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Note from the CEO

Mid-Year Update: Exploring Developments in the Cannabis Industry

WE ARE HALFWAY through 2024 and though many developments in the cannabis industry have already happened—such as the latest advancements in federal rescheduling and our Cannabis Science Conference in Kansas City, Missouri—much more is still ahead! Read on for a sneak peek into the latest ideas and innovations in our May/June 2024 issue.

In the "Cannabis Analysis" column, Brian C. Smith, brings us Part IV of his Calibration Science series with an article on calibration metrics, discussing how variance is measured, how to calculate the standard deviation of a data set, and more. In the "Extraction Science" column, Lo Friesen covers the differences between hemp and cannabis, the products that are on the market today, and why it is important for consumers to know where their products are coming from. This topic was voted most popular by our readers in a poll located on our LinkedIn page—you don't want to miss it! Next, in our "Cultivation Classroom" column, Editor Madeline Colli sits down with cannabis geneticist Adam Jacques and Zacariah Hildenbrand, for an in-depth interview on hop latent viroid (HLVd) and what cultivators can do to protect their crops. In the latest "Navigating the Labyrinth" column, Robert Thomas argues that based on evidence in the public domain, the panel of the "big four" heavy metals regulated by most states is not enough to protect consumer safety and should be increased. The "Tech Innovations" column from David Hodes discusses new methods and laboratory processes that do more targeted cannabis extraction, from simple DIY to lessons learned from mainstream agriculture to chemical discoveries. This issue's "Peer Reviewed" article by Westley Cruces, Maite L. Docampo-Palacios, Tesfay T. Tesfatsion, and Giovanni A. Ramirez explore the identification, binding interactions, and ADME properties of cannabinoid ethers, plus silico docking in CB1 and CB2 receptors, drug metabolism, and pharmacokinetics (DMPK) studies of methyl-, ethyl-, and pivaloy- ether cannabinoids analogs.

Be sure to check out our Feature article in this issue: our highlights of this year's Cannabis Science Conference! In May 2024, our editorial team conducted various insightful interviews with conference speakers and program chairs, plus provided onsite coverage of keynote speakers and session presentations. Read the full article to learn the latest in cannabis research and innovations from this event!

We hope you enjoy our May/June 2024 issue!

Mike Hennessy Jr
PRESIDENT AND CEO

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Calibration Science, Part IV: *Calibration Metrics*

By ***Brian C. Smith***

Now that we have discussed calibration lines, we now need to discuss how to measure their quality, that is, we need to study calibration metrics. We will discuss variance and how it is measured. Then, using illustrations and equations we will see how to calculate the standard deviation of a data set, which will give us a measure of accuracy; how to calculate the correlation coefficient, which was introduced in the last column; and the F for Regression, a measure of a thing called the robustness of a calibration, which is an obscure but important measure of calibration quality.

Variance

Hold on to your hats. To understand calibration metrics, we must talk about statistics. I will try to make this as painless as possible, using words and figures in addition to equations to explain things.

The mathematical concept of variance is also known as scatter or dispersion. In a set of data, variance is a measure of how the data are distributed with respect to the average of data. This is illustrated in **Figure 1**.

In Figure 1, the black dots represent data points, and the X represents the average of the data set. To put this all in concrete terms I will use the isopropanol (IPA) in water infrared spectroscopy calibration line example from last time (1). The peaks used in the peak area calculations are shown in **Figure 2**, the standard concentration and peak area data are in **Table 1,** and the actual calibration line is in **Figure 3**.

Note in Table 1 that the %IPA concentrations vary from 9% to 70%, this is an example of variance. The average of the %IPA values in Table 1 is 37%, this would be the X at the center of Figure 1. This variation in IPA concentrations listed in Table 1 is intentional: we varied the standard concentrations to obtain a concentration range that brackets those of the expected unknowns, a principle discussed before (2). Unintentional variance comes from random and systematic error, which we have also discussed (3). Imagine the data points in Figure 1 are the predicted values obtained by applying the calibration seen in Figure 3 to the spectra in Figure 2. Thus, in our example the total variance is equal to the sum of the variance from using standards with different concentrations AND from the error. Both these sources contribute to the variance seen in Figure 1. How do we go about quantifying this variance? Well, keep reading.

Variance in Pictures and Equations

Note that the outer circle in Figure 1 is labeled "total variance" and is obtained by simply drawing a circle that includes all the data points as shown. To measure the distance, that is, variance from any given data point i to the average, we can simply subtract their values as seen in **Equation 1**:

$$V_i = Y_i - Y \qquad [1]$$

where V_i is the variance of data point I; Y_i is the value of data point I; and Y is the average of all data points.

Remembering that in our example each Y_i is simply a predicted %IPA value.

The total variance then would be the sum of all variances for all the data points as given by **Equation 2:**

$$\text{Total Variance} = \Sigma_i(Y_i - Y) \qquad [2]$$

where Σ_i is the sum over all variances; Y_i is the value of data point I; and Y is the average of all data points.

Now the tricky bit here is some of the variances will have positive values, and some negative values, and when the sum is calculated they will cancel, not giving the true size of the variance. That is why rather than using Equation 2 to measure the total variance, we square each individual variance so all the values are positive, then add these numbers together as seen in **Equation 3**:

$$SST = \Sigma_i(Yi - Y)^2 \qquad [3]$$

where SST is the sum of squares total, the total variance; Σ_i is the sum over all variances; Y_i is the actual value of data point I; and Y is the average of all data points.

Now given the outer circle seen in Figure 1 spans the total variance, the SST is the calculated total variance, is the area

of this circle. Hence in Figure 1 it says "SST, Total Variance, Outer Circle".

Now, no model will predict all standard concentrations with 100% accuracy because of random and systematic error. In other words, for the calibration line seen in Figure 3 the line will never pass through the center of all the data points, although this calibration line is close to that ideal. This means calibration models can model some variance but not all of it. How then do we measure the variance accounted for by a model? That would be seen in **Equation 4**:

$$SSR = \Sigma_i(Y' - Y)^2 \qquad [4]$$

where SSR is the sum of squares explained by regression (the variance modeled); Y' is the predicted value; and Y is the average value.

The term SSR is annoying, but is what is used in the literature. It is simply the variance accounted for by a model, but the "regression" thing is assuming we are using a regression algorithm, which in this case we are: linear regression.

In our example, each Y' would be a predicted %IPA value as determined by applying the equation for the calibration line seen in Figure 3 to the peak area data seen in Table 1. Each (Y' – Y) is the difference between each predicted value and the average, then everything is squared to get all positive numbers, and all this is added together. In terms of the diagram in Figure 1, the SSR is the area of the inner circle, hence it says, "Variance Modeled, SSR, Inner circle." This makes sense because the SSR is based on the difference between the predicted values, the variance accounted for by the model, and the average.

What we have left to calculate is the variance not accounted for by the model. In other words, the magnitude of the total error in the model predictions. For the calibration line in Figure 3 this would be the space between the known %IPA standard values and the average. How do we calculate this? **Equation 5**, of course:

Figure 1: A set of data showing variance around the data average, denoted by **X**

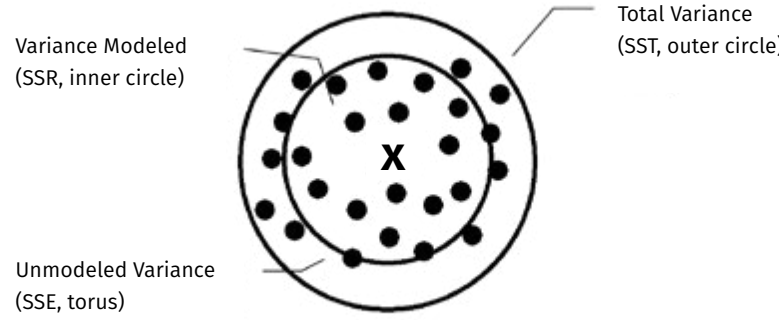
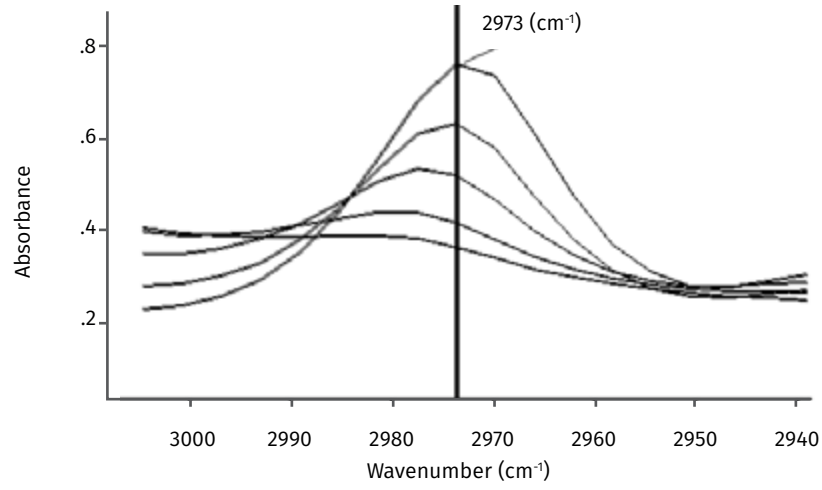


Figure 2: Infrared spectra of five IPA in water standard samples. The peak marked at 2973 cm^{-1} changes with IPA concentration and will be used to create a calibration.



$$SSE = \Sigma_i(Y_i - Y')^2 \qquad [5]$$

where SSE is the sum of squares due to error (variance not modeled); Y_i is the actual value; and Y' is the predicted value.

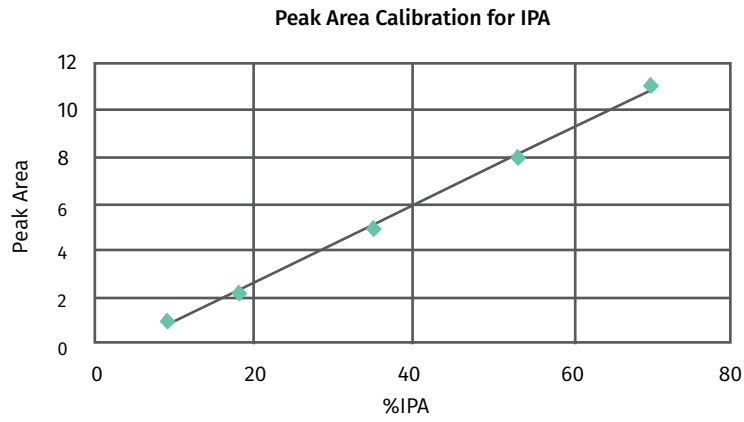
The SSE, the sum of squares due to error, quantifies the variance not modeled, and is the sum of the squares of the difference between the predicted and actual values. For our IPA example, Y_i would be the known IPA%, and Y' would be the predicted % IPA for each sample. The SSE corresponds to the area of the torus in Figure 1, where it says "Unmodeled variance, SSE, torus".

Table I: Volume percent IPA of standard samples and corresponding peak areas

%IPA	Area
9	1
18	2.2
35	4.9
53	8
70	11

Equations 3-5 are known as the "sums of squares" equations because in each case a quantity is calculated, it is squared, and then summed. To summarize, the SST is the sum of squares total, measures the total variance, and depends upon the actual values and their

Figure 3: Calibration line plot of peak area vs. volume percent IPA based on data in Table 1.



average. The SSR is the sum of squares accounted for by regression, measures the amount of variance accounted for by a calibration model, and depends upon the predicted values and the average. Lastly, the SSE is the sum of squares due to error, and depends on the actual and predicted values.

Calibration Metrics

Standard Deviation

Now we come to the reason why we slogged through all the math and statistics above, to be able to derive equations that tell us the quality of calibrations obtained with analytical instruments. The standard deviation, σ, is something we have discussed in the past (4). My way of explaining is that it is the average error per data point in a data set. In our %IPA example it would be the average error between the known and predicted %IPA values. If you look at the SSE equation (Equation 5) above, note that it also depends upon the known values, Y_i, and the predicted values Y'. So, can we use SSE to calculate the standard deviation? Yes, we can as given by **Equation 6**:

$$\sigma = (SSE/n-1)^{1/2} \qquad [6]$$

where σ is the standard deviation; SSE is the sum of squares due to error; and n is the number of data points.

If our data points are in units of %IPA, the SSE is in units of $(\%IPA)^2$, hence the need to take the square root of the term on the right-hand side in Equation 6. The number of data points, n, in terms of analytical instrument calibrations, is the number of standards used. For our IPA example, it is 5 as taken from the data in Table 1. Equation 6 is consistent with other equations I have presented to calculate the standard deviation as it has the total error in the numerator and the number of samples in the denominator.

Correlation Coefficient

The correlation coefficient, R, was mentioned in the last column (1). If you look closely at Figure 1 the big circle represents SST, the inner circle SSR, and the torus SSE. Visually, you can see that the area of the big circle is equal to the area of the inner circle and the torus. In other words, the total variance equals the sum of the variance modeled and the variance not modeled (due to error) as expressed in **Equation 7**:

$$SST = SSR + SSE \qquad [7]$$

where SST is the sum of squares total, the total variance; SSR is the sum of squares due to regression, the variance modeled; and SSE is the sum of squares due to error, the variance not modeled.

Recall (1) that the correlation coefficient is on a 0 to 1 scale, where 1 means a perfect correlation between actual and predicted values, and 0 means there is no correlation. Note that I said R depends upon actual and predicted values, well so does the SSE seen in Equation 5, thus we can write how R is calculated in **Equation 8**:

$$R = (SSR/SST)^{1/2} \qquad [8]$$

where R is the correlation coefficient; SSR is the sum of squares due to regression (variance modeled); and SST is the sum of squares total (total variance).

Note that in Equation 8, R is calculated as a ratio. What R really represents is the fraction of variance accounted for by a model. If SSR = SST there is no error, and we get R = 1. If none of the variance is modeled SSR = 0 and R = 0. Thus, we now know why R is on 0 to 1 scale.

A final calibration metric that is useful to calculate is the F for Regression (5). It is given by **Equation 9**:

$$F = (SSR/SSE)(n-m-1/m) \qquad [9]$$

where F is the F for regression; SSR is the sum of squares due to regression; SSE is the sum of squares due to error; n is the number of data points (number of samples); and m is the number of independent variables.

The first term in Equation 9, SSR/SSE, is the ratio of the variance modeled to the variance unmodeled, that is the error. This is a lot like a signal to noise ratio, which we have talked about before (3). The greater the variance modeled compared to the variance unmodeled, the better the model. The variable m is 1 for our %IPA example.

The F for regression is a measure of the robustness of a calibration, which is a measure of how strongly the model responds to changes in the input values. In the real world a robust stance is when you are standing on two feet, a small poke will

not topple you over. A non-robust stance would be standing on one foot, and the same small poke may be enough to topple you over. Similarly, calibrations may be accurate but not robust because a small change in conditions can have a huge effect on the results. Therefore, robust calibrations are always preferred, sometimes even at the expense of accuracy.

Conclusions

Variance is a measure of the spread or scatter in the data, and for calibrations the total variance equals the variance modeled plus the variance unmodeled. The three sums of squares equations measure the total variance, SST, the variance modeled, SSR, and the error, SSE. The calibration metrics of the standard deviation, correlation coefficient, and F for regression can be calculated from these quantities.

References

(1) Smith, B., Calibration Science, Part III: Calibration Lines and Correlation Coefficients, Cannabis Science and Technology, 2024, 7 (2), 6-11.

