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Differentiation of Group III Cannabis Cultivars into Novel Sub-Classes

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from the publisher

End of Year Reflections

WITH THE YEAR'S end creeping up on us and holiday festivities spurring kindness and cheer, it's a great time to gather with family and friends and reflect on the great moments this year brought us as well as some of the challenges. The cannabis industry was no stranger to good and bad moments this year—from increased legalization and bills to help those in need to challenges in regulations and testing guidelines, the industry was rife with change.

In our November/December issue, readers have the chance to dig in on some of these important topics that might spur even more change in 2022. This month's "Cannabis Analysis" column digs into how the pharmaceutical industry has better testing structures and capabilities than the cannabis industry, which allows for fewer mislabeling problems. In "Navigating the Labyrinth," we explore gas chromatography on a deeper level to increase understanding. Readers can learn various efficiency concepts and how to use those methods to develop circular cannabis systems in this month's "Cultivation Classroom" column. In "Cannabis Crossroads," Rylie and Janie Maedler, accompanied by Dr. Reggie Gaudino, discuss the challenges of growing hemp for medical grade cannabidiol (CBD) as well as their company Rylie's Sunshine. Our peer-reviewed article in this issue delves into the phytochemical composition of group III cannabis cultivars and how they are identified into novel sub-classes.

Also in this issue, we have three feature articles. The first article explores the effects of water and air movements in plants, and how it impacts their growing environments. In another piece, readers are able to divulge in if Carbon-14 testing can be used to determine the natural content of CBD products and ingredients to authenticate their true contents. And, finally, our last feature article provides an overview of standard hydrocarbon extract filtration equipment and media. This issue also features a wonderful portfolio of Supplier Profiles where you can learn more about the various companies servicing the industry.

We hope that you enjoy our November/December 2021 *Cannabis Science and Technology* issue and look forward to seeing you in the new year. Stay safe and happy holidays!

Mike Hennessy, Jr
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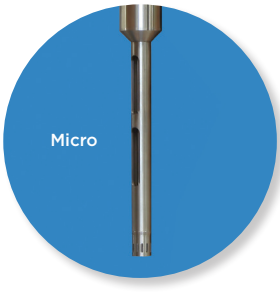

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The Need for In-House Testing:
I Hate to Say I Told You So But...

By ***Brian C. Smith***

Recently a batch of tincture bottles in Oregon labeled as containing cannabidiol (CBD) and no tetrahydrocannabinol (THC) were found to get people high (1,2). Subsequent investigation showed that the bottles had been mislabeled and contained THC. A number of people have reported adverse affects, and a lawsuit has been filed (2). This problem could have been avoided if proper in-house testing of final products had been implemented. I will review how the pharmaceutical industry tests and avoids problems like this, and will call again for more and better regulation of this industry to save our customers from potentially hazardous products.

Statement of the Problems

As some of you may know, my day job is as CEO and Chief Technical Officer of Big Sur Scientific, and my company makes a mid-infrared (IR) based cannabis analyzer (3). The company motto is "Cannabis is Medicine...Test it Like Medicine!" As I have also mentioned before, I almost gave this column series this name, and considering the headlines I have been reading lately, I wish I had.

Earlier this year some cannabis consumers in the Pacific Northwest started experiencing strange symptoms after consuming what was labeled as a THC-free CBD tincture (1,2). According to one man these symptoms included (2): "Not knowing where I was, if I was talking, my heart rate was north of 140 beats a minute and I thought I was dying."

(Before you scoff at the idea that a little THC could cause such a horrible reaction, hang on and read the next section on a similar experience I had.)

It turns out the Oregon company that manufactured the tinctures had mislabeled a batch of bottles as containing CBD but no THC, when in fact the bottles did contain THC. Additionally, the bottles containing CBD were mislabeled as containing THC. Thus, people expecting pain relief from CBD got high, while those expecting to get high experienced nothing because they got CBD and not THC. This mistake resulted in medical problems for a number of patients, and everyone was ripped off because they did not get what they paid for. The Oregon Liquor and Cannabis Commission, the state agency in Oregon tasked with regulating the cannabis industry, has issued a mandatory recall of the mislabeled bottles (1), but of course the damage has been done. What has surprised me is the lack of public outcry here. This has essentially been a local news story in Oregon and has received little coverage in the national media. Where's the outrage? Where is the concern for cannabis patient health?

Now let's put the shoe on the other foot and think about how things would have gone down if this had happened in the pharmaceutical industry. Imagine if the Dewey, Cheatham, and Howe pharmaceutical company had mistakenly added an ingredient to their aspirin tablets that made people nauseous and vomit. Once this became known it would be national news, the US Food and Drug Administration (FDA) would investigate, congressional hearings would follow, and certainly fines would be levied and people may even go to jail. The company responsible for the problems in Oregon is lucky they are getting off as easy as they are.

Now you might be thinking "Hah, such a problem could never happen in the cannabis industry" or "this is an isolated problem." It's not. In fact in an earlier report the same company that mislabeled the tincture bottles was fined $110,000 for "dishonest conduct" (5). The upshot is that they misrepresented what was in their vape products because apparently the people formulating the vape pens on their own added ingredients to the formulation not found in the recipe, and did it without permission and without telling the proper people in management!

THC and Me

Before you dismiss the man quoted above who had a negative experience with THC, let me tell you my own story. Like many of my generation when I was in college, back in the days of yore when dinosaurs roamed the earth, I experimented with marijuana. However, I found the effects of THC disagreeable, and too often found myself curled up

in a fetal position under the couch with paranoid delusions. I haven't touched the stuff since.

However, I am a big fan of the medicinal properties of CBD, and take a dropperful of tincture on a daily basis for arthritis pain. Earlier this year I took some tincture but instead of pain relief I ended up in bed in a fetal position for 4 hours, with my head spinning, wondering what was wrong with me, and thinking I might be dying . . . not unlike the gentlemen quoted above. After the effects wore off, I figured out there must have been THC in the tincture. I tell this story because some of you might think the experience detailed above wasn't true. What many marijuana devotees may not realize is that for those of us who do not regularly use THC, even a small dose can have a noticeable and unpleasant impact. Thus, I believe the gentlemen mentioned above, and understand his outrage.

The Pharmaceutical Industry Testing Paradigm . . . Here We Go Again

How could these problems have been prevented? Simple, in-house testing. There exist mid-IR based tincture analyzers that can measure the amounts of CBD and THC in tinctures quickly, easily, and accurately (4). By simply testing each day's production with an appropriate representative sampling plan these mislabeled products could have been caught. Since the manufacturer didn't catch the problem, they are not performing final product testing, aren't doing it right, or aren't doing it on a frequent enough basis. Why didn't they have an effective and robust in-house testing program in place? Because no one forced them to.

How do we prevent problems like this going forward? At the moment, to the best of my knowledge, no regulatory agencies require cannabis businesses to perform regular in-house testing. Instead, final products are sent to third party laboratories for analysis. The problem here is that the testing is not frequent enough. In the cases cited above, it is possible that only one batch or one day's production was bad, and if the law doesn't require each batch or day's production to be tested problems like these will continue to occur. We need a government regulatory body requiring the cradle-to-grave testing that is required of medicine manufacturers—as is done in the pharmaceutical industry.

> "Since the cannabis industry **appears incapable of policing itself,** it is time for regulators **around the country to step up**."

Multiple times in previous columns I have discussed the need for the cannabis industry to adopt the pharmaceutical industry testing paradigm (6-9), and have discussed what the industry needs to do to test correctly. To review:

1. In-house testing must be performed on each and every raw material for identity and purity.
2. After every manufacturing step every batch of material must be tested to make sure the correct product was produced. This is part of process control and quality control.
3. Every batch of every final product needs to be tested to insure the right ingredients in the right amounts were added to the formulation.
4. Every step of this process must have standard operating procedures to make sure things are done correctly, and management oversight to sign off on each step to insure accountability.

And yes, this testing paradigm probably would have prevented the problems discussed above.

We Don't Need No Stinking Testing!

I am shocked by the number of cannabis businesses I encounter who refuse to do in-house testing. Of course, the eternal excuse is that they can't afford it. However, as I have pointed out in previous columns (6-9), in-house testing is not only the right thing to do, but the profitable thing to do. Catching problem products before they go out the door saves time, money, and lawsuits. In-house testing also improves the efficiency of extraction and grow operations leading to increased profits (6-9). However, even when presented with these facts many companies do not bother to set up an in-house testing program.

Another part of the resistance is that some believe cannabis should be treated as a dietary supplement rather than a medicine. However, even the dietary supplement industry is required by the FDA to perform some

in-house testing, including identity testing of raw materials.

In-House Testing Must Be Required by Regulatory Agencies

Throughout the three years I have been writing this column I have been pointing out the problems with our industry in the hopes that it would police itself. Given the problems discussed above and the refusal of cannabis businesses to implement in-house testing programs, I am now of the belief that the industry cannot police itself. It is now time for government entities at the state or Federal level to require that cannabis businesses implement robust in-house testing and quality control programs. Current regulations are clearly not enough.

Part of the problem is that current regulations only require testing of the final product to be tested. To the best of my knowledge, no state or Federal agency requires cannabis businesses—particularly final product manufacturers—to implement in-house testing and quality control procedures.

Where's the FDA in all of this? That is a very good question. The FDA has taken its time to issue final regulations on cannabis products, and I thought they were taking their time so they could do it right. After all the time that has elapsed, I have to conclude they are taking their time because they don't know what to do. Perhaps for the first time in its history the FDA is faced with a medicine that has been approved in a specific indication, CBD, already being sold across the country in hundreds of different unregulated products without the usual rigor of FDA testing, approval, and oversight. The horse is out of the barn, so how do you go about regulating an industry that is so far ahead of the regulators? I am not a regulatory expert, but I can say that the FDA needs to do something now. It is probably too late to restrict the sale of CBD products around the country (and in no way would I support that), but they can at least make sure these products made are safe, effective, and properly labeled—and they can start by forcing the industry to perform more and better in-house testing. For starters, the FDA could implement the same level of oversight as they do for dietary supplements.

Conclusions

The cannabis industry is in the headlines again with mislabeled and potentially harmful products. This all could have been prevented by a robust in-house testing program. Since the cannabis industry appears incapable of policing itself, it is time for regulators around the country to step up. At minimum, the FDA should start regulating the manufacture of federally legal cannabis products at the same level they do dietary supplements to insure safety, efficacy, and proper labeling so as to protect the health of the American public.

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ABOUT THE COLUMNIST

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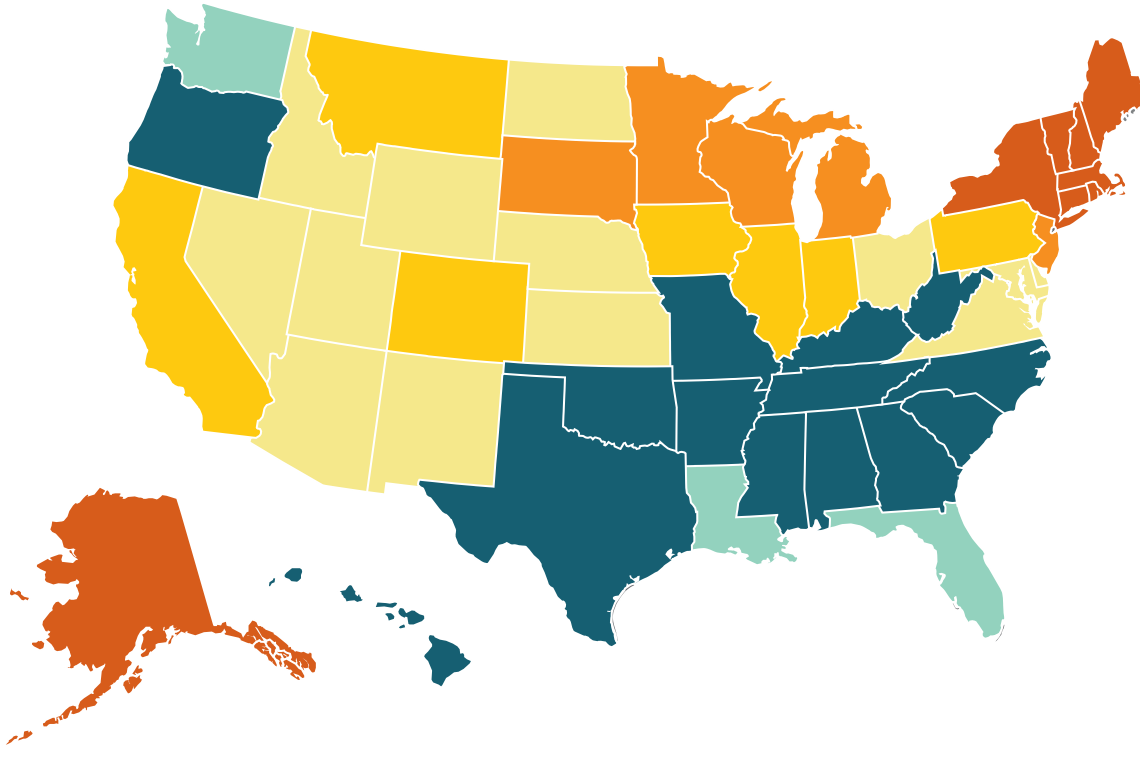

Looking with Light:
Understanding Gas Chromatography, Part I: Gases

By ***Patricia Atkins***

Gas chromatography (GC) is a well-established chromatography technique in analytical chemistry. Over its almost 50-year history the technology has improved and changed to accommodate a wide variety of compounds and targets. As we see in liquid chromatography, many analysts do not have a deep understanding of the functioning and chemistry of GC systems and often depend upon methods they find from manufacturers or technical sources and adapt them to their analyses. In this column, we will take a deeper look into the chemistry, physics, and methodology of each piece of GC instrumentation starting with the gases that compose the mobile phase. Over the next few columns, we will look at the different components of the GC system (form and function) to see what changes can be made to increase resolution, efficiency and produce better analyses.

Gas Chromatography Targets

There are several general conditions for samples to be amenable to the most common gas chromatography (GC) system configurations:

1. The samples and compounds must be volatile. *Volatility* is the ability of a substance to vaporize at a given temperature and pressure. Substances with high volatility usually exist as a vapor while low volatility samples exist as liquids or solids. Low volatility substances resist evaporation (liquids) or sublimation (solids) at atmospheric temperatures and pressures. Samples should be volatile at temperatures below 400 °C. The boiling points of these targets usually need to be less than maximum temperature of the GC columns or system in which they are used. So, if a GC column has a maximum temperature of 200 °C, then targets with boiling points over the 300 °C range will probably not be good candidates for analysis with that column.
2. Samples for gas chromatography must be volatile but also thermally stable. *Thermostability* is the ability of a substance to resist chemical, physical, or structural changes such as polymerization or decomposition when exposed to high temperatures, meaning the samples must be able to vaporize without breaking down in the high temperatures of the GC injection port.
3. Samples or substances should be unreactive with the GC system; they should not participate or cause reactions within the chromatographic pathway.

Boiling Points and Vapor Pressure

Volatility is not a defined measurement, but it is often a reflection of the vapor pressure or the boiling point of a substance. *Vapor pressure* is a measurement of how a substance changes from a condensed form to a vaporous form. Sometimes the term *vapor* is used interchangeable with gas or confused with an aerosol. Gases are one of the states of matter (solid, liquid, and gas) with a single form at room temperature. An aerosol is a gaseous form which contains both liquids and solids in fine particles or droplets. A *vapor* is the gaseous stage or state of a substance, which can be present with other states of the substance at the same time at the same temperature. *Vapor pressure* is the measurement of the equilibrium between vapor and condensed forms of a substance when confined in a sealed vessel. Volatile compounds exist more in the vapor phase of the system and therefore have a higher overall vapor pressure.

Boiling point, on the other hand, is related to vapor pressure in that it is the temperature at which the vapor pressure of a liquid is equal to the pressure around it and this

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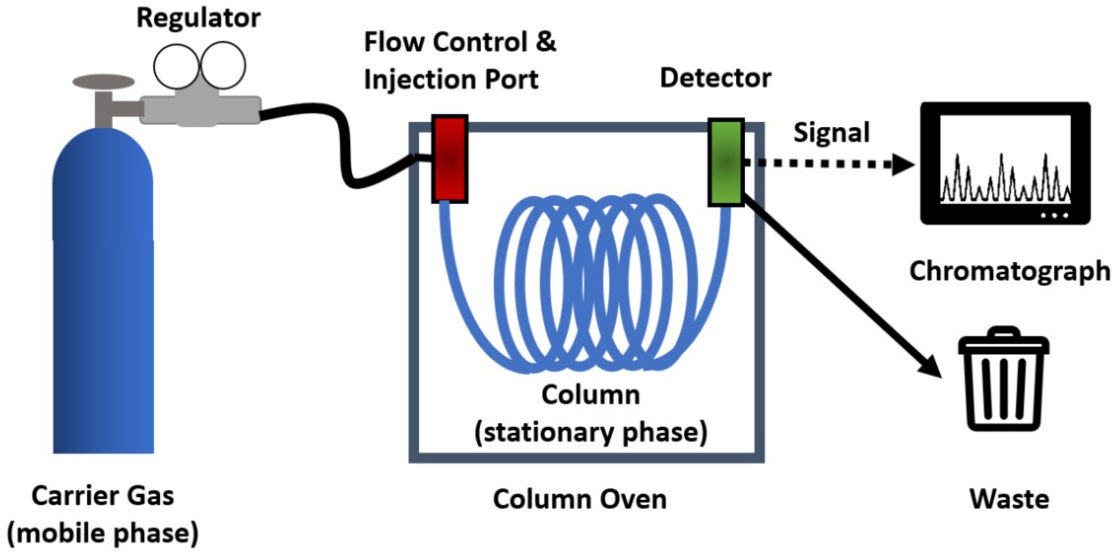
Figure 1: General schematic for a gas chromatography system.



temperature induces the liquid to evaporate quickly (boil). Most boiling points are recorded at atmospheric pressure. Boiling point can be affected by a variety of factors including polarity, molecular weight, and molecular or atomic interactions. The lower the boiling point of a substance, the more time it spends in the gaseous mobile phases and speeds more quickly through the chromatographic system. Very low boiling point substances like some solvents are virtually unretained on the stationary phase of columns, which make them good substances to dissolve GC samples. Generally, solvents with boiling points lower than the starting temperature of the GC method will quickly pass through the system as unretained, while solvents that have boiling points higher than the starting temperatures will possibly be retained on the stationary phase and produce a retained peak (**Table I**). Many GC column chemistries have retentions that reflect elution by either increasing molecular weights of the analytes or increasing boiling points which can help the chromatographer during method development.

GC System Configurations

The general configuration of a gas chromatography system starts with the carrier gas which is either contained in a cylinder or produced using a gas generator depending on the type of gas. Cylinders containing flammable gases such as hydrogen are commonly threaded in the opposite direction of nonflammable gas cylinders to prevent common mix-ups. The gas is controlled by a flow controller or a regulator and is plumbed into the GC system usually near the injection port. The injection port is the entry into the system for samples and connects to the column. Gas flows through the injection port and then the column allowing for the partitioning of analytes between phases. The final components are the detectors, which output an electronic signal to the chromatograph and the waste for the system (**Figure 1**).

