Simultaneous Determination of Cadmium and Lead in Medicinal Plants Using Graphite Furnace Atomic Absorption Spectrometry and Direct Slurry Sampling

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INTRODUCTION

Phytotherapy plays a major role in the healthcare and functional food markets (1). The global market of medicinal herbs and their products is close to 62 billion dollars, and it is estimated to reach 5 trillion dollars by the year 2050 (2). The establishment of modern and accurate methods to assess the quality and to ensure the safety of medicinal plants for humans is therefore very relevant (3). Plants store nutrients in their tissues to grow healthy but they may also accumulate hazardous elements due to environmental pollution (4,5).

Cadmium (Cd) and lead (Pb) are extremely toxic elements and normally occur at low concentrations in most plant materials (5). Thus, the determination of trace levels of Cd and Pb in real samples requires sensitive analytical methods. Among the spectrometric techniques, graphite furnace atomic absorption spectrometry (GF-AAS) offers the capability for trace analysis (6). Some of the main advantages of GF-AAS are high sensitivity and selectivity, the possibility to measure several elements simultaneously, and the feasibility for direct sampling of solids or slurries (7).

Single-element determinations of Cd or Pb in plant matrices by GF-AAS are largely found in the literature (5,8), but only a few papers deal with direct sampling of plant

ABSTRACT

A method is proposed for the simultaneous determination Cd and Pb in medicinal plants using direct slurry sampling by GF-AAS. The thermal behavior of analytes was checked in aqueous and slurry media using Ir permanent modifier. The optimized pyrolysis (1000 °C) and atomization (2200 °C) temperatures allowed calibration using aqueous standards.

For a 20- μ L injected volume, the calculated characteristic mass was 1.8 pg for Cd and 59 pg for Pb. The relative standard deviation ($n=12$) for Cd and Pb was 5.3% and 3.5%, respectively. The limit of detection was 0.12 μ g L⁻¹ for Cd and 1.22 μ g L⁻¹ for Pb. Accuracy was checked by analyzing the standard reference materials Ginkgo Biloba Leaves, Peach Leaves, and Pine Needles. The results obtained for the analytes were in agreement with the certified values at the 95% confidence level. Ten medicinal plant samples were analyzed and the found concentrations ranged from 0.19–0.34 μ g g⁻¹ for Cd and 1.02–1.91 μ g g⁻¹ for Pb.

slurries (7,9,10). Methods involving the simultaneous determination for Cd and Pb in plants using direct slurry sampling are not described in the literature.

The main purpose of this work was the development of a simple and fast method for the simultaneous determination of Cd and Pb in medicinal plants using direct slurry sampling and GF-AAS with a transversely heated graphite atomizer

and longitudinal Zeeman-effect background correction. The performance of the proposed method was checked after analyzing medicinal plant samples and three standard reference materials (SRMs).

EXPERIMENTAL

Reagents and Analytical Solutions

High purity water (resistivity 18.2 M Ω cm) was obtained using a Millipore® Rios 5 Reverse Osmosis™ and a Millipore Milli-Q™ Academic™ system Bedford, MA, USA). Suprapur® nitric acid (Merck, Darmstadt, Germany) was employed to prepare all working solutions.

The 1000 mg L⁻¹ iridium stock solution (IrCl₃·3H₂O; Fluka GmbH, Buchs, Switzerland) was used directly for permanent modification of the graphite furnace platform.

Five mixed analytical solutions (Cd: 0.5, 1.5, 2.5, 4.0, 5.0 μ g L⁻¹; Pb: 5.0, 15, 25, 40, 50 μ g L⁻¹) were prepared daily by diluting the individual 1000 mg L⁻¹ stock solutions (Merck, Darmstadt, Germany). All analytical solutions and samples were acidified to 0.14 mol L⁻¹ HNO₃. The autosampler washing solution was 0.1% (v/v) Triton® X-100 acidified to 0.14 mol L⁻¹ HNO₃.

