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Coming Soon:  “Cannabis Analysis” Column

Brian C. Smith, PhD, chief technical officer with Big Sur Scientific, has agreed to write a regular column for Cannabis Science and Technology called “Cannabis Analysis.” The column series will begin in our September/October 2018 issue. The goal of the column will be to introduce people in the cannabis industry to the importance of testing, how to interpret laboratory results, how different instruments work and what they are used for, and ultimately how to put all this together so they can run their businesses more profitably. Another goal will be to provide analytical testing novices the information they need to read the rest of the scientific content in our magazine with confidence. Finally, experienced analytical chemists will find the column useful because they will learn about techniques that are new to them or new applications for techniques they are already familiar with.

Thomas Jefferson University Announces Initiative to Study Health Outcomes in Medical Cannabis Patients

The Lambert Center for the Study of Medicinal Cannabis and Hemp at Thomas Jefferson University announced the creation of the mmj.org initiative in a press release on May 17 (1). The goal of the initiative is to bring together diverse stakeholders in the cannabis therapy space, advance scientific understanding of medical marijuana and its derivatives, and provide evidence-based resources for patients and their caregivers.

According to the press release, the mmj.org initiative is a national patient registry in which more than 100,000 medical marijuana patients will share their health outcomes to drive new understanding of the safety and medical utility of cannabinoids used as therapy. The registry will be the largest and most comprehensive clinical database yet accumulated in the field of medical marijuana. Patient recruitment into the registry will start early this summer via an online platform at www.mmj.org and with partnering patient and healthcare organizations and medical marijuana dispensaries across the country.

The mmj.org initiative is being managed through a public–private partnership between The Lambert Center and IoVita, a digital health company focused on technology to connect and empower patients living with chronic diseases.

“Millions of patients with chronic diseases are seeking health benefits from marijuana and various cannabinoids, and many are left to experiment with cannabis products on their own. These patients and their caregivers not only deserve our support, but they can help advance scientific understanding by sharing their experiences in a research registry designed with rigor and scale,” said Charles V. Pollack Jr., MD, director of The Lambert Center, in a press release.

Reference


First NFL Athlete Applies for Cannabis-Based Therapeutic Use Exemption

Mike James, a free agent with the National Football League (NFL) and former running back for the Tampa Bay Buccaneers and the Detroit Lions, is the first NFL athlete to apply for a therapeutic use exemption (TUE) for cannabis. James’s physician, Dr. Sue Sisley, completed the TUE application for medical cannabis on behalf of James back in March 2018. That application was recently denied by the NFL.

According to a press release from the nonprofit organization Doctors for Cannabis Regulation (DFCR) (1), after James suffered a devastating leg injury and surgery he was prescribed opioids to manage his pain. He developed a year-long dependency, which finally led him to seek alternative treatment: cannabis.

The DFCR works with a roster of current and former NFL players to persuade the league to change its regressive cannabis use policy. One of their primary reasons for advocating for this change is the fact that NFL players are four times more likely than the general population to develop an opioid dependency. Even though many states have legalized cannabis use either fully or for medical purposes, the NFL still refuses to give players a viable alternative to opioids.

In an interview with NJ.com (2), James said he was hopeful that he’d be able to keep playing football. “It is a game that I love very dearly,” he said. “I know right now I’m doing something that makes some people uncomfortable, and that I’m going against the establishment to push for a change in the way they look at this medicine. I know there’s a greater purpose here for a lot of guys in this league who I consider family members.”

References

1. www.DFCR.org
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One winter afternoon, a group of professionals and scientists came together to discuss the development of test methods for cannabis. These individuals represented a variety of organizations and viewpoints, from testing laboratories and standards providers to instrument manufacturers. Several government entities, while not officially sanctioned to work with cannabis, participated as interested voices in the process. Since then, this group, which is part of an internationally recognized standards organization, convenes on a regular basis to discuss and debate the testing of cannabis in ways that will eventually lead to standardized test methods for analytes of interest. Time and again the discussions progress down a similar path of proposal, debate, and compromise, which is an integral part of the consensus and peer-reviewed method development process.

Patricia Atkins

How is “good” science achieved when governments take no active role in establishing limits, validating methods, or issuing guidelines? How can standards organizations fill the void by not only trying to establish guidelines, but also to create policy in the face of the need for scientific accountability and the reality of a highly economically driven and price-conscious analytical environment? How does one demand “good” science and high accuracy when there is no legal reason for accountability, little consensus of official guidance or methods, and a driving force of economic competition between laboratories that rewards fast, cheap results that favor the manufacturers and distributors? The balancing act between “good” science and scientific accountability is widespread in the cannabis community, from the growers to the testing laboratories and beyond.
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Standardized analytical methods are constructed from templates or guides, specifying key areas that are integral to the method such as scope, applicability or use, preparation and analytical procedures, analytical needs (laboratory equipment, instrumentation, standards, and so forth), performance requirements, and statistical analysis (precision, bias, and validation). In addition, current or proposed applicable regulations and requirements are examined and incorporated into the methodology. There is an understanding that the methods will be built on the rules of “good” science; they will follow the scientific method, be proven reproducible, and represent the sample or the testing appropriately. The method should function in real-world use without any agenda other than producing accurate results, which achieve satisfactory precision and bias requirements (1,2).

These methods are commonly based on either governmental or industry-driven specifications and regulations. When a new analytical sample type or product enters the testing world, it is routed to a well-established market or industry (such as environmental, consumer safety, pharmaceutical, or food safety), which then takes over its governance. Once routed, the method creation procedure is followed and appropriate governmental and accreditation agencies put their marks on the process before it is incorporated into a laboratory workflow. As testing progresses over time, methods are updated and requirements are refined but, still, the consistency of the science and the data is expected to maintain its demonstrated level of quality. The methods are supposed to represent the best available test procedures for the intended use and reflect technological advances and requirements (2).

The steps of the standard process are well-proven. For many new industries the road to regulation and testing, despite some bumps or minor detours, is a fairly straightforward path. But, for an industry such as cannabis, these traditional guidelines pose difficulties and roadblocks because cannabis, by its very nature and position in culture, is not easily adopted by regulatory and governmental parent organizations that drive testing regulations.

Early Cannabis Testing

Less than a decade ago, the cannabis market moved from an underground black market business into the light of being a quasi-legal industry. Dave Egerton, the Vice President of Technical Operations for CW Analytical in California, saw the early testing for potency as a marketing tool, used by growers purely for economic reasons. The early years in the market were filled with small laboratories started by those people with an interest in cannabis as a new industry, but who also knew very little about laboratory testing. They were focused on taking in samples and putting out reports. It was difficult, in the beginning, to find veteran scientists willing to jump into an unknown and uncertain testing industry. “Experienced chemists are more risk averse, and it can be challenging to bring them into the market,” said Carl Carnagey, CEO of Juniper Analytics, a cannabis testing laboratory in Oregon.

The initial cannabis testing laboratories did not have methods to follow. They did not have to adhere to regulations or prove their competency. In many cases, procedures were created from literature searches, analysts’ personal experiences, or the type of instrumentation purchased (often times secondhand). In the United States, the federal regulatory agencies had no official guidance for the cannabis industry, which created a “wild west” mentality in the community. Laboratories were competing for the business of growers and dispensaries, whose primary focus at the start was not necessarily safety, but more often, low-cost testing and a “scientific” stamp of approval. Carnagey explained that those times were challenging for scientists. “Even labs that wanted to do things right were challenged because they did not know what ‘right’ was,” he said. “It became a quest to find what is right.”

The Future of Cannabis Testing

As the legalization of cannabis became part of the U.S. national and world debate, voices of concern grew louder regarding the safety and quality of cannabis products. The scientific and regulatory communities were recruited to help legitimize the safety and quality of these new products by the scientific method, without the benefit of procedures established in other legally recognized industries. Nearly a decade since the mainstream introduction of cannabis, the regulatory and standardization organizations are now stepping up to fill the void and standardize the testing of cannabis.

The goal of these cannabis method committees is not unlike their more established counterparts: to create methods that meet the needs and demands of the industry and analytical community. The difference between this industry and the ones that have come before it is that the method development process that is typical for other scientific fields creates challenges at every level, driven by commodity value, legal limitations, and financial burdens, that are not usually seen in other laboratory industries.

Take, for example, the first steps of a method development process—that is, defining the scope and applicability of a method. The definition of a method scope for a target such as potency is fairly straightforward. However,
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for other targets—pesticides, for example—it becomes a more difficult task. In the absence of regulations, the early laboratories did not know which pesticides to test for or what levels to consider as limits. States that legalized cannabis products began to separately issue some guidance for pesticide targets. Now, several years into cannabis testing, several states in the United States, along with Canada, have issued pesticide lists.

As the standardization organizations look at creating methods for cannabis, it becomes a question of which lists and limits most accurately reflect what is needed for the safety and testing of cannabis products; the lists generated are all very different from one another. One state list has less than a dozen pesticides, whereas the list from Canada is close to 100 target pesticides. Another state has a limit for a particular pesticide at 1 ppm, while a second state has a limit of 20 ppb.

Returning to that winter meeting of the cannabis committee, the debate continued without resolution. At the heart of the discussion: What pesticides should this international pesticide method for cannabis contain? Participants from the accreditation groups and the contributing members of governmental agencies worked from the perspective that pesticide testing in other industries, such as food, can screen for hundreds of pesticides. These members believed that the required testing list should encompass all the pesticides on all the available cannabis lists to date.

The opposing groups, mostly composed of members directly involved in cannabis testing or the cannabis industry, believed that requiring laboratories to test for a large list of pesticides would hurt the implementation of the method into widespread use. Their point of view was that most cannabis testing laboratories are only interested in meeting their own state’s requirements, or the requests of their customers. One member of the committee who works in a testing laboratory explained that his laboratory would not be allowed to develop methods beyond his own state’s requirements because of cost in both time and materials. This opinion was not unique to the cannabis laboratory members of the committee. When asked, both Carnagey and Egerton agreed that the focus of cannabis laboratories is meeting the regulations and rarely trying to exceed them.

Another point of contention within the standard methods process happens when it is time to standardize the sample collection and preparation processes. A food testing laboratory very rarely has to fight for adequate amounts of sample to conduct all the tests required. So, when a method is being written for a new food product, generally, previous methods can be adopted. Many of these methods dictate large sample sizes and multiple sampling and preparation schemes to ensure homogeneity and allow for multiple replicates. But when the product is a high-value commodity, it can be a fight with the sample supplier to obtain the necessary amount of material to ensure a homogenous sample and produce accurate results. Egerton explained that there has always been a hesitation of sacrificing product for testing. In the beginning, laboratories had to make do with what they were given to try to get an accurate result. As regulations change and more governments get involved, it becomes easier to justify the amount of sample needed. Even with increased input from regulatory bodies, it falls to the laboratories to educate the nascent industry on concepts such as variability, homogeneity, sampling tables, and representative samples.

The newly formed cannabis methods committees must weigh the economics of sample collection and sample replicates into the value of the method being produced. There is an economic cost to what most scientists would consider a sufficient analytical sampling and testing batch. There is a cost to multiple sample preparations and multiple sample testing replicates, which some cannabis suppliers and manufacturers are unwilling to incur, creating resistance to adopting methods requiring larger sample sizes.

At the point at which committees finally get to the actual analytical methods, there are then debates around performance requirements, instrumental and laboratory requirements, and reference materials. All of these requirements have challenges to the industry and unexpected associated economic impacts because of the gray areas in which the cannabis industry finds itself.

Carnagey believes that a large part of the cannabis testing business is dealing with the “gray” areas, such as finding leasable laboratory space that is willing to risk its mortgage or financing by renting or selling to a cannabis business. Cities will sometimes block attempts of cannabis businesses from renting or purchasing space, and governments charge these businesses and laboratories extra fees and require additional permits. Carnagey thinks that some of these extra requirements and fees are because of the misconception that the entire cannabis industry is “making tons of money.” In his experience, cannabis testing laboratories are operating on the same margins as other analytical laboratories, yet the cannabis laboratories face more hassles and fees. These financial burdens and legal limitations become part of the method development discussion when economics factor into analysis. The additional financial and legal challenges drain capital that would be better served by reinvesting in the equipment.
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Many modern analytical methods for targets such as pesticides require instruments that can quantitate smaller and smaller amounts of analytes and provide results with exacting performance requirements. Regarding equipment needs for cannabis, Carnegie said that pesticides in the cannabis laboratory should be done on a triple-quadrupole liquid chromatography–mass spectrometry (LC–MS/MS) system. However, many cannabis laboratories are struggling with the need for LC and gas chromatography (GC) triple-quadrupole instruments. It is not only the cost of these instruments that is an issue; the purchase of capital equipment and instruments can be a challenge in a gray-area industry, battling federal banking laws and facing boycotts by some scientific suppliers and instrument manufacturers.

Standards organizations do consider the cost of the method development process, but in many cases, especially in terms of safety, the cost incurred by a method is not a primary consideration (2). Many analytical method developers consider instruments such as LC–MS, GC–MS, inductively coupled plasma–mass spectrometry (ICP-MS), and in the past few years, triple-quadrupole versions of these, to be part of a laboratory’s standard resources—not an added burden for use in methods. The ideal balance is supposed to be “technically relevant results” proportional to the cost to determine those results (2).

This point brings up another hurdle in the methods process for cannabis testing: reference materials. There are a multitude of problems around securing and using relevant and appropriate reference materials in the cannabis industry. The first issue is the limited number of commercially available standards, specifically for cannabis potency. Each of the cannabinoids of interest is a U.S. federally scheduled compound, strictly regulated by the Drug Enforcement Agency (DEA). Reference material manufacturers must secure licenses to possess the materials and to manufacture the standards. There are strict limits imposed on the quantities of materials that can be purchased, and all aspects of the purchasing, manufacturing, and sale of the final products must be monitored and documented. The reference material manufacturers, like some of the cannabis laboratories, can be denied permits by local and state governments, or pay additional fees. The cost to manufacture a cannabis standard is much higher than that for most other type of standards. After licenses are obtained, there is the additional expense and difficulty of finding and purchasing quality starting materials for scientific use. All of the legal wrangling makes it difficult and expensive to produce cannabis reference standards, which creates higher costs passed down to the testing laboratories.

The second problem is that the transportation and sale of cannabinoid standards is restricted to concentrations of less than 1000 ppm, which does not necessarily match the analytical needs of potency testing for materials that have percent levels of cannabinoids. Additional dilutions and sample preparations must be employed to use standards at concentration levels that are hundreds, if not thousands of times more dilute than the actual samples.

Some analytical methods require a solid reference standard or a matrix-matched material to validate or confirm the analysis. In the case of cannabis, the transportation of a truly representative cannabis material containing high levels of tetrahydrocannabinol (THC) is often prohibited by federal law. This prohibition makes it difficult to impossible for standards manufacturers to sell a product into laboratories not located within their home state, or to the rest of the world. Substitute matrices, such as hemp, are available but are not identical to the ideal cannabis reference material.

A final point in any methods process revolves around the validation of the method and the definition of a method’s precision and bias. Precision and bias validation studies, in many cases, involve interlaboratory studies where a reference or study material is distributed to a variety of laboratories around the country—or, indeed, around the world—for analysis by the proposed method. In the case of cannabis, the studies would have to be conducted using a substitute material, or be confined to one state, because of the transportation prohibition.

**Conclusion**

For the time being, economics plays a significant role in the development of cannabis methods; at least until larger federal governmental agencies step in and take control of the regulation and reduce the prohibitions. Until such a time, the testing debates will continue with economics as part of the discussion. When the methods are finally agreed upon in some form it can be hoped that they will meet the needs of the cannabis industry and reflect the practices of good science—and are not just a reflection of the price this new industry is willing to pay.

**References**


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Medical Cannabis: Back to the Future
After a Brief Push to the Past

The recent policy reversals concerning medical education and a greater understanding about the evolution of cannabis from a dirty little secret to a shining new hope for safe and effective pain relief.

George Scorsis

One of the most promising new medicinal treatment options in modern history was quietly freed just months after it was roughly handcuffed and hauled off to jail by a Justice Department memo. Discreetly tucked into the omnibus spending bill recently passed by Congress, and largely overshadowed by the Deferred Action for Childhood Arrivals (DACA) policy and the threat of a government shutdown, the Rohrabacher-Blumenauer amendment restored federal protections for medical cannabis that had been placed in doubt following the Attorney General’s recision of the Cole Memorandum. The Rohrabacher-Blumenauer amendment specifically states that federal funds cannot be used to prevent states from “implementing their own state laws that authorize the use, distribution, possession or cultivation of medical marijuana.”

It’s critically important that both Congress and the White House have recognized the need to protect Americans’ access to medical cannabis, especially in light of the deadly opioid epidemic and the urgent need for alternatives to powerful, addictive painkillers. A recent study in the Journal of the American Medical Association (1) found that doctors in states where cannabis is legal prescribe more than 2 million fewer daily doses of opioids each year and have a 25% lower opioid death rate.

Cannabis has shown promise in treating symptoms for an ever-growing list of ailments including chronic pain, childhood epilepsy, post-traumatic stress disorder (PTSD), anxiety, cancer, and fibromyalgia. Ongoing research promises to find more uses for its many forms. Experience and education are slowly lifting the curtain of cannabis’s former smoky stigma.

The Justice Department’s brief attempt to allow federal prosecution of legal users was a chilling throwback to the past when most dismissed cannabis as a bad habit for slackers hiding in smoky back alleys. In those days, few understood the potential healing power and benefits of cannabis. Today, the future of medical cannabis looks increasingly bright. More and more people are realizing that medical cannabis is rarely smoked: it’s administered in oils, patches, energy bars, candies, tinctures, and even bath salts. In addition, more doctors and patients are embracing cannabis protections underscore the need for its value as a nonaddictive painkiller that has proven to help reduce dependency on many prescription medications. A recent study in the Journal of Alternative and Complementary Medicine (2) found medical cannabis users take fewer other medicines including opiates, anti-inflammatories, anticonvulsants, and over the counter pain relievers. One patient told the Chicago Tribune (3) that medical cannabis helped her win her battle against substance abuse because it was effective without being addictive.

