobic compounds from an acetonitrile extract that is still enriched for pesticides.

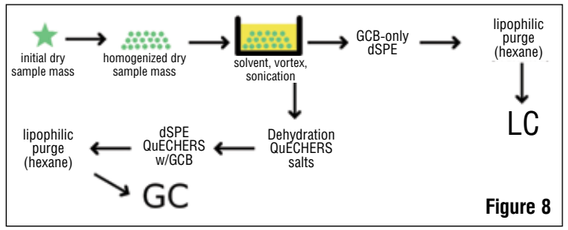


Figure 8

A combined extraction approach (Figure 8) involves a complex and coordinated clean-up. Sample are homogenized, dissolved in solvent, and the extract volumes are split for LC- and GC analyses. LC samples can be cleaned up with GCB and then purged of aliphatic compounds before LC injection. The GC samples are run through both stages of QuEChERS including dispersive solid-phase extraction with GCB added, and then treated with an aliphatic compound purge before injection.

QuEChERS cleanup is traditional in the regulated agricultural pesticide testing industry and an excess of validation documentation exists. The addition of the lipophilic purge was learned from the FDA-regulated olive oil industry. Olive oil has a massive hydrophobic component in the sample matrix, and analytical labs use GC and LC for quantifying pesticides contaminating the plants. [3] These hydrophobic interferences are similar to the cannabinoids, terpenes, and waxes seen in cannabis plants.

ANALYSIS

Other considerations for LC involve auto-sampler tray temperature, which can cause target or interference compounds to precipitate or clog vessels, injection volume, and needle washes. GC considerations involve the programmable temperature vaporizing (PTV) inlet and its necessity due to the range of optimal vaporization temperatures of certain analytes (captan, cypermethrin, cyfluthrin, etc.). These rapidly degrade when injected into a hot and constant temperature GC inlet, causing inconsistent results. With a PTV inlet, these analytes can be consistently delivered to the head of the GC column by slowly moving through the ideal vaporization temperature for each compound.

A thorough rinse of the GC sampling needle is also required to prevent jams which otherwise could frequently occur. An ideal rinse program incorporates hexane, isopropanol, and acetonitrile. This program works well because hexane and isopropanol are miscible, as are isopropanol and acetonitrile.

Hexane and acetonitrile's immiscibility is the basis for the biphasic lipophilic purge, but isopropanol provides an ideal middle-ground between the polarity of the two compounds to keep the injection needle free of clogs. The needle dwell time should be kept to a minimum to prevent burning of extract solvent on the needle exterior. This burnt material can re-circulate in extraction solvents and cause jams in the needle assembly.

When developing your chromatographic method, an initial rapid separation of analytes in solvent should be used as a baseline method. Further separation of analytes from matrix interferences can be performed for each matrix with unique chromatographic programs for each matrix class. Ion suppression and other variable "dark" influences on instrument response necessitate a matrix-matched calibration curve for each matrix type, even if no visible differences are identified on the chromatograms.

Some multiple reaction monitoring (MRM) channels show interferences in cannabis matrices, especially those with masses close to cannabinoids. Examples include myclobutanil, pyrethrins, acequinocyl, and spiromesifen. To preserve selectivity, additional chromatographic separation is necessary and which is why a separate chromatographic program may be necessary for each matrix class.

LC/MS-grade solvents are necessary for extraction and mobile phases because lower grades can be a source of organic contamination, which raises noise levels and causes some analytes to perform outside QC specifications. For LC-MSMS analytes revealing low response, such as acequinocyl and spiromesifen, organic mobile phase interference is a significant problem. Even among LC/MS-grade brands, some produce more noise than others and so experimentation is necessary to select optimal products.

LC/MS is prone to contamination from the glassware used in analysis. ESI+/- ionization, in particular, relies on cation adducts, often hydrogen adducts but also ammonium, sodium and potassium. Sodium adducts (Figure 9) can be formed from the trace amounts of sodium present in the glass of vials and other vessels holding mobile phase or sample. These ions form adducts which are less stable than hydrogen or ammonium adducts, and hence produce a lower MRM response than the other adducts, while simultaneously deprecating their responses. The suppression of sodium from glassware will boost signal and increase performance of analyte quantitation.

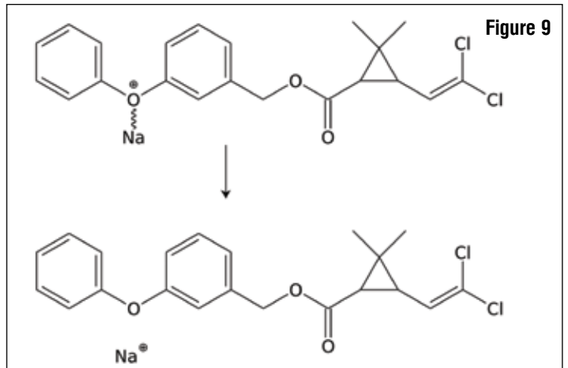


Figure 9

When alkali metallic cation adduct of charge of +1 is disturbed by collision event, cation rapidly dissociates taking +1 charge with it and leaving behind a neutral organic molecule, invisible to the mass spectrometer

MRMs are an excellent starting point for analyte detection in the mass spectrometer. For many analytes, Q1 and Q3 scans are necessary to discover ideal MRM for analyte detection. Q1 and Q3 are the names of the two quadrupoles that can be programmed to selectively filter ions by mass, and their operation in tandem is what allows for MRM scanning to occur. It is important to measure S/N and not just peak height in the MS. It is also important to determine the best S/N ratio in each matrix, as they are often not the same due to matrix and "dark" interferences. To compare potential fragments at different masses, a chromatogram (Figure 10) is preferred to just spectral output to see correlation with analyte retention time and S/N measurement.

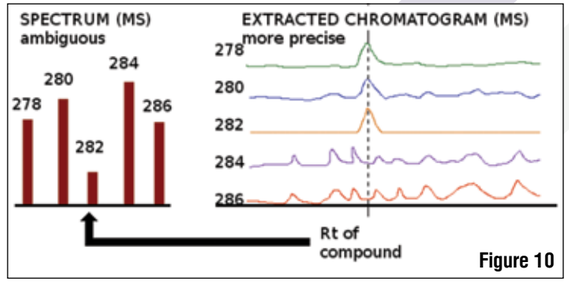


Figure 10

A component of mass spectrometry that is universal to LC and GC is the detection of certain compounds using isotopic abundances of specific elements. "A+2" elements, such as chlorine, bromine, and sulfur, are useful for identifying parent mass scans of compounds containing such atoms. "A+2" elements are commonly found in synthetically produced pesticides, making them particularly useful in pesticide analysis. Some typical ratios for "A+2" elements heavier isotopes are: 24.22% for ^{37}Cl; 49.31% for ^{81}Br; 4.29% for ^{34}S; and 0.21% for ^{18}O.

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Getting to the Root of Advanced Cannabis Pesticide Testing

By Toby Astill, PerkinElmer, Inc.

Today more than half of all US citizens -- and people from 44 other countries -- have access to legal medical cannabis. [1] As this market continues to grow and becomes more mainstream, calls for regulation and the need for the cannabis industry to ensure patient and consumer safety will similarly increase.

Be it in the form of flowers, oils or edibles -- or at the point of cultivation through production -- understanding and combatting pesticides as well as mycotoxins (not to mention heavy metals) is paramount.

Putting consumer safety first is particularly important for medicinal cannabis products, as they may be used by people with compromised immune systems.

Unique Requirements Call for Smart Science

Cannabis' unique testing and compliance environment demands that pesticide analysis solutions bring more than just an analytical technique.

