Understanding Cannabinoid Analytics

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The United States Food and Drug Administration currently prohibits the addition of CBD to food and dietary supplement product

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Introduction

Achieving high product quality remains a critical priority across the global cannabis industry. While each jurisdiction has adopted their own standards for testing, the definition of quality as defined by regulators remains fairly consistent between different markets (see footnote). In stark contrast to the pre-legalization quality descriptors (taste, smell, appearance, etc.), the modern quality standard of a cannabis product are now defined by:

1. The concentration of specific compound groups, such as the bioactive cannabinoids and terpenes.
2. The absence of contaminants including microbes, pesticides, residuals solvents, and heavy metals.
3. Attributes of the product that can affect the analysis of quality, such as the water content of the material.

The definition of these quantitative quality standards has continued to develop as the nascent cannabis industry has matured. While established testing procedures and post-process sterilization practices have been adopted from the food and pharmaceutical industry for the purposes of contamination testing (microbial, pesticide, heavy metal, residual solvent), there unfortunately remains a gap in the competency of sampling and analytical testing of cannabinoids and terpenes. This is of particular concern considering that it is these two compound groups that define the therapeutic potential of a cannabis product. The lack of standardized methodology for cannabinoid and terpene testing therefore continues to impact product quality across the industry, damages the therapeutic validity of cannabis products and poses a safety risk to consumers. This has been evidenced by numerous reports of inconsistent testing between different laboratories, accounts of wide discrepancies among product potencies and their respective label claims, and reports of significant adverse effects.

Stemming from the historic illegal cannabis market, consumers' perception of product quality was previously defined by sensory characteristics such as taste, smell, and appearance. These are qualitative metrics that are functionally different when compared to how regulatory agencies define quality. The issues with product testing, often reported by media outlets and sometimes resulting in product recalls, have increased consumers' awareness of the quality concerns surrounding the industry and helped to align the definition of product quality across consumer groups, product manufacturers, and regulators.

It is important that brands and consumers alike understand these defining quality attributes of cannabis products so that they can make informed decisions with respect to product manufacturing and purchasing. To aid in this effort, this paper provides an overview of analytical targets and current testing practices for cannabinoid products and focuses on the mainstream issues with cannabinoid and terpene analysis. Recommendations for improvements and ongoing efforts to alleviate these issues are also discussed with the hopes of encouraging the sector to develop an industry-wide analytical program that includes robust, reliable, and reproducible methods based on a sound understanding of analytical targets, best practices for sample preparation and testing, and method capabilities and sensitivities.

The Current State of Cannabinoid Product Testing

Over recent years, there have been various reports of discrepancies in results reported by different labs. One of the earliest warnings of these analytical issues was presented in 2011 following a comparison of potency results for flower and extract samples reported by 10 different labs in California[1]. Discrepancies in analytical methodology were observed, with some labs utilizing HPLC (high performance liquid chromatography) and others using GC (gas chromatography) to measure cannabinoid potency. While

Footnote: based on a comparison of regulatory frameworks for the Canadian, European, and US state markets.

Total THC and Total CBD values reported for flower samples were consistent between labs regardless of the employed methodology, the potency of extract samples varied significantly based on the type of analytical method used. THC measurements for two samples were 40% higher when measured using GC compared to HPLC, while CBD measurements obtained using GC were 20-33% higher compared to results obtained using HPLC. Additionally, some labs reported potency on a dry weight basis after taking into account the moisture content of the plant material, while others reported potency as-is. The authors found that the THC potency reported for three identical flower samples following flower homogenization varied by 20% of the mean, with potency results falling between 8.4% - 12.5% THC.

Unfortunately, more recent reports suggest that these analytical issues have not yet been resolved within the industry. A 2018 study involving 6 of the largest labs in Washington state identified large inter-lab variability in levels of THC and CBD reported for flower and concentrate products[2]. Interestingly, the labs that reported higher levels of THC for flower products also reported higher levels of CBD across flower products and THC levels for concentrate products. They attributed differences in the labs' reporting capabilities, particularly in instances involving low-dose CBD flower products, to differences in each lab's methodology and the associated limit of quantification (LoQ).

Labs in Seattle faced similar scrutiny after one report found that failure rates varied between 14 state-certified labs, with some labs accepting all batches they tested while others rejected between 12% - 44% of tested batches[3]. This brought into question the incentive that labs may encounter to return passing results in order to obtain more business from producers, as well as "cherry picking" sampling practices by producers.

Producers have also been criticized for their manufacturing practices after numerous reports found that tested edible and beverage products contain far less THC or CBD than claimed on the product label[4,5]. These label claim discrepancies likely stem from a lack of in-house analytical capabilities required for proper quality control and validation that batch mixing processes achieve homogenous distribution of the active cannabinoid ingredients throughout the entire lot. These capabilities are particularly important for products with complex ingredient profiles, such as beverages and chocolates, given the potential for phase separation between the oil-soluble (hydrophobic) cannabinoids and water-soluble (hydrophilic) ingredients. Furthermore, there is evidence that lipid-rich product matrices, such as those characteristic to chocolate products, maybe interfere with cannabinoid testing[6].

