Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 709–713
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Simultaneous determination of cadmium and lead in medicinal plants by anodic stripping voltammetry

Mónica Cecilia Vargas Mamani[a], Luiz Manoel Aleixo✠, Mônica Ferreira de Abreu[b], Susanne Rath[a,*]

[a] *Institute of Chemistry, Department of Analytical Chemistry, State University of Campinas, P.O. Box 6154, 13084-971 Campinas, SP, Brazil*
[b] *Centro de Solos e Recursos Agroambientais, Instituto Agronômico, P.O. Box 28, 13001-970 Campinas, SP, Brazil*

Received 19 September 2003; received in revised form 18 November 2004; accepted 19 November 2004
Available online 29 December 2004

Abstract

A simple method for the simultaneous determination of Cd and Pb in medicinal plants by differential pulse anodic stripping voltammetry, using a hanging mercury drop electrode, was developed. The pre-concentration of the metals was performed in 0.8 mol L^{-1} HCl at −0.73 V for 180 s. The sample preparation was carried out by dry-ashing 1.0 g of finely pulverized plant samples for 2.5 h at 500 °C. The determination limit of the method was 0.12 and 0.010 mg kg^{-1} for Pb and Cd, respectively. The method was applied to the quantification of cadmium and lead in samples of *Hypericum perforatum*, *Mikania guaco*, *Mikania glomerata* and *Peamus boldus*. The voltammetric method was shown to be useful for the control of contaminants in medicinal plants.

Keywords: Cadmium; Lead; Heavy metals; Medicinal plants; Stripping voltammetry

1. Introduction

Medicinal plants are consumed worldwide for the treatment of several diseases and are important raw materials for the pharmaceutical industry for the production of phytopharmaceuticals. In recent decades the use of phytopharmaceuticals and herbal medicines has increased worldwide, for several reasons, among them, that side-effects are often lower than those presented when synthetic drugs are employed, as well as due to the higher costs of many conventional pharmaceutical formulations [1].

As with other vegetation, medicinal plants are composed of many constituents and present great variability due to different growth, harvest, drying and storage conditions. Furthermore, they can be contaminated, as are other agricultural products, by pesticides, heavy metals and microorganisms [2]. Environment conditions in developing countries, pollution from irrigation water, the atmosphere and soil, sterilization methods and inadequate storage conditions [3] all play an important role in contamination of medicinal plants by heavy metals.

Ingestion of heavy metals through medicines and foods can cause accumulation in organisms, producing serious health hazards such as injury to the kidneys, symptoms of chronic toxicity, renal failure and liver damage [4,5]. Metals are probably the oldest toxins known to man.

The World Health Organization (WHO) has established standards for the quality control of medicinal plants including the classification, botanical identification, determination of active principles and identification of contaminants [6]. The WHO recommends qualitative and quantitative assays of heavy metals in phytotherapeutics, especially in raw materials of doubtful origin and plants produced by intensive agricultural means [7].

Several analytical methods have been reported for the quantitative determination of Pb and Cd in food,

* Corresponding author. Tel.: +55 19 37883084; fax: +55 19 37883023.
E-mail address: raths@iqm.unicamp.br (S. Rath).
✠ Deceased.

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.11.049

environmental and pharmaceutical matrices, including spectroscopic and electroanalytical methods. These methods comprise atomic absorption spectrometry [8–11], inductively coupled plasma optical emission spectroscopy [12], anodic stripping voltammetry [13], cathodic stripping voltammetry [14] and adsorptive voltammetry [15]. The electroanalytical methods present high selectivity and excellent detectability for the quantification of trace metals in complex matrices.

This paper describes the development of a simple method using anodic stripping voltammetry for the simultaneous determination of Cd and Pb in medicinal plants. The proposed method was applied to the determination of Cd and Pb in samples of *Hypericum perforatum*, *Mikania guaco*, *Mikania glomerata* and *Peamus boldus*, plants widely used in Brazil.

2. Experimental

2.1. Solutions and reagents

The reference samples R1 (sample 100, Grass GR94—*poaceae*) and R2 (sample 119, Rosa Plant, *Rose L.*) were furnished by the Agronomic Institute of Campinas (IAC) and were from the Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL), Plant Sample Exchange Programme (PSEP) January–March, 2001.

All chemicals used were of analytical-reagent grade. All solutions were prepared with water obtained from a Milli-Q purification system. Heavy metal standard stock solutions (1000 mg L^{-1}) of cadmium and lead were provided by TECLAB (São Paulo, Brazil).

