

Article - Engineering, Technology and Techniques

Method Validation Analysis of Toxic Elements As, Cd, Pb and Hg in Cannabidiol (CBD)-rich Oil by Inductively Coupled Plasma Mass Spectrometry

Eder José dos Santos^{1*}

<https://orcid.org/0000-0001-8202-9285>

Amanda Beatriz Herrmann¹

<https://orcid.org/0000-0002-1928-2699>

¹Instituto de Tecnologia do Paraná (Tecpar), Curitiba, PR, Brasil

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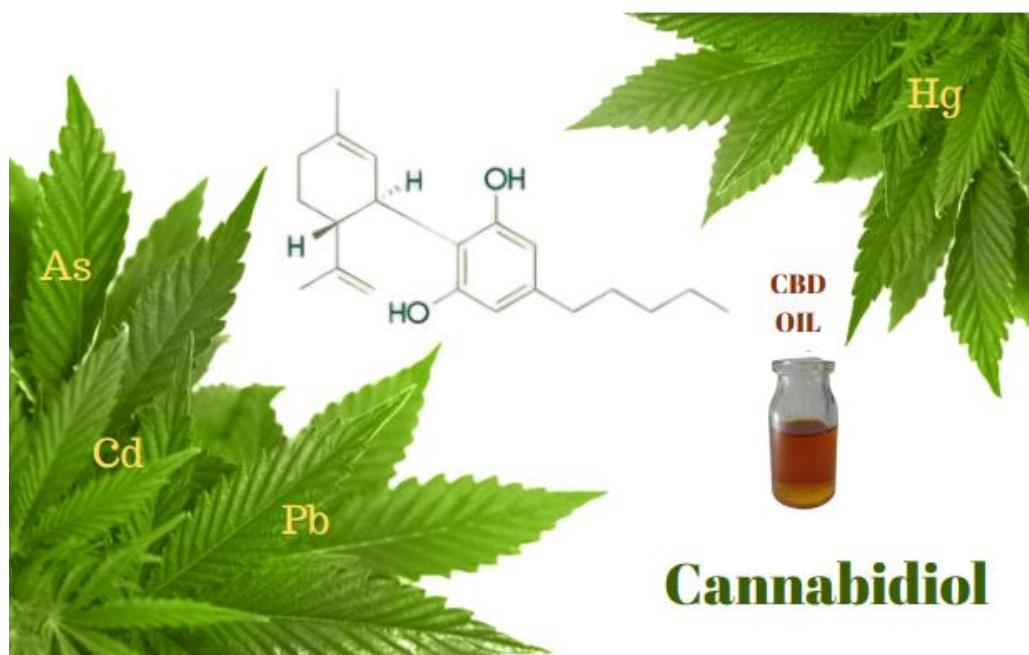
*Correspondence: eder@tecpar.br; Tel.: +55-41-33163100 (E.J.S.).

HIGHLIGHTS

- A robust method was developed for detection of As, Cd, Pb and Hg in Cannabidiol (CBD)-rich oil.
- Instrumental conditions were investigated by ICP-MS to selectively determine the toxic elements in Cannabidiol (CBD)-rich oil.
- Method validation demonstrated accuracy and precision.

Abstract: The development of a method and its validation for determination of toxic elements As, Cd, Pb and Hg in Cannabidiol-rich oil is presented. The samples, 500 mg, were initially treated with 5 mL HNO₃ and 4 mL H₂O₂ followed by digestion in a microwave system. The clean solutions were subsequently diluted with deionized water and the analytes quantitated by inductively coupled plasma mass spectrometry (ICP-MS). Instrumental conditions were carefully evaluated, including the use of correction equations and He collisional gas (KED) to avoid/correct for possible interferences such as ⁴⁰Ar³⁵Cl affecting ⁷⁵As. Limits of quantitation (LOQs, calculated from replicate measurements of method blanks) were: 0.01 µg g⁻¹ (As), 0.001 µg g⁻¹ (Cd and Pb), and 0.0005 µg g⁻¹ (Hg) based on processing 500 mg subsamples/50 mL. The accuracy of the method was confirmed by spiking the Cannabidiol-rich oil sample at different concentration levels and determining recoveries with acceptable results under repeatability and intermediate precision conditions. Eight commercially available formulations were analyzed and only one showed 0.14 µg g⁻¹ Pb. The present fit-for-purpose analytical method is robust and precise and was adopted for use by the Tecpar's laboratory of the Parana Institute of Technology (Curitiba, Brazil).

Keywords: Cannabidiol oil; toxic elements; ICP-MS; As, Cd, Pb and Hg.

