Analytical Cannabis
Extraction. Science. Testing.

TECHNOLOGICAL ADVANCES IN CANNABIS TESTING

WWW.ANALYTICALCANNABIS.COM

Contents

Foreword

Welcome to our latest eBook, *Technological Advances in Cannabis Testing*, a collection of features on some of the most exciting developments in marijuana testing, penned by some of the brilliant people making them happen.

Hear from Kimberly Ross, chief scientific officer at Peak Compliance, about the new and incoming regulations that could boost the efficiency of cannabis testing labs. Read an exclusive feature from Jini Curry, chief scientific officer at Modern Canna Laboratories, to learn about her lab's research into total yeast and mold tests, which illuminated just how useful this industry standard test is.

Further on, Toby Astill, global market manager for cannabis and hemp at PerkinElmer – the sponsor of this eBook – outlines how new data management technologies can boost every aspect of a lab's workflow. And in another feature, Adrijana Torbovska, a liquid chromatography application specialist, breaks down the many factors an analyst must consider before embarking on advanced cannabinoid analysis.

For anyone interested in the ever-evolving field of cannabis testing, this collection is a must-read.

By Leo Bear-McGuinness

The Promising Future of Cannabis Testing Laboratories

By Kimberly Ross, chief scientific officer at Peak Compliance

Thanks to the recent and rapid state-by-state adoption of regulated cannabis for medical and adult-use, the demand for cannabis testing services has boomed. In the US, regulations are currently promulgated at the state level, which results in inconsistencies state to state. But following the recent advancement of House Bill H.R.3617 to the Senate, federal legalized markets seem more imminent than ever before.

What can we expect for the future trajectory of cannabis testing, as the cannabis industry as a whole hurtles toward the changes likely to be ushered in by federal oversight?

The MORE Act advances to the US Senate

The Marijuana Opportunity Reinvestment and Expungement (MORE) Act, H.R.3617, passed by 220-204 votes in the US House of Representatives on April 1, 2022, and was received in the Senate three days later where it was referred to the United States Senate Committee on Finance. While the ultimate fate of this particular bill is still unknown, cannabis has gained broad public support and bipartisan sponsorship in Congress. The act, if signed into law, would remove cannabis from its current status scheduled under the Controlled Substances Act (CSA), eliminate many criminal penalties associated with possession, distributing and manufacturing, create an expungement process for previous federal offenses, put in place a framework for taxation, and remove barriers to basic financial services. All together, these provisions would have major implications for financial equity and access to traditional banking services for cannabis businesses.

In a future landscape of federally legal cannabis markets, where businesses have greater access to capital and other benefits, the laboratory testing segment of the industry will presumably see increased revenue growth based on the upward trajectory of cannabis wholesale and retail sales. Access to cannabis in the US will widen geographically, and sheer volume of cannabis produced will dictate more testing demand. Another potential effect to consider is consolidation, given that the expanded geographies will need testing services.

If cannabis can move freely cross state lines, testing services may not need to be stood up in every locale. Existing courier services such as USPS, Fedex, and UPS, which are currently prohibited from transporting cannabis products in most cases, would be allowed to deliver samples to cannabis laboratories, as is common for clinical and environmental testing services. As cannabis testing experiences some sort of standardization at the federal level, and with products able to cross state lines, the doors to consolidation at the nationwide level may fling wide open. The production and retail sides of the industry have certainly begun to experience consolidation, as hyper-successful brands look to expand their footprint and do so by acquiring smaller operations willing to sell. The testing services segment of the industry will likely follow suit.

More robust QA/QC to be required in future federal markets

The requirements for quality assurance (QA) and quality control (QC) surrounding routine cannabis product compliance testing are likely to align more with established testing industries for water quality or pharmaceuticals under a national regime. Federal oversight tends to standardize quality control, data integrity, and data traceability criteria across state lines. For now, some states require accreditation to the ISO 17025 international standard, which mandates certain quality system elements required for conformance with the standard. However, at the state level the overlay of QA/QC requirements are vastly inconsistent. With standardization of methods and associated method/analyte/matrix QC a likely outcome of federal regulation, many pre-existing labs will need to strengthen their QC procedures. Adding QC samples does add cost to testing by spending consumables, reagents, and human resources for preparation, analysis, interpretation, documentation and reporting of those additional samples. The current pricing structure seen across markets may therefore be a low estimate of what true costs will look like in a future scenario.

Modernized operations: innovations in automation and built-for-purpose software for data handling

Laboratory work for cannabis compliance testing involves a seemingly never-ending cycle of repetitive tasks carried out in a predetermined sequence according to standard operating procedures (SOPs). Once established SOPs are in place, the overarching goal is to execute these processes in the exact same manner, for all samples, all the time. Automation can play a key role here, simultaneously accomplishing the consistency necessary for repetitive steps of sample preparation while also freeing up human hands to direct efforts elsewhere, where human interaction is required. For a laboratory with ambitions of processing hundreds of samples per day, automated sample prep becomes not just a differentiator, but a necessity. At a time when all segments of the industry are introducing automation solutions to help ease the burden of manual labor shortages, labs that seek out innovative scientific equipment employed in other testing settings will increase sample volume capacity and throughput ahead of their competitors, without necessitating a corresponding increase in personnel.

The copious amounts of raw data produced by cannabis testing operations requires secure handling and storage, best accommodated with customized laboratory information management system (LIMS) software. The LIMS is the central nervous system of the lab, receiving data from instruments (and feedback from humans), processing information and making interpretations based on pre-defined calculations, and sending reports of the compiled inputs. The LIMS also acts as the memory of the laboratory, a vast repository for all data generated over time. Some degree of customization is necessary based on the specific suite of instruments in the lab, the list of state-required analytes, and their action limits. A thoughtfully built-out LIMS can also act as a dispatch system for lab personnel - surfacing bottlenecks and issues earlier than otherwise possible in a “paper and whiteboard” system. The value of the efficiency gained with a properly functioning, built-for-purpose LIMS cannot be overstated.

Beyond the basics: expanded scopes of testing to support industry needs

Cannabis cultivators and manufacturers are required to test according to state regulations, but what about meeting the needs of these entities beyond the scope of mandated testing? In addition to the demand for compliance testing of cannabis products in their final form, there are a plethora of opportunities to support cannabis operations in their endeavors to cultivate, formulate, innovate, and troubleshoot. In-process testing at various stages of cultivation or manufacturing can be extremely valuable and ideally includes screening of all inputs to the processes of cultivation (soil, water, nutrients, etc.) or manufacturing (raw ingredients other than cannabis). Environmental monitoring of facilities in accordance with good manufacturing practices (GMP) is also a beneficial value-added service to cannabis clientele. Screening for plant pests, nutrient deficiencies, infections, or other maladies further bolsters the types of offerings a lab can consider providing. Until cannabis businesses are able to bring capabilities and talent in-house to conduct GMP-style testing and facility and/or plant-health monitoring themselves, offering these services can help foster a strong relationship and build trust between the laboratory and its clients.

The future outlook: a promising trajectory

The cannabis testing space is dynamic in nature due to its recent indoctrination as a discipline in its own right, where method development and optimization are still ongoing, and new compounds are added to analyte testing lists as the industry as a whole expands and matures. The future outlook of cannabis testing is promising as it becomes standardized nationwide, automated, quality-controlled, expanded in scope, and market-valued. It is likely to arrive imminently, ancillary to the geographic broadening of the industry and increased demand resulting from federal legislation for legalization and regulation of cannabis and cannabis infused products.

Standard Testing Methods Are Finally Catching up to Cannabis Regulations

By Alexander Beadle

Those in the cannabis sector will know a familiar refrain: that cannabis is subject to a "patchwork of regulation" in the United States. The rollout of state-level cannabis legalization measures has resulted in very little harmonization from one state to another, both in terms of cannabis market regulation and analytical testing procedures.

But the desire to unify and follow some kind of collective standardization is there; many states require cannabis testing facilities to achieve ISO/IEC 17025 accreditation for this purpose. Independent standards organizations are also making significant progress in developing standard methods and bridging the gap between the regulatory, scientific, and business worlds.

In her recent *Analytical Cannabis* webinar, "Standard Test Methods Making Gains on the 'Runaway Train' of Regulations!", Dr. Susan Audino, chemistry laboratory consultant at S. Audino & Associates, LLC, presented an update on the development of standardized test methods for the cannabis industry.

