The Importance of Reliable THC Concentration and the Role of Analytical Data

The critical role of analytical data in achieving reliable concentration.

Jodi McDonald
President and Founder
Keystone Labs Inc.

INTRODUCTION

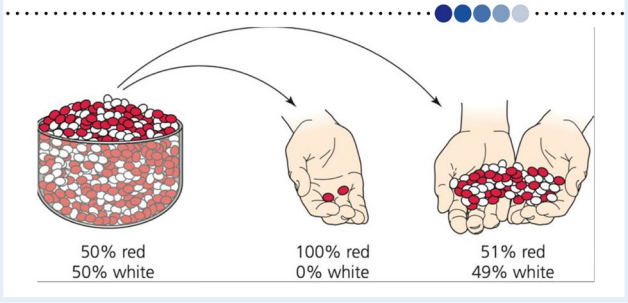
With the burgeoning production of cannabis products comes the heightened importance of reliable analysis and labeling. Tetrahydrocannabinol (THC), the primary active ingredient in cannabis, is a critical quality metric for producers and there is tremendous pressure to commercialize products with high concentrations of this natural chemical. This situation has led some producers to inflate their THC content and seek out analytical labs that would provide the highest possible THC quantification. As such, there is distrust between consumers and producers.

Cannabinoid concentration is not only a safety issue, but also a key market driver. Producers use it as a point of access to some markets or as a way to value the product for buyers. This is where business meets science. By utilizing analytical data, producers can control production and provide consistent products. The specifications and limits to which they adhere should ensure that the products they release are trustworthy. Thus, acquiring accurate data is vital to business success.

CRITICAL FACTORS FOR ANALYSIS

Consistent, reliable product labeling is essential for building industry confidence, and having high-quality data from the analytical laboratory is the key to this effort. Health Canada has specified that producers label total THC and total cannabidiol (CBD) within 10% of the product's actual value. Although the complexity of cannabis can introduce challenges to its analysis, there are straightforward approaches to achieving accurate, dependable results. The quality of data in cannabis analyses relies on three critical factors: sample collection, sample preparation, and performing the analysis. Failure to optimize all of these steps can lead to faulty data.

Figure 1: Larger sampling improves the chance it will be more representative of the whole batch.



Sample collection

Samples selected for analysis must represent the entire batch. A plant growing on one side of a room may be significantly different from one on the other side of the same room because they each grow and adapt to their environment differently. Thus, collecting a representative sample is critical and complicated. Take, for example, the red and white beads in FIGURE 1. The batch comprises 50% of each color. Quickly choosing a small sampling without a plan can result in biased sampling, as shown in the center image. Meanwhile, taking a larger sampling improves the likelihood that the sample will be representative of the batch, as depicted on the right. In Canada, the producer is responsible for outlining a sample plan. In some jurisdictions in the United States, the responsibility for taking a representative sample rests with the testing lab. Regardless of the regulations, the most important part of sampling is having a plan, as consistent sampling leads to consistent data.

A key variable in using a sampling plan is the batch, but Canadian legislation does not clearly define this term. In jurisdictions where *batch* is not defined by legislation, the decision about a sampling plan and the quantity of sample required all comes down to risk management. A batch defined by a smaller quantity leads to more product being used during the testing step. Although less material is used for testing if a larger batch is defined, more product would need to be rejected in the case of a failure.

To illustrate, a military sampling plan is shown in FIGURE 2. A tool like this is useful for trying

Figure 2: Sampling plan described in U.S. Department of Defense (1963), *Military Standard Sampling Procedure and Tables for Inspection by Attributes: MIL-STD-105D.*

MIL STD 105D

TABLE I
Sample size code letters

Lot or batch size	General inspection levels I	II (Normally Used)	III
2 to 8	A	A	B
9 to 15	A	B	C
16 to 25	B	C	D
26 to 50	C	D	E
51 to 90	C	E	F
91 to 150	D	F	G
151 to 280	E	G	H
281 to 500	F	H	J
501 to 1200	G	J	K
1201 to 3200	H	K	L
3201 to 10000	J	L	M
10001 to 35000	K	M	N
35001 to 150000	L	N	P
150001 to 500000	M	P	Q
500001 and over	N	Q	R

