

A Method Development Process for Analysis of Metals in Cannabis by ICP-MS

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Introduction

As cannabis is becoming legal in more states, testing requirements are also being passed to ensure safety. One of the tests that is required is metals testing, this is required since cannabis plants can pull heavy metals such as mercury and lead from the soil or water source used to grow the plants. The metals, testing requirements, and acceptable levels that are tested vary from state to state. Therefore, it is difficult to have a universal method that applies for all states. Here we will use a process for method development to develop a method for analyzing cannabis flower for Pb, Hg, Cd, and Ar.

Typical Method Development Process

1. Confirm State Regulations

Always check current regulations in your state regarding required metals testing. This will include which metals must be tested for, at what levels they must be tested, as well as other requirements that must be included in the method such as sampling, sample preparation, acceptable techniques, calibration requirements, etc. Because of the levels required by most states, the technique that will be used is ICP-MS due to its low detection limits. Most states will require the four heavy metals: Arsenic, Cadmium, Lead, and Mercury. However, some states will require testing of additional metals.

2. Sample Preparation

Microwave Digestion

Microwave digestion is the sample preparation technique that is most often used for preparation of cannabis samples. In this technique a sample and acid solution are added to a closed microwave digestion vessel. This is then heated in a microwave in a controlled fashion which allows rapid breakdown of the sample by the heated acid solution until the previously solid sample is completely digested in the acid solution.

Acid Concentration

Determination of the acid concentration is a balance of being strong enough to fully digest the sample into solution, but not so concentrated that it will require excessive dilution to bring the acid into typical analysis range. The final solution that is introduced into the instrument is typically 2-5% HNO₃. Therefore, dilution will be required to bring the acid concentration into an acceptable level.

Sample Amount

The sample amount used will be determined by the type of sample that is being analyzed (ie. flower, extract, etc.), or by state regulations. Some factors that may determine the sample amount may include the amount of sample available, the limits required for the sample form, or how easily the sample is digested. Typically, the sample should be large enough to help reduce the sample dilution factor and allow for quantitation by the ICP-MS, but not too large to prevent complete sample digestion.

Dilution factor

The dilution factor is dependent on the sample amount and amount of dilution required to bring the digested sample to an acceptable acid or analysis range.

3. Standard Preparation

Matrix Matching

All samples and standards should be matrix matched. This means that the final acid and additive concentrations of the standards and samples should be the same.

Concentrations

The standard concentrations are determined by looking at the regulated levels and adjusting for the dilution factor. These concentrations will then be included in the calibration curves. For example, a 1ppm target concentration with a dilution of 500 will mean the standard concentration will be 2ppb, and the calibration curve will encompass this concentration.

Internal Standards

Internal Standards should be included for quantitative analysis. Internal standards help adjust for increase or decrease in signal intensity due to various factors affecting signal intensity throughout an analysis. These standards should be chosen such that they are similar in mass to the analytes of interest, do not interfere with any of the analytes of interest, and are not likely to be present in the samples. The concentration of the internal standards should be similar to the levels being analyzed.

4. Other Considerations:

Acids

Trace metals grade nitric acid is commonly used for many ICP analyses, but HCl or other acids may sometime be required depending on the analytes.

Additives

Sometimes additives can be required to help with analysis of certain compounds. For example Hg tends to interact with the glass in the instrument's sample introduction system which will cause a memory effect. Addition of gold in the rinse solution helps disrupt these interactions and can reduce these memory effects.

5. ICP-MS Method Considerations

Element Masses

The state requirements will define the metals that are required for analysis, but within each element there may be multiple possibilities for the masses to be used for analysis. If not defined by state regulations, the element masses for analysis are usually chosen by selecting the highest intensity mass that is not prone to interference from other elements that may be present.

