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INFLUENCE OF ORGANIC ADDITIVES ON STIBINE  
GENERATION

Jožica Serafimovska, Sonja Arpadjan\*, Trajče Stafilov

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**Abstract**

The influence of EDTA, carboxylic acids, amino- and hydroxocarboxylic acids, monosaccharide and humic acids on the generation of stibine in hydride generation atomic absorption spectrometry (HGAAS) was investigated. EDTA, tartaric, citric and malonic acids, fructose and N,N-bis(2-hydroxyethyl)-glycine (BICIN) were found as appropriate reaction media for selective and sensitive determination of Sb(III). The pre-reduction of Sb(V) to Sb(III) in the presence of organic ligands is quantitative with L-cysteine (1% m/v) as reductant. An analytical application to the determination of Sb(III) and Sb(V) in waters, tea infusions, EDTA soil/sediment extracts using continuous flow HGAAS was demonstrated. The limit of detection was 0.08 µg/l Sb. Within-day precision was 5–20% in the range 0.2–1 µg/l Sb, and 4–12% in the range 1–5 µg/l Sb with recoveries between 94 and 102%.

**Key words:** antimony, speciation analysis, HGAAS, organic additives

**1. Introduction.** Antimony and its compounds are considered as pollutants of priority interest due to their high toxicity [1, 2]. Trivalent species are reported to be more toxic than pentavalent forms [3–5]. The maximum admissible concentration of antimony in drinking water is 5 µg/l [6]. The main sources of human exposing to Sb are water, air and urban dust. The accuracy, precision and reliability of the applied analytical methods are very important for better understanding and clarifying the toxicity of antimony in inorganic and organic form, its transformation and transport in the different environmental compartments as well as its biological function. Volatile hydride generation (HG) combined with

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atomic absorption spectrometric (AAS) determination is one of the most sensitive analytical methods for Sb [7, 8]. According to many publications Sb is one of the least problematic hydride forming elements since its hydride is generated instantaneously within a very broad interval of acidity and NaBH<sub>4</sub> concentration. These observations seem to be true for Sb (III). For pentavalent Sb the complication is that under any given set of hydride generation conditions not all Sb(V) compounds can form detectable volatile species. For determination of the total Sb most HG procedures involve a pre-reduction stage of any Sb(V) to Sb(III). But if the sample analysed contains masking agents, they change the conditional redox potential of Sb(V)/Sb(III), therefore this pre-reduction does not always proceed quantitatively. The effect of HF which strongly interferes with the reduction of pentavalent antimony to the trivalent state has been investigated [9, 10]. L-cysteine was used as pre-reducing and masking agent in order to control the interference effects arising from concomitant elements and inorganic acid mixtures [10, 11–13]. Biotic, food samples and pH buffers for biomedical research contain carboxylic acids, hydroxo- and amino-acids or monosaccharides (glucose, fructose) which form stable complexes with Sb(V). EDTA is a common extractant from soils and is routinely applied to assess the potential bioavailability of essential and toxic elements. The influence of organic substances on the stibine generation has not previously been studied in detail.

The aim of this study was to investigate the influence of EDTA, tartaric, citric, oxalic and malonic acids, BICIN (N,N-bis(2-hydroxyethyl)-glycine), glycine (NH<sub>2</sub>CH<sub>2</sub>COOH) and fructose on the efficiency of stibine generation using continuous flow (CF) hydride generation.

**2. Experimental. 2.1. Apparatus.** A continuous flow vapour generation accessory (VGA-77, Varian) connected to an atomic absorption spectrometer (SpectrAA 55B, Varian) was employed for HGAAS measurements. The instrumental parameters are given on Table 1.

T a b l e 1

Instrumental parameters for CF-HGAAS

Parameter	Setting
Radiation source	Varian hollow cathode lamp
Wavelength	217.6 nm
Bandpass	0.5 nm
Integration time	3 s
Delay time	140 s
Replicates	6
Quartz cell temperature	950 °C
Pre-reductant	L-cysteine (1% m/v)
Sample flow rate	7 ml/min
Acid flow rate	1 ml/min
NaBH <sub>4</sub> flow rate	1 ml/min

**2.2. Reagents.** All chemicals used were of analytical reagent grade. Double distilled water was used throughout the experiment. The antimony reagents used were as follows: antimony(III) chloride stock standard solution, 1000 mg/l Sb (BDH, Poole, UK), 1000 mg/l Sb(V) as kaliumhexahydroxoantimonat (V)  $K[Sb(OH)_6]$  (p.a. Merck). The working standard solutions (0.2–7  $\mu\text{g/l}$  Sb) for the two antimony species (Sb(III), Sb(V)) were prepared daily in distilled water alone and in the presence of different organics (EDTA, tartaric, citric, oxalic and malonic acids, BICIN, glycine and fructose). The carboxylic acids were chosen to cover a broad range of various types of functional groups as hydroxy-, amino-, dicarboxy-, polycarboxy-, polyhydroxy- acids. Sodium tetrahydroborate (p.a Merck) solution 0.6% m/v in 0.5% m/v sodium hydroxide was prepared prior to use.

