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Spectrophotometric determination of Sb(III) and Sb(V) in biological samples after micelle-mediated extraction

Tayyebeh Madrakian*, Elaheh Bozorgzadeh

Faculty of Chemistry, Bu-Ali Sina University, Hamedan, Iran

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ABSTRACT

This work presents a micelle-mediated extraction method for simultaneous preconcentration and determination of Sb(III) and Sb(V) species in biological samples as a prior preconcentration step to their spectrophotometric determination. The analytical system is based on the selective reaction between Sb(III) and bromopyrogallol red (BPR) in the presence of cetyltrimethylammonium bromide (CTAB) and potassium iodide at pH 6.4. Total Sb concentration was determined after reduction of Sb(V) to Sb(III) in the presence of potassium iodide and ascorbic acid. The optimal extraction and reaction conditions were studied and the analytical characteristics of the method (e.g., limit of detection, linear range, preconcentration factor, and improvement factors) were obtained. Linearity for Sb(III) and Sb(V) were obeyed in the range of 0.2–20.0 ng mL^{-1} and 0.4–25.0 ng mL^{-1}, respectively. The detection limit for the determination of Sb(III) and Sb(V) were 0.05 ng mL^{-1} and 0.08 ng mL^{-1}, respectively. The interference effect of some anions and cations was also studied. The method was applied to the determination of Sb(III) in the presence of Sb(V) and total antimony in blood plasma and urine samples.

1. Introduction

Antimony has received relatively little attention since it is non-essential for life and also because it content in most matrices is very low [1]. It is a cumulative toxic element and it has chemical and toxicological properties. Its persistence, fate, bioavailability toxicological and physiological effects in the environment strongly 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 [2] and might cause lung cancer and stibine is a highly toxic gas that can cause both serious injury to the central nervous system and hemolysis. Thus its determination in human blood plasma is important. Sb(V) 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 [3].

Total antimony concentration does not provide sufficient information to understand its toxicity, bioavailability, biotransformation and ways of circulation. Thus, in order to obtain correct information on toxicity and biotransformation of antimony, it is necessary not only to determine the total antimony in the different environmental and biological samples, but also to determine antimony in its different oxidation states. Different approaches have been reported for the speciation analysis of Sb being the combination of a powerful separation technique in environmental and biological samples such as electrothermal vaporization inductively coupled plasma atomic emission spectrometry (ETV-ICP-AES) [4], hydride generation (HG) and inductively coupled plasma optical emission spectrometry (HG-ICP OES) [5], hydride generation inductively coupled plasma atomic emission spectrometry (HG-ICP-AES) [6], electrothermal atomic-absorption spectrometry (ET-AAS) [7], atomic fluorescence spectroscopy (AFS) [8] kinetic-spectrophotometric determination [9], electrochemical [10], and hydride generation atomic fluorescence spectrometry (HG-AFS) [11].

The concentrations of Sb(III) and Sb(V) ions together and individually determined by atomic absorption spectroscopy using XAD-8 resin [12] and Chromosorb 102 resin [13] as retention medium.

However, due to the presence of antimony in environmental and biological samples at low levels, its separation from other elements present and also the use of a preconcentration step is usually necessary.

Cloud-point extraction (CPE), based on the clouding phenomena of surfactants, has become more and more attractive. CPE offers many advantages over traditional liquid–liquid extraction such as low cost, safety and a high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and high concentration factors [14]. For charged micelles, the phenomenon rarely occurs, presumably because electrostatic repulsion prevents phase separation in most cases. In the presence of salt, long-tailed cationic surfactants can self-assemble in aqueous solution into

* Corresponding author. Tel.: +98 811 8272404; fax: +98 811 8272404.
E-mail address: madrakian@basu.ac.ir (T. Madrakian).

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long, flexible wormlike micelles, thus rendering the solution viscoelastic [15,16]. Recently, we used the cloud-point extraction for preconcentration of trace quantities of some cations prior to their determination by spectrophotometry [17–21].

The aim of this work was to introduce a reliable method for the simultaneous preconcentration and determination of Sb(III) and Sb(V) in biological samples (urine and blood plasma) by spectrophotometry after preconcentration by the cloud point extraction technique. Preconcentration of sample solution allowed an improvement factor approaching 27.3 and 21.9 for Sb(III) and Sb(V), respectively, which compares favorably with other CPE methodologies.