Balancing regulation, scientific integrity, and the cannabis business

One important thing to note about the wild west world of cannabis regulations is that the current frame of reference for cannabis testing regulations was largely put together by non-scientists. Faced with the challenging task of developing a new suite of regulations for the nascent cannabis industry, regulators did their best to come up with sensible requirements by pulling on rules from other scientific sectors.

"Unfortunately, many of the cannabis testing requirements are really borrowed from other industries, be it wastewater or pharmaceuticals or dietary supplements or the food industry," Audino said.

"So we have to keep that in mind and realize that some of these regulations, if they seem pretty off the wall, may not be ours. They may be coming from a different industry, and although well intentioned, are inappropriately placed here."

Audino identified three driving forces that affect the general third-party testing laboratory: science, business decisions, and regulatory specifications. Operating as a cannabis testing laboratory means being able to find a sweet spot where all three of these conditions are satisfied, without compromising science or exploiting regulatory requirements, but while still supporting businesses and making customers happy.

"I used to think that the business decisions should be based solely on science and the soundness of that science. That was also when I believed that regulatory specifications were based on known empirical data for the cannabis industry," Audino added.

As an example, one of the biggest challenges to this sweet spot is the regulatory specification in place in some states that requires laboratories to test and report values that are at or around their limit of detection (LOD).

"When we have regulations that require not only a reporting levels at or near the LOD, but also a decision rule at or near the LOD, then it compromises the laboratories and compromises the science used to establish that method, because laboratories will do whatever they need to do to make that value as or that range as broad as possible," Audino said.

"But then it also compromises the regulations itself," she continued. "They are asking the laboratories to stick their necks out and claim confidence in something that, scientifically, they are just not able to be confident in."

Compromised science in cannabis testing labs:

In balancing regulatory requirements and business decisions, many laboratories end up operating in a gray zone where they are not necessarily operating with bad science, but where they are using science to manipulate the acceptance ranges prescribed by regulations.

For example, labs can cherry pick their quality control and laboratory control samples (LCSs) to give favorable results. Such controls are supposed to prove whether the methodology being used is able to make accurate and precise measurements.

"I've been in laboratories where a very well characterized substance such as olive oil is used as an LCS. But because it tests so well and it's characterized so well, it's used for every matrix: an edible, a concentrate, a flower," Audino said.

"Is that appropriate? Does that make for the best science? The answer is probably not."

Calibration is another area where laboratories often compromise scientific ideals. When making a calibration curve, it is important that the residuals – values that reflect the difference between the predicted response and actual measured response – are normally distributed.

"The mean of those residuals is zero. If it's not, it's not a good method. Very, very, very few laboratories actually do a residual analysis," Audino said.

Before a sample even approaches testing, scientists also need to think about the materials that they are using. If they are not careful, it can be easy to unintentionally introduce sampling bias when selecting which samples from a batch to test. Labs also need to ensure that their certified reference materials (CRMs) are suitable for use.

"CRM producers work very hard to develop their standards and to develop expiration dates for those standards. However, once that bio-container is cracked open by the laboratory, and the intention is to retain a remainder for some other use at a later time, the expiration date of that remainder must be determined by the laboratory," advised Audino.

Conflict with standards and protocols

Standardization and the development of standard test methods allow labs the opportunity to access detailed instructions, which in turn enable the interoperability of people across laboratories. This consistency also allows for better comparability between different laboratories.

With the goal of better standardizing laboratory procedures, several states now require cannabis testing laboratories to achieve ISO/IEC 17025 accreditation – a popular standard for all types of testing and calibration laboratories. However, in the cannabis industry, there can sometimes be conflict between these ISO standard requirements and the requirements of the state.

"Although not present in all states, at least one or two states say that all test methods must undergo full validation protocol, and this means a standard test method or compendial test method that has already gone through single-lab or multi-lab validation is still subject to going through full validation in the laboratory," Audino explained.

"[ISO/IEC] 17025 would refer to this as a verification; the benefit of using a standard method is that the method has already gone through rigorous evaluation, and the laboratory just needs to demonstrate their ability to follow that method and get the right result."

Standards organizations are catching up with cannabis regulation

Over the past several years, standards organizations such as AOAC International, ASTM, and the AHPA have begun to enter the space to help bridge this gap through fostering collaboration in developing standard methods specific to the cannabis industry.

"In the end, it is the standard test method that will provide a level playing field and take away the market edge of one laboratory versus another; where a client comes in and says, 'You either give me a value test report in this range, or I'm going to go to laboratory and get it tested where I know that their methods may not be as good as yours,'" Audino explained.

AOAC International, where Audino serves as a scientific advisor to the AOAC Cannabis Analytical Science Program, has formed several working groups tasked with

constructing standard method performance requirements (SMPRs) for the cannabis industry. These SMPRs are the first step in establishing a standard method.

Many organizations are now also producing specific guidelines for cannabis labs to follow, in a bid to remove some of the ambiguity that comes with solely following the broad, industry non-specific ISO 17025 guidelines.

Members of a working group from the American Council of Independent Labs (ACIL), under the direction of the Independent Laboratories Institute (ILI), recently produced a document titled "Guide to a Harmonized National Cannabis Laboratory Accreditation Program" which contains science-based guidance and best practices for cannabis labs. The AOAC Analytical Laboratory Accreditation Criteria Committee (ALACC) will also soon republish its "Guidelines for Laboratories Performing Microbiological and Chemical Analyses of Food, Dietary Supplements, and Pharmaceuticals" to include more specific guidelines for cannabis laboratories, Audino said.

Already, several cannabis-related methods have been approved for inclusion in AOAC International's "Official Methods of Analysis" compendium, including methods for the quantification of cannabinoids in dried plant materials, oils, and chocolate edibles. AOAC working groups have also produced many SMPRs for the cannabis industry, including quantifying cannabinoids and detecting microbiological hazards in different matrices.

"Why are all of these developments important? Well, as a scientific community, we continue to have to meet regulatory requirements," Audino said. "We are hoping that the regulatory bodies that are overseeing and dictating the cannabis landscape will become a little bit more knowledgeable about empirical science, what that means, and how we can apply that appropriately to the cannabis industry."

Although labs may feel like they have been caught in a gray area, the current state of collaboration between labs, industry experts, and regulatory bodies is perhaps more like the reaching of a crossroads. Continued development of standard methods and science-based guidance for cannabis laboratories should ensure that the cannabis industry is supported by analytical testing that is grounded in sound scientific principles.

The Benefits and Pitfalls of Total Yeast and Mold Counts in Cannabis Labs

By Jini Curry, chief scientific officer at Modern Canna Laboratories

The standard for yeast and mold testing

For years, the benefits of total yeast and mold (TYM) testing have been widely debated in cannabis industry. There is evidence that quantitative polymerase chain reaction (qPCR) is the better option since all DNA is amplified in the extraction process, which will avoid missing certain yeast and molds that do not culture well in plating methods. Alternatively, other evidence indicates that false negatives and low quantitation/ under estimation occur with qPCR tests. In which case, traditional plating methods may be the best choice.

So, regulators and laboratories are being forced to decide which method produces the most accurate results. However, the decisions tend to be made hastily and without extensive research to support the decision.

Our laboratory, Modern Canna, recently began looking at total yeast and mold methods closer, to better assess what the industry standard for this specific type of testing should be.

Throughout the investigation, we found several pieces of evidence that suggest, while there are benefits to total yeast mold testing in cannabis flower, there are also several pitfalls that may be leading labs to unintentionally report inaccurate results.

Which result is the correct answer?

One of the first questions that we hoped to answer was: which method is the most accurate and provides the most reproducible data? We began trying to find a certified reference material that could be used to determine which method matched the closest and was reproducible. During the process, we discovered that one of the only available reference materials for total mold in cannabis had two drastically different values (Part Number: FM-729, Lot Number: 220210rev1), one for each of the plating methods used. The certified activity is 152,000 colony forming units per gram (CFU/g) when Petrifilm Rapid Yeast/Mold Plates (P-RYM) are used, and 423,000 CFU/g when Sabouraud Dextrose (SabDex) Agar is the growth medium.

This fact alone poses an interesting question for the industry: which result is the correct answer? Or are both answers considered correct? In seeing this, the laboratory concluded that the next step to solving the mystery surrounding TYM was to run this analysis on cannabis samples using multiple different growth mediums and via qPCR to see how the results lined up with one another.

