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Technical Note

Sample Processing and Preparation for Cannabis Products

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Accuracy in analytical testing starts 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 are biased. The basis of accuracy of sampling and testing is based on two inter-related and fundamental concepts: representative samples and homogeneity. Representative samples are selected to accurately reflect the larger group as a whole. Ideally, representative samples are homogeneous or similar in nature, but when that is not possible, the best attempts must be made to achieve samples that represent the majority of the characteristics of the larger grouping.

The continued legalization of cannabis has started an onslaught of growing facilities and testing labs trying to shape law and regulation out of what was previously an unregulated illegal product. A vacuum of legal guidance forced testing laboratories and scientific organizations to create sampling schemes, preparation procedures and test methods. The scientific and regulatory communities are now working together to define these roles as well as redefine global strategies for sampling and testing harmonization worldwide.

Chemical Composition of Cannabis

Members of the Cannabaceae family are among some of the most structurally and chemically complex plants in the world. There have been over five hundred compounds identified in cannabis (many unique to the Cannabaceae family). The distribution of these compounds is varied and highly dependent on individual strains, the gender of the plant and location within the plant structures. 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. In some cases, it has been reported that higher THC concentrations are found in buds located high on the plant as opposed to buds located lower in the plant.

The majority of target cannabinoids, terpenoids and other compounds are secreted by glandular trichomes located in the floral calyxes and bracts of female plants. Cannabis is usually produced as dried flower buds, resin or various extracts or oils. These products are very complex matrices with high amounts of waxes, oils and other hard to process components in addition to the hundreds of active compounds. Many compounds of interest can be challenging to extract and analyze. The most abundant cannabinoids, CBDA and Δ9-THCA 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.

Sampling Methodologies

In addition to a lack of guidance and a complex heterogeneous nature, cannabis is a commodity of high economic value which inherently forces the grower to limit testing samples submitted to mandated sampling minimums. It is important to look at sampling from every point in the process to examine where the focus and perspective should be to ensure that cannabis sampling at every stage represents the whole being characterized. In the field, the key concepts on the larger scale or the grow side are: population, sampling frame and representative samples.

A population is the entire set of sampling targets, where a sampling frame is the entire set of target units potentially selected for sampling. A sample is one or more target units selected for inclusion in a sampling and a unit is the smallest discrete portion taken to make up a sample (see Figure 1).

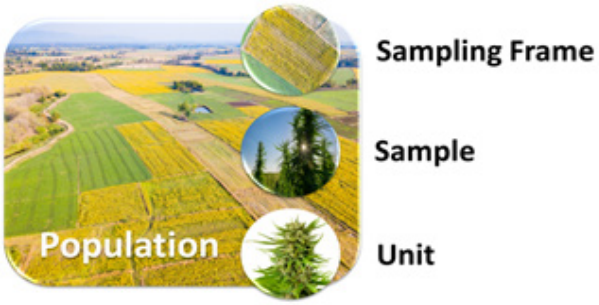

Figure 1. Examples of statistical sampling units

There are two basic types of sampling: probability sampling (random) and non-probability 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. Non-probability sampling is when samples are collected in a process where some samples are purposely selected and the selection processes do not give all of the possible samples an equal chance of selection.

Probability sampling has four basic methods of selection: simple random selection, systematic selection; stratified selection, or cluster selection (see Figure 2a).

- **Simple random selection**: random process which employs a random number generator or table.
- **Systematic selection**: collection method where every n^{th} member (i.e. the sampling interval (k)) of a population or sampling frame is taken as a sample.
- **Stratified selection**: the population or sampling frame is divided into subsets or strata such as different species or varieties within one population. This process is followed by another random selection process.
- **Cluster selection**: used for an extremely large population such as a national forest or a population of a state which must be grouped before sampling.

