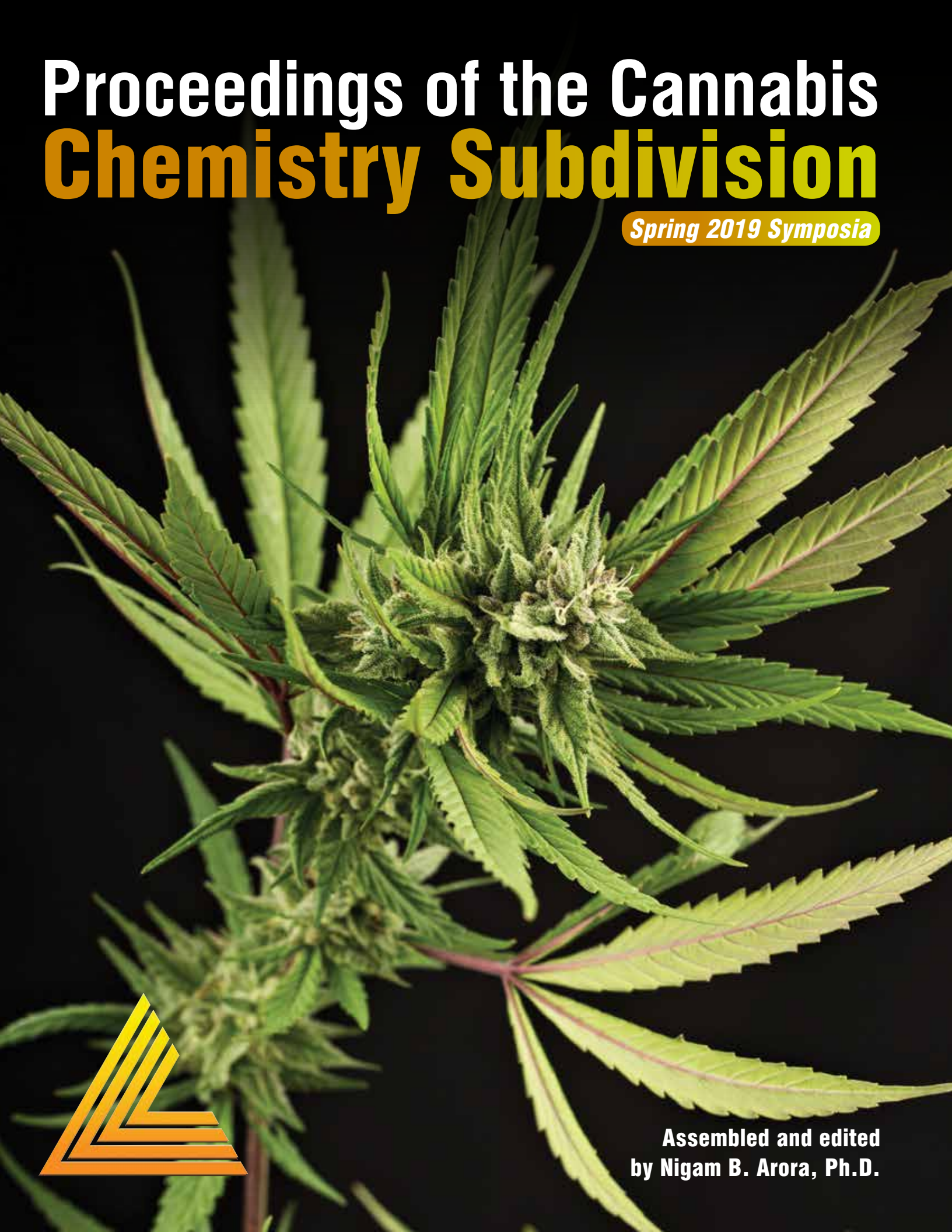
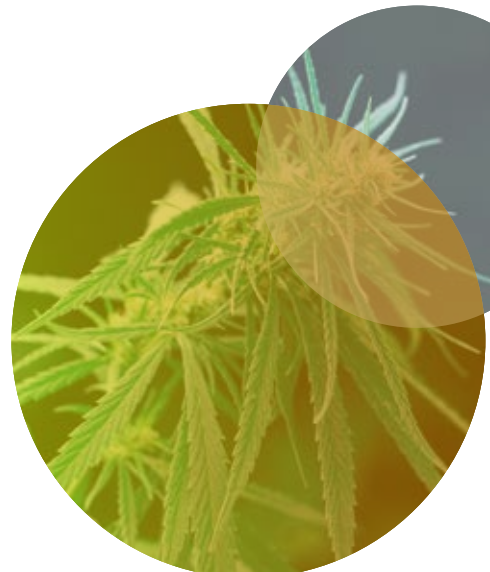


Proceedings of the Cannabis Chemistry Subdivision

Spring 2019 Symposia

Assembled and edited
by Nigam B. Arora, Ph.D.

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What **is CANN?**

The Cannabis Chemistry Subdivision (CANN) exists within the Division of Chemical Health and Safety (CHAS) of the American Chemical Society (ACS). Founded in 1876 and chartered by the U.S. Congress, the ACS has over 150,000 members from more than 140 countries, deeming it the world's largest scientific society. In its 140+ years of operation, the ACS has established itself as a leading resource for scientific standards, research, advancement, and publication. As a response to the nascent and rapidly growing cannabis industry in 2015, Ezra Pryor, Dr. Jahan Marcu, Melissa Wilcox, Dr. Joseph Payack, and Dr. Mark Scialdone approached the ACS with the vision of creating a strong network of scientists in the cannabis space to promote scientific innovation, safety, and development through education, research, and the empowerment of the scientific community. Naturally, CANN was formed.

CANN has since experienced immense growth, both in membership and outreach. To support its vision and initiatives, the CANN Executive Committee is comprised of nine units dedicated to building a foundational network of scientists and stakeholders to drive legitimacy, innovation, and academic liberty in the industry and related fields. CANN accomplishes this through programming cannabis related scientific symposia at ACS National Meetings, granting a scholarship named the ElSohly Award to leading cannabis scientists each year, hosting webinars showcasing novel information from leaders in the cannabis space, publishing scholarly and technical articles, holding trainings and workshops, and much more. As CANN continues to grow and expand our initiatives within the scientific and cannabis communities at large, we hope you will join us.



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Sincerely,
Julia Bramante
Chair of CANN

How Can You Keep Up with the Leading Research and **Discoveries in Cannabis Science?**

American Chemical Society (ACS) National Meetings are held twice annually and draw tens of thousands of the brightest chemical minds from industry and academia. Chemists from around the world look forward to these meetings as the ultimate forum for presenting on and learning about cutting-edge research. Since its inception in 2015, CANN has held cannabis focused symposia at every ACS National Meeting to date.