GRAPHICAL ABSTRACT:**INTRODUCTION**

The use of *Cannabis sativa* to produce industrial hemp, Cannabidiol (CBD)-rich oil and medical marijuana has grown significantly in recent years. Research concludes that the use of CBD-rich oil has shown encouraging results in the treatment of various diseases. An example is its use for childhood epilepsy as an alternative to traditional treatment based on antiepileptic drugs to achieve seizure-freedom [1]. Other promising results have also been obtained when treating Parkinson and Alzheimer diseases in addition to offering therapeutic potential for treatment of multiple sclerosis, anxiety, depression, cancer and diabetes, amongst others [2]. Since 2015, The National Health Surveillance Agency (ANVISA) has authorized the import of cannabis-derived products to Brazil. The most common forms of therapeutic administration are oils, ointments, extracts and even medications (some already available in pharmacies) [3,4]. *Cannabis sativa* cultivars, including hemp, are known to accumulate heavy metals such as arsenic, cadmium, lead and mercury and may remain during the process of preparing CBD extracts used for medicinal purposes [5,6]. Contamination by these and other toxic elements can have numerous origins, including environmental pollution from water, air and pesticide and fertilizer use as well as their naturally occurring presence in soil [6,7]. Human exposure to these metals is highlighted in the literature and is mainly associated with an increased risk of cancer and cardiopulmonary diseases; sources include cannabis products [8-10]. In this context, the development of analytical methods capable of identifying and quantifying these contaminants with precision, accuracy and adequate sensitivity is important to help ensure the quality of these ever more popular medicines, especially oil rich in CBD. Atomic spectrometric techniques such atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and ICP mass spectrometry (ICP-MS) are among the most used when dealing with elements at trace level concentrations [11]. Specifically, in the context of metal contaminants in Cannabis products, several studies are available in the literature, for example, the full interlaboratory study organized by the NIST Cannabis Laboratory Quality Assurance Program (CannaQAP) in 2022 [12]. ICP-MS techniques have become state-of-the art for cannabis testing. Standard test methods for analysis of multiple elements in marijuana and cannabis are described by the New Jersey Department of Health [13] and more recently, by ASTM D8469 [14] and include “The Big Four Heavy Metals” in Cannabis: As, Cd, Pb and Hg. For this purpose, most approaches, including those mentioned in the above standards, recommend processing samples using a microwave digestion system and quantifying the isotopes ^{75}As , ^{111}Cd , ^{208}Pb (or the sum of the three most abundant isotopes, ^{206}Pb , ^{207}Pb and ^{208}Pb) and ^{202}Hg [5,12-20]. The objective of the present work was to develop and validate a method for use in the Tecpar laboratory employing ICP-MS with automatic and rapid delivery of samples, as well as investigate the presence of As, Cd, Pb and Hg in CBD-rich oil in samples from local pharmacies in the Curitiba-PR region representing products from different authorized pharmaceutical producers.

MATERIAL AND METHODS

Instrumentation

Measurements were conducted using a PerkinElmer ICP-MS spectrometer (NexION 2000B) equipped with a S20 series autosampler and a vacuum-based, valve-driven HTS (High Throughput System) that is fully integrated with the software and quickly delivers sample to the plasma. The HTS was configured with a 1000 μL sample loop (1.0 mm ID), 0.75 mm ID pump tubing for de-ionized water carrier, 0.75 mm ID tubing for internal standard solution and 1.5 mm tubing for waste. The experimental conditions are summarized in Table 1. Argon of 99.999% purity was supplied by White Martins (São Paulo, Brazil).

Table 1. ICP-MS operating parameters.

Parameters	Specifications
Radiofrequency	24 MHz
Radiofrequency power	1600 W
Plasma gas flow rate	15.0 L min ⁻¹
Auxiliary gas flow rate	1.2 L min ⁻¹
Nebulizer gas flow rate	0.94 L min ⁻¹
Nebulizer type	Meinhard® glass concentric nebulizer
Spray chamber	Glass cyclonic
Peltier temperature (spray chamber)	3°C
Replicates	5
Torch type	Standard NexION quartz torch with 2.0 mm i.d. injector
Sample introduction system	PerkinElmer® HTS with autosampler S20 series
Collision Helium cell gas flow (KED)	3.5 mL min ⁻¹
Cell entrance voltage (KED)	-8 V
Cell exit voltage (KED)	-25 V
Isotopes of the monitored analytes	⁷⁵ As, ¹¹¹ Cd, ^{206,207,208} Pb, ^{200,201,202} Hg
Internal standard Isotope	¹¹⁵ In (95.7 % abundance)

An Ethos Easy microwave digestion system equipped with a model SK-10 high pressure segmented rotor (Milestone, Sorisole, Bergamo, Italy) was employed for sample preparation. A Shimadzu model AUW220D analytical balance (Kyoto, Japan) and hydrophilic PVDF 0.45 μm membrane filters (Millipore Millex, USA) were used during sample preparation.