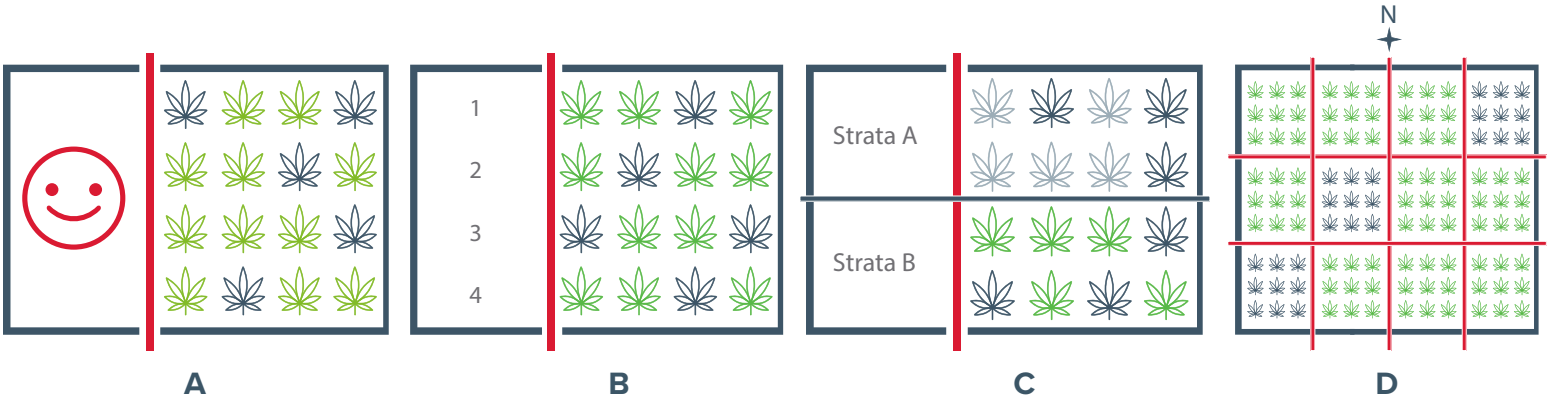

Figure 2a. Probability sampling methods: (a) random sampling, (b) systematic sampling, (c) stratified sampling, (d) cluster sampling

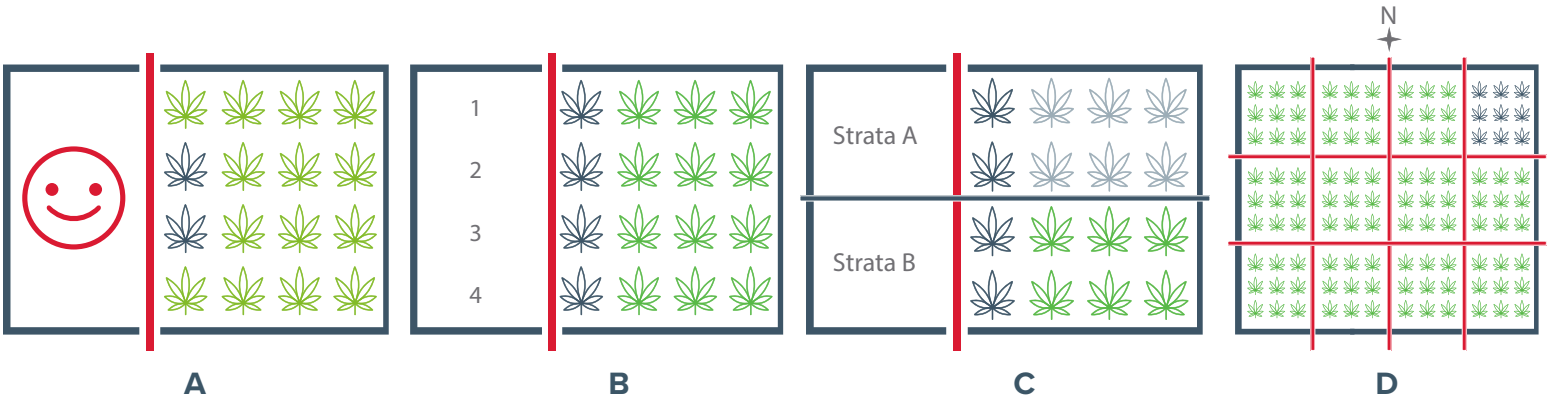

Figure 2b. Non-probability sampling methods:
(a) convenience, (b) consecutive, (c) quota sampling, (d) judgment sampling

In contrast to probability sampling, there is non-probability sampling where a non-random selection process for the purpose of obtaining targeted data or results can intentionally or unintentionally create bias. The four applicable basic types of non-probability sampling methods that are often used for obtaining analytical samples are: convenience sampling; consecutive sampling; quota sampling; and purposive or judgment sampling (see Figure 2b).

- **Convenience sampling**: samples with easy access but it can produce bias by under-representing the overall population.
- **Consecutive sampling**: samples are selected consecutively within or between units.
- **Quota sampling**: similar to stratified sampling by dividing into strata but the selection is not random
- **Judgment or purposive sampling**: sample is chosen based on what is needed for the study and is used for research in a small group or field to create a specific population and is biased to the selected purpose.

Most commercial agricultural operations do not do large scale sampling 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 preharvest sampling with terminology changes. Instead of defining population and sampling frame there is a harvest of all, or portions, of a crop. The harvest yields bulk material to be processed either at one time or in separate manufacturing events. The primary population becomes a lot or a batch.

Sample Preparation for Cannabis

Laboratory or analytical samples must be processed into a form which allows for extraction, digestion ultimately for an analytical instrument or chemical testing. Sample processing involves reducing the material size to ensure samples for homogeneity and extraction into a suitable matrix for analysis. The most common method for obtaining a homogeneous sample is grinding or comminution. Grinding samples allows for a reduced sample size in order to increase accuracy and decrease uncertainty. In a study by Thiex et al., it was shown that the smaller the particle size, the less sample was needed to achieve a lower amount of uncertainty in a sample (see Table 1).

