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Short communication

Liquid chromatography–hydride generation–atomic fluorescence spectrometry hybridation for antimony speciation in environmental samples

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Abstract

Atomic fluorescence spectrometry was used as an element-specific detector in hybridation with liquid chromatography (LC) and hydride generation for the speciation of Sb(III), Sb(V) and trimethylantimony dichloride ($TMSbCl_2$). The three species were poorly resolved in a single chromatogram but good results were obtained by anion-exchange chromatography, using a mobile phase with 20 mM EDTA and 8 mM hydrogenphthalate to separate Sb(III) and Sb(V) and 1 mM carbonate at pH 10 to separate Sb(V) and $TMSbCl_2$. Calibration graphs were linear between 2 and 100 $\mu g\,l^{-1}$. Detection limits were 0.9, 0.5 and 0.7 $\mu g\,l^{-1}$ for Sb(III), Sb(V) and $TMSbCl_2$, respectively. The method was applied to the speciation of antimony in environmental samples.
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Keywords: Antimony speciation; Liquid chromatography; Hydride generation–atomic fluorescence spectrometry; Environmental samples

1. Introduction

Antimony has a varied chemistry with many applications in industry and medicine [1]. Is an environmentally important element due to its toxicity and biological effects, and is non-essential for animals and plants. However, although sensitive analytical methods exist for the determination of Sb, very little is known about the speciation of the element. The toxicity of the element depends on its oxidation state, Sb(III) being more toxic than Sb(V), although its molecular form is also important, inorganic species being more toxic than organic compounds [2]. Antimony compounds are used for industrial purposes and are released to the environment and aquatic systems. The UE first established a maximum admissible concentration in drinking waters of 10 $\mu g\,l^{-1}$ [3] with typical values for the element in non-contaminated waters of <300 $ng\,l^{-1}$, the limit being posteriorly lowered to 5 $\mu g\,l^{-1}$. Sb(V) was the most common form in terrestrial water samples, although less clearly identifiable species were detected in sediment and plant extracts.

The variable toxicity of Sb species makes it necessary to develop new speciation methods [4]. Several procedures have been proposed using liquid chromatography (LC) coupled with spectroscopic techniques, such as hydride generation–atomic absorption spectrometry (HG–AAS) [5–9], inductively coupled plasma atomic emission (ICP-AES) [10,11], or mass spectrometry (ICP-MS) [1,5,6,8,12–18]. Atomic fluorescence spectroscopy (AFS) seems to be an appropriate alternative and the hybridation LC–HG–AFS offers good analytical characteristics as regards linearity and low detection limits; it is also relatively free of interference and memory effects. The technique is based on the selective determination of Sb(III) and, so

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it is necessary to carry out a reduction step to transform the organic compounds and Sb(V) into Sb(III). Very few papers concerning speciation of Sb by LC–HG–AFS have been published. Casiot et al. speciated Sb(III) and Sb(V) in spiked waters [19], Sayago et al. speciated Sb(III), Sb(V) and $TMSbBr_2$ in waters [20,21], Craig et al. speciated Sb(V) and TMSb in waters [22] and Miravet et al. speciated Sb(III), Sb(V) and trimethylantimony dichloride ($TMSbCl_2$) in waters [23].

A number of advances have been made in the determination of metal species using LC based upon the use of multidentate complexing agents [24]. EDTA has a very high complexing capacity, transforming the positive metal ion into a negatively charged complex. Potassium hydrogenphthalate (KHP) also produces neutral or negatively charged complexes. The use of a secondary equilibrium using complexing eluents improves the column efficiency for separating metal species. Normally the mechanism involved is ionic-exchange.

The present study is based on the speciation of Sb(III), Sb(V) and $TMSbCl_2$ using a LC–HG–AFS new hybridation procedure using anion-exchange LC with complexing agents in the mobile phase. The method was applied to the analysis of environmental samples.

