cannabis science and technology

LC GC

VIRTUAL SYMPOSIUM RECAP: Grow Your Skills in the Budding Cannabis Industry

Agilent

Introduction

Welcome to the follow up e-book to the virtual symposium, "Grow Your Skills in the Budding Cannabis Industry" held live on March 10th and 11th, 2022. The rapid growth of the cannabis industry has provided a rare opportunity for both entrepreneurs and scientists to be part of an emerging field. As new jurisdictions legalize the sale of cannabis and cannabis products, a disparate and constantly evolving compliance world has been created. Concerns and challenges related to the production, extraction, and chemical analysis of cannabis have increased with the industries expansion, which are compounded by the historical legal restrictions on cannabis research. This event brought together both academic and cannabis industry experts to highlight strategies to advance the scientific rigor in cannabis analysis by leveraging the research capabilities of academics to enhance problem solving and workflow optimization. Some of the key takeaways included:

- Strategies for building a strong team in a cannabis facility
- Approaches for addressing challenges of cannabis analysis
- Understanding the intersection of product quality and safety

I hope you find the presented ideas about building a successful team and robust methodology through industry and academic partnerships useful, and encourage you to contact either me or Agilent Technologies with any questions or inquiries about how we can partner to solve your cannabis analysis needs.

Thank you,

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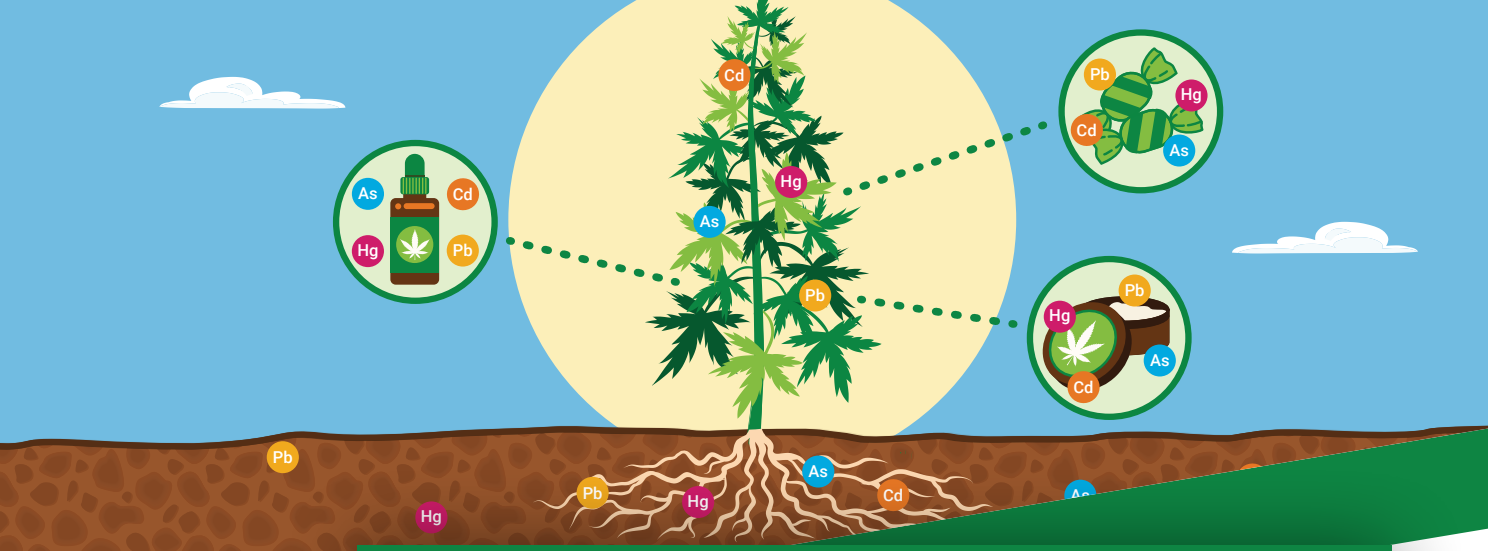

Trace Metals & Contamination: How Did it Get There?

Interview with Derek D. Wright, Ph.D.

How contamination in cannabis happens

In February's 2022 "Grow Your Skills in the Budding Cannabis Industry" two-day virtual symposium, hosted by Cannabis Science & Technology® and LCGC®, Derek Wright, associate professor at LSSU, presented on heavy metal contamination in cannabis. Here, he answers several questions about challenges with determining trace metals, metal speciation, recommendations for an analytical laboratory, and more.

What are some of the challenges with determining trace metals in cannabis?

The main challenges of determining trace metals in regulated cannabis products are:

1. Low detection limits
2. Multiple sample matrices
3. Heterogeneous samples
4. The significant potential for metal accumulation and contamination throughout the workflow—from the production phase to the analytical laboratory workflow.

It's difficult to think of any other regulated product that combines quite as many potential challenges as cannabis analysis. Cannabis is a plant thought to be an efficient metal accumulator, and it is incorporated into a wide variety of regulated and unregulated consumer products, which varies by jurisdiction. Cannabis products may be inhaled, ingested, applied topically, extracted, concentrated, or incorporated as ingredients in food and beverages. Cannabis is produced commercially both indoors and outdoors and by a wide variety of production methods. When you combine that with the various legal restrictions on cannabis (both historic and current), we have a situation where a regulated consumer product has broadly entered the marketplace, yet our scientific understanding and testing methodologies have lagged, and regulators have struggled to reach a consensus on appropriate product safety standards.

How well does cannabis accumulate metals?

While the scientific literature on metal accumulation in cannabis is limited, there are suggestions that the cannabis plant is an efficient accumulator of many metals, and its potential use in plant-based remediation of metal contaminated soils, i.e., phytoremediation, is being explored. As in most terrestrial plants, the primary metal uptake pathway is presumably through the roots. That said, adhesion of particulate matter—soil particle, atmospheric particles—is a confounding factor that may bias the measurement of metal uptake and can lead to the overestimation of (internal) metal accumulation. This issue is probably most significant in the roots, but it may be important in the cannabis flower due to its resinous nature. Additionally, mercury has been shown to accumulate in plant foliage by direct uptake through the stomata during gas exchange. The degree to which this particular uptake pathway might be important in cannabis is unclear.