2.2. Sample preparation

2.2.1. Sample preparation by dry-ashing

Samples of *H. perforatum*, *M. guaco*, *M. glomerata* and *P. boldus* were purchased from Homeopathic Pharmacies located in São Paulo, Brazil.

About 1.0 g of powdered vegetal material was placed in a porcelain crucible and mineralized in a muffle furnace for 2.5 h at 500 °C. After cooling, the ashes were humidified with water, dissolved with 2 mL of 6 mol L^{-1} HCl and the suspension was heated until complete evaporation of the liquids. The residue was dissolved in 5 mL of 2.0 mol L^{-1} HCl, heated and filtered. The filtered residue was washed with 5 mL of 2.0 mol L^{-1} HCl. The combined filtrates were diluted with water to 25 mL.

2.2.2. Wet digestion with HNO_3/H_2O_2

This treatment of the sample was based on the papers of Mingorance et al. [16] and Rodushkin et al. [17], with some modifications. Finely powdered leaf samples (1.0 g) were digested in concentrated HNO_3 (10 mL) and 30% (v/v) H_2O_2 (1 mL) for 2 h at 120 °C. The solution was evaporated to reduce the volume and then was cooled. The digested material was filtered and diluted to 25 mL.

2.3. Voltammetric determination of Cd and Pb in samples

A volume of 10 mL of the sample solution from the sample preparation was transferred to the voltammetric cell. After deaeration, Cd and Pb were determined by differential pulse anodic stripping voltammetry (DPASV), through the standard addition method. The voltammetric parameters comprise: deposition potential, −0.73 V; deposition time, 180 s; stirring speed, 300 rpm; scan rate, 5 mV s^{-1}; pulse amplitude, 50 mV and pulse duration, 40 ms. The current–potential curves were registered in the potential intervals of −0.54 to −0.29 V and −0.73 to −0.54 V, for Pb and Cd, respectively.

2.4. Instrumentation

All voltammetric measurements were performed using a Radiometer Copenhagen Polarograph, model POL 150, connected to a Radiometer Copenhagen stand, model MDE 150. A hanging mercury drop electrode (HMDE) and a platinum wire were used as working and counter electrodes, respectively. All potentials were recorded against an Ag/AgCl, KCl_{sat} reference electrode. Pure N_2 was bubbled through the sample solutions for 400 s before the measurements. The voltammetric cell was decontaminated in 6 mol L^{-1} HNO_3.

3. Results and discussion

Hydrochloric acid and ammonium citrate have been widely recommended for the determination of several heavy metals by anodic stripping voltammetry [18–20]. It was verified that in both supporting electrolytes, 0.10 mol L^{-1} HCl and 0.10 mol L^{-1} (pH 3) ammonium citrate, the corresponding peaks for Pb and Cd were well defined. However, the current peak intensities for Pb and Cd were greater in the HCl supporting electrolyte than with the citrate buffer, even after changing the pH of the ammonium citrate from 3 to 5. In addition to the better detectability obtained with the HCl medium, this supporting electrolyte would be particularly convenient considering that, in sample preparation, this acid is employed to redissolve the residues obtained after the digestion procedure. Changes in the HCl concentration over the range of 0.010–1.0 mol L^{-1} did not affect the current intensity and the peak potentials of either peak. The current intensities increase with the deposition time between 60 and 240 s and, for a solution containing 20 ng mL^{-1} of Cd and Pb, no deviation from linearity was observed. A time of 180 s was chosen, considering detectability and analytical frequency.

After establishing the optimized conditions for the quantification of cadmium and lead, the influence of the vegetal sample matrix on the voltammetric determination was

