White Paper

Our Journey to The Intuvo Trifecta of Gas Phase Analyses

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Abstract

Safety and quality testing of cannabis and cannabinoid products derived from cannabis or hemp requires a suite of analytical equipment. No single hardware platform can perform potency, pesticides, mycotoxins, residual solvents, terpenes, metals, and microbial analyses. Where chromatography is required, gas phase systems are used for three of these tests: pesticides, residual solvents, and terpenes. Gas phase systems could also be used for potency testing, but liquid phase systems are more common. Over the past several years, we have developed gas phase applications specific to cannabis testing. This article examines that journey and the lessons learned along the way. It describes the evolution of our testing workflows culminating in the Intuvo Trifecta methodologies that shift the paradigm of cannabis testing in the gas phase.

Background

Brief history

In November 2016, Agilent entered the cannabis regulatory testing industry as an instrument and consumables provider. At that time, only one other instrument vendor was active in the cannabis testing industry and just a handful of states in the U.S. had published regulatory requirements: Oregon, Colorado, Nevada, and Washington. It was understood that Canada would legalize adult recreational use of cannabis at the federal level in October 2017, but Health Canada had not yet published the testing regulations. It was further expected that California would legalize recreational cannabis, and in December of 2017, California published its first round of regulations.

The published testing of residual pesticides, mycotoxins, residual solvents, metals, terpenes, cannabinoids, and microbes were areas that Hewlett-Packard/Agilent had decades of experience in environmental, agricultural, clinical, forensic, food, and flavors industries. The big unknown however was the matrix, or better stated, the myriad of cannabis and cannabinoid products ranging from inflorescence, candies, oils, beverages, and concentrates, and the challenges each would present. As it turned out, the product matrices were extremely complex and sample preparation was quickly identified as a key need for proper cannabis testing methodologies.

With these facts in mind, a team of application scientists came together to prioritize the method development. Primarily through collaboration with active cannabis testing labs, the team targeted cannabinoids, terpenes, metals, and residual pesticides and mycotoxins as the applications with the most need. We pursued residual solvents as if it were traditional USP <467>, a decision we later determined to be incorrect but more on that later. Here is a quick recap of that first year and the development of the first-generation tests.

Cannabinoids testing

In 2016, the only other offering for cannabinoids testing was a self-contained, dedicated HPLC that required three methods to analyze a total of 11 cannabinoids. Working in conjunction with colleagues at CWC labs in Texas, we developed a fast and efficient method for the analysis of 11 cannabinoids on a self-contained HPLC-UV system. We also published one of the first applications for hemp testing and discovery, using liquid chromatography time-of-flight mass spectrometry (LC/Q-TOF) and another using LC-single quadrupole mass spectrometry for cannabinoid measurement in CBD oils.[1,2]

Comprehensive pesticide testing

As noted, when we entered the cannabis regulatory testing industry, only a handful of regulations had been published. With respect to residual pesticide testing, Oregon had the most comprehensive list, but it still only contained 59 pesticides. Our applications team felt a more comprehensive approach was required—one that targeted the regulated pesticides but also a larger list of common pesticides used in the agricultural industry. We further understood that to perform comprehensive testing, both LC-tandem quadrupole mass spectrometry (LC/MS/MS) and gas phase tandem quadrupole mass spectrometry (GC/MS/MS) would be required since, despite common mythology, electrospray (ESI) is not a universal ionization source. Lastly, we would require a streamlined sample preparation technique amenable to both platforms. Working with colleagues at Pacific Agricultural Laboratory, that is exactly what we accomplished.[3] Later in 2018, we set out to use the single stream sample preparation and two-platform approach specifically for Canada in collaboration with Canopy Growth Corporation and for California.[4,5,6]

Terpenes

There is little regulation pertaining to terpenes in cannabis and cannabinoid products. The interest in terpenes is mostly with respect to the quality of the product. There may also be instances where the product label makes a specific claim, e.g. 10 mg/g limonene, that require terpene identification and quantitation. Historically, terpenes have been analyzed using liquid injection on a gas phase GC system in the food and flavor industry, but the cannabis testing industry initially chose headspace for sample introduction. Why this is the case is unclear. It may have to do with a mindset of no sample preparation with headspace technologies or the loose classification of terpenes as volatiles, like residual solvents. In any case, in conjunction with a Nevada cannabis testing lab, we too developed a headspace gas phase mass spectrometry application that split the effluent to a Flame Ionization Detector (FID) for simultaneous collection of FID and MS signals.[7] Since that time, the cannabis testing industry has realized that headspace sample introduction for terpenes analysis is not appropriate and the current trend is moving towards liquid injection methodologies with mass spectrometry. This methodology mitigates problems encountered with headspace, such as loss of higher boiling sesquiterpenes. It also offers the ability to perform sample dilutions which may be necessary with cannabinoid products that have percent by weight concentrations of terpenes. And lastly, it provides speciation of the targets not attainable with nonselective FID. Again, more about this later.