Reagents and materials

All chemicals were of minimum analytical grade. High-purity water (18.2 M Ω cm resistivity) was de-ionized in a Milli-Q® IQ 7005 system (Bedford, MA, USA). The following reagents and standards were used: 65% HNO₃ distilled (Merck, Darmstadt, Germany), 30% H₂O₂ (Merck), NaCl (Merck) for preparation of a 1000 $\mu\text{g mL}^{-1}$ Cl⁻ standard solution; multielement standard stock solution containing 100 $\mu\text{g mL}^{-1}$ As, Cd and Pb from AccuStandard (New Haven, USA): Cannabis Metals Environmental Spike Mix (CP-MET-SPIKE-1) and a standard stock solution containing 1000 $\mu\text{g mL}^{-1}$ Hg also from AccuStandard: Cannabis Metals Mercury (CP-MET-HG-1) were used to prepare solutions for external calibration.

Samples and preparation procedure

Eight samples of CBD-rich oil, obtained from local pharmacies and representing products from different authorized pharmaceutical producers, were used in the present work. A nominal 500 mg of each sample was accurately weighed and placed into a microwave digestion vessel, to which 5 mL of HNO₃ (65%) and 4 mL of H₂O₂ (30%) were added and mixed well. The mixture was kept for 4h at room temperature with the vessels loosely capped to prevent any pressurization arising from any pre-reactions to safety occur. After securely capping, the samples were digested using the program outlined in Table 2.

Table 2. Microwave digester operating parameters.

Step	Ramp (min)	Target Temp (°C)	Hold (min)	Power (W)
1	20	200	--	1800
2	--	200	30	1800
3	--	cooling	10	--

The digestates were visually inspected for any undigested suspended particles. If present, they were filtered through a 0.45 μm filter before transferring the sample to 50 mL volumetric flasks and diluting to a final volume of 50 mL with high purity water. Blank solutions were run in parallel to the determinations and their results taken into consideration.

Calibration curve procedure

Multielement calibration standards were prepared by appropriate dilutions of an intermediate standard solution containing 0.50 $\mu\text{g mL}^{-1}$ As, Cd and Pb and 0.050 $\mu\text{g mL}^{-1}$ Hg to span the range of 0.50 to 10.0 $\mu\text{g L}^{-1}$ of As, Cd and Pb and 0.050 to 1.0 $\mu\text{g L}^{-1}$ of Hg, as summarized in Table 3.

Table 3. Calibration standard concentrations of analytes.

Analyte	Standard 1 ($\mu\text{g L}^{-1}$)	Standard 2 ($\mu\text{g L}^{-1}$)	Standard 3 ($\mu\text{g L}^{-1}$)	Standard 4 ($\mu\text{g L}^{-1}$)	Standard 5 ($\mu\text{g L}^{-1}$)
As	0.50	1.0	2.0	5.0	10.0
Cd	0.50	1.0	2.0	5.0	10.0
Pb	0.50	1.0	2.0	5.0	10.0
Hg	0.050	0.10	0.20	0.50	1.0

In parallel, an indium internal standard solution of 1.0 $\mu\text{g L}^{-1}$ was prepared and was added on-line.

Validation protocol

The validation process is based on method performance requirements prescribed in AOAC SMPR®2020.001 [21], USP General Chapter <233> [22] commonly used for evaluation of the levels of “heavy metals” in a variety of Cannabis and Cannabis-derived products, as well as those developed by INMETRO [23]. The following requirements were used to support validation: linearity; limit of detection (LOD); limit of quantification (LOQ); accuracy: repeatability and intermediate precision.

RESULTS AND DISCUSSION

Selection of suitable isotopes and instrumental conditions

The determination of metals by ICP-MS is generally subject to interferences. Those associated with sample introduction using a nebulizer and spray chamber are common and termed transport, physical or non-spectral interferences. Since these interferences can influence all elements, internal standard correction was used to effectively compensate for any signal changes. In the present study, ^{115}In was selected for this purpose [14,24]. The most severe interferences are typically polyatomic or spectral interferences due to molecular ions of the same nominal m/z as the analytes or in some cases derived from direct isobaric spectral overlaps from concomitant elemental isotopes. Table 4 summarizes some possible polyatomic ion interferences that are derived from plasma gas species, sample matrix and/or reagents that may impact the analytes of interest in this study [14,17,18,24].

