SPECIAL SECTION

Sample Processing and Preparation Considerations for Solid Cannabis Products

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Background: **The nascent cannabis industry is often challenged by a lack of guidance, unproven methodologies and conflicting legal challenges. The actual sampling and sample preparation of cannabis can be a difficult endeavor due the economic value, material complexity and heterogeneous nature of a plant based material.** ***Objective:*** **Examine the challenges of cannabis material sampling and sample processing as it relates to other similar materials.** ***Method:*** **In this paper, the complexity of sampling and sample preparation are reviewed for use in the cannabis industry. The mechanisms for sample processing are examined and compared for the best preparation techniques for targeted analytes in cannabis analysis.** ***Results:*** **Cryogenic grinding is one of the best methods for sample processing for the preservation of volatile compounds.** ***Conclusions:*** **Proper sampling techniques and procedures, including the use of standards, ensure homogeneity and improve analysis accuracy.** ***Highlights:*** **Methods of sample processing and preparation for cannabis plant material were examined to ensure homogeneity, accuracy and reproducibility.**

Scientific testing is often thought of, in popular culture, as a simple and straightforward process. The popularity of crime dramas has proliferated the idea that sample testing simply involves grabbing a pinch of a sample, running to an instrument, and pushing a button and then out pops an answer! We are sure many analysts wish it was just that easy! Although it is true that the evolution of standard methods and cutting-edge analytical instrumentation has made it possible to see a greater variety of compounds at very low concentrations, the reality of analytical sampling, preparation, and testing is far from a one-button answer. More often than not, laboratories are challenged with highly regulated and difficult sample schematics and sample preparation, extraction, and testing procedures.

Over the past 6 years, we have become witnesses to the birth, growth, and regulation of a brand new boom industry: cannabis. The continued legalization of cannabis, in certain U.S. states and around the world, has started a gold rush of growing facilities and testing labs, all trying to shape law and regulation out of what was previously an unregulated illegal product. However, its U.S. federal status as a Class I drug has prevented U.S. regulating bodies from issuing guidance, methods, and regulations. That vacuum of guidance is forcing testing laboratories and scientific organizations to lead in the creation of sampling schemes, preparation procedures, and test methods. The scientific and regulatory communities are now working together to define these roles as well as to redefine global strategies for sampling and testing harmonization worldwide.

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Complexity and Chemical Composition of *Cannabis*

Members of the Cannabaceae family [*Cannabis* (hemp, marijuana), *Humulus* (hops)] are some of the most structurally and chemically complex plants in the world. Originally, hops and *Cannabis* were grouped together by early botanists based on their appearance. The leaves of both the *Cannabis* plants and the hops plants are palmate-lobed (have veins, lobes, and leaflets that originate from a single point on the stem). Within the last two decades, molecular studies and DNA analysis confirmed that these similarities include more than just appearance. Hops and *Cannabis* plants were found to be closely related to each other, sharing structural characteristics, and belong to a single family, Cannabaceae.

Cannabis is thought to be one of the oldest agricultural crops grown for a variety of uses throughout history. Early civilizations used the tall, fibrous *Cannabis* plants for food, oils, textiles, and paper. The medicinal and psychoactive properties were also cultivated for medical and religious practices, which eventually led to strains and varieties we now consider marijuana. The prime difference between these two agricultural purposes was the development and breeding of different varieties for either industrial or psychoactive purposes. The early separation of the cannabis gene pool likely led to the different subspecies of *Cannabis* such as *indica* and *sativa*.

There have been over 500 compounds identified in *Cannabis* (many of which are unique to the Cannabaceae family). The distribution of these compounds is highly dependent on individual strains, the gender of the plant, and location within the plant structures. Plants in the genus *Cannabis* are annual dioecious flowering herbs. Most medicinal and recreational products are obtained from the resinous glandular trichomes of the floral calyxes and bracts of female plants. The majority of target cannabinoids, terpenoids, and other compounds are secreted by these glandular trichomes. As a drug, cannabis is usually produced as dried flower buds, resin, or various extracts or oils. These products are very complex matrixes with high amounts of waxes, oils, and other hard-to-process components in addition to over 500 active compounds.