Metals

The most commonly regulated metals in the cannabis testing industry are cadmium, lead, arsenic, and mercury. Some states have a few more such as chromium, barium, silver, and selenium. The team developed an ICP-MS Analyzer that tests for 25 metals encompassing the commonly regulated metals and others such as sodium, calcium, and magnesium.[8] This allows for a more comprehensive analysis of the metals content in cannabis products and for the analysis of other matrices such as water or growing media. A very nice component of metals analysis with ICP-MS is sample preparation. Most, if not all commonly encountered matrices in cannabis testing are prepared using microwave digestion.

Microbial screening

Ensuring cannabis products and inflorescence are not contaminated with potentially harmful microbes is essential for safety testing. A common method for microbial screening is culturing in plates with various growth media but these tests were not designed for use in complex matrices such as those encountered in the cannabis industry. The adaptation of culture-based methods for cannabis has led to false-positives, misidentification of bacterial species, and under-reporting of microbes such as *Aspergillus* spp.[9] To address the short-comings of culture-based methods, Agilent partnered with Medicinal Genomics to offer a highly selective quantitative polymerase chain reaction (qPCR) test to extract, purify, and identify amplified DNA in cannabis products.

The Intuvo Trifecta

Considering the chaos encountered in 2020 caused by the global outbreak of SARS-CoV-2, spring of 2019 may seem like a millennia ago. Nonetheless, as we entered that year, we recognized how the cannabis testing industry was maturing and began planning for the next generation of testing. This began with collating information from testing labs we were engaged with and understanding their pain points, bottle necks, and needs. There were at least five areas that needed to be addressed:

- Erroneous information in the general knowledge base concerning the use of atmospheric pressure chemical ionization (APCI) for the analysis of certain pesticides
- Problems encountered with electrospray negative ionization for the analysis of acid phytocannabinoids
- The need for a fast GC/MS/MS analysis for pesticides not amenable to liquid phase AP-electrospray ionization
- The need for a residual solvent analysis specific to cannabinoid products
- The need for a liquid injection terpenes analysis for cannabis and cannabis products

The first two bullets were fully addressed and published[10,11] and not in scope for this essay. Bullets three through five would eventually become the Intuvo Trifecta—the latest genre of cannabis testing in the gas phase.

The Purpose of the Trifecta

By late 2019 and into the spring of 2020, our colleagues working day-to-day in cannabis testing labs were defining what they want and need to keep their labs viable into the future. Paramount to these needs was accuracy, precision, and method robustness, especially for residual pesticide and residual solvent testing. By this time, we had learned that sample preparation was critical for successful pesticide trace analyses. Furthermore, treating residual solvent testing such as USP <467> was not appropriate since cannabinoid products are complex multichemical class extracts and edibles and typically not drug-like formulations with a single active pharmaceutical ingredient, excipients, or fillers. Lastly, it was becoming clear that headspace sample introduction for terpenes analysis suffered from losses of sesquiterpenes such as α-bisabolol in the presence of highly concentrated cannabinoids and Full Evaporation Technique (FET) was not appropriate for many cannabinoid matrices. Efforts to combine residual solvent and terpene testing into a single method resulted in analytical compromises. Based on this information, we knew that leveraging the unique features and ease-of-use capabilities of the Intuvo 9000 GC to build a laboratory-vendor partnership focused on leadership, process, and evidenced-based decision making was the right direction. The result was the Intuvo Trifecta: fully vetted, streamlined methodologies for residual pesticide, residual solvent, and terpene analyses.

Back to basics

One area of cannabis testing that changed little for us since 2016 was sample preparation for pesticide analysis. With the development of the first comprehensive method, we determined that QuEChERS was not appropriate for inflorescence. This was due to a pH spike, exotherm generation, and d-SPE scavenging certain pesticides resulting in poor recoveries. We also determined that techniques such as winterization or dilute and shoot were time-consuming, did not adequately remove matrix, and caused increased fouling of the electrospray source or inlet on a GC system.[12] Therefore, we stuck with the single stream sample preparation method followed by high dilution factors for pesticides analysis to ensure method robustness and significantly reduce instrument maintenance needs. Other basics were known best practices learned from decades of analytical experience including cold injection, analyte protectants, internal standards, backflush in the gas phase, injector programming in the liquid phase, using high quality reagents and standards, and matrix matching of calibrators and quality controls with samples. These best practices were also adapted for residual solvent and terpene testing.