Cannabis’s clean little secret is out: it contains more active ingredients than just tetrahydrocannabinol (THC), which creates the feeling of being high. A lesser known, but no less potent component called CBD, short for cannabinoid, functions as an anti-inflammatory, anticonvulsant, and antipsychotic agent that could hold potential in the treatment of neuroinflammation, epilepsy, nausea, anxiety, and schizophrenia.

The more we learn, the more the medical community and the general public are embracing this once forbidden substance. Its hidden healing powers are finally being examined, revealed, and legitimized.

Currently there’s a significant divergence between federal law, which includes cannabis on Schedule 1 of the Controlled Substances Act rendering it federally illegal, and the laws of 31 states or territories that have legalized either its medical or recreational use. Recent polling suggests that 93% of Americans support legalizing medical marijuana, while more than 60% of Americans now live in places where medical cannabis is legal, and about 30% live where adult recreational use is legal (4). But there’s a new groundswell of support for a number of reform laws to open the floodgates and untie the hands of more patients, doctors, cultivators and researchers:

• The U.S. Food and Drug Administration (FDA) is on the verge of giving final approval to a cannabis-based treatment for epilepsy. If approved, this would be the first time the FDA has confirmed that cannabis has a medical use, which would necessitate a rescheduling of cannabis from Schedule 1.
• Colorado Senator Corey Gardiner just struck a deal with President Trump, who agreed to support efforts to end the federal prohibition and let states decide which kinds of cannabis use they wish to sanction.

•
• Senator Minority Leader Chuck Schumer is working on a bill to deschedule cannabis from Schedule I to give states the freedom to decide and make it easier for scientists to study its potential benefits.

• Senate Majority Leader Mitch McConnell has fast-tracked a bill to remove hemp and hemp-related products including CBD with low THC content from Schedule I.

• Two bills would authorize the Veterans Affairs (VA) Department to advance medical cannabis research and allow VA physicians to recommend medical marijuana for veterans.

• The Safe Banking Act would authorize banks and other financial institutions to offer services to legitimate and licensed medical cannabis operators or service providers and prevent federal banking regulators from denying or limiting service to them.

• The Medical Cannabis Research Act would simplify the medical marijuana research process.

• The recently passed Rohrabacher-Blumenauer amendment restoring federal protection to medical cannabis in states where it’s legal brings medical cannabis back into the 21st century where it’s quickly gaining recognition as a game-changing painkiller.

As a leading player in the industry, Liberty Health Sciences is once again free to continue its mission to research, produce, and process the highest quality, pharmaceutical-grade cannabis and educate an ever-expanding community about its many benefits. Welcome back to the future, cannabis.

References


George Scorsis
is the CEO and director of Liberty Health Sciences in Alachua, Florida.
Cannabis-based products are available in a wide variety of formulations ranging from dry plant material, plant concentrates including waxes and distillates, and infused products such as foods and candies. Given the variety of sample matrices, existing sample preparation procedures developed for inductively coupled plasma–mass spectrometry (ICP-MS) and ICP–optical emission spectrometry (OES). A microwave acid digestion sample preparation procedure was verified using appropriate reference materials, and the digested cannabis samples were analyzed using both inductively coupled plasma–mass spectrometry (ICP-MS) and ICP–optical emission spectrometry (OES). The ICP-MS method used aerosol dilution to provide a highly robust plasma suitable for extended analysis of high-matrix sample digests. A fast ICP-OES method was also developed to analyze the same cannabis samples.

Jenny Nelson, Craig Jones, and Neli Drvodelic

As an increasing number of U.S. states and countries enact laws to legalize the use of medicinal or recreational marijuana, there is a critical need to ensure product quality and safety. Similar to other consumer products such as foods and pharmaceuticals, cannabis testing needs to include the analysis of metals, some of which may be toxic if ingested or inhaled. Existing analytical methods used for plant-based samples can be applied to cannabis plant material and other cannabis-based products.

Determiniation of Multiple Metals in Cannabis Samples Using ICP-MS and ICP-OES

Cannabis-based products are available in a wide variety of formulations ranging from dry plant material, plant concentrates including waxes and distillates, and infused products such as foods and candies. Given the variety of sample matrices, existing sample preparation procedures developed for inductively coupled plasma–based techniques can be applied to cannabis products. For example, trace–element analysis of plant and nutritional supplement materials is a well-established application (1). Following acidic digestion to break down the primary components of the plant-based samples, inductively coupled plasma–mass spectrometry (ICP-MS) or ICP–optical emission spectrometry (OES) is often used for quantitative analysis because of the multielement capability, speed, and robustness of each technique. ICP-OES is suited to the analysis of mineral and micronutrients such as K, Ca, Mg, Cu, Fe, Mn, Zn, Cu, Mo, and Ni—vital elements required for plant growth. When the required
analytes also include trace elements such as As, Se, Cd, Pb, and Hg, which may require lower detection limits, ICP-MS offers greater sensitivity, delivering detection limits and accurate analysis down to nanogram-per-liter (part-per-trillion) levels.

Most of the states that have legalized the use of marijuana for either medicinal or recreational use have enacted regulations for acceptable limits of toxic elements (Cd, Pb, As, and Hg) in cannabis and cannabinoid products (2). As shown in Table I (3), the limits can vary among states, and regulations governing the safety and quality of cannabis-based products are likely to evolve to include more elements.

### Experimental

#### Preparation of Cannabis Samples

Two cannabis plant samples were analyzed in this study. Approximately 0.15 g of buds from each cannabis plant was weighed into a quartz vessel. Then 4 mL of nitric acid (HNO₃) and 1 mL of hydrochloric acid (HCl) were added and the samples were microwave digested using a one-step program: ramp time of 20 min to a temp of 240 °C and hold time of 15 min. Hydrochloric acid was included to ensure the stability of Ag and Hg in solution.

The digested samples were diluted using a mix of 1% HNO₃ and 0.5% HCl. Natural Institute of Standards and Technology (NIST) 1547 Peach Leaves and NIST 1573a Tomato Leaves standard reference materials (SRMs) were prepared using the same method to verify that the digestion was complete and confirm the quantitative recovery of the analytes.

#### Instrumentation

The ICP-MS system used for the analysis was a standard 7800 (Agilent), which includes the High Matrix Introduction (HMI) system. The ICP-OES system used was a standard 5110 SVDV (Agilent) fitted with an Advanced Valve System (AVS) six-port valve. Both instruments were used with an SPS 4 autosampler (Agilent). The ICP-MS system was configured with the standard sample introduction system consisting of a Micromist glass concentric nebulizer, a quartz spray chamber, and a quartz torch with a 2.5-mm i.d. injector. The interface consisted of a nickel-plated copper sampling cone and a nickel skimmer cone. The ICP-OES sample introduction system consisted of a SeaSpray nebulizer, a double-pass cyclonic spray chamber, and a 1.8-mm i.d. injector torch. Table II lists the operating conditions used for the ICP-MS and ICP-OES systems.

The settings for the ICP-MS HMI were autotuned using an aerosol dilution factor of 4x, as appropriate for the matrix level of the sample digests. HMI enables the routine analysis of samples that contain high and variable matrix levels, while minimizing the need for conventional liquid dilution. By automating dilution in the aerosol...
phase, manual sample handling steps and the potential for contamination during sample preparation can be reduced, producing more accurate results. The 7800 ICP-MS system uses helium (He) collision–reaction cell (CRC) gas and kinetic energy discrimination (KED) to control all common polyatomic interferences. In plant samples such as cannabis, all the target metals can be measured at the required levels using He cell gas, so a very simple, single-mode method can be applied.

The 5110 ICP-OES system uses a vertical torch and solid-state RF generator to ensure robust, stable analysis of complex samples over extended

### Table III: Mean concentrations (n = 3, ppm) in two plant SRMs measured using ICP-MS and ICP-OES.

Results include percent recovery compared to certified or uncertified (information) values, where available.

<table>
<thead>
<tr>
<th>Element (Mass and Wavelength)</th>
<th>Certified Conc.</th>
<th>NIST 1547 Peach Leaves</th>
<th>ICP-MS</th>
<th>ICP-OES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Measured Conc. &amp; Recovery</td>
<td>Quality Criteria (80–120%)</td>
<td>Mean Measured Conc. &amp; Recovery</td>
<td>Quality Criteria (80–120%)</td>
</tr>
<tr>
<td>23Na</td>
<td>23.8</td>
<td>27.86</td>
<td>117</td>
<td>Pass</td>
</tr>
<tr>
<td>24Mg (279.078 nm)</td>
<td>4320</td>
<td>4264.2</td>
<td>99</td>
<td>Pass</td>
</tr>
<tr>
<td>27Al (396.152 nm)</td>
<td>248.9</td>
<td>251.7</td>
<td>101</td>
<td>Pass</td>
</tr>
<tr>
<td>37K (769.897 nm)</td>
<td>24330</td>
<td>24434.9</td>
<td>100</td>
<td>Pass</td>
</tr>
<tr>
<td>64Ca (315.887 nm)</td>
<td>15590</td>
<td>17371.6</td>
<td>111</td>
<td>Pass</td>
</tr>
<tr>
<td>51V</td>
<td>0.367</td>
<td>0.349</td>
<td>95</td>
<td>Pass</td>
</tr>
<tr>
<td>51Cr (205.560 nm)</td>
<td>1</td>
<td>1.118</td>
<td>112</td>
<td>Pass</td>
</tr>
<tr>
<td>55Mn (257.610 nm)</td>
<td>97.8</td>
<td>99.6</td>
<td>102</td>
<td>Pass</td>
</tr>
<tr>
<td>56Fe (239.563 nm)</td>
<td>219.8</td>
<td>222.8</td>
<td>101</td>
<td>Pass</td>
</tr>
<tr>
<td>59Co</td>
<td>0.071</td>
<td>0.069</td>
<td>99</td>
<td>Pass</td>
</tr>
<tr>
<td>60Ni (216.555 nm)</td>
<td>0.689</td>
<td>0.788</td>
<td>114</td>
<td>Pass</td>
</tr>
<tr>
<td>67Cu (324.754 nm)</td>
<td>3.75</td>
<td>3.649</td>
<td>97</td>
<td>Pass</td>
</tr>
<tr>
<td>64Zn (213.857 nm)</td>
<td>17.97</td>
<td>17.378</td>
<td>97</td>
<td>Pass</td>
</tr>
<tr>
<td>75As (188.980 nm)</td>
<td>0.061</td>
<td>0.059</td>
<td>98</td>
<td>Pass</td>
</tr>
<tr>
<td>78Se (196.026 nm)</td>
<td>0.121</td>
<td>0.108</td>
<td>90</td>
<td>Pass</td>
</tr>
<tr>
<td>95Mo</td>
<td>0.0603</td>
<td>0.054</td>
<td>90</td>
<td>Pass</td>
</tr>
<tr>
<td>107Ag</td>
<td>0.0261</td>
<td>0.028</td>
<td>107</td>
<td>Pass</td>
</tr>
<tr>
<td>111Cd (226.502 nm)</td>
<td>123.7</td>
<td>124.9</td>
<td>101</td>
<td>Pass</td>
</tr>
<tr>
<td>207Hg</td>
<td>0.0317</td>
<td>0.028</td>
<td>88</td>
<td>Pass</td>
</tr>
<tr>
<td>208Pb</td>
<td>0.869</td>
<td>0.846</td>
<td>97</td>
<td>Pass</td>
</tr>
<tr>
<td>233Th</td>
<td>0.051</td>
<td>0.050</td>
<td>100</td>
<td>Pass</td>
</tr>
<tr>
<td>238U</td>
<td>0.0151</td>
<td>0.014</td>
<td>93</td>
<td>Pass</td>
</tr>
</tbody>
</table>

*n = 3, replicate sample digestion, each in triplicate; 1FDA Elemental Analysis Manual (section 3.4 special calculations) 3.4 equation 20; 2QC Criteria FDA EAM 4.7 (80–120%) for NIST certified values (4); 3Noncertified reference value; 4Recoveries for As and Se are calculated relative to the original certified values (1991 revision). 5Elements have no certified or noncertified value for the associated SRM. These certified values have subsequently been removed from the certificate (2017 revision) so may not be reliable. NA since the calibration levels used for the ICP-OES measurements were too high to allow accurate recovery for Na.
run times. The integrated AVS valve system was used to improve the sample throughput and minimize argon gas usage.

Both instruments can perform a rapid screening measurement together with the quantitative analysis. Using a standard feature of the respective software, semiquantitative results can be reported for elements not included in the calibration standards.

### Calibration

Representative ICP-MS and ICP-OES calibration curves for the critical trace elements As, Cd, Pb, and Hg are shown in Figure 1. All curves show excellent linearity across the

<table>
<thead>
<tr>
<th>Certified Concentration</th>
<th>NIST 1573a Tomato Leaves</th>
<th>ICP-MS</th>
<th></th>
<th>ICP-OES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Measured Conc.*</td>
<td>Recovery†</td>
<td>QC Criteria (80–120%)‡</td>
<td>Mean Measured Conc.*</td>
<td>Recovery†</td>
</tr>
<tr>
<td>136</td>
<td>119.2</td>
<td>88</td>
<td>Pass</td>
<td>&lt;MDL</td>
<td></td>
</tr>
<tr>
<td>12000§</td>
<td>10213.1</td>
<td>85</td>
<td></td>
<td>10711.3</td>
<td>89</td>
</tr>
<tr>
<td>598</td>
<td>513.3</td>
<td>86</td>
<td>Pass</td>
<td>505.4</td>
<td>85</td>
</tr>
<tr>
<td>27000</td>
<td>25728.4</td>
<td>95</td>
<td>Pass</td>
<td>24886.7</td>
<td>92</td>
</tr>
<tr>
<td>50500</td>
<td>53983.3</td>
<td>107</td>
<td>Pass</td>
<td>48836.7</td>
<td>97</td>
</tr>
<tr>
<td>0.835</td>
<td>0.698</td>
<td>84</td>
<td>Pass</td>
<td>&lt;MDL</td>
<td></td>
</tr>
<tr>
<td>1.99</td>
<td>1.988</td>
<td>100</td>
<td>Pass</td>
<td>2.007</td>
<td>101</td>
</tr>
<tr>
<td>246</td>
<td>238.7</td>
<td>97</td>
<td>Pass</td>
<td>237.8</td>
<td>97</td>
</tr>
<tr>
<td>368</td>
<td>331.1</td>
<td>90</td>
<td>Pass</td>
<td>315.3</td>
<td>86</td>
</tr>
<tr>
<td>0.57</td>
<td>0.510</td>
<td>89</td>
<td>Pass</td>
<td>&lt;MDL</td>
<td></td>
</tr>
<tr>
<td>1.59</td>
<td>1.442</td>
<td>91</td>
<td>Pass</td>
<td>0.157</td>
<td>10</td>
</tr>
<tr>
<td>4.7</td>
<td>4.330</td>
<td>92</td>
<td>Pass</td>
<td>4.826</td>
<td>103</td>
</tr>
<tr>
<td>30.9</td>
<td>25.953</td>
<td>84</td>
<td>Pass</td>
<td>29.250</td>
<td>95</td>
</tr>
<tr>
<td>0.112</td>
<td>0.109</td>
<td>97</td>
<td>Pass</td>
<td>0.096</td>
<td>85</td>
</tr>
<tr>
<td>0.054</td>
<td>0.064</td>
<td>119</td>
<td>Pass</td>
<td>0.044</td>
<td>81</td>
</tr>
<tr>
<td>0.46§</td>
<td>0.445</td>
<td>97</td>
<td>Pass</td>
<td>&lt;MDL</td>
<td></td>
</tr>
<tr>
<td>0.017†</td>
<td>0.019</td>
<td>112</td>
<td>Pass</td>
<td>&lt;MDL</td>
<td></td>
</tr>
<tr>
<td>1.52</td>
<td>1.330</td>
<td>88</td>
<td>Pass</td>
<td>1.440</td>
<td>95</td>
</tr>
<tr>
<td>63§</td>
<td>56.500</td>
<td>90</td>
<td></td>
<td>59.660</td>
<td>95</td>
</tr>
<tr>
<td>0.034</td>
<td>0.033</td>
<td>97</td>
<td>Pass</td>
<td>&lt;MDL</td>
<td></td>
</tr>
<tr>
<td>0.12§</td>
<td>0.091</td>
<td>76</td>
<td>&lt;MDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.035†</td>
<td>0.028</td>
<td>80</td>
<td>&lt;MDL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
charged ions of the REEs 150Nd, 150Sm, 156Gd, 156Dy, 160Gd, and 160Dy therefore appear at half their true mass. Doubly charged ions (REE++) are problematic for the analysis of low rare earth elements (REEs), which can be problematic for the analysis of low concentrations of As and Se by ICP-MS. REEs have low second ionization potentials, so they readily form doubly charged ions (REE++). As the quadrupole mass spectrometer separates basions based on their mass-to-charge ratio (m/z), these doubly charged ions appear at half their true mass. Doubly charged ions of the REEs 150Nd, 150Sm, 156Gd, 156Dy, 160Gd, and 160Dy therefore appear at m/z 75, 78, and 80, potentially causing overlaps that can bias the results for As and Se in samples that contain high levels of the REEs. The ICP-MS system corrects for these interferences using the “half mass correction” setting in the ICP-MS MassHunter software. The mean results shown in Table III were in good agreement with the certified concentrations, where provided, including for As in NIST 1547 and Se in both NIST 1547 and 1573a.

Spike Recoveries
Quantitative results for the two cannabis samples showed that the concentrations of As (160.0 ppb), Cd (11.33 ppb), Pb (24.00 ppb), and Co (162.1 ppb) were relatively high in cannabis sample 1. Pb and Co were also high in cannabis sample 2, at 55.40 and 143.4 ppb, respectively. These concentrations were well below existing regulatory or guideline levels for As, Hg, Pb, and Cd, so a spike recovery test was carried out to check the accuracy of the ICP-MS and ICP-OES methods at the higher concentrations that may be encountered in actual sample analysis. The two cannabis sample digestes were spiked with a premixed standard (Environmental Mix Spike, Agilent) containing multiple elements at 200 ppb, Na, Mg, K, Ca, and Fe at 2000 ppb, and Hg at 4 ppb.

