Designation: D8493 – 23

Standard Guide for Sample Preparation of Cannabis and Hemp Inflorescence for Laboratory Analysis[1]

This standard is issued under the fixed designation D8493; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 In this guide, the basic steps in obtaining a test portion sample of either dried cannabis/hemp inflorescence are outlined.

1.2 Sample preparation depends on many factors including moisture (dryness) of the sample, the analyte to be measured, the concentrations/amounts, and the test method's precision and accuracy requirements. In this case, dried cannabis or hemp plant material require particle size reduction-comminution from a representative sample of which the final analytical testing portion is determined by the employed testing method. Local regulatory guidelines often dictate both the representative sample that is taken from the bulk material (harvest batch) and the final mass of the test portion (for example <1 g) for chemical analyses.

1.3 This guide will not purport to meet every local and state jurisdiction since different regulatory requirements vary; the local/state requirements are at the discretion of the user to follow and interpret.

1.4 *Units*—The values stated in SI units are to be regarded as the standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

D8197 Specification for Maintaining Acceptable Water Activity (a_w) Range (0.55 to 0.65) for Dry Cannabis Flower Intended for Human/Animal Use

D8270 Terminology Relating to Cannabis

D8282 Practice for Laboratory Test Method Validation and Method Development

D8334/D8334M Practice for Sampling of Cannabis/Hemp Post-Harvest Batches for Laboratory Analyses

E11 Specification for Woven Wire Test Sieve Cloth and Test Sieves

2.2 *Other Standards:*

ASTA Analytical Methods, Preparation of Samples, Method 1[3]

FDA Guide to Inspections Validation of Cleaning Processes[4]

3. Terminology

3.1 *Definitions:*

3.2 General definitions are in accordance with Terminology D8270 unless otherwise indicated.

3.3 *Definitions of Terms Specific to This Standard:*

3.3.1 *analytical sample, n*—prepared from the laboratory sample; the material from which the test portion is selected.

3.3.2 *ball mills, n*—ball mills pulverize by impact using hard balls inside an enclosed grinding jar, sample containers such as falcon tube (centrifuge tubes-high-clarity polypropylene), or a bowl with a secure lid.

3.3.2.1 *Discussion*—Types of ball mills include drum ball mills, jet mills, bead-mills, horizontal rotary ball mills, vibration ball mills, planetary ball mills. Feed material: soft, hard, brittle, fibrous (dry or wet); material feed size: <8 mm to 10 mm. Also referred to as an impact mill and rod, jar, or pebble

[1] This test method is under the jurisdiction of ASTM Committee D37 on Cannabis and is the direct responsibility of Subcommittee D37.03 on Laboratory.

Current edition approved Jan. 15, 2023. Published February 2023. DOI: 10.1520/D8493-23.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from the American Spice Trade Association (ASTA), 1101 17th St. NW, Suite 700, Washington, DC 20036, www.astaspice.org.

[4] Available from the U.S. Food & Drug Administration, www.FDA.gov.

mills. Use high-energy impact and frictional forces created by oscillating, rotational, and/or three-dimensional (3D) movement of vessels containing both the sample and one or more impactors. Material is placed in a bowl (or jar) with grinding balls and rotated. Throughput and milling efficiency can be affected by the sizes and shapes of grinding jars; the rotation speeds (r/min); the cycle time; and the number, weight, and size of balls added to the grinding jar.

3.3.3 *blenders, n*—use fixed blades rotating at high speeds inside a container to comminute materials and can be classified into two types: stationary and immersion.

3.3.3.1 *Discussion*—Devices labeled "blenders" or "homogenizers" usually include blades that are capable of high-speed movement but are small relative to the total volume of the container. The container ("blender jar") is designed to propel the material being mixed into a vortex so that it repeatedly comes into contact with the blades.

3.3.4 *bulk, n*—large sample that is representative of the lot and is prepared in some way (ground up, mixed, and so forth) to form the laboratory sample.

3.3.4.1 *Discussion*—Bulk material or gross sample or both aggregation of two or more increments taken from a lot. The portions may be either combined (composited or bulked sample) or kept separate (gross sample). If combined and mixed to homogeneity, it is a blended bulk sample.

3.3.5 *cannabis inflorescence, n*—fruiting tops of a cannabis plant (excluding the seeds and leaves when not accompanied by the tops) from which the resin has not been extracted by whatever name they may be designated by the authority having jurisdiction (AHJ).

