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# A new flow injection preconcentration method based on multiwalled carbon nanotubes for the ETA-AAS determination of Cd in urine

Jennifer Álvarez Méndez, Julia Barciela García, Rosa M. Peña Crecente, Sagrario García Martín, Carlos Herrero Latorre*

*Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias, Universidad de Santiago de Compostela, Campus de Lugo, 27002 Lugo, Spain*

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ABSTRACT

A new flow injection (FIA) procedure for the preconcentration of cadmium in urine using multiwalled carbon nanotubes (MWCNT) as sorbent and posterior electrothermal atomization atomic absorption spectrometry (ETA-AAS) Cd determination has been developed. Cadmium was retained in a column filled with previously oxidized MWCNTs and it was quantitatively eluted with a nitric acid solution. The parameters influencing the adsorption–elution process such as pH of the sample solution, amount of sorbent and flow rates of sample as well as eluent solutions have been studied. Cd concentration in the eluent was measured by ETA-AAS under the optimized conditions obtained. The results indicated the elimination of urine matrix effect as a consequence of the preconcentration process performed. Total recovery of cadmium from urine at pH 7.2 using a column with 45 mg of MWCNTs as sorbent and employing a $HNO_3$ 0.5 mol $L^{-1}$ solution for elution was attained. The detection limit obtained was 0.010 μg $L^{-1}$ and the preconcentration factor achieved was 3.4. The method showed adequate precision (RSD: 3.4–9.8%) and accuracy (mean recovery: 97.4–100%). The developed method was applied for the determination of cadmium in real urine samples from healthy people (in the range of 0.14–2.94 μg $L^{-1}$) with satisfactory results.



## 1. Introduction

Cadmium is a toxic metal without known positive physiological role in the human body, this element has been related to different damages in lung, kidneys and bones, according to the type of exposure [1]; cadmium accumulates in the liver and kidneys, and its half-life in this last one range between one and four decades. Tobacco smoking and diet are the main sources of cadmium for people unexposed to it in the workplace. High levels of Cd were associated with damages in central nervous system, injuries in immune system, fertility disorders and the occurrence of different types of cancer [2]. In addition other studies indicate a possible role for cadmium in coronary heart disease [3]. The amount of Cd in urine is correlated with its level in the kidney. Consequently, the urinary cadmium concentration is considered the best biomarker for total body burden past exposure [4,5], while cadmium levels in blood reflect both recent and cumulative exposures. Since Cd determination in environmental, biological materials and human body is an important screening procedure for the studies of environmental pollution and occupational exposure [6], therefore, urine samples become a common target to develop analytical methods for Cd measurement [7]. However, the very low analyte concentrations present in urine ([Cd] < 1 μg $L^{-1}$) as well as the severe matrix interferences due to urine complex composition make it difficult the correct determination of this element.

Inductively coupled plasma-mass spectrometry (ICP-MS) and electrothermal atomization atomic absorption spectrometry (ETA-AAS) appear as the most common analytical techniques for Cd determination in urine samples. ICP-MS allow detecting cadmium at nanograms per litter levels, however, it presents mass spectral interferences by molybdenum oxide (MoO) generated in hot plasma [8]. ETA-AAS provides enough sensibility for the determination of normal concentrations of these elements in urine (0.3–1.2 μg $L^{-1}$ for cadmium). Nevertheless, the determination of the element is a difficult task due to the complex composition of this type of sample, which contains considerable amounts of a high number of organic and inorganic components. Chlorides and phosphates, which occur in urine in large quantities, suppress the absorption of cadmium (chemical or matrix effects) and both causes considerable non-selective absorption. The use of diluted or pretreated samples, stabilized temperature-platform furnace (STPF) conditions, different matrix modifiers and Zeeman background correction may decrease or suppress both chemical effects and non-selective absorption. However, in a high number of cases,

* Corresponding author. Tel.: +34 982824064; fax: +34 982285872.
*E-mail address:* carlos.herrero@usc.es (C.H. Latorre).

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this approach is not enough to eliminate all the problems in the urine measurements of Cd [9].

