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New considerations about the separation and quantification of antimony species by ion chromatography–hydride generation atomic fluorescence spectrometry

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Abstract

A new method for the speciation of inorganic [Sb(III) and Sb(V)] and organic (Me_3SbCl_2) antimony species by using a polystyrene–divinylbenzene-based anion-exchange HPLC column (Hamilton PRP-X100) coupled to hydride generation atomic fluorescence spectrometry (HG-AFS) is presented. Several mobile phases were tested for the baseline separation of these three antimony species, investigating in detail experimental parameters such as concentration and pH. The best efficiency and resolution was achieved by using a gradient elution between diammonium tartrate 250 mmol l^{-1} pH 5.5 (A) and KOH 20 mmol l^{-1} pH 12 (B). The gradient programme used was 100% B for 1.5 min, decreasing to 0% B in 0.1 min and maintained the elution with 100% A for 5.5 min. Analysis time was less than 7 min. Equilibration of the column with the complexing mobile phase was found to be critical in order to avoid Sb(III) double peak formation. Dilution in diammonium tartrate medium was necessary in order to avoid Sb(III) oxidation at $\mu g\,l^{-1}$ concentration level. Detection limits of 0.06 $\mu g\,l^{-1}$ for Sb(V), 0.09 $\mu g\,l^{-1}$ for Me_3SbCl_2 and 0.04 $\mu g\,l^{-1}$ for Sb(III) as well as repeatability and reproducibility better than 5% R.S.D. (n = 10) and 9% R.S.D. (n = 30) (for 1 and 5 $\mu g\,l^{-1}$ of Sb(V) and Sb(III) and 5 and 10 $\mu g\,l^{-1}$ of Me_3SbCl_2) were obtained. Accuracy and recovery studies were carried out by analysing one river freshwater sample and two water certified reference materials. The proposed methodology can be considered reliable and straightforward for antimony speciation in fresh water samples.

Keywords: Antimony speciation; Methylantimony species; Ion chromatography; Gradient elution; Hydride generation; Atomic fluorescence spectrometry

1. Introduction

Antimony has been extensively used in various industrial applications for a long time [1]. However, it has received relatively little attention since such element is non-essential for life and also because its content in most matrices is very low [2]. Nevertheless, antimony is a toxic cumulative element with similar chemical and toxicological properties to arsenic [3]. Thus, the US Environmental Protection Agency (EPA) considers antimony and its compounds as priority pollutants, and the European Union has established a maximum admissible concentration of total antimony in drinking water of 5 $\mu g\,l^{-1}$ [4].

Most of the analytical methods for antimony assessment are based on the determination of total concentration. However, the determination of antimony species is fundamental for environmental studies because their toxicity and biological behaviour depend on the oxidation state. Thus, most of the proposed methods dealing with antimony speciation have been based on the determination of inorganic Sb(III) and Sb(V). Nevertheless, these methods can hardly be applied to complex environmental samples in which organic antimony species may exist. In this context, an increasingly attractive research area for antimony speciation has been the development of hyphenated techniques. The on-line combination of separation techniques with suitable element-specific detec-

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tors, permits the separation and determination of Sb(III) and Sb(V) but also of organoantimony species [5–12].

Ion chromatography using a strong anion-exchange column has been selected by most researches for the separation of antimony species [5,13–15]. However, simultaneous separation of inorganic and organic species still represents a big challenge. Thus, the main problems with separation stem primarily from the difficulty of finding a single chromatographic system due to the variation in chemical structure, charge, and subsequent retention time of these species. In the recent development of chromatographic systems, Sb(V) and trimethylated species are generally easily separated by using anion exchange columns while the elution of Sb(III) is considerably problematic [5,13,16]. Difficulties with the chromatographic separation of this species have been encountered, including irreversible retention and peak tailing [6,17]. These events are thought to occur as a result of on-column precipitation or the influence of double-charged anionic species which have a great affinity for the stationary phase [6]. The use of complexing mobile phases has been proposed as an alternative in order to alleviate the problems associated with the elution of Sb(III). However, this species is thought to present rather complicated equilibria partitions of agglomerations and non-defined species in the presence of those mobile phases [7]. Otherwise, aqueous interactions between Sb(V) and trimethylated species with complexing agents could lead to the formation of other complexes beyond those observed for Sb(III). Moreover, when using a gradient elution, some mixed complexes between the complexing agents and other eluent anions might also be formed during chromatographic separation [7].