(2) Smith, B., Quantitative Spectroscopy: Practicalities and Pitfalls, Part I, Cannabis Science and Technology, 2022, 5 (5), 8-13.

(3) Smith, B., Calibration Science, Part II: Systematic Error, Signal-to-Noise Ratios, and How to Reduce Random Error, Cannabis Science and Technology, 2024, 7 (1), 8-11.

(4) Smith, B., Statistics for Cannabis Analysis, Part I: Standard Deviation and Its Relationship to Accuracy and Precision, Cannabis Science and Technology, 2021, 4 (8), 8-13.

(5) Brian C. Smith, Quantitative Spectroscopy: Theory and Practice, 2002.

ABOUT THE COLUMNIST

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Decoding Cannabinoids:
Exploring the Differences Between Hemp and Cannabis

By ***Lo Friesen***

At one point in time, it was easy to make the statement that CBD comes from hemp and THC comes from cannabis. However, these parallel industries have been developing rapidly and the dividing lines have blurred. Products like "hemp-derived" THC gummies or high-CBN tinctures can be found in both hemp and cannabis marketplaces. This article will cover what the differences are between hemp and cannabis, the products that are on the market today, and why it is important to know where your product is coming from.

In the realm of cannabis science, understanding the distinctions between cannabinoids derived from hemp and those from cannabis is crucial. While both plants belong to the *Cannabis sativa* species, they exhibit significant differences in their chemical compositions and legal classifications. Moreover, the availability of hemp-derived tetrahydrocannabinol (THC) products online adds another layer of complexity to the conversation. Let's delve into the nuances of these cannabinoids and unravel why hemp-derived THC products have become increasingly accessible.

Cannabinoids are a class of chemical compounds found in cannabis plants, including both hemp and cannabis. These compounds interact with the body's endocannabinoid system, influencing various physiological processes, such as mood, memory, appetite, and pain sensation. Among the hundreds of cannabinoids identified, THC and cannabidiol (CBD) are the most well-known and studied.

Hemp vs. Cannabis: Understanding the Contrast

Hemp and cannabis share the same genus and species, but they differ significantly in their cannabinoid profiles and uses. Historically, hemp has been cultivated primarily for industrial purposes, such as textiles, paper, food, and wellness products. It contains high levels of CBD and low levels of THC, typically below 0.3% THC content by dry weight, as mandated by law in many jurisdictions.

On the other hand, cannabis, commonly referred to as marijuana, contains higher levels of THC. THC is the cannabinoid responsible for the "high" associated with cannabis consumption, along with varying levels of CBD and other "minor" cannabinoids. Cannabis strains can have THC concentrations ranging from a few percent to over 20%, depending on the cultivar.

Hemp-Derived THC Products: Legal and Regulatory Framework

The legality of THC products, whether derived from hemp or cannabis, hinges on the concentration of THC and the specific regulations governing their production and distribution. In many jurisdictions, hemp-derived products with less than 0.3% THC are considered legal, while cannabis-derived products may be subject to stricter regulations or outright prohibition.

The emergence of hemp-derived THC products online can be attributed to the Farm Bill of 2018 in the United States, which legalized the cultivation and commercialization of hemp and hemp-derived products with low THC content. This legislation opened doors for the production of a wide range of hemp-derived CBD and THC products, including oils, tinctures, edibles, and topicals.

However, it's essential to note that regulations surrounding hemp-derived THC products vary globally. These hemp-derived products that contain Delta-9 or Delta-8 THC are produced through chemical synthesis, which converts CBD into THC. While they are hemp-derived, the hemp

market is far less regulated and quality controlled than the regulated cannabis markets. This could result in less safe hemp-derived THC products available to the market, but more profitable for hemp-derived THC businesses as there are tax benefits and fewer regulatory boundaries. The accessibility of these intoxicating hemp products is concerning to regulatory agencies and the regulated cannabis industry.

Cannabinoids Derived from Hemp

While hemp is renowned for its high CBD content and has expanded offerings to include hemp-derived THC products, it also contains a plethora of other cannabinoids with potential therapeutic benefits. Some of the cannabinoids that can be derived from hemp include:

1. **Cannabidiol (CBD):** CBD is the most abundant cannabinoid in hemp and is known for its potential therapeutic properties, including anti-inflammatory, analgesic, anxiolytic, and neuroprotective effects. It has gained popularity for its purported role in alleviating various health conditions, such as anxiety, chronic pain, epilepsy, and insomnia.
2. **Cannabigerol (CBG):** CBG is considered a precursor to other cannabinoids, including THC and CBD. Although present in low concentrations in most cannabis strains, hemp cultivars bred for high CBG content are now available. Preliminary research suggests that CBG may have potential anti-inflammatory, neuroprotective, and appetite-stimulating effects.
3. **Cannabinol (CBN):** CBN is a degradation product of THC, formed as THC oxidizes over time. While typically found in trace amounts in fresh cannabis, aged or heated cannabis products may contain higher levels of CBN. Some studies suggest that CBN may have sedative effects and could potentially aid in sleep disorders.
4. **Cannabichromene (CBC):** CBC is another non-intoxicating cannabinoid found in hemp and cannabis. Research indicates that CBC may exhibit anti-inflammatory, antidepressant, and analgesic properties. It also shows potential in promoting neurogenesis, the formation of new neurons in the brain.
5. **Tetrahydrocannabinolic Acid (THCA):** THCA is the precursor to THC and is found in raw, unheated cannabis plants. When exposed to heat through decarboxylation, THCA converts to THC, the psychoactive compound responsible for the euphoric effects of cannabis.

In addition to these cannabinoids, hemp also contains trace amounts of other minor cannabinoids, terpenes, flavonoids, and phytonutrients, which collectively contribute to the entourage effect—the synergistic interaction of various cannabis compounds that may enhance therapeutic outcomes.

Minor cannabinoids are produced on the hemp market as isolates and supplied by a number of reputable manufacturers. Quality assurance and supplier vetting is an important step in formulating hemp-derived products. Access to a supply of minor cannabinoid isolates has facilitated a burst of product innovation on the hemp market. The market has become hyper-competitive and an opportunity for consumers to gain access to a wide variety of product types and cannabinoid ratios.

Conclusion

Understanding the differences between cannabinoids derived from hemp and cannabis is paramount for consumers, healthcare professionals, and policymakers alike. While hemp-derived THC products offer a legal alternative for those seeking the potential therapeutic benefits of cannabinoids, it's essential to navigate this rapidly evolving landscape with caution and awareness of regulatory frameworks.

As research into cannabinoids continues to expand, we can anticipate further insights into their mechanisms of action, therapeutic potential, and optimal applications. In the meantime, exploring the diverse array of cannabinoids derived from hemp underscores the multifaceted nature of cannabis science and its profound implications for health and wellness.

ABOUT THE COLUMNIST

LO FRIESEN is the founder, CEO, and Chief Extractor of Heylo. With a background in chemistry and clinical research, Lo was inspired to explore cannabis as a medicine and to enter the emerging industry. She joined Eden Labs, a leading CO_2 extraction equipment manufacturer to support and expand a Research and Development department. There she managed the development of their latest and greatest CO_2 extraction system. In 2017, after working with Eden Labs and another cannabis processor, Lo launched Heylo with a mission to help people get more out of life with cannabis.

HLVd: *The Menace to Cannabis Crops*

By ***Madeline Colli***

Sweeping and dismantling numerous cannabis crops throughout the cultivation space, you need to look no further than at the plant disease called hop latent virus (HLVd). Also known as Dudding Disease, the virus needs in order to survive and reproduce, a compatible host which it has done in several other plant species but has now made its entrance into cannabis cultivation.

In continuation of our cultivation education series, Adam Jacques, a world-renowned expert with more than 20 years' experience cultivating various unique strains of cannabis, as well as the Chief Geneticist at AgSense LLC, and Zacariah L. Hildenbrand, a research Professor at the University of Texas at El Paso, the principal founder of Inform Environmental, a partner of Medusa Analytical, and is a director of the Curtis Mathes Corporation, get to the root of the issue of experiencing an HLVd infestation and if anything can be done to salvage your crops.

Q: Can you tell us about the Hop Latent Viroid (HLVd) virus and how have you seen it affect cannabis crops?

A: Adam Jacques: These kinds of viruses started, we started noticing with this probably 15 years ago, right? And it got called everything tomato mosaic, hemp mosaic, you know, all of these, and everybody was considering it. Well, this must be a mosaic virus of some sort of tobacco mosaic, maybe. What we started seeing was what we call Dudding, the production of plant material wasn't great. You would see lower numbers, reduced yield, and would get worse over time. And so, you know, everybody was trying to come up with, 'well, what is this?' Obviously, now, we know it's HLVd, right? So once that got figured out, really, it was just, at that point, figuring out what are the metrics of damage that it's doing to the crops? It's not like hemp mosaic virus isn't a thing. It's a thing that exists and it expresses itself roughly in the same way. So, when we start talking about hop latent virus, we do to really know what you have, you're gonna need a lab test on it. I would say that, as of right now, I would be surprised if any number less than 50% of clones—and it's in this country—didn't have it.

Zacariah Hildenbrand: It's ubiquitous. From what I see, right, with so much of this poly hybrid desertion, with all these genetics, just being mixed and mingled together, there really hasn't been much concern for 'Are there any, is there any kind of genetic baggage associated with doing this', right? It's just like, 'Hey, this genetic was fire and this other genetic is fire, and I'm gonna put fire and fire together and it's gonna be 10 times better' and there's just no consideration for the genetic lineage and, and you can be stuck with hop latent viroid. The issue with this too, is you can have plants that appear asymptomatic for quite some time, and yet they're still carrying the virus. They're almost like a vector, if you will, and then boom, you then introduce that with another genetic. Based on the genetic background, maybe then the virus expresses into its actual phenotype and condition. And then you got a real problem. I think the folks at Medicinal Genomics probably have the best handle on this. They published a bunch of papers on this looking at hop latent viroid's prevalence across various genetics. And I mean, to Adam's point, it's well over 50%. I mean, it's everywhere. I think one of the questions I asked him is if you could detect this in the seeds? Or if or how long do you have to wait until this starts being detectable in the plant? And they were still working on that. But it's a ubiquitous issue with varying effects on client's outcome, and it still remains to be determined how to completely eradicate it.

Q: How does HLVd avoid detection?

A: Jacques: A lot of ways to do this in this industry is observational. It's not like I'm going to every plant and doing a test on it every day to see if it has a virus or an issue. A lot of the things that I'm doing is with my eyeballs. So, if I have spider mites or russet mites, or

something like that, I can visualize it. This is, it hides in the plant itself. So, it's not really expressing a lot of things that would cause me concern, especially in the vegetative state. I mean, you do things like stunted growth, reduced vigor, brittle stems, you know, less flower mass, those things happen. But in a newly planted clone and fresh soil who's real healthy, it may be using that energy fighting that virus, but it's in such a healthy state at this point that you're not really seeing any of the side effects. So, when you flip it into flower, and you start going and you're adding stress to the plant into a flowering cycle, and trying to push weights and things like that, that's when you really see the energy of the plant, focusing from production of the plant into fighting the virus in the plant. That's where you really start to see a lot of the side effects coming out at that point. It's too late, right? You've already wasted six weeks. Everything is stunted. Another awesome thing about this disease is that let's say you grow in aeroponics. Well then, your entire water supply and all of your lines are infected now because it will transfer through the roots. So, it hides by not being a big problem until it is.

Hildenbrand: It's a really good virus. Good viruses are one that really don't perturb their hosts much and their whole goal is just to spread its DNA. A really bad virus is one that's lethal, kills its host before it can spread on, but this one's actually quite effective in that it spreads so readily without having a significant impact on the host until it does. I've always seen it with the virus, that it's a really steep decline. It's like, 'Oh, there's something weird going on with this plant' and Adam's, right. You may see it initially in the veg, like, 'Ah, maybe it'll be okay here'. And then all of a sudden, it's just like, 'Wow, this looks terrible. We don't have any trichrome production, we have weird leaf morphology. Now we have discoloration, this plant is dead'. So, or it might as well be dead. You just end up sacking it anyways. So, it's definitely one of those things that if you're not on top of it, can be a major issue. And a lot of people to, kind of going back to my comment about the polyhybridism, people are so eager to get genetics from other folks. We've discussed about, you know, the prevalence of mites and bugs and pathogens, and but what about the prevalence of viruses that you're passing back and forth? You know, I think people need to be more cognizant of the provenance of the genetics that they're getting, before they get super excited about the new hype strain out of someone's garage.

Q: Why should cultivators and consumers be concerned of HLVd?

A: Jacques: Consumers don't need to be. It's an issue with the cultivators. It is bad for the cultivators, you're looking at a 10 to 15% loss in cannabinoids, you're looking at a 30% loss and weight off of the plants. You're looking at a much lower quality flower. So, something that may have been top shelf money, you're now getting B or C grade money for less and you're getting less and the THC percentage sucks. Stuff duds out. It's just terrible. It's gross, right? So, it turns good flower into [unusable] flower. On the consumer side, you know, I don't think we'll ever see a financial hit to them on it being that the industry is what it is right now. I don't think smoking something that had hop latent viroid is going to do anything to you. But, if you are getting cannabis from like let's say your grower or your caregiver or something that it is this, you're getting a much lower quality product than you should be getting. So, I guess you're damaged a bit in that way.

Hildenbrand: Yeah, I don't think the virus can transform from plant kingdom to eukaryotic animals. I don't think that's the thing. But to Adam's point, it's just it's going to result in a diminished product, it's going to be less efficacious. And so obviously is not going to be the same medicinal value, as if you had an uncontaminated sample.

Jacques: And as a grower, those things that I mentioned, that is your margin, right? You'll go bankrupt if you have it and don't deal with it.

Q: What can be done to prevent or contain the effects?

A: Jacques: So before I bring anything into my room, I would prefer to get a test on getting hop latent tests and getting tests isn't difficult, like I have to go to the university now to get something like that. You can actually buy testing kits online to test your plants for hop latent. Before you bring anything into your room, or if you're starting from seed or something new like that, it probably would behoove you to test for viruses in your plants. If they do have viruses in your plants, it's not a death sentence for the plant necessarily, right? We can fix this. But it's tough. Honestly, it's tough not to bring this into your space right now.

Hildenbrand: Yeah, it's just so ubiquitous. So, I think really, it just goes back to having a strong understanding of the reliability of the source of your genetics. I think that's your best course of action, getting it from people that take care of their plants, sterilize things that they can, and just run a clean operation. I think the worst mistake you can make is just haphazardly taking a clone from some bro that you met, and you just have no idea what sort of conditions they were dealing with there and then boom, you could have systemic termination of your entire facility.

Figure 1: Zacariah Hildenbrand shares his favorite tip when encountering an HLVd outbreak.

Q: Are there any other cannabis pathogens to be worried about? (Fusarium, Pythium, etc.)

A: Jacques: Mosaic viruses, I would keep an eye on, they can do damage and the damage looks a lot like this. So, it's roughly the same. That's the one everybody was looking out for. For a long time. Specifically, tobacco, hemp, and tomato mosaic viruses. As far as viruses go, that's about, you know, those are the worries that that I have.

Hildenbrand: And then just the fungi, you know, *Botrytis*, those sorts of things. *Aspergillus*.

Jacques: *Aspergillus*, even powdery mildews. That's tough because that's systemic? *Aspergillus* will kill you so it's a good one to worry about.