Rules for cannabis safety testing are literally all over the map. California labs test for 66 pesticides and 5 mycotoxins (with laws in the other 32 US states differing from one another), while Canadian scientists are required to test for 96 pesticides down to a level of 20 parts per billion (ppb). Testing solutions must, therefore, be able to cover a wide range of analysis while maintaining the ability to deeply mine data -- all while keeping an eye on testing efficiencies that run at the competitive pace of the market.

At the same time, controlling where and how much cannabis comes into contact with pesticides is not an exact science -- think overspray from other agriculture practices -- so analysis must be done across various product forms and at various stages of readiness for the market.

Further, the chemical composition of the cannabis plant itself is complex. The challenge of analyzing a nonhomogeneous plant are innate. Add pesticides and contaminants to the cannabis testing picture -- not to mention oils or other additives -- and it gets even more involved. Unlike potency or terpene tests, which play a key role in the final product but are more straightforward, pesticide testing is like looking for a needle in a haystack -- with the haystack being the extremely complex environment of the competing chemical composition of the cannabis plant. As a result, testing technologies must be able to tackle complexity with ease.

Finally, there is a strong demand in the market for testing solutions that are easy to use, as many cannabis organizations may still be in the early stages of developing in-house scientific expertise and teams. For example, having software that can step users through the typical workflow -- from preloaded MS and LC methods, to batch list creation and final results viewer -- is pivotal to successful implementation in emerging labs.

Taking a New Approach with Two Ion Source LC/MS/MS

All of these factors lead to the need for highly sensitive, flexible, scalable and intuitive testing and analysis options. This is where new innovations are coming into play.

Traditionally, two instruments would be used to test pesticides in cannabis -- an LC/MS/MS (high-performance liquid chromatography mass spectrometer) and a GC/MS (gas chromatography mass spectrometer), as many pesticides aren't detected well with traditional electrospray ionization (ESI) on an LC/MS/MS alone.

A new approach, however, involves a single LC/MS/MS instrument that contains two ion sources, meaning there are two different options for ionization of the pesticide before detection by the MS/MS (Tandem Mass Spectrometry).

The first ion source is the more traditional ESI, with the other being an atmospheric pressure chemical ionization (APCI) source. The APCI source is highly valuable in the cannabis industry, as it allows analysts to overcome the prior challenges associated with the more hydrophobic or chlorinated pesticides (i.e. chlordane or pentachloronitrobenzene). This dual source approach enables the analysis of all California state-regulated pesticides using a single instrument (including pesticides that are harder to detect at trace amounts such as captan, chlorfenapyr, cypermethrine, naled and more.)

With this new, single instrument capability, a single prep method is all that's required for a full pesticide work-up in as little as 22 minutes – delivering both rapid and reliable results. Sensitivity is also at an all-time high with a dual ion approach so pesticides can be found at present parts per trillion (ppt) – helping today's labs not only meet current regulatory demands, but also ensure they can meet even lower trace requirements in the future if needed.

Thinking Beyond to Standardization

In addition to thinking about advanced instruments and software, standardization is also important in

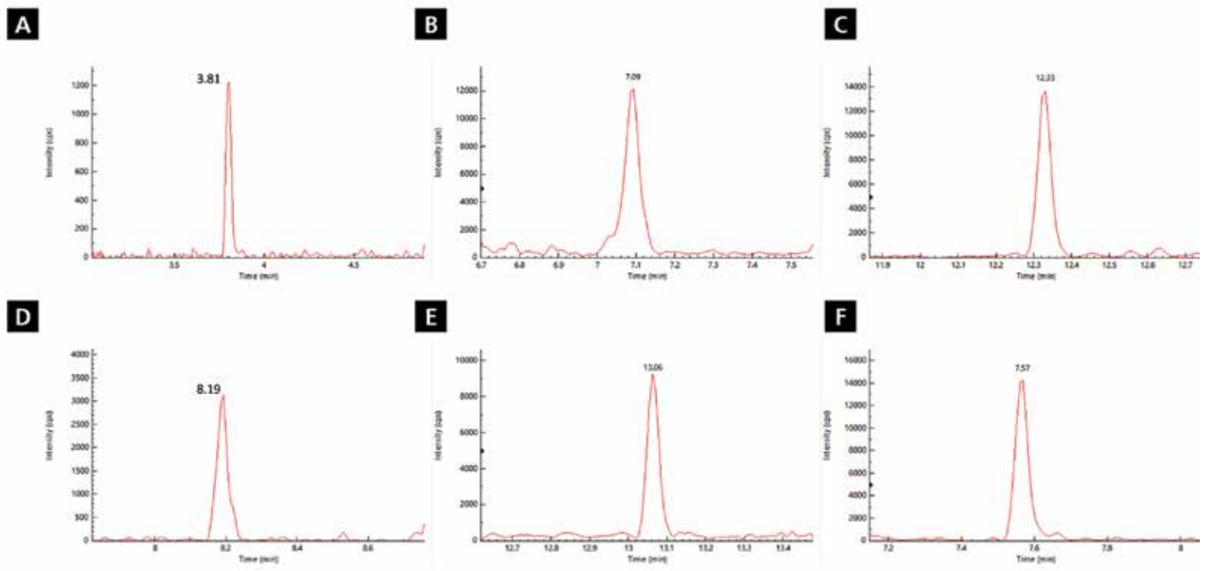

Figure Caption: MRM chromatogram from a sample cannabis matrix run on the PerkinElmer QSight ®420 with a representative set of pesticides:(a) oxamyl, (b) metalaxyl, (c) fenpyroximate, (d) myclobutanil, (e) Etofenprox and (f) Azoxystrobin.

cannabis testing. Labs utilizing standardized methods can help ensure safety, reproducibility, accuracy and compliance between products and geographies against an ever-changing backdrop of cannabis regulation.

As there is no federal oversight of cannabis in the US, however, no mandated methods or standardized proficiency tests exist today. There are, however, industry-led standards arising. Take for example, the Emerald Test™ from Emerald Scientific. This is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program that brings well-established testing practices and standards from industries like environmental, food, pharmaceutical and water testing to the cannabis space. PerkinElmer has already started moving itself and its cannabis lab customers towards standardization by being awarded Emerald Test badges for proficiency in analytical instruments and testing methods for the detection of pesticides, heavy metals, and residual solvents as well as the determination of product potency for cannabis.

Innovative Science is at the Heart of Compliance

As noted in the 2017 report by the US National Academies of Science, Engineering and Medicine, "this is a pivotal time in cannabis policy and research" [2], with the regulatory landscape for this market constantly evolving.

In turn, we need to empower today's cannabis labs and scientists with the most advanced solutions, thinking and standards to help them protect the safety of medicinal and recreational cannabis patients and consumers.
For more information on PerkinElmer solutions around cannabis please visit: **http://www.perkinelmer.com/category/cannabis-analysis**

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Not the UV/Vis You Grew Up with: Terpene Isomer Analysis with Gas Chromatography – Vacuum Ultraviolet Spectroscopy

By Alex Hodgson, M.S.,
Chris Hernandez, M.S., VUV Analytics

We can all agree that terpenes play a significant role in cannabis products. It is true that the total terpene profile contributes heavily to the aroma and flavor of a cannabis cultivar, but select terpenes also have powerful pharmacodynamic activity including anti-inflammatory, anxiolytic, antimicrobial, and anesthetic effects. These effects are potentially amplified in the presence of certain cannabinoids in a synergistic relationship referred to as the "entourage or ensemble effect" [1]. Therefore, the accurate analysis of terpenes in cannabis products is very important, not only to the producers and suppliers, but also to consumers. What's most astonishing about terpenes is that they are all built from units of the same molecule, isoprene (C_5H_8), leading to a high prevalence of structural isomers, each of which have different flavors, aromas, and pharmacodynamic effects.

