



# 107th AOCS Annual Meeting & Expo

May 1–4, 2016 | Salt Palace Convention Center | Salt Lake City, Utah, USA

## Cannabis Extraction and Analytics Interest Area Technical Program Abstracts

### Table of Contents

CEA 1: Advances in Extraction of Cannabis and Similar Plant Materials .....	2
CEA 2: Challenges and Recent Advances in Cannabinoid Analytics .....	3
CEA-P: Cannabis Extraction and Analytics Poster Session .....	6

*The presenter is the first author or otherwise indicated with an asterisk (\*).  
Abstract content is printed as submitted.*

## CEA 1: Advances in Extraction of Cannabis and Similar Plant Materials

Chair: C.L. Ludwig, AOCS, USA

### **Extraction of Cannabis and Hemp Using Sub- and Supercritical Fluids.** J.W. King, CFS, University of Arkansas, USA.

The use of sub- and supercritical fluids, particularly utilizing carbon dioxide, is becoming the method of choice for extracting ingredients from cannabis and hemp due to the favorable conditions employed and its environment-benign nature and consumer compatibility. In this presentation, the various processing modes and fluid options will be compared along with the multi-scaled equipment designed to effect the extraction and fractionation of the principle components derived from cannabis, such as the various cannabinoid moieties and terpene-based components. A solute-fluid solubility parameter approach will be presented offering criteria for the optimal extraction of the various cannabinoids, terpenes, as well as other compounds such as lipids found in hemp or cannabis. Also within this context, conditions will be suggested for separator-density based fractionation of these components and the differences inherent in accomplishing this using sub- or supercritical CO<sub>2</sub>, including sorbent-based technology on a preparative-level, to further fractionate-purify targeted components, including the removal of toxicant components, such as pesticide residues. The presentation will conclude with examples of several food-related and medicinal products that will be described in relation to the chosen extraction and processing conditions employed, with respect to CBD, CBC, CBN, THC and flavor/aroma components.

### **New Tools and Processes for Extraction and Isolation Workflow of Key Botanical Active Components and Essential Oils of Cannabis Necessary for Scientific Studies.**

J.A. MacKay, Waters Corp., USA.

Chemists and chemical engineers have synthesized and scaled up medically critical compounds, however the cannabis plant has yet to be cost effectively conquered. Until it has, it will be the source of active ingredients needed for essential scientific studies.

Preliminary results of novel tools and processes that have demonstrated promising increases in yield of active ingredients while minimizing the waxes and other compounds that typically contribute to time consuming post processing

steps. Having clear Design of Experiment (DoE) to obtain a time saving Standard Operation Procedure (SOP) is critical to the providing reproducible ratio of active botanical compounds needed for experiments.

### **Cannabis Extract Production: Trends and Developments in an Evolving Industry.** J.M. McCutcheon, Eden Labs, LLC, USA.

The cannabis plant has been utilized by humans for thousands of years as an entheogen, as a medicine, and for its valuable fibers. As legal barriers are lifted, ancient cannabis knowledge is reemerging from an underground movement into a new and exciting industry where classic and modern processing techniques are being applied to the production of a wide variety of interesting products. Many of these products are in the form of a plant extracts such as glandular solids, resins, and oils or extract formulations such as topical, edible, and smokeable products. The current cannabis extracts market is comprised of medical cannabis consumers and recreational cannabis consumers, each with their own expectations for extract quality, content, and appearance. In order to meet consumer expectations and evolving regulatory requirements, cannabis extract producers are utilizing mechanical, physical, and chemical extraction techniques, to produce whole plant extracts, and various separations techniques to remove impurities, isolate specific compounds, and enhance the tactile quality of the extracts. This presentation is a general overview of current trends and developments in cannabis extract production, examples of the products, and some of the challenges facing producers.

### **Immersion and Percolation Extraction for Fines and Specialty Products.** R.W. Ozer, Crown Iron Works Co., USA.

Fines in extraction plants hinder percolation and reduce recovery of oil. Fines are generally removed during prep and leave the plant with hulls.

Immersion extraction was developed to work with finer products that sink in the solvent. Small scale percolation extractors are also available for specialty applications such as cannabis or nutraceuticals where percolation is appropriate.

This talk will discuss applications such as the processing of fines from solvent plants or nutraceuticals such as cannabis, echinacea, etc. using hexane or other solvents.

## CEA 2: Challenges and Recent Advances in Cannabinoid Analytics

Chair: C.L. Ludwig, AOCS, USA

### No Longer Just for Hippies: What's the Buzz About Cannabis?

S.A. Audino, SA Audino & Associates, LLC, USA.

