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New Frontiers in Cannabis and Hemp Testing, Part 1

Agilent

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Introduction

Cannabis testing is a rapidly evolving field, and it's critical for laboratories to have a handle on the latest developments and techniques in the cannabis and hemp testing industry.

This *New Frontiers in Cannabis and Hemp Testing* ebook series will share stories of how Agilent, its partners, and its customers have taken the lead in developing innovative tools and approaches to cannabis testing and research. Industry thought leaders in the cannabis testing space will share insight intended to give labs a competitive advantage in the marketplace.

Part 1 will cover:

- Why proper sample preparation is such an important part of effective testing for potency and what key methods labs can employ to ensure accurate quantification of THC in edibles.
- Solutions and workflows that are shifting the paradigm of cannabis testing in the gas phase.
- Heavy metals testing: How to get started and what to look for.


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Simple, Accurate and Reliable Quantification of THC and CBD in Cannabis-Infused Chocolate Edibles, Pastries, and Candies

Interview with
Christophe Deckers
and Jean-Francois Roy

Why proper sample preparation is such an important part of effective potency testing.

As the use of medicinal cannabis has expanded, numerous edible products have been developed, thus creating a need to accurately quantify tetrahydrocannabinol (THC) and cannabidiol (CBD) amounts in diverse food matrices. Such work cannot be taken for granted. A 2015 study of 75 cannabis-infused edible products found that only 17% contained the expected range based on the label claim (1). Nearly one-quarter (23%) of products were under-labeled (more THC than advertised), and 60% were over-labeled (less THC than advertised). What can labs do to ensure accurate potency testing of cannabis edibles?

Here, Christophe Deckers, Application Scientist, Sample Preparation at Agilent, and Jean-Francois Roy, Mass Spectrometry Application Scientist at Agilent, answer some frequently asked questions about the best methods for quantifying THC and CBD in cannabis-infused edibles like chocolate, baked goods, and candies. Learn why proper sample preparation is such an important part of effective testing for potency and what key methods labs can employ to ensure customer safety in this growing market segment.

Taking the time to establish and validate an optimized chromatographic method will pay dividends in the long run with higher throughput, and less solvent consumption.

What makes potency testing in edible products a challenge?
Testing for THC, CBD, and other cannabinoids levels will be challenging if you don't take the nature of the food product into account. Think about how different edibles can be. Some foods, like chocolate or some baked goods, are sticky and full of lipids; others, like gummies, are mostly sugar. The diversity of these matrices is exactly why you cannot use the same sample preparation approach for every product to ensure good recovery and selectivity. For instance, high-fat or oil-rich foods like chocolate require separating the fat-soluble compounds from the fats. Moreover, these samples will require optimized cannabinoid extraction to accurately quantify THC and CBD.

In a nutshell, using the wrong sample preparation method for the material can mean incomplete recovery, interfering compounds in the separation, or degradation of the instrumental performance over time.

Finally, some edibles are very complex and when they are simply diluted and injected, they can clog the liquid chromatography (LC) column, leading to increased instrument maintenance and decreased accuracy over time.

Can you offer some examples of how labs can use improved sample preparation, chromatography, and detection methods to optimize potency testing for edibles?
Chromatography, detection, and sample preparation work together for an optimized and simplified procedure that lowers the cost per sample, increases lab productivity, and improves accuracy.

Sample preparation is the "forgotten dimension of chromatography." Again, much of the robustness, reliability, and precision of a method depends on the ability of the analyst to convert the complex sample into a relatively simple solution, free of interfering compounds. The downstream chromatography benefits from the ability to remove as much of the extraneous compounds from your sample as possible. Having cleaner samples means more reliable chromatography, with easier-to-interpret results, and instrumentation that requires less maintenance. Columns will last longer with cleaner samples, and peaks will be easier to unambiguously integrate.

Taking the time to establish and validate an optimized chromatographic method will pay dividends in the long run with higher throughput, and less solvent consumption. Having a more robust method means fewer

failed runs that need to be repeated. Whether using ultraviolet or mass spectrometry, having a detection method that is optimized for your compounds of interest will give you the maximum sensitivity and will minimize potential interference.