Terpene characterization has traditionally been carried out using the tried and true techniques of gas chromatography – flame ionization detection (GC-FID) or gas chromatography – mass spectrometry (GC-MS). To perform an accurate analysis, baseline resolution of chromatographic peaks is often needed, but due to the isomeric nature of terpenes, long GC analysis times are often required to avoid co-elution's. Additionally, many terpenes have identical mass spectra, making differentiation from fragmentation patterns alone impossible using GC-MS. Figure 1 shows the mass spectra of two such compounds. They are virtually identical, which makes reliance on the mass spectra troublesome.

Fortunately, there is a new GC analytical method available today which addresses the isomer differentiation and co-elution challenges commonly faced with terpene analysis. The technology is called gas chromatography - vacuum ultraviolet (VUV) spectroscopy, and it has been made available to the greater scientific community by VUV Analytics.

VUV Analytics' flagship instrument is the VGA-100 Vacuum Ultraviolet Detector. The VGA-100 can integrate with virtually any gas chromatograph and has just a single consumable - a deuterium lamp. And while the word "vacuum" is in the name, there are no vacuum pumps involved. The name "vacuum ultraviolet" applies to the wavelength range the VGA-100 operates within, 125 – 240 nm. Figure 2 shows an illustrated schematic of the instrumentation.

What differentiates GC- VUV from UV/Vis detectors are the shorter wavelengths utilized, which excite the electronic transition states in virtually all chemical bonds, allowing absorption and detection without the need for a chromophore [2]. For those unfamiliar, a chromophore is a part of a molecule responsible for its color and is very important for UV/Vis detection as it absorbs light at these wavelengths. Not all molecules have chromophores. In contrast, all molecules have electrons which absorb VUV light to achieve an exited state. The results are rich spectral fingerprints which are unique and repeatable for every compound analyzed, something not possible with the lower energy, longer UV/Vis wavelengths. Since VUV spectroscopy also accounts for the entire 3D electronic structure of the molecule, the absorbance spectra of structural isomers can be distinguished spectrally because all molecules have a unique absorbance spectrum. Figure 3 shows just a few examples of VUV spectra of terpene isomers. The subtle details in each spectrum, the individual peaks and valleys, are unique enough to tell each isomer apart, and those spectra never change. Utilizing these unique absorbance spectra, along with the fact that GC-VUV detector technology is not flow-rate limited (since there's no vacuum pump), offers the possibility of significant reductions of GC analysis times through flow rate-enhanced chromatographic compression and automated data analysis.

The isomeric nature of terpenes can naturally lead to quantitation difficulties during GC analysis when co-eluting analytes are present. Co-elutions happen when more than one analyte exits the GC column at the same time or at almost the same time, resulting in overlapping peaks which makes identification or quantitation difficult or impossible. Measured absorbance is linearly proportional to the amount of analyte passing through the flow cell, as stated by Beer's Law, which is an equation that illustrates the proportional relationship between analyte concentration and absorbance. When the concentration of an analyte increases, so does it's absorbance peak height and area.

Figure 4 shows five monoterpenes eluting in a 0.2-minute window, four of which are isomers (α-Terpinene, limonene, *cis*-ocimene, and *trans*-ocimene). Be aware that GC-VUV chromatographic data is just a representation of the analyte absorbance. Let's take a closer look at the absorbance data of coeluting analytes among this cluster of peaks.

The region highlighted in blue in Figure 5 shows that we are facing a co-elution of α-Terpinene and *cis*-ocimene, which are monoterpene isomers. The measured VUV absorbance spectrum (blue trace) is simply the sum of the α-Terpinene (red trace) and *cis*-ocimene (green trace) spectra. The peak fitting software included with the instrumentation, which operates by automatically (or manually) matching spectra to a library, provides the identity and concentration of the eluting terpenes, and indicates a spectral fit with an R^2 value of 0.999+.

References

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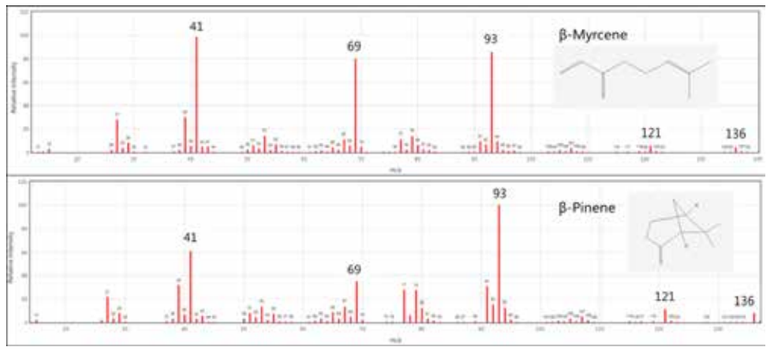


Figure 1: Mass spectra fragmentation patters of β-myrcene and β-pinene, two terpene isomers.

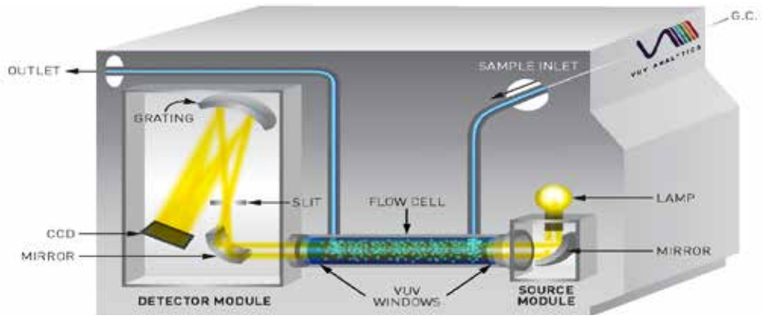


Figure 2: Illustrated schematic of the VGA-100 Vacuum Ultraviolet Detector from VUV Analytics.

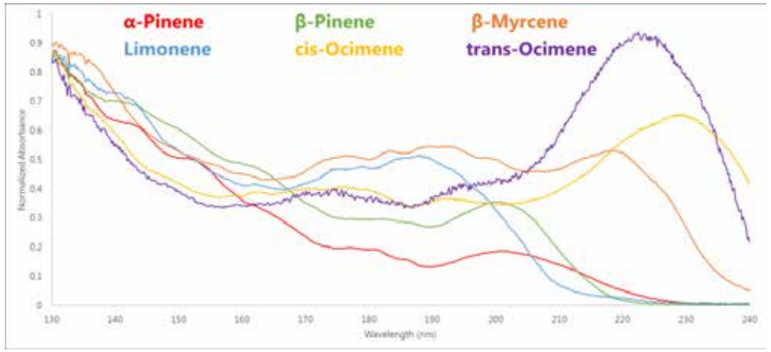


Figure 3: VUV spectra of six terpene isomers. The uniqueness of VUV spectra enable easy identification.

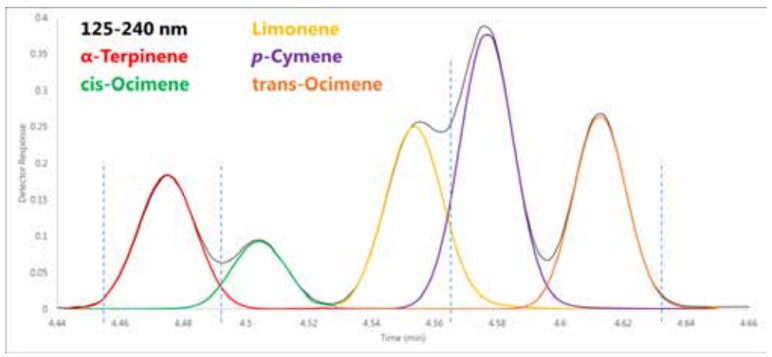


Figure 4: Chromatogram of five monoterpenes eluting within a 0.2-minute window.

