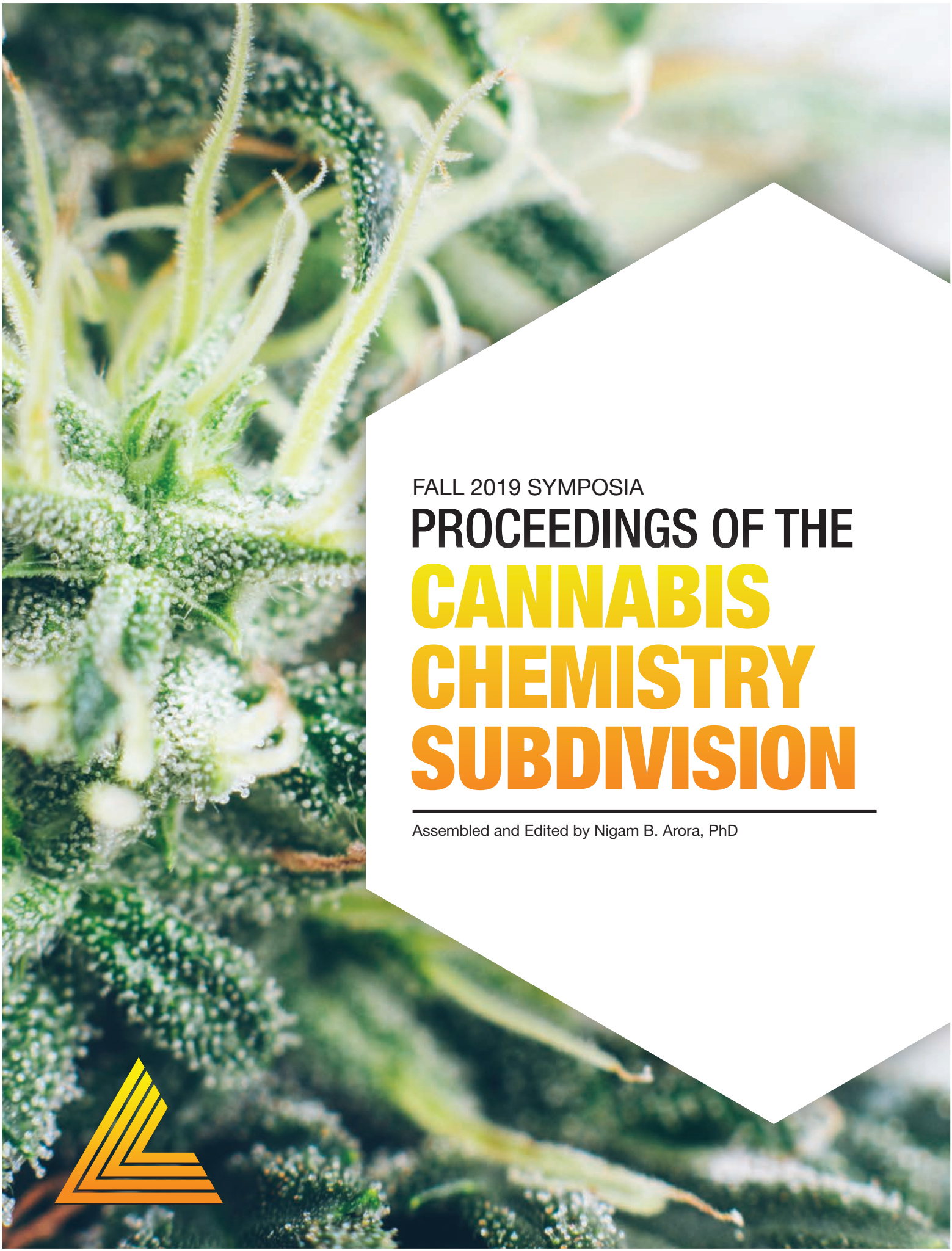

FALL 2019 SYMPOSIA

PROCEEDINGS OF THE CANNABIS CHEMISTRY SUBDIVISION

Assembled and Edited by Nigam B. Arora, PhD

FALL 2019 SYMPOSIA

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CANNABIS CHEMISTRY SUBDIVISION

Contents

Analytical Cannabis

State of CANN

CANN is proud to announce that after more than doubling its membership in 2019, it now stands at 705 members and continues to grow. Housing nine committees essential to CANN's mission of advancing the cannabis enterprise by driving scientific innovation and integrity, CANN is largely dedicated to generating and providing essential resources to its members and greater community.

Two of CANN's premier resources are highlighted below:

The ElSohly Award:

This award is granted for excellence in the field of cannabis science. The first of its kind, this award seeks to recognize leading cannabis scientists and provides a space for them to present their scientific works at the American Chemical Society's Spring National Meeting, providing up to $1500 per awardee. CANN has selected five award winners for 2020 and will be presenting awards this Spring.

CANN Journal Club:

CANN Journal Club is a monthly live webinar where leading cannabis scientists present a recent publication of theirs or a current hot topic in the industry. This is followed by a live question and answer session, providing a platform for the presenter and attendees to directly interact. I encourage you to join the conversation at CANN's next Journal Club session. Follow us on social media to find registration information for our next Journal Club and to stay informed about our various events and resources!

CANN ardently continues down the path set by its initiatives, values, and goals to best serve its many communities and the overarching cannabis enterprise.

Sincerely,

Julia Bramante
Chair of CANN

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Kyle Boyar

Senior Advisory Committee Chair
Russ Phifer

CANN Symposia at ACS San Francisco, August 16-20, 2020

One of the many ways CANN is able to share scientific knowledge and what is happening in this rapidly expanding industry is by hosting symposia at the biannual ACS National Meetings. For those unable to attend, CANN publishes conference proceedings. This proceedings issue covers the presentations we enjoyed from the symposia held at the San Diego meeting in August 2019. Big thanks to CANN's proceedings publishing partner Analytical Cannabis!

We are already planning for the San Francisco National Meeting August 16-20, 2020. The theme of the meeting is "Moving Chemistry from Bench to Market"—a perfect descriptor of the fast-moving cannabis industry. Abstract submissions are open now and have a hard deadline of April 20, 2020.

CANN is honored to be hosting a headliner session co-sponsored by the ACS's Multidisciplinary Program Planning Group to correspond to the overall meeting's "Bench to Market" theme. Our session is entitled "Cannabis, Soil to Oil: How *Cannabis sativa* L. Products go from the Benchtop to the Marketplace" and will feature high-profile leaders in cannabis research and industry.

CANN has hosted many interesting symposia and events in the past few years and we are very excited to continue to expand as the cannabis industry and related sciences grow. Consider submitting an abstract for San Francisco at https://callforpapers.acs.org/sanfrancisco2020/CHAS – for further information email me at amber.wise@gmail.com.

Sincerely

Amber Wise, PhD
Programming Committee Chair - CANN

Symposia Topics for August 2020 in San Francisco:

1. Advancements in Analytical Testing: Compliance, Environmental Concerns, & Consumer Safety
   Organizer – Nigam B. Arora, PhD
2. Advances in Cannabis Extraction & Purification
   Organizers – Ezra Pryor & Amber Wise, PhD
3. Cannabis & the FDA: Preserving Public Trust & Demanding Accountability
   Organizer – Jahan Marcu, PhD
4. Cannabis, Soil to Oil: How *Cannabis sativa* L. Products go from the Benchtop to the Marketplace
   Organizers – Ezra Pryor & Amber Wise, PhD
5. Dual Perspectives: Cannabinoid Dosing & Pharmacokinetics
   Organizer – Nigam B. Arora, PhD
6. Mind the Gap: Specialized Cannabis Formulations
   Organizer – Monica Vialpando, PhD

Abstract Submissions *Open until April 20, 2020!*

Degradant Formation in Cannabis Concentrate Aerosols

Jiries Meehan-Atrash and Robert M. Strongin, PhD
Department of Chemistry, Portland State University

Vaporizing, or vaping, cannabis by several methods have gained popularity among recreational and medical users as a consumption modality that is often purported to be healthier than smoking.[1] It is not currently known how vaporizer delivery of cannabinoids, terpenes and potentially toxic degradation products may affect vulnerable cohorts such as teens, pre-teens, or medical cannabis patients with compromised immune systems.[2]

Previously, our lab has investigated thermal degradation products of terpenes that are present in cannabis extracts when exposed to dabbing conditions.[3] Herein, we report the first time the chemical makeup of aerosol gas phases (GPs) obtained by dabbing and vaping using cartridge vaporizers (CV) have been investigated.[4] CVs have recently been implicated in the e-cigarette or vaping product use associated lung injury (EVALI) outbreak.[5] Pure THC was used for dabbing experiments and a synthetic recreation of distillate, a marijuana derivative commonly used in vaporizers, was used for CV vaping experiments. The synthetic distillate (SND) was created using analytical-grade THC (Cayman Chemical) with a terpene aromatherapy mix from cannabis cultivar Fire OG (Blue River Terpenes) in a ratio of 9:1 THC:terpenes. Dabbing was performed at a commonly used temperature (376 °C or 710 °F), and vaping was performed at three commonly used power levels (Table 1). Adsorption/thermal desorption gas chromatography-mass spectrometry (ATD-GCMS) was used to quantify volatile organic compounds (VOCs) of interest. Quantitative risk assessment (QRA) calculations were applied to estimate cancer and non-cancer risks from dabbing and cartridge vaporizer usage, results of which are compared to risks from smoking cannabis using quantitated cannabis smoke components from the literature.

Cannabinoids such as THC contain a terpene backbone, and it is not surprising that similar volatile products are generated from individually dabbing THC, SND, or terpenes. The significant levels of isoprene seen when dabbing THC alone indicate that the isoprene released may undergo oxidation to release methacrolein and methyl vinyl ketone, a mechanism for which has been described in the context of its atmospheric oxidation.[6-7] The nearly five-fold increase in isoprene released from SND with ~10% terpenes compared to THC alone suggests terpenes release isoprene more readily than THC. Indeed, all identified VOCs form in higher amounts per mg of product consumed when dabbing SND than from THC alone. Other minor components in cannabis extracts (waxes, fatty acids, flavonoids, phenols, etc.) may add to or alter GP degradants from other types of cannabis extracts.