Using the ICP-MS and ICP-OES systems’ direct analysis methods, excellent spike recoveries were achieved for most elements in the spiked samples. All recoveries were within ±20% for Cd, Pb, As, and Hg, as shown in Table IV. The spike recovery results for K, Ca, and Mn were invalid because the concentration levels of these elements in the mixed spike solution were much too low (20 times lower) relative to the levels present in the un-spiked cannabis digest samples.

Conclusions
Both ICP-MS and ICP-OES can be used for the quantitative analysis of multiple elements—including the four target toxic metals Cd, Pb, As, and Hg—in cannabis samples following acid digestion. The choice of which technique to use will depend on the required method detection limits, level of experience of staff in...
Both techniques are suitable for trace metal screening of medicinal and recreational cannabis, as well as related products. ICP-OES has a lower capital cost and is somewhat easier to use, while ICP-MS offers greater sensitivity and is more suitable for the ultratrace level analytes. To ensure that each method was simple enough to be applied to routine quality control (QC) and safety testing, the ICP-MS was operated in a single mode (helium collision mode) for all measurements. The ICP-OES was used with a 6-port valve system, suitable for high throughput applications. Based on the findings of the spike recovery test of two cannabis plant samples, both methods were found to be accurate for multiple elements over a wide concentration range. Suitability of the microwave-assisted sample preparation method was demonstrated by the good recovery results obtained for two plant-based SRMs.

References


Table IV: Quantitative and spike recovery results for two cannabis samples

<table>
<thead>
<tr>
<th>Element (Mass and Wavelength)</th>
<th>Cannabis Sample 1</th>
<th>Cannabis Sample 2</th>
<th>ICP-MS</th>
<th>ICP-OES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured Concentration, n = 3, ppb</td>
<td>Mean Recovery* ± 1σ QC Criteria (80–120%)*</td>
<td>Mean Recovery* ± 1σ QC Criteria (80–120%)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23Na (589.592 nm)</td>
<td>7.37 ppm 50.02 ppm</td>
<td>110 ± 4% Pass</td>
<td>115 ± 5% Pass</td>
<td></td>
</tr>
<tr>
<td>24Mg (279.078 nm)</td>
<td>542 ppm 5807 ppm</td>
<td>113 ± 10% Pass</td>
<td>107 ± 9% Pass</td>
<td></td>
</tr>
<tr>
<td>27Al (396.152 nm)</td>
<td>5.02 ppm 4.586 ppm</td>
<td>112 ± 12% Pass</td>
<td>101 ± 5% Pass</td>
<td></td>
</tr>
<tr>
<td>28K (769.897 nm)</td>
<td>4115 ppm 34101 ppm</td>
<td>NA NA</td>
<td>NA NA</td>
<td></td>
</tr>
<tr>
<td>40Ca (315.887 nm)</td>
<td>11394 ppm 9681 ppm</td>
<td>NA NA</td>
<td>NA NA</td>
<td></td>
</tr>
<tr>
<td>51V (292.401 nm)</td>
<td>37.04 25.09</td>
<td>116 ± 8% Pass</td>
<td>103 ± 2% Pass</td>
<td></td>
</tr>
<tr>
<td>52Cr (205.560 nm)</td>
<td>81.39 272.5</td>
<td>114 ± 5% Pass</td>
<td>100 ± 1% Pass</td>
<td></td>
</tr>
<tr>
<td>55Mn (257.610 nm)</td>
<td>11403 229650</td>
<td>NA NA</td>
<td>NA NA</td>
<td></td>
</tr>
<tr>
<td>56Fe (239.563 nm)</td>
<td>252188 219811</td>
<td>114 ± 6% Pass</td>
<td>105 ± 0% Pass</td>
<td></td>
</tr>
<tr>
<td>57Co (228.615 nm)</td>
<td>162.1 143.4</td>
<td>114 ± 5% Pass</td>
<td>102 ± 1% Pass</td>
<td></td>
</tr>
<tr>
<td>60Ni (216.555 nm)</td>
<td>108.2 185.7</td>
<td>113 ± 3% Pass</td>
<td>101 ± 3% Pass</td>
<td></td>
</tr>
<tr>
<td>62Cu (324.754 nm)</td>
<td>10865 13467</td>
<td>108 ± 1% Pass</td>
<td>104 ± 1% Pass</td>
<td></td>
</tr>
<tr>
<td>68Zn (213.857 nm)</td>
<td>72504 126816</td>
<td>96 ± 9% Pass</td>
<td>100 ± 9% Pass</td>
<td></td>
</tr>
<tr>
<td>74As (188.980 nm)</td>
<td>160.0 25.94</td>
<td>103 ± 6% Pass</td>
<td>98 ± 2% Pass</td>
<td></td>
</tr>
<tr>
<td>78Se (196.026 nm)</td>
<td>45.78 72.68</td>
<td>94 ± 13% Pass</td>
<td>96 ± 0% Pass</td>
<td></td>
</tr>
<tr>
<td>95Mo (202.032 nm)</td>
<td>3280 1589</td>
<td>118 ± 4% Pass</td>
<td>100 ± 2% Pass</td>
<td></td>
</tr>
<tr>
<td>109Ag (328.048 nm)</td>
<td>8.612 10.78</td>
<td>100 ± 10% Pass</td>
<td>97 ± 2% Pass</td>
<td></td>
</tr>
<tr>
<td>114Cd (226.502 nm)</td>
<td>11.33 7.471</td>
<td>110 ± 5% Pass</td>
<td>100 ± 2% Pass</td>
<td></td>
</tr>
<tr>
<td>116Ba (455.403 nm)</td>
<td>345.6 888.7</td>
<td>111 ± 12% Pass</td>
<td>104 ± 1% Pass</td>
<td></td>
</tr>
<tr>
<td>184Hg (184.887 nm)</td>
<td>29.19 27.06</td>
<td>107 ± 4% Pass</td>
<td>99 ± 9% Pass</td>
<td></td>
</tr>
<tr>
<td>201Pb (220.353 nm)</td>
<td>24.00 55.40</td>
<td>112 ± 4% Pass</td>
<td>104 ± 0% Pass</td>
<td></td>
</tr>
</tbody>
</table>

* n = 3, replicate sample digestion, each in triplicate, two separate cannabis samples. †QC criteria FDA EAM 4.7 (80–120%) for NIST certified values. NA indicates that spike levels were too low (<5%) relative to the unspiked concentration.

Jenny Nelson is an application scientist with Agilent Technologies in Santa Clara, California. Craig Jones is an application engineer with Agilent Technologies in Santa Clara. Neli Drvodelic is an application engineer with Agilent Technologies in Melbourne, Australia. Direct correspondence to: jenny_nelson@agilent.com
A Comprehensive Approach to Pesticide Residue Analysis in Cannabis

As the number of U.S. states allowing the adult use of cannabis and cannabis products increases, so does the need for product testing before retail sale. States that have legalized recreational use have specified testing requirements for pesticide residues in cannabis flower and cannabis products. Because the specific pesticides and action levels vary from state to state, a comprehensive approach to residue analysis can meet the requirements of multiple U.S. state regulations with a single analysis. The challenge of quantifying pesticide residues in cannabis is complex because of the high concentration of cannabinoids and terpenes relative to the levels of pesticides that may be present. Here we present a straightforward acetonitrile extraction using a solid-phase extraction (SPE) cartridge and targeted dispersive solid-phase extraction (dSPE) cleanup. The final dilute extract is analyzed with both gas chromatography–tandem mass spectrometry (GC–MS/MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) for a comprehensive target list (200+ compounds) that encompasses those identified on individual U.S. state lists. Limits of quantitation meet or exceed individual U.S. state requirements.

Rick Jordan, Lilly Asanuma, Dan Miller, and Anthony Macherone

For U.S. states that have legalized the recreational or medicinal use of cannabis and cannabis products, there are presale testing requirements meant to address quality and consumer safety (1–3). In the absence of guidance at the federal level, states must establish the scope of the required testing in addition to any minimum or maximum values before product release into the marketplace. These compliance efforts, combined with the lack of true consensus methods, allow individual laboratories to develop analytical methodology that typically only meet the regulatory requirements of the single state where the laboratory is located.

Conventional agricultural commodities are subject to U.S. maximum residue levels (MRLs) for pesticides labeled for use on each specific commodity. These MRLs are established by the U.S. Environmental Protection Agency (EPA) as part of the pesticide registration process. Commodities destined for international export will be subject to the MRLs established by the importing country. With a wide range of commodities, pesticides, and MRLs, a comprehensive approach to residue testing is necessary to ensure the commodity complies with all applicable regulations. This comprehensive approach to pesticide residue analysis can be applied to cannabis and cannabinoid products. U.S. states with approved cannabis sales have developed target lists for pesticides with associated “action levels.” An action level is not an MRL, but rather a subjective number assigned by state regulators to establish criteria for laboratory testing. These action levels were determined by a combination of factors such as laboratory capabilities, analytical techniques, and evaluating MRLs from other commodities (4,5). Additionally, U.S. states have language in statutes or administrative rules that prohibit any pesticide from being applied off-label (that is, not following product label instructions) to any crops. Pesticide products not included on a state list could potentially be applied during cannabis cultivation. Because U.S. state regulations require a limited scope targeted testing procedure, these residues would not be discovered during presale screening and would find their way into the final consumer products on retail shelves.

QuEChERS (quick, easy, cheap, effective, rugged, and safe) is a common approach for the extraction of pesticide residues (6,7), and has been applied to both cannabis and cannabis products. Although it generates acceptable results for most pesticides in cannabis, it was developed for samples that contain greater than 70% moisture. Cannabis flower typically contains 10–15% moisture so the addition of water to each sample is necessary to generate the desired partitioning that is the principle of the QuEChERS technique. Moreover, the addition of water and salts in the QuEChERS approach generates an exothermic reaction and an increase in pH—both of which can degrade sensitive pesticides. An alternate approach is a serial extraction using acetonitrile and a solid-phase cleanup step (8). This procedure results in less coextracted material and improved recoveries for the more polar pesticides.
The general trend among testing laboratories is to develop analytical methods using a single extraction followed by analysis using tandem quadrupole mass spectrometers. With the limited scope of many U.S. state regulatory lists, liquid chromatography–tandem mass spectrometry (LC–MS/MS) is often the choice when a single instrument is used. For a true comprehensive residue analysis, a dual-platform MS/MS scheme should be developed using both gas chromatography–tandem mass spectrometry (GC–MS/MS) and LC–MS/MS. This approach allows laboratories to evaluate compounds on each platform to optimize sensitivity and chromatographic performance. Many compounds can be identified and quantified using both techniques, giving laboratories orthogonal confirmation for difficult sample matrices.

The challenge with pesticide residue analysis in cannabis is the concentration difference between the pesticide residues and those of the coextracted cannabinoids and terpenes. These coextracted materials complicate residue testing by introducing matrix effects in the MS/MS system, and lead to increased system maintenance and instrument downtime. To address these issues, both MS/MS platforms need to have sufficient sensitivity to allow for large dilution factors that will reduce matrix effects and maintenance while meeting state-specific action levels.

**Sample Preparation**

In our laboratory, sample preparation includes a serial extraction with acetonitrile followed by a solid-phase cleanup. A 1.0-g aliquot of homogenized sample was accurately weighed into a 50-mL centrifuge tube, with 15 mL of acetonitrile added to each tube along with a ceramic homogenizer. The tubes were sealed and mechanically shaken for 2 min at 1500 strokes/min. The acetonitrile was decanted through a conditioned Strata-X (Phenomenex) 33-μm solid-phase extraction (SPE) cartridge (500 mg/12 mL), and the eluent was collected in a second 50-mL centrifuge tube. The primary extraction tubes were rinsed with two additional 5-mL portions of acetonitrile, and decanted through the SPE cartridge. All eluent fractions were combined in the second 50-mL centrifuge tube. The solvent extract was brought to a final volume of 25 mL with acetonitrile. After this step we have a dilution factor of 25x from the initial sample amount.

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Analytical Instrumentation
As previously discussed, the analyses are conducted with both LC–MS/MS and GC–MS/MS systems. The GC–MS/MS system was an Agilent 7890B GC equipped with an Agilent 7010 MS/MS system with a high efficiency source (HES). The GC system was configured with a multimode inlet (MMI) and a Purged Ultimate Union (PUU) to allow for column backflushing. The MMI is capable of fast temperature ramping, allowing for a “cool” inlet injection. The initial inlet temperature was 180 °C. The temperature was ramped to 280 °C at 400 °C/min post-injection. Combining the “cool” inlet condition with a pulsed splitless injection helped reduce degradation of thermally labile compounds in the GC inlet. To improve peak shape for more-polar analytes, the PUU was placed at the midpoint of two analytical columns of different stationary phases (column 1 = HP-35MS, 15 m x 0.25 mm, 0.25-µm df; column 2 = HP-5, 15 m x 0.25 mm, 0.25-µm df). The back-flush timing was configured to begin mid-run as determined by the retention time of the last compound exiting the PUU, and continued post-run to flush nonvolatile compounds from the injection port.

The LC–MS/MS system was an Agilent 1260 Infinity II with a Multisampler connected to an Agilent 6470 MS/MS system. A Poroshell 120 Phenyl-Hexyl column (100 mm x 2.1 mm, 2.7-µm df) was selected because of its chromatographic performance and robustness. The LC column provided excellent chromatography at standard (non-ultrahigh-pressure liquid chromatography [UHPLC]) pump pressures, again reducing instrument downtime. A feature of the Multisampler is the ability to perform an injection pretreatment before sample injection. A 2-µL sample was sandwiched between two 10-µL aliquots of water, and injected onto the analytical column. This sample injection sandwich is not a dilution, it simply creates a polar environment for the sample injection, which improves peak shape for the early eluted compounds. Mobile-phase A was 5 mM ammonium formate with 0.1% formic acid in water–methanol (95:5) and mobile-phase B was 5 mM ammonium formate with 0.1% formic acid in methanol–water (95:5).

Calibration
Both MS/MS systems share the same calibration technique. Calibration curves may use a linear fit (minimum five points) or a quadratic fit (minimum six points), with minimum correlation coefficients of 0.990 for all 215 compounds. The low calibrator concentration was 0.2 ng/mL for most compounds (92%), which corresponds to a sample concentration of 0.1 mg/kg after cleanup or final dilution factors. The upper range of the calibration curve was 10 ng/mL or 20 ng/mL depending on the individual analyte. All calibration curves used a 1/X weighting factor and exclude the origin. No internal standards were used, and all quantitative results were calculated using the external standard technique.

Analysis
GC–MS/MS extracts were prepared for analysis using a combination of dSPE cleanup and dilution. For the dSPE cleanup, 100 µL of sample extract was added to a 2-mL disposable tube containing 50 mg of primary secondary amine (PSA), 50 mg of C18, 7.5 mg of graphitized carbon, 150 mg of magnesium sulfate, and 900 µL of 1:1 hexane–acetone. These tubes were capped, vortexed for 30 s, and centrifuged for 2 min. A 300 µL aliquot was removed from the tube and added to an autosampler vial containing 300 µL of hexane–acetone (1:1). At this point, the sample extract consists of 5% acetonitrile–95% hexane–acetone (1:1). This solvent system gives much better performance with GC compared to 100% acetonitrile. The combined dilutions from the cleanup step (10x) and post cleanup (2x), with the initial extraction (25x) result in an overall dilution factor of 500x. Using this approach, background noise and matrix in the final extract was significantly reduced (Figure 1) and recoveries for the target pesticides were within acceptance limits of 70–120%.

The LC–MS/MS extracts were prepared for analysis using only a final dilution step. This final dilution of 50 µL of extract into 950 µL of acetonitrile results in a factor of 20x. Combined with the initial extraction, this dilution leads to an overall dilution factor of 500x. Again, the background matrix is reduced (Figure 1) and the recoveries for target pesticides are within acceptance limits of 70–120%.

Each cannabis strain will have its own unique profile of cannabinoids and terpenes, which may present different background interferences affecting pesticide determinations. If dilution alone does not adequately address matrix effects or interferences, there is an optional dSPE cleanup that can be applied to extracts for LC–MS/MS analysis. This optional step uses PSA as a sorbent. PSA is a powerful cleanup sorbent for challenging samples, but may result in low recoveries for some compounds. Recoveries for spinetoram, spinosad, spirotetramat, and spiroxamine can be reduced to <50% when subjected to a dispersive technique containing PSA, and daminozide is unrecoverable.