Comparison study of several growth mediums and qPCR analysis on cannabis samples

An initial study was conducted by analyzing ten cannabis flower samples for TYM using plating methods on various growth mediums (listed below) and qPCR technology.

- Compact Dry (CD)
- Petrifilm Rapid Yeast and Mold (P-RYM)
- Petrifilm Non-Rapid Yeast and Mold (P-NRYM)
- Potato Dextrose Agar (PDA)
- Potato Dextrose Agar with Chlortetracycline (PDAC)
- Dichloran Rose Bengal Chlorampenicol (DRBC)
- Sabouraud Dextrose (SabDex)

The results obtained from this experiment were inconclusive due to the large variations across the methods and the lack of reproducibility in results.

Therefore, the laboratory opted to have more control over the experiment by running two different studies. One that involved using a single sample and analyzing it five times at three different dilutions, and the second that looked at six different samples, processed in triplicate, (three of which were treated with radiation) at the same dilutions. This allowed the reproducibility of the methods to be measured when compared to itself and other methods. The experiment also involved documenting and counting colony growth at several time intervals (48, 72, 96, 120, and 144 hours), to determine incubation times for each type of plate used. True incubation times were established as the point when colonies no longer started appearing and instead just grew on the plates.

Plate/Agar	True Incubation Time (hours)
Compact Dry	72
Petrifilm Rapid Yeast and Mold	72
Petrifilm Non-Rapid Yeast and Mold	96
Potato Dextrose Agar	72
Potato Dextrose Agar with Chlortetracycline	96
Dichloran Rose Bengal Chlor-ampenicol	96
Sabouraud Dextrose	72

The conclusions obtained from the experiment suggest the following incubation times for the various growth mediums used:

The study revealed that while TYM numbers sometimes align from method to method, it is more common that the results will vary drastically, sometimes by more than 50%. This is one of the main pitfalls that cannabis methodologies face. Cannabis is a complex organic material that changes from sample to sample. Thus, the matrix plays a major role in the results being obtained. The initial studies conducted verified that more method development needs to be done to assess what assumptions can be made during the analysis regarding the matrices.

Moving forward – is there a need for a change?

While measuring the amount of yeast and mold is important for the cannabis industry, more emphasis needs to be placed on standardized methods for this analysis and a singular procedure should be selected. The variance seen from method to method indicates that while laboratories may not be intentionally passing failing products, this may occur depending on what technique they are using and how they are calculating their quantified results. Pitfalls, such as this one, can be avoided through more rigorous validation processes.

Throughout the investigation, the laboratory was able to confirm the previously determined benefits and pitfalls of various microbial testing techniques. In traditional plating methods, there is an indication that there is not enough selectivity and, as a result, bacterial colonies may grow on plates intended to detect TYM. To test this theory, we are in the process of having the colonies sequenced to determine how much bacterial growth is occurring. Additionally, our findings revealed that the typical dilutions performed in plating methods may cause data to be skewed. In most instances, calculated totals at each dilution were not duplicate numbers. Therefore, if an average of the three dilutions run is taken to determine the quantitative result, the true value may be increased or decreased depending on the skew seen.

Conversely, there are some concerns that qPCR may lead to false-negative results if there is too much contamination or if certain microbes are present. We are in the process of confirming if there is a threshold at which the contaminants present become too concentrated, thus skewing the qPCR data due to DNA saturation.

Additionally, it was determined that incubation time plays a pivotal role in the results obtained. During the experiment, it was noted that most of the agars used did not show complete colony growth in cannabis until at least 72 hours, even though some of the recommended incubation times are 48 hours. Therefore, if labs are processing samples based on

recommended incubation times, TYM counts may be severely underestimated. This is another indication that the methodology used for TYM may be lacking and that further studies need to be conducted.

It is our lab's suggestion that the industry consider moving away from including TYM as part of the standard cannabis testing panels and instead, test for additional yeast or mold species that commonly grow on cannabis and are dangerous to humans. Some of these microbes include Aspergillus species, Botrytis (bud rot), powdery mildew, Fusarium species (root rot), and other Penicillium species. One of the biggest pitfalls of TYM testing is that even if a sample contains less yeast and mold than the regulatory limit, there is still a chance that the microbes present could be extremely dangerous to humans. However, if speciation testing is not being performed to ensure that those microbes are not present, TYM analysis does not truly protect the consumer. It is important to note that some of these microbes do not culture well on some of the commonly used agars. As a result, labs may need to use molecular techniques, such as qPCR, to properly identify these contaminants.

There are clearly benefits and pitfalls to both plating and qPCR methodology. The quantitation of microbes can be extremely difficult based on the sample matrix and the homogeneity of the aliquot being used. While TYM analysis may be able to pinpoint when a grower should be concerned about the cleanliness of their processes, it may not be the best analysis to determine if products are safe for human consumption. The food and pharmaceutical industries have extensively researched how analyses such as TYM work for their products and ultimately, the cannabis industry needs to apply that same level of scrutiny to TYM in cannabis products.

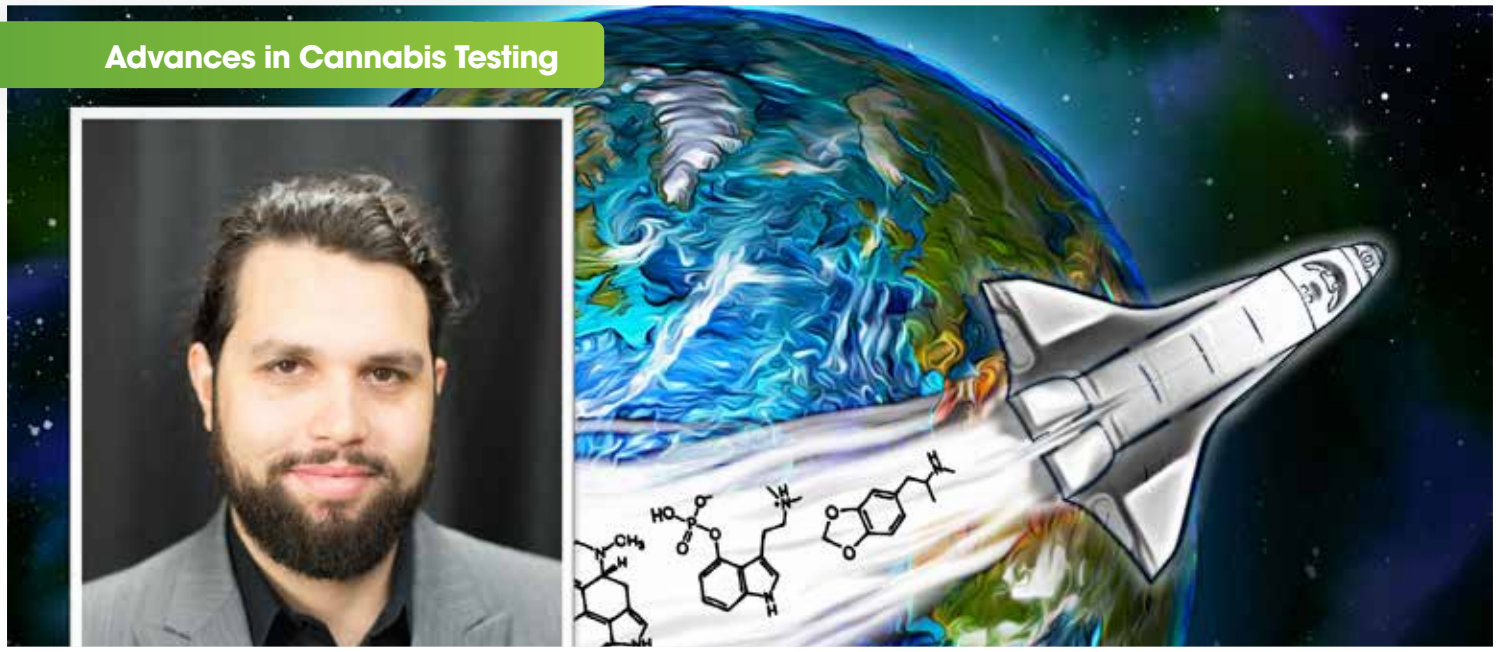

Image credit: Marcu & Arora

Launching the Cannabis and Psychedelics Industries: An Interview With Dr. Nigam B. Arora

By Leo Bear-McGuinness

Analytical Cannabis' Scientific Advisory Board grew by one new member earlier this year thanks to the addition of Dr. Nigam B. Arora, a founding partner of the consulting firm Marcu & Arora and co-host of the podcast How to Launch an Industry.

