# THE ROLE OF CANNABIS FLOWER SAMPLING IN QUALITY CONTROL IN MEDICAL CANNABIS INDUSTRY: A REVIEW

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**Abstract:** The purpose of cannabis flower sampling is to apply good practices for sampling cannabis that is strictly regulated, for analysis in order to examine the quality of the product. Every company involved in the production of cannabis has standard operating procedures for managing operations, sampling and testing. More than 500 bioactive components have been identified in the cannabis plant. The distribution and concentration of these compounds also depends on environmental conditions such as soil, water and light. A sample is a small part of something which represents a larger whole or grouping. It is important to look at sampling from the grow through to the laboratory to examine where the focus and perspective should be to ensure that cannabis sampling at every stage of the process represents the whole we are trying to characterize. For the cannabis industry, the population or sampling frame could be as large as an entire crop, just one variety within an operation, or as small as selected trimmed buds from specific plants, varieties, or just areas of growth, depending on the purpose for the sampling. There are two basic types of sampling: probability sampling (random) and nonprobability sampling. Probability sampling is when each unit of a population or a whole has the same chance of being selected to make up a sample and the probability of being selected can be calculated. Nonprobability sampling is when samples are collected in a process where some samples are purposely selected and the selection processes do not give all the possible samples an equal chance of selection. Dry plant material is not completely homogeneous. In sampling methods, sample size is very important, and laboratories often have to prepare smaller samples to meet all testing requirements. The most common method for obtaining a homogeneous sample is grinding. Grinding the samples increases the homogeneity, their surface area and reduces the particle size, which improves the extraction efficiency. In the end, the development of sampling plans-sample preparation methods that understand the importance of these concepts-will ultimately be the most important step in achieving good analyses.
**Keywords:** sampling plan, representative samples, homogeneity, grinding, cannabis flower.

## 1. INTRODUCTION

Most laboratories are challenged with regulated and difficult sample schedules, samples preparation, extraction in production facility, and testing SOPs that attempt to ensure testing accuracy and precision. Accuracy in analytical testing begins at the very beginning with sampling and sample preparation prior to testing. If the initial sample collection and preparation are flawed, then the final results will not meet the finished product specification (Smith, 2019). The foundation of sampling and testing accuracy is often based on two interrelated and basic concepts: representative samples and homogeneity. Representative samples are selected to accurately reflect the larger group and should represent the characteristics of the group as a whole. Ideally representative samples are homogeneous (Zana et al., 2012).

The purpose of the cannabis flower sampling procedure is to outline best practices for the sampling of regulated cannabis for analysis by a medical or retail cannabis testing facility. It is meant to provide the cannabis testing facility with samples representative of the harvest batch.

Agricultural samples can be some of the most difficult samples in the world to sample, prepare, and analyze because of their heterogeneous nature and complex matrices. Luckily for most of the agricultural testing world, the industry is equipped with detailed methods for operations, collection, and testing (Oregon Liquor Control Commission, 2018). There is also difficulty in the fact that cannabis is a very complex plant.

More than 500 bioactive components have been identified in the cannabis plant. The distribution of these compounds is highly dependent on the species of cannabis, the sex of the plant and the location of the plant. The concentration of these compounds also depends on environmental conditions such as soil, water and light (Zana et al., 2012; Sexton & Ziskind, 2013). Further complicating the analysis of bioactive components in cannabis is the fact that different amounts of compounds can occur at different locations in the plant. In some cases, it has been observed that higher concentrations of tetrahydrocannabinol are found in buds located high on the plant as opposed to buds located lower on the plant (Sexton & Ziskind, 2013). Different growing conditions, seasons, environmental,

and chemical exposure can also alter the chemical composition between growing cycles as well as the chemical distribution within an individual plant (Oregon Liquor Control Commission, 2018; Bureau of Cannabis Control).
Sampling for cannabis analysis raises the question of what the criteria are for sample homogeneity, as well as how much and what type of sample is sufficient to conduct representative sample testing. Many sample and test preparation methods depend on the establishment of representative samples and homogeneity to ensure accurate results. If sampling schemes are not designed to ensure representativeness and homogeneity of the entire culture up to the final analytical sample, then testing will not provide the required analytical data and results will not conform to the quality specification (Oregon Liquor Control Commission, 2018; Bureau of Cannabis Control).
The idea of a sample and sampling is commonplace to most of us. A *sample* is a small part of something which represents a larger whole or grouping. It is important to look at sampling from the grow through to the laboratory to examine where the focus and perspective should be to ensure that cannabis sampling at every stage of the process represents the whole we are trying to characterize (Bureau of Cannabis Control; Smith, 2019).