Collision Gas

The ICP-MS uses a collision cell with helium to help prevent diatomic molecules of the same mass as an analyte from passing through the mass spectrometer. As analytes and polyatomic interferences pass through the collision cell, polyatomic molecules will collide more frequently with the helium molecules which will decrease the kinetic energy of these molecules. These are then prevented from entering the quadrupole. During method development the method may be set-up as collision gas on and off for the same element/mass. Provided the detection limits are sufficient, the collision cell gas is typically used.

Method Optimization and Data Analysis:

Once the data is collected the results of each analyte's masses are compared to determine if masses have good isotope ratios and provide expected results. The mass list can be refined based on calibration linearity, y intercept, RSD's, and signal intensity. Select the mass that meets these requirements and has the lowest theoretical detection limit (3s).

Experimental

A method is developed using the outlined method development process. All samples were prepared by ACT Laboratories LLC and analyzed on a Shimadzu ICP-MS 2030. The samples were digested using an Anton Paar Multiwave GO.



Sample Preparation: The method development process was used for analysis of cannabis flower for the current Pennsylvania requirements. The sample was prepared by weighing 0.2 g cannabis flower and digesting it by microwave digestion with 10mL 60% nitric acid containing 1ppm Au. 1mL of the digested solution is then spiked with internal standard and diluted to 15mL with water for a dilution of 150. When accounting for the 0.2 g sample weight, the sample dilution factor becomes 750. With the dilution, the final sample matrix is 4% nitric acid and 0.66 ppm Au.

Spiked samples: Additional spiked samples were prepared as above and spiked prior to digestion for a final concentration of 0.5ppb. These will be used to determine the precision and accuracy of the method.

Standards: Calibration and internal standards were prepared in the sample matrix according to the Table 1.

ICP-MS Analysis: The masses and conditions in Table 2 were selected due to their high isotope ratios. The collision gas is used for all masses, and Y and TI are used as internal standards and associated with the appropriate analytes.

Table 1

Metal	State Regulation (ppm)	Sample Dilution Factor	Adjusted Concentration (ppb)	Standard Calibration levels (ppb)	Internal Standard and Concentration
As	0.4	750	0.533	0, 0.05, 0.1, 0.5, 1.0, 5.0	0.75ppb, Y
Cd	0.3		0.40		0.75ppb, Y
Hg	0.2		0.267		0.75ppb, TI
Pb	1		1.33		0.75ppb, TI

Table 2

Metal	Mass	Collision Gas	Internal Standard	Internal Std Mass
As	75	On	Y	89
Cd	114	On	Y	89
Hg	202	On	TI	205
Pb	208	On	TI	205

As Shown in Table 3, this analysis provided good linear calibration curves for all elements with linearities at 0.99999 and above. The detection and quantitation limits when adjusting for the sample dilution factor were also well below the regulated limits.