The extraction of cadmium from urine provides a way to concentrate the metal and simultaneously to remove it from the complex urine matrix, separation techniques for Cd removal include solvent extraction and solid phase extraction [4]. In the last few years, solid phase separation/preconcentration systems using flow injection (FI) are often chosen owed to their attractive features that involve lower risk of contamination, high pre-concentration factors and the easy possibility of coupling to spectro-analytical techniques [10]. Several type of materials have been reported as solid sorbent to construct on-line sorption systems for cadmium determination such as chelating resins with selective functional groups covalently attached to copolymer matrices, bonded silica with octadecyl groups (C18), activated carbon, cation exchange resins, macroporous ion-exchange resins as well as alumina [6]. Recently, different preconcentration methods have been developed by sorption on multiwalled carbon nanotubes (MWCNTs), because they have a large specific surface area and an excellent adsorption capability [11]. MWCNT oxidized can be exploited to develop sorption systems for trace analysis on the basis of their high adsorption efficiency towards metal ions [12–16]. The hexagonal arrays of atoms in graphene sheets of carbon nanotubes (CNTs) surface have a strong interaction with other molecules or atoms, which make CNTs a promising sorbent material substituted by activated carbon in many ways [17]. Such materials have generated a great deal interest (owing to their exceptional chemical and physical properties) and their application for preconcentration purposes has been studied in a small period of time (from 2004 to present days), however the most of the preconcentration methods were applied to water samples [18–23] and only in a few scarce cases the developed procedures were directed to complex matrix such as biological samples [23–26].

Bearing in mind all these considerations and taking into account that no preconcentration method was developed for Cd in urine based on the use of MWCNT, the aim of this work was to develop a FI on-line solvent extraction for the separation and preconcentration of cadmium in a biological sample like urine and its subsequent determination by ETA-AAS. The developed preconcentration method will be comparatively studied with other optimized method for the direct determination of Cd in urine. The applicability of MWCNTs packed into a microcolumn as solid-phase extractant for Cd preconcentration as well as the effectiveness and efficiency of the proposed FIA solvent extraction method will be demonstrated by measuring real urine samples from unexposed people.

## 2. Experimental

### 2.1. Instrumentation

An atomic absorption spectrometer, Varian-SpectrAA-600 with Zeeman correction, equipped with a Varian GTA-100 electrothermal atomizer linked to an automatic sample dispenser was used for this work. Measurements were performed using a Cd Varian Hollow Cathode Lamp operating at 238.8 nm, with a current intensity of 10 mA. The bandwidth was 0.5 nm in all cases. Argon was employed as inert gas at a flow equal to 3 mL $min^{-1}$. Pyrolitic graphite-coated tubes with platform atomization were used.

A FIA system comprised a Gilson Minipuls-3 peristaltic pump, equipped with Tygon pump tubing of 1.42 mm i.d. (Ismatec), was used to propel both sample and reagents. PTFE connecting tubing of 0.5 mm i.d. and various end-fittings and connectors (Omnifit) were employed. These connections were kept as short as possible to minimize the dead volumes. The FIA manifold, that was represented in Fig. 1, was designed to be as simple as possible in order to allow the fastest sample throughput.

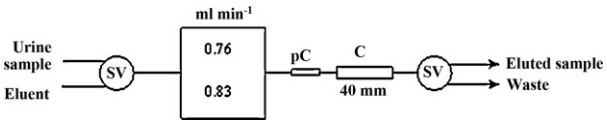


**Fig. 1.** Scheme of the FIA system employed in the present work. SV, selection valve; pC, precolumn; C, column.

### 2.2. Reagents

Cadmium stock standard solution (1.0 g $L^{-1}$) was obtained from Panreac (Barcelona, Spain) and diluted as necessary to obtain the working standard solutions. Palladium and magnesium nitrate and ammonium dihydrogenphosphate were obtained from Fluka (Switzerland), the working solutions were prepared by diluting the appropriate amount of a 10 g $L^{-1}$ stock solution. Wolfram permanent modifier solutions (1.0 g $L^{-1}$), was prepared from 179.4 mg $Na_2WO_4{\cdot}2H_2O$ powder (Sigma–Aldrich, Germany) dissolved in ultrapure water and diluted to 25 mL.

Untreated MWCNTs (purity > 95%, 10–20 nm o.d. and ~30 μm length) prepared using Chemical Vapour Deposition (CVD) of acetylene in hydrogen flow were supplied by Chengdu Organic Chemicals Co. Ltd., China. Before using, MWCNT were oxidized with $H_2SO_4$ and $KMnO_4$ according to the literature [27].

All sampler containers, autosampler cups and other materials were washed with nitric acid 10% (v/v) for a period of 24 h before rising with copious amount of ultrapure water and shaking dry prior to use. The cleaning solution employed to wash the sampling capillary contained 0.7% (w/v) $HNO_3$ and 0.2% (v/v) Triton X-100.

### 2.3. Statistical software

Plackett–Burmann and central composite designs as well as the surface and contour plots used in optimization of ETA-AAS programs were carried out using the statistical software package *Statgraphics 5.0 Plus*.