On the other hand, antimony speciation also needs specific and sensitive detection. Derivatisation by hydride generation and subsequent detection of the analyte by different spectrometric techniques is a reliable, simple and fast method of antimony determination at ultratrace levels. Among these techniques, atomic fluorescence spectroscopy has received growing attention because it offers good analytical performance in terms of detection limits and selectivity. Thus, determination of antimony by HG-AFS has been described in detail by several authors [10,12,16,17–19].

This paper presents a study of antimony speciation by ion chromatography-hydride generation-atomic fluorescence spectrometry (IC–HG-AFS). A method for the on-line determination of inorganic [Sb(III) and Sb(V)] and organic (Me_3SbCl_2) antimony species was developed. The hydride generation was optimised before studying the chromatographic separation. The conditions of separation were investigated in detail in order to achieve good efficiency and resolution in a short analysis time. Several mobile phases were tested. The dependence of the elution times on the pH and on the concentration of the mobile phase was assessed. The influence of a gradient elution with a complexing mobile phase in the chromatographic behaviour of Sb(III) was investigated rigorously. The stability of diluted Sb(III) standard solutions in several media was also tested. Finally, method validation by establishing the quality parameters and analysing fresh water certified reference materials was carried out.

2. Experimental

2.1. Instrumentation

2.1.1. Liquid chromatography system

A Perkin-Elmer 250 LC binary pump (CT, USA) and a polystyrene–divinylbenzene-based anion-exchange column Hamilton PRP X-100 (Reno, NV, USA) with ammonium quaternary salt with methyl groups as substituents, 10 μm particle size (250 mm × 4.1 mm), were used. A Rheodyne 7125 injector (Cotati, CA, USA) with a 100 μl loop was used for sample introduction.

2.1.2. Hydride generation system and AFS detection

Hydride generation was performed with a Hydride Generator module P.S. Analytical (Kent, U.K.), model 10.004. After reaction in a coil (75 cm × 1 mm i.d.), the generated stibine was driven by an argon flow (300 ml min^{-1}) to the AFS detector through the Type A gas-liquid separator. Before detection, the argon stream was passed through a Perma pure drying membrane (Perma Pure Products, Farmingdale, NJ, USA) which prevents droplets being transmitted into the transfer line. Air was used as drying gas at a flow rate of 2.5 l min^{-1}. Detection was carried out in a P.S. Analytical model Excalibur Atomic Florescence Spectrometer equipped with a diffusion flame and a Sb Boosted Hollow Cathode Lamp (Super Lamp, Photron, Teknokroma).

2.1.3. ICP-AES detection

A Perkin-Elmer Optima 3200 RL inductively coupled plasma atomic emission spectrometry (ICP-AES) system was used for standardisation of antimony stock standard solutions.

2.2. Reagents

All chemicals and reagents used in this study were of analytical-reagent grade or higher purity and de-ionized water obtained from a MiliQ System (USF Purelab Plus, Ransbach Baumbach, Germany, 18.2 MΩ cm^{-1}) was used throughout.

Two different 1000 mg l^{-1} stock standard solutions of Sb(III) were prepared by dissolving appropriate amounts of potassium antimonyl tartrate (Fluka, Neu-Ulm, Switzerland) and antimony(III) chloride (99.999%, Aldrich) in water and 6 mol l^{-1} HCl, respectively and diluting to 100 ml. Thousand milligrams per litre stock standard solutions of Me_3SbCl_2 and Sb(V) were prepared by dissolving trimethyl antimony dichloride (synthesized at the Research Centre Jülich, Institute of Applied Physical Chemistry, Jülich, Germany) and potassium hexahydroxyantimonate (Riedel-de Haën, Seelze, Germany), respectively in water and diluting to 100 ml. All stock standard solutions were stored in polyethylene bottles

in a refrigerator held at 4 °C. These solutions were standardised using a standard reference material (NIST 3102a, antimony standard solution) by ICP-AES measuring at three resonance wavelengths of antimony (206.8, 217.6 and 231.2 nm). Working solutions were prepared daily by diluting the stock standard solutions.