Table 4. Possible polyatomic interferences in typical Cannabis matrix samples.

Analyte	Polyatomic ion interferences
^{75}As	$^{40}\text{Ar}^{35}\text{Cl}$, $^{38}\text{Ar}^{37}\text{Cl}$, $^{36}\text{Ar}^{39}\text{K}$, $^{36}\text{Ar}^{38}\text{ArH}$, $^{40}\text{Ar}^{34}\text{SH}$, $^{40}\text{Ca}^{35}\text{Cl}$, $^{37}\text{Cl}_2\text{H}$, $^{59}\text{Co}^{16}\text{O}$,
^{111}Cd	$^{95}\text{Mo}^{16}\text{O}$, $^{94}\text{Zr}^{16}\text{OH}$, $^{39}\text{K}_2^{16}\text{O}_2\text{H}$
$^{206,207,208}\text{Pb}$	$^{190}\text{Pt}^{16}\text{O}$, $^{191}\text{Ir}^{16}\text{O}$, $^{192}\text{Pt}^{16}\text{O}$
$^{200,201,202}\text{Hg}$	-----

During daily performance monitoring of the ICP-MS instrument, the presence of interferences related to oxide ions such $^{59}\text{Co}^{16}\text{O}$, $^{95}\text{Mo}^{16}\text{O}$, $^{94}\text{Zr}^{16}\text{OH}$, $^{39}\text{K}_2^{16}\text{O}_2\text{H}$, $^{190}\text{Pt}^{16}\text{O}$, $^{191}\text{Ir}^{16}\text{O}$ and $^{192}\text{Pt}^{16}\text{O}$ was mirrored by the magnitude of the $^{156}\text{CeO}/^{140}\text{Ce}$ intensity ratio, which reflects the plasma's ability to dissociate the strongly bound Ce-O molecule, thereby providing a good indicator of the possible presence of other oxide molecular ions. In the present study, $^{156}\text{CeO}/^{140}\text{Ce}$ was typically around 3% [14,24]. It is evident from Table 4 that ^{75}As is the isotope that is most susceptible to polyatomic ion interferences, with $^{40}\text{Ar}^{35}\text{Cl}$ and $^{40}\text{Ca}^{35}\text{Cl}$ being the most likely to interfere since Cl and Ca are common elements in many environmental samples. Strategies to minimize and/or eliminate these interferences involve a variety of techniques, including use of a

thermoelectric (Peltier) cooled spray chamber, correction equations and/or collision/reaction cell (CRC) technology.

The use of a cooled spray chamber reduces water vapor loading in the plasma and signal drift caused by any temperature fluctuations, resulting in a hotter plasma which improves matrix tolerance, reducing the formation of oxides and other matrix-based polyatomic ion overlaps, increasing the degree of ionization and enhancing signal intensities. All experiments were conducted with the Peltier cooled spray chamber at a temperature of 3°C, following the manufacturer's recommendations.

The use of "correction equations" relies on being able to determine the signal intensity of an alternative isotope of the interfering element and subtracting its abundance corrected intensity from the analyte isotope signal. Thus, in the presence of high chloride concentrations, use of $^{40}\text{Ar}^{37}\text{Cl}$ intensity at mass 77 to calculate a correction for the presence of $^{40}\text{Ar}^{35}\text{Cl}$ at mass 75 As can be undertaken based on the isotopes of chlorine [25]. In this way, the corrected ^{75}As intensity = total intensity (75) – 3.127*(mass 77 – (0.874*mass 82)). For Pb, the sum of its 206, 207 and 208 isotopes were used. Similarly, for Hg the intensities of its 200, 201 and 202 isotopes were summed. The single ^{111}Cd isotope could be monitored without corrections [14-18].

Figures 1 (a) and (b) show the interference arising from increasing concentration of Cl in a simple solution of reagent blank and standard solution of $10\text{ }\mu\text{g L}^{-1}$ As, as well as the result of the application of the correction equation to eliminate the interference generated by $^{75}\text{ArCl}^+$ on the determination of $^{75}\text{As}^+$.

