available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/aca

A fully automated system for inorganic antimony preconcentration and speciation in urine

Pablo H. Pacheco[a,b], *Raúl A. Gil*[a,c], *Luis D. Martinez*[a,c], *Griselda Polla*[d], *Patricia Smichowski*[c,e,*]

[a] *Department of Analytical Chemistry, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, Chacabuco and Pedernera, 5700 San Luis, Argentina*

[b] *Fondo para la investigación científica y tecnológica (FONCYT), Avda. Córdoba 831 Piso 6 (C1054AAH) Ciudad de Buenos Aires, Argentina*

[c] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rivadavia 1917, CP C1033 AAJ, Ciudad de Buenos Aires, Argentina*

[d] *Comisión Nacional de Energía Atómica, Unidad de Actividad Física, Av. Gral. Paz 1499, B1650KNA-San Martín, Argentina*

[e] *Comisión Nacional de Energía Atómica, Unidad de Actividad Química, Av. Gral. Paz 1499, B1650KNA-San Martín, Argentina*

ARTICLE INFO

Article history:
Received 12 July 2007
Received in revised form
14 September 2007
Accepted 19 September 2007
Published on line 25 September 2007

Keywords:
L-Methionine
Antimony
Speciation analysis
Urine

ABSTRACT

A study was undertaken to ascertain the analytical capabilities of L-methionine immobilized on controlled pore glass for Sb preconcentration and speciation. A fully automated on-line system, implemented with hydride generation (HG) and inductively coupled plasma optical emission spectrometry (ICP OES), was used. Sb(III), at pH 10 was selectively retained in the column containing the immobilized aminoacid, while Sb(V) was not retained at all. A 30% HCl solution was used as eluent agent. Prior to total Sb determination, a pre-reduction step with thiourea was necessary. An on-line pH adjusting and pre-reduction of Sb(V) was achieved in a fully automated system. The detection limit for the preconcentration of 10 mL of an aqueous solution was 70 ng L^{-1} with a relative standard deviation of 2%. An enrichment factor of 20 was achieved when 10 mL of sample was passed through the system, reaching a throughput of 23 samples per hour. The method was successfully applied to the determination of Sb(III) and total Sb in urine.

1. Introduction

Antimony is an element that raises much concern from both environmental and human health standpoints. The potentially harmful effects of Sb have been recognized by health authorities to such an extent that this metalloid has been listed as a priority pollutant by the US Environmental Protection Agency and by the German Research Community [1,2]. It is a non-essential element for life, and it has chemical and toxicological properties similar to that of arsenic [3]. In spite of this, Sb has been much less studied than As and much less is known about its environmental fate and significance.

Among several industrial applications of Sb compounds, antimony trioxide (Sb_2O_3) is largely employed in the production of glassware and ceramics. Several Sb compounds are used as additives to metal coatings and to rubber, and others are added to textiles as flame retardant [4]. Lead-battery workers are exposed to antimony trioxide and stibine (SbH_3), resulting in elevated Sb concentrations in their urine [5]. The

* *Corresponding author*. Tel.: +54 11 6772 7873; fax: +54 11 6772 7886.
E-mail address: smichows@cnea.gov.ar (P. Smichowski).
0003-2670/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.aca.2007.09.034

determination of Sb in urine is important to study the level of exposure in workplaces.

In general, only limited information is available to accurately assess the impact of Sb on human health. As with other elements, the toxicological and physiological behaviour of Sb depends on its oxidation state. Elemental Sb is more toxic than its salts, and generally trivalent Sb compounds exert a ten times higher acute toxicity than pentavalent Sb species [4]. Subchronic toxicity of Sb on different tissues of rats after a 90-day exposure via drinking water was also observed [6].

In contrast, pentavalent Sb is used as an antiparasitical drug for the treatment of patients with leishmaniasis. One effective way of monitoring the administration of Sb(V) in these patients is through its determination in urine [7]. In this context, the development of new methods for its determination in this kind of samples becomes a matter of major relevance.

Krachler and Emons [2] reported the first study on the determination of Sb species in urine coupling on-line high performance liquid chromatography (HPLC) to an inductively coupled plasma mass spectrometry (ICP-MS) instrument. An ultrasonic nebulizer (USN) was used to improve sensitivity. Detection limits ranging from 8–20 ng L^{-1} were achieved for different Sb species. Only trimethylantimony dichloride ($TMSbCl_2$) at 0.09 μg L^{-1} was detected in non-exposed persons, while in exposed persons, Sb(III), Sb(V) and $TMSbCl_2$ were detected, Sb(V) being the predominant species.