Gas chromatography systems are plumbed to a variety of gases depending on the detectors and type of analysis. A carrier gas is common to all the most commonly used configurations. The carrier gas is the mobile phase, which carries analytes to the column for partition chromatography. Most gas lines are plumbed from either a cylinder, dewar, or generator with a series of filters

Table I: Common GC solvents and their boiling points

Solvent	Boiling Point (°C)
Dichloromethane	40
Acetone	56
Methanol	65
Tetrahydrofuran	65
Ethyl Acetate	77
Ethanol	78
Cyclohexane	81
Acetonitrile	82
Isopropanol	82
Water	100
Toluene	111

Table II: Common GC gas purities, designation, and uses

Designation	Nines	%	Maximum Impurities (ppm/volume)	Helium "Grades"	Nitrogen 'Grades'	Hydrogen "Grades"	Chromatography Applications	Analyte Range
N4	4	99.99	100	'Balloon' Grade	Food Grade	Prepurified	Do not use	-
N4.5	4.5	99.995	50	Industrial Grade	99.998 - Prepurified	HP (High Purity)	Do not use	-
N5	5	99.999	10	HP (High Purity)	UHP (Ultra High Purity)	UHP (Ultra High Purity)	General purpose chromatography use	%- ppm
N5.5	5.5	99.9995	5	UHP (Ultra High Purity)	Trace analytical or Semiconductor	UPC (Ultra-Pure Carrier)	Spectroscopy applications like GC–MS	<1000 ppm
N6	6	99.9999	1	~Pure Helium	Research, Chromatography	Research Grade	High Sensitivity Chromatography Applications and Research	Sub ppm

or traps intended to trap any contamination or potential compounds which could interfere with analysis.

The most common traps for GC systems include an oxygen trap, a moisture trap, and a hydrocarbon trap. The oxygen trap removes oxygen from the purified gas that could damage the GC column. Oxygen traps are often composed of metal and an inert reagent. The goal of the trap is to reduce oxygen concentrations to below 20 ppb. Many oxygen traps can remove small organic molecules and sulfur from the gases.

The moisture trap removes water and other moisture vapors from the system that could impede vaporization of the samples. Moisture or water traps can often be refilled with sorbent materials instead of being replaced. The body of a trap is plastic, glass, or some combination of both rated to withstand gas pressures of the GC system. Plastic traps may pose a risk of contamination, so for applications where this may be of concern use glass bodied moisture traps.

The hydrocarbon trap removes any potential hydrocarbon contamination from greases or oils which could be seen as peaks in the chromatogram. The trap sorbent is usually activated carbon or carbon media that removes organic solvents. The traps are in many cases metal and in some cases can be refilled instead of being replaced.

GC gas traps can be offered in a variety of configurations including manifolds with space for all the traps or combinations of different functionalities in one type of trap. There are traps that contain indicators which change color when the contamination is detected or when the traps are depleted. Each configuration has its own benefits (that is, ease of use, longevity, and so on) and drawbacks (cost, time). In the end, the best trap is the one that gives the user the highest purity of carrier gas through their GC system.

Gas Purity

The gas purity needed for a GC system depends on the type of system and the level of sensitivity needed by the system. A gas chromatography–mass spectroscopy (GC–MS) system measuring low parts-per-million targets have higher sensitivity and therefore need higher purity gases than a flame ionization detector (FID) that only measures percent levels of components in a mixture. The cost of gases increases as the purity of the gas increases, therefore it is best to use the grade of gases suitable for the type of system used and the analytical range of the targets you wish to detect. The nominally recommended purities for the common gases such as helium, nitrogen, and hydrogen should be between 99.995% and 99.999% for most GC applications.

Despite the grade of gas, the typical impurities of oxygen, water, and hydrocarbons should be below 1–2 ppm each. Other impurities such as carbon monoxide, carbon dioxide, and other gases are not often individually monitored but together with oxygen, water, and hydrocarbons should be less than 10 ppm. Gas suppliers can grade their gases in cylinders and containers in a variety of ways to indicate purity and potential use. Some designations of "grades" care more about marketing than industry standards, and the analyst needs to exercise care to distinguish the best product for their application. One company's ultra-high purity helium may be called ultra-pure carrier gas grade. The official designation should state a numerical purity like N4 or %99.99 (also known as four nines) (**Table II**). Compressed gas cylinders usually maintain their certified values for purity down to only 10% of the

Figure 2: Examples of eddy diffusion through (a) packed column and (b) tubular capillary column.

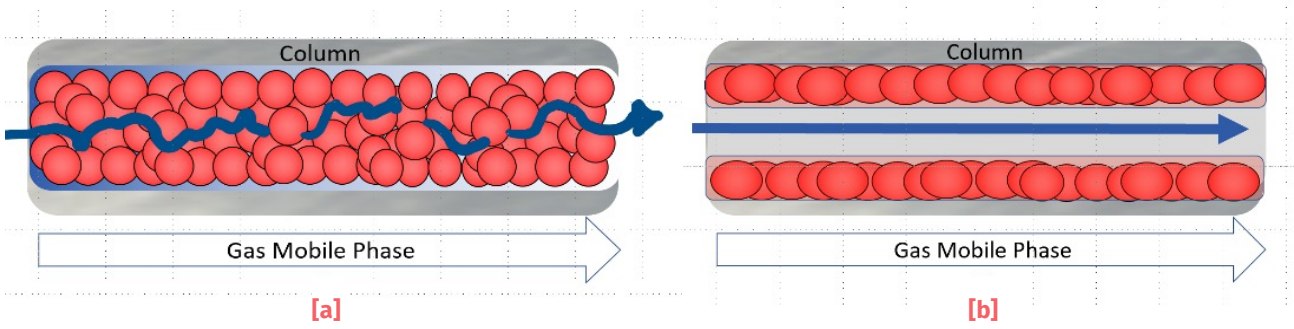
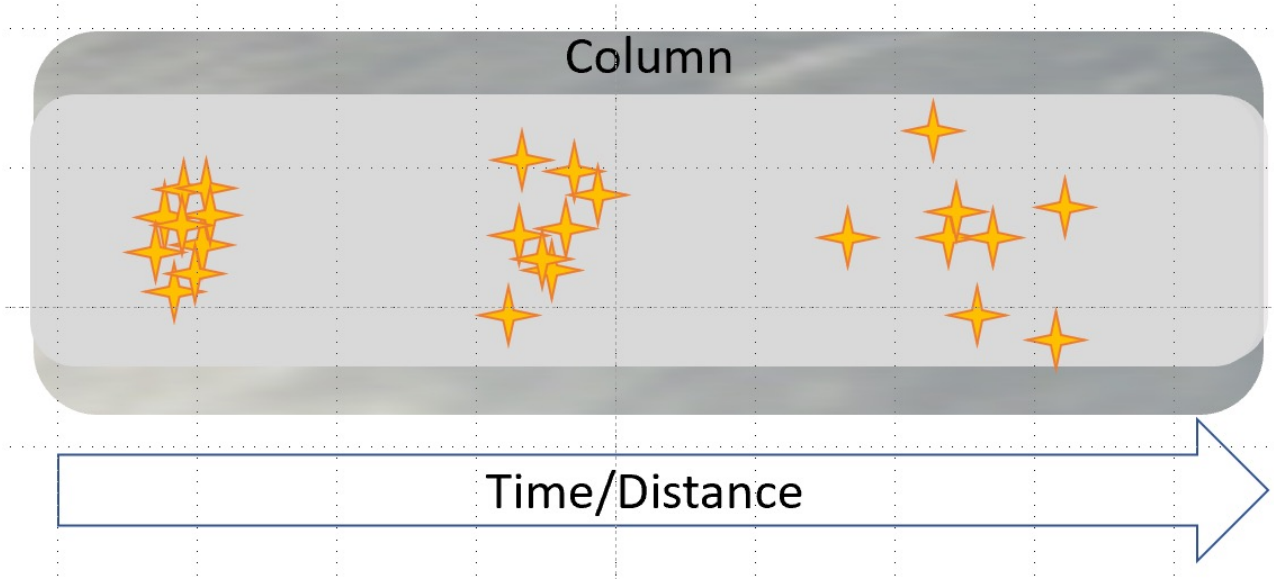


Figure 3: Example of longitudinal diffusion in a column.



original pressure. At lower pressures there could be higher levels such as impurities like water or hydrocarbons.

The number of impurities in a GC system can also increase with the improper choice of the gas handing equipment and plumbing. GC systems should always be plumbed using stainless steel or copper tubing and fittings. Gas regulators should have stainless steel components impervious to oxygen and moisture. Many companies have purity ratings similar to the gases to indicate the number of impurities that may be introduced to the gases from the regulator. Impurities from regulators may include off gassing of materials inside the regulator, leaks which allow air into the system, and reactions between the materials of the regulator and the gases. The purity of the regulator should match the purity of the gases used.

Regulators routinely used in the laboratory are either single stage or two or dual stage regulators. Single stage regulators are not used in GC systems but can be used in gas lines that require continue pressure adjustments. Dual stage regulators are actually two regulators in one piece of equipment fixed to the gas cylinder. The first stage controls the pressure from the cylinder and the second stage reduces the pressure further to plumb into the GC system. The second stage keeps the pressure continuous even when the overall pressure of the cylinder drops.

Selecting Carrier Gases

The carrier gases for most GC applications are helium, hydrogen, nitrogen, or argon. There are four main considerations in the choice of carrier gas:

1. Application;
2. Efficiency;
3. Availability; and
4. Cost

The application, type of column, and analysis can each dictate the type and grade of the required carrier or make-up gas. A make-up gas is an additional gas plumbed into the system to produce a specific effect on the detector or system. The lower in concentration of the potential target analyte, the higher the purity of carrier and make-up gas are needed for analyses. The higher the purity of gases, the higher the cost of the gas.

The next consideration of efficiency deals with the chromatographic efficiency of the system and theoretical plates as was discussed in our previous chromatography columns. *Efficiency* is a measure of theoretical plates in a chromatographic system. As has been discussed previously, chromatography columns do not have physical plates that can be measured so the plates are theoretical to describe the efficiency of the column. This is called height equivalent to a theoretical plate (HETP) and is measured by the van Deemter equation in gas chromatography analyses (**Equation 1**).

$$HETP = A + \frac{B}{u} + (C_s + C_m) \times u \quad [1]$$

where A is the eddy diffusion parameter, B is the longitudinal diffusion coefficient of eluting particles resulting in dispersion (m^2/s), C is the mass transfer coefficient of resistance of analyte between mobile (m) and stationary phase (s), and u is linear velocity or speed (m/s).

The first term of the equation is the eddy diffusion parameter and refers to the diffusion or mixing of substances by a turbulent, swirling motion around objects. In certain types of columns where the stationary phase is particulate in nature, currents or eddies can form and create forces that effect the HETP. These types of columns include packed GC columns (**Figure 2a**). Columns that are tubular in nature (that is, capillary columns) do not have the same forces so the term A of the equation is zero (**Figure 2b**).

The second term B is the longitudinal diffusion coefficient which is the constant proportion of the diffusion of one

Figure 4: Effects of the coefficients and terms of van Deemter equation on optimal velocity in a capillary column plotted in a van Deemter curve.

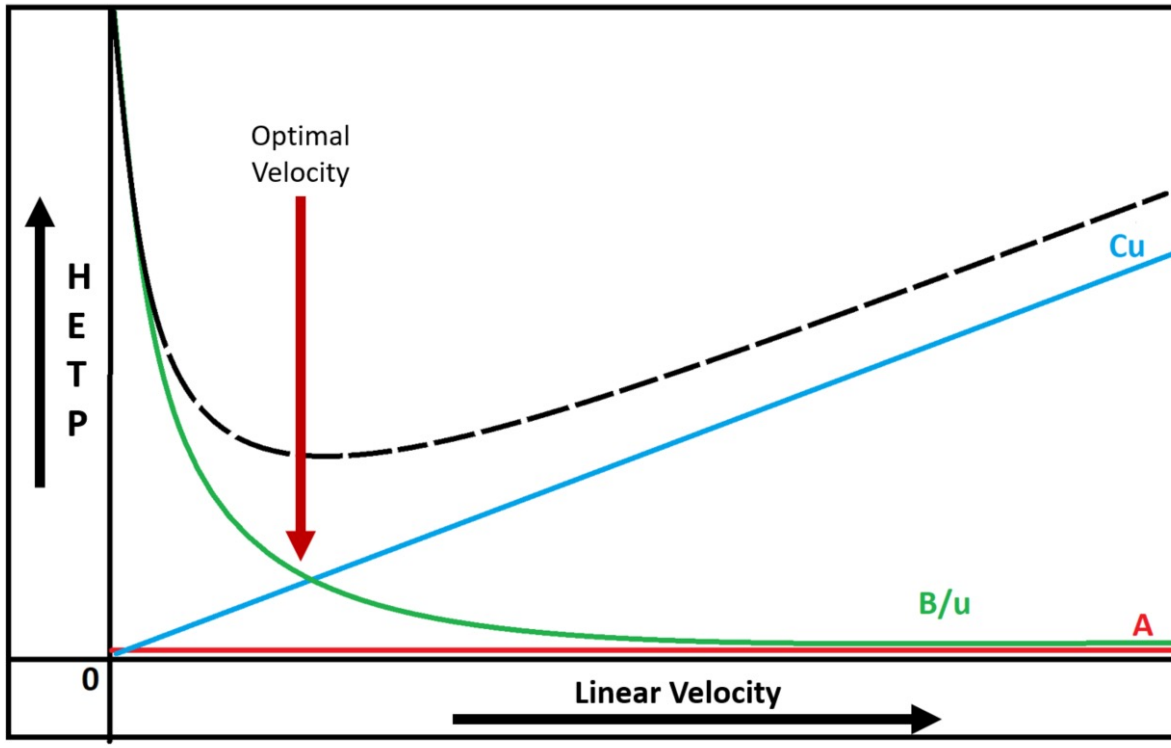
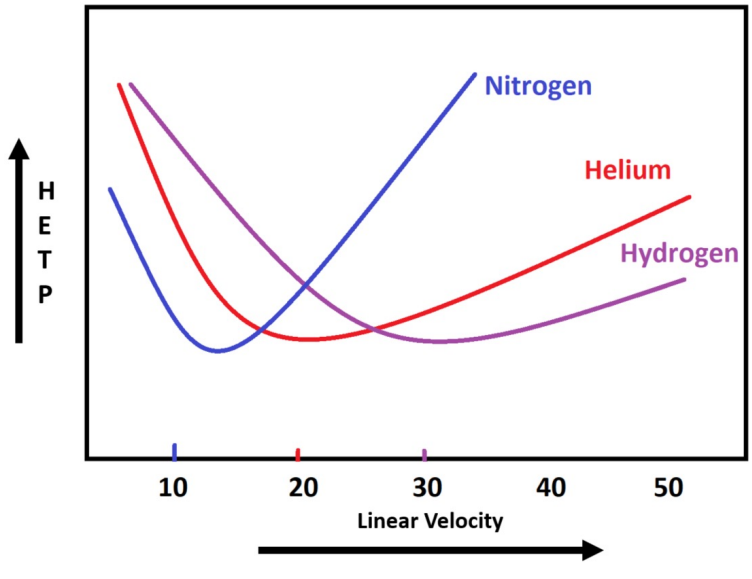


Figure 5: Relative comparisons of van Deemter curves for nitrogen, helium, and hydrogen carrier gases.



component into another divided by the average speed of diffusion. This term is a calculation of the dispersion as a solute travels down the pathway. In this situation the concentration of the solute is highest in the middle of the pathway and more diffuse on the edges with the solute diffusing more as it passes through the column and contributes to band broadening (**Figure 3**). The diffusion effects are more pronounced at lower flow rates. Gas chromatography is more effected by these forces because of the exponentially higher diffusion coefficients found in gases as opposed to liquids.

The third term is the mass transfer coefficient between the mobile and solid phase. Since the mobile phase (gas) is rapidly moving, an equilibrium may not be reached resulting in peaks being

either less retained or retained too highly on the solid phase depending on the depth or thickness of the solid phase (film). The mass transfer is most efficient at lower flow rates, which is the opposite of the longitudinal diffusion. The most effective set of conditions is a balance between these two coefficients resulting in a minimal value for the HETP and the optimized linear velocity (u) in a van Deemter curve (**Figure 4**).

The window of velocity for the optimal velocity can change with the type of carrier gas, solid phase (film) thickness, and the inner diameter of the column. Film thickness can influence the mass transfer while smaller diameter columns produce flattened curves with optimal velocity at lower velocities. The B/u diffusion term is greatly influenced by the type of carrier gas. The goal is to optimize the balance between the type of gas, the gas flow, and the column dimensions to produce an area of optimal practical gas velocities (~1.5 to 2x the optimal velocity) in which the system operates.

Carrier gases and their own van Deemter curves will influence the optimization of the GC system. Helium is the one of the most commonly used carrier gases for GC due to its safety and relatively good van Deemter curve with a range of practical optimum gas velocities (OPGV) between about (25–35 cm/s). The drawback to helium is that it is an expensive commodity which is dependent upon the finite reserves of natural gas. There are no easy was to produce helium (especially within the average laboratory) and helium's availability and cost in some cases offset its ease of use and safety.

Nitrogen is another potential GC carrier gas which unlike helium is fairly inexpensive and can be produced in-house with a liquid nitrogen dewar, or a nitrogen generator and purifier. Nitrogen is a fairly safe gas in the laboratory, but it is not the most optimal gas for GC with a smaller lower range OPGV from ~10 to 15 cm/s.

Finally, hydrogen, like nitrogen is cheap, accessible, and easy to produce but can cause some safety concerns (real and exaggerated) in the laboratory. Hydrogen can be produced using a hydrogen generator or from hydrogen cylinders. Hydrogen has the best OPGV range of values with higher velocities from 35 to 60 cm/s, but there can be possible hydrogenation reactions that may affect some target analytes (**Figure 5**).

As I said earlier, there are safety concerns regarding the use of hydrogen in the laboratory as a carrier gas. Hydrogen can be explosive at above ~4% volume in air but on the positive side, it quickly diffuses, and most modern GC systems regulate flow and have automated safety shutdowns to prevent accidents. Secondly, there is very little hydrogen flowing through any GC system so the amount of possible leakage at the system is minimal compared to other possible leaks in the gas plumbing system upstream of the chromatograph.

Final Thoughts

Each gas has its own positive and negative points for use in GC analyses. There can be applications that would benefit from a different type of carrier gas or even a mix of carrier gases. Many GC FID analyses may not need the purity and expense of helium and could perform better with hydrogen. In GC–MS analyses, the hydrogen may cause sensitivity issues or system reactions. In high-throughput laboratories, method run times could factor against using inherently slow running nitrogen (remember those optimal low velocities!). Some GC systems allow users to switch or blend gases, this is often controlled by the flow controllers in the inlet area of the GC which we will continue with in our next discussion of GC system components and functions.

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ABOUT THE COLUMNIST

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

Microwave Digestion and Trace Metals Analysis of Mixed Cannabis and Hemp Products

SAMUEL HECKLE AND LEANNE ANDERSON, CEM

In 1970, marijuana was designated a Schedule 1 drug, under the Controlled Substances Act, making it nearly impossible for laboratories to perform cannabis research. However, medicinal use of cannabis is now legal in Canada and 37 US states plus the District of Columbia, with more joining every year. With the passage of the Farm Bill in 2018, it is now federally legal to grow and process hemp in all 50 states. All of this interest in medical cannabis and CBD has highlighted the need for good analysis methodology in this relatively young market. Cannabis analysis is still developing standardized protocols, requirements, and acceptable testing practices. Typical testing requirements for cannabis and its products include heavy metal analysis, pesticide residue, and the potency of active ingredients such as tetrahydrocannabinol (THC). The terpene content of cannabis is also important. Terpenes have been shown to have beneficial uses for treatment of conditions ranging from cancer and inflammation to anxiety and sleeplessness. It is believed that the combination of terpenes and cannabinoids in cannabis produce a synergistic effect with regards to medical benefits, further elevating its popularity worldwide.

