Selective Determination of Antimony(III) and Antimony(V) in Blood Serum and Urine by Hydride Generation and Atomic Absorption Spectrometry

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São descritos métodos para determinação seletiva de antimônio (III) e (V) em soro sangüíneo e urina, fazendo-se uso, da dependência com a acidez, da evolução de estibina na redução com boroidreto de sódio. Coeficientes de variação de cerca de 2,0 e 2,8% foram obtidos para antimônio (III) e (V) respectivamente. Em cada caso o limite de detecção foi de 0,2 μg Sb/ℓ. A exatidão das análises foi avaliada pela determinação de antimônio total em amostras de padrões de referência de folhas de pomar (NIST SRM 1541) e pela determinação de antimônio (III) e (V) em amostras de soro sangüíneo e urina; em ambos os casos obteve-se uma boa concordância com as concentrações esperadas. Os métodos foram aplicados para a determinação de antimônio em soro sangüíneo e urina de pacientes com leishmaniose tratados com Glucantim.

Sensitive methods are described for the selective determination of antimony(III) and antimony(V) species in blood serum and urine, by exploiting the acidic dependence of the evolution of stibine on reduction with sodium borohydride. The coefficients of variation were about 2.0 and 2.8% for antimony(III) and antimony(V) determination, respectively. In either case, the detection limit was 0.2 μg Sb/ℓ. The accuracy of the analysis was evaluated by determining total antimony in NIST standard reference material Orchard Leaves (NIST SRM 1541) and by determining antimony(III) and antimony(V) in spiked blood serum and urine samples; in both cases results were in good agreement with expected concentractions. The described methods have been used to determine antimony speciation in a number of blood serum and urine samples from patients with leishmaniasis desease and treated with Glucantim.

Key words: *antimony, blood serum, hydride generation, atomic absorption spectrometry.*

Introduction

The ability to monitor trace levels of a number of heavy metals in a variety of samples is in important feature of modern environmental chemistry. Hence, sensitive analytical methods are required. Colorimetry[1], atomic fluorescence spectrometry[2], anodic-stripping voltammetry[3] and graphite furnace atomic-absorption spectrometry[4] have been reported to be suitable for ultratrace antimony determination, but they require lengthy sample preparation or pre-concentration procedures, which limit sample throughput to typically one per hour. The most commonly employed analytical approach involves hydride generation interfaced with some form of atomic spectroscopy, popularly atomic absorption spectrometry (AAS)[5]. Hydride methods have the advantage of being simple, rapid and relatively interference free.

Although, antimony is a potentially significant element for plants[6], it does not have any known essencial function in animals; on the contrary, its toxicity has been demonstrated. However, systemic antimonial therapy is still reccomended for multiple lesions caused by human leishmaniasis desease. As the pentavalent antimonials are much less toxic than the trivalent antimonials they are used for this type of therapy. The mode of action of pentavalent antimonials is not understood but nearly 5% is excreted in the urine in a trivalent form[7]. Due to this very different behavior in antimonials biological toxicity, speciation measurements are of fundamental importance for the study of their mode of action.

Colorimetry[8] and graphite furnace atomic absorption spectrometry[4] have been used for the determination of antimony(III) and antimony(V). However, as many experimental variables are involved with these techniques the most suitable and commonly employed analytical approach for antimony speciation involves hydride generation[9]. The hydride generation techníque includes the use of a reductant, such as sodium borohydride solution, to separate the volatile metal hydrides from the sample solution and the subsequent determination with AAS after decomposition of the hydrides in a heated quartz cell. For analytical purposes, one of the most frequently utilized reactions for stibine (SbH_3) generation involve the utilization of hydrochloric acid[2,9,10-12].

The present paper describes a simple and selective determination of antimonial compounds in urine and blood serum of humans (controls and with leishmaniasis disease) treated with Glucantim^R.

Experimental

A Varian-Techtron model AA-1475 atomic absorption spectrometer with a Varian-Techtron antimony hollow cathode lamp was used along with the following operating conditions: wavelength 217.6 nm; lamp current 7.0 mA; slit width 0.5 nm; and, flame composition 10.0/2.0 ℓ min^{-1} air/acetylene. A Varian-Techtron model 65 hydride generator with 3 ℓ min^{-1} nitrogen carrier gas was used for the hydride generation, whereas, absorbance-time signals were recordered with a Varian-Techtron 9176 potentiometric chart recorder.

All used reagents were of analytical grade chemicals and distilled deionised water was used througout. Stock standard solution (1000 mg ℓ^{-1}) for antimony(III) was prepared by a dissolution of $K(SbO)C_4H_4O_6 . 0.5H_2O$ in 0.5 M tartaric acid. In this way antimony(III) was protected from oxidation or interaction with antimony(V) species by complex formation with tartrate in aqueous solution. A 100 mg ℓ^{-1} standard solution of Sb(V) was prepared from a 1000 mg ℓ^{-1} antimony(III) solution: 10 mℓ of the antimony(III) solution were treated with 5 mℓ of 1% w/v potassium permanganate and 5 mℓ of 2M of sulphuric acid were added. This misture was heated during 30 min at 80°C, and after cooling, the excess of the produced potassium permanganate and manganese oxide was removed with a 15% v/v hydrogen peroxide solution and then diluted to 100 mℓ with a 0.5 M sulphuric acid solution. The antimony(V) solution was prepared just before use. 0.2 – 20 μ gℓ^{-1} antimony(III) and antimony(V) analytical solutions were prepared daily just before use with 2 M citric acid and 0.5 M sulphuric acid solutions, respectively.