Now legal in 24 states plus the District of Columbia cannabis is high demand. Children, adults, and even animals are “patients” who are demanding good quality and effective cannabis to address a myriad of diseases and disorders. Some states are imposing regulations to ensure dispensaries are providing safe or at least within specified limitations, cannabis and cannabis products. Cannabis products represent a wide array: raw plant material, extracts, oil, elixirs, infusions, etc. The problem all dispensaries, cultivators, and even consumers face is the overall lack of standardized test methods. This session will introduce participants to the cannabis consumers, provide a brief overview of the current status of legalization, and discuss the test methods that may or may not be ‘good enough’ for the industry.

### A Broad-based Analytical Perspective on West Coast Medical Cannabis.

J.C. Raber, The Werc Shop, LLC, USA.

*Cannabis sativa* L. is an exceptionally diverse plant which is rapidly rising in popularity as a physiologically useful tool in alleviating a number of chronic ailments and disease states. Large numbers of cannabis based products within a broad array of different types of product classes are currently available, and those numbers continue to expand rapidly. An analytical perspective within California and Washington has shown a vast number of different plant product are currently being brought to market, sometimes being misnamed and misidentified, with popularized strain names often most abused. For patients who are critically ill, or ailing with chronic debilitating conditions, selection of the right cannabis product can be exceptionally important. Advancing analytical techniques coupled to evolving regulations requiring the use of independent testing laboratories, consumers can now also use analytical data to further inform themselves about their selections. The importance of broad based analytical methods to fully profile cannabis flowers, including both cannabinoid and terpene components of interest will be described and discussed. Additionally, infused products continue to rise and a cannabinoid content survey of diverse infused products acquired in California and Washington will be detailed and discussed, describing the extent of mislabeling and inaccuracy of dosages observed for these products.

### Levels of the Sesquiterpenes Beta-caryophyllene and Alpha-humulene are Highly Correlated in Cannabis Varietals.

E.P. Miller<sup>1</sup> and J.S. Abrams<sup>2</sup>, <sup>1</sup>Phytophile Consulting, USA, <sup>2</sup>Abrams BioConsulting, USA.

The sesquiterpenes beta-caryophyllene (BCP) and alpha-humulene (AHum) are ubiquitous in all tested Cannabis

strains. We have analyzed three independent databases of Cannabis terpenoids and observed a very tight relationship between levels of BCP and AHum in Cannabis flower samples in two out of the three databases. Their concentrations are highly correlated. We have used the tight relationship between BCP and AHum as a Quality Assurance (QA) parameter when analyzing terpene profiles in Cannabis flower samples. Data is presented demonstrating the validation and interpretation of datasets using this parameter. This tight relationship between BCP and AHum production in Cannabis suggests that they are coordinately regulated. They are likely produced by the same sesquiterpene synthase enzyme (or complex) thereby using the same precursor (farnesyl pyrophosphate) as substrate to produce both sesquiterpene products. This phenomenon has been previously reported for the closely related Cannabaceae family member *Humulus lupulus* (hops). We observe that BCP is produced at ~3X higher rate than AHum in Cannabis, in contrast to the inverse ratio of approximately 0.3 produced by hops. We present a hypothetical mechanism for this observation based on available sequence data for the homologous sesquiterpene synthases (STS1) protein sequence from these two species. We propose that the tight ratio of BCP to AHum can be used as an identity parameter for Cannabis flower samples. Furthermore, we propose that this parameter be used as a day-to-day metric to evaluate historical terpene assay performance via control charting.

### Expectations vs. Realities—Analytical Labs and Supply Manufacturers Persevere in an Unstable Industry.

K. Blake, Emerald Scientific, USA.

Cannabis analytical testing facilities are faced with a lack of standardized methods and constantly changing regulations, and manufacturers of the materials they require grapple with extensive application processes, strict DEA oversight, ever changing demands and unrealistic expectations. Working together and supporting programs and organizations that strive for industry best practice, testing standards, and validated methods will pave the way to consistency and legitimize the industry.

### Cannabinoids Analysis: How Short an HPLC Method Can Be.

R. Kachadourian, CMT Lab., USA.

One major challenge of the cannabis testing industry is a rapid turn-around time; when dozens of samples are analyzed in an instrument every day, the length time of a run is crucial. Published methods for the LC analysis of plant cannabinoids (over a hundred have been identified) are up to 30 min runs. On the other hand, in Colorado, five cannabinoids (CBD, CBDA, CBN, THC, and THCA) are regulated. Hence, how short a method can be for a reliable analysis of these five

cannabinoids taking into account possible interferences, between these cannabinoids, but also other cannabinoids in significant amounts (CBGA, CBG, CBNA, CBCA, THCAC<sub>4</sub>, THCVA) and other metabolites? This presentation discusses the advantages and limitations of several LC columns (Phenomenex, Restek, Agilent Poroshell, Agilent Poroshell PFP) commonly used for cannabinoids analysis, depending on the type of product analyzed (flowers vs. decarboxylated products).