2. Experimental

2.1. Instrumentation

The LC system consisted of an Agilent 1100 (Agilent, Waldbronn, Germany) liquid chromatograph operating at room temperature with a flow-rate of 1 ml min^{-1}. The solvents were degassed using an on-line membrane system (Agilent 1100). Aliquots of 100 μl were injected manually using a Model 7125-075 Rheodyne injection valve (Rheodyne, CA, USA). Separation was performed on a Hamilton PRP-X100 (Teknokroma, Barcelona, Spain) strong anion-exchange column (150 mm × 4.1 mm, 10 μm).

Hydride generation–atomic fluorescence spectrometry was performed using a PSA Millenium Excalibur continuous flow system (PS Analytical, Orping, UK) with a PSA 10570 UV cracker. Measurements were carried out using a boosted discharge hollow cathode lamp for antimony (Photron PTY Ltd., Australia) at the 217.6 nm line, with a 17.5 mA primary current and a 15 mA boost current. The conditions for HG–AFS were: reagent flow-rates of 2.5 ml min^{-1} for both the 1.5 M hydrochloric acid and the 2.5% (m/v) sodium tetrahydroborate solutions. The U-shaped gas liquid separator was flushed with argon gas and the volatile hydride produced was swept by the stream of argon (270 ml min^{-1}), passed through a Perma Pure hygroscopic membrane (Farmingdale, NJ, USA) and atomised using a hydrogen diffusion flame. Valves and T-pieces were obtained from Omnifit (Cambridge, UK). Fig. 1 shows the on-line system used.

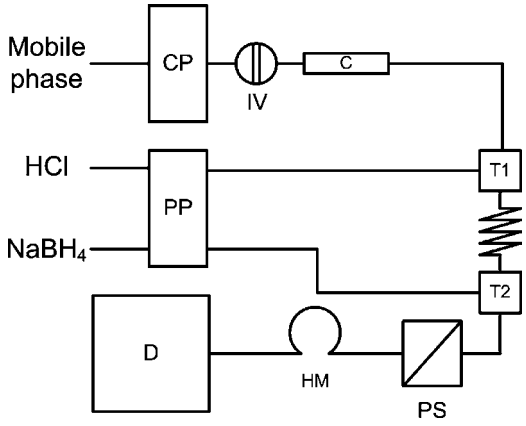

Fig. 1. Chromatographic system coupled with detection by HG–AFS. CP, chromatographic pump; IV, injection valve; C, chromatographic column; PP, peristaltic pump; T_1 and T_2, T-pieces for mixing reagents; PS, gas–liquid phases separator; HM, hygroscopic membrane and D, HG–AFS detector.

An UP 200 H ultrasonic processor (Dr. Hielscher, Germany) and an EBA 20 centrifuge (Hettich, Germany) were also used.

2.2. Reagents

All the solutions were prepared with deionised water (18 MΩ cm) purified through a millipore purification system (Millipore, Bedford, MA, USA). The glassware was thoroughly acid-washed with a 10% (v/v) nitric acid solution and rinsed with deionised water prior to use.

Stock solutions of 1000 μg ml^{-1} were prepared by dissolving potassium antimonyl tartrate hydrate (99.95% purity), potassium hexahydroxyantimonate (99.99%) and trimethylantimony dichloride (96%) (Aldrich, Milwaukee, USA) from the commercial products using water. Diluted solutions were prepared daily with water. The mobile phases were an 8 mM EDTA–2 mM potassium hydrogenphthalate (Panreac, Barcelona, Spain) solution prepared daily and a 1 mM potassium carbonate solution of pH 10 prepared daily from the commercial product (Fluka, Buchs, Switzerland) and pH was adjusted by adding dropwise 30% (m/v) potassium hydroxide (Panreac). The 2.5% (m/v) sodium tetrahydroborate solution was prepared daily by dissolving sodium tetrahydroborate (Aldrich) in 1% (m/v) sodium hydroxide solution and the 1.5 M hydrochloric acid solution was diluted from the concentrated acid (Fluka). Nitric acid (65%, m/v, Aristar, Poole, Dorset, UK), citric acid (Fluka) and sodium hydroxide pellets (Fluka) were also used.

2.3. Samples

The waters collected were 11 tap, river and residual samples. The four soil samples were certified reference materials supplied by the National Institute of Standards and Tech-

nology, San Joaquín (NIST 2709) and Montana (NIST 2711) and the China National Analysis Center (NCS DC 73323 and NCS DC 73324).

