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Article

Simple GFAAS Method for Determination of Pb, As, and Cd in Cannabidiol Extracts Used for Therapeutic Purposes

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Cannabis sativa has many promising medicinal applications for the mitigation, or even cure, of chronic diseases. The high bioaccumulation potential of *C. sativa* enables its use for the phytoremediation and detoxification of soil, may increase the levels of inorganic elements in products such as cannabidiol (CBD) extracts being necessary the evaluation/monitoring of the level of inorganic components, mainly the toxic species in these formulations. An analytical method employing graphite furnace atomic absorption spectrometry (GFAAS) was developed for the quantification of Pb, As, and Cd in commercial CBD extracts and homemade samples. The alkaline solubilization was employed in agreement with the external analytical curve, in a robust method. The limits of quantification were 0.26 (Pb), 0.067 (As), and 0.011 μg g^{-1} (Cd), with satisfactory accuracy (80-120%) and relative standard deviation (RSD) values < 7%. The CBD extracts presented levels of Pb, As and Cd below the maximum limits established by regulatory agencies.

Keywords: *Cannabis sativa* L., medicinal plant, inorganic quantification, toxic elements, alkaline solubilization

Introduction

The *Cannabis sativa* plant is known for its therapeutic potential, as well as for being an efficient bioaccumulator of soil chemical elements, enabling its use for soil remediation.[1,2] However, its bioaccumulation capacity leads to concerns about possible adverse health effects of products derived from this plant that are ingested by humans, in the form of food, seeds, butters, and oils, as well as cannabidiol extract (CBD) used for medicinal purposes.

The organic composition of *C. sativa* has been the focus of many studies, especially concerning the characterization and quantification of the compounds tetrahydrocannabinol (THC), cannabidiol (CBD), and other cannabinoids present, together with their effects on human health.[3-14] THC is one of the most well-known cannabinoids of the *C. sativa*, being considered the main substance responsible for the psychoactive effects of the plant. Studies[15] report that high levels of THC can induce anxiety, panic, and psychosis, especially for new users. CBD is another of the main chemical substances present in *C. sativa* and has been studied in terms of its structural formula and therapeutic effects.[16] In contrast, there have been few studies concerning characterization of the inorganic components of *C. sativa* extracts and their potential effects on human health.[17-19]

In Brazil, cannabidiol products (rich in CBD and low in THC) were officially recognized by the Brazilian Health Regulatory Agency (ANVISA)[20] in 2014 as medicine for the treatment of diseases. The World Health Organization (WHO)[21] now recognizes the therapeutic potential of cannabidiol for a wide range of conditions such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, anxiety, depression, cancer, diabetes, inflammatory syndromes, among others.

In fact, the plants naturally can absorb soil chemical elements through their roots or from the atmosphere. Consequently, the final medicinal product may contain high amounts of inorganic chemical elements, some of which are considered essential, while others are toxic to humans.[22] Given the potential of *C. sativa* to bioaccumulate inorganic compounds present in the soil,[1,23,24] studies are needed in order to understand the chemical compositions of *C. sativa*

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products used for therapeutic purposes, characterizing them in terms of their contents of elements toxic to humans (such as Pb, As, and Cd). These extracts are not classified as medicines in their countries of origin, because they are not submitted to the chemical analyses generally used for the quality control of traditional medicines.

The elements Pb, As, and Cd are considered toxic in the environment and towards humans. They have a wide range of industrial applications. However, their effects in biological and environmental systems only began to be elucidated at the end of the 20th century. This was largely due to the development of analytical methods that enabled the study of chemical elements at ultra-trace (ng kg^{-1}) levels, as well as studies related to the synergistic effects of these elements, even at low concentrations, in biological systems. They are classified by the US Food and Drug Administration (FDA)[25] as Class 1, which means that they are not desirable in medicines produced by pharmaceutical industries, as they have no benefit to human health and may be harmful. Therefore, both ANVISA[26] and FDA[25] regulate the maximum values of these elements and other species that may be present in medicines and foods. Pb, As, and Cd can cause various types of damage to human health and are considered carcinogenic by the International Agency for Research on Cancer (IARC).[25-29]

The total contents of inorganic elements in plant materials are usually determined using wet digestion procedures for decomposition of the matrix.[30] The literature[31-35] reports studies employing sample digestion in closed systems, assisted by microwave radiation heating, which can reduce the interference of the matrix in the analyte signal. Acid digestion is a conventional procedure widely used in routine analysis, which converts the sample into a suitable solution that can be analyzed effectively by a range of analytical techniques. However, sample preparation by acid digestion can be time-consuming, tedious, and sometimes costly. Some of these disadvantages can be allayed by using sample preparation strategies that are faster and require minimum manipulation of the sample, hence reducing possible analyte losses and sample contamination.