2. Experimental

2.1. Apparatus

A Perkin-Elmer Lambda 45 UV–vis spectrometer was used for recording absorbance spectra. Absorption measurements at λ_{max} were performed using a Shimadzu UV-mini-1240 V spectrophotometer with a 1-cm quartz cell (0.5 mL). A Metrohm pH meter (model 713) with a combined glass electrode was used for pH measurements. A centrifuge with 10-mL calibrated tubes (Superior, Germany) was used to accelerate the phase separation process.

2.2. Reagents

All chemicals were of analytical grade purchased from Merck Company (Darmstadt, Germany). A 1000.0 mg L^{-1} (1.11×10^{-2} mol L^{-1}) stock solution of Sb(III) was prepared in concentrated HCl using $K(SbO)C_4H_4O_6 \cdot 0.5H_2O$ (Merck). Working solutions were prepared by appropriate dilution of the stock solution with doubly distilled water daily. A 1000.0 mg L^{-1} stock solution of Sb(V) was prepared in concentrated HCl using $SbCl_5$. A 1.79×10^{-5} mol L^{-1} Bromopyrogallol red (BPR) solution was prepared by dissolving an appropriate amount of BPR in 50% v/v aqueous ethanol. This solution is stable for several weeks. A 2.0% (w/v) cetyltrimethylammonium bromide (CTAB) solution in water was prepared. A 0.1%(w/v) KI solution in water was prepared. Phosphate buffer solution of pH 6.4 was prepared from 0.1 mol L^{-1} potassium dihydrogenphosphate and 0.1 mol L^{-1} sodium hydroxide. The 10% (w/v) ascorbic acid reductant solution was prepared by dissolving appropriate amounts of ascorbic acid in 25 mL of water. This solution was also prepared daily just before use.

2.3. Procedure

An aliquot of the solution containing 2.0–200.0 ng of Sb(III) ion was transferred into a 10 mL tube containing 0.1 mL of 1.79×10^{-5} mol L^{-1} BPR solution, 2.0 mL of pH 6.4 phosphate buffer solution and 1.0 mL of 2.0% (w/v) CTAB solution. The solution was diluted to approximately 9 mL with water and allowed to stand for 10 min at room temperature. Then 1.0 mL of 0.3 mol L^{-1} KI solution was added and made up to the mark with water. Separation of two phases was accelerated by centrifugation for 5 min at 3000 rpm. Then, the aqueous phase could be separated by inverting the tube. The surfactant-rich phase of this procedure was dissolved and diluted to 0.5 mL with the ethanol and transferred into a 0.5-mL quartz cell. The absorbance of the solution was measured at 574 nm. A blank solution was also prepared in the same way except that distilled water was used instead of antimony solution. The blank solution was submitted to the same procedure and its absorbance was measured at 574 nm. The difference between the absorbance of the blank and sample solutions (ΔA) was then calculated. A calibration graph for Sb(III) was constructed by measuring the ΔA for the solutions containing different concentrations of Sb(III) at 574 nm.

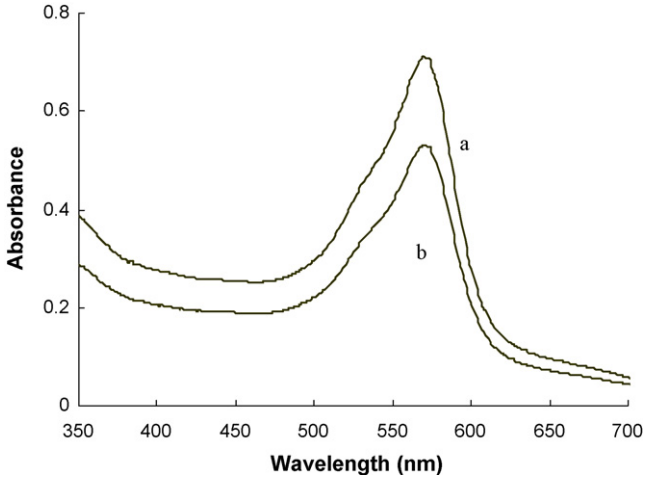

Fig. 1. Absorption spectra for BPR (a) and its complex with Sb(III) (b) in surfactant-rich phase. Conditions: BPR, 4.0×10^{-6} mol L^{-1}; Sb(III), 10.0 ng mL^{-1} (8.21×10^{-8} mol L^{-1}); KI, 0.03 mol L^{-1}; CTAB concentration, 0.2% (w/v) (5.49×10^{-3} mol L^{-1}); pH, 6.4.