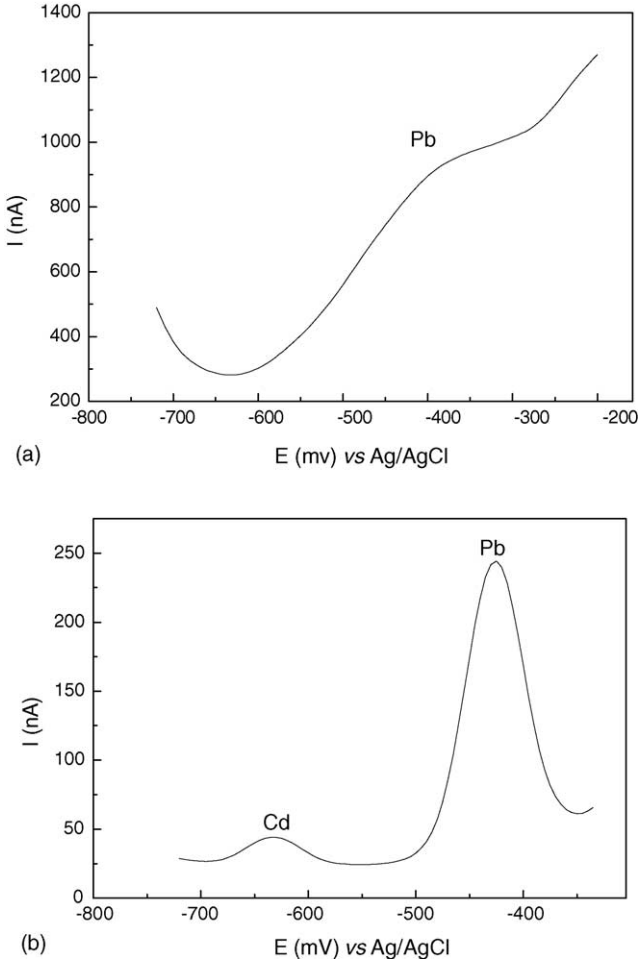

Fig. 1. Voltammograms for the determination of Cd and Pb in the reference material R1 employing: (a) wet digestion—HNO_3/H_2O_2; (b) dry-ashing in a muffle furnace. Experimental conditions: Supporting electrolyte, 0.8 mol L^{-1} HCl; t_d, 180 s; t_r, 15 s; ΔE, −50 mV and E_d, −730 mV; ν, 5 mV s^{-1}.

evaluated. It is well known that the complete mineralization of the sample is the most important requirement for accurate voltammetric analysis. Thus, two sample mineralization procedures were evaluated: (i) wet digestion with HNO_3/H_2O_2 and (ii) dry-ashing. For these studies the reference material R1 was employed and the voltammograms for the two processes are presented in Fig. 1.

It was verified that, when the plant sample was submitted to the wet digestion according to the procedure described in Section 2.2.2, the peaks corresponding to Cd and Pb are not well defined in the voltammogram (Fig. 1a) and the quantitation of these ions is not possible. This could be explained if the organic matter was not completely destroyed under this sample preparation procedure and some of these substances adsorb on the mercury surface during the pre-concentration step. Employing the dry-ashing procedure these interferences were not observed and the peaks for Cd and Pb were well defined (Fig. 1b). Furthermore, this procedure is simpler than the wet digestion, requires less sample manipulation, dispenses the use of acids and, consequently, is more adequate for routine analysis.

Table 1
Validation parameters

	Pb	Cd
Linear range (ng mL^{-1})	0.10–50	0.25–20
Linearity[a]	0.999	0.999
Sensitivity (nA mL ng^{-1})[b]	4.59 ± 0.05	9.89 ± 0.14
Repeatability (% R.S.D., $n = 10$) 20 ng mL^{-1}	2.14	0.28
Limit of detection (μg kg^{-1})[c]	36	3
Limit of quantification (μg kg^{-1})[c]	120	10

[a] Linearity is expressed by the correlation coefficient of the calibration graph.
[b] Average value with a confidence interval of 95%.
[c] Plant sample weight = 1.00 g.

With the experimental conditions for the voltammetric method established, the method was in-house validated for the Cd and Pb determination in medicinal plants using the following performance criteria: linearity and range, sensitivity, precision (repeatability), limit of detection (LOD), limit of quantitation (LOQ) and accuracy. The results are presented in Table 1.

The linearity, range and sensitivity were obtained from a calibration graph with five concentration levels, with triplicate analysis, employing 0.80 mol L^{-1} HCl as supporting electrolyte and a deposition time of 180 s at −730 mV.

The repeatability of the method was evaluated through the relative standard deviation of replicate measurements of a solution with a concentration of 20 ng mL^{-1} (within-run precision).

The detection limit ($LOD = k\, s_{y/x}/m$) was calculated according to Miller and Miller [21], where $k = 3$, $s_{y/x}$ is the residual standard deviation of the regression line and m is the slope of the calibration graph. The quantitation limit (LOQ) was calculated through the same equation as for the detection limit, however the value of k was taken as 10. Considering a sample of 1.0 g of the vegetal sample, the determination limit was 0.12 mg kg^{-1} for Pb and 0.010 mg kg^{-1} for Cd.

