The Big Four Heavy Metals in Cannabis:

Sample Preparation and Analysis via ICP-MS

BY STEPHAN ALTMAIER

The legalization of cannabis in various countries has created a hype around the plant and resulted in the commercialization of cannabis and numerous cannabis-containing products in the food and beverage, cosmetics, and personal care products markets. This development made testing of products for their levels of cannabinoids, pesticides, or heavy metals necessary to prove they are harmless to consumers. This work describes an inductively coupled plasma–mass spectrometry (ICP-MS) method for the analysis of various heavy metals in cannabis sativa plant material. Cannabis is known to accumulate metals in various parts of the plant to a different extent. As a consequence, several protocols for the homogenization of cannabis buds were developed. For comparison, cannabis buds were separated into seeds, stems, and leaves and the plant parts were subjected to ICP-MS analysis.

VARIOUS COUNTRIES HAVE legalized or decriminalized cannabis, thus a new and constantly growing cannabis industry came into being. This industry is marketing a vast amount of products covering numerous customer needs in the area of food and beverages, cosmetics, and personal care products. Dispensaries focus on high tetrahydrocannabinol (≥ 0.3% THC) consumables such as marijuana flowers, capsules, vaping cartridges, tinctures, gummies, chocolates, or soda, while grocery stores offer an extended range of products that contain cannabis with less than 0.3% THC (for example, cereals, cookies, or pasta). In addition, a strong trend towards goods such as oils, creams, or tinctures containing high amounts of cannabidiol (CBD) is visible.

Cannabis is a plant genus that consists of three different species (*Cannabis sativa, indica,* and *ruderalis*—also referred to as strains, varieties, or subspecies). All of these are known to accumulate heavy metals such as arsenic, cadmium, lead, or mercury in parts of the plant (roots, leaves, seeds, and so forth). Due to this ability, cannabis has been used for the remediation of contaminated soil (phytoremediation and phytoextraction) (1–4). On the other hand, this inclination can hinder the use of cannabis in the food or medical industry. As a consequence, all plant material utilized in either food or pharmaceutical products needs to be tested regarding its heavy metal content.

As of October 2020, 24 US states and Canada issued regulations for the testing of heavy metal content in cannabis, and all of them provided limits for arsenic, cadmium, lead, and mercury (those metals are referred to as the "big four"). In addition, several states set limits for one or more of these metals: chromium, barium, silver, selenium, antimony, copper, nickel, and zinc (limits not shown).

Dried cannabis plant material is a very inhomogeneous matter that consists of leaves, buds including resin, stems of various thickness, and seeds. **Figure 1** displays images of cannabis leaves, seeds, and stems from one single bud.

All of these plant parts accumulate heavy metals to a different extent. As it was shown in numerous studies, the heavy metal uptake depends on both the plant part and the element analyzed (1,5,6). In addition, uptake is influenced by external factors such as fertilization and liming (1), ultimately causing an uneven distribution of metals throughout the plant. Hence, if the focus of studies is on the overall

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Figure 1: *Cannabis sativa* "Santhica" separated into (a) leaves, (b) stems, and (c) seeds.

heavy metal content of cannabis, the material needs to be thoroughly homogenized before sample analysis. The recommended process for this sample breakdown is grinding. Various milling techniques exist, differing in their technical complexity: mills (knife, cutting, rotor, or ball mill), mortar and pestle, and rolling pin.

The above-mentioned grinders can be discriminated in terms of their milling speed, feed quantity (and therefore sample representation), and final sample fineness. It can be advantageous to, for example, combine knife mills and ball mills for a quick premilling step for coarse homogenization and subsequent fine grinding, respectively.

No matter what type of grinding method is chosen, because of the sticky, smearing appearance of cannabis resin it is recommended to freeze samples prior to milling. Freezing can either be accomplished at -20 °C in a refrigerator, or by using dry ice (-78 °C) or liquid nitrogen (-196 °C) as a cooling agent.

The setup of any grinder must be performed according to the target analytes. For the analysis of the "big four" heavy metals, stainless steel tools can be used, no matter what type of grinder is chosen for sample preparation. The advantage of this approach is its high speed and often high throughput (high feed quantity). In contrast, if the abundance of additional metals such as Cr or Ni in a sample is of interest, knife mills with titanium blades or mills with grinding tools made of ZrO_2 or polytetrafluoroethylene (PTFE) need to be selected. As these tools are normally smaller in size, the milling process will be more time consuming.