How should I adjust my sample prep method for the matrix I'm analyzing?

Consider the form of the material (hard, soft, or liquid), as well as the matrix components, like a high amount of fats. Hard samples must be ground and extracted in solvent. Liquid samples are probably the simplest, but might require a step that will concentrate the sample, such as extraction, or QuEChERS. Solid samples typically need to be chopped up or ground. The best process (e.g., manual chopping, blender, cryomilling, mechanical grinding) may depend on the material and its tendency to stick to containers. Chocolate and baked goods will often have high-fat concentrations that must be removed. Captiva EMR-Lipid is well suited for this. High-sugar foods, like gummies or hard candies, will not dissolve in organic solvents, so an aqueous dissolution step is needed. As a general rule, acetonitrile extractions have been found to give cleaner samples than methanol extraction.

Does it make sense to adjust chromatography conditions and detection settings for specific matrix matrices like gummies versus chocolate?

A well-cleaned sample will not require a great deal of adjustment to the separation or detection methods. An effective sample cleanup will remove compounds that may coelute and/or interfere with the separation or detection of the compounds of interest. Once the sample preparation has been optimized for your sample, it's unlikely that any new peaks will show up in the chromatogram that will cause problems for the separation or data analysis. In the unlikely event that a novel ingredient makes it through the cleanup process and interferes with the quantitation, consider a change to the chromatography or mass spectrometry or revisit the cleanup method.

What are some challenges typically encountered when testing high-fat edibles like chocolate and baked goods?

The biggest challenge comes from the fact that cannabinoids have a similar hydrophobicity to fats. Unless the fats and oils have been effectively removed, they are highly likely to coelute and cause issues with the column and the instrument. Even with a mass spectrometer that can theoretically differentiate fats from cannabinoids, the fats can cause ion suppression, which will impact your quantitation. Fats can also be difficult to get off the column, resulting in longer run times and shorten column life. They may also accumulate on materials in the instrument requiring higher maintenance and cleaning time. Fortunately, there are off-the-shelf solutions, like Captiva EMR-Lipid, that can effectively and selectively remove fats from a sample. You will often get better results if your initial extraction uses

acetonitrile, rather than methanol. The higher polarity results in a cleaner sample without sacrificing extraction efficiency. Acetonitrile works best because it is an aprotic solvent, compared to MeOH that is protic and hence captures more matrix effect because of hydrogen bonding.

One issue with baked goods is that they can have nuts, chips, or other non-homogenous pieces in them. When breaking them down, you need to be sure that the mixture is rendered homogeneous. No chunks should remain after grinding or blending.

Might QuEChERS sonication or winterization during sample prep help overcome these issues?

QuEChERS come in a variety of chemistries. Some are ideal for removing polar interferences like salts, buffers, and sugars. The C18 QuEChERS chemistry is effective at removing lipids, but it has a limited capacity. It will become saturated when a large amount of lipid is present. Worse, C18 does not have sufficient selectivity between fats and cannabinoids, resulting in some loss.

Winterization is an effective way of removing lipids, and works well when the compounds of interest are hydrophilic. Unfortunately, cannabinoids are fat soluble, and will tend to partition into the fats as they separate, resulting in losses and high variance in the method.

What are the best ways to improve sample prep for optimized testing of high-fat edibles?

The ideal sample preparation method will remove interfering species (in this case fats) while leaving behind the cannabinoids. Methods based solely on hydrophobicity (such as QuEChERS C18) suffer from a lack of selectivity because cannabinoids have a similar hydrophobicity to fats. Captiva EMR-Lipid particles use a patented technology to remove fats selectively, while leaving other hydrophobic compounds. They provide an effective method of cleaning high-

fat samples while maintaining sensitivity and precision for cannabinoids. They have a higher capacity, meaning they're well suited for high-fat foods like chocolate or some baked goods.

What is Captiva EMR-Lipid Cleanup and how does it compare with QuEChERS for baked goods?