CERTAIN HEAVY METALS CAN cause adverse effects on human health. Toxic heavy metals such as arsenic, cadmium, lead, and mercury are persistent once released into the environment and can accumulate in cannabis plants. Since hemp is a strain of cannabis that contains very little THC, it is susceptible to bioaccumulation of heavy metals like its THC containing counterparts. Cannabis-based products such as foods, oils, tinctures, and lotions should be tested for the presence of heavy metals to ensure consumer safety and product quality. Cannabis and CBD infused products have grown in popularity in states that allow medicinal and recreational use of cannabis and hemp. This application note details the digestion and analysis of various forms of hemp and cannabis products for determination of trace metals content.

FIGURE 1: Samples selected for digestion and analysis.

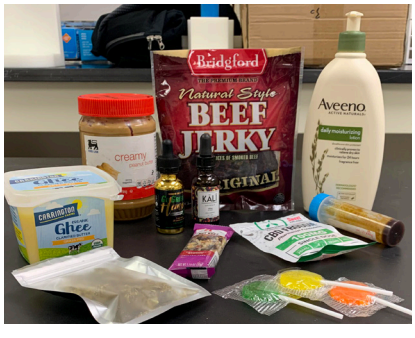

TABLE I: Shimadzu ICPMS-2030 operational conditions

PARAMETER	SETTING
Radio freq. power	1.20 kW
Sampling depth	5.0 mm
Plasma gas	8.0 L/min
Auxiliary gas	1.10 L/min
Carrier gas	0.70 L/min
Mix gas	0.00 L/min
Cell gas	6.0 mL/min
Cell voltage	-21 V
Energy filter	7.0 V
Chamber temp.	5 °C

INSTRUMENTATION

A CEM MARS™ 6 microwave digestion system was used to digest varied cannabis and hemp sample types. The MARSXpress™ Plus vessels uses a vent and reseal design, which allows for acid vapors to be exhausted while maintaining the sample integrity and elements within the vessel. The 110 mL vessel volume provides for a larger headspace, allowing a larger sample size for a more homogenous sample. The MARSXpress Plus vessel design uses only three pieces that are easily assembled and placed in a 24-position turntable, prior to placing into the MARS 6 system.

All samples were analyzed by ICP-MS on a Shimadzu ICPMS-2030.

PROCEDURE AND METHOD

A variety of 11 hemp and hemp containing samples were obtained from local retailers. Samples ranged from edibles to lotions to plant material. Solid samples required minimal particle size reduction in order to obtain a representative sample. Liquid and semi-liquid samples were digested as received.

A 0.5 g sample was weighed into a MARSXpress Plus vessel taking care to deposit the entire sample onto the bottom of

TABLE II: Spike recovery results

		^{75}As	^{111}Cd	^{200}Hg	^{208}Pb
Blank	Mean value	n.d.	n.d.	n.d.	n.d.
	RSD (n = 3)	---	---	---	---
Fortified blank	Mean value	5.23	5.23	5.1	5.08
	RSD (n = 3)	2.03	0.76	3.57	1.59
Recovery (%)		105	105	102	102
Hard candy	Mean value	n.d.	n.d.	n.d.	0.0116
	RSD (n = 3)	---	---	---	1.79
Fortified hard candy	Mean value	5.12	4.92	5.05	5.13
	RSD (n = 3)	3.51	1.68	0.19	1.74
Recovery (%)		102	98	101	102
Granola bar	Mean value	n.d.	0.125	n.d.	0.0186
	RSD (n = 3)	---	1.63	---	3.02
Fortified granola bar	Mean value	4.55	5.07	5.09	5.06
	RSD (n = 3)	13.53	1.61	1.58	1.15
Recovery (%)		91	99	102	101

TABLE II (continued): Spike recovery results

		^{75}As	^{111}Cd	^{200}Hg	^{208}Pb
MCT oil	Mean value	n.d.	n.d.	n.d.	n.d.
	RSD (n = 3)	---	---	---	---
Fortified MCT oil	Mean value	5.39	5.04	5.13	4.94
	RSD (n = 3)	1.36	1.54	1.24	1.89
Recovery (%)		108	101	103	99
Ghee	Mean value	n.d.	n.d.	n.d.	n.d.
	RSD (n = 3)	---	---	---	---
Fortified ghee	Mean value	5.46	5.05	5.15	5
	RSD (n = 3)	3.8	2.01	1.19	1.73
Recovery (%)		109	101	103	100
Hemp oil	Mean value	n.d.	n.d.	n.d.	0.0723
	RSD (n = 3)	---	---	---	1.6
Fortified hemp oil	Mean value	5.27	5.18	5.2	5.09
	RSD (n = 3)	11.91	1.53	2.1	1.5
Recovery (%)		105	104	104	100
Lotion	Mean value	n.d.	n.d.	n.d.	n.d.
	RSD (n = 3)	---	---	---	---
Fortified lotion	Mean value	4.96	5.09	5.16	5.03
	RSD (n = 3)	11.08	1.21	3.12	2.62
Recovery (%)		99	102	103	101
Froggy	Mean value	n.d.	n.d.	n.d.	0.0407
	RSD (n = 3)	---	---	---	0.66
Fortified froggy	Mean value	4.76	4.9	4.83	5.07
	RSD (n = 3)	0.95	0.74	1.46	1.92
Recovery (%)		95	98	97	101
Hemp flower	Mean value	0.0231	n.d.	n.d.	0.163
	RSD (n = 3)	6.07	---	---	1.09
Fortified hemp flower	Mean value	4.81	5.05	5.11	5.15
	RSD (n = 3)	9.68	1.22	0.6	1.48
Recovery (%)		96	101	102	100
Beef jerky	Mean value	n.d.	0.0482	n.d.	0.057
	RSD (n = 3)	---	0.77	---	1.65
Fortified beef jerky	Mean value	4.81	5.07	5.06	5.22
	RSD (n = 3)	7.54	1.17	1.25	1.86
Recovery (%)		96	100	101	103
Peanut butter	Mean value	n.d.	0.111	n.d.	0.0511
	RSD (n = 3)	---	3.92	---	1.32
Fortified peanut butter	Mean value	5.22	5.28	5.13	5.11
	RSD (n = 3)	5.32	1.08	1.45	2.93
Recovery (%)		104	103	103	101
Conc. CBD oil	Mean value	n.d.	n.d.	n.d.	0.0662
	RSD (n = 3)	---	---	---	0.78
Fortified Conc. CBD oil	Mean value	5.41	5.17	4.93	5.05
	RSD (n = 3)	10.43	0.7	2.03	2.74
Recovery (%)		108	103	99	100

the vessel. A combination of 9 mL HNO3 and 1 mL HCl was added to each vessel before the vessels were sealed and placed into the microwave. Using the preprogrammed Cannabis One Touch Method, the samples were digested at 210 °C for a total run time of 45 min including a 20 min ramp to temperature, a 15 min hold at 210 °C, and a 10 min cool down. Upon completion of the run samples were diluted to 50 mL with DI H2O. All samples were run in duplicate with duplicate blanks.

A second batch of samples was run identically to the first except for the addition of a spike solution containing 5 ppb Pb, As, Cd, and Hg to all samples, duplicates, and blanks. **FIGURE 1** shows the 11 samples analyzed in this study.

All samples were analyzed on a Shimadzu ICPMS-2330 to determine spike recoveries and actual sample content of the "big four" heavy metals of As, Cd, Hg, and Pb. Instrument conditions are shown in **TABLE I**.

RESULTS AND DISCUSSION

The MARS 6, with MARSXpress Plus vessels was able to successfully digest all 24 samples and blanks in a single batch. All of the samples were completely digested, yielding clear and particulate-free solutions upon dilution with deionized water. The MARS 6 is an ideal digestion system for the cannabis industry because it is able to successfully digest batches of mixed materials, including foods, oils, tinctures, creams, and plant materials, in as little as 45 min.

The clear and particulate-free digestate solutions that were analyzed by ICP-MS showed excellent spike recovery and RSDs, as shown in **TABLE II**, indicating complete matrix decomposition by microwave digestion.

CONCLUSIONS

The MARS 6 with MARSXpress Plus vessels was able to digest a wide variety of cannabis and hemp samples in mixed batches producing digestate that was suitable for analysis. Analysis of all samples showed excellent spike recovery and repeatability. Labs faced with a large number of samples will be able to confidently digest a mixed batch of samples in under 1 h.

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Circular Cannabis Systems:
Tracking and Minimizing Waste and Emissions Impacts

By ***Gretchen Schimelpfenig***

Cultivation operations use resources such as energy and water and also generate waste products like wastewater, biomass waste, and other solid waste such as growing supplies and packaging materials. Growers can benefit from learning about circular processes that recapture waste streams to improve operational efficiency and reduce impacts on surrounding communities and sensitive environments. Specialized waste benchmarks help businesses track emissions of cultivation facilities to quantify the costs and benefits of implementing circular best practices. Compare year-over-year performance and contrast key performance indicators against industry averages to value and prioritize strategies. Minimize environmental impacts by controlling horticultural lighting to improve yields, recapturing, treating, and reusing drained irrigation water, and minimizing crop loss through effective integrated pest management (IPM). In this final "Cultivation Classroom" column of 2021, bring efficiency concepts together to create circular cannabis systems.

Emissions associated with cannabis operations include greenhouse gas emissions from production processes, wastewater, and solid waste. For growers in some regions such as Illinois and Colorado, there may be regulations requiring the management of certain types of emissions. It is crucial for cannabis businesses to stay competitive in changing markets by understanding the opportunities for circularity in their facilities. Circular economy metrics benchmark the emissions generated by facilities to measure progress towards regenerative practices and reward validated sustainability performance.

Resource Innovation Institute (RII) gathers Technical Advisory Council key stakeholders and subject matter experts in the Emissions Working Group (1) to standardize methodology for quantifying emissions associated with indoor agriculture, determine data the market needs to understand the emissions impacts and benefits of controlled environment agriculture, and motivate the industry to utilize low-carbon approaches across a range of cultivation methods and geographies to increase resilience and reduce vulnerability.

Avoiding Greenhouse Gas Energy Emissions

The greenhouse gas (GHG) emissions impacts of cannabis production include emissions from cultivation and post-harvest supply chain processes. In cultivation and processing, cannabis demands energy and creates associated emissions impacts. Globally, nearly 80% of all GHG emissions come from the production and consumption of energy. Growers of all kinds use systems that need energy in order to increase yields.

Outdoor farms use energy for pumping for cultivation, environmental control, and on-farm transportation and hauling. Irrigation systems require electricity for pumping water. Equipment for heating and farm vehicles require fuel. Greenhouse and indoor facilities can have higher energy demands than field farms. Indoor vertical farms use high-intensity sole-source horticultural lighting systems to optimize growth and high-capacity HVAC systems to maintain climate and airflow. Greenhouses use electricity for supplemental lighting, and in colder climates where greenhouses seek to supply year-round harvest, greenhouses also use fuel for heating.

Energy used for cannabis cultivation has different emissions impacts depending on fuel choice and source energy for regional electric grids (2). Facilities can minimize GHG emissions by using energy-efficient lighting and HVAC equipment, producing on-site renewable energy, and minimizing use of fossil fuels.

Closing the Water Loop

Climate change, resource constraints, and environmental disasters impact cannabis businesses along with human and ecosystem health. During peak drought cycles approximately one-quarter of the US experiences extreme or exceptional drought (4). A future of rising costs and tightening access to water due to prolonged and historic droughts make water efficiency an increasingly urgent priority.

In field farming, water that is not retained by plants is evaporated back into the surrounding environment or drains as

runoff, carrying soil and fertilizer into groundwater, rivers, and lakes. Water recirculation systems used by greenhouses and indoor cultivation facilities design out waste and keep a constrained resource in use. Both kinds of facilities present opportunities to recapture, treat, and reuse irrigation water (5). Indoor farming can also recycle condensate captured from HVAC and dehumidification systems. Regenerative practices are phenomenal opportunities for sustainable water practices; recirculating indoor farms can use 90–95% less water than field farms (6). In some regions, cannabis operations must implement wastewater treatment plans to recapture condensate and treat and reuse drained irrigation water.

Recycling Waste

Solid waste emissions from cannabis facilities include biomass, cultivation supplies, and packaging waste. Used substrate and root balls from grow rooms and branches, stems, and leaves from trimming are examples of cannabis biomass waste. In some regions, cannabis biomass cannot be composted, or is required to be mixed with other kinds of waste. Supplies to support optimal plant growth and development such as trellis netting can be challenging to reuse and impossible to recycle. Many growers and consumers are also concerned about packaging waste. Regulations in some areas can make recycling packaging materials difficult, and consumers are often left responsible for finding sustainable options.

Benchmarking Cannabis Emissions

Accounting for the emissions impacts of cannabis is possible with specialized benchmarking tools like Resource Innovation Institute's PowerScore (7) that can calculate key performance indicators (KPIs) for resource efficiency and productivity for outdoor, greenhouse, and indoor cultivation operations. PowerScore has KPIs for energy-associated greenhouse gas emissions, water, and solid waste. RII's Emissions Working Group peer reviews changes to the PowerScore platform related to emissions and prioritizes new KPIs to measure both emissions impacts and benefits (see **Figure 1**).

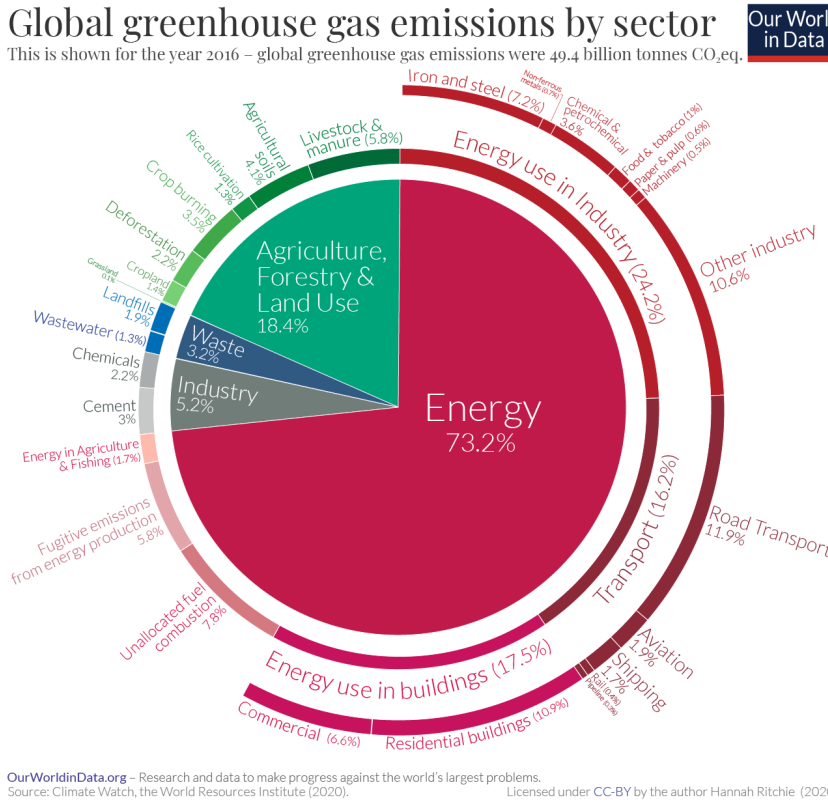
Cannabis businesses can be recognized for excellence with environmental reporting and certification programs. Certification programs help address the need to measure progress toward circularity as climate change, resource constraints, and environmental disasters impact human and ecosystem health. Benchmarking tools such as PowerScore can be used as inputs to the certification process to prove circularity of resources like energy and water. Certification programs and benchmarking tools like PowerScore evaluate the resource efficiency and productivity of controlled-environment agriculture (CEA) facilities while rewarding regenerative practices like keeping materials in use, conserving water, and reducing energy consumption.

Greenhouse gas emissions impacts from energy are measured in equivalent kilograms of CO_2 (CO_2e). Comparing the emissions of greenhouses with identical energy productivity, a Massachusetts facility would produce nearly 50% less CO_2e than a Colorado facility (3).

Creating a Circular Cannabis Economy

Greenhouse and indoor cultivation approaches offer pathways to a circular cannabis economy and are increasingly important strategies as extreme weather events increase in frequency, resources like water are further constrained, and demand for cannabis continues to increase globally. Energy use and associated greenhouse gas emissions of cannabis production should be measured with benchmarking tools to understand ecosystem impacts so certification programs reward circular practices in the cannabis industry.

Figure 1: Contribution to carbon emissions impacts from major industries.



Download RII's latest free "Best Practices Guide on Automation & Controls for Cannabis Cultivation" (8). Access RII's guides on *LED Lighting, HVAC, and Controls for Cannabis Cultivation* at ResourceInnovation.org/Resources. Check out RII's past "Cultivation Classroom" columns on their author page (9-13).

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ABOUT THE COLUMNIST
GRETCHEN SCHIMELPFENIG, PE
As Technical & Operations Director, Gretchen manages the PowerScore resource benchmarking platform, facilitates RII's Technical Advisory Council Working Groups, and manages RII's continuing education program for producers, efficiency programs, and design and construction communities. She works with members and subject matter experts to publish technical guidance for the production of plants in controlled environments, develops and delivers curriculum, and supports PowerScore users with resource benchmarking analysis and reporting compliance. She authors RII's Best Practices Guides for controlled environment agriculture. Gretchen is a licensed Civil Professional Engineer (Construction) in California and Vermont. She also has a specialty in analyzing the interactive effects between HVAC and lighting systems and commissioning controls systems. Gretchen grows vine crops, cannabis, and herbs in her veggie garden, greenhouse, and basement in her Vermont farmhouse and is constantly using her HVAC and lighting knowledge to optimize her grow environment.

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Inspiration, Move Me Brightly:
A Closer Look at the Rylie's Sunshine Journey Led by the Youngest CEO in Cannabis

By ***Josh Crossney***

We have all met amazing people within the cannabis industry—pioneers that bring brightness to our lives. In this installment of "Cannabis Crossroads," I sat down with three inspirational forces to discuss the journey of the Rylie's Sunshine brand. This is an ongoing story of not only fighting stigma, but also one of perseverance, change, family, and community. I greatly admire and respect Rylie and Janie Maedler and Dr. Reggie Gaudino. They are shining examples of what can be achieved through community and collaboration when hearts lead the way and work together to overcome fear.

Q: We have been following your journey for several years. For readers not familiar with Rylie's Sunshine, can you please tell us a little about the brand and explain why it was created?

A: Janie Maedler: Rylie created a 501c3 in 2015, Rylie's Smile Foundation, after she achieved no evidence of disease (NED) from her bone tumors. Through Rylie's Smile Foundation she helped create safer legal access to medical cannabis for pediatric patients by steadily creating legislation in her home state of Delaware. Beginning legislatively in 2015, she was able to pass bills for medical marijuana (MMJ) for pediatric patients, administration of MMJ on school property, added pediatric autism as a qualifying condition, and inspired the Compassionate Use Program which allows doctors to prescribe MMJ for conditions not listed on the states qualifying conditions if they have scientific evidence that it will benefit the patient medically. We knew that these laws would set a precedent if Rylie could accomplish these changes legislatively in her state—enacting these laws would help other states do the same.

Within the first two years, Rylie's Smile Foundation became well known for not only legislative improvements for pediatric patients but it also became an advisory for out of state legislators and many families navigating medical cannabis for the first time for their severely sick children. This is where we not only saw a drastic need for quality cannabinoid medicine but we were seeing that this was a bigger issue then anyone could ever imagine! There was no education in the general public nor the regulation of products. Many patients or consumers who were desperate for improved health were being taken advantage of. We examined countless sources of medical cannabis. We were horrified at some of the products' lab reports and sales tactics to get "rid of subpar products" at high cost to unsuspecting desperate parents. It came down to Rylie and our family wanting to create a company that genuinely cared where these families' medicines were coming from, it's cannabinoid levels from plant to finished product, how it was grown, and how it was processed. Simple transparency. Since the 501c3 could not cultivate and process cannabis, Rylie's Sunshine was created to assist in this area.