Q: Is there any products you use/recommend to detect or use to deal with an HLVd infestation?

A: Hildenbrand: Yeah, I would just say again, it just goes back to the analytical techniques, [polymerase chain reaction] PCR. I think it's becoming so readily available, that you could have kind of at home kind of garage enthusiast, if you will, running their own PCR as a supplement to their grow. And it's been nice. I mean, we've seen the entire industry, like the folks at Shimadzu or the folks at Agilent. They're trying to take their cannabinoid analysis and their terpene analysis and make it kind of miniaturized so that the at-home user, maybe it's more accessible, and that the instruments are becoming cheaper. I think in five years' time, if you're a commercial outfit, you're going to have your own [high-performance liquid chromatography] HPLC to do cannabinoids, you'll have your own [gas chromatography mass spectrometry] GC-MS, to do terpenes, and you'll have your own PCR to do virus testing. And that's just going to be part of your daily protocol.

Jacques: I would say as far as products go to deal with this, no. There's not something you can water in or spray on the plant that's going to save it. If you have a virus in real life, same idea, right? You need to find something that's a directed cure for that thing, which just doesn't exist yet for this. You can make your plants healthier, which is going to help mitigate the damage that this virus is doing. But then you're just putting cost on top of costs to try and keep a sick plant alive. But no, there's no answer to fixing it in a product or a bottle that you can buy.

If you know somebody who's really good at tissue culture, you could go in and do a sanitizing tissue culture on it and that has been proven to remove the virus from plants. So, if there's a plant that you really want to keep in your stable, you can put out the financial

needs to get something tissue cultured and completely cleansed like that. It does work, I've seen it. It's a lot to do for, let's say, 500 plants in a grow. Let's say it's some killer genetic that's unique to you, and you want to keep it and it has an illness, there are ways to remedy this. But the plant doesn't like it. Plants don't like getting bleached necessarily. And I would, there's certain genetics that if that issue came up with them, I would find a way to save them. I would go that far to save those genetics. But I would say, for the average home grower at home, if all of a sudden you figure out that's what's happening, just rip it out and start again. Getting your plant tissue cultured and cleaned, like that's going to cost more than grow in that plant's worth.

Hildenbrand: If anyone experiences this for the first time, just start over. Don't try to play Sherlock Holmes to figure out where it came from and how to solve it. Just start over with new stuff and hopefully go back to the basics.

Q: What are the risks of clone sharing?

A: Jacques: I think clone sharing in general is as old as growing this plant is. It's like baseball cards amongst each other. Right? Who's got the new, unique elite thing? When me and Christian West started out, that was the thing, right? You would go and you go around, you collect all the clones from the different areas and that's how you got genetics. Now, I don't think my number 50% + is wrong of clones out there, have this. Generally, you're not going to see this expressing itself in a clone. So, you have no way of knowing with clone sharing whether you're getting a clean clone or not. Could there be systems put into place to make that easier, like maybe a certificate of testing on it or something, but then all of a sudden that clone's cost is not $20 anymore. It's hundreds of dollars to get a clean clone. I can't fault people for sharing clone and seed. That's a tough thing to do and it's exciting thing to do. And then you get new things. Turns out that can come with a lot of issues.

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Understanding Sources of Heavy Metals in Cannabis and Hemp Consumer Products, Part I: *Is the Fractured Nature of State-based Regulations Ignoring the Evidence?*

By ***Robert Thomas***

Introduction

In this month's column, guest contributor, Rob Thomas takes a critical look at the big four heavy metals which are regulated by the vast majority of US states and suggests that there is compelling evidence in the public domain that the panel should be increased because there is not a good understanding of sources of elemental contaminants in the production of cannabis consumer products. As a result of the inadequate nature of these state-based regulations, consumer safety is most likely being compromised.

Part 1 of this column examines the fractured nature of state-based regulations and compares it with the federal limits for pharmaceutical products and dietary supplements. Part 2 will look at specific examples in the public domain of elemental contamination of cannabis consumer products, which shows that other elements are worthy of consideration and should be an integral part of the regulatory framework.

The Regulatory Framework

Even though cannabis is in the process of being rescheduled from a Schedule I to a Schedule III drug by the Drug Enforcement Administration (DEA), uncertainty about federal oversight of cannabis consumer products in the US has left individual states to regulate its use. Cannabis is legal for medicinal purposes in 39 jurisdictions, while 19 states and Washington, D.C. allow its use for adult recreational consumption (1). However, the cannabis plant is known to be a hyper-accumulator of toxic metals in the grow medium and other manufacturing pathways, so it is critical to monitor levels of elemental contaminants to ensure cannabis products are safe to use. Unfortunately, there are many inconsistencies with heavy metal limits in different states where cannabis is legal. The vast majority of these states set limits for the "big four" heavy metals: lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg). New York State also requires the testing for chromium (Cr), nickel (Ni), copper (Cu), and antimony (Sb), while Michigan requires inorganic As (not total) and also adds Cr, Ni, and Cu to the big four. Maryland and a few other states also include Cr as well as the big four. Some states base their limits directly in the cannabis, while others are related to human consumption per day. Some take into consideration the body weight of the consumer, while other states do not even have heavy metal limits. To complicate the situation, certain states only require heavy metals in the cannabis plant/flower, while some give different limits for the delivery method such as oral, inhalation, or transdermal (2). This review article will provide evidence from publicly-available sources that a wider panel beyond the "big four" heavy metals are worthy of consideration, suggesting that the industry does not have a good understanding of sources of elemental contaminants in the cultivation and production of cannabis consumer products and as a result the fractured nature of state-based regulations could

potentially be compromising consumer health and safety.

State Inconsistencies

The inconsistencies of state-based limits would make it extremely complicated to implement at the federal level, unless there was a completely fresh assessment of the regulations. For example, why does New York State currently have action limits for eight elemental contaminants whereas the vast majority of the other states only require the big four? In other words, what do New York regulators know about the toxicological impact of the four additional elements, compared to California that only regulates four? Or why does Michigan require inorganic arsenic while all other states just list total arsenic? Furthermore, how can some states justify no heavy metal action limits at all? It might all become a moot point when federal regulators eventually have oversight of the industry, but what will that regulatory panel look like? The disparity in state-based limits might not be a good indicator, but there could also be clues in the way federally approved cannabidiol (CBD) drug formulations have historically been regulated, together with an understanding of how standards and reference material organizations have approached developing standardized analytical methodology for measuring heavy metals.

Disparity with Federal Limits

To highlight the disparity between state-based limits and federal guidelines for pharmaceutical formulations, the Food and Drug Administration's (FDA) Botanical Review Team in the Office of New Drug Products recently published a study entitled, "Quality Standards in State Programs Permitting Cannabis for Medical Uses" (3), which compared the maximum allowable limits of the big four heavy metals for states that had Medical Cannabis Programs (MCP) with *United States Pharmacopeia* (USP) Chapter <232> permitted daily exposure (PDE) concentrations in inhalation and oral drug formulations routes of administration (ROA) and *USP* Chapter <2232> Elemental Contaminants in Dietary Supplements (4). Although the actual states are not mentioned, it can be seen from **Table 1** that in the majority of cases, the eight medical programs reported are very different to the *USP* limits. In fact, it can be seen by much of the data in bold, that they are significantly higher than the federal limits. There is no obvious reason as to why the state programs action limits are so different, but it was interesting that the researchers' critique was three-fold:

- Testing for the four heavy metals in inhalation and oral products was lacking in the majority of state medical programs.
- Even when present, it was inconsistent across the different jurisdictions.
- It did not always align with USP recommendations.

Table I: Comparison of state Medical Cannabis Programs (MCP) with USP Chapter <232> permitted concentrations in inhalation and oral drug formulations and USP Chapter <2232> elemental contaminants in dietary supplements (3). Note: Data in red indicates they are higher than federal (USP) values

Inhalation ROA						
	USP <232> (µg/g)	MCP1 (µg/g)	MCP2 (µg/g)	MCP3 (µg/g)	MCP4 (µg/g)	MCP5 (µg/g)
Arsenic	0.2	0.2	**0.4**	**2.0**	**10.0**	0.2
Cadmium	0.2	0.2	**0.5**	**0.82**	**4.1**	0.2
Lead	0.5	0.5	**1.0**	**1.2**	**10.0**	0.5
Mercury	0.1	**0.2**	**0.2**	**0.4**	**2.0**	0.1
Oral ROA						
	USP <232> (µg/g)	MCP6 * (µg/g)	MCP7 (µg/g)	MCP8 (µg/g)	MCP5 (µg/g)	
Arsenic	1.5	1.5	**10.0**	1.5	1.5	
Cadmium	0.5	0.5	**4.1**	0.3	0.5	
Lead	0.5	**1.0**	**6.0**	**1.0**	0.5	
Mercury	3.0	1.5	2.0	0.5	3.0	

MCP – Medical Cannabis Program

ROA - Route of Administration

*USP Chapter <2232> limits

How Many Metals are Enough?

So clearly there is a need for more consistency across state lines, particularly as the industry inevitably moves in the direction of federal oversight. This is further compounded by the fact that there is a great deal of evidence in the public domain that only monitoring the big four heavy metals is not enough to ensure consumer safety. But how many metals should there be in an expanded panel, particularly as there is no comprehensive understanding of the sources of elemental contaminants

Table II: USP Chapter <232> and ICH Q3D guidelines permitted daily exposure (PDE) limits for elemental impurities in drug compounds per method of administration (4, 6)

Element	Class	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalational PDE (µg/day)	Proposed Transdermal PDE (µg/day)
Cd	1	5	2	3	20
Pb	1	5	5	5	50
As	1	15	15	2	30
Hg	1	30	3	1	30
Co	2A	50	5	3	50
V	2A	100	10	1	100
Ni	2A	200	20	6	200
Tl	2B	8	8	8	8
Au	2B	300	300	3	3000
Pd	2B	100	10	1	100
Ir	2B	100	10	1	100
Os	2B	100	10	1	100
Rh	2B	100	10	1	100
Ru	2B	100	10	1	100
Se	2B	150	80	130	800
Ag	2B	150	15	7	150
Pt	2B	100	10	1	100
Li	3	550	250	25	2500
Sb	3	1200	90	20	900
Ba	3	1400	700	300	7000
Mo	3	3000	1500	10	15000
Cu	3	3000	300	30	3000
Sn	3	6000	600	60	6000
Cr	3	11000	1100	3	11000

in the cannabinoid cultivation and production processes? Moreover, unlike drug products, there has been no risk assessment studies carried out with regard to heavy metal contaminants in cannabis consumer products and for that reason, consumer health is likely being compromised.

Regulatory Evidence

So, assuming that only monitoring the big four heavy metals is inadequate to ensure consumer safety, what is a realistic panel of elemental contaminants that should be used for state regulatory purposes? The only solid evidence we have at this current time for what could be a federally regulated panel is with the FDA approved CBD-based drug Epidiolex, which is available in the US to treat childhood seizures. Manufactured by UK-based GW Pharmaceuticals (now a division of Jazz Pharmaceuticals), it went through the regulatory process prior to its introduction in 2018 to get it approved in the US (5) and had to show compliance by meeting permitted daily exposure (PDE) limits for up to 24 elemental impurities as defined in *USP* Chapter <232> (3) and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q3D guidelines (6). In practice, because eight of those metals are associated with the drug synthesis process using platinum group catalysts which was not used in the Epidiolex manufacturing process, only 16 were used for manufacturing quality assurance purposes.

Furthermore, USP recently published a draft monograph for CBD as an active pharmaceutical ingredient (API) for a federally approved drug formulations which stated that (7):

"Elemental impurities in official drug products are controlled according to the principles defined and requirements specified in Elemental Impurities—Limits, Chapter <232>, as presented in the General Notices 5.60.30."

In the long term, this could possibly indicate that the FDA will regulate CBD products for up to 24 elemental contaminants when it eventually has oversight of the cannabis industry. But more importantly, in the short term it implies that CBD being manufactured in the US for recreational or medicinal purposes does not meet the purity requirements for federally approved drugs, because currently it only has to comply with the state's maximum limits for heavy metal contaminants, which in most US states is typically only Pb, Cd, As, and Hg.

However, it's important to stress that a panel generated by pharmaceutical regulators isn't necessarily one that should be used by the cannabis industry, as the process for manufacturing cannabinoids is very different to drug products. In addition, many consumer products

are not intended for medicinal purposes, but can be classified as foods, beverages, candies, or snacks so perhaps an argument could be made that they should be treated more like food products than drug substances. However, based on recent reports, it is clear the FDA is not doing such a good job of keeping us safe from the effects of toxic metals in our food supply as exemplified by a reprimand from Congress in 2021 for failing to identify processed baby food cereals with elevated levels of heavy metals (8) and more recently, for not flagging a case of intentional adulteration of cinnamon with lead chromate (Pb CrO4) for economic gain (9). So, until there is a mandatory requirement for food manufacturers to test their products on supermarket shelves, there is always the potential for incidents like this. So irrespective of what regulated limits are used, at some point the cannabis industry needs to carry out a comprehensive risk assessment study of its own to provide evidence as to what metals should be monitored, similar to what regulators did to characterize elemental impurities in drug products and formulations (10). To better understand this risk assessment process and how it might have a bearing on a cannabis panel of heavy metal contaminants, let's take a detailed look at how regulations for pharmaceutical and supplements came into existence, which eventually became the basis for state-based oversight of the cannabis industry.

Federal Regulations for Drug Substances

The pharmaceutical industry began the process to overhaul regulations and methodology for elemental impurities over 20 years ago when it updated its 100-year-old semi-quantitative sulfide precipitation colorimetric test for lead and a small suite of heavy metals to eventually arrive at a method to monitor 24 elemental impurities in drug products using plasma spectrochemistry. Moreover, they completely reassessed the toxicological impact of these elemental contaminants based on well-established animal models and defined them by permitted daily exposure (PDE) limits according to the mode of administration (oral, parenteral, inhalation, transdermal) and classified them by toxicity and the probability of finding them in the drug manufacturing process.

Table III: Toxicological classification of the regulated panel for drug products, and the suggested testing frequency (6)

Regulated Elemental Impurity	Testing Frequency
Class 1 metals - Pb, Cd, As, Hg	These are human toxicants that have no purpose in the manufacture of pharmaceuticals, and should be evaluated at all times, without exception.
Class 2A metals – Co, V, Ni	Have high probability of occurrence in the drug product and should also be monitored at all times.
Class 2B metals - Au, Pd, Ir, Os, Rh, Ru, Se, Ag, and Pt	Have a reduced probability of occurrence related to their low abundance and as a result, can be excluded unless they are intentionally added during the manufacture of the drug product (For example: metal catalysts used in drug synthesis).
Class 3 metals - Li, Sb, Ba, Mo, Cu, Sn, and Cr	Have relatively low toxicities by the oral route of administration but could warrant serious consideration for inhalation and intravenous routes.

> "Moreover, unlike drug products, there has been **no risk assessment studies carried out** with regard to heavy metal contaminants in cannabis consumer products and for that reason, **consumer health is likely being compromised**."

These limits were described in USP <232> - Elemental Impurities (4) and ICH Q3D Guidelines (6), together with USP Chapter <2232> for dietary supplements (4). While the measurement procedures were defined in USP Chapter <233> which describes the plasma spectrochemistry methodology (inductively coupled plasma optical emission spectroscopy [ICP-OES] or inductively coupled plasma mass spectrometry [ICP-MS]),

microwave digestion procedure and a full set of validation protocols (11). **Table 2** represents the full list of USP/ICH elemental PDE limits in microgram per day (µg/day) per delivery method and toxicological classification, which are explained in greater detail in **Table 3**.