1. *Popova, L.; McDonald, E. A.; Sidhu, S.; Barry, R.; Maruyama Richers, T. A.; Sheon, N. M.; Ling, P. M., Perceived harms and benefits of tobacco, marijuana, and electronic vaporizers among young adults in Colorado: implications for health education and research. Addiction 2017, 112 (1821-1829).*
2. *Meehan-Atrash, J.; Korzun, T.; Ziegler, A., Association of cannabis inhalation with voice disorders. JAMA Otolaryngol Head Neck Surg 2019.*
3. *Meehan-Atrash, J.; Luo, W.; Strongin, R. M., Toxicant Formation in Dabbing: The Terpene Story. ACS Omega 2017, 2 (9), 6112-6117.*
4. *Meehan-Atrash, J.; Luo, W.; McWhirter, K. J.; Strongin, R. M., Aerosol Gas-Phase Components from Cannabis E-Cigarettes and Dabbing: Mechanistic Insight and Quantitative Risk Analysis. ACS Omega 2019, 4 (14), 16111-16120.*
5. *Butt, Y. M.; Smith, M. L.; Tazelaar, H. D.; Laslo, T. V.; Swanson, K. L.; Cecchini, M. J.; Boland, J. M.; Bois, M. C.; Boyum, J. H.; Froemming, A. T.; Khoor, A.; Mira-Avendano, I.; Patel, A.; Larsen, B. T., Pathology of Vaping-Associated Lung Injury. NEJM 2019, 381 (18).*
6. *Atkinson, R.; Arey, J., Atmospheric degradation of volatile organic compounds. Chem Rev 2003, 103 (12), 4605-4638.*
7. *Teng, A. P.; Crounse, J. D.; Wennberg, P. O., Isoprene Peroxy Radical Dynamics. J Am Chem Soc 2017, 139 (15), 5367-5377.*

Component, unit	THC dab	SND dab	Vape 3.2 V	Vape 4.0 V	Vape 4.8 V
Methacrolein, µg	2.7 ± 0.8	12 ± 0.82	5.6 E-3	3.2 E-2	1.9 E-1
Benzene, ng	33 ± 14	360 ± 120	9.9 E-1	2.7	3.6 E+1
Xylenes, µg	0.33 ± 0.20	0.85 ± 0.30	1.0 E-3	1.5 E-2	1.8 E-1
Toluene, µg	0.44 ± 0.22	1.4 ± 0.42	7.0 E-4	1.0 E-2	1.6 E-1
Styrene, ng	0.88 ± 0.72	27 ± 14	9.3 E-2	2.7 E-1	ND*
Ethylbenzene, ng	1.5 ± 0.99	55 ± 30	3.7 E-2	2.5 E-1	2.7
Isoprene, µg	9.6 ± 1.7	44 ± 3.5	3.0 E-2	8.3 E-1	6.0
Other HCs,† µg	5.3 ± 0.7	21 ± 11	4.2 E-2	7.2 E-1	7.9
Total VOCs, ‡ µg	2.0 E+01	7.7 E+01	9.4 E-2	1.5	1.2 E+1

*Table 1: Selected GP components identified in dabbing and CV vaping using ATD-GCMS. For THC and SND dabbing, these were quantified based on duplicate samples and are presented for a single 40 mg dab ± SEM (standard error of the mean). GP components for vaping at 3 voltages are from single puff. *Styrene was not detected in CV vaping at 4.8 V due to overlap of alkenic terpene degradation products. †Non-target hydrocarbons (HCs) not otherwise specified on this table. ‡Total of all VOCs quantified.*

Combined LC-DAD-MS/MS and FT-IR Platform for the Analysis of Cannabis and Cannabis-related Products

Laura Mercolini, PhD
Research Group of Pharmaco-Toxicological Analysis, University of Bologna

Cannabis sativa L. represents one of the most widely used sources of drugs worldwide. Its active compounds are cannabinoids, the main ones being Δ9-tetrahydrocannabinol (THC) to whom the majority of psychoactive effects are attributed, tetrahydrocannabinolic acid (THCA), cannabidiol (CBD), cannabidiolic acid (CBDA) and cannabinol (CBN). Medical and recreational cannabis, sometimes known as hashish or marijuana, are available in multiple markets. Additionally, new products (mainly fiber-type cannabis) have been recently released and are often characterized by low THC levels and high CBD content.

In this study, samples belonging to the categories mentioned above were analyzed, i.e. cannabis products in their commercial (legal) or seized (illegal) forms. The analytical approach involved an effective preliminary screening method without the need for any sample preparation, represented by an attenuated total reflectance (ATR) Fourier transform infrared (FT-IR) methodology. The aim was to identify unknown samples, also quantifying their active compounds after development and validation of proper calibration curves. ATR FT-IR results were then compared to those obtained by means of an advanced LC-DAD-MS/MS method coupled to an originally developed solid-liquid extraction procedure (Figure 1).

This was done by exploiting multiple reaction monitoring (MRM) acquisition mode, through an electrospray ionization (ESI) source, by means of a triple-quadrupole mass analyzer also coupled to a diode array detector (DAD).[1]

Satisfactory validation results were obtained in terms of linearity, precision (RSD% <6.0) and accuracy. Both the developed ATR FT-IR and LC methods have been applied to medical and also to fiber-type plant varieties to monitor the content of non-psychoactive cannabinoids for pharmaceutical and nutraceutical applications (Figure 2).

This combined strategy of ATR FT-IR and LC-DAD-MS/MS represents a versatile, fast and reliable tool to assess cannabinoid levels in regular or illicit samples, for quality control and toxicological or forensic purposes.

Academia and industry can benefit from the fast and easy to perform, but still fully validated and reliable approach presented herein, in order to characterize and discriminate different types of cannabis and related products.

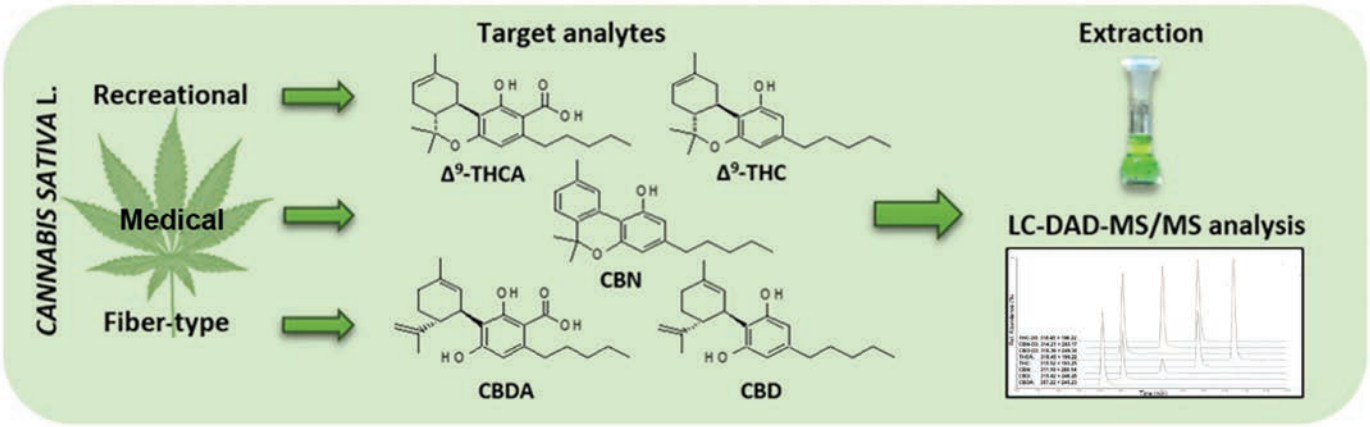


Figure 1: Analytical method based on LC-DAD-MS/MS

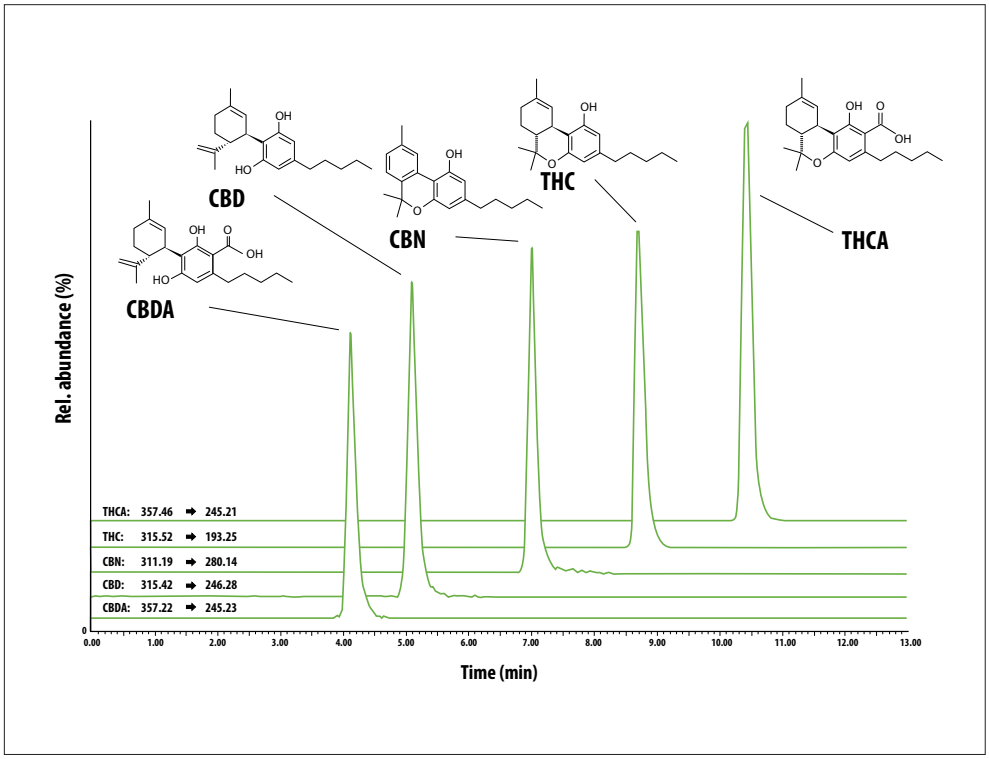


Figure 2: LC-ESI-MS/MS MRM chromatogram of a recreational hashish sample

1. *Protti M, Brighenti V, Battaglia MR, Anceschi L, Pellati F, Mercolini L "Cannabinoids from Cannabis sativa L.: A New Tool Based on HPLC-DAD–MS/MS for a Rational Use in Medicinal Chemistry" ACS Medicinal Chemistry Letters., 2019, Volume 10(4):539-544*

Homogeneity, Formulation, and the Shelf Life of Cannabis-infused Beverage Emulsions

M. Vanden Eynden, PhD[1] and Scott Riefler[2]
(1) Formulaction Inc. & (2) Tarukino

Testing within the cannabis industry involves multiple types of analysis on different kinds of products. While testing on flower and oil extracts are the most common and important, clear testing rules and regulations are not yet in place for all areas of the market and a new wave of testing is likely to commence with edibles becoming increasingly legal in the U.S. and Canada. Cannabis-infused edibles pose a different set of questions and concerns than that of standard inhalation-type consumption (smoking, vaping). Bioavailability and metabolism of active cannabinoids render a much different type of psychoactive effect and typically possess longer onset times. Dosages of materials need to be strictly controlled and the consumers must also be informed through proper packaging, labeling, product descriptions, and overall knowledge of this different method.