A concept developed by Schenck and Wong (9) to address the loss of planar pesticides when using
Table I: Comprehensive pesticide target list: GC–MS/MS (bold) LC–MS/MS (italic)

<table>
<thead>
<tr>
<th>Pesticide Name</th>
<th>GC–MS/MS</th>
<th>LC–MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxycarbofuran</td>
<td>Cyanazine</td>
<td>Fenamiphos sulfoxide</td>
</tr>
<tr>
<td>Abamectin*</td>
<td>Cyrantranilprole</td>
<td>Fenamiphos sulfoxide</td>
</tr>
<tr>
<td>α-BHC</td>
<td>Cyrazofamid</td>
<td>Fenarimol</td>
</tr>
<tr>
<td>Acephate</td>
<td>Cycloate</td>
<td>Fenazaquin</td>
</tr>
<tr>
<td>Acequinocyl-hydroxy</td>
<td>Cyflufenamid</td>
<td>Fenbuconazole</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>Cyflometofen</td>
<td>Fenoxycarb</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>Cyfluthrin</td>
<td>Fenpropathrin</td>
</tr>
<tr>
<td>Aldicarb sulfoxide</td>
<td>Cymoxanil</td>
<td>Fenpyroximate</td>
</tr>
<tr>
<td>Aldicarb sulfoxide</td>
<td>Cypermethrin</td>
<td>Fenvalerate</td>
</tr>
<tr>
<td>Aldrin</td>
<td>Cyprodnil</td>
<td>Fipronil</td>
</tr>
<tr>
<td>Allethrin</td>
<td>Cyromazine</td>
<td>Flonicamid</td>
</tr>
<tr>
<td>Ametocdradin</td>
<td>Dacthal</td>
<td>Fludioxonil</td>
</tr>
<tr>
<td>Atrazine</td>
<td>Diminozide</td>
<td>Flumeturan</td>
</tr>
<tr>
<td>Azinphos-methyl</td>
<td>ß-BHC</td>
<td>Flupicolidide</td>
</tr>
<tr>
<td>Azoxytrobin</td>
<td>DCPMU</td>
<td>Flupyriram</td>
</tr>
<tr>
<td>ß-BHC</td>
<td>Deltamethrin</td>
<td>Fluxastrobion</td>
</tr>
<tr>
<td>Bendiocarb</td>
<td>Diazinon</td>
<td>Flupyradifurone</td>
</tr>
<tr>
<td>Benfluralin</td>
<td>Diazoxon</td>
<td>Fluridone</td>
</tr>
<tr>
<td>Bensulide</td>
<td>Dichlobenil</td>
<td>Flutolanil</td>
</tr>
<tr>
<td>Bifentrazate</td>
<td>Dichlorofenthion</td>
<td>Flutiafol</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>Dichlorvos</td>
<td>Flulazine</td>
</tr>
<tr>
<td>Bolstar</td>
<td>Diclofol-methyl</td>
<td>Flupropyramid</td>
</tr>
<tr>
<td>Boscalid</td>
<td>Dicloran</td>
<td>Formetanate HCl</td>
</tr>
<tr>
<td>Bromacil</td>
<td>Dicofol</td>
<td>γ-BHC</td>
</tr>
<tr>
<td>Bromopropylate</td>
<td>Diflubenzuron</td>
<td>Heptachlor</td>
</tr>
<tr>
<td>Captain¹</td>
<td>Dimethoate</td>
<td>Heptachlor epoxide</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>Dimethomorph</td>
<td>Hexachlorobenzene</td>
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<td>Carbendazim</td>
<td>Dinotefuran</td>
<td>Hexazinone</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>Diphenamid</td>
<td>Hexythiazox</td>
</tr>
<tr>
<td>Carfentrazone-ethyl</td>
<td>Disulfoton sulfone</td>
<td>Imazalil</td>
</tr>
<tr>
<td>Chlorantranilprole</td>
<td>Dithiopyr</td>
<td>Imidacloprid</td>
</tr>
<tr>
<td>Chlordane</td>
<td>Diuron</td>
<td>Indaziflam</td>
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<tr>
<td>Chlorfenapyr</td>
<td>d-Phenothrin</td>
<td>Indoxacar</td>
</tr>
<tr>
<td>Chlorfenapyr</td>
<td>Estenvalerale</td>
<td>Isoxaben</td>
</tr>
<tr>
<td>Chloroneb</td>
<td>Ethalfluralin</td>
<td>Kresoxim-methyl</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>Ethofumesate</td>
<td>λ-Cyhalothrin</td>
</tr>
<tr>
<td>Chlorpropham</td>
<td>Ethophosphos</td>
<td>Linuron</td>
</tr>
<tr>
<td>Chlorpyrifs¹</td>
<td>Ethoxyquin</td>
<td>Malaoxon</td>
</tr>
<tr>
<td>Chlorpyrifs-methyl</td>
<td>Etofenprox</td>
<td>Malathion</td>
</tr>
<tr>
<td>cis-Nonachlor</td>
<td>Etoxazole</td>
<td>Mandipropamid</td>
</tr>
<tr>
<td>Clethodim</td>
<td>Etridazone</td>
<td>Mefenoxam</td>
</tr>
<tr>
<td>Clofentezine</td>
<td>Famphur</td>
<td>Metconazole</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>Fenamidone</td>
<td>Methamizidophs</td>
</tr>
</tbody>
</table>

*%RSD exceeds 15% (abamectin = 17.9%, phosalone = 19.2%, triadimefon = 20.1%).
†Recovery falls outside 70–120% (captan = 133%, chlorpyrifos = 122%).
graphitized carbon as a cleanup sorbent can be applied to compounds strongly retained by PSA. To improve recoveries of planar pesticides when using graphitized carbon, toluene (25–30%) is added to sample extract to push planar pesticides off the sorbent. When using a dSPE designed for fatty samples (50 mg of PSA, 50 mg of C18, 150 mg of magnesium sulfate), a similar approach can be used with methanol.

Adding methanol at the dSPE step will improve the recoveries for compounds retained by PSA. The data indicate that 10–20% methanol addition will increase recoveries for the problematic compounds (Figure 2). This approach will improve recoveries to acceptable levels with minimal matrix returning to the extract. Keep in mind that no amount of methanol will recover daminozide from PSA, and typical reversed-phase chromatography is not optimal for measuring daminozide residues. A more appropriate technique would be an extraction optimized for polar compounds and using hydrophilic-interaction chromatography (HILIC) chromatographic techniques (10).

Results and Discussion
A solvent extraction with a pass through solid-phase cleanup gives an extract with less coextracted materials when compared to extracts from a QuEChERS technique. Having a single extraction procedure that can be split to both MS/MS platforms streamlines the workflow, resulting in higher sample throughput. Dispersive cleanup and dilution techniques can be optimized for each analytical technique and compound list to give the necessary precision and accuracy. The cleanup and dilution procedure for GC–MS/MS analysis allows the laboratory to place the sample extract into a solvent that is more amenable to GC (hexane–acetone). An optional dispersive cleanup procedure for LC–MS/MS analysis can be used if adverse matrix effects or interferences are encountered.

This procedure was validated for 215 pesticides (Table I) split between the two MS/MS platforms. A set of five replicates was prepared at the limit of quantitation (LOQ) for each compound. The signal-to-noise ratio (S/N) criterion of 10:1 (quantitation ion) was met for each compound at
the LOQ. For the GC–MS/MS-amenable compounds, 72 of the 74 pesticides had recoveries of 70–120%, and the percent relative standard deviation (%RSD) was less than 15% for all 74 compounds. For compounds analyzed by LC–MS/MS, all 141 pesticides had recoveries of 70–120%, and the %RSD was below 15% for 138 of 141. The LOQ was validated at 0.1 mg/kg for all compounds except those listed in Table II.

**Conclusion**

The key to successful pesticide residue analysis in cannabis and cannabinoid products is understanding and gaining control of the background matrix. With a reduced matrix load in the final extract, GC issues like fouled or plugged syringes, dirty inlets or liners, and contaminated sources are greatly reduced. LC issues such as fouled or dirty valves, decreased column performance, contaminated spray shields and sources are less likely with the reduced matrix present in the sample extracts. With fewer potential interfering isobars, detector conditions are more stable and performance increases, resulting in increased sensitivity and stability in both baselines and compound response. All these factors combined provide a laboratory with increased sample throughput and a reduction in downtime because of maintenance and repairs. The use of high sensitivity MS/MS systems will allow laboratories to utilize calibration curves at sub-parts-per-billion levels.

By using a comprehensive approach to residues analysis, as is traditionally applied to conventional agricultural crops, a laboratory will be able to screen for the compounds required by regulation in addition to pesticides not included on a U.S. state list. There are many pesticides with similar chemistries and modes of action as those on a state specific list that could go undetected and enter the marketplace unless a more comprehensive approach is taken.

**Acknowledgment**

The authors would like to thank Melissa Churley with Agilent Technologies and Dr. Joan Stevens for their assistance with this work.

**References**

1) Oregon Administrative Rules 333-007-0400.
2) California Code of Regulations, Title 16, Division 42. Bureau of Cannabis Control, Chapter 11, § 5719.
4) D.G. Farrer, “Technical report: Oregon Health Authority’s Process to Decide Which Types of Contaminants to Test for in Cannabis” (Oregon Health Authority, 2015).
7) CSN EN 15662, Foods of Plant Origin - Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-Up by Dispersive SPE - QuEChERS-Method.

**Table II:** Compounds with LOQs above 0.1 mg/kg

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOQ (mg/kg)</th>
<th>Compound</th>
<th>LOQ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captan</td>
<td>0.5</td>
<td>Pyrethrin</td>
<td>0.5</td>
</tr>
<tr>
<td>Clethodim</td>
<td>0.2</td>
<td>Pirimicarb</td>
<td>0.2</td>
</tr>
<tr>
<td>Cycloate</td>
<td>0.2</td>
<td>Sethoxydim</td>
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</tr>
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</tr>
<tr>
<td>Dicloran</td>
<td>0.5</td>
<td>Tebuconazole</td>
<td>0.2</td>
</tr>
<tr>
<td>Fenbuconazole</td>
<td>0.2</td>
<td>Triadimefon</td>
<td>0.2</td>
</tr>
<tr>
<td>Flubendiamide</td>
<td>0.2</td>
<td>Triadimenol</td>
<td>0.2</td>
</tr>
<tr>
<td>Methidathion</td>
<td>0.2</td>
<td>Trifloxystrobin</td>
<td>0.2</td>
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<tr>
<td>Propargite</td>
<td>0.2</td>
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<td></td>
</tr>
</tbody>
</table>

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Cannabis Quality Control Testing Using Gas Chromatography

The cannabis industry has been struggling to keep pace with the changing attitudes toward the drug. With no standardized regulations for quality control between states or countries, there is a huge gap to fill in terms of rigorous testing. The industry is considerably different than the pharmaceutical industry, where drugs pass through extensive quality control to meet stringent safety standards. The challenge for global cannabis producers and processors is how to combat the lack of rigorous testing and oversee quality control to ensure reliable, safe, and consistent products. Gas chromatography (GC) is a successful analytical technique for cannabis testing that provides opportunities to advance the cannabis industry.

Andrew James

The lack of standardized regulations means that producers of cannabis and cannabis-derived products can supply goods unhindered, potentially carrying unsafe levels of pesticides, mold, fungi, and bacteria that can be hazardous to human health. But the cannabis industry is constrained by a legal framework that makes it very difficult to find cannabis with tested cannabinoid content.

This article highlights the work that is being done by Shamanics, a cannabis oil extraction company based in Amsterdam (1). The company works with cannabis, in particular the cannabinoid cannabidiol (CBD), to produce high quality CBD oil. Unlike many of its competitors in the Netherlands, Shamanics conducts key analyses on products before they’re taken to market to provide tested, quality products to meet consumer demand.

Effects of Cannabis

Cannabis consists of the dried flowers of the female Cannabis sativa L. plant, also known as hemp or marijuana, and contains a number of active substances including Δ⁹-tetrahydrocannabinol (THC) and CBD (2). Through selective breeding, growers have developed strains with different sensory, psychoactive, and medicinal properties.

During the past decade, evidence has been presented that cannabis could have therapeutic effects in a number of major diseases such as epilepsy, cancer, and Gilles de la Tourette syndrome (3). However, as a result of the restrictions
imposed on cannabis research, it is thought that many studies were carried out without adequate control trials or sample sizes. For patients who rely on cannabis for medicinal use to ease their symptoms, safety and consistency are difficult to guarantee. To be considered as a drug for medicinal use, cannabis shouldn’t be different from any other pharmaceutical. In the pharmaceutical industry, consumers trust that prescribed medication has been tested to contain no harmful contaminants and a precise dose of an active pharmaceutical ingredient. As the cannabis industry becomes increasingly transparent, there is scope for the increased testing and research that consumers demand.

The Growing Legal Market
Traditionally, there have been blurred lines between countries and states on their rules and regulations around cannabis, for both medicinal and recreational use. In the United Kingdom, the possession and supply of cannabis is illegal but there have been a number of cases where cannabis has been prescribed for medicinal use. One example of cannabis

Figure 1: Decarboxylation of THCA in the GC injector.

Figure 2: Derivatization of THCA.
prepared for medicinal use is the drug Sativex, which has THC as an active compound. It is also possible to buy CBD oils from high street stores in the UK, but those products have usually been extracted from hemp rather than cannabis (4).

In contrast, 30 states in the U.S. have legalized cannabis for medicinal use (5). California’s legalization of recreational cannabis in January 2018 was a huge milestone in the industry. And with legalization comes regulation; the state has imposed stringent rules around standardized quality testing of cannabis products including pesticide testing. All legal cannabis must be tested for residues of 66 pesticides listed by the Bureau of Cannabis Control.

Despite this movement, cannabis isn’t classified as a medicine because it remains illegal under federal law, set at the U.S. government level. Subsequently, the U.S. Food and Drug Administration (FDA) has not approved the plant or plant extracts as a medicine. However, Δ9-THC, the main psychoactive ingredient in the Cannabis sativa L plant, has been an FDA-approved drug for more than 25 years.

Navigating the landscape of cannabis testing in comparison to the imposed regulations is complex. For example, Massachusetts legalized cannabis for medicinal use but required safety and potency testing by a third-party for all products despite it being illegal to operate a cannabis testing laboratory in the state.

Despite this backdrop, the cannabis market is forecasted to continue to grow. A recent report suggested that medical marijuana sales will grow to $13.3 billion in 2020 and adult recreational sales are estimated to reach $11.2 billion by 2020 (6).

The Need for Testing

It’s important that imposed regulations aren’t time-consuming and expensive to implement, or there is a risk of people turning to the black market to get around them, offsetting the initial aim of the regulations in keeping the public safe. New testing laws increase costs for manufacturers on top of the license and permit fees they are required to pay. Push back from producers is imminent with the implementation of new regulations, but this interaction is necessary to upgrade the cannabis industry and ensure that high-quality products are being made.

In the past, testing wasn’t typically carried out in-house because of the perception that analytical instrumentation is too expensive and complex, requiring a deep understanding of analytical chemistry. However, there are technologies and instrumentation available that are easy to use, cost-effective, and adapted for users with minimal experience. Gas chromatography (GC) is a widely used analytical tool for cannabis testing that enables potency testing, terpenes profiling, pesticide screening, and residual solvents analysis—knowledge that can significantly benefit the cannabis industry. With access to these robust testing techniques, companies can accurately determine the active cannabis ingredients, providing vital information in a competitive marketplace. This, in turn, builds consumer confidence and aids market growth.

Typically, the primary cannabinoids of interest for potency testing are THC, CBD, and cannabiol (CBN). A key goal in cannabis analysis is positive identification and quantification of the THC:CBD ratio. For medicinal cannabis, CBD is the primary component of interest because it is often characterized by high levels of CBD and low levels of THC. In contrast, recreational cannabis typically has high levels of THC—the main psychoactive ingredient—and low levels of CBD.

Figure 3: Chromatogram showing the terpenes and cannabinoids separated in a single run.

Table I: GC conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
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</thead>
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<tr>
<td>Injector temperature</td>
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<tr>
<td>Detector type</td>
<td>FID</td>
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<tr>
<td>Detector temperature</td>
<td>280 °C</td>
</tr>
<tr>
<td>Carrier gas type</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>Detector range</td>
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</tr>
<tr>
<td>Carrier flow</td>
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<tr>
<td>Split flow</td>
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<tr>
<td>Injection volume</td>
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<td>Stabilization time</td>
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<td>Column type</td>
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<td>Initial temperature</td>
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<tr>
<td>Hold</td>
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<tr>
<td>Temperature 1</td>
<td>200 °C</td>
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<tr>
<td>Ramp 2</td>
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</tr>
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<td>Total run time</td>
<td>17 min</td>
</tr>
</tbody>
</table>
Cannabis Testing in the Home of the Coffee Shop

Despite the perceived relaxed laws around cannabis consumption in the Netherlands, where tourists can legally consume cannabis in coffee shops, restrictions remain on growing and purchasing the drug (7). As a result, many consumers, including coffee shops, have to turn to the black market to acquire the product, where testing remains very rare.

Shamanics’s goal is to upgrade the cannabis scene in the Netherlands using GC to protect and grow the market. The company uses GC for quality assurance of its products and offers a testing service for coffee shops in Amsterdam to test the quality of their cannabis. The company conducts terpene analysis and potency testing. When testing for potency, they analyze total THC and CBD. When testing by GC, the acidified versions of the cannabinoids are converted to the neutral forms in heat of the GC injector (Figure 1), so the results of the neutral THC and CBD peaks will represent the total values. If the levels of the acidic and neutral cannabinoids are

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Response</th>
<th>Amount (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Myrcene</td>
<td>2.463</td>
<td>0.635</td>
<td>2.536</td>
</tr>
<tr>
<td>2</td>
<td>Δ-3-Carene</td>
<td>2.737</td>
<td>1.167</td>
<td>9.605</td>
</tr>
<tr>
<td>4</td>
<td>Geraniol</td>
<td>5.636</td>
<td>0.363</td>
<td>5.496</td>
</tr>
<tr>
<td>6</td>
<td>β-Caryophyllene</td>
<td>5.981</td>
<td>0.478</td>
<td>7.793</td>
</tr>
<tr>
<td>7</td>
<td>α-Humulene</td>
<td>6.524</td>
<td>0.212</td>
<td>15.551</td>
</tr>
<tr>
<td>8</td>
<td>CBD</td>
<td>13.356</td>
<td>0.709</td>
<td>26.620</td>
</tr>
<tr>
<td>9</td>
<td>Δ9-THC</td>
<td>14.284</td>
<td>18.523</td>
<td>601.286</td>
</tr>
<tr>
<td>10</td>
<td>CBG</td>
<td>14.843</td>
<td>0.811</td>
<td>24.035</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>692.922</td>
</tr>
</tbody>
</table>

No need to go to ICP/ICP-MS for heavy metal analysis in Cannabis, PF7 is specialize to analysis the heavy metals (especially As,Hg,Pb,Cd) in ppt level, high speed and throughput (10~20 seconds for each sample), much lower running cost, simple to use and easy to maintenance.

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required separately, they can be tested with GC by derivatizing the sample before analysis (Figure 2). This process protects the acidic cannabinoids from converting to their neutral forms in the GC injector. The total cannabinoid value is then calculated by adding the amount of neutral version present together with the amount of neutral version that could be created by the decarboxylation of the acidic version of the cannabinoid present.

**Experimental**

A typical analysis completed at Shamans's laboratory in Amsterdam is shown in Figure 3. The chromatogram shows the concentration and profile of the terpenes and cannabinoids in a cannabis sample to establish the potency, the flavor profile, strength, and therefore, quality.

**Materials**

First, 0.1 g of cannabis was added to 30 mL of methanol at ambient, shaken for 30 s and left to extract for 30 min. An aliquot of the extractant liquid was then collected through a syringe filter and placed in a sample vial ready for analysis. The analysis was performed using a 200 Series GC system with flame ionization detection (FID) (Ellutia) and a 30 m x 0.25 mm, 0.25-μm film thickness EL-5 column (Ellutia). The standards used for the calibration were a standard three-component cannabinoid mix (Restek Cat.# 34014: Cannabinoids Standard) and a 19-component terpene mix (Restek Cat.# 34095: Medical Cannabis Terpenes Standard #1).