It's also important to emphasize that the data in Table 2 are maximum limits per day. So, for a suggested daily dosage of 10 g, these limits should be divided by ten to calculate the maximum allowable limits in the drug products in microgram per gram (µg/g). However, even though 10 g is a typical maximum daily dosage for drugs, we have no way of knowing in what quantities consumers use cannabis products. So, if larger or smaller quantities are being used these PDE limits will be different, based on the weight consumed. Furthermore, the mode of administration will also impact the regulated limit, so inhalation PDEs in most cases are significantly lower than the oral ones. In addition, the classification number will impact the frequency of testing with Class 1 and 2A metals of higher priority than the Class 2B and 3 metals. In fact, the classification can offer clues as to what elements from this list would be worthy of inclusion in an expanded panel to regulate cannabis and hemp consumer products. For example, Class 1 and 2A would warrant inclusion in any regulated panel, whereas Class 2B metals would likely not be required at all, because they are not used in the cannabinoid manufacturing process. On the other hand, Class 3 metals may not be required for all oral products but would definitely be required for inhalation products such as vaping devices.

Final Thoughts

Part 1 was essential background information to the topic of this review article. Part 2 of the column takes a closer look at some of the evidence in the public domain, which underscores that the industry does not have a thorough understanding of heavy metal contaminants in consumer products which could support the measurement of a wider panel of elemental contaminants in cannabis materials.

Further Reading

(1) Marijuana Policy by State https://www.mpp.org/states.

(2) Thomas, R., The Importance of Measuring Heavy Metal Contaminants in Cannabis and Hemp, Analytical Cannabis, 2021, https://cdn.technologynetworks.com/ac/Resources/pdf/the-importance-of-measuring-heavy-metal-contaminants-in-cannabis-and-hemp-312957.pdf

(3) Pruyn, S.A.; Wang, Q.; Wu, C.G.; and Taylor, C.L., Quality Standards in State Programs Permitting Cannabis for Medical Uses, Cannabis and Cannabinoid Research, 2022, https://doi.org/10.1089/can.2021.0164

(4) United States Pharmacopeia (USP) General Chapter <232> Elemental Impurities in Drug Products and Chapter <2232> Elemental Contaminants in Dietary Supplements, https://www.usp.org/chemical-medicines/elemental-impurities-updates

(5) FDA Approves First Drug Comprised of an Active Ingredient Derived from Marijuana to Treat Rare, Severe Forms of Epilepsy, FDA News Release, 2018, https://www.fda.gov/news-events/press-announcements/fda-approves-first-drug-comprised-active-ingredient-derived-marijuana-treat-rare-severe-forms

(6) ICH Guideline Q3D on Elemental Impurities (R1), European Medicine Agency https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-32.pdf

(7) USP Draft Monograph for CBD, Pharmacopeial Forum (PF) 48(1), January 2022, https://www.gmp-compliance.org/gmp-news/usp-draft-monograph-for-cbd

(8) Thomas, R. Regulating Heavy Metals in Baby Food: The Challenges of Food Manufacturers and the FDA Being on the Same Page, AP Column, Spectroscopy Magazine, 2021, 36 (7), 10-16, https://www.spectroscopyonline. com/view/regulating-heavy-metals-in-baby-food-the-challenges-of-food-manufacturers-and-the-fda-being-on-the-same-page

(9) FDA Takes Steps to Ensure Safety of Cinnamon Products Sold in the US, FDA News Release, 2024, https://www.fda.gov/news-events/press-announcements/fda-takes-steps-ensure-safety-cinnamon-products-sold-us

(10) Thomas, R.; Destefano, A. Understanding Sources of Heavy Metals in Cannabis and Hemp: Benefits of a Risk Assessment Strategy, Analytical Cannabis, https://www.analyticalcannabis.com/articles/how-to-better-understand-sources-of-heavy-metal-contaminants-in-cannabis-part-1-what-can-we-learn-313862

(11) United States Pharmacopeia General Chapter <233> Elemental Impurities – Procedures: Second Supplement to USP 38–NF 33, 2015, https://www.usp.org/chemical-medicines/elemental-impurities-updates

ABOUT THE GUEST COLUMNIST

ROBERT (ROB) THOMAS is the principal scientist at Scientific Solutions, a consulting company that serves the educational needs of the trace element user community. He has worked in the field of atomic and mass spectroscopy for almost 50 years, including 24 years for a manufacturer of atomic spectroscopic instrumentation. Rob has written over 100 technical publications, including a 15-part tutorial series entitled, *A Beginner's Guide to ICP-MS*. He is also the editor and frequent contributor of the "Atomic Perspectives" column in *Spectroscopy* magazine, as well as serving on the editorial advisory board of *Technology Networks*. In addition, Rob has authored six textbooks on the fundamental principles and applications of ICP-MS. His most recent book is entitled *A Practical Guide to ICP-MS and Other AS Techniques*, which was published in September 2023. Rob has an advanced degree in analytical chemistry from the University of Wales, UK, and is also a Fellow of the Royal Society of Chemistry (FRSC) and a Chartered Chemist (CChem).

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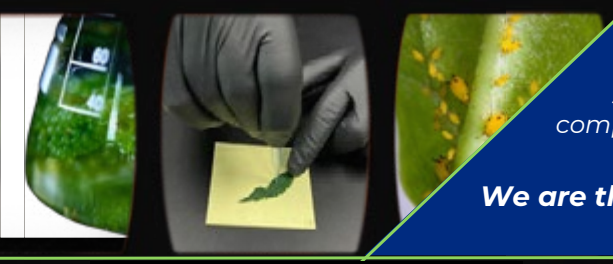

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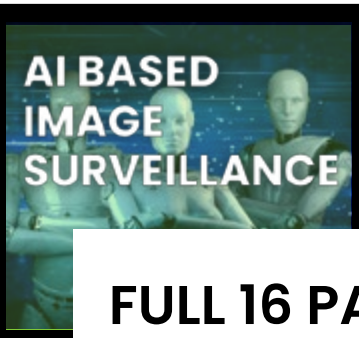

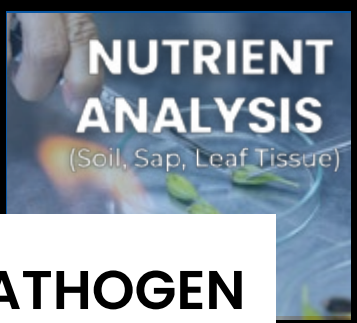

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Extraction Tech Inspires New Product Development

By ***David Hodes***

From simple DIY to lessons learned from mainstream agriculture to chemical discoveries, extraction techniques are driving more product innovation.

The search to isolate more and more cannabinoids is driving extraction development. That's been a trend for years (1).

But there is also a search for better methods of extraction (read: cleaner, greener) and more focus on extracting specific cannabinoids, as their value to human wellness is explored. Newer concentrate products, such as badder and cured resin—made using a solvent within a pressurized closed-loop extraction system—are showing up at dispensaries. They are among the top sellers in the California, Oregon, and Nevada markets (2).

The *Journal of Cannabis Research* points to the use of extraction methods such as ultrasonic-assisted, microwave-assisted, supercritical fluid, and pressurized liquid extraction processes as greener options than conventional extraction processes, because they reduce the need for synthetic and organic solvents, cut down on operational time, and produce a better-quality extract with a higher yield (3). Less solvent is used, and extraction times are reduced from conventional methods.

That's all good for the recreational market. But the potential recent rescheduling of cannabis from Schedule I to Schedule III, announced in April 2024, will drive the search for a reproducible, pharmaceutical-quality cannabis product that will drive further innovation.

This installment of the "Tech Innovations" column will discuss new methods and lab processes to do more targeted cannabis extraction. There is also a growing need to accurately measure homogeneity in the cannabis product, especially for medicinal cannabis, and that begins in the extraction process.

Sustainability and Extraction

Extraction technology in the cannabis industry is becoming part of the global sustainability efforts, with the goal of developing medicinal preparations from the biomass of cannabis. That biomass extraction technology is becoming standard operating procedure for mainstream cash crops, with interesting product payoffs. But the road to getting there in the cannabis industry is full of obstacles.

The amount of research into cannabis waste is extremely small relative to the research into cannabis itself, in part because of the need to control that biomass from getting into the black market. Cannabis biomass has to be destroyed or otherwise disposed of (landfill, composting, anaerobic digestion) instead of recovered as a resource (4).

Waste valorization (5) has been a research interest of Josh Katz, senior research and development scientist at Trulieve (6). "We grow all of this biomass," Katz explained. "We reduce the volume of the mass by a certain amount, but it's still a gigantic amount of biomass that we either have to throw away or compost or whatever. There's a lot of interesting chemistry out there that can take this spent material and digest it into commercially interesting products that the cannabis industry hasn't even thought of."

The biomass of cannabis are the so-called non-utilized components of the plant, such as the roots, which can be rich in polyphenols, and other bioactive compounds, which can be used for health promoting products.

To get an idea of what can be created through extraction of biomass, the cannabis industry should take note of how it works for some mainstream cash crops.

The annual yield of banana peels is 60 million tons; orange peels 10 million tons; avocado pits and peels 4 million tons; and wine by-products more than 14.6 million tons, according to researchers (7).

PeelPioneers, in the Netherlands processes citrus peels into oil, D-limonene. Garden of Natural

Solution Co., Ltd. uses an ultrasonic extraction method to produce citrus peel extract with antioxidant effects as a cosmetic ingredient (8). Lianyuan Kangbiotech Co., Ltd. extracts various fruits and fruit peels to obtain sweetener, oils, and polyphenols (9).

Keracol, a company formed by the University of Leeds (10), extracts resveratrol, a natural molecule found in the outer skins of red grapes, which is an antioxidant and known to have protective anti-ageing properties.

Biomass extraction progress is coming to cannabis. There is an extraction method for working with biomass that comes from Milestone on their Ethos platform (11). "It doesn't even extract cannabinoids," Katz said. "It's a terpene extractor. It allows you to take flower immediately after harvest, extract the terpenes out of it, dry the biomass out, re-extract the cannabinoids and you can basically make a vape cart out of the biomass of flower that was harvested yesterday." They have used it in a couple of their products so far, he said. "From a dollars and cents point of view, it's a substantially cheaper route to cannabis-derived terpenes than CO_2 extraction is. Throughputs are comparable, and the total terpene content in what comes out is higher than CO_2 extraction. It uses a pretty interesting strategy for getting the terpenes out of material that is not unique to cannabis by any stretch of the imagination."

Aside from biomass, another extraction method in the sustainable camp is pressurized liquid extraction (PLE) (12), a high-throughput and green extraction technique for the sustainable extraction of bioactive compounds from natural sources.

PLE—also called accelerated solvent extraction (ASE), pressurized fluid extraction (PFE), pressurized hot solvent extraction (PHSE), high-pressure solvent extraction (HPSE), high-pressure high-temperature solvent extraction (HPHTSE), and subcritical solvent extraction (SSE) (13)—is one method of extraction that is easy to use and efficient, using elevated temperatures and moderate to high pressures.

PLE works with a reduced amount of solvent, with the ability to extract a wide range of compounds—ideal for a plant like cannabis where there are more than 550 chemical compounds in cannabis, with more than 100 phytocannabinoids being identified in addition to tetrahydrocannabinol (THC) and cannabidiol (CBD) (14).

Compared to a conventional extraction performed at ambient pressure, the PLE high pressure maintains the solvent in liquid-phase. This improves mass transfer of the extraction by increasing the solubility and decreasing limiting factors such as viscosity and surface tension.

Low Tech to High Tech Review

As new extraction ideas and technologies gain traction, it's important to understand that simple THC extraction and infusion into an edible product arose from an at-home DIY process nearly anyone with basic kitchen know-how can whip up.

First important point to know is that preparing cannabis infused foods is not a simple matter of mixing in crushed cannabis to a brownie mix, for example, and loading that mixture into the oven.

The reason is that THC is not water-soluble, and the human body is composed of up to 60% water (15). But THC is fat soluble and can bind to fat molecules, such as those in oils and other fatty ingredients. THC oil can be mixed or baked into product such as a brownie or cake by making it bind with an oil—generally olive oil, butter, or coconut oil.

To begin the extraction process and get the THC to bind with oil, the cannabis is first ground up, then put on a baking sheet and baked at 230 °F for about an hour to decarbolize it and to activate the compounds (THC and CBD). It is then taken out and allowed to cool.

A separate glass jar with a lid is then put into a large pot. The pot is filled up halfway with water. The now decarbed cannabis is put into the jar, the jar is filled up with the chosen oil nearly to the top, and the lid is screwed on fairly tightly. Then the water in the pot is heated to about 190° F and left to simmer for about three hours. It's left to cool.

The decarbed cannabis is then filtered out of the oil using some sort of strainer (cheesecloth works), and the oil is poured into a container for future use as a cooking oil to make any THC-infused meal or treat.

Extraction from flower into a dab is also a simple procedure. There are YouTube videos demonstrating how anybody can extract rosin using a simple hair straightener and some parchment paper (16). And old tech is the foundation to some new cannabis extraction tech. One example is short path distillation (17). A short path distillation looks very similar in execution to what most people imagine to be a simple distillation apparatus, Katz said. "You have a big flask, you're heating it. It's boiling, compounds are evaporated. You get a vapor, and the vapor contains a mixture of what was in what you started boiling. The composition of that vapor will change over the course of the distillation. And when I say it changes, I mean it has higher or lower relative fraction of the thing that you're interested in, for example, the cannabinoids."

Short path distillation is a dynamic process, starting roughly at room temperature, then heating up the cannabis. "The more volatile things come off first, such as any residual

solvents, and the terpenes. When they stop coming over, there's usually overlap between the first part of the cannabinoids coming over and the last part of the terpenes coming over," Katz mentioned. "Part of the skill of extraction is knowing when to make the cut at the overlap."

A wiped film evaporator (18) is a variation of short film distillation that reduces oil exposure to heat. Wiped-film short path molecular distillation is common in the cannabis industry for separating cannabinoids from terpenes and heavy compounds. It's a process to get cannabinoid-rich distillate from the molecular distillation process without affecting cannabinoid quality.

"With the wiped film distillation process, you have basically set your parameters so that only the thing that you're interested in, distills over," Katz explained. "Everything that is more volatile than that will get caught in a cold trap. Anything less volatile than that will get passed on to the residue. So wiped film evaporators are an industrialized approach to short path evaporation. A wiped film evaporator allows you to process a lot more oil a lot faster."

Another high-tech process is the use of a microwave (MW) reactor developed by Ethos Lean specifically for the decarboxylation of the acidic cannabinoids in cannabis inflorescences prepared for extraction into oil. Both of the steps to produce the oily extract with the MW system were carried out in the Ethos Lean's cavity with special accessories: a rotating drum for the decarboxylation process and a glass reactor with a stirrer for the extraction step, according to an article in *Sustainable Chemistry and Pharmacy* (19).

In collaboration with a hospital pharmacy, the efficiency of the MW device was evaluated by comparing the results obtained with those of exhaustive decarboxylation in a conventional oven and ethanol extraction. A comparison was also made with conventional procedures in olive oil. "Thanks to the rotating drum, which is sensitive to dielectric heating, the complete and homogeneous decarboxylation of phytocannabinoids was rapidly achieved (30 min, 120 °C) without releasing the characteristic intense odor into the laboratory," the article concluded.