2.4. Procedures

2.4.1. Water samples

Aliquots were centrifuged at 6000 rpm for 5 min, filtered through a 0.45 μm nylon millipore chromatographic filter and injected into the chromatograph.

2.4.2. Soil samples

Soil amounts (0.1–0.25 g) were weighed into polypropylene bottles and 3 ml of 30 mM citric acid was added. The sample was sonicated for 3 min at maximum energy and maintained at room temperature for 10 min. After centrifugation at 6000 rpm for 10 min, the residue was subjected to two further extractions, adding 3 ml of citric acid. The supernatants were mixed and aliquots were filtered through a 0.45 μm nylon millipore chromatographic filter and injected into the chromatograph.

3. Results and discussion

3.1. Optimal conditions for the HG–AFS hybridation

The only compound which generated the hydride was Sb(III) and so the use of an UV cracker to reduce all the species was assayed. Similar fluorescence signals were obtained for Sb(III), Sb(V) and $TMSbCl_2$ without and with the cracker when using hydrochloric acid as reductor. Consequently, the UV cracker was deemed unnecessary. The use of an additional reductor, such as potassium bromide or potassium iodide was tried in an attempt to improve the reduction efficiency. However, the signals again remained constant and so hydrochloric acid was the only reductor used.

The dependence of the fluorescence on the concentration of hydrochloric acid was investigated between 0.5 and 5 M. As can be seen from Fig. 2a, the fluorescence rapidly increased with increasing acid concentrations up to 1.5 M, after which the responses were constant for Sb(III) and Sb(V), although quite different for $TMSbCl_2$ whose signal decreased strongly. Thus, a 1.5 M concentration was chosen, which also provided the maximum signal-to-background ratio for all the species. When the sodium tetrahydroborate concentration was varied between 1 and 4% (m/v), the behaviour of the three Sb species was similar (Fig. 2b), with maximum sensitivity achieved with a 2.5% (m/v) which was selected because higher percentages led to high noise. The flow-rates for the hydrochloric acid and the sodium tetrahydroborate solutions were varied between 1 and 5 ml min^{-1}, and a value of 2.5 ml min^{-1}, which provided the maximum peak area and an appropriate flame stability was selected for both channels.

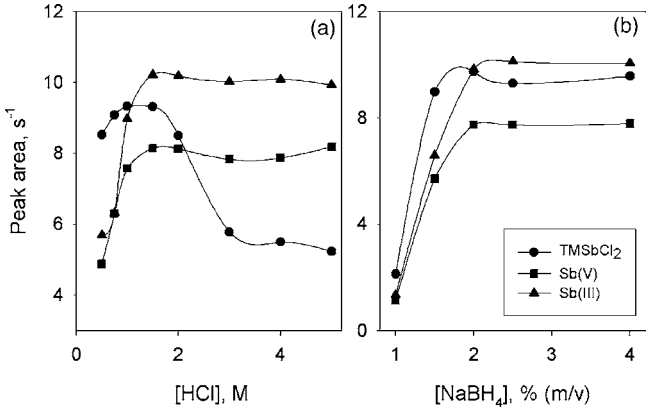

Fig. 2. Influence of the hydrochloric acid (a) and the sodium tetrahydroborate (b) concentrations on the signals of Sb(III), Sb(V) and $TMSbCl_2$ (50 μg l^{-1} each). Reagent flows, 2.5 ml min^{-1}; argon flow, 270 ml min^{-1}.

3.2. Optimization of the liquid chromatographic separation

The distribution equilibrium of the metallic species depends on the pH of the mobile phase, the concentrations of all the ligands and the complex formation constants. Thus, to optimize chromatographic resolution, it was necessary to select a ligand concentration range and the pH of the mobile phase at a flow-rate of 1 ml min^{-1}. The two inorganic Sb species could be separated by means of a secondary equilibrium using complexing eluents such as EDTA and potassium hydrogenphthalate. Addition of EDTA between 0 and 16 mM showed that the retention of the three Sb species decreased at higher EDTA concentrations (Fig. 3a), although $TMSbCl_2$ eluted as a very broad peak. An 8 mM EDTA concentration allowed good separation of Sb(V) and Sb(III), the former being eluted at the solvent front, because both formed negatively charged species. This concentration was selected and the addition of other competing ligand, potassium hydrogenphthalate was assayed between 0 and 4 mM. This anion produced a substantial reduction in both the retention and