Li et al. [3] combined an on-line cloud point extraction (CPE) method with electrothermal vaporization inductively coupled plasma optical emission spectrometry (ETV-ICP OES), which allowed reaching a limit of detection of 0.09 μg L^{-1}. Sb(V) was the only species detected in human urine samples at a concentration of 0.94 μg L^{-1}.

Overall reference concentrations for Sb in urine as reported in the literature are in the range 0.10–1.8 μg L^{-1} [8]. In this context, the expected levels of Sb species in urine are far below those achieved directly by inductively coupled plasma optical emission spectrometry (ICP OES) and the detection power had to be improved by introducing a preconcentration step. Although hydride generation (HG) associated with ICP OES or graphite furnace-atomic absorption increases its detection power, it is not sufficient for Sb determination in urine. This situation is more difficult when the identification of Sb species is required.

Novel materials have been proposed and successfully employed for the preconcentration and/or speciation of different analytes [9,10]. The development of on-line systems for the preconcentration and separation of trace metals has promoted the investigation of a variety of immobilized chelating agents ranging from the well characterized 8-hydroxyquinoline to more heterogeneous binding agents such as algae, bacteria and metal-binding proteins. Peptides, aminoacids and polyaminoacids have been immobilized onto controlled pore glass [11–14] and in all instances they have proven to be effective metal chelators [11–16]. Malachowski and Holcombe compared the metal binding capabilities of poly-L-glutamic acid and poly-L-aspartic acid [17].

In this work, we exploit the capabilities of L-methionine immobilized on controlled pore glass (CPG) and packed in a minicolumn for the on-line selective preconcentration of Sb(III), followed by HG-ICP OES detection. By on-line adjusting the solution pH in the preconcentration step, it was possible to retain selectively Sb(III). Thiourea was added to convert Sb(V) to Sb(III) and total content of Sb was determined in a fully automated system. To the best of our knowledge, it is the first time that L-methionine immobilized on CPG is employed for the identification and quantification of Sb species.

2. Experimental

2.1. Apparatus

Measurements were performed with a sequential ICP spectrometer Baird ICP 2070 (Bedford, MA, USA). The ICP operating conditions are listed in Table 1. The flow injection (FI) system used is shown in Fig. 1. A Minipulse 3 peristaltic pump Gilson (Villiers-Le-Bell, France) was used. Sample injection was achieved using a Rheodyne (Cotati, CA, USA) Model 50, four-way rotary valve. The hydride generator unit used was from PS Analytical Ltd. A Watson Marlow 303X peristaltic pump controlled the flow rate of reagents. About 60 mg of L-methionine immobilized in CPG (as it is described in Section 2.3) was loosely packed into an empty conical micropipette tip made of polypropylene. To avoid loss of filling when the sample solution passed through the conical minicolumn, a small amount of quartz wool was placed at both ends of column. A conical shaped column (40 mm length, 4.5 mm internal upper-diameter and 1.5 mm internal lower-diameter) was used to give better performance when samples were introduced at thin end during loading, and later eluted from the same end into the ICP OES. In addition, the conical shape allows a higher enrichment factor due to a diminution of the dispersion. It was then connected to a peristaltic pump with PTFE tubing to form the preconcentration system. Tygon-type pump tubing (Ismatec, Cole Parmer, Vernon Hills, IL, USA) was employed to propel the sample, reagents and eluent. The Sb 217.581 nm spectral line was used.

2.2. Reagents

Unless otherwise stated, the chemicals used were of analytical grade, and therefore no further purification was required. A 0.6% (w/v) sodium borohydride solution (Aldrich Chemical Co. 98%) was prepared in 0.5% (w/v) sodium hydroxide solution and was filtered through Whatman No. 42 filter paper to remove undissolved solids. This solution was prepared daily.