### 2.4. Urine samples

Different urine samples were collected into acid-washed polypropylene bottles from healthy people. These samples were treated as follows: 100 mL of each sample were acidified by addition of 0.35 mL of concentrated $HNO_3$ and they were diluted to 30% (v/v) before preconcentration and analysis. When the analysis was not carried out immediately the urine samples were stored at 4 °C in the darkness (the storage period was kept as short as possible).

### 2.5. Analytical procedures

#### 2.5.1. Direct ETA-AAS method for cadmium determination in urine

500 μL of urine were mixed in the autosampler cup with 100 μL of $HNO_3$ (0.5 M) and made up to 1000 μL with ultrapure water. 20 μL of this solution were introduced in the graphite tube and were subjected to ETA-AAS under the optimized conditions summarized in Table 1, using W as permanent modifier.

#### 2.5.2. ETA-AAS method for Cd determination in urine after preconcentration in MWCNT column

For an aliquot of urine sample treated as indicated in 2.4, the pH was adjusted to 7.2 using the appropriate amounts of NaOH or HCl solutions. 8 mL of this aliquot were passed through the microcolumn with a peristaltic pump at a 0.76 mL $min^{-1}$ flow rate.

**Table 1**
Furnace heating programs for ETA-AAS determination of Cd.

| Step | Temperature (°C) | | Ramp (s) | Hold (s) |
|---|---|---|---|---|
| | Urine | Eluent | Urine/eluent | Urine/eluent |
| Dry | 100 | 100 | 10 | 10 |
| Ash | 500 | 400 | 10 | 10 |
| Atomization | 1200 | 1100 | 4 | 4 |
| Clean | 2400 | 2400 | 2 | 1 |
| Cold | 40 | 40 | 20 | 15 |

Afterwards, retained cadmium ions were eluted employing 1.5 mL of $HNO_3$ 0.5 M (with a flow rate of 0.83 mL $min^{-1}$). 20 μL of the eluted solution sample were subjected to ETA-AAS under the optimized conditions indicated in Table 1 using a W-metallic layer deposited on the graphite tube inner surface on the L'vov platform as permanent modifier [28].

## 3. Results and discussion

### 3.1. MWCNTs pretreatment and microcolumn preparation

The sorption capabilities of metal ions by raw CNTs are very low but they are significantly increased after oxidation by different oxidizing agents, this fact could be explained because the tips of CNTs treated were opened and the fractures took place at the location where defects such as pentagons and heptagons existed after oxidation. Therefore, the oxidation process improves their dispersion and increases a large amount of oxygen-containing functional groups like —COOH, —OH, or —C=O on the surface site of CNTs. These functional groups cause a rise in negative charge on carbon surface and the oxygen atoms in functional groups donate single pair of electrons to metal ions, consequently the cation exchange capability was increased [29]. Different oxidizing agents and different kinds of energies (such as $HNO_3$ [12–17,19–27,30,31], $H_2SO_4$ [15], $HNO_3/H_2SO_4$ [15,32], and $H_2SO_4/KMnO_4$ [15,27]) were proposed in literature to pretreat the MWCNTs; it was also observed that there is no direct correlation between the metal ion sorption capabilities in relation with the specific surface area, pore specific volume and mean pore diameter. However, the sorption ability was demonstrated to be strongly dependent upon its total acidity surface [16].

In the present work, according to our previous experiences and following the literature [27], 0.5 g of MWCNTs were oxidized using a mixture $H_2SO_4$–$KMnO_4$ (10 mL $H_2SO_4$ 1 M and 0.5 g $KMnO_4$) and employing microwave energy (400 W) for 15 min. The total acidity surface of the oxidized MWCNTs obtained by this procedure was quantitatively measured using the Boehm's titration method [33]. An adequate total acidity surface was achieved (3.02 mmol $g^{-1}$) compared to other treatment methods (2.52–4.04 mmol $g^{-1}$). Furthermore, it should be considered that the use of the more aggressive oxidation treatments could produce higher surface total activity but it also causes the broken of CNTs, and the resulting pieces of carbon nanotubes could produce an obstruction and overpressure in the FIA system. In the present case, employing the chosen treatment with $H_2SO_4$–$KMnO_4$ and microwave energy, the mentioned problems were avoided.

The preconcentration microcolumn was made from a PTFE tube with a length of 40 mm and inner diameter of 4 mm. This column was packed with 45 mg of treated MWCNT, and plugged with a small portion of glass wool at both ends to avoid sorbent losses during the preconcentration/elution steps. Before use, a 1.0 M $HNO_3$ solution followed by Milli-Q water were passed through the column in order to clean and to condition it.