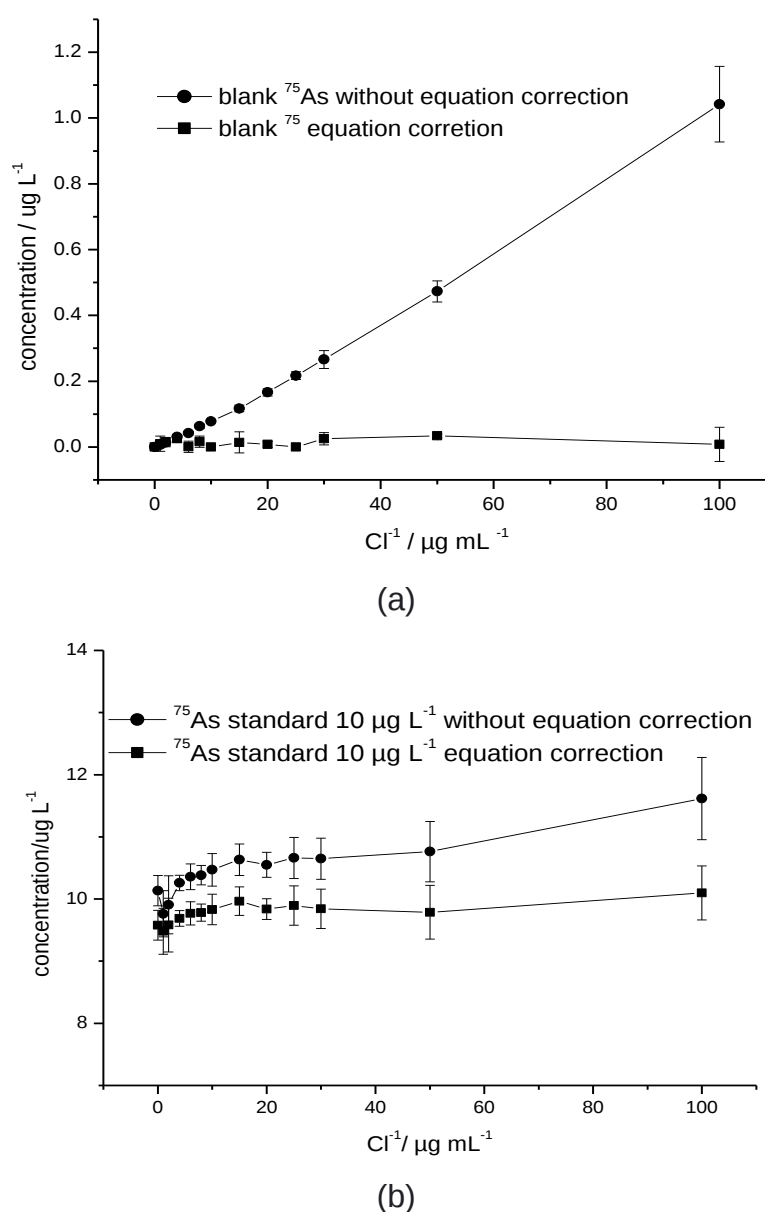


Figure 1. Influence of chloride on the determination of ^{75}As : (a) blank solution (HNO_3 10% v/v) spiked with 0 to $100\text{ }\mu\text{g mL}^{-1}$ Cl^{-1} with and without correction equation; (b) standard solution of $10\text{ }\mu\text{g L}^{-1}$ As in 10 % v/v HNO_3 spiked with 0 to $100\text{ }\mu\text{g mL}^{-1}$ Cl^{-1} with and without correction equation. Error bars represent standard deviation of three replicate measurements.

It is observed that beyond only a few $\mu\text{g mL}^{-1}$ Cl^{-1} does the interference of $^{75}\text{ArCl}^{+}$ on the determination of ^{75}As effectively begin; however, it is also evident that the simple mathematical correction procedure allows for the precise determination of ^{75}As . Titrimetric analysis results for Cl^{-1} (Mohr method) revealed that it was present at a level of 2 mg L^{-1} in all commercial CBD-rich oil samples used in this work. Accounting for sample preparation ($500 \text{ mg} / 50 \text{ mL}$), it can be concluded that Cl^{-1} is not present at levels that cause this type of interference. However, higher levels of Cl^{-1} may be encountered with other Cannabis matrices (hemp, flower and cannabis-related products) and for this reason the developed procedure was selected as the most suitable for the determination of As in the CBD-rich oil samples.

CRC technology employing various gases (typically He) in combination with kinetic energy discrimination (KED) or use of reactive gases such as hydrogen, ammonia and/or oxygen in dynamic reaction mode (DRC) technology are the most frequently used with ICP-MS. Specifically, He has been the most commonly used for method development studies involving Cannabis and its products [14-18]. In the present work, a CRC approach was undertaken, starting with the optimization of He flow rate. Figure 2 presents results for optimization of He gas flow rate acquired during introduction of a solution containing $100 \mu\text{g mL}^{-1}$ Cl^{-1} and a standard solution of $10 \mu\text{g L}^{-1}$ As, both in 10% v/v HNO_3 .