TABLE II-A
Single sampling plans for normal inspection (Master table)

Sample size code letter	Sample size	Acceptable Quality Levels (normal inspection)	0.010	0.015	0.025	0.040	0.065	0.10	0.15	0.25	0.40	0.65	1.0	1.5	2.5	4.0	6.5	10	15	25
		Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re	Ac Re
A	2																			1 2
B	3																			2 3
C	5														0 1		0 1		1 2	3 4
D	8													0 1		0 1		1 2	2 3	5 6
E	13												0 1		1 2		1 2	2 3	3 4	7 8
F	20											0 1		1 2		1 2	2 3	3 4	5 6	10 11
G	32										0 1		1 2		2 3	3 4	5 6	7 8	10 11	14 15
H	50									0 1		1 2		2 3	3 4	5 6	7 8	10 11	14 15	21 22
J	80								0 1		1 2		2 3	3 4	5 6	7 8	10 11	14 15	21 22	
K	125							0 1		1 2		2 3	3 4	5 6	7 8	10 11	14 15	21 22		
L	200						0 1		1 2		2 3	3 4	5 6	7 8	10 11	14 15	21 22			
M	315					0 1		1 2		2 3	3 4	5 6	7 8	10 11	14 15	21 22				
N	500				0 1		1 2		2 3	3 4	5 6	7 8	10 11	14 15	21 22					
P	800			0 1		1 2		2 3	3 4	5 6	7 8	10 11	14 15	21 22						
Q	1250	0 1			1 2		2 3	3 4	5 6	7 8	10 11	14 15	21 22							
R	2000			1 2		2 3	3 4	5 6	7 8	10 11	14 15	21 22								

Ac Acceptance number.
Re Rejection number.

Use first sampling plan below arrow. If sample size equals, or exceeds, lot or batch size, do 100 percent inspection.

Use first sampling plan above arrow.

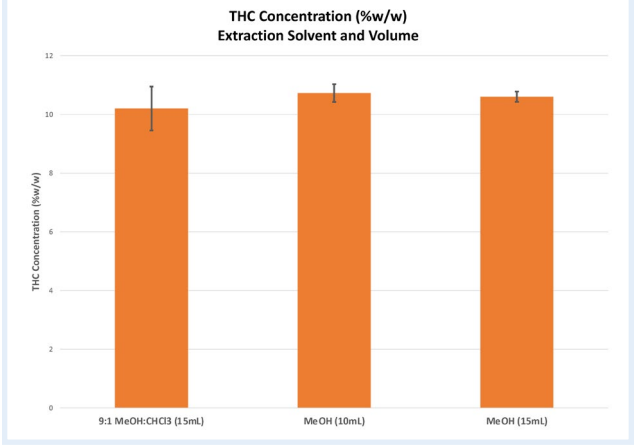
The definition of a batch is of great significance. In any case, the sampling plan should include consistent batch sizes for reduced batch-to-batch variability.

to decide if a batch of received material is acceptable for use. Assume, for example, that there is a batch of 50 boxes of surgical masks. Using a normal inspection level, eight boxes would need to be inspected for defects (denoted as D). If the acceptable quality level is set at 1%, then, according to the table in Figure 2, if only one defect is found, the whole batch must be rejected. Sampling a larger batch size offers the opportunity for more defects to be accepted in the batch. The military sampling protocol has become the basis of many sampling plans that are used in cannabis testing.

Meanwhile, the state of Oregon has clearly legislated what is required in terms of cannabis sampling and has defined many parameters that are key to executing a good sampling plan. They have specified that a batch is not more than 15 pounds or 6.8 kg, and that samples must be collected in increments of not less than 0.5% of the total batch. Thus, the minimum sample size is 34 grams. The total number of samples are outlined in a table attached to the legislation, which also clearly states that enough sample increments must be collected to determine whether a batch is homogenous.

In a hypothetical batch of 6.8 k (comprising 170 packages of 40 g each), Oregon's legislation indicates that eight packages must be collected

Figure 3: Effect of adjusting the composition and volume of extraction solvent for THC in cannabis.



and sent to the lab for testing. If there were a failure in this batch, all product must be rejected. If that batch were split into three smaller batches of 56 packages each, five samples per batch must be collected. In this case, if one batch fails, two-thirds of the original product would still be available for release and sale. For that reason, the definition of a batch is of great significance. In any case, the sampling plan should include consistent batch sizes for reduced batch-to-batch variability.

