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Research Article

HUMAN NUTRIENT METHODS

Determination of Heavy Metals in a Variety of Cannabis and Cannabis-Derived Products, First Action 2021.03

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Abstract

Background: The legalization of medicinal or recreational marijuana across many states in the United States and other countries has driven demand for cannabis, hemp, and related products.
Objective: In the absence of federal regulations to ensure the product quality and safety of these products, each state issues its own guidance and sets its own regulations. Like food and pharmaceuticals, cannabis testing should include the analysis of heavy metals, which may be toxic if ingested or inhaled.
Methods: Based on established methods for the preparation and multi-elemental analysis of plant materials, a range of cannabis and cannabis-related products were prepared for analysis using microwave-assisted acid digestion followed by testing with inductively coupled plasma-mass spectrometry (ICP-MS). The sample preparation procedure was validated by measuring arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) in four plant-based certified reference materials (CRMs).
Results: The mean ICP-MS results for As, Cd, Hg, and Pb were in good agreement (85–110%) with the certified concentrations. The accuracy and precision of the ICP-MS method for the determination of As, Cd, Hg, and Pb, as well as other elements, were tested by spiking the various cannabis samples at different concentration levels and determining the spike recoveries. The spike recoveries for As, Cd, Hg, and Pb in all spiked samples met the AOAC *Standard Method Performance Requirements* (SMPR®) for Determination of Heavy Metals in a Variety of Cannabis and Cannabis-Derived Products (SMPR 2020.001) of 60–115% for low-level spike and 80–115% for mid- and high-level spikes.
Conclusion: Microwave-assisted acid digestion and ICP-MS are suitable for trace metal preparation and analysis of cannabis, as well as related products, as shown by the results of this method. The repeatability and recovery results for As, Cd, Hg, and Pb met the method requirement criteria in the AOAC SMPR 2020.001. This method can be used for all stages of production to ensure safety with respect to toxic metals.
Highlights: A wide range of cannabis and hemp samples, from raw materials to finished products, are within scope for analysis using the microwave-assisted acid digestion/ICP-MS method.

Marijuana remains a Schedule I controlled substance at the federal level in the United States, despite most U.S. states having legalized medical and/or recreational marijuana (cannabis) for adult use (1, 2). Currently, there are no regulations at the federal level to limit the concentration of heavy metals in cannabis products, but U.S. states that permit the use of medicinal and recreational marijuana require rigorous testing of cannabis and associated products to ensure safety from contaminants,

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especially the toxic elements arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb; 3). Heavy metals can accumulate in plants grown in polluted soils (4). Contamination can also occur during the manufacturing process, so analysis of cannabis and its derived products is necessary at all stages of production. The analysis of mineral and additional trace elements is of regulatory interest in some jurisdictions, and is therefore included in this method.

Trace element analysis of plant and nutritional supplement materials is a well-established application (5). Following acidic digestion to break down the plant-based samples' primary components, inductively coupled plasma-mass spectrometry (ICP-MS) is often used for quantitative analysis because of its multi-element capability, high sensitivity, speed, robustness, and wide linear dynamic range that allows major and trace elements to be determined in a single analysis. Since there are currently no AOAC *Official Methods of Analysis*℠ for the determination of trace metals in cannabis or related products, the method outlines a procedure for using microwave-assisted acid digestion and ICP-MS for the routine monitoring of As, Cd, Hg, Pb, and other elements of regulatory interest in cannabis and cannabis matrices.

A single quadrupole ICP-MS with collision/reaction cell (CRC) technology such as the Agilent 7850 ICP-MS or Agilent 7900 ICP-MS was used in this study. Previous generation instruments (Agilent 7800 and 7700x ICP-MS) could also be used. The method can also be run using a triple quadrupole ICP-MS instrument such as the Agilent 8900 or 8800 if the instrument is operated in single quadrupole mode. Once a method has been set up, the ICP-MS can be operated with or without browser-based instrument control software (such as Agilent ICP Go software). The browser software limits access to method parameters, simplifying the operation of the instrument for the routine monitoring of samples by inexperienced or nonexpert analysts.

Typical minimum specifications of the ICP-MS instrumentation required for the method are shown in Table **2021.03A**.

Table 2021.03A. Typical performance requirements of the single quadrupole ICP-MS used in the method

Sensitivity (Mcps/ppm)	
7 Li	110
89 Y	270
205 Tl	340
Background (cps at *m/z* 9)	<0.3
Detection limits, ppt	
9 Be	<0.1
115 In	<0.04
209 Bi	<0.04
Stability	
Oxides, %	<1.8
Doubly charged, %	<2.5
Stability 20 min RSD, %	<1.0
Stability 2 h RSD, %	<1.2

In addition, the ICP-MS must be capable of scanning *m/z* range 5–240 u with a resolution better than 0.5 u at 10% peak height. The ICP-MS must have a CRC that can be pressurized with helium for the removal of polyatomic interferences using kinetic energy discrimination (KED). Since the cannabis method was developed using an Agilent 7850 ICP-MS model, many of the directions given in the method are specific to Agilent-branded equipment, so procedural modifications may be needed if other branded instrumentation is used. Any such modifications must be validated according to AOAC *Standard Method Performance Requirements* (SMPR®) for Determination of Heavy Metals in a Variety of Cannabis and Cannabis-Derived Products (SMPR 2020.001) guidelines and method quality control elements (6). The SMPR requirements are listed in Table 1.

Summary of Validation Study Design

The procedure is based on a single laboratory validation and the local State Cannabis Testing Manual in the United States and Canada (7) and is applicable for the 'big four' elements, total arsenic (CAS No. 7440–38-2), total cadmium (CAS No. 7440–43-9), total mercury (CAS No. 7439–97-6), total lead (CAS No. 7439–92-1), and other elements of regulatory interest in cannabis and cannabis matrixes. This procedure is suitable for cannabis and cannabis-related products and is based on complying with medical and adult-use cannabis laws. This method should be used by analysts experienced in the use of microwave digestion and ICP-MS, matrix interferences and procedures for their correction, and should only be used by personnel thoroughly trained in the handling, preparation, and analysis of samples for the determination of trace elements in cannabis and cannabis products.

Analytical Limits

Analytical limits were established per the SMPR 2020.001 requirements (Table 1). The limits of detection (LODs) and background equivalent concentrations (BECs) for As, Cd, Hg, and Pb were obtained from external calibration curves. The ICP-MS was calibrated using a calibration blank and at least four multi-element standards using a linear curve fit. The limits of quantitation (LOQs) were calculated by analyzing a low-level spike solution: LOQ = 3 × SD (low-level spike) × (dilution factor).

Analytical Quality Control

To monitor instrument performance over the analytical cycle, an initial calibration verification (ICV) standard was run and a mid-level calibration standard comprising mineral elements at 5 mg/L (ppm), Hg at 1 μg/L (ppb), and all trace elements at 50 μg/L (ppb) was used as the continuing calibration verification (CCV) solution. The CCV was analyzed every 10 samples throughout the run.