QuEChERS and Bond Elut EMR-Lipid are both dispersive extraction techniques, meaning that particles are mixed with the sample and then separated by centrifugation or filtration. The chemistries are quite different, however. EMR-Lipid stands for Enhanced Matrix Removal for Lipids. This advanced technology uses both size exclusion and unique chemistry to only capture fats and not cannabinoids. For edibles, we use the Captiva EMR-Lipid format that is not a dispersive but in convenient filtration tubes. You can't achieve this specificity with QuEChERS because the C18 groups in dispersives are not selective enough and will also capture THC and CBD. The problem becomes more pronounced when there is a large amount of fat.

In high-fat samples, EMR-Lipid provides the most effective and selective cleanup. The capture of lipids is based both on hydrophobicity and size exclusion. EMR-Lipid also has a higher absorbing capacity to deal with high-fat samples, like chocolate.

Is QuEChERS a good option for gummies and candies?

Gummies and hard candies need water to dissolve, which makes QuEChERS an excellent candidate for sample cleanup. While those foods are mostly sugars, there is also a relatively small amount of oil and fat in those edibles. We can therefore use QuEChERS extraction salts to remove that water and sugars but we found that using dispersives to further cleanup the acetonitrile supernatant was not significantly beneficial. A simple PTFE filtration of the resulting acetonitrile extract was sufficient. In beverages, however, after a QuEChERS water partitioning step, we do need to perform an EMR cleanup step to remove micro-emulsions/emulsifiers that can interfere with detection and buildup in your instrumentation

Reference

1. R. Vandrey et al., "Cannabinoid Dose and Label Accuracy in Edible Medical Cannabis Products," *JAMA*. 313(24), 2491–2493 (2015).

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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.

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The Intuvo Trifecta. One Instrument, Three Analyses—Pesticides, Residual Solvents, and Terpenes

Interview with Anthony Macherone, PhD

GC solutions and workflows for cannabis testing.

The typical cannabis testing lab needs key three things to survive and thrive in this rapidly expanding and changing market:

1) Fast gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis for pesticides not amenable to electrospray ionization;
2) Residual solvent analysis specific for cannabinoid products; and
3) Liquid injection terpenes analysis for cannabis and cannabis products.

Here, Anthony Macherone, PhD, senior scientist at Agilent Technologies, Inc., reviews some solutions and workflows that are shifting the paradigm of cannabis testing in the gas phase.

How can I increase the lifetime of my column and keep my source clean longer? A lot of junk (like late eluters) collects and causes issues with productivity, not to mention we spend a lot of time cleaning our system. Heavy sample matrix and interfering chemicals can be a maintenance and throughput headache. Some of these problematic compounds will be sitting at the head of the column even as the compounds of interest are entering the detector. The Guard Chip on the Agilent Intuvo 9000 GC system

provides the first line of defense against highly retained matrix and interferences that can affect chromatography, quantitation, and potentially ruin a column. It functions as a sacrificial section of column that will capture such compounds. It can be easily and quickly replaced, preserving the life of the column and overall method performance.

High boiling and late eluting compounds can be a different problem. They slow down the run, requiring extra time to bake off. Worse, they tend to collect on the source, leading to increased downtime for cleaning and maintenance. Traditionally, once a compound has entered the column, little can be done other than to wait for it to come out the other end. Agilent Capillary Flow Technology that can be added to the Agilent GC systems enables backflush, which reverses the column flow at a specified time, to quickly remove unwanted compounds that have not traveled far from the inlet. Because they are exiting from the head of the column, the source is never exposed to them.

Regarding cannabis methods application notes, why not just run a calibration curve and write an app note?

Obtaining results that can be translated effectively to other labs or other analysts requires that all the conditions the sample encounters be reproducible, from collection, to storage, and especially sample preparation.

Simply running a calibration curve isn't enough to establish that a method is robust enough to stand up to regulatory guidelines. There must be some level of validation of the method to establish such parameters as the minimum detectable level, the limit of detection, the limit of quantitation, accuracy, and precision among other performance characteristics. If a method is going to be published and replicated by many labs, it should also be reliable day in and day out and be very detailed, so that laboratories can accurately replicate the process.