#### **Sample Preparation in On-site Cannabis Potency Analysis.**

D. Wilks and S. McArdle, Orange Photonics, Inc., USA.

Sample preparation is often cited as a major source of error in laboratory analytical techniques for cannabis potency analysis. On-site testing adds another layer of complexity since instrument users are not typically lab technicians. Additionally, complicated extraction mechanisms with potentially toxic chemicals are not possible. Several techniques were tested and analyzed for repeatability and accuracy on extracts, flowers and marijuana infused foods. Accurate, repeatable results were obtained for extracts and flowers. Marijuana infused foods require further testing, however some foods were successfully measured through a simple extraction technique.

#### **Quality Assessments for Organically-complex Botanical**

**Extracts.** B.G. Rohrback<sup>1</sup> and P. Gibson<sup>2</sup>, <sup>1</sup>Infometrix, Inc., USA, <sup>2</sup>GW Pharmaceuticals Ltd., UK.

The quality control of botanically-derived pharmaceutical products represents a significant challenge and drove the development of a unique chemometric approach. US FDA Botanical Guidelines describe the requirements of a fingerprint for a botanical drug product, but this edict is not prescriptive in outlining a mechanism for creating and qualifying these fingerprints. As a result, we developed API and Finished Product specifications that have two sections: the first based on a conventional, comprehensive quantitative data evaluation; and the second a qualitative chemometric assessment using the raw chromatographic profiles. The chromatograms of new batches are aligned using statistical methods to provide peak retention time consistency and then are compared against standard models using principal component analysis models. Four chromatographic assays are used to provide the chromatograms that describe the entire content of the botanical material which are then integrated into a single, comprehensive fingerprint. The methodology has been developed for routine QC use by the inclusion of interpretation software that generates a single drug-release metric: a traffic light type “pass-warn-fail” system for each new batch based on this full fingerprint.

#### **Mass Spectrometry Applications to Cannabis Testing.**

S.A. Kuzdzal<sup>1</sup>, D. Wang<sup>1</sup>, J.H. Dahl\*<sup>1</sup>, J. Edwardsen<sup>1</sup>, W. Bankert<sup>1</sup>, J. Kowalski<sup>2</sup>, J. Zitzer<sup>3</sup>, and K. Kovash<sup>4</sup>, <sup>1</sup>Shimadzu Scientific Instruments, USA, <sup>2</sup>Restek Corp., USA, <sup>3</sup>Trace Analytics, USA, <sup>4</sup>G.I. Grow, USA.

Thirty-seven states have pro-medical cannabis laws, including 23 states (and DC) that have medical laws, and four states that have full legalization. There are now over 2 million legal medical marijuana patients in the USA. Cannabis testing laboratories have emerged to accurately determine cannabinoid potencies in cannabis products as well as ensure that these products are free from contaminants.

HPLC and GC are important cannabis testing laboratory separation techniques. Mass spectrometry is playing an increasingly important role in cannabis testing laboratories for the analysis of cannabinoids and terpenoids in cannabis products, including extracted oils. MS is also being employed for the ultra-low level quantitation of contaminants including pesticides and heavy metals. This presentation will provide an overview of LC-MS/MS and GC-MS/MS applications to cannabis testing, including ultra-fast polarity switching for the simultaneous detection of approximately 200 positive and negative pesticides. As MS adoption continues to grow and its utility expands, it is critical that mass spectrometry knowledge gained from more established scientific disciplines, including food safety, be applied to cannabis testing laboratories. This presentation will also discuss future MS-based opportunities for techniques like MALDI-TOF MS, SFE-LC-MS, direct ionization methods and integrated “cannabis analyzers”.

**Analyzing Cannabis: What is “Good”?** S.A. Audino, SA Audino & Associates, LLC, USA.

The cultivation and use of medicinal and recreational cannabis has brought together an interesting constellation of proponents and debunkers alike. Alliances among physical scientists, botanists, herbalists, researchers, and non-science trained regulatory bodies continue to navigate a myriad of challenges as they pave a road in unfamiliar territory. Analytical testing laboratories are emerging every day, many without the scientific acumen to run them. Other laboratories come from a position of developing test methods based on sound validation practices. In no other industry or sector, however, is there such a profound lack of official and/or standard test methods. This session will briefly discuss the current and ever-changing landscape of the cannabis sector as it applies to analytical testing.

#### **Agrochemicals in Cannabis Oil: A Novel Approach for Large Scale Flash Chromatographic Pesticide Remediation from Cannabis Oil.**

A. Martinez, R. Murphy, M. Rubinsky, and A. Conn, Metagreen Inc., USA.

The presence of agrochemical contamination in cannabis oil products is a serious threat to human health as well as representing a huge monetary product liability. Initial

investigations have exposed the rampant and excessive use of pesticides in cannabis. This work has demonstrated a significant pathway from plant to product; irrespective of extraction and refining method (supercritical carbon dioxide, hydrocarbon or ethanol), pesticides are preserved – and in fact concentrated by most extraction methods – to an alarming degree in the refined oil.