What are the typical concentrations of metals in soils?

Soils, in general, are primarily composed of a mixture of mineral particles and organic residues: plant detritus and humus. The elemental composition of soils is dominated by silicon and oxygen, with carbon, aluminum, iron, etc., often found in high abundance. While the big four elements that are a concern to consumer safety , i.e., the most commonly regulated elements: arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), are typically found as minor or trace constituents of most soils, but this is not true in all cases. Geochemical processes and human activities can act to concentrate these, and other potentially hazardous elements, to elevated concentrations in both soil and irrigation water.

Are metals mostly sequestered in the roots, or are they transferred into the foliage?

Again, the scientific literature here is limited, but it's probably true that most metals are found at higher concentrations in root tissue. Some metals—manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), aluminum (Al), etc.—appear to accumulate to significant degrees in flower/foliage even when cannabis is grown

in uncontaminated soils. This is unsurprising, as most of these are also essential elements required for plant growth. That said, there is also the potential for the same pathways that transport required nutrients to potentially transport chemically similar elements (i.e., P/As, Zn/Cd, and Hg, etc.). All of this is with the caveats that there are relatively few studies on the cannabis plant specifically and that most studies have not attempted to quantify potential bias due to surface contamination and similar issues, so there is much more work needed to better understand metal distribution within the cannabis plant.

What is metal speciation, and what does it have to do with metals in cannabis?

Metal speciation refers to the specific chemical forms of the metals as they exist in their molecular environment. Metals may exist as elements, metal salts, free ions in water, aqueous complexes with either organic or inorganic ligands, in various redox states, etc. The specific chemical species present have a profound effect on the behavior of metals in the environment with regards to solubility, metal uptake and transport, bioavailability, and bioaccumulation. Speciation is also dependent on pH, so the addition of acids, bases, nutrients, etc., to soil/growth media each has the potential to alter speciation and potentially modify the solubility and uptake of metals.

What are potential sources of metal contaminants from cultivation inputs? What about from facilities and equipment?

Due to the importance of metals in society, as well as their natural abundance, potential contamination sources are too numerous to provide an exhaustive list. What is critical is to be thoughtful about the design of the entire production process and to carefully screen all production inputs. For instance, many people are aware of potential issues related to lead contamination and plumbing, which has the potential to impact the quality of irrigation water.

Fewer people are aware that many regions in the world, including many parts of the United States, have groundwater that is naturally enriched in arsenic, or that some types of phosphate fertilizers and liming materials may contain elevated metals such as As, Cd, and Pb. For jurisdictions that regulate chromium or nickel, stainless-steel production equipment could be an issue, depending on how it is utilized and the quality of the equipment. For producers, prescreening soil, growth media, nutrient additives, and irrigation water should be standard practice. Indoor grow operations should give careful thought to their selection of construction materials and should investigate for the presence of potentially problematic materials, i.e., lead paint, when renovating or repurposing older structures.

What are your recommendations for growers and processors/producers?
My primary recommendations for growers and producers are:

1. Careful design of the production process
2. Prescreen production inputs
3. Utilize standard operating procedures (SOPs) and keep detailed records
4. Perform as much in-house quality control as possible.

While I discussed the first two recommendations above, the other two merit some additional explanation. The advantages of SOPs and detailed records may not be obvious until a problem is identified, usually when a product batch fails testing. At that point, correcting the problem is of utmost urgency. Producers who have carefully documented their operations will often be able to rapidly identify the issue and apply corrective action. For instance, if a fertilizer supplier switches sources, and the new lot of fertilizer results in elevated metals, good records may help identify the new fertilizer lot as the issue. At the very least, detailed records should help to narrow the list of potential issues. This approach works best in conjunction with pre-screening production materials and issues are often identified even more quickly when appropriate in-house quality control is utilized.

In my opinion, every production facility should, at a minimum, inspect their product visually for foreign debris with a stereomicroscope. The presence of Foreign Particulate Matter (FPM) alone may result in product failure in some jurisdictions, but in any case, its presence indicates potential contamination sources within the production process. FPM inspection is also useful as a qualitative screening technique for the early identification of issues with mold and/or agricultural pests. A final recommendation is to prepare for the future regulation of a broader suite of elements—by applying these best practices to currently unregulated elements, you will have a good, advanced understanding of your operational performance and won't be caught off guard by regulatory changes.

What is a typical laboratory workflow?
The typical laboratory workflow involves several steps:

1. Representative sampling
2. Sample homogenization
3. Sample digestion by microwave acid digestion
4. Analysis by inductively coupled plasma - mass spectrometry (ICP-MS).

Of these steps, the greatest potential to introduce bias or error into the analysis is in the representative sampling and sample homogenization steps due to the highly heterogeneous nature of many cannabis samples—both batch to batch, and within batch variability. Additionally, most standard homogenization methods utilize metal-containing apparatuses, potentially yielding false-positive results and loss in customer confidence. It is also important to emphasize

that closed-vessel digestions are necessary to avoid loss of analyte, especially with Hg and As, and that dry-ashing procedures are not appropriate for determining these elements.

What are some things to be mindful of when using ICP- MS-based analysis methods?