To further complicate analysis of chemicals in *Cannabis* is the fact that different amounts of compounds can occur in different locations within the plant. In some cases, it has been reported that higher tetrahydrocannabinol (THC) concentrations are found in buds located high on the plant, as opposed to buds located lower on the plant. Different growing conditions, seasons, and environmental and chemical exposure can also alter the chemical composition between growing cycles as well as the chemical distribution within an individual plant.

Cannabis contains over 70 cannabinoids, 150 terpenes, and hundreds of other compounds such as hydrocarbons, nitrogen-containing compounds, carbohydrates, flavonoids, fatty acids, and phenols. Many of the primary compounds of interest can be very challenging to extract and analyze. For example, the most abundant cannabinoids, cannabidiolic acid and Δ9-tetrahydrocannabinolic acid, are biosynthesized as the acid form and decarboxylate into other forms. These acidic cannabinoids can decompose under conditions of light and heat, making them unstable during some common sample preparation, extraction, and testing methods.

Challenges in Solid *Cannabis* Plant Sampling

In the world of agricultural testing, growers are often equipped with detailed methods and monographs describing all the details on their growing operations. Sampling for a crop farmer is a simple process of removing set amounts of samples at designated volumes or intervals and testing those samples for the prescribed list of chemical and biological targets. Unfortunately, for the *Cannabis* grower, there is a void of guidance to which to refer to manage operations, sampling, and testing. In addition to a lack of guidance, there is the added concern that the crop itself is a commodity of high economic value, which inherently forces the limitation of the amount of samples submitted for testing.

Sampling of *Cannabis* brings into question how much and what type of sample is enough to conduct representative sample testing, and what are the criteria for sample homogeneity. Many sample preparation and test methods depend on the foundation of representative samples and homogeneity to provide accurate results. If sampling schematics are not designed to ensure representation and homogeneity of the entire crop then the testing will be biased. In some states, cannabis facilities are required to provide pounds of material for testing, after which the unused portion is returned to the provider. This procedure raises not only economic concerns for the provider but also issues of homogeneity, and finally of contamination when the material is returned.

Another set of questions arises from the debate over the contents of a sample. Is a representative sample a representative sample of the whole plant or primarily just the bud product? Is it necessary for all testing to be conducted on the bud material, or can some nonpotency testing be conducted on other parts of the *Cannabis* plant? Can a discussion be made for using the leaf material to test for pesticide residue instead of the more economically valuable bud material?

Sample Preparation Challenges for Solid Cannabis Materials and Products

An important issue in sample preparation methods for *Cannabis* is homogeneity. Homogeneity is the state of a material being uniform in composition or character (i.e., size, shape, weight, distribution, etc.). By its very nature, dried plant material is not particularly homogeneous. Most of the target analytes (cannabinoids and terpenes) in *Cannabis* plant material exist in the trichomes, which are surrounded by the outer bracts, stems, and seeds of the bud. In sampling methods in which the sample being tested is a high-value commodity, sample size matters, and laboratories are often tasked with preparing smaller samples to meet all the testing demands. Small samples, however, increase potential bias and error unless it can be reasonably assured that the samples are homogeneous.

The most common method for obtaining a homogeneous sample is grinding or comminution to reduce the particle size of the material. Particle size reduction allows for a smaller sample of dry material to more accurately represent the entire batch. In a study by Thiex et al., the particle size was a large factor in the amount of material needed to achieve homogeneity, as seen in Table 1 (1). Grinding samples has many benefits for sample preparation because it increases homogeneity, increases surface area, and decreases particle size, which improves extraction efficiency. Some of the negatives regarding grinding samples are potential contamination, increase in moisture, evaporation, loss or alteration of volatile compounds or labile constituents, and safety issues regarding grinding.

Principles of Grinding

In many cases, the method of grinding is particular for the character or nature of either the sample or the type of studies conducted. In general, sample size reduction is accomplished by either crushing or grinding using forces of impact, attrition, shearing, or compression. Impact force is the striking of one object or material against another. One object may be stationary or both may be in motion. Attrition forces are created by materials rubbing against each other, usually in opposite directions or planes. Shearing force is the cleaving or cutting of a material by some cutting implement or blade. Compression force is the slow application of force against a solid to crush it into smaller pieces, usually between two solid surfaces.