**GC Conditions**

The GC conditions are listed in Table I.

**Results and Discussion**

The results show that this sample has 601.226 μg/mL of THC. This results in the sample having a total THC content of 18%.

Figure 4 shows a high level of THC and suggests that if consumed it would have more of the psychoactive effects. The results include the ratio of THC and CBD, providing information about the potency of the product. Figure 5 shows the concentration and profile of the terpenes that are present, providing information about the flavor profile that guides users about the sort of flavor characteristics that can be expected from the cannabis when consumed.

The variations in terpenes present and their subsequent levels define how that strain of cannabis will smell and taste. As an example, a strain showing high levels of limonene may have a citrus lemon aroma (limonene is also commonly found in lemons). Similarly, a high level of myrcene will give flavors similar to beer (myrcene is also commonly found in hops). The levels of terpenes can also be used as a guide to understand the quality of the product. For example, low levels could indicate that it has not been dried correctly. This information enables manufacturers to verify the quality of the final product.

**Conclusion**

It is evident that the cannabis industry still has a distance to go in terms of quality control. With no centralized regulatory body responsible to ensure stringent safety standards, the responsibility lies with the
grower, manufacturer, and even the individual consumer.

Access to affordable, robust analytical techniques, such as GC, enables products to be tested to measure the cannabinoid and terpene content and determine its overall quality, providing reassurance to consumers throughout the supply chain.

In the case above, GC testing determined the overall THC content to be 18%. This is a high level of THC and can therefore be expected to have psychoactive effects, providing crucial information for commercial purposes. It is critical to comply with fast-changing regulations to provide consistent, tested products in a highly competitive marketplace.

Ultimately, if cannabis wants to be considered a medicine, it needs to be manufactured and tested with the same care that is given to all medicines in the pharmaceutical market.

References
1) https://shamanics.nl/.

Andrew James
is the marketing director at Ellutia. James has worked at Ellutia for more than 20 years, during that time he has been involved with many aspects of the business from product development to strategic planning. Through this wide range involvement, he has developed an extensive wealth of knowledge and experience in the chromatography industry. James has been in charge of the company’s marketing for the last eight years, working to continually grow both the Ellutia brand and company as a whole. Direct correspondence to: Andrew.James@ellutia.com

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The Quantitative Determination of Phytocannabinoids in Hemp Oils Using HPLC with UV Detection

Medical marijuana generally possesses high levels of the psychotropic tetrahydrocannabinol (Δ9-THC) and lower levels of the nonpsychotropic cannabidiol (CBD). Pain mitigation and reduced severity of nausea and seizures are just a few of the therapeutic benefits reported by medical cannabis patients. Conversely, hemp contains high levels of CBD and lower levels (generally less than 0.3%) of THC. CBD has been reported to reduce or eliminate pain, stress, depression, inflammation, and headaches.

Medical marijuana generally possesses high levels of the psychotropic tetrahydrocannabinol (Δ9-THC) and lower levels of the nonpsychotropic cannabidiol (CBD). Pain mitigation and reduced severity of nausea and seizures are just a few of the therapeutic benefits reported by medical cannabis patients. The main source of CBD-rich oil is carbon dioxide or butane extraction of industrial hemp. Hemp is a robust crop containing high quantities of CBD and minor quantities of other cannabinoids. Like cannabis, hemp oil may be analyzed easily and effectively for its cannabinoid content. Presented herein is a procedure for the quantitative determination of 11 important cannabinoids, including CBD, in hemp oil using high performance liquid chromatography (HPLC) with ultraviolet (UV) detection.

Craig Young and Bob Clifford

To complicate matters, there is evidence that a combination of CBD, a host of other minor cannabinoids, and a complex array of terpenoids may provide the most benefit—called the entourage effect (1). Both free and esterified sterols and triterpenes have been identified in
cannabis and hemp, among which β-sitosterol and β-amyrin have been shown to have antibacterial properties (2). CBD-rich oil has become increasingly popular and is administered via sublingual drops, gel capsules, or as a topical ointment.

The main source of CBD-rich oil is industrial hemp. Hemp is considered a rustic plant because it is frost resistant, adapts to poor soil, reproduces easily, and does not require chemical fertilizers, pesticides, herbicides, or fungicides to thrive. A hemp crop tends to resist mildew and requires less water than cotton. Hemp textiles are considered softer than cotton.

CBD oil is derived as concentrate from carbon dioxide or butane extraction of hemp, sometimes followed by steam distillation or ethanol distillation for purification. The Farm Bill of 2014 distinguishes hemp from marijuana, yet interpreting the law is difficult in that “CBD oil” may be classified as marijuana. Various forms of hemp oil may be purchased online.

The U.S. Food and Drug Administration (FDA) has issued warning letters against unproven claims to some companies that market products containing CBD. As part of these actions, the FDA has determined the cannabinoid content of some hemp products and many were found to contain levels of CBD that are very different from the label claim. It is important to note that such products are not approved by the FDA for the diagnosis, cure, mitigation, treatment, or prevention of any disease (3).

The literature indicates more than 90 phytocannabinoids in cannabis and hemp (4). Only a handful are common targets of analysis for medical potency because they are the most abundant. Like cannabis, hemp oil may be analyzed easily and effectively for its cannabinoid content. This work presents

**Figure 1:** Cannabinoids found in hemp and marijuana.

**Figure 2:** Hemp oil 1, black label, label claim: 23 mg per serving; 100 servings per 100 mL. Calculation of label claim: 23,000 μg/mL or 2.3%.

Hemp oil 2, blue label, 500 mg per 30 mL, calculation of label claim: 16,666 μg/mL or 1.7%.

Hemp oil 3, green label, 15 mg per 1 serving per 0.5 mL = 15 mg/0.5 mL, calculation of label claim: 30,000 μg/mL or 3.0%.

Hemp oil 1
Black label
Label claim: 23 mg per serving; 100 serving per 100 mL
Calculation of label claim: 23,000 μg/mL or 2.3%

Hemp oil 2
Blue label
500 mg per 30 mL
Calculation of label claim: 16,666 μg/mL or 1.7%

Hemp oil 3
Green label
15 mg per 1 serving per 0.5 mL = 15 mg/0.5 mL
Calculation of label claim: 30,000 μg/mL or 3.0%
the quantitative determination of 11 important cannabinoids, including the predominate CBD (Figure 1), in various hemp oils.

**Standard Curves**

Using a comprehensive mixture of 11 cannabinoids (Shimadzu Part #220-91239-21; 250 μg/mL), standard curves were prepared for each target analyte with a minimum acceptable correlation coefficient ($R^2$) of 0.999 over six standard levels. A linear dynamic range was established at 0.5–100 mg/L (ppm) in each analyte.

**Experimental**

**Instrument and Chromatographic Conditions**

Instrument: Shimadzu LC-2030C UV (Cannabis Analyzer for Potency)

Column: Shimadzu NexLeaf CBX for Potency (150 mm x 4.6 mm, 2.7-μm $d_p$)

Guard column: Shimadzu NexLeaf CBX Guard

Mobile-phase A: 0.09% phosphoric acid in water

Mobile-phase B: 0.09% phosphoric acid in acetonitrile

Gradient: B concentration 70% (initial) → 95% (8 min)

Flow rate: 1.5 mL/min

Column temperature: 35 °C

Injection volume: 5 μL

Detection: 220 nm

**Hemp Oil Sample Preparation**

Hemp oils are typically rich in CBD, with relatively minor concentrations of other cannabinoids. All cannabinoid targets have a linear dynamic range, above which the detector response ceases to be linear with concentration. Accurate quantitation relies on the detector response to the analyte lying within the calibration range. Therefore, two dilution factors were used, depending on the quantitative goal. One dilution factor yielded the appropriate detector sensitivity to the array of minor cannabinoids. A second, higher dilution factor was established for the most accurate quantitation of CBG/CBDV, THC/CBN, and THCA/CBC.

**Table I: Summary of CBD quantitative determination for five hemp oils**

<table>
<thead>
<tr>
<th>ID Number</th>
<th>Name</th>
<th>1 Label Claim (CBD): 23,000 μg/mL</th>
<th>2 Label Claim (CBD): 16,666 μg/mL</th>
<th>3 Label Claim (CBD): 30,000 μg/mL</th>
<th>4 Label Claim (CBD): 8000 μg/mL</th>
<th>5 Label Claim (CBD): 8333 μg/mL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μg/mL (ppm)</td>
<td>%</td>
<td>μg/mL (ppm)</td>
<td>%</td>
<td>μg/mL (ppm)</td>
</tr>
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<td>5</td>
<td>CBD</td>
<td>21880.06</td>
<td>2.188</td>
<td>15242.8</td>
<td>1.524</td>
<td>24210.45</td>
</tr>
<tr>
<td>CBD % of label claim</td>
<td>95</td>
<td>92</td>
<td>81</td>
<td>122</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>
quantitation of the major CBD component so that its response was within the established quantitative dynamic range established for that analyte. In practice, it was found that the two approaches yielded quantitative values for CBD that agreed within 0.2%.

A. Quantitative Total Cannabinoids
1. Add 400 μL of isopropanol to a 2-mL glass vial
2. Add 10 μL of hemp oil sample and completely dissolve
3. Agitate for 30 s
4. Add 400 μL of methanol to the mixture
5. Agitate for 30 s
6. Filter the mixture through a 0.2-μm PTFE syringe filter into a high performance liquid chromatography (HPLC) vial
7. (Note: Total dilution factor 81X)

B. Quantitative CBD Only
1. Add 800 μL of methanol to a 2-mL glass vial
2. Add 200 μL of the part A mixture
3. Agitate for 30 s
4. (Note: Total dilution factor 405X)

Five hemp oils were tested in this study; they were purchased from various mail-order vendors. The appearance and label information for three of the five appear in Figure 2, referenced as black, blue, and green. The two other samples tested but not pictured are referred to as red and yellow.

Qualitative Analysis of Hemp Oils
Chromatograms for hemp oils 1 (black), 2 (blue), and 3 (green) appear in Figure 3. Peak labels appear for only those cannabinoids identified in the sample.

Quantitative Results
Summary for Hemp Oils
The astute reader may correctly ask about the noncannabinoid constituents in the sample and how they may influence the chromatography. As the sample preparation presented here is essentially a simple dilution of the oil sample into injectable form, nothing is done to separate cannabinoids from terpenes, phenolics, or pigment compounds. Under the reversed-phase chromatography conditions used here, any pigment compounds emerge early in the chromatography.

The sum total terpene content of hemp and cannabis is 1–3% (2), comprising possibly hundreds of terpenoids. As for chemistry, many triterpenes and sesquiterpenes have similar polarities to the cannabinoids and are expected to retain on the
analytical column to similar degrees as the cannabinoids. Within the detection limits of this work, any terpenoids are undetectable, essentially lost in the baseline.

Discussion

Tables I and II summarize the quantitative findings for the samples studied. Table I reflects the accurate quantitation of CBD using the higher dilution factor (405X). Table II reflects the quantitation of the comprehensive target list.

As a general sample observation, hemp oils 1 (black) and 2 (blue) exhibited a transparent, weak-yellow or green coloration. Our assumption was that each of these is a product of multistep purification after extraction; for example, carbon dioxide or butane extraction followed by steam distillation. Notably, hemp oil 3 (green) was opaque brown or green and gritty in appearance. It also had the most intense smell—a distinctly “earthy” odor. Accordingly, our assumption was that the sample was the result of crude extraction only, with no further refinement.

It is important to note that it has been reported in the literature that the whole plant can be more beneficial to the consumer because it contains not only the cannabinoids, but also an array of terpenes providing a synergistic whole plant benefit. The whole plant can also provide essential fatty acids, plant sterols for lowering cholesterol, and antioxidants chlorophyll and vitamin E.

Hemp oils 1 (black) and 2 (blue) showed high ratios of CBD to total cannabinoids, both at 92%, and the lowest quantity of other cannabinoids. This finding supported the assumption, along with transparency and color, that these oils were the more highly purified samples. Both samples also tested close to label claim at 95% and 92%, respectively.

Hemp oil 3 (green) revealed the highest content of CBD and total cannabinoids, yet exhibited the

<table>
<thead>
<tr>
<th>Table II: Summary of total cannabinoids quantitative determination</th>
</tr>
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<tbody>
<tr>
<td>ID Number</td>
</tr>
<tr>
<td>Number</td>
</tr>
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<tr>
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<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>Total cannabinoids %</td>
</tr>
<tr>
<td>CBD % of total</td>
</tr>
</tbody>
</table>
lowest ratio of CBD to total cannabinoids (59%). This observation is consistent with the assumption that its crude appearance reflected the least amount of post-extraction purification. Although its CBD percent of label claim tested the lowest (81%), this sample did contain the highest level of CBD compared to all other oils tested.

Hemp oils 4 (red) and 5 (yellow) tested higher than label claim at 122% and 200%, respectively. The observation is consistent with FDA findings for CBD products, perhaps calling into question the type and accuracy of testing used to justify label claims.

In summary, all samples contained less $\Delta^2$-THC than the amount allowed by law (0.3%). Also, all samples showed an array of other cannabinoids, but the minor component, THC-V, was not detected in any of the hemp oil samples. From a quality control point of view, two samples were within a reasonable range of the label claim at ±10%. One sample was well below label claim and two other samples were well above the label claims, one by as much as 200%. When purchasing CBD oils, one should consider

• label claim,
• actual concentration,
• the quality control from batch to batch,
• other cannabinoids of importance,
• whole plant complexity, and
• the selling price.

References

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Holding Data to a Higher Standard, Part I: A Guide to Standard Production, Use, and Data Validation

Testing, manufacturing, and research laboratories face more challenges and regulations than ever before. Many accreditation bodies issue increasing numbers of guidelines. Regulatory agencies increase the number of compounds and elements that need to be reported while the levels of detection required are being decreased. There is often a lot of time, effort, and money invested in deciphering the data and determining its validity and accuracy. Here, we explore the accreditation, regulation, and guidelines around the manufacture and use of standards and certified reference materials (CRMs) and discuss the variables from accreditation to uncertainty involved in producing and using standards and reference materials. We also discuss the changing field of chemical analysis and the challenges that arise from the pursuit of increasingly smaller levels of detection and quantitation, including clean laboratory techniques to reduce analytical error and contamination in standards use and data collection.

Patricia Atkins
or standard will retain its chemical properties within the designated “shelf life” or within its expiration date if it is maintained under the expected and outlined stability conditions. A material is considered to be unstable if it can decompose, volatilize (burn or explode), or oxidize (corrode) under normal stated conditions.

Homogeneity is the state of being of uniform composition or character. Reference materials can have two types of homogeneity: within-unit homogeneity or between-unit (or lot) homogeneity. Within-unit homogeneity means there is no precipitation or stratification of the material that cannot be rectified by following instructions for use. Some reference materials can settle out of solution, but are still considered homogeneous if they can be redissolved into the solution by following the instructions for use (that is, sonicate, heat, shake). Between-unit or lot homogeneity is found between separate packaging units.

Traceability is the ability to trace a product or service from the point of origin through the manufacturing or service process through to final analysis, delivery, and receipt. Reference materials producers must ensure that the material can be traced back to a primary or secondary standard.

Uncertainty is the estimate attached to a certified value that characterizes the range of values where the "true value" lies within a stated confidence level. Uncertainty can encompass random effects such as changes in temperature, humidity, drift accounted for by corrections, and variability in performance of an instrument or analyst. Uncertainty also includes the contributions from within-unit and between-unit homogeneity, changes because of storage and transportation conditions, and any uncertainties arising from the manufacture or testing of the reference material.

Types of Uncertainty
There are two basic classifications for types of uncertainty: Type A and Type B uncertainty. Type A uncertainty is associated with repeated measurements and the statistical analysis of the series of observations. Type A uncertainty is calculated from the measurement’s standard deviation divided by the square root of the number of replicates.

Type B uncertainty is based on scientific judgement made from previous experience and manufacturer’s specifications. Type B normal distribution is used when an estimate is made from repeated observations of a randomly varying process and an uncertainty is associated with a certain confidence interval. This type of uncertainty distribution is often found in a calibration certificate with a stated confidence level. Reference materials providers accredited by ISO 17025 and ISO 17034 provide certificates using combined and expanded uncertainties within a normal distribution. These certificates contain stated values and the uncertainty associated with that value as well as the contributions to those uncertainties.

True Value, Accuracy, and Precision
Uncertainty is not error or mistakes. Error causes values to differ when a measurement is repeated and is the difference between the stated measurement and the true value of the measurand. True values are never absolute. The nature of a true value is that it contains uncertainty and error, which make it somewhat indeterminate. True values are obtained by perfect and error-free measurements, which do not exist in reality. Instead, the expected, specified, or theoretical value becomes the accepted true value. Analysts then compare the observed or measured values against that accepted true value to determine accuracy or “trueness” of the data set.

Often, accuracy and precision are used interchangeably when discussing data quality. In reality, they are very different assessments of data and the acquisition process. Accuracy is the measurement of individual or groups of data points in relationship to the “true” value. In essence, accuracy is how close your data gets to the target and is often expressed as either a numerical or percentile difference between the observed result and the target or “true” value.

Precision, on the other hand, is the measurement of how closely the data points within a data set relate to each other. It is the measure of how well data points cluster within the target range and is often expressed as a standard deviation. Precision is an important tool for the evaluation of instrumentation and methodologies by determining how data is produced after varied replicates (Figure 1). Repeatability and reproducibility measure the quality of the data, method, or instrumentation by examining the precision under the same (minimal difference) or different (maximal difference) test conditions. Repeatability (or test-retest reliability) is the measurement of variation arising when all the measurement conditions are kept constant. These conditions typically include location, procedure, operator, and instrument run in repetition over a short period of time.

Reproducibility is the measurement of variation arising in the same measurement process occurring across different conditions such as location, operator, instruments, and over long periods of time.
Estimating Error

Another way of looking at accuracy and precision is in terms of measuring the different types of error. If accuracy is the measurement of the difference between a result and a “true” value, then error is the actual difference or the cause of the difference. The estimation of error can be calculated in two ways, either as an absolute or relative error. Absolute errors are expressed in the same units as the data set and relative errors are expressed as ratios, such as percent or fractions.