A: Rylie Maedler: We knew we would need help to accomplish this to the degree of quality that I wanted for families. I immediately brought on medical and research advisors who were well versed in medical cannabis research. Among them, I asked Dr. Reggie Gaudino to join me and he accepted. This has been a dream come true for me since we share the same values of clean quality cannabis medicines and transparency. I value his input tremendously and it's great to have him be such a big part of my life now. I admire what he has accomplished and the things he has set out to do to advance cannabis research.

A: Janie: Dr. Gaudino shared Rylie's goals with Front Range Biosciences who in turn decided they would love to help Rylie achieve these goals. Each year we plant approximately 35+ varieties of Front Range Bioscience's genetics in order to study how they perform in our region, our specific soil conditions, and how well they achieve expected cannabinoid profiles. This helps ensure that we grow the healthiest and best plants that we possibly can. Front Range is present from the months leading up to planting our seedlings and clones to ensure our soil and irrigation testing are taken into consideration when making amendments. We then keep extremely detailed data on each variety, which includes tissue sampling the varieties to look at their cannabinoid performance as they mature. With this data we can plan what we will plant the following year to target certain medical conditions and exactly when to harvest for the highest cannabinoid levels we are looking for. We cannot imagine

doing it any other way now! We put the utmost care from the soil all the way to the end product to ensure it is something we can confidently give to our own medically compromised family members.

Q: As the youngest CEO in the cannabis industry, what inspires you on a daily basis?

A: Rylie: The main thing that has inspired me has always been the patients that I've helped and the friends that I've made during my journey of educating and advocating for better cannabis access. Every person I've met has had their own journey and their own struggles to overcome, which inspires me to be like them and help them continue thriving.

Q: Today you operate your own farm out of Virginia. Can you share with us what it has been like to grow, harvest, and produce your own products? What lessons have you learned along the way?

A: Rylie: Yes, we have 25 acres of farm land on the Eastern Shore of Virginia. In the beginning there is a lot of trial and error. Thankfully, we've had help along the way from our amazing advisors, fellow farmers, a great farm crew, many friends who have experience, the assistance of Front Range Biosciences, and Dr. Gaudino. It is a lot of responsibility and physical hard work, to say the least, from laying the irrigation, planting, weeding, collecting three to four dozen tissue samples every couple of weeks

Figure 1: Rylie Maedler in her hemp field.

and taking note of how each variety is doing in detail all the way through to harvest and post processing. We are steadily learning and working to continuously improve. Since I suffer from seizures, the hardest part for me is when the field gets hot, so I am in the field early in the evening, also when it's cloudy or rainy I'm out there. I've done everything from driving the tractors to hanging the drying poles from our ceiling. My favorite part is the tissue sampling of the plants. This gives me a chance to look at the differences in their odor, color, leaf pattern or size, the flowering stage, and even what type of pests are present. I find a lot of lady bugs still since we released so many a few years ago. I also enjoy taking photos of the plants. The biggest lesson I've learned is that there are a multitude of plant varieties and they have different characteristics. You can get amazing results from a little plant sometimes and poor results from a huge plant. They also all have different odors due to their varying terpenes. It's fun to learn about the tissue sample results when we come across one that has a really pleasing smell. Last year my favorite happened to be a high cannabichromene (CBC) variety, which made sense because of my seizures.

A: **Janie:** For me, I enjoy being in the middle of our field and looking up to see my children, my husband, father, stepmother, extended family, our crew, and pediatric families who come to volunteer, in the field with smiles on their faces. We all are in awe that we are nurturing a plant that will improve someone's quality of life. All of us talk to the plants but I think I might do it the most. There have been times that a family desperate for help has reached out to us and sometimes their stories break you to the core. It's hard to not let it weigh on your mind heavily so I will sit in the middle of the field and just try to take in the role these plants will have. Often families will come for a couple of days to help us and be a part of the creation of their loved one's medicine. I often see them talking to a plant or two as well. The biggest lesson I have learned is that in cultivation you never have complete control over Mother Nature, so do not expect it.

Q: **The legal and commercial landscape has changed in many ways since we first met. What are some of the biggest challenges you continue to face?**

A: **Janie:** We have had a lot of obstacles from the very beginning in 2013 when I started Rylie on cannabis oils for her tumors. She used both tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA) and cannabidiol (CBD), all full spectrum. Back then it was nearly impossible to find full extract cannabis oil (FECO) anywhere much less CBD rich FECO! When I did find a safe source, it was extremely expensive and highly secretive because nobody wanted to get caught. I had many yard sales to be able to afford her cannabis oil each month. It's unbelievable how expensive it was.

When Rylie passed MMJ for pediatrics in our state, everyone was scared to speak up . . . except Rylie. That tiny nine-year-old girl stood up before all of those big legislators and convinced them, even the ones who originally objected, to make a change they'd never forget and be proud of. Rylie continued making changes to help children so that they would not have to go through all of the things she did to gain access. It's hard to imagine the difficulty resulting from negative stigmas these days but they are still very present in many corners of society. I've watched Rylie in action before legislators and going up against six figure lobbyists. I can tell you that she fights for quality access legislatively from the heart and sometimes when she is done, I feel like I need to catch her and protect her but she won't have that. She is like a cute lion.

Q: **Front Range Biosciences (FRB) is a leader in cannabis and hemp genetics and analytical testing. How is FRB working with Rylie and Janie? In what ways do you help improve product quality?**

A: **Dr. Reggie Gaudino:** We've partnered in a way that benefits both companies. Rylie's family has a fair bit of land that has been used for agriculture. Janie had grown about an acre a couple of years back and had done really well. I was already an advisor to the "Rylie" brand, they were nice enough to allow me to work with them, so we found a way that FRB could run field trials on their land. And then all the biomass goes into products for both Rylie's Smile and Rylie's Sunshine. Some of the varieties we test have been bred for specific terpenes or minor cannabinoids, and so all that gets to potentially help kids and their families. So, it's kind of a perfect match. Our stuff will end up potentially helping someone, and having potentially been introduced into at least a few use case studies. We are also taking a look at the actual concentrate made from the variety that helped Rylie. Our chemistry team has taken an initial look at it, but we need to go back and use some more sensitive instruments to get a bit deeper.

As for the product quality, it depends on whether or not you mean the quality of the plants or the oils. For the plants, we tested all our breeding product progeny at first on just a few acres in 2020 and then Janie must have been on some amazing training program over the winter, because she went from 4 acres last year to 13 acres this year, without a lot of additional help, if any. How that helps us improve is to get metrics on things like performance in that environment, since the performance of any given genetic will not be the same everywhere. It allows us to identify which varieties from our germplasm should be sold in which regions to allow us to make sure our product is helping the cultivator do his best. If it is a genetic that is suited to that set of conditions, then you start out with a stronger base. It also helps us get a feel for the spectrum of photoperiod response when you have crosses that include plants that have different light trigger requirements. So, that's how it helps

us. I'll be honest, that region is a tough region to grown in. In general, the things that do well there do well anywhere else I put them. So, it definitely helps us.

As far as helping improve the quality of their products, with that much biomass they can do a lot of experimenting and formulating, and get up to speed with their new extraction facility, so hopefully that is useful to them. Also, the partnership, when the weather cooperates, means that when we do find those good genetic combinations, with respect to minor cannabinoids or other compounds, they get to put that into their formulations too, so I think we help there as well.

Q: What goals are you looking to achieve by working with Rylie's Sunshine and other farms?

A: Dr. Gaudino: I really want us to be able to help Rylie figure out what compound or combination of compounds helped her overcome the disease that nothing else could. Then I want to breed that up so more people have access to it and we can continue to show why this plant is, in fact, a miracle.

As for other farms, the partnerships are all about mutual benefit. They help us produce some of the best genetics on the planet and we in turn help them maximize the return on their investment and labor. We're starting to create those partnerships on the regulated cannabis side now as well. The hemp industry collapse in the US has hurt our development on the hemp side. We've had to reduce the extent of our hemp field trials. But Rylie's is a partnership that will continue because of the number of ways we work together.

This interview has been edited due to space limitiations, to read the full version, please visit: https://www.cannabissciencetech.com/columns/cannabis-crossroads.

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Reducing Your Carbon Footprint with Advanced LED Lighting Solutions

LED lighting offers significant advantages and benefits over traditional lighting—from sustainability and energy conservation to operation efficiencies, cost reduction, and higher yields.

Mikhail Sagal
President
TSR Grow

Gary Arnold
VP of Sales & Marketing
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Sponsored by

TSRgrow™

CST: What are the benefits of LED lighting vs. traditional lighting in cannabis cultivation?
SAGAL: LED lighting offers significant advantages and benefits over high-pressure sodium (HPS) lights. Energy consumption is almost half of HPS, and the heat produced in a room with LED lights is 50% less than in a room with HPS lights. Spectrums are matched to cannabis crops to optimize growth characteristics, and you don't have radiated heat emitting from a light source as with HPS—LED intensities and photosynthetic photon flux density (PPFDs) are also dialed in to increase yields with half the amount of power and a higher nutrient intake. There is also a lot more granularity and capability in terms of dimming, controls, and monitoring.

CST: What makes TSRgrow LED lighting solutions different from traditional LED lighting?
ARNOLD: TSRgrow has taken commercial cultivation to the next level by integrating functionality that gives a competitive edge. Our lighting fixtures do not require a driver or ballast on the fixture—some conventional LED lights can have as many as six drivers per light, multiplying failure points. Our digital remote power server technology is centrally located outside of the grow chamber, which offers considerable advantages:

- Reduced heat in the grow room
- Reduced weight on the supports or racking
- Reduced installation costs by eliminating all two-wire dimming circuits
- No contactors or relays needed to turn the lights off
- No step-down transformers: our remote power servers can take 600/480v directly

Each light or group of lights can be controlled separately with unlimited zones. Our digital remote power servers monitor and manage scheduling, micro-climates, lighting control, and environment, and they produce current historical reporting for process improvement and regulatory adherence. Additionally, tracking and scheduling can be remote or local.

Additional controls and monitoring modules are customizable for each cultivator's needs.

CST: How do your LED lighting solutions reduce the carbon footprint of the grow facility?
SAGAL: TSRgrow Advanced LED Solutions are just that: advanced for the cultivator not only to gain tighter control of their greenhouse and indoor grows but also help them reduce energy and emissions through our lighting platform integrated monitoring and control. This allows cultivators to increase production and have a long-term sustainable cultivation that provides capabilities to address future changing regulations—which we know will happen—through simple programming rather than fixture changes.

We can deliver the highest performance and yield while delivering the lowest possible operating costs because we can optimize energy usage through advanced control of our lighting, integrated with heating, ventilation, air conditioning, and dehumidification (HVACD). CO_2 is more efficiently utilized, and water is conserved, enabling the lowest carbon footprint.

Our digital remote power server technology plays a key role in this, as it not only centralizes the lighting system's power and operation, but it can also be integrated into microgrids, which are combined heat and power (CHP) systems. We can also connect directly to regulated DC power generated from solar, battery storage, and wind generation. Direct DC power input represents the most efficient conversion of power possible—sustainability at its best.

We're giving growers and cultivators the ability to reduce their carbon footprint and be sustainable now with the ability to adjust and transition to new technologies and new power-generation capabilities in the future without having to change any TSRgrow lighting infrastructure. We can integrate into CHP systems or solar, which is adaptable and programmable in the future. Our lighting solution is sustainable and adaptable to local regulation changes, saving costly equipment changes and leading to long-term, efficient operations, giving growers the highest yields and lowest operating costs well into the future.

CST: What other ways do your LED lighting solutions enable growers to be environmentally friendly?
ARNOLD: Because we can monitor all the variables and use lighting as a platform, we're able to prevent, diagnose, and perform maintenance accordingly. For example, if you have an outage, be it a motor, fan, or air handling system, oftentimes you don't notice until it's too late and lose a crop. When that happens, there's a lot of clean up because you can't throw it in the compost pile; it has to be discarded according to regulations. Our lighting platform can detect and alert you about problems in HVACD or CO_2 and make adaptive changes before it's too late and a crop is lost. We can help prevent things that cause environmental contamination.

One of the most important things is making sure that people are doing their job. Our tracking and reporting allow shift managers to see whether they're on track or they need to change things up to avoid inefficient use of energy, materials, and manpower, all while maintaining the optimal environment.

Regulations are changing almost monthly in every state for stricter environmental control, and that puts a cultivator at risk if they don't have a flexible and adaptable system like TSRgrow. We can change, modify, and adapt to the rigorous regulations that are coming. We tell every customer, "You don't know what you don't know, so be prepared by having TSRgrow flexibility."

CST: In what ways do these LED lighting solutions help growers maximize efficiency and help profit margins?
SAGAL: TSRgrow is a partner, not a supplier. As such, we look through the cultivator's lens to monitor, manage, and proactively respond to the operation, planning, and production capacities. We are an intuitive network throughout the facility that can not only track and validate but alert and inform the activities for continuous efficient operation. We are all starting on a long road toward highly regulated cultivation. There will be more restrictive requirements on energy, emissions, quality, environment, and sustainable responsibilities. TSRgrow is not waiting; we are there now and have the platform to continue to embrace the changes coming. We are excited about what we are doing and with our customers/partners.

Differentiation of Group III Cannabis Cultivars into Novel Sub-Classes

ZACARIAH L . HILDENBRAND, TIFFANY LIDEN, MATTHEW SPURLOCK, ADAM M. JACQUES, CHRISTIAN WEST, ORIAH LOVE, ANDREW GROSELLA, R . EDWARD WESTERFIELD, AND KEVIN A . SCHUG

The chemical characterization of different Cannabis sativa spp. cultivars has important regulatory and potential pharmacodynamic implications. While Groups I-III cultivars are distinguishable on the basis of total tetrahydrocannabinol concentrations and the relative ratio to cannabidiol, the expression of other secondary metabolites, such as the terpene family of phytoconstituents, has a more significant bearing on the potential therapeutic outcomes for medicinal end users as a result of the synergies associated with the "entourage effect." In the work presented here, 131 Group III cannabis cultivars were analyzed for cannabinoid and terpene composition. Principal component and hierarchical cluster analyses were used to assess multidimensional relationships in the overall phytochemical content. Weak correlations were found between individual cannabinoid and terpene species; however, the relative expression of monoterpenoids and terpenoids were the most discriminative variables in terms of differentiation of individual cultivars into two distinct subclasses. The identification of distinct sub-classes within Group III cannabis on the basis of cis-2- pinanol, fenchol, and alpha-terpineol expression, suggests that these classifications can be used to guide more targeted therapeutic outcomes.

With the passage of the 1970 Controlled Substances Act, research into the medicinal properties and phytochemical constituents of cannabis was brought to a halt in the United States. As various states have legalized the use of cannabis within their borders, nascent research is being conducted, but remains fraught with legal peril, as cannabis is still prohibited at the Federal level. The study of cannabidiol (CBD)-rich hemp, now legal to grow in the United States as a result of the 2018 Farm Bill, is not hampered by these restrictions and research on the chemical composition and phytochemical diversity of commercially available cannabis can be performed.

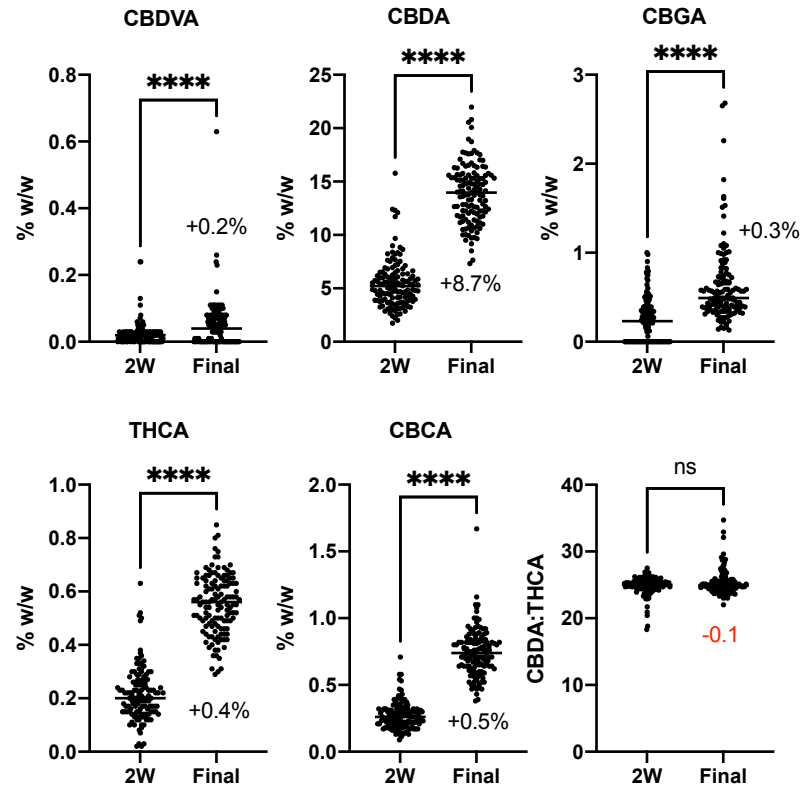
From a regulatory perspective, cannabis is a Schedule 1 controlled substance that differs from hemp on the basis of total Δ^9-tetrahydrocannbinol (Δ^9-THC) concentration, with the bifurcation occurring at 0.3% weight/ weight (w/w) total THC. This particular metric is the sum of the amount Δ^9-THC plus the amount of its acidic precursor, tetrahydrocannabinolic acid (THCA), after accounting for the loss of mass during decarboxylation (that is, total THC = Δ^9-THC + 0.877*THCA). However, and more specifically, cannabis cultivars can be classified into one of three groupings. Group I cannabis cultivars are THC-dominant strains (>20:1 THC/CBD); Group II cannabis cultivars have approximately equal amounts of THC and CBD (that is, ~1:1 THC/ CBD); and Group III cannabis (hemp) cultivars are CBD-dominant (that is, >20:1 CBD/THC). Remarkably, Groups I-III cannabis cultivars generally demonstrate indistinguishable morphological structures, with comparable flavonoid,

terpene, and ancillary cannabinoid profiles (1). This lack of discernable phytochemotaxonomy is likely the result of extensive interbreeding and hybridization (2). However, minute differences in phytochemical composition may produce or affect different therapeutic outcomes for patients using medicinal cannabis.

Terpene molecules are ubiquitous hydrocarbons that are naturally abundant in cannabis or hemp and can stimulate a wide array of physiological responses (3). There are currently 20,000+ known quantifiable terpenes, with cannabis producing more than 120 different terpenes species (4). Most terpenes exhibit antimicrobial and anti-inflammatory properties, but other terpenes have been found to modulate blood glucose and attenuate pain (3). Additionally, terpenes such as beta-myrcene, myrcene, and limonene are all perceived as invigorating; whereas linalool, also found in lavender, is perceived to induce a sedative effect. Other terpenes, such as alpha- and beta-pinene produce bronchodilatory effects (5).

In this work, targeted cannabinoid and terpene analyses, in combination with multivariate statistical analysis, were used to identify and select hemp cultivars that produced increased amounts of cannabidiolic acid (CBDA). Using gas chromatography–mass spectrometry (GC–MS), two distinct clusters within Group III cannabis were identified on the basis of their cannabinoid and terpene expression profiles. A parallel analysis by high performance liquid chromatography (HPLC) also resulted in the separation of unique phytochemical clusters, albeit with less discrimination than was afforded by the GC–MS analysis. These results suggest that separate sub-classes within Group III cannabis can be identified on the basis of ancillary phytochemical expression; the elucidation of these discriminatory and bioactive features can possibly be used to guide medicinal end users towards pharmacodynamically more favorable treatment outcomes.