3.3.6 *comminution, n*—reduction of solid material particle size by fracture via grinding, milling, or similar processes.

3.3.6.1 *Discussion*—Such mechanical methods are aimed at increasing the accessible surface area.

3.3.7 *compositional heterogeneity, n*—arising from differing composition among individual elements (that is, particles) in a decision unit.

3.3.8 *cryogenic milling, n*—mechanical milling process (often of the impact type) in which temperature-sensitive samples and samples with volatile components are milled in a cryogen [liquid nitrogen (LN_2), liquid carbon dioxide (CO_2) dry ice-solidified carbon dioxide].

3.3.8.1 *Discussion*—Cryogenic milling takes advantage of both cryogenic temperatures and conventional mechanical milling. Cryogenic or cold grinding involves grinding aids such as dry ice and liquid CO_2 (–78 °C) or liquid nitrogen (–196 °C) that are capable of embrittling the sample by cooling and making it break more easily. Low-temperature freezing also promotes the formation of much smaller ice crystals, which causes less damage to a plant's cellular structure. In addition, the lower temperature of dry ice or liquid nitrogen or both significantly lowers the high vapor pressure of the components and embrittles the sample matrix in which the volatile components (that is, terpenes and pesticide residues) are not largely affected by the relative temperature increase that occurs during the mechanical grinding process. The extensive particle size reduction by cryogenic milling on a laboratory scale results in a higher surface area quickly homogenizing samples into a fine powder.

3.3.9 *cutting/shearing mills, n*—use blades or rotors to shear or cut the material and cutting mills can be subcategorized by: (*1*) rotating blades and stationary blades or (*2*) rotating blades and either a sieve screen or an abrasive grinding ring.

3.3.9.1 *Discussion*—Size reduction in cutting mills is affected by cutting and shearing forces. The sample passes through the hopper into the grinding chamber where it is seized by the rotor and is comminuted between the rotor blades and the stationary cutting bars inserted in the housing.

3.3.10 *decision unit, n*—material from which a primary sample is collected and to which an inference is made.

3.3.11 *distribution heterogeneity, n*—refers to the differences in how the pieces (fragments, particles, or molecules) are distributed spatially, that is, how well mixed or segregated the material is due to density, particle size, or other factors.

3.3.12 *food processor, n*—similar to a blender, with the exception that blades and disks (attachments) are interchangeable.

3.3.12.1 *Discussion*—Modern food processors, in commercial sizes, are also capable of chopping commodities into sufficiently fine pieces to provide homogeneity. Commercial-size food processors are smaller than the 20 qt cutter-mixers, processing of several batches, followed by thorough mixing, may be necessary.

3.3.13 *fundamental error, FE/fundamental sampling error, FSE, n*—error that results from compositional heterogeneity.

3.3.13.1 *Discussion*—FSE random error is caused by computational heterogeneity (CH) and controlled through the selection of an adequate mass.

3.3.14 *fundamental sample size (sample mass), n*—mass of the sample intended to represent the entire population sometimes termed the composite sample.

3.3.14.1 *Discussion*—Number of samples taken with a mass sufficient to evaluate, compare, or provide independently confidence to ensure reproducibility of the composite or the uniformity of the population.

3.3.15 *homogenous, adj*—ingredients appear in the same proportions in any sample taken at any point of a mixture.

3.3.16 *increment, n*—randomly chosen portion from the bulk material from which the primary sample is assembled.

3.3.16.1 *Discussion*—An increment is a correctly delineated, materialized unit of the lot that, when combined with other increments, provides a multi-increment sample and a group of elements collected by a single operation of a sampling device and combined with other increments to form a sample. For some finite element materials, an increment may consist of a single element.

3.3.17 *knife mill, n*—laboratory purpose design similar to food processors designed with more matrix-tolerant materials.

3.3.18 *laboratory sample, n*—original test sample as received by the laboratory.

3.3.18.1 *Discussion*—When the laboratory sample is further prepared (reduced) by subdividing, quartering, mixing, grinding, or by combinations of these operations, the result is the test sample. When no preparation of the laboratory sample is required, the laboratory sample is the test sample. A test portion is removed from the test sample for the performance of the test or analysis.

3.3.19 *mass reduction, n*—subdivision of the bulk material to obtain a sample size suitable for analysis.