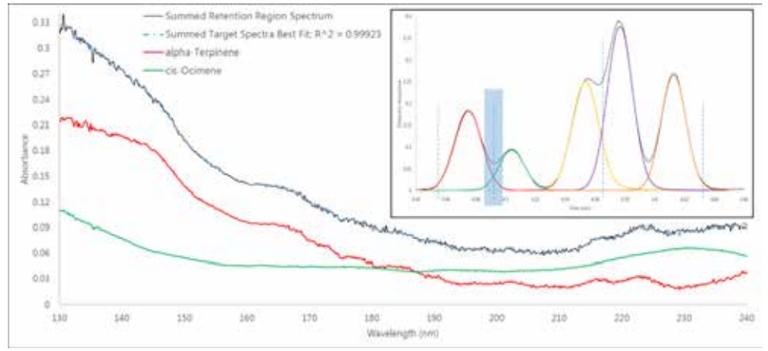


Figure 5: The coelution and spectral deconvolution of α-terpinene and *cis*-ocimene.

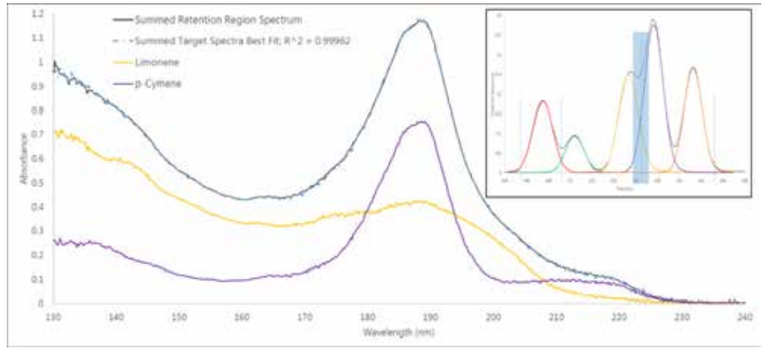


Figure 6: The additive nature of VUV spectra simplifies deconvolution of co-eluting chromatographic peaks.

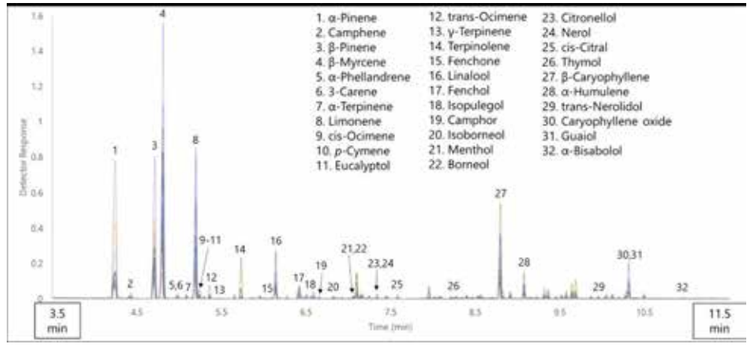


Figure 7: An overlay of steam-distilled terpene extracts from 17 cannabis samples within an 11-minute elution time for 32 target terpenes. Coelutions are common with many of these analytes, but spectral deconvolution is possible with GC-VUV.

The third highlighted region is another co-elution, this time between limonene and *p*-cymene with a 0.999+ R^2 value. While these are not isomers, the deconvolution demonstrated in Figure 6 does highlight again the additive nature of VUV absorbance spectra. We can see that the measured spectrum (blue trace) is the sum of the two contributing terpenes, limonene (yellow trace) and *p*-cymene (purple trace).

By utilizing the power of unique absorbance spectra with the ability to deconvolve co-eluting peaks, it is possible to deliberately compress the chromatographic separation using GC-VUV. Figure 7 shows an overlay of steam-distilled terpene extracts from 17 cannabis samples which is an 11-minute elution time for 32 target terpenes. Each one of these analytes can be identified spectrally, and co-eluting peaks can be deconvolved if necessary. Chromatographers typically try to avoid the burden of co-elutions, but with GC-VUV, co-elutions are no longer troublesome.

GC-VUV spectroscopy is a relatively new analytical technique which is proving to be a powerful solution to the challenges faced in the growing field of cannabis analysis, challenges that can limit GC-FID and GC-MS. With the data richness of repeatable spectra combined with the fundamental simplicity of spectral deconvolution, terpene analysis can be accelerated and automated with GC-VUV.

Microbes: Why You Don't Want Them in Cannabis and What's the Best Way to Test for Them

By Milan Patel & Carl Yamashiro, PhD., PathogenDx

E. coli and Salmonella – these names strike fear in the heart of any food producer around the world. These microbes should strike even more fear for those who use medicinal cannabis for their health. The Food and Drug Administration (FDA) or United States Department of Agriculture are not monitoring cannabis, therefore there is no federal regulatory structure for cannabis currently in place.

In addition to this lack of regulatory framework, it is up to the individual states in the US to create and implement testing regulations. The reality is that individual states are setting different standards, and outside the US, regulatory structures also differ by country. Thus, microbe regulation is confusing to many cannabis and terpene producers.

Another lesser known fact important to the cannabis industry is that fungal species also produce terpenes and terpenoids. [1] These molecules can have a wide range of effects on humans, beneficial and harmful. For instance, some of the fungal terpenes and terpenoids are actually mycotoxins produced by fungal species such as Fusarium, which includes fumonisins. [2] As such, one must ensure the purification of the desired terpenes/terpenoids and must remove the fungal terpenes that can include mycotoxins such as fumonisins, which could cause adverse health events. [3]

Most states are generally striving for a clean food standard in cannabis – but some countries outside the US are striving for pharma-level standards for cannabis testing. In fact, the only cannabis product approved for use in the US by the FDA, Epidiolex® by GW Pharmaceuticals, had to pass a pharmaceutical standard for approval. So how can cannabis products and brands make the right decisions regarding how to test for microbes when there are so many different regulations to follow?

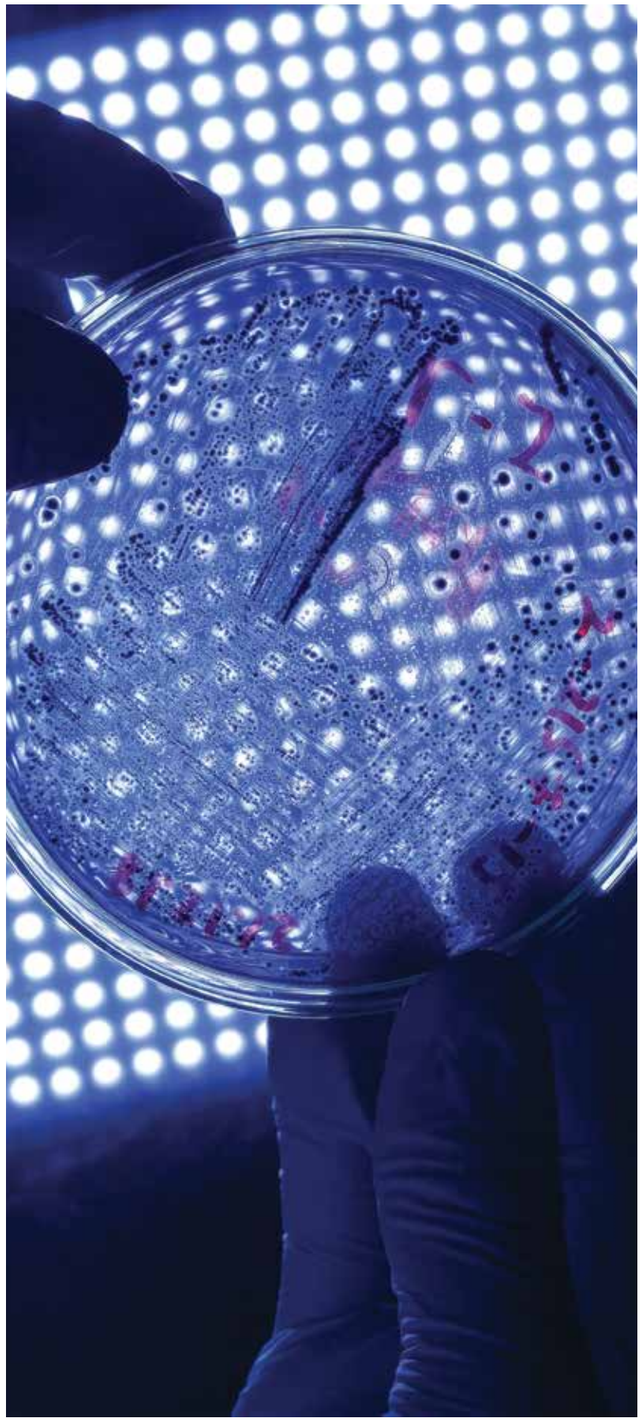
Even US federal regulations have not prevented outbreaks of *E. coli, Salmonella* and certain pathogenic species of *Aspergillus* in the general food supply. Without standardization of testing standards state to state, one can imagine that the cannabis supply is even more vulnerable to these contaminants. In fact, in 2019, according to the California Bureau of Cannabis Control, 10% of California's tested cannabis supply (expected to be a $5.1B market) is still tainted with these microbes, despite best efforts to regulate for appropriate safe levels.