One study reported that the magnitude of signal interference caused by a chocolate matrix is related to the number of phenolic hydroxyl groups present on the cannabinoid molecule[7]. They reported that cannabinoids with two hydroxyl groups, such as CBD and CBG, experience fewer matrix effects and higher recovery rates, whereas cannabinoids containing only one hydroxyl group, such as THC and CBN, experience stronger interaction effects and consequentially lower recovery rates. They confirmed that the explanation for this phenomenon is that a cannabinoid with a single hydroxyl group is less preferentially solvated by methanol as a chromatography solvent, and therefore is distributed between both the chocolate phase and the column solvent phase. In comparison, cannabinoids featuring two hydroxyl groups are more favourably solvated by the column solvent, and therefore recover at higher rates. These issues can be circumvented with proper analytical method development involving rigorous evaluation of solvent and matrix effects.

More recently, several federally licensed Canadian cannabis producers have been implicated in a lawsuit claiming that the potency of several oil products are almost 50% lower than their label claims. This has

drawn attention to the large discrepancies between release testing and independent labs, which likely result from the use of different sample preparation and analysis methodologies, and also suggest possible stability issues with cannabis oil products based on the magnitude of the reported discrepancies[8].

There have also been concerns in both the United States and Canada about the presence of pesticide residues in plant material. One lab in California reported that 84.3% of cannabis samples received by their lab tested positive for pesticide residues[9]. Of particular concern was the pesticide Myclobutanil, a carcinogen known to convert into the lethal toxin hydrogen cyanide when combusted or heated, which was detected in excess quantities in over 65% of samples tested. In Canada, several licensed producers were required to recall batches of cannabis flower and oil products in 2016 and 2017 after traces of myclobutanil and bifenazate were detected in the products[10,11]. Following these events, Health Canada implemented more stringent pesticide testing requirements that have since reduced the number of samples with positive pesticide test results to less than 5%[12].

This collection of incidences highlights the need for stringent and standardized testing programs. Standardization should focus on sampling procedures and sample preparation, as well as analytical calibration, methodology and results reporting. Methods should take into account possible interference effects caused by the product matrix, as well as differences in product potency between different product classes, such as refined extracts and isolates (high potency) and edibles (lower potency), as a one-size-fits-all method may not be sensitive enough to evaluate this exceptionally broad range of product formats.

Analytical Targets in Cannabis Products

Testing regulations for cannabis products encompass a wide range of analytical targets. These targets aim to substantiate product safety and regulatory compliance. While the thresholds for these targets varies by region, analytical targets include compounds that are innate to the cannabis plant, such as cannabinoids, terpenes, water content, and microbial contamination, as well as targets that are introduced during cultivation and product manufacturing, such as pesticides, heavy metals and residual solvents. Identifying the numerous analytical targets associated with cannabis product testing is essential for understanding the scope, capabilities, and limitations of current testing practices.

Cannabinoids

The cannabis plant and its derivatives are variable heterogeneous specimens containing a range of compounds with diverse physiochemical properties. For instance, over 100 cannabinoids have been identified within the cannabis plant, most of which are present at concentrations well below 1.0% (w/w). Cannabinoids represent the primary analytical target for cannabis product testing, as their presence is often associated with the product's therapeutic effects and intoxication potential. Furthermore, some cannabinoids such as THC are scheduled as controlled substances within specific markets of sale. It is common practice to report both the concentration of THC as well as its acidic precursor THCa so that the potential of THCa to convert into THC is considered. This is often reported as Total THC. Accurate reporting of both scheduled and unscheduled cannabinoids is essential for evaluating product potency, complying with local regulations, and ensuring label claim accuracy.

Water Content

The water content of plant material is another target often evaluated in cannabis testing. While water content is correlated to the potential for microbial growth, it also has a dilution effect on the potency of the tested plant material. As such, the potency of a sample will increase as the water content is reduced through drying. Considering the marketing potential of high-THC products, drying may be used as a strategy for artificially increasing the label claim potency of flower products. It is therefore helpful to establish drying requirements for cannabis plant material prior to testing to promote standardized testing and reporting of results. For example, the potency of cannabis flower products sold in Canada are reported as-is, meaning that the water content is not taken into account when calculating cannabinoid potency. The justification for reporting potency on an as-is basis is that factoring out the water content and reporting the potency of the dry material only (i.e. on an anhydrous basis) increases the cannabinoid content and is not reflective of the potency that a consumer or patient would encounter when consuming the product. In Canada, flower material is typically dried to a maximum water content of 14% to prevent mould growth.