Investigations in several jurisdictions have resulted in product recalls in both Colorado and Washington as well as opened a public forum for scientific study and debate. Currently regulations to address agrochemical issues in cannabis are on a state by state basis, and are handicapped by an unclear legal as well as a complex political landscape. This suggests that the problems of agrochemical contamination are only beginning to be understood.

We report an initial study that addresses the use of potentially large-scale preparative flash (normal and reverse phase) chromatographic methodology to remediate cannabis oil products containing agrochemical contamination. A panel of organochlorine and organophosphorus pesticides were used to “spike” various extracts. These agrochemicals were reduced to a highly significant degree. Specifically, the reduction of levels of common, persistent, and problematic agrochemicals (with reductions of several target pesticides below detection thresholds for typical Maximum Residue Levels in other commodities), suggests these methods may represent a novel and scalable remediation and normalization process for the cannabis industry.

## CEA-P: Cannabis Extraction and Analytics Poster Session

Chair: C.L. Ludwig, AOCS, USA

### 1. Cannabis, Hemp, and Hops: Sample Comparative Results with Qualitative Strip Test Analysis for the Determination of Total Aflatoxin. C. Borbone, J. Yu, and N. Zabe, Vicam, a Waters Business, USA.

Many states throughout the United States have enacted laws stipulating a 20ppb action level for total aflatoxins in cannabis. In these experiments, lateral flow test strip technology was used to analyze total aflatoxin qualitatively in hemp, hops, and cannabis flower material. Hops were evaluated as a non-regulated surrogate as well as hemp as a possible surrogate to cannabis. A lateral flow qualitative strip test with a methanol-based extraction solvent was used to determine four different spiked aflatoxin levels visually in cannabis, hemp and hops samples equal to and below the regulatory cutoff level of 20ppb. The visual data generated and recorded for each strip test showed stable control lines as expected as well as test lines of various intensities in direct correlation with the four different aflatoxin spike levels. This procedure was shown to be an effective and sufficient way of screening the amount of total aflatoxin in hemp, hops and cannabis at 20ppb. Due to current US state to state regulations and laws that restrict the transport of any cannabis and hemp samples across state borders, it is concluded that alternative samples such as hemp containing less than 0.3% THC or hops could be used in place of cannabis for research purposes.

### 2. The Fate of Cannabinoids and Contaminants Upon Consumption of Cannabis Flowers and Oils by Inhalation. S. Elzinga, N. Sullivan, and J.C. Raber, The Werc Shop, LLC, USA.

In this poster we present data on pesticide contamination and cannabinoid contents in cannabis flowers and extracted oils as well as their volatilization upon use by a consumer. The presented study was conducted in order to quantify to what extent cannabis consumers may be exposed to pesticides and cannabinoids through consumption by inhalation. A setup consisting of two sequential cooled

methanol traps and a computer controlled vacuum system was used to simulate the human lung. Various flowers and oils with known quantities of cannabinoids and/or pesticides were smoked or vaporized utilizing various devices that are commercially available to cannabis consumers. The cannabinoids and pesticides volatilized during the chosen method of consumption were trapped in the cooled methanol. Analyzes of the contents of the methanol traps allowed for the quantification of the amounts that volatilized. The results of this study show that up to 69.5% of the pesticides present could be recovered in the smoke stream of contaminated cannabis flowers. The smoking of traditional cannabis cigarettes showed a recovery of 27.5-46.3% of the cannabinoids in the smoke stream. The vaporization of extracted cannabis oils showed a recovery of up to 70.0% of the cannabinoids.

### 3. Ensuring the Quality and Safety of Cannabis Products Through Multi Analytical Technique Analysis. R.J. Packer and K. Kuhr, PerkinElmer Inc., USA.

The legal cannabis industry is growing at great rate in the USA, up 17.4% from 2014 to 2015 to \$5.4B. This fast growth however presents a challenge to producers and regulators to ensure that the products are safe and of expected quality. In terms of safety, during the growing and harvesting phases, heavy metals, pesticides, and mycotoxins may contaminate plant products making them unfit for consumption. In post-harvest production, harmful chemicals, otherwise known as residual solvents, may be left in extracted oils contaminating subsequent products. In terms of quality, monitoring the potency of cannabis (the cannabinoid levels) is useful for optimizing growing conditions, maximizing product value, determining harvest time and vital for ensuring the product is of specified strength. This work shows the wide variety of analytical techniques that can be used to test for both the quality and safety of cannabis products.