Designation: D8469 – 22

Standard Test Method for Analysis of Multiple Elements in Cannabis Matrices by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)[1]

This standard is issued under the fixed designation D8469; 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 This test method uses inductively coupled plasma mass spectrometry (ICP-MS) to determine multiple trace elements in cannabis and cannabis-related matrices following sample preparation using microwave-assisted acid digestion. This test method is applicable to the quantification of trace levels of elements in dried plant materials, concentrates, oils, extracts, tinctures of cannabis and cannabis-related products. Other matrices may be added provided that the lab validates the extra matrices using Practice D8282. Details are provided on the validation of both the sample preparation procedure and analytical method using certified reference materials (CRMs) and validation of the analytical method using spike recovery testing of several cannabis based samples.

1.2 This test method should be used by analysts experienced in the use of microwave digestion and ICP-MS, matrix interferences, and procedures for their correction or reduction, and should only be used by personnel trained in the handling, preparation, and analysis of samples for the determination of trace elements in cannabis and cannabis products (**1**).[2] This test method was developed using a single quadrupole ICP-MS equipped with a collision/reaction cell (CRC) that can be pressurized with helium (He) gas for the removal of polyatomic interferences using kinetic energy discrimination (KED). This test method can also be run using a triple quadrupole or "tandem" mass spectrometer (MS/MS) ICP-MS instrument, which is fitted with CRC technology. The ICP-MS method accounts for polyatomic interferences, which are the most common spectral overlaps in ICP-MS, isobaric interferences, and any potential doubly-charged ion interferences (M^{2+}) that may arise from the presence of rare earth elements (REEs) in the samples, as the REE^{2+} ion interferences can affect the accuracy of the measurement of arsenic (As) and selenium (Se) in the samples. Table 1 lists elements for which the test method applies along with recommended analytical masses, and secondary masses for some elements. The priority toxic elements arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), also sometimes referred to as the "big four" toxic trace elements are listed separately because of their toxicity, as discussed in 5.1.

1.3 Certified reference materials (CRMs) should be matrix matched as closely as possible to the cannabis/plant matrix. In-house reference materials (RMs) are acceptable if no CRM is available and/or the in-house RM is well characterized, but CRMs are preferred. NIST 1575a Pine Needles, NRC HEMP-1, and a NIST hemp sample (NIST number not assigned yet) were analyzed to verify method bias. The RM/CRMs should have a recovery between 80 % to 120 % when concentrations are above the limit of quantification (LOD) or within the concentration uncertainty (converted to percent relative uncertainty) supplied on the certificate, whichever is greater. If acceptable values are not obtained, the analytical solution may be reanalyzed once. If acceptability is still not met, recalibrate and reanalyze the entire analytical sequence and/or prepare and digest new analytical portions.

1.4 *Multi-laboratory Validation (MLV)*—This test method was tested by analyzing an NRC hemp CRM, a NIST hemp SRM, and a NIST plant control sample (NIST 1575a Pine Needles) by four laboratories each running a single quadrupole ICP-MS.

1.5 Gravimetric dilution (weight/weight), volumetric dilution (volume/volume), or a combination of the two techniques (weight/volume) are acceptable methods for ICP-MS sample preparation.

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

1.7 *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.*

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the*

[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.

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

TABLE 1 Recommended Masses for Each Element and Some Secondary Masses

Priority Elements	Recommended Analytical Mass	Secondary Analytical Masses
Arsenic (As)	75	...
Cadmium (Cd)	111	114
Lead (Pb)	208[A]	...
Mercury (Hg)	201	200, 202
Other Elements	**Recommended Analytical Mass**	
Sodium (Na)	23	...
Aluminum (Al)	27	...
Potassium (K)	39	...
Vanadium (V)	51	...
Chromium (Cr)	52	53
Manganese (Mn)	55	...
Iron (Fe)	56	54
Cobalt (Co)	59	...
Nickel (Ni)	60	58, 62
Copper (Cu)	63	65
Zinc (Zn)	66	67, 68
Selenium (Se)	78	77, 80
Molybdenum (Mo)	95	98
Silver (Ag)	107	...
Antimony (Sb)	121	...
Barium (Ba)	137	...
Thallium (Tl)	205	...
Thorium (Th)	232	...
Uranium (U)	238	...

[A] Lead is measured as the sum of the three most abundant isotopes, 206, 207, and 208: (208) = M(206) + M(207) + M(208).