Specifically, cannabis beverage emulsions have the propensity to become unstable over long periods of shelf storage. This physical instability can come in a variety of mechanisms such as clarification and sedimentation of pulp and other particles that are purely aesthetic, aside from brand appreciation and quality. More critical is the oiling out effect of the active THC and CBD components, causing potency variants within the beverage. Prediction of this phenomenon is worthwhile as manufacturers should be aware if the entire dosage of their beverages is to be contained in a small oil layer on top of a product in a few months, risking customers to imbibe the entire dosage in a small amount of product.

The results of our study (Figure 1) show that utilizing Static Multiple Light Scattering techniques using the Turbiscan device (Formulaction, France) can be used to quantify and predict such destabilization mechanisms in an effort to provide more advanced and detailed information to formulation scientists. A long-term shelf life study was done with a THC emulsion concentrate at three different storage temperatures that shows the stability of the sample is acceptable at more than 200 days of storage, with no more than 2% of the dosage separating within the still-opaque emulsion. This facile and quantitative technique can provide results that may be tedious or inaccurate through other methods of testing while giving critical data feedback to the formulators in the R&D stage. This will only improve the stability, appearance, and performance of a product over time and allow the entire industry to put quality and safe products on the shelf.

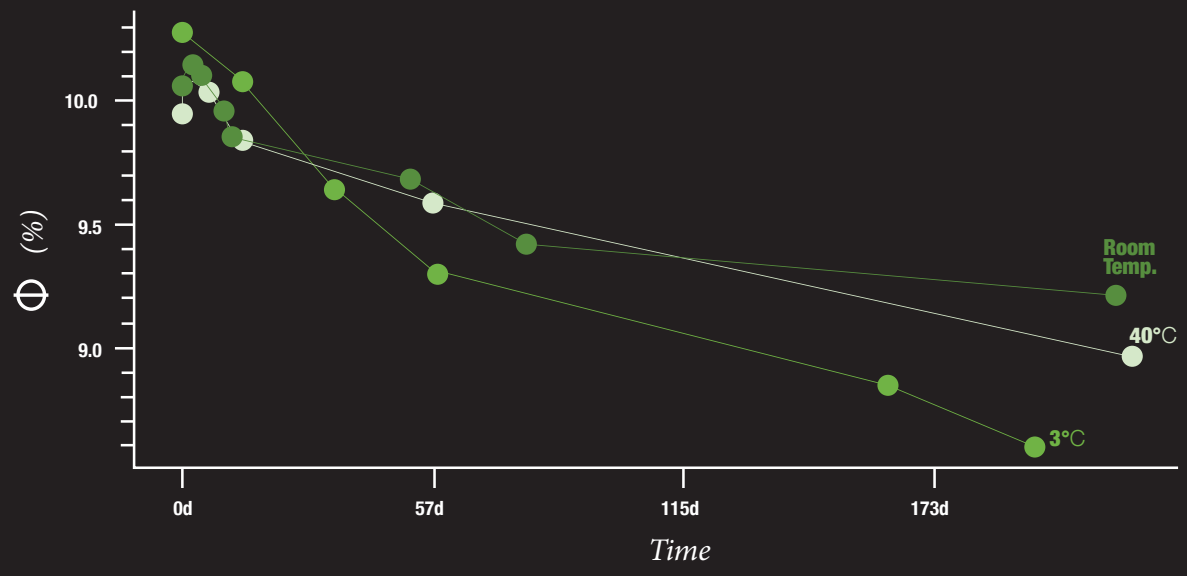


Figure 1: Monitoring of THC emulsion stability over time using Static Multiple Light Scattering

FEE, QbD, HACCP, GMP, OSHA:
Industry Jargon or Hidden Treasures

David Vaillencourt, MSc
The GMP Collective

As the legal cannabis industry continues to evolve and mature, it continues to be stymied by disparate and ever changing regulations, regulators and business owners that are uninformed, poor or non-existent planning, existing stigmas by sectors of the public, and a lack of trust due to the culture of some black market competition, among others. A lack of consistent or accepted terminology further complicates the ability for the industry to mature through established evidence of best practices. Figure 1 depicts the stages of industry based on levels of information and trust. If one dives into the expertise of other similar industries, a clear vision to provide consistent, high quality, and safe products that is financially competitive begins to emerge. The predominant industries are agriculture/farming, consumer packaged goods (CPGs), dietary supplements and natural health products, pharmaceutical, and food production.

Incorporating concepts of risk management, quality by design (QbD) principles, and project and product management best practices that have been developed in parallel industries can provide new businesses significant savings through operational efficiencies. By investing time and money and performing due diligence in the planning stages, one can avoid costly changes during later stages of commissioning and operation (Figure 2). This is known in construction projects as front end engineering (FEE). For food and edible products, Hazard Analysis and Critical Control Points (HACCP) plans have been instrumental in minimizing food related illnesses and deaths while mass producing food that is shipped globally. In-process testing, qualification of equipment, and validation of processes are all part of good manufacturing practices (GMPs) which are required to operate in the pharmaceutical industry. As space where products must conform to tight specifications in order to provide therapeutic value which is proven through a series of clinical trials.

Regulatory bodies including the FDA and OSHA will ultimately have their say in the degree of regulation in our industry. The cost of compliance can be offset through investments in innovation and adoption of best practices from analogous industries that allow companies to front load their costs and remain competitive and resilient in the long-term. At the end of the day, both consumers and businesses alike will be rewarded through benefits in efficiencies of mass production through reduction in production costs per unit, as well as consistent quality and product safety.

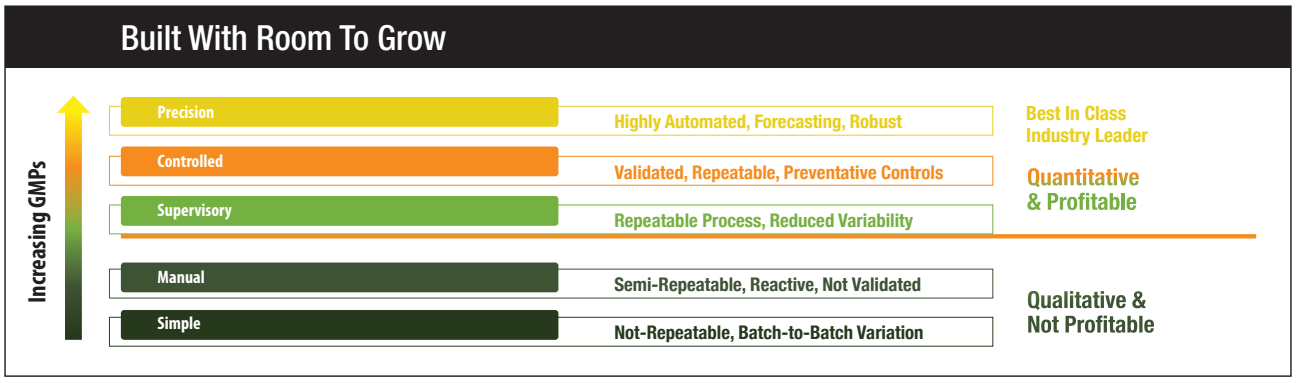


Figure 1: Stages of industry based on levels of information and trust

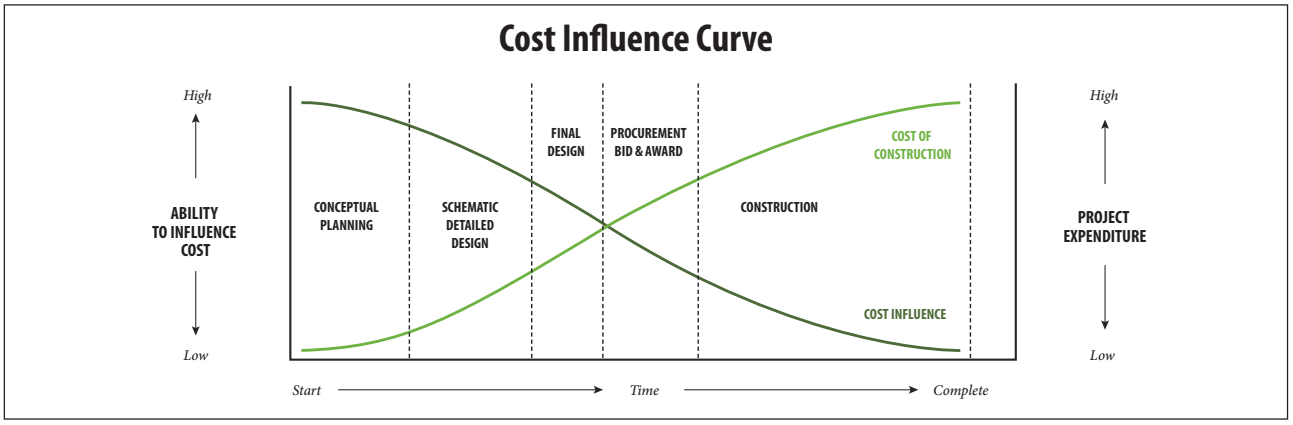


Figure 2: General project evolution in relation to costs

Raising the Bar for Cannabis Extraction Methods: Introducing a Novel, Safe, Efficient, and Environmentally Friendly Approach to Extracting High Quality Cannabis Resins

Tyrell R. Towle, PhD
Easy Extracts, LLC

Cannabis extracts have become exceedingly popular around the world. They can be consumed orally, through infusion into edible products, or by inhalation. Historically, the hydrocarbon gas butane has been among the most popular extraction solvent. Use of this solvent began due to its availability and ease of use. A glass tube and a coffee filter are essentially all that is needed in its simplest manifestation, a technique dubbed open-blast. However, this is also an extremely dangerous way to extract cannabis as butane is highly flammable. Many apartments, houses, and other structures have exploded due to improper handling of butane during illegal extraction operations. More recently, particularly in legal marketplaces, closed-loop butane has gained favor over open-blast.