Other New Extraction Methods

Another new method of extraction was discussed at the 2024 Cannabis Science Conference by Anthony Repay, Laboratory Director of Method Testing Labs (20), involving the use of polysorbate 80 to extract cannabis and distill it into solution.

Polysorbate 80 is a synthetic compound that is commonly used as an emulsifier in a variety of foods, cosmetics, and pharmaceutical products. It's made from ethylene oxide, which is petroleum-based compound. "Why on earth would we go with the cosmetics method when we're dealing with something that is inhaled?" Repay said. "It's about applicable methods, and things that are similar."

He demonstrated the mixing process via a video during his presentation, showing where there was a significant association with polysorbate 80 percentage and cannabinoid concentration in a 1-in-10 dilution. "So as the relative percentage of polysorbate 80 in the solution increases, there's a corresponding increase in the amount of Delta-9 THC extracted," he explained. "Based on this data, we can conclude that there's a statistically significant relationship between the concentration of polysorbate 80 and the extraction of Delta-9 THC, confirming the success of the extraction process."

"For me, I think we need to get to a point where we get to an extraction efficiency and really see where it starts to tail off," Repay expressed. "But I'd love to see at what level we start to see a true extraction efficiency where it comes into a linear kind of an algorithmic growth," he mentioned. "The microbes, like *Aspergillus flavus* or *E. coli* or others, showed no significant impact by increasing concentration of polysorbate 80. At the end of the day, I think we all agree that science needs more data points."

Schedule III and Extraction Tech

The news coming down from the US Drug Enforcement Administration (DEA) in late April 2024 about rescheduling cannabis (21) appeared at first to be a shot in the arm for the industry. While it is generally welcomed news, and practically speaking, is likely to be years away from implementation, it will cause tech innovators from cultivators to extractors to keep a wary eye on what it means to their operations, and to the designs of the processors and equipment they use.

Conclusion

With a rescheduled cannabis, making compliant cannabis products using one of the currently available extractors could be a dodgy prospect if science, the regulatory process, and physicians who may soon be able to prescribe cannabis, try to understand different medical cannabis extract products. "Unfortunately, the labs are going to continue to be in a position where they have to make a choice between maintaining scientific integrity, or doing what they need to do to chase regulations that may or may not make sense, but in a way to keep their doors open," explained Susan Audino, a chemist/chemometrician and independent consultant to chemical and biological laboratories, during the 2024 Cannabis Science Conference which took place in Kansas City, Missouri. "'So how do I cut corners to meet these regulations that don't make

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any sense and still maintain a customer base so I can be not just a profitable business, but an open business?' I see that conflict is going to continue."

"Opening up this opportunity for research will begin to provide more data to support the contentions from the medical field right now that are using cannabis at any level," Audino said. "Right now, we know that it works. But if I asked to see the empirical data to demonstrate why and how it works, there's been a teeny tiny amount of information out there, if any. So, I hope rescheduling will open up the opportunity for better research to improve the vast knowledge of empirical data to help support what we've been working with and knowing for a long time."

But there is still an issue about how regulators do their job right now.

For example, with Delta-8, regulators are looking at the input—what is left over from the synthesis of Delta-8—not the output, Audino said. "That's the detrimental part to the regulatory structure and the interface between regulations and laboratory testing," Audino explained. "They have to be in alignment. Regulations need to be founded and justified by appropriate science, not the other way around. Because right now science is chasing its tail."

"We don't know what is going to be rescheduled," Katz said. "Because Delta-9 THC is a tetrahydrocannabinolic acid (THCA). It's going to be really hard to say if we should change our workflows until we know what is actually going to get rescheduled. I understand why everybody's up in arms right now, because it seems like the sluice gate has been open. But I just caution people not to get too squirrely."

References

(1) Hodes, D. New Extraction Technologies Lining Up to Be Game-Changers. *Cannabis Science and Technology*. **2020**, 3 (4), 14–27. https://www.cannabissciencetech.com/view/new-extraction-technologies-lining-be-game-changers.

(2) Best Selling Cannabis Concentrates in California https://www.headset.io/the-best-selling-cannabis-products/california-concentrates.

(3) Lazarjani, M.P., Young, O., Kebede, L. et al. Processing and Extraction Methods of Medicinal Cannabis: A Narrative Review. *J Cannabis Res*, **2021**, 3, 32. https://doi.org/10.1186/s42238-021-00087-9

(4) Baroutian, S.; Bror, R.; Randhawa, P.; Robertson, K.; Star, C. Opportunities and challenges in waste management within the medicinal cannabis sector. *Industrial Crops and Products*, **2023**, 197. https://doi.org/10.1016/j.indcrop.2023.116639

(5) Waste Valorization https://www.aiche.org/topics/energy/waste-valorization. (accessed April 30, 2024).

(6) https://www.linkedin.com/in/katz-jd00/ (accessed April 30, 2024).

(7) Laibach, N, Chapter 5. Extraction of Valuable Components From Waste Biomass. Waste to Food. 2022, 147-168. https://doi.org/10.3920/978-90-8686-929-9_5

(8) http://www.naturalsolution.co.kr. (accessed April 30, 2024).

(9) https://www.kangbiotech.com (accessed April 29, 2024).

(10) https://www.keracol.co.uk/ (accessed April 29, 2024).

(11) https://www.milestonesci.com/microwave-extraction-systems/ (accessed April 29, 2024).

(12) Alvarez-Rivera, G.; Ballesteros, D.; Bueno, M.; Ibañez, E.; Mendiola, J.; Chapter 13 - Pressurized Liquid Extraction. *Handbooks in Separation Science*, **2020**, 375-398. https://doi.org/10.1016/B978-0-12-816911-7.00013-X

(13) Barp, L.; Moret, S.; Visnjevec, A. Pressurized Liquid Extraction: A Powerful Tool to Implement Extraction and Purification of Food Contaminants. *Foods*, **2023**, 12 (10), 2017; https://doi.org/10.3390/foods12102017

(14) Parker, L.; Rock, E. Constituents of Cannabis Sativa. *Adv Exp Med Biol*, **2020**, 1-13. doi: 10.1007/978-3-030-57369-0_1

(15) https://www.usgs.gov/special-topics/water-science-school/science/water-you-water-and-human-body#:~:text=Up%20to%2060%25%20of%20the,bones%20are%20watery%3A%2031%25. (accessed April 30, 2024).

(16) https://www.youtube.com/results?search_query=rosin+extraction+and+simple+hair+straightener

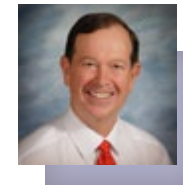
(17) https://precisionextraction.com/2021/05/guide-to-short-path-distillation/ (accessed May 1, 2024).

(18) Addo, P.; Bilodeau, S.; Gladu-Gallant, F.; Lefsrud, M.; MacPherson, S.; Orsat, V.; Paris, M.; Sagili, S. Optimization of Wiped-Film Short Path Molecular Distillation for Recovery of Cannabinoids from Cannabis Oil Using Response Surface Methodology. *Industrial Crops and Products*, **2023**, 195. https://doi.org/10.1016/j.indcrop.2023.116442

(19) Barge, A.; Binello, A.; Carnaroglio, D.; Cravotto, G.; DiFranco, M.; Gandlevskly, N.; Rosso, E.; Visinoni, F. A New Prototype Reactor for the Fast Microwave-Assisted Decarboxylation and Extraction of Cannabinoids in Olive Oil from Cannabis Inflorescences. *Sustainable Chemistry and Pharmacy*, **2023**, 36, https://doi.org/10.1016/j.scp.2023.101303

(20) https://www.linkedin.com/in/anthony-repay-m-s-2b201696/ (accessed May 10, 2024).

(21) Miller, Z.; Goodman, J.; Mustian, J.; Whitehurst, L. US Poised to Ease Restrictions on Marijuana in Historic Shift, But It'll Remain Controlled Substance https://apnews.com/article/marijuana-biden-dea-criminal-justice-pot-f833a-8dae6ceb31a8658a5d65832a3b8. (accessed May 4, 2024).



ABOUT THE COLUMNIST
DAVID HODES has written for many cannabis publications, and organized or moderated sessions at national and international cannabis trade shows. He was voted the 2018 Journalist of the Year by Americans for Safe Access, the world's largest medical cannabis advocacy organization.

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Cannabinoid Ethers: Identification, Binding Interactions, and their ADME Properties

BY MAITE L. DOCAMPO-PALACIOS, GIOVANNI A. RAMIREZ, TESFAY T. TESFATSION, AND WESTLEY CRUCES

New cannabinoid analogs are constantly being synthesized and marketed to the public as ways to increase psychotropic activity or to remain competitive within the market. The issue with new analogs is the lack of proper testing and characterization—companies are misrepresenting what they sell, and customers are consuming unknown research compounds. Our group performed in-silico docking in CB1 and CB2 receptors, drug metabolism, and pharmacokinetics (DMPK) studies of methyl-, ethyl-, and pivaloy- ether cannabinoids analogs. A dataset of eight cannabinoid ethers was docked to the model to further analyze key interacting residues on the CB1 and CB2 receptors. In addition, the methyl ether derivatives of Delta 8-tetrahydrocannabinol (D8-THC) and hexahydrocannabinol (HHC) were synthesized and the full nuclear magnetic resonance (NMR) characterization of these compounds was achieved.

1. Introduction

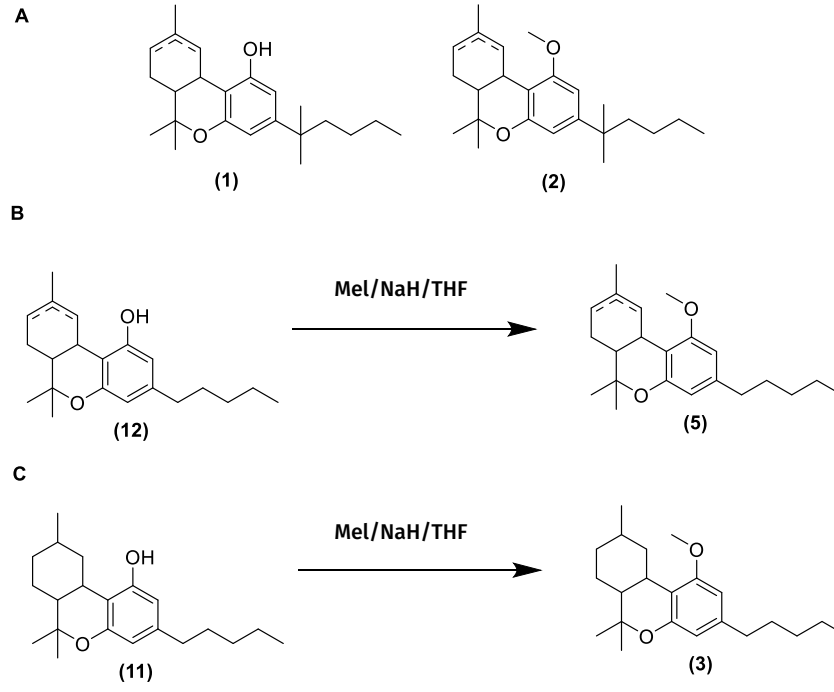
Within the volatile cannabinoid market, the need to stay relevant and competitive grows: new cannabinoid derivatives are being introduced to circumvent regulations or to increase efficacy, with safety being called to attention. Pre-clinical testing of compounds can be expensive and without the correct staff to interpret and present results, can make the data null. Computer-aided in-silico techniques that can perform ADME (absorption, distribution, metabolism, and excretion) computation (1-4), can assist in cost-effective analysis to generalize indicative characteristics of the tested compounds to pose health hazards (5). Some popular cannabinoids such as Delta 8-THC, Delta 9-THC (D9-THC), and HHC are popping up with prodrug moieties, which include methyl, ethyl, and pivaloyl (PV) prodrug moieties being attached to

the essential hydroxy group within the aromatic ring that contributes to the important cannabimimetic activity (6). The prodrug moieties are typically used to create a stronger "psychedelic high" specifically the -O acetate (7), which does not increase smokable highs but might delay metabolism and survive the first pass leading to increased "high" orally, though nothing substantial. The motif is followed through the changing of the groups, which can enact different effects, as well as delay metabolism increasing and modulating the "high" effects. Cannabinoid derivatives in which the hydroxyl group in the resorcinol core was removed or replaced by an alkyl chain to generate an ether group significantly decreased ligand binding to CB1 exhibiting better selectivity for the CB2 receptor. Phenol hydroxy (PH) is believed to participate in the hydrogen bonding interaction with the CB1 cannabinoid receptor (8). For example, tested dimethyl compounds such as 6,6,9-trimethyl-3-(2-methylhexan-2-yl)-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (**1-Figure 1**) has significant affinities for both CB1 and CB2 receptor, showing 0.83 nM and 0.49 nM as affinity constant (Ki) of CB1 and CB2, respectively. However, its corresponding methyl ether analog, 1-methoxy-6,6,9-trimethyl-3-(2-methylhexan-2-yl)-6a,7,10,10a-tetrahydro-6*H*-benzo[c]chromene (**2-Figure 1**) presented high CB2 selectivity with K*i* (CB1) >20,000 and K*i* (CB2) =19 nM (8,9). This led us to investigate several other linkers on the market attached to HHC and THC scaffolds as the primary focus due to their upcoming popularity within the market and identify their binding relationships as well as their ADME characteristics to help shine a light on an ever-changing market and provide a semblance of information on potential toxicities if any present.

2. Materials and Methods

General Remarks: All commercial acids and solvents were American Chemical Society (ACS) and high-performance liquid chromatography (HPLC) grade, respectively. Acids and solvents were all purchased from Sigma Aldrich and were used without further purification. Cannabidiol (CBD) isolate was purchased in bulk from GVB Biopharma. Quantification was performed using high-performance liquid chromatography (HPLC) on an Agilent 1100 series equipped with Next Leaf CBX for Potency C_{18} RP column 2.7uM, 150x4.6mm. MeOH with CH_2O_2:H_2O with CH_2O_2 is used for quantification of reactions. Reagents and solvents were ACS and HPLC grade, respectively. Reagents and solvents were all purchased from Sigma Aldrich and were used without further purification. CBD isolate was purchased in bulk from Columbia Basin Bioscience, OR. Quantification was performed using high-performance liquid chromatography (HPLC) on an Agilent 1100 series equipped with Next Leaf CBX for Potency C_{18} RP column 2.7uM, 150x4.6mm. MeOH with CH_2O_2:H_2O with CH_2O_2 is used for quantification of reactions. Computation chemistry was performed according to methodology from Cruces et al. (10).

