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Simultaneous determination of Cd and Pb in antibiotics used in sugar-cane fermentation process by GFAAS

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Abstract: A method has been developed for the simultaneous determination of Cd and Pb in antibiotics used in sugar-cane fermentation by GFAAS. The integrated platform of transversely heated graphite atomizer was treated with tungsten to form a coating of tungsten carbide. Six samples of commercial solid antibiotics were analyzed by injecting 20 μL of digested samples into the pretreated graphite platform with co-injection of 5 μL of 1000 mg L^{-1} Pd as chemical modifier. Samples were mineralized in a closed-vessel microwave-assisted acid-digestion system using nitric acid plus hydrogen peroxide. The pyrolysis and atomization temperatures of the heating program of the atomizer were selected as 600°C and 2200°C, respectively. The calculated characteristic mass for Cd and Pb was 1.6 pg and 42 pg, respectively. Limits of detection (LOD) based on integrated absorbance were 0.02 μg L^{-1} Cd and 0.7 μg L^{-1} Pb and the relative standard deviations (n = 10) for Cd and Pb were 5.7% and 8.0%, respectively. The recoveries of Cd and Pb added to the digested samples varied from 91% to 125% (Cd) and 80% to 112% (Pb).

Keywords: cadmium; lead; antibiotic; permanent modifier; GFAAS.

Introduction

The efficiency of ethanol production by sugar-cane fermentation is high dependent on bacterial contamination degree. Selective antibiotics have been available to control foreign bacteria in fermentation process of ethanol production without affecting the performance of the yeasts [1].

The accurate determination of trace inorganic contaminants in agro industrial antibiotics is very important since these products are employed in large scale in fermentation processes and can contaminate the sugar, ethanol or final residues which are either usually released to crop areas or used as ingredient in animal food. Among inorganic contaminants, cadmium and lead are of public health interest due to their high toxicity after accumulation in multiple organs in the human body [2]. As inorganic contaminants are usually present at low levels in most samples, sensitive analytical techniques are desirable, such as graphite furnace atomic absorption spectrometry (GFAAS). This is a suitable and widely used technique for the determination of trace elements due to its high selectivity, sensitivity and ability to directly determine several analytes in different samples [3]. Besides solid or slurry sampling is a reality in GFAAS, some samples require solubilization before analysis.

Microwave-assisted sample preparation has been used for solubilization of a large variety of workable samples. It presents among main

advantages high performance, security, short waiting time, low contamination and minimum analyte losses [4]. The main benefits of microwave-assisted sample digested with dilute acid for GFAAS include reduced blank values, lower reagent consumption, risky to the analyst and aggressive media for the platform of the graphite tube, mainly when the platform is pretreated with permanent modifiers [5].

Among advantages of using permanent modifiers are the reducing in time required for sample dispensing, leading to simpler and faster heating programs for GFAAS; lowering modifier blanks due to the elimination of volatile impurities during graphite treatment, resulting in better detection limits, longer signal term stability, reducing the number of recalibrations during routine analysis; and remarkable improvement in the tube lifetime, lowering analytical costs [6]. Reports of simultaneous determination of Cd and Pb by GFAAS are found in the literature [2, 7-12]. However, little attention has been given to the use of permanent modifiers in simultaneous determination. Likewise, reports of preparation of antibiotic samples for determination of Cd and Pb are lacking in the literature.

This study reports on a development of a simple and fast method for the simultaneous determination of Cd and Pb in antibiotics by GFAAS using a tungsten coating on platform of graphite tube with co-injection of palladium nitrate as modifier. Samples were pre-treated in a closed microwave oven system using concentrated or diluted nitric acid plus hydrogen peroxide.

Materials and methods

Apparatus

A Perkin-Elmer SIMAA™ 6000 simultaneous graphite furnace atomic absorption spectrometer with longitudinal Zeeman-effect background corrector and an AS-72 autosampler were used. The instrumental conditions applied were the recommended by the manufacturer. End-capped THGA tubes with integrated platforms were used in all measurements. High-purity argon (99.999%, White Martins, Brazil) was used as the purge gas. The experiments were carried out under STPF conditions [13]. The heating program of atomizer used for the simultaneous determination of Cd and Pb is shown in Table 1.