A sodium borohydride solution (0.25 w/v) was prepared from sodium borohydride powder (Merck) by dissolving it in 0.1M sodium hydroxide; this solution was prepared daily just before use.

A 2M citric acid solution was prepared in water for pH adjustment.

Blood samples (10 – 15 mℓ) were obtained from the forearm veins. These samples were collected, stored and treated as previously described by Alarcón *et al.*[13] and Burguera *et al.*[14]. Whereas urine samples were collected and treated as indicated by Burguera and Burguera[15].

All the investigations were carried out under the optimum conditions described in Table 1.

To determine total antimony, 5 mℓ of the antimony(V) solution in 0.5M sulphuric acid were injected in the hydride generator, followed by the addition of 1 mℓ of 10% w/v potassium iodide in order to ensure complete reduction of antimony(V) and antimony(III). Purging was started by flowing 3 ℓ min^{-1} nitrogen during 30 s under continuous stirring. Then 5 mℓ of 0.25% w/v sodium borohydride in 0.1M sodium hydroxide were added.

To determine antimony(III), 5 mℓ of the antimony(III) solution were injected in the hydride generator, followed by the addition of 2 mℓ of citric acid at pH 2.5. Thereafter, the procedure was identical to that used for the determination of total antimony.

In either case the volatilised hydride was swept into the flame heated quartz cell aligned vertically and horizontally to allow maximum absorbance. The mean value of five readings of absorbance was determined, unless otherwise stated, and the deuterium background correction was not necessary.

Results and Discussion

Previously published works[10,17,18] have reported that the use of hydrochloric acid accelerated the irreversible devitrification of the quartz cell due to the formation of antimony chlorides. Also, in our preliminary studies, it was visually observed a rapid devitrification (in about one month) of the quartz cell when hydrochloric acid was used. Eventually, after two month the cell broke-down. Thus, in this work the use of hydrochloric acid was avoided. Our results showed that the response for antimony(V) was almost unaffected by using sulphuric acid instead of hydrochloric acid. On the other hand, antimony(III) effectively reduced to stibine in either 0.5M tartaric acid, 1M malic acid and 2M citric acid. Whereas, antimony(V) did not give significant signal in either of these acids. These results were in agreement with those previously reported by the literature[16,19]. Therefore, in this work, sulphuric and citric acids were used to adjust the acidity of the media for the selective determination of antimony species (see below).

For total antimony, the most suitable acid concentration with respect to sensitivity, reproducibility and blank values was selected by evaluating the absorbance signals obtained in 0.1 – 1.0 M sulphuric acid media. Higher sulphuric acid concentrations than 1.0 M supressed about 10 – 20% the absorbance signals. Within the range 0.1 – 1.0 M sulphuric acid the sensitivity remained unchanged. Therefore, the optimum sulphuric acid concentration was considered to be 0.5 M. The selective determination of antimony(III) in the presence of antimony(V) was confirmed by monitoring its signal response over a wide range of pH. The results indicated that the antimony(III) signal response was relatively unaffected over the pH range 1 to 3 in agreement with previously published works[16,18]. However, the hydride yields was greatly reduced above pH 3.

Different volumes and concentrations of $NaBH_4$ solution in sodium hydroxide were studied, and the optimum values

Table 1. *Details of optimum experimental conditions**

Conditions commom to antimony(III) and total antimony determination	
Sample volume, mℓ	5
Sodium borohydride solution, mℓ	5 (0.25% w/v)
Nitrogen flow-rate, ℓ min^{-1}	3
Purging time, s	30
Total antimony determination	
Potassium iodide solution, mℓ	1 (10% w/v)
Reaction medium, sulphuric acid	0.5 M
Antimony(III) determination	
pH	2.5
Citric acid, mℓ	2 (2M)

*Instrumental conditions as specified in the text.

were found to be 5 mℓ of a 0.25% w/v solution in 0.1 M sodium hydroxide.

By averaging absorbances of five readings and plotting them against antimony concentration, the following analytical curves were obtained:
$A_{Sb\ (III)} = 0.012 + 0.016\ X_{Sb\ (III)}$, r = 0.9997 and $A_{Sb\ (T)} = 0.006 + 0.015\ X_{Sb\ (T)}$, r = 0.9995, where $A_{Sb\ (III)}$ and $A_{Sb\ (T)}$ represent absorbance and $X_{Sb\ (III)}$ (1 – 20 μg ℓ^{-1}) and $X_{Sb\ (T)}$ (1 – 15 μg ℓ^{-1}) represent antimony(III) and total antimony concentrations, respectively. The amount of antimony(V) was calculated from the difference between the total antimony and antimony(III) concentrations. Within-batch, the precisions of the proposed methods were excellent, with an approximate coefficient of variation of 2.0 and 2.8% for antimony(III) and antimony (V) determination, respectively. Detection limits based on three times the standard deviation of the blank was in either case 0.2 μg Sb/ℓ

The accuracy of the analytical methods was established by successful determination of total antimony in certified materials and antimony(III) and antimony(V) in spiked blood serum and urine samples.

