CAN 1: Lab Proficiency Programs and Reference Samples

Chairs: Steven P. Bennett, Evolab, USA; and Doug Rennie, AOCS, USA

State of Cannabis - A Bird's Eye View Steven P. Bennett*, *Evolab, USA*

Building Bridges: Cannabis Science Education and Medical Reform Josh Crossney*, *jCanna & Cannabis Science Conference, USA*

After nearly a century of misinformation and stigma surrounding cannabis plants, there is a great need for stronger interaction amongst cannabis industry experts, analytical scientists, medical professionals, patients, regulators, and teachers. We must bridge the gap between analytical sciences and the medical cannabis industry to help improve the quality of medicinal cannabis products being used by patients. This presentation will take a closer look at specific, recent activities to educate, empower and inform, including: articles, conferences, symposia, conferee networking sessions and “Canna Boot Camp” hands-on workshops for a new cross-disciplinary perspective. While these efforts help lay a solid foundation for information sharing, we have a very long way to go towards the development of courses incorporating new pedagogical cannabis approaches, including the revision of existing medical curricula and the development of courses bridging cannabis and teacher education. We will examine the driving forces that promote cannabis change and the restraining forces. The current landscape of cannabis testing is improving, but it is fragmented and proprietary. Cannabis labs use different analytical instrumentation and methods, as well as different sample preparation techniques. HPLC methods measure various cannabinoids, whereas GC methods measure “Total THC”. Pesticide lists vary from state to state, if testing is required at all. Federal laws prohibit labs from working with cannabinoids standards above concentrations of 1 mg/ml. Testing rules and regulations vary from state to state, further complicating matters. We must work together to move beyond regional or even national standardization toward international cannabis standards and methods.

Cannabis Contaminants: Practical Considerations vs. Regulatory Requirements Christopher J. Hudalla*, *ProVerde Laboratories, Inc., USA*

The cannabis industry is currently one of the fastest growing industries in the United States, with 28 states permitting medicinal use, as well as 8 states that permit adult-use. This growth is fueled by recent revelations of the benefits of cannabinoid therapies for many health conditions. One challenge that has emerged is the ability to ensure consumer safety, providing accurate dosing and products that are free from potential contaminants. Analytical testing is a necessary component to ensure patient/consumer safety for products that are being consumed both medicinally as well as recreationally. Yet, many states have minimal or no regulations in place to require analytical testing. For the states that do mandate testing, there is little synchronicity between requirements from state to state. In an effort to address this, several organizations have begun to develop and validate methods that can be used as a basis for this testing. These methods not only encompass testing for the active phytochemical constituents (cannabinoids and terpenes), but also for potential contaminants including heavy metals, residual solvents (VOCs), pesticides, mycotoxins, and microbiological contaminants. Many of these contaminants are the simple result of human handling and processing of the products. The methodologies that are being used to address these testing requirements include a wide variety of chromatographic techniques in addition to mass spectrometry and a variety of approaches to address microbiological contaminants. Standardization of these methods for the industry

will give regulators the resources they need to include sensible requirements for regulation and legislation that is being crafted to monitor and control the use of cannabis within the US medical and adult-use markets.

Novel Regioselective Extraction of CBD by Modified Microemulsions. Rotem Edri, Sharon Garti- Levi and Nissim Garti* *Casali Center of Applied Chemistry The Hebrew University of Jerusalem, Israel*

Today, most of cannabis bioactives are extracted from the cannabis flowers by solvents. The two commons extractions solvents of CBD or mixtures of CBD and THC (along with other smaller components) are ethanol or supercritical CO2 extractions which is expensive and non selective. A new " novel solvent mixtures" are specially modified microemulsions (ME or modified NSSL- nano sized self assembled vehicles) that facilitate high extraction yields (up to three fold from the solvent extraction) and are exhibiting low cost production and enhanced structural regioselectivity selectivity for CBD over THC. This process, will not only offer a better and more efficient alternative for CBD extraction but will allow the use of non-engineered strains which are cheaper. MEs are homogeneous nanometric monodispersions; thermodynamically stable capable to solubilize high amount of both lipophilic compounds. The novel ME systems are patented as "solvent media" technology for a direct and selective extraction of CBD from the plant. Various extraction parameters and their effect on extraction yields and selectivity with a comparison to common extractions with ethanol will bediscussed. In a multiple steps extraction, in which the novel medium was reused, for the extraction of new batches of flowers, we were able to load the mixture with up to 4 wt% of CBD and achieved average yield of >60% of the CBD in the plant. However one can carry out three extractions with fresh ME on the same flowers and will get CBD levels in the extracted product of > 6%, and extraction yield of 80% of the cannabis bioactives. This extraction yield over two fold more CBD compared to 12-24 hours extraction with ethanol.