These symposia serve as a nexus for discussions on cannabis-related research, technology, safety, and thought leadership and have been host to numerous subject-matter experts in cannabis science. In congruence with CANN's vision, the Publication Committee was formed to promote dissemination of the knowledge presented at CANN symposia and, furthermore, to provide a public outlet for knowledge sharing by the cannabis chemists and scientists that make up the CANN membership.

The proceedings presented here are the inaugural work from the CANN Publication Committee and review the presentations made at the ACS National Meeting in Orlando during April 2019. This includes presentations from the First Annual ElSohly Award Symposium, sponsored by Heidolph North America. For publication, CANN elected to partner with T&T as they have risen to the top as the most widely read cannabis science magazine. Going forward, readers can expect a proceedings publication to follow each ACS National Meeting. CANN Publication Committee has several additional projects in the works so be sure to stay tuned for more!

JOIN CANN

Want to join the Cannabis Chemistry Subdivision of the largest scientific society in the world? Visit us at cannachem.org to learn more
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Sincerely,
Nigam B. Arora, Ph.D.
CANN Publication
Committee Chair

Frontiers in the Development of Cannabis Based Therapeutics

By Mahmoud A. ElSohly, Ph.D., National Center for Natural Products Research Department of Pharmaceutics and Drug Delivery School of Pharmacy, University of Mississippi; ElSohly Laboratories, Inc., Oxford, MS

Cannabis is one of the oldest plants known in medicine with a reported use for treating a wide variety of ailments for centuries.

However, it was not until the last part of the 20th century when the first cannabis-based therapeutic was introduced into modern medicine, Marinol®. Marinol is formulated in a soft gelatin capsule in a sesame seed oil solution of Δ^9 -tetrahydrocannabinol (THC), and was first approved for controlling nausea and vomiting in cancer patients receiving chemotherapy and later for appetite stimulation of AIDS patients suffering from the wasting syndrome.

Over the last few years, interest in *Cannabis* and *Cannabis*-based medicines have intensified, especially in the United States, resulting in several states legalizing *Cannabis* for medical use, despite its being illegal on the Federal level.

Cannabis is rich in phytochemicals belonging to a variety of chemical classes, most characteristic of which are the cannabinoids. In our efforts to develop cannabis-based therapeutics, we have embarked on research efforts aimed at developing derivatives and formulations for the most studied cannabinoids, namely THC and CBD (cannabidiol). These efforts culminated in the development of one derivative (a prodrug) of THC and one derivative of CBD.

The THC derivative has substantial bioavailability from suppository formulations (while THC is not bioavailable from suppositories) with the advantages of lower dosing, bypassing the first-pass effect, extended duration of action (more like a slow-release preparation) and lack of abuse potential. The



product is able to deliver THC from a transmucosal delivery system. Furthermore, formulated in an ophthalmic preparation (eye drops), it penetrated into the inner compartments of the eye and lowered the intraocular pressure (IOP), lending itself to be a good candidate for the treatment of glaucoma.

The CBD derivative, on the other hand, is not a prodrug, but does have activity of its own, much like CBD, but better efficacy and better bioavailability. Therefore, the CBD derivative holds a great promise for development to treat a variety of medical conditions.

Finally, to take advantage of the total cannabis constituents, a CBD-rich extract has been formulated into an oil solution (sesame seed oil) under good manufacturing practices and an investigational new drug application has been filed and approved by the FDA. The Clinical Study has been ongoing for approximately six months for the treatment of intractable childhood epilepsy with a promising outcome.

It is hoped that these preparations and others from cannabis will make it to market as FDA approved pharmaceuticals.

Chemical and Genetic Origins of Cultivar-specific Aroma in *Cannabis sativa* L.

By Avery N. Gilbert, Ph.D., Headspace Sensory LLC

Aroma is one of the defining sensory features of *Cannabis* flower. As legalization of its production and sale continues to expand, so does public and professional attention to the nuances of its characteristic scent. Online product reviewers and judges at “cannabis cup” competitions offer informed opinions in the manner of wine experts, yet they do so without the sommelier’s training or shared vocabulary. At the same time, consumers express distinct aroma-based cultivar preferences, even as they struggle to verbally describe exactly what it is they like or dislike.

A long history of chemical analysis has focused attention on a set of common terpenes as the source of *Cannabis* aroma, and recent surveys of chemotypic variation across cultivars have identified large-scale patterns in cultivar-specific terpene content. While extremely informative, these chemical studies have not directly addressed the impact of terpene content on olfactory perception.

By using quantitative sensory evaluation methods, I established for the first time that untrained consumers can reliably discriminate the scent of different *Cannabis* cultivars. Statistical analysis of the cultivar aroma profiles reveals large-scale patterns, namely two basic clusters: cultivars that smell woody, earthy, and herbal, and those that smell citrus, lemon, sweet, and pungent. Recent work suggests that within a given cultivar, genetically anomalous samples produce aroma profiles that differ from those of genetic consensus samples. The fact that genetic and chemical variations in *Cannabis* phenotype can be detected by the human nose has commercial implications for growers, manufacturers, and retailers. It opens the door to sensory market segmentation, precision control of brand identity, and quantitative end-user feedback for product innovation.



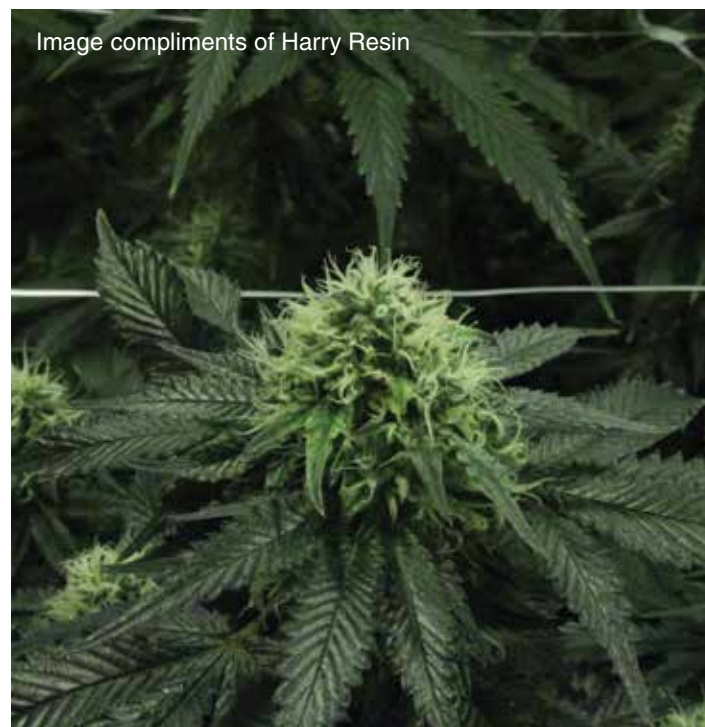
Old Wine in New Bottles: Cannabis and the Rx Drug Epidemic

By Jahan Marcu, Ph.D., International Research
Center on Cannabis and Mental Health

The endocannabinoid system (ECS) is an adaptive system that responds to stress and is stimulated by compounds made by the body (the endocannabinoids), as well as ingredients found in the cannabis plant and elsewhere in nature. Endocannabinoids are synthesized on demand in response to internal and external triggers, which governs physiological processes involved with eating, sleeping, relaxing, forgetting, and protecting our body from damage. There are two main receptors, CB₁ and CB₂, which THC and endocannabinoids interact with. The endorphin or opioid system (the term endorphin is an abbreviation of endogenous morphine) is similar to the ECS, in that both are systems important for mammals, with medicinal plants and licensed pharmaceuticals targeting those systems.