Table 1. Standard method performance requirements for As, Cd, Pb, and Hg

LOQ	≤ 10 ppb, μg/kg		
Range	Repeatability (RSD_r), %	Reproducibility (RSD_R), %	Recovery, %
>10 ppb to 100 ppb	15	32	60–115
>100 ppb to 1 ppm	11	16	80–115
>1 ppm to 10 ppm	7.3	8	80–115

Reference Materials

Four products from the National Institute of Standards and Technology (NIST), standard reference material (SRM) 1547 peach leaves, SRM 1573a tomato leaves, SRM 1575 pine needles, and SRM 1515 apple leaves, were analyzed to verify the digestion process. SRMs or 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. RM/CRMs should have a recovery between 80–120% when concentrations are above the LOQ 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.

Repeatability and Spike Recoveries

To check the accuracy of the method for actual sample analysis, a spike recovery test was carried out per the SMPR 2020.001 requirements (Table 1). Final concentrations of spiked elements in 50 g aqueous solution are shown in Table 2. Spike recovery tests can fail due to inappropriate fortification, so the cannabis sample should be analyzed initially as a pre-test and then fortified at an appropriate level. After determining the native level of the target elements in the sample, a duplicate portion is fortified at a "low", "mid" and "high" level, depending on the calibration range. The spiked element final concentrations given in Table 2 were defined by SMPR 2020.001 and adjusted for the sample preparation dilution factor of 100. The concentration added by fortification into the microwave digestion vessel with the analytical portion should be at the level of interest, or 50–300% of the native elemental concentration, whichever is greater. In this study, a spike at a low-level standard analytical solution concentration was made to ensure that the spike level is appropriate when the native level is considered. During the method validation, and measurement of many different cannabis flower samples, it was found that native levels of Cd in cannabis flower plants can be at high levels, therefore a low-level spike might not be appropriate at the 0.1 ppb, µg/kg level. Pre-screening the Cd levels in cannabis flower samples, and determining the best spiking level is recommended, to meet the 50–300% of the native elemental concentration range. As shown in Table 2, spiking at the high end of the high spike range (very high spike) was done.

Table 2. Spiked element final concentrations

Spike solution	Trace Elements: Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, U	Major elements: Fe, K, Ca, Na, Mg
Low spike	≥0.1 ppb, µg/L to 1 ppb, µg/L	≥1 ppb, µg/L to 10 ppb, µg/L
Mid spike	>1 ppb, µg/L to 10 ppb, µg/L	>10 ppb, µg/L to 100 ppb, µg/L
High spike	>10 ppb, µg/L to 100 ppb, µg/L	>100 ppb, µg/L to 1 ppm, µg/L

AOAC *Official Method*SM 2021.03

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[Applicable to the quantification of heavy metals: arsenic (As), CAS No. 7440–38-2; cadmium (Cd), CAS No. 7440–43-9; mercury (Hg), CAS No. 7439–97-6; and lead (Pb), CAS No. 7439–92-1, at trace levels in dried plant materials, concentrates, oils, extracts, tinctures of cannabis, and cannabis-related products. Examples of samples analyzed by this method include: hemp flower, cannabinoid (CBD) vape oil, hemp isolate extract, full spectrum softgel capsules, full spectrum tincture, isolate tincture, CBD coffee grounds, hemp butter, hemp seed oil, CBD beef jerky, CBD hard candy, CBD pineapple drink, full spectrum balm, CBD pain relief cream, CBD balm, CBD topical oil, hemp soap, hemp biomass, spent hemp biomass, trichomes, CBD crude extract, and CBD distillate.]

Caution: The use of laboratory equipment and chemicals exposes the analyst to several potential hazards. Good laboratory technique and safety practices should be used at all times. Safety glasses and acid resistant gloves should be worn at all times when handling samples or reagents, or when in the vicinity of others handling these items, especially when handling standards containing toxic elements such as As, Cd, Hg, Pb. Also, proper ventilation and other physical safeguards should be in place when handling these standards. Analysts should consult, and must be familiar with, their laboratory's chemical hygiene and safety plan and safety data sheets for all reagents and standards listed.

Warning: Cryogenic and suffocation hazard: ICP-MS instruments require a supply of argon gas. This can be provided from compressed argon gas cylinders or bottles, or from a liquid argon dewar. Liquid argon represents a potential cryogenic and suffocation hazard. Leaks from a compressed argon cylinder can also represent a suffocation hazard. Safe handling procedures should be employed at all times when handling compressed gas cylinders, liquid argon tanks, and fittings, and appropriate gas monitoring equipment (e.g., O_2 sensors) should be installed in laboratories where such gases are stored and used. The Agilent ICP-MS instruments are fully interlocked to prevent user exposure to harmful electrical voltages, radio frequency emissions, UV radiation, high temperatures, and other hazards. At no time should the operator attempt to disable these interlocks or operate the instrument if any safety interlocks are suspected to have been disabled. Refer to instrument manuals for safety precautions regarding use.

All additional company safety practices and procedures should be followed at all times. Spilled samples and reagents should be cleaned up from instrument and laboratory surfaces immediately. Acid spills should be neutralized with sodium bicarbonate solution before cleanup. The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification (and disposal) of samples should be done in a fume hood.

A. Principle

Cannabis samples should be homogenized if necessary, before preparation by microwave digestion. The elements present in the aqueous cannabis-sample digestate are then analyzed by single quadrupole ICP-MS. A sample aerosol is introduced into an argon plasma that functions as an ion source. The quadrupole mass spectrometer separates the elemental ions based on their mass-to-charge ratio (m/z). By scanning the quadrupole

rapidly across the mass range (2–260 u), each mass (isotope) of interest passes sequentially to the electron multiplier detector, creating a mass spectrum. The magnitude of each peak is directly proportional to the concentration of an element in a sample. Samples are quantified by comparing signal intensities measured in the samples to those generated from standards of known concentration.

(a) *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 reference list of polyatomic ions that are derived from the plasma gas (Ar), reagents, or sample matrix can be found in U.S. Environmental Protection Agency (EPA) standard methods (8, 9). 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 CeO molecule. Since the CeO 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 low CeO/Ce ratio (typically <1.8) indicates a robust plasma. Helium mode uses KED to filter out polyatomic ions while allowing atomic ions to pass through the cell. 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. Correction equations within ICP-MS software can be used to correct for polyatomic interferences, although they can be less reliable than using a CRC in KED/He mode of operation. Interference equations involve determining the signal for another isotope of the interfering element and subtracting the appropriate signal from the analyte isotope signal.