**2.3. Sample preparation.** For determination of total antimony, the natural water samples and aqueous tea extracts were pre-reduced with L-cysteine (1% m/v) for 45 min at room temperature. For preparation of aqueous tea extracts the teacup bags (1.5g dry tea substance; Dilmah Ceylon black tea) were placed for 5 min in 50.0 ml boiled distilled water. Prior to measurement of tea extracts under conditions presented on Table 1, a few drops of 30% m/v silicone antifoaming emulsion in water were added.

**2.4. Preparation of EDTA extracts of soil and sediment.** Extractions with EDTA (0.05 mol/l, pH 6–7) were performed with 500 mg samples of air-dried soil or sediment. The sample was weighed in a 15 ml centrifuge tube. Then 5 ml of 0.05 mol/l EDTA solution was added. The tube with the mixture was placed in an end-over-end shaker for 1 h at  $23 \pm 2^\circ\text{C}$ . After shaking, the sample was filtered through a filter paper (narrow pore “blue ribbon” paper filter, previously EDTA and water washed) into a 25 ml volumetric flask and the residue was washed with 10 ml water. Before adjusting to the mark, 5 ml HCl (35% m/v) were added to the volumetric flask. After homogenization, two 10 ml aliquots from the filtrate were transferred to 15 ml centrifuge tubes. To the one of these tubes 0.1 g L-cysteine was added, the sample was homogenized and left for 45 min at room temperature. The non-treated sample aliquot was used for determination of extracted Sb(III) fraction. The L-cysteine treated solution was used for determination of the total extracted fraction of antimony by CF-HGAAS using the same experimental parameters (Table 1). The content of Sb(V) was obtained by difference.

**3. Results and discussion. 3.1. Effect of EDTA and dicarboxylic acids on the generation of stibine.** The effect of various carboxylic acids was studied at fixed concentration of  $\text{NaBH}_4$  (0.6%  $\text{NaBH}_4$  in 0.5%  $\text{NaOH}$ ) in the reductant channel and at fixed concentration of HCl (1.2 mol/l) in the acid channel. EDTA, tartaric, citric, oxalic and malonic acids were investigated as representatives of organic acids, encountered in food and environmental samples. The observed effects on the generation of stibine are shown in Fig. 1. As it

can be seen, EDTA, tartaric, citric and oxalic acids (0.2 mol/l) provide the same sensitivity for Sb(III) as compared to pure aqueous solutions. Antimony(III) gives 1.3-fold higher absorption signal in the presence of malonic acid (0.2 mol/l). Atomic absorption signals for Sb(V) are detected only for oxalic acid solutions but the response for Sb(V) is 5-fold lower vs. Sb(III) species. EDTA, tartaric and citric acids totally suppress the generation of volatile Sb(V) hydride, i.e. no absorption signal for Sb(V) is registered. It means that these three organic acids allow quantitative determination of Sb(III) (100% recovery in comparison to pure aqueous Sb(III) standard solution) at total suppression of the signal for Sb(V). EDTA, tartaric and citric acids can be successfully applied for speciation of inorganic antimony in water solutions and drinks. Our results agree well with previously reported data for tartaric and citric acids [14–17].