Sample Preparation

Sample preparation is the most important step for ensuring that the results will be reflective of the samples received. Specifically, the quantity of sample used in the analysis and the cannabinoid extraction parameters play key roles in the analysis. FIGURE 3 demonstrates the effect of adjusting the composition and volume of extraction solvent for THC in cannabis. Note that, in addition to THC concentration, the confidence intervals for each measurement are dramatically impacted by the modifications. Using a method from the American Herbal Pharmacopoeia as a starting point, extraction solvent was adjusted incrementally until extraction efficiency and repeatability were maximized. It was discovered that using 20 mL of 100% methanol with 0.2 g of flower was optimal.

Figure 4: Comparison of manual (left) versus mechanical (right) grinding techniques.

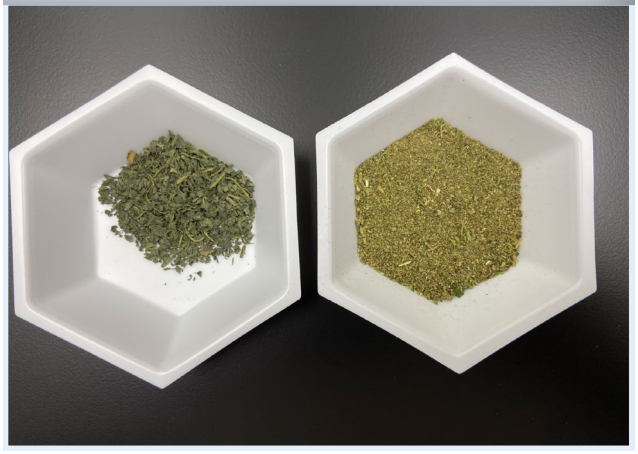
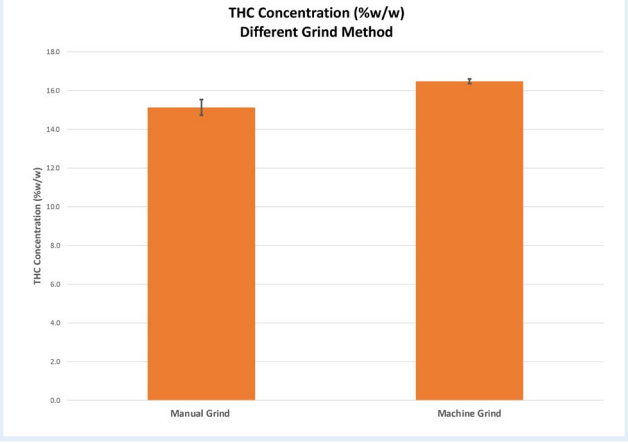


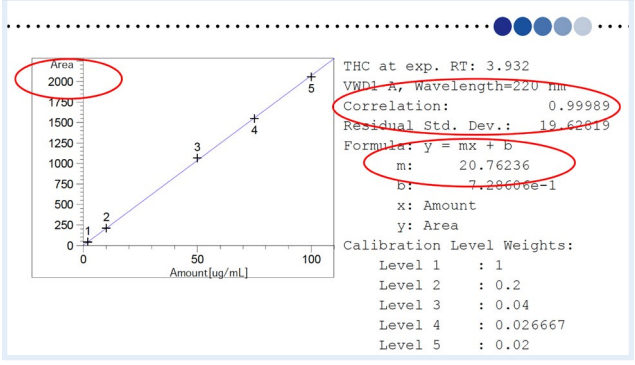
Figure 5: Mechanical grinding improves the recovery of cannabinoids from dry flower.



Other parameters have been explored, including the size of the grind of the dry flower. The photo in FIGURE 4 compares the results of a mortar and pestle manual grinding technique to that of a mechanical grinding instrument. As depicted in FIGURE 5, using a smaller grind improved the recovery of the cannabinoids from the dry flower by almost 10%. Just as noteworthy is the decreased confidence interval from the smaller machine grind, which demonstrates significantly improved repeatability of the measurement.