In addition to polyatomic ion overlaps, isobaric and doubly charged ion interferences can affect ICP-MS measurements. Isobaric overlaps occur when an analyte is measured at an isotope mass where an isotope of a different element is also present. Most isobaric interferences that could affect ICP-MS determinations have been well studied in the literature and are not unique to this method (8–10). These overlaps are easy to avoid by choosing the default, preferred analytical isotope for each element of interest. Doubly charged ions can affect quadrupole ICP-MS measurements because a quadrupole mass spectrometer separates ions based on their *m/z*, rather than their true atomic mass. So, if an atom loses two electrons—giving it a double positive charge (M^{2+}) rather than the usual single positive charge (M^+), it will appear at half its true mass. Arsenic can suffer a doubly charged ion interference from the rare earth elements samarium ($^{150}Sm^{++}$) and neodymium ($^{150}Nd^{++}$), *see* Table 2021.03B. The Agilent ICP-MS MassHunter software (version 5.1 and later) includes an automatic routine (known as M^{2+} Correction) to correct for doubly charged ion interferences. The same correction can be set up manually in version 4.6 or earlier of ICP-MS MassHunter, following manufacturer guidelines.

Table 2021.03B. Possible interferences for As, Cd, Hg, and Pb

Analyte isotope	Polyatomic interferences	Elemental interferences
^{75}As	$^{40}Ar^{35}Cl$, $^{59}Co^{16}O$, $^{40}Ca^{15}Cl$, $^{36}Ar^{38}ArH$, $^{38}Ar^{37}Cl$, $^{36}Ar^{39}K$	$^{150}Sm^{++}$ $^{150}Nd^{++}$
^{111}Cd	$^{95}Mo^{16}O$, $^{94}Zr^{16}OH$, $^{39}K_2^{16}O_2H$	
^{201}Hg		
^{208}Pb	$^{192}Pt^{16}O$	

B. Apparatus and Materials

(a) *Mass spectrometer*.—Agilent 7850 ICP-MS (Tokyo, Japan) equipped with an Octopole Reaction System (ORS^4) CRC or equivalent instrument. The single quadrupole ICP-MS instrument was used with a standard sample introduction system comprising a glass concentric nebulizer, quartz double-pass spray chamber, 2.5 mm injector quartz torch, and nickel (Ni) interface cones. The plasma was automatically optimized using the autotune function in the ICP-MS MassHunter instrument control software (version 5.1). Plasma optimization is critical for the analysis of varied sample digests to ensure less suppression, better sensitivity, lower interferences, better stability, and less maintenance. Ultra High Matrix Introduction (UHMI) was used as part of the standard sample introduction hardware on the 7850 ICP-MS. UHMI adds automated and fully calibrated aerosol dilution capability, using an additional argon gas flow to dilute the aerosol before it reaches the torch. UHMI further increases plasma robustness, enabling samples with percent level total dissolved solids (TDS) to be analyzed routinely over extended periods of time. To control common polyatomic ion interferences using KED, the ORS^4 cell was operated in collision mode using helium cell gas. Operating the CRC in He/KED mode enables the same cell conditions to be used for multiple elements in varied sample types, access to confirmatory isotopes, and the ability to do full mass screening for additional elements.

(b) Instrument startup routine and initial checks should be performed per the manufacturer's recommendations. Ignite the plasma and the peristaltic pump will start automatically. Allow the plasma and system to stabilize per the manufacturer's recommendations. Tune the ICP-MS instrument according to the guidelines in the manufacturer's tuning guide. During the tuning step, the internal standard tubing is placed in reagent water. Optimize plasma parameters for best sensitivity counts per second (cps)/ppm, as outlined in Table 2021.03A), while maintaining acceptable oxide and double-charged ratios per manufacturer recommendations (*see* Table 2021.03A). The instrument must exceed minimum manufacturer specifications. Perform a daily check for instrument sensitivity, oxide formation ratios, double-charged element formation ratios, and background. This daily check can be done automatically using the instrument's pre-run performance check routine, as recommended by the manufacturer. If the performance check is not satisfactory, additional optimization or maintenance actions may be necessary per manufacturer recommendations.

(c) Typical ICP-MS operating conditions for the analysis of cannabis and cannabis-related samples are shown in

Table 2021.03C. Typical ICP-MS operating parameters for the analysis of cannabis and hemp-matrices.

Parameter	Value
RF power, W	1600
Sampling depth, mm	10
Carrier gas, L/min	0.80
Dilution (HMI) gas, L/min	0.15
UHMI setting	4
Helium cell gas, mL/min	4.3
Energy discrimination, V	3.0

Parameters are automatically optimized by the ICP-MS MassHunter software during startup for the 4x UHMI conditions

Table 2021.03C. UHMI was used to dilute the sample aerosol using argon gas, allowing unknown high-matrix samples to be analyzed without the need for matrix matched calibrations and enabling varied samples to be analyzed in the same batch. For this method, a UHMI dilution factor of 4 was used. If different acid levels are used to prepare samples and standards, UHMI will help to reduce the suppression effects from the sample matrix and the different acid composition. However, large differences in matrix and acid levels can lead to physical sample transport and nebulization effects that will need to be corrected using appropriate internal standards. If UHMI aerosol dilution technology is not available on the ICP-MS, dilution of high matrix samples using conventional liquid dilution is required. Equivalency can be ensured when all quality control parameters listed in this procedure are met. For the analysis of unknown plant materials, M^{2+} Correction should be selected in the Method Wizard for the analysis of As, Se, and Zn in case the samples contain rare earth elements (REEs) and barium (Ba) at a high enough concentration to interfere with those elements.

(d) *Microwave digestion system*.—CEM MARS 6 microwave with dual magnetrons, iWave contactless in-situ temperature sensor technology, Teflon-coated 316 stainless steel cavity, safety features including vessel recognition and counting, and continuous cavity monitoring (capable of disabling the magnetrons when it senses a vessel event in the cavity), and 24 position MARSXpress Plus vessels or equivalent system capable of reaching 210°C when a minimum of 24 vessels are used.

(e) *Balances*.—Precision balance XPE1203S (Mettler-Toledo, Switzerland) or equivalent, for sample preparation and capable of measuring to 0.1 mg.

(f) *Pipettes*.—Calibrated mechanical pipettes and trace metal grade pipette tips in the following ranges: (0.5–10 µL, 10–100 µL, 100–1000 µL, and 1000–5000 µL).

(g) *Common laboratory plasticware*.—All labware used in the experiment was plasticware, including Corning 50 mL polypropylene (PP) centrifuge tubes and Corning 15 mL PP centrifuge tubes. Glassware should be avoided for all trace metal applications.

C. Reagents

(a) *Deionized water (DIW)*.—Resistance >18 MΩ•cm Milli-Q Element system (Millipore, Bedford, MA, USA) or equivalent was used for all dilutions.

(b) *Nitric acid (HNO_3)*.—Concentrated (UltraPure or equivalent recommended) was used for microwave digestion and preparing diluted acid for sample preparation and analysis.

(c) *Hydrochloric acid (HCl)*.—Concentrated (UltraPure or equivalent recommended) was used for microwave digestion and preparing diluted acid for sample preparation and analysis.

(d) *Diluent*.—1% (v/v) HNO_3 and 0.5% (v/v) HCl solution in DIW.

(e) *Stock solutions and tuning solution*.—Table 2021.03D shows Agilent stock standards used for the preparation of the samples and standards.