Terpenes

Cannabis plant material and raw extracts contain a wide variety of terpenes which are broadly classified as light (C10) and heavy (C15) based on the number of isoprene (C5) units present in the molecule[13]. This difference in molecular weight accounts for the range of boiling points observed among the terpene class, whereby light terpenes possess lower boiling points than their larger-molecule counterparts. Additionally, while most terpenes are hydrophobic (oil soluble), a subset are partially hydrophilic (water soluble) and thus present different solubility behaviours. These properties are important to understand when developing extraction and refinement processes for cannabinoid extracts or formulating finished products with terpene-rich raw or refined extracts.

In general, terpenes are associated with the taste and aroma of the cannabis plant and its derivative products, and therefore terpene testing is generally not mandatory. However, terpenes are known to exert a range of biological activity when consumed and therefore warrant testing, particularly by manufacturers who tout that their products contain terpenes in support of the “entourage effect”. In these situations, both brands and consumers should be aware that terpene testing, which is typically conducted using gas chromatography (GC), is not indicative of the abundance of individual terpenes since the value reported is a relative percentage based on mass, rather than the molecular count.

Microbial Contamination

Microbial testing is critical for determining product safety. Being a living species, cannabis plants always foster a diverse community of microorganisms. However, stringent cultivation practices and quality controls can be implemented to successfully reduce the microbial burden and prevent colonization from harmful species. These precautions are most effective when implemented in controlled cultivations environments such as indoor and greenhouse facilities given the ability to stabilize environmental conditions and shield the plants from foreign airborne pathogens. Common microbial targets include yeasts and molds, total aerobic count, Bile-tolerant Gram-negative bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp*., and *Pseudomonas aeruginosa*. Testing for aflatoxins, which are produced by molds such as *Aspergillus flavus* and *Aspergillus parasiticus*, is also now mandatory in several jurisdictions. Irradiation is often used as a post-process sterilization method to reduce bioburden levels to acceptable regulatory thresholds. However, the heat produced during this process and the

potential for free radical formation can vaporize volatile terpenes, alter the chemical structure of the material, and further reduce its water content.

Endogenous Contaminants: Heavy Metals, Residual Solvents, and Pesticides

Heavy metals such as arsenic, cadmium, mercury, and lead are another source of plant contamination. These elements are commonly absorbed from the soil or water during cultivation. Furthermore, some varieties of *Cannabis sativa*, including hemp cultivars, are known to be efficient phytoremediators, having the ability to efficiently concentrate heavy metals from contaminated soil[14]. Due to their toxicity to human health, heavy metal testing is required for cannabis plant material and derivative products.

Additional contamination targets include residual solvents and pesticides. The former includes solvents such as ethanol, butane, and propanol which are often utilized during the production of cannabinoid extracts. Pesticide testing requirements vary by region, with Health Canada mandating the testing of 96 pesticides for Canadian cannabis products[15].

Defining the Analytical Testing Process

The process of analytical testing, also referred to as a "method," involves several steps, all of which are vital to the accuracy and validity of the final result. These steps include:

1. Sampling of the product material
2. Handling and storage of the sampled material
3. Preparation of the sampled material for analysis
4. Analysis
5. Data processing
6. Data reporting

The current limitations with cannabinoid product testing stem from a lack of a standardized approach that encompasses all the above steps. Harmonization through the entire analytical process is required reduce inter- and intra-lab variability and product methods that are robust, reliable, and reproducible.

Sampling of the Product Material

Cannabinoid levels are known to vary across plant tissue. As an example, two flower clusters (colloquially referred to as a bud) from the same plant can have a different cannabinoids concentration. When it comes to sampling plant material from a bulk lot for potency testing, it is important that sufficient sample material is obtained from various flower clusters in order to establish both the mean potency and an accurate range about the mean. While this is less of an issue for extracted products, which are inherently more homogeneous, this example highlights the risk of how low sampling or non-random sampling can lead to errors in analytical results, even if the remaining steps in the method are standardized and accurately followed.

Handling and Storage of the Sample Material

Method development usually focuses on mid and downstream operations, such as instrumentation and data analysis, and fails to sufficiently consider upstream activities such as sampling, sample stability,

sample storage, and chemistry before analysis. However, analytical testing requires the most extreme care from the analyst, which is often a source of variability and even contamination, especially in situations where analytes within a sample are prone to degradation or when dealing with trace and ultra-trace analyses. Therefore, sample storage and handling procedures should involve validated exposure thresholds for light, oxygen, and heat to prevent sample degradation or contamination. This applies to samples stored at the site of manufacturing and those received by third-party testing laboratories.

Preparation of the Sampled Material for Analysis

Sample preparation methodology refers to the ways in which a sample is treated prior to its analysis. Preparation is the most important first step in most analytical procedures because the target analytes are often not responsive to the analytical technique in its matrix-associated form and may yield distorted results due to interference effects that further impact the recovery of analytes and their determination at high sensitivity and specificity. Treatments such as dissolution, extraction, reactions, filtering, comminution and dilution are commonly utilized to prepare the sample into a form ready for analysis by specified analytical equipment.