For determination of total Sb, a mixture of potassium iodide (2 mL of a 1% (w/v) solution) and ascorbic acid (2 mL of a 10% (w/v) solution) as reducing agents were used for reduction Sb(V) to Sb(III) [22] and reduction of produced I_2 to I^-, respectively.

3. Results and discussion

Bromopyrogallol red as an anionic indicator shows maximum absorbance at 574 nm at pH 6.4, Bromopyrogallol red is often used as a chromogenic reagent for the determination of a large number of metals [23,24]. Sb(III) in HCl medium reacts with BPR in the presence of CTAB and the absorbance of solution decreases at 574 nm. The solution became turbid after addition of the iodide ion. Therefore the ternary complex of Sb(III)–BPR–CTAB can be extracted by CPE method. At the same conditions, Sb(V) does not react with BPR. Fig. 1 shows the absorbance spectra for Sb(III)–BPR–CTAB complex and the blank solution in surfactant-rich phase. After separation of surfactant-rich phase, the absorbance was measured at 574 nm against a reagent blank as the reference.

Sb(V) can be reduced to Sb(III) by iodide. Therefore Sb(V) can also be analyzed after reduction to Sb(III). In order to remove the interference from the iodine color, it can be reduced to iodide by the addition of ascorbic acid [22].

3.1. Optimization of the system

To take full advantage of the procedure, the reagent concentrations and reaction conditions must be optimized. Various experimental parameters were studied in order to obtain optimized system. These parameters were optimized by setting all parameters to be constant and optimizing one each time.

The effect of pH on the absorbance at a constant concentration of complex in surfactant-rich phase was investigated in the range of 2.0–7.2. The results are shown in Fig. 2. As Fig. 2 shows the difference between the absorbance of blank and sample solutions (ΔA) increased by increasing pH up to 6.4 and decreased at higher pHs. The complexation reaction at pH values lower than 2 is incomplete due to protonation of BPR and complexation reaction is incomplete. The decrease in ΔA at pH values higher than 6.4 could be due to the hydrolysis of Sb(III).

Effect of BPR concentration on the extraction and determination of Sb(III) was investigated in the range $(0.40–2.20) \times 10^{-6}$ mol L^{-1}. The sensitivity of the method increased by increasing BPR concen-

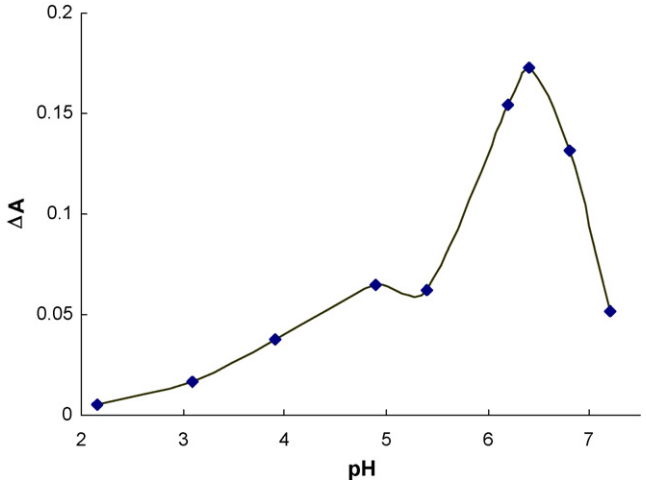

Fig. 2. Effect of pH on signal of Sb(III)–BPR complex. Conditions: 10 ng mL^{-1} (8.21×10^{-8} mol L^{-1}) Sb(III); 0.2% (w/v) (5.49×10^{-3} mol L^{-1}) CTAB; 0.03 mol L^{-1} KI; 1.60×10^{-6} mol L^{-1} BPR.