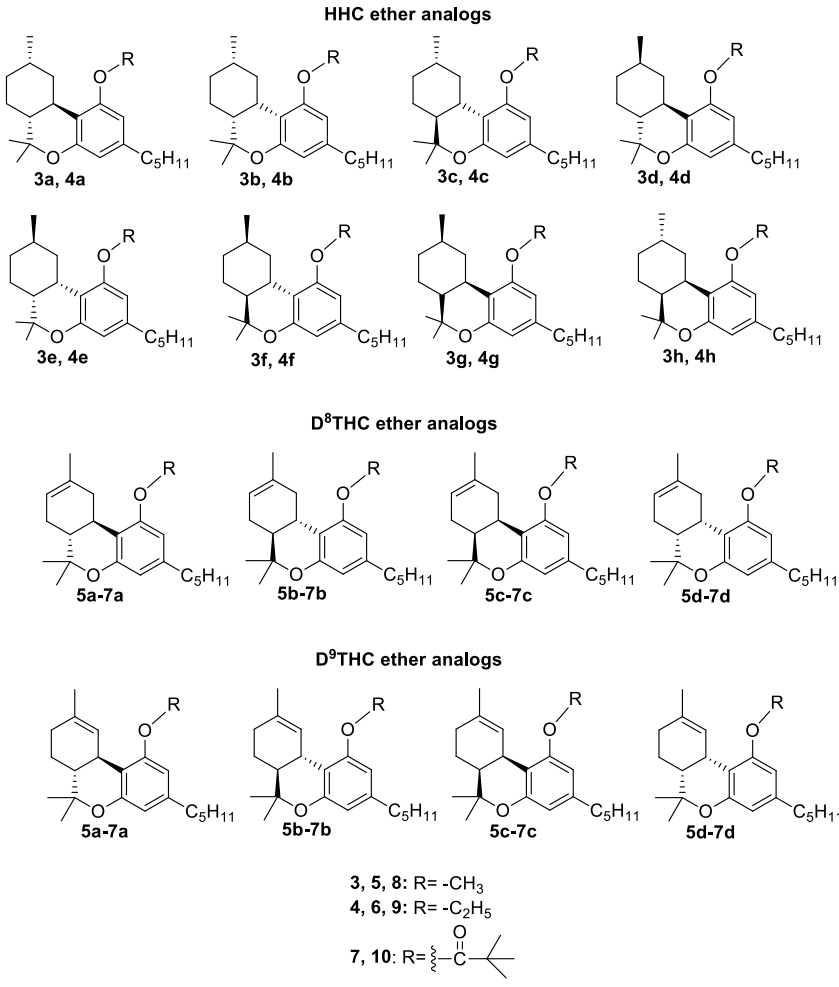
Figure 1: A) Structures of 6,6,9-trimethyl-3-(2-methylhexan-2-yl)-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-1-ol (1) and 1-methoxy-6,6,9-trimethyl-3-(2-methylhexan-2-yl)-6a,7,10,10a-tetrahydro-6H-benzo[c]chromene (2). B) Is the chemical transformation of D8/D9-THC into the methyl ether analog. C) Is the chemical transformation of HHC into the methyl ether analog.



2.1 Proteins and Ligands Preparation

All molecular docking experiments were achieved on CybertronPC CLX 13th Gen Intel(R) Core(TM) i9-13900KF at 3.00 GHz comprising 24 computing cores. Schrödinger Release 2023-3: Glide software was used as the docking program (11,12). Crystal structures of CB1, CB2, were retrieved from the RCSB Protein Data Bank. CB_1 [(PDB: 7V3Z]/, CB2 [(PDB: 5ZTY)]. The proteins were prepared using a protein preparation workflow tool on Schrödinger Protein Preparation Wizard (3,4). The external water molecules and ions were removed. Polar Hydrogens were added. Missing

Figure 2: HHC, D9-THC, and D8-THC ether derivatives that were screened.



side chains were filled using Epic and PROPKA. Het states were generated at pH 7.4 (+/- 2.0). Heavy atoms converged to RMSD 0.30Å. 3D structures of cannabinoids and hydrogenated cannabinoids were established in 2D sketcher which was then exported as an SDF file and imported and prepared using LigPrep, to form 3D conformers, including the various 3D chiral conformations.

2.2 In Silico Molecular Docking

The grid parameter was generated covering the CB1 pockets for (PDB:7V3Z) [-42.91, -163.58, 306.7] for x,y,z coordinates. The ligand diameter midpoint box follows a 10Å x 10Å x 10Å x,y,z dimension. The grid parameter was generated covering the CB2 pockets for (PDB:5ZTY) [9.09, -0.17, -55.72] for x,y,z coordinates. The ligand diameter midpoint box follows a 10Å x 10Å x 10Å x,y,z dimension.

2.3. Prediction of ADMET Properties

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) (13) properties of the cannabinoids were performed using QikProp version 4.4 integrated into Maestro (Schrödinger, LLC, New York, 2015) which predicts the widest variety of pharmaceutically relevant properties: QPlogS (predicted aqueous solubility), QPlogHERG (Predicted IC_{50} value for blockage of HERG K+ channels), QPPCaco (predicted apparent Caco-2 cell permeability. Caco-2 cells are a model for the gut-blood barrier), QPlogBB (predicted brain/blood partition coefficient), and % Human Oral Absorption (predicted human oral absorption in gastrointestinal tract on 0 to 100% scale). The calculated physicochemical descriptors are displayed in Table 4-SI. QikProp bases its predictions on the full 3D molecular structure and the global minimum energy conformer of each compound was used as input for ADMET properties.

2.4. Characterization Data

For the NMR analysis, the synthesized compounds were dissolved in $CDCl_3$ (99.8 % atom D; Cambridge Isotope Laboratories, Andover, MA) and then transferred to a normal NMR tube. The proton and carbon nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR, respectively) spectra were recorded using on a 500 MHz Bruker AVANCE II system at 25°C. 1H and 13C data sets were analyzed to yield complete 1H and 13C peak assignments. Coupling constants (*J*) are given in Hertz (Hz), and spin multiplicities are shown by the following symbols: s (singlet), d (doublet), dd (doublet of doublets), td (triplet of doublets), dt (doublet of triplets), dq (doublet of quartets), tt (triplet of triplets), and m (multiplet).

3. Experimental

General Procedure for the Methylation of Cannabinoids

To a solution of NaH 60% in mineral oil (152 g, 3.79 mol) in dry dimethylformamide (DMF) (2000 ml) cooled in an ice bath was added the corresponding cannabinoid derivative (3.1 mol) in DMF (2000 ml) dropwise as the reaction is very exothermic. The reaction was stirred in the ice bath until bubbles subsided, and then methyl iodide (236 ml, 3.79 mol) was added, stirring overnight at room

Table I: Results of threshold regression with hinge effect

Compound	Interacting Residues	Interaction Types	Docking Score
		5ZTY	
AM10257	*Phe87, Phe116 Phe183, Trp405, Trp194, H2O*	*π-stacking, π-stacking, π-stacking, π-stacking, H-bonding, H-bonding*	*0.00*
Me HHC **(3a)**	Phe183, His85, Trp194	π-π stacking, π-π stacking, π-π stacking	-10.770
Me HHC (**3b**)	Phe183	π-π stacking	-9.091
Me HHC (**3c**)	Phe183	π-π stacking	-9.580
Me HHC **(3d)**	No interaction		
Me HHC (3e)	Phe183, Phe87	π-π stacking, π-π stacking	-9.672
Me HHC **(3f)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.579
Me HHC **(3g)**	Phe183	π-π stacking	-9.874
Me HHC **(3h)**	Trp194	π-π stacking	-8.964
Et HHC **(4a)**	Phe183	π-π stacking	-9.266
Et HHC **(4b)**	Phe183	π-π stacking	-9.321
Et HHC **(4c)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.849
Et HHC **(4d)**	No interaction		
Et HHC **(4e)**	Phe183	π-π stacking	-9.137
Et HHC **(4f)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.979
Et HHC **(4g)**	No interaction		
Et HHC **(4h)**	No interaction		
R-HHC	No interaction		
S-HHC	Phe183, Trp194	π-π stacking, π-π stacking	-7.121
Me D8THC **(5a)**	Phe183, Phe87	π-π stacking, π-π stacking	-10.167
Me D8THC **(5b)**	Phe183, Phe87	π-π stacking, π-π stacking	-10.167
Me D8THC **(5c)**	Phe183	π-π stacking	-9.684
Me D8THC **(5d)**	His85	π-π stacking	-9.558
Et D8THC **(6a)**	Phe183	π-π stacking	-9.425
Et D8THC **(6b)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.756
Et D8THC **(6c)**	No interaction		
Et D8THC **(6d)**	His85	π-π stacking	-8.685
PIV D8THC **(7a)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.712
PIV D8THC **(7b)**	No interaction		
PIV D8THC **(7c)**	No interaction		
PIV D8THC **(7d)**	Phe183	π-π stacking	-9.415
D8THC	No interaction		
Me D9THC **(8a)**	No interaction		
PIV D8THC **(7d)**	Phe183	π-π stacking	-9.415
D8THC	No interaction		
Me D9THC **(8a)**	No interaction		
Me D9THC **(8b)**	Phe183	π-π stacking	-9.173

Compound	Interacting Residues	Interaction Types	Docking Score
Me D9THC **(8c)**	No interaction		
Me D9THC **(8d)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.288
Et D9THC **(9a)**	Phe183	π-π stacking	-9.642
Et D9THC **(9b)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.964
Et D9THC **(9c)**	No interaction		
Et D9THC **(9d)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.905
PVI D9THC **(10a)**	No interaction		
PVI D9THC **(10b)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.709
PVI D9THC **(10c)**	No interaction		
PVI D9THC **(10d)**	Phe183, Phe87	π-π stacking, π-π stacking	-9.341
D9THC	Phe87, Phe183	π-stacking, π-stacking	-7.147
7V3Z			
ORG27569	*Phe72, Phe170, Phe268, Ile169, Ser75, Lys94, Ser407, Ser505, His80*	*π-stacking, π-stacking, π-stacking, H-bonding, H-bonding, H-bonding, H-bonding, H-bonding, π-cation*	-
Me HHC **(3a)**	Phe170, Phe268	π-π stacking, π-π stacking	-6.710
Me HHC **(3b)**	Phe170, Phe268	π-π stacking, π-π stacking	-6.912
Me HHC **(3c)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.455
Me HHC **(3d)**	Phe170, Phe268	π-π stacking, π-π stacking	-8.561
Me HHC **(3e)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.643
Me HHC **(3f)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.201
Me HHC **(3g)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.109
Me HHC **(3h)**	Phe170, Phe268	π-π stacking, π-π stacking	-6.626
Et HHC **(4a)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.113
Et HHC **(4b)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.259
Et HHC **(4c)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.086
Et HHC **(4d)**	Phe170, Phe268	π-π stacking, π-π stacking	-8.399
Et HHC **(4e)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.879
Et HHC **(4f)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.566
Et HHC **(4g)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.137
Et HHC **(4h)**	Phe170	π-π stacking	-6.677
S-HHC	No interaction Type		
R-HHC	Phe170, Phe268	π-π stacking, π-π stacking	-9.146
Me D8THC **(5a)**	Phe268	π-π stacking	-6.724
Me D8THC **(5b)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.959
Me D8THC **(5c)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.714
Me D8THC **(5d)**	Phe170, Phe268	π-π stacking, π-π stacking	-6.942
Et D8THC **(6a)**	Phe268	π-π stacking	-4.174
Et D8THC **(6b)**	Phe170, Phe268	π-π stacking, π-π stacking	-8.193
Et D8THC **(6c)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.295

Compound	Interacting Residues	Interaction Types	Docking Score
Et D8THC **(6d)**	Phe170, Phe268	π-π stacking, π-π stacking	-8.229
PVI D8THC **(7a)**	Trp279	π-π stacking	-4.258
PVI D8THC **(7b)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.081
PVI D8THC **(7c)**	No interaction Type		
PVI D8THC **(7d)**	Phe170, Phe268	π-π stacking, π-π stacking	-7.031
D8THC	Phe268, Phe170	π-π stacking, π-π stacking	-6.095
Me D9THC **(8a)**	Lys178	π-π stacking	-6.364
Me D9THC **(8b)**	Phe170, Phe268	-π stacking, π-π stacking	-7.531
Me D9THC **(8c)**	Lys178	π-π stacking	-5.443
Me D9THC **(8d)**	Phe170, Phe268	-π stacking, π-π stacking	-7.760
Et D9THC **(9a)**	No interaction		
Et D9THC **(9b)**	Phe170, Phe268	π-π stacking, π-π stacking	-8.135
Et D9THC **(9c)**	Phe170, Phe268	π-π stacking, π-π stacking	-6.206
Et D9THC **(9d)**	Phe170, Phe268	π-π stacking, π-π stacking	-8.010
PVI D9THC **(10a)**	No interaction		
PVI D9THC **(10b)**	Phe170, Phe268	π-π stacking, π-π stacking	-6.765
PVI D9THC **(10c)**	No interaction		
PVI D9THC **(10d)**	Phe170, Phe268	π-π stacking, π-π stacking	-5.731
D9THC	Phe170, Phe268, Ser505	π-stacking, π-stacking, H-bonding	-9.497

temperature. HPLC after 18h showed no starting material. The reaction was quenched with water and extracted with dichloromethane (DCM). The organic layer was washed five times with water, dried over sodium sulfate, and concentrated in vacuo to give a dark red oil. The oil was purified by silica gel plug with hexanes: EtOAc (95:5) to afford the cannabinoid methyl ether.

1-methoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c] chromene methyl ether (3)

Yellow oil. 938 g (92% of yield).1H NMR (400 MHz, CDCl3) δ (ppm): 6.31 (1H, d, J=1.7 Hz, H2); 6.25 (0.70H, d, J=1.7 Hz, H4, R-HHC-Me: 70%); 6.23 (0.30H, d, J=1.7 Hz, H4, S-HHC-Me: 30%); 3.80 (2.04H, s, OCH3_8'-R-HHC-Me: 70%); 3,78 (0.94H, s, OCH3_8', S-HHC-Me: 30%); 3.02 (0.30H, dt, J=2.8 Hz, 11.0 Hz, H10a, R-HHC-Me: 70%); 2.86 (0.30H, dq, J=1.5 Hz, 2.8 Hz, 11.2 Hz, H10a, S-HHC-Me: 30%); 2.65 (0.30H, td, J=2.8 Hz, 11.2 Hz, H6a, S-HHC-Me: 30%); 2.52 (2H, td, J=2.8 Hz, 8.2 Hz, H1'); 2.43 (0.70H, td, J=2.8 Hz, 11.0 Hz, H6a, R-HHC-Me: 70%); 1.88-1.82 (1H, m, H10-1); 1.66-1.59 (3H, m, H9, H8-1, H7-1); 1.51-1.42 (1H, m, 10-2); 1.40-1.31 (8H, m, H7-2, H8-2, H2', H3', H4'); 1.15-1.07 (6H, m, CH3_6', CH3_7'); 0.97-0.89 (6H, m, CH3_5', CH3_9'). 13C NMR (100 MHz, CDCl3) δ (ppm): 159.1 (C1); 154.6 (C5); 142.5 (C3); 111.8 (C5a); 110.3 (C4); 103.2 (C2); 76.9 (C6); 55.8 (OCH3_8'); 49.4 (C6a); 39.3 (C10a); 36.1 (C10); 35.8 (C1'); 33.0 (C8); 31.8 (C2'); 30.9 (C3'); 29.7 (C9); 28.3 (C7); 27.9 (C4'); 22.8; 22.7 (CH3_6', CH3_7'); 19.1 (CH3_9'); 14.2 CH3_5').