All solutions were stored in Nalgene® high-density polypropylene bottles (Rochester, NY, USA). Plastic bottles and glassware materials were cleaned by soaking in 10% (v/v) HNO₃ for at least 24 hours and then rinsed abundantly in deionized water before use.

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Preparation of Samples and Certified Reference Materials

Medicinal plant samples of *Annona muricata*, *Baccharis trimera*, *Echinodorus grandiflorus*, *Equisetum arvense*, *Matricaria chamomilla*, *Melissa officinalis*, *Panax ginseng*, *Peumus boldus*, *Pimpinella anisum*, and *Mikania glomerata* were purchased at a local market in Araraquara city, São Paulo, Brazil.

All samples were dried at 50 °C for 48 hours in a forced air oven and ground in a cutting mill fitted with a 20-mesh screen at the bottom of the cutting chamber. Thereafter, the samples were powdered in a Spex 6750 cryogenic mill (Metuchen, NJ, USA) using a program developed for plant materials (11). A mass of 50 mg of samples cryogenically powdered was dispersed in 10 mL of 0.2% (v/v) HNO_3 + 0.05% (v/v) Triton® X-100 solution. Slurries of 3464 Ginkgo Biloba Leaves, 1547 Peach Leaves, and 1575a Pine Needles SRMs (National Institute of Standards and Technology, Gaithersburg, MD, USA) were similarly prepared.

Instrumentation

A PerkinElmer SIMAA™ 6000 simultaneous multi-element atomic absorption spectrometer was used, equipped with a transversely heated graphite atomizer (THGA™), longitudinal Zeeman-effect background (BG) correction, and an AS-72 autosampler (PerkinElmer, Inc., Shelton, CT, USA). A PerkinElmer electrodeless discharge lamp (EDL) was used for Cd (228.9 nm) and a PerkinElmer Lumina™ hollow cathode lamp (HCL) was used for Pb (283.3 nm). The lamp current was 240 mA and 10 mA for Cd and Pb, respectively. The atomic signals were measured in peak area mode. High-purity argon (99.99%, White Martins, Sertãozinho, Brazil) was used as the protective and purge gas at the 250 mL min⁻¹ flow rate.

It should be pointed out that the experiments were carried out using the stabilized temperature platform furnace, the so-called STPF conditions (12). A PerkinElmer USS-100™ ultrasonic slurry sampler with titanium tip was used to promote homogenization of the particles during sampling (7).

Analytical Procedure

Iridium was thermally deposited onto the integrated platform of the THGA by using the features of the original software of the spectrometer. The platform was coated with 500 µg Ir modifier as previously described (13).

Slurries of the samples and SRMs were transferred to autosampler cups. Immediately before sampling, the Ti ultrasound probe at 40% ultrasound amplitude was introduced into the slurries for homogenization during 10 seconds. The use of ultrasonic agitation is important to provide a uniform distribution of particles in an autosampler cup (7).

The thermal behavior of Cd and Pb was evaluated by means of pyrolysis and atomization temperature curves in order to select the optimum pyrolysis and atomization temperatures. Pyrolysis and atomization curves were built up in the following media: (a) 0.2% (v/v) HNO_3 aqueous solution containing 2.5 µg L⁻¹ Cd + 50 µg L⁻¹ Pb, and (b) 0.5% (m/v) plant slurries of Ginkgo Biloba Leaves (Pb) and Pine Needles (Cd) containing 0.2% (v/v) HNO_3 + 0.05% (v/v) of Triton X-100. In both situations, 20-µL aliquots of samples were dispensed into the pre-treated graphite platform.

The matrix effects were evaluated by comparing the slopes of the analytical curves (0.5–5.0 µg L⁻¹ Cd and 5.0–50 µg L⁻¹ Pb) built up in 0.2% (v/v) HNO_3 aqueous solution and 0.5% (m/v) slurries containing 0.2% (v/v) HNO_3 + 0.05% (v/v) Triton X-100.