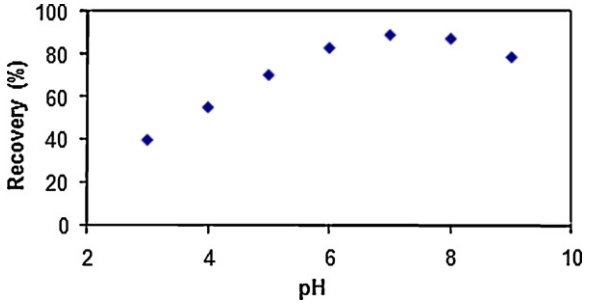


**Fig. 2.** Effect of pH on Cd recovery from MWCNT ($n = 3$): [Cd]: 1.0 μg $L^{-1}$.

### 3.2. Optimization of extraction/preconcentration step

#### 3.2.1. Effect of pH

After oxidation of carbon nanotubes, some active groups, such as —COOH and —OH, were formed on the surface of MWCNT. Hence, it is clear that the pH of the solution passed through the column affects the structure of the sorbent and degree of ionization and speciation of the adsorbates: the capability for ion adsorption on MWCNT is highly dependent of the pH [26]. The surface charge depends on the pH of the surrounding electrolyte, and there is a pH value, called "point of zero charge" (PZC), at which the surface has zero net charge [22]. When pH of the solution is higher than $pH_{PZC}$, the negative charge on the active groups on the surface provides favorable electrostatic interactions for the adsorption of cationic species. Therefore, in order to work at pH higher than $pH_{PZC}$ and to evaluate the effect of pH on the adsorption of cadmium ions on carbon nanotubes treated, a study of the Cd recovery at different pH values was conducted according to Stafiej and Pyrzynska [29]. A series of sample solutions containing the same Cd concentration (1 μg $L^{-1}$) were adjusted to a different pH in the range of 3.0–9.0 employing the appropriate amounts of different solutions of NaOH (0.1–0.01 M) and/or HCl (0.1–0.01 M). The obtained solutions were processed according to the recommended preconcentration procedure. The adsorption percentage was calculated on the basis of the difference between the amounts of cadmium in the starting solution and the one eluted from the column. The effect of pH on the cadmium recoveries is shown in Fig. 2. As it can be seen, Cd was poorly absorbed at pH < 4, and a quantitative recovery (higher than 90%) was obtained in the pH ranging from 6.0 to 8.0, pH values above 8.0 or higher showed again a low recovery of Cd. Thus, a pH value of 7.2 was selected as optimum for future extractions. Urine samples were not buffered because the extraction step was the only stage in which a pH control was necessary. Therefore, in all cases, the pH was adjusted to the optimum value of 7.2 immediately before the introduction of the urine sample in the column. In addition, the unemployment of buffer solutions avoids the introduction of more salts in such complex matrix as urine.

#### 3.2.2. Influence of sample flow rate

The retention of cadmium on the MWCNTs and the duration of the complete analysis are influenced by the sample flow rate through the column. The effect of sample flow rate on adsorption percentage of Cd was investigated by passing 8 mL of sample through the microcolumn with a peristaltic pump, at different flow rates comprised between 0.5 and 1.5 mL $min^{-1}$. The optimum recoveries for cadmium (higher than 90%) in the studied range were obtained at 0.76 mL $min^{-1}$. Higher flow rates produced an important decrease in the recovery level due to a diminution of the adsorption kinetics. Therefore, a flow rate of 0.76 mL $min^{-1}$ was selected as the optimum value in order to perform the quantitative extraction of cadmium.