## 2. CLASSIFICATION OF SAMPLING TYPES FOR ANALYSIS

The key concepts on the larger scale or the grow side are: population, sampling frame, and representative samples. A *population* is the entire possible group of objects that are being sampled or a subset of those objects. A sampling frame is a possible source material taken from the population where samples will be obtained. So if a single variety of cannabis in a crop of multiple varieties is to be harvested, then the population can be seen as the entire crop of all varieties and the sampling frame is all the harvested plants of a single variety or strain (Potter & Duncombe, 2012). The *sampling frame* is composed of primary samples which in turn are composed of sample units. *Sample units* are the smallest discrete portions that are taken to form the whole or part of a primary sample. For the cannabis industry, the population or sampling frame could be as large as an entire crop, just one variety within an operation, or as small as selected trimmed buds from specific plants, varieties, or just areas of growth, depending on the purpose for the sampling (Ghodki & Goswami, 2016; Ghodki, et al., 2016; Singh & Goswami, 1999).
There are two basic types of sampling: probability sampling (random) and nonprobability sampling. *Probability sampling* is when each unit of a population or a whole has the same chance of being selected to make up a sample and the probability of being selected can be calculated. *Nonprobability sampling* is when samples are collected in a process where some samples are purposely selected and the selection processes do not give all the possible samples an equal chance of selection (Singh & Goswami, 1999).
Probability sampling has four primary methods of selection: simple random selection; systematic selection; stratified selection; or cluster selection. *Simple random selection* is, by its own title, a random process which sometimes can use a random number generator or table to obtain samples. *Systematic selection* uses a collection method where every $n^{th}$ member (that is, the sampling interval [$\kappa$] of a population or sampling frame is taken as a sample. The sampling interval ($\kappa$) selected to use is dependent on the size of the population or sampling frame and the number of samples to be collected. To calculate the sampling interval ($\kappa$); the total population size *(N)* is divided by the targeted sample set size (n) or $\kappa = N/n$. For example, if a grower has 100 plants and wants to have a sample size of 20, then they would have to sample 100/20 = 5; or every $5^{th}$ plant (CFR - Code of Federal Regulations, 2019; Bureau of Cannabis Control, 2018; Smith, 2019; Thiex et al., 2008).
The third type of probability sampling is *stratified sampling* which is when the population or sampling frame is divided into subsets or strata. This type of sampling can be used to differentiate between samples of different types such as different species or varieties within one population. Once the strata are established then another random selection process is used to select samples. The final type of probability sampling is *cluster sampling* where physical areas or geography are designated into clusters that are then sampled. Cluster sampling is best used when there is an extremely large population, such as a national forest or a population of a state, which must be examined (Ghodki et al., 2016). Focusing on reducing the sample frames down to smaller groups reduces the amount of time, energy, and money to represent the target population.
In contrast to probability sampling, there is nonprobability sampling where a nonrandom selection process for the purpose of obtaining targeted data or results can intentionally or unintentionally create bias. *Convenience sampling* means that the sampler takes samples with easy access. Convenience sampling is easy, cost effective, and fast but it can produce bias by under-representing the overall population (Shailendra et al., 2015). Similar to convenience sampling is *consecutive sampling*, where the samples are selected consecutively within or between units, and in a typical crop, selection setting is essentially the same as convenience selection. *Quota sampling* is similar to stratified sampling by dividing into strata, but the selection is not random. Finally, there is *judgement* or *purposive* sampling where the sample is chosen based on what the sampler or researcher thinks is needed for the study. This technique is used for research in a small group or field to create a specific population and is biased to the selected purpose (Sreenarayanan & Mathew, 2007). For example, if a grower wants to know the

highest concentration of THC found in his crops, and the grower knows that a certain area of the crop, or certain parts of the plants, have traditionally the highest THC concentration because of variety, environment, water access, and other conditions, then sampling of only those areas have a purpose to create data on the population of the plants with the highest THC concentration.

Each method of sampling we have examined so far has both advantages and disadvantages. There are places for each type of sampling method and the choice of sampling method often comes down to purpose and intent. In some cases, different methods of sampling may be combined at different points in a process to achieve either an overall representative sample, or a sample with a specific purpose and intent. Most commercial agricultural operations do not perform sample collection for testing directly from the field but wait until harvest to partition and sample their batches or lots. The methods for post-harvest sampling remain the same as pre-harvest sampling, just some of the terminology changes. Instead of defining population and sampling frame, there is a harvest of all or portions of a crop (Ghodki et al., 2016; Singh & Goswami, 1999; Shailendra et al., 2015). That harvest yields bulk material to be processed either at one time or in separate manufacturing events.