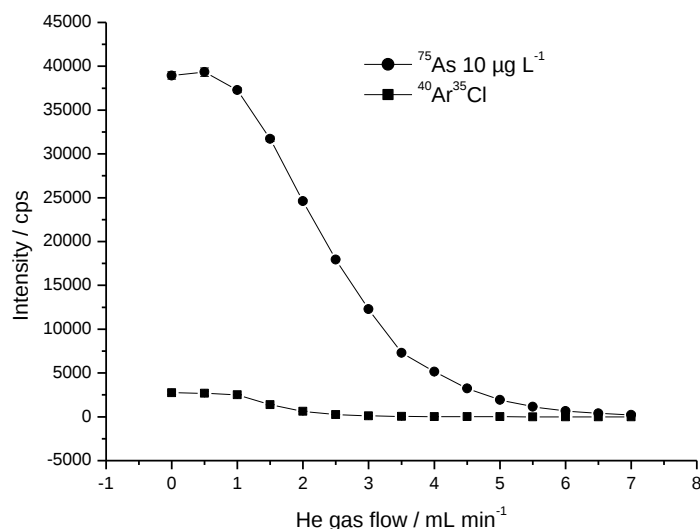
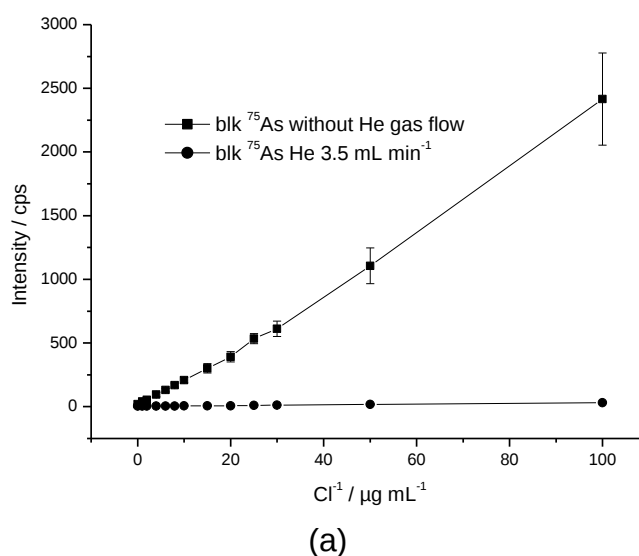


Figure 2. Impact of He gas flow on CRC optimization for introduction of a solution of $100 \mu\text{g mL}^{-1}$ Cl^{-1} in 10% v/v HNO_3 and standard solution of $10 \mu\text{g L}^{-1}$ As in 10% v/v HNO_3 . Error bars represent standard deviation of three replicate measurements.

The influence of He collision gas on the interference reveals not only attenuation of the polyatomic ion intensity, but also that of the analyte ion. It is evident that a flow of 3.5 mL min^{-1} He generates a constant intensity for the polyatomic anion. This flow rate was adopted for subsequent experiments.

Figures 3 (a) and (b) show the impact of increasing concentrations of Cl^{-1} in solutions of both a reagent blank and standard containing $10 \mu\text{g L}^{-1}$ As using a flow of 3.5 mL min^{-1} He.



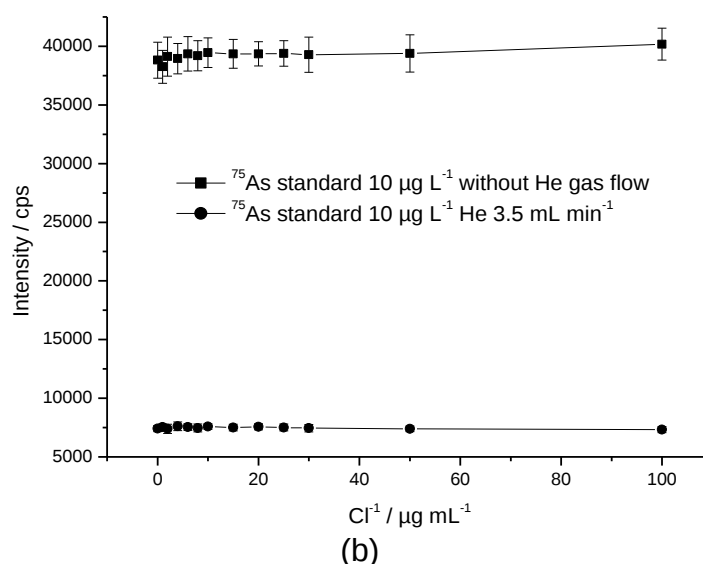


Figure 3. Study of the influence of the Cl^- ion on the determination of ^{75}As : (a) blank solution (10% v/v HNO_3) spiked with 0 to 100 $\mu\text{g mL}^{-1}$ Cl^- with and without He collisional gas at 3.5 mL/min; (b) standard solution of 10 $\mu\text{g L}^{-1}$ As in 10 % v/v HNO_3 spiked with 0 to 100 $\mu\text{g mL}^{-1}$ Cl^- with and without use of He collision gas. Error bars represent standard deviation of three replicate measurements.