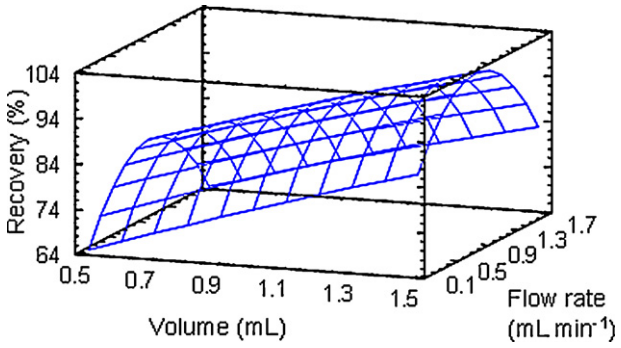


**Fig. 3.** Response surface for the effect of flow rate and volume eluent on the Cd recovery.

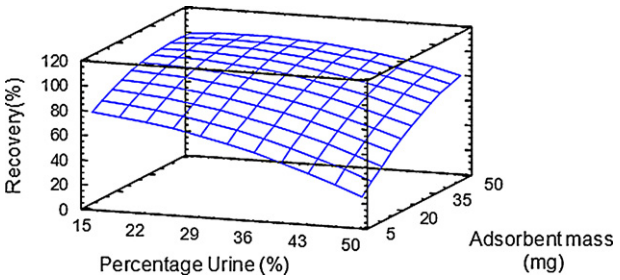


**Fig. 4.** Response surface for the effect of sorbent mass and percentage of urine on the Cd recovery.

### 3.2.3. Effect of elution parameters

Different mineral acid solutions (such as $HNO_3$, HCl or $H_2SO_4$) could be used to produce the elution of the metal ions from the preconcentration column. However, in the present work, nitric acid was directly selected for this task because it was demonstrated to be the most suitable eluent and also because it was proved that $HNO_3$ produces a very low *background* in subsequent ETA-AAS analysis [34]. For the complete elution of Cd from the column, the concentration of the eluent ($HNO_3$), flow rate and the volume required need to be optimized; therefore, a central composite $2^3$+ star design in 17 randomized runs was employed to optimize this three parameters. A statistical test was applied in order to evaluate the influence of the considered parameters by comparing the mean square against an estimate of the experimental error. From the results obtained it can be concluded that volume ($P = 0.0009$) and concentration ($P = 0.009$) of eluent, and its interaction ($P = 0.005$), presented $P$-values <0.05, indicating that they are significant influence in the analytical signal, at a 95% confidence level. On the basis of the response surfaces obtained from the central composite design previously described, the optimum values for the studied parameters were as follows: the eluent concentration (investigated in the range between 0.0 and 1.5 mol $L^{-1}$) was 0.5 mol $L^{-1}$; for the eluent volume it was found that bigger volumes produced better recovery percentages. 1.5 mL was chosen for future elutions because with this eluent volume 100% recovery was achieved. The flow rate of eluent was also studied in the range of 0.1–1.5 mL $min^{-1}$ and 0.83 mL $min^{-1}$ was selected as optimum. As it can be seen in Fig. 3, good recoveries can be obtained (~100%) using 1.5 mL of eluent with a flow rate of 0.83 mL $min^{-1}$.

### 3.2.4. Effect of urine factors (percentage of urine, sample solution volume) and sorbent mass

The preconcentration and analysis of urine samples using a MWCNT column was found to be more complex than in water samples. In order to prevent the column blocked or overpressure in FIA system, it was considered filtering the urine, but problems of recovery were arising. Better results were obtained when the samples were diluted, and when a precolumn with glass fiber to retain solid particulates of the sample was used. Keeping in mind that cadmium in urine sample is present in very low concentrations and with the target to improve the sensitivity of the procedure; the percentage of pure urine in the sample solution was studied. Also, the sample solution volume to be passed through the column to obtain better performance was evaluated. On the other hand, the increase of volume and percentage of urine is directly related to the quantity of MWCNTs packed in the column, higher percentage and volume of urine contain higher ion amounts that need higher mass of sorbent mass to be retained. Then these three factors were evaluated together in the following ranges: the sorbent mass was investigated from 5 to 45 mg, the percentage of urine in the sample in the interval 15–50% (v/v), and sample volumes from 2 to 10 mL. It is clear that a relative large sample volume improves the sensibility, but the time to pass the sample through the column increase. Therefore, after different experiences with different volumes, 8 mL of sample solution were selected as an intermediate optimum because this volume provides Cd enough for an appropriate sensitivity and it can be passed through the column in an adequate time (10.5 min were needed to pass 8 mL of sample at the 0.76 mL $min^{-1}$ flow rate established). Also, the flow rate could influence the contact time between sample and sorbent. However, in this case, the equilibrium was reached faster because the studied concentrations are low (the adsorption site trap the available metal ions more rapidly for lower concentrations [15]).

The mass of sorbent was studied for a sample volume of 8 mL and for percentages of pure urine in sample solution higher than 20%, under these conditions the use of 15–20 mg of sorbent produced very low recoveries of Cd, indicating that a great part of the cadmium present was not retained (Fig. 4). Considering that the chemical interaction between the metal ions and the surface functional groups of CNTs is the major mechanism for the ion adsorption, a higher mass of sorbent was evaluated. For 45 mg of sorbent and 30% (v/v) of urine in the sample an optimum recovery was attained. High sorbent mass and higher percentages cause worse recoveries because that larger mass of sorbent need more eluent volume to complete the elution of all metal ions. Therefore, 8 mL of sample volume, 45 mg of sorbent and a 30% (v/v) of urine in sample were selected as optimum values for future extractions.