D. Preparation of Test Samples/Standard Solutions

(a) *Blank solutions*.—Assemble or prepare the following blank solutions on the same day as analysis:

(1) *Calibration blank*.—Either 1% (v/v) HNO_3 and 0.5% (v/v) HCl in DIW or 2% (v/v) HNO_3 and 0.5% (v/v) HCl in DIW.

(2) *Rinse blank*.—1% (v/v) HNO_3 and 0.5% (v/v) HCl in DIW or 2% (v/v) HNO_3 and 0.5% (v/v) HCl in DIW.

(b) *Calibration standard solutions*.—Prepare these weekly in a 50 mL polypropylene centrifuge tube:

(1) *Working environmental calibration standard*.—1 mg/L Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, U, and 100 mg/L Fe, K, Ca, Na, Mg in 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution. Prepare by adding 5 mL of environmental calibration standard (5183–4688) to 45 mL 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution.

(2) *Mercury (Hg) stock calibration standard A*.—10 mg/L Hg in 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution. Prepare by adding 0.5 mL 1000 mg/L Hg standard (5190–8485) to 49.5 mL 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution.

(3) *Mercury (Hg) working solution A*.—0.1 mg/L in 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution. Prepare by adding 0.5 mL Hg stock calibration standard A (*see* above) to 49.5 mL 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution.

(c) *Calibration standards*.—Prepare the multi-element calibration standards listed Table 2021.03E from the calibration stock solutions. Prepare the standards on the same day as sample analysis, make each standard in a 50 mL polypropylene centrifuge tube.

(d) *Internal standard and QC standards*.—Prepare the following 11 standard solutions and sample solutions in 50 mL polypropylene centrifuge tubes:

(1) *Internal standard working solution for on-line addition*.—2 mg/L ^{6}Li, Sc, Ge, Lu, In, Tb, Rh, Bi in 1% HNO_3. Prepare by adding 1 mL internal standard mix (5188–6525) to 49 mL 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution.

(2) *Mercury (Hg) working solution B*.—1.0 mg/L Hg in 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution. Prepare by adding 5.0 mL 10 mg/L Hg standard (8500–6941) to 45 mL 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution. Prepare independent solutions from working Hg solution A. Use independent Hg standard from Hg stock calibration standard A.

(3) *Initial calibration verification (ICV) solution*.—0.05 mg/L Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, V, Zn, Th, U; 5 mg/L Fe, K, Ca, Na, Mg; and 0.001 mg/L Hg in 2% (v/v) HNO_3 and 0.5% (v/v) HCl solution. Prepare by adding 0.25 mL initial calibration verification standard (5183–4682) and 0.05 mL 1 mg/L working Hg solution B to 49.7 mL 1% (v/v) HNO_3 and 0.5% (v/v) HCl solution. Prepare independent solutions from the continuing calibration verification solution.

Table 2021.03D. Details of standard and calibration solutions

Solution	Elements	Agilent Part No.
Initial calibration verification standard (second source standard from the environmental calibration standard)	10 μg/mL Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, U; 1000 μg/mL Fe, K, Ca, Na, Mg, Sr	5183-4682
Environmental spike mix	100 μg/mL Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, U. 1000 μg/mL Fe, K, Ca, Na, Mg	5183-4687
Environmental calibration standard	10 μg/mL Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, U; 1000 μg/mL Fe, K, Ca, Na, Mg	5183-4688
Internal standard	100 μg/mL ^{6}Li, Sc, Ge, Lu, In, Tb, Rh, Bi	5188-6525
Tuning stock solution	10 μg/mL Li, Co, Ce, Y, Tl	5188-6564
Mercury	1000 μg/mL Hg	5190-8485
Hg calibration solution	10 μg/mL Hg	8500-6941

Table 2021.03E. Concentration of calibration standards

	Volume to add						
Solution	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6[a]	Std 7[a]
Working environmental calibration standard (1 mg/L trace, 100 mg/L major)	0	5 μL	25 μL	50 μL	500 μL	2500 μL	5000 μL
Working Hg solution, A (0.1 mg/L Hg)	0	5 μL	25 μL	50 μL	500 μL	2500 μL	5000 μL
Calibration blank	50.00 mL	49.99 mL	49.95 mL	49.90 mL	49.00 mL	45.00 mL	40.00 mL
Final volume	50.00 mL	50.00 mL	50.00 mL	50.00 mL	50.00 mL	50.00 mL	50.00 mL
Final concentrations							
Trace elements (μg/L)	0	0.1	0.5	1	10	50	100
Major elements (μg/L)	0	10	50	100	1,000	5,000	10,000
Hg (μg/L)	0	0.01	0.05	0.1	1	5	10

[a] Std 6 and Std 7 are for optional use, should any of the element concentrations prove higher than 5 μg/L, or 10 μg/L for Hg

(4) *Continuing calibration verification (CCV) solution.*—Use a mid-level calibration standard (Std 5 in Table 2021.03E).

(5) *Method blanks (MBKs).*—A minimum of two MBKs must be included in each digestion batch to verify the absence of contamination that may arise from the vessels.

(6) *Reference Materials (RMs).*—Analyze at least one reference material such as NIST SRM 1547 peach leaves, NIST SRM 1573a tomato leaves, or NIST SRM 1575 pine needles. Digest them with the other samples.

(7) *Laboratory duplicates (DUP).*—Randomly select a sample from the batch to be analyzed. Remove two 15 mL aliquots of the sample solution and place each aliquot in a separate 50 mL polypropylene test tube.

(8) *Spike solution.*—Prepare the spike solutions and add to the samples before closed-vessel microwave digestion. Prepare the standards on the same day as sample digestion; detailed information is shown below.

(9) *Mercury (Hg) spike stock solution* A.—100 mg/L Hg in 1% (v/v) HNO_3 solution. Prepare by adding 5 mL 1000 mg/L Hg standard (5190–8485) to 45 mL 2% (v/v) HNO_3 solution in a 50 mL polypropylene test tube. The Hg spike stock solution is prepared without HCl and should therefore be freshly prepared each month or more frequently if the stability of the standard cannot be confirmed.

(10) *Working spike solution—high.*— 1 mg/L Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, U, and 10 mg/L Fe, K, Ca, Na, Mg in 2% (v/v) HNO_3 solution. Prepare by adding 0.5 mL environmental spike mix (5183–4687) and 0.5 mL Hg spike stock solution A (*see* above) to 49 mL 2% (v/v) HNO_3 solution in a 50 mL polypropylene test tube.

(11) *Working spike solution—low.*—100 μg/L Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, U, and 0.1 mg/L Fe, K, Ca, Na, Mg in 2% (v/v) HNO_3 solution. Prepare by adding 5 mL of working spike solution—high (see above) to 45 mL 2% (v/v) HNO_3 solution in a 50 mL polypropylene test tube.