The cannabis industry presents several product forms, which include dried plants material, extracts, and formulated products ranging from simple oil blends to more complex edibles like chocolates or gummies. Each of these product forms dictates the requirement for individualized sample preparation prior to analysis. Comminution or size reduction is commonly performed on dried plant material to ensure homogenization of the sample. Filtration and extraction by combinations of solvents is prescribed to isolate the analyte and further dilute it as required for accurate instrumental analysis.

Analysis

In addition to sample preparation, the development of an analytical method that can accurately quantify the presence of a specific analyte is dependent on several factors, including the availability of high-purity standards, statistical validation of results, and considerations towards the chemistry of the target analyte. It is important that the reference standard is high purity with well characterized descriptions of the trace minor components and any known interference effects. Additionally, the reference standards should be commercially available at scale so that analytical process are not negatively impacted dues to standard shortages.

Statistics plays an important role in method validation and ensuring that the analytical process functions within acceptable operational parameters. A method's robustness can dictate the level of replicates that need to be run to ensure that matrix effects, sample non-homogeneity, and analytical equipment instabilities are correctly accounted for[16]. As mentioned, this may be of particular importance when testing cannabinoid products with complex matrices, such as chocolates.

One item seldom considered in the cannabis industry is the concept of molecular chirality. Chirality is an important concept for stereochemistry and biochemistry. Most substances relevant to biology are chiral, such as carbohydrates (sugars, starch, and cellulose), amino acids and de-facto proteins, and the nucleic acids. In biological systems, it is typical to find only one of the two enantiomers of a chiral compound. When organisms consume a chiral compound, they can usually metabolize only one of its enantiomers. By extension, when dealing with nutraceuticals, pharmaceuticals and cannabinoids, it is of paramount importance to have the correct enantiomer present as they usually have vastly different potencies or biological effects[17]. As extensive processing typically occurs in producing the various forms of cannabis

products, there are several unit operations where interconversions or biasing of enantiomers may occur, such as the use of improperly degassed solvents, specific solvents themselves, and thermal methods such as those encountered during decarboxylation and distillation.

Data Processing

Analytical chemistry is dependent on the reproducibly of measurements. However, this represents only a snapshot of the phenomenological surface and are indirectly linked to what one is aiming to determine. As an example, what is perceived as an “off-white” color in a bulk material can be attributed to any form of weak absorbance in the visible spectrum range of 400 to 800nm. For this reason, the application of statistics in analytical sciences necessitates chemical experience, full appreciation of what happens between sampling, the instrument providing numbers on the screen and understanding which theories apply before one can even think of crunching numbers[18]. Most modern instrumentation enables the rapid acquisition of vast amounts of information on a particular sample, however; the instruments and the highly trained staff needed to run them are expensive. Often, samples are not cheap either, this is particularly true if they are pulled to confirm the quality of production lots. A manufacturing process yields a product that is usually characterized by one and up to several specifications, each in general calling for a separate analytical method. Several points that directly affect data and consequently how data is processed are:

1. The number of samples is often restricted for cost reasons (few replicates)
2. Careless random sampling and workup can easily skew concentration-signal relationships
3. The dynamic range of an instrument can be overwhelmed leading to signal distortions and poor signal to noise ratios (S/N) for the observed analyte.
4. Specificity is often inadequate (the instrument cannot sufficiently differentiate between species of interest and others not being investigated)
5. Duplicates can be justified, but several repeats do not improve the interpretation.
6. Impurities on complex matrices affect the repeatability and resolution and consequently the figure of merit.
7. Sample collection and workup artefacts lead to problems with inexperienced workers.
8. Non-statisitical decision criteria are the norm because specification limits are frequently prescribed (ex. 95%-105% of nominal) which maybe be perfectly acceptable for some methods however are beyond the expectations of what statistical common sense might suggest for the specific analyte understudy.

Data Reporting

A certificate of analysis (CoA) is a document generated by laboratories in which they report product-specific analytical results. For each analytical target or quality metric (i.e. water content), it is good practice to specify the method used during testing, as well as any acceptance thresholds specific to the method or target. Additionally, it is recommended that triplicate testing is conducted on critical analytes such as cannabinoids, and that their potency is reported as a mean and standard deviation. Where applicable, the limit of detection (LoD) and limit of quantification (LoQ) should also be reported so that method sensitivities and results obtained from different labs or using different methods can be compared.

Overview of Different Methods for Cannabinoid Product Testing

A variety of methods are currently employed for the testing of cannabis products (Table 1). Regulators have encouraged the adoption of established testing practices from other developed industries, particularly in the case of contamination testing related to residual solvents, heavy metals, pesticides, and microbial contamination. As a result, the methods typically used for evaluating these quality indicators are often compendial methods developed by the United States Pharmacopeia (USP) or European Pharmacopeia (EP), or in some cases methods developed and validated by reputable industry groups such as Association of Official Analytical Chemists (AOAC)[19,20,21]. These rigorously validated methods also include instructions on sample preparation with the intent of reducing human variability to ensure results reproducibility.