### 3.2.5. Column reuse

The long-term stability of MWCNTs was examined by passing successively through the column samples of 8 mL of the urine prepared as indicated in Section 2.4. The stability and potential regeneration of the column was assessed by measuring the recoveries of the analyte in the eluent of the column, and by controlling the overpressure problems which could appear. After each step, the column was regenerated with 2 mL of 1 M $HNO_3$ and Milli-Q water until to obtain neutral pH after pass and elute each sample. The regenerated column allowed more than 100 adsorption–desorption cycles without decreasing its adsorption capability, recovery of the analysis and without changing the FIA operation mode.

## 3.3. Optimization ETA-AAS programs

### 3.3.1. Optimization ETA-AAS program for cadmium in urine

The elimination of organic and inorganic matrix components during the mineralization step present certain difficulties in the cadmium ETA-AAS determination, because Cd is a volatile element that can be lost from the graphite atomizer at temperatures higher than 200 °C in absence of chemical modifier [35]. Taking into account other works in the literature concerning the use of different chemical modifiers [36–39] and considering our previous studies

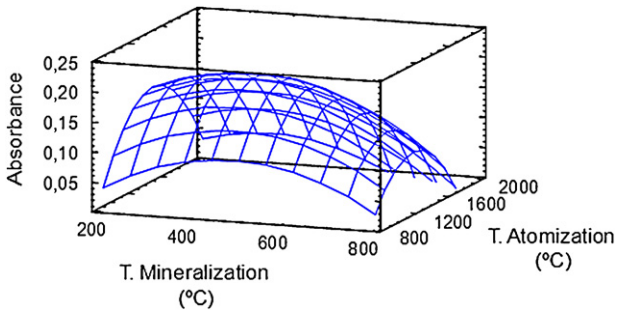


**Fig. 5.** Response surface for the effect of mineralization and atomization temperatures on the Cd absorbance.

[28], in this work an ETA-AAS method for the Cd determination using W as permanent chemical modifier has been chosen as the most suitable procedure.

The effect of mineralization and atomization temperatures on the cadmium integrated absorbance for urine sample was studied in the range of 200–1000 °C and 800–1000 °C, respectively. As it can be observed in Fig. 5, for this type of samples, the integrated absorbance of Cd was observed to decrease at mineralization temperatures above 500 °C. The effects of atomization temperature on the integrated absorbance for urine samples were also studied in the same design. It was verified that the integrated absorbance for the Cd in urine sample decreases gradually for the atomization temperatures higher than 1200 °C (see Fig. 5). On the basis of the response surface obtained, a mineralization temperature of 500 °C and an atomization temperature of 1200 °C were chosen as the optimum ones. The rest of furnace conditions such as temperatures ramps and holds were also studied from the values obtained in previous studies [28]. However due to the fact that they do not have significant influence in analytical signal, no changes were required. Thus, the established furnace operation conditions optimized have been listed in Table 1.

Several authors indicated that the matrix effect can be corrected with a supplementation to the sample with a $HNO_3$ solution in order to help in the mineralization process [28,40,41]. Therefore, in the present study, the nitric acid addition for Cd determination in urine samples was studied. Different $HNO_3$ concentrations were assayed and it was observed that a single addition of 100 μL of 0.5 M $HNO_3$ contribute effectively to the complete elimination of the matrix effect.

### 3.3.2. Optimization ETA-AAS program for eluent

Similar optimization strategy as the previous case was used to optimize the ETA-AAS program for the eluent from preconcentration column and similar results were obtained. The only significant factors influencing the analytical signal were atomization and ash temperatures. Therefore, the effects of the mineralization and atomization temperatures on the determination of cadmium on the eluted sample solutions were investigated. It was observed a significant loss of analyte for mineralization temperatures higher than 400 °C, producing an ETA-AAS reduction of the absorbance signal. So, 400 °C were selected as the better mineralization temperature. The optimal atomization temperature was found to be 1100 °C, producing the highest analytical signal and also well-shaped peaks. The rest of the optimum conditions, temperatures ramps and holds were the same than in the case of ETA-AAS for urine, previously described in Section 3.3.1. The optimum conditions are shown in Table 1.

### 3.3.3. Evaluation of interferences

Interference experiments were carried out under the optimized conditions described in Section 2.5.2 employing a urine sample solution containing 1 μg $L^{-1}$ of Cd. The interference, that was evaluated individually, was considered positive if the foreign ions cause a change greater than ±5% in the recovery of analyte. Interferences from representative alkali metal ions: K(I), Na(I); alkaline metal ions: Mg(II), Ca(II); and typical transition metal ions such as Fe(III), Zn(II), Cr(III), Ni(II), Co(II), Cu(II) and Pb(II), were examined for concentrations between $10^3$ and $10^4$ times the Cd concentration at mg $L^{-1}$ levels. The results are shown in Table 2. All the ions studied were tolerated at concentrations considerably higher than the levels present in urine samples. Therefore the developed method allowed the interference free-determination of cadmium in urine samples.