Particle reduction of solids occurs in multiple stages, starting with the accumulation of defects or stresses in a concentrated location increasing the strain on a solid or particle. The stress forms microcracks, and in crystal lattices, it will disrupt the crystal lattice in several cells or locations. The microcracks then join to form a larger major disruption or crack, which ultimately divides the solid into pieces (Figure 1).

Different applications and quantity of throughput and final end products often designate the method employed to grind materials (Figure 2). Crushers are commonly shearing or compression disruptors and are used to create larger particles in the 50–100 mm range. These particles are often just a primary step in some processing scheme. Most crushers are able to process either a large continuous stream of materials or larger

Table 1. Sample size (g) required to ensure homogeneity with their uncertainties with different particle sizes of material (1)

Uncertainty, %	5 mm	2 mm	1 mm	0.5 mm
15	56	4	0.4	0.06
10	125	8	1	0.13
5	500	32	4	0.5
1	12 500	400	100	12.5

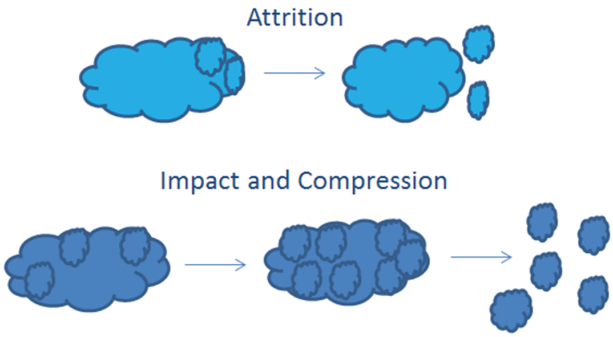

Figure 1. Attrition: Smaller particles break off larger particles through rubbing against surface or each other. Impact and compression: Faults or micro-cracks within the larger particle increase with increasing force, causing the larger particle to break apart.

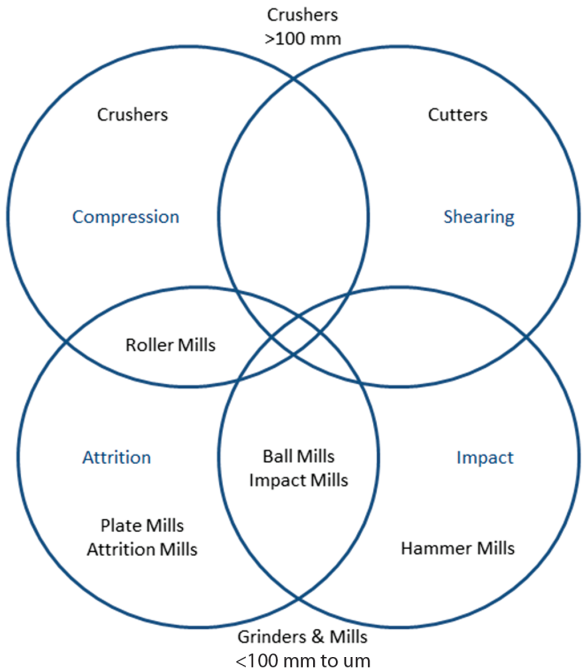

Figure 2. Types of crushing and grinding machinery for laboratory use.

batches. Grinders often produce smaller particles in smaller scales. There are many types of grinders based on the method of grinding and the forces used to grind materials.

Ball mills or ball-medium mills are very popular laboratory tools that grind through impact of a grinding media such as balls, rods, etc. The grinding media and the material to be ground are moved around the mill body or grinding container. These mills are often named for the motion imparted: tumbling mills, vibration mills, and planetary (plate) mills. Ball mills can be used for both wet and dry systems. The rotation of the mill and the impact of the media create a mixture of impact and attrition forces. The advantages of ball mills are multiple product applications, ability to obtain fine particles, safety, simplicity, and reliability. The disadvantages of the ball mills are that they are bulky, heavy, use high energy consumption, and they generate heat, noise and sample contamination from balls or other grinding media.