Cannabis and opium have been used as drugs since antiquity and still exist today as modern-day medical preparations and illicit street drugs. Cannabis-based licensed pharmaceutical drugs include Marinol® (synthetic THC approved by the FDA since the 1980s, cannabis extracts such as Sativex® (licensed in over 30 countries), and cannabis flos (a licensed preparation of whole plant flowers available in pharmacies in several countries). Unlike opioids, there are no reported deaths from cannabis-based drugs, and standardized preparations of cannabis offer a uniquely low abuse and diversion potential. [1]

Many of the societal problems with opioids became well-documented with the commercialization of opium by the Dutch East India company. The opium trade and the opium wars reached their heights in the 1880s with over 4 million kilos being shipped to China each year, and with it, creating over 12 million addicts. Opioid receptors are largely targeted



for pain. Stimulating the μ -opioid receptor is associated with analgesia, respiratory depression, gastrointestinal (GI) motility, physical dependence, sedation, and euphoria. Stimulation of δ -opioid receptors is associated with analgesia, respiratory depression, GI motility, behavior, and reinforcing actions. And κ -opioid receptors are associated with analgesia, respiratory depression, GI motility, dysphoria, physical dependence, and sedation. Common pharmaceuticals include morphine, codeine, fentanyl, and tramadol.

The endorphin system and the ECS interact with each other in ways that are clinically relevant; the impact is a factor

with public health implications. Research has shown that administering cannabis/hemp and opioids together does not appear to alter their drug abuse liability. [2, 3] The availability of medical and adult-use cannabis in the United States is linked to: decreased numbers of opioid prescriptions, numbers of overdoses, and lower opiate-positive screens in fatal car accidents. [4, 6]

The ECS and endorphins “talk” to each other. For example, animals deficient in CB₁ receptors have reduced opioid reward. Opioids regulate the release of endocannabinoids, and endocannabinoids alter opioid peptide levels. ECS and opioid receptors are expressed in the same cells in discrete neuronal pathways highly implicated in addictions. The key point is that the significant convergence for opioid/cannabinoid interactions opens up opportunities to assess potential cannabinoid interventions for Opioid Use Disorder (OUD).

CBD has been shown to modulate anxiety, reduce amygdala activation, reduce heroin-related cue-induced craving and was recently shown to be safely administered with fentanyl in a clinical setting. [7-10] CBD did not exacerbate adverse effects associated with intravenous fentanyl administration. These properties translate into the potential modulation of addiction-related outcomes. While studies such as those mentioned above seem to pour a foundation for building OUD-related treatments with hemp, many questions remain. For example, little is known about what stage of the drug addiction cycle that CBD would or would not be effective. And like THC, CBD has limitations, including potential side-effects from chronic, heavy, long-term use. Concerns are quietly being discussed, such as increases in IOP (Intraocular Pressure), effects on hormone levels from long-term use, and drug-drug interactions from oral administration — CBD is metabolized by the same enzymes as 60% of pharmaceutical drugs. [11, 12]

Focused clinical trials are needed to determine the most appropriate formulations, routes of administration, and treatment regimens for the use of THC or CBD-preparations for OUD-related issues. More data focused on the co-administration of opioids with cannabis preparations would help guided patients, doctors, and regulators interested in the effective use of cannabis or hemp in the treatment or prevention of addiction and mitigation of the Opioid Epidemic. Presently, the only clinically approved use for CBD is for a specific form of epilepsy. Although CBD’s lawful status with respect to the Controlled Substances Act appears to be expanding, its future regulatory status with respect to the FDA is difficult to predict.



Novel Isomers of THC in Cannabis Distillates

By Zack Iszard, Confidence Analytics

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Compliance cannabis testing provides strong opportunities for research — access to the full variety of materials produced in the market. More than a cursory review of the many sample types reveals curiosities too numerous to count. The topic presented is an example of one of the many unturned stones in cannabis science, which has only resulted from innovation in the space since commercial legalization. These findings promote the need for continuous improvement in a rapidly developing — and maturing — field of analytical chemistry, and demand a higher degree of rigor than merely developing a method from an application note and reference standards alone.

Through careful HPLC-UV analysis and quality assurance, the team at Confidence Analytics observed a trend among cannabis extracts processed with vacuum distillation — missing THC. In the place of the expected 90% or greater THC composition, several new peaks of considerable size showed up in the HPLC-UV data. Others working in this space noticed the mystery interferences, too. Spectral and chromatographic assessment revealed these peaks to be different than any reference material we had, though the interfering peaks often resemble cannabichromene (CBC) in retention time and UV spectrum. We thus termed these compounds "faux-CBC".

From the data available, and observation of the short-path vacuum distillation (SPVD) processes employed, simple advice for processors emerged: reduce heat, increase vacuum strength, and the target THC could be better preserved. These findings were delivered locally, via the meetup group Cannabis Science, Technology, Engineering and Math (Cann-STEM), and online via the American Chemical Society (ACS) Cannabis Chemistry Subdivision (CANN) Journal Club. Word spread among the curious community, and we observed a decrease in the prevalence and abundance of these "degradants". Success?



Method development with more powerful chromatography revealed that there are a number of peaks in this region, requiring very high power to resolve. Mass spectrometric assessment reveals molecular weight and product ion data for these "faux-CBC" peaks. Here I present the newest data we have regarding these mysterious compounds — ostensibly isomers of d9-THC — describe conditions of formation, and promote the further characterization of these compounds with stronger tools such as high-resolution fraction collection and high-frequency NMR. Understanding the chemistry at play, and characterizing the phenomenon, takes far more resources and time than a compliance method specialist has available.

Fundamental Chemistry Research from a Collaborative Cannabis Research Venture

By Markus Roggen^a, Ph.D., Glenn Sammis^b, Ph.D.

^aComplex Biotech Discovery Ventures (CBDV)

^bUniversity of British Columbia, Department of Chemistry

During our work in process optimization of cannabis production, we noticed a rate difference of decarboxylation for tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA). The noticeable rate difference between the decarboxylation reactions of THCA & CBDA has been noted but is generally overlooked. [1a, 1b] We were curious to study and understand those rate differences. We took a two-pronged approach. First, we investigated the reaction mechanism in-silico. Secondly, we developed an in-process analytical tool to track decarboxylation in real time.