Laboratories that do not have extensive experience with trace element analysis need to recognize the critical importance of maintaining a dedicated trace metal workflow, the need to minimize possible contamination sources at each step, and the importance of working closely with instrument vendors to develop an optimized and validated method for their sample types. The use of dedicated metal-free workspaces, acid-cleaned plasticware, and high-purity trace-metal-grade reagents are standard practices in the trace metal analysis community, but their importance may not be recognized by analysts and lab managers from other disciplines. Also, the ICP-MS method developed and implemented for cannabis product testing must be both sensitive and robust. It must be able to control/correct for key polyatomic interferences ($ArCl^+$ on $^{75}As^+$) as well as doubly charged interferences from rare earth elements ($^{150}Sm^{2+}$ and $^{150}Nd^{2+}$ on $^{75}As^+$). Additionally, we find the use of a chilled spray chamber, nickel-plated cones, and 4x aerosol dilution provides a substantial improvement in matrix tolerance, leading to more robust method performance. In our lab, we utilize an Agilent 7800 ICP-MS for cannabis analysis, as well as the analysis of water, soils, fertilizers, and soil amendments. When combined with appropriate sample preparation and a high-performance microwave digestion system, we can successfully determine a large suite of elements in various cannabis products under a single set of analytical conditions.

What are a few recommendations for an analytical laboratory?

The need to invest in the correct infrastructure and to hire qualified personnel is clear, as is the need for a robust, validated workflow. Having a close working relationship with instrument vendors for technical assistance and the timely performance of recommended maintenance can help ensure robust and reliable method performance. One current challenge is the lack of externally validated certified reference materials (CRMs) for method validation; however, non-cannabis plant-based reference materials are widely available and should be utilized until a cannabis reference material is available. A final recommendation for both existing and new cannabis testing labs is to prepare for the possibility of additional elements becoming regulated in the future and to begin developing methods and providing data on additional elements to customers so that they can improve the management of their process.

DEREK D. WRIGHT, Ph.D.
Associate Professor
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Part 1: What's Next for Potency Testing?

Interview with Christophe Deckers

Potency testing in challenging applications

In February's 2022 "Grow Your Skills in the Budding Cannabis Industry" two-day virtual symposium, hosted by Cannabis Science & Technology® and LCGC®, Christophe Deckers, application scientist of sample preparation at Agilent Technologies, presented on sample preparation as it's related to the future of potency testing. Here, he answers several questions about processing different samples, potency testing in difficult formats such as gummies and beverages, and more.

How can I process very different samples in the most simple and cost-effective way to get good, accurate results with, for example, chocolate?

It is tempting to extract all samples with methanol, filter, and inject. However, we have demonstrated in our application notes that dirty samples increase cost per sample due to extra sample re-runs, column changes, and instrument downtime. Different samples like chocolates, gummies, and beverages also have very different consistency and need different sample processing. Chocolate and baked goods need to be milled; gummies need to be melted in water; and beverages are in a very diluted liquid form—so right there, it calls for different sample processing, even before you start

extracting cannabinoids to quantify them by liquid chromatography (LC) UV or LC – tandem mass spectrometry (LC-MS/MS).

For chocolate and baked goods, more specifically, the most simple and efficient extraction was to use cold acetonitrile and then to filter on a Captiva EMR-Lipid filter to remove proteins, lipids, and flavonoids in cocoa that seem to negatively affect the quantification of cannabinoids.

> **Gummies were one of the first edibles infused with cannabinoids, but to this day, they are still one of the most difficult samples to work with.**

What about gummies—why is potency testing in gummies so challenging?
Gummies were one of the first edibles infused with cannabinoids, but to this day, they are still one of the most difficult samples to work with. Even the most established laboratories regularly report inconsistencies in their testing methods when it comes to gummies. Why is that? The answer probably lies in the great variety in consistency and formulations. Some gummies are made of gelatin; others are vegetarian and pectin-based; others are made from starch.

What we have found is that all gummies melt better in water, and sonication and heat can help to speed the extraction. High molecular weight sugars like starch don't dissolve well in solvents and become viscous, increasing column pressure during chromatography. We also discovered that some gummies, in particular vegetarian gummies, melt better at a high pH. We optimized a water extraction followed by an Agilent QuEChERS extraction in acetonitrile. That procedure is very robust and works for all gummy types. Note that it's better to use the inside of the gummies for testing, as the outside is often sanded with sugar and covered with oil or resins. This process has a significant impact on how manufacturers establish and report the theoretical cannabinoid concentration in their products.

What challenges are associated with potency testing in beverages? What are some solutions to these challenges?
Beverages have much lower concentrations of THC and CBD, so you need to make sure that you have good sample preparation to reach lower detection limits by LC-UV. The second challenge, which is also a problem by LC-MS/MS, is that oils and emulsifiers additives in these beverages can cause

analytical problems. They build up in LC columns/instruments and negatively impact method accuracy. We compared different sample preparation techniques and found that a simple Agilent QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction followed by filtration on Captiva EMR-Lipid cartridges provides excellent analytical performance and lab productivity. If you just filter your beverage in a regular filter without QuEChERS extraction, you will lose cannabinoids and get very variable results. That's why it is not uncommon to get different potency measurements on the same sample but sent to different labs. It is also important to degas carbonated beverages before testing.

What's the role of sample preparation in reaching accuracy and robustness?

Cannabis testing is a relatively nascent industry and methods are not fully mature. It is not uncommon to send the same sample to different labs and get different potency results. These differences may have to do with the analytical instrument and its method settings, the quality of the chromatography, or sample preparation.

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Potency Testing for Cannabis and Hemp

First, to be accurate, you need to have a representative portion/sampling to ensure that what you are testing is relevant. Sample weight also has an impact on sampling. The bigger the sample, the better it represents the entire batch. Secondly, you need to physically process the sample to ensure optimal extraction of cannabinoids in the next step. For example, harder samples need to be milled while gummies must be melted before extraction. If the sample is not ground finely enough or melted completely, you can't expect complete and consistent extraction.

The next sample preparation step is to extract THC and CBD using the appropriate solvents. Cleaner extracts like candies can just be filtered, but more complex samples need to be cleaned so that they meet the desired method robustness and accuracy.

If you inject dirty extracts from topicals, baked goods, or beverages, you will have a lot more instrument maintenance, which decreases lab productivity. You will also need to change LC columns more frequently and be forced to perform more sample-runs. All these elements induce more variability in potency testing results and end-up impacting method accuracy and precision. In other words, with good sample preparation, you can reduce your overall cost per sample and expect to be more accurate and for longer.