The use of alkaline solubilization with tetramethylammonium hydroxide (TMAH) can provide highly effective sample preparation, besides being compatible with spectrometric techniques such as graphite furnace atomic absorption spectrometry (GFAAS).[36] As reported by Nóbrega *et al*.,[36] TMAH has been successfully used for the preparation of different sample matrices. Examples of matrices treated with TMAH are edible oils,[37] as well as fish liver, spleen, gills and muscle,[38] and other biological samples.[39] Samples solubilized in TMAH solution can usually be analyzed directly by spectrometric techniques. In the case of determination by GFAAS, sample preparation with TMAH also has the advantage of greater durability of the graphite tube, since TMAH is less aggressive to the tube, compared to typical acidic media. Ribeiro *et al*.[40] found that the use of TMAH increased the graphite tube lifetime to 1000 firings when the technique was used to determine Cd in hair samples. The lifetime of a graphite tube typically varies in the approximate range of 600-700 firings when sample preparation is performed using an acidic medium.

Eboh and Thomas,[17] Ghani *et al*.,[18] and Khan *et al*.,[19] proposed the use of flame atomic absorption spectrometry (FAAS) for the quantification of inorganic elements present in the *C. sativa* plant. This technique has modest sensitivity, achieving detection at mg L^{-1} levels.[41] However, the maximum limits recommended for inorganic elements by regulatory agencies are generally at μg L^{-1} concentrations, so it is necessary to employ analytical techniques that provide sufficient sensitivity to achieve the level of detection required.

The present work describes the development of a simple analytical methodology for the quantification of Pb, As, and Cd in CBD extracts. The elements were determined by GFAAS, with satisfactory sensitivity achieved by careful optimization of the GFAAS heating program, together with the use of chemical modifiers and a background correction system. The instrumental and sample preparation conditions led to appropriate thermal treatment of the matrix, reducing interferences and enabling accurate and reproducible quantification of the elements.[42]

Experimental

Reagents, solutions, and samples

Prior to the analyses, all the glassware and plastic vessels were immersed in a solution of 10% (v v^{-1}) HNO_3 (Carlo Erba Analyticals, Barcelona, Spain) for 24 h, followed by rinsing with ultrapure water from a Milli-Q system (Millipore Inc., Bedford, USA). All the working solutions were prepared with 1% (v v^{-1}) HNO_3 that had been previously distilled in a Teflon sub-boiling system (Distillacid, Berghof/Analítica, São Paulo, Brazil).

Aqueous standard solutions were prepared from 1000 mg L^{-1} stock solutions of the individual elements Pb, As, and Cd (SpecSol, Curitiba, Brazil) in 1% (v v^{-1}) HNO_3, by appropriate dilutions to the concentration levels required. Stock standard solutions of 10 μg L^{-1} $Pd(NO_3)_2$ (palladium matrix modifier, Sigma-Aldrich, St. Louis, MO, USA) and 10 μg L^{-1} $Mg(NO_3)_2$ (magnesium matrix

Table 1. GFAAS instrumental parameters for the quantification of Pb, As, and Cd in the CBD extracts

Parameter	Pb	As	Cd
Measurement wavelength / nm	283.3	193.7	228.8
Lamp	HCL	HCL	HCL
Lamp current / mA	20	12	8
Slit / nm	1.0	1.0	1.0
Background correction	D_2 lamp	D_2 lamp	D_2 lamp
Measurement mode	peak area	peak area	peak area
Calibration mode	concentration / (g L^{-1})	concentration / (μg L^{-1})	concentration / (μg L^{-1})
Replicate	3	3	3
Graphite tube type	heated pyrolytic graphite atomizer	heated pyrolytic graphite atomizer	heated pyrolytic graphite atomizer
Sample injection volume / μL	20	20	20
Chemical modifier injection volume / μL	5	5	5