Other types of laboratory mills for solid sample processing include roller mills, vibratory and shaker mills, attrition and colloid mills, and impact and hammer mills. Roller mills reduce particle size by the movement of materials between various rollers to crush and pulverize the samples into smaller particles. Vibratory or shaker mills use high-speed vibrations and grinding media to combine multiple grinding forces to reduce materials to fine powders. Attrition mills use multiple grinding surfaces, usually as opposing plates that move in opposite directions. Some attrition mills use ball media and stirring to create the attrition forces that cause the media and material to rub against each other to reduce particle size. Impact mills have a moving impactor that pulverizes a sample through repetitive motions. Hammer mills are a form of impact mills in which material is passed between moving hammers. They are good for processing different types of material and are easy to operate. The drawbacks to hammer mills are heat buildup, which can cause sample degradation, and sticky materials, which can clog or damage the mill. Impact mills employ an impactor, which moves in a repetitive movement and crushes the stationary material (Figure 3).

Understanding Material State

The selection of the correct type of mill depends on the material and the factors that will affect size reduction. The most important factors that must be considered when selecting a grinding method are as follows:

(1) Hardness or toughness: Particularly hard samples will need stronger and more energy-intensive grinding methods such as crushers or cutters. Screens or filters are often used to sort by particle size.

(2) Material structure: Samples that are abrasive will cause wear of the grinding system and higher amounts of contamination. Sticky samples can clog grinding heads and screens. Low-density samples or powders may not enter the grinding media area or may float above the grinding surfaces. In some cases, density of powders becomes a safety issue, in which the grinding of powders can lead to flash ignition of materials suspended in the air.

(3) Moisture content: Samples with more moisture are harder to grind and cause more clogging of the systems. High-moisture samples are more often ground in ball medium–type mills or closed systems without filters or screens.

(4) Melting or softening temperature: Grinding generates energy and heat, which can cause material to soften or melt. In laboratory analysis, the heat from grinding can degrade samples or volatilize organic compounds. In the thermally labile products or samples, additional cooling of the material or grinding system is sometimes needed to prevent sample loss or promote efficient grinding.

(5) Purity of required material: Grinding methods often create exposure to other materials of the grinding system or other previously ground materials. Wear metals from some systems slowly transfer into ground samples or products.

The most efficient grinding system is a system that applies the minimum amount of energy to rupture the material without adding excess energy or heat. Energy is required to reduce particle size, but it also generates heats, which can change the sample state or degrade materials. It also then applies that ability to reduce heat generation or negate the effects of heat on the grinding system, which allow

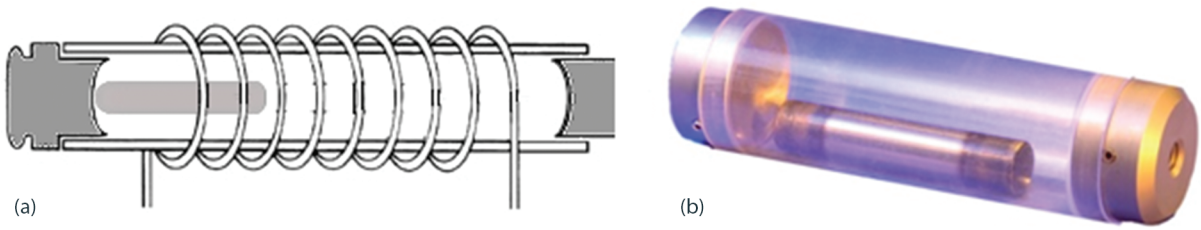

Figure 3. Illustrations of (a) cross-section of solenoid magnetic impact mill and (b) grinding container (SPEX SamplePrep).

for application of more energy into the system to create a more efficient particle reduction. The application of cooling to grinding systems would therefore also be beneficial by (*i*) reducing degradation of materials from heat stress by creating a physical state for the material that promotes efficient grinding, and (*ii*) increasing the retention of important thermally labile constituents.