How does Captiva EMR-Lipid filters help with extract cleanup?

Cannabinoids are very fat soluble, and as a result, it is very difficult to extract them for potency testing without co-extracting lipids, oils, surfactants, and emulsifiers. Therefore, you end up with very dirty samples that negatively impact your lab productivity and method accuracy.

For the very first time, it is possible to remove these interferences from your sample with a filtration on Captiva EMR-Lipids. Proteins, oils, and emulsifiers are captured on the filter while cannabinoids pass through. This is a simple patented technology that enables robust potency testing for the most challenging samples.

CHRISTOPHE DECKERS
Application Scientist, Sample Preparation
Agilent Technologies

What can we expect from future regulations regarding accuracy and robust regulations?

We expect regulations to evolve and become more demanding over time. We have seen regulation increase in environmental testing over the years, and it is expected to be similar for the cannabis industry as it matures. Adopting good SOPs that include effective sample preparation will not only ensure that you remain compliant as regulations evolve, but it will also ensure that you keep a competitive edge over other laboratories that will have to change their protocols to meet new accuracy and precision requirements.

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Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

Part 2: What's Next for Potency Testing?

Interview with Jean-Francois Roy

Simple, Accurate and Reliable Quantification of THC and CBD

In February's 2022 "Grow Your Skills in the Budding Cannabis Industry" two-day virtual symposium, hosted by Cannabis Science & Technology® and LCGC®, Jean-Francois Roy, application scientist for chromatography and MS at Agilent Technologies, presented on chromatography and detection ion exchange analytical methodologies in cannabis potency testing. Here, he answers several questions about the three pillars of potency testing, various techniques for potency testing and their advantages and disadvantages, and more.

What are the three pillars of potency testing, and what variables arise when performing potency testing?

A well-designed, and ultimately accurate, potency testing methodology will include optimized sample preparation, chromatography, and detection. Each respective pillar can potentially impact the two others, so it is imperative to work along a few key variables when optimizing a potency method, among which are found:

1. The number of targeted cannabinoids and their respective concentration ranges.
2. The type of materials (matrices) for which potency will be tested.
3. The type of information that is sought: qualitative, semi-quantitative, or quantitative.
4. The level of expertise of the staff performing potency testing.

Why is liquid chromatography (LC) UV the most popular technique for potency testing?

First and foremost, it can be used to analyze a large number of cannabinoids in a rather wide concentration range. It is also compatible with a large number of matrices, ranging from plants and concentrates to edibles and personal care products. The quantitative results generated with LC-UV are typically accurate and reproducible, although highly dependent on sample preparation. Finally, LC-UV does not require extensive expertise nor training for successful operation.

The quantitative results generated with LC-UV are typically accurate and reproducible, although highly dependent on sample preparation.

What are the advantages of adding mass spectrometry (MS) detection? What are the drawbacks, if any?

LC-MS is typically more sensitive than LC-UV, so if targeted levels are lower and can't easily be achieved by LC-UV, MS will serve well. It can also potentially offer some extra separation for compounds that co-elute but that differ in mass. Finally, if looking at tandem MS (MS/MS), it can give extra specificity to the signal, i.e., produce a signal for a given compound that is free of interference from the matrix from which it has been extracted out.

Some drawbacks associated with MS, compared to LC-UV, are the additional expertise required to operate, the typical restrictions in the choice of compatible mobile phase modifiers, and the additional acquisition and operation costs.

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How to Select the Right Equipment for Cannabis Potency Testing

How important is column temperature?
It was somewhat surprising to witness the impact of a slight column temperature change on the elution time of certain cannabinoids during method development. It is therefore highly recommended to use a thermostated column compartment with a constant temperature setting when doing potency testing by LC-UV or LC-MS.

What are the advantages and disadvantages of using gas chromatography (GC) for potency testing?
In some regions, GC may be required by regulation to generate potency results. GC has been used for a long time in analytical chemistry; therefore, it may be the only available chromatographic technique is some cannabis labs. It could potentially be complimentary to LC-UV in some matrices. Its major drawback is its incompatibility with acidic cannabinoids without derivatization. Depending on the targeted cannabinoids and the materials from which they are extracted, GC could be ruled out as a useful technique for accurate potency testing.

What's next for potency testing?
MS will become the preferred detection technique for potency testing, due to the extreme diversity of materials on which potency testing is performed. Also, due to the expanding number of targeted cannabinoids and the fact that many cannabinoids are isobaric, i.e., they have the same mass, more powerful and orthogonal separation techniques will be required, such as two-dimensional chromatography and chiral chromatography.

JEAN-FRANCOIS ROY
Application Scientist, Chromatography and MS
Agilent Technologies

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Trusted Answers

Getting it Right: From Sample Preparation Through Data Analysis, Best Practices for Cannabis Testing Labs

In this series of brief videos, Agilent cannabis testing experts define the dos and don'ts for robust and reliable sample preparation, testing methods for cannabinoids in challenging matrices, customized tips for edibles sample preparation, and residual pesticide analyses using both LC/MS/MS and GC/MS/MS.

We invite you to get a cup of coffee, relax, and watch these light-hearted but highly educational videos with your colleagues. Enjoy!

Watch these videos now: www.agilent.com/chem/cannabis-tips

Topics presented:

Potency Testing in Fatty Edibles, Like Chocolate and Baked Goods

Potency Testing in Cannabis Infused Beverages

Potency Testing Cosmetics

Potency Testing in High Sugar and Low-Fat Edibles

Troubleshooting tips for analysis of Captan

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Cannabis Testing Best Practices: Making a Case for Understanding Fundamentals

Interview with Julie Kowalski, Ph.D.

Avoid chaos and discover cannabis testing opportunities with analytical chemistry basics.

In February's 2022 "Grow Your Skills in the Budding Cannabis Industry" two-day virtual symposium, hosted by Cannabis Science & Technology® and LCGC®, Julie Kowalski, Ph.D., acting lab director at 1st Choice Labs and consultant at JA Kowalski Science Support, presented on making the case that understanding the fundamentals of techniques is critical to establishing suitable methods that will not stumble and fail when implemented for daily use.