Due to the hazards of butane extractions of cannabis, alternative methods have been developed and have also become mainstream. These include subcritical and supercritical carbon dioxide, ethanol, and heat press (rosin) extractions. Each of these has its own advantages and disadvantages. For example, supercritical carbon dioxide extractions do not leave any residual solvents, but they have long extraction times, high energy costs, and are expensive to scale up. For ethanol, the advantages are the ability to skip winterization (if ethanol is cooled to a low enough temperature during extraction) and its scalability. However, cooling ethanol to cold enough temperatures to prevent wax extraction is hazardous and expensive. Cold ethanol extractions can be performed at temperatures as low as -80°C which can damage exposed skin on contact. On the other hand, ethanol extractions performed at higher temperatures result in inferior extracts with high levels of lipids and chlorophyll.

Research on new cannabis extraction methods was performed to address the limitations and drawbacks of these commonly used extraction methods, particularly the safety hazards surrounding butane extractions and extracts. Ultimately, ethyl acetate has proven to be a safe, efficient, and environmentally friendly approach to extracting high quality cannabis resins. Extracting cannabis with ethyl acetate is protected under US Patent #9,937,218, currently owned by Easy Extracts, LLC. Ethyl acetate is a naturally occurring compound commonly found in fruit and wine. It is FDA approved for use in food as a flavor/fragrance enhancer and solvent. Ethyl acetate is also used in perfumery, non-acetone nail polish removers, coffee decaffeination, and other applications. Ethyl acetate has a similar safety profile to ethanol in an industrial setting and poses no health risk to consumers in residual levels. Like ethanol, the main hazard of ethyl acetate is its flammability. Ethyl acetate also happens to be an excellent solvent for selectively extracting cannabinoids and terpenes at room temperature (Figure 1). With proper starting material, cannabinoid plus terpene purities have reached as high as 98% in crude extract. This is far above the average level of purity achieved by any competing method.

Using ethyl acetate extraction versus the popular supercritical carbon dioxide extraction leads to higher throughput, the ability to skip days worth of pre-processing and post-processing (no need to dry in a vacuum oven, decarboxylate prior to extraction, grind finely, or winterize), lower cost of capital investment, and lower cost per gram of extract produced. Ethyl acetate is recovered about twice as fast as ethanol during rotary evaporation or other distillations due to its higher vapor pressure. It is also suitable for re-use in future extractions when water removal is performed immediately after the extraction and before solvent recovery. Additionally, the entire process is performed at room temperature under atmospheric pressure and the final extract has only touched food grade materials.

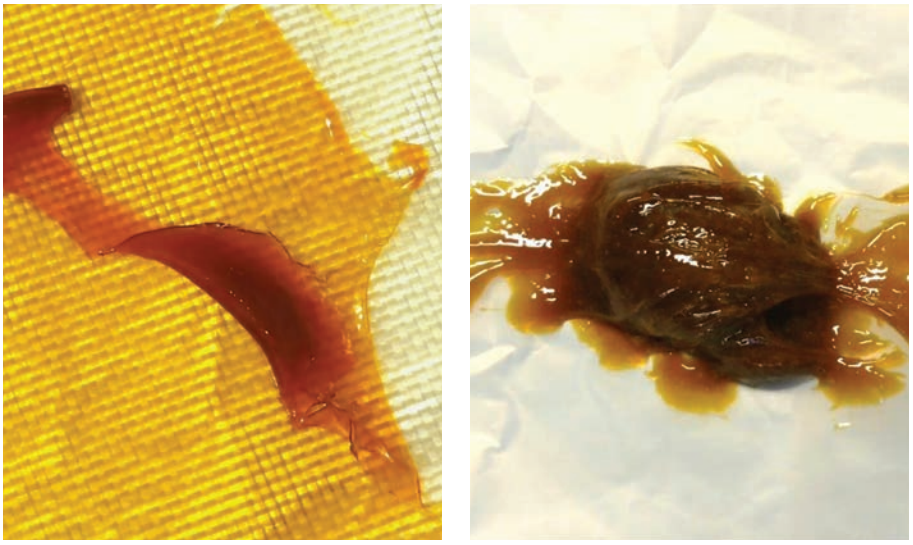


Figure 1: Shatter made from B grade cannabis flower (left) and extract made from extremely fresh A grade cannabis flower (right) resulting in a sugary, terpene rich concentrate that tested at 81% total cannabinoids and 16% total terpenes.

HPLC-UV Method Development for the Baseline Resolution of 17 Cannabinoids

Edward Franklin, PhD and Melissa Wilcox
Regis Technologies, Inc

As relevant agencies continue to formulate regulatory frameworks around medical and recreational cannabis programs, it is important for testing laboratories to have robust analytical methods in place for the determination and quantitation of cannabinoids and potential contaminants. To that end, High Pressure Liquid Chromatography (HPLC) with ultraviolet (UV) or mass spectrometric (MS) detection has emerged as the preferred technique for cannabis potency analysis. Herein, HPLC-UV method development for the baseline resolution of 17 cannabinoids is described with particular attention paid to resolution of all 17 cannabinoids and analysis speed.

Analytical reference cannabinoid standards (1 mg/mL) were combined to a final concentration of approximately 59 µg/mL of cannabidivarinic acid (CBDVA), cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabidiol (CBD), tetrahydrocannabivarin (THCV), tetrahydrocannabivarinic acid (THCVA), cannabinol (CBN), cannabinolic acid (CBNA), exo-tetrahydrocannabinol (exo-THC), Δ9-tetrahydrocannabinol (Δ9-THC), Δ8-tetrahydrocannabinol (Δ8-THC), cannabicyclol (CBL), cannabichromene (CBC), tetrahydrocannabinolic acid A (THCA-A), and cannabichromenic acid (CBCA). Method development was performed using an Evoke C18, 15 cm x 4.6 mm column packed with 3 µm particles from Regis Technologies, Inc. Reversed-phase conditions were screened using both acetonitrile and methanol as organic modifiers in both isocratic and gradient modes, and the addition of formic acid to mobile phases was important for achieving good peak shape of carboxylated cannabinoids, such as CBCA and CBNA. Chromatographic conditions were found that provided baseline resolution for most cannabinoids, but still resulted in some coelutions. The addition of ammonium formate to the mobile phase provided a means to affect the retention of acidic compounds relative to the neutral species, and an optimized buffer concentration resulted in baseline separation of the 17 cannabinoids in the test mixture (Figure 1). The method was subsequently transferred to a 10 cm x 2.1 mm column packed with 1.8 µm particles, and runtime was effectively halved.

Peak IDs:

1.	CBDVA	7.	THCV	13.	Δ8-THC
2.	CBDV	8.	THCVA	14.	CBL
3.	CBDA	9.	CBN	15.	CBC
4.	CBGA	10.	CBNA	16.	THCA-A
5.	CBG	11.	exo-THC	17.	CBCA
6.	CBD	12.	Δ9-THC		

Column:	Evoke C18; 15 cm x 4.6 mm; 3µm	
Mobile phase A:	H_2O + 0.1% HCO_2H + 7.5 mM NH_4HCO_2	
Mobile phase B:	CH_3CN + 0.1% HCO_2H	
Flow:	2.0 mL/min	
Gradient:	Time (min.)	%B
	0.00	75
	15.00	90
Oven Temp:	30°C	
Detection:	228 nm	

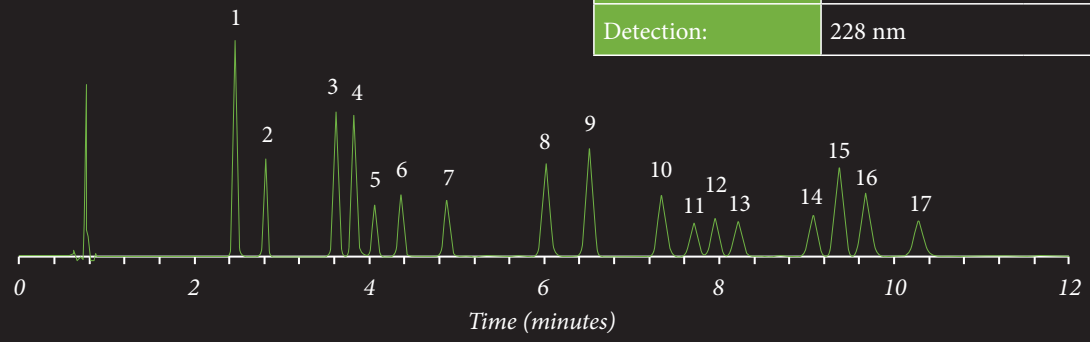


Figure 1: HPLC-UV chromatogram (bottom) and conditions (top right) for the baseline resolution of 17 cannabinoids (top left)