Table 1. Effect of particle size on amount of material needed to ensure various uncertainty levels for representative samples

	Uncertainty			
Particle Size	**15%**	**10%**	**5%**	**1%**
5 mm	56	125	500	12,500
2 mm	4	8	32	400
1 mm	0.4	1	4	100
0.5 mm	0.1	0.1	0.5	12.5

Grinding Principles

Sample size reduction is accomplished by either crushing or grinding using forces of impact, attrition, shearing, or compression.

- **Impact force**: the striking of one object or material against another. One object may either be stationary or both may be in motion.
- **Attrition force**: created by materials rubbing against each other usually in opposite directions or planes.
- **Shearing force**: cleaving or cutting of a material by some cutting implement or blade.
- **Compression force**: 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 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.

Different applications, quantity of throughput and final end products often designate the method employed to grind materials from large scale crushers to finer impact mills.

- **Crushers** are commonly shearing or compression disruptors that process a large continuous stream of materials or larger batches into larger particles.
- **Grinders** produce smaller particles in smaller scales using abrasive grinding forces.
  - **Ball mills or ball-medium mills** grind through impact of a grinding media such as balls, rods, etc.
    - Grinding media and material are moved around the mill body or grinding container.
    - Used for both wet and dry systems.
    - Create a mixture of impact and attrition forces.
    - See Appendix I & II for methods of using ball mills for dry cannabis plant samples.
- **Roller mills** move 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.
- **Hammer mills** are a form of impact mills where material is passed between moving hammers.
- **Impact mills** have a moving impactor which pulverizes a sample through repetitive motions.
  - Impact mills employ an impactor which moves in a repetitive movement and crushes the stationary material (see Figure 3).

A B

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

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:

- **Hardness or toughness**: particularly hard samples will need energy intensive grinding methods such as crushers or cutters.
- **Material structure**: samples that are abrasive will cause wear of the grinding system and cause 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 float above the grinding surfaces.
- **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.
- **Melting or softening temperature**: grinding generates energy and heat that can cause material to soften or melt which can degrade samples or volatilize organic compounds. In the thermally labile products or samples sometimes additional cooling of the material or grinding system is needed to prevent sample loss or promote efficient grinding.
- **Purity of required material**: grinding methods often create exposure to other materials of the grinding system or other previously ground materials.

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 heat 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 allows for application of more energy into the system to create a more efficient particle reduction.

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 which shrinks the crystal lattice of the solids to be ground. This shrinking causes microscopic cracking which in turn uses less energy to fracture reducing heat capacity and increasing efficiency. The reduction in temperature of a material also can cause the material to become embrittled and makes it easier to grind.

If liquid nitrogen (LN_2) is used, there are added benefits of a further temperature reduction which prevents temperature excursions, a more uniform particle size as opposed to ambient grinding and increases safety where the inert nitrogen keeps oxygen low in the grinding system protecting against powder or dust explosions. For many solids, especially materials with crystal lattices or metals, once it is cooled below its embrittlement temperature (called ductile-brittle transition temperature (DBTT) in metals) there it has a much greater risk of shattering during impact or grinding rather than to bend or deform. 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. Moisture level in products effects 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. 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 for 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. 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.

In spice milling, the objective is to have a powdered product with small particle sizes that retain all of 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 which can cause loss of critical aromatic components. 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 to the cryogenically ground samples. The refrigeration and pre-cooling 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.

Another group of compounds that can be damaged by heat and oxidation are pesticides. While 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 at high temperatures and oxidation. In cases where 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

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 endpoint for calculation but is as equally, if not more, important as the monitor for the sample preparation processes. The International Organization for Standardization (ISO) recognizes different types of standards from the point of origin in 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 created by national metrological institutions (NMI) such as 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. These types of standards refer back to a primary standard within their certification (traceable to NIST, for example).

Correct Use of Standards

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 to be used to determine statistical variables such as uncertainty, accuracy and precision.

Error is the difference between an actual measurement and the true value of the measurand. Error does not include mistakes which 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 which 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 which 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. Precision is how close the results fall to one another (see Figure 4). Many precision calculations are based on relative standard deviation or (RSD) or percent (RSD). Relative standard is a measure of dispersion of a probability distribution or frequency distribution. Often it is expressed as a percentage. Precision confidence increases and the percent RSD decreases.