While the decarboxylation reaction has been studied [1a, 1b], [2a, 2b], [3a], and a mechanism has been proposed [3b], most of this work has been based on basic THCA analogs. Our calculations [4] for decarboxylation of THCA and CBDA show that:

- The rate determining step is the intramolecular protonation process from the carboxylic group to benzene ring.
- The rate determining step energy barrier could be significantly decreased with the help of one water/alcohol molecule.
- The rate difference is due to steric effects caused by meta substitute rather than electronic effect from para substitute.
- The steric effect was achieved by blocking the occurrence of an orthogonal carboxylic group conformation of the transition state (TS2, Figure 1).

For the in-process analytics we developed a Fourier transform infrared attenuated total reflectance (FT-IR-ATR) method in collaboration with PerkinElmer. We utilized cannabis extracts of different cannabinoid concentrations and decarboxylation ratios in the reaction setup. The extracts were heated in an oil bath equipped with an overhead stirrer.

During an 80-minute reaction time we recorded extract temperature in regular intervals, measured mid-IR spectra every five minutes and collected aliquots for high-performance liquid chromatography (HPLC) analysis. For the IR spectra (Figure 2) principal component regression (PCR) was used to generate quantitative models for THCA and THC.

Over the course of the decarboxylation reaction, the change in THCA and THC concentration can be observed (Figure 3). Cannabinoid concentrations predicted from IR spectra are overlaid with the HPLC reference values.

The excellent fit between our IR model and the reference data demonstrates the utility and power of a low-cost, in-process analytical tool for real-time reaction analysis and control.

In conclusion, we showed our multi-level approach to reaction analysis and process development to support the cannabis industry in their production challenges.

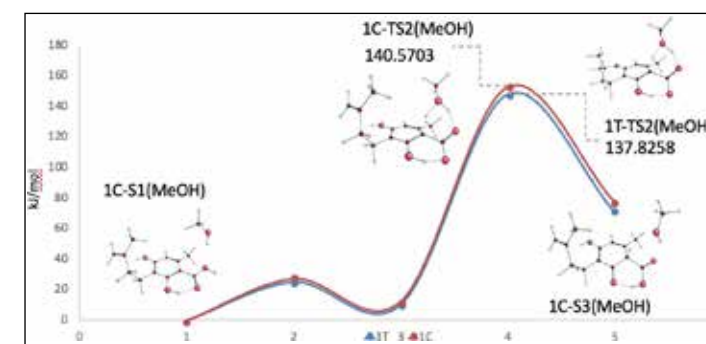


Figure 1: Potential Energy Graph of Decarboxylation: Calculations show that the decarboxylation is bi-molecular between the cannabinolic acid (CBDA) and methanol. The alkene side chain of the CBDA analog, 1C, poses steric hindrance for the attack of methanol.

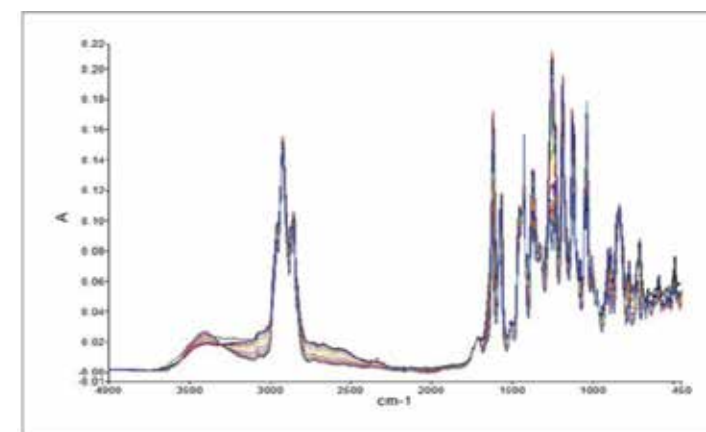


Figure 2: Mid-IR Spectra: Example spectra of cannabis extract throughout the course of decarboxylation by the application of heat.

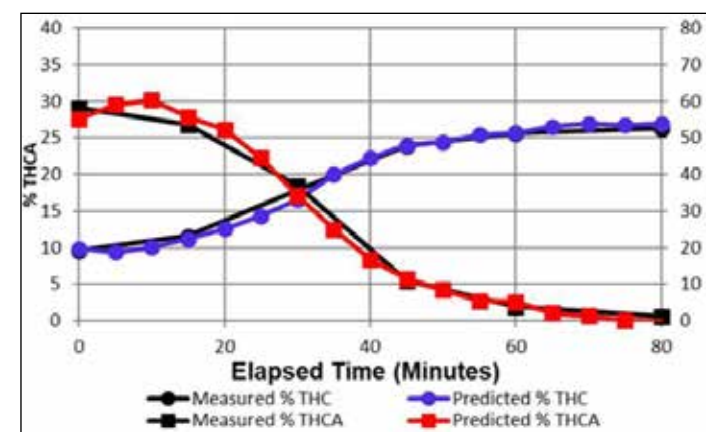


Figure 3: Fit of IR spectra, based on PCR model, to HPLC reference data: Cannabinoid concentration plots over the course of decarboxylation for three separate decarboxylation reactions.



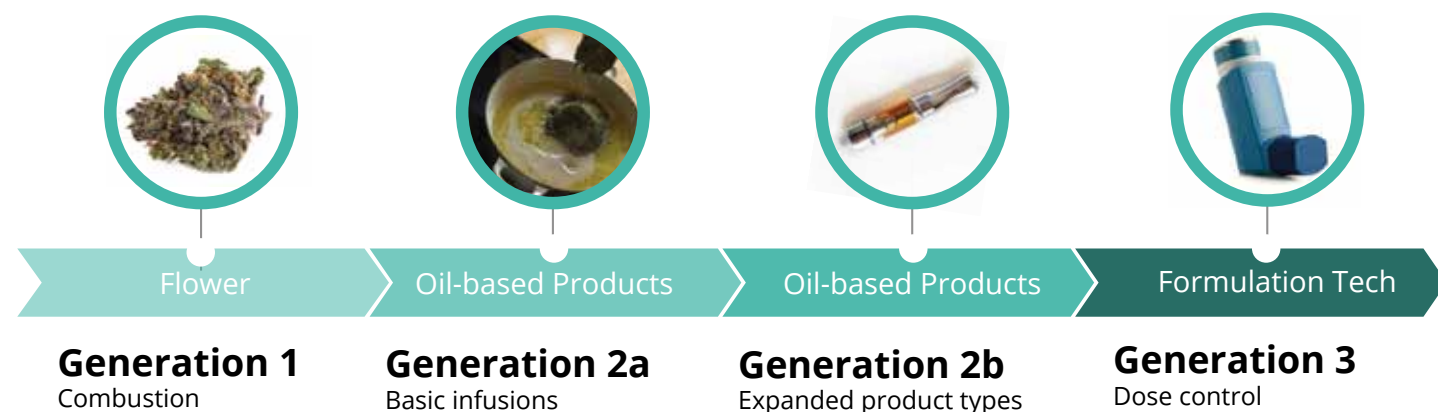
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Innovation Follows Inspiration