In the harvest and processing of cannabis material, the samples are no longer grouped by which plant is sampled or area is sampled, but by which parts of the harvested and processed materials are sampled and the method by which the samples and units are selected. The same methods of sampling still apply, for example, if systematic sampling is used-instead of sampling every $5^{th}$ plant, every fifth container of harvested buds has to be sampled, or every $5^{th}$ bud processed is taken, depending on the sampling plan design (Sreenarayanan & Mathew, 2007; Murthy & Bhattacharya, 2008).

Once the lot is sent for testing, the material is subdivided many times according to the laboratory or state regulations to create representative samples for testing. Primary samples can be taken from the lot and composited together to create a bulk testing sample. The laboratory samples can either be processed at this point-or extracted, ground, and so forth, depending on final testing-and purpose or the material can be further subdivided, processed, and sampled for individual analytical samples, such as portions and aliquots. In each step where there is further subdivision of the material, an appropriate sampling method must be used to try and achieve the homogeneous representative sample of the lot (Oregon Liquor Control Commission, 2018; Bureau of Cannabis Control, 2018; CFR - Code of Federal Regulations, 2019).

## 3. SAMPLE MATERIAL PROCESSING CHALLENGES FOR CANNABIS

Laboratory samples must be processed in a form that allows for analytical, instrumental and chemical testing. Most of the time, this process involves grinding the samples to homogeneity and processing (extraction) for analysis. With each division of the material, the samples become smaller and smaller until by the last step only a small amount of material is actually tested (Bureau of Cannabis Control, 2018). This fact raises the questions of how much and what type of sample is enough to conduct representative sample testing, and what are the criteria for sample homogeneity. It can be difficult in the cannabis industry to determine if what is thought to be representative by the state regulations actually fits the bill scientifically for accurate results. If sampling schematics are not designed to ensure scientifically valid sample representation and homogeneity of the lot, then the end testing will be biased.

A fundamental issue in cannabis sample preparation methods is homogeneity. Dried plant material is not homogeneous. In sampling methods where the sample being tested is a high-value commodity, sample size is important, and laboratories are often tasked with preparing smaller samples to meet all testing requirements (Thiex et al., 2008).

Methods used for preparing of a homogeneous sample is grinding or milling. Sample grinding has many benefits for sample preparation as it increases homogeneity, increases surface area and reduces particle size, which improves efficiency in productions processes (etc. extraction). Sample grinding also allows for a reduced sample size to increase accuracy and reduce uncertainty (Thiex et al., 2008; Ghodki & Goswami, 2016). A particle size of 5 mm is about the size of a pencil eraser. If a particular laboratory needed to have results within 5% uncertainty, they would have to use 500 g of material for testing. But, if the laboratory reduced the particle size to less than 0.5 mm (the size of a fine point pen tip), the amount of sample needed to ensure 5% uncertainty would drop to less than 0.5 g (Thiex et al., 2008; Ghodki & Goswami, 2016; Ghodki et al., 2016; Singh & Goswami, 1999).

The grinding of cannabis plants and products presents a number of challenges in terms of physical condition and efficient grinding. The plants are very fibrous and resist methods that use cutting or filtering in further production processes such as extraction. Cannabis material contains waxes, lipids and oils that adhere to the grinding blade (Singh & Goswami, 1999).

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*

(Ghodki & Goswami, 2016). Moisture level in products affects a material's glass-transition temperature. Tg 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 (Ghodki & Goswami, 2016; Ghodki et al., 2016).

Another area is cryogenic applications for sample preparation that can aid in laboratory analysis and for improved stability of materials and retention of important labile or volatile compounds, such as terpenes. Some active components in cannabis can be broken down by high temperatures and oxidation. The grinding processes generated heat and energy and cause the loss of aromatic components (Singh & Goswami, 1999).

Studies of ground cannabis flowers have shown that cryogenically ground cannabis contains almost 40% more volatile compounds and essential oils than ambient ground. (Shailendra et al., 2015). Low temperatures do not lead to the decomposition of volatile compounds (Sreenarayanan & Mathew, 2007; Murthy & Bhattacharya, 2008). Pesticides can also be damaged by heat and oxidation. Many pesticides commonly used and monitored for cannabis analysis are easily degraded by high temperatures and oxidation. In cases where potentially important compounds may be lost during processing, it becomes necessary to be able to prevent the loss and calculate the loss using standards.

## 4. CONCLUSION

Sampling for the analysis of cannabis material is of crucial importance for the quality control of the plant material as well as for further production processes. The approach to sample processing must however remain the same as prescribed in the manufacturer's standard operating procedures and protocols. Rational sampling, processing, grinding, extraction, as well as testing of cannabis products are of great importance to the controls at the production stage and the taking of samples for analysis at each stage of the processes. Accurate sample analysis begins with the two fundamental concepts of representative samples and homogeneity. Ultimately, developing sampling plans - sample preparation methods that understand the importance of these concepts is the most important step in achieving good analyses.

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