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

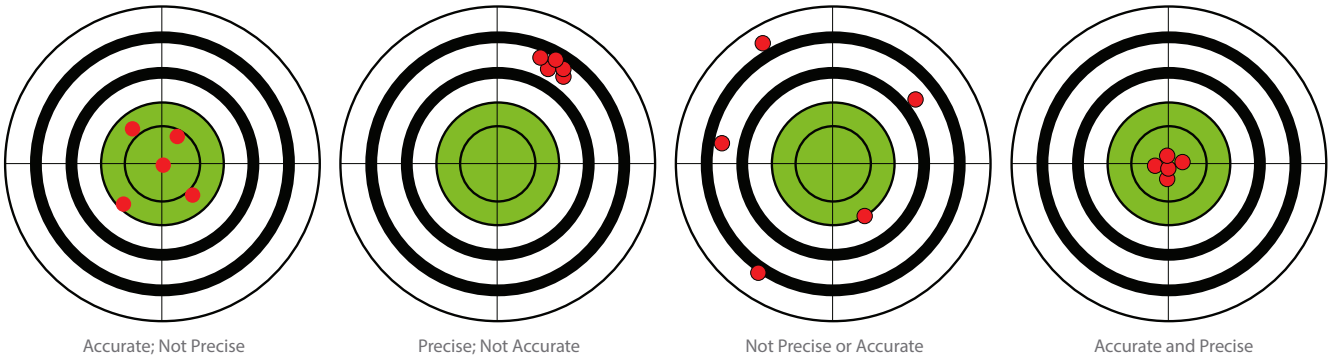

Figure 4. Examples of accuracy and precision

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 which prove that instrument's reliability and reproducibility. Standards are necessary to calculate both accuracy and precision of sample preparation processes and analysis.

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Role of Standards

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 the preparation process to compensate for complex matrices or calculate and adjust for bias or recovery. These types of standards are called internal standards (IS), recovery standards and spiking solutions. The use of standards for extremely complex products such as cannabis is necessary to ensure accurate results. In an industry where 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, recovery standard added to a blank sample matrix or a certified solid 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 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. Internal standards need to be added in the range that the target analyte is expected above the expected level of detection (LOD) and expected level of quantitation (LOQ) in the instrument where the analysis is being performed. Level of detection (LOD) is the lowest amount of an analyte detected by an instrument or method. Level of quantitation (LOQ) is the calculation of the lowest amount of an analyte that can be reliably quantitated within an instrument or method.

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 either be matrix-matched (i.e. having the same matrices as the sample) or unmatched. External standards are most often used to create calibration and response curves, identify analysis, or compare to 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 same way as 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 the 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

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

Accurate sample analysis starts with the two fundamental concepts of representative samples and homogeneity. In the end, the development of sampling plans, sample preparation methods which understand the importance of these concepts, will ultimately be the most important step in achieving good analyses. The accuracy also must follow through with the choices of standards and how they are used to produce accurate and precise data.

References

Available upon request.

Appendix I: Ball Grinding Methods for Cannabis Samples

A. Grinding protocol for flower, bud, leaf, and hemp: small sample

- Equipment: 2010 Geno/Grinder® & 1600 MiniG®
- Sample size: up to 2 grams

1) Place two 11 mm stainless steel balls into a 50 mL centrifuge tube.
2) Weigh up to 2.0 grams of flowers or cuttings into the tube.
3) Shake at 1500 rpm for 1-2 minutes with the SPEX 2010 Geno/Grinder®.
4) Add half of the desired volume of extraction solvent (methanol or acetonitrile, etc.) to the tube.
5) Swirl contents of the tube until steel balls are free of any residual material and remove balls with magnetic pick up tool, rinsing balls with solvent into the sample matrix to ensure minimal sample loss.
6) Discard grinding balls for cleaning and reuse.
7) Add remaining solvent to reach desired volume.
8) Reseal the centrifuge tube and shake at 1000 rpm for 30 seconds to ensure complete extraction and homogeneity.
9) For samples with an exceptionally high moisture content or “sticky” characteristics, the following approach may aid in grinding:
   a) Place flower or cuttings in a freezer for 1+ hours prior to grinding or place sample in liquid nitrogen/ dry ice for several minutes to sufficiently reduce the temperature of the material.

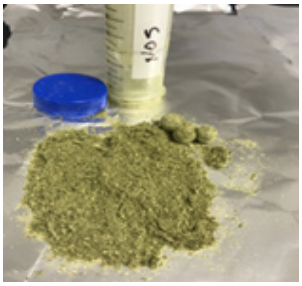
B. Grinding protocol for flower, bud, leaf, and hemp: mid-size sample

- Equipment: 2010 Geno/Grinder® & 1600 MiniG®
- Sample size: 2 to 5 grams

1) Chill samples before homogenization.


2) One 11 mm and two 9.5 mm stainless steel balls are added to the bottom of a 50 mL tube before weighing the cannabis sample and adding to the tube.

3) Add > 2.0 grams up to 5.0 grams of material in a 50 mL centrifuge tube.

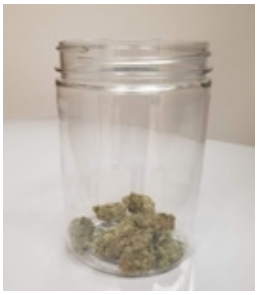
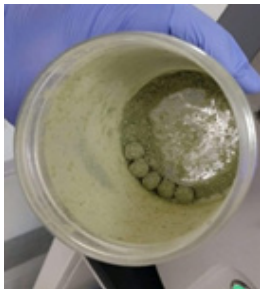




