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Note from the CEO

March of Progress

AS CANNABIS AWARENESS Month comes to a close, it's important to reflect on the enormous progress that has been made in overcoming the stigma of cannabis. So far, 39 states have legislation that makes cannabis legal for either medicinal purposes or both medicinal and recreational use. While some states have not legalized cannabis, they have decriminalized its possession. In fact, there are only four states in which cannabis is fully illegal, according to *USA Today*.

While states have led the way for cannabis legalization, progress on the federal level has been slow. The process of rescheduling cannabis from Schedule I to Schedule III under the Controlled Substances Act of 1970 had begun, but has stalled since January after a motion was filed alleging bias on the part of the Drug Enforcement Agency against rescheduling. More recently, Congressman Dave Joyce (OH-14) and House Democratic Leader Hakeem Jeffries (NY-08) reintroduced bipartisan legislation designed to "better and immediately prepare the federal government for the cannabis reforms seen across the country and inevitably at the federal level." The bill, titled "Preparing Regulators Effectively for a Post-Prohibition Adult Use Regulated Environment (PREPARE) Act," hopes to "equip lawmakers with the information necessary to establish a safe and effective federal regulatory system." Legislation such as this is not a sure thing, and it's a far cry from federal legalization, but it is indicative of a regulatory environment in which the federal government is feeling pressure from the states.

In the meantime, the best thing the cannabis industry can do is build upon cannabis science and push for high standards. The more we know about the plant, its benefits, and the best ways to test and process the materials, the better prepared industry will be once the federal government comes around on cannabis. This issue of *Cannabis Science and Technology* tackles challenges and offers fresh insights. For example, on pages 20, Julie Kowalski, PhD, provides solutions for common challenges in cannabis pesticide samples when using gas chromatography-mass spectrometry. Overcoming these challenges allows for more accurate testing and safer products.

Offering fresh insights, on page 10 we have a peer-reviewed study titled "Impact of Water Activity on the Chemical Composition and Smoking Quality of Cannabis Flower: The Science of Smokability Phase I Results." The study's findings demonstrate how water activity can be optimized to balance chemical, sensory, and financial factors in cannabis production.

Cannabis forges on despite a challenging regulatory environment, and the continued progress on the scientific side only lends industry greater legitimacy.

Mike Hennessy Jr
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contents

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Mass Spectroscopy Primer, Part II:
Data Interpretation

By ***Brian C. Smith***

Part II of this series on mass spectroscopy explains how mass spectral data is interpreted to determine molecular weight, molecular formulas, molecular structures, and functional group information. Two examples are provided to illustrate the process mass spectroscopists use to draw conclusions.

Mass spectrometers, particularly when interfaced to gas chromatographs and liquid chromatographs, are particularly useful in cannabis labs for the analysis of pesticides and potency. In the previous installment of this series, we provided an introduction to mass spectroscopy [1]. In this installment, we will cover mass spectral data interpretation. We will discover the power of mass spectra to give us molecular weights, molecular formulas, molecular structures, and functional group information.

A Comment on Ion Stability

Previously, we discussed how electron impact ionization is used to generate ions [1]. The process can be thought of as a chemical reaction as seen in **equation 1**, where M=molecule and e^-=electron:

$$M + e^- \Rightarrow M+ \,\&\, 2\, e^- \quad (1)$$

This is a process in which the impinging electron knocks an electron off from our analyte molecule generating a positively charged ion called a *cation*, so it is correct to say that electron impact ionization generally gives positively charged organic cations. Recall [1] from the block diagram of a mass spectrometer seen in **Figure 1** that the ions formed in the ion source are gathered into a beam and then travel some distance over the course of time through the instrument to get to the detector.

This means there is a time lag between when the ions are formed and when they are detected. Now, organic cations are fragile things or else our universe would be full of them. Instead, our universe is full of electrically neutral organic molecules. Thus, for a cation to be detected in mass spectroscopy it must survive long enough to make the trip from the ion source to the detector. Some cations are more stable than others and these more stable ions will tend to have greater abundance than unstable ions that fall apart before being detected. For example, molecular ions [1] are often times the biggest peaks in mass spectra because they can be the most stable ions formed from an analyte molecule in the ion source.

Over time, and based on the observed mass spectra of thousands of molecules, some rules have been developed wherein we can predict, based on a molecule's structure, what its most intense m/z peaks will be in its spectrum [2]. This then allows us to do the reverse, interpret the peak positions and intensities in a mass spectrum to determine what molecular fragments make up a molecule. A recital of the details of relative organic cation stability and mass spectral interpretation rules is beyond the scope of this article. In general though, since cations have a positive charge, the presence of heteroatoms in an ion such as nitrogen or oxygen containing lone pairs of electrons will stabilize these ions and lead to an increase in their detected abundance [2]. Similarly, functional groups with high concentrations of bonding electrons such as triple bonds including C≡N, double bonds such as C=O and C=C bonds, and aromatic rings can stabilize organic cations leading them to be detected in significant quantities in a mass spectrometer. We will make use of these ideas in the mass spectra we interpret below.

A Simple Mass Spectrum

An example of a simple mass spectrum used in the previous column [1] was that

of carbon dioxide, as seen in **Figure 2**.

Note that the x-axis is in "m/z" units, where m stands for mass and z for charge, thus the term m/z is pronounced "mass to charge ratio." A mass spectrometer does not separate ions based solely on their mass but by their mass to charge ratio. It can happen, for example, that an ion with a mass of 100 and a charge of 1 and hence with an m/z of 100 will be detected at the same time as an ion with a mass of 200, a charge of 2, and hence also have m/z = 100. Note that the y-axis here is "% Relative Intensity." If we plotted the raw signal this scale would be labeled "ion abundance" which is a direct count of the number of ions detected at a specific m/z. It is convenient though to divide the intensity of each individual peak by that of the largest peak. In this case, m/z = 44, multiply by 100, then plot the y-axis in % Relative Intensity units as seen.

Assuming carbon has an atomic mass of 12 and oxygen 16, then CO_2 has a molecular weight of 44. Note in Figure 2 that the peak with the highest m/z, and also the most intense peak, has m/z = 44. This is called the *molecular ion* peak, M^+ peak, or parent ion peak [1]. This peak is from a CO_2 molecule with a single positive charge on it. Molecular ion peaks are often seen in mass spectra and are very useful because they tell us the molecular weight of an analyte. Note also that there are peaks with m/z values of 16 and 12. The former is a from a positively charged oxygen ion, O^+, and the latter from a positively charged carbon ion, C^+. Note then that the value of a m/z peak by itself can tell us what chemical species gave rise to that peak.

The peak at m/z = 28 is more interesting. Its peak position tells us this molecular fragment has a mass of 28, however if we subtract its mass from that of the molecular ion we get 44 – 28 = 16, and we appropriately call this peak a "M-16" peak and it is due to a CO^+ ion. We saw above that the atomic mass of oxygen is 16, so the *difference* in m/z between the molecular ion peak and our peak of interest can be used to deduce what fragments were given off by the molecular ion and hence what functional groups comprised our original analyte molecule. The CO^+ ion was detected because it owes its stability to the lone pairs of electrons on the oxygen atom as pointed out above.

Figure 1: A block diagram of a mass spectrometer.

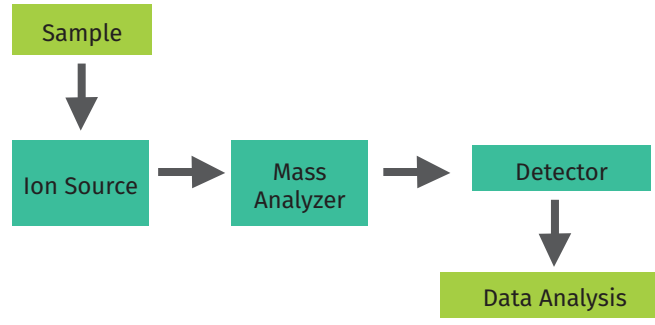
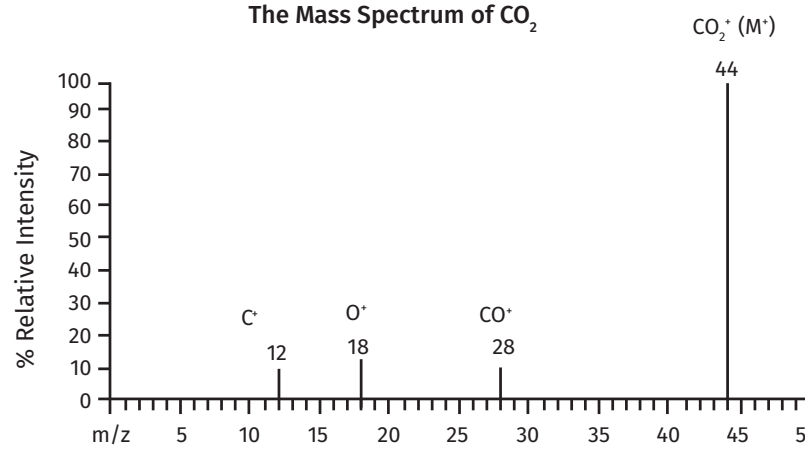


Figure 2: The mass spectrum of carbon dioxide (CO_2).



A More Complex Mass Spectrum

The mass spectrum of benzoic acid, $C_7H_6O_2$, is seen in **Figure 3**.

Note that benzoic acid contains a mono-substituted benzene ring and a carboxylic acid or -COOH group. The y-axis of the spectrum in Figure 3 is ionic abundance, a count of the number of ions detected at each m/z.

Assuming again that carbon has an atomic mass of 12, oxygen 16, and that hydrogen's is 1, we can calculate the molecular mass of benzoic acid as seen in **equation 2**:

$$(7x12) + (16x2) + (6x1) = 122 \quad (2)$$

Note that there is a molecular ion peak in Figure 3 at 122 and that it is large. We can attribute this ion's stability to the presence of two oxygen atoms and a benzene ring in its structure.

In both Figures 2 and 3 the molecular ion peaks are the biggest peaks in the

Figure 3: The mass spectrum of benzoic acid.