Calibration Curves

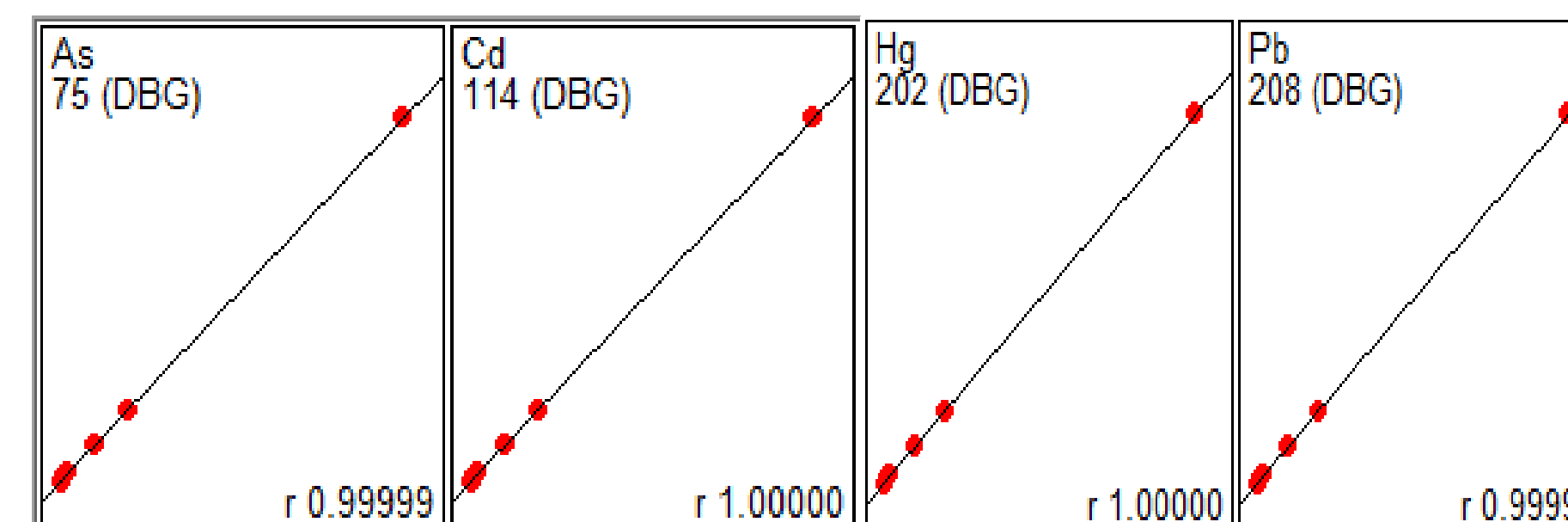


Table 3

Metal	Calibration Linearity	Detection Limit (ppb)	Adjusted Detection Limit (ppb)	Quantitation Limit (ppb)	Adjusted Quantitation Limit (ppb)
As	0.9999	0.00097	0.7275	0.0032	2.4
Cd	1.0000	0.00029	0.2175	0.00096	0.72
Hg	1.0000	0.0027	2.025	0.0091	6.825
Pb	0.9999	0.00098	0.735	0.0033	2.475

The sample along with a 0.5ppb spiked sample were tested to confirm accuracy. The recovery for the spiked sample was between 96.6% and 101.6%, this is shown in Table 4. An additional spiked sample was prepared and run 10 times to confirm precision. Table 5 shows these replicate runs produced standard deviations below 0.01 ppb and relative standard deviations below 2%.

Table 4

Sample	As (ppb)	As Recovery	Cd (ppb)	Cd Recovery	Hg (ppb)	Hg Recovery	Pb (ppb)	Pb Recovery
Unspiked	0.012		0.029		0.13		0.070	
Spiked (0.5ppb)	0.513	100.1%	0.528	99.7%	0.521	101.6%	0.553	96.6%

Table 5

	As	Cd	Hg	Pb
Run 1	0.531	0.555	0.558	0.573
Run 2	0.544	0.568	0.556	0.557
Run 3	0.538	0.565	0.557	0.563
Run 4	0.556	0.574	0.556	0.57
Run 5	0.539	0.567	0.552	0.57
Run 6	0.537	0.571	0.551	0.563
Run 7	0.534	0.562	0.558	0.569
Run 8	0.542	0.564	0.555	0.564
Run 9	0.555	0.577	0.548	0.562
Run 10	0.554	0.576	0.56	0.559
Average	0.543	0.568	0.555	0.565
SD	0.009	0.007	0.004	0.005
%RSD	1.668	1.204	0.666	0.929

Conclusion

There are many factors when developing an ICP-MS method for cannabis analysis. Regulations that vary by state and can change over time make it difficult to create a single method that applies to all testing laboratories. The process detailed here provides a good roadmap for these laboratories to develop a method that is suitable for their required regulations. An example method was developed using this approach providing accurate and repeatable results for this analysis.

References

"Analysis of heavy metal contaminants in cannabis flower using the Shimadzu ICPMS-2030", Shimadzu application note.

Acknowledgements

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