Sodium tetrahydroborate solutions ranging from 1.3 to 2% (m/v) were prepared daily from $NaBH_4$ 97% "purum" (Fluka, Buchs, Switzerland) in $NaOH{\cdot}H_2O$ "suprapur" (Merck, Darmstadt, Germany) 0.2 mol l^{-1} aqueous solutions. Solutions of HCl ranging from 1.5 to 3.5 mol l^{-1} were prepared from HCl fuming analytical-reagent grade 37% (Merck).

Potassium hydroxide and Tetramethylammoniumhydroxide mobile phase solutions were prepared by dissolving in water KOH "pellets" 99.99% (Aldrich) and TMAOH pentahydrate 97% (Aldrich), respectively. Diammonium tartrate (Fluka) and ethylenediaminetetraacetic acid (99.999%, Aldrich) were also used as mobile phases for HPLC. Both were prepared by dilution in water. All the buffer solutions assayed were filtered off through a 0.22 μm nylon membrane before use.

2.3. Samples and certified reference materials

River freshwater from the area of Barcelona (Spain) was used in recovery studies by spiking it with different concentrations of Sb species. Thus, the river freshwater sample described here was from a river that crosses a high industrial activity area (estimated flow rate 885 l s^{-1}) and had the following composition: CO_3^{2-}: 404, SO_4^{2-}: 194, Cl^-: 509, Ca^{2+}: 112, Mg^{2+}: 30, Na^+: 314, K^+: 44 (all in mg l^{-1}). The sample was stored in a polypropylene bottle at 4 °C until analysis.

The standard reference material SRM 1640 (trace elements in natural water) from the National Institute of Standards and Technology (Gaithersburg, MD, USA) as well as TMDA-54.3 (trace elements in water) from National Water Research Institute (Burlington, Canada) were analysed for method validation. The antimony certified values in these standard reference materials are 13.79 ± 0.42 μg Sb kg^{-1} and 25.1 ± 3.38 μg Sb l^{-1}, respectively.

2.4. Procedures

2.4.1. Hydride generation conditions

Solutions of $NaBH_4$ and HCl ranging from 1.3 to 2% (m/v) in NaOH 0.2 and 1.5–3.5 mol l^{-1}, respectively were tested. Lower concentration of both reagents causes the extinction of the flame whereas a higher made it unstable. Three independent replicates of standard solutions containing 15 μg l^{-1} of Sb as both Sb(V) and Me_3SbCl_2 were analysed under every condition tested. Data acquisition of the signal from the spectrometer was performed with a microcomputer by using software (Avalon 2.0) from P.S. Analytical. The analytical response was evaluated as the peak height.

2.4.2. Chromatographic separation

A 100 μl aliquot of the sample solution was injected into the LC system. A 1.5 ml min^{-1} mobile phase flow rate was used in all the analyses. The eluate then reached the hydride generation system, where 2 mol l^{-1} HCl at 7.2 ml min^{-1} and 1.7% (m/v) $NaBH_4$ at 3.2 ml min^{-1} was added for stibine generation. Data acquisition of the signal was carried out with a microcomputer by using home-made software (Pendragon 1.0). Peak areas were calculated from custom-developed software running on Matlab language [20].

2.4.2.1. Isocratic elution of Sb(V) and Me_3SbCl_2. Different KOH solutions at two pH values (11 and 12) with five concentrations ranging from 2 to 40 mmol l^{-1}, were tested. Moreover, four TMAOH solutions ranging from 5 to 20 mmol l^{-1} at pH 12 were also studied as mobile phase. Five independent replicates of solutions containing 10 μg l^{-1} of both Me_3SbCl_2 and Sb(V) were analysed in every condition tested.