HCL: hollow cathode lamp.

modifier, Sigma-Aldrich, St. Louis, MO, USA) were used to prepare chemical modifier solutions at different concentration levels, with final volumes of 10 mL, in 1% (v v^{-1}) HNO_3 solution. The GFAAS rinsing solution employed in each analysis was prepared with 0.1% (v v^{-1}) Triton X-100 (Vetec, Duque de Caxias, Brazil) diluted in 1% (v v^{-1}) HNO_3. Sample preparation in an alkaline medium was performed using an aqueous solution of 25% (m v^{-1}) TMAH (Sigma-Aldrich, St. Louis, USA).

The CBD extracts analyzed were either pharmaceutical grade or were homemade samples provided by patients who used the extracts for the relief of undesirable symptoms.

Instrumentation

All measurements of Pb, As, and Cd in the CBD extracts were carried out using a GFAAS instrument (model AA 6800, Shimadzu, Japan) equipped with a deuterium background correction lamp and pyrolytic graphite tubes that were heated longitudinally. Analytical grade argon (99.999%) was employed as the purge and protective gas. The GFAAS instrumental parameters are shown in Table 1.

Optimization of instrumental parameters and sample preparation conditions

The GFAAS instrumental conditions were investigated for each inorganic element, employing a univariate approach to optimize the pyrolysis and atomization temperatures, in order to achieve the levels of accuracy required by regulatory agencies as European Communities Directive 96/23.[43] Analyses were performed using reference solutions of each analyte in 1% (v v^{-1}) HNO_3. The levels of the reference solutions employed were 20 μg L^{-1} (Pb), 15 μg L^{-1} (As), and 10 μg L^{-1} (Cd), different concentration levels were employed according to the sensitivity of the GFAAS for each element, providing better evaluation of the signal-to-background ratio (SBR). The optimum analytical conditions were then applied in analyses of the CBD extracts in 0.1% (m v^{-1}) TMAH solution. The modifier optimization was evaluated employing solutions containing Pd and Mg as well as solutions containing only Pd at different concentration levels in 1% (v v^{-1}) HNO_3 (Table 2). In these experiments, the physicochemical characteristics of the analytes were considered, as well as the thermal stability of these analytes at high temperatures, as reported in the literature.[42] In these tests, the SBR parameter was used to indicate the most suitable atomization conditions for analyte quantification.[44]

Table 2. Chemical modifier concentrations levels studied for quantification of Pb, As and Cd in CBD extracts by GF AAS

Chemical modifier	Concentration / (μg *per* 5 μL)
Pd / Mg	5-3
Pd / Mg	7.5-4.5
Pd / Mg	2.5-1.5
Pd	7.5
Pd	5.0
Pd	2.5

For the analysis of the CBD extracts, portions of about 0.1000 g were solubilized using different concentrations of 25% (m v^{-1}) TMAH solution. For Pb, which was the first element studied, a full factorial design (2^3) was employed in order to achieve a better evaluation of the sample preparation variables, as well as their interaction effects. The variables studied were the concentration of

(m v^{-1}) TMAH, the sample solubilization time (min), and the temperature (°C). These experiments were conducted in random order. The software used was Statistica 7.0[45] and the variables were studied at lower (−) and higher (+) levels, as shown in Table 3.

Table 3. Full factorial design (2^3) applied for optimization of sample preparation for Pb determination by GFAAS

Variable	Level	
	Minimum (−1)	Maximum (+1)
[TMAH] / (% m v^{-1})	0.2	5.0
time / min	5	10
Temperature / °C	25	80

[TMAH]: tetramethylammonium hydroxide concentration.

In these experiments, the CBD extracts were weighed directly into 15 mL Falcon tubes, followed by addition of the TMAH solution. The solubilized extracts were diluted to final volumes of 5.0 mL. All the samples were previously spiked with the analyte (Pb), followed by vortex homogenization and application of the reaction conditions of the 2^3 factorial design. The analyte recovery was the dependent variable used to select the most suitable conditions for preparation of the CBD extracts in TMAH medium. The optimized sample preparation condition found for Pb was applied for the other analytes.