C. Grinding protocol for flower, bud, leaf, and hemp: larger samples

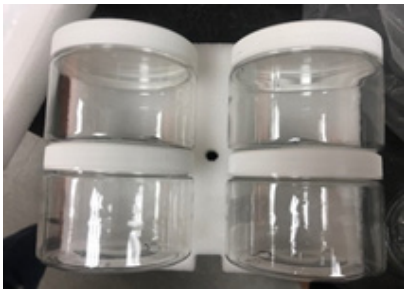
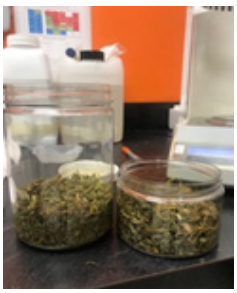
- Equipment: 2010 Geno/Grinder®
- Sample size: > 5.0 gram to 30 gram up to 4 samples can be run at a time in 12 oz jars

1) Using either 12 oz or 25 oz PET jars (PN 2248, 2258) in conjunction with our new large format foam holder (PN 2259), add sample to jar with up to (15) 11 mm stainless steel grinding balls.
2) Grind sample at 1500 rpm for 1-5 minutes.
3) The number of balls will depend on the volume of the sample:
   a) Samples between 5 gram and 30 gram: 3-6 balls.
   b) Samples between 40 gram and 80 gram: 10-15 balls and a run time of 3-5 minutes.
4) For samples with an exceptionally high moisture content or “sticky” characteristics, the following approach may aid in grinding:
   a) Place flower or cuttings in a freezer for 1+ hours prior to grinding or place sample in liquid nitrogen/ dry ice for several minutes to sufficiently reduce the temperature of the material.
5) The grinding balls must be cleaned prior to extraction to prevent sample loss. This can be accomplished by rinsing the beads or by using liquid nitrogen to freeze the adhered sample and scrape it from the grinding balls into the bulk sample.


Example: 30.0 gram sample of cannabis trimmings at 2 minutes at 1500 rpm, 8 x 11 mm stainless steel balls

Example: 10.0 gram sample of cannabis buds ground at 1 minute at 1500 rpm, 5 x 11 stainless steel balls

Examples of high volume sample jars and foam holder for use with cannabis (hemp) buds/flower/leaves/stem samples

Appendix II: R & D Scale Ball Grinding Methods for Cannabis Samples

A. Grinding protocol for flower, bud, leaf, hemp, and infused products: R & D or very small QC samples

- Equipment: 1200 GenoLyte®: flower/buds/leaf/hemp/infused
- Sample size: up to 2 grams

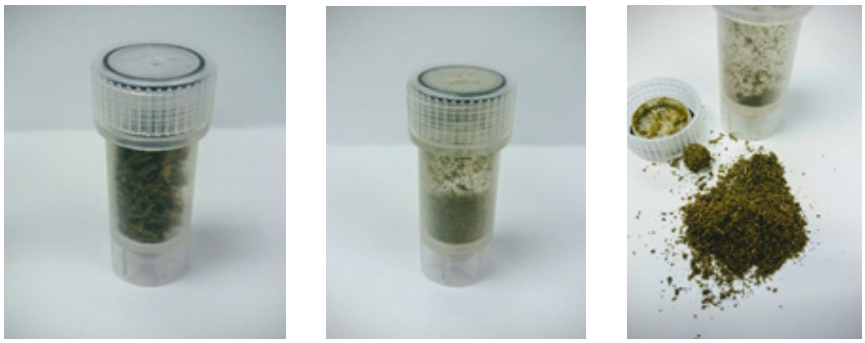
1) Place 1-2, ¼ inch (6.35 mm) stainless steel balls into 7 mL centrifuge tubes (PN 2142-PE).
2) Weigh up to 0.5 gram of flower or cuttings into the tubes.
3) Shake at 3000 rpm for 15-30 seconds with SPEX SamplePrep 1200 GenoLyte®.
4) Add the desired volume of extraction solvent (methanol or acetonitrile, etc.) to the tube.
5) Place tube(s) back into the GenoLyte®and shake for an additional 10 seconds at 750 rpm or until steel balls are free of any residual material and remove balls with magnetic pick up tool, rinsing balls with solvent into the sample matrix to ensure minimal sample loss.
6) Discard grinding balls for cleaning and reuse.
7) Add remaining solvent (if necessary) to reach desired volume.
8) Reseal the centrifuge tube and shake at 750 rpm for 30 seconds to ensure complete extraction and homogeneity.