The length of extraction and filtration has also been evaluated. In testing several different filters, it was discovered that some of them decreased the cannabinoid concentration

Figure 6: Correlating the concentration of a standard with area response using linear regression.



and increased analysis variability. The most reproducible results were observed with filters made of polytetrafluoroethylene (PTFE).

SAMPLE ANALYSIS

After sample preparation, the analytical instrumentation must allow for high sample throughput and minimal operator intervention for expedient reporting of results. Hence, high performance liquid chromatography (HPLC) is the ideal tool for routine cannabinoid concentration measurements. Robust method validation is vital, followed up with tools to compare the consistency of analysis from run to run. FIGURE 6 shows a typical standard curve from Keystone Labs correlating the concentration of a standard with its area response using linear regression. Fresh curves are generated for each cannabinoid, and the peak areas are compared to historical data for a supplemental check on the system's stability. In addition, the slope of the curve is monitored, as it can indicate an error in the preparation of standard solutions. Use of a QC standard obtained from a different vendor than the primary standards enables verification of the standards from run to run. Spike recovery and sample duplicates can also help ensure that the sample preparation and analysis are repeatable. By optimizing sampling, sample preparation, and analysis, accurate results can be achieved routinely.

Producers receiving the analytical data need the results to be representative of the entire batch, which stems from establishing and following an appropriate sampling plan. Determining whether the data are within the expected range of concentrations can be beneficial, as well as monitoring trends of the data from batch to batch. The more information a producer has about their production, the better. It helps them understand what the measured concentrations mean in terms of labeling, trending, and process control at their facility.

FUTURE CONSIDERATIONS

In Canada, topics of industry-wide discussions include whether or not a single data point represents an entire batch, and whether the industry and regulators should consider a range of concentrations rather than a single discrete data point to represent the packaged cannabinoid concentration. In addition, what is acceptable for reporting and what variation is appropriate for product label concentration are also being deliberated. These conversations have been spurred by a class-action lawsuit against Canadian producers for mislabeling the cannabinoid concentration in products. The suit will likely affect the choice of sampling plans and which laboratories are selected for analysis.

Accordingly, analytical laboratories should be considering future accountability of their data. That accountability may include lab accreditation, lab proficiency, and standardization of methods. While the current focus on cannabis quality is primarily centered around cannabinoid concentration, other measurable qualities of cannabis can indicate consistency and overall safety of products. For example, scientists are beginning to develop more data on the synergies between cannabinoids and terpenes and the impact of terpenes and flavonoid profiles. Many metrics could be used to set the bar of cannabis quality without relying solely on cannabinoid concentration. Producers and analysts can expect the requirements to evolve as the industry grows.

CONCLUSION

As producers face pressure to sell cannabis products with increased cannabinoid concentration, and mislabeling has caused distrust with consumers, reliable analyses are critical to their success. Accurate labeling of products will help producers establish a favorable reputation and contribute to consumer confidence and, ultimately, repeat business. Since labeling relies on testing results, the steps of the analytical process for this complex matrix must be optimized, including sampling, sample preparation, and analysis. A robust method combined with instrument performance checks ensure that the data is trustworthy. This holistic approach to testing enables cannabis producers to offer veracious labeling with their products, thereby offering promising outcomes for their businesses.


The Colonel/stock.adobe.com

FAQs: Reliable THC Concentration and Analytical Data

Here, Jodi McDonald, president of Keystone Labs answers several questions asked during a recent *Cannabis Science and Technology–LCGC* webcast on "The Importance of Reliable THC Concentration and the Role of Analytical Data."

What trends are you seeing in the lab when testing for potency?

We have seen so much innovation. THC has been the focus for the last two years, but now, I'm seeing more importance placed on novel cannabinoids. For example, tetrahydrocannabivarin and cannabinol.

What is the most difficult part of the potency validation process?

The whole validation undertaking is complicated. Unlike pharmaceutical products that are very clean with little interference during a method validation, cannabinoids have a lot of interferences. In terms of potency, the most complicated part is the small details, but sample preparation is the most important.

Do you run a standard curve for each cannabinoid you are testing?

Yes. In our lab, background air temperature has a large impact on retention time, so we relocated our primary instrument for cannabinoid concentration. We wouldn't have known that without running a standard curve for each cannabinoid and doing a fresh curve every time.