Table 1 – ICP instrumental parameters

RF generator power (kW)	1.0
Frequency of RF generator (MHz)	40.68
Plasma gas flow rate (L min^{-1})	8.5
Auxiliary gas flow rate (L min^{-1})	1
Carrier gas flow rate (mL min^{-1})	90
Observation height above load coil (mm)	15
Analytical line: Sb (nm)	217.581

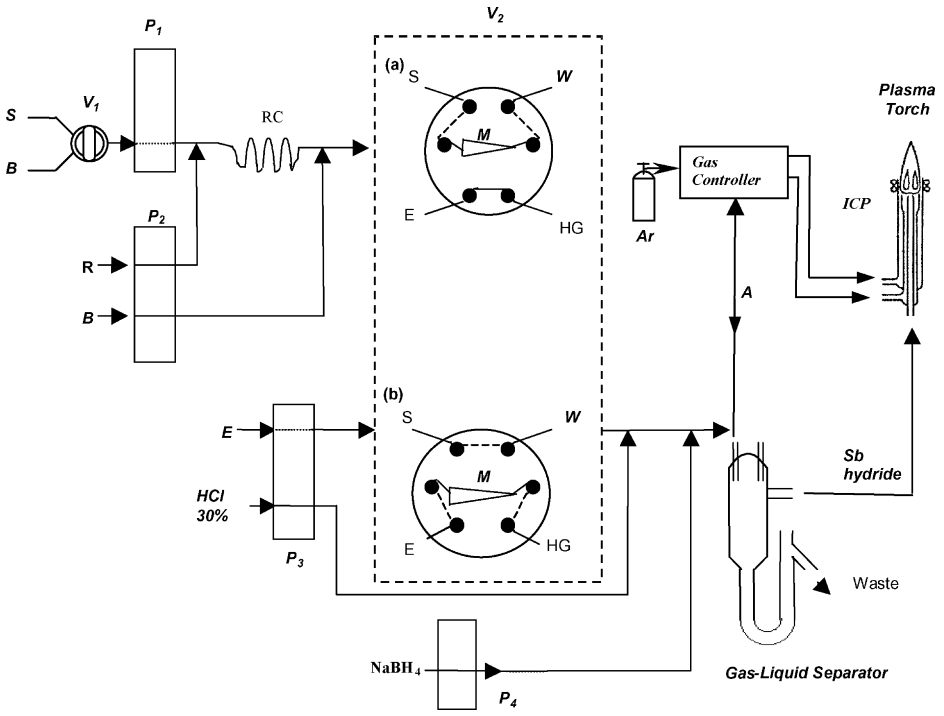

Fig. 1 – Schematic diagram of the instrumental set up. S, sample (flow rate: 3.0 mL min^{-1}); B, buffer; R, reductant/water; E, eluent (flow rate: 1 mL min^{-1}); RC, reaction coil; W, waste; P_1, P_2, P_3 and P_4, peristaltic pumps; M, minicolumn packed with L-methionine immobilized on CPG; V_1 and V_2, injection valves. Valve positions: (a) sample loading; (b) injection.

To prepare the Sb(III) standard stock solution at 1000 mg L^{-1}, 0.1334 g of antimony potassium-(C) tartrate salt (A.R., BDH) were dissolved in 1000 mL of a 3.0 mol L^{-1} HCl solution. For the 1000 mg L^{-1} Sb(V) standard stock solution, 1.05 mL of $SbCl_5$ (99.999%, Alfa) was added to a 1000 mL volumetric flask and made to volume. This solution was 5 mol L^{-1} in HCl.

Thiourea (99% pure) was obtained from Sigma–Aldrich Laboratories (St. Louis, USA). L-Methionine was obtained from Fluka A. G., (Switzerland). Controlled pore glass (CPG, pore diameter 240 Å, mesh size 240–400), 8-aminopropiltriethoxysilane and glutaraldehyde were supplied by Sigma (St. Louis, USA).

2.3. Immobilization procedure

A 0.2 g portion of L-methionine was suspended in 15 mL of 0.1 mol L^{-1} phosphate buffer at pH 7.0. Silanisation of the CPG using 8-aminopropyltriethoxysilane and the use of the bifunctional property of glutaraldehyde to prepare the glutaraldehyde-treated CPG was the same as used previously [18]. Glutaraldehyde allows that L-methionine binds to CPG, acting as a cross linker. The glutaraldehyde-treated CPG was filtered and washed with ultrapure water. To the beaker containing the L-methionine solution, 1.0 g of the treated glass was added and N_2 was flushed for 15 min. The mixture was kept at 4 °C for 24 h under a N_2 atmosphere and then air-dried filtered. Fig. 2 shows the support after the immobilization process of the L-methionine by scanning electron microscopy (SEM).