Why is it important to understand the fundamentals of analytical chemistry?

I have been fortunate in my career to have opportunities and mentors that focused on making sure I understood how everything functioned instead of just pushing a button on a piece of instrumentation. Having that basic knowledge can help you establish strong methods and fix problematic methods.

Because cannabis testing is still relatively new, technical experience is not as great as it is in other industries, and anyone who works in this industry is aware of that. This is a new market, so there is a lot of urgency around establishing businesses and getting methods up and running. The urgency can produce a certain amount of pressure to shortcut method development and validation and not investigate the science thoroughly. Without understanding the fundamentals of analytical chemistry, developing and applying methods in this new, high-pressure environment can result in poor performance, missed information, and, unfortunately, wrong answers.

There is a lot of opportunity, however, not just from a business perspective but from a scientific perspective. We are doing things that had been largely illegal in the past, at least for most of us that try to be on the bench as much as possible, and certainly domestically in the United States. All of this creates an opportunity for us to work on new methods and matrices and improve on what is out there. It is an exciting time.

Have you noticed, specifically, that some cannabis testing labs did not have a core understanding of basics? What sort of problems can that lead to?

There are methods published by vendors and other groups with a lot of valuable information. Many people want to pick up that information, go into the lab, and do *that* method. Sometimes that works out, and sometimes it doesn't. It is something that I have never been one-hundred percent comfortable with because those methods were not created for *your exact situation*—for your staff, your instruments, your regulations. Without an understanding of the fundamentals of analytical chemistry and vetting method performance carefully, you can create problems that could have been avoided.

In any analysis of a natural product, the sample matrix is not the same every time. If you use a method that is not a standardized method, you do not know the extent to which that method has been vetted. An example of this is the analysis of cyfluthrin and cypermethrin, which are two pesticides often regulated for cannabis testing. If you consider gas chromatography – mass spectrometry (GC-MS/MS) analysis, they can have similar retention times especially for compressed chromatography. And complicating this, commonly transitions (m/z 163 to 127 and m/z 163 to 91) are used for both sets of compounds. In this situation, you have to know that this possible analyte-to-analyte interference exists and build your chromatographic method to avoid issues.

Identifying this possible analyte-to-analyte interference is something you can do on paper if you understand GC-MS/MS and find some published transitions.

What is the biggest mistake you see during method design and development? How can those mistakes be avoided?
The biggest mistake is not vetting the methods in a stepwise fashion—an application note or a journal article is reviewed, and the lab jumps right in and tries to follow the full method instead of approaching it in a stepwise fashion.

What I mean by this is, is your instrument working the way it should be? Is your chromatography working the way it should be? Is your detector working the way it should be? What does the accuracy look like? How does your calibration curve look? What's your calibration range? Are you recovering the analyte?

So, stepwise vetting of your instrument and acquisition and processing methods first and then adding sample preparation in is the best way to avoid mistakes.

In addition, you are also learning a method or a technique that you maybe have not done before. Trying to get all those different parts of a method correct on the first attempt can be challenging, even for experienced people. If you build up each section more slowly, you will spend less time struggling to do an entire method out of the gate, not having it work the way you are expecting it to. Then you are troubleshooting an entire method. So, I do advocate for a more of a stepwise approach.

How can cannabis laboratories build for robustness?
Having established methods that you have vetted for your applications will benefit you in the long term if you are a business owner. Doing things to a certain level of vigilance, training staff, and bringing in people with experience may sometimes take a longer and cost up front, but I can guarantee that it will pay off, saving you money and time.

Building for robustness is the best way to get into and stay in this business. The other thing it does is prevent you from nightmare situations where you have established methods and set up a laboratory very quickly and now, you are in chaos mode. You are fighting fires every day; methods are not working, or they are not working the way they should be, and it causes a lot of chaos. That leads to unhappy staff, a high-stress situation, and you start to make mistakes and it can cause a lot of repeat analyses, and it is going to affect your business. So, preparing is going to benefit you long term if you are a business owner.

What are some of the best practices or resources for analysts to use while gathering information to develop their own methods?
Start by understanding the properties of the analytes you are looking at. What is the solubility, the thermal behavior? Are you going to use MS as the identification? What does the mass spectrum look like?

Do a thorough review of the literature. Get the chemical formulas and CAS numbers of the analytes. Are there other names for them that might appear if you do a text search? Do you expect to see isomers? Check the properties of the compounds of interest such as molecular weight, solubility, and if there are heteroatoms present. The NIST webbook has a lot of information on chemical compounds, and it is a trusted source.

Other things to consider are whether there are published methods for the analyte? Can the published methods be modified to meet your needs? But be careful about modifying and make sure you understand why you are changing something.

Gather up all the information you can before you start and keep all the information on each analyte. You may find, too, that an analysis that worked at one point no longer works. With all the information, you can go back and see if there are indications of what changed.

How does gathering information on your analytes help prepare you to run analytical tests and prevent interference?

When you understand the chemistry of the analytes, you can anticipate where there might be problems. Cyfluthrin and cypermethrin, again, are a good example of why you need to understand the analytes. Each compound has isomers, and their structures look very similar—they both have chlorine, which has two stable isotopes at about 25% - 75% abundance. That 25% is a significant number. If you consider the structures, you could have three different masses to worry about for the same compound.

If you start to think about the ions that result, the math is simple. I have a table that shows this (TABLE).

TABLE

Compound	monoisotopic masses (u)	[M+H]+, (u)	[M+NH4]+, (u)	[M+Na]+, (u)
Cyfluthrin	433.0647	434.073	451.099	456.055
Cyfluthrin	434.06815	435.076	452.103	457.058
Cyfluthrin	435.0618	436.070	453.096	458.052
Cypermethrin	415.0741	416.070	433.070	438.070
Cypermethrin	416.0775	417.085	434.112	439.067
Cypermethrin	417.0712	418.079	435.106	440.061

In the table are two compounds that are relatively close to each other in mass. They both have two chlorine atoms, so they are going to potentially have multiple ions at different values. My concern is, do we have any potential analyte-to-analyte interferences in this situation?