1-methoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c] chromene (5)

Yellow oil. 915.1 g (90 % yield). 1H NMR (400 MHz, CDCl3) (ppm): 6.32 (1H, d, J=1.7 Hz, H2); 6.26 (1H, d, J=1.7 Hz, H4); 5.43 (1H, dd, J=4.8 Hz, 9.0 Hz, H10); 3.81 (3H, s, OCH3_8'); 3.16 (1H, dt, J=4.1 Hz, 11.0 Hz, 9.0 Hz, H10a,); 2.67 (1H, td, J=4.8 Hz, 11.0 Hz, H6a); 2.51 (2H, td, J=2.7 Hz, 8.2 Hz, H1'); 2.18-2.10 (1H, m, H8-1); 1.90-1.81 (1H, m, H8-2); 1.80-1.77 (2H, m, H7-1, H7-2); 1.71 (3H, s, CH3_9'); 1.61 (2H, tt, J=2.2 Hz, 8.2 Hz, H2'); 1.37; 1.10 (3H, s, CH3_6', CH3_7'); 1.35-1.33 (4H, m, H3', H4'); 0.90 (3H, s, CH3_5'). 13C NMR (100 MHz, CDCl3) δ (ppm): 159.1 (C1); 154.5 (C5); 142.6 (C3); 135.1 (C9); 119.4 (C10); 112.0 (C5a); 110.4 (C4); 103.2 (C2); 76.6 (C6); 55.3 (OCH3_8'); 45.2 (C6a); 36.4 (C10a); 36.2 (C1'); 32.0 (C8); 31.8 (C2'); 30.9 (C3'); 28.1 (C7); 27.7 (C4'); 23.7; 22.7 (CH3_6', CH3_7'); 18.6 (CH3_9'); 14.2 CH3_5').

4. Results and Discussion

4.1. Molecular Docking of Cannabinoid Ethers

Our team used a virtual screen of eight cannabinoid ethers to explore the binding interaction and binding energies

Table II: General ADME Bio Scores

Compound	MW	QPlogS[a]	QPlogHERG[b]	QPPCaco[c]	QPlogBB[d]	% Human Oral Absorption[e]
Me HHC **(3)**	330.509	-8.701	-4.169	9906.038	0.717	100
Me D8THC **(5)**	328.494	-9.006	-5.070	9906.038	0.672	100
Me D9THC **(8)**	328.494	-9.106	-5.303	9906.038	0.737	100
Et HHC **(4)**	344.536	-9.662	-4.373	9906.038	0.796	100
Et D8THC **(6)**	342.520	-9.196	-5.031	9906.038	0.786	100
Et D9THC **(9)**	342.520	-9.702	-5.003	9906.038	0.806	100
PVC D8THC **(8)**	398.584	-7.150	-5.007	5519.052	-0.052	100
PVC D9THC **(10)**	398.584	-7.582	-5.064	6040.273	-0.036	100
HHC	316.483	-6.709	-4.705	4524.042	-0.092	100
D8THC	314.467	-6.621	-4.821	4719.169	-0.073	100
D9THC	314.467	-6.708	-4.828	4350.853	-0.112	100

Range of 95% drugs: a: Predicted aqueous solubility [-6.5 to +0.5]; b: HERG K+ Channel Blockage (log IC50) [concern below –5]; c: Apparent Caco-2 cell permeability in nm/s [<25 poor; >500 excellent]; d: Predicted log of the blood/brain partition coefficient [-3.0 to +1.2]; e: Human Oral Absorption in GI [<25% is poor].

between cannabinoid ethers and CB1 (7V3Z) and CB2 (5ZTY) receptors. The compounds that were screened included HHC-OMe (**3**), HHC-ethyl ether (**4**), D8THC-OMe (**5**), D8THC-ethyl ether (**6**), D8THC-Pivaloyl-ether (**7**), D9THC- OMe (**8**), D9THC-ethyl ether (**9**), D9THC-Pivaloyl-ether (**10**), and their diastereomers (**Figure 2**). Using Jaguar to perform minimizations and calculate the density functional theory (DFT) for given scaffolds, the then minimized scaffolds were docked within 7V3Z and 5ZTY proteins that were prepared using the Schrödinger protein preparation workflow.

For protein 5ZTY, which is bound with AM10257 an antagonist of the CB2 receptor, the results show that all docked cannabinoid ethers interacted with the amino acid residues of this protein (**Table 1**), and the most common residues were Phe183 and Phe87 with ϖ-ϖ stacking interaction. The best docking score is -10.770 kcal/mol which corresponds to S-Me-HHC (**3a**) and the worst is -8.685 kcal/mol for Et-D8-THC (**6d**) (Table 1). In addition, Me-HHC (**3a** and **3h**) were the only compounds that exhibited an interaction with Trp194, which is a key residue in the binding pocket of the CB2 receptor. It is interesting to point out that the *R*-HHC (**3d**, **4d**) ethers and R-HHC did not display any interaction with the amino acids of the 5ZTY protein. Also, D8-THC methyl ether (**5a**) showed a good docking score (-10.167 kcal/mol), meanwhile, D9-THC methyl ether (**8a**) did not exhibit any interaction. For protein 7V3Z as a CB1 receptor with a negative allosteric modulator ORG27569 bound, the specific interactions among the docked cannabinoids and 7V3Z residues and the docking scores are disclosed in Table 1. The most important amino acids found in the binding pocket that interact with cannabinoids include Phe170 and Phe268 via ϖ-ϖ stacking. Most of the docked compounds displayed good docking scores between -8.561 (R-Me-HHC-compound **3d**) and -4.174 (Et-D8-THC-compound **6a**). It is remarkable to observe that the best docking scores were obtained for protein 5ZTY (CB2 receptor) compared to protein 7V3Z (CB1 receptor). We selected S-HHC methyl ether (**3a**) and D8-THC methyl ether (**5a**) as the compounds that revealed the best docking affinity for the CB2 receptor.

The use of in-silico software to identify the binding affinities allows us to hypothesize the effects of compounds prior to running in-vitro or in-vivo experiments to determine their physiological or behavioral effects. Experiments done from 1991-1992 using radioligand assays to determine effects of methyl ether cannabinoids, D9-tetrahydrocannabinol methyl ether (D9-THCME) was shown to have a Ki value of >10,000nM meaning the compound did not bind to CB1 receptor unless a large concentration was taken. The reference ligand to test the affinity was ^{3}H-CP-55,940, a ligand with high affinity for CB1 and CB2 at 0.58nM and 0.68nM respectively. The ligand is used in in-vivo experiments to mimic the effects of THC (14). Behavioral experiments were performed using D8-tetrahydrocannabinol methyl ether (D8-THCME), D9-THCME, and D8-THC. D8-THCME from the invitro studies had a 3200nM binding affinity with the tail flick and behavioral characteristics being completely diminished compared to the weaker D8-THC (15). The study also acknowledges that

Table III: NMR spectroscopic data for and 1-methoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromene (5)

	Compound 4		Compound 6	
Position	**1H-NMR (δ ppm, J Hz)**	**^{13}C-NMR (δ ppm)**	**^{1}H-NMR (δ ppm, J Hz)**	**^{13}C-NMR (δ ppm)**
1	-	159.1	-	159.1
2	6.31, d, *J*=1.7	103.2	6.32, d, *J*=1.7	103.2
3	-	142.5	-	142.6
4	6.25, d, *J*=1.7 (R-HHC, 70%) 6.23, d, *J*=1.7 (S-HHC, 30%)	110.3	6.26, d, *J*=1.7	110.4
5	-	154.6	-	154.5
5a	-	111.8	-	112.0
6	-	76.9	-	76.6
6a	2.65, td, *J*=2.8, 11.2 (S-HHC, 30%) 2.43, td, *J*=2.8, 11.0 (R-HHC, 70%)	49.2	2.67, td, *J*=4.8, 11.0	45.2
7	7-1:1.66-1.59, m 7-2: 1.40-1.31, m	28.3	7-1: 1.80-1.77, m 7-2: 1.80-1.77, m	30.9
8	8-1: 1.66-1.59, m 8-2: 1.40-1.31, m	33.0	8-1: 2.18-2.10, m 8-2: 1.90-1.81, m	32.0
9	1.66-1.59, m	29.7	-	135.1
10	10-1: 1.88-1.82, m 10-2: 1.51-1.42, m	36.1	5.43, dd, J=4.8, 9.0	119.4
10a	3.02, dt, *J*=2.8, 11.0 (R-HHC, 70%) 2.86, dq, *J*=1.5, 2.8, 11.2 (S-HHC, 30%)	39.3	3.16, dt, *J*=4.1, 11.0, 9.0	36.4
1'	2.52, td, *J*=2.8, 8.2	35.8	2.51, td, *J*=2.7, 8.2	36.2
2'	1.40-1.31, m	31.8	1.61, tt, *J*=2.2, 8.2	31.8
3'	1.40-1.31, m	30.9	1.35-1.33, m	28.1
4'	1.40-1.31, m	27.9	1.35-1.33, m	27.7
5'(CH3)	0.97-0.89, m	14.2	0.90, s	14.2
6', 7'(CH3)	1.15-1.07, m	22.8, 22.7	1.37, s; 1.10, s	23,7, 22.7
8' (OCH3)	3.80, s (R-HHC, 70%) 3,78, s (S-HHC, 30%)	55.2	3.81, s	55.3
9'(CH3)	0.97-0.89, m	19.1	1.71, s	18.6

through the use of rat and mouse testing the D8-THCME and D9-THCME are inactive and would need much higher doses to possibly create effects (15).

4.2. In Silico ADME Properties of Cannabinoid Ethers

Optimization of the ADME properties of the drug molecule is often the most difficult and challenging part of the whole drug discovery process. The ADME profile will also have a major impact on the likelihood of success of a drug.

The drug-likeness and physiochemical properties of cannabinoid ethers were analyzed via Maestro's QikProp Schrödinger software (11). The predicted ADMET properties and descriptors for the compounds are presented in **Table 2**. The aqueous solubility (QPlogS) is critical for the estimation of absorption and distribution of the compounds within the body and ranges between -7.150 and -9.702. These values were out of the recommended range and had poorer solubility than their analogs with the free OH group. The solubility of cannabinoids is a challenge due to their lipophilic character. QPlog-hERG is another parameter that is out of the recommended range for D8-THC and D9-THC ethers. It is predicted IC_{50} value for blockage of hERG K channels. Most other descriptors are within the recommended range by QikProp for 95% of known oral drugs. These results suggest that HHC-ethers exhibited acceptable physiochemical properties, however, D8-THC and D9-THC ethers can induce cardiotoxicity and result in arrhythmia (16-18), according to the hERG (Human Ether-a-go-go related gene) scores. Studies have been published showing HHC can cause a failed hERG but pass Nav/Cav, meaning although inhibition is reported of the hERG pathway there is no inhibition of the ion channels allowing for regular beating of the heart. CBD also has shown to cause issues with hERG and can cause arrythmia and cardiac adverse events, with the use of Schrödinger we can identify possible issues based off the structure of the compound and known medicinal chemistry principles with a score of -5 or higher possibly be concern for failing hERG and the need for further testing.

The predicted solubilities of the cannabinoids are well outside the calculated ranges, which proves that the cannabinoids are extremely lipophilic with no feasible solubility in water. The decreased solubility reduces the absorption, digestion, and metabolism of the compound if taken as pure compound with the bioavailability scores being abysmal (<5%). In studies the uptake of cannabinoids in fats, increases the bioavialability and affecting the absorption, digestion and metabolism of the cannabinoid, in an increased manner (19).

4.3 Synthesis and Characterization of HHC-Me and D8THC-Me

Our group was able to synthesize the methyl ether of HHC, starting with 6,9-dimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-*6H*-benzo[*c*]chromen-1-ol (**11**) using methyl iodide as methylation agent, and sodium hydride as base in tetrahydrofuran (THF) to obtain 1-methoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-*6H*-benzo[*c*]chromene (**3**) with excellent yields (Figure 1b-c).

HHC (**11**), the starting material, and HHC-OMe (**4**), the finished product, can be identified via ^{1}H-NMR and ^{13}C-NMR. ^{1}H-NMR: In the region between 3.5-4.0 ppm, compound **3** shows a singlet corresponding to the hydrogens of the methoxy group. This peak does not appear at the ^{1}H-NMR spectrum of HHC. ^{13}C NMR: For Me-HHC (**3**), in the region between 50-60 ppm appears a peak corresponding with the methyl carbon in the methoxy group, which does not appear in the HHC (**11**). Table 2 provides NMR spectroscopic data for the Me-HHC (**4**) and D9-THC-ME (**5**).

Our group also synthesized the methyl ether of THC, starting with 6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-*6H*-benzo[*c*]chromen-1-ol (**12**) using the procedure mentioned above to obtain 1-methoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[*c*]chromene (5) with excellent yields. THC (12), the starting material, and Me-D8THC (5), the finished product, could be identified by 1H-NMR and 13C-NMR. 1H-NMR: At 3.8 ppm, compound 5 shows a singlet corresponding to the hydrogens of the methoxy group. This peak does not appear at the 1H-NMR spectrum of THC. 13C-NMR: For Me-D8THC (5), in the region between 55-56 ppm appears a peak corresponding with the methyl carbon in the methoxy group, which does not appear in the THC (12). Table 2 above provides NMR spectroscopic data for the Me-D8THC (5).

5. Conclusion

Our team synthesized both the THC and HHC methyl ethers in high yield using NMR to verify the addition of the methyl ether, since HPLC can cause false identification of desired compound. Molecular docking studies show that the cannabinoid methyl ethers bind favorably to CB2 over CB1 similar to the literature. The best docking affinities were shown by S-HHC methyl ether and D8-THC methyl ether. ADME results show that these compounds have low aqueous solubility and a potential for hERG K+ Channel Blockage. The production of this data provides an in-silico assessment of the binding interactions of these pro-drug moiety analogs and an importance in identifying potential issues that would need further testing to ensure safety for consumers such as the hERG values which are pertinent in the pharmaceutical fields in whether or not drugs can be safe to use.