2.4.2.2. Gradient elution of Sb(V), Sb(III) and Me_3SbCl_2. Separation was achieved by using a gradient elution between two mobile phases (solutions A and B). KOH in conjunction with diammonium tartrate or EDTA was tested for this purpose. Seven diammonium tartrate solutions with concentrations ranging from 200 to 500 mmol l^{-1} at four different pH values (from pH 5 to 6.5) were investigated. Six EDTA solutions from 2 to 40 mmol l^{-1} at pH 4.7 were also tested. Five independent replicates of solutions containing 10 μg l^{-1} of antimony in each form [Me_3SbCl_2, Sb(V) and Sb(III)] were analysed in every condition tested.

2.4.3. Short term stability of diluted standard solutions

Two Sb(III) standard solutions of 10 and 100 μg l^{-1} in the presence of Sb(V) at the same concentration level, were prepared in different media: HCl 1% (v/v); HCl 1% (v/v) deoxygenated for 10 min with an argon stream; 250 mmol l^{-1} diammonium tartrate pH 5.5 and 250 mmol l^{-1} diammonium tartrate pH 5.5 deoxygenated for 10 min with argon. Twenty independent injections of both solutions were carried out consecutively, so about 150 min elapsed between the first and the last injection.

2.4.4. Samples and certified reference materials

River freshwater was filtered through a 0.45 μm nylon filter (Osmonics, USA) and then through a C_{18} cartridge (Lida, USA) conditioned with 5 ml of methanol and then with 10 ml of water. Certified reference materials were not subjected to any pre-treatment. Five millilitres of sample (both river freshwater and certified reference materials) were spiked with 5 and 10 μg l^{-1} of each one of the antimony species studied [Sb(V), Sb(III) and Me_3SbCl_2] and then diluted to 10 ml with diammonium tartrate 250 mmol l^{-1}. A

100 μl aliquot of the sample solution was injected into the LC system.

2.4.5. Proposed procedure for Sb speciation in fresh water samples

Both working standard solutions and water samples were diluted in 250 mmol l^{-1} diammonium tartrate medium to ensure the stability of antimony species. A 100 μl aliquot was injected into the chromatographic system.

Separation was achieved by using a gradient elution of two mobile phases at 1.5 ml min^{-1}, diammonium tartrate 250 mmol l^{-1} pH 5.5 (solution A) and KOH 20 mmol l^{-1} pH 12 (solution B). The gradient programme used was 100% B for 1.5 min, decreasing to 0% B in 0.1 min and maintained the elution with 100% A for 5.5 min. The column was equilibrated with solution A for at least 60 min before analysis and for 1 min after each run. The solution was then poured into the hydride generation system, where the operating conditions described in Section 2.4.2 were followed.

For water samples containing particulate matter or complex matrices, a clean-up procedure such as that described in Section 2.4.4 for the river fresh water sample is recommended.

3. Results and discussion

3.1. Hydride generation

In order to obtain the maximum yield of stibine generation, the hydride generation step was optimized without chromatographic separation. The parameters studied were the effect of hydrochloric acid and sodium tetrahydroborate concentration on the fluorescence signal (Section 2.4.1). The hydride generation was optimized for both Sb(V) and Me_3SbCl_2 species.

Fig. 1 shows the results obtained for all the conditions tested. The signal obtained for a Sb(III) standard solution was considered as reference since this species generates stibine quantitatively. Therefore, the relative net fluorescence signal intensity was calculated as the ratio between the signal of an Sb(V) or Me_3SbCl_2 and a Sb(III) standard solution at the same concentration level. As is shown, the relative net fluorescence intensity of Sb(V) increased with increasing of sodium tetrahydroborate concentration in all cases (Fig. 1b). However, in the case of Me_3SbCl_2, $NaBH_4$ 2% (m/v) produces a decrease of the relative net fluorescence intensity with respect to those signals obtained when using 1.5 and 1.7% (m/v) $NaBH_4$ (Fig. 1a). Thus, sodium tetrahydroborate 1.7% (m/v) was adopted as the best suitable concentration for reduction of both antimony species. At this $NaBH_4$ concentration, 2 mol l^{-1} HCl provided the maximum yield of both Sb(V) and Me_3SbCl_2 stibine generation (Fig. 1). Therefore, those conditions enable a good overall performance in the hydride generation step for both antimony species. They were thus adopted for further investigations.