3.3.20 *measurement uncertainty, n*—parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand.

3.3.21 *mortar and pestle, n*—used to grind up solids into a fine powder and crush solids into smaller pieces.

3.3.21.1 *Discussion*—The mortar's interior if unglazed is more effective for grinding. The substance is ground between the pestle and mortar by rubbing or pounding the substance with the pestle against the wall of the mortar, which creates a fine powder. Friction and pressure grinding is the impact of two hard surfaces, "mortar grinder."

3.3.22 *planetary mills, n*—use a two-way planetary action to comminute rapidly using high impact resulting in a very narrow particle size range.

3.3.22.1 *Discussion*—Material to be comminuted is placed in a bowl (or jar) with grinding balls and placed on a rotating platform. In planetary action, balls rotate opposite to the direction of the bowl platform rotation and centrifugal forces alternately add or subtract.

3.3.23 *primary sample, n*—initial or gross sample.

3.3.23.1 *Discussion*—The collection of one or more increments or units initially taken from a population. The portions may be combined (composited or bulked sample).

3.3.24 *representative sample, n*—correctly extracted material from the lot or batch, which can only originate from an unbiased, representative sampling process.

3.3.25 *rotor mills, n*—multi-toothed blade rotating at high speed (3000 r⁄min to 28 000 r⁄min) near an enclosing screen that combines impact, pressure, friction, cutting, shearing, and sieving forces to reduce particle size.

3.3.26 *test portion/sample, n*—quantity of material taken for measurement (from the analytical sample) typically by performing quartering in which the withdrawn portion remains as representative as possible.

3.3.26.1 *Discussion*—Also known as the analytical portion. The part of the sample that is actually tested by the laboratory.

3.3.27 *theory of sampling, TOS, n*—sampling errors including incorrect sampling errors (ISEs) not included in the measurement uncertainty framework.

3.3.27.1 *Discussion*—TOS deals comprehensively with sampling, defines representativity, defines material heterogeneity, and furthers all practical approaches needed in achieving the required representative test portion.

4. Summary of Guide

4.1 The measurement process begins with a primary lot (decision unit) that is typically characterized by extensive material heterogeneity and is the main source of all sampling errors, a concept that is fully defined by the theory of sampling (TOS) as shown in Fig. 1. The following relationships are also defined in TOS: error to mass, error to increments, and error to sample correctness, with the ultimate goal of mitigating and estimating the total error in sampling. Heterogeneity contributes on all scales from constituent particles to full lot scale and, thus, securing a representative primary sample is often a challenging first step. The heterogeneity of a primary lot material can be split into constitutional or compositional (CH) and distributional heterogeneity (DH) or spatial heterogeneity. The chemical or physical differences or both between individual "constituent units" are known as fragments in which each fragment can exhibit any analyte concentration between 0 % to 100 %. DH is the irregular spatial distribution of the constituents (stratification or layering). The greater the difference in composition of each fragment CH, the greater the possible DH. The random error in the overall selection process includes: FE or FSE because of the CH and grouping or segregation error (GSE) because of DH (**1, 2**)[5]. Since the CH of a primary lot increases when the difference between individual fragments increases, only comminution, grinding/milling of the material reduces the CH of the primary lot. The FE is the minimum error generated when a sample is collected

[5] The boldface numbers in parentheses refer to a list of references at the end of this standard.

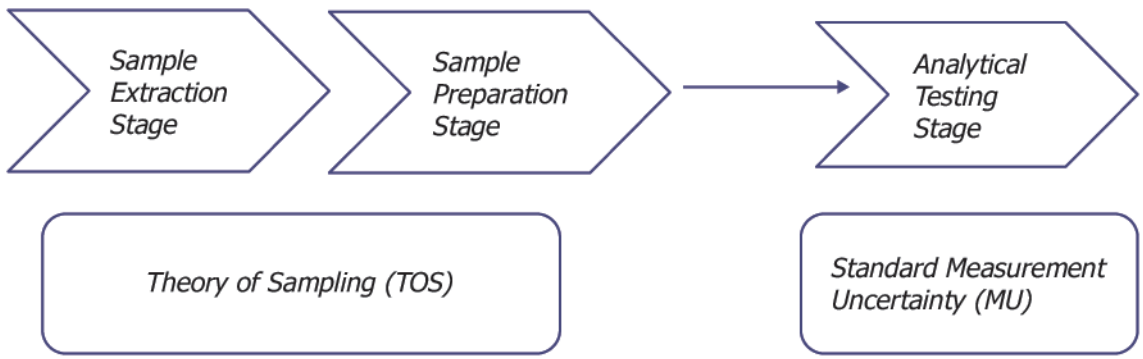

FIG. 1 Overview of TOS

of a given weight and is influenced by particle size. FE expressed as variance (**3**):