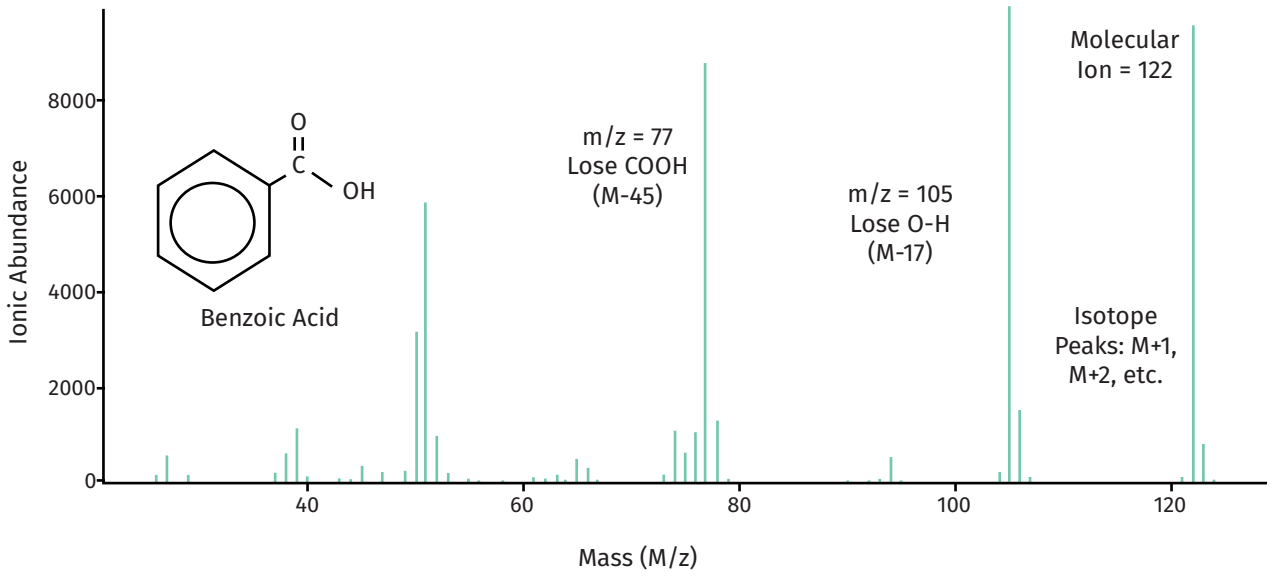
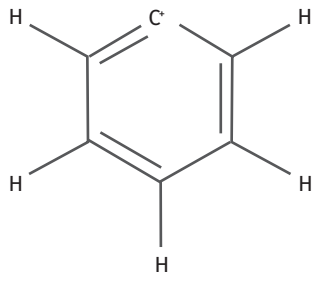


Figure 4: The chemical structure of the phenyl ion, C_6H_5+, with m/z = 77.



spectrum. This is commonly seen but is not necessarily always the case, it all depends upon the stability of the molecular ion. If a particular molecule forms a M^+ ion that is stable, a significant number of them will survive the trip through the mass selector and be detected. On the other hand, some molecules may form particularly unstable molecular ions, and fragments with m/z values less than that of the molecular ion may be the most abundant.

Note in Figure 3 there is a particularly large peak at m/z = 105. Now, we could try to draw molecular fragments with this molecular mass, but this could be a long and drawn out process as the number of fragments with this mass value may be many. What is more interesting is that the m/z = 105 peak is 17 mass units less than the mass of the molecular ion of 122 or in other words is an M-17 peak. This peak formed when the molecular ion lost something that weighed 17 to preferentially form m/z = 105 ions, and the ion formed must be particularly stable for so many of them to have formed. Trying to deduce what ions might have a mass of 17 is easier than trying to deduce what ions might have a mass of 105 because there are simply fewer of the former. Amongst the chemical species with a mass of 17 is an isotope of oxygen or ^{17}O, however its relative abundance is less than 0.1% than that of ^{16}O, so it's doubtful this rare isotope is responsible for our M-17 peak. Another chemical species with a mass of 17 is the hydroxyl or O-H group where the oxygen weighs 16 and the hydrogen weighs 1. The loss of an OH group to give the M-17 peak here makes sense since OH groups are commonly found in organic structures. In general then, any molecule with a M-17 peak may contain an OH group.

Notice in Figure 3 that there is a peak at m/z =77 and that it is an M-45 peak. Rather than trying to brute force the calculation of what chemical species might have a mass of 45 we can simply rely on the literature [2]. It is well known that the carboxylic acid group, -COOH, weighs 45 (12 + 32 +1) and that these peaks are commonly seen in the mass spectra of these molecules. Thus the presence of a M-45 peak is suggestive of a molecule containing the carboxylic acid functional group. A value of m/z = 77 also corresponds to that of a mono-substituted benzene ring or phenyl ion, $C_6H_5^+$, whose structure is seen in **Figure 4**.

This ion is relatively stable because of the presence of the electron rich aromatic ring. Two of the biggest peaks then in the mass spectrum of benzoic acid correspond to the molecular ion falling apart into $-COOH^+$ and phenyl

cations, both of which are stabilized by the presence of electron rich moieties. Note again that the difference in mass between a peak of interest and the molecular ion gives functional group information. This is one of the strong points of mass spectrometery [3].

Molecular Formulas from Mass Spectra

In addition to obtaining molecular weight information from the molecular ion and functional group information from the peak positions in a mass spectrum, the molecular formula of an analyte can be obtained as well. For mass spectrometers of high enough resolution, typically good to several decimal places, the exact m/z for a molecular ion can be used to calculate molecular formulas from readily available tables and computer programs.

However, many labs do not have the budget for a high resolution mass spectrometer, and often times instruments with a mass resolution of 1 are all that are available. The problem with these instruments is that molecules with different chemical structures but the same mass will give measured molecular ions with the same m/z value. For example, carbon monoxide, C≡O, and nitrogen, N_2, both have molecular weights of 28 and hence have molecular ions of the same value. How would we distinguish between them using a mass spectrometer?

We can make use of the fact that different elements have different isotopic abundances. For example, in carbon for every 100 atoms of C^{12} there is about 1 atom of the stable isotope C^{13}. Thus for every 100 $C^{12}O$ molecules there is one $C^{13}O$ molecule. This means that in the mass spectrum of carbon monoxide there will be what we call an M+1 peak, a peak with an m/z value one more than that of the molecular ion due to the $C^{13}O$ molecules, whose size will be 1% that of the parent ion peak.

For nitrogen, the stable isotope N^{15} has a natural abundance of 0.4% that of N^{14}. Thus for every 100 $N^{14}N^{14}$ molecules there are about 0.4 $N^{14}N^{15}$ molecules. We would then expect the M+1 peak for nitrogen to be about 0.4% the size of the molecular ion peak. The point here is that even though carbon monoxide and nitrogen have molecular ion peaks with the same m/z values, their M+1 peaks will be of different sizes allowing them to be distinguished from each other. In general, the size of M, M+1, and M+2 peaks can be used to determine molecular formulas. Tables [2] and computer programs exist to allow these calculations to be made.

> "**Molecular ions** are often times the biggest peaks in mass spectra because they can be the most stable ions formed from an **analyte molecule** in the **ion source**."

Conclusions

Mass spectra can give molecular weight, functional group, and molecular formula information on analyte molecules. The m/z value of the molecular ion can give the mass of a molecule. The m/z values of peaks in a mass spectrum and the difference between their m/z and that of the molecular ion provides functional group information. Lastly, for the typical mass spectrometer with a mass resolution of 1, molecular formulas can be obtained by measuring the size of the M+1 and M+2 peaks.

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(3) Fred W. McLafferty, *Interpretation of Mass Spectra*, University Science Books, Herndon VA, 1980.

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Impact of Water Activity on the Chemical Composition and Smoking Quality of Cannabis Flower: The Science of Smokability Phase I Results

A. JUSTICE, R. KIRK, A. MANNING, M. ROGGEN, AND M. SHIELDS

Smoking remains the most common method of cannabis consumption, particularly for patients seeking rapid relief. Despite this, little is known about what defines a high-quality smokable product. This study investigated how varying water activity levels (0.45 aW, 0.65 aW, 0.85 aW) affect cannabis flower's chemistry and perceived smoke quality. Chemical analyses showed that 0.65 aW yielded the highest terpene content and comparable cannabinoid delivery to 0.45 aW, while 0.85 aW significantly reduced cannabinoid levels. Sensory panelists noted minimal differences between 0.45 and 0.65 aW samples, though harshness and ash color varied. Higher water activity increased moisture and product weight—suggesting economic benefits for producers. These findings offer insights into optimizing water activity to balance chemical, sensory, and financial factors in cannabis production.

Introduction

The growing interest in cannabis for the expanding medical and recreational markets has created an immediate need for enhanced understanding of the factors that influence product quality, consumer safety, and the overall user experience. Despite research focusing heavily on the negative effects of smoking cannabis, there has been limited research on the effects of various cultivation and post-harvest processing practices on the final perceived quality of the cannabis smoke.(1-4) It is well-known community knowledge that a majority of medical patients prefer high-temperature, combustion conditions of flower as a therapeutic method of administration.(5,6) Smokeable products remain the most sold and consumed products on the cannabis market. This may be due to multiple factors including traditional use, unique chemical composition, social impact, and increased therapeutic value.(1,7) Investigating the quality of this medicinal formulation should be an immediate priority for the optimization of practices, increased understanding of therapeutic benefits, and as public education and risk reduction.

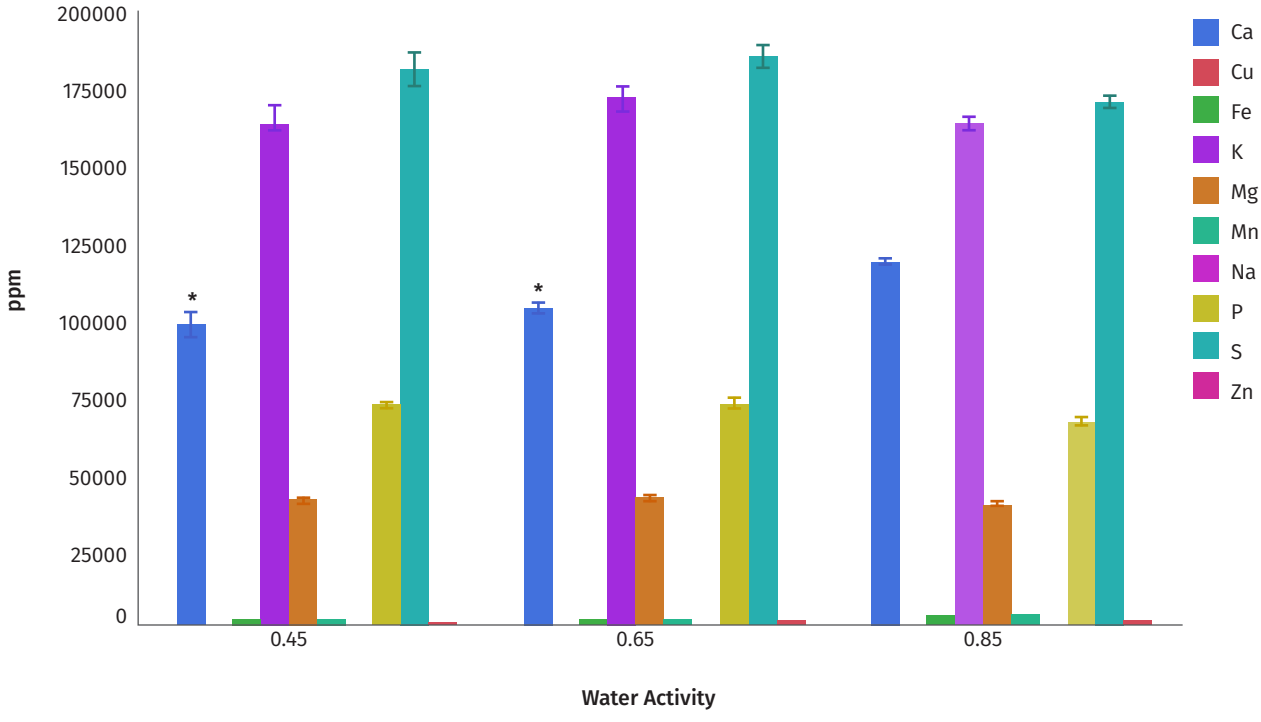
Many factors could ultimately affect the chemical diversity of the delivered product. Moisture content

Table I: Analysis of total and specific cannabinoids and terpenes delivered in the smoke from pre-rolls with three different water activity levels

mg/pre-roll (g)											
Cannabinoids						Terpenes					
Water Activity	Total Cannabinoids	CGB	CBD	D9 THC	CBC	Total Terpenes	a-pinene	b-myrcene	b-pinene	d-limonene	b-caryophyllene
0.45	19.26 ab[z]	0.42 ab	15.27 ab	1.62 a	0.80 ab	2.256 b	0.054 b	0.078 b	0.022 b	0.034 b	0.394 ab
0.65	22.27 a	0.44 ab	17.68 a	1.82 a	0.90 a	3.562 a	0.146 a	0.878 a	0.076 a	0.124 a	0.438 a
0.85	6.59 b	0.12 b	5.18 b	0.71 a	0.26 b	1.732 b	0.036 b	0.362 b	0.028 b	0.068 ab	0.134 b
Non-Combusted Flower	118.4	1.15	107.56	4.43	5.24	35.08	4.82	19.11	2.15	2.15	2.68

[z] Data followed by the same letter within the same column do not differ at p<0.05 using Tukey's HSD. Data are means, (n=3).