Figure 1: Changes in cannabinoid expression during the final two weeks of cultivation. The paired, nonparametric Wilcoxon test was used to generate statistical comparisons. **** represents p<0.0001, ns symbolizes no statistical significance.



Material and Methods

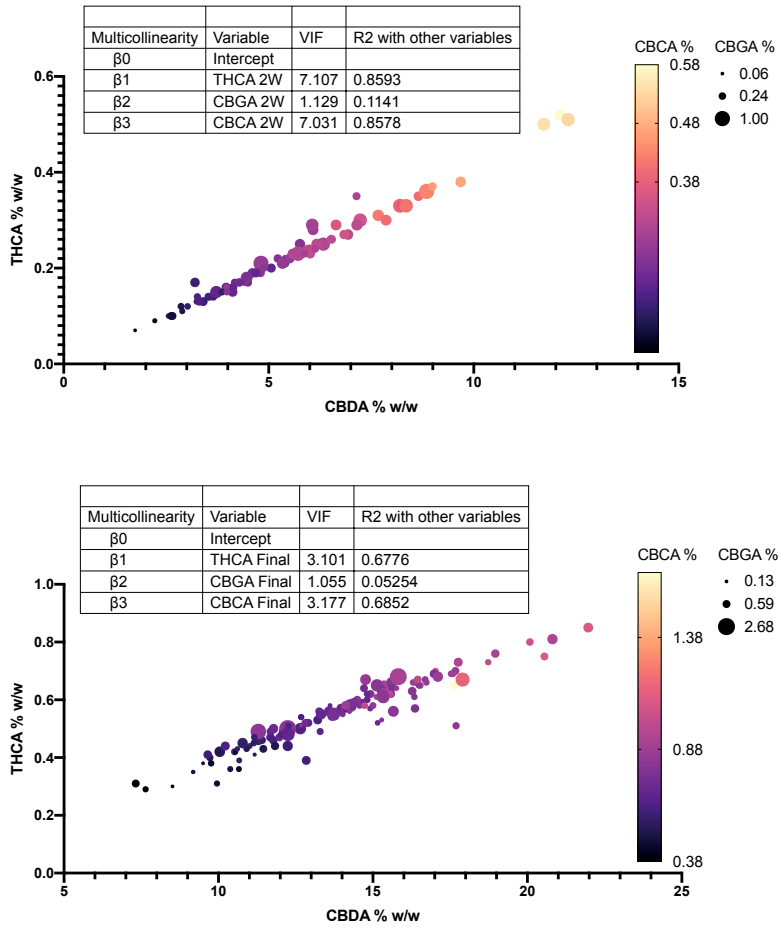
Group III Cannabis Genetics

In this study, 131 novel Group III cannabis (hemp) cultivars created by ZED Therapeutics were included. These cultivars were selected on the basis of CBDA expression and the absence of discernible genetic abnormalities or poor performance characteristics. These included hermaphrodism, variegation, extremely long flowering periods, elongated internodal spacing, poor cannabinoid production, and susceptibility to insects, molds, and plant viruses. All plants were grown indoors in an organic blended soil medium (compost, worm casting, perlite, and cocoa coir) under ceramic metal halide (CMH) and high-pressure sodium (HPS) and lighting technologies. During the vegetative cycle, 1000 W CMH lights were used on a 16:8 light:dark regimen, and 1000 W HPS lights were used on a 12:12 light:dark regimen during the flowering cycle.

GC–MS Analysis

Samples of final cured flower material were analyzed for cannabinoid and terpene content by GC–MS methods performed by Sunrise Analytical, using the Environmental Protection Agency's 8270 method. In this work, 10 and 50 different cannabinoid and terpene species were quantified, respectively (please see Supplemental Dataset online). Concentrations of the acidic pre-

Figure 2: A multivariable bubble plot illustrating the relationships amongst CBDA (ß0), THCA, CBCA, and CBGA in hemp samples analyzed two weeks before harvest (top) and those of final cured material (*bottom*).



cursor cannabinoids (that is, THCA and CBDA) were determined via GC–MS after derivatization (6). Calibration was performed with a quadratic calibration curve with 8 reference points and a linearity greater than 0.995. Quantitation was performed within 85–115% of sample matrix reproducibility.

HPLC Analysis

Samples collected two weeks before harvest and of final cured flower material were analyzed by HPLC methods developed by ZED Therapeutics. Briefly, samples were treated with methanol and syringe filtered, prior to separation on a 2.2 µm, 3.0 x 75 mm C18 column and an acetonitrile gradient using Shimadzu's Cannabis/Hemp Analyzer. Certified reference standards from Cerilliant were used to identify and quantify cannabidivarinic acid (CBDVA), cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabidial (CBD), tetrahydrocannabivarin (THCV), tetrahydrocannabivarinic acid (THCVA), cannabinol (CBN), Δ^9-tetrahydrocannbinol (Δ^9-THC), Δ^8-tetrahydrocannbinol (Δ^8-THC), cannaicyclol (CBL), cannabichromene (CBC), tetrahydrocannabinolic acid (THCA), and cannabichromenic acid (CBCA). Calibration was performed using a set of five serial dilutions of each reference standard (2.5, 12.5, 25.0, 125.0, and 250.0 µg/L) with a linearity greater than 0.998. The limits of detection and quantitation were determined experimentally to be 0.5 and 1.0 µg/L, respectively.

Data Processing and Statistical Analysis

Pairwise and correlative analyses were performed using the Prism software suite by GraphPad. Principal component analysis (PCA), hierarchical cluster analysis (HCA), and partial least squares discriminant analysis (PLS-DA) were performed using MetaboAnalyst 4.0 online. Prior to statistical analysis, the data were auto-scaled to CBD and CBDA concentrations to normalize the dataset so that individual constituents could be compared based on their correlation to each other. This is referred to as unit variance scaling where the highest and lowest concentrations are represented by 1 and -1, respectively, with all other concentrations being relative (7). No further data transformation was performed.

Results and Discussion

GC–MS and HPLC Determinations

The concentrations of CBDA and THCA in Group III cultivars are of particular interest in the hemp industry due to efforts to maximize the former while still maintaining regulatory compliance with the latter. Current United States Department of Agriculture (USDA) regulations specify that in order to fit the classification of hemp, Group III cultivars must exhibit a total THC (Δ^9-THC + decarboxylated THCA) equal to or less than 0.3 % w/w on a dry weight basis. Therefore, we determined the major acidic cannabinoid precursors (that is, CBDVA, CBDA, CBGA, THCA, and CBCA) in flower material collected two weeks before harvest, as is required by USDA regulations, and in the final

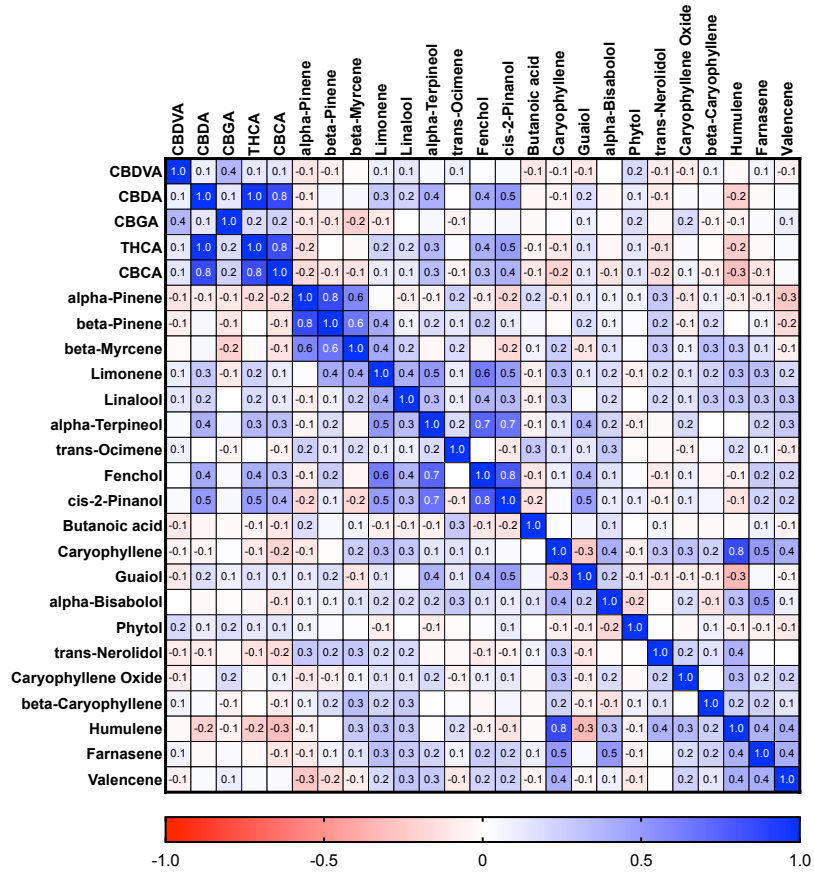
cured material (**Figure 1**). All 131 cultivars were devoid of detectable Δ^9-THC two weeks prior to harvest, whereas 10 of the 131 cultivars demonstrated total THC levels above 0.3% w/w once THCA decarboxylation was accounted (mean and median values of 0.19% and 0.18%, respectively). The concentrations of all five major acidic precursor cannabinoid species increased significantly ($p<0.0001$) in the final two weeks of cultivation, while CBDA:THCA ratios remained relatively unchanged (25.0 versus 24.9, $p=0.55$) between the two collections points. CBDA concentrations rose the most dramatically in the final two weeks of harvest, with a median increase from 5.22% to 13.97% ($p<0.0001$).

Correlation Analyses

CBDA, THCA, and CBCA concentrations exhibited multicollinearity at both time collection points, whereas CBDA and CBGA exhibited poor correlation two weeks before harvest ($R2=0.11$), which was accentuated in the final cured flower ($R2=0.05$) (**Figure 2**). This was expected since CBGA is converted into CBDA, THCA, and CBCA, respectively, via a series of synthase enzymes. Interestingly, the strength of the aforementioned multicollinearity amongst CBDA, THCA, and CBCA decreased over time, and was attributed to the substantial increase in CBDA expression in the final two weeks of cultivation.

An additional two-tailed Pearson correlation analysis was performed to assess the relationships between the major cannabinoids and prominent terpenes in the final cured flower materials (**Figure 3**). This analysis revealed several weak correlations between individual cannabinoid and terpene species (that is, CBDA and fenchol, CBDA and cis-2-pinanol, $r \leq 0.5$), compared to stronger correlations between individual terpenes. For example, humulene concentrations strongly correlated with caryophyllene concentrations ($r=0.8$, $p<0.0001$). Similarly, fenchol concentrations were correlated strongly

Figure 3: Pearson correlation matrix illustrating the relationships between the five most prevalent acidic cannabinoids and 20 prominent terpene molecules detected in cured hemp material. Red and blue colorations represent negative and positive correlations, respectively.



with cis-2-pinanol concentrations ($r=0.8$, $p<0.0001$) and alpha-terpineol ($r=0.7$, $p<0.0001$); and the concentrations of the two pinene isomers were strongly correlated ($r=0.8$, $p<0.0001$).

Identification of Group III Sub-Classes

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to evaluate overall individual phytochemical profiles in relation to each of the Group III cannabis cultivars (see Supplemental Table I available online). Samples were evaluated based on their terpene content, cannabinoid content, and the combination of the two compound classes. For analysis of the terpene content (see Supplemental Figure 1 available online), the first four principal components (PCs) had a total variance of 57.8% with PC 1 and 2 explaining 20.8% and 15.0% of the total variance, respectively. Samples were additionally evaluated based on the cannabinoid content. PC1 and PC2 accounted for 58.9% of the total variance, and the first three PCs for 69.2% of the total variance (see Supplemental Figure 2 available online). Cannabinoids were evaluated using HPLC.

PCA results were similar to those determined by GC–MS. The total variance explained by the first three PCs was 68.7%. PC1 accounted for 30.8% and

Figure 4: Principal component analysis (PCA) (left) and biplot (right) for the combined terpene and cannabinoid data of the hemp samples evaluated. Sub-classes 1 and 2 are represented by the colors red and green in the left-hand PCA plot, respectively.

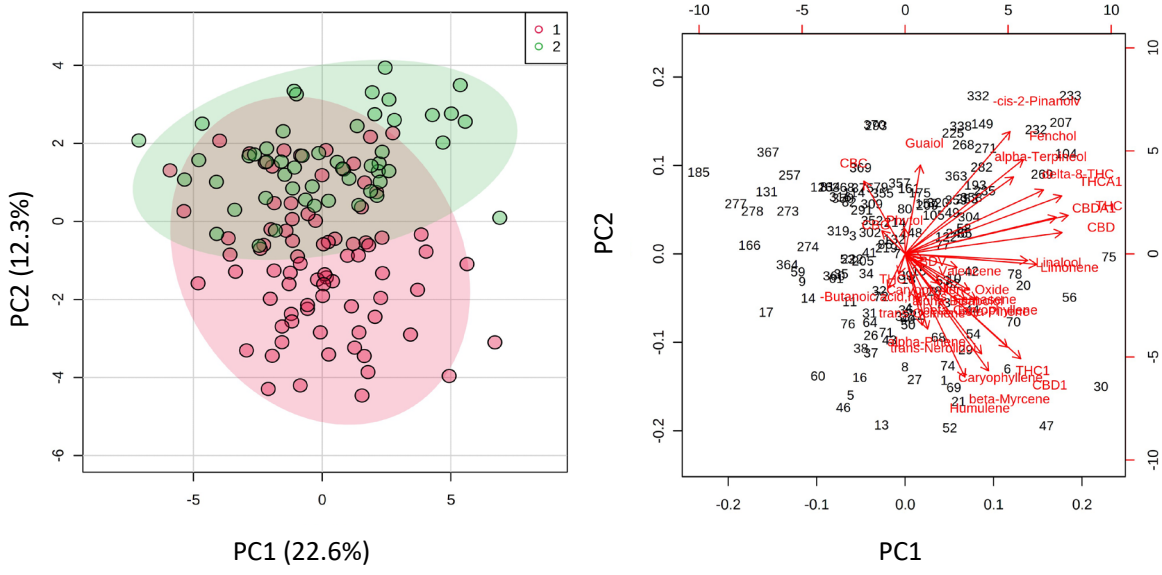
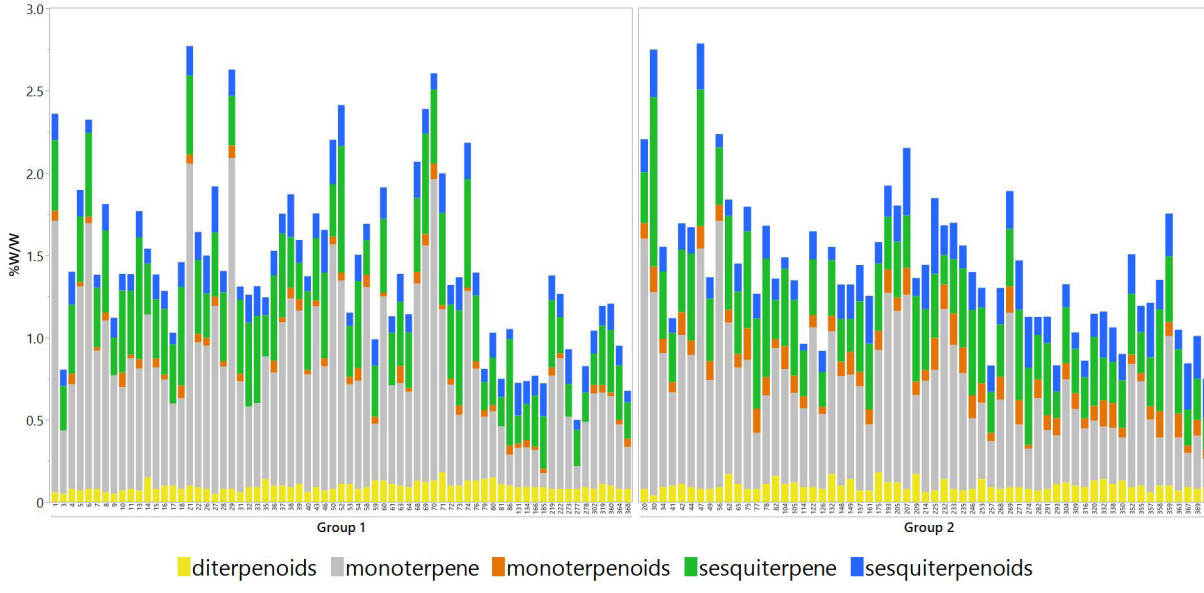


Figure 5: Bar graphs representing the concentration (% w/w) for the terpene concentrations for the hemp samples analyzed. Each bar, which represents a sample, is in sequential order based on the sample number.



PC2 accounted for 24.9%. The terpene and cannabis data were combined, which yielded total variance of 52.6% for the first four PCs with PC1 and 2 explaining 22.6% and 12.3%, respectively (**Figure 4**).

Those compounds with the greatest influence in differentiating the two sub-classes were selected using the following statistical tests: t-test (p-value <0.05), partial least square discriminant analysis (PLS-DA), and random forest (RF). While the t-test is a univariate approach that does not consider correlation between features, PLS-DA and RF are multivariate approaches that can be

used with collinear features. The compounds commonly identified by the three approaches for the terpene analysis were cis-2-pinanol, fenchol, α-terpineol, and guaiol—all of which are terpenoids (see **Figure 5** and Supplemental Table II available online). The results of the GC–MS analysis of cannabinoids indicated that CBD, THC, Δ^8-THC, and THCA were the most influential compounds based on all three statistical tests and that CBD had the greatest influence. The evaluation of the HPLC data revealed that the acid components THCA, CBDA, and CBCA, were the most influential because of the elevated levels in sub-class 1. Based on the RF analysis, CBCA, CBC, and THCA ranked as the top three features because of the low concentration of CBC in sub-class 1; CBDA and THCA were elevated in sub-class 2. When the combined results from the terpenes and cannabinoids quantitation were evaluated, the same sub-class differentiation was evident that was determined as with terpene data alone. The results of the t-test, RF, and PLS-DA statistical tests indicated that cis-2-pinanol and fenchol were the most influential with respect to class differentiation due to lower concentrations in sub-class 1 samples. To a lesser extent, α-terpineol and CBD also affected the differentiation between sub-classes based on the t-test and RF evaluations. However, PLS-DA ranked α-terpineol and THCA third and fourth in significance, respectively.

While the results indicate that THCA concentrations influence the structure of the all-encompassing model space, overall, the variability in terpenoid concentrations, primarily those of cis-2-pinanol, fenchol, and to a lesser degree, alpha-terpineol had the greatest effect on sub-class differentiation. Interestingly, all three of these terpene species are classified as monoterpenoids and contain a hydroxyl (OH) functional group with either a cyclic or bicyclic structural backbone (see Supplemental Table II available online). Concentrations of terpenoids were 2.44 times higher in sub-class 2 than in sub-class 1. Additionally, the monoterpene concentration for sub-class 1 was 1.32 times higher than that in sub-class 2.