(e) *Sample homogenization.*—This method assumes that all samples have been collected and homogenized accordingly per production batch size and physical form of the product. The aim of sample collection is to obtain a representative sample of the entire production batch while the aim of homogenization is to obtain an analytical sample representative of the collected sample. Follow state protocol for cannabis sampling and homogenization, or refer to U.S. Food and Drug Administration, *Elemental Analysis Manual (EAM)* 2.2, Food Homogenization (11) for reference on homogenization approaches. Depending on the type of sample, the homogenization procedure should be such that it provides uniform and repeatable results, using instrumentation that is easy to clean and maintain, is adapted to hold various sample sizes, and, preferably, that is metal free, to reduce the risk of contamination of the sample.

(f) *Closed-vessel microwave digestion sample preparation.*—This sample preparation is designed for samples that must be digested in a concentrated acid using closed-vessel

Table 2021.03F. A typical microwave digestion program suitable for preparation of cannabis samples

Example digestion	
Sample weight, g	0.5
HNO_3, mL	9
HCl, mL	1
Spike solution (for spiked samples only), µL	50–500
Low spike, µL	50
	Working spike solution—low
Mid spike, µL	500
	Working spike solution—low
High spike, µL	50
	Working spike solution—high
Very high spike, µL[a]	500
	Working spike solution—high
Oven program	
Ramp (to 210°C), min	20
Hold (at 210°C), min	15
Cool-down, min	10
Final dilution	
Reagent water	Add to 50 g
Total dilution factor	100 ×

[a] Very high spike is done using the high end of the high spike range

Table 2021.03G. Cannabis and hemp samples analyzed in the study (data available upon request)

Sample category	Sample
Inhaled	Hemp flower
	CBD vape oil
	Hemp isolate extract
Oral	Full spectrum softgel capsules
	Full spectrum tincture
	Isolate tincture
	CBD coffee grounds
	Hemp butter
	Hemp seed oil
	CBD beef jerky
	CBD hard candy
	CBD pineapple drink
Topical	Full spectrum balm
	Pain relief cream
	CBD balm
	CBD topical oil
	Hemp soap
Manufacturing	Hemp biomass
	Spent hemp biomass
	Trichomes
	CBD crude extract
	CBD distillate
	CBD isolate

digestion apparatus. Most samples can be digested completely using a mixture of HNO_3 and HCl, but all concentrated acids introduce safety issues. Therefore, appropriate safety precautions should be employed at all times including use of personal protective equipment (PPE) and following lab and microwave manufacturer safety protocols. This standard does not purport to address all of the safety concerns associated with its use. It is the responsibility of the analyst using this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations before use.

(1) Table 2021.03F shows an example of a microwave digestion procedure that has been shown to have broad applicability. These digestion conditions were optimized for batches of mixed samples of various degrees of decomposition difficulty. If digesting only plant material, for example, less acid volume may be used. When using less acid volume, a complete digestion should still be achieved. In most cases, a clear and colorless solution upon dilution with no particulate constitutes a complete digestion. Check for matrix enhancements and suppressions by monitoring spike recovery data and by examining the internal standard signal. If digestion is incomplete, additional concentrated HNO_3 may be required. H_2O_2 (1 mL or 2 mL) can be added to reduce the total acid concentration and also breakdown the sample, but do not use less than 5 mL concentrated acid in the digestion vessel. Do not use more than 2 mL H_2O_2 to avoid diluting the acid mixture. Refer to microwave manufacturer for optimum conditions for digestion.

(2) All reagents used for the preparation of samples and standard solutions should be free of elemental impurities, as appropriate for the level of the target analytes. For the preparation of calibration standards and sample stabilization, ultra-pure grade acids are recommended.

(3) For MBKs, add acids as noted in Table 2021.03F and omit the sample. Optionally, 0.5 g reagent water may be added instead of the sample if samples are high in water content, i.e., tinctures, beverages.

(4) If the analyte list includes chloride-soluble elements such as Hg, Ag, or Sn, 0.5% HCl should be used for the stabilization of acidic samples prepared for analysis by ICP-MS.

(5) Cannabis regulations do not specify that post-digestion sample stability must be determined. All samples and standard solutions are to be diluted using the reagent water.

(g) *Samples*.—A range of cannabis and products containing cannabis and hemp were analyzed in this study (Table 2021.03G). HCl was included to ensure the stability of Ag and Hg in solution. The digested samples were diluted using the same acid mix as the standards. The SRMs were prepared using the same method to verify that the digestion was complete and confirm the analytes' quantitative recovery.

E. Determinations

(a) Check calibration performance.

(1) Linear regression correlation coefficient (r) must be $\geq$0.9975.

(2) Run an ICV solution to verify calibration. The ICV recovery must be 100 ± 10% to proceed. If ICV fails, reanalyze one more time. If ICV fails again, determine the source of problem and remedy before proceeding.

(b) External standard calibration curves shown in Figure 2021.03A were obtained using the following protocol:

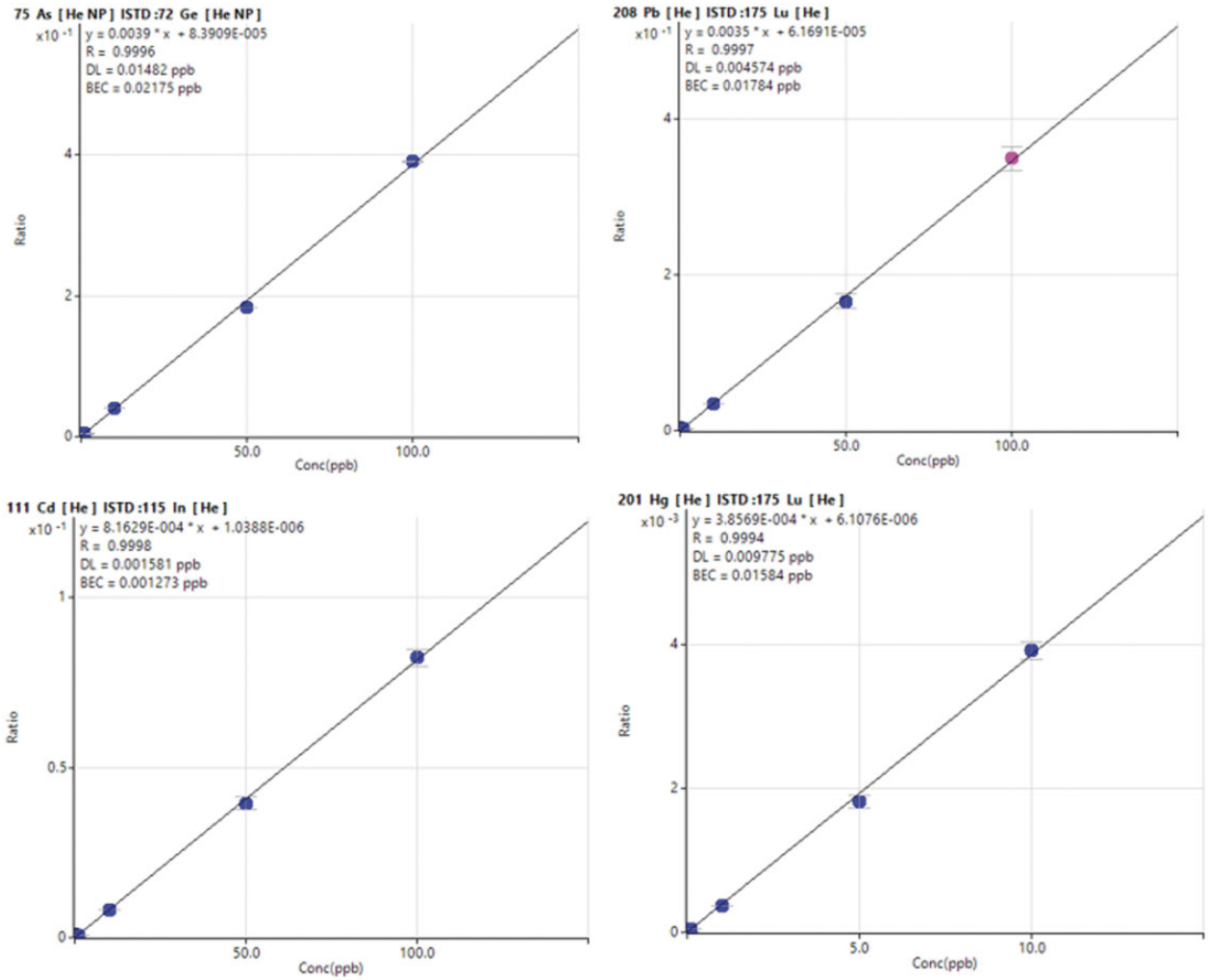

Figure 2021.03A. Representative calibration curves for As, Cd, Pb, and Hg, showing good linearity ($R > 0.999$) across the calibration range.

(1) Calibrate using the standard blank and at least four multi-element standards (*see* Table 2021.03E).

(2) Include the calibration blank as a point on the calibration curve (0 μg/kg calibrant); in the ICP-MS MassHunter software that would be Level 1.

(3) For the origin use "Blank Offset", and a linear curve fit.

(c) Analyze instrument checks and analytical solutions.

(1) A continuing calibration verification (CCV) solution must be analyzed at a frequency of every 10 samples and at the end of the analytical sequence. Recovery must be 100 ± 10% to proceed. If CCV fails, reanalyze one more time. If CCV fails again, reanalyze samples analyzed after the last acceptable CCV. If CCV fails a third time, restart the analytical sequence and/or prepare new digests, standards, and QC solutions.

(2) The RSD of replicate concentrations must be <10% for all solutions when instrument response is >5 times LOQ. If the RSD exceeds 10%, determine the source of noise and remedy before proceeding.

(3) Analyze the continuing calibration blank (CCB) every 10 samples and at the end of the analytical sequence. It must be below the LOQ to proceed. If CCB fails, reanalyze one more time. If CCB fails again, reanalyze samples analyzed after the last acceptable CCB. If CCB fails a third time, restart the analytical sequence with a longer washout time and/or prepare new digests, standards, and QC solutions.

(4) Carryover/memory effects arise when elements in a previously run sample contribute to the signals measured in the next sample in the sequence. These effects have been well studied in the literature and other standard methods and are not unique to this method (8). Memory effects can result from sample deposition on the sampler and skimmer cones, and from the buildup of sample material in the plasma torch and spray chamber. These effects can be minimized by ensuring the elements are stabilized in solution (for example by adding HCl) and by flushing the system with a rinse blank(s) between samples. The possibility of memory effects should be acknowledged, and suitable rinse times should be employed. The required rinse time for a particular element varies and should be determined prior to analysis. This can be accomplished by aspirating the highest calibration standard containing the elements of interest for a normal sample analysis period, followed by analysis of the rinse blank multiple times afterwards. Track the rinse blank

for any carryover. The length of time required to reduce analyte signals to within a factor of 10 of the method limit of detection should be noted. Another option is to use the Intelligent Rinse function in the ICP-MS MassHunter software. If the analytical solution concentrations are higher than the highest standard concentration, dilute the analytical solution. The new concentration should be at the midpoint of the calibration curve for that element. If the concentration of the injected unknown sample is higher than the highest calibration standard, then check all samples following that high sample. Make sure to check for carryover by checking the RSD of replicate concentrations of all samples following the sample that was higher than the highest calibration curve. If the RSD exceeds 10%, determine if it is due to carryover and remedy before proceeding.

(d) Check the Internal Standard (ISTD) response.

(1) The ISTD response measured in the samples must be within 60–125% of the reference signals measured in the calibration blank. Monitor internal standard signals and dilute any analytical solution where % recovery falls below 60% or exceeds 125%.

(2) It is helpful to monitor the total matrix solids (TMS) levels in all cannabis/plant samples. Reviewing the samples' TMS levels can lead to insights on why samples react differently in the plasma and allows suitable dilutions or the adjustment of method parameters (e.g., aerosol dilution setting). TMS information is also useful when running new sample types or troubleshooting results, for example identifying when data may have been affected by unusually high matrix levels. TMS data can be automatically collected using the IntelliQuant quick scan function in the software.

F. Calculations

(a) A summary of the calibration data, including BECs, LODs, and LOQs, is given in Table 2021.03H. To compensate for natural variation in the abundances of the three isotopes of Pb between samples and standards, the reported concentration for Pb is calculated from the sum of the signals at the three main Pb isotopes at *m/z* 206, 207, and 208. The summation is done automatically in the software using the following equation:

Equation for summation of signals at *m/z* 206, 207, and 208 for lead :

$$\mathrm{Mc}(208) = \mathrm{M}(206) + \mathrm{M}(207) + \mathrm{M}(208)$$

(b) In ICP-MS there are possibilities for interferences, as explained in the Principal section of the method. Table 2021.03B lists the possible polyatomic and isobaric interferences for the elements As, Cd, Hg, and Pb.

Polyatomic ion overlaps are by far the most common spectral overlaps in ICP-MS. They are controlled through a combination of robust, high temperature (low CeO/Ce ratio) plasma operation, and He KED collision cell mode. Any residual interferences that may occur when the interfering element is very high and the interfered analyte is very low can be corrected using mathematical equations. An equation for the residual MoO interference that can affect ^{111}Cd should be included. Interference correction equations can be applied in the ICP-MS software, or they can be calculated using the following equations.

Interference correction equation for ^{111}Cd

$$\mathrm{Mc}(111) = \mathrm{M}(111) - \mathrm{M}(108) \times 1.18 + \mathrm{M}(106) \times 0.712$$

where the last two terms correct for any $^{95}MoO^{+}$ contribution at *m/z* 111 and for any Pd that may be present.

Isobaric interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal *m/z*. Such overlaps are usually easy to avoid, but some cannot be avoided, such as ^{115}Sn on In at *m/z* 115. Also, if isotopically enriched ^{6}Li is used as an internal standard for low mass correction, an equation should be used to correct for any naturally occurring ^{7}Li in the ISTD solution.