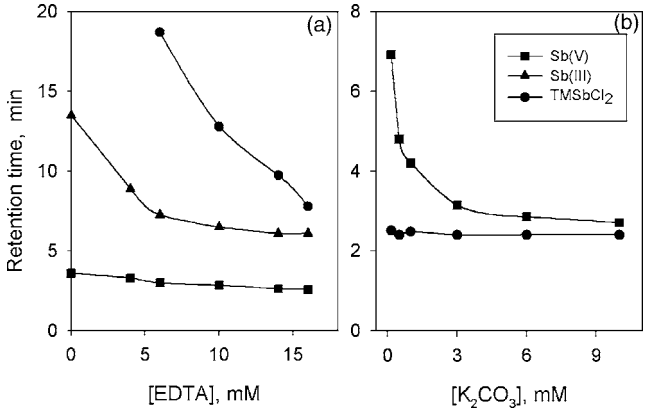

Fig. 3. Influence of the mobile phase composition on the chromatographic retention of Sb species. (a) Effect of EDTA concentration; (b) effect of potassium carbonate concentration. Flow-rate, 1 ml min^{-1}.

Table 1
Analytical characteristics of the chromatographic separations

Mobile phase	Compound	Intercept	Slope	Correlation coefficient	Linearity range ($\mu g\,Sb\,l^{-1}$)	Detection limit ($\mu g\,Sb\,l^{-1}$)	R.S.D. (%)
EDTA/KHP	Sb(V)	−0.057	0.115	0.9999	2–100	0.58	1.9
	Sb(III)	0.102	0.070	0.9999	5–100	0.91	3.0
Carbonate	$TMSbCl_2$	−0.351	0.076	0.9999	5–100	0.72	2.4
	Sb(V)	−0.131	0.093	0.9999	2–100	0.55	1.8

the peak width of Sb(III) and so a 2 mM concentration was selected since this produced sharper peaks. However, the $TMSbCl_2$ peak width was not modified and its separation did not improve. This behaviour of $TMSbCl_2$ has been attributed to molecular rearrangements by several authors [9,25]. The separation of Sb(V) and $TMSbCl_2$ was optimized using an alkaline mobile phase containing potassium carbonate at pH 10, varying the concentration of carbonate in the 0.5–10 mM range. Fig. 3b shows that with this mobile phase, the retention of Sb(V) rapidly decreased at higher carbonate concentrations, while the retention times for $TMSbCl_2$ were not affected since it was eluted almost at the void time, while Sb(III) remained in the column. Thus, a 1 mM concentration was selected, which also provided narrower peaks. When the pH of buffer was varied in the 8–11 range, a similar behaviour was observed, the retention time of Sb(V) decreasing with higher buffer pH values. A pH of 10 was chosen, because separation was optimal at this value. Thus, the three species could not be resolved with sharp and symmetric peaks for all the Sb species in a single chromatogram. Fig. 4 shows the profiles obtained in the two chromatographic conditions selected (a) for Sb(V) and Sb(III) and (b) for Sb(V) and $TMSbCl_2$.

3.3. Calibration, repeatability and detection limits

Calibration graphs were made by plotting peak area against concentration ($\mu g\,Sb\,l^{-1}$), following linear regression analysis. Table 1 shows the equations obtained for the calibration graphs and the regression coefficients using the two different mobile phases containining EDTA/KHP and carbonate, respectively. The detection limits were calculated on the basis of 3σ (σ being the residual standard deviation around the regression line), using the regression lines for the standards. The precision of the method was demonstrated by repeated analyses, calculating the average relative standard deviation (R.S.D.) for 10 replicate injections of the same sample at the 20 $\mu g\,l^{-1}$ concentration level. Values are given in Table 1.