Absolute accuracy error is the true value subtracted from an observed value and is expressed in the same units as the data. Errors in precision data are most commonly calculated as some variation of the standard deviation of the data set. An absolute precision error calculation is based on either the standard deviation of a data set or values taken from a plotted curve. A relative precision error is most commonly expressed as relative standard deviation (RSD) or coefficient of variance (CV or %RSD) of the data set (Table I).

The most common error in regards to data are observational or measurement errors, which are the difference between a measured value and its true value. Most measured values contain an inherent aspect of variability as part of the measurement process, which can be classified as either random or systematic errors.

Random (or indeterminate) errors lead to measured values that are inconsistent with repeated measurements. Systematic (or determinate) errors are introduced inaccuracy from the measurement process or analytical system. There are some basic sources for systematic error in data such as operator or analyst, apparatus and environment, and method or procedure. Systematic errors can often be reduced or eliminated by observation, record-keeping, training, and maintenance.

Understanding and Compensating for Indeterminate Error

Random or indeterminate errors arise from random fluctuations and variances in the measured quantities and occur even in tightly controlled analysis systems or conditions. It is not possible to eliminate all sources of random error from a method or system. However, random errors can be minimized by experimental or method design. For instance, while it is impossible to keep an absolute temperature in a laboratory at all times, it is possible to limit the range of temperature changes. In instrumentation, small changes to the electrical systems from fluctuations in current, voltage, and resistance cause small continuing variations that can be seen as instrumental noise. The measurement of these random errors is often determined by the examination of the precision of the generated data set. Precision is a measure of statistical variability in the description of random errors. Precision analyzes the data set for the relationship and distance between each of the data points independent of the “true” or estimated value of the data to identify and quantify the variability of the data.

Accuracy is the description of systematic errors and is a measure of statistical bias that causes a difference between a result and the “true” value (trueness). A second definition, recognized by ISO, defines accuracy as a combination of random and systematic error that then requires high accuracy to also have high precision and high “trueness.” An ideal measurement method, procedure, experiment, or instrument is both accurate and precise with measurements that are all close to and clustered around the target or “true” value. The accuracy and precision of a measurement value is a process validated by the repeated measurements of a traceable reference standard or reference material.
Using Standards and Reference Materials

Certified standards or CRMs are materials produced by standards providers that have one or more certified values with uncertainty established using validated methods and are accompanied by a certificate. The uncertainty characterizes the range of the dispersion of values that occurs through the determinate variation of all the components that are part of the process for creating the standard.

Each of the components in the creation of the standard have a calculated uncertainty, which then are all combined to create a combined uncertainty associated with the certified value. For example, in the creation of a chemical standard there could be separate uncertainties for all the volumetric glassware used in the production of the standard as well as uncertainty from the purity of the starting material, variations in the balance, temperature of the laboratory, and purity of the solvents. Each uncertainty for individual components is calculated and added together to form the combined uncertainty for the standard. To be clear, the uncertainty listed on a standard certificate is the measured uncertainty for that standard’s certified value and not the expected range of results for an instrument or test method. Each test method or instrument carries its own set of uncertainty calculations that determine the accuracy and precision of that analytical method, which is independent of the value on the certificate of the standard.

CRMs have a number of uses, including validation of methods, standardization or calibration of instruments or materials, and quality control and assurance procedures. A calibration procedure establishes the relationship between a concentration of an analyte and the instrumental or procedural response to that analyte. A calibration curve is the plotting of multiple points within a dynamic range to establish the analyte response within a system during the collection of data points. One element of the correct interpretation of data from instrumental systems is the effect of a sample matrix on an instrumental analytical response. The matrix effect can be responsible for either analyte suppression or enhancement. In an analysis where matrix can influence the response of an analyte, it is common to match the matrix of analytical standards or reference materials to the matrix of the target sample to compensate for matrix effects.

Different approaches to using calibration standards may need to be employed to compensate for the possible variability within a procedure or analytical system. Internal standards are reference standards that are either similar in character or analogs of the target analytes that have a similar analytical response are added to the sample before analysis. In some cases, deuterated forms of the target analytes are used as internal standards. This type of standard allows the variation of instrument response to be compensated for by the use of a relative response ratio established between the internal standard and the target analyte. A second type of internal standard is a standard addition or spiking standard. In some analyses, the matrix response, instrument response, and analyte response are indistinguishable from each other because the analyte concentration nears the lower limit of detection or quantitation. A target standard can then be added in known concentration to compensate for the matrix or instrument effects to bring the signal of the target analyte into a quantitative range.

External standards are multiple calibration points that contain standards or known concentrations of the target analytes and matrix components. Depending on the type of analytical techniques, linear calibration curves can be generated between response and concentration that can be calculated for the degree of linearity or the correlation coefficient ($r$). An $r$ value approaching 1 reflects a higher degree of linearity, most analysts accept values of $>0.999$ as acceptable correlation.

Calibration curves are often affected by the limitations of the instrumentation. Data can become biased by calibration points
influenced by instrument limits of detection, quantitation, and linearity. Limit of detection (LOD) is the lower limit of a method or system at which the target can be detected as different from a blank with a high confidence level (usually over three standard deviations from the blank response). The limit of quantitation (LOQ) is the lower limit of a method or system at which the target can be reasonably calculated where two distinct values between the target and blank can be observed (usually over 10 standard deviations from the blank response) (4) (Figure 2).

A second method of determining levels of detection and quantitation can be considered using the signal-to-noise ratio (S/N). The signal-to-noise ratio is the response of an analyte measure on an instrument as a ratio of that response to the baseline variation (noise) of the system. Limits of detection are often recognized as target responses that have three times the response of baseline noise or S/N ≥ 3. Limits of quantitation are recognized as target responses which have 10 times the response of baseline noise or S/N ≥ 10.

Limits of linearity (LOL) are the upper limits of a system or calibration curve where the linearity of the calibration curve starts to be skewed creating a loss of linearity (Figure 3). This loss of linearity can be a sign that the instrumental detection source is approaching saturation. The array of data values between the LOQ and the LOL is considered to be the dynamic range of the system where the greatest potential for accurate measurements will occur.

The understanding of a system’s dynamic range and the accurate bracketing of calibration curves within the range and around the target analyte concentration increase the accuracy of the measurements. If a calibration curve is created that does not potentially bracket all the possible target data points, then the calibration curve can be biased to artificially increase or decrease the results and create error.

**Conclusion**

The elimination of error from analytical methods is an ongoing process that forces the analytical laboratory to examine all their processes to eliminate sources of systematic error and mistakes. It is then a process of identifying the sources of random error in the analysis and sample preparation procedures to calculate the uncertainty associated with each source. Standards and certified reference materials give an analyst the known quantity, character, or identity to reference against their samples and instruments to further eliminate error and increase accuracy and precision to bring them closer to their goal of determining the true value.

**References**


Applying Ultrasonic Energy to Cannabis Production and Quality Control

Ultrasonic energy, broadly defined as sound above the range of human hearing (approximately 20,000 cycles/s or 20 kHz), can be used in several ways during the production of cannabis to ensure that the product is of the highest quality. In addition to providing a tutorial on how ultrasonic equipment works, this article describes how the equipment is applied in laboratory practices and manufacturing. Examples include extraction procedures, cleaning ion targets in mass spectrometers, preparing oil emulsions for edible or drinkable products, degassing oil (removing trapped air) to maintain stable oil volume during selling, degassing high performance liquid chromatography (HPLC) solvents, bubbling off ethanol before oil distillation, and removing gums and waxes from glassware used in production.

Robert Sandor and Kirsten Blake

Ultrasonic energy is commonly associated with ultrasonic cleaning. As explained by Edward W. Lamm in his article "The Development of Ultrasonic Cleaning" (1), its history dates to the early 1930s and work done at Radio Corporation of America (RCA) laboratories in New Jersey. The first practical applications, according to Lamm's article, were introduced in the 1950s, and were operated at 18–40 kHz. "Up until the late 1980s most of the commercially available systems operated at 25–40 kHz," Lamm stated.

Today, ultrasonic cleaners are available in several frequencies, including 25, 45, 80, and 130 kHz. Units are also available offering dual-frequency options.

Ultrasonic energy is used in research, product development, and manufacturing operations. Typically, these involve homogenizing, emulsifying, dispersing, dissolving or mixing difficult samples, and degassing liquids to remove trapped air.

Extraction Procedures

Ultrasonic energy is a proven technique to achieve fast, safe extraction. For example, it is a method often specified in United States Pharmacopeia (USP) monographs to extract active pharmaceutical ingredients from carriers for content uniformity and potency assay tests.

In cannabis production, most regulated markets require all cannabis products to be tested for efficacy (active ingredients, such as cannabinoids and terpenoids), as well as for contaminants (such as pesticides, mycotoxins, heavy metals, microbes, and residual solvents).

Cannabis products include plant material (mostly flowers and trimmed leaves), concentrated extracted essential oils (concentrates, waxes, and oils), and infused products (edibles such as candies, chocolates, baked goods, transdermal patches, suppositories, and beverages).

Clearly, accurate test results depend on efficient, reproducible extraction from these often complex matrices, and sonication is one way many cannabis laboratories seek to achieve those goals. Sonication is valuable because it deposits energy into the solvent–matrix system, effectively speeding the process of extraction and dissolution.

Mass Spectrometry

Mass spectrometers are typically the workhorse instruments in a cannabis laboratory. Many laboratories have inductively coupled plasma–mass spectrometry (ICP-MS), liquid chromatography–tandem mass spectrometry (LC–MS/MS) and gas chromatography (GC)–MS/MS instruments all in the same laboratory.

Since cannabis contains viscous oils and resinous compounds of moderate to high molecular weight, ion sources and associated components can get contaminated with organic residues that are difficult to remove. In particular, contamination of electrodes that steer the ions leads to defocusing and loss of signal.

Sonication is often the most efficient method to clean these parts. Likewise, the chromatography injectors and inlets can also become contaminated and clogged with the resins and residues and sonication in a nonpolar solvent is often the method of choice to clean these components as well.

General Cleaning with Sonication

For cleaning applications, ultrasonic energy is used to remove contaminants from the surfaces of virtually any product that can be safely immersed in a water-based
biodegradable ultrasonic cleaning solution. Cleaning solution formulas, dilution recommendations, and operating procedures are available for specific cleaning tasks.

Components of Ultrasonic Cleaning Equipment
There are multiple manufacturers of ultrasonic cleaning and processing equipment. Regardless of the manufacturer, common components include:

- A tank, usually stainless steel, to hold the cleaning solution or water that is typically mixed with a surfactant
- A generator to supply power
- Ultrasonic transducers bonded to the bottom of the tank
- A mesh-bottomed basket (standard or optional) to hold products being cleaned or processed without contacting the bottom of the tank
- A lid (standard or optional) to reduce cleaning solution evaporation and noise

Selection criteria then can move to:
- Cleaning tank size (length, width, and depth)
- A control panel that can range from a simple on–off switch to highly sophisticated options including timers, thermostats, and features including sweep, normal, pulse, degassing, and adjustable ultrasonic power and frequency.

One might ask about the need for a more sophisticated unit. The answer is simple. It provides cannabis processors with the ability to develop and customize optimum processing steps to achieve consistent, high-quality product from a variety of sources.

How the Process Works
When activated, the equipment’s generator powers the transducers to vibrate at their designed ultrasonic frequency. This vibration causes the tank bottom to vibrate as a membrane that produces countless microscopic vacuum bubbles.

In applications such as cleaning glassware, these bubbles implode with tremendous force in a process called cavitation. This cavitation quickly and safely blasts loose contaminants and carries away even the most tenacious residue. Products are cleaned with a solution formulation designed for the application.

In a processing application, products are contained in Erlenmeyer flasks, test tubes, or beakers. These containers are lowered, but not fully immersed, into a water or surfactant solution. Ultrasonic energy passes through the glass walls of the containers to act on the contents.

This approach achieves the homogenizing, emulsifying, degassing, and other cannabis processing steps in a fast, efficient, and environmentally friendly way.

Comparing Ultrasonic Cannabis Production and Quality Control to Alternative Methods
In 2015, the National Hemp Association published an article in Hemp News titled “Five Major Types of Cannabis Extraction” (2). In the article, Rien Havens, PhD, CTO, Really Helping, PBC, stated that in the winter of 2014 he began research to develop the optimal methods of hemp extraction. “It was quite a ride. I had in mind three main goals,” said Havens. “No use of fossil fuels, low energy footprint, and cost effectiveness.”

Here, we paraphrase Havens’ findings as published in the article (2). Readers may wish to access the full article for additional details.

- Ethanol produced a relatively good quality extract, but the solvent and energy costs were high. It did not produce the desired results of volume and speed. Residual solvent in the final product was also a drawback.
- A closed-loop hydrocarbon extractor is inexpensive to set up, but uses fossil fuels, almost always contains cancer-causing components like benzene, and often there are metal filings and welding debris in the solvent tanks.
- A supercritical carbon dioxide extractor was able to produce a high quality extract with very high terpene retention, a great color, taste, speed, and selectivity. The downside of this approach includes high overhead and unruly energy consumption.
- Critical water extraction is “green” with no added solvents but clean water. There is no solvent loss, or cost, and the volume and cost of the extractor makes it a good candidate for industrial hemp extraction.
- A truly solventless method is sonic and ultrasonic waves in the plant matter that push the product out through vibration. This method can also be scaled up, like water extraction on a budget, and produces a very nice, high-quality extract.

A Closer Look at Ultrasonic Equipment for Cannabis Production
Ultrasonic cleaner tanks are available in multiple sizes in terms of length, width, and depth. When processing in flasks and beakers, a shallow-depth tank is a good choice with a length and width that allows the processing of several containers at once.

The following sections provide a more detailed illustration of how the process works. This example describes the use of a 37-kHz ultrasonic cleaner based on its tank configuration and operating features.

Remember that the transformation or extraction process avoids chemical degradation that can be caused by excessive heat or mechanically induced damage.

Extraction and Processing Steps
Product is placed in flasks along with a recommended solvent. Flasks are
partially immersed in a sonicator bath containing a surfactant.

The tank configuration of the ultrasonic unit used in this process is especially designed to quickly and safely accomplish extraction and further processing. The inside dimensions of the shallow basket, 17.9 x 9.8 x 2.2 in. (LxWxH), facilitate positioning of multiple smaller containers or larger beakers. Flask clamps are used to affix flasks to the mesh-bottom basket; test tube holders are also available.

The equipment described was also selected because of its high ultrasonic power per unit volume. This feature permits the preparation process to be completed before heat buildup, a natural result of ultrasonic energy, which can degrade product. If heat is a concern, a useful accessory is a cooling coil to prevent temperature increase. The cooling coil must be attached to a source of recirculating cold liquid such as a laboratory chiller.

Another suggestion for producers is to look for an ultrasonic unit equipped with a microprocessor-controlled ultrasonic generator that adjusts to the load; a degas mode to remove trapped air, and a timer that displays set and remaining time.

Other useful features include the ability to operate in a fixed frequency (also called normal) mode that is ideal for breaking up product and a sweep mode that provides uniform distribution of ultrasonic energy when it is used to clean glassware and other processing equipment (see below). The sweep mode delivers a small positive and negative fluctuation in ultrasonic frequency throughout the bath.

**An Extraction Sequence**

Water and a surfactant are added to the fill line of the sonicator tank. The unit is turned on and the degas function is activated to both mix the solution and drive off trapped air. This step should take about 10 min.

The product is lowered into the bath and the unit is set to operate in the normal mode. The generator provides ultrasonic energy in the bath that passes through flask walls. This step mixes, disperses, emulsifies, homogenizes, and dissolves the samples. The unit will shut down at the end of the timed cycle.

Operators will soon develop their own “techniques” or “standard operating procedures” for their processing cycles.

**Cleaning Processing**

**Glassware and Other Equipment**

Substantial investments may be made in cannabis processing glassware and other tools. Because of the nature of the process, difficult-to-remove deposits adhere to the inside of flasks, test tubes, and beakers. Overall, cleaning is also recommended to ensure a quality product.

Cleaning internal surfaces can be accomplished by filling the container with a suitable biodegradable cleaning solution and, as with the extraction and processing steps, placing the container in the water–surfactant solution and activating the degas mode and ultrasound. Cavitation passes through the glass walls to loosen and remove the strongly adhering residues. These residues are then discarded and the containers can be rinsed for further use.

Small instruments can be placed in the mesh tray. In this case, the water–surfactant solution is removed and replaced with a degassed biodegradable formulation designed for glassware. In this instance, cleaning should be accomplished using the sweep mode to provide more-uniform cleaning.

To thoroughly clean internal and external surfaces of processing equipment, a larger ultrasonic cleaner is required with a suitable depth to enable full immersion of the equipment. Biodegradable concentrates for labware are available in acidic, basic, and neutral formulations depending on the nature of the contaminants to be removed. All of the formulations come with material safety data sheets and use instructions including dilution recommendations and cleaning temperatures.

**Acknowledgment**

The authors acknowledge with thanks the contributions of Donald P. Land, PhD, to this article. He is Chief Scientific Consultant for Steep Hill, a leading cannabis testing and research and development (R&D) company with locations across the world.

**Reference**


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Pioneering Spirits: Three Women Leaders in Cannabis Science Share Their Trials, Tribulations, Successes, and Advice

In this month’s “Cannabis Crossroads,” we share inspiring stories of passion and persistence by highlighting the amazing efforts of some truly pioneering women: Shannon Hoffman, the Regional Director of Operations and Certifying Chemist for Steep Hill Maryland, Pennsylvania, and Washington, D.C.; Dr. Jacklyn R. Green, Chief Executive Officer of Agate Biosciences; and Dr. Sue Sisley, M.D., President of Scottsdale Research Institute, on faculty at Colorado State University and Humboldt State University, and Senior Fellow at Thomas Jefferson University Lambert Center. These three women, each with a diverse background, have become leaders in cannabis science.