Is it possible to do one sample preparation for consumables that works for both liquid chromatography (LC) triple-quadrupole and GC triple-quadrupole systems?

The primary purpose of any sample preparation method is to remove as much of the matrix as possible while leaving the compounds of interest. An effective sample prep method will therefore result in a sample that can be

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Our Journey to the Intuvo Trifecta of Gas Phase Analyses

injected into a wide variety of instruments for analysis. At most, a final dilution step may be needed to mechanically remove matrix and improve overall signal-to-noise.

Is electrospray ionization suitable for captan?

Getting reliable results for captan is challenging with electrospray ionization. The molecule does not have an efficient site to gain or lose a proton and form a quasi-molecular ion. Captan can be run with positive electrospray ionization but it will lack the required selectivity and sensitivity under these conditions.

Captan degrades quickly and has a finite shelf life. How can we overcome these challenges in the lab?

First, it's important to follow the storage instructions for your captan reference materials and pay close attention to expiration dates. Captan is pH and thermally labile. Captan is most stable under acidic conditions. At room temperature, captan will last about a day at a pH of 4, but will disappear faster than it can be measured at pH >9. Higher temperatures will accelerate degradation. It's also important to use clean, high-grade solvents, water, standards, and reagents. Lower grade materials have been found to have contaminants that catalyze degradation.

Did you try degrading captan in the inlet and measure 1,2,3,6-tetrahydrophthalimide (THPI) as an end product?

We've performed studies in which we deliberately degraded captan in the inlet. This can be done with a hot-inlet injection, which will result in close to 100% conversion of captan to THPI. Deliberate degradation and then quantification of THPI is a viable way of measuring captan but this approach may not be allowed in certain jurisdictions.

Deliberate degradation and then quantification of THPI is a viable way of measuring captan but this approach may not be allowed in certain jurisdictions.

Can you talk about cold solvent vent type injection for captan?

The thermal reactivity of captan can be a challenge for GC analysis. The idea of the cold solvent vent-type injection is to minimize the exposure of the captan in the samples to high temperatures. The inlet starts at 60°C at the time of injection, and then very quickly ramps up to the injection temperature. Much of the solvent and matrix is eliminated through the split vent, and the captan is transferred into the column for analysis. The use of analyte protectants can also mitigate degradation of captan.

What is the sample prep for residual solvents?

A common misconception about residual

solvent analysis in cannabinoid products is that it's another application of *USP* <467>. Although various state or country target compound lists are like those found in *USP* <467>, cannabinoid products can be much more complex than single API drug formulations and therefore must be treated differently. A primary difference is sample preparation. It is most common to use Full Evaporation Technique (FET) with headspace GC systems. FET considers the sample volume (or mass) as insignificant and thusly the phase ratio is large and all the analytes partition into the headspace. However, given the vast array of cannabinoid products, the assumptions of FET are not always true, and accuracy and precision of the test results can be negatively affected. In our work, we determined that dissolving the samples into an appropriate solvent like dimethyl acetamide, the use of saturated saline solution and an internal standard, greatly improved accuracy, precision, and overall method performance.

Have you tried different matrix like resins, food, or beverages?

Yes, we have evaluated concentrates, distillates, oils, tinctures, chocolates, hard candies, gummy bears, and beverages.

What is chemical noise mitigation, and how do I need to account for it in sample prep?

Recovery is a measure of how efficiently analytes of interest are removed during sample preparation procedures. Part of this is removing chemical noise that can interfere with the analysis and affect selectivity and quantitative results. Another aspect of reducing chemical noise is dilution of the sample after extraction. While this may seem counterintuitive for residual pesticides analysis, one must consider how tandem mass spectrometry works. Essentially, MS/MS mitigates chemical noise by focusing on specific precursor-product ion pairs and ignoring other chemical signals. The addition of mechanically reducing chemical noise through sample dilution when using MS/MS results in an overall improved signal-to-noise for target analytes.

Can I use QuEChERS for cannabis flowers?