**3.2. Influence of aminocarboxylic acids.** The influence of two aminocarboxylic acids glycine (aminoacetic acid) and BICIN (N,N-bis(2-hydroxyethylglycine)) on the generation of stibine is presented in Fig. 1. There is no significant difference in the absorption signals for Sb(III) before and after addition of glycine (0.2 mol/l) and BICIN (0.025 mol/l). At higher BICIN concentrations (0.05–0.2 mol/l) the response for Sb(III) is 1.6-fold higher vs. pure aqueous Sb(III) solutions. It means that with addition of BICIN to the aqueous standard and sample solutions, we can achieve a 1.6-fold enhancement of the sensitivity of the CF-HGAAS determination of antimony. Taking into account that BICIN does not allow the formation of volatile Sb(V) hydride at all, this aminocarboxylic acid

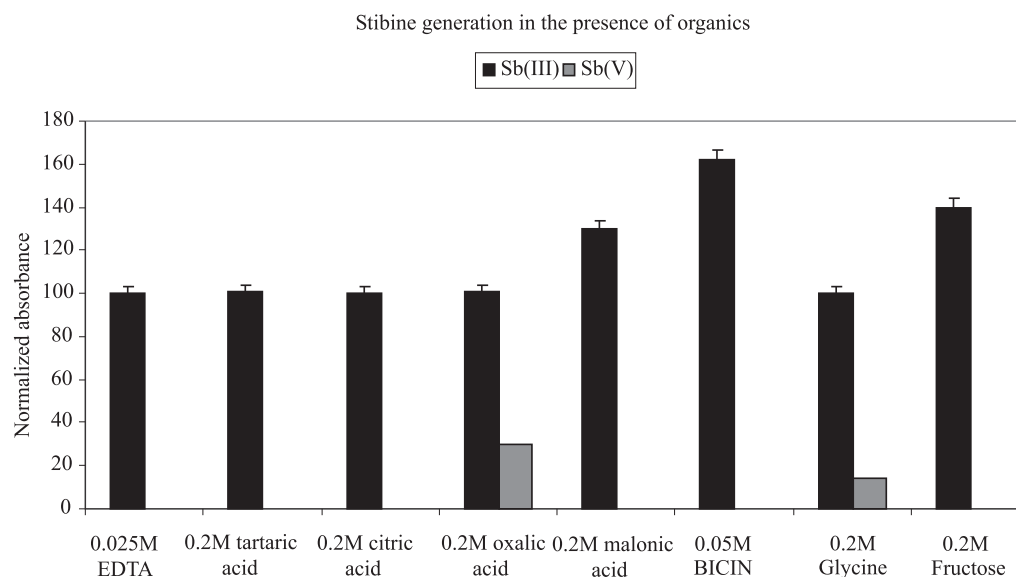


Fig. 1. Influence of organic media on the hydride generation of Sb(III) and Sb(V). Concentration of Sb: 4 µg/l; reductant channel: 0.6% m/v NaBH<sub>4</sub> in 0.5% m/v NaOH; concentration of HCl in the acid channel: 1.2 mol/l HCl

could be applied for more sensitive determination of inorganic Sb(III) and total antimony species after pre-reduction.

**3.3. Influence of monosaccharide.** Fructose effect on stibine generation was examined (Fig. 1). In the presence of 0.2 mol/l fructose the signal for Sb(III) was 1.4-fold higher in comparison with pure aqueous Sb(III) standard. No absorbance signal was detected for Sb(V) which means that the Sb(V) species do not form volatile hydrides in the presence of fructose. A pre-reduction step is necessary for determination of total antimony in fructose containing samples.

**3.4. Optimization of the pre-reduction procedure.** L-cysteine (1% m/v), KI (2% m/v) + ascorbic acid (1% m/v) and  $\text{NH}_2\text{OH}\cdot\text{HCl}$  (2% m/v) were studied as reductants for pre-reduction of pentavalent antimony to the trivalent form. The reduction was carried out off-line at room temperature. The efficiency of the reduction was examined in dependence on the concentration of HCl (0.5–6 mol/l HCl) in the sample solution and on the pre-reduction time (5–60 min). The recovery of Sb(V) compared with Sb(III) was the highest with L-cysteine as reductant. Full recovery was obtained in the presence of 1.5 mol/l HCl after standing for 45 min (Figs 2 and 3). Potassium iodide required higher acid concentration in the sample (6 mol/l) and the precision of the obtained recoveries was not sufficient. With  $\text{NH}_2\text{OH}\cdot\text{HCl}$  as reductant the recovery of Sb(V) in the presence of the investigated organics was lower than 70%.