To mark the addition, we caught up with Dr. Arora to discuss his podcast, his role as an advisor to cannabis and psychedelic businesses, and what the future holds for both sectors.

Leo Bear-McGuinness (LBM): How did you come to specialize in cannabis and psychedelics?

Nigam B. Arora (NBA): Traditional and alternative medicine has been an interest for as long as I can remember. Eastern and indigenous cultures have used a variety of plant, fungi, and other natural remedies for millennia. The value of the modern western medical model is also undeniable; my PhD pursuit in interdisciplinary life science with a focus on organic and analytical chemistry was directly influenced by these perspectives. I trained in separations/extractions of compounds from natural products, synthesis of novel molecules, as well as the translational process – developing innovations from the lab into products and technologies that impact the field of health and medicine. These experiences primed me well for work in cannabis and psychedelics as both fields seem to be converging at the nexus of ancient knowledge and modern medicine. Since then, I've been working day in day out in a variety of roles in the cannabis space, including building and operating state licensed business infrastructure, formulating novel products, and working on a variety science-based projects for Marcu & Arora's clients.

The deep dive has continued into the psychedelics industry: reading all the peer reviewed literature in the space and following both major and emerging players. This includes a variety of entities. The work going on at well-funded and publicly traded companies like Compass, Atai, Cybin, MindMed, etc. are of course of interest. There is equally interesting science and major potential at smaller companies working on niche technologies or their own suite of novel chemical entities. There are some very meaningful non-profits in the space as well, Chacruna Institute, Decriminalize Nature, Psychedelics Bar Association, among others, are doing important work.

Beyond reading, studying, and doing hands-on work, the opportunity to interact and work with a variety of leaders in both the cannabis and psychedelics industries has been invaluable. Many of these folks have been guests on our podcast, How to Launch an Industry, so interested readers can check out howtolaunchanindustry.com to learn more.

LBM: What type of work does your firm Marcu & Arora do?

NBA: Our firm is highly active in all things life science related in cannabis and psychedelics. In the cannabis space

we've worked with multi-state operators (MSOs) and multinational cannabis companies supporting their R&D and safety initiatives. This can take the shape of designing highly specialized laboratories and experimental protocols or technical reporting on a variety of niche scientific topics that are of interest to our clients. In the psychedelics space we've worked closely with manufacturers who are supplying active pharmaceutical ingredients (APIs) for research studies and clinical trials. We also provide valuable resources to investors and venture capital firms by performing scientific due diligence.

LBM: How did you come to start your podcast? And how has it been received?

NBA: We started the podcast in conjunction with the launch of Marcu & Arora. We hold an abundance of knowledge about the science and policy of cannabis and psychedelics at our firm and believe in the importance of starting open access and fact-based conversations in those areas. The podcast is our way of doing just that. The show has been well received and we've attracted numerous prominent industry experts as guests including founders, medical doctors, attorneys, and of course PhD scientists. For our most recent season, several of our guests have joined the cast of the show, allowing us to offer even more in depth and diverse perspectives to our listeners.

LBM: With so many podcasts out there, what makes How to Launch an Industry unique?

NBA: There are several unique aspects of our show. For each episode we curate a conversation among a diverse group of experts. Episodes are not focused on a particular individual or company, but rather on the group discussion of hot topics in the industries. Each episode is structured into three segments, we share a fun and educational game with the listener, followed by news, and we conclude with Rapid Fire Science, where we perform a critical review of a recent peer reviewed publication.

Another unique aspect: we collaborate with an artist to create custom cover art for each and every episode. We believe in the power of art to help convey and interpret ideas and feelings. We've worked with some incredible artists and encourage everyone to check out the fantastic album covers for each episode.

LBM: In the podcast, you regularly discuss good practices for cannabis businesses to keep moving forward. These tips may be more useful to business in certain locations. California, for example, still has a thriving illicit cannabis market four years on from the legal market's opening. How can businesses affected by such stiff competition keep moving forward?

NBA: Different markets certainly have individual concerns but there are good practices that standards organizations and the literature would suggest are applicable across the board. I'll share some examples that have come up over the last couple years on the podcast: standardized and ethical practices in analytical testing of biomass and products; consistency and transparent reporting in the manufacturing, storage, and handling of products; product labeling and consumer-focused education that is easy for consumers to understand and use to consume in an informed fashion. As the industry matures, these will become a requirement for companies that want to maintain trust and brand loyalty with their customer base.

California is unique in several ways. For one, some analyses suggest it is the world's largest cannabis market and that it was that way long before recreational legalization. Your question is a good one, though. There is no silver bullet here but there are a few key things we know from other sectors that the industry and regulators can strive for to improve the legal market's ability to compete with the illicit market. The first, and one that advocates and business owners alike have been actively calling for, is a reduction in cannabis specific taxes. Cannabis is currently one of the most highly taxed consumer goods. This level of taxation has been, and will continue to be, prohibitive to companies seeking to put quality products on the market at a price that's palatable to consumers. There is some potentially good news on that front recently in the form of Assembly Bill 195.

The second is for cannabis companies to continue to implement some of the items mentioned above, which lowering the tax burden will be a boon to. These types of changes can improve consumer trust in products from the legal market and provide strong differentiation compared to products from the illicit market. It should also be mentioned, there are likely some shifts regulators could make to licensing structures for outdoor cultivation that would allow more legacy growers, whose shoulders we all stand on, to participate in the legal market rather than being pushed out of it. To sum up, improving quality, lowering costs, and including the folks who built the industry in the first place would all benefit the legal market in California.

LBM: You speak to so many cannabis experts but seem to have a growing focus on psychedelics. In your view, how far behind is the field of psychedelics testing from the cannabis testing sector? What has to be figured out?

NBA: Whenever speaking about psychedelics, I segment the industry into two very broad buckets: the

pharmaceutical path and the natural whole plant or whole fungi path. For the pharma path, folks working in that area generally have the dual benefit of licensure for working with scheduled substances and analytical chemistry capabilities of pharma at their disposal. Because of this they are able to move more rapidly in the testing and development of compounds. Folks working on the natural path are commonly at the reciprocal disadvantage of not having licensure to work with scheduled substances and not necessarily having a suite of high-quality analytical instruments at their disposal. In either path there is significant work to be done on making standards for a broad range of molecules as well as protocols for analysis widely available. Currently, on the natural path, those doing the work seem to have enabled access to their own instruments and the know how to put together workable protocols. As the industry matures and decriminalization efforts continue, it would be great to see more analytical testing companies be able to offer services for natural psychedelics. Our team would love to work with groups interested in moving those types of analytical services forward.

LBM: You've recently launched a new season of the podcast. Is there anything new on the horizon listeners can look forward to?

NBA: We are planning a few live recordings at events and conferences. Make sure to follow How to Launch an Industry and Marcu & Arora on social media to stay up to date on those occasions. And if you're planning an event, let us know and we might bring our team! Feel free to reach us at hli@marcu-arora.com.

Dr. Nigam B. Arora, founding partner of the Marcu and Arora firm and co-host and co-producer of the How to Launch an Industry podcast, was speaking to ***Leo Bear-McGuinness***, science writer at *Analytical Cannabis*.

Image credit: PerkinElmer

How Strategic Cannabis Testing and Analysis Data Management Can Help Transform Product Quality and Compliance

By Toby Astill, global market manager for cannabis and hemp at PerkinElmer, Inc.

Cannabis labs today experience no lack of challenges. From high staff turnover or shortages to geographically diverse regulations and labeling inaccuracies, the industry is constantly on its toes to keep up with demand for innovative products and increasing sample complexities and volumes.

However, the thread running through each of these areas is the same one that can open opportunities to help tackle industry pain points: data.

Creativity and the unique growth mindset of the cannabis industry has led to staggering product innovation and advancements within how technologies are leveraged. This same mindset has been instrumental in revolutionizing testing and analytical practices and is now leading the charge in data management.

If cannabis labs can quickly, completely, and accurately capture and leverage their testing and analysis data, they can make more timely and effective data-driven decisions. This can also reduce human error, get new staffers up to speed more quickly, and ensure increased data traceability and integrity. All this points towards delivering more innovative and compliant products that hit the mark for regulators and consumers alike.