Results and Discussion

Evaluation was made of the effects of different concentration levels of the universal Pd-Mg modifier and the modifier containing only Pd. The levels were based on satisfactory results previously reported in the literature. Ribeiro *et al.*[40] obtained recovery values for As and Cd of 100 and 93%, respectively, employing alkaline solubilization of hair samples with TMAH. Welz and Sperling[42] found that the Pd-Mg modifier provided the best results for Pb determination, while palladium nitrate provided stabilization of both inorganic and organic arsenic, resulting in satisfactory accuracy and precision of GFAAS analyses. For analysis of Cd, Bulska *et al.*[46] reported that the pyrolysis temperature could be increased when palladium was used as a chemical modifier. In this study, for Pb determination, the use of the universal chemical modifier (5 µg Pd / 3 µg Mg) in a volume of 5 µL provided the most suitable analysis conditions, considering the SBR values. For As and Cd, the chemical modifier containing only Pd (2.5 µg) provided the most satisfactory SBR values.

The low concentration of 0.1% (m v^{-1}) TMAH and the optimized GFAAS heating program provided a homogeneous and representative slurry. These conditions avoided the formation of precipitate, as observed by Ribeiro *et al.*,[40] for Pd in contact with samples in an alkaline TMAH medium.

The purpose of this study was to develop a simple method that could be easily applied in routine analyses. The quantification method was investigated using the analyte in 1% (v v^{-1}) HNO_3 solution, in order to find the optimum instrumental conditions. However, the sample preparation was performed in alkaline medium, using TMAH as reagent, which minimized manipulation and improved throughput. Despite the differences between these media, it was possible to quantify the analytes with satisfactory accuracy and precision, after adjustment of the instrumental parameters (pyrolysis and atomization temperatures) and the sample preparation procedure. Assays were performed with the CBD extracts in order to evaluate the influence of matrix interferences on quantification of the analytes.

The pyrolysis and atomization temperatures were studied for each analyte in 1% (v v^{-1}) HNO_3 solutions previously spiked with 20 µg L^{-1} of Pb, 15 µg L^{-1} of As, and 10 µg L^{-1} of Cd (Figure 1).

The highest absorbance values for Pb and As were obtained using pyrolysis temperatures of 900 and 850 °C, with atomization temperatures of 1700 and 2200 °C, respectively. These conditions provided well-defined and symmetrical peaks, with satisfactory SBR values. The satisfactory transient signal shapes indicated that both the thermal decomposition of the matrix (for reduction of matrix interferences) and the atomization stage (for analyte quantification) were satisfactory, resulting in accurate results.

In contrast, for Cd analyte signals with suitable SBR were not obtained under the optimal instrumental conditions employed. It can be seen from Figure 1a that when atomization temperatures higher than 1600 °C were used, the Cd signal did not return to the baseline, leading to inaccurate values, since the absorbance value was calculated by integrating the peak area. For atomization temperatures lower than 1600 °C (Figure 1b), the Cd signals presented multiple peaks. The anomalous behaviors of the Cd signals observed using these temperatures could be explained by the presence of precursors, such as oxides and carbonates, which were produced before formation of the atomic cloud and were released faster during the atomization step. In addition, some chemical elements may form carbides due to interaction with the surface of the graphite tube.[47] The pyrolysis and atomization temperatures that provided the most satisfactory absorbance and SBR values for Cd quantification were 700 and 1600 °C, respectively (Figure 1c).