Cannabis Extraction and Refinement in Colorado: A 5,280 ft. View Christian F. Sweeney*, *Cannabistry Labs, USA*

In Colorado, and many other legal states, a number of techniques have been adopted for extraction and refinement of cannabis. Much like traditional botanical extractions, the aim of these techniques is to provide the desirable qualities of cannabis in a more readily usable form for the delivery method of choice. Ideally, these techniques minimize the loss of bioactive and flavor compounds while removing undesirable components. The most commonly applied extraction and refinement techniques in Colorado will be discussed and reviewed for these attributes. Techniques discussed will include, Supercritical Fluid Extraction, Light Hydrocarbon (Butane/Propane) Extraction, Organic Solvent Extraction, Aqueous Extraction, Expression, and Distillation. The core principles of each technique will be discussed as well as their strengths and weaknesses at achieving the goals of extraction. In addition to covering the basics of each technique, their current uses will be discussed and the challenges associated with finding best fits for various delivery applications will be reviewed. Lastly, the potential hazards to both consumers and operators will be discussed for each technique, as well as basic strategies for hazard mitigation.

Advances in Extraction: Double Yield in Half the Time by Focusing on Essential Principles and Practices John A. MacKay*, *Waters Corporation, USA*

During this time, we will examine the reality of extraction for a wide spectrum of formulated products. It is tantamount to maximize profits in a market that is demonstrating a significantly diminishing margin model. Over the past six years the progress of extraction has advanced

significantly. Many of the anecdotal “u-tube and urban legend university facts” has been proven to align with scientific theory, but just as many have been refuted. There are many examples of both, but we will focus on three current ones. One example is that flavors and fragrances have been extracted for eons from plants as evidenced by the robust perfume and food industries. So, it is not too surprising that even the most volatile mono hydrocarbon terpenes (like alpha pinene) are extremely well extracted by sub and supercritical CO_2, butane, ethanol, Freon, etc. The early days of thinking this couldn’t be done was more due to poor methods versus the instruments. Having a car go off the road on a sharp corner is more often about the driver’s skill versus the specifications of the car. A second example is the urban legend about how high temperature and high pressure are detrimental to extraction is again not based on scientific facts and design of experiments. The last example that will be addressed is the misplaced emphasis on huge extraction vessels for mid-range extraction processes are also finally being displaced by the reality of extraction science principles and practices. During this time will look at how to accelerate the accurate urban legends and dismantle the ones that have no basis in science principles and certainly not practice.

CAN 2: Method Development

Chairs: Steven P. Bennett, Evolab, USA; and Douglas Rennie, AOCS, USA

Analyzing Cannabis: What has Changed? Susan Audino*, *Audino & Associates LLC, USA*

This session will look at the changing requirements for analytical testing in the US, and in particular the lack of consistency from state to state. Many states are now requiring not only analytical testing, they mandate those testing laboratories to be ISO/IEC 17025 accredited. Discussion will include dispelling myths associated with the ISO/IEC 17025:2005 standard for laboratory accreditation, particularly as many testing labs and regulatory bodies have incorrect information about its requirements. Finally, the session will provide an update on the respective progress of AOAC and ASTM on the development of official/standard analytical methods for the cannabis testing laboratory.

Method Validation and Quality Control: Overview and Strategies for Cannabis-Specific Analytical Methods Amanda Rigdon*[1], Fred Hill[2], and Frank Dorman[3], *[1]Emerald Scientific, USA; [2]Emerald Scientific, USA; [3]Pennsylvania State University, USA*

The cannabis industry's explosive growth and continued federal scheduling of cannabis have forced cannabis analytical laboratories and non-scientific state regulatory bodies into a quest for state level self-regulation in the interest of consumer safety. Currently laboratory data quality is highly variable both within and between states, which is a cause for concern in terms of consumer safety. Several states are attempting to address this issue by requiring third-party certification (e.g. ORELAP, ISO) as a condition of licensing for cannabis labs. Additionally, several professional societies (AOCS, AOAC, etc.) are in the process of developing compendial methods for cannabis analysis in order to standardize laboratory data collection. Regardless of whether or not laboratories choose to implement compendial methods or to pursue and validate their own methods, some level of method validation and ongoing QC is required. This talk will present the validation steps required to implement compendial methods as well as explore validation strategies for performance-based methodologies. Cannabis-specific data will be presented for validation experiments for cannabis potency and residual solvents methods. Validation methodologies presented will include determination of potency extraction recovery and evaluation of matrix interference for residual solvent analysis.