Figure 1: Elemental concentration in ppm of ash from three combusted test groups.

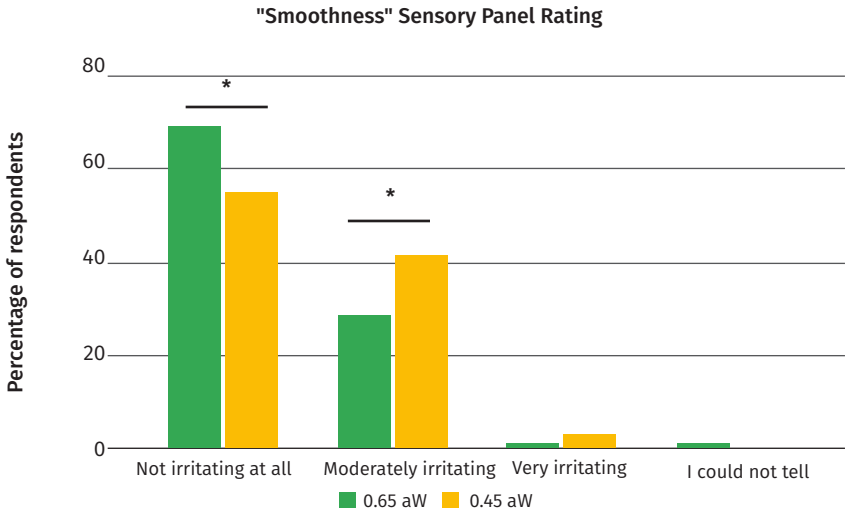


(*) indicates statistically significant differences between water activities per elemental group (p<0.05, ANOVA). Data are means, (n=3).

(MC) is a critical factor that plays a role in determining the desirability, smokability, and safety of cannabis flower. Water activity (aW) measures the availability of free water in a product and differs from total moisture content by directly influencing microbial growth, chemical stability, and combustion behavior. Furthermore, water activity is likely a key contributor to consumer satisfaction, product performance, and medical value.(8-11)

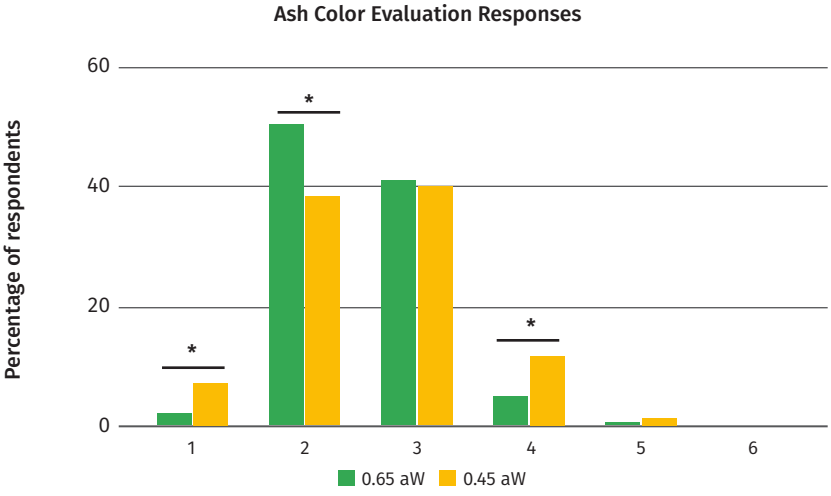
The water activity is known in both legacy and industrial practices to affect the quality of cannabis during storage, packaging, and consumption.(12) It is standard practice in the cannabis industry to dry and cure to water

Figure 2: SurveyMonkey responses noting the irritability of the cannabis smoke at 0.65 aW (n= 178). SurveyMonkey responses noting the irritability of the cannabis smoke at 0.45 aW, (n= 132).



**Results for "Not irritating at all" and "Moderately irritating" are significant by proportional z-test analysis with p-values of 0.0127 and 0.0169, respectively.*

Figure 3: SurveyMonkey results ranking the observed ash color from participants smoking the pre-roll at 0.65 aW, (n= 178). SurveyMonkey results ranking the observed ash color from participants smoking the pre-roll at 0.45 aW, (n= 132).



***Results for the color number ratings 1, 2, and 4 are significant by proportional z-test analysis with p-values of 0.0255, 0.0371, and 0.0239, respectively.*

activity levels around 0.65 aW to avoid microbial growth and contamination, in this experiment we chose to study levels below 0.85 aW and above 0.45 aW for a better understanding of the role of water activity when evaluating smokeability.(13) This study aims to establish standards for discrete metrics like water activity as well as contribute to optimizing the overall smokability of cannabis flower by directly involving consumer preferences.

Other industries such as food science and tobacco have well-documented research on the effects of water activity on product stability, microbial growth, and sensory attributes including aroma and texture.(14-21) Additionally, the tobacco industry has identified the effect of water activity on nicotine availability and chemical diversity of the smoke chemistry and end-user experiences including the creation of harmful byproducts.(22-27) It is apparent that these factors should be investigated with as much scientific rigor for cannabis, particularly as the legal markets and consumer demands continue to rapidly change and evolve. The rapid growth of the cannabis industry has only emphasized previous research limitations that have prevented an accurate assessment of quality due to regulatory and financial barriers. These shortcomings have created the need for rigorous validation of all prior scientific literature conducted within this framework not representative of the real-world formulations and experiences.

The Science of Smokability (SOS) studies aim to bridge critical knowledge gaps in understanding how cultivation and post-harvest processes affect the overall quality of cannabis smoke, including the user experience. By integrating analytical tools with real-world data from consumers, these experiments not only advance the science of cannabis smoke chemistry but also empower the community with evidence-based knowledge and practices. Public education is a cornerstone of this project, including the creation of accessible, community-focused educational resources to promote harm reduction and actively involve the public in shaping and participating in scientific practices in the industry. This first

phase has already served as an exploratory investigation into the complexities of cannabis smoke chemistry, highlighting the need for research on the variables that contribute to its chemical diversity and the downstream impact on health and therapeutic use.

Experimental:

- **Smokability Survey**
  An online questionnaire was designed using SurveyMonkey to evaluate the smokability of cannabis pre-rolls. Two surveys were created and sent to participants: one for general consumers (n=315) and another for cannabis experts, or Ganjiers (n=38). Ganjiers are certified cannabis experts trained to assess flower, concentrates, and cartridges using a rigorous Systematic Assessment Protocol (SAP) that evaluates appearance, aroma, flavor, and anticipated effects. Through a combination of in-depth online coursework, hands-on training, and a comprehensive certification exam, Gangiers develop mastery in product evaluation, client service, as well as cannabis history, science, and ethics.

  Both surveys featured identical questions, but data were analyzed separately for experts and consumers. Participants were blinded to the water activity level of the pre-rolls they received. A video tutorial provided instructions on completing the survey and determining when to answer each question. The survey consisted of 13 questions developed collaboratively by the SOS research team and a panel of Ganjiers, who applied established methods for evaluating the smoking experience. Only pre-rolls with water activity levels of 0.45 and 0.65 aW were distributed; those with 0.85 aW were excluded from consumer testing due to potential microbial risks associated with higher water activity. The survey participants were notified and recruited via social media platforms TikTok and Instagram.
- **Hemp Flower**
  The hemp flower used for the smoking experiments as well as the pre-rolls that were used for smokability analysis was the variety 'FunDip'. 'FunDip' is a cannabidiol (CBD) dominant variety bred and grown by a licensed hemp company in South Carolina, The Hemp Mine. The dominant terpenes in 'FunDip' are myrcene and alpha-pinene (**Table I**).
- **Pre-Roll Preparation, Packaging, and Water Activity Stability Testing**
  Once the flower was dried to a water activity of 0.65 in a drying room at a temperature of 65 F and 60% relative humidity, flowers were removed from stems and ground. Whole cannabis flower was ground using a Futurola OG Original Shredder (Hawthorne, California) for 15 seconds, and manually sifted to remove stems using 10 mesh. Quality checks ensured all stems were removed. One gram Custom Cones (109 mm, Natural Brown; Renton, Washington) were loaded into a Knockbox, which uses vibration to evenly pack ground cannabis into pre-rolled cones. Ground flower was evenly distributed into the cones over a 2-minute run. Fill weight and packing uniformity were regularly checked for consistency. Pre-rolls were hand-twisted at the top and individually weighed to ensure uniformity.

  Prepared pre-rolls were stored in mason jars for water activity treatments targeting levels of 0.45 aW, 0.65 aW, and 0.85 aW. Water activity was adjusted using distilled water on paper towels within sealed containers, monitored with a digital hygrometer. Once stabilized at the target water activity, pre-rolls were placed in plastic tubes with rubber gasket seals, vacuum-sealed with a commercial-grade vacuum sealer and heavy-duty 4 mil vacuum sealer bags to maintain water activity integrity. Stability assessments confirmed that the process preserved target water activity levels for at least 30 days. Vacuum-sealed pre-rolls were shipped to participants within 24 hours, arriving within 3 business days, with participants completing their smoke study within 5 days of receipt. Ground flower material that was analyzed for cannabinoid and terpenes content was sent as ground loose flower not inside a preroll cone.
- **Smoking Machine**
  A Cambustion Smoke Analyzer SCS (Cambridge, United Kingdom) was employed to measure the pressure drop across each pre-roll, collect smoke condensate for chemical analysis, and retain the remaining ash for organic analysis. The smoking method utilized was adapted from the Health Canada protocol for tobacco use (ISO 3308, Health Canada Intense).(28) Temperature was monitored 1 cm downstream from the pre-roll, simulating a consumer's mouth position, using a thermocouple. Smoke condensate was captured in a 50 mL glass impinger containing 10 mL of HPLC grade ethanol, which was maintained on ice to minimize evaporation. All impingers, beakers, and associated equipment were cleaned via rinsing in ethanol, cleaning with laboratory grade soap (Alconox), rinsing with water, followed by a 2 minute sonication with ethanol and air drying between samples. The smoking machine's orifice and plastic tubing were similarly cleaned between samples and sample types to prevent cross-contamination.
- **Dry Ash Analysis**
  Ash was collected from pre-rolls used in the smoking machine and

analyzed at the Clemson University Agricultural Service Laboratory. Reagents included 1 N hydrochloric acid, prepared by diluting 83.3 mL concentrated HCl to 1 L with deionized water (dH_2O), and 6 N hydrochloric acid, prepared by diluting 50 mL concentrated HCl to 100 mL with dH_2O. Apparatus used included a muffle furnace, "high form" porcelain crucibles, 100 mL volumetric flasks, and 13 × 100 mm flint glass test tubes. For the procedure, 1.000 g of sample was weighed into a porcelain crucible and ashed in a muffle furnace by gradually increasing the temperature to 500°C and maintaining for 3 hours. The ashed sample was wetted with a small volume of dH_2O, treated with 5–10 mL of 6 N HCl, and evaporated to near dryness on a hot plate. The residue was dissolved in 10 mL of 1 N HCl, quantitatively transferred into a 100 mL volumetric flask, and washed down with dH_2O. The solution was diluted to volume with dH_2O, shaken, and an aliquot was transferred into an ICP test tube for analysis.