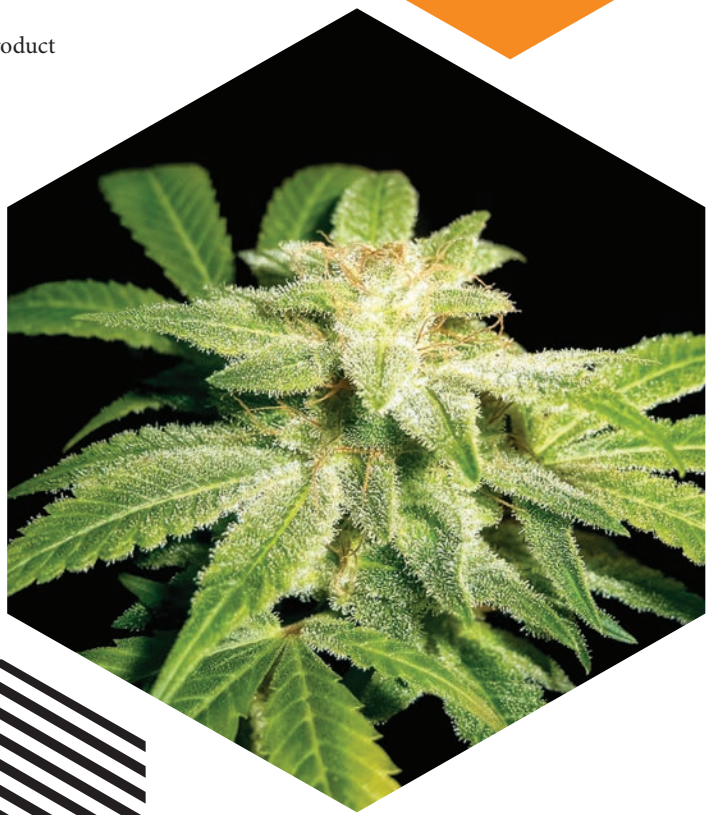
Cannabis Product Critical Safety Attributes, Critical Quality Attributes, and Good Distribution Practice

Andrew Samann
Orion GMP Solutions

Critical quality attributes are economically useful specifications agreed upon across cannabis products in the supply chain. Distribution of cannabis flower products from Canada to Germany, for example, exposes the drug product to varying environmental conditions that can negatively affect usable shelf-life over time. Some specifications, such as water activity and water content of cannabis flower, can adversely influence the stability of a product, and become a root cause of non-conformance leading to batch rejection at the port of entry.

In order to properly evaluate transportation, delivery, and storage risks, adequate attention to the factors that affect product stability must be understood. Stability studies specific to product packaging and temperature cycles encountered during stages of distribution and storage evaluate how to properly prepare and handle cannabis throughout the post-processing product lifecycle.

Nomenclature is important for describing a state of control over product stability areas such as: loss or increase in active ingredients (or potency), degradation products (e.g. THC to CBN), change in functional relevance or bioavailability of products, increase in microbiological bioburden, and/or loss of package integrity. In most cases, these factors of stability can be measured in advance through on-going stability programs with validated analytical methods. Case by case evaluation of specific product stability establishes reasonable shelf life and guides temperature and humidity monitoring/controls during transportation and storage.



How to Create a Consistent Water Soluble Cannabinoid Bulk Ingredient

Harold Han, PhD
Vertosa

What does the consumer look for in a cannabis drink? It boils down to two words: consistency and predictability.

Consistency points to ensuring precise potency. If the label says 10 mg of THC or CBD per bottle, consumers should be able to trust that this amount will remain the same no matter what state they're purchasing it in and no matter how long the product has been sitting on the shelf. Consistency requires the water compatible cannabinoids to be compatible with the beverage base, manufacturing device, pasteurization process, and packaging material. It also requires the potency of cannabinoids to be tested accurately using analytical methods (Table 1). A good water compatible cannabinoid provider should ensure those requirements are resolved.

Predictability points to bio-availability – or how quickly the consumer will feel the effects of the cannabinoids. If someone drinks a beverage containing 10mg of cannabinoids, what amount will their body absorb and how quickly? Understanding the pharmacokinetics is very important for determining the bioavailability of cannabis beverages. Only by understanding and controlling consistency can unpredictability be eliminated (Figure 1). By focusing on both consistency and predictability, cannabis manufacturers and brands can build confidence with consumers and make cannabis beverages a product that the mainstream masses can enjoy – and trust.

At Vertosa, we focus on understanding and delivering the best water compatible cannabinoid technology. We do not believe in a one size fit all approach – rather, we develop different and distinct emulsion solutions to fit the goals and needs of our partners' products. For example, the cannabinoid emulsion that we formulate for a canned cold brew coffee may not work for a bottle of red wine. We always start by focusing on the final product and what each client wants to achieve in their beverage or topical, and then work backwards to find the best emulsion solution.

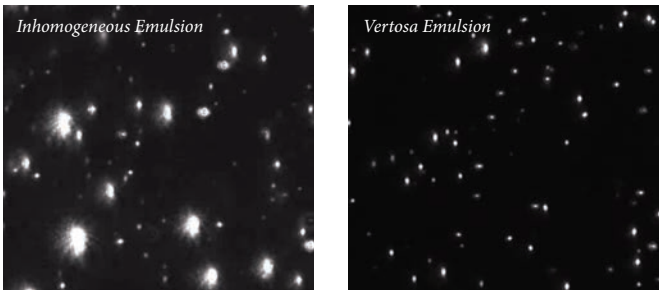


Figure 1: Comparing Vertosa's emulsion droplet size and distribution (right) with a third party's emulsion (left). Here we use light scattering to visualize emulsion droplet size and distribution. A poorly designed or processed emulsion (left) will show high polydispersity of droplet size, which may cause potential problems of emulsion instability after infusing into a beverage.

Sample	Repeats	Target Potency (mg/g)	Tested Potency (mg/g)	% Difference	
1 x HH221	#1	20.000	19.938	0.31%	Raw Emulsion
	#2		19.932	0.34%	
	#3		19.944	0.28%	
10 x HH221	#1	2.000	1.991	0.45%	Topical
	#2		1.989	0.55%	
	#3		1.986	0.70%	
100 x HH221	#1	0.200	0.196	1.85%	High Dose Beverage
	#2		0.193	3.50%	
	#3		0.197	1.50%	
500 x HH221	#1	0.040	0.039	2.50%	Low Dose Beverage
	#2		0.038	5.00%	
	#3		0.038	5.00%	

Table 1: All cannabis infused products have to ensure accuracy and consistency in potency. Lab testing is the key. Receiving reliable results can be challenging because each lab typically uses their own testing methods. To address this issue and help our clients build confidence in their products, Vertosa offers our own SOP for potency tests. This SOP is developed to work with our emulsions, which offer high consistency and accuracy. The testing results are shown in the table above.

Fundamental Research for In-process Analytics to Control Cannabis Formulations

Markus Roggen, PhD[1] and Glenn Sammis, PhD[2]

(1) Complex Biotech Discovery Ventures (CBDV) & (2) Department of Chemistry, University of British Columbia

The cannabis industry has come a long way since legalization of recreational use in Colorado and Washington in 2012[1,2] and Canada in 2018.[3] Production has increased[4], new product types have hit the market,[5] and even established players from other industries have entered the space.[6] Nevertheless, cannabis production continues being an under-researched field with many knowledge gaps. For example, scientists have yet to catch up with hyped products, and fully understand the effects of CBD.[7] Even more fundamental is the incomplete tracking of cannabis metabolites in the plants and processed goods.[7] Currently, licensed analytical laboratories offer tests for around ten cannabinoids and about 50 terpenes.

We have started tracking known metabolites of the cannabis plant and degradation products commonly observed throughout the cannabis production pipeline. Currently we recognize 24 different compound categories and around 800 individual compounds. We have built a database that tracks dozens of physical and chemical data points about each compound from exact mass to boiling point, from chemical structure to fragmentation pattern in MS. While it is not complete, we are diligently working on filling in those gaps and we do share the database with fellow researchers to support their work. In the future, we hope to be able to share the database with the public as well.

Knowing what to look for helps us greatly in building the right tools to monitor production processes. The first in-process monitoring analytics we developed was an FT-IT-ATR method in collaboration with PerkinElmer (Figure 1). We already described this technology at length in another publications.[8] We are currently working on additional monitoring tools and hope to present on those at upcoming American Chemical Society events.

Having the datasets to identify and the tools to track a plethora of cannabis compounds, we turned to a third pillar of CBDV's research expertise, big-data analytics for extraction optimization. In the past for extraction parameters through Design of Experiment (DoE). We recently expanded on this by incorporating analytics of historical data (Figure 2). We can now work with extraction companies to analyze their past data to spot inefficiencies (Figure 3). Additionally, we can use our large datasets to fine tune a host of extraction parameters to react to a variety of factors, such as water content for input material or desired product makeup (Figure 4).

We look forward to further expanding on our in-line monitoring tools and data analytics to bring cannabis production forward. We hope that many of you will join us in these efforts.