2.4. Evaluation of the retention step

Antimony retention and filling flow rate were studied off-line. About 10 mL of an aqueous solution containing Sb(III) or Sb(V) buffered to different pHs were loaded on the column filled with L-methionine immobilized on CPG at a flow rate of 2 mL min^{-1}. Finally, the retained metalloid was eluted with 2.5 mL of HCl at different concentrations. Prior to hydride generation, the final acid concentration was adjusted to 30%, leaving a final volume of 5 mL. Antimony concentration was determined by HG-ICP OES. Recoveries were calculated against the theoretical concentration.

2.5. Sample preparation

Urine samples were collected and stored in Sb-free polyethylene containers without adding preservatives and were filtered through a 0.45 μm pore size membrane. Samples were analyzed immediately to avoid possible species transformation or interconversion, as reported by Lindemann et al. [19].

2.6. Procedure for the preconcentration and separation of Sb(III) and Sb(V)

FI system used for preconcentration, separation and subsequent determination of Sb is shown in Fig. 1. Before loading the sample, the column was conditioned for preconcentration at the optimized pH (valve V_1 in position B) with B (NH_3). A fixed volume of sample was then loaded onto the conical minicolumn (M) with valve V_1 in position S, valve V_2 in position (a).

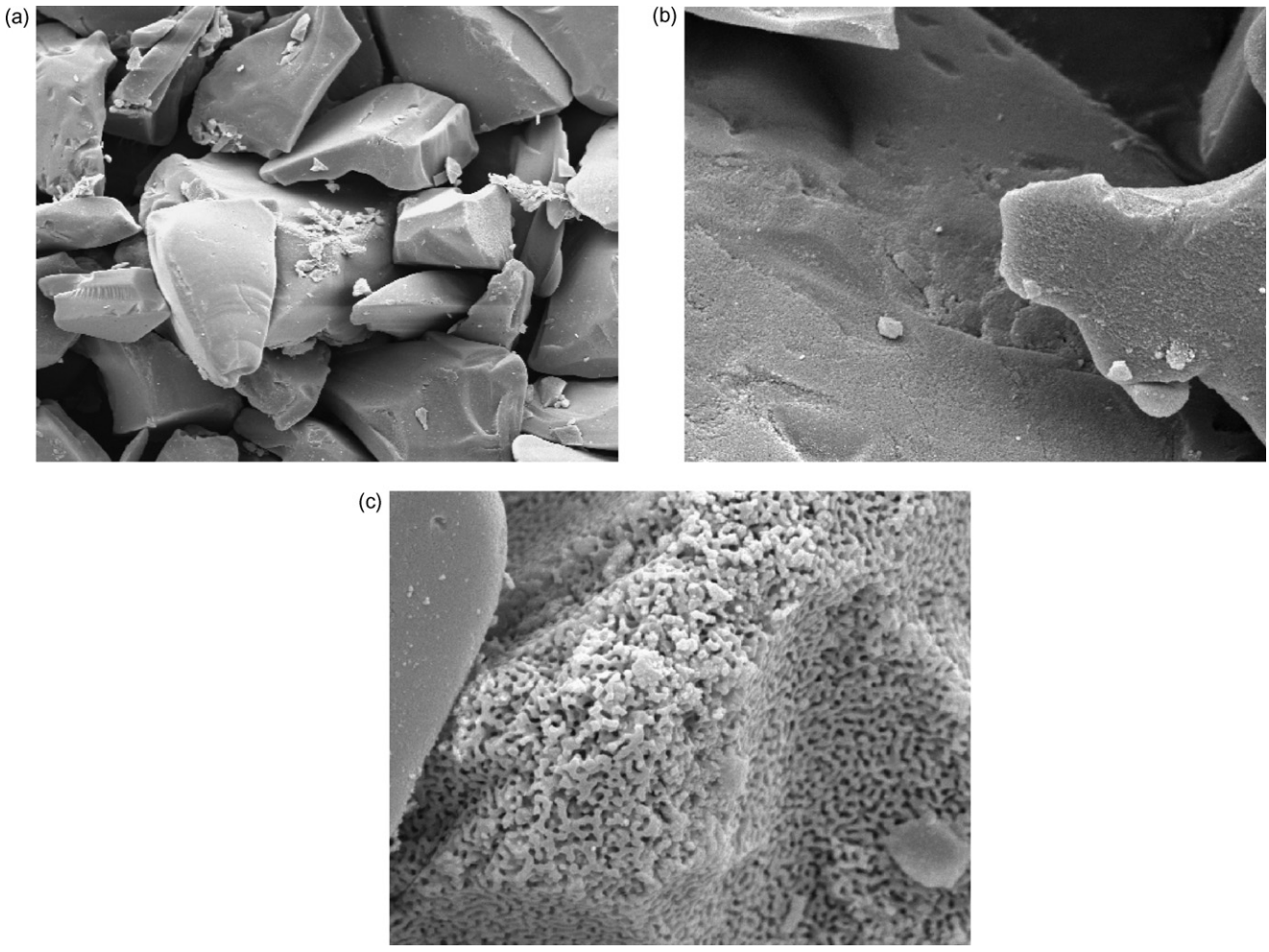

Fig. 2 – Electron micrographs of L-methionine immobilized on CPG at different magnifications: (a) 500×; (b) 2400×; (c) 5200×.

Prior to Sb total determination, a pre-reduction step was necessary. This was achieved by injecting a thiourea solution as reductant agent (R) (2 mol L^{-1} thiourea + 4 mol L^{-1} HCl) with pump P_2. The acid, in excess, was neutralized with NH_3 (c) (B), to reach the optimized pH (pH 10) for loading. The reductant agent (R) and buffer (B) were injected at a flow rate of 1 mL min^{-1}; sample (S) was injected at a flow rate of 3 mL min^{-1}; leaving a net sample flow rate of 5 mL min^{-1}. For Sb(III) determination, the above procedure was followed, but the reductant was changed by ultrapure water in order to reproduce the flow rates of total Sb preconcentration and determination.