By Monica Vialpando, Ph.D., Vialpando LLC

Evolution of medical cannabis products



The use of cannabis has been documented throughout history for myriad indications such as inflammation, pain relief and nausea. Recent changes in regulation surrounding the production and consumption of cannabis have led to the commercial launch of a wide variety of cannabis-based products such as tinctures, capsules, and topical formulations. While these products are typically manufactured from crude starting materials such as cannabis-based extracts, market demand and regulatory requirements are driving progress toward more medicinal-like products. These more advanced products typically rely on starting materials of defined purity (e.g. extracts with known composition) and are developed with much more focus on dose uniformity, batch-to-batch consistency and stability, to ensure user safety and reproducible efficacy.

Development of products that meet these more stringent quality criteria presents specific challenges for cannabinoids. Most cannabinoids are highly lipophilic, which makes them poorly soluble in water and therefore difficult to formulate

into products that generate reproducible absorption after oral administration. The low metabolic stability of many cannabinoids may further contribute to low bioavailability after oral intake. Another complexity is that the properties of starting materials may vary greatly with composition. For example, the viscosity of *delta*-9-tetrahydrocannabinol increases 20-fold when purity is increased from 70% to 85%. Such a dramatic viscosity difference may require an entirely different formulation approach.

Beyond just the technical challenges, the development of cannabis-based products is compounded by an ever-evolving regulatory landscape, the apprehensiveness of suppliers to sell to cannabis companies and the market's preference for all-natural products. In spite of these challenges, a great many of new products will be put on the market in the coming years. Market demand and regulatory requirements are anticipated to drive an evolution toward more medicinal-like products with well-defined quality and demonstrated safety and efficacy.

Cannabis Microbiome Sequencing: Implications for Cannabis Safety Testing

By Kyle Boyar, Medicinal Genomics

Microbial testing is a crucial component of quality control for cannabis products. This type of analysis is especially critical in the context of immunocompromised patients whose bodies cannot readily fight off infection from opportunistic pathogens. There are various methods currently available to detect and/or quantify microbial contaminants in cannabis but not all of them are suitable for the diverse variety of cannabis matrices. Furthermore, there are some inherent flaws in many of these technologies that can produce false negatives and jeopardize consumer safety.

Our work investigates the cannabis microbiome of multiple cultivars. Conserved organisms of concern for certain patient populations that were revealed by sequencing are reviewed. Regulatory requirements in certain regions are also discussed and how the battery of tests required in these states are not necessarily producing meaningful data while hindering products from going to market. Additionally, experiments performed using the various methodologies available and how they compare to quantitative PCR (qPCR) are discussed, specifically focusing on *Aspergillus* spp. and examples of where traditional culture-based methods fall short are presented.

This work demonstrated a number of distinct differences between culture-based methods and molecular methods and also unveiled some distinct flaws. Specifically, it was shown that 3M Total Aerobic and Total Coliform counts were severely suppressed when *E. Coli* was spiked into cannabis-infused gummy matrices. This was in stark contrast to the no matrix controls using just tryptic soy broth (TSB) where we saw the proper enumeration. We believe this to be due to the presence of citrate and citric acid which is listed as an inhibitor to the plate's reporter chemistry. Additionally, sequencing the



microbiome of the various culture cartridges and plates demonstrated a poor consistency in operational taxonomic units (OTUs) between the 3M and Biomerieux platforms. Furthermore, where inconsistencies were seen between culture and qPCR, the presence of an endofungal bacterium *Ralstonia* was the common denominator and was present in nearly all of these discordant samples. This research highlights the importance of choosing the appropriate and specific methodology in order to achieve accurate microbial testing data for cannabis.

Chemistry of Cannabis Concentrate Aerosolization

By Jiries Meehan-Atrash and Robert Strongin, Ph.D., Portland State University

Image compliments of Harry Resin

Reference

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Previously in our lab, we have reported and quantified gas phase toxicants in the aerosol of vaporized terpenes, specifically methacrolein and benzene a known pulmonary irritant and carcinogen respectively. More recently, we have performed experiments that closely recreate the cannabis extract vaporization experience seen when using cartridge vaporizers. Traditional e-cigarette technology consists of a resistance-heated wire wrapped around a cotton or fiberglass wick. During functioning of an e-cigarette, we can distinguish *wet* and *evaporative* layers of the wick; the wet layer of the wick supplies e-liquid (traditionally a mixture of glycerin and propylene glycol) to the evaporative layer near the coil through capillary action. Cartridge vaporizers for usage with cannabis extract are designed to handle the high viscosity of cannabinoids, which are typically spiked with cannabis-derived terpenes at ~10% for flavor enhancement, and in an attempt to decrease the viscosity. Despite the best effort to enhance delivery of cannabis extract to the evaporative layer through specialized atomizer design, we hypothesized that overheating would occur and lead to the formation of degradation products.

In order to test this hypothesis, we mixed analytical-grade THC with a terpene mixture designed to recreate the scent

of the cannabis at 10% in the final product. This was then introduced into a vape pen atomizer and used for subsequent vapor collection experiments that used a standard puffing parameter (3 s puff period, 30 s puff interval, and 55 mL puff volume). Using a previously published vapor collection and analysis method [1] several degradation products, such as *m*-, *o*-, and *p*-xylene, toluene, isoprene and *p*-cymene were tentatively identified in the gas phase by gas chromatography-mass spectrometry (GCMS). The presence of benzene was confirmed, and its levels quantified at 0.26 ± 0.08 g benzene per puff, or 1.5 ± 0.4 ppm in the inhalation volume. The battery of the vaporizer was set at 4.8 V, the upper limit of the typical operating range of 3.3–4.8 V for all experiments. No carbonyls were detected in the gas phase using a standard method for their analysis. THC delivery to the aerosol was also quantified by high performance liquid chromatography and it was found that each puff delivers 3.5 ± 0.6 mg of THC, and that THC consists of ~86% of the total particulate matter of the inhaled aerosol. Future experiments will attempt to track the origin of the degradation products and their mechanism of formation. Additionally, voltage and puff parameter dependencies on degradant release will be assessed.

Cannabis Horticultural Research in Academia

By Bryant Jones, University of Minnesota

Until recently, cannabis has not been the focus of extensive breeding programs due to the federally enforced Schedule I drug classification associated with the plant. This aspect may have led to cropping and breeding methodologies which follow no standard operating procedures (SOPs). As other areas of scientific study can draw from centralized knowledge upon which respective scientists can progress the discipline, cannabis science should follow suit.