The accuracy of the method was evaluated by the determination of Pb and Cd in two reference material samples (R1 and R2). The results obtained are presented in Table 2. The mean values obtained by the proposed method and the reference values for both samples do not differ significantly ($P < 0.05$).

The validated voltammetric method was employed for the quantitation of Pb and Cd in the following medicinal plants: *H. perforatum*, *M. guaco*, *M. glomerata* and *P. boldus*. All determinations were carried out by the standard addition method. The results obtained in these heavy metal determinations are summarized in Table 3.

The results indicate that the highest mean level of Pb (11.4 mg kg^{-1}) and Cd (1.08 mg kg^{-1}) were found in *H. perforatum* samples from Bulgaria and China, respectively. The heavy metal content in plants depends on anthropogenic con-

Table 2
Results of the voltammetric analysis of Pb and Cd in the samples of reference R1 and R2

Metals	Reference value ($mg\,kg^{-1}$)	Determined value using ASV ($mg\,kg^{-1}$)	
		s	$X \pm t/(n)^{1/2}$
Cd	0.085 ± 0.022[a]	0.011	0.08 ± 0.02
	0.120 ± 0.013[b]	0.006	0.12 ± 0.01
Pb	1.259 ± 0.343[a]	0.156	1.2 ± 0.2
	2.126 ± 0.196[b]	0.015	2.17 ± 0.03

s: Estimated standard deviation ($n = 5$), X: average value, t: Student's t ($P < 0.05$).
[a] Reference material R1.
[b] Reference material R2.

tributions, environmental factors and on the plant species. The *H. perforatum* samples analyzed presented higher levels of Pb and Cd than the *P. boldus* and *Mykania* samples. The high Pb and Cd levels encountered in the *H. perforatum* samples corroborate with other studies that this plant is a potent metal bioaccumulation species. The lowest levels of these metals determined in samples of *P. boldus* were 0.27 and $0.007\,mg\,kg^{-1}$, of Pb and Cd, respectively.

Comparing the results obtained in this research with results found in the literature, the lead concentration is near to that reported by Pluta et al. ($10.02\,mg\,kg^{-1}$) [8] for an extract of medicinal plants from Poland. The values of Cd are about three times the values reported by Abou-Arab et al. [4] and Kim et al. [22] for medicinal plants cultivated in locations without pollution.

The establishment of the maximum tolerable limit for the heavy metal content in medicinal plants has been the object of discussion in the recent years. Some authors have suggested the employment of the maximum limits established for food products, others recommend the guidelines of the Pharmacopoeias. The US Pharmacopoeia establishes a maximum limit of 0.005%, i.e. $50\,mg\,kg^{-1}$, for Pb in medicinal plants. The World Health Organization indicates that the content in phytotherapeutic formulations should not exceed 10 and $0.3\,mg\,kg^{-1}$ for Pb and Cd, respectively. Considering these recommendations, one sample of *H. perforatum* analyzed herein would exceed these tolerance limits.

Table 3
Results obtained in the determinations of Cd and Pb in some medicinal plants

Medicinal plant	Origin	Content of Cd and Pb ($mg\,kg^{-1}$) $X \pm t/\sqrt{n}$	
		Cd	Pb
H. perforatum	Bulgaria	0.27 ± 0.05	11 ± 3
	China	1.1 ± 0.2	1.46 ± 0.07
	China	0.56 ± 0.01	3.8 ± 0.9
P. boldus	Chile	0.014 ± 0.005	0.7 ± 0.4
	Chile	0.009 ± 0.002	0.27 ± 0.04
	Brazil	0.007 ± 0.005	0.33 ± 0.06
M. guaco	Brazil	0.12 ± 0.02	1.2 ± 0.4
M. glomerata	Brazil	0.21 ± 0.04	0.43 ± 0.04
M. guaco	Brazil	0.13 ± 0.02	0.5 ± 0.1

X: average value ($n = 5$), t: Student's t ($P < 0.05$), s: estimate of the standard deviation, n: number of determinations.

Nevertheless, the estimated weekly intake of the metals, considering the consumption of the therapeutic dose of the medicinal herbs under study, did not exceed the provisional tolerable weekly intake (PTWI) of 25 μg lead/kg body weight [23] and 7 μg cadmium/kg body weight [24], established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). However, human exposure to lead and cadmium from the diet and drinking water can reach up to 60% of their respective PTWI in some regions and, consequently, the possible contribution from medicinal herbs might become important [25].

These results reinforce the need for harmonization of the threshold limits of heavy metals in medicinal plants as well as and the importance of quality control of these heavy metals in phytotherapeutic formulations.