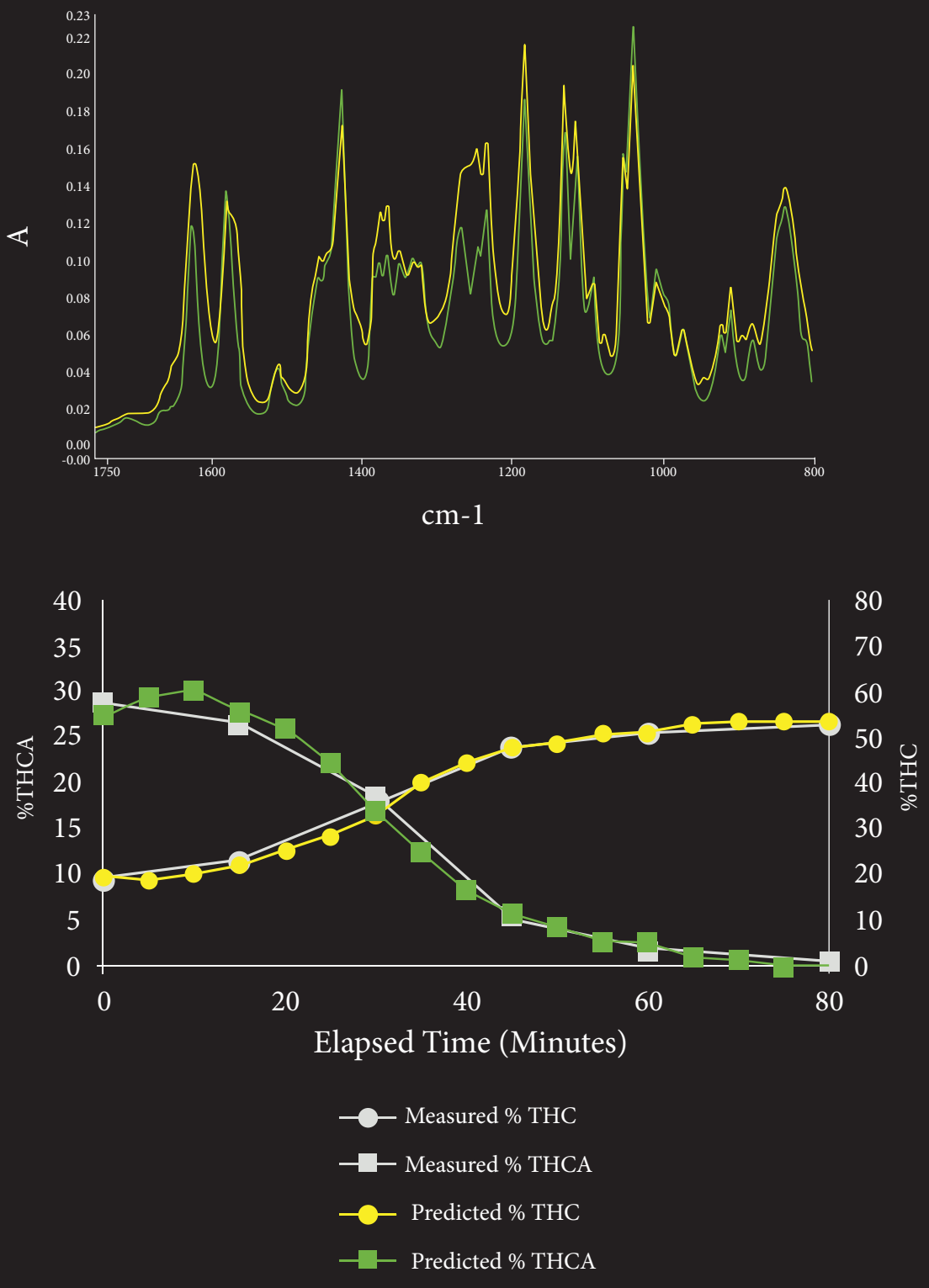


Figure 1: Mid-IR Spectra and Fit of IR Data Example spectra of a cannabis extract throughout the course of decarboxylation by the application of heat (upper panel). Cannabinoid concentration plots over the course of the decarboxyalation reactions. The IR model tracks very well with the LC reference data (lower panel).

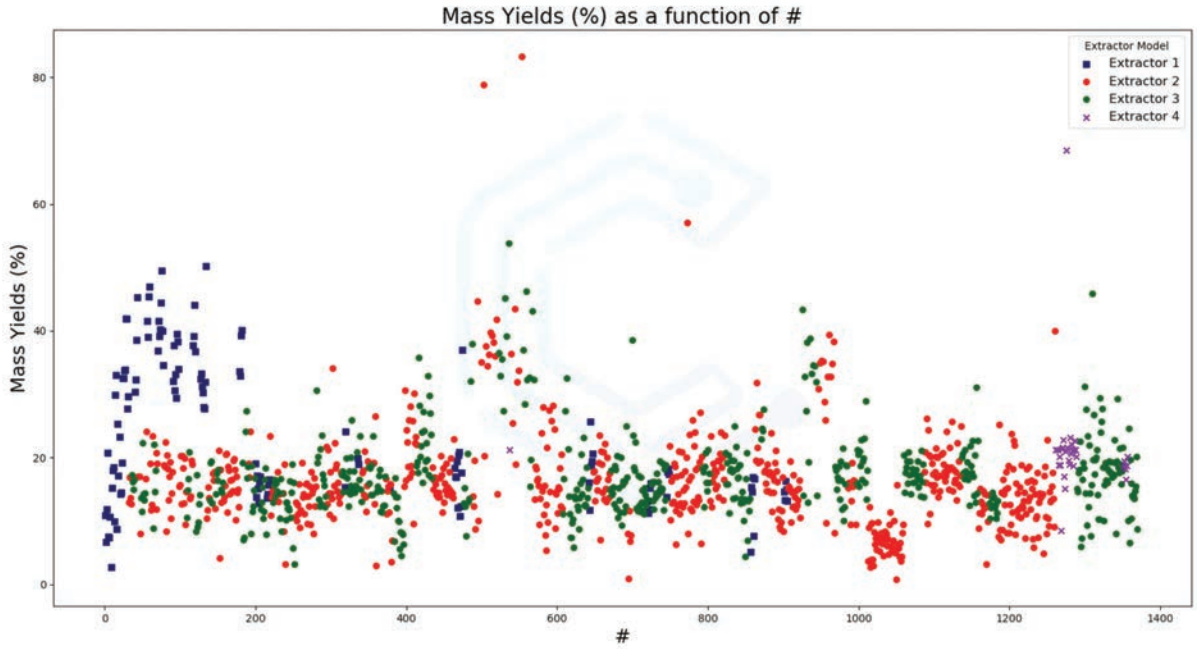


Figure 2: A selection of extraction run datasets. Every individual extraction run is plotted along the x-axis vs. its yield on the y-axis. The data set included four different extractor models, marked by different colors.

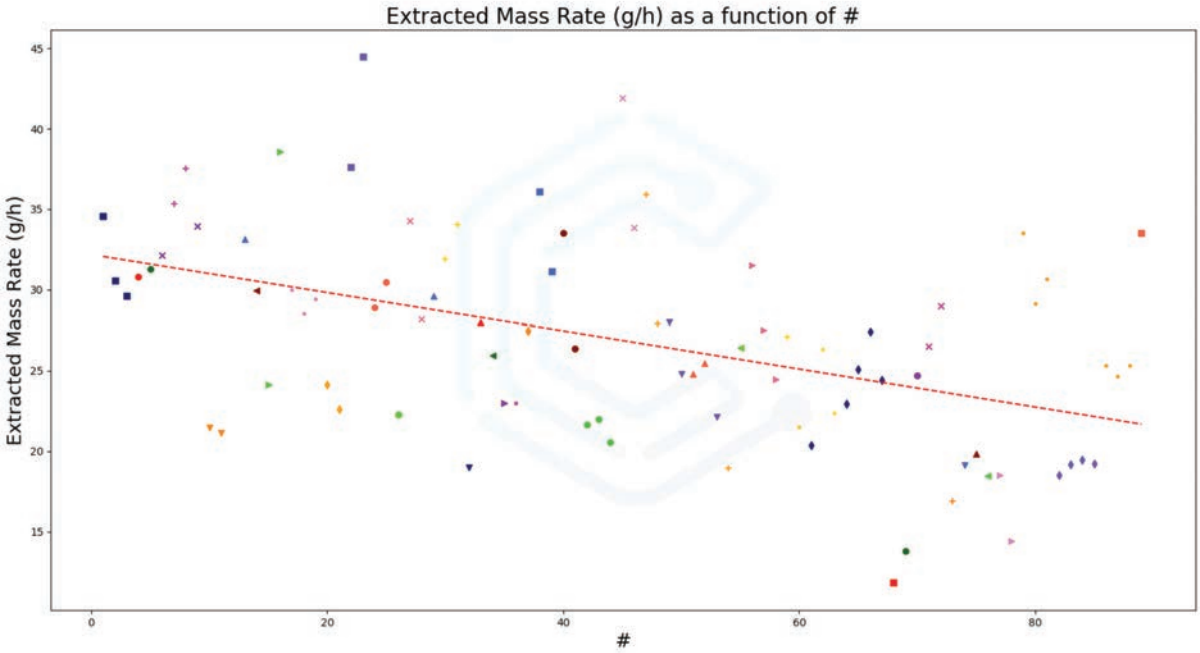
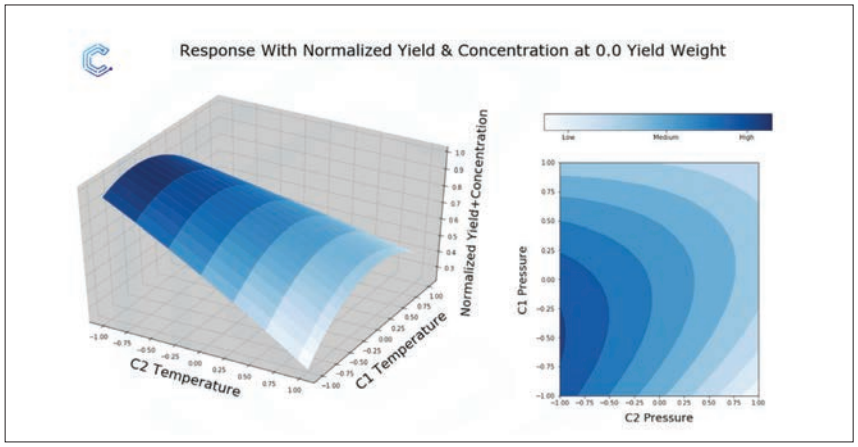
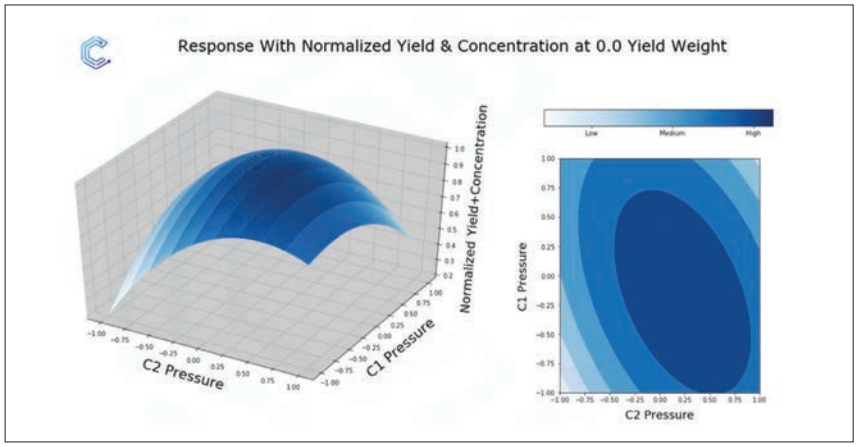
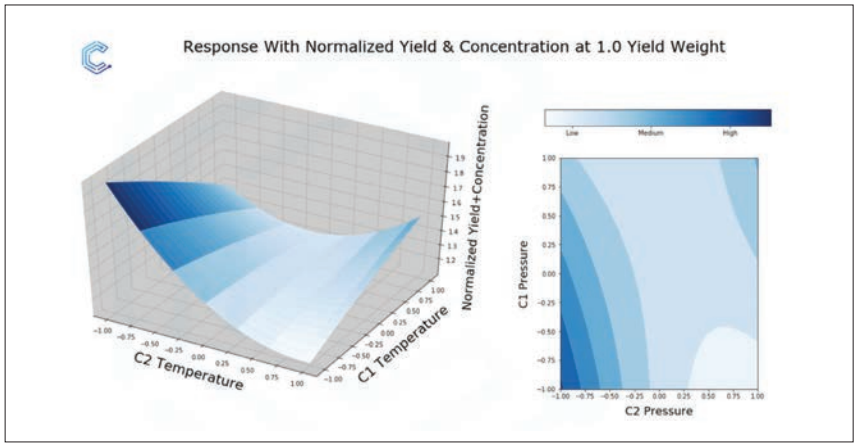


Figure 3: Extraction run data for one extractor model plotted as run number vs. mass yield in grams per hour. This overcomes input variabilities of mass input and run length to clearly show a reduction in extraction efficiency. The different colors identify different cannabis cultivars that were extracted.