An Anton Paar Multiwave microwave oven equipped with 6 pressure decomposition TFM vessels of 50-mL capacity was employed for antibiotic digestion.

Reagents, Reference Solutions, and Samples

High-purity water (resistivity 18.2 MΩ.cm) obtained by using a Millipore Milli-Q academic™ deionizer system was used throughout the work.

Nitric acid and hidrogen peroxide Suprapur® (Merck, Damstadt, Germany) were used throughout for preparing the solutions and samples.

Tungsten stock solution (1.0 g L^{-1} W) was prepared by dissolving 0.1794 g $Na_2WO_4.2H_2O$ (Merck, Darmstadt, Germany) in 100 mL of water.

Palladium nitrate solution (1.0 g L^{-1} Pd) used as chemical modifier was prepared by appropriate dilution of the 10.0 g L^{-1} Pd stock solution (Merck, Darmstadt, Germany).

Table 1. Heating program* of atomizer** for the simultaneous determination of Cd and Pb in antibiotics

Step	Temp. (^oC)	Ramp time (s)	Hold time (s)	Gas flow (mL min^{-1})
1	110	5	25	250 (Ar)
2	130	10	20	250 (Ar)
3	600	5	10	250 (Ar)
4	2200	0	5	0 (reading)
5	2400	1	3	250 (Ar)

* Total time: 84 s. ** Injection temperature: 20°C; sample injection speed: 100%.

Pyrolysis and atomization curves were built-up after measuring integrated absorbance of a 0.2% v/v HNO_3 solution spiked with 2.0 μg L^{-1} Cd and 10 μg L^{-1} Pb.

Five multielement calibration solutions within 0.5-2.5 μg L^{-1} Cd and 5.0-25.0 μg L^{-1} Pb were prepared daily in 5% v/v HNO_3 by diluting the individual 1000 mg L^{-1} stock solutions (Carlo Erba, Normex®). For each measurement, a volume of 20 μL of sample or reference solution was used together with 5 μL Pd modifier solution. All measurements of integrated absorbance were made at least in triplicates.

Solid antibiotics samples (m ~ 0.25 g) were digested in a closed vessel microwave oven system by two procedures: a) 3 mL of concentrated HNO_3 plus 2 mL of H_2O_2 30% (v/v) and b) 4 mL of HNO_3 20% (v/v) plus 1 mL of H_2O_2 30% (v/v). The digests were transferred for a 10 mL volumetric flask and topped up with deionized water. The optimized heating program used in the sample decomposition is describe elsewhere: (step 1: 100-400 W for 3 min; step 2: 400-800 W for 3 min; step 3: 1000-1000 W for 4 min and step 4: 0-0 W for 10 min).

The graphite platform was recoted with tungsten automatically by using the facilities provided by the original software of the autosampler and graphite furnace. A mass of 250 μg W was termally and sequentially deposited on the integrated platform as described elsewhere [14,15].

Results and discussion

Pyrolysis and atomization curves were carried out in order to define the compromise conditions for simultaneous determination of Cd and Pb, since in simultaneous detection the heating program of the atomizer is the same for all analytes. The pyrolysis temperature is limited by the most volatile element, but should ideally be as high as possible in order to assurance the matrix elimination. On the other hand, the atomization temperature should be lower as possible to avoid faster graphite tube deterioration, but it is limited by the more refractory element. Besides the heating program, the modifiers, the flow rate and composition of purge gas and the restricted linear response interval of GFAAS are among other parameters that may taken into consideration in the simultaneous detection [2]. Nevertheless, several works have been published on literature on simultaneous determination of different analytes in wide-ranging samples [16,17].