Analyte	Typical Methods	Examples of Analytical Targets	Standardized Method Available
Cannabinoids	UPLC-MS, HPLC-UV/Vis, SFC, Chiral HPLC	THCa, THC, CBDa, CBD	N
Terpenes	HS-GC-MS GC-MS GC-FID	Limonene, myrcene, beta-caryophyllene	N
Pesticides	HPLC-MS/MS GC-MS/MS GC-ECD	Organophosphates, carbamates, organochlorines, pyrethroids	Y
Residual Solvents	HS-GC-MS GC-MS	Ethanol, butane, iPrOH, propane	Y
Metals	ICP-OES ICP-MS	As, Hg, Pb, Cd, etc.	Y
Moisture	Loss on drying (LOD) Karl-fisher titration Near infrared (NIR)	Water	Y
Microbiology (bacteria, fungi, viruses)	qPCR Traditional culture methods (TCM)	Coliforms, fusarium, aspergillus,	Y

Table 1: Analytes and methods typically used for the testing of cannabis and cannabis derived products.

In contrast to the standardized methods utilized for contamination testing, there are currently no compendial or industry-recognized methods for evaluating cannabinoid potency or terpene content. Rather, individual labs must develop and validate their own procedures for sample preparation, analysis, data processing, and controlled reporting. Furthermore, the ownness of obtaining representative samples from bulk material remains with the manufacturer, which introduces further variability into the testing process. While consensus has developed on the type of analytical tool to use for cannabinoid testing (HPLC) and terpene testing (GC), the fact remains that testing of these two target groups varies across labs, as evidenced by the numerous reports of discrepancies between labs and inaccurate label claims.

The lack of standardized methodology for cannabinoid and terpene testing therefore continues to impact product quality across the industry while posing a safety risk to consumers and hindering the maturation of the market. To this end, current practices for cannabinoid and terpene testing warrants further discussion with the goal of identifying the limitations to current approaches in testing, best practices

established to date, and opportunities for improvement. More specifically, an overview of chromatography as an analytical tool will be provided, followed by a discussion on the significance of the limits of detection (LoD) and limits of quantification (LoQ) for method validation and interpretation of results. This information will provide much needed insight into the root causes of error observed with current cannabinoid and terpene testing practices, and hopefully aid industry stakeholders in their efforts to resolve these existing issues with testing.

Understanding the Science of Chromatography

Column chromatography is an important physical technique that enables the separation, identification, and purification of individual components within a mixture for qualitative and quantitative analysis. Chromatography is a method in which the components of a mixture are separated based on their differential interactions with two chemical or physical phases: a mobile phase and a stationary phase. The mobile phase travels through the system and carries sample components with it once the sample has been applied or injected (Figure 1). The stationary phase is held within the system by a support and does not move. As a sample's components pass through this system, the components that have the strongest interactions with the stationary phase will be more highly retained by this phase and move through the system more slowly than components that have weaker interactions with the stationary phase and spend more time in the mobile phase[21]. This leads to a difference in the rate of travel for these components and their separation as they move through the chromatographic system.

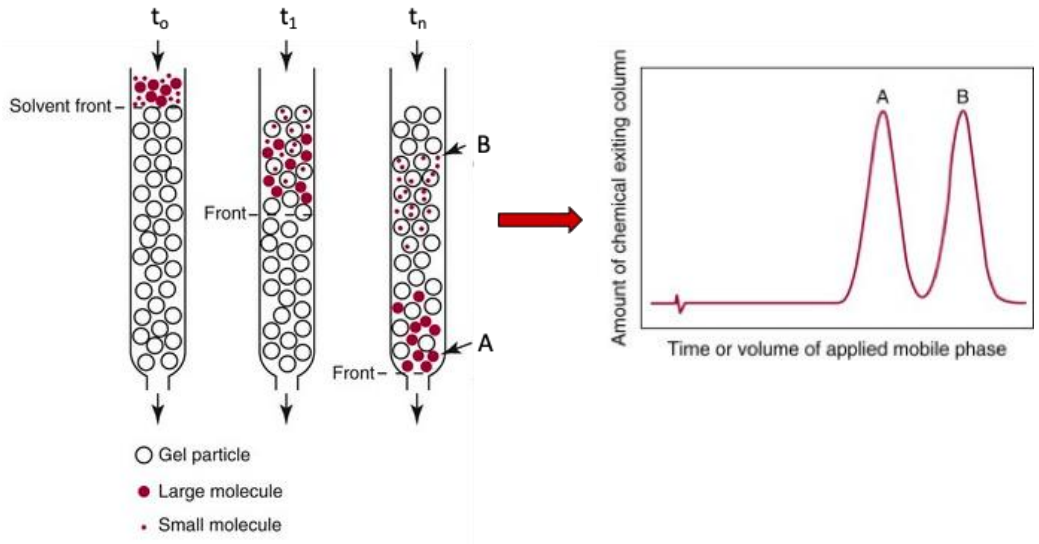

Figure 1: Components of a mixture are separated based on their differential interactions between the mobile phase and a stationary phase, resulting in elution peaks which can be quantified.