Cannabis seed is the plant part that is most widely used as a food additive, for example, in cereal mixtures, chocolate bars, or pasta. In this case, inductively coupled plasma-mass spectrometry (ICP-MS) analysis of heavy metals must focus on seeds only and a homogenization of the entire plant sample prior to analysis is undesired.

In this work, four different grinding methods were applied for the milling of three different *Cannabis sativa* strains. ICP-MS was used to determine the heavy metal content of the samples and the homogenization efficiencies of all approaches were compared based on the results.

In a second set of experiments one cannabis variety was separated into seeds, leaves, and stems and ICP-MS was utilized to identify possible variations of heavy metal concentrations in the different plant parts.

Experimental

Sample Homogenization and Milling

Three different *Cannabis sativa* varieties ("Finola", "Felina," and "Santhica") purchased from a German drugstore were analyzed. All of them were industrial hemp (according to German regulations THC content <0.2%) and were sold as "hemp flowers" in 25 g batches of dried buds.

The experiments for the analysis of the homogenization efficiency were performed by applying four different grinding methods to each sample. Four aliquots of each variety were prepared and underwent the grinding processes described below. The milling techniques utilized were:

- Rolling pin (RP)
- Mortar and pestle (MP)
- Knife mill (KM)
- Cryo ball mill (CM)

All four procedures are very different in terms of their speed, throughput, and ability to provide homogeneous samples in a reproducible way.

Some vendors of grinding equipment offer mills that allow for permanent cooling of the sample during grinding with (cold) water or liquid nitrogen, for example. The advantage of this setup is it's consistency and the ease of chopping a cold and brittle material. In contrast to other techniques, sample temperature is maintained low during the milling process and loss of sample components by evaporation (such as terpenes) or change of sample composition because of thermal decomposition (such as decarboxylation of cannabidiolic acid [7,8]) can be eliminated. Sample precooling or intermittent grinding are alternative, but comparably tedious procedures.

The detailed proceedings for each of the milling techniques applied are described in the following sections.

Rolling Pin

Approximately 10 g of dried buds were weighed into an airtight zip bag and cooled in a refrigerator at -20 °C for 1 h.

Subsequently the bag was placed on a hard support and cannabis was ground using a wooden household rolling pin. After 3–5 min no further sample breakdown was observed and the process was stopped. Cannabis stems needed to be broken into shorter pieces of approximately 10 mm length by hand. An image of the final sample revealed rather large pieces of stem segments and undamaged seeds in an overall inhomogeneous material (**Figure 2**).

Mortar and Pestle

Approximately 10 g of aliquoted, dried buds were weighed into an airtight zip bag and cooled in a refrigerator at -20 °C for 1 h. Then one or two buds were withdrawn from the bag, placed in a china mortar and ground for 5 min utilizing a porcelain pestle. The resulting coarse powder still contained large pieces of rigid cannabis stems, which needed to be broken by hand to obtain shorter pieces of approximately 10 mm length. The entire process was repeated until a 10 g sample had been prepared. This method leads to a material comparable to the one obtained after rolling pin grinding, but with seeds broken up. Rather large pieces of stem material were visible in the otherwise homogeneous, final sample.

Knife Mill

This mill can be utilized to quickly grind a large sample batch and to produce a representative sample. The resulting sample can subsequently be subjected to cryomilling.

For knife milling, 18 g of cannabis buds were placed in a mill equipped with titanium blades and ground for 30 s at 4000 rpm (one cycle) or 10,000 rpm (two cycles), respectively (**Figure 3**). The first setup resulted in a sample that looked similar to the sample obtained utilizing the rolling pin. It contained large pieces of stem segments and intact seeds. In contrast, the milling process generated a coarse powder comparable to ground coffee beans and no single plant parts could be discriminated any longer.

Figure 2: Cannabis buds "Finola" after a 3 min grinding process using a wooden rolling pin.

(Cryo) Ball Mill

One cannabis bud (approximately 2.5 g) was manually broken into pieces and filled into a 50 mL stainless steel milling beaker. A 25 mm stainless steel milling ball was added and the sealed beaker was mounted to a cryo ball mill equipped with a liquid nitrogen filling system for permanent coolant supply. The grinding parameters were as follows: precooling at 5 Hz, two cycles of 90 s at 30 Hz and 30 s at 5 Hz (for intermediate cooling). This process resulted in a very fine powder with a sample particle size of 100 µm or smaller. Depending on the sample properties, one cycle or two cycles with reduced grinding time can be sufficient to obtain identical results.

As an alternative, for example, 25 mL zirconia milling beakers and 15 mm zirconia milling balls can be utilized. This setup leads to decreased throughput because of limited sample capacity and increased milling times.