Finally, valve V_2 was switched to the injection position (b) and the Sb retained in the minicolumn was eluted with the eluent E at a flow rate of 4.5 mL min^{-1}. After that, the eluted Sb(III) was merged with a pump tubing containing 30% HCl at a flow rate of 4.5 mL min^{-1}; leaving a net flow rate of 9 mL min^{-1} (4.5 mL min^{-1} of eluent + 4.5 mL min^{-1} 30% HCl) prior to the hydride generation process. After this, it was introduced into the hydride generator and subsequently to the ICP torch. The process (including analytical determinations) was completed in no more than 3 min for 10 mL of sample. This fully automated system avoided excessive manipulation of the sample conducting to reduce or eliminate contamination and improving sample throughput.

3. Results and discussion

3.1. *Parameters affecting Sb(III) preconcentration*

Commercially available single amino acids and biohomopolymers consisting of a single type of amino acid were successfully attached to solid supports for elemental binding studies [16]. These studies reported that immobilized amino acids and peptides have high capacity for metal cations and metal oxoanions retention, being selective and showing specific chelation of target elements. This selectivity is primarily based on the side group functionality of the amino acid.

In order to optimize the sorption conditions for the retention of Sb on L-methionine/CPG, the intensity of Sb signal was monitored by ICP OES as a function of the pH of the solution that passed through the conical minicolumn. Between pH 3 and pH 11, a maximum retention of Sb(III) is observed in Fig. 3. This feature is an advantage of the method because a strict control of the pH was not necessary

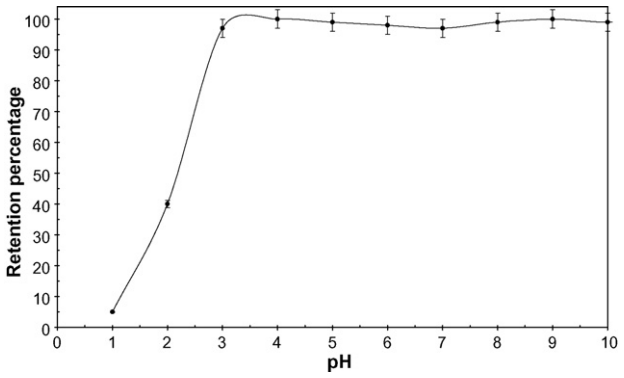

Fig. 3 – Dependence of Sb(III) retention on pH of loading solution. Volume of sample: 10 mL; Sb concentration: 0.5 mg L^{-1}.

during the preconcentration step. At lower pH values the binding is lower, which is attributed to the protonation of the aminoacid. In the reduction step, a 30% HCl solution was added, and due to the high acidity generated, NH_4OH was merged in the flow injection analysis (FIA) system to reach an adequate alkaline media prior to Sb(III) retention. For further experiments pH 10, obtained with NH_3/NH_4^+ buffer, was used to achieve retentions close to 100%. On the other hand, Sb(V) was not retained on the L-methionine/CPG at any pH value. The species of Sb(III) present in solution at this pH value, which could be interacting with L-methionine, are hydroxo-complexes and oxo-complexes, such as SbO_2^- and $Sb(OH)_4^-$ [20]. It is plausible to think that an electrostatic interaction between L-methionine and Sb species may occur [21].