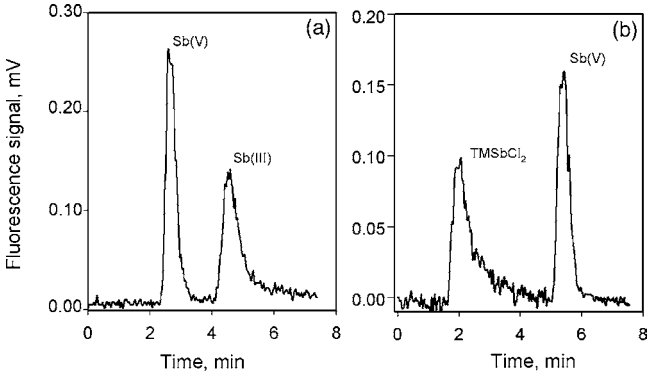

Fig. 4. Elution profiles obtained (a) for Sb(V) and Sb(III) using a mobile phase containing 8 mM EDTA and 2 mM potassium hydrogenphthalate (pH 4.4) and (b) for Sb(V) and $TMSbCl_2$ using a mobile phase 1 mM potassium carbonate (pH 10). Flow-rate, 1 ml min^{-1}. Standard concentrations, 20 $\mu g\,l^{-1}$.

3.4. Recovery studies and environmental applications

The proposed method was evaluated by the analysis of Sb species in spiked water samples because there are no certified reference materials. Eleven water samples were analyzed and no antimony was found above the detection limits. A recovery study was carried out by spiking the samples with the Sb species at the 10 $\mu g\,l^{-1}$ level. No sample matrix interferences were found because the slopes of standard additions were similar to those of aqueous calibration graphs and recoveries were practically quantitative (Table 2).

Four certified reference soil samples with different Sb contents ranging from 8 to 60 $\mu g\,g^{-1}$ were analyzed. These samples contained the total Sb certified, because there are no CRM with the different Sb species certified. The samples were extracted using three different chemicals in order to compare overall extraction efficiencies: water, 50 mM EDTA (pH 7) and 30 mM citric acid. Both EDTA and citric acid were good extractants by complexing antimony and the EDTA-extractable fraction of metals has been established as a plant-available fraction. Samples were homogenized using different procedures: ultrasonic bath, ultrasonic processor and vibratory automatic stirrer. The best results were obtained using the ultrasonic processor, which was used for 3 min at the maximum energy. Extraction was repeated three times to improve overall recoveries. For the Montana soil sample

Table 2
Recovery of Sb species from water samples

Samples	Recovery (%)		
	Sb(V)	Sb(III)	$TMSbCl_2$
Tap water	99 ± 4	99 ± 2	98 ± 3
River water	97 ± 4	98 ± 3	100 ± 2
Residual water 1	96 ± 2	98 ± 4	97 ± 3
Residual water 2	98 ± 4	97 ± 2	96 ± 3

(NIST 2711), only 4.3% of Sb was water-soluble and recoveries of 18.3 and 40% were obtained with EDTA and citric acid, respectively, showing that other fractions were not soluble. Similar results were obtained for the San Joaquín soil (NIST 2709). In all the soil samples analyzed, Sb(V) was the only species found; however, recoveries were not quantitative and clearly depended on the oxide and organic matter content of the soil samples. This dependence of recoveries on sample matrices has already been mentioned by other authors [4,13,17] because Sb tends to be associated with immobile hydrous oxides of Mn, Fe and Al, and can be adsorbed to humic acid. Poor recoveries were obtained for samples NCS DC 73323 and 73324, which contained high amounts of Al, Fe and Mn oxides. Consequently, this procedure could only be used to speciate antimony in the soluble fraction, where it determined the content which estimates the maximum plant-available amount.

4. Conclusion

The hybridation LC–HG–AFS appeared to be a good alternative to LC–HG–ICP-MS and provided good analytical characteristics regarding linearity and low detection limits. Anion-exchange chromatography with complexing agents in the mobile phase could be used for the speciation of Sb(V), Sb(III) and $TMSbCl_2$. Reduction of Sb(V) did not require UV irradiation or additional reducing agents. The procedure was applied to the speciation of Sb species in environmental samples. The method is simpler than other hybrid atomic systems, allowing speciation of Sb with low detection limits.

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