Conclusion

The data presented here document the phytochemical composition of a large dataset (n = 131) of Group III cannabis cultivars and allow for the identification of distinct sub-classes based on the cannabinoid and terpene concentrations expressed in individual cultivars. Weak correlations between individual cannabinoid and terpene species were detected; however, the relative concentrations of monoterpenoids and terpenoids (cis-2- pinanol, fenchol, and alpha-terpineol) were the most discriminative factors in differentiating individual cultivars into two distinct sub-classes. This may be important within the context of pharmacodynamics and potential therapeutic outcomes. For example, fenchol is an antioxidant, anti-inflammatory, and anti-microbial agent (8). Additionally, fenchol and other monoterpenoids have been found to have analgesic activity (9). Therefore, cannabis users looking for pain relief in Group III cannabis may benefit from selecting cultivars with the phytochemical signature of sub-class 2 hemp, which is characterized by higher concentrations of fenchol and other analgesic monoterpenoids compared to subclass 1 hemp. Ultimately, the more resolution that can be provided by cannabis analysis and the resulting classification, the better-informed cannabis users can be when selecting products for the treatment of their individual illnesses. Additional efforts to characterize and quantify different flavonoid species in Group III cannabis may provide additional insight and further sub-class differentiation.

Supplemental information

Several tables and figures mentioned in the text as supplementary information can be found online at: www.cannabissciencetech.com/view/differentiation-of-group-iii-cannabis-cultivars-into-novel-sub-classes.

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Part 4: Need Speed and Simplicity for Metals Analysis in Cannabis? High Throughput and a Full Cannabis Analyzer Package

In the fourth part of this six-part series, Cannabis Science & Technology sat down with Anthony Macherone, senior scientist and cannabis technical lead for Agilent Technologies to speak with Jenny Nelson and Craig Jones, both from Agilent's ICP-MS scientist team, to discuss tools that address metals testing in the vast array of cannabis and cannabinoid products and a new cannabis ICP-MS testing method for the Association of Official Analytical Chemists (AOAC).

Jenny Nelson
ICP-MS Application Scientist
Agilent Technologies

Craig Jones
ICP-MS Application Scientist
Agilent Technologies

Anthony Macherone
Senior Scientist
Cannabis Technical Lead
Agilent Technologies

MACHERONE: I heard you are both working on an AOAC method for metals in cannabis. Can you explain that process and how far along you are in that journey?
NELSON: Craig and I started the process of developing an AOAC metals in cannabis method over a year ago. We had worked on a method for metals in cannabis a few years back, but when we saw the AOAC call for a method—which is a determination of heavy metals in a variety of cannabis and cannabis-derived products—we decided to submit our method to AOAC since it met their requirements for the standard method performance requirement (SMPR) 2020.001.

The submitted method manuscript, which includes validation data, is reviewed by an AOAC expert review panel for consideration for the AOAC first action official method of analysis and is then adopted and published by AOAC International. For this metals in cannabis method, we needed to determine total cadmium, total arsenic, total lead, and total mercury. We have now submitted our method and it is in the electronic review process (ERP). Craig and I are meeting our expert review panel at the end of August, and it will hopefully be accepted for first-action status soon. After it's accepted, we are required to do a reproducibility study.

MACHERONE: You mostly talked about the big four elements. How many other elements can you measure?
NELSON: We can measure a lot of elements with our method. Some additional elements we included are antimony, barium, chromium, copper, nickel, silver, selenium, and zinc. We included these as supplementary data in the method submitted to AOAC; however, our ICP-MS software has a feature called IntelliQuant that screens for the entire periodic table—to give our users an idea of how many different elements can be in their cannabis and cannabis-derived products.

MACHERONE: That makes a lot of sense. What are the different types of samples you studied?
NELSON: We tried to cover four different sample categories: inhaled products, oral products, topical products, and manufactured products.

For the inhaled category, we studied hemp flower, CBD day oil, and hemp isolate extract. For oral products, we studied soft-gel capsules, tinctures, isolate tinctures, CBD coffee grounds, different butters, and seed oil. We also did different food products such as beef jerky, hard candy, and a pineapple drink, which I didn't even know were options on the market. As part of the topical category, we looked at different balms and pain-relief creams as well as topical oils and different soaps. For the manufactured category, we looked at biomass, spent biomass, crude extracts, and refined extracts distillates (i.e., distillates and isolates).

MACHERONE: What is the sample preparation for these different products for ICP-MS testing?
NELSON: For ICP-MS, we use microwave digestion. A combination of different acids is used to digest the cannabis products into a clear solution that we can then analyze on our ICP-MS.

For the most part, our acid combination of nitric acid and hydrochloric acid works well for the majority of these different products. We haven't run into any issues yet; we're confident with our method and being able to digest these huge, different sample types in our lab.

MACHERONE: Since you are both in the field, what are your experiences with Agilent customers and the metals testing cannabis community in general?
JONES: Our customers were excited that we could analyze more than the big four elements—we could analyze other metals on the periodic chart at the same time, which means we could give them a comprehensive view of their sample. They could also analyze for other nutrient elements such as phosphorus, magnesium, and calcium that might be in the soil, and then determine if any of those nutrients have been taken up by the plant material.

With the IntelliQuant feature, even if they don't calibrate that multitude of elements, they have the complete story of what's in any sample they analyze.

MACHERONE: What advice can you offer folks on how to get started with metals testing and add it to their lab's capabilities?
JONES: The Agilent system has a cannabis analyzer package. In addition to MassHunter, we have ICP Go, a software platform that runs on top of MassHunter with predefined methods. ICP Go allows an entry-level user to set up the analysis, set up the calibration standards to the associated PC, fill up racks with unknown samples, and press go.

The idea behind this is a lot of cannabis labs are ramping up and want to get going quickly. We can get them started with validation within a week. Also, this is a browser-based platform software, i.e., if you're a lab manager, you can see where the analysis is and that customer samples are running right now. Plus, it gives you the ability to tell the analyst to put another set of samples on because, "It is a priority, and we need to get that data out the door." It makes things so much simpler and allows the customers to get up and running quickly.

Providing Sufficient Airflow for Plant Growth Environments

SAMUEL BURGNER

This article series explores some of the more technical aspects of cannabis cultivation that growers should understand in order to run their businesses more efficiently and more profitably. Part IV builds on the concepts of leaf temperature, vapor pressure deficit, and light spectrum effects on water movement in plants. This is the most technical information so far and it is recommended to read the prior articles in this series to gain the most in-depth knowledge and understand the core concepts.

IN NATURE, PLANTS are typically exposed to bulk movement of massive amounts of air as wind passes through the canopy. At times they may be exposed to gusts, which are sudden spikes in wind speed characterized by peaks and lulls. In response to gusts, plants build hard fibers to prevent bending or breaking, which would put stress on the plant and inhibit the ability to harvest light. While this is an intelligent adaptation, it consumes quite a bit of energy. When available energy is consumed to build harder stems, growth of valuable organs such as fruits, flowers, or tender leaves is slower.

In the case of cannabis cultivation, controlled, gust-like conditions can be useful for building structure when it comes to density and resistance to breakage, but most end users are not interested in buying stems. In cannabis cultivation facilities, tools like trellis arrays can be used to control breakage. Hard stem fibers can increase product density as less material is required to form buds, increasing potency, but total production of floral oil typically decreases. In addition, optimum photosynthetic rate and light absorption is achieved by allowing the leaf to remain perfectly placed under the light source instead of fluttering around. Bulk air movement is critical for plants, and as long as it is consistent and mild (leaves fluttering intensely indicates too much air movement), carbon dioxide uptake and transpiration will occur at an optimum rate for growth.

Understanding the Boundary Layer

Plant leaves build up a "boundary layer" around the stomata where humidity can be higher and carbon dioxide concentration can be lower if there is no air movement. This effect is more pronounced in the inner canopy where leaves might block the movement of air, which is why mold growth is more common in these parts of the plant if left unpruned (we'll cover this in more detail in the final article in this series).

The boundary layer needs to be broken regularly by air movement to ensure high carbon dioxide uptake, however the effect on plant transpiration is not quite as intuitive because water vapor is also a form of heat. When water evaporates, it cools the object from which it came. This cooling effect will reduce the temperature of the leaf, which will slow down transpiration as the water molecules become less energetic and more heat must be provided by the light source or air. Increasing air velocity can displace moisture in the boundary layer, increasing transpiration, but if sufficient airflow is already provided the temperature of the air must be higher than the leaf to further increase transpiration.

"Imagine what it feels like to **sit right under the air vent** in a conference room or classroom—while everyone else in the room feels fine, **you are probably freezing.** Your plants feel the same way when **cold or hot air** is being supplied **directly on top of the canopy**."

Light Intensity and Spectrum

Light intensity and spectrum tend to be the most impactful plant-growth factors, followed by air movement, because they control the effects of every other factor. Consider the light spectral impacts on plant transpiration, as discussed in our previous article in this series (1-3). The low blue/green ratio of high-pressure sodium (HPS) lamps paired with an intense near-infrared peak causes plants to generally be at a higher temperature than the grow room air (+1-3 °C). Light emitting diode (LED) fixtures have a higher blue/green ratio and little-to-no infrared, which results in plants staying cooler than the grow room air (-2-4 °C, depending on proximity to the light source). These spectral differences can hijack the vapor pressure deficit (VPD) that would control transpiration in a natural setting.

As previously explained, plants create a boundary layer around their leaves that needs to be broken with air movement and the air conditions necessary for this task will differ depending on the light source. Plants grown under HPS lamps need cool air just below the room temperature and relative humidity in order to keep the plants from getting too hot, whereas those grown under LED fixtures are more complex. Because you'll need to add more heat to the plant to compensate for the increased transpiration rate (spectral effect), the room setpoint needs to be higher than the target leaf temperature to compensate for this cooling.

Generally speaking, HPS-grown plants are always getting hotter, while LED-grown plants are always getting colder because of the spectrum of the lamp. In one situation you are cooling your plants and in the other you are warming them. To get this warming effect, airflow needs to be high because you rely on the action of convection to warm the plant. The longwave infrared emission from the objects in the room is not strong enough to combat the cooling effect of transpiration in the lower canopy. Depending on spectrum, LED-grown plants can require much more dehumidification (this primarily applies to fully-grown canopies) and you need as much heat as possible to avoid overcooling the space.

Keep in mind that the effect of VPD is more pronounced at higher temperatures due to the water holding capacity of the air, so if the leaf is heated or cooled too much, the transpiration rate changes exponentially. This impacts the management of both the air quality and movement in a space.

Managing Air Quality and Movement

Consider a traditional commercial cannabis grow room with four pieces of equipment: standard off-the-shelf heat pumps, standalone dehumidifiers, oscillating wall fans (which are a constant source of stressful gusts, not bulk air movement), and can fans for air filtration. These systems work together to supply plants with conditioned air, but the plants are living inside the mixing chamber as opposed to air mixing happening inside the equipment and then being supplied to the room. Light adds heat and plants add moisture, while the heat pump supplies cold saturated air, the dehumidifier supplies hot, dry air and the oscillating fans attempt to mix this air and the filters attempt to clean it before it reaches the plants. This setup was originally created to deal with the black market and leads to energy inefficiency and inconsistent quality.

These four primary pieces of equipment discussed above can easily be purchased, installed, and replaced by cultivators, making them an attractive choice, however all of this equipment can be combined into one system (with built in redundancy if desired) and separate the plants from the mixing chamber. Why is this important? Imagine what it feels like to sit right under the air vent in a conference room or classroom—while everyone else in the room feels fine, you are probably freezing. Your plants feel the same way when cold or hot air is being supplied directly on top of the canopy. The photosynthetic response decreases quickly when the temperature drops. To mitigate this response, you do not need wall fans, you instead need to create a path of air movement through the room from the supply that will move through the canopy evenly. Ideally, air in the space should be constantly exchanged so that plants and lights don't push humidity and temperature values out of range.

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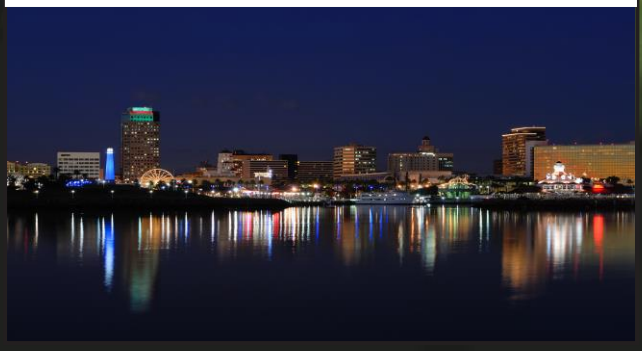

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"All light sources, **due to the inefficient nature** of turning electricity into photons, **produce large amounts of heat,** but the form of that heat can **change everything about the grow room**."

Another benefit to a high air turnover rate is the amount of exposure to your filtration equipment, whether that is simple particle filtration, photocatalytic oxidation, or both.

If you take an infrared thermometer and check the temperature of your leaves just under the light and compare it to those lower in the canopy, you will see why air movement is critical to maintain the overall productivity of your plant. Stomata develop in response to their environment. The mature lower leaves of a plant were at some point the upper leaves, so the light source they developed under will have determined the number and size of stomata.

The internal temperature of a plant in most grow rooms is somewhere between the supply temperature and the room temperature. Light source greatly influences how you manage this temperature and air movement controls how closely your plant is held to its desired temperature for light intensity and carbon dioxide level. This is critical because if air movement is not sufficient, the plant will quickly get too hot (HPS) or too cold (LED). All light sources, due to the inefficient nature of turning electricity into photons, produce large amounts of heat, but the form of that heat can change everything about the grow room. Keep in mind that you have to cool the room air to remove moisture (with the exception of desiccants), so you need to be able to manage your environmental control systems to reheat and regulate moisture in the air returning to the room.

Final Thoughts

We have painted a picture of what an ideal situation would look like, but what is it that inhibits our ability to create these conditions and what does it mean for yield if we do not provide them? When racking systems are used to increase planting density on a set floor space, obstructions to airflow are created in the grow room. There are multiple ways to approach this issue. When plants are crowded into a small space to grow, humidity levels build up quickly and heat can get trapped within the shelves. Inline metal ducts with independent air movers can be placed above the lighting system paired with well-planned supply and return to ensure that each level of the room is receiving the same airflow. Another way to approach this issue is to use fabric or plastic inflatable ducts below the plant canopy to supply air directly into the canopy. This is a much cheaper option, but it neglects to address the heat buildup just below the lights. Each option has its pros and cons.

Product consistency is extremely important to success and microclimates have a significant impact on the outcome. Plants are only interested in the air being supplied to them, and unless you separate the plants from the air-mixing process, you have no control over what they are exposed to or the efficiency of your system (electrical costs). Your supply and return locations should be coordinated with an airflow path through your grow room that considers any obstructions and how that might change as plants mature.

Installing environmental control equipment isn't just centered around feeling good about your setpoints, it is about increasing carbon dioxide consumption (the biggest factor driving growth) and decreasing the energy load on your heating, ventilation, air conditioning and dehumidification (HVACD) system by using less water and electricity while still encouraging plants to grow larger and more uniformly. Consider the impact of your preferred lighting source on your cooling and dehumidification requirements, and most importantly, give all of your plants an air flow pattern that suits the optimum growth of your desired end product. Sufficient air velocity means just breaking through the boundary layer around the stomata—any further and you risk decreasing yield.

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Authenticating the Natural Content of CBD Products

JORDAN TURNER

Here we describe the applicability of Carbon-14 testing for determining the natural content of cannabidiol (CBD) products and ingredients. Carbon-14 natural product testing is useful for the detection of adulteration of natural ingredients in materials such as plant extracts, flavors, fragrances, supplements, cosmetics, and essential oils.

Image credit: Kindel Media/ Pexels.com

THE NATURAL CONTENT of cannabidiol-based (CBD) ingredients can be validated via carbon-14 analysis. The method measures levels of carbon-14 to identify the percentage of naturally sourced material in an ingredient or product (1). The results can be used by manufacturers and distributors to reliably confirm that their CBD extract and products are naturally sourced. Testing also alerts to the presence of petrochemical-derived adulterants in product ingredients. Verifying the content of CBD products using carbon-14 testing substantiates "natural" claims on product labeling and in marketing schemes.

Carbon-14 Analysis as a Tool for Authenticating Your Natural CBD Products

Carbon-14 is an isotope present in all materials that are sourced from nature of plant or animal origin. All living organisms possess carbon-14 in known amounts while materials that are petrochemical-derived do not contain carbon-14 because they decayed out of the carbon cycle long ago. The percentage of carbon-14 present in an ingredient or product can be measured using natural product analysis, revealing how much of the material is natural-sourced or biobased. Less expensive, synthetic alternatives originating from petroleum-based sources can be distinguished from natural-derived materials because they no longer contain carbon-14.

Carbon-14 analysis is the only way to determine if a material contains petroleum-derived adulterants as opposed to natural-sourced ingredients (2). A result of 100% biobased content proves a material is completely natural-sourced. A product with a low or zero percentage of biobased content is made either in whole or in part of petrochemical-derived alternatives.

Why Verify the Natural Content of Your Products?

Authentication of the natural content of your CBD extract and products is a beneficial tool in support of quality control and marketing efforts. Products containing CBD have been attracting more and more interest in recent years. The market size is expected to increase at a growth rate of more than 20% annually between 2021 to 2028 (3). Demand for CBD extract in a wide variety of personal care, cosmetics, supplements, and food and beverage products creates the temptation for manufacturers to take shortcuts in product development by replacing more costly natural ingredients with cheaper, synthetic alternatives (4). Additionally, the popularity of natural products and an increase in consumer interest and demand heightens the possibility for falsification of product labeling. This leads to claims that products are made with natural ingredients when they are actually formulated with petroleum-derived ingredients.

> "Confirming your ingredients **are natural-derived via** natural product analysis dispels **uncertainties in marketing and labeling claims** and ensures CBD products **are free of synthetic adulterants.**"

Natural product testing provides scientific validation to ensure your CBD extracts and product ingredients are truly natural. You can be certain your products and ingredients are not adulterated with petroleum-derived synthetics by measuring their carbon-14 content. Quality assurance teams can rely on natural product analysis to identify the presence of natural versus artificial ingredients in their products before they reach consumers.

Conclusion

Measuring the natural content of your CBD products with carbon-14 testing provides reliable verification of the product's natural source. Confirming your ingredients are natural-derived via natural product analysis dispels uncertainties in marketing and labeling claims and ensures CBD products are free of synthetic adulterants.

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The Correct Filtration Tools for Hydrocarbon Extracts

DOMINICK MONACO

This article provides an overview of standard hydrocarbon extract filtration equipment and media. Due to the quality products they produce, closed-loop hydrocarbon extraction methods are widely popular in the cannabis industry. To make the purest extracts possible, producers opt to filter cannabis oil to remove excess plant materials. Filtration methods vary according to the chosen extraction equipment and the type of products being made. In this tutorial, we review extract filtration processes for inline dewaxing systems versus those that utilize winterization. By assessing and comparing these essential elements in hydrocarbon extraction, we help current and future cannabis companies better understand their options for extract filtration.

HYDROCARBON EXTRACTS ARE immensely popular with both medical and adult-use cannabis consumers. As the cannabis space has advanced, it has developed good manufacturing practices (GMP) akin to other industries such as food production.

Hydrocarbon extraction processes for cannabis have expanded with scope and sophistication. Yet, with so much available information on the topic today, cannabis companies sometimes have difficulty knowing what methods and equipment are best for their needs. This tutorial will review hydrocarbon extract filtration to better clarify this critical step in the manufacturing process.

Today's commercial producers used closed-loop extraction systems to produce hydrocarbon extracts safely. Closed-loop extractors utilize either an inline dewaxing system or an additional winterization to chill materials to the required temperatures for filtration. Each of these practices has its filtration equipment and media.

What are Hydrocarbon Extracts?

Hydrocarbon extracts are typically made from butane or propane. Consumers enjoy hydrocarbon extracts because of their high quality and purity. Some of the most popular hydrocarbon extracts on the market are sauces, batters, shatters, crumbles, waxes, and sugars. Hydrocarbon extracts are also commonly infused in cannabis edibles.