$$s_{FE}^2 = \frac{c \times d_{max}^3}{mass} \quad (1)$$

where:

d = Diameter of the maximum-sized particles (cm),
m = Mass (in g) of the sample collected (or comminuted), and
c = Constant (g/cm^3) related to compositional characteristics that are specific to the nature of the analyte/matrix in the lot.

4.2 As shown in Table 1, the FSE is controlled by adequate mass selection. The larger the mass, the smaller the variance of the FSE.

4.3 A representative sample (primary sample) is often selected randomly with a set number of increments, which when combined makes up a composite sample. A larger number of increments is more representative but often not practical, as well the number of increments is often dictated by the local jurisdiction. Once the primary sample is collected, the laboratory receives the sample (laboratory sample) and further processes the "analytical sample" from which the final test portion is taken, see Fig. 2 (**4, 5**). The main goal of the sample comminution process and selection of the final test portion is to reduce the FE (sample test portions of smaller particle size) and provide a test portion that accurately represents the original sampled primary lot.

4.4 Sample processing begins with a sufficient collected sample mass before comminution and is further subsampled for analysis to represent the varying composition of the analytes of interest in the sample. In addition, all pre-analysis sampling steps (primary sample selection, laboratory mass reduction (with subsampling steps), sample splitting, and sample preparation procedures that lead to the final test portion are the primary contributors to the total uncertainty budget, which will need to be included to ensure the validity of measurement uncertainty estimates as shown in Fig. 3.

4.5 Particle size reduction is the mechanical process of grinding (also referred to as milling, cutting, shearing, granulating, comminuting, and so forth) to obtain particles in a material, reduce their average size, reduce the FE, and increase overall sample surface area as to improve extraction efficiency before sample analysis. Dried plant material requires comminution to reduce the particle size to between 250 µm to 5000 µm (see Table 2). Homogeneity and analytical fineness are often dictated by the analysis method in which the amount analyzed is only a small fraction of the original material. There is no definitive particle size that a sample shall have to be homogeneous; however, in practice, sizes of 500 µm has been previously reported, which is also dependent on the extraction method (**6-8**).

TABLE 1 Sample Mass Requirements: Fundamental Sampling Error Versus Particle Size (4)

	Minimum Mass: Relative Standard Deviation (RSD) from Laboratory Subsampling				
FSE (expected RSD) maximum particle size	15 %	10 %	5 %	2 %	1 %
0.5 mm	0.06 g	0.13 g	0.5 g	3 g	12.5 g
1 mm	0.4 g	1 g	4 g	25 g	100 g
2 mm	4 g	8 g	32 g	200 g	400 g
5 mm	56 g	125 g	500 g	3130 g	12 500 g

4.6 Cryogenic milling of other commodities such as food and feed is well documented. The cooling of samples preserves the sample in its entirety by shrinking the crystal lattice of the solids to be ground. Shrinking can cause "microscopic" cracking and, in turn, reduce the amount of energy required for fracturing (**9**). When using cryogenic milling for particle size reduction of solids-fibrous materials such as cannabis/hemp, particle size reduction occurs in multiple stages starting with the accumulation of defects or stresses in a concentrated location, which increases strain and eventual divide of the material into pieces. The most efficient particle size reduction system is one that applies the minimum amount of energy to rupture the material without adding excess energy or heat. Low-temperature homogenization, therefore, can prevent the degradation of volatile analytes and product uniform particle size with decomposition of less stable analytes and improves retention of volatile components.

4.7 Consistency in the mean diameter of cryogenically milled samples has been reported for other commodities compared to ambient milling. During grinding, the temperature of the material may rise to a level in the range of 42 °C to 95 °C dependent on the oil and moisture content of the material (**10**). The use of cryogenic grinding with cryogens such as liquid nitrogen (LN_2), liquid carbon dioxide (LCO_2), and solid carbon dioxide (CO_2/dry ice) reduces the loss of volatile analytes (pesticides, terpenes/terpenoids) (**11-14**).