While QuEChERS is a powerful and widely used sample preparation technique, it does not provide the best results for cannabis flower. The addition of water, salts, dispersive raise the pH, cause exotherm, and co-scavenge certain pesticides. The dispersed phase also has a limited capacity that can be overwhelmed by the common fat-soluble compounds found in cannabis.

Why use both LC-MS/MS and GC-MS/MS for pesticides and where are they both needed (meaning which states)?

There is a lot of overlap between what can be measured with LC-MS and GC-MS analysis, but they are not identical. Some compounds are better tested with GC methods. Canada

and states like California, Nevada, and Florida have testing mandates that will require GC analysis. Furthermore, having both platforms gives you the ability to perform confirmatory redundancy for those compounds that can be tested with both.

What are the ESI compounds to consider for GC-MS/MS? Why not APCI?

The compounds that cause the biggest problems with ESI are those that don't have inherent chemical moieties amenable to gaining or losing a proton. These include pentachloronitrobenzene (PCNB), chlordane, captan, and others. APCI, unfortunately, is not selective in its ionization of compounds like PCNB and tends to be less sensitive compared to electron ionization.

With the Intuvo Trifecta, are the hardware and software for residual solvents and terpenes identical?

Yes. The injector, column, ionization, and mass spectrometry components are all the same, as is the software package used to do the analysis.

Can you talk about the use of liquid injection for terpenes versus headspace?

The use of headspace injection techniques for terpenes is not appropriate and primarily results in losses of higher molecular weight terpenes in matrices high in cannabinoid content. Like residual solvents, the use of FET headspace for terpenes analysis is not amenable for the vast array of sample types. Liquid injection for the analysis of terpenes has been a standardized method for decades in industries like flavors and fragrance.

Do I need a suite of analytical equipment when performing safety and quality testing of cannabis and cannabinoid products derived from cannabis or hemp? Or can I just have an LC system?

The nature of the regulated testing requires laboratories to have HPLC for potency testing, LC/MS/MS for pesticides and mycotoxins, ICP-MS for metals, GC/MS for residual solvents and terpenes, qPCR for microbial screening, and in many cases GC/MS/MS for pesticides. No single analytical system can measure the various chemical classes required by regulations.

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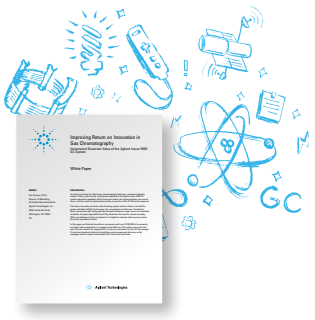

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Indo Laboratories: How the Agilent ICP-MS is Helping Create a New Standard for Laboratory Testing

Interview with Nick Bilotti and Nicholas Masso

Heavy metals testing: how to get started and what to look for.

Indo Laboratories is a cannabis laboratory with a highly educated staff with extensive experience within academia, biotechnology, and the marijuana business landscape. Indo pays rigorous attention to detail within the thoughtfully developed testing and the most advanced technologies to provide customers with the results they can trust.

Indo Laboratories is the brainchild of Nick Bilotti, chief operating officer and co-founder, and Nicholas Masso, CEO, and co-founder. While studying Huntington's and Parkinson's disease at MGH, Masso became deeply interested in the science behind the use of cannabis for medicinal purposes. Likewise, Bilotti grew up in California, the first state to legalize medicinal marijuana back in 1996. He witnessed family members and friends afflicted with cancer and neurodegenerative disease use cannabis for pain support, and became even more interested in the field in his graduate work. The pair met while working at a laboratory solutions company, and sensing the growth of the cannabis industry, felt there was a major bottleneck in meeting the regulatory requirements for cannabis product manufacturing and distribution.

Indo Laboratories uses the Agilent ICP-MS for heavy metal testing at their facilities. This instrument has been crucial for the detection and quantification of toxic heavy metals that might be present in flowers, concentrates, tinctures, topicals, edibles, and other cannabis-infused products. With state-of-the-art equipment combined with automation and scientific rigor, Indo Laboratories is bringing credibility to the science of cannabis by creating a new standard for laboratory testing.