Sample Preparation using Cryogenic Grinding Sandy Mangan*, *SPEX SamplePrep LLC, USA*

Abstract not available.

Analysis of Terpenes in Cannabis by Liquid Chromatography Dylan Wilks* and Don Lavery, *Orange Photonics, Inc., USA*

Savvy medical and recreational cannabis consumers understand there is more to Cannabis than potency. Cultivators that are sensitive to customer preferences are turning to science to improve quality and develop a competitive edge. At the forefront of this shift is the recent uptick in requests for terpene analysis. Terpene profile can be used as an indication of quality, and some evidence indicates that terpenes may interact with cannabinoids and alter uptake in the brain. While terpene analysis in a lab setting is now a routine analysis typically involving gas chromatography, a liquid chromatography analysis technique was developed to provide a rapid field-based analysis. Preliminary results and discussion will be presented.

Rapid Field and Laboratory Methods for Precise Detection of Aflatoxin and Ochratoxin A in Plant Tissue, Extracts and Edible Products Across the Botanicals Industry Lanny Smith*, *VICAM / Waters Corporation, USA*

Abstract not available.

Analysis of Pesticide Residues in Cannabis Regulated by Oregon State using QSight LC/MS/MS Jason Weisenseel*, *PerkinElmer, USA*

Abstract not available.

Validation of a Rapid Analytical Method for the Measurement of *E Coli* in Cannabis Surrogates Edward F. Askew*, *Cannabinoid Technical Group, USA*

The current microbiological methods used in cannabis testing for pathogenic bacteria rely on classical media growth, isolation of colonies and separate bacteria confirmation. This type of analyses was also prevalent in water testing until the innovation of enzymatic Most Probable Number tests. These enzymatic tests reduced the time to colony quantitation and the labor required to reach these values. Modification of these water tests to include a sterile extraction of cannabis surrogate matrixes (Nettles, raw soy oil and chocolate) in water and quantitation of the results will be presented along with the proposed single laboratory validation study for AOCS method consideration.

High Throughput Microarray Analysis of Multiple Pathogens on Cannabis Michael E. Hogan*[1], Rick Eggers[1], Melissa May[1], Kevin Obrien[1], Carl Yamashiro[1], Reggie Gaudino[2], Christian Cizek[2], and Anthony Torres[2], *[1]PathogenDx, USA; [2]Steep Hill Labs, USA*

We have developed a rapid, low cost microarray technology that can quantify and detect dozens of pathogens (bacteria, fungi, viruses) in parallel. The most steps in the process can be performed at room temperature with simple off-the-shelf lab equipment, with a capability of processing several hundred samples per day. Four significant “game changing” advantages were developed and have been deployed in its technology, to advance microbial testing in cannabis and more broadly, in food and agriculture: 1. Bypass microbial incubation and enrichment steps. 2. Raw Sample genotyping (RSG) to bypass DNA extraction and quantitation. 3. Scalable, low-cost microarray technology: analyze dozens of microbes in parallel. 4. Simplified testing protocol that can be done at ambient conditions with inexpensive test equipment, and does not need highly trained lab personnel. The combination of those 4 advantages reduces the time to conduct the pathogen test, reduces cost, improves scalability and specificity of the test compared to competing alternatives. This microarray based test has now been validated by multiple cannabis labs. Some of those cannabis validation data will be presented. Highlighted system performance parameters include: 1. Detection of multiple DNA containing pathogens in parallel on cannabis, without DNA purification. 2. 16 different samples processed in parallel, in a simple microscope-slide-like format. 3. Turnaround time 8 hours, for up to 96 samples, processed by 1 technician. 4. High sensitivity without pre-enrichment. 5. Accuracy and specificity to match gold-standard plate counting. 6. 30% lower cost and 10 times higher throughput than qPCR on multiple pathogens. 7. Testing can be performed on the lab bench at ambient conditions.

CAN-P: Cannabinoid Analytics Poster Session

Endogenous Solvents in Cannabis Extracts David W. Egerton*, *CW Analytical, USA*
Solvent-extracted oil from Cannabis sativa has become increasingly popular among Cannabis users in states that permit its use. Quality testing regulations of this material has recently focused on the presence of residual solvents that may be carried over during the extraction and purification process. Many of these regulations exhibit low thresholds and cumulative thresholds that do not account for the endogenous solvents found in all plant material, such as acetone and lower alcohols. This study demonstrates the prevalence of these compounds in both the native plant material and the concentrated oil, despite their lack of use during the production process. An analysis was performed on Cannabis plants and oils by HS-GC-MS, and found an average of 50-250 ppm of methanol, ethanol, isopropanol, and acetone in plant and extract materials.