Interference correction equation for ^{115}In

$$\mathrm{Mc}(115) = \mathrm{M}(115) - \mathrm{M}(118) \times 0.0149$$

The coefficients should be verified experimentally using the procedures or coefficients provided by the instrument manufacturer.

Some plant materials may contain high levels of REEs, also known as lanthanides (LA). These elements have low second ionization potentials, so they form a higher proportion (up to a few percent) of doubly charged ions than other elements. As the quadrupole mass spectrometer separates ions based on *m/z*, these doubly charged ions appear at half their true mass. For example, doubly charged REE ions (REE^{++}) from ^{150}Nd, ^{150}Sm, ^{156}Gd, ^{156}Dy, ^{160}Gd, and ^{160}Dy appear at *m/z* 75, 78, and 80, potentially causing overlaps that can bias the results for As and Se in samples that contain high levels of the REEs. Samples with a high concentration of barium can also lead to an interference from $^{132}Ba^{2+}$ on $^{66}Zn^{+}$. He mode alone is not effective at resolving doubly charged ion overlaps. In these cases, M^{2+} Correction, which is applied automatically in the ICP-MS MassHunter software, is beneficial.

Taking the example of REE^{2+} overlaps on As at *m/z* 75, Sm and Nd both have several isotopes, ^{147}Sm, ^{148}Sm, ^{149}Sm, ^{150}Sm, ^{152}Sm, ^{154}Sm, and ^{142}Nd, ^{143}Nd, ^{144}Nd, ^{145}Nd, ^{146}Nd,

Table 2021.03H. Calibration data, LODs, BECs, and LOQ = 3 × SD (low-level spike) × 100 (dilution factor)

Tune mode	Mass (m/z)	Element	Units	MassHunter Software			Calculated
				R	LOD	BEC	LOQ ≤10 ppb, µg/kg
He	75	As[a]	ppb	0.9996	0.0148	0.0217	9.18
He	111	Cd	ppb	0.9998	0.0016	0.0013	6.25
He	201	Hg	ppb	0.9994	0.0098	0.0158	2.16
He	206, 207, 208	Pb	ppb	0.9997	0.0046	0.0178	7.85

[a] Data for As was obtained using half mass correction.

^{148}Nd, and ^{150}Nd, and they all form doubly charged ions in the plasma. As the production rate of doubly charged ions is constant for all isotopes, the M^{2+} ions should form with the same natural isotopic abundance ratio as the singly charged M^{+} ions. However doubly charged ions from odd-number isotopes will appear at half masses, so $^{147}Sm^{2+}$ appears at *m/z* 73.5 and $^{145}Nd^{2+}$ appears at *m/z* 72.5, for example. The half-mass REE^{2+} ions are therefore free from direct overlap by any singly charged ions. Normally in ICP-MS measurements, the quadrupole is operated with a peak width of about 0.7 u, so peaks at half masses (such as $^{145}Nd^{2+}$ at *m/z* 72.5 and $^{147}Sm^{2+}$ at *m/z* 73.5) would be overlapped by the "tails" of the adjacent elemental ion peaks at *m/z* 72, 73 and 74. Selecting M^{2+} Correction in the ICP-MS MassHunter software (version 5.1 or later) automatically sets the quadrupole to a narrow peak mode to allow measurement of the doubly charged ions at half mass positions allowing the measurement of ions at *m/z* = 72.5 and 73.5 without any overlaps from the adjacent masses. A high-performance hyperbolic quadrupole is needed to measure narrow peaks with high ion transmission to enable the automated doubly charged ion correction to be effective.

For ICP-MS instruments without the "half-mass correction" routine, it is possible to manually calculate and correct for the $^{150}Sm^{2+}$ and $^{150}Nd^{2+}$ contribution at *m/z* 75 (provided the quadrupole is capable of separating half masses). Based on the isotopic abundance ratio for Sm (^{147}Sm:^{150}Sm = 14.99:7.38), $^{150}Sm^{2+}$ should produce 0.4923 times the counts at $^{147}Sm^{2+}$. Similarly, from the isotopic abundance ratio for Nd (^{145}Nd:^{150}Nd = 8.3:5.6), $^{150}Nd^{2+}$ should produce 0.6747 times the counts at $^{145}Nd^{2+}$. Using this information, a correction equation can be used to calculate the counts of $^{150}Sm^{2+}$ (at *m/z* 75) based on the counts of $^{147}Sm^{2+}$ (measured at *m/z* 73.5) using the known Sm isotope abundance ratio. In the same way, the counts of $^{150}Nd^{2+}$ (at *m/z* 75) can be calculated from the counts at $^{145}Nd^{2+}$ (measured at *m/z* 72.5) as shown below. The equation can then be applied to subtract the contribution of $^{150}Sm^{2+}$ and $^{150}Nd^{2+}$ on $^{75}As^{+}$.

Interference correction equation for ^{75}As

$$Mc(75) = M(75) - M(72.5) \times 0.6747 - M(73.5) \times 0.4923$$

(c) The ICP-MS MassHunter software will calculate the concentrations of all analytes in the samples based on the individual element's calibration curve. The user does not need to do any hand calculations. However, the calculation of the concentration (mass fraction) of the analyte in the measured samples is calculated according to:

$$\text{Concentration (ppb, } \mu g/kg) = S \times DF \times (M/m)$$

where S = concentration of analyte in the analytical solution (ng/g); M = mass (g) of analytical solution; = mass of analytical portion (g); and DF = dilution factor (1 if the analytical solution is not diluted).

(d) Spike recoveries can be set up to be calculated by the ICP-MS MassHunter software. However, the calculations as follows:

$$\%\text{Recovery} = [(C_{x+s} - C_x)/(C_s M_s/M_x)] \times 100$$

where C_{x+s} = concentration determined in spiked sample (μg/kg); C_x = concentration determined in unspiked sample

Table 3. ICV and CCV recovery tests for As, Cd, Hg, and Pb

Mass (m/z)	Element	Recovery of ICV %	Mean recovery of CCV ± 1σ, *n* = 6, %	Recovery range of CCV, %
75	As[a]	96	93 ± 3	90–97
111	Cd	97	95 ± 3	92–99
201	Hg	—[b]	95 ± 4	92–101
206, 207, 208	Pb	98	98 ± 2	94–100

[a] Data for As was obtained using half mass correction.
b — = No value.

