APPLICATION NOTE

ICP - Mass Spectrometry

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To further validate the performance of this method for the industry, The Emerald Proficiency Test (PT) for Heavy Metals was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test Badge seen on the right.
https://pt.emeraldscientific.com/

Digestion, Testing, and Validation of Heavy Metals in Cannabis

Introduction

Owing to the toxicity of heavy metals, it is increasingly important to test cannabis flowers and other cannabis derivatives so that patient and consumer safety is maintained as the use of cannabis becomes more common. This need has translated into an increasing demand for testing cannabis flowers and other cannabis derivatives for toxins such as the heavy metals cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg). Similar to federal pharmaceutical and nutraceutical requirements in the US[1-5], states like California[6], Oregon, and Colorado have published action limits for heavy metals.

Each jurisdiction where cannabis is permitted has published required maximum allowable heavy metals in cannabis and related products. Many of these limits are based on USP <232>/ICH Q3D recommendations. The limits differ based on the route of administration, similarly to what is set out in the ICH Q3D recommendations. Currently, Canada has not set regulations around metals in cannabis products, but is referring to USP <232> and <233> for guidance. Some of the currently known limits for heavy metals are provided in Table 1. For the purpose of this study, the California limits on "all inhaled cannabis goods" were used as they are the most stringent and most applicable to cannabis flower.

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Table 1. A list of the heavy metals and their limits based on jurisdiction and route of administration.

	Canada (Based on USP <232>)	California[6]		Colorado	Connecticut, Maryland, Nevada, New Mexico	Massachusetts		Minnesota	Washington
Heavy Metal	Inhaled Cannabis Goods (µg/g)	All Inhaled Cannabis Goods (µg/g)	Other Cannabis Goods (µg/g)	Flower, Concentrates and Infusions (ppm)	"µg/kg of body weight per day"	All Uses (µg/kg)	Ingestion Only (µg/kg)	PPM in Final Product	µg/Daily Dose (5 grams)
Cadmium (Cd)	0.2	0.2	0.5	0.4	0.09	200	500	0.3	4.1
Lead (Pb)	0.5	0.5	0.5	1	0.29	500	1000	1.0	6.0
Arsenic (As)	0.2	0.2	1.5	0.4	0.14	200	1500	1.5	10.0
Mercury (Hg)	0.1	0.1	3	0.2	0.29	100	1500	0.5	2.0

Several challenges arise in the elemental analysis of cannabis. Of primary consideration is the required sample preparation and digestion. To account for the wide variety of cannabis sample types (flower, concentrates, edibles, extracts, tinctures, waxes, and oils etc.), a robust sample preparation scheme must be employed. Typically, preparation consists of homogenization followed by microwave digestion to break down the complex matrix and extract the heavy metals. Therefore, specific sample prep protocols, microwave digestion conditions, and ICP Mass Spectrometry (ICP-MS) methodology were developed and employed to offer a robust method for all cannabis sample types.

ICP-MS is a very effective technique for trace metal analysis. Due to its ability to see low levels in complex matrices, it is the ideal tool for the determination of trace metals in cannabis samples, especially since normal levels for some analytes are extremely low (sub-ppb).

In this application note, we present data to illustrate the successful validation of the Titan MPS™ Microwave Sample Preparation System and the NexION® ICP-MS for the determination of heavy metals in cannabis flower according to the validation protocols set in USP General Chapter <233>, which are commonly used for evaluation of the levels of elemental impurities in samples.

Experimental

Sample Preparation Procedure

In this work, all samples were digested using microwave digestion (Titan MPS System: PerkinElmer Inc., Shelton, Connecticut, USA) with standard 75 mL TFM vessels. Approximately 3-5 grams of cannabis flower was ground and homogenized. The California-proposed regulations require that "the laboratory shall analyze at minimum 0.5 grams of the representative sample of cannabis goods or cannabis product to determine whether heavy metals are present".[6] Therefore, 0.50 ± 0.05 g of each sample was weighed on a weight boat and then transferred into a digestion vessel, followed by 7 mL of nitric acid (70%), and 3 mL of hydrogen peroxide (30%). The vessels were left uncapped for ten minutes to allow for any pre-reactions to occur safely before being capped and digested following the program in Table 2. To evaluate the effect of the sample preparation on analyte recovery, spikes were added to the microwave vessel prior to the addition of the reagents. To stabilize mercury, 200 ppb gold (Au) was added to each sample.