**3.5. Determination of inorganic antimony species in natural waters and aqueous tea extracts.** From each examined water and aqueous tea extract two parallel 40 ml aliquots were placed in two 50.00 ml volumetric flasks. Then 0.4 g BICIN and 6 ml conc. HCl were added. After dissolution of the BICIN, to one of the flask 0.5 g L-cysteine was added and the flasks were filled up to the

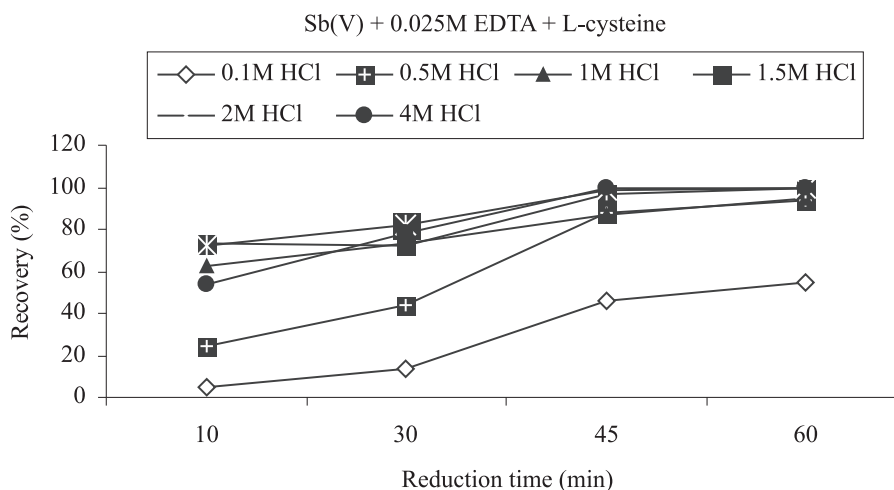


Fig. 2. Efficiency of reduction of Sb(V) to Sb(III) in 0.025M EDTA with 1% L-cysteine vs. hydrochloric acid concentration in the sample solution and reaction time

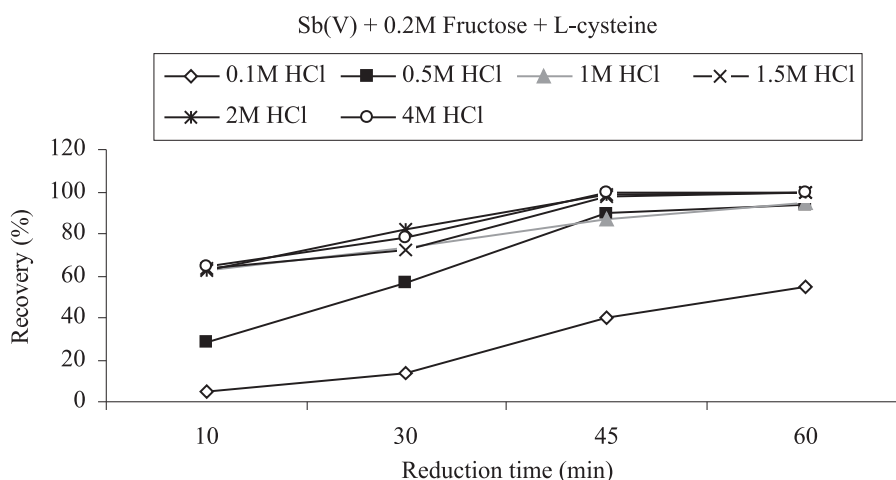


Fig. 3. Efficiency of reduction of Sb(V) to Sb(III) in 0.2M fructose with 1% L-cysteine vs. hydrochloric acid concentration in the sample solution and reaction time

mark with doubly distilled water. The samples were left at room temperature for 45 min. The concentration of total antimony was determined by CF-HGAAS (Table 1) in the pre-reduced with L-cysteine sample, and the Sb(III) content – in the sample without L-cysteine. In the presence of BICIN only Sb(III) forms volatile hydride and can be measured under conditions presented on Table 1. The calibration standards for natural waters were prepared in the same way as the samples using aqueous Sb(III) standards. For tea samples standard addition calibration method was applied. Blank was prepared dissolving 0.4g BICIN in 50.00 ml doubly distilled water.

**3.6. Analytical performances.** The accuracy of the proposed procedures was checked by spiking the samples with Sb(III) and Sb(V). The mean recoveries were between 94 and 102%. Additionally the accuracy of the procedure for total antimony determination in natural waters was estimated analysing two standard

T a b l e 2

Analytical characteristics of CF- HGAAS procedure for determination of total antimony in natural waters and tea aqueous extracts