**Table 2**
Effect for several foreign ions in the developed method for Cd determination.

| Foreign ion | Concentration of foreign ion (mg $L^{-1}$) | Recovery (%)[a] |
|---|---|---|
| K | 10 | 101.2 |
| Na | 10 | 99.9 |
| Mg | 10 | 101.5 |
| Ca | 10 | 98.9 |
| Fe | 5 | 98.2 |
| Zn | 5 | 98.5 |
| Ni | 5 | 99.8 |
| Cr | 1 | 99.5 |
| Co | 1 | 98.6 |
| Cu | 1 | 99.2 |
| Pb | 1 | 98.6 |

[a]Recovery of a urine sample solution containing 1 μg $L^{-1}$ of Cd.

### 3.4. Analytical figures of merit

Under the optimized conditions described in Section 3.3, the proposed method provided a linear calibration range from LOD up to 3.0 μg $L^{-1}$, with a correlation coefficient higher than 0.999. The calibration equation obtained by the flow preconcentration procedure coupled to ETA-AAS was: Abs = (0.415 ± 0.004) [Cd (μg $L^{-1}$)] + (0.166 ± 0.024). The enhancement of ETA-AAS sensibility by inserting the preconcentration step with MWCNT is attested by calculation of the preconcentration factor (PF). This parameter was calculated as the ratio between the slopes of analytical curves with and without preconcentration step. The calibration curve obtained without the concentration phase was: Abs = (0.122 ± 0.006) [Cd (μg $L^{-1}$)] + (0.004 ± 0.003). Therefore, using the preconcentration step developed in this work, a 3.4 increase in the sensitivity was achieved [19].

In addition, it is necessary to keep in mind that the most of ETA-AAS Cd determination methods in urine samples need the employment of addition graphs to avoid interference matrix. However, in the proposed method, the preconcentrated samples can be read directly in the calibration graph because no significant differences between the slope of calibration and addition graphs were detected. This matrix effect has been eliminated as a consequence of the preconcentration process performed. This fact can be verified by observing the peak profiles of the cadmium and background for both urine and preconcentrated urine in Fig. 6. It can be observed an appropriate high peak without background for preconcentrated sample, while for urine sample the peak attained was lower and it presented a significant background.

Detection (LOD) and quantification (LOQ) limits were calculated according to the IUPAC definition [42], LOD = 3SD/$m$, where $m$ is the slope of the addition graph and SD the standard deviation of 10 consecutive measurements of blank solutions, LOQ is determined as 10SD/$m$. In this work the limits of detection (10 ng $L^{-1}$) and quantification (36 ng $L^{-1}$) achieved allows the appropriate measurement of Cd level in human urine. The LOD is comparable with those reported by Vasil'eva et al. [9] (10 ng $L^{-1}$) and Aranda et al.

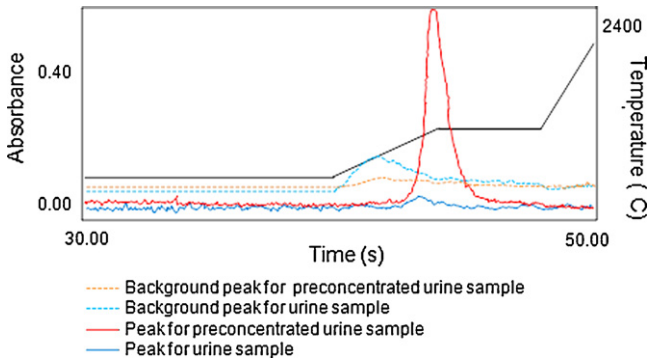


**Fig. 6.** Cadmium and background peaks for urine sample and preconcentrated urine samples.

**Table 3**
Accuracy of the proposed method.

| Added ($\mu g L^{-1}$) | Found ($\mu g L^{-1}$) | RSD (%) | Cd adsorption (%) |
|---|---|---|---|
| 0.25 | 0.25 ± 0.02 | 8.8 | 97.4 |
| 0.50 | 0.50 ± 0.03 | 5.7 | 99.1 |
| 1.00 | 1.00 ± 0.03 | 3.4 | 99.0 |
| 1.50 | 1.50 ± 0.12 | 7.9 | 100.0 |

[43] (8 ng $L^{-1}$) for the Cd determination in urine by ETA-AAS after extraction of microwave-digested urine. Other methods with better LOD (2.0 ng $L^{-1}$ [44], 2.9 ng $L^{-1}$ [45], and 5.0 ng $L^{-1}$ [46]) can be found in literature, but in all these cases, the proposed method needed of a combination of two steps: a preconcentration stage after a previous digestion procedure.