This principle of separation applies to most analytical process, namely gas chromatography (GC), supercritical fluid chromatography (SFC), and high-performance liquid chromatography (HPLC). It is of value to note that separation based on the physical size or molecular weight of the molecule is not the driving force for solute separation in these cases. However, the molecular weight plays an indirect role in the separation profiles by effectively increasing the number of available interaction sites with the stationary phase material. Peak detection uses methods that examine spectral properties (UV/Vis), mass properties (MS), specific chemical species (ECD) and electrochemical properties (EC).

In column chromatography, the stationary phase may be a pure silica or polymer, or it may be coated onto or chemically bonded to support particles. The coatings can be either symmetric or asymmetric (chiral) in nature, with the latter being of valuable importance in the analysis of chemical compounds of biological origin or designed to function in a biological application. These compounds have an inherent geometric property called chirality or “handedness”. Column chromatography includes both GC, liquid chromatography (LC), and SFC, with the classification dependent on whether the mobile phase is in the gas, supercritical or a liquid state.

Operationally, the instrument used to perform a GC or LC separation is known as a gas or liquid chromatograph. When the stationary phase in LC consists of small-diameter particles, the technique is known as high-performance liquid chromatography (HPLC). When a GC, SPC or HPLC is connected to a mass spectrometer, the combined or “hyphenated” techniques are gas chromatography–mass spectrometry (GC-MS), (SPC-MS) and liquid chromatography–mass spectrometry (HPLC-MS).

Limits of Detection and Quantification

Analytical laboratories are continually challenged with appropriately determining the Limits of Detection (LoD) and Limits of Quantitation (LoQ) while validating a specific method. The LoD is the point where a true signal is distinguishable from the noise of the detector being employed. Scientifically sound determination of LoDs and LoQs is something that few laboratories employ, and most often choose to define the LOQ as a single low-level standard. These performance parameters are related to the ability of the method to detect, identify, and quantify low analyte levels in samples.

Limit of detection (LoD) is the smallest amount or concentration of the analyte that can be reliably distinguished from zero[23]. The LoD is used for two purposes:

1. To estimate whether the analyte is detected by the measurement procedure by comparing the LoD value and the result obtained from analysis of the sample
2. To characterize the analytical method in terms of its ability to detect low levels of analytes and compare it to other methods, laboratories, or standards

There are several different approaches that can be used to estimate LoD. Guidelines often suggest different approaches and it is up to the analyst or method developer to choose which approach to use. If a specific approach is not demanded by the guideline, this choice must be made based on the necessities and properties of the analytical method. In many cases, regulations set maximum allowed limits for analytes (e.g. pesticides) in certain matrices (e.g. food products)[24].

The limit of quantitation (LoQ) is defined as the lowest analyte concentration in the sample that can be determined with an acceptable repeatability and accuracy. LoQ is not a limit set by nature, as quantitative information at analyte concentrations below the LoQ do exist and can be meaningful in some analyses. However, such quantitative information should be used with care due to the relative uncertainty of results below an LoQ.

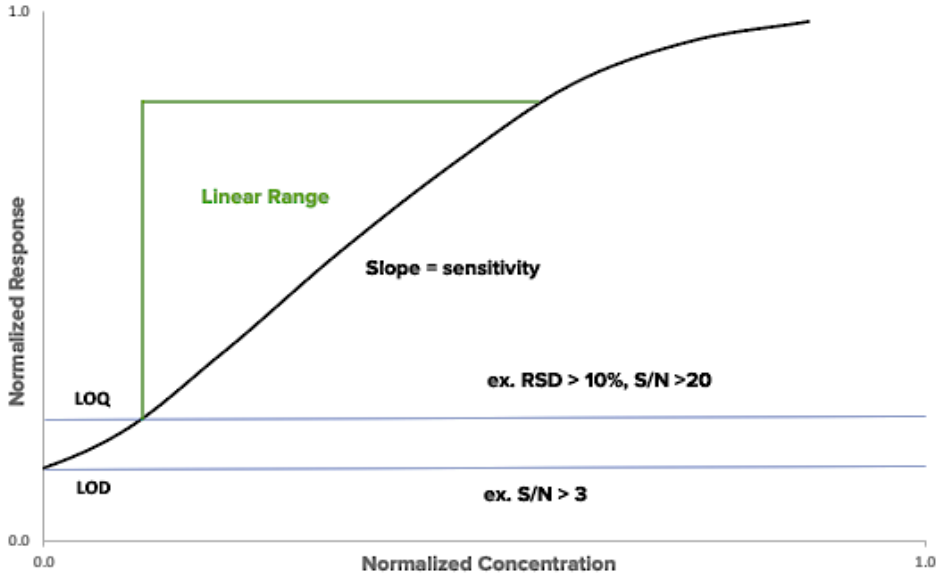

Figure 2: Generalized graphic indicating key attributes of a typical calibration-validation curve encountered during analytical method development (LOQ = Limit of Quantification, LOD = Limit of Detection, S/N = signal to noise ratio, RSD = relative standard deviation).