Because butane and propane are highly volatile, hydrocarbon extracts can be hazardous if not handled properly. However, modern extractor companies have worked tirelessly to develop safe equipment for the hydrocarbon extraction process (1). Today, hydrocarbon extracts are commercially manufactured under the heavily regulated auspices of state cannabis compliance agencies and accepted industry GMPs.

Closed-Loop Extraction Machines

Closed-loop extractors are the industry standard for hydrocarbon extraction methods. With closed-loop systems, volatile hydrocarbons such as butane and propane never make contact with the open-air. By keeping these volatile compounds contained, closed-loop machines make hydrocarbon extraction much safer than in the early days of the industry.

Most closed-loop hydrocarbon extractors on the market today are certified for safety standards. Popular certifications for hydrocarbon extraction equipment are 3-A Sanitary Standards, Inc. (3-A), National Fire Protection

With the establishment of **GMPs** in **hydrocarbon extraction**, our focus is beginning to shift from **safety** to **product quality**.

Association (NFPA), Pressure Safety Inspectors (PSI), and The American Society of Mechanical Engineers (ASME) (2–5).

What Does it Mean to Filter Hydrocarbon Extracts?

To make the purest cannabis products possible, producers filter hydrocarbon extracts during manufacturing. Filtration methods and equipment vary with the cannabis biomass in question and the desired end product type. Nonetheless, the fundamental scientific principles for filtering hydrocarbon extract remain the same regardless of equipment, biomass, and product.

Hydrocarbon extracts are filtered to remove excess plant materials (6). Notably, chlorophyll, lipids, and other plant particles negatively affect the taste and color of hydrocarbon extracts. With a growing interest in product purity in cannabis, effective filtration has taken on a new level of significance in recent years.

The filtration process for hydrocarbon extracts begins after the solvent has passed through the biomass. With the solvent housing, both valuable cannabinoids and excess materials such as chlorophyll, it is ready to be filtered.

Extremely cold temperatures are required for the filtration of hydrocarbon extracts to take place. For the best results, temperatures should be at a consistent -60 °C for both the solvent and biomass; when the extract reaches this temperature, fats and lipids containing unwanted plant materials are not solvated by the hydrocarbons. Any frozen fats and lipids that may be extracted can then be filtered from the rest of the hydrocarbon extract.

If done correctly, the filtration process should result in a clear, amber-colored extract free of chlorophyll and other particulates that will give it a bitter taste (6).

Inline Dewaxing and Hydrocarbon Extraction and Filtration

Extractors with inline dewaxing systems can extract, cool, and filter hydrocarbon extracts without the added step of winterization. Whether with butane or propane, inline extractors can accomplish the hydrocarbon extraction process without mixing an additional solvent.

The most identifiable element of extractors with inline dewaxing systems are dewaxing columns. Dewaxing columns are the part of extraction machines responsible for chilling the solvents and biomass to -60 °C. The columns are located between the solvent tank and collection plate on closed-loop extractors.

The dewaxing columns are surrounded by a jacket packed with extremely cold materials such as dry ice, chilled alcohol, or liquid nitrogen. When the hydrocarbon extract passes through the dewaxing column, it reaches -60 °C and freezes fats and lipids in preparation for filtration.

Certain manufacturers prechill their biomass to -80 °C before extraction, on top of chilling the dewaxing column themselves.

The cannabinoid-rich solvent can then be passed through a filtration media such as activated carbon, bentonite clay, silica, or diatomaceous earth to remove unwanted materials. The required media varies depending on the type of biomass and desired end product.

The Correct Filtration Tools for Inline Dewaxing

Extremely cold temperatures are essential when it comes to filtering hydrocarbon extracts. The correct filtration tools for the process are dedicated to either cooling the solvent or filtering it after adequately cooling it.

Products made from extractors with inline dewaxing systems will utilize a combination of the below filtration equipment and media.

Filtration equipment for inline dewaxing:

- Column remediation columns
- Cooling materials (dry ice, chilled alcohol, liquid nitrogen)
- Inline baffle and bead kits
- Micron and submicron mesh screens

Filtration media for inline dewaxing:

- Diatomaceous earth
- Activated carbon
- Bentonite
- Silica
- Alumina
- Molecular sieves

Winterization and Hydrocarbon Extraction and Filtration

Extractors that aren't integrated with dewaxing columns must add the additional winterization step to get solvents and biomass down to -60 °C for filtering. Unlike those made with dewaxing columns, hydrocarbon extracts made via winterization must be mixed with another solvent—commonly ethanol.

To winterize a hydrocarbon extract, it is moved to a reactionary vessel after the solvent has passed through the solvent tank and into the collection plate. The entire vessel is then moved to a winterization freezer and brought down to -60 °C. In time, a layer of fats and lipids form on top of the ethanol, and the mixture is ready for filtration.

To filter the extract after it has undergone winterization, it is poured through micron filters. After that, processors utilize vacuum pumps to pull extracts through a Buchner/Hochstrom device and filter paper. To catch the finest particulates, some manufacturers line filtration paper with diatomaceous earth.

The Correct Filtration Tools for Winterization

As seen with inline dewaxing systems, extremely cold temperatures are also vital in filtering through winterization. The primary difference between inline dewaxing and winterization has to do with how the cold temperatures are achieved.

Hydrocarbon extracts created through winterization generally utilize a combination of the below items. Nonetheless, each manufacturer likely has additional pieces of equipment they deem necessary in the process of winterization.

Filtration equipment for winterization:

- Ethanol
- Reactionary vessel
- Winterization freezer
- Micron and submicron plates or paper
- Vacuum pump
- Buchner/Hochstrom device

Filtration media for winterization:

- Filter paper
- Diatomaceous earth
- Activated carbon

Conclusion

With the establishment of GMPs in hydrocarbon extraction, our focus is beginning to shift from safety to product quality. As equipment manufacturers and regulatory agencies have worked together to provide safe parameters for hydrocarbon extraction, there is now ample room for producers to master their craft.

While the filtration of hydrocarbon extracts was often deemed unnecessary in the industry's early days, it is growing increasingly important today. Whether with edibles or pure extracts, consumers are more interested in cannabis product purity than ever before.

To achieve the best results possible with hydrocarbon extraction, producers must have the correct equipment and media for the job. Looking at both inline dewaxing systems and winterization, some careful study can guide cannabis businesses to utilize the best tools possible.

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about the author

DR. DOMINICK MONACO is the Director of Laboratory Operations for CLS Holdings' newly opened approximately $4 million laboratory, and is responsible for all day-to-day operations inside the North Las Vegas facility. Dr. Monaco brings more than 8 years of licensed and regulated cannabis experience, starting back in 2012 when medical marijuana first opened in Arizona, he has held numerous positions, with escalating responsibilities year over year. He graduated from the University of Arizona College of Pharmacy, in Tucson, Arizona, with a Doctor of Pharmacy in 2010. Direct correspondence to: dominick@citytrees.com.

ANALYTICAL INSTRUMENTS

CEM Corporation

COMPANY DESCRIPTION

CEM Corporation is a leading global company specializing in scientific solutions for critical laboratory applications. We design and manufacture systems for analytical laboratories, life sciences, and processing plants worldwide. Our product portfolio includes innovative instrumentation for sample preparation for analysis of heavy metals, pesticides, potency, and mycotoxins.

We have been deeply involved in the cannabis industry for many years and are familiar with testing procedures and regulations. We are founding members of many cannabis committees that are working on standardization of testing and reporting of data. We are passionate about transcribing this information into systems to help your business grow and have the knowledge to support you along the way.

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France, Germany, Ireland, Italy, Japan, United Kingdom, Local agency representatives

www.cem.com

CHIEF SERVICES SUPPORTED

World-class application and service support are what set CEM apart from other instrument manufacturers. Our mission is to simplify your testing procedures with easy-to-use systems that do the work for you. With built in methods, onboard support videos, and live applications and service support ready to help when you need it most, CEM is your partner in sample prep.

MAJOR PRODUCTS

- **MARS 6:** Microwave digestion system for trace metals sample prep of all cannabis and cannabis containing products.
  The simple-to-use system has a built in cannabis method that applies the appropriate amount of power to digest your entire batch of mixed cannabis and hemp products giving the highest throughput of any digestion system available. The MARS 6 is not only easy to use but it is easy on your bottom line.
- **EDGE:** Automated extraction system for potency, pesticides, terpenes, and mycotoxins. The EDGE automatically performs solvent addition, sample extraction, filtration, and cooling in as little as 5 minutes.

STATES SERVED

Nationwide

ANALYTICAL INSTRUMENTS | MANUFACTURING & PROCESSING | OTHER SERVICES

Chem Service, Inc.

COMPANY DESCRIPTION

Established in 1962, **Chem Service** is accredited for ISO 17034 and ISO 17025 and registered and certified to the ISO 9001 Quality Management System for the design, development, production, distribution, and servicing of organic neat and synthetic reference materials.

CHEM SERVICE, INC.

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www.chemservice.com

CHIEF SERVICES SUPPORTED

Chem Service, Inc., produces high purity chemicals for use as reference materials and for other laboratory purposes. More than 95% of their standards grade materials have a certified purity of 98.0% or greater. Standards-grade chemicals are clearly labeled with an expiration date that is based on years of experience in handling and testing. Products are packaged in small quantities to minimize storage, waste, and disposal requirements. Organic and inorganic chemicals, solutions, and mixtures are available to meet a wide range of specialized laboratory needs.

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- DIN standards
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- Petroleum hydrocarbons
- Fatty acid methyl esters
- High purity inorganics
- Surfactants
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- Ultraviolet inhibitors
- Plasticizers
- Biological stains
- Phthalate standards
- Polynuclear aromatics
- Vitamins
- Carbohydrates standards
- Forensic standards
- Personal care product standards

STATES SERVED

US and international

ANALYTICAL INSTRUMENTS | ANALYTICAL TESTING LAB | EXTRACTION | MANUFACTURING & PROCESSING | CULTIVATION/ GROWING | CONSULTING | OTHER SERVICES

Emerald Scientific

Scientific supplies, equipment, and technical expertise

COMPANY DESCRIPTION

Emerald Scientific is dedicated to promoting collaboration, transparency, and the evolution of cannabis science by supporting research, testing, production, and extraction. We source and distribute the widest variety of scientific products, supplies, and equipment specifically tailored to the cannabis and hemp industries.

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Wes Burk, President
Kirsten Blake, Vice President

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Fritsch Milling & Sizing, Inc.

COMPANY DESCRIPTION

Since 1920, **Fritsch** has engineered and manufactured a wide range of milling and grinding systems for agricultural, food, and pharmaceutical applications. The PULVERISETTE 19 Precision Cutting Mill System sets a standard with controllable particle size output, reproducible performance regardless of user, throughput of 1–8 lb/min avg., heat mitigation, dust-free, easy cleaning design. Scientific studies demonstrated no loss of cannabinoids or terpenes. Oil extractions and preparation of pre-rolls or other ingredients can be optimized. For the analytical testing laboratory, Fritsch offers several cryogenic milling options for individual flower or edible samples. For high-throughput the VHF hopper feeder system can be added to the P19.

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locations of other offices and facilities:
USA headquarters and applications laboratory in North Carolina. Global headquarters in Germany. Additional company offices in China, Asia Pacific, Russia, and France.

SERVICES SUPPORTED

North Carolina based proof of concept lab. Sample trials in our instruments to Identify the ideal instrument configuration for your application.

MAJOR PRODUCTS

Pulverisette 19 Precision Cutting Mill, Pulverisette 11 Knife Mill with cryo-options, Pulverisette 14 Rotor Mill, Analysette 22 & 28 Particle analysis instruments.

STATES SERVED

Worldwide

ANALYTICAL INSTRUMENTS

Restek Corporation

COMPANY DESCRIPTION

Restek is a leading developer and manufacturer of chromatography columns, sample preparation and collection products, reference standards, and instrument accessories. We are an independent, international, and diverse team of employee-owners not bound to a specific brand of instrument or geographic region. And our passion extends beyond our science and making innovative products that enable great chromatography; we are just as committed to providing exceptional "Plus 1" customer service that exceeds your expectations.

From LC and GC columns to sample preparation, reference standards to accessories, Restek is your first and best choice for chromatography products, applications, and expertise.

RESTEK
Pure Chromatography

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Rob Freeman, Director of Business Development

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CHIEF SERVICES SUPPORTED

Restek provides the industry with accurate, fast, and reliable analytical testing workflows. We support this in Restek blogs, application notes, presentations at conferences and seminars, customer visits, and webinars.

MAJOR PRODUCTS

Restek offers cannabis workflow testing solutions and analytical consumables for potency, terpenes, pesticides, residual solvents, and mycotoxins. These products include CRMs, sample prep, columns, and application notes for various testing methodologies.

STATES SERVED

International

ANALYTICAL INSTRUMENTS

SepSolve Analytical Ltd

COMPANY DESCRIPTION

SepSolve Analytical Ltd is a specialist in providing product and application packages for GC analysts, which extract maximum analytical data and deliver faster throughput for both research and routine GC applications. Addressing the whole workflow process, from sampling to detection, SepSolve provides instrumentation, accessories, and know-how for every step of the process. Its wide range of options include sample preparation equipment, robotic autosamplers, thermal desorption instruments, GC×GC technology, TOF mass spectrometers with Tandem Ionisation® (simultaneous hard- and soft-ionisation technology), and powerful data analysis software packages. SepSolve is part of the Schauenburg Analytics Ltd group of companies.

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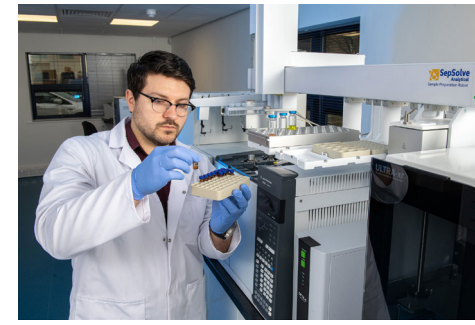
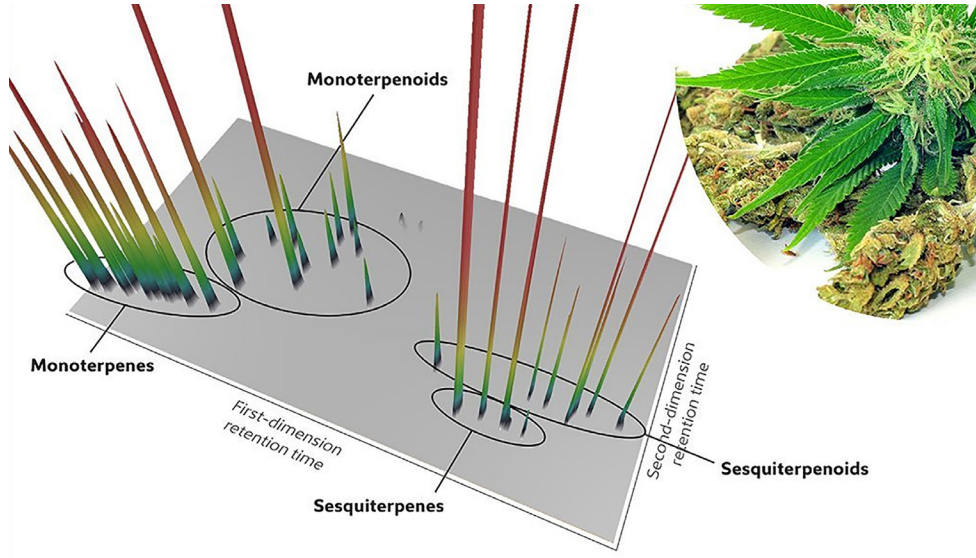
MAJOR PRODUCTS

Amongst others, SepSolve offers a product package specifically for the profiling of cannabis terpenes. This comprises INSIGHT® GC×GC flow modulation, which resolves co-elutions between terpenes and terpenoids; BenchTOF2™ mass spectrometer, which provides reliable identification and quantitation of terpenes, while also screening for other aroma-active compounds (e.g. esters); Tandem Ionisation® for BenchTOF™ , which simultaneously acquires both 'soft EI' and regular 70 eV mass spectra, to aid identification of challenging compounds, such as isomers; ChromCompare+™ software, which provides fully automated sample comparisons for easy visualisation of trends and differences across cannabis strains.

STATES SERVED

Global





ANALYTICAL INSTRUMENTS

Technobis Crystallization Systems BV

COMPANY DESCRIPTION

Technobis Crystallization Systems is a world leading technology provider for solid-state research, process development, and formulation. Our mission is to help research succeed!

Since 2005, platforms for accelerating crystallization research have been successfully developed and installed in nearly every pharmaceutical research lab in the world. With many sold instruments in the field, a large community of scientists have been enabled to make a significant contribution to the accelerated development of new pharmaceuticals. In the last few years, new applications have been developed in agrochemical, fine chemical, military and (bio) fuel research, food and personal care creating new markets focused on understanding the importance of crystallization research.

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SERVICES

With the Technobis Crystallization Systems workflow, scientists are now able to perform well controlled crystallization studies from hit and lead identification up through process scale up and formulation. Combine the CrystalBreeder, Crystal16, and Crystalline in a flexible configuration to optimize solid-state success!

Drug substance development is continuous troubleshooting and risk management. In the initial R&D phase, crystallization is used as purification and isolation method for the compound. Next, in parallel with the process development, the solid-state chemistry manages your drug's physchem properties by finding the polymorph that will give the perfect balance between stability, solubility and permeability. Once the first GMP batches have been produced and the first clinical studies successfully completed, a full IP screen will provide the overview of additional crystalline polymorphs, salts, co-crystals and amorphous forms. Technobis crystallization instruments help scientists around the world speed their molecules through development for commercial success!

PRODUCTS

The *CrystalBreeder* is the first crystallizer dedicated for both development and discovery, carrying out complete crystallization screens with as little as 1mg of sample.

The *Crystal16®* parallel crystallizer is a multiple reactor station providing a screening solution for solubility determination, solid-state research and process development: medium-throughput crystallization studies at a 1ml scale.

With 8 independently controlled reactors and additional analytical capabilities, the *Crystalline™* is a unique modular product line at a working volume of 5 ml for solid-state research, process development and formulation research.

STATES SERVED

Nationwide for the US

ANALYTICAL INSTRUMENTS | EXTRACTION | CONSULTING

UCT, Inc.

COMPANY DESCRIPTION

UCT, LLC UCT is a vertically integrated manufacturer and supplier of chromatography products for the cannabis testing industry. We offer the largest selection of QuEChERS, solid-phase extraction products, UHPLC and HPLC columns. Featured products for the cannabis industry, unique to UCT, include Chlorofiltr®, for the selective removal of chlorophyll from cannabis extracts, SpinFiltr®, which combines dSPE clean-up and 0.2 µM filtration in a single step, and, LipiFiltr®, our targeted cleanup cartridge for the analysis of pesticides in oil-based cannabis products.

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CHIEF SERVICES SUPPORTED

UCT has 30+ years of experience as the premier supplier for chromatography consumables. We combine this with world class technical support and application development.