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:*[3]

D1193 Specification for Reagent Water

D8270 Terminology Relating to Cannabis

D8282 Practice for Laboratory Test Method Validation and Method Development

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

2.2 *Other Standards:*

U.S. Environmental Protection Agency (EPA) standard methods 200.8 and 6020B **(2, 3)**

FDA EAM 2.2 Food Homogenization **(4)**

3. Terminology

3.1 For definitions of terms related to cannabis, see Terminology D8270.

3.2 *Definitions:*

3.2.1 *analytical solution, n*—a cannabis sample that has been prepared for analysis per 11.3.

3.2.2 *calibration blank (CalBK), n*—volume of Type 1 reagent water containing the same amount of acid matrix as is in the calibration standards.

3.2.3 *calibration standards (CalSTD), n*—a standard having an accepted value (reference value) for use in calibrating a measurement instrument or system.

3.2.4 *calibration stock solution, n*—solution prepared from the stock standard solution(s) to verify the instrument response with respect to analyte concentration.

3.2.5 *cannabidiol (CBD), n*—a cannabinoid found in cannabis plants.

3.2.6 *collision/reaction cell (CRC), n*—device used to remove polyatomic interfering ions through reaction or collision with a gas added to the cell.

3.2.7 *interlaboratory study (ILS) or multi lab validation (MLV), n*—study in which collaborators in multiple laboratories use a defined method of analysis to analyze identical portions of homogeneous materials to assess the performance characteristics obtained for that method of analysis.

3.2.8 *internal standard (ISTD), n*—pure element(s), which is not one of the analyte elements and is not present in the sample, added in known amount(s) to a solution.

3.2.9 *laboratory duplicate (DUP), n*—two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures; analyses of laboratory duplicate 1 and laboratory duplicate 2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.2.10 *limit of detection (LOD), n*—the minimum concentration of an analyte that can be identified, measured, and reported with 99 % confidence that the analyte concentration is greater than zero.

3.2.11 *method blank (MBK), n*—use 0.5 g Type 1 water for method blanks.

3.2.12 *relative standard deviation (RSD), n*—the measure of deviation of the measured analytical concentrations around the mean—used to express the precision of the results.

3.2.13 *spike solution, n*—an aliquot of cannabis sample to which known quantities of the method analytes are added in order to check if the sample matrix contributes bias to the analytical results; the background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the spiked sample corrected for background concentrations—see 14.2.

3.2.14 *tuning solution, n*—a solution which is used to determine acceptable instrument performance before calibration and sample analyses—see 13.1.

4. Summary of Test Method

4.1 This test method describes the multi-element determination of multiple trace elements by ICP-MS in cannabis and cannabis-based samples and matrices. The digested sample is introduced into the ICP (plasma) as an aerosol by passing the liquid sample through a simple pneumatic nebulizer. The largest aerosol droplets are removed from the gas stream by a

[3] 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.

spray chamber, and about 2 % to 3 % of the remaining smaller droplets are swept into the central channel of the plasma via the torch. It is recommended to use a thermoelectric (Peltier) cooled spray chamber to reduce signal drift caused by localized temperature change. A cooled spray chamber also reduces the water vapor content of the aerosol, resulting in a hotter plasma and so improving matrix tolerance, increasing ionization, reducing suppression, and reducing formation of oxides and other matrix based polyatomic ion overlaps. The sample aerosol enters the plasma, which is generated in a stream of argon contained in a torch. The torch is surrounded by a coil or plate to transfer RF energy into the plasma. The argon plasma temperature (up to 10 000 K maximum and around 7500 K in the central channel) rapidly dries the aerosol droplets, which are then decomposed, vaporized, atomized, and ionized by the removal of one electron from each atom. The positively charged ions that are produced in the plasma are extracted into the vacuum system via a series of interface cones. To maintain the high vacuum around the mass spectrometer, the orifices of the cones are small, typically 1 mm diameter or less. An ion focusing system or "lens" is used to focus the ions into the entrance aperture of a quadrupole mass spectrometer (MS). The quadrupole uses a combination of DC (direct current) and AC (alternating current) electrical fields to separate the ions based on their mass-to-charge ratio (*m/z*). The electron multiplier (EM) detector detects each ion as it exits the quadrupole. Singly charged ions (M^+) with an *m/z* equal to the ion's mass are detected by the EM detector. The detector electronics count and store the total signal for each mass (*m/z*), creating a mass spectrum.