But where to start? When thinking about tapping into the power of testing and analysis data, there are some key things to keep in mind.

Defining data management and thinking beyond the cannabis industry

Whether a cannabis or hemp lab is processing 10 or 10,000 samples a day, standardized data management can benefit every step of the workflow. At a minimum, data management refers to how results are recorded and stored. However, other factors such as accurate data capture also play an important role. From plant to beverage, by looking at all of these areas holistically, data analysis, results reporting, and effective R&D collaboration can be enhanced.

It's also helpful to look toward more traditional industries, such as food and pharmaceuticals, where data management has always been a top priority. In the same way that it's vital to ensure that medicines going to patients or food to our tables is accurately tested and labeled, cannabis and hemp products require careful tracking.

The medicinal cannabis market has already seen standardization. Furthermore, across Canada, all

cannabis labs (recreational and medicinal) must be good manufacturing process (GMP) compliant. With more and more companies using medical trials for their pharmaceutical-grade products, the necessity to comply with the FDA's structured and stringent requirements has risen. Although most labs already meet ISO-17205 or GMP guidelines, these only specify the steps needed to be taken in order for a product to be approved, and not which instruments, systems, or technology should be used.

Four key ways to enhance your cannabis data management

When analyzing cannabis, there are up to 15 different points along the workflow – from sample preparation to final quality control – where capturing and passing along accurate data is critical to the final result. Even just one error can become a major headache for labs as it's compounded throughout the value chain. Therefore, accurate recording and movement of data is critical for reducing errors. In order to ensure better data management and integrity, below are four solutions and technologies for consideration to improve how you handle data in the lab:

1. Higher throughput and more data: how automated workflows can help

To handle the increased volume of samples that may enter an in-house or contract lab on any given day, many cannabis scientists are turning towards automation technologies. These solutions, comprised of instruments with robotic capabilities and software, can help alleviate many issues for cannabis and hemp labs in terms of throughput. They can also generate better data at a faster rate. Automation touchpoints can span across customer management; order entry, sample prep and analysis, post-analysis review and test result verification, sample reporting and certificate of analysis (COA) generation – they can all be integrated in one data management package.

Today, cannabis and hemp labs are heavily reliant on manual manipulation of samples, the use of multiple reagents, and the dedication of several staff scientists. Implementing automation technology improves sample turnaround time, enhances data accuracy, increases overall personnel efficiency, and supports easier compliance by providing the tools that remove the need for human intervention. Additionally, the intuitive software solutions that are included mean tasks such as inventory management can also be taken care of. This includes monitoring the number of samples a lab needs to process in a day and keeping track of the volume and expiry dates of reagents needed for that workflow. By letting software handle these more menial tasks, technical staff have more time to dedicate to developing more innovative products.

2. Growing as an industry: from spreadsheet to digital data capture

Many labs currently face challenges moving hundreds of samples (if not more) a month across the multiple steps. In a budding industry such as the cannabis and hemp sector, there's still a huge range of data management techniques in play, from the cumbersome use of noting down values with pen and paper, to more elegant solutions such as digital balances that can send data values directly to the cloud. With pen and paper, there is no guarantee of data integrity, the incidence of unforced error is much higher, and there's an increased risk of data being altered along the way. However, electronically controlled formats often come with built-in software privileges that only allow some people to access and edit the data. Not only does this ensure the integrity and accurate transfer of better data in a more time-efficient manner, but it also negates the risk of intentional data manipulation.

As many staff don't have the programming or IT knowledge to move data efficiently, the implementation of standardized data management protocols in contract testing labs across the industry would help assure initial data accuracy and reduce unknown, compounded errors. More defined data management guidelines could empower cannabis scientists to confidently make data-driven decisions. Software tailored for each step along the workflow means that the burden of dealing with complex data input and analysis is removed. Furthermore, knowing test results have not been altered by accidental or deliberate changes, labs can also enhance efficiency, costs, and resource leverage while reducing waste – ultimately delivering higher quality products to consumers.

3. Needle in a haystack: data visualization to improve efficiency

Data visualization is becoming pivotal to the analytical side of cannabis testing. With the growing number of cannabis-infused products and varying matrices, scientists can now generate an abundance of data from just a single sample. To help scientists pick out the most important data points and assess how they relate to one another, graphs and pie charts can be used to paint a visual picture of the data. This not only benefits customers but also consumers. For example, there are programs that have been developed to visually display the cannabinoids and terpenes present in a sample. This allows producers to create more diverse and nuanced products and for consumers to, in turn, be able to choose more personalized product options and experiences.

Lab stakeholders are also empowered not only by the volume of analytical data they can obtain from analyzes, but by ease of interpretation. Standardization in this area would ensure all labs follow the appropriate analysis and

visual reporting along the workflow, implementing an effective overall approach to product safety and quality control while also improving risk management.

▲Cannabis automation. Image credit: PerkinElmer.

4.Thinking outside the box: looking at all areas of the workflow

An old saying goes: a workperson is only as good as their tools. In a similar vein, for cannabis labs, analytical accuracy can only be as good as the instruments being used. Although data recording and analysis can be improved by implementing the practices and solutions discussed above, individual value accuracy is still reliant on the use of sensitive, high-quality instruments. Furthermore, standardization in other areas of the workflow, such as the use of CRM-certified standard reagents, can improve data integrity and management.

When cannabis and hemp labs take a moment to zoom out and examine the wider picture of their workflow, they can better identify the areas in which errors could occur in relation to data management – similar to how a risk assessment would be carried out when handling a certain reagent. After identifying potential sources of error, labs can ensure they have the most up-to-date instruments and standardized, in-date solvents to carry out effective sample analysis. This helps reduce the incidence of error outside of data handling to continually ensure integrity of data and confidence in results.

As the industry continues to innovate, evolve, and grow, cannabis labs must work towards building new methods, best practices, and more uniform quality and safety standards. Increasingly educated and diligent consumers want to be assured that the products they reach for are what they claim to be. Cannabis labs and producers, tasked with quality assurance and regulatory compliance testing, are therefore entrusted to ensure the quality and safety of their products. Rigorous and smart data management can help meet consumer and regulatory pressures.

In cannabis, data are incredibly important and pivotal. Small changes can alter whether a product is fit for market or not and there may be millions of dollars riding on individual samples. When labs can demonstrate data accuracy and integrity, it provides them with a significant advantage in the industry and an easier path to compliance. Strategic data management provides labs with the tools they need to reach product integrity and support traceability. As such, the practice is a vital framework for labs and their business.

With the implementation of automated technology and a review of data management procedures, more accurate data are recorded and move through the workflow. The right approach towards laboratory automation and digitization is unique for every lab and driven by the requirements of the business. For cannabis labs looking to expand into new areas of application or digging deeper into their core strengths, a process of better managing data is the tool to empower their business.

Heavy Metals Analysis in Hemp Extract Products

By Alexander Beadle

Following the federal descheduling of hemp under the 2018 Farm Bill, many US state legislatures were quick to develop their own hemp crop programs and legalize the cultivation and sale of hemp within their state. In Florida, a 2019 statute legalized the growth and sale of hemp and gave the Florida Department of Agriculture and Consumer Services (FDACS) regulatory authority over the local hemp industry.

Speaking at *Analytical Cannabis'* The Science of Cannabis Extraction 2021 online symposium, Diane Pickett, chief of the laboratory within the FDACS' Division of Food Safety, and Serena Giovinazzi, PhD, environmental manager of the Bureau of Quality Management at the FDACS Division of Food Safety, detailed new results from their laboratory's research into heavy metal contamination in hemp extracts.

Hemp and food safety in Florida

As defined by the 2018 Farm Bill, the term hemp refers to any plant from the Cannabis sativa species containing less than 0.3 percent THC by weight. Hemp products are therefore any product or substance that is derived from or contains hemp.

"Because hemp extracts are intended to be ingested, they are regulated as food in Florida," Pickett explained. "And there are many benefits to this; the food industry has been established for over 100 years, this lets us apply all of the knowledge that we've already gained and the lessons we've learned on contamination, sourcing, and packaging issues."

"This also helps the hemp industry, which is relatively new to food regulations, to not have to reinvent the wheel."

Just like any other food product, hemp products in Florida must be tested to ensure that they are safe for ingestion. This involves routine testing to ensure the absence of any harmful residual pesticides or biological pathogens in the edible product, as well as cannabinoid-specific testing.