- **Cannabinoid and Terpene Analysis**

All samples were analyzed at MCR Labs, MA. Cannabinoid reference standards were acquired from Cerilliant Corporation and Cayman Chemical Company. Terpenes standards were acquired from LGC Standards.

UHPLC Conditions: Samples were either directly injected or diluted 1:10 in Methanol and injected directly. Roach samples were agitated with 10mL MeOH for 10 minutes at room temperature and then centrifuged to remove particulates and diluted between 1:2 and 1:10 in MeOH for direct HPLC injection. Reversed-phase chromatography was conducted using an Agilent 1290 UHPLC system with OpenLab CDS Rev C.01.10, including an autosampler with thermostat, binary pump, column oven, and diode array detector. Peak integration was performed with Agilent ChemStation. The final analysis was carried out on a Restek Raptor ARC-18 column (100 mm × 3.0 mm, 1.8 µm) using gradient elution with 5 mM ammonium formate (0.1% FA) in water and acetonitrile (0.1% FA) as the organic phase. The injection volume was 2.00 µL, with a column temperature of 30 °C, the autosampler is equipped with a chiller kept at 4 °C, and a flow rate of 1.0 mL/minute. Cannabinoids were monitored at λ=228 nm (reference λ=360), with spectra acquired from 190–400 nm at a step size of 2 nm. Integration was performed using Agilent's standard parameters.

GC-MS Conditions : Samples were either directly injected or diluted 1:10 in Methanol and injected directly. Gas chromatography was conducted using an Agilent 7980 GC system, including an automated liquid sampler and an Agilent 5975 inert XL MSD. Agilent MassHunter software and processing was used to acquire and analyze the data. Analysis was done on a Restek Rxi-624Sil MS column (30 m, 0.25 mm ID, 1.40 µm) with He as the carrier gas at a 100:1 split ratio and a constant flow of 1 mL/minute. The injection volume was 1.00 µL, with an inlet temperature of 250 °C. The column oven started at 60 °C, ramped to 320 °C, with a total runtime of 23 minutes. The MSD source temperature was set to 230 °C, and the quad temperature was set to 150 °C. Single ion monitoring for terpenes was done with full scan data from 30–750 Da for untargeted analysis.

- **Statistical Analysis of SurveyMonkey Data of Ash Color, Smokability and Potency**

The raw survey data were downloaded from SurveyMonkey and cleaned using Python's pandas library. A statistical analysis to compare the proportions of survey responses between two groups categorized by water activity levels (aW = 0.45 and aW = 0.65) was performed utilizing python329 and statsmodels 0.14.430. For each response category, percentages were calculated using the observed counts divided by the total responses for each group. A two-proportion Z-test was applied to assess whether the differences in response proportions between the two groups were statistically significant (p value less than 0.05). The test results, including Z-statistics, p-values, and group percentages, were compiled into a summary table to identify significant differences. This methodology provides a robust framework for evaluating proportional differences in survey responses across distinct groups. Cannabinoid, terpene and ash statistical analyses were performed using JMP® statistical software (Version 18, SAS Institute Inc., Cary, NC, USA). Mean comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test, with statistical significance established at $p < 0.05$.

Results

QUANTITATIVE RESULTS

Cannabinoids

The flower material in the pre-rolls contained primarily acidic cannabinoids, such as cannabidiolic acid (CBDA). However, the smoke analysis revealed no significant amount of acidic cannabinoids, indicating complete decarboxylation during combustion. The cannabinoid content in the flower and pre-rolls was quantified in milligrams per unit, converted from weight percent values (Table I). For the flower sample, 'FunDip', the acidic cannabinoid content was converted to that of the

fully decarboxylated or neutral form by multiplying by the ratio of molecular masses (0.877 in the case of CBDA and THCA). Since CBDA was not detected in the smoke and only CBD was detected, the results are better described in terms of total CBD content.

CBD was the dominant cannabinoid in both the flower (as CBDA) and the smoke (as CBD). Additionally, cannabinoids such as cannabigerol (CBG), cannabichromene (CBC), and D9-tetrahydrocannabol (Δ9-THC) were present in flower and the smoke. The pre-rolls with a water activity of 0.65 produced the highest cannabinoid concentrations in the smoke, followed closely by those with a water activity of 0.45. In contrast, the cannabinoid concentrations of the 0.85 aW samples were approximately 30% of those observed in the 0.65 aW samples (Table I).

Variability in cannabinoid content was large but consistent with previous findings,(1,3) emphasizing the inherent variability in preparing pre-rolls. The percentage yield of the amount of each cannabinoid transferred from the pre-roll to the impinger indicated that the lowest percentage yields were observed in samples containing 0.85 aW, consistent with their reduced smoke cannabinoid concentrations.

Terpenes and other secondary metabolites

The terpene content in the flower material, expressed in milligrams per pre-roll, reports total terpene concentrations and the five most abundant terpenes. The percentage yield of terpenes was omitted due to consistently poor results across all samples. Among the tested pre-rolls, the 0.65 aW samples demonstrated the highest terpene delivery to the smoke, a difference that was statistically significant (Table I). Compared to cannabinoids, the distinction between 0.65 and 0.45 aW samples was more pronounced, suggesting stronger water activity-dependent effects for terpene transfer in this range. In contrast, the 0.85 aW sample percentage yields were relatively better for terpenes than for cannabinoids but still delivered lower amounts overall.

Individual terpene patterns revealed intriguing trends. The highest concentrations of all terpenes were consistently obtained from 0.65 aW samples. However, the relative ranking between 0.45 and 0.85 aW samples varied depending on the terpene. For alpha-pinene, beta-pinene, myrcene and limonene, there was no statistically significant difference between 0.45 and 0.85 aW samples. Additionally, d-limonene was delivered at the highest concentration at 0.65 but was not statistically different from the highest water activity. Beta-caryophyllene had similar concentrations at the two lower water activities and was significantly lower than 0.85 aW. Based on these findings, the 0.65 aW samples are expected to deliver the most pronounced flavor profile due to their greater terpene yield.

Abstrax Tech investigated how water activity affected other non-terpenoid and non-cannabinoid secondary metabolites by 2D GCxGC chromatography (**Figure S2**), including substances not typically characterized in the smoke.(31-33) Qualitative differences were identifiable in the monoterpene, sesquiterpene and cannabinoid regions. Further characterization of these and other substances is needed to determine and establish more quantitative metrics of quality for cannabis smoke.

Elemental Analysis

The ICP analysis (Inductively Coupled Plasma Spectroscopy) of the resulting ash from the three water activity samples showed minimal variability in elemental concentrations across the groups. Elemental concentrations in the ash were consistent and comparable between the 0.45, 0.65, and 0.85 aW samples, except for calcium. Calcium concentrations in the 0.85 aW treatment group were significantly greater than the lower water activity treatments (**Figure 1**). The underlying cause of this difference remains unclear and warrants further investigation.

When compared to the elemental composition of tobacco ash as reported by Dumas, the cannabis ash samples exhibited similar levels for most elements, with the exception of markedly elevated sulfur and phosphorus concentrations and a significantly reduced calcium content.(26) These deviations are likely attributable to differences in cultivation practices, particularly fertilization and pesticide use. Elemental sulfur is commonly applied during the early stages of cannabis growth as a treatment against russet mites (*Aculops lycopersici*), which may contribute to the elevated sulfur levels observed in the ash. Likewise, excessive phosphorus application is a frequent practice in commercial cannabis production, potentially explaining the increased phosphorus content.(34,35)

Qualitative Results

Sensory attributes were investigated in different water activity (aW) groups. For aromatic profile and flavor intensity, the results were similar between the 0.45 and 0.65 aW pre-rolls with no statistical differences. Notably, these groups were ranked differently by the general population compared to certified Ganjiers, but the variability exceeded the numerical differences. Overall desirability slightly favored the 0.65 aW pre-rolls, though large variability rendered this distinction inconclusive (data not shown).

Regarding the "smoothness" of the smoke, responses ranged from not irritating to very irritating with the 0.45 aW pre-rolls producing a more irritating experience, and less noted it was not irritating at all (**Figure 2**).

For the rating of "not irritating at all" and "moderately irritating," the difference between the two groups was statistically significant with p values of 0.013 and 0.017, respectively.

Similarly, ash color was evaluated by comparing ash color to a provided 'ash color guide ranging from 1-6' with 1 being the lightest and 6 being the darkest color (**Figure S1**). The results indicated that the ash color was consistently closer to white for both water activities. However, the color of the ash from the 0.65 aW samples was lighter than that from the 0.45 aW samples (**Figure 3**), suggesting ash color may be affected by water activity. Comparison of the percentages of total responses of ash color by Z-test for the 0.45 and 0.65 aW groups found that the difference in response numbers 1, 2, and 4 were significantly different with p-values of 0.025, 0.037, and 0.024, respectively. The top response for 0.65 aW ash color #2, and for 0.45 aW ash color, #3 was the most frequent response. These findings suggest that sensory attributes were generally stable across the tested conditions, but that there was an observed variation between the 0.45 and 0.65 samples in ash color.

These results indicate that while some attributes of the sensory and consumer experience are not affected by water activity, others such as irritability and ash color may be affected.

Discussion

While there may be additional variables and observable changes depending on the methodology of drying and curing to reach a desired water activity, this study provides novel insights into the chemical composition and user experiences associated with cannabis smoking, emphasizing the influence of water activity on cannabinoid and terpene delivery and user experiences.

Terpene analysis revealed that the 0.65 aW samples consistently delivered the highest concentrations of all terpenes analyzed, indicating that optimal water activity enhances the terpene yield during smoking. This finding is important, as terpenes contribute to the flavor and aroma of cannabis and may modulate its psychoactive effects.(36-40) Furthermore, the variability in delivered terpene profiles between the three test groups suggests that water activity not only influences overall yield but also relative volatilization of these compounds, with potential implications for differences in consumer experience.