Here, Bilotti and Masso talk about challenges and best practices when opening a new cannabis laboratory.

What are some things to look for when selecting a cannabis laboratory's location?
There are legal challenges to transporting cannabis across state borders, and the testing requirements can be very different from state to state. Life is much easier for both the laboratory and the producers if they are both in the same state. As cannabis is legalized in each new state, there is an opportunity for more labs to open to service their testing needs. While it would require a certain amount of redundancy, a multistage state testing company would be best organized to have a facility in each state that it services.

What happens in a testing lab? What types of different tests do you/can you run?
Cannabis testing is still an industry in flux. Unlike other kinds of testing areas (like environmental testing), there are no compendia of agreed-upon methods for cannabis analysis. In addition to testing, a cannabis lab is also in the business of establishing suitable testing methods, finding and establishing standards, communicating the need for sufficient testing for growers and consumers and working with regulators to help establish meaningful and achievable regulations. A multidisciplinary approach is often needed. Right now, there is a shortage of testing companies, and many producers are facing testing backlogs.

> **It puts a lot of pressure on the testing lab to ensure that they can back up their results, including having quality control standards to provide continuous process validation.**

The types of tests to run are also changing rapidly. In addition to heavy metals, needs may arise for testing microbiological contamination, mold toxins, potency, flavor components, and also stability. Cannabis is a biologically active product and will change over time. Cannabis can be a challenging and complex matrix, and the inclusion in edibles means that labs must deal with even more and varied matrices.

What does the metal-testing process look like? How do you get set up for metal testing? What specific metals are you looking for?

We typically start with raw flower, which we homogenize to a fine powder. A portion of the powder is weighed out and digested in a high-pressure microwave digester with strong acids. Then, the sample is loaded onto the Agilent 7900 ICP-MS to be measured. Internal standards are used for quantitation, and quality control checks are built into the method. At the end, the data are evaluated, and a certificate of analysis is generated.

The target analyte are dictated by federal and state regulation and guidance. They include arsenic, cadmium, mercury, and lead. Other elements may need to be added depending on regulatory requirements. Both the market and the testing requirements are still growing. It's a good bet that a testing lab will always need more equipment and instrumentation than first planned for.

Can you talk about developing methods for heavy metals testing?

Cannabis testing is a new area, and there aren't EPA or other compendial methods already written. This makes it necessary to develop methods in house, which can be a time-consuming process, and can depend on the hardware and instrumentation available. We developed a method that could detect the target metals well below the regulatory requirements so that clients can learn about potential problems before they become important.

If a product is deemed unsafe or non-compliant, what do you do?

It's a big deal for the client if a sample fails. The typical production batch is 15 pounds, which is a costly loss if it can't be sold. There is not much they can do with that sample other than to selectively extract the active ingredients and sell it as a concentrate. It puts a lot of pressure on the testing lab to ensure that they can

back up their results, including having quality control standards to provide continuous process validation. A failed result also provides an opportunity for consulting with the client on ways of locating the source of contamination and preventing it in the future.

If you compare a cannabis product sold on the black market to one that is grown in a certified-organic farm, what kind of differences do you see? Do you test both?

Licensed growers have to take a lot of precautions to ensure they are adhering to guidance from the US Department of Health and other regulatory agencies. Generally, home growers and black-market producers don't even know about the necessary precautions. Testing black-market materials can pose a legal liability as well as a risk of contamination of your facility. Cannabis has been used as a phytoremediator of contaminated soils because of its tendency to pull toxic elements out of the soil and into the plant. A plant grown in an uncontrolled environment could potentially concentrate harmful levels of contaminants.

How do you share the data and information you obtain, meaning is it proprietary because your growers don't want you to share it?

Data can be published if the customers provide releases. Some customers do not wish to sign releases. That data can still be used, but must be blinded so no identifying information is published. The data is valuable as a way of tracking potential dangers to the consumer. Unfortunately, there currently is no similar testing of the mass-produced cartridges used for vaping. This is an important opportunity for cooperation and sharing information to create a vibrant and stable industry.

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.

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