Giving credit to the cannabis plant as a viable therapeutic and medicinally important crop with a sundry of beneficial interactions in the body highlights the necessity for further critical analysis through all scientific scopes, including that of chemical analysis, genomics, and pharmacokinetics. I argue the precursor to all cannabis science begins first in applied horticultural science which purposefully produces a plant significantly different than those found in the wild in order to satiate demand.

Analysis of this crop through the foundations of horticultural science is significant to advancing this crop into public familiarity. The need for university level experiments involving cannabis and peer-reviewed results is immense. In line with this paradigm, my aim is to further fill in this gap of applicable cannabis propagation knowledge and bolster the general horticultural sense surrounding a plant which has become so infamous and enigmatic in society.



Cannabis: the Old and the New

By Allegra Leghissa, Ph.D., Zacariah Hildenbrand, Ph.D., Kevin A. Schug, Ph.D., University of Texas-Arlington

Cannabis is a hot topic in the United States, both in research and in marketing. Daily, many more people are starting to see the medicinal and therapeutic benefits of this plant. This is leading to a more concerted effort in researching its nature and components. Cannabis carries two main classes of bioactive constituents: cannabinoids and terpenes. [1] The former are largely responsible for the therapeutic properties, while the latter confer fragrances and aroma.

Cannabinoids have been widely analyzed, both with gas and liquid chromatography (GC and LC). [1] For a simple potency testing, an ultraviolet detector (Photodiode Array, PDA, for LC, and Vacuum Ultraviolet Detector, VUV, for GC) is sensitive enough to obtain reproducible and accurate results, but when trace analysis is required, especially from complex samples like edibles [2], the coupling with a mass spectrometer (MS) is mandatory. For this purpose, Leghissa et al. created a GC-MS/MS multiple reaction monitoring (MRM) library for the eight predominant cannabinoids, leading to limits of detections of picograms on the chromatography column. [3] The same process was carried out for the two main Δ^9 -THC metabolites, 11-hydroxy-9-THC, and 11-nor-9-carboxy- Δ^9 -THC. [4]

Terpenes, on the other hand, are more challenging to analyze; their similarity in structure makes their separation and analysis difficult. Therefore, the most suitable approach to their characterization by chromatographic techniques has included their confirmation of identification through retention indices. [1] That said, the new VUV detector allows for a fast and easy characterization of isobaric compounds thanks to the highly discriminating gas phase absorbance spectra measured from 120-240 nm. [5,6] This new technique resulted in a fast qualification of the compounds of interest, thanks to the ability of the detector to differentiate among terpenes even post-run, not requiring a long chromatographic separation. [5]

References

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Analytical Expressions for Viscosity of Concentrated Cannabis Oils

By Michael Coffin, Bloom Farms



Physical characteristics of high potency cannabis oil impact formulation and general workability of the materials. Current post-extraction processing methods often involve some type of molecular distillation to produce concentrates in excess of 85% cannabinoids, commonly known as "distillate". As the concentration of THC approaches 99%, the material becomes an amorphous solid, while CBD will readily crystallize at concentrations above 40%.

The highly viscous THC oils prove problematic in "vape pens" as flow to the heating element and atomizer is impaired by the viscosity. Methods to decrease viscosity include the introduction of diluents which include propylene glycol (PG), polyethylene glycol (PEG), plant-derived terpenes,

and cannabis-derived terpenes. The end user's aversion to "chemicals" in inhalable products has resulted in use of terpenes as diluents.

We have studied the effect of terpenes on the viscosity of distillate which follow an exponential decrease in viscosity with percent terpene addition. It has been observed that terpene structure and size have a dramatic impact on viscosity. In simple systems, myrcene (an acyclic monoterpene) has a much greater viscosity decreasing effect than *beta*-caryophyllene (a bicyclic sesquiterpene). The efficacy of viscosity reduction from terpenes extracted from cannabis flowers depends greatly on the specific makeup of extract with generalizations hard to draw. Strangely, 84%+ CBD solids added to THC distillate follow this trend as well.

Automated Image Analysis with ImageJ of Yeast CFUs from Cannabis Flowers

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Cannabis sativa was studied in this report due to the many testing regulations required for its use in the medical and recreational sectors. Cannabis is federally illegal in the United States. However, on a state level, more states have legalized the use of cannabis for medical, recreational or both purposes. Therefore, high-throughput assays are necessary to analyze the yeasts and molds present on cannabis plants to determine if the product is safe for consumer use.

In Colorado, the Marijuana Enforcement Division requires testing for yeast and molds on cannabis flowers, medical cannabis-infused products, water-based, heat/pressure-based, and food-based medical cannabis concentrates. The Code of Colorado Regulations, 1 CCR 212-1 states that levels less than 10,000 colony forming units (CFU) per gram are considered acceptable for total yeast and mold. A comparison of manual and automated counting was studied utilizing the software tool ImageJ for analysis. The automated image analysis methods allowed for quick counting of CFUs from yeast and mold. Counts as high as 500 CFUs, which is considered too numerous to count (TNC), were achieved, demonstrating the ability of this method to function at the upper limit of the plating technique.

The performance of this newly developed automated CFU counting method was demonstrated by comparing manual and automated counts from cannabis flower samples spiked with *Candida albicans* as well as samples that tested positive for yeasts and molds. Fifteen plates of various concentrations of yeasts and molds were studied by 13 different users at two different institutions. The counts from the automated



ImageJ procedure were within 5% of those obtained manually. Based on manual and automated CFU counts of 13 users, no significant difference was found between the automated and manual counts independent of sample dilution. This data indicated that the automated counting protocol could be used as an additional or alternative method, thereby increasing laboratory efficiency by reducing transcription time and counting errors.

An Efficient Method for the Extraction and Isolation of Cannabidiol using Pressurized Liquid Extraction (PLE) and Flash Chromatography

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A variety of techniques for extraction of cannabinoids from cannabis have been developed and reported. The use of pressurized liquid extraction (PLE) for this purpose has not been widely reported. Pressurized liquid extraction was developed as an efficient alternative to Soxhlet extraction, and operates at elevated pressure and can operate at elevated temperatures. In this study, a Thermo Scientific Dionex ASE 350 system was used. The ASE 350 operates at a pressure of about 10,300 kPa and temperatures up to 200°C. Sample cell volumes for the ASE 350 range from 10 mL to 100 mL. Initial work with the PLE method was developed for optimal cannabidiol (CBD) recovery. However, evidence gathered during the development of this method suggests that PLE is effective at extracting a range of cannabinoids found in cannabis. Furthermore, PLE like most extraction techniques will extract a variety of components, phytochemicals in this case, from samples in addition to the target analyte(s), which may require that a post-extraction isolation technique be employed to separate the desired analyte(s) from other extracted components (matrix). For this purpose, flash chromatography was employed.