Surprisingly, our findings indicate that aW had no effect on cannabinoid yield for consumer-relevant water activities. The highest cannabinoid concentration was measured in smoke from pre-rolls with a 0.65 aW but were not significantly different from the 0.45 aW samples. The 0.85 aW samples, which are not safe to consume for users due to potential microbial growth, showed substantially lower concentrations of both cannabinoids and terpenes. This is likely due, at least in part, to the increased pull resistance experienced at 0.85 aW. This suggests that moisture content affects cannabinoid yield during smoking, potentially due to improved combustion efficiency or aerosol formation. Notably, THC and CBG demonstrated higher yields compared to CBD and CBC for all measured water activities, though the underlying mechanisms remain unclear.

The complete decarboxylation of acidic cannabinoids, such as CBDA, during combustion is known from previous reports, confirming that heating during smoking decarboxylates acidic cannabinoids to their neutral forms. This decarboxylation of the acidic cannabinoids is crucial, because the pharmacological effects of cannabinoids differ between their acidic and neutral forms.(40,41)

Elemental analysis of the ash showed minimal variability between different aW samples, with most elements exhibiting approximately a seven-fold increase in concentration when the flower was combusted to produce ash. However, the high sulfur and phosphorus content in cannabis ash is atypical and not observed in tobacco ash. This could be explained by differences in cultivation and fertilization practices between crops. This warrants further investigation to validate the implications of elevated elemental concentrations for consumer health and product quality.

Ash color is anecdotally an important factor in determining quality cannabis from a community perspective with the common perspective being darker ash is correlated with a lower quality smoking experience.(42) Despite the small differences in chemical profiles in the smoke from pre-rolls with different water activities, the qualitative survey results indicate that consumers experience differences between them. This may affect the medicinal value of the products. Consumers who smoked the pre-rolls at 0.45 aW reported a more irritating experience than at 0.65 aW (**Figure 2, Figure 3**) as well as differences in ash color with 0.65 aW samples having lighter ash color compared to 0.45 aW samples. The increased irritation at lower water activity may have implications in user experience and inflammatory response which could affect the medicinal potential of the products. The overall experience users noted between the 0.45 samples and 0.65 samples was 45.2 vs 54.2, respectively; noting that 0.45 aW samples were reported more frequently as 'moderately irritating' while 0.65 aW samples leaned more toward 'not irritating at all'. These results suggest that water activity affects some components of the smoking experience, although individual preferences and perceptions may vary.

This study represents one of the first to investigate cannabis smoking by measuring both subjective measurements of quality as reported by consumers and objective measurements from chemical analysis. Our findings underscore the importance of water activity in influencing the chemical composition of cannabis smoke, with potential implications for both product quality and consumer health and experience. Future research should explore the mechanisms underlying the observed differences in cannabinoid and terpene yields from pre-rolls at different water activities, as well as the health implications of elevated elemental concentrations in cannabis ash. Additionally, studies incorporating larger sample sizes and diverse consumer demographics would provide a more comprehensive understanding of the factors influencing cannabis smoking experiences.

The smoke machine puff profile method used in this study was developed by Health Canada for the analysis of tobacco smoke. The smoking profiles of tobacco versus cannabis smokers are likely different in the time between puffs, the duration of the puff and the intensity of the puff. Future research by the authors will present a more accurate method for the Cambustion smoke machine that will use consumer data to implement a more accurate method of smoke analysis.

Financial Impact of Water Activity

Water activity and moisture content (MC) are closely related parameters but are not directly interchangeable. In this study, differences in weight and potential quality were evaluated between two water activity levels: 0.45 aW and 0.65 aW, which represent typical variations encountered in commercial cannabis processing. To quantify financial implications, it is essential to translate water activity values into corresponding moisture content levels;

SUPPLEMENTAL METHODS:

Comprehensive Two Dimensional **Gas Chromatography**

GC × GC ANALYSIS WAS PERFORMED USING THE INSIGHT reverse fill flush flow modulator (SepSolve Analytical). This was coupled with an Agilent 7890B GC equipped with a BPX5 (20 m × 0.18 mm ID × 0.18 µm film thickness) first dimension column and Mega Wax HT (4.8 m × 0.32 mm ID × 0.25 µm film thickness) second dimension column and BenchTOF Select mass spectrometer (Markes International). ToF-MS was used to identify the compounds. Quantification of all non-sulfur-containing analytes was performed using a flame ionization detector. Sulfur-containing analytes were quantified using a sulfur chemiluminescence detector. Sample introduction was done using direct injection with an Agilent 7693 Injector Tower (G4513A). The syringe was washed three times with isopropyl alcohol and hexanes before and after injection. The injection volume used was 5 µL. The inlet split flow and temperature were 20:1 and 280 °C, respectively. The TOF-MS ion source was held at 280 °C and a transfer line temperature of 260 °C. Mass spectral data were acquired at 60 Hz with a scan range of 40–350 m/z with a solvent delay of 6 min.

The GC × GC configuration includes two columns: apolar to polar setup. The GC oven ramp rates were programmed as follows: the temperature was initially set to 45 °C and held for 3 minutes. The temperature was then ramped at 3 °C per minute to 98 °C, followed by a 6 °C per minute ramp rate to 140 °C, followed by an 8.5 °C ramp rate to 170 °C followed by a 2 °C ramp rate to 190 °C, followed last by a 15 °C ramp to 260 °C, and held for 13 minutes. The modulation period set for the flow modulator was 6.00 seconds. Data were collected, integrated, and analyzed using the ChromSpace software platform (Sepsolve Analytical). Integration, statistical analysis, and data transformations were done using Terplytics and Python 3.

FIGURE SI: Ash color guide sent to participants to evaluate the color of the ash while smoking.

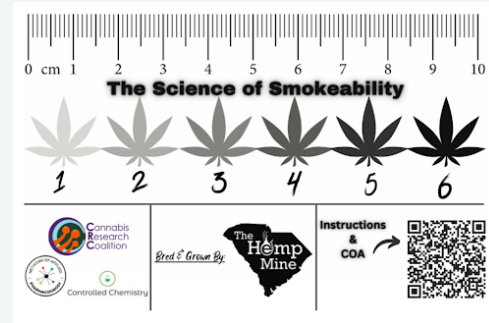
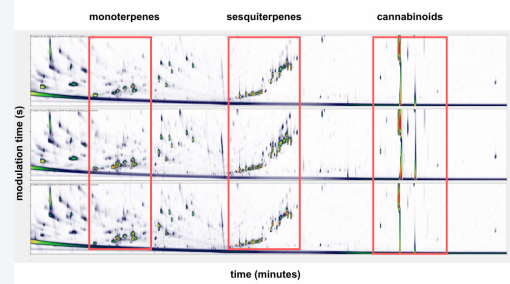


Figure S2: 2D-GCxGC chromatograms of smoke extract samples of 0.45 aW (top), 0.65 aW (middle), and 0.85 (bottom) aW.



a water activity of 0.45 equates to approximately 5% MC, whereas 0.65 corresponds to roughly 9% MC. This conversion reveals an actual dry matter weight difference of 18.14 grams per pound of cannabis flower between the two moisture content levels. At an assumed market price of $1.50 per gram, this weight differential represents an estimated revenue loss of $27.20 per pound for material at the lower water activity level (0.45 aW, 5% MC).

These results emphasize the substantial economic impacts associated with precise water activity management during post-harvest cannabis processing. Future research should investigate the complex interplay between water-driven weight variations, quality parameters, and consumer preferences to deliver evidence-based guidance that enables cultivators to maximize both product quality and profitability.

Conclusion

In conclusion, our research highlights the critical role of water activity in modulating the chemical constituents of cannabis smoke. By optimizing water activity levels, it may be possible to enhance the delivery of desirable compounds, such as cannabinoids and terpenes, while minimizing the presence of potentially harmful elements. Additionally, consumers could not tell the difference between pre-rolls prepared at 0.45 aW and 0.65 aW suggesting lower water activity may be safer for shelf life and avoiding microbial growth. These findings contribute to the growing body of knowledge aimed at improving the safety and quality of cannabis products in an increasingly widespread context of use.

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Thank you for your unwavering support, dedication, and shared vision for a more sustainable, informed, and consumer-focused cannabis industry. This research is a testament to the power of partnership in driving meaningful progress.

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Easy GC Injection Set-Up and How to Avoid Peak Splitting *of Cannabis Pesticide Samples*

By ***Julie Kowalski, PhD***

Gas chromatography-mass spectrometry (GC-MS) pesticide testing of cannabis will increase as the number of required pesticides increases. GC analysis of pesticides in cannabis matrices is challenging due to low quantification levels required in dirty sample extracts, which places high demands on the GC inlet. The typical splitless inlet limits sample injection volume and fouls quickly from nonvolatile components in extracts. Popular cannabis sample preparation methods use polar acetonitrile solvent, which is difficult to gas chromatograph on standard non-polar GC columns. This article will briefly introduce GC splitless inlet anatomy and show a simple example for how optimizing injection type and volume, initial GC oven temperature, and extract dilution can improve GC peak shape.

Introduction

Gas chromatography-tandem mass spectrometry (GC-MS/MS) is a powerful analytical technique used for the separation and trace-level analysis of pesticides. Optimizing GC inlet parameters is crucial to ensure accurate and repeatable results, especially since most problems in GC for cannabis samples happen at the start of the analysis. One particular issue that occurs when injecting an acetonitrile extract of cannabis is chromatographic peak splitting, which reduces pesticide detectability, and leads to quantitative inaccuracy due to improper peak integration. This article will show how simple adjustments for two parameters, GC initial oven temperature and sample dilution with toluene, can mitigate peak splitting. Additionally, pulsed splitless injection is demonstrated as a way to improve pesticide detectability by injecting a higher volume of sample.

Injection of a Pesticide Sample into a GC

A liquid solvent sample containing pesticides is syringe injected into a hot GC inlet containing a glass liner, which is often packed with deactivated glass wool. The solvent is vaporized in the GC inlet and a carrier gas sweeps the sample into a cooler GC column where the solvent and pesticides, ideally, condense along a narrow length of the column as a smooth film, **Figure 1**. In short, the goal is to introduce the sample to the column by going from liquid to vapor and back to liquid. Subsequently, the GC oven is heated to elute the solvent and separate the pesticides for detection.

Solvent and Analyte Focusing on a GC Column for Good Peak Shape

Two key techniques for focusing the analytes (e.g., pesticides) from a sample injection onto a GC column are solvent focusing and analyte focusing. In solvent focusing, the GC oven temperature start is below the boiling point of the solvent (typically, about 20°C below) to condense it on the stationary phase, which helps to focus more volatile components for better peak shapes. Analyte focusing, which can only be used if less volatile pesticides are being analyzed, is accomplished at a temperature above the boiling point of the sample solvent, such that the solvent never condenses. Analytes are "cold trapped" on the GC stationary phase, focusing them for good peak shapes.

Sample Solvent and GC Stationary Phase Polarity Mismatch

A common issue in cannabis pesticide residue analysis by GC is the polarity mismatch between the polar acetonitrile solvent used for extraction and the non-polar GC stationary phase, which is most commonly a 5-phase column. This mismatch can lead to split peaks and inaccurate results. To visualize this issue, imagine water beading on a recently waxed car surface, then consider acetonitrile acting the same on a GC column. These discrete populations of acetonitrile in the GC column, each containing pesticides in solution, result in distorted or split peaks, especially for earlier eluting compounds. Split peaks

result in detectability and integration issues, leading to poor quantification and repeatability.