Parameter	Natural waters	Tea aqueous extracts
Limit of detection ( $3\sigma$ ), $\mu\text{g/l}$	0.08	0.12
Limit of determination ( $6\sigma$ ), $\mu\text{g/l}$	0.14	0.2
Linear range, $\mu\text{g/l}$	0.14–7	0.2–7
Recovery, %	$97 \pm 3$	$95 \pm 4$
Precision, RSD %		
Concentration range (0.2 – 1) $\mu\text{g/l}$	5–15	6–20
Concentration range (1–5) $\mu\text{g/l}$	4–10	4–12
Combined uncertainty		
Concentration range (0.2 – 1) $\mu\text{g/l}$	0.03–0.1	0.04–0.1
Concentration range (1–5) $\mu\text{g/l}$	0.1–0.2	0.1–0.2

T a b l e 3

Antimony contents in natural waters from Bulgaria (river and tap waters), and Macedonia (mineral, spring and river waters)

Type of samples	Number of samples	Sb(III) in $\mu\text{g/l}$	Sb(V) in $\mu\text{g/l}$
Mineral water	3	0.1–0.2	0.18–0.45
Mineral water	10	< 0.08	0.08–0.27
Spring water	1	< 0.08	$0.14 \pm 0.01$
Tap water	5	< 0.08	< 0.08
River water	9	< 0.08	0.08–0.24
River water	3	0.08–0.22	0.2–0.83

T a b l e 4

Antimony species in soil/sediment extracts ( $\mu\text{g/g}$  as Sb in solid sample,  $n = 3$ )

Sample	total Sb	Sb(III) in EDTA extract	Sb(III) + Sb(V) in EDTA extract
CRM MESS-3	$1.02 \pm 0.09^*$	$0.49 \pm 0.05$	$0.91 \pm 0.06$
Black sea sediment (Burgas)	$0.95 \pm 0.08$	$0.42 \pm 0.06$	$0.75 \pm 0.05$
Industrially polluted soil (Pb-Zn factory)	$4.2 \pm 0.3$	$1.3 \pm 0.1$	$2.9 \pm 0.2$
Industrially polluted soil (coal burning power plant)	$2.8 \pm 0.1$	$0.8 \pm 0.1$	$1.5 \pm 0.1$

\*Certified value for total Sb.

reference materials for river water SLRS-1 and SLRS-3 with reported values for Sb  $0.63 \pm 0.05 \mu\text{g/l}$ , and  $0.12 \pm 0.01 \mu\text{g/l}$ , respectively. The obtained values ( $0.57 \pm 0.04 \mu\text{g/l}$  Sb, and  $0.14 \pm 0.02 \mu\text{g/l}$  Sb) showed acceptable agreement. The analytical characteristics of the proposed procedure are summarized on Table 2.

**3.7. Antimony in natural waters and black tea infusion.** The proposed method was applied for the determination of Sb(III) and Sb(V) in mineral water (commercially bottled), spring water (commercially bottled), tap and river waters (Table 3). None of the analysed samples contained antimony above  $1 \mu\text{g/l}$ , i.e. much lower than the permitted level. In the black tea infusion only Sb(III) species were detected ( $0.23 \pm 0.02 \mu\text{g/l}$ ).

**3.8. Determination of EDTA extractable antimony in soils and sediments.** Extracts from soils and sediment CRM were prepared as described. The calibration was performed with aqueous standard solutions of Sb(III) containing  $0.025 \text{ mol/l}$  EDTA (pH 5–6). The calibration curve is linear ( $r = 0.99$ ) in the range  $0.5\text{--}10 \mu\text{g/l}$  Sb in diluted extracts. The procedure allows detection of  $0.8 \text{ mg/kg}$  EDTA extractable Sb in a soil/sediment sample. The within-day precision was 3–10% RSD (three independent replicates) The recovery of spikes of Sb(III) and Sb(V) ( $0.5$  and  $5 \text{ mg/kg}$  Sb), passed through the whole extraction and measurement procedures were between 92 and 102%. The results obtained for EDTA

extracts from some soil samples and marine sediment reference material MESS-3 are presented on Table 4.

**Conclusion.** EDTA, glycine, tartaric, citric and oxalic acids give the same sensitivity for Sb(III) as compared to pure aqueous solutions. Malonic acid, BICIN and fructose enhance the absorption signals for Sb(III). L-cysteine is the appropriate reductant for determination of Sb(V) in all studied media. The addition of BICIN to water samples permits simple, sensitive, precise and accurate speciation of antimony.

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*Institute of Chemistry  
Faculty of Natural Sciences and Mathematics  
Sts. Cyril and Methodius University  
1000 Skopje, Macedonia*

*\*Faculty of Chemistry  
St. Kl. Ohridski University of Sofia  
1, J. Bourchier Blvd  
1164 Sofia, Bulgaria*