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References

(1) Durán-Iturbide, N. A.; Díaz-Eufracio, B. I.; Medina-Franco, J. L. In Silico ADME/Tox Profiling of Natural Products: A Focus on BIOFACQUIM. ACS Omega 2020, 5 (26), 16076–16084. https://doi.

org/10.1021/acsomega.0c01581.
(2) Paul Gleeson, M.; Hersey, A.; Hannongbua, S. In-Silico ADME Models: A General Assessment of Their Utility in Drug Discovery Applications. Curr. Top. Med. Chem. 2011, 11 (4), 358–381. https://doi.org/10.2174/156802611794480927.
(3) Criscuolo, E.; De Sciscio, M. L.; Fezza, F.; Maccarrone, M. In Silico and in Vitro Analysis of Major Cannabis-Derived Compounds as Fatty Acid Amide Hydrolase Inhibitors. Molecules 2020, 26 (1), 48. https://doi.org/10.3390/molecules26010048.
(4) Docampo-Palacios, M. L.; Ramirez, G. A.; Tesfatsion, T. T.; Okhovat, A.; Pittiglio, M.; Ray, K. P.; Cruces, W. Saturated Cannabinoids: Update on Synthesis Strategies and Biological Studies of These Emerging Cannabinoid Analogs. Molecules 2023, 28 (17), 6434. https://doi.org/10.3390/molecules28176434.
(5) Matter, H.; Schmider, W. In-Silico ADME Modeling. In Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays; Springer Berlin Heidelberg: Berlin, Heidelberg, 2013; pp 1005–1052.
(6) Bow, E. W.; Rimoldi, J. M. The Structure–Function Relationships of Classical Cannabinoids: CB1/CB2 Modulation. Perspect. Medicin. Chem. 2016, 8, PMC. S32171. https://doi.org/10.4137/pmc.s32171.
(7) Kruger, D. J.; Bone, C. C. B.; Meacham, M. C.; Klein, C.; Kruger, J. S. THC-O-Acetate: Scarce Evidence for a Psychedelic Cannabinoid. J. Psychoactive Drugs 2023, 1–5. https://doi.org/10.1080/02791072.2023.2230573.
(8) Khanolkar AD, Palmer SL, Makriyannis A. Molecular probes for the cannabinoid receptors. Chem Phys Lipids. 2000; 108 (1–2):37–52. http://dx.doi.org/10.1016/s0009-3084(00)00186-9
(9) Han, S.; Thatte, J.; Buzard, D. J.; Jones, R. M. Therapeutic Utility of Cannabinoid Receptor Type 2 (CB2) Selective Agonists. J. Med. Chem. 2013, 56 (21), 8224–8256. https://doi.org/10.1021/jm4005626.
(10) Docampo-Palacios, M. L., Ramirez, G. A., Tesfatsion, T. T., Pittiglio, M. K., Ray, K. P., Cruces, W., In Silico ADME, binding affinities, and properties of hydrogenated cannabinoids, Invited Submission to Pharmaceuticals (MDPI) 2023. https://doi.org/10.26434/chemrxiv-2023-7jxvh
(11) Schrödinger, LLC . Schrödinger Release 2021-4: QikProp. Schrödinger, LLC; 2021.
(12) Grant, B. J.; Gorfe, A. A.; McCammon, J. A. Large Conformational Changes in Proteins: Signaling and Other Functions. Curr. Opin. Struct. Biol. 2010, 20 (2), 142–147. https://doi.org/10.1016/j.sbi.2009.12.004
(13) Guan, L.; Yang, H.; Cai, Y.; Sun, L.; Di, P.; Li, W.; Liu, G.; Tang, Y. ADMET-Score – a Comprehensive Scoring Function for Evaluation of Chemical Drug-Likeness. Medchemcomm 2019, 10 (1), 148–157. https://doi.org/10.1039/c8md00472b
(14) Compton, D. R.; Rice, K. C.; De Costa, B. R.; Razdan, R. K.; Melvin, L. S.; Johnson, M. R.; Martin, B. R. Cannabinoid Structure-Activity Relationships: Correlation of Receptor Binding and in Vivo Activities. J. Pharmacol. Exp. Ther. 1993, 265 (1), 218–226.
(15) Compton, D. R.; Prescott, W. R., Jr; Martin, B. R.; Siegel, C.; Gordon, P. M.; Razdan, R. K. Synthesis and Pharmacological Evaluation of Ether and Related Analogs of .DELTA.8-, .DELTA.9-, and .DELTA.9,11-Tetrahydrocannabinol. J. Med. Chem. 1991, 34 (11), 3310–3316. https://doi.org/10.1021/jm00115a023
(16) Orvos, P.; Pászti, B.; Topal, L.; Gazdag, P.; Prorok, J.; Polyák, A.; Kiss, T.; Tóth-Molnár, E.; Csupor-Löffler, B.; Bajtel, Á.; Varró, A.; Hohmann, J.; Virág, L.; Csupor, D. The Electrophysiological Effect of Cannabidiol on hERG Current and in Guinea-Pig and Rabbit Cardiac Preparations. Sci. Rep. 2020, 10 (1). https://doi.org/10.1038/s41598-020-73165-2
(17) Garrido, A.; Lepailleur, A.; Mignani, S. M.; Dallemagne, P.; Rochais, C. hERG Toxicity Assessment: Useful Guidelines for Drug Design. Eur. J. Med. Chem. 2020, 195 (112290), 112290. https://doi.org/10.1016/j.ejmech.2020.112290
(18) A. Collins, T. Tesfastion, G. Ramirez, K. Ray, and W. Cruces, Cannabis Science and Technology®, 2022. 5 (7), 23-27.
(19) Perucca, E.; Bialer, M. Critical Aspects Affecting Cannabidiol Oral Bioavailability and Metabolic Elimination, and Related Clinical Implications. CNS Drugs 2020, 34 (8), 795–800. https://doi.org/10.1007/s40263-020-00741-5

about the authors

WESTLEY CRUCES co-founded Colorado Chromatography Labs and joined as their Chief Science Officer in the spring of 2020. Together with Kyle Ray, he commercialized HHC manufacturing, built out multiple laboratories, returned 100% ROI within 18 months, and has authored over 10 publications and patents while at CCL. After screening various compounds against cancer cell lines, he had two hits with solid IC-50 values. The CCL team spun off BlackStone Therapeutics and set out to complete the preclinical studies needed to apply to Phase 1 Clinical Trials. Direct correspondence to **wes@coloradochromatography.com**.

MAITE L. DOCAMPO-PALACIOS has developed technologies applying the regioselective catalysis as a cost-effective alternative for the commercial manufacture of cannabinoids such as CBD and THC, as well as the rare cannabinoids CBDV, THCV, CBDP, THCP, CBN, CBC, CBT, and many more under mild conditions. She successfully brought more than 10 synthetic cannabinoids to market for the nutraceutical industry. Now, she is the Lab Director of Colorado Chromatography Labs. Her role includes leading the R&D team through the entire cycle of cannabinoids and research, development, and production from designing and optimizing the total and partial synthesis of rare cannabinoids, feasibility assessment, risk analysis, and process validation to large-scale production with low cost.

TESFAY T. TESFATSION has worked on many CDMO projects delivering final products in a timely manner. Including validating final compounds with COA and full characterizations as needed. Currently, Tesfay is a senior scientist at Sunflower Wellness, formerly Colorado Chromatography, developing novel methods and synthesizing cannabinoids from bench to pilot scale. Including closely working the chief scientific officer in in reaching production goals and processes.

GIOVANNI A. RAMIREZ is currently an R&D chemist at Sunflower Wellness, formerly Colorado Chromatography, where he focuses mainly on computational chemistry and medicinal chemistry while also assisting in organic bench work. He also participates in writing manuscripts, patents, and technical reports.

Cannabis Science Conference 2024:

Advancing the Latest in Cannabis Research and Innovations

BY ERIN MCEVOY

Cannabis Science Conference Spring was held in Kansas City, Missouri from May 7-9, 2024. Here, we provide highlights from this exciting event.

FOR THE FIRST time in its history, the Cannabis Science Conference headed to the Midwest, an area comparatively new to the regulated cannabis industry. Led by the four program chairs and an educational steering committee, the three-day event facilitated thought-provoking discussions and collaborations on cannabis analytical methods, cultivation technology, research, patient care, compliance, and much more. One topic of discussion that permeated the event was the recent developments with the Drug Enforcement Administration and the potential of rescheduling cannabis from a Schedule I to a Schedule III drug. Read on for our coverage from this year's Cannabis Science Conference, including links to insights from Program Chairs, onsite interviews, and presentation highlights for you to dive even deeper into the experience!

Pre-Conference Workshops

Leading up to the conference, two pre-conference workshops offered attendees the opportunity for interactive learning. In one workshop, four ASTM International subcommittee meetings covered a variety of topics. Darwin Millard, vice-subcommittee Chair with ASTM, helped direct two of the meetings with Jimmy Farrell, Standards Development Manager at ASTM International: ASTM Subcommittee D37.04 Initiative on Equipment & Facility Cleaning and Joint ASTM Subcommittee Initiative on Vape Device Safety & Testing. Jini Glaros, Chief Scientific Officer at Modern Canna Laboratories, led the ASTM Task Group on developing a Standard Guide for Cannabis/Hemp Laboratory Out-of-Specifications (OOS) and Retesting (WK85874), and Scheril Murray Powell, Esq., Cannabis, Agricultural, and Dietary Supplement Attorney, led the ASTM Subcommittee D37.93 on Diversity, Equity, and Inclusion Meeting.

Additionally, Susan Audino, Kate Evans, Julie Kowalski, and David Vaillencourt, hosted the workshop, "Cannabis Science, Testing and Troubleshooting with Experts: Turning Mountains into Molehills," an afternoon of discussion between laboratory scientists, technicians, laboratory managers, scientific directors, non-science laboratory owners, GMP professionals, and anyone interested in cannabis science and testing. In this onsite interview, Julie Kowalski, Technical Program Director of the Cannabis Science Conference and the Program Chair for the Analytical Track, shares her insights from this workshop.

Cannabis Science Conference 2024: Day One

Kicking off the two-day conference was keynote speaker, Jamila Owens-Todd, Naturopathic Doctor, Formulations Consultant and Director of Clinical Education, and an Ad-


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"Led by the **four program chairs** and **an educational steering committee**, the three-day event facilitated **thought-provoking discussions** and **collaborations** on cannabis analytical methods, cultivation technology, research, patient care, compliance, and much more."

junct Professor of Cannabis Pharmacology at St. Louis University's Cannabis Science and Operations Course. In her presentation, "The Evolution of Cannabis As Medicine—Keys to Cannabis Manufacturing," Owens-Todd discussed the various ways to approach plant medicine and manufacturing, examining how innovations in cannabis production and technology can still adhere to the original integrity and healing of the plant.

Next, Kim Stuck from the Canna Consortium, moderated the General Session discussion panel, "Federal Rescheduling: Opinions and Impacts," which featured David Vaillencourt of S3 Collective, Ken Sobel, Esq., and Susan Audino of S.A. Audino & Associates, LLC, who shared their various perspectives on the potential reclassification of cannabis to a Schedule III drug.

Following these presentations were sessions in the four tracks for this conference: Analytical, Medical, Cultivation, and, new this year, the Compliance track. Each session included time for attendees to ask questions of and engage with the presenters.

Notable presentations from the Analytical Track included "Polysorbate 80: An Emulsifying Agent in Cannabis Microbiology," by Anthony Repay, Laboratory Director of Method Testing Labs, and "Innovative Method Development to Meet the Needs for the Cannabis Industry," presented by Patrick Bird, Senior Manager of Scientific Affairs at bioMérieux. Other topics in this track also included molecular biology, onsite laboratory validation, and moisture analysis for cannabis flower. Some of these tracks were joint presentations in the Compliance Track, based on the subject explored.

The first presentation in the Cultivation Track was given by Antonio Timoteo Jr., PhD, research assistant at the University of Maryland Eastern Shore, who presented, "Maximizing Terpene Yields with Precise Harvest Timing in *Cannabis sativa L.*" Two other notable presentations included Bernie Lorenz, Chief Science Officer of ProKure Solutions, who discussed "How COVID could revolutionize the way we grow cannabis," and James Wylde, President and CEO of Greenlight Analytical, who presented "Maximizing Quality & Revenue with Daily Analytics." Other topics in the track included horticulture lighting and phytochemical content, critical materials recovery, and performance metrics.

The Medical Track covered a variety of topics including cannabidiol (CBD) and receptor promiscuity, the endocannabinoid system, cannabinoid applications, microbiology risks and mitigation for consumers, psilocybin, patient perspectives of cannabis, and geriatric risk with cannabis. Several of the presenters were from the Cannabis Nurses Network, and a special recognition was given at the beginning of the conference to honor their work (and the work of all nurses) during National Nurses Week (May 6–12, 2024). One of the nurses, Marcie Cooper, MSN, RN, AHN-BC, HWNC-BC, GHNA, presented "Reducing Polypharmacy for the Aging Population," to close out the first day of the track. "I have been a hospice and palliative care nurse for about 20 years now," Cooper explained in an interview with *Cannabis Science and Technology*. "And a lot of times we do see really horrible side effects that patients have from the polypharmacy. Working as a hospice nurse in Colorado for the past 15 years has been really eye opening, being able to see how cannabis can help relieve a lot of those interactions that happen with multiple drugs."

While some of the Compliance Track sessions were joined with the Analytical Track, the standalone presentations featured these instructive discussions: "Nailing Compliance: Building and Maintaining an AI-powered ISO 17025-Compliant Cannabis Testing Lab," "Enhancing Cannabis Product Quality through Quality Management Systems," plus an hour-long panel presentation, "Impurities, Policy, and Progress: Charting the Future of Cannabinoid Product Safety," which was moderated by Program Chair Kim Stuck and featured panelists David Vallencourt, GMP Collective; Robert Welch, University of Mississippi; and Chris Hudalla, ProVerde Laboratories, Inc.

The breaks in between sessions—plus the opening night reception—provided ample opportunity for networking and poster presentations in the Exhibit Hall. Here, Danielle Lenoir explains her research in the poster presentation, "Proficiency Test Program Expands to Oil Matrix to Provide Additional Performance Assessment for Hemp and Cannabis Testing Laboratories."

Cannabis Science Conference 2024: Day Two

The second day of the Cannabis Science Conference featured a much-anticipated presentation of research from Allison Justice, PhD, and Riley Kirk, PhD, who discussed, "The Science of Smokeability: Insights on Cultivation and Post-Harvest Techniques to Enhance the Quality of the Smoking Experience." Dr. Allison Justice is the Founder and CEO of Hemp Mine, and Dr. Riley Kirk is the Co-Founder of Network of Applied Pharmacognosy. As Dr. Kirk summarized, "This research is about learning more about the smoking experience because our industry has not done any of this really baseline fundamental research in understanding from plant to smoke entering the body. What is happening on a biochemical level? What is happening with these different molecules? Are there different levels of transformation happening? And can consumers actually pick up on these little nuances? Or can they not?"

After the keynote presentation, the Cannabis HR Council hosted the General Session presentation, "The Role of DEI and Social Impact in Cannabis Science and Innovation," with Scheril Murray Powell, Esq., and John Calloway Jr., Founder of Calloway Venture & Associates. This presentation explored the many facets of diversity in the cannabis workplaces and leadership, including the social impact diversity has on cannabis science, research, and innovation. As Calloway Jr. summarized, "diversity is going to fuel your innovation, inclusion is going to drive your productivity, and social impact will improve and enhance your reputation and the sustainability of your organization."

The second day of the conference continued the informative session discussions and presentations from the day prior. The Analytical Track covered a wide variety of subjects including Hansen Solubility Parameter and mixture entropy; cannabinoid and terpene content in homemade butane hash oil and rosin extracts; mitigating baseline toxicity of inhaled cannabis; total yeast, mold, and viable bacteria results in cannabis flower grown outdoors in New York; and the analysis of metal nanoparticles in legal and illegal cannabis vapes.

The Cultivation Track explored new developments in postharvest touchpoints and pre-roll quality, bioaerosols in indoor cannabis manufacturing, climate control systems, the influence of mycorrhizal amendment on hemp, and optimizing lighting strategies to increase yield and chemical uniformity.

Building on to the topics discussed during Day One, the Medical Track covered scientific insights into sales and customer practices, post-traumatic stress disorder (PTSD) and trauma-informed care, nurses leading change in the industry, integrating medical cannabis into nursing practice, and holistic approaches in cannabis therapeutics.

Finally, the Compliance Track featured two standalone presentations before joining with the Analytical Track to round out the end of the day: "Product Safety Standards for CBD Products," by Cristelle Santos, a toxicologist with the Broughton Group, and "Colorimetric Quality Control for CBD, Cannabis, and Hemp-based Consumer Products," by Charles Steele, founder and president of CBF-Forensics, lecturer of Physical Science, and Forensic Science Coordinator at Purdue University Northwest.

Thank you to our sponsors, exhibitors, presenters, attendees, and everyone behind the scenes who made this event successful! See you in 2025 for the next Cannabis Science Conference!

To stay up to date on more cannabis innovation and future conferences, follow us on social and please visit www.cannabisscienceconference.com.

> "**Thank you** to our sponsors, exhibitors, presenters, attendees, and everyone behind the scenes who made this **event successful**! See you in **2025** for the **next Cannabis Science Conference**!"

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