GC Oven Start Temperature

Figure 2 shows a series of GC oven start temperatures and the first part of chromatograms for a pesticide standard in acetonitrile. Dichlorvos is the most volatile pesticide in the standard mix, which means it will be most sensitive to poor peak shape caused by solvent and GC stationary phase mismatch when trying to use the solvent focusing approach mentioned above. The boiling point of acetonitrile is around 82°C. If using a non-polar solvent on a non-polar stationary phase, a good GC oven start temperature for solvent focusing would be around 60°C, but as you can see in the figure, dichlorvos is split into several peaks (all shaded red in the first chromatogram). At 70°C and 80°C initial oven temperatures, incrementally less peak splitting is noted, but the results are still unacceptable. Finally, at 90°C, analyte focusing, where acetonitrile is not condensed, promotes suitable peak shape for the relatively volatile pesticide dichlorvos.

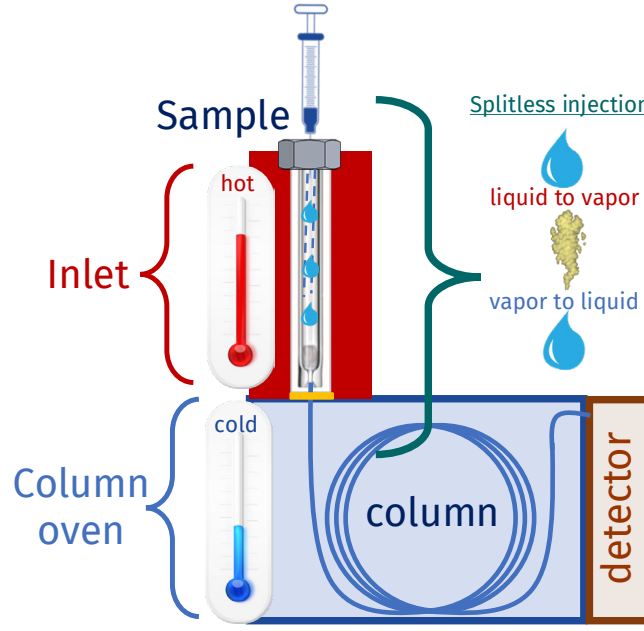
Sometimes, as it was in this case, experimental testing is the best way to determine what oven temperature start will provide acceptable pesticide peak shapes, especially when acetonitrile is the sample solvent.

Adding Toluene to Acetonitrile Sample and Employing Pressure-Pulsed Splitless Injection

Another way to mitigate the polar solvent and non-polar GC stationary phase mismatch problem that results in pesticide peak splitting, is to do a solvent exchange. An acetonitrile cannabis extract could be heated while streaming dry gas on the extract to reduce it to almost dryness and then adding the non-polar solvent. However, this approach can cause loss of volatile pesticides and is time intensive. An easier way is to dilute an acetonitrile extract by half with toluene, a solvent miscible with acetonitrile that chromatographs well on non-polar GC stationary phases.

Figure 3 shows a chromatogram (**A**) with a one microliter injection of dichlorvos in acetonitrile at an oven start temperature of 90°C, which was previously shown to eliminate peak splitting on the non-polar GC stationary phase. Even with some tailing, the dichlorvos peak can be integrated successfully. The next chromatogram (**B**) for dichlorvos may show a slight improvement for a 50:50 acetonitrile:toluene mix, but detectability has been halved because of the dilution. It is possible to inject 2 µL by employing a temporary increase in the GC column pressure during injection (pressure-pulsed splitless injection mode). Under pressure-pulsed injection conditions, the acetonitrile only dichlorvos peak shape is quite respectable as shown in chromatogram (**C**). The dichlorvos peak shape for the 50:50 acetonitrile:toluene was significantly improved by using a pressure-pulsed splitless injection, as seen in chromatogram (**D**). The injection volume was two microliters, which recoups the detectability lost by the toluene dilution. Also, the peak is almost symmetrical, which increases detectability even further.

Figure 1: Illustration of a gas chromatograph highlighting the hot inlet and the cooler column oven which allows sample to vaporize in the GC inlet, travel the inlet via carrier gas and condense on the GC column in the cooler oven.



Conclusion

Optimizing GC inlet and injection parameters is essential for accurate and reliable pesticide residue analysis in cannabis. Understanding the challenges and employing the appropriate techniques can significantly improve the performance of the GC system. By using solvent or analyte focusing,

Figure 2: Injection of a dichlorvos standard in acetonitrile at increasing initial oven temperatures. GC-MS/MS, plot of one MRM transition, data using a 5-phase column. All solid color filled peaks are dichlorvos.

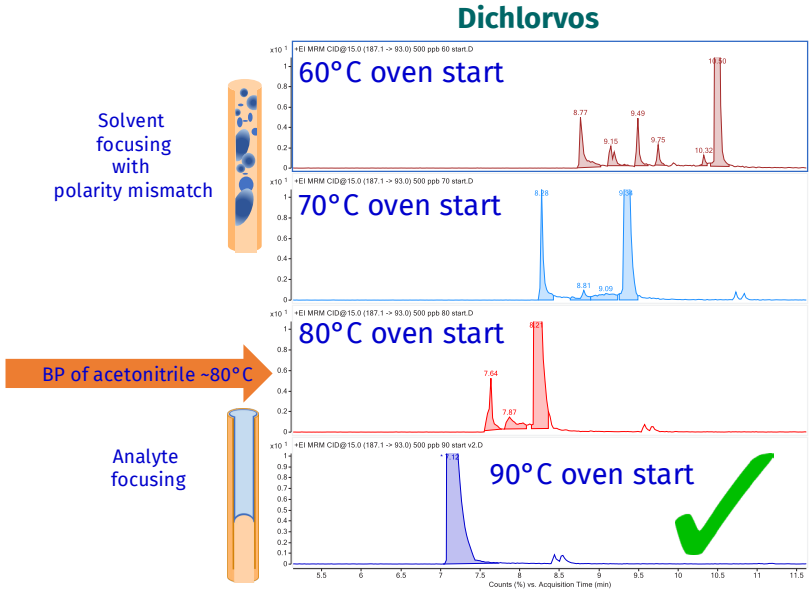
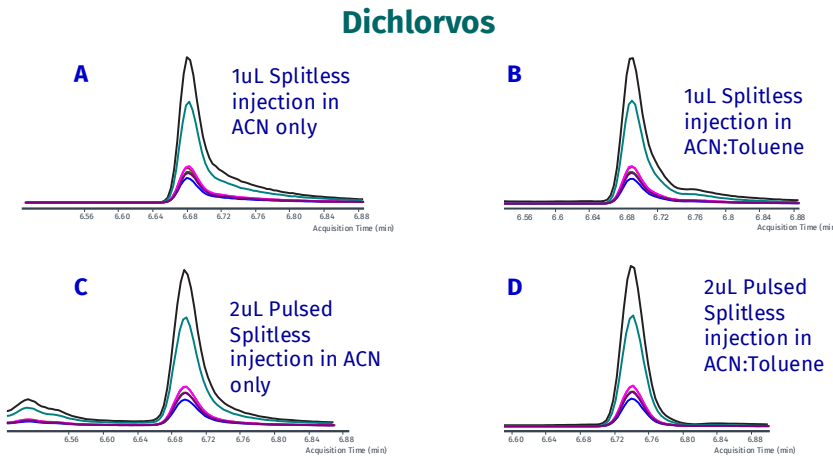


Figure 3: Plot of multiple MRM transitions for dichlorvos. Example dichlorvos peaks injected in acetonitrile or 50:50 acetonitrile:toluene solutions, splitless or pulsed splitless injection modes and 1 or 2 µL injection volume. Plot of multiple MRM transitions for dichlorvos. Peak height normalized to allow easier visual comparison of peak shape. Acetonitrile (ACN).



addressing solvent and GC stationary phase polarity mismatch, and using a pressure-pulsed splitless injection, analysts can achieve better confidence in their results.

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ABOUT THE COLUMNIST

JULIE KOWALSKI is a technical consultant primarily serving the cannabis and hemp testing market. She earned her graduate degree in Analytical Chemistry. Her professional experience includes troubleshooting, method development and validation for GC, GC-MS, LC, and LC-MS/MS in addition to extensive pesticide residue analysis experience and chromatography method development. With over ten years at a technology provider, she has served as Scientific Director and Chief Scientific Officer for a cannabis testing lab. She has served as the President of the North American Chemical Residue Workshop, served on AOAC Expert Review Panels, the Cannabis Scientific Task Force for Washington State, chaired the AOAC CASP Chemical Contaminants Working Group and is currently chairing the AOAC CASP Pesticide Think Tank. Julie is a part of Saturn Scientific, LLC, an interdisciplinary team of experienced scientists that leverage their collective expertise to perform technical investigations for the cannabis and hemp industries.

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cannabis science and technology

Engineering Efficiency:
Optimizing Energy Systems in Indoor Cannabis Cultivation Facilities

ERIN MCEVOY

As cannabis production continues to increase in the US, focus on efficiency in cultivation has also increased. Indoor cultivation strives to create an ideal ecosystem for cannabis plants, but this effort comes with inherent challenges. Focusing on lighting, climate control, water usage, and air circulation, this article compiles three different perspectives – a scientist, a grower, and an engineer – on how to optimize cannabis growing from an energy usage standpoint while still supporting healthy plant growth.

A **STUDY PUBLISHED** in 2025 outlined the effects of indoor cannabis cultivation on greenhouse gas emissions and climate change, noting that indoor cultivation increased from about 33% in 2012 to 65% in 2023, as legalization occurred throughout the country (1). Industry-wide emissions are around 44 Mt CO_2e/year (from both legal and illegal operations), the equivalent of emissions from 10 million cars. Compared to outdoor operations, "cultivating a given amount of cannabis indoors results in approximately 30 times more emissions per kilogram than cultivating outdoors," the study explains. "When incorporating emissions from all other stages of the life cycle, cannabis cultivated in plant factories is 7 times more emissions intensive."

Indoor cannabis cultivation consists of several necessary sources of energy: lighting, water and fertilization, climate control, and air circulation, to name some. Each of these sources contains ways to reduce energy consumption while optimizing the grow operation.

Lighting

Lighting technology in cannabis cultivation has evolved to be more efficient over the years, but a balance is needed between energy conservation and providing the plants with the energy they need. As Zacariah Hildenbrand, PhD, partner of Medusa Analytical, and a director of the Curtis Mathes Corporation (OTC:CMCZ) explained in an interview with *Cannabis Science and Technology*, "If you don't have enough light intensity, your plants are not going to thrive, and maybe they don't even survive. If you have too much intensity, you could get photo bleaching." Plants will need more light in flower phase than in vegetative phase, he adds, which does require increasing the watts regardless. Lighting technology has evolved from metal halides and high-pressure sodium lights to efficient LEDs, he explains, which provide the full spectrum of lighting that plants need yet do not produce as much waste heat.