4.8 Liquid nitrogen, for example, provides a "refrigerated" environment that prechills the cannabis/hemp inflorescence and maintains a lower temperature by absorbing the heat generated during the milling operation. Additionally, a precooling step is often recommended before feeding the material into the milling equipment, which ensures that the material is at or below its brittle point. As the liquid nitrogen vaporizes into the gas state, an inert and dry atmosphere is created that additionally protects the milled material preventing further loss of both volatiles and moisture.

4.9 If liquid nitrogen is not available, dry ice can be used to improve sample homogeneity and the prevention of labile analyte loss such as pesticides. One major disadvantage of using dry ice, particularly for bulk samples, is the need to chop and pre-freeze the cannabis/hemp before the addition of dry ice or CO_2 fog along with small pieces of the sample that will be generated in the milling equipment (**14**). In addition, the CO_2 takes several minutes to sublime after it mixes with the sample, which adds additional time before a test portion can be taken. In humid climates, condensation of water may occur on the dry ice itself, that is, from the laboratory room air, and reduce the purity of dry ice. The addition of water to dry ice can form carbonic acid and change the pH of the sample that may have downstream implications on the analytical test method versus liquid nitrogen, which is inert.

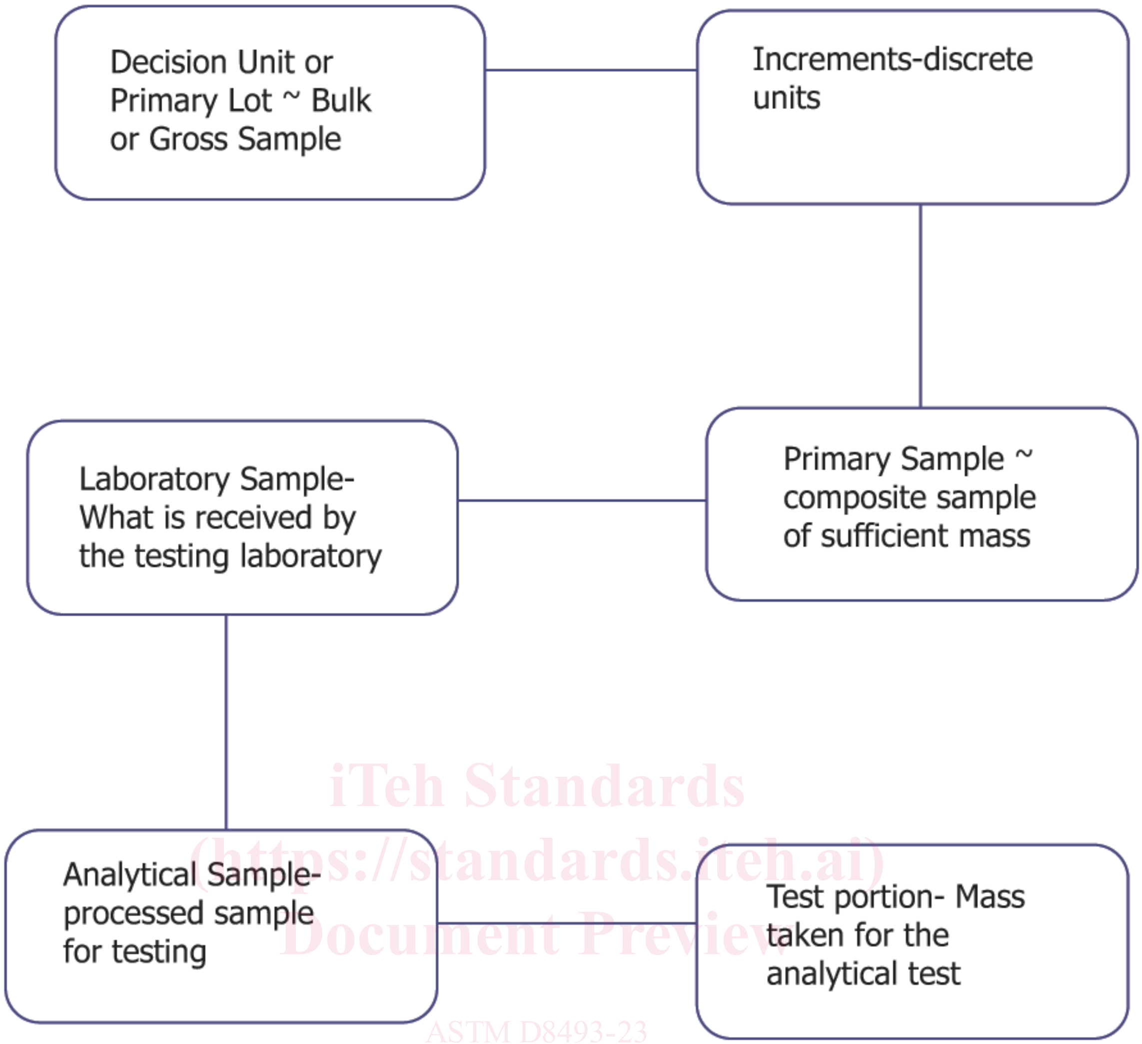

FIG. 2 Sampling Stages to get to the Final Test Portion

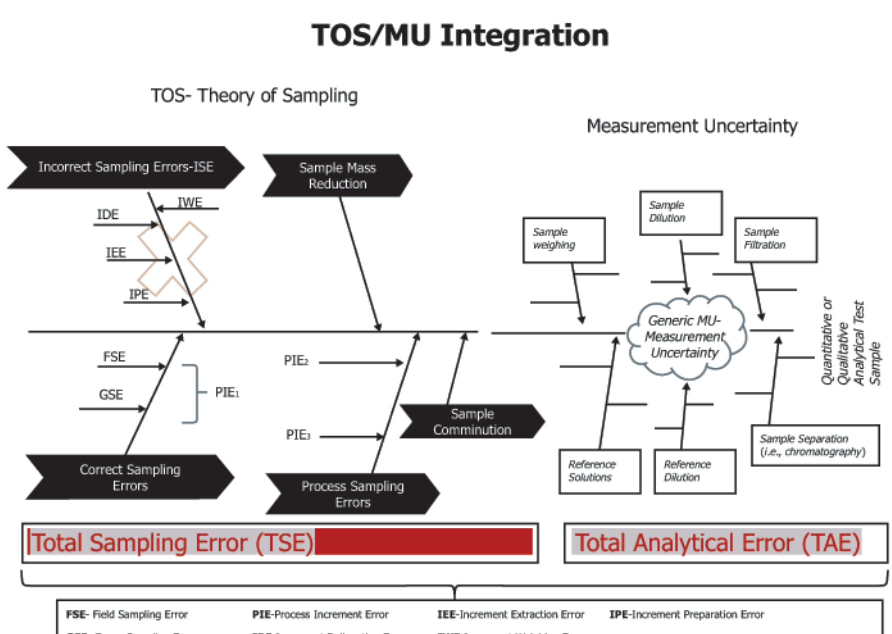

FIG. 3 Error Contributions Sampling, Sample Preparation, and Analytical Test Sample