Leyden et al. [22] reported that alkoxysilanes immobilized onto CPG are useful for oxoanion removal. For this reason, the sorption capacity of CPG, including silanisation and glutaraldehyde treatement, for Sb(III) retention without L-methionine was tested and no retention was observed, concluding that only L-methionine was responsible for Sb retention.

The sample flow rate through the column packed with CPG/aminoacid is an important parameter to optimize, since this is one of the steps that controls the time of analysis. We could verify that with flow rates up to 5.0 mL min^{-1}, there was no effect on the analyte recovery (98–100%). Higher flow rates were avoided because the recovery decreased and the back-pressure generated could damage the FI system. This is an important point, because a flow rate of 5 mL min^{-1} allowed (i) the on-line pre-reduction and pH adjusting steps, (ii) subsequent elution of the retained analyte, prior to hydride generation. In addition, a better sampling throughput is reached.

3.2. *Elution*

The type and acid concentration is important for an easy and quantitative elution of the species. This fact permits to enhance the preconcentration ratio and to achieve complete column regeneration. Miller et al. [23] demonstrated that acids cause a reversible change in the tertiary structure, which provide efficient and rapid release of the metal from the binding cavity.

After some screening experiments with different inorganic acids, HCl was chosen as eluent. Antimony signal was monitored by ICP OES up to HCl concentrations close to 30%. We could verify that the recoveries increased with HCl concentrations up to at least 30% (v/v). Malachowski and Holcombe [16] also observed that while the cation metals are easily stripped from a CPG/aminoacid column, the oxoanions are not removed as easily. Higher concentrations were not tested because the maximum recoveries were achieved and to avoid irreversible damages to the column even when the substrate resulted to be chemically and mechanically stable. The same column was used during the development of this study.

The elution of the retained analyte was made at a flow rate of 4.5 mL min^{-1}, then it linked with HCl 30% at a flow rate of 4.5 mL min^{-1} (Fig. 1), obtaining a final flow rate of 9 mL min^{-1} and a final acid concentration of 30%, prior to hydride generation.

3.3. *Study of possible interferents*

The effects of potential interference species at the concentration levels at which they may occur in the studied sample were tested. As(III), Cr(III), Cr(VI), Cu(II), Fe(III), Ni(II), Se(VI) and V(V) were tolerated up to at least 1000 μg L^{-1}. Other concomitant elements such as alkali and alkaline elements were not retained on the immobilized L-methionine under the working conditions. Since anions may also impact Sb binding, the effect of Cl^-, CO_3^{2-} and SO_4^{2-} were evaluated and we observed that they were tolerated up to at least concentrations of 2500 μg L^{-1}. This fact evidences that CPG/L-methionine has the greatest capacity for Sb(III). These characteristics of the system are important because it avoids that possible components of urine could interfere in the determination of Sb species.

3.4. *Performance of the system*

The overall time required for the on-line reduction and preconcentration of 10 mL of urine sample (2 min, at a flow rate of 5 mL min^{-1}), elution (0.16 min, at a flow rate of 4.5 mL min^{-1}), and washing and conditioning (0.4 min) was about 2.6 min; hence, throughput was approximately 23 samples h^{-1}. The calibration graph using the preconcentration system for Sb was linear with a correlation coefficient of 0.9993 from concentrations close to the detection limit up to at least 100 μg L^{-1}. The detection limit (LOD) was calculated as the amount of Sb required to yield a net peak that was equal to three times the standard deviation of the background signal (3σ). As the preconcentration of Sb is regarded, the detection limit depends, among others, on the sample volume loaded onto the column. It resulted to be 70 ng L^{-1} when 10 mL were passed through the column. The overall procedure exhibited good repeatability of measurements as shown in Table 2, where the relative standard deviations (R.S.D.s) turned out to be better than 2% at low Sb concentrations. A concentration efficiency of 7.5 min^{-1} was achieved. According to Fang [25], the concentration efficiency (CE) is used for the evaluation and comparison of the efficiency of various systems. CE is defined as the product of the enrichment factor EF and the sampling frequency in number of samples analyzed per minute, expressed in min^{-1}.