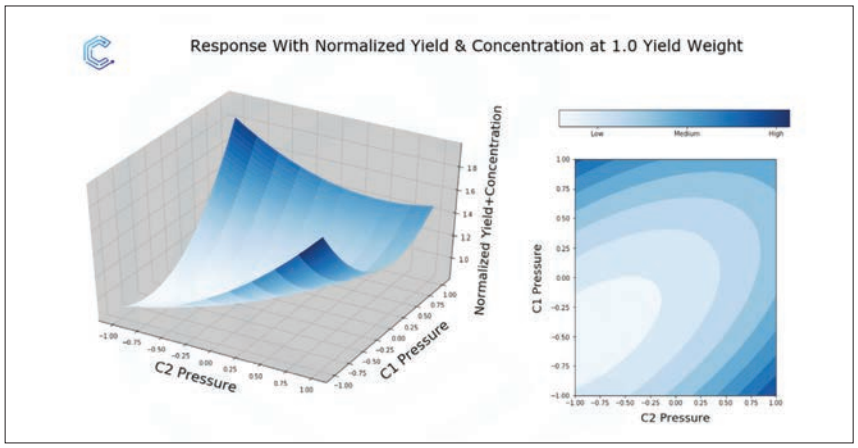


Figure 4: Response surfaces showing the effects for both temperature and pressure in two separator vessels on the product outcome, either optimized for cannabinoid yield or concentration.

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Scaling Production for a GMP Cannabis Facility

Stefanie Maletich
MedPharm Holdings

Creating a new product can be a daunting task. By dividing the process into proof of concept, bench scale, and full production scales of manufacturing, it is easy to achieve any production goal.

At the proof of concept stage, the product design should include active ingredients (cannabinoids, terpenes, caffeine, etc.) and dosage form (topical, pill/capsule, etc.). The goal is to create something tangible for evaluation. Depending on the product, this can be as small as a single dose or unit. Aim for a low investment and try different options. Experiment with flavors, colors, ingredients, and processes. If possible, reference another industry, such as food, pharmaceutical, or cosmetic. Use current equipment or purchase small scale commercial equipment or tools to create the product. For GMP environments, consider the impact of allergen ingredients early; avoid them if possible, or plan to mitigate cross-contamination. For cannabis products, it's critical to consider local laws and regulations. Don't scale up a product that works just to find that it's in violation of a local rule for dosage, ingredients, or other factors. Cannabis can drastically affect formulations, whether it's distillate, oil, isolate, or flower, so be sure to create the product with and without cannabis. This will ensure a successful active product.

Bench scale is the stage to create a repeatable, consistent product. This is typically the most time-consuming stage of production. Develop necessary processes such as equipment, ingredients, and cleaning. Front-load on troubleshooting to make the transition to production scale easy. Investigate suppliers for ingredients and equipment, identifying bottlenecks and limiting reagents. Explore R&D scale or bench scale equipment for options that can grow to meet larger production demands. Invest time in equipment operation and set quality acceptance criteria to be ready for production scale. For cannabis products, it's critical to obtain user feedback, and this can be the best time to do it. Proof of concept products may not be as consistent or ready for feedback, and changes are difficult to implement at production scale. Since cannabis can affect people differently, it's important to get multiple people to try the product and assess its performance.

Production scale is the stage to ramp up quantities and get as much product as possible within the desired scale of production. At this point, ingredients, equipment parameters, and other components should be set, and production can commence easily. Purchase and store large quantities of ingredients from approved suppliers. Invest in larger, faster equipment, or push the limits of bench scale equipment. Observe equipment operation during continuous use; sometimes machines will exhibit new issues when running for 3 hours of production scale vs. 15 minutes in bench scale. Maintain quality acceptance/quality assurance criteria to ensure consistent products from batch to batch, and all necessary documentation including SOPs, batch logs, cleaning logs, etc. This is especially important for GMP facilities. All the hard work from earlier stages will pay off in full production batches of new product.

We use this process when implementing new products at MedPharm for our Batch, Become, and Aliviar brands. Taking into consideration each product's unique characteristics, especially for cannabis products, during production will ensure a successful operation and a quality product.

AuditProHBX, A Tool for Regulatory Compliance in a Field That Needs Modern Tools to Flourish

Ezra Pryor
Heidolph North America

The cannabis industry presents a myriad of new challenges to operators, regulators and consumers. Often the magnitude of these challenges is not understood until insufficient systems are already in place. An excellent example is the regulatory oversight for the states of California and Oregon, just to name a few. Oregon in 2018 was only able to inspect less than 10% of licensed facilities, with a significant number of those facilities coming up as deficient or even at risk of closure. California is another example of a state whose cannabis regulatory agency is failing to be proactive about regulatory oversight. Not only is this a shortsighted strategy but it also lends itself to be a tool of malevolence, as competitors can use the complaint hotline to attack their neighbors.

The solution to this issue is to employ resources that make these challenges more manageable. One such resource is AuditProHBX. This tool takes the burdensome task of inspecting a facility and makes it much easier and faster. With an easy to use tablet application, this software comes with state and local regulations preloaded. What is more, the software keeps track of each issue so that they can be addressed, and liability can be tracked. Not only have growers, extractors, and distributors been able to make use of it, state regulators have also been able to use it to facilitate their regulatory oversight program. Delaware, for example, is using AuditProHBX to inspect the licensed facilities in their state, making the task quicker, more effective, and supported by instant generation of reports.

The rapid growth of the cannabis industry presents many modern and unexpected challenges. In order to address these challenges, we also need modern solutions. AuditProHBX is an example of the inspection process improved with modern technology. With the use of such technologies on both the parts of the operators and the regulators, the cannabis industry can overcome these challenges and flourish.

Extraction Methods and Their Effect on Terpene Retention

Gene Ray
Garden Remedies Inc.

The range of phytochemicals from cannabis is vast and contains several medicinal benefits and effects. There are over 400 identified compounds in cannabis of which 113 are grouped as phytocannabinoids and at least 140 are terpenes[1] that are markers for certain effects.[2] Isolating such compounds can be beneficial to the research world and the cannabis industry. Separating these phytochemicals from one another to experiment with specific formulations can create useful data for development of products. Separating compounds via extraction or distillation can also create a process that is continuous, reproducible and undisruptive.

Terpenes are volatile, yet they are essential organic compounds that come into consideration during both the extraction and distillation processes. These compounds are ubiquitous in nature and can be found in juniper, ginger, black pepper, citrus rinds, and mangoes, to name a few. Terpenes are responsible for the unique flavor and synergistic effects of cannabinoids. Their unique effects depend on the overall composition of the individual terpenes and additional phytochemicals.[3] Due to their relatively small molecular size, weak intramolecular forces and heat sensitivity, terpenes are volatile by nature[4] and regulated conditions are needed to remove and preserve them.[5]

Filtering terpenes along with phenols, chlorophyll, fats and waxes via extraction prior to refinement is ideal in order to remove and preserve the individuality of a strain and to mitigate post extraction problems.[6]

There are three major types of solvent extractions: supercritical CO_2, hydrocarbon, and alcohol. All of these extraction methods have different capabilities and can produce an array of concentrates. However, they follow the same principle, which is to increase extraction efficiency via time, temperature, pressure, polarity and/or agitation.

Preparation via filtration, solvent removal and decarboxylation allows the concentration of targeted cannabinoids to increase with less energetic competition during processing. Residual terpenes in crude extract reduces as the extract is properly prepared for refinement via molecular distillation. An over-abundance of terpenes in extracts can cause disruptive distillation which includes fluctuations in pressure, problematic vacuum operations and irreproducible processing conditions. Proper refinement of crude extracts can capture the specific cannabinoids in the form of concentrated refined oil, free of terpenes.[9] Because terpenes are removed prior to distillation, the marketed effects of the terpenes in the plant are often not present in an extract of the plant. Preserving individual terpene extracts can contribute to future innovation in research that can educate the general public and create properly formulated products.

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Establishing Robust Quality-based Systems for Cannabis Production

Karen Peña
The GMP Collective

Quality is a word that is used frequently to describe cannabis products yet rarely is that "quality" defined in any specific manner that is consistent. This comes as no surprise in the US where cannabis regulations heavily focus on security and not quality. Currently, the major distinction between compliance-based systems and quality-based systems is that compliance-based systems only require final product testing. Quality based systems on the other hand, require final product testing, in-process testing, in-process controls, risk assessments, data collection/trending, process monitoring, process/equipment validation, continuous improvement, to name a few essentials. Quality based systems benefit both the product manufacturer and consumer with a focus on preventative versus reactive approach, as efficiencies are improved, and non-compliant events are detected before they occur.

Setting up a quality-based system within an organization allows companies to build facilities and create products with quality designed into every critical aspect. This begins with identifying requirements that come from the following main sources: regulatory requirements, user requirements, and company requirements. These requirements are the foundation for designing a robust quality-based system. From requirements, specifications are derived, and specifications define how processes are designed and mapped. Once products have been designed and processes are mapped, the associated risks can be identified, assessed, and analyzed in order to mitigate and reduce their impact.

Quality-based systems enable preventative versus reactive actions which are capable of adapting to shifting cannabis regulations as the regulations continue to mature towards risk identification and mitigation. The pharmaceutical and food industries evolved similarly and as cannabis derivatives continue to be infused into consumables, many of the same regulations will likely apply. These features render quality-based systems an ideal design strategy for both new and established businesses within the emerging cannabis sector.