Commonalities

The Intuvo Trifecta refers to residual pesticide, residual solvent, and terpene analysis in cannabis and cannabinoid products built around the Agilent Intuvo 9000 GC platform.[13,14,15] The MultiMode Inlet (MMI), Guard Chip, midcolumn backflush, matrix matched calibration, and the use of internal standards are common to all three methods. For residual solvents and terpenes, the hardware and consumables (column, liner, etc.) are identical and both methods can be run on the same system without any changes with the addition of the optional Agilent XLSI Transfer Line Interface and Agilent 7650A Automatic Liquid Sampler. These commonalties coupled with the fact that Agilent MassHunter software is used for all three tests, significantly reduce expenses on consumables and supplies and personnel training needs.

Honesty in method performance: is "fit for purpose" fit for purpose?

Analytical testing must meet certain performance characteristics to be considered reliable. This is sometimes referred to as "fit for purpose" but is this reference appropriate for analytical testing where safety is concerned? Yes and no. Fit for purpose should be considered, as should system suitability, in the design and implementation of an analytical testing method but testing in the cannabis industry should be more than just "good enough".[16] At a minimum, quantitative chemical testing methods should be rigorously evaluated for accuracy, precision, method detection limits (MDL), limits of quantitation (LOQ), selectivity, range, linearity, and robustness.[17] To this end, we established a vetting paradigm for the Intuvo Trifecta and other applications development since 2019 that would evaluate method performance in cannabis matrices. The program included a minimum of two interday MDL studies comprised of eight replicate injections of multiple calibration levels prepared in matrix. MDL was determined with Equation 1.

$$\text{MDL} = (\text{SD}) \times (\text{Student t-value, n} - 1 \text{ degrees of freedom, 99\% confidence})$$

Equation 1.

where SD is the sample standard deviation shown in Equation 2. The appropriate concentration level for MDL calculation was determined by the logical test: Calculated MDL < Spike Level < 10 × Calculated MDL.

$$\text{SD} = \left[\frac{\Sigma(x - \bar{x})^2}{n - 1} \right]^{1/2}$$

Equation 2.

Determinations of LOQ were performed through a minimum of three independent analyses of five replicate injections of eight calibrator levels prepared in matrix (n ≥120). Interday and intraday data were used to determine LOQ through Equation 2.

$$\text{LOQ} = 10 \times \text{SD}$$

Equation 3.

Interday and intraday accuracy and precision (%RSD) were determined using Equations 4 and 5 through multiday analyses comprised of a minimum of three independent studies of five replicate injections over eight calibrator levels prepared in matrix. Range, linearity, and robustness were also determined with the same datasets and statistical results.

$$\text{Accuracy} = [(\text{spiked concentration} - \text{calculated concentration/spiked concentration})] \times 100$$

Equation 4.

$$\%\text{RSD} = (\text{SD/Average}) \times 100$$

Equation 5.

In our reporting of the Intuvo Trifecta methods, we disclosed all data and illustrated exactly how method performance was determined. We further shared data where the results were not as expected and discussed how the issues were addressed. For us, including the blemishes and how they were resolved, best exemplified the scientific method and illustrated some of the pitfalls of method development and validation.

Conclusion

Herein we provided a brief review of Agilent's journey in the cannabis testing market. This resulted in a very specific method development and vetting paradigm that enabled rapid implementation and robust analyses. This is where we are after undertaking evidenced based decision making and a willingness to work outside the expected norms of the industry. We've proven that liquid phase APCI is not a good choice for pesticides that do not ionize or ionize poorly with ESI, we've demonstrated that acid phytocannabinoids decarboxylate using ESI in negative mode and we've shown how time-of-flight mass spectrometry and statistical software can be used in comparative analysis of cannabinoid products.[18] We realized that residual solvent testing for cannabinoid products should not be based on USP <467> and addressed problems encountered with headspace sample introduction for the analysis of terpenes. A primary lesson learned in our journey was to be agile in response to an ever-changing cannabis testing industry enveloped in a world prone to stochastic—even unfathomable—events. We also learned that working in collaboration with testing labs toward a common goal of improving results by using new and novel methodologies that lead the industry into the future. The Intuvo Trifecta exemplifies this philosophy. These new directions became the model for our method development and implementation as we evolved with the industry. Along with cannabis testing kits and eMethods, we see nothing but a blue ocean in front of us.

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Disclaimers

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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