The grinding of cannabis plants and products produces a fair amount of challenges in regard to the physical state and efficient grinding. The plants are very fibrous and resist methods that employ cutting or may clog filters and screens. The bud material contains high amounts of waxes and oils that stick to grinding media. Cannabis edibles could also be sticky and have difficulty in most grinding apparatus. The moisture content can vary greatly with the type of product, as would its melting or softening temperature. Finally, when looking at metals analysis required in some states, it is important to ensure that wear metals or other contaminants were not entering the samples from processing. One way to unify some of this material complexity is cryogenically cooling the samples before or during grinding.

Cryogenic Effects on Material State and Grinding Efficiency

Using reduced temperatures to grind materials has become a common method to process thermally labile samples and products. In addition to preserving important compounds, the cooling of samples often shrinks the crystal lattice of the solids to be ground. This shrinking causes microscopic cracking, which in turn uses less energy to fracture. The reduction in temperature reduces the heat capacity of the material. Heat capacity is the amount of energy needed to change the temperature of a material by one degree. Through the use of thermal cooling during grinding, the heat capacity of the material is lowered and less energy is needed to drop the temperature of the material. The grinding system becomes more efficient.

The reduction in temperature of a material can also cause the material to become embrittled and in some cases change to a glass state that is easier to grind, which increases throughput and efficiency while reducing particle size. Higher efficiency in a grinding system also means less wear on the grinding system and lower contamination from wear metals in the sample. If liquid nitrogen (LN_2) is used, there are added benefits of a further temperature reduction, which prevents temperature excursions and maintains a more uniform particle size as opposed to ambient grinding; finally, there is an additional element of safety in which the inert nitrogen keeps oxygen low in the grinding system, protecting against powder or dust explosions.

The use of liquefied gases to reduce temperature is called cryogenics. The most popular of the cryogenic liquids in laboratories is liquid nitrogen because of its wide availability and relatively low cost. Liquid nitrogen causes rapid freezing and has a boiling point of –195.79°C. The low temperatures that LN_2 can achieve become important in understanding embrittlement temperature and glass state of materials. Embrittlement is the process by which a material loses its ductility or malleability. This process can be caused by a number of mechanisms, including temperature. For many solids, especially materials with crystal lattices or metals, once it is cooled below its embrittlement temperature (called ductile–brittle transition temperature in metals), there is a much greater risk of shattering (rather than bending or deforming) during impact or grinding.

The glass state of a material, or the glass-transition temperature (T_g), is the range of temperatures over which amorphous materials or semi-crystalline materials transition from a viscous or rubbery state to a hard and brittle glassy state. The process of a viscous liquid or semi-solid transitioning to a glass state through super cooling is often referred to as vitrification. Many industries have documented T_g values to aid in the grinding and material analysis of products. In particular, the plastic industry uses the glass-transition temperature in materials testing and failure analysis. Most plastic and rubber compounds have T_g between –70 and 145°C, meaning that in order to achieve embrittlement, the use of liquid nitrogen can cool to below –190°C and create the glass state for the plastics.

Moisture level in products affects a material's glass-transition temperature. T_g decreases with increased moisture levels. A study of food products including cassia showed that water in the food had a plasticizing effect, which resulted in needing lower temperatures to achieve the glass-transition temperature in food items with higher water content (2, 3). In cannabis products with high moisture content, it becomes especially important to negate the effect of the moisture to ensure efficient grinding.

Cryogenic Effects on Chemical Stability and Compound Retention

The second area where cryogenic applications to sample preparation can aid in laboratory analysis is in the stability of materials and the retention of important labile or volatile compounds or elemental species. As has been discussed previously, cannabis products have a multitude of volatile compounds that must be retained during sample processing. The approach to the sample preparation and grinding of cannabis should mimic another similarly economically valuable group of products—spices. In many ways, spices are very similar to cannabis. Spices are full of the same highly aromatic compounds

as cannabis (terpenes, volatile oils, etc.), which contribute to taste, aroma, and medicinal attributes. Some spices and cannabis also contain many different fats and oils that can be degraded by high temperatures and oxidation.