The precision of the method assessed as relative standard deviation (RSD) of ten independent preconcentration cycles adding 0.0, 0.25, 0.50, 1.00 and 1.50 $\mu g L^{-1}$ of Cd were 9.8%, 8.8%, 5.7%, 3.4% and 7.9%, respectively.

The reproducibility for column preparation was studied in order to evaluate if the construction of the column influenced the results. Thus, ten columns with the same dimensions (40 mm length and 4 mm i.d.) were prepared and packed with 45 mg of the same pretreated MWCNTs according to the procedure indicated in Section 3.1. The obtained results of the Cd concentration measured for the same sample using the ten columns prepared were compared using the corresponding Anova-test. Since the *P*-value (0.37) is greater than 0.05, the factor evaluated has not statistically significant effect in the analytical signal (at the 95% confidence level). Thus, the reproducibility of the results obtained with different columns assembled in the same way was demonstrated appropriate.

The accuracy of the proposed method was evaluated through recovery test. Different urine samples were supplemented with different quantities of Cd in the range of 0.25–1.50 $\mu g L^{-1}$ and they were measured under the conditions described in Section 2.5. As it can be seen in Table 3, appropriate results were obtained in the range of 97–100%.

The entire preconcentration/elution/regeneration cycle for one sample requires 10.5 min. Thus the sample throughput was found to be 5.7 samples per hour. However, it is possible to improve the operation time using several columns simultaneously to preconcentrate several samples employing a simple multichannel FIA.

The analytical figures of merit obtained were comparable to those reported for other Cd determination ETA-AAS methods in urine with different separation and/or preconcentration procedures including microwave-digestion and posterior cloud point extraction [43], electrodeposition followed by in situ stripping [47], on-line solvent extraction with a gravitational phase separator [48], among others. However, the method developed is simpler and more automated. The elimination of the matrix and the concentration of Cd are achieved in a single step, avoiding the combination of two steps (digestion and separation, electrodeposition and stripping, etc.).

### 3.5. Analysis of real samples

In order to demonstrate its applicability in real cases, the preconcentration method developed in this work has been applied for the determination of Cd content of seven urine samples obtained from healthy smoker and non-smoker people. It was demonstrated that the proposed preconcentration procedure achieved enough sensitivity (LOQ = 0.036 $\mu g L^{-1}$) to determine the Cd content in all samples; the concentrations measured ranged between 0.14 and 2.94 $\mu g L^{-1}$. For non-smoker group the concentrations attained were comprised in the range of 0.14–1.60 $\mu g L^{-1}$, that can be considered as normal levels. The concentrations found in urine of smokers or people usually exposed to tobacco smoke were higher, ranging between 1.96 and 2.94 $\mu g L^{-1}$. Therefore, the direct method (LOQ = 0.49 $\mu g L^{-1}$) would only allow the determination of Cd in urine at high levels, in both exposed or smoking people.

## 4. Conclusions

In this research, a microcolumn preconcentration system using MWCNT as sorbent material for the ETA-AAS determination of Cd in urine has been developed and its validity for real cases has been demonstrated. Multiwalled carbon nanotubes (MWCNT) were treated previously with $H_2SO_4$–$KMnO_4$ using microwave energy to introduce some functional groups on their surfaces in order to improve their adsorption capability of cations. Cd retained on MWCNT can be easily desorbed (without carryover observed in the next analysis) and measured by ETA-AAS with appropriates figures of merit. The new method developed presented advantages in comparison with other direct methods for the determination of Cd in urine: (i) the preconcentration step produced an increase in the detectability of Cd with a preconcentration factor of 3.4 reaching the possibility of determining Cd in urine at low levels; (ii) the elimination of the matrix permitted the measurement of Cd in the urine samples avoiding the necessity of a previous digestion procedure; and (iii) the absence of matrix effect allowed the measurement of preconcentrated samples directly in calibration graph eluding the requirement of employing standard-addition procedure. The developed method was demonstrated useful by its successful application to the determination of urine Cd level in healthy unexposed people. Also, the proposed method offered benefits by its simplicity and capability of automatization compared to other ETA-AAS methods for Cd determination in which the combination of two steps was needed, a previous digestion procedure followed of a preconcentration stage.

The great potential of multiwalled carbon nanotubes as sorbent for preconcentration and matrix effect elimination in the determination of trace/ultratrace elements by ETA-AAS for complex samples has been demonstrated. Since the presence of carboxyl groups at the MWCNT surface provides an anchor for the attachment of a variety of groups, other applications of this approach with higher sensitivity, selectivity and specificity developing different nanotubes suitable for particular applications are expected in the future.

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