A NIST Standard Reference Material, Orchard Leaves (NIST SRM 1541), which yielded the result 3.1 ± 0.4 μg g^{-1} (n = 5) as compared to recommended value 2.9 ± 0.3 μg g^{-1} (difference not significative). In this case the digestion of the samples was performed following the procedure described by Narasaki[20]. The accuracy of the methods were further verified by determining antimony(III) and antimony(V) in spiked blood serum and urine samples. Each sample was split into two aliquots: antimony(III) was selectively determined in one aliquot and total antimony was determined in the other. Good recoveries were obtained using the total antimony and antimony(III) methods (Table 2). Sample storage is an important and often neglected factor. In this work, recoveries for both antimony species varied with time of storage of the spiked biological samples. While, antimony(III) recoveries were lower (95, 92 and 89%) antimony(V) recoveries were higher (102, 104 and 105%) for 1, 2 and 3 days of storage at 4°C, respectively. The reason for the different recovery values for both antimony species under different conditions was based on a change in the valence state. However, no possible explanation of the oxidative process of antimony(III) to antimony(V) in blood serum and urine with time of storage can be yet be provided. If delay are unavoidable prior to analysis, then storage by deep freezing is advised.

The described methods have been used to determine antimony speciation in a number of blood serum and urine samples from human patients with leishmaniasis desease[7] and treated with Glucantim^R and some typical results are given in Table 3. Antimony(III) has always been a minor specie in the matrices studied. The found antimony values before the treatment was started (day 0) could likely be considered as the basic concentractions of both antimony species in blood serum and urine of humans. After five days of treatment, there was a marked increase of both antimony species in blood serum and urine. However, concentrations in urine were much higher than the respective concentractions in blood serum, indicating the urine role in the clearance of antimony species from the blood. Thirty days

Table 2. *Recovery of antimony(III) and antimony(V) added to pooled blood serum and urine samples**

Concentration of antimony in sample, μg ℓ^{-1}						Recovery, %			
		Found							
Added to blood and urine		Blood		Urine		Blood		Urine	
Sb(III)	Sb(V)	Sb(III)	Sb(V)	Sb(III)	Sb(V)	Sb(III)	Sb(V)	Sb(III)	Sb(V)
5	0	8.3	4.7	7.3	0.9	96	-	98	-
10	0	13.4	4.7	12.2	0.9	99	-	98	-
5	2	8.4	6.7	7.4	2.9	98	100	100	100
0	2	3.5	6.8	2.4	2.9	-	105	-	100
0	4	3.5	8.8	2.4	5.0	-	102	-	102

*Endogenous antimony(III) and antimony(V) were of 3.5 and 4.7 (in blood serum) and of 2.4 and 0.9 (in urine) μg ℓ^{-1}, respectively. All data are averages of triplicates.

Table 3. *Concentration of antimony (III) and antimony (V) in blood serum and urine of patients with leishmaniasis injected intravenously with Glucantim^R*

Days*	Concentration of Sb(III), μg ℓ^{-1}		Concentration of Sb(V), μg ℓ^{-1}	
	Blood	Urine	Blood	Urine
0	1.8 ± 0.2 (1.3 – 1.7)	1.6 ± 0.1 (1.4 – 1.8)	1.8 ± 0.2 (1.5 – 2.1)	1.8 ± 0.1 (1.5 – 2.0)
5	330.2 ± 36.0 (294.3 – 360.5)	10250.8 ± 2630.9 (5750 – 15410.2)	1737.4 ± 720.0 (1155.0 – 2501.5)	19625.5 ± 3600 (14000.2 – 25000.3)
40	3.9 ± 0.5 (3.5 – 4.3)	38.5 ± 3.0 (36.3 – 43.5)	10.0 ± 2.6 (7.0 – 12.4)	241.5 ± 22.8 (205.3 – 283.4)

* From 0 to 10 days daily dosis of 17 mg $\ell\ g^{-1}$ Sb(V) (as Glucantim^R) were injected to each patient. Apropriate dilution of the samples was performed to bring antimony concentrations within the linear range of the methods. Numbers in parentheses indicate concentration ranges. n = 10.

after the end of the treatment (day 40) with Glucantim^R some antimony(III) and antimony(V) were still present in the samples.

Our described methods provided a simple, rapid, precise and accurate way of determining Sb(III) and Sb(V) in blood serum and urine samples. The use of hydrochloric acid medium is avoided, thus in this way antimony chlorides are not formed and the accelerated irreversible devitrification of the quartz cell does not occur.

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