The effect of slurry amount on precision and accuracy was evaluated by analyzing slurries of Ginkgo Biloba Leaves (Pb) and Pine Needles (Cd) in 0.5–2.0% (m/v) slurries in 0.2% (v/v) HNO_3 + 0.05% (v/v) Triton X-100.

For sample analyses, aliquots of 20 µL of a blank, analytical solutions, and slurries were automatically injected in sequence onto the atomizer platform by using the facilities provided by the original software of the autosampler and graphite furnace. The optimized heating program of the graphite tube is listed in Table I. All atomic absorption measurements were made at least in triplicate and based on peak area mode.

The proposed method was applied to the determination of Cd and Pb in 10 medicinal plants and three SRMs in order to assess the capability of the method in terms of accuracy and precision.

Limits of detection (LOD) and limits of quantification (LOQ) were calculated according to the IUPAC recommendations (14).

RESULTS AND DISCUSSION

Volatile elements such as Cd and Pb can be easily released from the graphite platform at temperatures below ca. 500 °C in the absence of a modifier (9). Direct analyses of solids or slurries usually require elaborate heating programs comprising two pyrolysis steps or air-assisted pyrolysis in order to remove the matrix entirely. It should be mentioned that an air-assisted pyrolysis at 600 °C was included as the first pyrolysis step (Table I) to help the complete removal of the organic matrices. And the use of permanent modifiers may help matrix removal by increasing the volatilization temperature of the analyte or by catalytic decomposition, resulting in low blank values and increased graphite

tube lifetime. Taking into consideration the benefits mentioned above, an Ir-coated platform was selected for the direct analysis of the plants.

Electrothermal Behavior

In simultaneous determinations by GF-AAS, all analytes are similarly exposed to the single heating program of the atomizer. Consequently, the optimized pyrolysis and atomization temperatures are frequently not the best for each analyte but the most favorable to furnish required sensitivity, accuracy, and precision. The electrothermal

behavior of Cd and Pb in aqueous solution and vegetal slurries using an Ir-coated platform are illustrated in Figure 1. Analysis of the figure reveals that the pyrolysis temperature reduced from 1200 °C to 1000 °C when the medium changed from aqueous to slurries, respectively. The most volatile element (Cd) defined the pyrolysis temperature at 1000 °C. The atomization was fixed at 2200 °C. Although these temperatures did not furnish the highest integrated absorbance for Cd, narrow transient peaks (fast appearance and baseline restoration times) and suitable sensitivity

and precision (RSD < 2.5% for Cd and < 3.5% for Pb) were observed.

Matrix Effects

Matrix effects were evaluated by comparing the slopes of the calibration curves built up in aqueous solution and slurries of *Matricaria chamomilla*, *Panax ginseng*, and *Peumus boldus*. For that sequence, good linear correlation coefficients (r^2) were obtained for Cd (0.9984, 0.9918, 0.9955, 0.9951) and for Pb (0.9974, 0.9965, 0.9995, 0.9980); and the slopes of the analytical curves were 0.0329, 0.0310, 0.0339, 0.0329 (Cd) and 0.00144, 0.00150, 0.00151, 0.00144 (Pb). These data expect errors of < 6% and 4% for Cd and Pb, respectively, when aqueous standard calibration is used. Matrix matching or analyte addition calibrations would minimize matrix effects. However, taking into consideration that errors of < 6% are acceptable for trace analysis using direct slurry sampling, aqueous calibration is straightforward and was adopted for further studies.