Upon completion of the digestion, all samples were diluted with deionized water to a final volume of 50 mL. This resulted in a total dilution factor of 100x with a reagent matrix of 14% HNO_3. Calibration standards were prepared in this same matrix. Figure 1 shows the cannabis flower and the resulting clear solution after digestion and preparation for analysis.

Table 2. Titan MPS System microwave digestion program for dissolution of cannabis samples.

Step	Target Temp (°C)	Pmax (bar)	Ramp (min)	Hold (min)	Power (%)
1	160	30	5	5	90
2	200	30	5	20	100
3	50	30	1	30	0

Figure 1. Cannabis flower before and after digestion.

Instrumentation

A PerkinElmer NexION ICP-MS, which includes the proprietary Universal Cell Technology™ (UCT) as well as the All Matrix Solution (AMS) system, was used for the analysis. The NexION ICP-MS was configured with the standard SMARTintro™ sample introduction module consisting of a MEINHARD® glass concentric nebulizer, glass cyclonic spray chamber, and a quartz torch with 2 mm id injector.

The instrument operating parameters are shown in Table 3. To reduce the matrix loading in the plasma and provide robust plasma conditions for the high sample matrix, an AMS dilution factor was set to approximately 3x. All analytes were acquired in Collision mode using helium. Using this simple methodology, the UCT reduces or eliminates all common polyatomic interferences using kinetic energy discrimination (KED).

Calibration

To cover the wide range of concentrations for all cannabis sample types, including concentrates and extracts, a calibration was developed using a blank and four calibration standards. The elements, masses, and standard concentrations are shown in Table 4. As stated in the previous section, the calibration blank and standard were prepared in 14% nitric acid to matrix match with the samples. To stabilize mercury, 200 ppb gold (Au) was added to the calibration blank and each standard.

To monitor the instrument response from sample to sample, internal standards (Ge, In, and Tb) were added on-line.

Table 3. NexION ICP-MS Operating Conditions.

Parameter	Value
RF Power (W)	1600
Nebulizer Flow (L/min)	0.88
Dilution Gas Flow (L/min)	0.11
Sample Uptake Rate (mL/min)	0.20
Collision (He) Gas Flow (mL/min)	4

Table 4. Elements and standard concentrations.

Analyte	Mass	Standard 1 (µg/L)	Standard 2 (µg/L)	Standard 3 (µg/L)	Standard 4 (µg/L)
Cadmium (Cd)	110.90	0.5	1	5	10
Lead (Pb)	207.98	1.25	2.5	12.5	25
Arsenic (As)	74.92	0.5	1	5	10
Mercury (Hg)	201.97	0.1	0.2	1	2

Table 5. PDEs and Spike Levels.

Analyte	PDE for Inhaled Products	Spike Level (µg/L)		
		50% PDE	100% PDE	150% PDE
Cadmium (Cd)	0.2	1.00	2.00	3.00
Lead (Pb)	0.5	2.50	5.00	7.50
Arsenic (As)	0.2	1.00	2.00	3.00
Mercury (Hg)	0.1	0.50	1.00	1.50

Results and Discussion

Method Validation

USP General Chapter <233> defines the following requirements for method validation:

Accuracy: The matrix and materials under investigation must be spiked with target elements at concentrations that are 50%, 100%, and 150% of the maximum permitted daily exposure (PDE). Mean spike recoveries for each target element must be within 70%-150% of the actual concentrations.

To calculate the appropriate spike levels, we used the California inhalational limits for all inhaled cannabis goods. The 50%, 100%, and 150% spike levels were calculated based on a nominal preparation factor of 100. The limits and spike levels used for this study are shown in Table 5.

Repeatability: Six independent samples of the material under investigation must be spiked at 100% of the target limits defined and analyzed. The measured percent relative standard deviation (%RSD) must not exceed 20% for each target element.

Ruggedness: Carrying out the repeatability measurement testing procedure by analyzing the six repeatability test solutions either on different days, either with a different instrument or by a different analyst. The %RSD of the 12 replicates must be less than 25% for each target element.