B. Alternate grinding protocol for flower, bud, leaf, hemp, and infused products: R & D or very small QC samples.

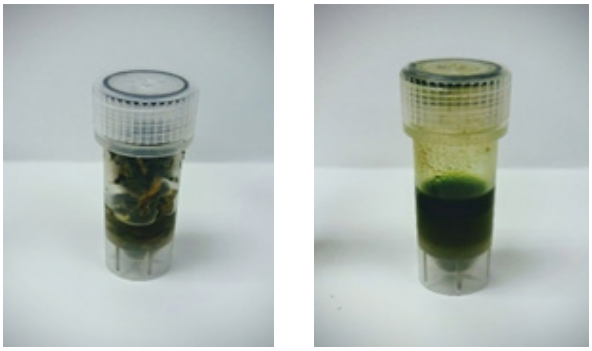
- Equipment: 1200 GenoLyte®: flower/buds/leaf/hemp/infused
- Sample size: up to 2 grams

1) Place 1-2, ¼ inch (6.35 mm) stainless steel balls into 7 mL centrifuge tubes (PN 2142-PE).
2) Weigh up to 0.5 gram of flower or cuttings into the tubes.
3) Add extraction solvent to tubes volumetrically, seal tubes and grind at 3000 rpm for 15-30 seconds with the SPEX SamplePrep 1200 GenoLyte®.
4) For samples with an exceptionally high moisture content or “sticky” characteristics, the following approach may aid in grinding:
   a) Place flower or cuttings in a freezer for 1+ hours prior to grinding or place sample in liquid nitrogen/ dry ice for several minutes to sufficiently reduce the temperature of the material.

Example: GenoLyte® configured for 2 x 7 mL tubes loaded with 0.5 gram of cannabis (hemp)



Example: 0.5 gram of cannabis (hemp) before and after being ground in the GenoLyte® configured for 7 mL tubes with one ¼ inch stainless steel bead. Sample was ground for 30 seconds at the 3000 rpm setting.



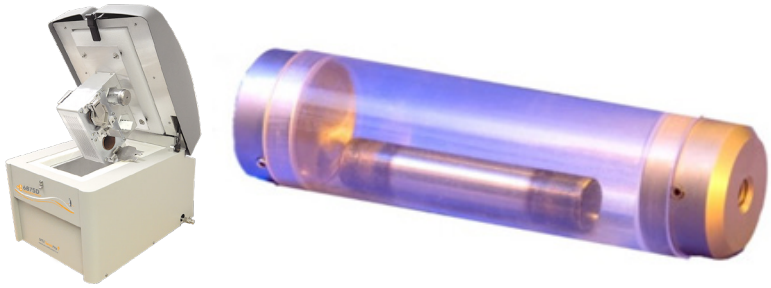
Example: 0.5 gram of cannabis (hemp) before and after being ground in the GenoLyte® configured for 7 mL tubes with one ¼ inch stainless steel bead and 3 mL of EtOH. Sample was ground for 30 seconds at the 3000 rpm setting.

Appendix III: Cryogenic Impact Mill Grinding Methods for Cannabis Samples

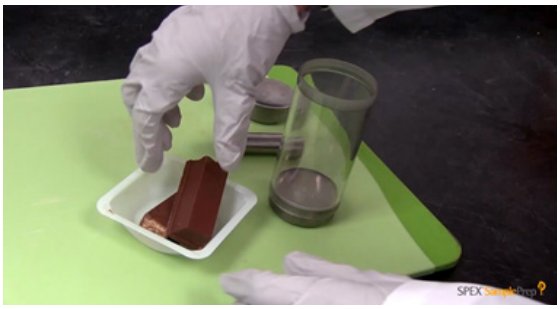
A. Grinding protocol for edibles, concentrates, infused consumables

- Equipment: Freezer/Mill® 6875/6875D

1) The Freezer/Mill® has a magnetically driven impactor.
   a) Insert impactor into the vial along with the sample to be ground.
   b) Ensure that the impactor has free movement after the sample is placed into the vial and the endcap is replaced.
   c) If the impactor becomes lodged or obstructed from movement, the sample will not be ground.



2) Using the large 6801 grinding vials, add sample to vial and insert vial into the grinding chamber (please refer to the instruction manual for loading and operation of the instrument).



Example: large sized Freezer/Mill vial and edible sample loading

3) Depending on the sample viscosity, consistency and moisture content, it may be necessary to pre-chill the sample in liquid nitrogen before inserting the impactor,
   a) Set the pre-cool time to approximately 5 minutes (up to 10 minutes depending on the sample) and the run time between 1-2 minutes.
4) Set the rate/cycle per second to 12.
5) For most samples in this category a single cycle will be necessary, you may set the cycles to 1.

6) The instrument will start grinding as soon as the pre-cool period has elapsed and the impactor movement will be audible. The run can be paused or canceled at any time to facilitate inspection of the sample for the desirable particle size reduction.
7) Once run is complete, remove the vial from the instrument and immediately transfer the contents (ground sample) to a container for testing or storage.
   a) If the sample is going to be stored, it is advisable that it be kept as cool as possible to prevent agglomeration.
   b) For later testing, most samples will retain their powder consistency if transported to and stored at -80°C immediately after grinding.

Example: Before and after results using the Spex SamplePrep Freezer/Mill® for several representative matrices commonly encountered with regard to infused/edible products

8) The cleaning procedure is crucial for longevity of the vials.

   a) Vials have center cylinders made of polycarbonate and are incompatible with organic solvents (alcohols, cyclohexanes, etc.).
   b) Polycarbonate is not able to be autoclaved or exposed to high temperatures.
   c) Clean tubes with mild laboratory grade detergents and lukewarm water or a 5% bleach solution.
   d) These tubes can be decontaminated with RNAse/DNAse solution, UV and gaseous decontaminants (ethylene oxide).
   e) The end plugs and impactor, that are made from stainless steel, do not require these special cleaning considerations.