Cannabis Batch Testing
Certificates of Analysis as of March 15, 2019

Certificates of Analysis Received	Tested Batches	Failed Batches	%
39,634	39,634	4,392	11.1
Tested Batches By Category	**Tested Batches By Category**	**Tested Batches By Category**	**%**
Flower	20,120	1,479	7.4
Inhalable (cartridges, waxes, etc.)	12,651	1,541	12.2
Other (edibles, tinctures, topicals, etc.)	6,863	1,372	20.0
Total	39,634	4,392	11.1
***Reasons For Failure**	**Failed Batches By Category**		**%**
Label Claims	2,379		52.9
Pesticides	1,135		25.2
Microbial Impurities	428		9.5
Residual Solvents	272		6.0
Homogeneity	36		0.8
Foregin Material	15		0.3
Moisture	71		1.6
Heavy Metals	140		3.1
Water Activity	5		0.1
Cannabinoids	18		0.4
Total	4,499		

*Batches can fail for multiple categories

Reference: https://bcc.ca.gov/serp.html?q=Cannabis+Batch+Testing+Certificate+of+Analysis+March+15&cx=00177922245372747843:lujxsa6khxm&cof=FORID:10&ie=UTF-8&nojs=1



Like the traditional US food supply, these plant-borne pathogens can cause illness, disease and death, especially in medical patients who use cannabis for their health. In 2017, Dr. George Thompson at University of California-Davis Medical Center and other researchers published their conclusions on the concern of the infectious risks of cannabis in *Clinical Microbiology and Infection* and released the data to the media, alerting regulators, consumers, producers and growers to the potential deadly threat of microbes in cannabis. [4, 5]

The publication of this research is compelling. Cannabis must be tested for microbes to be safe. The discovery of *E. coli* or *Salmonella* in one's cannabis products can cause large losses of a high value crop, harming a company's brand, its investors, its customers and certainly patients. What are the most reliable methods for testing cannabis?

Plating Methods

Traditional microbial diagnostics, viable plate counts, for example, have been generally regarded as the standard pathogen testing method in the food industry for over 140 years since the discovery of the Petri dish. Plating methods for the detection of bacteria, fungi, yeasts and molds use non-selective and/or selective enrichment of samples to identify pathogenic organisms.

Microbial plating has several limitations such as, limited sensitivity, specificity, and dynamic range. In addition, plate-based methods for pathogen detection require a heavy workload, *both time and trained scientists*, which creates a bottleneck in testing for cannabis and food safety laboratories. These limitations have led to the need for the development of faster and more sensitive methodologies for pathogen detection.

DNA-Based Methods

The field of microbiology was rapidly advanced through the discovery of DNA and subsequent development of molecular diagnostics. Molecular diagnostics, including but not limited to, quantitative-polymerase chain reaction (qPCR), DNA Microarrays, and Next-Generation Sequencing (NGS) have expanded our understanding of the microbial community, improved sensitivity and specificity of detection and greatly improved the time and cost associated with pathogen detection. Several companies offer this technology to their customers, including Agilent, BioMeriuex, Bio-Rad and Medicinal Genomics.

MICROBIAL TESTING REGULATIONS

PathogenDx

	Alaska	Arkansas	California	Canada	Colorado	Connecticut	Delaware	Florida	Hawaii	Illinois	Maryland	Massachusetts	Michigan	Montana	Nevada	New Jersey	New Mexico	New York	Ohio	Oklahoma Tentative Regs	Pennsylvania	Rhode Island Tentative Regs	Vermont	Washington	West Virginia
Detection Assay																									
Pathogenic *E.coli* < *1CFU*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	
Salmonella < 1CFU	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Aspergillus Niger < 1CFU	x		x					x	x				x		x	x		x	x		x		x		x
Aspergillus Flavus < 1CFU	x		x					x	x				x		x	x		x	x		x		x		x
Aspergillus Terrus < 1CFU			x					x							x	x					x				x
Aspergillus Fumigatis < 1CFU	x		x					x	x				x		x			x	x		x		x		x
Staphlococcus Aureus											x										x				x
Bacterial Screening Assay																									
Total Aerobic < 10^5				x		x			x		x					x	x		x		x	x			
Total Aerobic < 10^4	x						x			x		x			x										
Total BTGN < 10^4																x								x	
Total BTGN < 10^3				x		x	x		x			x			x		x	x	x			x			
Total BTGN < 10^2										x															
Total Coliform < 10^3												x			x	x						x			
Total Coliform < 10^2							x			x															
P Aeruginosa < 10^3																		x			x				x
Total Enterobacteriaceae		x																							
Fungal Screening Assay																									
TY&M < 10^4	x				x		x				x	x			x	x				x	x	x	x		x
TY&M < 10^3				x		x			x	x							x		x						
Penicillium Species																		x							
White Powdery Mildew																									
PDx KIT TYPE																									
Detect*- Bacterial	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Detect*- Fungal	1		1						1				1		1	1		1	1		1		1		
Quant*- Fungal	1	1		1	1	1	1		1	1	1	1			1	1	1	1	1	1	1	1	1		1
Quant*- Bacterial (H)				1		1			1	1					1	1			1			1		1	1
Quant*- Bacterial (L)										1												1			1

qPCR Method

Real-time qPCR technology has been in existence for over 28 years and was originally developed for both detection and quantification of specific DNA sequences. [6] This method relies on real time detection of a fluorogenic dye that is indicative of the total amount of amplified product at the time of measurement. This method requires sample preparation protocols and products to remove contaminants which can interfere with PCR and fluorescence detection. Upon initiation of the PCR, the quantification of the input amount is rapid and straightforward. However, for qualitative detection, not quantification of microbial pathogens, it should be noted that most methods require an enrichment step. The enrichment process requires that the sample be placed in volume of liquid growth medium and incubated one day or more prior to taking an aliquot for sample preparation processing.

This enrichment process is primarily used to increase the level of sensitivity of the assay enhancing the detection of very low copy numbers of the microbes of interest. In theory, enrichment increases the starting number of cells after many cycles of cell division, enhancing the level of detection. Due to the nature of the current instrumentation measuring fluorescence signals, the ability to multiplex reactions and measure multiple organisms is limited and can be challenging. Of course, individual analytes can be tested in multiple reactions, but this adds cost to the overall testing in jurisdictions that regulate a panel of micro-analytes.