Investigation of Matrix Effects in Cannabis-infused Chocolates

David D. Dawson, PhD
CW Analytical

In the state of California, where cannabis legalization went into effect January 1st, 2018, every legal cannabis product must pass stringent testing requirements before it can be sold to consumers on the legal market. In addition to testing for contaminants (e.g. pesticides, residual solvents, heavy metals, microbials), all cannabis products must be tested for the presence of six cannabinoids, a class of biologically active compounds that can induce the desired psychoactive and/or medicinal effects.[1,2] A label claim of the expected potency must be printed on every product's package, and the potency analysis performed by laboratories must fall within +/- 10% of the stated label claim for each cannabinoid. If a product tests below 90% of the stated label claim, the entire batch must be re-labeled at cost to the producer; if it tests above 110%, the entire batch must be destroyed. The nature of these testing requirements have resulted in high financial stakes associated with state level compliance potency analysis. Further complicating matters is the ever-expanding number of cannabis-infused matrices developed by cannabis producers, which are quickly moved to state level compliance testing in order to reach shelves as fast as possible. Thus, third party cannabis testing laboratories are placed in the precarious position of having to provide accurate and precise testing methods for matrices that sometimes have no research and development periods, and yet the producers have a vested financial interest in the product passing compliance testing. With no prior literature on cannabis testing, fractured scientific requirements from state to state, and a constant stream of new product types, it falls on the cannabis testing laboratories to research the analyses of these disparate matrices and establish scientific standards for cannabis product testing. This research is beneficial to all levels of the legal cannabis market, as consumers, producers, and the scientific community all benefit from sound analytical testing research on cannabis-infused products.

Our research on potency testing of complex cannabis-infused matrices begins with cannabis-infused chocolates, which are widely available on the legal market. Chocolate is a notoriously difficult food matrix for analyte extraction and detection, as a high fat content and presence of polyphenolic compounds can frustrate precise analytical testing.[3,4] We began our investigation into cannabis-infused chocolates by looking at the effect of sample loading across several solvent volumes. Commercially packaged cannabis-infused milk chocolate and dark chocolates were prepped at two sample sizes (1000 mg and 2000 mg) and four solvent volumes (10, 20, 30, 40 mL methanol); each chocolate bar had a label claim of 100 mg Δ9-THC per package (Figure 1). A striking feature of these plots is that for each solvent volume, the 1000 mg sample loading provided higher calculated potencies of Δ9-THC than the 2000 mg sample loading. This is surprising, as it is expected that the more material placed in the vial should lead to a more representative sample, and likely a higher value. Additionally, the data in Figure 1B illustrates the effects of sample prep on a product's ability to pass label claim requirements in the state of California. This cannabis-infused dark chocolate, when tested 1000 mg / 20 mL, provided an average calculated potency value of 93.55 mg [n = 10], which is within +/- 10% of the stated '100 mg Δ9-THC' label claim and thus passes according to California state law. The same product,

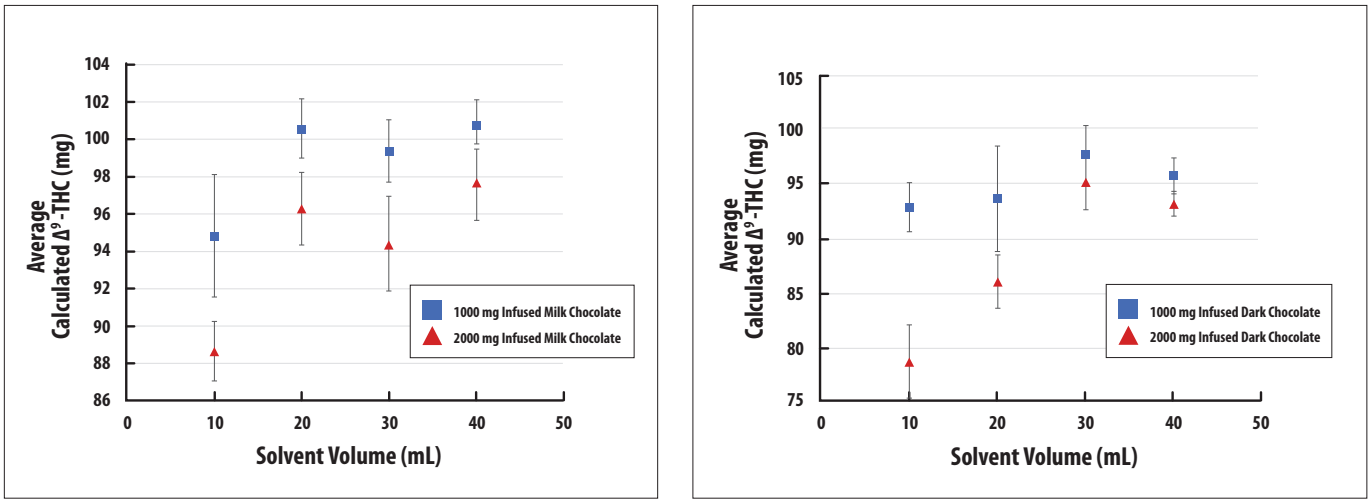

Figure 1: Sample loading comparisons of cannabis-infused milk chocolate in methanol (left), and dark chocolate in methanol (right). All data points [n = 10].

when tested as 2000 mg / 20 mL, afforded an average calculated potency value of 85.97 mg [n = 10], which falls outside of the +/- 10% range of the stated label claim and would result in the product failing label claim and necessitating batch relabeling. By simply increasing the amount of infused-chocolate tested from 1000 mg to 2000 mg, the product can go from a passing label claim value to a failing label claim value. This remarkable finding underscores the importance of performing detailed studies of all cannabis-infused complex matrices, for an unexpected phenomenon such as this may exist in any number of product types, and has the potential to dramatically alter testing results.

Based on our findings that the amount of chocolate tested is related to the overall calculated potency, we developed a model system to investigate this trend in a controlled manner. This investigation utilized undosed milk chocolate and dark chocolate made by the same cannabis chocolate manufacturer, and a stock solution of Δ9-THC in methanol (103.05 µg/mL Δ9-THC). Various sample loadings of both milk and dark chocolates (1000 mg, 2000 mg, 3000 mg) were added to 20 mL of the Δ9-THC stock solution; by testing the recovery of Δ9-THC, we could quantify the effects of chocolate on cannabinoid analysis (Figure 2). The data in Figure 2 clearly show that increased chocolate quantities correlate with lower recovery rates of Δ9-THC, which reinforces the trends observed in cannabis-infused chocolates in Figure 1. Additionally, the decrease in Δ9-THC recovery is essentially negligible when only 1000 mg of chocolate is used, which likely explains why 1000 mg sample loadings afford higher values than the corresponding 2000 mg sample loadings seen in Figure 1.

Taken as a whole, these findings underscore the need for rigorous scientific investigations of complex cannabis-infused matrices, to ensure that the products sold to consumers have been tested in highly precise and accurate manner. Although this study focused only on one analyte in one product type, it underlines the pressing need for thorough scientific research on all cannabis products.

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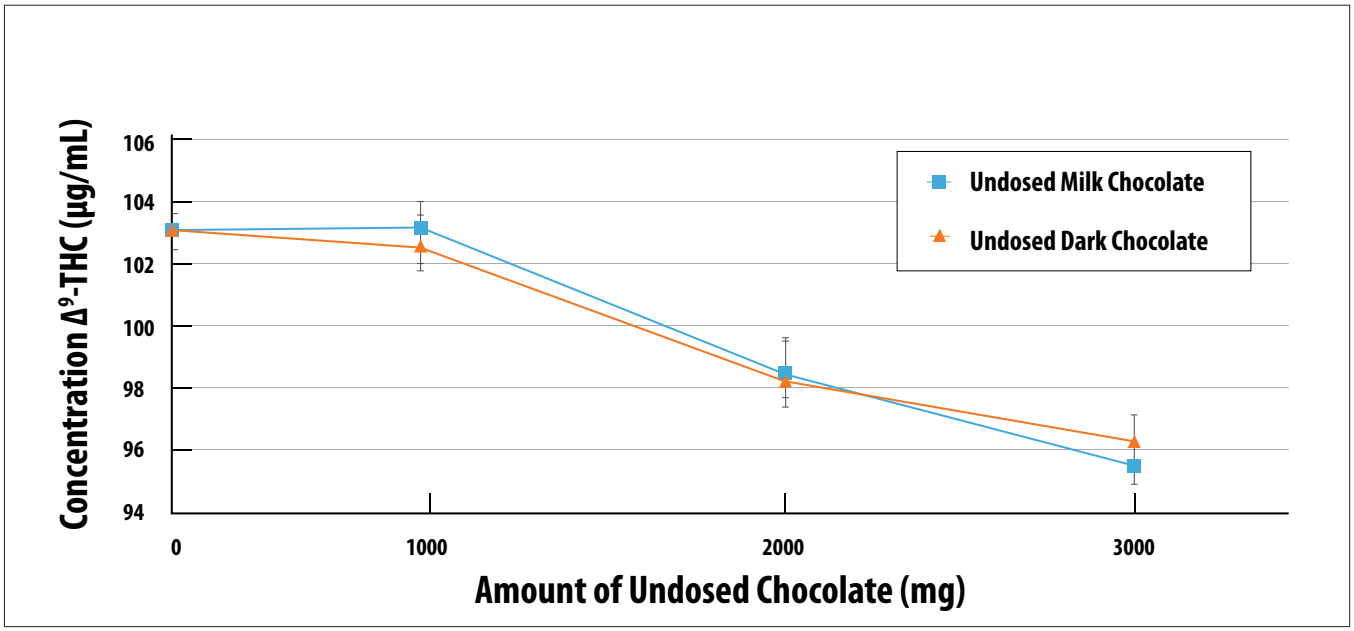

Figure 2: Effect of undosed chocolate on Δ9-THC recovery. Samples dissolved in 20 mL methanol-based stock solution. All data points [n = 10].

CANN

CANNABIS CHEMISTRY SUBDIVISION

VISION

Serving as a pillar of legitimacy for the cannabis enterprise through promoting scientific advancement and empowering community

MISSION

Advancing the cannabis chemistry enterprise by driving scientific innovation and integrity

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