Josh Crossney

In this month’s “Cannabis Crossroads,” I would like to share inspiring stories of passion and persistence by highlighting the amazing efforts of some truly pioneering souls: Shannon Hoffman, the Regional Director of Operations and Certifying Chemist for Steep Hill Maryland, Pennsylvania, and Washington, D.C.; Dr. Jacklyn R. Green, Chief Executive Officer of Agate Biosciences; and Dr. Sue Sisley, M.D., President of Scottsdale Research Institute, on faculty at Colorado State University and Humboldt State University, and Senior Fellow at Thomas Jefferson University Lambert Center. These three women, each with a diverse background, are true leaders in cannabis science.

Please explain how long you have been working in the cannabis industry and what first got you interested in cannabis science.

Shannon Hoffman: I’ve been working in cannabis for close to a year. I started off doing some consulting last summer before joining Steep Hill in December 2017. I’ve been casually interested in cannabis science for some time. A merger at my previous employer led to my decision to resign at just the time when the industry was on the brink of opening in Maryland; it seemed fated for me to make the jump into cannabis.

Dr. Jacklyn R. Green: I was first introduced to cannabis science by my best friend, Dr. Claudia Jensen, in 1996, when the California laws changed. She was one of the very first cannabis doctors in California. I was supportive of cannabis, although I did not partake. Through my conversations with Claudia, I became much more attuned with the role of cannabis in helping people with a wide variety of conditions. At that time, I was working full time as a planetary scientist at The National Aeronautics and Space Administration’s (NASA) Jet Propulsion Laboratory. My husband, Dr. Roger Kern, a plant microbiologist, and I developed a concept for a small, autonomous greenhouse for the surface of Mars to test technologies that we would need for larger greenhouses for future astronauts exploring the Red Planet. This project gave us our first steps in developing controlled environment agriculture (CEA) systems. As the years flew by, we continued our work in CEA—and as cannabis laws changed across the country, we found ourselves in an environment that allowed us to bring our scientific knowledge, systems engineering approach, and project management experience from NASA to the cannabis industry to help solve some of the most challenging problems facing us to ensure safe and consistent products for people across the country.

Dr. Sue Sisley: It’s been almost 10 years since I began studying cannabis as a medicine and fighting for public policy reform. Military veterans prompted my interest in studying cannabis. They claimed they were benefiting, but I was highly skeptical. I’m a lifelong Republican and had been brainwashed by big pharma and the medical profession to view cannabis as a dangerous addictive drug. I first began paying attention because of the social justice issues. We launched a charity called Doctors for Cannabis Regulation to try to create a safe harbor for physicians to speak openly about their views on ending cannabis prohibition. We’ve also created a veterans charity called the Battlefield Foundation, which recently received its 501(c)(3). We are busy trying to raise money for rigorous controlled trials as well as to create jobs in the cannabis industry for veterans. So we do a matchmaking
service and it’s been very gratifying to create meaningful employment for veterans within the cannabis space.

What is one lesson that you had to learn the hard way, or a major obstacle that you had to overcome in cannabis science?

Green: Explaining the role of science, technology, engineering, and math (STEM) subjects to long-term cannabis industry participants is an ongoing challenge. Cannabis is coming out of the shadows into the bright light of scientific investigation, well-implemented systems engineering, and expert project management. The challenge is that people, in general, believe they know how to grow plants and specialists know how to grow cannabis in high-tech environments indoors. However, most cannabis insiders perform their work by the seat of their pants, by the look and feel of things. We must have rigorous procedures, exactness in standards, and intelligent planning for projects of all sizes that allow optimization, at the systems level, to create a safe and reliable supply chain and a consistent, regulated distribution chain for legal cannabis products.

Sisley: I learned that public universities are terrified of the word cannabis. Cannabis has become so politically radioactive that many universities are still convinced they will lose their federal funding if they do even U.S. Food and Drug Administration (FDA)-approved research.

Can you please share with me your biggest achievement or favorite memory from working in the cannabis industry?

Hoffman: My biggest achievement was leading our laboratories in Maryland and Pennsylvania through ISO 17025 accreditation. I’ve worked in the regulated industry with demanding quality systems, but ISO is quite rigorous. It required our entire team to come together and work tirelessly to meet tight timings. You really see what people are made of when you face challenges and I couldn’t ask for a more talented or dedicated team at Steep Hill.

Green: I am a competitive person, so I find it intensely gratifying to win licenses and permits for our clients. I love to start with them in our brainstorming workshops in which we use design thinking and creative problem-solving techniques to identify and tackle their most challenging problems. We go through an intense phase of concept development and then dig deep into the systems engineering for requirements and risk analysis. Finally, we go into the hard-core project management to get the full concept finalized and written up into a compliant, compelling, and beautiful application. I find that delivering a set of documents to our clients to be my favorite moment. I love to see their faces as they thumb through the documents. Then, of course, hearing that they have won through the official governing bodies is a moment of great satisfaction.

Sisley: The biggest achievement is perseverance in the face of constant government blockades. Over the course of the past 10 years we have managed to overcome some really onerous hurdles to get efficacy research started. The U.S. government has systematically impeded cannabis efficacy trials for so many decades. But we are slowly learning how to navigate these waters and teaching other young scientists how to pick up the baton!

What excites you most about the future of cannabis science?

Hoffman: There is so much yet to learn about how this amazing plant can benefit our health. Having more and more access to research is going to enable us to target therapy in ways we couldn’t imagine. I’m excited to be a part of Steep Hill because we can help to ensure that patients are getting safe, efficacious medicine now and in the future as we learn more about what this plant can do.

Green: Cannabis science—it is a subject just waiting for exploration! As an astronomer, I love to explore and see what is new in the universe, or closer to home, in our own solar system. Who would imagine that there is a plant with special medicinal properties that any hobbyist could grow but that has been forbidden to us to explore and learn about? It defies logic that we would not explore and advance our knowledge for the good of humanity. There is a wide variety of potential studies, across the many disciplines of STEM, that would provide fundamental knowledge about cannabis. Unfortunately, we are not able to tackle those scientific studies at this time! We need federal funding for robust research programs, university research programs, and grants for private companies to conduct unbiased, zero conflict-of-interest studies. For that to happen, the laws will need to change at the federal level.

Sisley: I think it’s the opportunity to possibly work with other growers besides the University of Mississippi. Since 1968 the University of Mississippi has enjoyed a government-enforced monopoly on the only federally legal supply of cannabis for research. But thanks to relentless lobbying efforts from the Multidisciplinary...
What advice do you have for readers interested in starting a career in cannabis science?

Hoffman: You have to love it. I think anyone who feels a sense of purpose about cannabis can find a place for themselves in the industry, but you will have to work hard and should be prepared for challenges along the way. It is a volatile industry in the sense that it is varied and evolving and we are all learning together the best way to do this. There is a need for a diverse skill set in cannabis, so there are opportunities for anyone who feels that drive to be involved to work to their strengths and contribute to building something amazing.

Green: I am very clear in my advice to readers interested in starting a career in cannabis science—get as much education as you possibly can. Think of every degree you obtain as a ticket—a ticket to a journey that will take you far in life and in knowledge. As a scientist you have an element of wonder in your life. You see and experience nature in ways that most people don’t ever contemplate. You can become a world expert in cannabis across many scientific disciplines—from biology, chemistry, physics, engineering, math, computer science, numerical modeling, medicine, plant science, microbiology, and more. I am grateful beyond words that I became a scientist. Whether I am an astronaut or a cannabis consultant, my skills as a thinker and explorer serve me well every single day. I know that no problem is too difficult to tackle. We may make small steps in progress, but each small step opens up a new horizon. With a life in science, I always want to see the next horizon.

Sisley: The first step is volunteering with the team and your local area. Try to identify scientists who are already doing this work and see if they will mentor you or let you apprentice there. Even if you’re an unpaid intern for a few months, they will likely recognize your skill and enthusiasm and probably hire you on!

About the Authors

Shannon Hoffman is the Regional Director of Operations and Certifying Chemist for Steep Hill Maryland, Pennsylvania, and Washington, D.C. Steep Hill, a leading cannabis science and technology company, was the first commercial cannabis lab in the U.S. and has expanded its best practices in cannabis testing to the mid-Atlantic with physician-led laboratories in Maryland, Pennsylvania, and Washington, D.C. Hoffman is an analytical chemist with 18 years of experience in research and development and manufacturing of consumer products, cosmetics, and over the counter pharmaceuticals. She has a bachelor’s degree in chemistry from the University of Maryland, Baltimore County and a master’s degree in analytical chemistry from the Illinois Institute of Technology.

Dr. Jacklyn R. Green is a project manager and systems engineer focused on commercial businesses in the cannabis industry. With her PhD from The University of Texas at Austin, she leads, manages, and delivers compliant, compelling, and selectable applications for city- and state-legal commercial cannabis businesses. She spent 27 years at NASA’s Jet Propulsion Laboratory as a scientist and project manager, leading advanced technology development projects and proposal development in the complex government-regulated environment. Now, as the Chief Executive Officer of Agate Biosciences, a consulting firm for project management, systems engineering, and science for controlled environment agriculture, she brings the best practices of management and systems engineering to cannabis businesses, allowing them to be successful in a complex, government-regulated environment.

Dr. Sue Sisley, M.D., serves as President of Scottsdale Research Institute and Site Principal Investigator for the only FDA-approved randomized controlled trial in the world examining safety and efficacy of whole plant marijuana in combat veterans with treatment-resistant post-traumatic stress disorder (PTSD). Dr. Sisley is on the faculty at Colorado State University and Humboldt State University, and a Senior Fellow at Thomas Jefferson University Lambert Center, assisting their Institutes of Cannabis Research. Dr. Sisley has been a Member of Nevada ILAC Medical Cannabis Commission for the past three years, outlining regulations for laboratory testing including limits on pesticides, residual solvents, and other guidelines that are currently being used as a model for other states medical cannabis laws. Her other areas of current institutional review board (IRB)-approved research include supervising studies evaluating cannabis for pain management, cannabis as a substitution therapy for opioids, and also a safety study looking at cannabis. Dr. Sisley is also the Principal Investigator on a Colorado State University project to build a robust nationwide medical cannabis patient registry.

About the Columnist

Josh Crossney is the columnist and editor of “Cannabis Crossroads” and a contributing editor to Cannabis Science and Technology magazine. Crossney is also the president and CEO of CSC Events. Direct correspondence to: josh@jcanna.com
Rapid Heavy Metal Screening of Cannabis

Kimberley Russell, Bruker

The portable, battery operated CTX™ is utilized to quickly, easily and nondestructively screen cannabis products for heavy metals with minimal sample preparation. The small footprint and lightweight portability enable it to be within reach when actionable information is needed.

Lab methods such as ICP which require extensive sample preparation for heavy metal analysis of cannabis as well as operation by skilled technicians are extremely important, especially when toxic metals are at very low, sub PPM levels. However, portable X-ray fluorescence (pXRF) technology can be used to quickly screen plants like cannabis, soils, liquids, and even solids for metals with little to no sample preparation. PXRF can also quantitatively analyze cannabis for heavy metals from PPM levels up to dangerously high percentage levels with minimal, straightforward sample preparation.

Portable X-ray fluorescence (pXRF) spectroscopy has been used to monitor heavy metals in soil for many years now. The 2005 US EPA published Method 6200 and the later ISO 13196:2013 describe the use of field portable XRF for soil and sediments to guide screening inspections and to perform compositional analysis of sites for regulatory clearance purposes. As described in these methods, pXRF technology saves time and cost of elemental analysis compared to the lab without destroying the sample. There are no requirements to digest, liquify, or dilute samples.

The more recent interest in plant uptake of nutrients and heavy metals has lead many organizations to utilize pXRF. It is the method of choice for this type of analysis because it can be taken into the field where plants are grown to monitor real-time uptake in the matrix it’s grown in; and, it can be used in mobile or impromptu labs.

PXRF can screen samples as they are; but, for accurate analysis some sample preparation is required. Buds, leaves, or stems should be dried, ground, sieved, and packed tightly into a sample cup to present fully homogeneous, small particle, and flat surface samples to the pXRF. Factory installed calibrations using certified standard reference materials in similar matrices provide the most precise and accurate pXRF measurements possible. Results can be correlated with ICP data to assure consistency in results. pXRF is nondestructive which means samples can be saved, used, or analyzed again.

PXRF analyzers can present data as identified elements, spectra, composition, or as preset pass/fail results based on user defined thresholds and ranges. Calibrations can be fine-tuned with user specific standards to better match unique sample matrices. Additionally, users can develop their own standard reference material calibrations using EasyCal™ PC software.

Bruker’s newly introduced CTX™, countertop XRF, is a derivative of the popular TITAN handheld XRF used in a desktop stand. The CTX™ was designed for customers who primarily measure small or prepared materials in sample cups instead of in-situ samples.

Conclusion
Adequate QA/QC methods which include nondestructive pXRF screening of heavy metals in cannabis products at any stage of development help protect customers, brand and investments without having to send samples to a lab.
Cannabinoid Sample Preparation
Increased Accuracy and Time
Savings for HPLC Analysis
Hamilton Company

Demand for Accurate Measurements
In today’s growing cannabis industry, customers and state regulations demand accurate measurements of active compounds in cannabis products. The Microlab® 600 Diluter/Dispenser makes measurements more reliable, allows the method to use less consumables, and cuts down on preparation time.

“My biggest highlights of the Microlab 600 are improved throughput, reduced consumables, and significantly reducing analyst to analyst variability.” — Lucas Mason, Aurum Labs

Faster Sample Prep and Less User Variation with the Microlab 600 Diluter/Dispenser
- Dramatic time savings (reduction in overall labor and greater throughput)
- Reduced consumable cost
- Virtually eliminates analyst-to-analyst variability
- Inert flow path

Pipettes can be problematic in any laboratory setting. Common problems include: significant variation between users, inconsistencies with different solution types, and performance variation at different elevations. These problems are eliminated with the Microlab 600. The Microlab 600 positive displacement system allows for extremely accurate dilution/dispense volumes on demand. The Microlab 600 can reduce %RSD by more than 50% compared to hand pipetting methods. See Figure 1.

Cannabinoid Method Summary

Faster Sample Prep and Less User Variation with the Microlab 600 Diluter/Dispenser

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Cannabinoid %RSD

**Figure 1:** %RSD testing comparison between the ML600 and traditional hand pipetting methods for CBD-A and THC-A.
Reduced User-to-User Variation
The Microlab 600 Diluter/Dispenser automates method steps prone to user variation.

In addition, methods can be saved as favorites for quick and easy access. The instrument performs all the precision work using high resolution syringe pumps, so it is not necessary to use a special technique. Once programmed, the instrument can be handed off to a new user with minimal training and the same results will be obtained when compared to a more experienced user. The instrument utilizes an encoder that ensures the positioning of the syringe drive mechanism, reducing variations between users, equal or greater than 99% accuracy.

Eliminate Expensive Consumables
The Microlab 600 Diluter/Dispenser requires less solution to attain the correct dilution concentration for a sample, which means spending less on solvents and reagents. The instrument remains accurate down to the microliter volume range so the user can get the exact concentration needed at the exact volume desired. Reduce consumables consumption by 50% or more when using the Microlab 600.

Increase Throughput
Because the Microlab 600 Diluter/Dispenser does not require additional glassware to prepare a sample or standard solutions, the time it takes to prepare solutions and clean up afterwards is reduced considerably. Making different dilution concentrations on the fly is as easy as inputting a different solvent volume in the Quickstart or the Dilution Wizard. These improvements to workflow create significant time savings, freeing up your schedule for more important tasks.

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<td>Microlab 615 Basic Dual Syringe Dispenser</td>
<td>ML615-DIS</td>
</tr>
<tr>
<td>Microlab 625 Advanced Dual Syringe Dispenser</td>
<td>ML625-DIS</td>
</tr>
</tbody>
</table>
Cannabis testing is critical to the success of growers and processors. In addition to providing vital information on product quality and safety while complying with state regulations, certain cannabis tests can help growers to maximize the efficiency of their yield prior to harvest. At the same time, an increased number of samples submitted for testing, combined with time-consuming manual testing methods, creates a backlog in the testing laboratory that, in turn, delays the sale of the crop.

Assay protocols vary widely, and many older methods are not specifically validated for use with cannabis samples, are not scalable, and do not include internal cannabis controls to confirm test accuracy. Additionally, labor-intensive protocol steps such as sample preparation limit sample throughput, and risk error due to human variability and subjective interpretation. Automated cannabis testing methods, using assays specifically developed for cannabis testing, can increase throughput and reduce bottlenecks without sacrificing the quality of results. Here, we demonstrate automated workflows using medicinal genomics (MGC) assays specifically developed for cannabis testing. The SenSATIVAx Plant/Microbial DNA Purification Kit provides fast, easy isolation of plant and microbial DNA from freshly cut or cured leaf or flower material without the need for organic extraction, and can be used to prepare samples for use with the PathoSEEK and FemINDICAtor assays. The PathoSEEK Microbial Safety Testing Platform uses a quantitative polymerase chain reaction to search for and quantify the unique DNA sequences of target organisms in cannabis flower, extracts and infused product samples, and includes an internal cannabis control. The FemINDICAtor qPCR Plant Gender Detection Assay accurately identifies male cannabis plants significantly faster and earlier than visual inspections so that growers can remove them from the crop, thereby improving yields.

The workflows were automated on the Microlab NIMBUS automated liquid handler from Hamilton Robotics (Figure 1). The Microlab NIMBUS utilizes patented pipetting technology to provide high accuracy and precision in a variety of low-to-medium throughput liquid handling steps such as aspirating, dispensing, washing, and transferring. The combined, automated system of assays and equipment significantly reduce active labor time compared to manual methods without negatively impacting results.

Automated Method Features and Key Benefits
The Microlab NIMBUS software was preprogrammed with the SenSATIVAx, FemINDICAtor, and PathoSEEK workflow steps as a standard solution to facilitate user-friendly operation and reduce operator input errors. The intuitive User Interface (Figures 2–3) allows for selection of customizable workflow options. The user may process between 1–32 samples when running SenSATIVAx and can set up 1–96 qPCR reactions when running either PathoSEEK or FemINDICAtor. When running qPCR setup (PathoSEEK or FemINDICAtor), the user can run the full method including both decontamination setup and qPCR setup, or run either separately. Options selected via the User Interface will automatically update future User Dialogs that prompt the user to set up reagents to account for number of samples being processed. A full 32-sample extraction can be processed in approximately 90 min and a full 96 qPCR reaction can be set up in approximately 30 min compared to approximately 3 h for the same using manual methods.