Using LEDs for lighting is a significant factor in saving energy in other areas, explained Adam Jacques, owner of AgSense, LLC. "As far as saving money goes, there's nothing that can touch an LED. When you're looking at the environmentals, you're halving everything, as far as heat dissipation and humidity issues and those types of things. It fixes so much in the grow without you having to change too much of your infrastructure that financially, it makes no sense to use anything else." Additionally, he explains, energy audits with a PAR (photosynthetically active radiation) meter can be useful as well. A critical concept in lighting, PAR "encompasses the range of light wavelengths that drive photosynthesis, typically between 400-700 nm," as explained

in the January/February Cultivation Classroom Column co-authored by Hildenbrand, Hannia Mendoza-Dickey, and Robert Manes, in *Cannabis Science and Technology* (2). "PAR is quantified in µmol (micromoles) per square meter per second, representing the number of photons within the PAR range reaching a given area in a specific time frame," the authors explained. PAR meters can help identify hot or cold spots in lighting and ensure an even PAR over the canopy. Also important to note, different genetics will require different levels of lighting, Jacques adds.

At the same time, the energy conservation from lighting can only be taken so far. As Nadia Sabeh, PhD, president and founder of Dr. Greenhouse, explains, the law of diminishing returns applies to cannabis cultivation. "There's going to be a point at which [the lighting] efficacy that we're driving towards, micro moles per joule, is going to reach a limit, because there's only so many red diodes that we can use to grow a plant. We're going to hit a ceiling at some point." Occasionally she sees growers negate the energy saved from more efficient lights by increasing light levels or adding more light fixtures to increase plant production, which in turn requires more air conditioning. She offers other options for optimizing lighting in an indoor facility, namely dimmable fixtures, ideal distribution of light, and the greatest use of white surfaces to reflect photons back to the leaves.

Climate

Climate control in indoor grows is another significant source of energy. Combined with lighting, managing temperature and humidity comprises about 70 to 75% of the cost to cultivate, explains Hildenbrand, and though HVAC systems are a significant initial expenditure, they are crucial for maintaining the vapor pressure differential (VPD). "You're trying to be in an optimized range of temperature and humidity, where the plants are happy, not too hot, not too humid, not too dry, not too cold," he explains. Energy usage of the HVAC system is about equivalent in amount of energy as lights, Sabeh explains, though it can be slightly less because while the HVAC system is removing energy generated by the lights, some light is converted into plant biomass through photosynthesis. Some HVAC systems use hot gas reheat to recover the heat of compression. For systems that do not, an additional heat source—electrical or gas—will need to be used, Sabeh added, which results in higher energy, possibly more than from lighting.

Additionally, negative air pressure in smaller grows may even be enough to offset the heat generated from LEDs, Jacques explains. When looking at insulating the operation, building construction and location needs to be taken into account, such as wood or steel frame, potential radiant heat, and region climate, for example. Jacques explains he has found spray foam to be the easiest because it avoids mold issues other insulations can cause due to the humidity in the facility.

Insulation for most indoor grows are at an R rating between 10 and 30, depending on climate zone and region, explains Sabeh. Every inch of an insulated panel represents R5, so a four-inch-thick structural insulated panels (SIP) which would represent R20, and a correct building envelope would add R10 to equal R30. "You need to make sure that you are meeting your local building codes," she adds. "Because every state, even local jurisdictions within states, use a different energy code or mechanical code. As time has moved on, those R values have mostly increased."

Water

Similar to lighting in indoor cultivation, water conservation also has its tradeoffs. A fully closed loop system can theoretically reduce water consumption and loss by recirculating it, though managing the recycled water can be challenging. Hildenbrand explains that the cost to filter the recaptured water is something to consider, especially depending on your soil matrix. Nutrients like nitrogen, phosphorus, and potassium need to remain part of the soil, though the soil could also contain heavy metals or other contaminants that need to be removed. A reverse osmosis filter can be used, yet it is inherently energy intensive, he adds.

Jacques notes that UV lighting or activated carbon filters are being used by some growers. "What we're trying to do is get that water back down to a zero PPM state," he states. Consistency is also a must, he adds, though the overall payoff of the system needs to be taken into account. "Once you start paying for all of these things to keep your water clean in a closed loop, you're throwing the baby out with the bath water because you're spending so much additional energy and money and time to reclaim this water." Closed-loop systems may be less feasible for smaller operations, and also depend on your location, Jacques explains. Water scarcity issues or hydroponic grows with drip lines are examples to consider when weighing water conservation. "I want to save as much natural resources as humanly possible, but with something like a closed loop watering solution, I would say that your money could be spent better somewhere else in the process," he explains. "You can do it, it's great long term water care if you're willing to do the investment. At a corporate scale where you're growing an acre of closed loop indoor greenhouse, that might start making a lot more sense."

Sabeh also advocates for careful water consumption methods that do not generate waste. She explains that currently, with reverse osmosis,

nutrients and fertilization need to be added back into the recaptured water, the pH may need to be rebalanced, and at most 70% of the water is recaptured. Ultimately, she states, energy can come from renewable resources, but fresh water is a limited resource and focus on reducing its consumption across agriculture in general is important.

Air Circulation

Air circulation is a crucial component of maintaining VPD, Hildenbrand explains. Structural engineers can assess facilities and recommend improvements that can make a significant impact, he explains. Sabeh emphasizes design over technology with air circulation efficiency. To avoid hot spots, she recommends the traditional horizontal airflow fans (HAF) air circulation strategy, common in greenhouses, using the racetrack design to create a funnel to pull hot air to the plant canopy. She cautions against vertical air flow because when cannabis is being grown densely, it can result in low velocity and air becoming trapped on top of the canopy. Likewise, under-bench airflow can be inconsistent and increase the transpiration rate of the plants, increasing their water intake and reducing water conservation.

The air exchange rate is going to be different for each grower, Jacques explains, depending on the volume of the space. Wall-mounted oscillating fans in a grid pattern can help reduce dead spots, and using negative pressure through an extraction fan can be an added help with cooling in space with no open CO_2. Implementing environmental sensors can help adjust power usage in real time. "The fans go on to X amount of speed when we hit X humidity or X temperature," he explains. "So instead of running all of your HVAC and dehumidification and fans at the same level all the time, we can use some sort of controller to have those change their power usage based on what's specifically happening in the room at that point." Manual or automated energy audits that track highs and lows of humidity and temperature and adjust as needed can also help optimize energy usage, he adds, saving on electricity bills.

Final Thoughts

According to Sabeh, when monitoring the environmental conditions of a grow operation, placement of a sensor such as a thermostat or relative humidity sensor is crucial and must be representative of the environment as this affects the energy efficiency of the equipment. Another common impact on the environment Sabeh sees in facilities is doors being left open. "I can look at a data set, a plot, a graph, and I can point out almost every single time when the doors were open, because you see a rapid change in that environment." This also exposes the plants to mold spores.

Hildenbrand says that it's important to advocate for environmental stewardship and be a good neighbor to everyone on the same power grid. "If you can afford the initial capital expenditure of solar panels and you live in a favorable locale, you should absolutely go that route as a supplemental measure," he explains.

Jacques warns that while there are important measures growers can take to maximize efficiency and create an ideal environment, some things may be out of one's control. "The plants have a mind of their own, believe it or not, and they're going to do what they want to do," he explains. "It's like raising a kid, right? Every genetic is different. They're not going to react the same to your environment, your watering schedule, your nutrients, and so it's just trying to build the best environment you can so they can become themselves."

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Result Manipulation: *From THC Inflation to Aspergillus*

BY SEBASTIAN KRAWIEC

Economic incentives are driving dishonest laboratory test results, and there's data to prove it.

THERE'S A PERCEPTION among cannabis consumers that higher THC content in cannabis products is synonymous with value. Yasha Kahn, co-founder of MCR Labs, explained a recent episode of the Noid Knowledge podcast (1–3) that consumers are more likely to purchase products with high THC content because they're generally thinking in terms of cents per milligram of THC. "If you have an option of 30% THC flower or 20% THC flower, you're more likely to go for the 30%. And you're more likely to pay more for that 30% and be happy with it," he explained.

This perception breeds the incentive for industry to cultivate and sell high-THC products. That potency is verified though laboratory testing, but if a cultivator tests their product at two different labs and the results of one of the labs shows higher THC content, the cultivator is more likely to use those results because that will help them sell to the retailer who is looking for higher THC product. While, in isolation, differences in the results from competing labs do not necessarily indicate manipulation of test results, on a larger scale, there is an observable pattern in which labs feel pressure from clients to provide preferable results. If they don't, someone else will. This has been dubbed "lab shopping," and it's something Khan has experienced first-hand.

"We had a lab in Pennsylvania, an excellent lab performing fast and accurate testing. Then some of our clients, cultivators, would come to us and say, you failed us, but this other lab offered not to fail us or didn't fail us for anything," explained Kahn. Instead of trying to figure out the discrepancies or remediate any issues such as mold, it was easier for those cultivators to just use the labs that passed

them. Similarly, Kahn said that in Massachusetts, clients began coming to them demanding better potency results. "[They] would say that another lab is giving them much higher potency results. If we don't do the same, if we can't match the potency results that they get at the other lab, they're going to leave us for that lab," said Kahn. At the end of the day, while THC inflation is deceptive, there is also a larger public health concern in which some labs are passing products that are in excess of the threshold for contaminants such as mold.

This anecdotal experience is being verified with data. In the case of THC inflation, a 2023 study published in *PlosOne* (4) found that when testing the THC potency of 23 samples purchased from 10 different Colorado dispensaries, the tested potency was substantially lower than the label claims. The researchers found that on average, the observed THC potency was 23.1% lower than the lowest values reported on the label, and 35.6% lower than the highest values reported on the label. Seventy percent of the samples overall were greater than 15% lower in THC than label claims. While the researchers acknowledge that the exact source of the discrepancies is difficult to pin down, they explain that there are a number of factors at play, including economic incentives for high-THC products, as well as a lack of standardized testing protocols and limited regulatory oversight.

Jeff Rawson, president of the Institute of Cannabis Science, explained in a presentation (5) titled, "Market Audits Combat Cannabis Misinformation" for ASTM International's D37 Virtual Symposium in 2023, that a big problem is the lack of analysis of compliance testing. An article of the same name co-authored by Rawson and Kahn was published in the *Journal of Testing and Evaluation* in July of 2024 (6). "Compliance testing happens at a different stage than consumption. It's one step of quality control, but it's not a quality assurance program," Rawson explained. "Furthermore, the compliance testing that's done is not evaluated systematically, or rarely. I don't see a lot of people doing big data analysis on it right now, and products from the retail space aren't measured at all. This is an engineering principle: You don't control what you don't measure. So, if you're never measuring the consumer experience by checking products in the marketplace, then you're not really controlling the quality of those products."

Rawson offered Washington state as an example, in which seven of the 25 licensed labs have been suspended or cited for some kind of infraction, and all these infractions, he says, leave signature in the data they produce. "In fact, some of these infractions were discovered by actively monitoring compliance testing data and noticing that some of the labs had discrepant practices," he explained. For example, among these Washington labs with discrepant practices, Rawson pointed out a pattern in which labs with above-average THC values saw a monthly increase in market share, indicating that more cultivators were going to this lab each month for preferable results. This illustrates how lab shopping happens and hurts honest labs. Some of the labs shown by Rawson to have increased their market share with high THC values were eventually shut down.