"We have analytical methods for determining cannabinoid content, which we use to evaluate two different potential issues," Pickett said. "First, we measure the THC, because to be considered hemp it must have less than 0.3 percent THC. We also measure the amount of other cannabinoids, for example CBD, to determine if the label claims are accurate."

This sampling by FDACS is done at retailers across Florida, collecting products from store shelves and testing their contents. This is different to the tests that producers may do following manufacture, Pickett emphasizes, and is designed to reflect the state of products as they reach the consumer.

Heavy metals in hemp extract products

Heavy metals are a particularly important aspect of hemp product testing. The cannabis plant is a known bio-accumulator, meaning that it readily absorbs minerals and nutrients from the soil it is planted in and accumulate these nutrients in its leaves. While this property makes hemp a

useful crop for soil remediation, as it will suck heavy metals and pesticides out of contaminated soil, it proves a problem when the hemp is intended for human use.

Effective heavy metals testing protocols are crucial to protecting consumers from the harms of accidental contamination. As Dr Giovinazzi explained, heavy metal poisoning can result in severe nerve damage, neurological issues, cardiovascular problems, and greatly increase the incidence of certain cancers.

The FDACS Division of Food Safety uses inductively coupled plasma mass spectrometry (ICP-MS) to inspect the products it collects for heavy metal screening. In a recent examination of 206 hemp extract products, the FDACS Division of Food Safety found 10 products to contain lead above the 0.5 parts per million (ppm) regulatory safety limit; two samples contained high enough levels of lead to violate the Resource Conservation and Recovery Act (RCRA) limits for hazardous waste.

"To be honest with you, we have found a greater number of samples than expected to contain toxic heavy metals, lead in particular," Giovinazzi said. "So, as you can imagine, we immediately started investigating all the possible sources. Is it the plant? Is it arising from the processing? Or the action of manufacturing? Is it the packaging material or container?"

Heavy metal leaching raises concerns

The FDACS Division of Food Safety carried out an additional investigation into the sources of this contamination using information provided by three manufacturers. Presenting data from this ongoing investigation for the first time at the *Analytical Cannabis* symposium, Giovinazzi revealed that this preliminary data indicates heavy metals are leaching into hemp extract products over time.

For example, one representative product tested in January 2019 returned a lead content of less than 0.01 ppm. When tested again in April, this level had risen to 0.45 ppm, reaching 0.8 ppm by January the following year.

"This prompted us to look more in depth into this issue," Giovinazzi said. "Our laboratory's division for inorganic chemistry investigated the leachability of lead in hemp extract packaging material. The study was designed to test packaging components separately – bottles, caps, and graduated droppers – which were produced from two manufacturers."

"The leaching [study] was conducted with two commonly used carrier oils, hemp seed oil and MCT oil, and measured lead concentration over time."

In both carrier oils, samples which had been exposed to the graduated droppers showed elevated levels of lead over time, insinuating that these could be a source for lead leaching in finished hemp extracts. FDACS research into the potential sources and preventative measures for heavy metal leaching in hemp oils is still ongoing.

"We do not have all the answers for all contamination identified so far. We are still working on gathering information on all potential sources, such as plant accumulation or extract processing," Giovinazzi said.

Working with industry to eradicate heavy metal contamination

All of the products identified by FDACS in its market studies were removed from commerce in Florida; two brands also chose to issue nationwide recalls. Moving forward FDACS are continuing to raise awareness about the potential dangers of heavy metal contamination in hemp extracts and will work closely with the industry to identify possible sources.

"We cannot stress enough how important it is to test your extract and packaging material. And we need to make the consideration that testing [...] the finished product after production may not account for lead leaching," Giovinazzi said.

"We are always seeking ways to collaborate with industry, and with today's [sic] webinar we raise awareness on this issue. Definitely we urge you to consider the quality and safety testing of your packaging materials, not only your extracts."

Five Ways Cannabis Testing Labs Can Leverage Data

By Patrick Callahan and James Brennan, director and sales and marketing specialist, respectively, at LabWare Analytics

As more states initiate medical and adult-use marijuana commerce, the industry is working continuously to establish credibility and produce high-quality products to satisfy consumer demand. Knowledgeable consumers, including many patients, are concerned about cannabis product safety and consistent quality. Overall, the cannabis industry realizes the value of proper regulations and stringent third-party analytical testing. Testing services ensure cannabis products meet consumer expectations for quality, safety, and compliance. Cannabis testing laboratories help growers, cultivators, and processors to:

- Build consumer and healthcare provider trust.
- Meet numerous state regulatory requirements.
- Mitigate risks in a rapidly expanding market.
- Focus on superior products.

At the top of many efforts in the cannabis industry is the use of quality standards. Many cannabis testing laboratories receive accreditation for their conformance to the ISO/IEC 17025:2017 standard, which provides "General requirements for the competence of testing and calibration laboratories." But some organizations have gone further. The ASTM Committee D37 on Cannabis has developed standards to address quality control, safety, and compliance in the cannabis industry. AOAC INTERNATIONAL also created the Cannabis Analytical Science Program (CASP) to promote analytical excellence with cannabis testing standards.

Cannabis industry standardization is becoming even more essential as the US Food and Drug Administration (FDA) increases its attention on this growing industry because patients use marijuana for numerous medical conditions. Committed to protecting public health, the FDA has offered guidance documents for cannabis products with an eye toward CGMPs (current good manufacturing processes). A recent article authored by members of FDA's Botanical Review Team evaluated how closely the current state-level regulations follow CGMPs. They found some of the written state regulations to be insufficient to support a submission to FDA for human clinical trials.

To compensate for the current and future guidelines, many cannabis testing labs, from start-ups to seasoned multistate operators, are relying on data to monitor the performance of the lab, reduce the variability in testing, automate lab testing, and, as a whole, align with growers to meet the need of any future requirements from both consumers and regulators.

State of the industry:

Today's cannabis industry lacks harmonized regulations but is evolving towards quality-centric operations. In addition to maintaining compliance with various state laws, many organizations are developing standard operating procedures across state lines to gain efficiencies through standardization. Cannabis companies are starting to embrace the benefits of CGMPs, purpose-built laboratory informatics that support cannabis regulatory compliance, and advanced data practices to maintain this level of quality and meet the growing complexity in the market.

Five ways to leverage data to meet today's and tomorrow's challenges

1. Laboratory data integrity is an essential practice for high-quality cannabis.

Producing high-quality cannabis products relies on making sound business decisions based on laboratory data. The integrity of these data should address their completeness, consistency, and accuracy. The FDA issued guidance in 2016 explaining the principles of complete, consistent, and accurate data that should be attributable, legible, contemporaneously recorded, original or a true copy, and accurate (ALCOA). These data integrity concepts have expanded to ALCOA+ and similar acronyms to include complete, consistent, enduring, and available data. Data integrity is essential for any system that receives, stores, processes, or reports data generated during cannabis testing. Robust laboratory information management systems (LIMS) and practical data analysis tools can monitor laboratory performance and provide business intelligence to the laboratory and its clients. Systems that support data integrity concepts can ensure cannabis testing laboratories manage, analyze, and deliver high-quality data.

2. Take a data-first approach to reducing variability in cannabis testing.

Rigorous data management should be at the top of the testing laboratory infrastructure list. The process starts when a customer places an order for testing with the lab. Adherence to the principles of good laboratory practice (GLP), ISO/IEC 17025 accreditation, and a high-quality LIMS can effectively reduce data errors and variability in cannabis testing laboratories.

3. Meet demand without sacrificing high-quality results.

Cannabis testing laboratories can realize a connected ecosystem of data management technology applications: client test ordering portal, LIMS, quality management system (QMS), and data analytics to enable cost reduction, efficiency, and optimization.

As testing laboratories mature with the entire cannabis industry, they must rely on automation to meet client demand and deliver high-quality results. Various commercial solutions support the automation of the many processes in a cannabis testing lab. Selecting commercial systems developed with quality built-in and features that support regulatory compliance is crucial.

4. Leveraging data to analyze what happened.

Data automation should alert users to out-of-specification (OOS) results and support complete traceability of all events as the data moves through the laboratory. Data flagging can bring focus to a test failure that requires further action. Audit trails support data integrity and provide insight into the steps taken, who took the steps, and when. Inspection of the audit trail will show if any system user added or removed tests and if they edited test results. Audit trail review after results authorization and through to the final step of sample COA release can identify any irregularities and implement corrective action to improve laboratory processes or initiate personnel retraining. Leveraging audit trail data throughout the process is a good quality practice and builds credibility for the laboratory.