In spice milling, the objective is to have a powdered product with small particle sizes that retain all the compounds that contribute to aroma and flavor. In ambient temperature processes, heat and energy are generated, which can raise the temperature of spices to almost 100°C, causing the loss of critical aromatic components (4).

Studies of ground spices showed that spices ground under cryogenic grinding conditions contained increased amounts of volatile compounds and essential oils. The loss of some volatile oils during ambient grinding was found to be almost 40% compared with the cryogenically ground samples (5). The refrigeration and precooling of the spices maintained low temperatures and absorbed the heat generated by grinding, which prohibited the breakdown of volatile compounds. In one study, it was found that grinding black pepper under cryogenic conditions showed better retention of monoterpenes (myrcene, limonene, and pinene) than grinding at ambient temperature. These monoterpenes are the same primary monoterpenes in many cannabis varieties (6, 7).

Oxidation of aromatic compounds, which is inherent in open-air systems, is reduced in the closed environment of cryogenic grinding vessels. Cryogenic grinding reduces compound loss, and the vaporization of the LN_2 creates an inert environment to reduce oxidation (7). In addition, the extreme low temperatures generated by solutions such as liquid nitrogen solidified the fats and oils of the spices, leading to a more finely ground sample of consistent particle size with increased surface area, allowing for better extraction during further sample processing. Cooling can also reduce the clogging of equipment by products with high fat and oil content.

Another group of compounds that can be damaged by heat and oxidation are pesticides. Although most growers would be happy that pesticides were degraded during sample preparation and analysis, the health of the end users and the practice of good science cannot allow critical areas of analysis to be damaged by sample processing. Many pesticides that are commonly used and monitored for cannabis analysis are easily degraded by high temperatures and oxidation. In cases in which potentially important compounds (i.e., terpenes, THC, volatile oils, or pesticides) could be lost to processing, it becomes necessary to be able to prevent the loss and calculate for the loss by processing by using standards.

Standards as Sample Preparation

An important but often overlooked step in sample preparation and processing is the use of standards. Many analysts only think of standards at the point in which they are ready to introduce their processed sample to their instrument for analysis. The role of standards is not only important as the end point for calculation but is equally if not more important as the monitor for the sample preparation processes.

The basic definition of a standard is a "known" with which an "unknown" is compared. The American Heritage Dictionary states that a standard is "An acknowledged measure of comparison for quantitative or qualitative value." The International Organization for Standardization (ISO) recognizes different types of standards from the point of origin with respect to their accuracy—primary and secondary standards.

A primary standard has the highest metrological quality and is a value that is accepted without reference to other same quality standards. Primary standards are the first order of standards that are accepted without comparison. Primary standards are created by national metrological institutions in each country, such as the National Institute of Standards and Technology (NIST) in the United States.

A secondary standard is a standard whose value is assigned by comparison with the same quantity of a primary standard. Many accredited standards offered by standards manufacturers are secondary standards. These types of standards refer back to a primary standard within their certification (traceable to NIST, for example).

Some other common terms associated with standards are reference material, certified reference material (CRM), traceability, and stability. A reference material is a material or substance whose property value(s) are sufficiently homogeneous and well established for use in the calibration of an apparatus, assessment of a measured method, or for assigning values to materials (ISO). A reference material accompanied by a certificate is called a certified reference material or certified standard. The accompanying certificate must have many points of information to be accredited by a certifying body such as ISO. An ISO-compliant certificate has one or more property values that are certified by a procedure that establishes traceability and uncertainty. The certificate also must contain methods of measurement, materials of preparation, and statistical treatment of results, product stability, and homogeneity, as well as other supplementary information.

Traceability is the ability to trace a product from its origin, through manufacture through to delivery and receipt of final product. For CRM, that ensures that a material can be traced to a primary standard. Stability of a product means that the product or standard is not reactive during normal use and retains its properties in an expected time scale in the presence of the expected conditions.

Correct Use of Standards

The first step in using standards as a part of sample preparation and processing is to understand what standards are and how they are used. Standards can be used for qualitative analysis (identity) and/or quantitative analysis (numerical results). Standards can also aid in the identification or elimination of error or be used to determine statistical variables such as uncertainty, accuracy, and precision.