Table 2 – Recovery data. Recovery is expressed as percentage

Aliquot	Base value ($\mu g L^{-1}$)	Sb(III) added ($\mu g L^{-1}$)	Sb(III) found ($\mu g L^{-1}$)	Total Sb found ($\mu g L^{-1}$)	Recovery (%)[a]
1	0.42	0.5	0.90	–	96.0
2	0.42	1.0	1.42	–	100.0
3	0.42	5.0	5.43	–	100.2
4	0.65	0.5	–	1.14	98.0
5	0.65	1.0	–	1.63	98.0
6	0.65	5.0	–	5.66	100.2

[a] 100 × (found − base)/added.

Therefore, if f is the sampling frequency expressed in samples analyzed per hour, CE = EF × (f/60). Table 3 summarizes the analytical performance of the system.

A comparison of our detection limit (70 $ng L^{-1}$) with that recently reported by Li et al. [3] (90 $ng L^{-1}$), who employed an on-line cloud point extraction combined with ETV-ICP OES for inorganic Sb speciation, evidences a slight improvement.

3.5. *Recovery study*

Unfortunately, there are no standard reference materials with a certified content of individual species of Sb available. In this case, a recovery study can be considered as a validation alternative. Urine samples were spiked with 0.5, 1.0 and 5.0 $\mu g L^{-1}$ of Sb to calculate Sb recovery. Human urine was divided in portions of 10 mL each. The proposed method was applied to six portions (three for total Sb and three for Sb(III)). Base value was established and increasing quantities of Sb(III) were added to the other aliquots of the sample and the analyte was determined by the same method. As shown in Table 2, the recovery values were close to 100% for Sb(III). The results were compared with the t-test and no significant differences were observed at 95% confidence level.

3.6. *Analysis of real samples*

The method was applied to antimony determination in urine samples. Concentrations were in the range 0.40–0.44 $\mu g L^{-1}$ for Sb(III) and 0.62–0.68 $\mu g L^{-1}$ of Sb(V). These results were obtained by the standard addition method and are shown in Table 2.

The urine of normal individuals may contain from 0.35 to 1.8 $\mu g L^{-1}$ of Sb [24]. According to Caroli et al. [8], the overall reference concentrations for Sb in urine are in the range 0.19–1.8 $\mu g L^{-1}$. Krachler and Emons [2] found levels of total Sb <0.12 $\mu g L^{-1}$ for non-exposed persons. For exposed individuals, they reported concentrations of 8.3 ± 0.3 $\mu g L^{-1}$ and 5.1 ± 0.4 $\mu g L^{-1}$. A comparison of our results with those reported above, indicates that the samples analyzed correspond to non-exposed persons, even when they work in an industrial area.

Table 3 – Analytical performance of the preconcentration system

Preconcentration time (s)	120
Calibration range ($\mu g L^{-1}$)	~LOD–100
Sample consumption (mL) (sample flow rate = 5 mL min^{-1})	10
Sample throughput (samples h^{-1})	23
Relative standard deviation (%) (n = 6, at 1.0 $\mu g L^{-1}$ Sb(III)–Sb(V) level)	1.1–1.5
Detection limit ($ng L^{-1}$)	70
Enrichment factor (EF)	20
Concentration efficiency (CE) (min^{-1})	7.5

4. Conclusions

The determination of Sb(III) and Sb(V) in urine samples is a difficult task, especially due to the high chloride concentration that can severely hamper chromatographic separations. In this context, we propose a simple, sensitive and reliable method using L-methionine immobilized on CPG for Sb preconcentration and speciation that allows the determination of individual inorganic species of this metalloid in exposed and non-exposed person.

An issue that arose during this study was the need for improving the detection limit for Sb species due to the low expected levels for this element in human urine. This problem was solved by using ICP OES in a fully automated on-line preconcentration system. The on-line combination of FI, HG and ICP OES for Sb determination allowed reaching a detection limit of 70 $ng L^{-1}$.

The detection power afforded by this analytical approach was adequate for the selective and reliable determination of Sb species in human urine with a retention efficiency for Sb(III) of 96–100% and a throughput of 23 samples h^{-1}. The reproducibility of the whole procedure (at 1 $\mu g L^{-1}$) using six measurements was satisfactory.