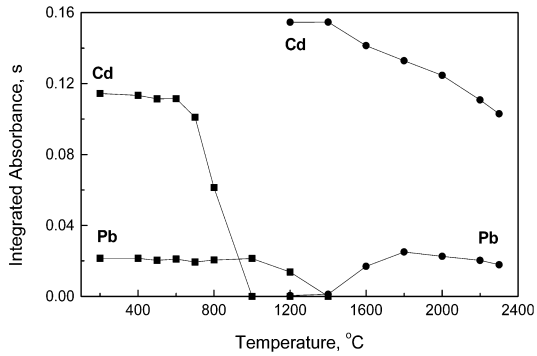

Figure 1. Pyrolysis (n) and atomization (h) curves obtained simultaneously for 40 pg Cd and 200 pg Pb in HNO_3 medium using 250 μg W permanent coating plus co-injection of 5 μg Pd as modifier.

Shown in Figure 1 are the pyrolysis and atomization temperature curves for 40 pg Cd and 200 pg Pb in 0.028 mol L^{-1} HNO_3 medium using platform of graphite tube pre-treated with 250 μg W and co-injection of 5 μL of Pd modifier solution. The pyrolysis temperature chosen was 600°C (limited by Cd) while the atomization temperature was fixed at 2200°C. Regardless of lower sensibility for Cd, this temperature furnished well defined and narrowest peak profile for Pb and Cd (Figure 2).

It should be commented that platinum group metals – PGM (Pd, Ir, Rh and Ru) were also tested as modifiers co-injected on W pre-treated platform. Slight differences in atomic peak profiles were observed, but there was an evident increase in the sensibility for Pb and Cd when co-injecting palladium. This was attributed to an increased catalytic action of the W + Pd mixture in the matrix degradation, confirmed by an accentuated decrease in the backgound values. No specific study was done about the lifetime of the pre-treated tube, but it remained proper for use even after *ca*. 820 firings.

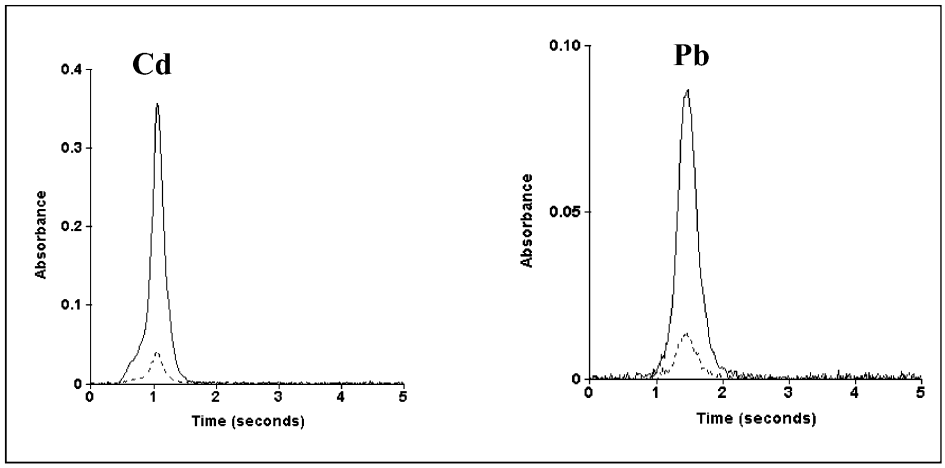

Figure 2. Absorbance (solid lines) and background (dotted lines) peak profiles for 40 pg Cd and 200 pg Pb in nitric acid using 250 μg W permanent modifier + 5 μg Pd co-injected modifier. The heating program of Table 1 was employed throughout.

Using 600°C and 2200°C as pyrolysis and atomization temperatures, respectively, the calculated characteristic mass (m_o) values were 1.6 pg Cd and 42 pg Pb, respectively. These values are slightly higher when compared with typical m_o values for single-element conditions for THGA furnace (1.3 pg Cd and 30 pg Pb using $NH_4H_2PO_4/Mg(NO_3)_2$ as modifiers) [18]. Comparing with other works found in literature for simultaneous determination of Cd and Pb in different matrices such as wine [12] and foodstuffs [2], and using different mixtures of modifiers [$Pd(NO_3)_2$ + $Mg(NO_3)_2$ and $NH_4H_2PO_4/Mg(NO_3)_2$, respectively] it was observed a small deterioration in sensitivity mainly for Pb. This reveals that the use of a W-coated platform with Pd co-injection did not impair the simultaneous determination of Cd and Pb in antibiotics. Although the small decrease of the Pb sensitivity observed in this work, the main advantage found was the increase lifetime of the graphite tube in *ca*. four times.