As part of the PLE method development, the solvent, temperature, solvent volume, and extraction time were optimized. Using water as a solvent under PLE conditions



resulted in decarboxylation of cannabidiolic acid (CBD-A) to CBD in the extraction cell without extraction of the CBD from the sample. Using water in a pre-extraction step, followed by PLE with an organic solvent resulted in greater neutral CBD recovery than extraction using an organic solvent alone (Figure 1). Ultimately ethanol was selected as the solvent for the second PLE step to recover CBD. During method development, temperature was the most important PLE parameter influencing CBD recovery.

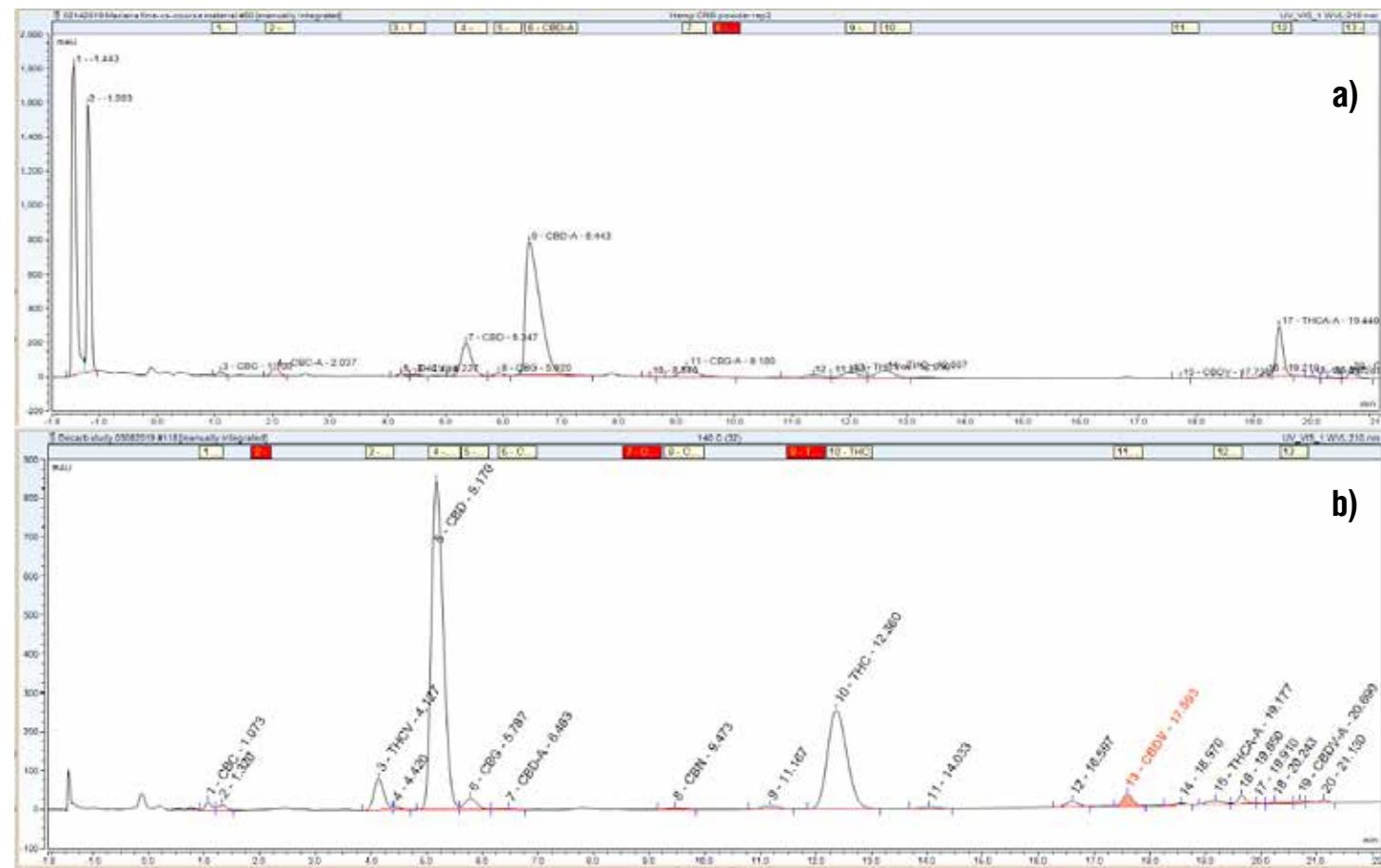


Figure 1: a. HPLC chromatogram of an extract of industrial hemp produced using the United Nations Office of Drug and Crime liquid extraction method. This extract represents the native cannabinoids present in the industrial hemp used in this study. b. HPLC chromatogram of an extract of industrial hemp produced using the dual pressurized liquid extraction method described in this work. The PLE extract demonstrates that acidic cannabinoids, such as CBD-A have undergone decarboxylation during PLE to their neutral form.

While not the focus of this project, a variety of cannabinoids other than CBD were extracted using the PLE method briefly described here. Further, the water pre-extraction step appears effective at decarboxylation of other acidic cannabinoids present in the industrial hemp used during this project. To isolate CBD from the rest of the phytochemicals extracted during PLE, a multi-step flash chromatography method was developed. A two-step flash chromatography process employing silica cartridges with a gradient of hexane and ethyl ether was effective at near isolation of the CBD from other components present in the extract (Figure 2). Further processing select fractions recovered from the silica cartridge through a C-18 cartridge on the flash chromatograph should result in isolation of CBD. Analysis of other fractions collected during isolation of CBD suggested that other cannabinoids in the PLE extract can be effectively isolated using flash chromatography.