TABLE I
Heating Program of Atomizer for the
Simultaneous Determination of Cd and Pb

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Argon Flow (mL min ⁻¹)
1	110	1	30	250
2	130	10	30	250
3	600	10	40	250 (air)
4	40	5	35	0
5	1000	10	10	250
6	2200	0	5	0 (read)
7	2500	1	5	250

Fig 1 (A&B). Pyrolysis (■: Cd; ●: Pb) and atomization (□: Cd; ○: Pb) temperature curves in aqueous solution (A) and slurry samples (B) using Ir-coated platform. Curves in A correspond to 2.5 µg L⁻¹ Cd + 50 µg L⁻¹ Pb in 0.2% (v/v) HNO₃. Curves in B correspond to 0.5% (m/v) slurries of *Ginkgo Biloba* Leaves (Pb) and *Pine Needles* (Cd) prepared in 0.05% (v/v) Triton X-100 + 0.2% (v/v) HNO₃.

Fig 2. Influence of slurry concentration on simultaneous determination of Cd (A) and Pb (B) by GFAAS.

Effect of Slurry Concentration

Homogeneity is crucial in direct slurry sampling analysis. So, the influence of slurry concentration on accuracy was performed in the 0.5–1.5% (m/v) range. The results for Cd obtained in all concentrations were in agreement at the 95% confidence level (Student's *t*-test) with the certified value of the SRM Pine Needles (Figure 2A). Concentrations > 1.5% (m/v) produced clogging of the autosampler capillary, deteriorating sample delivery and precision. For Pb, better results were obtained for a 0.5% (m/v) slurry concentration, which produced concordant results at the 95% confidence level with the certified value of SRM Ginkgo Biloba Leaves (Figure 2B). With the aim of direct and simultaneous determination of Cd and Pb, 0.5% (m/v) slurry concentration for medicinal plants was selected.

Analytical Performance

The main figures of merit relating to the simultaneous and direct determination of Cd and Pb in slurry using an Ir-coated platform modifier were evaluated and are compiled in Table II. A 20-µL volume of slurries was injected and

TABLE II
Main Figures of Merit for Simultaneous Dermination of Cd and Pb in Medicinal Plants by the Proposed Method

Parameters	Cd	Pb
LOD	0.12 µg L ⁻¹	1.22 µg L ⁻¹
LOQ	0.40 µg L ⁻¹	4.04 µg L ⁻¹
m ₀	1.8 pg	59 pg
RSD	5.3%	3.5%
Slope	0.04695	0.00147
R ²	0.9982	0.9997
Pyrolysis temperature	1000 °C	
Atomization temperature	2200 °C	
Injected volume	20 µL	
Slurry	0.5% (m/v)	
Modifier	500 µg Ir	

LOD: limit of detection; LOQ: limit of quantification; m₀: characteristic mass; RSD: relative standard derivation; R²: correlation coefficient.

the calculated characteristic mass for Cd was 1.8 pg and for Pb was 59 pg. The relative standard deviation (n=12) for a sample (*Melissa officinalis*), containing 0.19 µg g⁻¹ Cd and 1.91 µg g⁻¹ Pb, was 5.3% for Cd and 3.5% for Pb. The limit of detection, calculated according to IUPAC recommendation (14), was 0.12 µg L⁻¹ and 1.22 µg L⁻¹ for Cd and Pb, respectively.

TABLE III
Comparative Results (Medium $\pm$ SD, n=3) of the Simultaneous Determination of Cd and Pb in SRMs by the Proposed Method

SRM	Cd		Pb	
	Found ($\mu\text{g g}^{-1}$)	Certified ($\mu\text{g g}^{-1}$)	Found ($\mu\text{g g}^{-1}$)	Certified ($\mu\text{g g}^{-1}$)
Pine Needles	0.218 ± 0.020	0.233 ± 0.004	< 0.8	0.167 ± 0.015
Peach Leaves	< 0.08	0.026 ± 0.003	0.87 ± 0.03	0.81 ± 0.03
Ginkgo Biloba	< 0.08	0.020 ± 0.001	0.99 ± 0.03	0.99 ± 0.07

TABLE IV
Results (Medium $\pm$ SD, n=3) for Cd and Pb in Medicinal Plants Samples