Experimental Design and Results
As defined in the SenSATIVAx procedure, 0.25 g of cannabis flower sample was weighed into a Whirl-Pak bag (Nasco #B01385WA, Fort Atkinson, WI) followed by the addition of 3.55 mL of Tryptic Soy Broth (TSB, MGC #420205). The flower sample was then manually homogenized for 1 min. Following homogenization, 285 μL of the homogenized flower/TSB mixture was transferred into one well of the extraction plate (Perkin Elmer #6008290, Waltham, MA). This process was repeated for each of four flower samples tested. The DNA extractions using SenSATIVAx reagents were processed on
the Microlab NIMBUS. After setting up the Microlab NIMBUS deck with necessary reagents and materials, the SenSATIVAx DNA Extraction method was selected from the Method Manager screen, and user and sample information was input. Microlab NIMBUS then automatically added lysis buffer to the wells and incubated for 2 min. The plate was then manually placed in a centrifuge to pellet lysed cellular material and trichomes. After returning the plate to the Microlab NIMBUS deck, the supernatants of each sample were transferred to fresh wells of the extraction plate. MGC binding buffer was then added to the samples and tip mixed. DNA from the lysed samples was bound to the beads, and was then separated via a magnet on the Microlab NIMBUS deck. The samples were then washed with 70% ethanol to remove any non-DNA material, and eluted in aqueous buffer. The purified DNA was then transferred to separate wells of the

Table I: Detection of total yeast and mold, and total aerobic count PathoSEEK qPCR results

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample</th>
<th>Processing Method</th>
<th>Target</th>
<th>Cq</th>
<th>Target</th>
<th>Cq</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Bubba Kush</td>
<td>NIMBUS</td>
<td>Total yeast and mold</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>25.35</td>
</tr>
<tr>
<td>B1</td>
<td>Bubba Kush</td>
<td>Manual</td>
<td>Total yeast and mold</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>25.13</td>
</tr>
<tr>
<td>C2</td>
<td>Erk Train</td>
<td>NIMBUS</td>
<td>Total yeast and mold</td>
<td>33.85</td>
<td>Cannabis DNA</td>
<td>26.45</td>
</tr>
<tr>
<td>D1</td>
<td>Erk Train</td>
<td>Manual</td>
<td>Total yeast and mold</td>
<td>33.33</td>
<td>Cannabis DNA</td>
<td>25.55</td>
</tr>
<tr>
<td>E1</td>
<td>Harlox</td>
<td>NIMBUS</td>
<td>Total yeast and mold</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>27.21</td>
</tr>
<tr>
<td>F1</td>
<td>Harlox</td>
<td>Manual</td>
<td>Total yeast and mold</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>26.59</td>
</tr>
<tr>
<td>G1</td>
<td>Rugburn OG</td>
<td>NIMBUS</td>
<td>Total yeast and mold</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>25.80</td>
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<tr>
<td>H1</td>
<td>Rugburn OG</td>
<td>Manual</td>
<td>Total yeast and mold</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>25.18</td>
</tr>
<tr>
<td>A2</td>
<td>TYM Positive Ctrl</td>
<td>NIMBUS</td>
<td>Total yeast and mold</td>
<td>11.49</td>
<td>Cannabis DNA</td>
<td>N/A</td>
</tr>
<tr>
<td>C2</td>
<td>NTC</td>
<td>NIMBUS</td>
<td>Total yeast and mold</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>N/A</td>
</tr>
<tr>
<td>E2</td>
<td>Bubba Kush</td>
<td>NIMBUS</td>
<td>Total aerobic count</td>
<td>37.42</td>
<td>Cannabis DNA</td>
<td>25.44</td>
</tr>
<tr>
<td>F2</td>
<td>Bubba Kush</td>
<td>Manual</td>
<td>Total aerobic count</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>25.13</td>
</tr>
<tr>
<td>G2</td>
<td>Erk Train</td>
<td>NIMBUS</td>
<td>Total aerobic count</td>
<td>35.00</td>
<td>Cannabis DNA</td>
<td>26.69</td>
</tr>
<tr>
<td>H2</td>
<td>Erk Train</td>
<td>Manual</td>
<td>Total aerobic count</td>
<td>34.42</td>
<td>Cannabis DNA</td>
<td>25.95</td>
</tr>
<tr>
<td>B2</td>
<td>Harlox</td>
<td>NIMBUS</td>
<td>Total aerobic count</td>
<td>27.41</td>
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<td>27.37</td>
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<td>A3</td>
<td>Harlox</td>
<td>Manual</td>
<td>Total aerobic count</td>
<td>27.16</td>
<td>Cannabis DNA</td>
<td>26.79</td>
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<tr>
<td>D2</td>
<td>Rugburn OG</td>
<td>NIMBUS</td>
<td>Total aerobic count</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>25.68</td>
</tr>
<tr>
<td>C3</td>
<td>Rugburn OG</td>
<td>Manual</td>
<td>Total aerobic count</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>25.07</td>
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<tr>
<td>E3</td>
<td>Total Aerobic Positive Ctrl</td>
<td>NIMBUS</td>
<td>Total aerobic count</td>
<td>12.57</td>
<td>Cannabis DNA</td>
<td>N/A</td>
</tr>
<tr>
<td>G3</td>
<td>NTC</td>
<td>NIMBUS</td>
<td>Total aerobic count</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Microbial detection of total yeast and mold as well as total aerobic count using MGCs PathoSEEK qPCR reagents using manual and automated methods from multiple flower samples.
Table II: Cannabis plant gender detection FemINDICAtor qPCR results

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample</th>
<th>Target</th>
<th>Cq</th>
<th>Male or Female?</th>
<th>Target</th>
<th>Cq</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Male 1</td>
<td>Y Chrom</td>
<td>29.14</td>
<td>M</td>
<td>Cannabis DNA</td>
<td>30.20</td>
</tr>
<tr>
<td>B1</td>
<td>Male 2</td>
<td>Y Chrom</td>
<td>30.64</td>
<td>M</td>
<td>Cannabis DNA</td>
<td>31.64</td>
</tr>
<tr>
<td>C1</td>
<td>Male 3</td>
<td>Y Chrom</td>
<td>30.66</td>
<td>M</td>
<td>Cannabis DNA</td>
<td>31.48</td>
</tr>
<tr>
<td>D1</td>
<td>Female 1</td>
<td>Y Chrom</td>
<td>N/A</td>
<td>F</td>
<td>Cannabis DNA</td>
<td>30.07</td>
</tr>
<tr>
<td>E1</td>
<td>Female 2</td>
<td>Y Chrom</td>
<td>38.19</td>
<td>F</td>
<td>Cannabis DNA</td>
<td>26.98</td>
</tr>
<tr>
<td>F1</td>
<td>Female 3</td>
<td>Y Chrom</td>
<td>N/A</td>
<td>F</td>
<td>Cannabis DNA</td>
<td>27.89</td>
</tr>
<tr>
<td>G1</td>
<td>XY Positive Ctrl</td>
<td>Y Chrom</td>
<td>13.54</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>N/A</td>
</tr>
<tr>
<td>H1</td>
<td>Neg Control</td>
<td>Y Chrom</td>
<td>N/A</td>
<td>N/A</td>
<td>Cannabis DNA</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Six leaf hole punch samples run using MGCs FemINDICAtor qPCR reagents on the Microlab NIMBUS. A Cq value less than 35 indicates a male plant.

Table III: Comparison of manual versus automated PathoSEEK qPCR results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Automated or Manual</th>
<th>Fluor</th>
<th>Target</th>
<th>Cq</th>
<th>Fluor</th>
<th>Target</th>
<th>Cq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry Cheesecake</td>
<td>Automated</td>
<td>FAM</td>
<td>E. coli</td>
<td>22.34</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>27.63</td>
</tr>
<tr>
<td>Blueberry Cheesecake</td>
<td>Automated</td>
<td>FAM</td>
<td>E. coli</td>
<td>22.27</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>27.51</td>
</tr>
<tr>
<td>Blueberry Cheesecake</td>
<td>Automated</td>
<td>FAM</td>
<td>E. coli</td>
<td>22.28</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>27.56</td>
</tr>
<tr>
<td>Blueberry Cheesecake</td>
<td>Automated</td>
<td>FAM</td>
<td>E. coli</td>
<td>22.24</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>27.44</td>
</tr>
<tr>
<td>Blueberry Cheesecake</td>
<td>Automated</td>
<td>FAM</td>
<td>E. coli</td>
<td>22.34</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>27.66</td>
</tr>
<tr>
<td>Blueberry Cheesecake</td>
<td>Manual</td>
<td>FAM</td>
<td>E. coli</td>
<td>21.60</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>26.92</td>
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<tr>
<td>Blueberry Cheesecake</td>
<td>Manual</td>
<td>FAM</td>
<td>E. coli</td>
<td>21.54</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>27.06</td>
</tr>
<tr>
<td>Blueberry Cheesecake</td>
<td>Manual</td>
<td>FAM</td>
<td>E. coli</td>
<td>21.70</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>27.44</td>
</tr>
<tr>
<td>Blueberry Cheesecake</td>
<td>Manual</td>
<td>FAM</td>
<td>E. coli</td>
<td>22.05</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>27.61</td>
</tr>
<tr>
<td>Blueberry Cheesecake</td>
<td>Manual</td>
<td>FAM</td>
<td>E. coli</td>
<td>21.97</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>27.34</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Automated</td>
<td>FAM</td>
<td>E. coli</td>
<td>14.27</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>N/A</td>
</tr>
<tr>
<td>Negative Control</td>
<td>Automated</td>
<td>FAM</td>
<td>E. coli</td>
<td>N/A</td>
<td>HEX</td>
<td>Cannabis DNA</td>
<td>N/A</td>
</tr>
</tbody>
</table>

When comparing cycle quantification (Cq) values obtained from identical homogenized sample via manual and automated methods, consistency among results is seen in line with expectations. Results from automated methods are reliable and robust, with lesser variability observed between technical replicates.

Figure 3: Microlab NIMBUS User Interface indicating MGC PathoSEEK qPCR Master Mix Reagent set up volumes and instructions. Automated methods using preprogrammed steps can reduce or eliminate human errors and variability while automatically scaling volumes to meet any throughput needs.

extraction plate for subsequent assay analysis. Four flower samples were also processed manually. Samples were then tested for total yeast and mold and total aerobic bacteria contamination via qPCR using the PathoSEEK qPCR Master Kit, Total Aerobic detection assay and Total Yeast and Mold detection assay. Total Yeast and Mold and Total Aerobic Count assay master mixes and positive controls were prepared by the user and added to the Microlab NIMBUS deck when prompted. DNA samples and positive and negative controls were automatically transferred to a clean qPCR microplate followed by prepared qPCR master mixes, which include assay specific primers. Samples were automatically mixed then placed in the Bio-Rad CFX96™ Real-Time PCR Detection System (Hercules, CA) for qPCR analysis. See Table I for the results. For testing FemINDICAtor, three known male and three known female plant leaves were hole punched using a 4.0 mm biopsy punch (Integra LifeSciences Corporation, #33-34-P/25, Plainsboro, NJ). Each sample punch was transferred to a 96 well PCR plate (USA Scientific 1402-9300, Ocala, FL). The DNA extraction using SensaTIVAx Flower/Leaf DNA Extraction Kit was then processed on the Microlab NIMBUS using the procedure outline above adapted to plant leaves. The samples were decontaminated following the assay kit manufacturer’s recommended protocol, followed by addition of prepared qPCR master mix which includes assay detection primers. The samples were
mixed, then placed in the qPCR instrument to test for the presence or absence of Y chromosome DNA. See Table II for results. Finally, a comparison of manual versus automated qPCR workflows were assessed for repeatability in sample handling. Identical homogenized flower sample was extracted five times manually and on the Microlab NIMBUS using the SenSATIVAX assay kits. Purified DNA samples were then processed using the PathoSEEK assay kit to detect E. coli and cannabis DNA primer target sequences, similarly as was done above following the manufacturer’s recommended protocol. See Table III for results.

Conclusion
The Medicinal Genomics SenSATIVAX DNA Purification Kits, PathoSEEK Microbial Safety Tests, and FemINDICAtor sex testing kits automated on the Microlab NIMBUS liquid handling platform provide a robust, flexible, and efficient DNA extraction and qPCR set up workflow in less time than manual methods. Thirty-two samples can be extracted, and a full 96-well plate of qPCR reactions can be set up in approximately 66% less time than manual methods without sacrificing sample results. The intuitive user interfaces guide the user through the setup process, which allows for seamless set up and method execution without errors or variability from user-to-user, or run-to-run. The combined automated solution allows testing labs to quickly and accurately deliver results to their customers while maximizing time and labor resources.

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Preview of the 2018 Cannabis Science Conference

The 2018 Cannabis Science Conference will be held August 27–29, in Portland, Oregon. With the conference quickly approaching, we sat down with Josh Crossney, president and CEO of CSC Events, for a preview of what this year’s show will offer attendees.

Megan L’Heureux

What are some of the key topics that this year’s Cannabis Science Conference will address?

Crossney: Some of the key topics include advances in academic research and quality control (QC) testing, genomics, efforts to improve standardization, improvements in analytical instrumentation and testing methods, as well as greater medicinal cannabis information sharing, including physician and patient presentations. In the medical track, I also see increasing focus on cannabis as a more effective alternative to opiates. Opiates kill nearly 100 Americans a day, whereas no one has ever died as a direct result from a cannabis overdose. What could be a more relevant and important discussion than advancing alternative medicines that put an end to the terrible opioid epidemic?

We have also added a cultivation track this year, which differs greatly from other conferences because the focus is more technical. This year we also have technical updates from several cannabis testing labs, with topics ranging from sample preparation and method development to microwave-assisted extractions and informatics.

Can you tell us more about the addition of a cultivation track? Why did you feel there was a need to add that track?

Crossney: At last year’s Canna Boot Camp, there was great interest from all participants in a phenomenal cultivation session hosted by Autumn Karcey (Cultivo, Inc.) and Ken Kovash (GI Grow). Cultivation techniques greatly impact cannabis science, both in terms of therapeutic efficacy as well as potential introduction of contaminants.

Additionally, many QC and research scientists lack a fundamental understanding of cannabis cultivation, so we felt there was a strong need to help bridge this gap by introducing a cultivation track.

What do you hope for people to learn or gain from your conference this year?

Crossney: My hope is that attendees walk away from this year’s conference feeling educated, empowered, inspired, and connected. By empowered, I mean that the Cannabis Science Conference has become the annual pilgrimage for key opinion leaders like Tracy Ryan, Professor Jack Henion, Professor Dedi Meiri, Dr. Scott Kuzdzal, Dr. Uma Dhanabalan, Dr. Sue Sisley, Janie Maedler, Sharlene Mavor, and Dr. Jahan Marcu. These truly inspirational people have so much knowledge to share, and at the Cannabis Science Conference we work very hard to maximize networking with these experts; including opportunities ranging from discussions at poster sessions and exhibit booths to participation in interactive panel discussions. Attendees will walk away with new knowledge and collaborations that will help catalyze and advance cannabis science for years to come.

Can you give us any hints as to who your plenary speaker will be?

Crossney: I think attendees will be thrilled with this year’s plenary speaker, who is a well-known celebrity, and is also someone that has battled cancer and walked away not only as a survivor, but someone that created a foundation to help women be more aware of the early signs of cancer. I mentioned previously that I want this year’s attendees to walk away inspired: our plenary speaker will most certainly inspire people.

Is there anything else you’d like to add about this year’s conference?

Crossney: This year we received hundreds of scientific abstracts. In addition to adding a cultivation track, attendees will see expansion in our poster sessions as well as the number of vendors on our exhibition floor. We are anticipating more than 160 sponsors, exhibitors, and media partners this year!

I am excited to announce that our Canna Boot Camp full-day, hands-on workshop will return to Chalice Farms again this year. By the time this article goes to print there will likely be only a few tickets remaining, but I strongly encourage everyone interested in a more hands-on experience covering everything from cannabis cultivation and preprocessing to extractions, analytical testing, edibles manufacturing, and dispensary operation to attend a future Canna Boot Camp.

In closing, I would like to thank Cannabis Science and Technology for your continued support. Your sister publications, LCGC and Spectroscopy, have been our premier media partner now for years, and I look forward to working with your team for years to come. There are many exciting things ahead for us!
Use Promo Code **CSC25** for 25% Off Registration!!!

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The **World’s Largest Cannabis Science Conference** returns to downtown Portland for an incredible gathering of analytical scientists, medical professionals, patients, cannabis industry experts and novices interested in learning more about cannabis science!

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RETSCH Mills for Cannabis Sample Preparation

RETSCH GRINDOMIX Knife Mills
The new GRINDOMIX GM 200 and GM 300 Knife Mills are designed to take on very difficult samples with material properties that are difficult for conventional sample prep milling systems to handle. High moisture, fat, starch, and high heterogeneity of sample components can all be processed in the GM Knife Mill units to homogeneous sample; thus, making them ideal for preparing cannabis samples for analysis.

RETSCH CryoMill
The CryoMill is designed to process samples under constant cryogenic temperatures with an integrated LN₂ connection to the unit to ensure constant temperatures and operator safety. For sample preparation procedures of materials where heat generation compromises analysis, such as analysis of volatile components as pesticides, the CryoMill is ideal. Additionally, the CryoMill also serves as a tool to process fatty and sugar-filled edible samples such as gummy bears and candies in order to obtain a homogeneous powder.

RETSCH Cutting Mill SM 300
The SM 300 Cutting Mill is designed to pre-grind initial lab samples that have particle sizes that are too large to fit into a conventional mill for sample preparation. For the cannabis industry, the unit is well-suited for the task of processing buds, stalks, and can even be used for homogenizing waste material for disposal. With a large infeed hopper, collection canisters, and optional cyclone collection system for dust mitigation and lower heat generation, the SM 300 Cutting Mill can handle your pre-grinding needs.

If you are looking for a complete line of products for sample preparation and particle analysis, look no further than RETSCH.

- Mills and grinders for all solid sample types
- Widest range of sieve shakers in the market
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