Microarray Method

DNA microarray is an established methodology and has been in use for over 40 years. [7] The microarray-based technology offered by PathogenDx for cannabis testing is a novel application for microbial detection. The assay is a DNA-based methodology that is both rapid and precise in identifying microorganisms that are present (viable or non-viable). When detecting some pathogens like *Aspergillus spp.* where mycotoxins may be present, but cells are no longer viable, traditional culture-based assays fall short because they will not recover non-viable cells. However, the DNA-based applications will detect DNA of these pathogenic organisms if present via specific endpoint microarray hybridization and detection. An advantage for the DNA microarray platform is flexibility which allows for the rapid addition of new content, as regulatory jurisdictions

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Top 5 Products for ANXIETY

1. **Northern Lights**
   Rhythm - Chesapeake Alternatives
   Type: Concentrate # Sessions: 129
   Average Rating: 4.5
2. **Cookies & Cream**
   Culta
   Type: Flower # Sessions: 26
   Average Rating: 4.5
3. **Harle-Tsu Distillate**
   Verano Brands
   Type: Concentrate # Sessions: 55
   Average Rating: 4.4
4. **Rhythm Shock**
   Rhythm - Chesapeake Alternatives
   Type: Concentrate # Sessions: 30
   Average Rating: 4.3
5. **ACDC**
   Curio Wellness
   Type: Concentrate # Sessions: 58
   Average Rating: 4.3

Top 5 Products for CHRONIC PAIN

1. **Ace Of Spades**
   Chesapeake Alternatives
   Type: Concentrate # Sessions: 16
   Average Rating: 4.1
2. **Synergy**
   Dixie
   Type: Edible # Sessions: 13
   Average Rating: 3.9
3. **Ace Of Spades**
   Curio
   Type: Concentrate # Sessions: 14
   Average Rating: 3.5
4. **Harle Tsu**
   Culta
   Type: Flower # Sessions: 20
   Average Rating: 3.5
5. **ACDC**
   Rhythm - Chesapeake Alternatives
   Type: Concentrate # Sessions: 21
   Average Rating: 3.4

Top 5 Products for INSOMNIA

1. **Bubba Kush**
   Curie Cultivation LLC
   Type: Flower # Sessions: 14
   Average Rating: 4.9
2. **Harle-Tsu Distillate**
   Verano Brands
   Type: Concentrate # Sessions: 42
   Average Rating: 4.3
3. **Northern Lights**
   Rhythm - Chesapeake Alternatives
   Type: Concentrate # Sessions: 102
   Average Rating: 4.3
4. **Rhythm Shock**
   Rhythm - Chesapeake Alternatives
   Type: Concentrate # Sessions: 25
   Average Rating: 4.2
5. **Plushberry**
   Rhythm - Chesapeake Alternatives
   Type: Concentrate # Sessions: 21
   Average Rating: 4.1

Top 5 Products for DEPRESSION

1. **Cookies & Cream**
   Culta
   Type: Flower # Sessions: 22
   Average Rating: 4.8
2. **Northern Lights**
   Rhythm - Chesapeake Alternatives
   Type: Concentrate # Sessions: 122
   Average Rating: 4.5
3. **Harle-Tsu Distillate**
   Verano Brands
   Type: Concentrate # Sessions: 40
   Average Rating: 4.5
4. **Rhythm Shock**
   Rhythm - Chesapeake Alternatives
   Type: Concentrate # Sessions: 15
   Average Rating: 4.5
5. **ACDC**
   Curio Wellness
   Type: Concentrate # Sessions: 45
   Average Rating: 4.4

Top 5 Products for PTSD

1. **Bubba Kush**
   Curie Cultivation Llc
   Type: Flower # Sessions: 14
   Average Rating: 4.9
2. **Deadhead OG**
   Culta
   Type: Flower # Sessions: 36
   Average Rating: 4.9
3. **Cookies & Cream**
   Culta
   Type: Flower # Sessions: 66
   Average Rating: 4.7
4. **Northern Lights**
   Rhythm - Chesapeake Alternatives
   Type: Concentrate # Sessions: 233
   Average Rating: 4.4
5. **Harle-Tsu Distillate**
   Verano Brands
   Type: Concentrate # Sessions: 97
   Average Rating: 4.4

Terpenes and Testing Magazine
Terpenes, Horticulture, Chemistry & Analytics

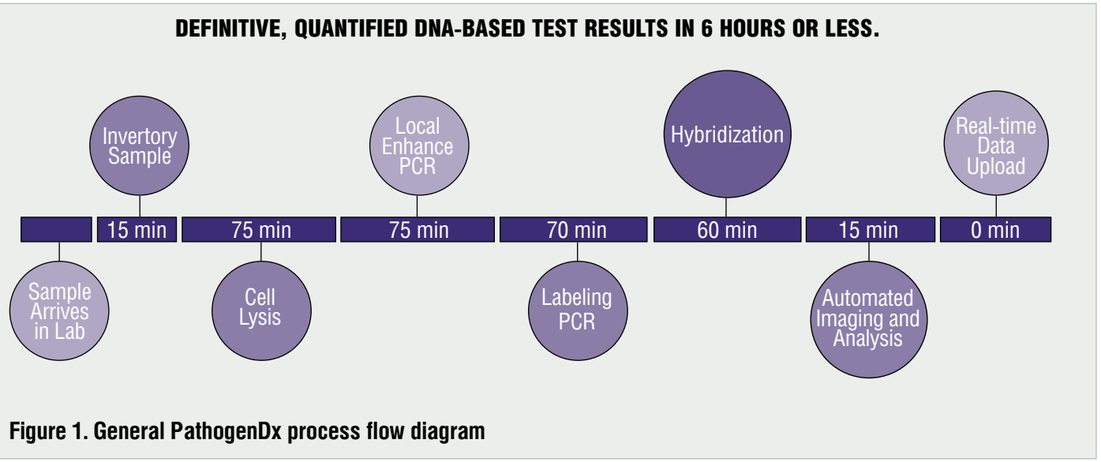

Figure 1. General PathogenDx process flow diagram

modify their regulations, while a challenge has been microarray slide manufacturing costs and therefore has not allowed for expansion and adoption of the technology previously.

Validating Data

Each method is capable of being validated and has a significant number of validation studies performed in matrix to ensure that the clients are receiving quality cannabis. Depending on design, each method is sensitive down to 1 CFU/gram, according to different state regulations. The difference becomes clear on the specificity of each method and whether labs want to do multiple tests to get one specific, appropriately sensitive result. In addition, each of the laboratories that adopt systems perform independent validation to acquire ISO 17025 certification.
For PathogenDx, the goal of these validation studies was to demonstrate that the current assay meets the regulations for cannabis testing, with minimum turnaround time, correlation with plating using enrichment and non-enrichment of samples, and blinded validation using a panel of NSI unknown standards. Many labs have validated the PathogenDx methodology including Brightside Scientific in Long Beach, CA, Steep Hill's Corporate Lab in Berkeley, CA and Caliva in San Jose, CA.

The comparative results of the microarray technology to the well-established plating method meets the state's threshold limit regulations for cannabis quality assurance testing. In this three-step validation, a master inoculum was prepared which contained the six organisms of interest with known CFU values so that the traditional plating method could be compared to the technology. Clean matrix samples were obtained and spiked with and without 16-24 hours enrichment. The master inoculum used was tested both by lab and plate enumeration methods to confirm the spiked CFU values. The second portion of this validation was to assay known genomic DNA reference material in serial dilutions with known concentrations to demonstrate the sensitivity and specificity of the assay. A series ranging from 100,000 genome copies down to 1 copy was run to show the sensitivity of the assay down to 1 copy number per organism. The third step in the validation was to obtain NSI proficiency samples and correctly identify which of the blinded samples contained the target organism.

The Future

Each method discussed have their merits and challenges. The microarray assay has been demonstrated to provide the sensitivity to detect live organisms in the 1 CFU/gram range and the purified genomic DNA down to 1 copy number with extraordinary specificity. This was achieved even in the presence of competitor organisms exceeding the 1 CFU/gram concentration.