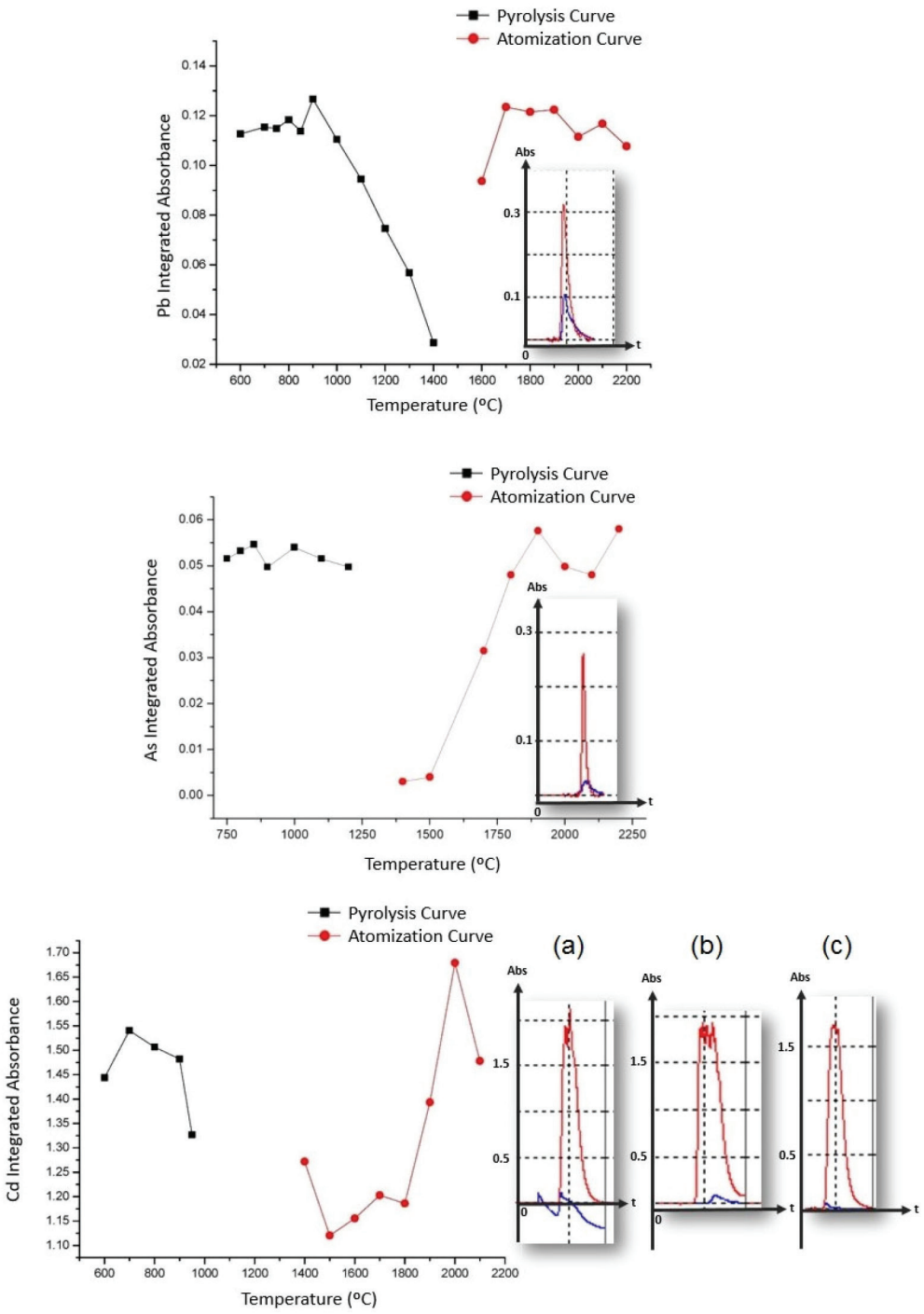

Figure 1. Pyrolysis temperature (T_p) and atomization temperature (T_a) curves for (top) Pb, (middle) As and (bottom) Cd. For Cd quantification: (a) $T_a > 1600$ °C; (b) $T_a < 1600$ °C; (c) $T_p = 700$ °C and $T_a = 1600$ °C. The red plots are the analyte signals and the blue plots are the background (transient) signals. The analyses were performed using 20 µL aliquots of standard solutions of each analyte in acid medium (1% (v v^{-1}) HNO_3), in the presence of 5 µL of chemical modifier (5 µg Pd / 3 µg Mg for Pb; 2.5 µg Pd for As and Cd).

After optimization of the instrumental conditions for an acidic medium, it was necessary to adjust the drying temperature of the GFAAS heating program, when the CBD extracts solubilized in alkaline medium were analyzed. TMAH is a more viscous reagent, compared to the aqueous acidic medium for which the GFAAS was programmed. Due to the viscosity difference of these solutions, it was observed that during the drying step, the CBD extract in 0.1% (m v^{-1}) TMAH was expelled out of the graphite tube, hence affecting the analytical measurements. Therefore, adjustment of the drying step was required, in order to ensure smooth drying of the CBD extracts, avoiding any

Table 4. GFAAS heating programs developed for the determination of Pb, As, and Cd in the CBD extracts solubilized in alkaline medium (0.1% (m v^{-1}) TMAH)