Samples	Cd ($\mu\text{g g}^{-1}$)	Pb ($\mu\text{g g}^{-1}$)
<i>Annona muricata</i>	< 0.08	< 0.8
<i>Baccharis trimera</i>	< 0.08	1.28 ± 0.07
<i>Echinodorus grandiflorus</i>	< 0.08	< 0.8
<i>Equisetum arvense</i>	0.34 ± 0.01	1.02 ± 0.12
<i>Matricaria chamomilla</i>	< 0.08	< 0.8
<i>Melissa officinalis</i>	0.19 ± 0.01	1.91 ± 0.06
<i>Mikania glomerata</i>	0.20 ± 0.01	< 0.8
<i>Panax ginseng</i>	< 0.08	< 0.8
<i>Peumus boldus</i>	< 0.08	< 0.8
<i>Pimpinella anisum</i>	< 0.08	1.06 ± 0.19

Accuracy was checked for Cd and Pb determination in three plant SRMs (Table III). A paired *t*-test showed that the results found with the proposed GF-AAS method were in agreement with the certified values for Cd and Pb at the 95% confidence level.

The analysis of 10 medicinal plants (Table IV) presented concentrations in the range of $0.19\text{--}0.34 \mu\text{g g}^{-1}$ for Cd and $1.02\text{--}1.91 \mu\text{g g}^{-1}$ for Pb. These concentrations are comparable to media values ($0.5 \mu\text{g g}^{-1}$ Cd and $1 \mu\text{g g}^{-1}$ Pb) usually found for medicinal plants (5).

CONCLUSION

This work presents a simple and fast method for the simultaneous determination of Cd and Pb in medicinal plants by GF-AAS using direct slurry sampling. Matrix effects did not impair aqueous solution calibra-

tion. The relative standard deviations were 5.3% for Cd and 3.5% for Pb. The LOD was $0.12 \mu\text{g L}^{-1}$ for Cd and $1.22 \mu\text{g L}^{-1}$ for Pb. The proposed slurry sampling-based method did not require sample preparation with hazardous reagents, offers reduced risk of contamination or sample losses, less analysis time and waste, which are desirable characteristics of a clean analytical method.

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REFERENCES

1. P. Kalny, Z. Fijałek, A. Daszczyk and P. Ostapczuk, Sci. Total Environ. 381, 99 (2007).
2. N.S. Chauhan, Medicinal and Aromatic Plants of Himachal Pradesh, 1st ed., Indus Pub., New Delhi, India (1999).
3. WHO, Quality control methods for medicinal plant materials. WHO, Geneva, Switzerland (2007).
4. S.S. Ražić, S.M. Dogo, L.J. Slavković, Microchem. J. 84, 93 (2006).
5. M.E. Farago, Plants and the Chemical Elements, 1st ed., Wiley-VHC, Weinheim, Germany (1994).
6. G.P.G. Freschi, C.S. Dakuzaku, M. Moraes, J.A. Nóbrega and J.A. Gomes Neto, Spectrochim. Acta, Part B 56, 1987 (2001).
7. N.J. Miller-Ihli, At. Spectrosc. 13, 1 (1992).
8. B. Welz and M. Sperling, Atomic Absorption Spectrometry, 3rd ed., Wiley-VHC, Weinheim, Germany (1999).
9. H. Chen, W. Hu, S. Li and M. Wang, Microchim. Acta 166, 133 (2008).
10. I. Karadjova and M. Karadjov, Fresenius J. Anal. Chem. 360, 246 (1998).
11. B. Ambrozini, V.R.A. Filho, S.R. Oliveira, L.V.S. Sacramento, J.A. Gomes Neto, Food Chem. 116, 1024 (2009).
12. W. Slavin, D.C. Manning, G.R. Carnrick, At. Spectrosc. 2, 137 (1981).
13. G.P.G. Freschi, C.D. Freschi, S.R. Oliveira and J.A. Gomes Neto, At. Spectrosc. 27, 179 (2006).
14. L.A. Currie, Anal. Chim. Acta 391, 105 (1999).