The chart shows the ammonium adducts of cyfluthrins are higher than the highest adduct of cypermethrin, so I am not worried about it. Same with sodium adducts. Cypermethrin protonated ions are m/z 416, 417, and 418, which are lower than the lowest adducts of cyfluthrin. These are not going to be concern either. But when I look at the remaining options, we have m/z 434 (for the protonated cyfluthrin ion), and m/z 433 (for the ammonium adduct of cypermethrin), and that is close. We also have ions atm/z 436 and m/z 438—two units apart, which could be a concern.

> **Cannabinoids are more amenable to an LC system, and terpenes and residual solvents are more appropriate for GC.**

We have some ions that are, under unit mass resolution setting, typically 1-1.5 amu, going to potentially generate signal at the same position for both m/z 434 and 435. If we are forming both the protonated cyfluthrin ion and the ammonium cypermethrin ion, we could have a signal from the sum of the contribution of two different analytes. If we open our mass resolution setting up to "wide," that is typically close to 2 amu, now we would have more issues with these compounds. So, knowing this situation before I even collect a single datafile, I would avoid setting my mass spectrometer to a wide-resolution setting.

Of course, you also need to look at interferences among analytes and the impact of the matrix on the separations, but this is a good example why you need to gather information before you start analysis.

What factors need to be considered when running methods on LC versus GC systems?

There are a number of things to consider when choosing between LC and GC, but the first thing to consider is thermal stability: If the analyte decomposes on heating, GC is not the answer. Second, consider how you will detect it: If MS is not available, then what detectors are available, and will your analyte be detected properly?

Cannabinoids are more amenable to an LC system, and terpenes and residual solvents are more appropriate for GC.

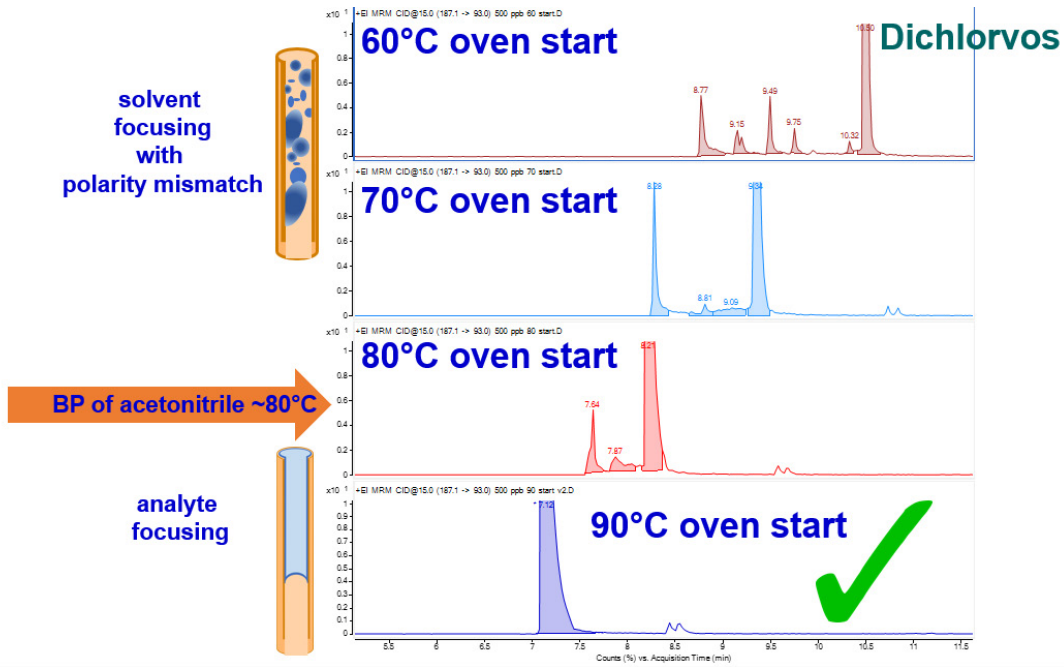
It is important to remember that you can use more than one tool. For example, I prefer to use both GC- and LC-based methods for pesticides testing when possible. It may not be possible to test for all the required pesticides at the regulatory limits on both platforms, but you can cover a substantial portion on both instruments. You can use the secondary method for confirmation of positive hits, which adds to the defensibility of results. This is especially valuable for complex matrices like cannabis biomass and concentrates. The use of a confirmation method can become invaluable when results are questioned, which is a common occurrence in the cannabis industry.

The example of cyfluthrins and cypermethrins continues to be good one here as well. We discussed testing these pesticides on LC-MS/MS, but you can also test these using GC-MS/MS. You can easily find GC-MS/MS transitions for these compounds, and interestingly, transitions (Q1/Q3) of m/z 163/127 and m/z 163/91 are commonly used for both. They do elute closely, so additional and unique transitions are beneficial. In this case, we optimized two additional transitions: one transition specific to cyfluthrin and one specific to cypermethrin. The overall method is built on both LC- and GC-based methods and with more specific, even if less common, transitions for both analytes. In this case, a positive hit for one of the analytes would be backed by retention times on GC and LC systems and multiple ion ratios. One can have more confidence in the positive detection, as this is now highly defensible data.

You spent a lot of time explaining how a GC inlet works. Why is it so critical to establishing GC-based methods?