Step	Temperature / °C			time / s			Heating mode	Air flow / (L min^{-1})
	Pb	As	Cd	Pb	As	Cd		
Drying	120	50	50	100	50	20	ramp	0.1
	200	120	120	20	70	90	ramp	1.0
	–	200	200		20	20	ramp	1.0
Pre-pyrolysis	700	700	550	10	10	10	ramp	1.0
Pyrolysis	900	850	700	10	10	3	hold	1.0
Atomization	1700	2200	1600	2	2	2	hold	0.0
Cleaning	2500	2500	2500	2	2	3	hold	1.0

loss or contamination of the sample. The GFAAS heating programs developed for analysis of the inorganic elements studied are shown in Table 4.

The applicability of preparation of the CBD extracts in TMAH medium was evaluated in order to determine whether the reaction medium suitable for sample treatment was compatible with the analytical method developed using an acidic medium. For this, recovery assays were performed, employing the standard solutions of Pb, As, and Cd in acidic medium (1% (v v^{-1}) HNO_3) and the CBD extracts in alkaline medium previously spiked with the standard solutions at the same concentration levels.

A 2^3 full factorial design was employed in order to determine the effects of the different solubilization factors on the quantification of Pb. The results are shown in the form of a Pareto chart (Figure 2). The 2^3 full factorial design matrix is provided in Table S1 (Supplementary Information (SI) section).

Improvement in the extraction efficiency was observed when a higher temperature was employed (T ca. 80 °C). As reported in the literature,[36,48-50] higher temperatures generally enhance solubilization of samples, including those with matrices similar to the present one.

An important finding was that the interaction between temperature and the TMAH concentration presented a negative effect, indicating that the best conditions for efficient extraction of the analyte from the matrix involved the simultaneous effect of high temperature and low TMAH concentration. In fact, a lower concentration of TMAH should lead to better results, since this reagent (with the formula $(CH_3)_4NOH$) has a high content of carbon, which could interfere in the GFAAS signal and increase the absorbance of the analytical blank. The nature of the

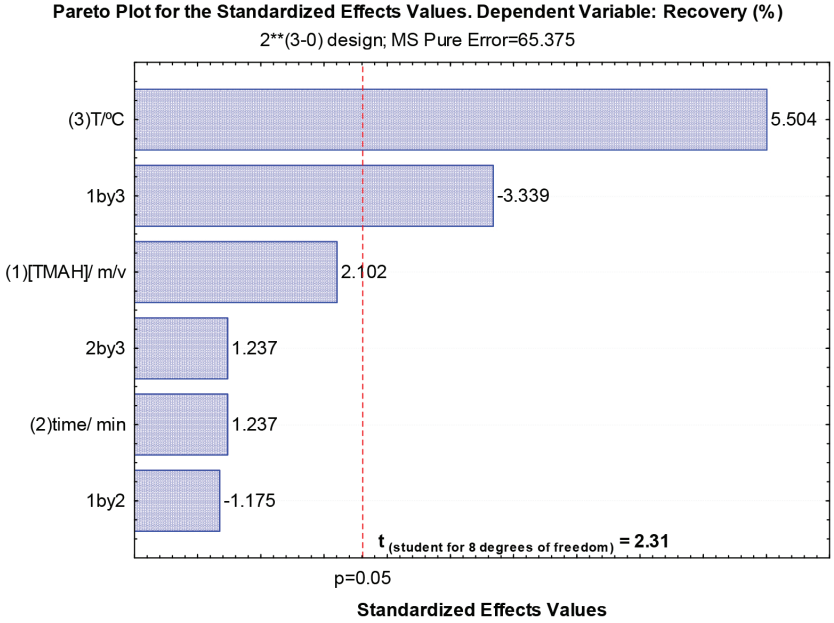

Figure 2. Pareto chart for the CBD extraction in TMAH medium, obtained from the 2^3 full factorial design, for the quantification of Pb by GFAAS.

chemical interferences in the condensed phase has not yet been fully elucidated, but it is noted that carbide and/or intermediate compounds may be formed, which can lead to incomplete atomization of the analyte or changes in its volatilization kinetics.[51] Hence, in order to overcome these concerns, it was necessary to find a compromise condition that provided effective solubilization of the CBD extracts employing a low concentration of TMAH.