TABLE 2 Sieve Sizing Specification E11

Sieve No. (U.S Standard Number)	Size, µm
N/A	5000 (ISO)[A]
No. 4	4750
No. 6	3350
No. 10	2000
No. 18	1000
No. 20	850
No. 25	710
No. 30	600
No. 35	500
No. 40	425
No. 45	355
No. 50	300
No. 60	250

[A] 5 mm sieve is ISO compliant, no Specification E11 equivalent sieve number.

4.10 Other sample processing steps may include mechanical pretreatment such as:

4.10.1 Two-step communition (primary bulk sample homogenization followed by fine milling of subsample to get to a reasonably sized test portion), and

4.10.2 Cleaning, drying, grinding, sieving, vortexing, and filtering before instrumental methods of analysis. This approach extends to chemical methods, for example, digestion, decomposition, extraction approaches, separation, and enrichment required for a wet chemical procedure.

5. Significance and Use

5.1 The sample preparation procedure for comminution impacts other downstream processes such as extraction and sonication, which ultimately affects the total analytical error (TAE) and measurement uncertainty.

5.2 Factors that may influence the sample preparation process include the prevention of cross-contamination (carryover) from a prior sample and an inadequate cleaning procedure between preparation of samples, poor sample handling, storage (sample preservation), and moisture content (drying methods) of plant material being greater than 15 % (**15**). Samples with high moisture content are hard to process completely and may yield lower analyte (that is, cannabinoid) concentration during extraction and further processing. Lastly, water activity Specification D8197 is recommended, activity (aw) range (0.55 to 0.65) for dry cannabis or hemp flower or both.