Unmilled Samples

For a second set of experiments, the cannabis variety "Santhica" was manually separated into seeds, leaves, and stems to identify possible variations of heavy metal concentrations in the different plant parts (see also Figure 1). These samples were directly subjected to digestion (without a preceding grinding step) and then analyzed by ICP-MS.

Solvents, Reagents, and Preparation of Standard Solutions

All solvents, acids, and reagents were Suprapur or Ultrapur quality and were obtained from MilliporeSigma/Supelco. Ultrapure water was tapped from a Milli-Q IQ 7005 ultrapure water purification system. Single element standards for ICP Certipur and TraceCERT and various certified reference material (CRM) heavy metal mix TraceCERT standard solutions were purchased from MilliporeSigma/Cerilliant.

To compensate for sample matrix effects, a standard addition approach utilizing various CRM heavy metal mix TraceCERT standard solutions was applied for the preparation of all calibration curves. The final calibration curves were comprised of four data points (three standard addition solutions plus sample solution).

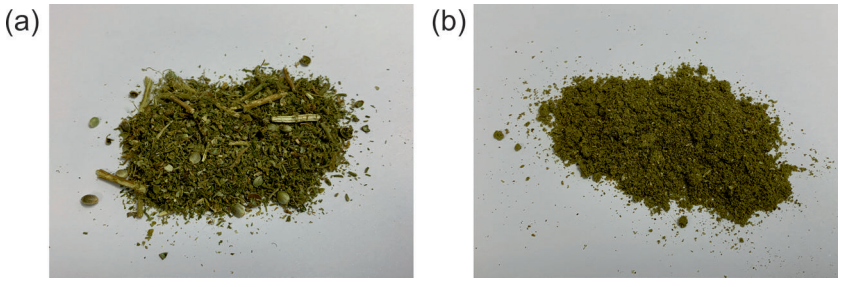
Alternatively, standard solutions were also prepared by using single element standards for ICP Certipur and TraceCERT (data not shown). Indium was utilized as an internal standard in all experiments. For accuracy reasons, the composition of addition solutions 1–3 was adjusted to the heavy metal concentration in each of the three samples. In detail, the standard solutions were prepared as listed in **Table I** (for further information please also see reference 9).

The calibration curves for As, Cd, Hg, and Pb reveal an excellent linearity over the entire calibration range (exemplary for As in **Figure 4**, variety "Finola"). All standard addition solutions were prepared utilizing heavy metal mix TraceCERT VIII standard solution.

Table I: Preparation and composition of all standard solutions utilized in the ICP-MS analysis of cannabis samples

Standard Solutions	Compounds
Indium standard solution, 100 mL	3 mL nitric acid 60%, 1000 µL of indium ICP standard (1000 mg/L), fill up to 100 mL with ultrapure water. Final concentration 10 µg/mL.
Blank solution, 50 mL	3 mL nitric acid 60%, 1 mL H_2O_2 31%; after digestion and fill up to 50 mL with ultrapure water.
Sample solution, 50 mL	50 ±1 mg ground sample, 3 mL nitric acid 60%, 1 mL H_2O_2 31%; after digestion + 50 µL indium standard solution and fill up to 50 mL with ultrapure water.
Addition solutions 1-3, 50 mL each	50 ± 1 mg ground sample, 3 mL nitric acid 60%, 1 mL H_2O_2 31%, different volumes of a CRM heavy metal mix TraceCERT standard solution; after digestion + 50 µL indium standard solution and fill up to 50 mL with ultrapure water. Heavy metal concentrations in final solution 1–20 µg/g for initial sample weight of 50 mg. The resulting sample was subsequently subjected to ICP-MS analysis.

Figure 3: (a) Cannabis buds "Santhica" after grinding in a knife mill for 30 s at 4000 rpm and (b) cannabis buds "Felina" after grinding in a knife mill for 30 s at 10,000 rpm (two cycles).



Equipment

The following equipment was used:
- Microwave digestion system turboWAVE, MLS (Germany)
- ThermoFisher Element 2 or Element XR

Digestion and ICP-MS Conditions

The conditions applied for sample digestion and ICP-MS analysis are shown in **Table II**.

Results and Discussion

The recovery rates for the big four heavy metals are listed in **Table III**. The determined recovery rates for all heavy metals were excellent and within the range of ±10%.

Milling Methods

The heavy metal contents of three cannabis varieties, that were subjected to the different milling processes, are listed in **Table IV**.