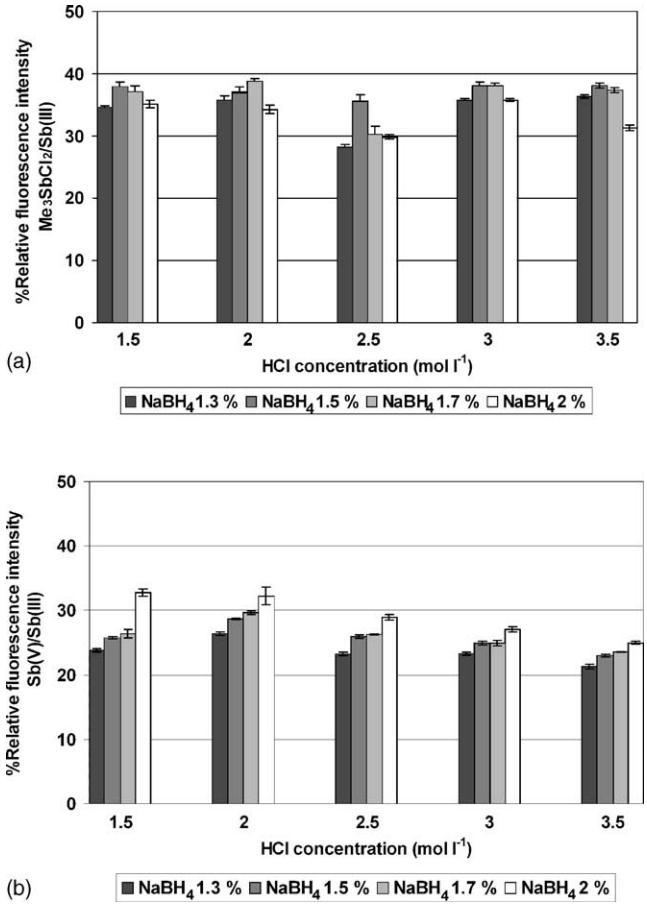

Fig. 1. Optimization of the hydride generation conditions for (a) Me_3SbCl_2 and (b) Sb(V).

3.2. Chromatographic separation

First, we studied the separation of Me_3SbCl_2 and Sb(V) by using an isocratic elution with basic mobile phases. Fig. 2 shows the chromatograms obtained by using KOH 20 mmol l^{-1} and TMAOH 12 mmol l^{-1} both at pH 12.0, since these conditions provided the best peak shapes. Taking into account that 85 s is the time of unretained components, it can be deduced that Me_3SbCl_2 does not interact with the anion-exchange groups of the stationary phase. In the literature [21], Sb(V) is proposed to have one negative charge in water solution representing $Sb(OH)_6^-$ (pK_a 2.7) as the predominant species in basic media for potassium hexahydroxyantimonate. This behaviour matches the results of the present study in which Sb(V) elutes rather early ($k' = 0.67$). KOH was selected because Me_3SbCl_2 retention was increased beyond the void volume (Fig. 2a).

Otherwise, in the literature [6,22,23] is reported that Sb(III) is supposed to be strongly retained in the anion-exchange groups of the stationary phase under basic conditions so the use of complexing mobile phases under acidic conditions has been proposed as a good alternative. Taking this into account, separation of the three antimony species in a short analysis time would require a gradient elution with