The He CRC approach eliminates interference generated by $^{75}\text{ArCl}^+$ on the determination of $^{75}\text{As}^+$, but significantly decreases the sensitivity for ^{75}As . This instrumental procedure can be used as an alternative method for the determination of As, since the He CRC approach was used only for this analyte, especially for such samples containing high concentrations of chlorides.

Figures of merit: linearity and LOD/LOQ

As noted in Table 1, multiple isotopes were monitored for Pb (at m/z 206, 207 and 208) and Hg (at m/z 200, 201 and, 202). For these two elements, responses from all three isotopes were summed, whereas for Cd only m/z 111 was monitored and, of course, As is monoisotopic. Correlation coefficients > 0.999 characterize the linear portions of all calibration functions spanning 0.50 - 10.0 $\mu\text{g L}^{-1}$ As, Cd, Pb and 0.050-1.00 $\mu\text{g L}^{-1}$ Hg, in a 10% w/v HNO_3 demonstrating adequate linearity. Accounting for sample dilution (500 mg / 50 mL), limits of detection (LOD), defined as 3 times the standard deviation of 10 replicate measurements of the method blank divided by the slope of the calibration function, and limits of quantification (LOQ), defined as 10 times the standard deviation of 10 replicate measurements of the method blank divided by the slope of the calibration function, are summarized in Table 5 [14,21-23,26].

Table 5. Regression coefficients, limits of detection and quantification of analytes.

Analyte	Linear regression coefficients	Slope ($\mu\text{g g}^{-1})^{-1}$ cps $^{-1}$	Limit of detection* $\mu\text{g g}^{-1}$	Limit of quantification* $\mu\text{g g}^{-1}$
^{75}As	1.000	3023	0.003	0.01
^{111}Cd	0.999	4134	0.0002	0.001
206,207,208Pb**	1.000	56517	0.0005	0.001
200,201,202Hg***	0.999	9232	0.0001	0.0005

* accounting for sample preparation 500 mg / 50 mL.

** Pb results were based on the sum of the signals measured at mass 206, 207, and 208

*** Hg results were based on the sum of the signals measured at mass 200, 201, and 202

To achieve the lowest LOQ performance, characterized by method blanks, requires careful attention to operating conditions and the highest level of quality control. In this context, fit-for-purpose conditions specified by AOAC SMPR®2020.001 [21] defines limit of quantification as a “minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result”. For this purpose, seven replicates of the CBD-rich oil sample were spiked at the time of weighing with concentrations of each analyte close to the experimentally obtained LOQ characterizing the method of sample preparation and quantification by ICP-MS. The results obtained are presented in Table 6, which also summarizes legislated upper limits for the analytes in Cannabis Flower.

Table 6. Spiked element in a sample of oil rich in CBD at quantification limit levels (n=7).

Analyte	Nominal Spiked Conc. $\mu\text{g g}^{-1}$	Conc. Obtained * $\mu\text{g g}^{-1}$	RSD, %	Recovery, %	Limits from EU Pharm** $\mu\text{g g}^{-1}$ maximum
⁷⁵ As	0.02	0.022 $\pm$ 0.001	5.1	110	0.2
¹¹¹ Cd	0.005	0.0051 $\pm$ 0.0001	2.7	102	0.3
^{206,207,208} Pb	0.02	0.021 $\pm$ (0.0004)	1.9	105	0.5
^{200,201,202} Hg	0.002	0.0021 $\pm$ (0.0001)	6.1	105	0.1

*accounting for sample preparation 500 mg / 50 mL.

**European Pharmacopeia, supplement 11.5: Cannabis Flower, 2024 [27].

The values obtained show excellent precision and accuracy for spike recovery, demonstrating the technique's ability to detect these toxic elements at low concentration levels. Calibration linearity and the LOQs are fit-for-purpose for the determination of these analytes in the CBD-rich oil sample as they meet the limits set by the European Pharmacopeia [27].