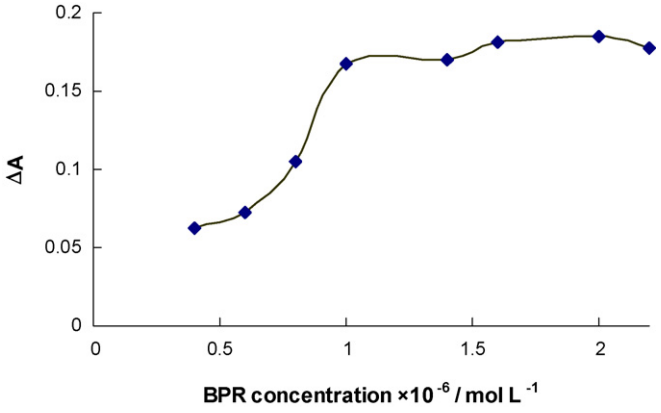

Fig. 3. Effect of BPR concentration on signal of Sb(III)-BPR complex. Conditions: 10 ng mL^{-1} (8.21×10^{-8} mol L^{-1}) Sb(III); CTAB concentration, 0.2% (w/v) (5.49×10^{-3} mol L^{-1}); 0.03 mol L^{-1} KI and pH 6.4.

tration up to 0.89×10^{-6} mol L^{-1} and remained constant at higher concentrations. Therefore, 1.60×10^{-6} mol L^{-1} of BPR was used in further works. The results are given in Fig. 3.

Effect of CTAB concentration on the extraction and determination of Sb(III) was investigated in the range 0.0–0.3% (w/v) (0.00–8.23×10^{-3} mol L^{-1}). The results are shown in Fig. 4. Signal (ΔA) increased by increasing in CTAB concentration up to 0.15% (w/v) (4.12×10^{-3} mol L^{-1}) and remained constant at higher concentrations. Therefore, 0.2% (w/v) CTAB was chosen as the optimum.

As described in introduction section, addition of salts can cause cationic surfactant solutions to separate into immiscible surfactant-rich and surfactant-poor phases. Therefore, iodide was added to induce micellar growth and extraction of complex. The effect of iodide concentration was studied in the range 0.005–0.040 mol L^{-1}.

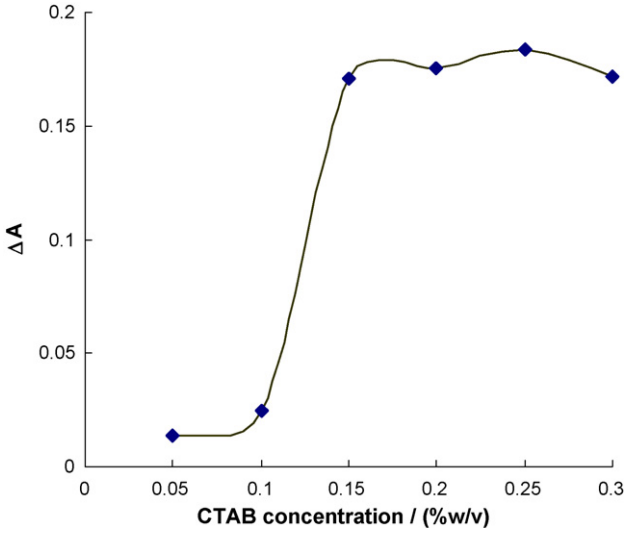

Fig. 4. Effect of CTAB concentration on signal of Sb(III)-BPR complex. Conditions: 10 ng mL^{-1} (8.21×10^{-8} mol L^{-1}) Sb(III); 1.60×10^{-6} mol L^{-1} BPR; 0.03 mol L^{-1} KI and pH 6.4.

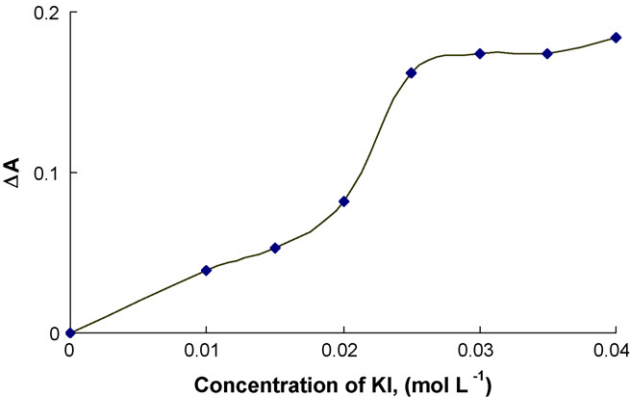

Fig. 5. Effect of iodide concentration on the extraction and determination of Sb(III). Conditions: Sb(III), 10.0 ng mL^{-1} (8.21×10^{-8} mol L^{-1}); BPR 1.60×10^{-6} mol L^{-1}; CTAB concentration, 0.2% (w/v) (5.49×10^{-3} mol L^{-1}); pH, 6.4.