Injection conditions for both GC and LC are important to correctly starting the chromatographic process. The goal is to introduce the sample and analytes to the head of the column in a narrow band. For LC, there are a couple of common and easy steps to follow options: match your sample solvent with the initial mobile phase or inject a small volume. There are other, more exotic options like injection programs that can be used as well. In GC, the injection process is more complicated and very critical. For liquid injection on a split/splitless inlet, you are injecting a liquid sample, vaporizing it, and then condensing it back to a liquid on the head of the column. Understanding this process and how to establish proper injection parameters seems to be lacking in education and training programs. That is why I wanted to show people that if you understand how it works then you can sort out parameters. One of the challenges with injection and inlet parameters is testing pesticides samples because the extraction solvent is often acetonitrile. Acetonitrile is a relatively polar solvent, but GC methods almost exclusively use a nonpolar GC column, like a 5 phase. This creates a polarity mismatch resulting in poor, sometimes split peak shapes of early eluting compounds like acephate and dichlorvos. You can think of these split or distorted peaks as resulting from acetonitrile beading on the stationary phase like water on wax. Some scientists simply assume that you have to rely only on LC for some of these small

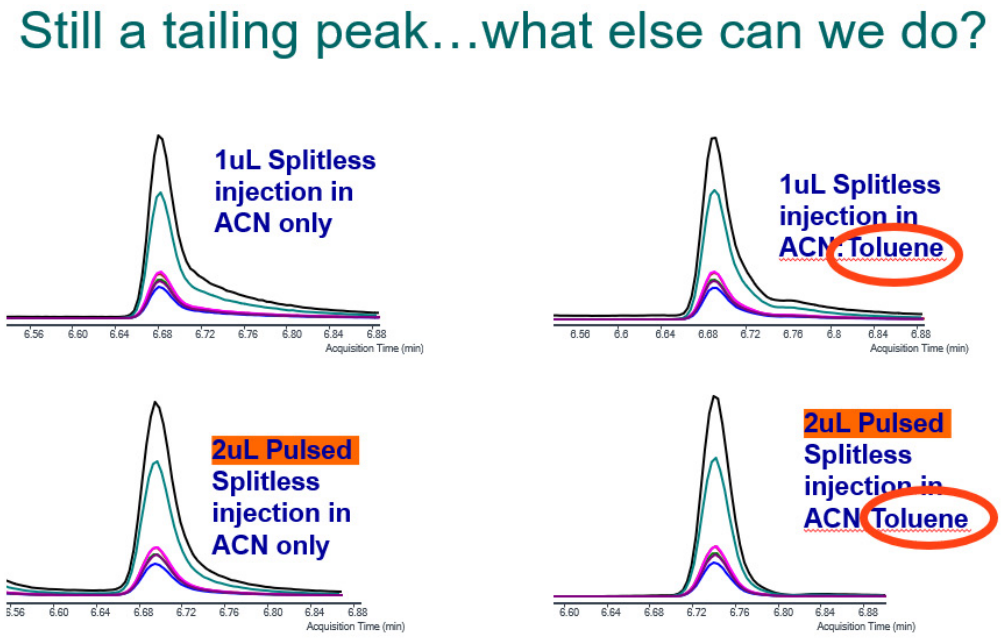
FIGURE 1



polar pesticides because of the peak shape/splitting issue. One can largely mitigate this issue if you understand the polarity mismatch and analyte focusing scheme for setting initial run parameters. The figure below shows that the peak shape of dichlorvos improves greatly when the initial oven temperature is 90°C. This is above the boiling point of acetonitrile, so when acetonitrile is injected and vaporized in the hot inlet, it never recondenses and elutes from the column. Meanwhile, the analyte is cold trapped or condenses on the column stationary phase.

Another approach to diminish the poor peak shape is to change the polarity of the sample solvent. Basic chemistry tells us that we need to make the injection solvent less polar and more nonpolar. This can be accomplished by diluting the sample with a nonpolar solvent like toluene. We diluted our sample 1:1 (v/v) with toluene. This results in half the concentration being injected, so it directly affects our detectability. But now that we are in a favorable sample solvent, we can take advantage of pulsed splitless injection. This mode transfers the injected sample through the inlet liner more quickly, which preserves active pesticides, improves peak shape, and allows the injection of more than 1 microliter of sample. Below, we show a symmetrical peak for dichlorvos injected with 2 microliters (lower right) (FIGURE 2).

FIGURE 2



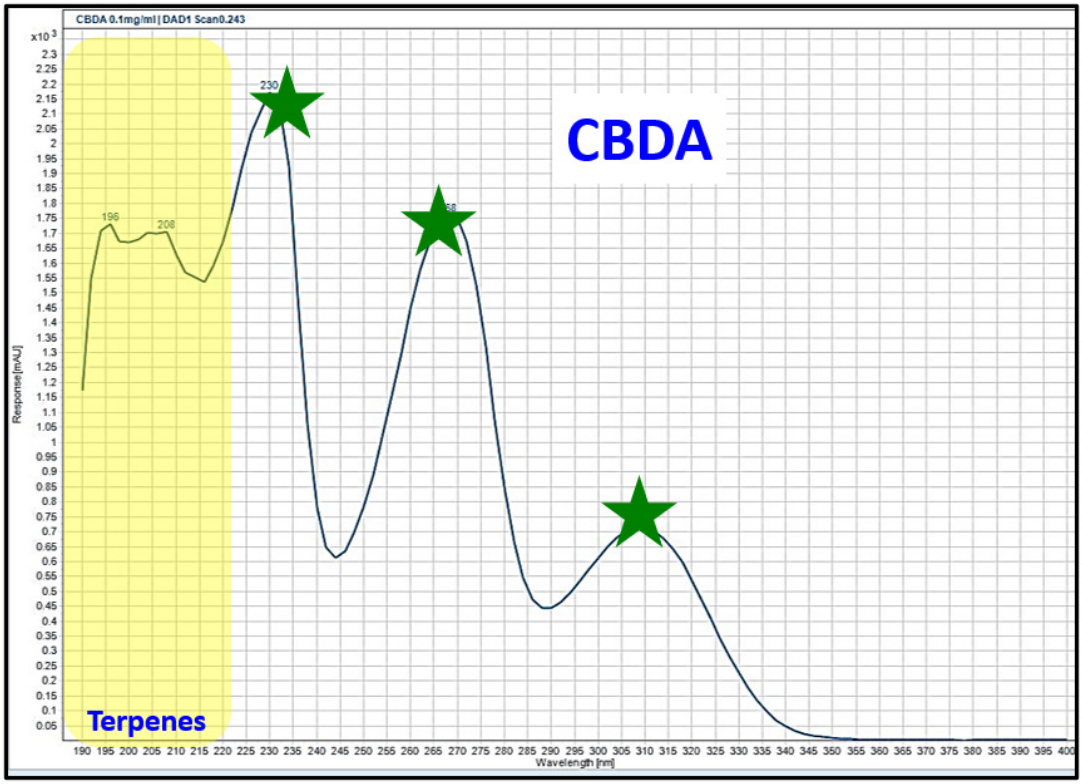
This development work took advantage of optimizing GC sample introduction parameters, resulting in the ability to have reproducible injections and peaks, adding LC-able pesticides to a GC method for confirmation, and the ability to inject 2 microliters.