Interpreting Analytical Results Using LoD and LoQ

The interpretation of analytical results obtained for a specific sample based on LoD and LoQ parameters can be achieved in the following way:

1. When the analyte cannot be detected or its concentration in the sample is found to be below LoD it can be said that the analyte content is below LoD.
2. When the analyte content is above LoD but below LoQ, the analyte can be said to be present at trace level, but usually no quantitative data can be reported.
3. When the analyte content is at or above LoQ, then the result can be quantified.

A result below LoD does not indicate that there is no analyte in the sample, but only that the analysis method is not capable of detecting the analyte in this sample. If the analyte is found to be above LoD, but below LoQ, there is more information within the results than is presented by only stating the analyte's presence, hence the importance of the LoD value for a given analytical method. Based on this knowledge it can be selected whether a simple approach to estimate LoD is enough or a more complex approach that makes less assumptions is more appropriate.

There are cases where the analytical method has properties that do not allow the use of some LoD estimation approaches. For example, it can be difficult to estimate the standard deviation of the blank for LC-MS/MS methods as the noise can be zero due to signal processing. As the blank values all give intensity of 0, the LoD value cannot be calculated from them but the standard deviation at 0 can be still estimated by other approaches, such as from the standard deviation of intercept value or from standard deviation of residuals.

The approach most commonly used in chromatographic methods involves estimating LoD by using the signal-to-noise ratio (S/N). Most modern chromatography programs determine this value automatically. The signal value is determined from the height of the peak while the noise value is determined from either

the standard deviation of the noise or from the so-called peak-to-peak value calculated as the difference between the highest and lowest points in the noise[25]. S/N ratio can be found for only one measurement of a sample. However, a single measurement does not consider the variability between measurements and therefore LoD should not be evaluated from this result. A more typical approach utilizes 10 samples that are measured at different concentration levels and the lowest concentration where all 10 are detected is taken as LoD. The decision that an analyte has been detected can be made from the fact that the S/N is equal to or greater than 3. This means that many measurements must be made to estimate LoD.

Another property that must be considered is homo- and heteroscedasticity. Homoscedasticity means that the variance of the signal is constant even as the concentration changes, while heteroscedasticity means that the variance changes with concentration. Analytical methods are often heteroscedastic – the signal variance increases as the concentration of the sample increases. If it is shown that the calibration data collected is heteroscedastic, then with the intent of being fully rigorous, weighted linear regression (WLS) should be used in order to accurately take into account the variance of the slope and intercept. In conclusion, the method developer and analyst must understand which approaches and considerations can and cannot be used for a given analytical method. This is one of the underlying reasons why results and specifications of LoQ and LoD may be drastically different between laboratories.

Shaping the Future of Cannabinoid Product Testing

There is an apparent need for harmonized and standardized testing processes for cannabinoid-based products. In particular, cannabinoid and terpene testing face growing scrutiny across the industry due to numerous accounts of label claim inaccuracies and inter-lab discrepancies. The consensus within the analytical community and broader industry is that these shortcomings could be resolved by developing standardized testing procedures similar to those adopted by the food, pharmaceutical, and cosmetic industries. To this end, various organizations have already begun to pioneer efforts aimed at developing validated methods for cannabinoid and terpene testing with the hopes that such methods may be approved by regulators and adopted by the cannabis industry. While these efforts are a step in the right direction, they are only the start of a long journey towards remedying the quality issues that continue to burden this growing sector.

Industry Group Efforts to Standardize Testing

Several scientific organizations have initiated their own efforts to provide solutions for standardized testing. In 2017, the American Society for Testing and Materials (ASTM) formed Committee D37 on Cannabis with the goal of developing voluntary consensus standards for the cannabis industry that include test methods and practical guidance for a range of activities, such as cultivation, quality assurance, laboratory testing, packaging and site security[26]. To date, they have proposed 27 new standards for the industry[27]. Of particular note, several of the proposed standards involve sampling procedures as well as analytical methodology for evaluating cannabinoid potency, terpene content, pesticide residue, and trace element contamination. The Association of Official Agriculture Chemists (AOAC) has made similar efforts by establishing three working groups to develop standardized methods for the cannabis industry that address cannabinoid, chemical contamination, and microbial contamination analysis[28]. They are also working towards providing guidance on training, education, and proficiency testing needs for the industry.