The results show that at zero point the micellization process does not take place and the amount of ΔA is zero. Addition of 0.030 mol L^{-1} iodide sufficed for maximum extraction of the complex and the signal remained constant at higher concentrations. A concentration of 0.035 mol L^{-1} iodide was selected for further works. The results are shown in Fig. 5.

3.2. Analytical characteristics

Table 1 summarizes the analytical characteristics of the optimized method including regression equation, linear range, limit of detection, preconcentration and improvement factors. The limit of

Table 1
Analytical features of the proposed method.

	Sb(III) (ng mL^{-1})	Sb(V) (ng mL^{-1})
Regression equation (n = 16)	$\Delta A = 0.0149C + 0.095$, $r^2 = 0.9878$	$\Delta A = 0.0117C + 0.0065$, $r^2 = 0.9845$
Regression equation (n = 10) before preconcentration (μg mL^{-1})	$\Delta A = 0.5464C + 0.0402$, $r^2 = 0.9845$	$\Delta A = 0.5352C + 0.0173$, $r^2 = 0.9937$
Linear range	0.2–20.0 (20.0–1000.0)[a]	0.4–25.0 (40.0–500.0)[a]
Limit of detection (n = 5)	0.05	0.08
Maximum preconcentration factor	20	20
Improvement factor	27.3	21.9

[a] Linear range before preconcentration

Table 2
Tolerance ratio of diverse ions on the determination of 10 ng mL^{-1} of Sb(III) or 10 ng mL^{-1} of Sb(V).

Ion	Tolerance ratio
NO_3^-,SO_4^{2-},Cl^-	1000
Ca^{2+},Cu^{2+}	1000[a]
Ni^{2+}, Pb^{2+}, Al^{3+}, Co^{2+}, Mg^{2+}, Mn^{2+}, Sr^{2+}, Cd^{2+}, Ce^{3+}, Zn^{2+}	300[a]
As^{3+},Fe^{2+}, Fe^{3+}	100[a]
MoO_4^{2-}	10[a]

[a] After removing as described in text.

detection, defined as $C_L = 3S_B/m$ where C_L, S_B, and m are the limit of detection, standard deviation of the blank and slope of the calibration graph, respectively, were 0.05 ng mL^{-1} and 0.08 ng mL^{-1} for Sb(III) and Sb(V), respectively. Because the amount of Sb(III) in 10 mL of sample solution is measured after preconcentration by CPE in a final volume of 0.5 mL, the maximum preconcentration factor of the solution is 20. The improvement factor, defined as the ratio of the slope of the calibration graph for the CPE method to that of the calibration graph in aqueous media, for Sb(III) and Sb(V) were 27.3 and 21.9, respectively.

The relative standard deviation (R.S.D.) for five replicate measurements of 0.5 ng mL^{-1} of Sb(III) and Sb(V) were 2.7% and 2.2%, respectively, and for 10.0 ng mL^{-1} of Sb(III) and Sb(V) were 2.3% and 1.9%, respectively.

3.3. Selectivity

The effect of different cations and anions on the determination of 10.0 ng mL^{-1} Sb(III) or 10.0 ng mL^{-1} Sb(V) by the proposed method was studied. An ion was considered to be an interferent when it caused a variation greater than ±5% in the absorbance of the sample. For the determination of 10 ng mL^{-1} Sb(III) by this method, the foreign ions can be tolerated at the levels given in Table 2. Ni^{2+}, Pb^{2+}, Al^{3+}, Co^{2+}, Mg^{2+}, Mn^{2+}, Sr^{2+}, Cd^{2+}, Ce^{3+} and Zn^{2+} interfered at 1000 ng mL^{-1} level. Their interfering effects up to 3000 ng mL^{-1}, were completely removed by the addition of 1.0 mL of 0.1 mol L^{-1} EDTA. As(III), Fe(II) and Fe(III) interfered at 100 ng mL^{-1}. Their interfering effects up to 1000 ng mL^{-1} were completely removed by the addition of 1.0 mL of 0.1 mol L^{-1} EDTA. Copper(II) interfered at 1000 ng mL^{-1}. Its interfering effect up to 10000 ng mL^{-1} was removed in the presence of 0.01 mol L^{-1} cyanide ion. Cyanide ion was added before the addition of EDTA [25]. Molybdenum interfered extensively, but it could be tolerated at moderate concentrations (100 ng mL^{-1}) in the presence of 0.5 mL of 0.01 mol L^{-1} fluoride ion.