A recent study[52] using Fourier transform (FT)-Raman spectroscopy reported the action of TMAH in solubilizing proteins and other organic compounds such as lipids. The mechanism of action of TMAH in solubilizing organic matter and releasing analytes is not well understood. However, in the studies of Aranha *et al.*,[53] Ghisi *et al.*,[49] and Nóbrega *et al.*,[36] it was found that the use of low concentrations of TMAH solution enabled homogeneous and representative suspensions to be obtained for different types of samples, with reduced chemical interferences.

For Pb, the solubilization time was not significant (Figure 2). However, for extraction of As and Cd, a longer sample alkaline treatment time was desirable. Tests at different temperatures showed that an extraction time of 15 min provided the best recovery values for As and Cd. Therefore, quantification of all the analytes employed an optimized sample preparation condition consisting of 0.1% (m v^{-1}) TMAH, at 100 °C, for 15 min.

The analytical quality and applicability of the method were evaluated considering the linear ranges and limits of detection (LOD) and quantification (LOQ), according to accepted international[43] and national criteria,[54] as shown in Table 5. The LOD and LOQ values were calculated using the ratios of 3 and 10 times the standard deviation of the blank, respectively, and the angular coefficient (slope) of the analytical curve. Two analytical curves were constructed using the different media, in order to evaluate the effect of matrix interferences on the analyte signal and the sensitivity of the method using these media (Figures S1-S3, SI section).

The analytical curves constructed using acidic and alkaline media showed similar sensitivities for all the inorganic elements studied, indicating that it was possible to employ analytical curves obtained using external standards in acidic media for quantification of the elements in the CBD extracts. This was possible due to the detailed study of the experimental parameters for the sample treatment and the quantification method, for the different reaction media, together with adjustment of the pyrolysis temperature according to the chemical modifier concentration and the reaction medium. Optimization of the instrumental conditions, using the chemical modifiers at suitable concentration levels, enabled efficient thermal treatment of the matrix and satisfactory atomization of the analytes.

Due to the unavailability of certified reference materials (CRMs), addition/recovery experiments were performed at three concentration levels within the range of the analytical curve (Table 6).

For Cd, the concentration in the sample (0.6 μg L^{-1}) was lower than the first point of the analytical curve (2.0 μg L^{-1}), but this value was considered in the addition/recovery experiments, resulting in accurate quantification by the proposed method, as can be seen in Table 6. The recovery values obtained were within the range considered acceptable by the European Community Directive 96/23[43] (80-120%).

The pharmaceutical grade (CBD PG) and homemade (CBD H) extracts were analyzed by the proposed method. The results for quantification of Pb, As, and Cd are shown in Table 7. The sample numbers indicate the different origins of the pharmaceutical grade samples and those produced locally.

Only samples CBD PG 01 and CBD H 07 presented arsenic contents above the LOQ of the method (Table 7). The values were lower than the maximum limit of 1.5 μg g^{-1} recommended by regulatory agencies (FDA[25] and ANVISA),[26] for oral medications where daily doses do not exceed 10 g *per* day.

Table 5. Figures of merit of the proposed method for quantification of Pb, As, and Cd in the CBD extracts by GFAAS

	Medium	Analytical curve parameter	Linear range / (μg L^{-1})	R^2	LOD / (μg g^{-1})	LOQ / (μg g^{-1})
As	acid	$y = -0.00442 + 0.00649x$	2.0-10.0	0.999	0.022	0.067
	alkaline	$y = -0.00128 + 0.00719x$	2.0-10.0	0.998		
Cd	acid	$y = -0.03970 + 0.14222x$	2.0-6.0	0.995	0.0035	0.011
	alkaline	$y = -0.05614 + 0.15090x$	2.0-6.0	0.997		
Pb	acid	$y = -0.00881 + 0.00478x$	5.0-13.0	0.998	0.090	0.26
	alkaline	$y = -0.01028 + 0.00405x$	5.0-13.0	0.997		

R^2: coefficient of determination; LOD: limit of detection; LOQ: limit of quantification.