Error and uncertainty are not the same concept. Error is the difference between an actual measurement and the true value of the measurand. Error does not include mistakes that can be explained and excluded. Error causes values to differ when measurement is repeated. It is impossible to completely eliminate error, but it can be controlled and characterized.

Uncertainty is a statistical estimate attached to a certified value that characterizes the range of values where the true value lies within a stated confidence interval. Uncertainty estimates the effect of short-term fluctuations, variables in the performance of an analyst or piece of instrumentation, or accounts for bias or drift that can be corrected or calculated. The uncertainty on a standard's certificate shows the user the certainty of the true value being within the stated range.

The most common role of standards is to be that known to calculate the unknown. The goal of most laboratories is accurate and precise results that reflect the true value of the analytes. Accuracy and precision are different concepts. Accuracy is an expression of how close an analysis gets to the true value. For example, if the true value for a THC concentration is 5% and the instrument result is 5.5%, this value may be considered accurate if it fits within the criteria established by the laboratory. The criteria for accuracy are usually determined within an individual laboratory based on the levels of detection and quantitation of an instrument.

If the sample is repeated several times and the results are 4.5, 5.0, 5.5, and 5.2%, then whereas those results may be accurate, they may not be precise. Precision is how close the results fall to one another. Many precision calculations are based on RSD or RSD, %. Relative standard is a measure of dispersion of a probability distribution or frequency distribution. It is often expressed as a percentage. Precision confidence increases and the RSD percent decreases.

RSD, % = 100 (sample standard deviation/sample mean)

Looking at the same example of a 5% THC concentration, if the laboratory gets results such as 4.5, 4.4, 4.3, 4.3%, then those results could have excellent precision (RSD, % = 2.19%) but would not fit the criteria for accuracy. These results would be precise but not accurate (Figure 4).

In much instrumental analysis, the instrument manufacturer will often provide guidance as to their instrument's precision. In general, most instruments have acceptable working precision values that prove that instrument's reliability and reproducibility. Some common instruments and autosamplers such as HPLC and UV/diode-array detectors often have very low RSD values under 1–5%. Instrument processes with higher uncertainty such as GC/MS or LC/MS detectors may have higher acceptable performance RSD percent in the range of 10% or under.

Standards are necessary to calculate both accuracy and precision of sample preparation processes and analysis. The questions then often arise of which standards are used and at what point are standards introduced into a sample preparation and analysis process.

Role of Standards

The second important step in using standards in sample processing and preparation is to understand the role standards play within a designated process or analysis. Standards are often designated by either their location in the sample or their intended role in the process. Standards are often added to the sample or to the preparation process to compensate for complex matrixes or calculate and adjust for bias or recovery. These types of standards are called internal standards (IS), recovery standards, and spiking solutions. The uses of standards for extremely complex products such as cannabis are necessary to ensure accurate results. In an industry in which concentrations of the active drug components matter, it is essential that the sample preparation and extraction process is monitored to correct for recovery rates and error using matrix-matched standards.

An internal spiking standard or recovery standard (added to a blank sample matrix or a certified reference material) can be used at the beginning of sample preparation to monitor the efficiency of a sample grinding or preparation process. A recovery standard added before extraction can calculate the recovery percentage of that extraction. An internal standard added to a sample will combat instrument bias.

The best types of internal standards are compounds that are similar to the target analyte. These compounds can be labelled analogues or similar species of compounds not already present in the sample. Internal standards need to be added in the range that the target analyte is expected, above the expected LOD and expected LOQ in the instrument in which the analysis is being performed. LOD is the lowest amount of an analyte detected by an instrument or method. LOQ is the calculation of the lowest amount of an analyte that can be reliably quantitated within an instrument or method. Internal standards can be added to a variety of types and sizes of batches, from bulk samples to laboratory sample sizes. In each case, homogeneity of the application and distribution of the standard must be ensured. There are larger chances for error to occur in the application of internal standards to larger batches of dry materials than there are in application to smaller batches or liquid batches of materials. The most common application of internal standards to larger-scale or laboratory batches is the creation of an IS solution that is either sprayed in a controlled container or fashion to the dry material, which is then subsequently agitated, shaken, or tumbled to distribute the internal standard to the dry materials. Multiple lot samples need to be taken and analyzed to ensure homogeneity of the application.