Knowing the importance of data and what it can reveal, Kahn and MCR Labs used Freedom of Information Act (FOIA) requests to acquire testing lab data from 20 of the 39 states that have cannabis regulations. This compilation of data includes information from 2.4 million flower samples, 144 labs, and has nearly 68 million data points. To give credit to policy makers and regulators, this is all thanks to the fact when they drafted regulations, they not only made testing mandatory but also required third party labs to submit all their test results to the state. That makes it public information. Now, to what extent these states monitor and use this data is another story.

Data has the ability to reveal an inconvenient truth. Regulators set limits on factors like total yeast and mold, for example, but these limits are not relevant when you're dealing with result manipulation. "There are labs you can find that will pass you in almost every state, not every, but almost every state. And so…limits are just not a relevant topic up until the result manipulation issue [is addressed]," says Kahn. "Once we deal with that problem…then we should address action limits. And there needs to be public health officials that make that decision on what the limits should be. I can see in the data, in labs that test honestly, around 12 to 16% of flowers will fail at the 10,000 colony forming units action limit. Around 4 % will fail at the 100,000 colony forming units action limit."

Dealing with recalls not only costs money for stakeholders, but also tax revenue, which is another layer of incentive that can explain how labs and cultivators get away with result manipulation. Kahn explains that when filing FOIA request, some states were easier to work with than others. "The states that I had to sue are the ones with typically the worst data despite robust data. As in, clearly they know that there's something to hide and they do not want this out in the open," said Kahn. "I think it's also that regulators are in a position where they

will be held responsible if problems are found. Even if it's not their fault. Maybe they're not given the ability, the resources, or the jurisdiction to act on some of those things, and it's better to like, you know, stay silent, let's hope this blows over and then move on… In other states they really are doing as good a job as they can under the circumstances and are just not allowed to do anything. The market's making money for the state, don't touch it."

As an example of the type of information these FOIA request provided, MCR Labs provided *Cannabis Science and Technology* with figures demonstrating the purported discrepancies between labs. These particular figures focus on Mississippi. **Figures 1 and 2** show a distribution of total THC measured by two different testing facilities. In Figure 1, we see a pretty standard bell curve, while in Figure 2 we see a significant cliff in the number of products testing above 30% THC. Why such a steep cliff at 30% THC? Well, Mississippi law states that the potency of cannabis flower and trim cannot exceed 30% total THC. Looking at *Aspergillus*, **Table 1** (available online) depicts the detection rate for *Aspergillus* from three different testing facilities. Out of 1,636 samples, facility 1 has a 0% detection rate. Facility 2 had a 0.23% detection rate out of 2,151 samples, while facility 3 had a 2.50% detection rate out of 320 samples tested. That means that statewide, Mississippi's detection rate is 0.32% out 4,107 samples. Compared to other states, Mississippi has among the lowest detection rates for *Aspergillus* (**Figure 3**, available online). There's so much more data that can be dug into and interpreted to find patterns of manipulation, and finding these discrepancies is a great first step in solving the problem.

Figure 1: Analysis of cannabinoids from biological fluids using HPLC

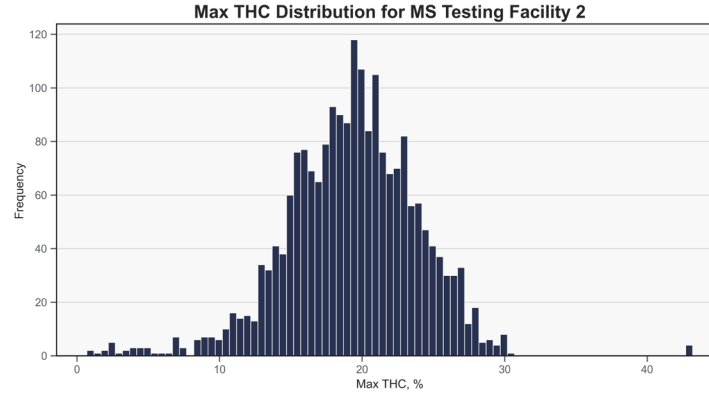
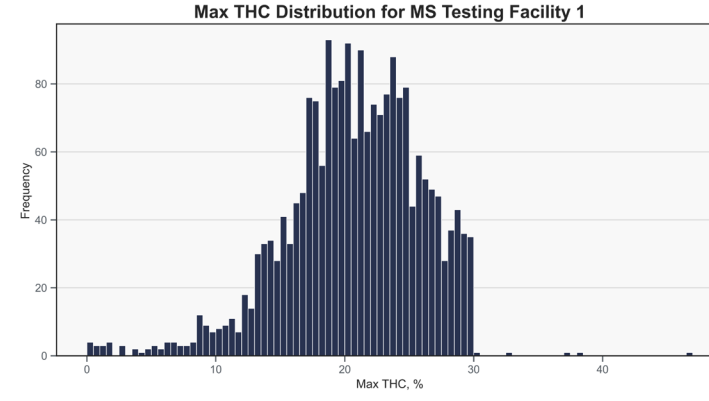


Figure 2: Analysis of cannabinoids from biological fluids using HPLC



On the bright side, some states have already taken action to address manipulation. In the case of Mississippi, regulators did shut down one of the biggest offenders, and pulled product tested by that company off the shelf (7). California's Department of Cannabis Control, for example, implemented Business and Professions Code section 26100(f)(2) that established standard cannabinoid test methods, "including standardized operating procedures, that shall be utilized by all licensed testing laboratories in California." That means that January 1, 2024, all testing labs had to use this method to test for cannabinoids and there has been a noticeable dip at the retail level in the THC levels of cannabis products (8). While this does create a more level playing field for labs, this action does not address other factors such as total yeast and mold, which can affect the quality of products and pose a safety risk to consumers. There's definitely more work that needs to be done to prevent result manipulation, and a data driven approach may be the best way to develop effective policies.

Full charts and references can be viewed online.

From Pain to Pimples:
The Growing List of Topical Cannabinoid Applications

BY MADELINE COLLI

Topically applied cannabinoids such as CBD and THC may offer significant benefits for people experiencing localized pain and dermatological conditions. This article explores these potential benefits as well as their mechanisms.

CANNABIS PRODUCTS OFFER a variety of consumption options for users to choose from, including edibles, joints, vapes, and suppositories. These dosage formats fit different needs and consumer preferences, but cannabis products can also be administered externally through formats such as creams and balms.

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The most popular cannabinoids used in topical products are cannabidiol (CBD) and tetrahydrocannabinol (THC). THC is well-known for being intoxicating when ingested orally, but there is not a great deal of research on THC's psychoactive effects when applied on the skin. That said, it is commonly accepted that THC is not intoxicating when applied topically. One study published in *Advances in Therapy* asked subjects applying a topical containing 100 mg CBD: 100 mg THC whether they were experiencing psychoactive effects at multiple timepoints. All participants said no (1).

There is a growing body of scientific literature demonstrating that topically applied cannabinoids may provide therapeutic benefits by either relieving pain or alleviating symptoms of skin conditions. "There have been human studies showing benefits of topical cannabis preparations in a variety of conditions, including musculoskeletal symptoms like TMJ pain and sports-related pain, as well as dermatological conditions like acne, eczema, and wound healing," said Dustin Sulak, DO, Founder of Healer. "There is also clinical evidence of systemic absorption of topically-applied cannabinoids for systemic conditions like peripheral neuropathy and Fragile X syndrome (2). Preclinical studies show many more benefits, including UV light protection, anticancer effects, and more."

Looking at musculoskeletal pain, a review recently published in February 2025 in *Pharmaceutics* called, "The Therapeutic Potential of Cannabidiol in the Management of Temporomandibular Disorders and Orofacial Pain," investigated CBD's potential for alleviating temporomandibular disorders (TMDs) (3). TMDs are are a group of conditions that affect temporomandibular joint (TMJ) and other related muscles (3). The review explains that CBD has been shown to decrease inflammation, reduce pain, and provide muscle relaxation. Dermatological conditions such as eczema may also benefit from topical cannabinoids due to their anti-inflammatory and antipruitic properties (4). "The mechanisms by which cannabinoids decrease inflammation and pruritus are diverse and involve CB1/CB2 receptors, chemokines, and an interaction between the endocannabinoid system and the immune system," write Filipuic et al. (4) Other studies show that acne, characterized by high production of sebum and chronic inflammation, may be treated by inhibiting the CB2 receptors, resulting in suppression of basal lipid production. (4)

Cannabis topicals work via the endocannabinoid system, which can be found throughout the body. The two main receptors for the endocannabinoid system, mentioned earlier, are the CB1 and CB2 receptors (5). The expression of these receptors in human skin has been well documented, and elements of the endocannabinoid system have been found in "epidermal keratinocytes, melanocytes, mast cells and cutaneous immune regulatory system." (5) When applied on the skin, topicals provide a more localized approach for users so that they can place the topical onto a specific area of the body. Topicals come in a variety of options such as, salves, lotions, balms, roll-ons, creams, and massage oils to name a few. With the various methods of cannabinoid consumption, users may feel concerned that the cumulative exposure to cannabinoids through smoking, edibles, and topicals can lead to adverse effects or interaction issues, but there is little risk of this.

"I don't think it's likely to cause a problem in most cases, and spacing out wouldn't make a big difference because both oral and topical/transdermal have long durations of action. Conversely, there's evidence that people who use multiple routes of delivery, such as oral + topical, get better results when treating pain," Dr. Sulak explained.

Although THC and CBD are the most commonly used cannabinoids in cannabis products, other cannabinoids are beginning to be explored for their medicinal properties. Cannabinoids such as cannabigerol (CBG) and cannabidiolic acid (CBDA) are some of the lesser-known cannabinoids that are rising up in popularity for their anti-inflammatory, pain, and other health benefits, says Dr. Sulak. "I'm most interested in CBDA, which is not commonly found in cannabis topical products. One human study (6) showed impressive wound healing with CBDA," he explained. "Also, based on animal data, CBDA is absorbed much better than CBD. Other animal data suggests that CBDA and THC have synergistic effects on inflammatory pain."

"As usual, there's a lot of overlap among the cannabinoids, but also some important differences," Dr. Sulak added. "For example, THC and CBD can both help with pain and spasticity, but THC appears to be better for itching while CBD is better for preventing scarring."

There are a number of potential synergies that can be leveraged when formulating topical cannabis products, both from within cannabis and from other source. Some terpenes, for example, have been found to have skin permeation enhancer abilities. "Beta caryophyllene, also found in some cannabis varieties, has its own benefits and is likely the therapeutic component of copaiba oil, which has its own body of literature suggesting beneficial topical effects. It's a CB2 agonist and could be very complimentary with CBD and CBDA. Healer topicals also contain copaiba oil for this reason," said Dr. Sulak. "Also, one study (7) found that a combination of CBD and ginger worked well together for eczema. I'm sure we have a lot more to learn about combining cannabinoids and other botanicals in topical preparations."

The potential benefits of topical cannabinoids are vast, and current research warrants further investigation to elucidate their mechanisms and develop effective treatments.

Full charts and references can be viewed online.

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