The cannabis industry is poised to be the leading industry of new and exciting technologies to be utilized for broader medical, food and agricultural markets to better educate and diagnose human and plant pathogens. The promise to not only expand our understanding of trends in food outbreaks, allowing researchers to predict and prevent pathogen contamination, but the potential is within reach to provide information to growers and farmers related to plant pathogens present in soil, water, or air that in many cases stunt growth and limit sizable harvests is a goal for all methods. Most notably is that these applications were developed and validated for cannabis testing and will soon solve problems for food and agriculture as well.

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Increasing Return on Investment for Pesticide Analysis in Cannabis

By Bob Clifford, PhD., Heather Reece, Jeff Dahl, PhD., Vikki Johnson, Shimadzu Scientific Instruments

After measuring cannabinoid concentrations, also called potency analysis, pesticide testing is the most in-demand application in the cannabis lab according to SDi report 18-025, "Pot of Gold – Opportunities for Analytical Instruments in Cannabis Testing". [1] Potency accounts for 44% of testing, pesticides 15%, microbial 14%, heavy metals 12%, terpenes 9%, and residual solvents 6%. Since all labs in the cannabis industry have analyzers for quantifying potency, this article will focus on the return on investment (ROI) for pesticide analysis by mass spectrometry, an analysis that brings in the most revenue per sample.

The first question is what type of mass spectrometer (MS)? Should it be a single or triple quadrupole system? For pesticides in cannabis, the recommendation is a triple quadrupole MS in order to obtain superior sensitivity and selectivity. The next question is which technique is required: LC-MS/MS, GC-MS/MS, or both. In general, it depends on a number of factors, including the molecular weights of the compounds, polarity, volatility, thermal stability, and ionization efficiency. Also, as this industry continues to evolve, the list of pesticides continues to grow with more compounds added. For example, Colorado started with 15 pesticides. Oregon quadrupled the number of pesticides to 59. California used the previous states' regulations plus additional pesticides to increase the number to 66. Canada used some states' regulations in the USA, plus additional pesticides to increase the number to 95. Finally, AOAC International is developing a method using every state in the USA with requirements and Canada's list for a total of 104 pesticides. In addition, this method specifies maximum residual limits (MRLs) that are half of what's currently listed, making analysis more difficult.

The Venn Diagram in Figure 1 shows select pesticides that are easiest to analyze by LC-MS/MS and by GC-MS/MS, and an overlapping area where either technique will suffice. The darker the blue circles on the Venn Diagram, the easier the analysis is by LC-MS/MS; conversely, the darker red circles indicate analysis that is easier by GC-MS/MS. It can be seen that imidacloprid would have high sensitivity by LC-MS/MS and endosulfan would have high sensitivity by GC-MS/MS. And cyfluthrin could be measured by either instrument.

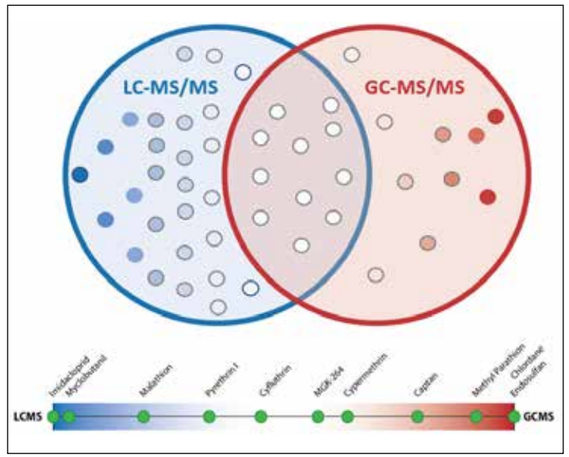

Figure 1: Venn Diagram of pesticides analysis by LC-MS/MS & GC-MS/MS

High-throughput contract laboratories are in business to make money and hopefully to protect consumers, especially immunocompromised patients, from contaminants such as pesticides. Some laboratories want to analyze the entire California pesticide list by LC-MS/MS only, often to minimize initial capital equipment costs, but is that the most efficient way with the highest ROI?

To attempt such an analysis on an LC-MS/MS requires an optional dual ionization source platform with electrospray ionization (ESI) typically used for "LC-MS/MS pesticides", and an atmospheric pressure chemical ionization (APCI) source, which costs additional money, may be able to be used for pesticides that are historically analyzed by GC-MS/MS. The measurements by dual ionization sources are analyzed sequentially; in other words, the ESI measurement run time may be 19 minutes followed by the APCI measurement over 6 minutes for a total analysis time of 25 minutes. Thus, the bottleneck is the sequential analysis.

The other approach uses both LC-MS/MS and GC-MS/MS for a simultaneous analysis. The LC-MS/MS is a 15-minute analysis, while the GC-MS/MS analysis is only 6 minutes since only a handful of pesticides are analyzed. Thus, all the results are completed in 15 minutes utilizing both instruments compared to 25 minutes using only LC-MS/MS. The GC-MS/MS method requires an addition sample preparation step compared to the LC-MS/MS method with the addition of dSPE and syringe or vial filtration.

Shown in Table 1 are the ROI calculations utilizing both approaches. The revenue per day for the LC-MS/MS is $12,825, compared to $21,600 by LC-MS/MS and GC-MS/MS, for an advantage of $8,775 for the two-instrument approach.

Method	LC-MS/MS (ESI) + GC-MS/MS	LC-MS/MS (ESI + APCI)
Instruments Cost	$500,000	$400,000
Price Difference	$100,000	($100,000)
ESI Time (min)	15	19
APCI (min)	X	6
GC-MS/MS (min)	6	X
Time (max)	15	25
Revenue/sample	$225	$225
Min/Day	1440	1440
Samples/Day	96	57
Revenue/Day	$21,600	$12,825
Additional Revenue	$8,775	($8,775)
Break Even (Days)	23	31
Profit Per Year (365 Days)	$7,884,000	$4,681,125
Profit Difference/Year	$3,202,875	($3,202,875)

Table 1: Return on Investment (ROI) for pesticide analysis utilizing two approaches

Reference

[1] "Pot of Gold – Opportunities for Analytical Instruments in Cannabis Testing", Report # 18-025, Strategic Directions International, 2018. https://strategic-directions.com/product/cannabis-testing-market-data/

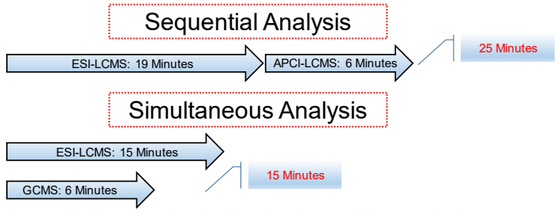

Figure 2: Diagram illustrating the time difference between a sequential analysis and a simultaneous analysis.

The capital cost shown in Table 1 is more expensive with the two-instrument approach, but payoff days for the LC-MS/MS only approach is 31 days compared to 23 days for the two-instrument approach. So, the return on investment is eight days less for the two-instrument approach. After the instruments are paid off, the two-instrument approach will continue to earn an additional $8,775/day. Over a one-year period, that amounts to an extra $3,202,875 in revenue compared to the single instrument, dual ionization source LC-MS/MS.

The additional revenue provided by the two-instrument approach is not limited to the total shown above because GC-MS/MS can also be used to analyze terpenes or residual solvents since the analysis time is 9 minutes faster than the LC-MS/MS method. As mentioned above, terpenes account for 9% of the cannabis analysis while residual solvents account for 6% of the analysis. While the power of GC-MS/MS is not required for residual solvent analysis, the instrument can be operated in the single quadrupole GCMS mode or the GC/FID mode if equipped. Shown in Table 2 is an example of the ROI for residual solvent analysis using the free time on the GC-MS/MS. This will result in an additional revenue of $1,244,842/year for residual solvent analysis. The combined extra revenue for the two-instrument approach for pesticides and residual solvents would be $4,447,717/year.