Accuracy: repeatability and intermediate precision

Since certified reference materials for this type of sample do not exist, twenty-one replicates from a CBD-rich oil sample were digested after being spiked with low-, mid- and high-level concentrations of each analyte. Spikes encompassed the linear working range of the method. The samples were then analyzed under conditions of repeatability. The intermediate precision was performed under the same conditions of repeatability but on a different day with a different analyst. The results were combined with the repeatability analysis, so the total number of analyses was 14. Results are presented in Table 7.

Table 7. Repeatability and intermediate precision achieved with CBD-rich oil samples.

Analyte	Repeatability conditions (n=7)			Intermediate precision conditions (n=14)*		
	0.050 $\mu\text{g g}^{-1}$ 0.0050 $\mu\text{g g}^{-1}$ (Hg)	0.50 $\mu\text{g g}^{-1}$ 0.050 $\mu\text{g g}^{-1}$ (Hg)	1.00 $\mu\text{g g}^{-1}$ 0.10 $\mu\text{g g}^{-1}$ (Hg)	0.050 $\mu\text{g g}^{-1}$ 0.0050 $\mu\text{g g}^{-1}$ (Hg)	0.50 $\mu\text{g g}^{-1}$ 0.050 $\mu\text{g g}^{-1}$ (Hg)	1.00 $\mu\text{g g}^{-1}$ 0.10 $\mu\text{g g}^{-1}$ (Hg)
As	0.053 $\pm$ 0.004	0.51 $\pm$ 0.03	1.10 $\pm$ 0.06	0.057 $\pm$ 0.004	0.52 $\pm$ 0.02	1.15 $\pm$ 0.07
Cd	0.047 $\pm$ 0.001	0.48 $\pm$ 0.02	0.96 $\pm$ 0.03	0.047 $\pm$ 0.001	0.48 $\pm$ 0.01	0.94 $\pm$ 0.03
Pb	0.055 $\pm$ 0.005	0.46 $\pm$ 0.01	0.92 $\pm$ 0.03	0.048 $\pm$ 0.008	0.47 $\pm$ 0.02	0.96 $\pm$ 0.05
Hg	0.0046 $\pm$ 0.0003	0.051 $\pm$ 0.001	0.096 $\pm$ 0.006	0.0041 $\pm$ 0.0005	0.046 $\pm$ 0.006	0.089 $\pm$ 0.009
**RSD %	2.1 – 9.1	2.0 – 5.9	3.1 – 6.3	2.1 – 16.6	2.1 – 13.0	3.2 – 10.1
**Rec %	92-110	92-102	92-110	93-113	90-108	94-115

* results were combined with the repeatability analysis.

**Method performance requirements: AOAC SMPR®2020.001 [21]: RSD%: 11% and Rec %: 80-115; USP General Chapter, 233 [22]: RSD%: 20% and Rec %: 70-150; INMETRO [23]: RSD%: 11% and Rec %: 80-110.

The results show relative standard deviations of 2.0 - 7.5% under repeatability conditions and demonstrate fit-for-purpose accuracy, in accordance with AOAC SMPR®2020.001 [21], USP General Chapter <233> [22] and INMETRO criteria [23]. Spike recoveries also meet the requirements of standard method performance with 92-110% for low-level, 92-102% for mid-level and 92-110% for high-level spikes [21-23]. Similarly, acceptable recoveries and relative standard deviations were obtained under intermediate precision conditions, validating the accuracy of the method and demonstrating the low variability of results within the laboratory, taking into account that two different analyst participated in the study.

Application to real samples

All eight commercial CBD-rich oil samples examined presented analyte concentrations below the limits set by the European Pharmacopeia [27] and only one had a Pb concentration as high as 0.14 $\mu\text{g g}^{-1}$ Pb. Despite values being below the maximum limits defined by the most recent legislation, this study demonstrates the importance of implementing effective quality control to avoid the presence of contaminated or falsely labeled products on the market, potentially placing consumers' health at risk.

CONCLUSION

As natural elements or sources of contamination, As, Cd, Pb and Hg may be present in the soil in which crops are grown and cannot be avoided. Cannabis, being a hyperaccumulator, can always synthesize oils rich in CBD to contain these contaminants, making quality control of these products essential. The validated method developed herein presents adequate precision, accuracy and sensitivity to effectively be used to assess quality control of identified heavy metal (i.e., As, Cd, Pb and Hg) contaminants in such samples. It has been adopted for use by the Paraná Institute of Technology – Tecpar (Curitiba, Brazil) laboratory for this purpose.

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Conflicts of Interest: The authors declare no conflict of interest.

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