The achievements of this study indicate that the method is a valid alternative to other methods for detecting Sb species at expected levels in urine, avoiding complex sample pretreatment and excessive preconcentration time, which in turn may result in possible changes of relative concentration of individual Sb species, contamination or loss of the analytes.

To the best of our knowledge, it is the first time that CPG/L-methionine is used for Sb preconcentration and speciation analysis. The possibility of using other aminoacids should be also investigated and compared. Work is now progressing in this direction.

Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional

de Promoción Científica y Tecnológica (FONCYT) (PICTBID), Universidad Nacional de San Luis (Argentina) and Comisión Nacional de Energía Atómica (CNEA).

REFERENCES

[1] A.G. Leyva, J. Marrero, P. Smichowski, D. Cicerone, Environ. Sci. Technol. 35 (2001) 3669.
[2] M. Krachler, H. Emons, Trends Anal. Chem. 20 (2001) 79.
[3] Y. Li, B. Hu, Z. Jiang, Anal. Chim. Acta 576 (2006) 207.
[4] M. Kentner, M. Leinemann, K.H. Schaller, D. Weltle, G. Lehnert, Int. Arch. Occup. Environ. Health 67 (1995) 119.
[5] R. Poon, I. Chu, P. Lacavalier, V. Valli, W. Foster, S. Gupta, B. Thomas, Food Chem. Toxicol. 36 (1998) 21.
[6] N. Miekeley, S. Mortari, A. Schubach, J. Anal. Bioanal. Chem. 372 (2002) 495.
[7] M. Krachler, Emons, J. Anal. At. Spectrom. 16 (2001) 20.
[8] S. Caroli, A. Alimonti, E. Coni, F. Petrucci, O. Senofonte, N. Violante, Crit. Rev. Anal. Chem. 24 (1994) 363.
[9] S. Moyano, R.G. Wuilloud, R.A. Olsina, J.A. Gasquez, L.D. Martinez, Talanta 54 (2001) 211–219.
[10] P. Smichowski, M.B. de La Calle, Y. Madrid, M.G. Cobo, C. Cámara, Spectrochim. Acta, Part B 49 (1994) 1049.
[11] M. Howard, H.A. Jurbergs, J.A. Holcombe, Anal. Chem. 70 (1998) 1604.
[12] J.L. Stair, J.A. Holcombe, Microchem. J. 81 (2005) 69.
[13] E. Gutierrez, T.C. Miller, J.R. Gonzalez-Redondo, J.A. Holcombe, Environ. Sci. Technol. 33 (1999) 1664.
[14] T.C. Miller, J.A. Holcombe, J. Hazard. Mater. 83 (2001) 219.
[15] H.A. Jurbergs, J.A. Holcombe, Anal. Chem. 69 (1997) 1893.
[16] L. Malachowski, J.A. Holcombe, Anal. Chim. Acta 495 (2003) 151.
[17] L. Malachowski, J.A. Holcombe, Anal. Chim. Acta 517 (2004) 187.
[18] H.A.M. Elmahadi, G.M. Greenway, J. Anal. At. Spectrom. 6 (1991) 643.
[19] T. Lindemann, A. Prange, W. Dannecker, B. Neidhart, Fresenius, J. Anal. Chem. 368 (2000) 214.
[20] F. Burriel Martí, F. Lucena Conde, S. Arribas Jimeno, J. Hernández Méndez, Química Analítica Cualitativa, Editorial Paraninfo, Madrid, 1994.
[21] P.H. Pacheco, R.A. Gil, P. Smichowski, G. Polla, L.D. Martinez, J. Anal. At. Spectrom. 22 (2007) 305.
[22] D.E. Leyden, G.H. Luttrell, W.K. Nonidez, D.B. Werho, Anal. Chem. 48 (1976) 67.
[23] T.C. Miller, E.-S. Kwak, M.E. Howard, D.A. Bout, J.A. Holcombe, Anal. Chem. 73 (2001) 4087.
[24] C.G. Elinder, L. Friberg, Antimony Toxicology of Metals, vol. 2, National Technical Information Service, Springfield, VA, 1977, p. 15.
[25] Z.L. Fang, Flow Injection Preconcentration and Separation, VCH Publishers, Inc., New York, 1993.