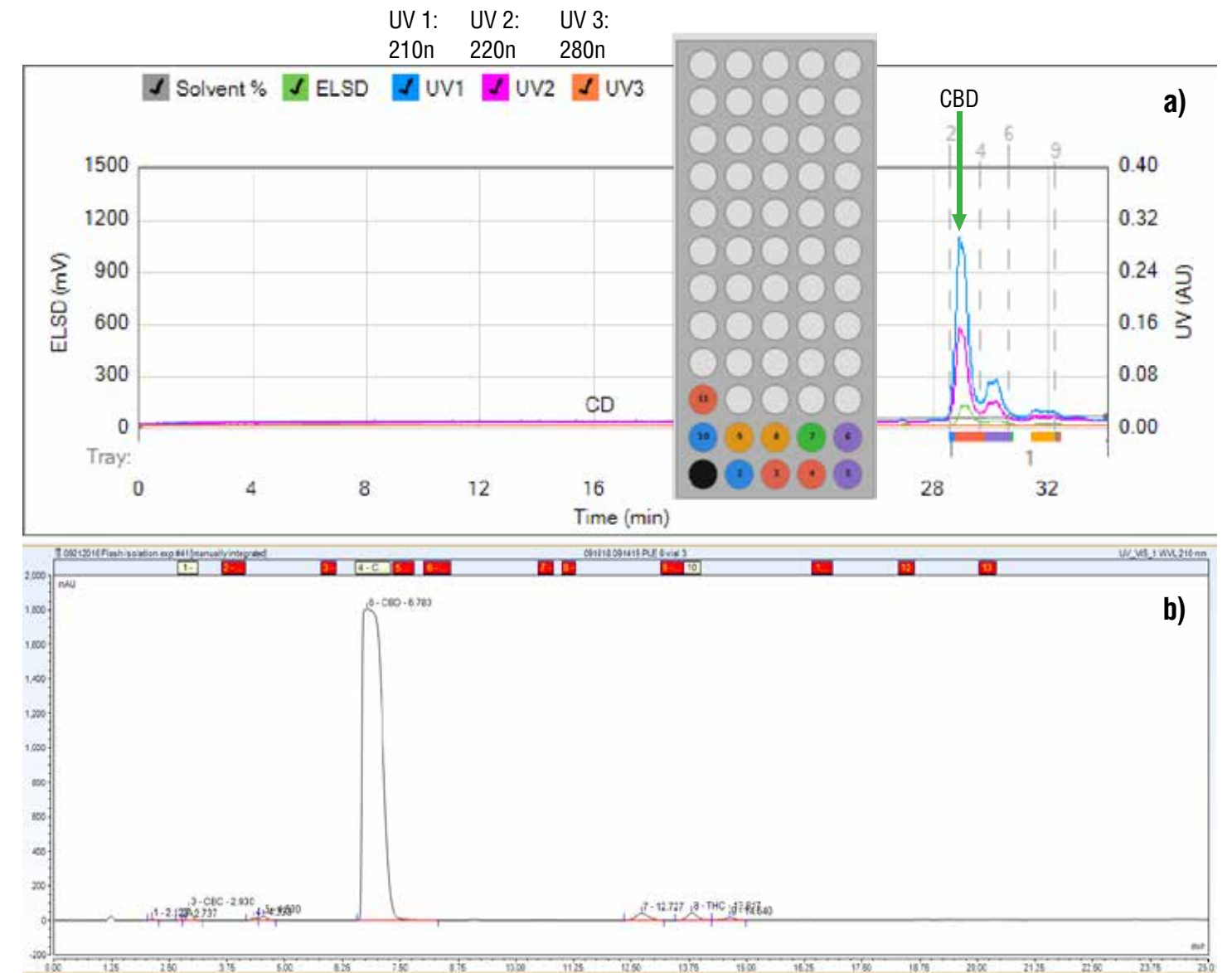


Figure 2: a. Chromatogram from the second flash chromatography step using a silica cartridge. b. HPLC chromatogram of the flash chromatography fraction/peak highlighted by the green arrow in part a of this figure.

Constituents of Cannabis — **Less is More?**

By Stephen Goldman, PhytaTech

Cannabis is a complex plant matrix consisting of many small molecules such as cannabinoids, terpenes, flavonoids, and lipids. Most states in the USA mandate specific testing of cannabinoids and terpenes but none have investigated the composition, or medical benefit, of the lesser known constituents.

As the Laboratory Director for a certified testing facility, I have access to a database of over 130,000 chemotype tests making it easy to screen interesting phenotypes. Methods for anthocyanin screening and lipid (cholesterol) content was presented along with data correlating these markers with the more famous cannabinoid/terpene profiles. Increasing anthocyanin concentrations may compete with cannabinoid production and yield lower potency values.

Microbiological screens to determine “normal” cannabis microbiomes vs “diseased” cannabis microbiomes and the dangerous pathogens to plants and humans that typically appear in “passed” cannabis products in the state of Colorado was presented. Data show that pathogen screening at grows can be a successful tool for growers to maximize yield. Interestingly, some microorganisms can act as biomarkers for other, hard to sample, plant pathogens. These studies are starting to present a typical microbiome for healthy vs. diseased plant production cycles.

Formulation of Customized Cannabis Concentrates in **the Vertical Supply Chain**

By Taylor Trah, Outco

The majority of scientists may find themselves focused on one aspect of this infinitely complex plant and its products during their dive into the rapidly evolving cannabis industry. Some will work as part of a vertically-integrated supply chain where they will have the ability to influence finished product formulations from a seemingly endless array of choices to be made starting even before the production cycle.

Whether you are tasked with selecting the genetics to be cultivated, building the room that plants will be processed in, or creating recipes for edibles, vertical supply chain integration requires all processes to work towards a common goal from the start. In order to take full advantage of this additional degree of organizational control, a thorough understanding of the integrated production practices is necessary.

The production pipeline is often segmented: cultivation, post-harvest, extraction, formulation, packaging, and distribution. However, vertical integration reunites the singular process to work in seamless unison. Select cultivars with confidence, understanding the environment that plants will be subject to and the products that they will yield. Treat pests with caution, selecting biological controls or preventative measures that will not leave residues, paying particular mind to concentration during extraction. Handle harvested plants with great care, be cognizant of the delicate trichome heads that will be separated via wet sieving or the volatile terpenes that will be captured with supercritical carbon dioxide. Do not forget your end-consumer's desire to have both clean, consistent medicine and a diversity of experiences through quality formulations. No matter where your role starts, recognize the importance of an open dialogue amongst all facets of the supply chain to create the best possible user experience.



Standardizing the Entourage Effect: Regulating Terpenes & Flavor Additives for Inhalable Cannabis Products

By Brad Douglass, Ph.D., The Werc Shop

Global regulatory bodies are in the process of developing safety standards for nicotine vapor products, including what additives to permit, prohibit, and otherwise regulate. Some stakeholders are advocating for a one-size-fits approach to *all* vapor products, whether they include nicotine or not.

Although cannabis vapor and oil products share similarities with other vapor modalities, they also exhibit unique differences. Foremost is the polypharmacy of cannabis — often referred to as the Entourage Effect (cf. Ensemble Effects). Terpenes and terpenoids are contributors to these pharmacological, modulating effects and are at least partly responsible for the advent of medical cannabis systems despite the availability of FDA-approved dronabinol: *trans*- Δ^9 -tetrahydrocannabinol. However, many of these same terpene compounds are regulated as flavor additives in food and other products and are at risk of becoming the baby thrown out with the bathwater via nicotine vape flavor regulation.

A cannabis-specific approach to flavor additives, including terpenes, is required. Cannabis vapor and oil products offer a means to standardize and harness the multitudinous effects of whole-plant cannabis. To succeed in ensuring that Ensemble Effects do not find themselves regulated out of manufactured cannabis products altogether, industry must engage in self-regulation to establish standards and to address the potential hazards of inhaled aerosolized constituents.