How do you determine method uncertainty?

It depends on the method validation. First, we start with optimal conditions. We look at the standard curve as it looks with nothing in it. We look at replicate analyses of a single sample. Additionally, we have a QC sample that we prepare in house and run multiple times through the course of an analysis and then over time.

Did you try to extract the sample with reagents?

We tried several solvents for extraction, simply looking for a repeatable, inexpensive, easy-to-use solvent. Methanol worked really well with the chromatography we were running.

Could we quantify more analytes using the same extraction process, for example, terpene profiles?

Yes, in our lab, one benefit of using our methanol extraction is we're able to use that same extracted sample for terpene profiling.

Can you share any info on the move away from using chloroform in the extraction solvent, less interferences, other observations?

Chloroform didn't add anything to the extraction or the chromatography. At the same time, we were working on developing our terpenes method and found that by taking the chloroform out, we were able to use the same extract for potency as well as for the terpene analysis.

When checking the quality of your extraction, how did you determine the amount of analyte in the unknown sample?

We prepare a standard curve, and then we have a standardized sample preparation method—we measure the response in the unknown in the prepared sample against the standard curve.

We do two dilutions on each sample. The first solution, the low dilution, allows us to quantify the low concentration and cannabinoids such as cannabigerol (CBG) and cannabigerolic acid. Then, we do a higher dilution to get THC, tetrahydrocannabinolic acid (THCA), cannabidiol (CBD), and cannabidiolic acid into the range of our standard curve.

Is it possible to use the extract for a concentration of cannabinoids for other analysis?

Yes. We've only scratched the surface on what would be possible in our lab, but the two analyses we have synergy with—our cannabinoid concentration and the terpenes—we're able to do a single extraction and get two completely different instruments and two completely different analyses done on them.

Do you offer a separate method for isolate testing other than standard potency?

Our chromatography doesn't change depending on the sample. The key part of this is sample preparation. We have a specific method for sample preparation of isolate, and it requires a much larger

dilution because most of the mass weighed into your sample for dilution is pure isolate.

What types of sample prep techniques are used in your lab?

For cannabinoid concentration, we use a solvent extraction. When the sample arrives, we weigh the sample for microbial first, then everything else gets processed or other tests that have been requested on the same sample are processed after we've taken the microbial sample out. We grind the rest of the sample and sub-sample for each of the analyses requested. For potency, once we take out the amount needed for extraction, we begin that step of the sample preparation. Next is filtration and dilution, then onto the instrument.

How many samples do you run in one batch?

It varies from day to day. The sample tray we have in our high-performance liquid chromatography (HPLC) has space for 96–100 samples. We do two dilutions for each sample, so we can look at the low concentration occurring cannabinoids as well as a higher dilution; the high-level cannabinoids show up within the range of the standard curve that we run. With all of our QCs, standards, and blanks, we run about 40 samples in an assay.

Is manual integration permitted when performing HPLC analysis?

It comes down to controls. In our lab, it is absolutely not allowed. We have our integration parameters before analysis, and it's based on the information and data that we collected during our robust validation protocol. If we do any manual integration, we need the quality-assurance approval, as this is a deviation in our standard operating procedure (SOP).

Does your group use LC-MS for THC analysis? How do you get the matrix effect over?

We have been approached by a couple of companies conducting research and development on product. Liquid chromatography (LC) and HPLC don't have the sensitivity needed to make decisions about process and product, so we started to look at mass spectrometry. But we're only at the early days of figuring out how to optimize it.

How do you control the chromatography in each assay?

We prepare an external standard curve in the mobile phase. When we're looking at new products or trying to understand what the matrix impact is, we do a standard addition to the samples.

What is the biggest indicator of difference in THC between samples?

The biggest indicator is the amount of moisture in the product you're analyzing. When we first worked on this method, we dried everything until it was bone dry and dusty, and we got good, repeatable results.

The biggest difference in THC among various samples from a single producer and a single batch is material taken from the top of the plant produces higher THC than samples that get less light. There are a lot of studies to support this.

We have had several clients think they can just send leftover material at the end of packaging and trimming, and that doesn't usually give them the data they want.

Have you noticed a difference in ratio of THC to THCA in hash sample?