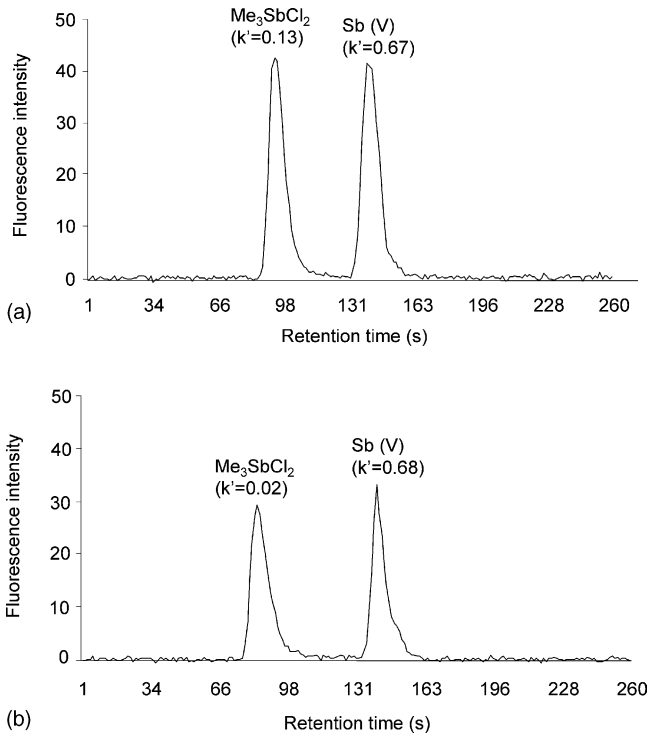

Fig. 2. Isocratic elution of Sb(V) and Me_3SbCl_2 standard solution by using (a) 20 mM KOH pH 12 and (b) 12 mM TMAOH pH 12 as mobile phases.

considerable changes in pH. So, the elution by using KOH and a complexing mobile phase (diammonium tartrate and EDTA) was investigated in the present study.

By way of example, two of the chromatograms obtained are shown in Fig. 3. As can be observed, the retention times of Me_3SbCl_2 and Sb(V) were reversed with respect to those obtained previously when using an isocratic elution. This is in agreement with the formation of a complex between tartrate and thrimethylantimony species. Moreover, by using diammonium tartrate as mobile phase, two peaks were eluted in the retention time supposed for Sb(III), since this species was expected to be the most strongly-retained on the column. In order to confirm that point, standard solutions of each of the three antimony species were injected separately and the double peak was only obtained in the case of Sb(III) when the gradient elution was used (Fig. 4).

On the other hand, Table 1 shows the influence of the complexing mobile phase concentration on the retention time and on the asymmetry factor. As can be observed, the retention times of Me_3SbCl_2 and Sb(V) were only slightly affected by the concentration of both complexing mobile phases. Otherwise, Sb(III) eluted in a very broad peak when EDTA was used for all the conditions assayed, so it was discarded. Contrary, the elution time of both peaks attributed to Sb(III) decreased by increasing the concentration of the diammonium tartrate solution. Thus, a 300 mmol l^{-1} diammonium tartrate solution was considered optimum since only slight different behaviour for higher concentrations was observed. Consequently, a gradient elution between KOH 20 mmol l^{-1} pH