5.3 There are many different types of hardware technologies that address the comminution of dried cannabis or hemp; however, the list of devices is exhaustive and thus beyond the scope of this guide. See Table 3 and Table 4 (**16-18**) for a summary of different milling technologies. Distinctions among various pieces of equipment often relate to the type, mass, and size/shape of the sample (dry, fibrous) for which each is most effective. In addition, there may be economic reasons for mill selection, that is, the sample throughput of the testing laboratory (number of samples per day), access to cryogenics, and sample mass requirements.

5.4 In addition to sampling devices, this guide does not include the sample preparation of edibles, tinctures, oils/concentrates, beverages, and so forth in which the sample diversity poses significant sample preparation challenges to be put forward in additional work items.

TABLE 3 Classification of Milling Equipment and Description (16, 17)

Classification of Milling Equipment	Description
Rotor mills (ultra-centrifugal mills, cyclone mills, rotor beater mills)	Rotor mills have a multi-toothed blade rotating at high speed ~3000 r/min to 28 000 r/min in close proximity to an enclosing screen that combines impact, pressure, friction, cutting, shearing, and sieving forces.
Knife mills	Similar to food processors, designed with more matrix tolerant materials, accessories—different knives, types of containers (stainless steel, polycarbonate), reduction lid.
Impactor mills (bead and ball mills, mixer mills, and some cryogenic mills (specialized vessels) and disc mills)	Impact and frictional forces created by oscillating, rotational 3D movement of vessels containing both the sample and impactors.
Cutting mills parallel section rotor (axe) sixdisc rotor (shredder) V-rotor (scissor)	Cutting and shearing forces in which the sample passes to a hopper into a grinder chamber where the rotor "grabs" and further reduces the particle size between the rotor blades and stationary cutting bars.
Blender	Kitchen appliance—top of the vessel is a cap (sealing) and a bottom vessel with a fixed blade assembly where the base sits on a motor, multiple speed capable.
Food processor	Similar to blender, swappable blades and discs versus fixed blades.
Mortar grinder (mortar and pestle)	Mortar grinders force the samples against two hard surfaces, grinding the sample by combining pressure and friction.

TABLE 4 Common Types of Cryogenic Mills (18)

Type of Mill	Feed Size and Maximum Feed Amount	Notes
Mixer mill	<8 mm 2 × 20 mL	Sample is placed into stainless-steel grinding jars tightly sealed and immersed into liquid nitrogen before grinding.
Cryogenic (cold) mill	<8 mm 2 x 20 mL	Continuous feed of LN_2 (direct connection to source), user is never in contact with the LN_2.
Knife mill	<40 mm 2000 mL	Dry ice embrittlement, use of full metal knife, stainless-steel grinding container and reducing lid.
Ultra-centrifugal (rotor) mill	<10 mm 4000 mL	Embrittlement with dry ice or LN_2. Dry ice is preferred if the sample material is <1 mm or has low thermal capacity.
Cutting mill	<80 mm 4000 mL	Cryogenic grinding with dry ice or LN_2. Use of six-disc rotor or cyclone or both mandatory, including sieves of 2 mm to 20 mm.

5.5 The sample size for comminution purposes is limited as the analytical testing portion required is often 500 times smaller than the bulk sample lot and not every testing laboratory is equipped to handle large sample sizes (that is, greater than 100 g of dried cannabis inflorescence/hemp).

5.6 The particle size is determined by passing the milled cannabis/hemp material through standard sieves, for example, starting with #18 (1000 µm) for the optimum particle size is further determined by extraction efficiency, where <1 mm for cannabis and hemp has been previously reported.

5.7 Preparing multiple analytical samples from one comminuted primary sample and their parallel analysis gives information about the repeatability of the corresponding analytical process (including sample preparation, injection, and integration) and reflects on the homogeneity of the primary sample.

5.8 Moisture removal is critical before any comminution. This step can be accomplished by "drying" at various temperatures ranging from freeze drying to ambient room temperature, as well as vacuum oven drying and forced air oven drying. Some of the active compounds in the product are temperature sensitive, and thus, freeze drying before primary and secondary drying steps is expected to be advantageous in reducing quality deterioration.