4.2 A weighed portion (approximately 0.5 g is typical) of a thoroughly homogenized cannabis sample is digested using a mixture of HNO_3 and HCl using closed-vessel digestion apparatus. The digests are then diluted using Type 1 reagent water to 50 g. Blanks and CRMs are prepared in the same way. Internal standards are added to the solutions to compensate for variations in sample introduction efficiency and element ionization efficiency in the plasma. The solutions are typically introduced to the ICP-MS instrument via an autosampler. By comparing measured *m/z* peak intensities of elements in the sample with *m/z* peak intensities measured with the calibration standards, the concentrations of elements in the sample can be calculated.

5. Significance and Use

5.1 Medical and/or recreational marijuana (cannabis) has been legalized for adult use in many countries and states within the USA (**5**). Many jurisdictions that permit the use of medicinal and recreational marijuana require testing of cannabis and associated products to ensure safety from contaminants, especially the toxic "big four" elements such as As, Cd, Hg, and Pb (**6**), and other metals worthy of consideration (**6**). These heavy metals can accumulate in plants grown in polluted soils or contamination can occur during the manufacturing process (**7**). In addition to ensuring product safety, the analysis of mineral and other trace elements is required for labeling purposes when these products are sold as nutritional supplements. Trace element analysis of plant and nutritional supplement materials is a well-established application (**8**). Following acidic digestion to break down the plant-based samples' primary components, ICP-MS is often used for quantitative analysis because of its multi-element capability, high sensitivity, speed, robustness, and wide dynamic range.

5.2 This test method covers the rapid determination of multiple elements in cannabis sample digests. The elements include the priority toxic elements (As, Cd, Hg, and Pb), as well as elements required by some states and elements of interest in the cannabis community (V, Cr, Cu, Zn, Sb, Ba, Se, Ag, Na, Al, K, Mn, Fe, Co, Ni, Mo, Tl, Th, and U). Irrespective of the number of elements being measured, test times are approximately a few minutes per test specimen, and detectability for most elements is in the low- to sub-ppb range.

6. Interferences

6.1 *Spectral Interferences*—Polyatomic ions that have the same *m/z* as an analyte ion are the main source of spectral interferences in ICP-MS. A list of typical polyatomic ions that are derived from the plasma gas (Ar), reagents, or sample matrix is shown in Table 2. If a high plasma temperature is maintained, the level of many polyatomic interferences will be reduced. The robustness of the plasma can be determined by measuring the CeO/Ce ratio. The oxide ratio shows the plasma's ability to break apart the Ce-O molecule. Since the Ce-O molecule is strongly bound, it is a good indicator of decomposition of the sample matrix and other molecular ions. Comparing the signal for CeO^+ at *m/z* 156 to Ce^+ at *m/z* 140 allows a quick assessment of the plasma's capability to decompose the matrix. A more robust plasma (lower CeO/Ce ratio) is beneficial for analyzing the high and variable matrix levels encountered in typical batches of cannabis samples. The typical CeO/Ce ratio that can be achieved varies for different ICP-MS designs, but operators should be aware of the analytical benefits of better matrix tolerance, higher ionization, and lower levels of polyatomic interferences that are provided by optimizing to a lower CeO/Ce ratio, ideally as close as possible to 1 %. Often polyatomic ion interferences can be avoided by the selection of an alternative analytical isotope. If that is not appropriate, some ICP-MS systems provide a simple and reliable solution to resolve common polyatomic interferences using a CRC that is operated in helium collision mode, often referred to as He mode. He mode uses KED to filter out polyatomic ions while allowing atomic ions to pass through the cell to the detector. KED is a physical process that makes use of the fact that polyatomic (molecular) ions have a larger ionic cross-section than the atomic ions at the same *m/z*. Due to their larger size, the polyatomic ions collide more frequently with the helium cell gas, and so lose more energy than the (smaller) analyte ions do. Because of their greater energy loss during passage through the cell, the polyatomic ions can be rejected using a bias voltage at the cell exit. He mode is effective for many polyatomic ion overlaps, so it works for many elements, and it can be applied to a wide range of typical ICP-MS sample types, even varied samples with complex and unknown matrices. Common, matrix-based polyatomic interferences are removed, allowing access to the preferred isotopes of all