The United States Pharmacopeia (USP) is also working with industry, academia, health care experts, and regulators to establish a harmonized framework for cannabis testing and characterization[29]. Thus far, they have developed 6 analytical reference standards and offer several general chapters for reference

standards (USP <11>), Articles of Botanical Origin (USP <561>), and Chromatography (USP <621>) as relevant considerations for testing medical cannabis. Most recently, their Cannabis Expert Panel published a paper in the Journal of Natural Products outlining their recommended specifications for defining cannabis flower quality attributes[19]. Their overarching recommendation was that specifications should differentiate between THC-dominant chemotypes, intermediate chemotypes containing both THC and CBD, and CBD-dominant chemotypes, with subclassifications for each chemotype depending on the minor cannabinoid and terpene profiles. They also suggested that identification and quantitative analysis should be based on morphological and chromatographic tests, while toxicological data combined with USP methods and cut-offs should be considered while establishing limits for pesticides, microbial contamination, mycotoxins, and heavy metals. They suggested that these recommendations are in agreement with efforts to standardize testing made by other scientific groups such as ASTM and AOAC, as well as the American Herbal Pharmacopeia (AHP) which issued a monograph in 2013 that details nomenclature, identification, commercial sourcing, analytics, and handling of cannabis[30].

Proposed Solutions to Challenges with Cannabinoid Product Testing

Industry-wide standardization of testing will require collaboration from regulators, manufacturers, and laboratories given their respective roles in the development and commercialization of cannabinoid-based products. Methods for cannabinoid and terpene testing, including those currently under development by the various aforementioned organizations, will require approval from regulators as recognition of the methods' reliability, robustness, accuracy, and reproducibility. Industry will then be tasked with adopting such methods and adhering to identified best practise for product testing.

In order for an industry to adopt a standardized analytical methodology, the proposed method should meet a several design criteria. More specifically, consideration should be given to the method's accuracy, precision, sensitivity, selectivity, robustness, ruggedness, scale of operation, analysis time, availability of equipment, and cost. From a practical perspective, the method should also meet the following criteria:

1. The sample preparation should be fairly simple and robust to allow for minor variations in analyst lab techniques and differences in environmental factors such as temperature, pressure and humidity.
2. The method should not be overly complex, nor use exotic or cost-prohibitive chemicals in the process.
3. The method should use common analytical equipment to avoid the requirement of specialists to execute the analysis (i.e. HPLC-UV/Vis for cannabinoids).
4. Automation should be used when possible to eliminate human variability.

In the cannabis industry, HPLC is typically the go-to equipment for cannabinoid analysis. Indeed, this piece of equipment meets many of the described selection criteria, such as availability, usability, complexity and throughput. However, the major flaw of HPLC is not the equipment, but rather the variability in the developed analytical methodologies. Cannabis extracts are chemically complex, and the industry is primarily interested in a handful of the compounds therein. Many existing methods are haphazardly developed to quantify these compounds of interest and are not validated to account for potential interference effects between different cannabinoids and their surrounding product matrices.

The likelihood of two different cannabinoid compounds within the same extract displaying similar characteristics in the analysis is a common occurrence. These interactions often present as peak overlaps (non-resolved peaks) that create artificially higher signals (i.e. CBDa and CBGa) and misidentifications (i.e. CBC and Δ^{10}-THC, sCBG and THCa). Other interferences and overlap

combinations can occur and, being governed by the mobile phase compositions, the column material and isocratic vs. gradient schemes, are method specific. One of the largest drivers of this problem is that analytical method developers attempt to create protocols that can rapidly quantify as many compounds as possible in a single run.

To this end, method developers should consider the value in developing methods that target a narrow range of compounds. A sound understanding of cannabinoid and terpene chemistry is required to anticipate possible interference effects between compounds of similar structure and molecular weight. Similarly, developers should consider devising methods that include instructions for sample preparation to eliminate variability and that are unique to specific product formats and thus take into account potential interference effects between cannabinoids, terpenes, and their surrounding matrix (i.e. chocolate)[7].

The credibility of the cannabis industry will continue to be questioned until testing practices are standardized. The current shortcomings of analytical testing for cannabinoid-based products jeopardizes consumer safety and cause consumers and regulators alike to lose confidence and trust in the industry. On a global scale, they are a barrier to the globalization of the cannabis industry, creating a gap in efforts to promote cross-border movement of cannabinoid-based products. Maturation of the industry is therefore dependent on the harmonization of quality standards and the methodologies used to qualify cannabis products as compliant and safe. This harmonization must happen across plant products and their derivatives, including refined extracts and isolates, which have already infiltrated into food, pharmaceutical, veterinary, and cosmetic supply chains[31,32].

LAVVAN's Cannabinoid Solutions

At the forefront of cannabinoid cellular agriculture, LAVVAN utilizes yeast fermentation technology to produce high-quality, reliably sourced, natural cannabinoid ingredients. LAVVAN will provide cannabinoids with unparalleled purity, consistency, potency and sustainability at a scale capable of serving a range of industries including health, beauty, food and beverage, and pharmaceuticals[33]. LAVVAN's cannabinoids are identical to those found in nature and produced in a cGMP facility in accordance with the most stringent standards, including being devoid of pesticides, mold, bacteria, and other contaminants often found in traditional cannabis agriculture. In addition to providing high purity cannabinoid ingredients, LAVVAN will leverage its cannabinoid formulations expertise to support its industry partners with integrating cannabinoids into formulations for various end products that require specific utility.

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