The mercury content of all samples was below the limit of detection (LOD), and only one out of 14 samples displayed a cadmium level above the detection limit (0.1 µg/g). For arsenic the results were similar, with five samples containing As close to the LOD (0.1 µg/g). The findings for lead were a bit different, and detected concentrations ranged from 0.3 to 1.0 µg/g. It is noteworthy, that these values do, in part, exceed the limits of various US states and Canada by a factor of 2–3 (depending on the intended use). The analysis of the "big four" elements was reproducible and except for one deviation (Pb content of cannabis "Felina" ground with mortar and pestle) the milling technique did not affect the detected heavy metal concentrations.

Cryo milling was performed utilizing stainless steel equipment and resulted in the detection of elevated levels of chromium in all cryo-ground samples. Though nickel is also a content of stainless steel alloys, increased amounts (compared to rolling pin and mortar and pestle milling) were only found in the cannabis "Finola" sample. The cause of this result can be a difference in grinding time. Some US states (as of now MI, MD, MO, and NY) issued regulations that make the analysis of chromium in cannabis necessary. In this case, it is essential to utilize a cryo milling approach and zirconia or PTFE grinding equipment to avoid sample contamination. In contrast to cryo milling, knife milling was performed using titanium blades and therefore did not affect the Cr (and Ni) content of cannabis samples.

Plant Part Analysis

The heavy metal content of stems, seeds, and leaves of the *Cannabis sativa* variety "Santhica" and the respective recovery rates are listed in **Table V**. All results except the lead content of seeds are in line with the data shown in the previous section. This finding corresponds to results published in various publications, that also reported the Pb concentration in seeds being lower than in other cannabis plant parts such as leaves, stems, flowers, or roots (5,6).

Conclusion

This work demonstrates a comprehensive ICP-MS workflow, using the

Table II: Conditions for sample digestion and ICP-MS analysis

Digestion				
Digestion program	Nitric acid digestion at 280 °C			
Microwave vial	Quartz glass			
Basic load	110 mL ultrapure water and 5 mL nitric acid or 115 mL ultrapure water			
Charging pressure	40 bar			
Deflation rate	5 bar/min (from T < 80 °C)			
Vessel cooling	Yes (> 40 °C)			
Program Parameters				
Time (h)	Microwave Output (W)	Temperature 1 (°C)	Temperature 2 (°C)	Pressure (bar)
00:03:00	700	70	60	100
00:15:00	1000	180	60	120
00:30:00	1200	280	60	120
After digestion the obtained solution was clear and particle free. Above-mentioned conditions can be adapted in order to enable complete sample digestion.				
ICP-MS Conditions				
Plasma output	Approx. 1300 W			
Plasma gas flow	Approx. 16 L/min			
Assist gas flow	Approx. 1 L/min			
Nebulizer gas flow	Approx. 1 L/min			
Sample delivery	Peristaltic pump (or equivalent), delivery volume approx. 1 mL/min			
Nebulizer	Quartz spray chamber (or equivalent) / Meinhardt nebulizer (or equivalent)			
Mass resolution	4000 + 10000			
Calibration	Standard addition			
The analysis was performed in the sequence: Blank, sample 1 – x, additions.				

Table III: Recovery rates for three cannabis varieties (RP and MP grinding was applied to each sample) using CRM Heavy Metal Mix TraceCERT standard solutions I, II, or III

Element	Cannabis Finola		Cannabis Santhica		Cannabis Felina	
	RP Mix I (%)	MP Mix I (%)	RP Mix II (%)	MP Mix II (%)	RP Mix III (%)	MP Mix III (%)
As	104	102	98	99	104	98
Cd	98	97	99	94	102	100
Hg	90	91	108	110	100	97
Pb	94	95	96	102	98	99

standard addition calibration method, for the determination of heavy metals in *Cannabis sativa* plant materials. Critical elements in the process include homogenization of samples and use of accurate traceable CRM mixes, that are tailored to state specific regulations for heavy metals in cannabis. Reproducible samples were prepared by grinding cannabis with different mill types and techniques. Samples were then digested utilizing a specific digestion protocol optimized to provide clear digestion solutions. The resulting solutions were subjected to ICP-MS analysis.