5. Leveraging data to predict quality

Cannabis testing laboratories overflow with data that can provide actionable insights to help achieve performance goals. They present the testing results in many ways as an offering to the producers. A roadmap for cannabis analytics starts with getting consistent quality data at the beginning of the testing process. Maintaining data integrity at all steps will ensure that any subsequent reporting or analytics are the highest quality. The lab can offer its customers reliable analytics beyond the CoA, such as trending cannabinoid makeup by strain or presenting outliers in heavy metals results.

Modern statistical methods coupled with the correct data collection and technical strategies offer a means to develop predictions that can inform optimizations in the sample testing process at the lab and the production process for the client. The system that captures sampling results is the operating system of the lab, so keeping all information in an environment with clean and consistent data is imperative to leveraging advanced analysis and predictive tools. To ensure the analysis is effective, the first step should always be to evaluate how the data will be operationalized (or used) and how to implement changes directed by the predictions from the operator's perspective. Ensure you have a team that understands why a prediction is showing a specific quality measure. Finally, a solid and tested lab information management system is key to managing the data and the process.

Conclusion

The demands of running a complex laboratory as a successful business are immense, but the effort to support cannabis product safety and quality is vital. Looking to data can help the cannabis industry proactively address challenges and be ready for inevitable changes. Cannabis testing labs can learn from other sectors with existing

processes to leverage data for efficiency and quality. Efforts to develop standards for cannabis and hemp continue to be developed and contribute to credible and reliable results. While the federal enforcement priorities for cannabis remain unclear, the industry can deliver cannabis quality now by looking to established regulated industries, such as environmental, food, and pharmaceuticals, where quality is built into harmonized processes and systems from the start and not added later. There is little doubt that high-quality data collection and analysis will continue to impact the future growth, credibility, and acceptance of the cannabis industry.

Waste Stream Management for Cannabis Operations

By Alexander Beadle

Cannabis is an extremely popular product, there's no denying it. So the idea of significant volumes of cannabis products going to waste might even seem laughable.

But the retail market is only one half of the equation. Getting cannabis from the fields and onto dispensary store shelves is a significant undertaking, and one that requires lots of processing and diligent quality testing. These cannabis operations also produce a notable amount of waste product, and operators must dispose of it accordingly. But what does that involve?

The diverse world of cannabis waste

The term cannabis waste might bring to mind the off-cuts of some cannabis plants that need to be dealt with. But in reality, the pool of items that can be classified as being cannabis waste is extremely broad.

"If you're in the cultivation facility it means one thing, if you're in the production lab or production facility it means something else. In the testing laboratory, it has yet another meeting. In all of those places waste is defined, and sometimes it's defined for them by the regulatory body," Dr Susan Audino told *Analytical Cannabis*. Audino is a chemistry laboratory consultant at SA Audino & Associates, LLC, and a scientific advisor to AOAC International's Cannabis Analytical Science Program (CASP).

"In general, in the laboratory, cannabis waste is the leftover product that a client drops off for testing. They drop off this much, but the lab only uses that much, that means we have an amount that becomes raw product waste," Audino explained.

"Then there's also the waste product – you mix up your cannabis in a bottle of something and now we have to get rid of that bottle that happens to contain some cannabis."

Every different stage of cannabis production will have some kind of waste product, Audino explained. A cultivation facility might have plant material that has been contaminated by pesticides or mold that needs to be disposed of, and a dispensary might have products that have gone past their sell-by date. Hazardous cannabis waste usually refers to that which is contaminated with pesticides or residual solvents. In the case of retail waste, this could also mean items such as spent vape pens containing lithium-ion batteries.

Naturally, cannabis operators cannot dispose of their plant off-cuts in the same way that they would this hazardous waste. Improper disposal of cannabis waste could cause serious harm to the environment, but also to human and animal welfare – if products are not disposed of in a way that makes them undesirable and/or unusable, it heightens the odds of stray animals or "dumpster divers" coming across cannabis materials and unknowingly consuming them.

Waste disposal methods

Given cannabis' status as a controlled substance under federal law, improperly disposed of waste material could bring an operator to the attention of the US Drug Enforcement Administration (DEA) and lead to hefty penalties. Other federal statutes such as the Clean Water Act also need to be considered when an operator is drawing up their disposal plan.

But federal prohibition also means that disposal procedures have largely been left up to the individual states to decide. Proper disposal techniques can therefore vary significantly between states, and operators should always check with their local regulatory body to confirm what is allowed.

The four most common disposal methods for cannabis waste are composting, landfill, incineration, or in-vessel digestion. All of these intend to render the cannabis waste as "unusable and unrecognizable," thus limiting the risks that this waste might pose.

Compostable waste, such as plant waste, cannot be composted immediately. First, it must be mixed with at least 50 percent non-cannabis compostable waste, such as food waste, yard trimmings, manure, or wood chips. The same is true for non-compostable materials that are sent to landfill – these need to be mixed with other wastes such as cat litter, sawdust, or plastics.

"I believe that those scenarios were put in place to prohibit people from or dissuade people from going through the trash and pulling out unused flower," Audino explained. "Who wants to go through kitty litter? Early on, many facilities were faced with people going through their trash."

Incineration is another common method where the waste is sent to a licensed municipal solid or hazardous waste incinerator to be burned, depending on state regulations, or burned in a special incinerator on-site. The open burning of cannabis plants can cause other public health concerns, whereas disposal at a municipal facility means that the emissions from the waste can be more carefully controlled.

In-vessel digestion systems and facilities can also be used for the disposal of organic cannabis. Here, waste is decomposed using bacteria or other biological elements within a sealed vessel under aerobic or anaerobic conditions to render it unusable. Once the digestion process has run its course, the remaining material can normally be repurposed as compost or disposed of at landfill.

"Another [method] is to chemically degrade it," Audino added. "It is getting recycled in an attempt to remediate or to create a different product. So if the material fails on some particular level, they say okay, we'll just repurpose that and use it for something else where the specification isn't this low and I have more opportunity to do more things with it."

"From seed to destruction"

One of the biggest issues concerning cannabis waste stream management today is not so much a lack of regulation, but a lack of effective oversight.

"It is very, very difficult to monitor," Audino said. "With seed to sale, you type in a lot number and you can track that down the food chain. With waste, there's no way to track it [...] so they are taking laboratories and cultivators and product managers and dispensaries at their word when they say, yes, we destroyed it."

"Not everybody is going to lie," Audino added. "But I know that the black market is thriving in response to this."

This feeds back into the idea of needing to make cannabis waste unusable and unrecognizable, so that it cannot be salvaged by bad actors. But as things stand, it is difficult for operators to prove to regulators that they are in fact doing this transformation and disposing of their waste responsibly.

"I am aware of a new piece of equipment that has not yet hit the market, that should be hitting the market here very soon, but that provides an audit trail of exactly how much waste has been deposited into the machine with a date and timestamp, and then how it's been destroyed by an external independent third party," Audino said. "That can then prove that the cannabis waste has been destroyed."

"It was developed to provide that audit trail of objective destruction that can then tie right back into the seed to sale. The goal with this is to extend the philosophy from seed to sale to seed to death, or seed to destruction."

Until this equipment arrives and becomes commonplace, Audino believes that improved oversight should be a key focus for the nation's regulators.

"It is a problem that, I hope, is on the priority list of CANNRA [the Cannabis Regulators Association]," said Audino. "I hope that they are looking at this as a problem and that they're trying to solve that problem. Because if black market materials end up going into the wrong hands, people will get sick, and then it reflects badly on the legitimate industry."

Tips for Advanced Cannabinoid Analysis

By Adrijana Torbovska

Cannabis sativa is used across the world for its therapeutic properties, provided through its 144 cannabinoids, THC and CBD being the most well-known.

Under EU Regulation 1308/2013, it is legal to cultivate and supply cannabis plants if the THC content is limited to 0.2 percent weight per weight (w/w). On the other hand, medicines containing THC can be prescribed in the quantity necessary for treatment up to 30 days but not exceeding 7.5 grams.

Under the US's 2018 Farm Bill, cannabis plants and derivatives that contain no more than 0.3 percent THC on a dry weight basis are not considered to be controlled substances under federal law. Instead, the Food and Drug Administration (FDA) has the authority to regulate products containing cannabis or cannabis-derived compounds.