The calculated limit of detection (LOD) for cadmium (0.02 μg L^{-1}) and lead (0.7 μg L^{-1}) was calculated as three times the standard deviation (n = 20) of the blank solution by the slope of the calibration curve [19, 20]. The typical relative standard deviations were 5.7% and 8.0% for Cd and Pb, respectively.

The determination of analytes was carried out in the antibiotic samples digested by the two procedures studied (concentrated and 20% v/v nitric acid). The levels of analytes in all samples were always lower than the LOD. The accuracy was then evaluated by addition / recovery tests. Six digested antibiotic samples were spiked with 1.0 μg L^{-1} Cd and 15 μg L^{-1} Pb. Recoveries varied from 91% to 125% Cd and 80% to 97% Pb for concentrated nitric acid and from 99% to 120% Cd and 82% to 112% Pb for diluted nitric acid. The choice of the diluted acid medium in the sample preparation is the best option, since it provides lower blank values and reagent consumption and reduced graphite tube deterioration.

The heating program of graphite tube takes 84s, but the total time of the cycle was *ca*. 120 s due to the extra time for sampling and data acquisition. For samples analyzed in routine works in duplicate, the analytical frequency is calculated as 30 determinations per hour.

Conclusions

This work presents a simple, fast and accurate method for the simultaneous determination of Cd and Pb by GFAAS in solid antibiotic

samples digested with diluted nitric acid. Exception to the slightly reduction in sensitivity for Pb, the performance of the tungsten coating plus co-injection of $Pd(NO_3)_2$ was similar to the $NH_2H_2PO_4 + Mg(NO_3)_2$ modifiers recommended for Cd and Pb in single-element conditions or when analyzing Cd and Pb simultaneously in several types of matrices and modifiers mixtures. Nevertheless, the W-coating increased considerably the lifetime of the graphite tube in almost four times.

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V. R. Amorim Filho, W. L. Polito, S. R. Oliveira, G. P. G. Freschi, J. A. Gomes Neto. Determinação simultânea de Cd e Pb em antibióticos usados no processo de fermentação da cana de açúcar por GFAAS.

Resumo: Um método foi desenvolvido para a determinação simultânea de Cd e Pb em antibióticos usados na fermentação da cana de açúcar por GFAAS. A plataforma integrada do atomizador de grafite aquecido transversalmente foi tratada com tungstênio, formando um depósito de modificador permanente de carbeto de tungstênio. Seis amostras de antibióticos sólidos comerciais foram analisadas injetando-se 20 μL das amostras digeridas em meio ácido (sistema de forno de microondas fechado) dentro da plataforma de grafite pré-tratada seguido de 5 μL do modificador químico Pd 1000 mg L^{-1}. As amostras foram digeridas em frascos fechados com auxílio de radiação microondas empregando ácido nítrico concentrado e peróxido de hidrogênio. As temperaturas de pirólise e atomização do programa de aquecimento do atomizador foram fixadas em 600°C e 2200°C, respectivamente. As massas características calculadas para cádmio e chumbo foram 1,6 pg e 42 pg, respectivamente. Limites de detecção (LOD) baseados na absorbância integrada foram 0,02 μg L^{-1} Cd e 0,7 μg L^{-1} Pb e os desvios padrões relativos (n = 10) para Cd e Pb foram 5,7% e 8,0%, respectivamente. As recuperações de Cd e Pb adicionadas às amostras digeridas variaram de 91% a 125% (Cd) e 80% a 112% (Pb).

*Palavras-chave***:** cádmio; chumbo; antibiótico; modificador permanente; GFAAS.

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