Yes, there are so many things about the profile that we can tell in the lab such as how new the sample is. Hash samples typically are higher in THC than THCA.

Do you have a separate method for hemp versus cannabis testing?

We have different dilutions that we prepare for a dry hemp sample versus a dry flower sample, and it goes directly to the calibration range. We have a standard calibration range that we run all the time.

Again, it doesn't matter what samples we're running; the chromatography never changes. What changes for us and our lab is the sample preparation. For samples that we could expect a high concentration, we expect to do larger dilutions. Samples such hemp, where it's very low-level concentrations, we do very little sample manipulation, very low dilutions before running them in our chromatography.

For pre-rolled products, do you receive the finished product or the milled flower for testing?

We receive both; it depends on the license holder. Some test the material before they put it into a final packaged product for the pre-roll; other companies will do some analysis in-house, then send out only their final packaged product. We have familiarity with both types of samples.

What wavelength do you recommend for quantitative testing of cannabinoids?

We played with wavelengths a lot. The German DAB has a monograph for measuring potency or cannabinoid concentration in dry flower; they recommend using two wavelengths, one in the UV and one in the visible or in the high UV. We've optimized our analysis for 220 nanometers. It's the best of both worlds. We get good measurements of the neutral cannabinoids, THC, CBD, CBG, CBC, and cannabimovone (CBM). For the acidic form, the chromatography is less clean, but linear. It still meets all the criteria for the rest to report.

THC is present in buds in the form of THCA, so do you have to measure both and add them up?

Yes, using LC we have the benefit of being able to separate the neutral and acidic forms of all the cannabinoids. At the end of the day, we report all the cannabinoids individually, so right now, there are 10 cannabinoids we commonly see and report to our clients. For THC and CBD, we do stoichiometric calculation to convert the acidic form to a neutral form and report a total THC and a total CBD.

How much sample do you collect from LP for the required Health Canada potency? Do you grind the entire sample?

As a Canadian lab, this is a pain point for us and the license holder. There is not a quantity requirement that's defined in the regulations—the responsibility for making an appropriate sample lies 100% with the license holder. They have to do a risk analysis to know whether to sample only one bud and use that to release hundreds of kilos of product. When we receive samples, we are not only doing potency testing, but we're doing contamination testing as well.

For potency, we use a small amount—0.2 grams of the total milled sample. Microbial sampling happens first because we don't want to contaminate the remaining sample. Then we grind the remaining samples for all the other analyses.

Do you use whole bud or only top part for Genogrinder?

In Canada, we test the samples provided by the license holder as they are received; we don't go into a facility and sample for our clients. The clients do the sampling according to their defined sampling plan and provide them to the lab.

We essentially work as a sample receipt desk and label them with an identification number and throw them into the lab. If the license holder only selects the top part of the plant or the sweepings off their floor, that's what we use.

How was cannabinoid trichome loss controlled for the mechanical grind? Was this checked for higher THCA—25–30% THCA content?

The key part is simple preparation. The United States Pharmacopoeia (USP) says how to do a subsample, which means dumping the whole grind onto a surface, then quartering it until you have the appropriate amount of sample you're using for the extraction. So, how do we control for it? We take an appropriate representative sample of the ground sample.

What happens if a lab report presents dried weight versus as received—can the cultivator use the value of percent dried weight for THC/CBC values?

This comes down to the regulatory authority that's overseeing the industry you're operating in. The Canadian guidance document, The Good Production Practices, says no Canadian lab shall test the product in a dried form—you must test it as received.

Someone can use the results if the lab report is using a dried weight—in our lab, when we have a request from a client to dry it, we have a statement on the final report that says we didn't test it according to the regulations but according to the requirements the client laid out for us. It is possible there's a way to use a calculation to include a hypothetical moisture content. The lab can work with you on items like that.

How do you report results when you perform a duplication of sample?

We don't often repeat analysis. We have a validated method and controls in the assay. If we repeat an analysis because of a lab-identified error, we indicate that on the final test summary. We talk to the client to make sure we're reporting data that is meaningful to them. Regarding duplicate sample analysis in the assay as part of your assay control, we only report the first analysis and then use the duplicate sample analysis as our control points. We make sure that it meets all the criteria for the assay reporting.