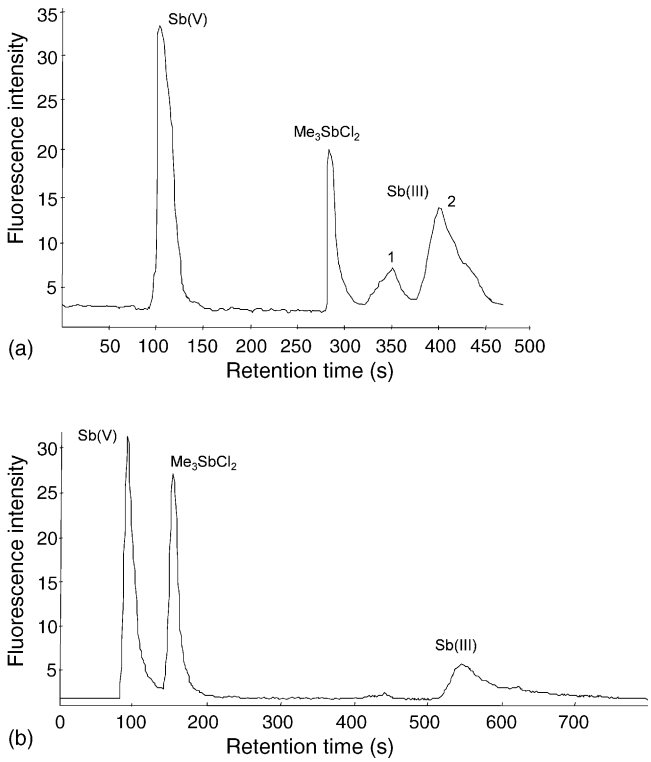

Fig. 3. Gradient elution of Sb(V), Me_3SbCl_2 and Sb(III) standard solution by using 20 mM KOH pH 12, (a) 300 mM diammonium tartrate pH 5.0 and (b) 20 mM EDTA pH 4.7 as complexing mobile phases.

12.0 (solution A) and diammonium tartrate 300 mmol l^{-1} pH 5 (solution B) was further investigated.

Fig. 5 shows the effect of diammonium tartrate solution pH. As can be seen, the double peak associated with Sb(III) was eluted in all cases whereas the ratio between the net area of the first and the second peak was decreased from 0.15 to 0.06 by increasing the pH.

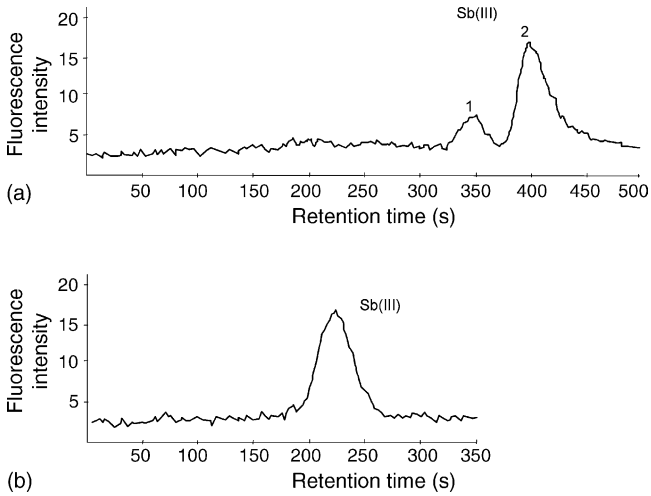

Fig. 4. Chromatogrames of a 10 μg l^{-1} Sb(III) standard solution by using (a) a gradient elution with 20 mM KOH pH 12 and 300 mM diammonium tartrate pH 5 and (b) an isocratic elution with 300 mM diammonium tartrate pH 5.

Table 1
Effect of the concentration of the complexing mobile phase on the separation of antimony species

Mobile phase	Concentration (mmol l^{-1})	Sb(V)		Me_3SbCl_2		Sb(III)		
		t_R(s)	A_s[a]	t_R (s)	A_s	t_{R1} (s)	t_{R2} (s)	A_s
Diammonium tartrate, pH 5	200	116	1.6	279	2.3	518	563	
	250	111	1.6	282	2.5	439	453	
	300	106	1.4	289	2.5	356	403	
	350	108	1.5	279	2.4	358	399	
	400	108	1.5	281	2.3	367	385	
	450	108	1.3	290	2.6	365	380	
	500	103	1.4	293	2.5	358	373	
EDTA, pH 4.7	2	98	1.5	148	1.4	632		4.7
	5	95	1.4	149	1.4	610		4.5
	10	95	1.7	152	1.1	581		4.0
	20	92	1.6	154	1.2	542		3.8
	30	99	1.7	156	1.3	537		4.1
	40	93	1.6	154	1.4	540		4.0

All values calculated from five measurements.
[a] A_s: asymmetry factor (calculated at 10% of the peak height).