The extensive interference of calcium was also eliminated in the presence of mixture of masking agent EDTA–fluoride. Sb(V) do not interfere up to 10000 ng mL^{-1} on the determination of Sb(III).

3.4. Determination of Sb(III) and Sb(V) in urine sample

In order to evaluate the analytical applicability of the proposed method, it was applied to the determination of Sb(III) and Sb(V) in a urine sample. The tested urine was found to be free from antimony and so antimony was determined after addition to urine sample before pretreatment. On 25 mL of urine sample, 10 mL of 1:1 H_2O:HNO_3 mixture was added and heated for 10 min. The sample was cooled and then 5 mL concentrated nitric acid was added. The sample was filtered and diluted to the mark with distilled water [26]. After pretreatment for the determination of Sb(III) concentration, 2 mL of digested sample was transferred into 10-mL tubes then proposed method by the standard addition method was applied. For total antimony (Sb(III) + Sb(V)) determination, after reduction of Sb(V) to Sb(III), proposed method was applied. The results are given in Table 3. As could be seen, the recoveries for the spiked samples were in the acceptable range.

3.5. Determination of Sb(III) and Sb(V) in blood plasma

For analysis of plasma, 2 mL of acetonitrile was added to 10 mL of plasma and the sample was centrifuged for separation of proteins. Two millilitre of sample solution was transferred into a 10-mL tube then proposed method was successfully applied to the determination of Sb(III) concentration.

After reduction of Sb(V) to Sb(III), proposed method was successfully applied to the determination of Sb(III) and Sb(V) in blood plasma. The results are given in Table 3. As could be seen, the satisfactory results were observed.

4. Conclusion

The proposed procedure gives a simple, very sensitive and low-cost spectrophotometric procedure for preconcentration of Sb(V)

Table 3
Analytical data of antimony species determined in urine and blood plasma sample (mean ± S.D., n = 3).

Sample	Added (ng mL^{-1})	Determined (ng mL^{-1})	Recovery (%)	
	Sb(III)	Sb(V)	Sb(total)	Sb(total)
Urine	0.00	11.00	11.37	103.4
	2.00	2.00	4.10	102.5
	11.0	0.00	10.96	99.6
Blood plasma	0.00	0.00	0.23	–[a]
	0.60	0.60	1.42	99.3
	0.80	0.80	1.83	100.0

[a] Not detected.

Table 4
Preconcentration procedures for determination of Sb(III) using sorbent or reagent for extraction.

Sorbent or reagent	Analytical method	Detection limits for Sb(III) (ng mL^{-1})	Detection limits for Sb(V) (ng mL^{-1})	Ref.
Pyrrolidine dithiocarbamate	ETV-ICP-AES	0.09	–	[4]
L-proline	HG -ICP	0.09	–	[5]
PSTH-Dowex	FI-ETAAS	2	–	[27]
S. cerevisiae-PUF	HG-ICP-OES	0.15	–	[28]
IDAEC	GFAAS	0.18	0.25	[29]
L-methionine	HG-ICP OES	0.07	–	[30]
Proposed method	CPE	0.05	0.08	

and Sb(III) and determination of trace quantities of them that can be applied to real samples. Further, in comparison with solvent extraction methods, it is much safer, since only small amounts of surfactant, which has a low toxicity, is used. A comparison between the proposed method with the previously reported methods [4,5,27–29] for preconcentration and determination of Sb(III) and Sb(V) (Table 4) indicate that this method has a lower detection limit and wider linear range. The method is, safe, simple, rapid and inexpensive for the determination of trace quantities of antimony to real samples.

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