Table 4. Mean concentrations (ppm) of three repeat measurements of four SRMs, including comparison data for certified elements, where appropriate, and recovery data

	NIST 1547 peach leaves				NIST 1573a tomato leaves			
Element	Measured concn[a]	SD	Certified concn	Recovery, %	Measured concm	SD	Certified concn	Recovery, %
^{75}As	0.06	0.01	0.06	99	0.109	0.021	0.112	97
^{111}Cd	0.025	0.001	0.026	96	1.39	0.01	1.52	91
^{201}Hg	0.034	0.003	0.031	108	0.032	0.002	0.034	96
Pb[b]	0.78	0.02	0.87	90	0.555	0.098	[c]	
	NIST 1575 pine needles				NIST 1515 apple leaves			
^{75}As	0.047	0.008	0.039[d]		0.029	0.009	[c]	
^{111}Cd	0.210	0.006	0.233	90	0.014	0.003	0.013	108
^{201}Hg	0.0380	0.0010	0.0399	96	0.0420	0	0.0432	98
Pb[b]	0.144	0.002	0.167[d]		0.41	0.01	0.47	86

[a] *n* = 3. Triplicate replicate sample digestion.
[b] Pb results were based on the sum of the signals measured at mass 206, 207, and 208.
[c] Elements have no certified or non-certified value for the associated SRM.
[d] Non-certified reference value.

Table 5. Spike recovery results for As, Cd, Hg, and Pb in four cannabis-samples

Each sample digested in triplicate by CEM (~100x dilutions)				Method Performance Requirements								
				(Low) ≥10 ppb to 100 ppb			(Medium) >100 ppb to 1 ppm			(High) >1 ppm to 10 ppm		
Mass	Element	*Limit of quantitation (LOQ) ≤10 ppb, μg/kg	Native Level in Matrix ppb, μg/kg	Repeatability (RSDr), % 15	Nomial Spiked Conc. (in sample, μg/kg)	Recovery, % 60–115	Repeatability (RSDr), % 11	Nomial Spiked Conc. (in sample, μg/kg)	Recovery, % 80–115	Repeatability (RSDr), % 7.3	Nomial Spiked Conc. (in sample, μg/kg)	Recovery, % 80–115
Fortified Method Blank												
75	As	9.171		5.398	10	107	1.943	100	105	0.667	1000	101
111	Cd	6.247		1.220	10	102	2.651	100	101	1.060	1000	98
201	Hg	2.155		14.165	10	102	1.796	100	106	0.449	1000	104
208	Pb	7.849		6.707	10	114	3.416	100	105	1.279	1000	102
Flower (Inhaled)												
75	As	9.171	91.247	13.299	10	87	3.849	100	95	1.706	1000	107
111	Cd	6.247	209.214	11.366	100	99	3.193	1000	101	1.048	10000	100
201	Hg	2.155	16.875	14.370	10	95	2.991	100	94	1.508	1000	102
208	Pb	7.849	305.946	3.806	10	66	9.290	100	109	1.566	1000	100
Hemp Butter (Oral)												
75	As	9.171	0.481	7.298	10	108	3.756	100	103	2.009	1000	102
111	Cd	6.247	0.157	4.231	10	98	2.289	100	100	1.054	1000	95
201	Hg	2.155	<LOQ	5.476	10	103	3.253	100	104	0.935	1000	101
208	Pb	7.849	3.726	3.419	10	95	2.697	100	102	0.464	1000	98
Pain Relief Cream (Topical)												
75	As	9.171	11.804	4.417	10	64	0.373	100	97	0.945	1000	100
111	Cd	6.247	2.256	1.555	10	93	0.434	100	99	1.646	1000	98
201	Hg	2.155	6.864	5.538	10	78	1.431	100	89	2.032	1000	103
208	Pb	7.849	12.442	11.622	10	69	1.199	100	100	1.575	1000	102
CBD Crude Extract (Manufacturing)												
75	As	9.171	3.147	0.167	10	88	0.770	100	99	1.761	1000	100
111	Cd	6.247	1.105	2.560	10	98	0.947	100	97	2.592	1000	98
201	Hg	2.155	5.757	14.329	10	85	1.724	100	91	1.769	1000	94
208	Pb	7.849	188.103	1.982	10	63	2.082	100	89	1.755	1000	100

*LOQ = 3 * SD (low level spike) x 100 (dilution factor), ($n = 10$). Data for As was obtained using M^{2+} Correction.

(μg/kg); C_s = concentration of spiking solution (μg/kg); M_s = mass of spiking solution added to an analytical portion (g); and M_x = mass of analytical portion (g).

Results and Discussion

The analytical limits listed in Table 2021.03H are presented as an example of results achievable for cannabis and cannabis products when using the ICP-MS method and equipment specified herein. The AOAC SMPR 2020.001 requirements for LOQs of ≤10 ppb were met for As, Cd, Pb, and Hg. Analytical limits will vary depending on instrumentation and actual operating conditions used. Also, per the SMPR requirements to check the instrument performance, an ICV standard was run and the CCV was analyzed six times throughout the run. The results given in Table 3 show that the recoveries for all the certified elements present in the standard were excellent, ranging from 93–104%. The mean recoveries and range are also given in Table 3. All CCV recoveries were within ±10% of the expected value.

Analysis of Reference Materials

The results for the analysis of the four SRMs, SRM 1547 peach leaves, SRM 1573a tomato leaves, SRM 1575 pine needles, and SRM 1515 apple leaves, are given in Table 4. The mean ICP-MS results were in good agreement with the certified concentrations where certified values are provided.

Analysis of Cannabis Samples

Using the ICP-MS method, excellent spike recoveries were achieved for As, Cd, Hg, and Pb in all spiked samples, covering three spiked levels (low, mid, and high), in four different matrices, demonstrating the accuracy of the method. Recoveries for all elements in the hemp flower (inhaled), hemp butter (oral), pain relief cream (topical), and CBD crude extract (manufacturing), are shown in Table 5, and met the SMPR requirements for repeatability (RSD_r) and recovery. Reproducibility will be collected during the multilaboratory study at a later date.

Conclusions

ICP-MS is suitable for the analysis of cannabis and associated products and the analysis is easily performed using a single quadrupole instrument with a robust plasma, such as the Agilent 7850, following microwave acid digestion. Measurements were obtained by controlling polyatomic ion interferences using helium collision gas and KED in the instrument's CRC. The same helium/KED cell conditions were used for the determination of all elements in the varied sample types. The aerosol dilution capability of the UHMI technology of the Agilent ICP-MS enabled the routine analysis of samples that contain high and variable matrix levels while minimizing the need for conventional liquid dilution. UHMI enabled samples and standards with varying matrices to be analyzed in the same batch without matrix matching the standards. The method met the method requirements for LOQs for As, Cd, Hg, and Pb of ≤10 ppb.

The suitability of the microwave-assisted sample preparation method was demonstrated by the good recovery results obtained for four plant-based SRMs. It is possible that plant-based samples contain high concentrations of REEs, which would lead to doubly charged ion interferences affecting the measurement of As and Se. These potential interferences were corrected using the M^{2+} Correction routine in the instrument software (ICP-MS MassHunter), which allowed As, (Se, and Zn) to be determined with good accuracy.

ICP-MS is suitable for trace metal screening of medicinal and recreational cannabis, as well as related products, as shown by the spike recovery test results. The repeatability and recovery results for As, Cd, Hg, and Pb spiked at three spike levels in a range of samples met the method requirement criteria. The analysis can be carried out at all stages of production to ensure product quality and products that are free of toxic metals.

Conflict of Interest

None declared.

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