Table 6. Recovery values obtained for GFAAS analysis of CBD extract (sample CBD PG 01) solubilized in 0.1% (m v^{-1}) TMAH (mean ± standard deviation, n = 3)

	Concentration in the sample / (μg L^{-1})	Concentration level added / (μg L^{-1})	Value found / (μg L^{-1})	Recovery / %
Pb	< LOQ	5.0	4.5 ± 0.3	90
	< LOQ	9.0	8.8 ± 0.4	98
	< LOQ	13.0	12.7 ± 0.6	98
As	3.8 ± 0.2	[a]	1.8 ± 0.1	91
		1.5	5.3 ± 0.3	101
		5.0	8.4 ± 0.06	99
Cd	0.6 ± 0.03	2.0	2.5 ± 0.1	96
		4.0	5.0 ± 0.1	109
		6.0	6.6 ± 0.1	100

[a]Diluted sample for the As lowest level of 1.8 μg L^{-1}. CBD PG: pharmaceutical grade cannabidiol extract; LOQ (limit of quantification) for Pb: 0.26 μg g^{-1}.

Table 7. Concentrations of Pb, As, and Cd in the CBD extracts from different origins, determined using the GFAAS method (mean ± standard deviation, n = 3)

Sample	Pb / (μg g^{-1})	As / (μg g^{-1})	Cd / (μg g^{-1})
CBD PG 01	< LOQ	0.18 ± 0.01	< LOQ
CBD PG 02	< LOQ	< LOQ	< LOQ
CBD H 01	< LOQ	< LOQ	< LOQ
CBD H 02	< LOQ	< LOQ	< LOQ
CBD H 03	< LOQ	< LOQ	< LOQ
CBD H 04	< LOQ	< LOQ	< LOQ
CBD H 05	< LOQ	< LOQ	< LOQ
CBD H 06	< LOQ	< LOQ	< LOQ
CBD H 07	< LOQ	0.12 ± 0.01	< LOQ
CBD H 08	< LOQ	< LOQ	< LOQ
CBD H 09	< LOQ	< LOQ	< LOQ

CBD PG: pharmaceutical grade cannabidiol extract; CBD H: homemade cannabidiol extract; LOQ (limit of quantification) values (in μg g^{-1}): 0.26 (Pb), 0.067 (As), 0.011 (Cd).

Sample CBD PG 01 was digested using conventional treatment with an acidic medium and heating by microwave radiation.[22] The concentration obtained for As was 0.18 μg g^{-1}, while the concentrations of Pb and Cd were below the LOQ of the method, in agreement with the results obtained using the alkaline solubilization.

The concentrations of chemical elements in plants may vary depending on the environment in which the plant was grown.[2,13] The inorganic elements contents found in this work were compared to other studies that reported the presence of As, Cd and Pb in leaves of the *C. sativa* plant, once CBD extracts are obtained from leaves of this plant. In fact, the contents for the elements investigated in the CBD extracts, were lower than the ones found previously in the leaves as reported by Eboh and Thomas,[17] and Khan *et al*.[19] In these studies, the contents found in the leaves were: 1.58-6.37 μg g^{-1} for Pb; 13.6 μg g^{-1} for As (only in Eboh and Thomas)[18] and 4.40-3.41 μg g^{-1} Cd.

In the same way as the studies carried out on *C. sativa* plants, which presented levels within the acceptable range, this work shows that in relation of inorganic composition, the CBD extracts did not present any risk to the health of patients using them for the treatment of different diseases.

Conclusions

The sample treatment employing alkaline solubilization with TMAH reagent was efficient, enabling accurate quantification of Pb, As, and Cd in the CBD extracts by GFAAS. Optimization of the instrumental parameters for analysis of sample extracts in an acidic medium, together with adjustment of the sample treatment conditions, was an effective strategy for method development, resulting in an optimized quantification methodology. This enabled the use of an analytical curve constructed with external standards, without any significant matrix effects. The alkaline solubilization produced homogeneous and representative slurries, resulting in accurate analyses.

The CBD extracts presented safe levels of Pb, As, and Cd, without any risk to human health, especially considering individuals with poor health and who regularly use these extracts. The findings of this study contribute to a better understanding of the inorganic chemical profile of CBD extracts, providing a methodology suitable for the monitoring of non-essential chemical elements in these matrices. Two samples containing As presented values of 0.18 ± 0.01 μg g^{-1} (commercial sample) and

0.12 ± 0.01 μg g^{-1} (homemade CBD extract), although these concentrations are not considered harmful to health.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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