A second important type of standard is external standards. External standards are not added to the sample. These types of standards are usually run with the actual samples in the same method as the samples. External standards can be either matrix-matched (i.e., having the same matrixes as the

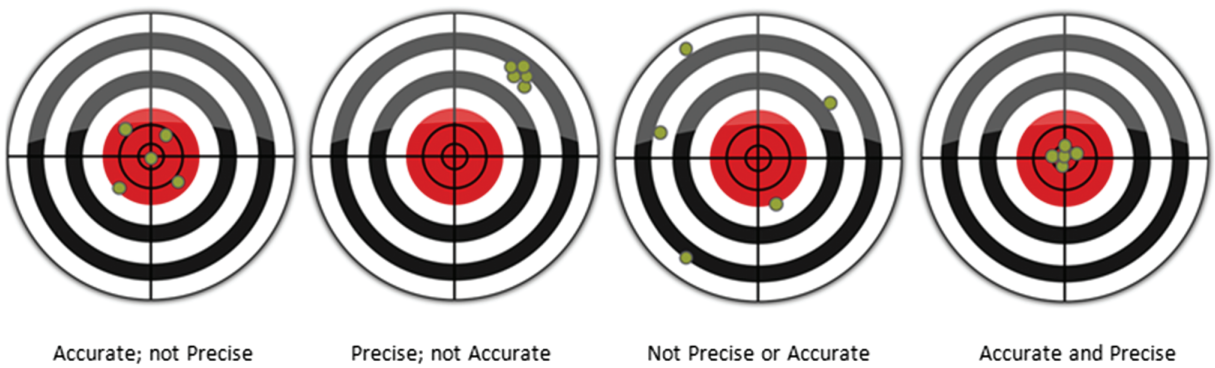

Figure 4. Examples of accuracy and precision.

sample) or unmatched. External standards are most often used to create calibration and response curves, identify analytes, or to compare with the response of an internal standard to correct for differences in sample preparation, extraction, or instrumental drift. External standards cannot compensate for volume variations of injections in the way of internal standards. External standards are often duplicates of the target analytes prepared in the expected range of the target analytes. The most common use of external standards is for calibration curves and quantitation of analytes.

A calibration curve is made up of several known points created by standards of different concentrations. An effective calibration curve has over three points, with five-point curves being common. The points of the curve bracket the low and high points of the expected analyte concentrations. Lack of bracketing can force calculation against imaginary points of the curve, thereby creating error. The most accurate point for quantitation in the curve is the point closest to the target value.

Standards are a critical step in the sample preparation process of cannabis; the challenge becomes obtaining and using standards in an industry that is still considered illegal in the majority of countries around the world. The logistics of finding, purchasing, and using reliable and accurate standards becomes a challenge of availability, legality, and transportation. In the interim of widely available and legal standards and reference materials, scientists and manufacturers continue to design methods and processes to accommodate the state of the current climate.

Conclusions

The approach to sample preparation and processing for the new cannabis industry is as truly complex as the cannabis plants and products themselves. The growers are faced with uncertain legalities and a lack of guidance. The laboratories are also faced with those uncertainties in addition to a lack of proven validated methodologies for a very complex and economically valuable product. Not only do the regulatory bodies and laboratories have to worry about good science but they have to worry about good economics. Sometimes the pursuit of a good scientific result (i.e., adequate sample volumes for homogeneity and quality certified reference materials and standards) is hindered by physical, legal, and economic stumbling blocks that seem to fight against good science.

Everything about the new cannabis industry is challenging, from the actual structure and chemistry of the plant itself down to how samples are transported for testing. Despite all the complexity, the approach to sample processing and testing still must remain along the same lines as other similarly complex agricultural products (i.e., hops, spices, etc.). The rationale for sampling, processing, grinding, extraction, and testing of these similar products can be used as a roadmap for the cannabis sampling and processing methods.

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