Appendix IV: Product List

Sample Preparation Equipment	
Description	**Part #**
Geno/Grinder	2010-115
Foam Holder for 50 mL Centrifuge Tubes	2196-16-PE
Foam Holder for 12 oz and 25 oz PET Jars	2259
12 oz PET Jars	2248
25 oz PET Jars	2258
9.5 mm Stainless Steel Grinding Beads	2155
11 mm Stainless Steel Grinding Beads	2156
Freezer/Mill - Dual Chamber Autofill (preferred)	6875D-115
Freezer/Mill - Single Chamber Autofill	6875A-115
Large Grinding Vial Set	6801
End-plug Extractor, Large Vials	6808
Small Grinding Vial Set	6751
Small Vial Adapter	6807
End-plug Extractor, Small Vials	6756
Long Cryo-Transfer Hose, 6 ft (includes fitting and adapters)	6907

Product demo videos are available. Check out the links below:

Geno/Grinder: Automated Tissue Homogenizer and Cell Lyser	https://bit.ly/2yz97Tt
GenoLyte: Hemp Sample Preparation Using the GenoLyte	https://bit.ly/3bxK0i9
Freezer/Mill: Grinding Cannabis Edibles in the Freezer/Mill	https://bit.ly/3bJfxOB

Organic Certified Reference Materials	
Description	**Part #**
Pesticide Residues	
Organochlorine Pesticides Mix A, 18 compounds	5252-PA
Organochlorine Pesticides Mix B, 15 compounds	5252-PB
Nitrogen-Phosphorus Pesticides Mix D, 9 compounds	5252-PD
Nitrogen-Phosphorus Pesticides Mix E, 3 compounds	5252-E

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Technical Note

Terpenes	
Borneol at 1,000 µg/mL in Methanol-P&T, 1 mL	S-4570
Eucalyptol at 1,000 µg/mL in Methanol, 1 mL	S-4352
(R)-(+)-Limonene at 1,000 µg/mL in Methanol-P&T, 1 mL	S-4021
Linalool at 1,000 µg/mL in Methanol, 1 mL	S-5133
alpha-Pinene at 1,000 µg/mL in Methanol-P&T, 1 mL	S-4172
beta-Pinene at 1,000 µg/mL in Methanol-P&T, 1 mL	S-3142
Residual Solvents	
Residual Solvent Mix, 24 compounds	USP-RS-C3A
Acetone at 1,000 µg/mL in Methanol-P&T, 1 mL	S-140
n-Butane at 1,000 µg/mL in Methanol-P&T, 1 mL	S-605
Ethane at 1,000 µg/mL in Methanol-P&T, 1 mL	S-1880
Ethanol at 1,000 µg/mL in Methanol-P&T, 1 mL	S-1885
n-Hexane at 1,000 µg/mL in Methanol-P&T, 1 mL	S-2190
Methane at 1,000 µg/mL in Methanol-P&T, 1 mL	S-2379
2-Methylbutane at 1,000 µg/mL in Methanol-P&T, 1 mL	S-2462
2-Methylpropane at 1,000 µg/mL in Methanol-P&T, 1 mL	S-2555
n-Pentane at 1,000 µg/mL in Methanol-P&T, 1 mL	S-2975
Propane at 1,000 µg/mL in Methanol-P&T, 1 mL	S-3145
2-Propanol at 1,000 µg/mL in Methanol-P&T, 1 mL	S-3165
CAN-TERP Mixes	
CAN-TERP Mix 1 at 100 µg/mL in Methanol, 21 compounds	CAN-TERP-MIX1
CAN-TERP Mix 1 at 1,000 µg/mL in Methanol, 21 compounds	CAN-TERP-MIX1H
CAN-TERP Mix 2 at 100 µg/mL in Methanol, 21 compounds	CAN-TERP-MIX2
CAN-TERP Mix 2 at 1,000 µg/mL in Methanol, 21 compounds	CAN-TERP-MIX2H
CAN-TERP KIT: Kit contains CAN-TERP-MIX1 and CAN-TERP-MIX2	CAN-TERP-KIT
CAN-TERP KIT (High Level): Kit contains CAN-TERP-MIX1H and CAN-TERP-MIX2H	CAN-TERP-KIT-H
DEA Controlled Substances	
Cannabidiol (CDB) at 1,000 µg/mL in Methanol, 1 mL	S-10241
Cannabinol (CBN) at 1,000 µg/mL in Methanol, 1 mL	S-10242
Cannabidivarin (CBDV) at 1,000 µg/mL in Methanol, 1 mL	S-10245
Cannabigerol (CBG) at 1,000 µg/mL in Methanol, 1 mL	S-10246
Cannabigerolic acid (CBGA) at 1,000 µg/mL in Acetonitrile, 1 mL	S-10247
Cannabichromene (CBC) at 1,000 µg/mL in Methanol, 1 mL	S-10248
Cannabidolic acid (CBDA) at 1,000 µg/mL in Acetonitrile, 1 mL	S-10249
(-)-delta9-THC at 1,000 µg/mL in Methanol, 1 mL	S-10260
(-)-delta8-THC at 1,000 µg/mL in Methanol, 1 mL	S-10261
Cannabidivarinic acid (CBDVA) at 1,000 µg/mL in Acetonitrile, 1 mL	S-11055
Tetrahydrocannabinolic acid (THCA) at 1,000 µg/mL in Acetonitrile, 1 mL	S-11056
Tetrahydrocannabivarin (THCV) at 1,000 µg/mL in Methanol, 1 mL	S-11057
Tetrahydrocannabivarinic acid (THCVA) at 1,000 µg/mL in Acetonitrile, 1 mL	S-11058
Cannabichromenic acid (CBCA) at 1,000 µg/mL in Acetonitrile, 1 mL	S-11059