Sample Analysis

All quantitative sample data were less than the lowest calibration standard and, as a result, were less than the target limits for the heavy metals in inhalable cannabis products.

Table 6. Sample Results.

Element	Sample Results			Units (µg/g)			Pass/Fail
	1	2	3	Mean	SD	Limit	
Cadmium (Cd)	0.029	0.037	0.042	0.036	0.006	0.2	Pass
Lead (Pb)	0.009	0.021	0.010	0.013	0.007	0.5	Pass
Arsenic (As)	0.027	0.030	0.045	0.034	0.010	0.2	Pass
Mercury (Hg)	0.056	0.044	0.044	0.048	0.007	0.1	Pass

Meeting the Validation Criteria

All quantitative sample data were less than the lowest calibration standard and, as a result, were less than the target limits for the heavy metals in inhalable cannabis products.

Accuracy

The accuracy data of the methodology is exemplified in Table 7, which shows that the pre-digestion spike recovery test in the sample matrix passes at all three spike levels (50%, 100%, and 150% of the target limits) with the mean spike recoveries for each target element well within the 70-150% acceptance criteria.

Repeatability

Six independently prepared samples of a cannabis flower were digested and then spiked at 100% of the target limit and analyzed. As shown in Table 8, the %RSDs for all target elements were within 3%, which is well under the 20% acceptance limit.

Ruggedness

The six samples used for the repeatability study shown in Table 7 were prepared by two different analysts. The RSDs for these twelve measurements are all < 2.5% (as shown in Table 9), well below the method requirement of 25%.

Table 7. Accuracy Test Results.

Element	Mean Unspiked Sample (µg/g)	Mean Recovery (%)			Pass/Fail
		50%	100%	150%	
Cadmium (Cd)	0.036	87	94 %	91	Pass
Lead (Pb)	0.013	81	85 %	84	Pass
Arsenic (As)	0.034	94	96 %	98	Pass
Mercury (Hg)	0.005	97	95 %	107	Pass

Table 8. Repeatability Test Results.

Element	Sample 1 (µg/g)	Sample 2 (µg/g)	Sample 3 (µg/g)	Sample 4 (µg/g)	Sample 5 (µg/g)	Sample 6 (µg/g)	Mean (µg/g)	%RSD	Pass/Fail
Cadmium (Cd)	0.22	0.21	0.23	0.22	0.23	0.23	0.23	2.90	Pass
Lead (Pb)	0.22	0.21	0.23	0.22	0.23	0.23	0.23	2.90	Pass
Arsenic (As)	0.43	0.43	0.44	0.43	0.45	0.47	0.44	1.10	Pass
Mercury (Hg)	0.23	0.22	0.22	0.23	0.24	0.24	0.23	1.10	Pass

Table 9. Ruggedness Test Results.

Element	Sample 1 (µg/g)	Sample 2 (µg/g)	Sample 3 (µg/g)	Sample 4 (µg/g)	Sample 5 (µg/g)	Sample 6 (µg/g)	Sample 7 (µg/g)	Sample 8 (µg/g)	Sample 9 (µg/g)	Sample 10 (µg/g)	Sample 11 (µg/g)	Sample 12 (µg/g)	Mean (µg/g)	% RSD
Cadmium (Cd)	0.22	0.21	0.23	0.22	0.23	0.23	0.20	0.21	0.19	0.21	0.19	0.21	0.21	7.07%
Lead (Pb)	0.43	0.43	0.44	0.43	0.45	0.47	0.38	0.43	0.38	0.42	0.38	0.43	0.42	6.85%
Arsenic (As)	0.23	0.22	0.22	0.23	0.24	0.24	0.20	0.22	0.21	0.22	0.21	0.22	0.22	5.26%
Mercury (Hg)	0.10	0.10	0.10	0.10	0.10	0.11	0.09	0.10	0.09	0.11	0.09	0.10	0.10	4.96%

Conclusion

This work has demonstrated the ability of PerkinElmer's NexION ICP-MS coupled with the Titan MPS Sample Preparation System to perform accurate and reproducible analyses of cannabis flower samples. Using PerkinElmer's AMS and Universal Cell Technology, a robust method was developed. All quantitative sample data were less than the target limits for heavy metals in "Inhaled Cannabis Goods". This work easily passed the acceptance criteria for the testing protocols described in USP General Chapter <233>.

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