MAJOR PRODUCTS

- ENVIRO-CLEAN® C18 SPE cartridges
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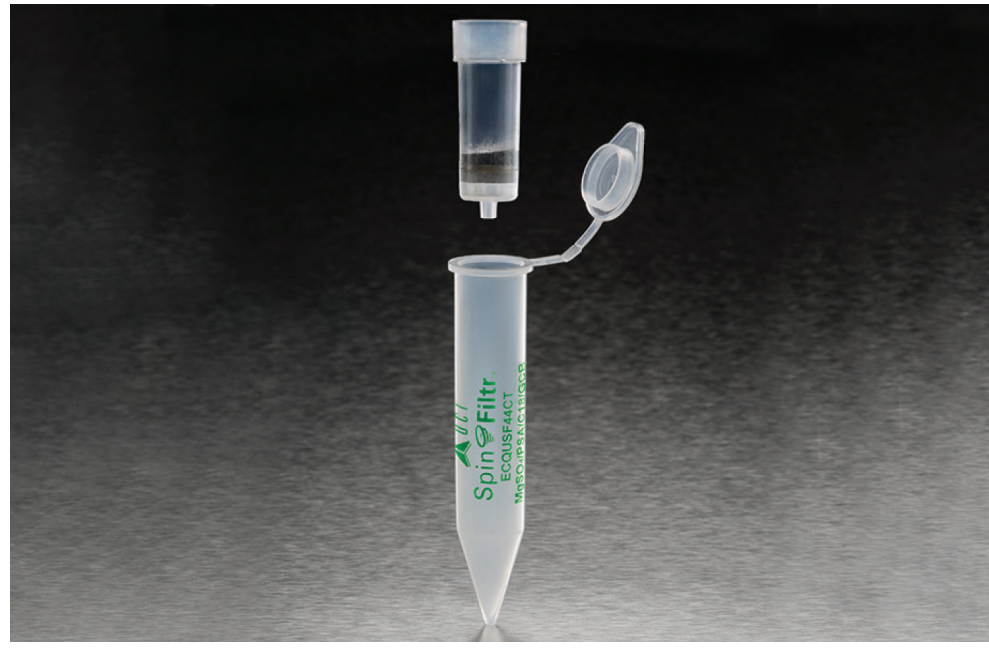
STATES SERVED

Nationwide and international through distribution.



CONSULTING | OTHER SERVICES

ASTM International

COMPANY DESCRIPTION

ASTM International is the standards leader in 150 global industries. Leveraging our 120-year history and a consensus-based approach, ASTM develops standards that give equal weight to the needs of all industry stakeholders. Over 12,500 ASTM standards operate globally. Defined and set by us, they improve the lives of millions every day. Combined with our innovative business services, they enhance performance and help everyone have confidence in the things they buy and use. Our volunteer members are producers, users, consumers, government, and academia from more than 140 countries. ASTM International offers global access to fully transparent standards development, resulting in the highest technical excellence in standardization.

Cannabis Standards and Services

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CHIEF SERVICES SUPPORTED

The cannabis industry is growing – so is the need for quality assurance and safety standards. Our comprehensive approach to standards development allows businesses to improve quality control while also enhancing safety. With services ranging from training, proficiency testing, and certification programs to advisory, sales, and marketing solutions, our holistic outlook provides confidence and credibility for industry stakeholders. ASTM International Committee D37 on Cannabis includes 1000+ members from 30 countries – experts from all parts of the industry. We welcome anyone with interest in creating safety standards for the cannabis industry to join us. Learn more at www.astmcannabis.org.

MAJOR PRODUCTS

- Cannabis Standards – 30+ standard test methods, practices, specifications, and guides for cannabis and hemp including indoor and outdoor agriculture, processing and handing, security and transportation, quality management, personnel training and credentialing, plus 100+ standards in development
- Hemp Flower Proficiency Testing Program - Labs worldwide turn to ASTM International for all their proficiency testing needs. Our testing solutions reflect real-world processes, helping cannabis and hemp businesses gain actionable insights and produce quality products. Use our proven statistical quality assurance (SQA) tool and standards developed by ASTM International Committee D37 on Cannabis to measure your performance in testing samples for various factors, including potency (cannabinoids), pesticides, residual solvents, moisture content, water activity, terpenes and terpenoids, mycotoxins, trace elements, and more.
- CANNQ/HEMPQ Certification Program - We help all cannabis and hemp producers meet and exceed best practice standards by following Good Manufacturing Practices (GMP), ensuring only consistent and safe products are available in the marketplace. A growing list of states are requiring GMP systems in cannabis regulations. Getting certified with ASTM gives you independent scientific evidence that your products and facilities adhere to the strictest industry standards and meet regulations. This unique program features full quality audits of policies and facility operations. All aspects of your production operation plus quality and compliance will be examined.

STATES SERVED

US and international

CONSULTING | EXTRACTION | MANUFACTURING & PROCESSING | CULTIVATION/ GROWING | OTHER SERVICES

Extractcoa, A Division of Weldcoa

COMPANY DESCRIPTION

Extractcoa is a new division of **Weldcoa**; a long standing, trusted leader in cryogenics, high pressure liquids and compressed gas. We are a US manufacturer that designs, engineers, manufactures, programs, and tests our equipment and automation in-house. Since 1968 we've engineered solutions, manufactured equipment, and provided uncompromising service to our customers, who use gases in the production of their products. If it involves gases, we can help. Our history of innovation has always been driven by the needs of our customers. As new applications for high pressure gases, cryogenics, high pressure liquids and supercritical fluid began to emerge, we met the challenge with effective solutions to deliver on the growing demand in emerging markets. Constant innovation has become our hallmark, one which our customers have grown to depend upon, and so can you.

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A DIVISION OF WELDCOA

EXTRACTCOA, A DIVISION OF WELDCOA

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Robert Ranc, Director of Sales

www.extractcoa.weldcoa.com

SERVICES

- Project Management
- Engineering and Design
- Installation and Start-Up
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PRODUCTS

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- Bulk Supercritical Phase CO2 Supply System best suited for Extraction Equipment of 40 Liters or Larger.
- CO2 Linear Manifold and Stand System is best suited for Extraction Equipment of 10 Liters to 40 Liters

STATES SERVED

USA

MANUFACTURING & PROCESSING

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COMPANY DESCRIPTION

As the world's largest and most experienced manufacturer of mixing and blending systems, **Ross** has helped thousands of companies to expand their product lines and perfect their processes. With a diverse line of equipment, our products can serve in a variety of cannabis-related applications, and we can often ship immediately from stock. Choose from R&D and small-batch models, scaling-up to full production models. Specialty vessels and control systems are also available. Call to arrange a no-charge test in our NY-based lab, or a trial rental in your facility.

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MAJOR PRODUCTS

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- **Ribbon Blenders** — lab and production blending for baked goods, beverages and supplements. Available with optional spray bar.

STATES SERVED

Worldwide

MANUFACTURING & PROCESSING

High Yield Solutions Corp.

COMPANY DESCRIPTION

Recognizing the need for industrial duty cannabis processing and handling equipment, **High Yield Solutions Corp.** was formed. We are a leading US manufacturer of industrial cannabis shredding and processing equipment. We have significant experience with cannabis applications paired with a reputation for providing quality equipment that will stand up to the demands of the industry. We look forward to bringing high quality equipment to the industry to help our customers with all their processing equipment needs.

High Yield Solutions Corp.™

HIGH YIELD SOLUTIONS CORP.

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SERVICES

We are here to help make your business more successful by assessing your processing needs and recommending a solution. From standard to customized systems and a range of equipment in between, we can help with industrial cannabis processing equipment to meet everyday demands.

PRODUCTS

Cannabis waste shredders and equipment, automated cannabis processing systems, industrial hemp shredders and equipment, premium finishing equipment

STATES SERVED

North America

MANUFACTURING & PROCESSING | PACKAGING & SUPPLIES | OTHER SERVICES

Oakwood Chemical, Inc.

COMPANY DESCRIPTION

Oakwood Chemical is a leader in selling high quality reagents to academic, chemical, and pharmaceutical customers for almost thirty years. Located in the South Carolina Lowcountry, we have over 10,000 square feet of laboratory space, as well as two large warehouses totaling 200,000 square feet with over 150,000 chemicals in stock. We offer benchtop to production quantities of inorganic and organic reagents, solvents, salts, catalysts, and biochemicals in numerous grades (reagent, anhydrous, ACS, HPLC, LCMS, NMR, USP). Let us help you in succeeding in this high growth industry.

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Vice President, Operations
Wilson Butler, Director of Business Development

www.oakwoodchemical.com

PRODUCTS

Oakwood Chemical offers an array of reagents and solvents for every step of the cannabinoid preparation process: isolation, synthetic isomerization, purification, and analysis. Our catalog contains chemicals needed for extraction of cannabinoids from plant material in different grades (reagent, ACS, and USP) and in different amounts (from lab bench to industrial drum sizes). We also sell reagents to convert the various isomers of Tetrahydrocannabinol to the desired Delta-9-THC, as well as other isomers. Oakwood sells numerous types of stationary phase materials for purification of the extracted material from unwanted plant by -products. Finally, we have a wide range of high purity analytical solvents for HPLC and LC/MS in quality control.

STATES SERVED

We serve every state in the United States and ship internationally worldwide.

OTHER SERVICES

A2LA

COMPANY DESCRIPTION

Established in 1978, **A2LA** is among the largest accreditation bodies in the world and the only independent, 501(c)3, non-profit, internationally recognized accreditation body in the US that offers a full range of comprehensive conformity assessment accreditation services. We are industry experts and a leader in Cannabis accreditation that insures confidence in the testing results from accredited testing labs on the safety of medical and recreational cannabis for public consumption.

A2LA

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www.A2LA.org

SERVICES

Accreditation is the most appropriate way to ensure a laboratories performance to international standards. We provide programs to fit those needs:

- ISO/IEC 17025 standard and additional state specific regulations, including requirements from the Americans for Safe Access (ASA) and Patient Focused Certification (PFC)
- ISO/IEC 17043 for Proficiency Testing Providers (PTPs) to ensure that providers are competent to run quality proficiency testing programs
- ISO 17034 for Reference Material Producers (RMPs) to produce quality reference materials to be used in the testing process.
- Exceptional customer service, offering a single point of contact to guide laboratories through the process and answer any questions.

MAJOR PRODUCTS

A2LA can accredit laboratories for either hemp or cannabis testing, allowing us to serve laboratories in states which have not yet legalized cannabis. Laboratories that test both hemp and cannabis would be required to setup separate legal entities. Appropriate testing is critical to demonstrate that these products do not contain harmful levels of contaminants or adulterants and insures confidence for public consumption for medical and recreational cannabis.Specific testing may include, but is not limited to, the following:

- Cannabinoid testing and content (CBC, CBD, CBDa, CBG, CBN, THC, THCa, THCv, etc.)
- Terpene profile
- Pesticides/fungicides/plant growth regulators
- Residual solvents
- Heavy metals
- Microbiological contaminants (mold, insects, bacteria, etc.)

STATES SERVED

Global

PACKAGING & SUPPLIES

True Liberty® Bags

COMPANY DESCRIPTION

TRUE LIBERTY® BAGS is a family-based business founded on the principle that the best products should not only be the strongest, safest, and highest quality in an industry, they should also be made by a company which honors it's responsibility to customers, community, and the environment.

TRUE LIBERTY® BAGS

5900 Pruitt Ave. #230
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USA

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key personnel:
Jennifer Diaz, Co-Founder & Owner
Kimberly Crews, Accounting & Site Supervisor
Ciel Pierce, Warehouse Supervisor

www.truelibertybags.com

SERVICES

True Liberty® is the original All Purpose Bag. Industrial-strength nylon, plastic and bpa-free, reusable eco bags have many garden and outdoor uses

- Harvest, cure, preserve
- Transport, biomass disposal
- Fresh frozen, decarboxylation
- Prevent cross-contamination & mineral build-up
- Long-term storage
- Quick & easy clean-up
- Line industry standard containers

PRODUCTS

- 2 Gallon Bags (12" x 20")
- 3 Gallon Bags (18" x 20")
- 4 Gallon Bags (18" x 24")
- 8 Gallon Bags (24" x 40")
- Can Liner (30" x 48")
- Bin Liner (48" x 30")
- Drum Liner (36" x 48")
- Pallet Container Liner (55" x 44" x 90")
- Rolling Bench Table Liner (80" x 500')

STATES SERVED

Nationwide

cannabis
science and technology®
advancing research, quality & education

CALL FOR PAPERS

Cannabis Science and Technology®
2022 Issues

Cannabis Science and Technology® magazine is seeking contributed manuscripts for the upcoming 2022 issues.

Manuscript scope and format

Cannabis Science and Technology broadly covers science and product quality issues in the cannabis industry. We welcome manuscripts that describe analytical methods for product quality control; the development of standard (consensus) methods; proper laboratory techniques and laboratory best practices; laboratory accreditation, proficiency testing, and inter-laboratory comparison testing; equipment and technology for testing and processing; regulatory issues; current good manufacturing practices; research on cannabinoids and terpenes; cultivation and extraction best practices, challenges, and tips; and topics related to processing/manufacturing. We also offer a blinded, peer-review process for manuscripts.

TECHNICAL ARTICLES

Technical articles describe improvements in methods or techniques. Papers should be ~3500-4500 words long and should be of immediate relevance to the analytical and quality issues facing the cannabis industry. Authors should not make comparisons between commercially available products from different manufacturers. We urge authors to submit a proposal to the editor before completing a manuscript.

TUTORIAL ARTICLES

Tutorial articles should be presented as a how-to on a technique, application, or method related to cannabis analysis or processing (for example, QuEChERS extraction). Papers should be ~2000-2500 words long and should be of immediate relevance to the analytical and quality issues facing the cannabis industry. We urge authors to submit a proposal to the editor before completing a manuscript.

REVIEW ARTICLES

Review articles survey recent developments and the state of the art of current techniques or emerging technologies. Manuscripts should be ~3500-4500 words long. We urge authors to submit a proposal to the editor before completing a manuscript.

Deadlines

Submission deadlines for the April 2022 issue are:

Abstracts: February 2, 2022
Completed manuscripts: March 1, 2022

Submission deadlines for the May 2022 issue are:

Abstracts: March 7, 2021
Completed manuscripts: April 1, 2022

Where to submit

Send all proposals and completed articles to Editor **Meg L'Heureux, at mlheureux@mjhlifesciences.com.**

HIGH-THROUGHPUT ANALYSIS OF CANNABINOIDS BY LC-UV

- Increase sample throughput with this fast, 9-minute analysis.
- Baseline separation of 16 cannabinoids provides more accurate and comprehensive potency and profile data.
- Simple, isocratic method is more easily transferable between instruments and labs than gradient methods.

As the cannabis market grows, interest in more detailed analysis of cannabinoid profiles is expanding because more comprehensive data can be used for strain identification as well as to ensure more accurate potency testing. More than 100 cannabinoids have been isolated from cannabis to date, and these compounds can interfere with the five most commonly analyzed cannabinoids: tetrahydrocannabinol (THC), delta-9-tetrahydrocannabinolic acid A (THCA), cannabidiol (CBD), cannabidiolic acid (CBDA), and cannabinol (CBN). The LC-UV method shown here uses a Raptor ARC-18 column to fully resolve 16 major and most frequently observed minor cannabinoids for which commercial standards are available. Baseline separation ensures positive identification and accurate quantitation. As shown, all compounds were resolved in a fast 9-minute analysis, making this method suitable for high-throughput cannabis testing labs. In addition, this analysis uses a simple isocratic mobile phase so it is more easily transferable between instruments, compared to more complex methods that incorporate atypical mobile phase gradients or additives.

RESTEK CORPORATION
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Website:www.restek.com

TABLE I: EXP Direct Connect Holder

Description	qty.	cat.#
EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules)	ea.	25808
Maximum holder pressure: 20,000 psi (1,400 bar)		

TABLE II: Raptor ARC-18 LC Columns (USP L1)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 µm Columns			
30 mm	9314232	---	---
50 mm	9314252	931425E	---
100 mm	9314212	931421E	---
2.7 µm Columns			
30 mm	9314A32	9314A3E	9314A35
50 mm	9314A52	9314A5E	9314A55
100 mm	9314A12	9314A1E	9314A15
150 mm	9314A62	9314A6E	9314A65
5 µm Columns			
30 mm	–	931453E	–
50 mm	9314552	931455E	9314555
100 mm	9314512	931451E	9314515
150 mm	9314562	931456E	9314565

FIGURE 1: Baseline resolve 16 cannabinoids with a Raptor ARC-18 column and a simple, easily transferable isocratic method.

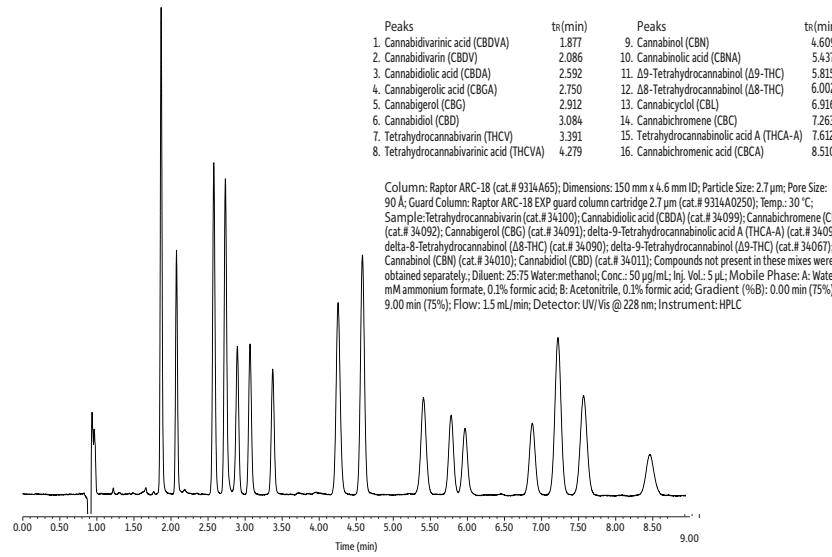


TABLE III: Medical Marijuana Singles

Compound	CAS #	Solvent	Conc.	cat.#
Cannabichromene (CBC)	20675-51-8	PTM	1,000	34092
Cannabidiol (CBD)	13956-29-1	PTM	1,000	34011
Cannabidiolic Acid (CBDA)	1244-58-2	ACN	1,000	34099
Cannabigerol (CBG)	25654-31-3	PTM	1,000	34091
Cannabinol (CBN)	521-35-7	PTM	1,000	34010
delta-8-Tetrahydrocannabinol (D8-THC)	5957-75-5	PTM	1,000	34090
delta-9-Tetrahydrocannabinol (D9-THC)	1972-08-3	M	1,000	34067
delta-9-Tetrahydrocannabinolic acid A (THCA-A)	23978-85-0	PTM	1,000	34093
Tetrahydrocannabivarin	31262-37-0	M	1,000	34100

ACN = acetonitrile; M = methanol; PTM = purge-and-trap grade methanol

Separation of Eight Cannabinoids

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

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

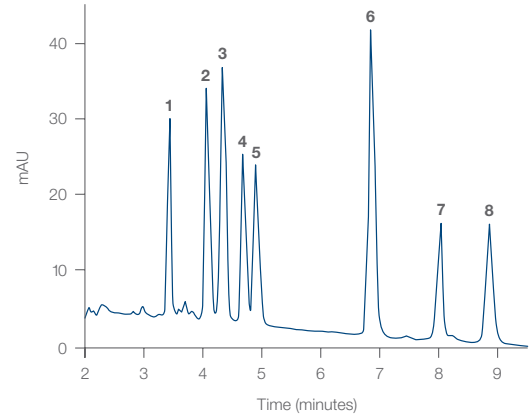
Column Information

Packing Material	HxSil, 3 µm
Part Number	79641

Chromatographic Conditions

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

Separation of Eight Cannabinoids



Compounds:

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

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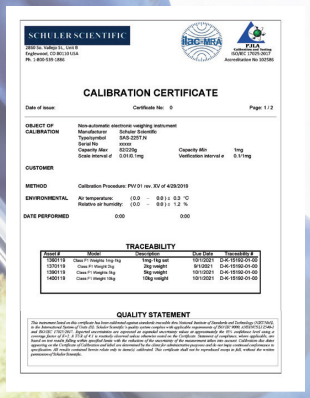
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The SH-Series high-capacity balances are ideal for anywhere that accuracy and portability is required.

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