You spoke about possible opportunities that are based on fundamental thinking. Can you provide an example?

It is important to think through everything from a basic level, even if you want to implement an established method. This does take time and resources, but the benefit is that you get to know a method intimately, and you may see an opportunity for improvement. For example, most cannabinoids methods quantify concentration using a single wavelength—228 nm is popular—on an LC-UV (DAD) system. Cannabis commodities have a wide range in cannabinoid concentrations, resulting in the need to perform different dilution schemes to be within the calibration curve range. But if you look at the full UV spectra of cannabinoids, you will notice that acids have a couple of apexes and neutral cannabinoids have a sort of apex region. Wavelengths near these apexes are at relatively low intensity. (FIGURE 3)

Does this allow us to use a low intensity quant wavelength, like 308 nm, for high concentrations of acidic cannabinoids or 274 nm for neutral cannabinoids where signal at 228 nm would be saturated? This could result

FIGURE 3



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in fewer dilution schemes or repeating sample preparations at different dilutions. Could this also provide alternative quant wavelengths if a matrix interference occurs? Opportunities like this could make the methods more efficient and robust, but you need to build this type of work into the scope of method development. Providing the time and resources and keeping the analytical lab skills up to date is the only way to build a stable technical program and avoid operating in a "firefighting" mode.

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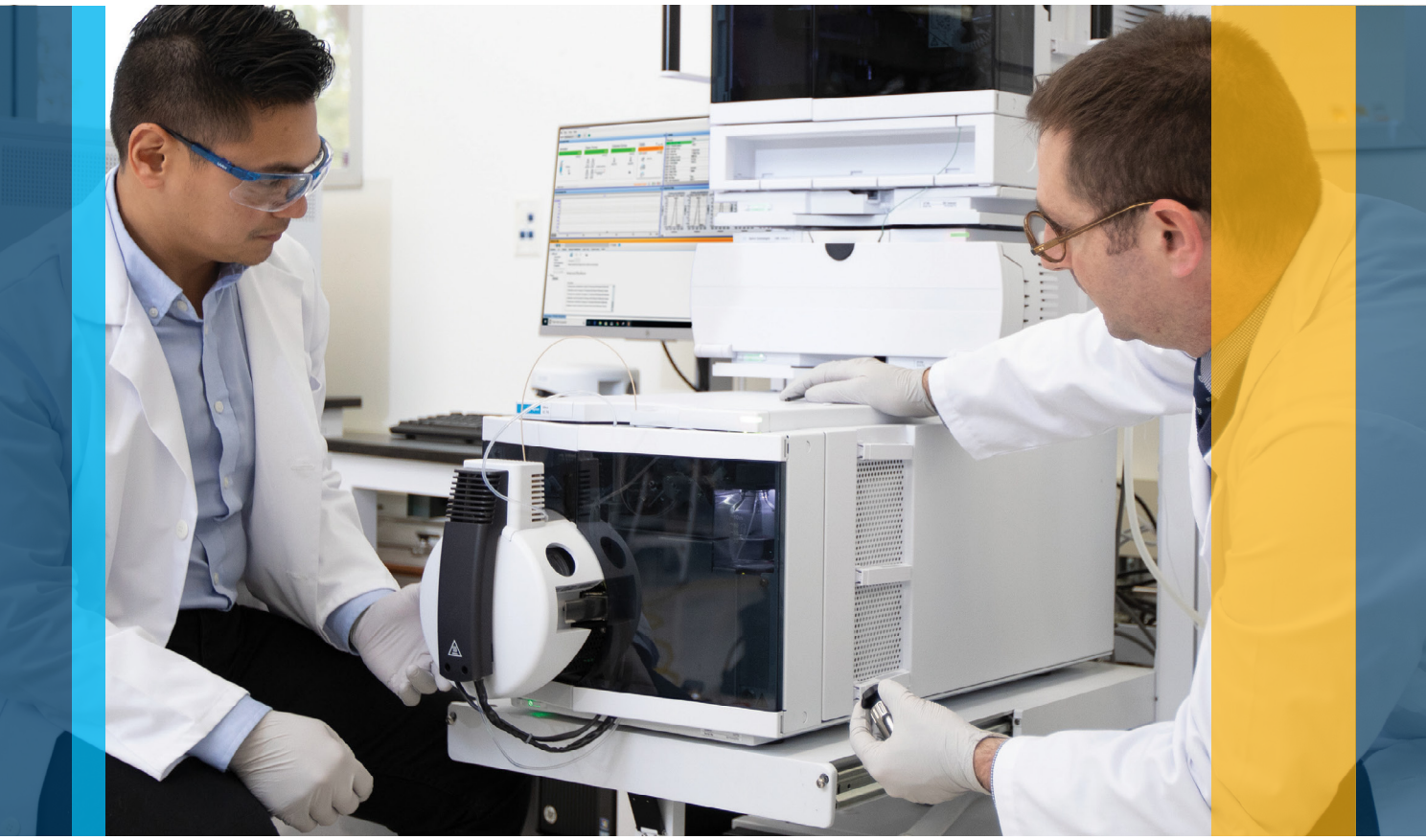

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