5.9 Freeze drying is often a fast approach when a vacuum holds the cannabis plant at temperatures below –40 °C, which retains the high-quality phytochemicals, for example, volatile compounds (terpenes/terpenoids) and acidic forms of cannabinoids. In addition, embrittlement is accomplished before comminution by freezing (–80 °C freezer, for example) for a set time period or by adding cryogen dry ice or liquid gases, such as liquid nitrogen.

5.10 Cryogenic milling may be the preferred way of particle size reduction because of the fact that cannabis/hemp inflorescence exhibits many material properties that can be challenging (that is, moisture content, oil/resin content, and fibrosity). In cannabis milling applications, it is common to add liquid nitrogen directly into the mill to reduce the heat of grinding. Another step may include placing the sample into a liquid bath or immersion sample assembly that pre-cools the material in advance of feeding the material into the cryogenic mill. If available, cryogenic cooling of hemp and cannabis inflorescence samples before and during the grinding process can be an efficient way to prevent thermal degradation during grinding. Lower temperatures may achieve:

5.10.1 Induces microscopic fractures in hemp/cannabis inflorescence before grinding, reducing the energy required to grind it.

5.10.2 Makes the plant material brittle, which is easier to grind mechanically.

5.10.3 It can lower the heat capacity (the amount of heat supplied to a given mass of material to generate a change of unit temperature) and decrease the amount of energy needed to change the temperature of the hemp/cannabis inflorescence, which increases the efficiency of grinding. Depending on the material of the cutting zone, the lowest cutting capability may be achieved with LCO_2 followed by LN_2 correlated to the material feed flow rate.

6. Apparatus

6.1 Sample splitter capable of reducing samples into equal portions.

6.2 *Milling Apparatus (See Table 3 and Table 4 for Available Types of Technologies)*—Select a size-appropriate stainless-steel blade grinder, blender, ball mill (jars or sample container), and ball-type stainless steel; ball size dependent on sample mass of test portion and capable of size reduction to a particle size of <1 mm. Often a single type of reduction equipment is not adequate to obtain the desired particle size.

6.3 *Examples:*

6.3.1 Rotary screen several diameters, 20 cm; screens, 3.15 mm and 2 mm mesh size; screen deck; lid; centrifugal grinder with 0.5 mm to 1 mm sieve insert with rotor and lid (**19**).

6.3.2 Primary or bulk homogenization equipment: knife mill or vertical cutter mixer, blender or food processor (exchangeable blades, fine serrated s-shaped) for use with large sample sizes (for example >100 g) up to 2 L volume, and stainless-steel container with size-reduction lid capable of cryogenic milling with the addition of dry ice and or liquid nitrogen directly to sample container. Secondary or fine milling of subsamples can be conducted with a cryogenic freezer mill, ball mill, or other size-reduction equipment.

6.3.3 *Ball Mill*—Polypropylene (PP) centrifuge tubes (50 mL) or equivalent for aliquoting sample test portion and stainless-steel 11 mm and 9.5 mm balls or similar active grinding media. Polyethylene terephthalate (PET) jars (various sizes, 5 oz or 12 oz) and screw-top grinding jars ranging from 1.5 mL to 50 mL in materials such as hardened steel, stainless-steel agate, tungsten carbide, zirconium oxide, and polytetrafluoroethylene (PTFE).

6.3.4 Consideration shall be made to minimize contamination of the sample by reducing the formation of metal shavings from the grinder blades. If using a blade-type milling apparatus, may substitute with ceramic, zirconia, plastic grinding blades, and/or balls may be required for heavy metals testing, alternatively. If stainless-steel milling apparatus is used, an evaluation should be done to ensure no metal contamination is carried over to the sample.

6.4 If using a mortar/pestle, the material construction shall be nonporous marble, agate material, and/or stainless steel, best if used with cryogenic media.

6.5 Calibrated analytical balance capable of readability to 0.1 mg to 0.01 mg for weighing test portion.

6.6 Calibrated weight set (appropriate for balance minimum and maximum weight).

6.7 Measuring sieves U.S Standard number, see Table 2 to select the appropriate size sieve.

6.8 A diamond tap sieve (tap sieve shaker) for particle size analysis.

6.9 *Optional*—Sample splitter for reducing samples into equal portions.

6.10 Stiff brush.

6.11 Storage container (impervious to light, amber-colored glass) with tight lid in various sizes.

6.12 PP centrifuge tubes (50 mL) or equivalent for aliquoting sample test portion.