4. Conclusions

Differential pulse anodic stripping voltammetry was shown to be a suitable method for Pb and Cd determination in medicinal plants. Sample preparation is an important step to be considered and dry-ashing was shown to be adequate for organic matter destruction prior to the voltammetric determination of these heavy metals.

The highest Pb and Cd levels were determined in a sample of *H. perforatum*, results which corroborate with other studies that this species is a potent metal bioaccumulator. The heavy metal content in medicinal plants could be due to anthropogenic sources or environmental factors, as well as to the plant species.

The results obtained in this work reinforce the need of harmonization of the tolerance limits of heavy metals in medicinal plants in order to permit an adequate quality control of phytotherapeutics to assure safety and quality and indicate the need for a systematic control of toxic heavy metals in medicinal plants.

Acknowledgements

The authors acknowledge the support of this research by FAPESP and thank Professor Carol Collins for English language revision.

References

[1] S.M.K. Rates, Toxicon 39 (2000) 603–613.
[2] L. Kabelitz, Pharmazeut. Ind. 60 (1998) 444–451.
[3] World Health Organization (WHO), Quality Assurance of Pharmaceuticals. A Compendium of Guidelines and Related Materials. Good Manufacturing Practice and Inspection, vol. 2, WHO, Geneva, 1999.
[4] A.A.K. Abou-Arab, K.M. Soliman, M.E. El Tantawy, B.R. Ismail, K. Naguid, Food Chem. 67 (1999) 357–363.
[5] L.S. Goodman, A.G. Gilman, As Bases Farmacológicas da Terapêutica, eighth ed., Guanabara Koogan, Rio de Janeiro, 1990.

[6] World Health Organization (WHO), Monographs on Selected Medicinal Plants, vol. 1, WHO, Geneva, 1999.
[7] World Health Organization (WHO), Quality Assurance of Pharmaceuticals. A Compendium of Guidelines and Related Materials, vol. 1, WHO, Geneva, 1997.
[8] J. Pluta, B. Figura, K. Lorenz, L. Wendt, Pharmazie 39 (1984) 63.
[9] I. Baranowska, K. Srogi, A. Wlochowicz, K. Szczepanik, Pol. J. Environ. Stud. 11 (2002) 467–471.
[10] M. Tuzen, Anal. Lett. 35 (2002) 1667–1676.
[11] I. Karadjova, S. Girousi, E. Iliadou, I. Stratis, Mikrochim. Acta 134 (2000) 185–191.
[12] E. Vassileva, N. Furuta, Fresenius J. Anal. Chem. 370 (2001) 52–59.
[13] M.M. Ghoneim, A.M. Hassanein, E. Hammam, A.M. Beltagi, Fresenius J. Anal. Chem. 367 (2000) 378–383.
[14] C. Locatelli, G. Torsi, Talanta 50 (1999) 1079–1088.
[15] Z. Ali, A. Zuhri, W. Voelter, Fresenius J. Anal. Chem. 360 (1998) 1–9.
[16] M.D. Mingorance, L.M. Peréz-Vasquez, M.J. Lachica, Anal. At. Spectrom. 8 (1993) 853–858.
[17] I. Rodushkin, T. Ruth, A. Huhtasaari, Anal. Chim. Acta 378 (1–3) 4 (1999) 191–200.
[18] W. Wasiak, W. Ciszewska, A. Ciszewski, Anal. Chim. Acta 335 (1996) 201–207.
[19] J.L. Han, H.Y. Chen, H. Gao, Electroanal. 5 (1993) 619–622.
[20] U. Greulach, G. Henze, Anal. Chim. Acta 306 (1995) 217–2223.
[21] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 3a ed., Ellis Horwood Limited, Chichester, England, 1993.
[22] B.Y. Kim, K.S. Kim, S.H. Lee, S.H. Yoo, J. Agric. Sci. Soil Fertilizer 36 (1994) 310–330.
[23] JECFA, Joint FAO/WHO Expert Committee on Food Additives, 55th Meeting, Summary and Conclusions, World Health Organization, Geneva, 2000.
[24] JECFA, Joint FAO/WHO Expert Committee on Food Additives, 55th Meeting, Summary and Conclusions, World Health Organization, Geneva, 2003.
[25] R.V. Bath, G.G. Moy, Monitoring and Assessment of Dietary Exposure to Chemical Contaminations, Rapport Triemestriel de Statistiques Sanitaires Monidales 50 (1997) 132–148.