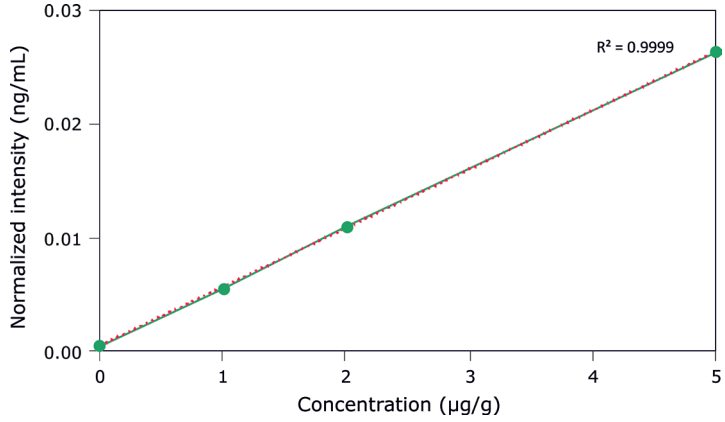
Table IV: Heavy metal content of three cannabis varieties as determined by ICP-MS. Four different grinding procedures were applied. Roman figures indicate the use of a specific CRM Heavy Metal Mix TraceCERT standard solution (III to VIII) for the preparation of respective addition solutions.

Element	Cannabis Finola				Cannabis Santhica					Cannabis Felina				
	RP (µg/g)	MP (µg/g)	CM-VII (µg/g)	CM-VIII (µg/g)	RP (µg/g)	MP (µg/g)	KM (µg/g)	CM-V (µg/g)	CM-VI (µg/g)	RP (µg/g)	MP (µg/g)	KM (µg/g)	CM-III (µg/g)	CM-IV (µg/g)
As	0.1	0.1	0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cd	< 0.1	< 0.1	< 0.1	<0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cr	0.3	0.4	12	12	0.2	0.3	0.3	3.6	2.5	0.3	0.5	0.6	3.7	4.3
Hg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Ni	0.8	1.1	2.0	2.1	0.4	1.7	0.4	0.7	0.6	0.5	0.6	0.6	0.7	0.7
Pb	0.6	0.7	0.7	0.7	0.3	0.5	0.5	0.4	0.4	0.5	1.0	0.5	0.5	0.5

Table V: Heavy metal content of cannabis "Santhica" stems, seeds, and leaves as determined by ICP-MS (duplicate determination) and respective recovery rates (RR) using CRM heavy metal mix TraceCERT standard solution II. No grinding was performed prior to digestion.

Element	Stems			Seeds			Leaves		
	#1 (µg/g)	#2 (µg/g)	RR (%)	#1 (µg/g)	#2 (µg/g)	RR (%)	#1 (µg/g)	#2 (µg/g)	RR (%)
As	< 0.1	< 0.1	99	< 0.1	< 0.1	96	< 0.1	< 0.1	99
Cd	< 0.1	< 0.1	100	< 0.1	< 0.1	98	< 0.1	< 0.1	102
Hg	< 0.1	< 0.1	96	< 0.1	< 0.1	103	< 0.1	< 0.1	100
Pb	0.4	0.4	97	< 0.1	< 0.1	101	0.4	0.4	99

Figure 4: Exemplary calibration data for arsenic and cannabis buds "Finola" utilizing heavy metal mix VIII.



Calibration data was obtained by the preparation and analysis of standard addition solutions obtained by diluting various different heavy metal CRM mixes containing arsenic, cadmium, lead, and mercury. The final results were consistent for all samples and revealed an As, Cd, and Hg concentration of <0.1–0.1 µg/g. The detected lead content of the three cannabis varieties ranged from 0.3–1.0 µg/g.

References

(1) M. Griga and M. Bjelkova, in *Plant-Based Remediation Processes*, D.K. Gupta, Ed. (Springer, Berlin, 2013), pp. 199-238.

(2) P. Linger, A. Ostwald, and J. Haensler, *Biol. Plant.* **49**, 567–576 (2005).

(3) R. Ahmad, Z. Tehsin, S.T. Malik, S.A. Asad, M. Shahzad, M. Bilal, M.M. Shah, and S.A. Khan, *Clean Soil Air Water* **44**, 195-201 (2016).

(4) G. Shi, C. Liu, M. Cai, Y. Ma, and Q. Cai, *Appl. Biochem. Biotechnol.* **168**, 163-173 (2012).

(5) V. Angelova, R. Ivanova, V. Delibaltova, and K. Ivanov, *Ind. Crops Prod.* **19**, 197–205 (2004).

(6) L.O. Eboh and B.E. Thomas, *Pak. J. Nutr.* **4**, 349–351 (2005).

(7) M. Taschwer and M.G. Schmid, *Forensic Sci. Int.* **254**, 167–171 (2015).

(8) C. Citti, B. Pacchetti, M.A. Vandelli, F. Forni, and G.Cannazza, *J. Pharm. Biomed. Anal.* **149**, 532–540 (2018).

(9) SigmaAldrich.com/Cannabis.

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