Interesting to note is that the cannabis class of compounds are not analyzed in equal numbers in some facilities. For example, if 96 pesticides are analyzed/day (Table 1), which

Additional Revenue Method	GC-MS/MS (Residual Solvents)
Minutes/Day	1440
Required GC-MS/MS Pesticides/Day	96
GC-MS/MS Pesticide Analysis Time (min)	6
Total Pesticide Time by GC-MS/MS (min)	576
Open Time on GC-MS/MS (min)	864
Analysis Class of Compounds	Residual Solvent
Number of Compounds (CA)	21
Analysis Time (min)	19
Analysis/Day	45
Revenue/sample	$75
Revenue/Day	**$3,411**
Profit Per Year (365 Days) - Solvents	$1,244,842
Profit Per Year -Pesticides & Solvents	**$4,447,717**

Table 2: Additional Revenue earned by using the free time on the GC-MS/MS for residual solvents

equals 15% of a total cannabis analysis, and residual solvents require 6% of the time, then 38 residual solvents would have to be analyzed per day to keep the ratios even because 96 x (6/15) =38. Table 2 shows 45 residual solvents per day are possible, which is more than the 38 required. Also showing the combination of LC-MS/MS and GC-MS/MS provides a better ROI.

Conclusion

On the surface, using a single LC-MS/MS instrument for analysis of a single class of compounds (i.e. pesticides) appears to provide the best ROI. In reality, though, the use of a combination of both LC-MS/MS and GC-MS/MS for a single class of compounds provides the greatest ROI because this dual approach enables simultaneous analysis, resulting in a potential of up to $3 million more in revenue per year. In addition, up to $1 million in extra revenue per year could be earned for additional compound classes such as residual solvents with the open time on the GC-MS/MS. The two-instrument simultaneous approach could provide up to an additional $4 million in revenue over the single instrument sequential method. LC-MS/MS is the most expensive instrument in a cannabis lab and has the highest revenue per sample, so it is important to have the highest throughput to analyze the most samples per day and obtain the best ROI. It should also be noted that aflatoxins B1, B2, G1, G2 and the mycotoxin ochratoxin A can be analyzed during the LC-MS/MS pesticide analysis with the two-instruments approach.

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In-House Analytical Testing: Spending Money to Save Money

By Dylan Wilks, PhD., Orange Photonics

If you ask anyone that grows or processes cannabis about analytical testing, they will likely say the same thing: testing is a necessary evil. It's expensive and doesn't necessarily add value to a product. We all want safe, effective products, but anyone making those products needs to make money in order to survive long term. Many processors are spending tens of thousands of dollars per month on testing. How do you reduce that cost? Ironically, the answer may be to *spend more money*, at least initially, by investing in in-house testing capabilities.

It's possible to spend anywhere from $10K to $1 Million on in-house testing depending on what you want to test, and the level of accuracy and sophistication needed. Let's start with some of the lower cost options, and how they might help your business and build up from there.

Cannabinoid Profile Testing

Cannabinoid profiling, or potency testing, is one of the most common and lowest cost in-house testing options. This is because the process is relatively simple compared to other analytical tests and there's an obvious value proposition. Portable cannabinoid testing solutions can be in the $10-20K range and can come close to laboratory accuracy. For example, LightLab, by Orange Photonics, mimics laboratory instrumentation by using similar technology, liquid chromatography (LC), but doesn't require a full-time laboratory technician. Full laboratory high performance liquid chromatography equipment typically can cost around $50-100K, with added installation and ongoing costs such as maintenance, method development, and calibration.

The value proposition for in-house potency testing is often two-fold: First, it saves time since you don't have to wait for lab results. Second, the per-test cost is orders of magnitude less than sending to a lab. This combination opens up areas of testing that can vastly improve efficiencies. For example, if you process cannabis into oil, there's several places you might test:

- **Trim procurement:** avoid poor purchasing decisions.
- **Extraction set-up:** evaluating your starting material can elucidate the best extraction parameters.In-Process: can fine tune an extractor and get the most efficiency possible.
- **Crude oils:** ensure your crude oils contain the profile and potency you expected.
- **Waste materials:** this is an often overlooked but important area to test. If your waste material still contains significant cannabinoid content, something went wrong, and you're leaving money on the table.
- **Distillation or post-processing:** ensure efficient processing, and that no degradation products are created during the process. We have seen several cases where post-processing results in damage to cannabinoids and lower overall potency.
- **Final product:** know before you send a sample for expensive lab testing whether your product is going to meet your own specifications and your customer's expectations.

Terpene Profiling and Residual Solvents

Terpenes are not far behind cannabinoids in importance for both medicinal efficacy and consumer demand. While the current gold standard in terpene profiling, gas chromatography (GC), is relatively low in cost ($20-$50K for a typical gas chromatograph), the calibration, sample handling and general know-how required is often higher than potency testing. This is in part because terpenes are volatile, so they can change very quickly and are more difficult to sample accurately. If you don't have a dedicated technician or at least someone knowledgeable with GC operation, this might not be a good bet for in-house testing. There are simpler terpene profilers that are either on the market or in development that offer simpler testing alternatives, but these have yet to be validated. Analyzing terpene profiles can have similar value propositions to cannabinoid profiling- it's difficult to make or grow a particular profile if you can't monitor your samples from early plant through final product. As terpenes gain more popularity, in-house testing of terpene profiles will likely grow.
Another area GC can help with is residual solvents. Ensuring your extracted product meets state requirements here is an added bonus, though you will still have to send a final sample out for lab verification in most states.

Pathogens and DNA

Recent advances in DNA testing capability have made it possible to determine the genetics of your particular plant

as well as target some of the major microbial contaminants. Phylos Bioscience, for example, has products that allow you to determine the genetic make-up of any plant for a few hundred dollars in addition to highly accurate sex tests. Neither are technically in-house since a prepared sample is sent to their lab for processing, but their approach of having a user prepare and send the sample in their self-contained kit means the samples can be processed fast.

Other companies, like Medicinal Genomics and PathogenDX, have created DNA based pathogen detection systems that are relatively simple to use when compared to traditional plate counting techniques, though the process is still something that will require at least a moderate level of laboratory experience and $20K+ worth of equipment.

Pesticides

When growers and processors think about testing, they are often thinking about pesticide testing. Pesticide testing is often required by the state and can cost hundreds of dollars per test. False positives and false negatives are often in the news and can cripple a company when recalls are required. The pesticide limits set by most states are extremely low, often just a few parts per billion. To put that in another context, one part per billion is akin to three seconds in a century- it's an exceedingly small amount.

These extremely low levels are not easy to measure, especially not without sophisticated equipment. A typical laboratory that evaluates cannabis products for pesticides will have two pieces of equipment called a GC-MS and an LC-MS, the MS standing for mass spectrometry. The price tag on both is around $400K, and both require a PhD level technician to mine the data. This means measuring pesticides in-house is unfortunately out of the question for all but the largest operations. Best practices with pesticide use and understanding where pesticides could get into your products is likely a more economical alternative to in-house testing in most cases.

Summary

Analytical testing will often be viewed as an expensive but necessary step even outside the cannabis industry. In many cases, sending samples for lab testing can't be avoided, but investing in some in-house testing capabilities can massively reduce that cost, as well as provide information and insight into where your process can be optimized. If the pharmaceutical industry is a guide, in-house testing will become more important as the cannabis industry matures. Nearly every drug maker has in-house testing capability. The right balance of in-house and laboratory testing will depend on your particular products and processes, but a balance of both is likely to provide the most value and best product possible.