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Technical Note

Heavy Metals	
Heavy Metals Mix, 4 Metals	USP-TXM2
Arsenic at 1,000 µg/mL in 2% HNO_3, 125 mL	PLAS2-2Y
Cadmium at 1,000 µg/mL in 2% HNO_3, 125 mL	PLCD2-2Y
Chromium at 1,000 µg/mL in 2% HNO_3, 125 mL	PLCR2-2Y
Lead at 1,000 µg/mL in 2% HNO_3, 125 mL	PLPB2-2Y
Mercury at 1,000 µg/mL in 10% HNO_3, 125 mL	PLHG4-2Y
Nickel at 1,000 µg/mL in 2% HNO_3, 125 mL	PLNI2-2Y
Silver at 1,000 µg/mL in 2% HNO_3, 125 mL	PLAG2-2Y
Thallium at 1,000 µg/mL in 2% HNO_3, 125 mL	PLTL2-2Y
Canadian Pesticide Components, Mixes and Kits	
Canadian Pesticide Mix 1 at 100 µg/mL in LC/MS Acetonitrile, 1 mL - 14 components	CAN-CAN-1
Canadian Pesticide Mix 1A in LC/MS Acetonitrile, 1 mL - 14 components	CAN-CAN-1A
Canadian Pesticide Mix 2 at 100 µg/mL in LC/MS Acetonitrile, 1 mL - 16 components	CAN-CAN-2
Canadian Pesticide Mix 2A in LC/MS Acetonitrile, 1 mL - 16 components	CAN-CAN-2A
Canadian Pesticide Mix 3 at 100 µg/mL in LC/MS Acetonitrile, 1 mL - 8 components	CAN-CAN-3
Canadian Pesticide Mix 4 at 100 µg/mL in LC/MS Acetonitrile, 1 mL - 19 components	CAN-CAN-4
Canadian Pesticide Mix 4A in LC/MS Acetonitrile, 1 mL - 19 components	CAN-CAN-4A
Canadian Pesticide Mix 5 at 100 µg/mL in LC/MS Acetonitrile, 1 mL - 26 components	CAN-CAN-5
Canadian Pesticide Mix 5A in LC/MS Acetonitrile, 1 mL - 26 components	CAN-CAN-5A
Canadian Pesticide Mix 6 at 100 µg/mL in LC/MS Acetonitrile:LC/MS Methanol (50:50), 1 mL - 11 components	CAN-CAN-6
Canadian Pesticide Mix 6A in LC/MS Acetonitrile, 1 mL - 11 components	CAN-CAN-6A
Canadian Pesticide Mix 7 in LC/MS Acetonitrile, 1 mL - 9 components	CAN-CAN-7
Dibrom (Naled) at 1,000 µg/mL in LC/MS Acetonitrile, 1 mL	LCS-2650
Dibrom (Naled) at 100 µg/mL in LC/MS Acetonitrile, 1 mL	LCS-2650-100
Dimethomorph at 1,000 µg/mL in LC/MS Acetonitrile, 1 mL	LCS-3970-ACN
Canadian Pesticide Kit: Kit contains CAN-CAN-1, CAN-CAN-2, CAN-CAN-3, CAN-CAN-4, CAN-CAN-5, CAN-CAN-6, LCS-2650	CAN-CAN-KIT
Canadian Pesticide Kit 2: Kit contains CAN-CAN-1A, CAN-CAN-2A, CAN-CAN-4A, CAN-CAN-5A, CAN-CAN-6A, CAN-CAN-7, LCS-2650-100	CAN-CAN-KIT-2
Canadian Residual Solvent Mix 1 at 1,000 µg/mL in Methanol-P&T, 100 mL - 25 components	CAN-CAN-RS-1
Cannabis Inhalation Standard - Inhalation Metal Mix for Cannabis Metal Analysis	CANN-INHL1
Cannabis Impurities Standard - Impurities Metal Mix for Cannabis Metal Analysis	CANN-EDBIL

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