Whatever side of the Atlantic you are on, it is vital that cannabis products are tested with rigorous methods, so that consumers can be certain they will be provided with safe products that elicit their advertised effects. Thankfully, advances in analytical methods have resulted in impeccable detection and quantification of a wide range of cannabinoids in the last decade.

With all of that in mind, this guide aims to provide quality control analysts and R&D method developers an overview of the key considerations relating to advanced cannabinoid analysis.

LC-DAD or LC-MS

Cannabinoids are chemically quite similar, and all have a maximum absorption at around 228 nanometers (nm). So, achieving a perfect peak purity when developing an analytical method for cannabinoid analysis in complex matrices can be quite challenging.

Liquid chromatography coupled with a photo diode array detector (LC-DAD) is the standard instrument configuration for potency testing of cannabis and hemp products. The instrument provides acquisition of full UV-Vis spectra for better identification of compounds, especially ones with similar spectral signatures.

On the other hand, liquid chromatography coupled with mass spectrometry (LC-MS) offers some sensitivity and selectivity advantages as it provides identification based on mass. As such, LC-MS offers mass identification that provides total peak purity when analyzing cannabis complex matrices. The most common ones are single quadrupole (SQ) MS and triple quadrupole LC-MS/MS instruments. In the first case, the fragmentation of the precursor ion is known as source-induced dissociation (SID), which is used for mass identification of cannabinoids. SID is also often referred to as "in-source" fragmentation. LC-MS/ MS also provides precursor ion fragmentation, but the process takes place in the collision cell. Thus, it is known as collision-induced dissociation (CID) and used for the structural

determination of analytes. Therefore, these instruments provide near-perfect solutions when dealing with cannabinoid analysis in complex matrices.

In summary, LC-MS provides far better selectivity and sensitivity for cannabinoid analysis compared to LC-DAD. Pricewise, however, LC-DAD is around ten times cheaper than LC-MS.

Selecting the right column

Columns with a C18 phase are the most common choice for cannabinoid analysis. The C18 stationary phase is excellent for separation of non-polar or slightly polar compounds, which have a minimum ratio of three carbon atoms for every heteroatom. THC and other relevant cannabinoids possess these characteristics. However, if you are looking to resolve structural isomers, then columns with a polyaromatic stationary phase should be a perfect choice. These columns rely on hydrophobic and a p-p interaction mechanism and are compatible with highly aqueous mobile phases.

Common column lengths range from 50 to 100 millimeters (mm), which is sufficient for sample separation and enable a relatively short run time. One thing that is key when selecting the right column length is achieving good resolution between the critical cannabinoid pairs THCV/CBD, CBD/CBG and delta 9-THC/delta 8-THC, without extending run time.

Column particle sizes range from 1.8 to 5 micrometers (mm), depending on the sensitivity and resolution needed for your laboratory. Smaller particle sizes provide higher sensitivity and resolution. However, one should keep in mind that there is a more popular option nowadays, core shell technology. This technology, which consists of a column filled with particles with a hard core and a stationary phase placed on their outer layer, help keep the pressure lower in the HPLC system, which maintains the quality of the method's resolution and shortens the run time. One thing to keep in mind, though: the core shell technology limits your injection volume to less than 5 microliters (mL), as the analyte only travels through the pores of the outer layer of the stationary phase particles. A column pore size of 100 to 120 angstrom (Å) is quite sufficient for cannabinoid analysis.

Selecting the right mobile phase

When samples contain ionizable compounds, mobile phase pH is one of the most important variables in the control of retention in reverse-phase HPLC separation. With a low pH mobile phase, acids will be better retained. In freshly harvested cannabis material, all cannabinoids are in their acidic forms. When the acid cannabinoids are exposed to heat, over time, the acid molecule degrades, producing CO2, and we are left with the neutral cannabinoid. The number of cannabinoids in acidic form present in the analyzed cannabis plant provides the analyst with information on whether the cannabis plant is freshly harvested or not. Thus, one of the main reasons for acidifying the mobile phase is to maintain a good retention of the acidic forms of the cannabinoids as they are important in every cannabinoid analysis.

The most common acidifiers of the mobile phase in cannabinoid analysis are formic acid and ammonium formate. Formic acid is a common additive component of mobile phases. It is a perfect buffer component when pH (values around pKa of 3.75) must be controlled to achieve separation of target compounds with acidic-basic properties, such as cannabinoids in their two forms.

In most cases, formic acid is present at 0.1 percent (v/v) in the organic and inorganic part of the mobile phases, while ammonium formate is present at a maximum of 10 millimolar (mM) solution in the inorganic part. These ratios and concentrations provide good retention of the cannabinoids in their acidic forms and are safe for the column. Furthermore, the presence of only a low concentration of formic acid in the mobile phase is known to improve peak shape of the separated cannabinoids.

Gradient or isocratic

Gradient elution is a technique where the mobile phase has a varying concentration throughout the run. The mobile phase usually has two components: a weak nonpolar solvent and a strong more polar solvent. The weak solvent predominately consists of water or some kind of buffer with a defined pH. Whereas the strong solvent is usually an organic solvent such as methanol, acetonitrile, or a mix of both. The weak solvent allows the analyte to elute slowly, while the strong one causes more rapid elution of the analyte. The most positive thing about the use of a gradient technique is the ability to allow later eluting analytes to elute faster, by shifting the mobile phase composition from lower to higher concentration of the strong solvent. The second benefit of the gradient method is the improvement in peak shape and peak height. However, the downside of this approach is the elution order changes that happen when changing column dimensions. Another drawback is the need for pre-run equilibration at the beginning of every consecutive run, as the concentration of mobile phase is different at the end of the run compared to its beginning. Lastly, gradient analysis is at its lowest robustness when transferred from one vendor HPLC to another, so it is important to readjust the mobile phase concentration to make up for the difference in dwell volume.

Isocratic elution is a technique where the mobile phase has a constant concentration throughout the chromatographic

process. This method is simpler and faster than the gradient method, as there is no need for pre-run equilibration between sample runs. The positive side of this technique is that the selectivity does not change according to the column dimensions. Therefore, the peaks elute in the same order even if the column length and diameter change to lower or higher values. Isocratic methods are very robust when transferred from one vendor HPLC to another, with almost no additional adjustments needed. With isocratic methods, the peak width increases with retention time linearly in the chromatogram. This leads to a disadvantage for late eluting peaks, which get very flat and broad, becoming very difficult to recognize as peaks.

Sample prep

There are various sample types for cannabinoids analysis, including cannabis plant material, CBD oil, crude oil, and extracts. In this final section we will discuss a few of the most common samples for cannabinoids analysis and share some tips for their sample preparation and extraction.

Cannabis plant samples include aerial parts of the cannabis plant such as inflorescences, leaves, roots, resins, buds, and flowers. The most important thing with these samples is to initially grind the plant material into a fine homogenized powder, which is easier to weigh and provides a proper representation of the whole plant sample. The next step is sample clean-up with solvent extraction. The most commonly used solvents are ethanol, methanol, and acetonitrile. Ethanol is usually the first choice because it is considered more eco-friendly, even if it is more viscous than methanol. Also, it has a high extraction efficacy because of its high affinity for the molecular structure of cannabinoids.

Cannabis plant extraction is usually accompanied by dynamic maceration, vortex or stirring at ambient temperature. To achieve faster and higher extraction rates, many scientists rely on ultrasound-extraction, microwave-assisted extraction, and supercritical fluid extraction. One technique that is not advisable is the QuEChERS method, which involves adding water to the cannabis plant sample. Upon this addition, the suspension becomes very basic, which ultimately converts THCA to THC and may cause additional breakdown products, such as the conversion of CBDA CBD. Furthermore, QuEChERS salts cause an exothermic reaction, which, in the presence of water, will also cause decarboxylation of THCA to THC.

Crude oil and cannabis extracts are highly viscous. Thus, these samples first need to be heated to maximum of 80°C (127°F) before weighing them. This doesn't apply to CBD oils because they have low viscosity as they consist of CBD diluted in different carrier oils, such as olive oil, medium chain triglyceride oil, hemp seed oil, and black cumin seed oil. Oils cannot be injected directly into the HPLC due to their high viscosity. Therefore, an efficient extraction procedure is required. Just like cannabis plant samples, cannabis oil samples are most commonly extracted using organic solvents.