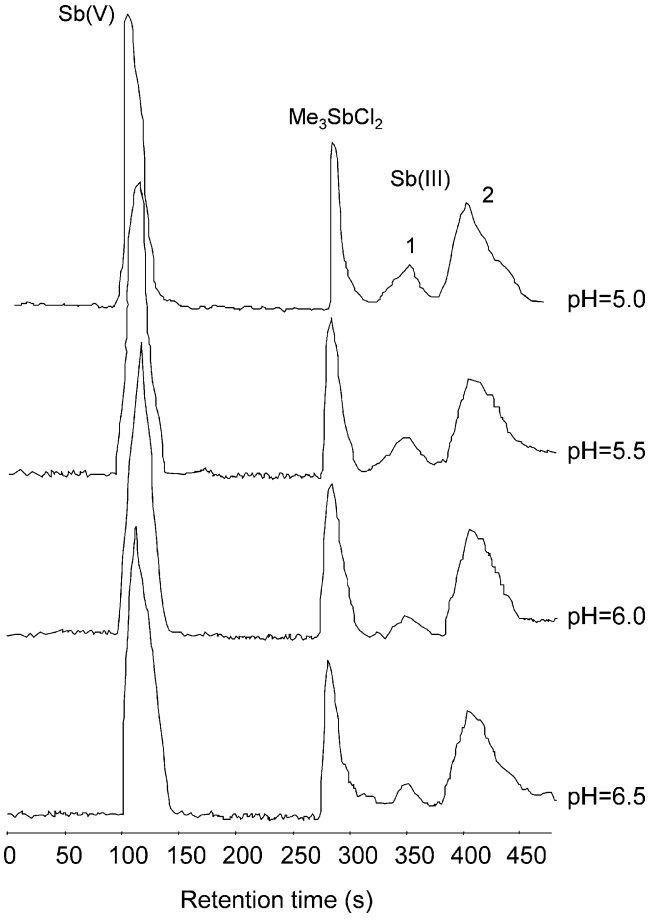

Fig. 5. Gradient elution of Sb(V), Me_3SbCl_2 and Sb(III) standard solution by using 20 mM KOH pH 12 and 300 mM diammonium tartrate at the studied pH values.

Moreover, two different Sb(III) stock standard solutions diluted in several media [deionized water, 1% (v/v) HCl and diammonium tartrate] were tested. From the results, the double peak was eluted in all cases. However, the dilution in diammonium tartrate was the best way to reduce its formation.

According to the literature [24], different chromatographic behaviour was observed for Sb(III) when using basic mobile phases. Taking this into account, in the present study the column was equilibrated after each run with diammonium tartrate instead of KOH since it was thought that the equilibration of the column with the latter could favour the equilibrium between different Sb(III) complexes. KOH was then passed through the column only for a short time to achieve the elution of Sb(V) and Me_3SbCl_2. In these conditions, the double peak was not observed when Sb(III) from both stock standard solutions diluted in all three different media tested previously was injected. Otherwise, the retention time obtained for Sb(III) and Me_3SbCl_2 evidenced that using the new separation conditions, the complexing effect of diammonium tartrate was increased. Therefore, both pH and concentration of the diammonium tartrate solution were optimized again. Table 2 summarizes the results obtained. Diammonium tartrate 250 mmol l^{-1} at pH 5.5 was adopted as the most suitable concentration since good resolution was obtained in a short analysis time and the overall salt concentration was not so high. Fig. 6 shows the chromatogram obtained under optimised conditions. Baseline separation of Sb(V) (k' = 0.32, A_s = 1.1), Me_3SbCl_2 (k' = 2.70, A_s = 1.6) and Sb(III) (k' = 3.54, A_s = 2.0) was obtained.

3.3. Short term stability of Sb(III) standards

It has been reported in the literature [17] that at high concentrations no changes in the composition of inorganic antimony standard solutions are observed. Nevertheless it is known [25] that Sb(III) is easily oxidised to Sb(V) within a short time at low concentration level. In the present study, the short term stability of Sb(III) diluted solutions was investigated. Hence, 20 consecutive injections of a solution

Table 2
Effect of the concentration and the pH of the diammoniumtartr tartrate solution on the separation of Me_3SbCl_2 and Sb(III)

Diammonium tartrate (mmol l^{-1})	pH											
	5.0			5.5			6.0			6.5		
	Me_3SbCl_2, t_R(s)	Sb(III), t_R (s)	R_s	Me_3SbCl_2, t_R (s)	Sb(III), t_R (s)	R_s	Me_3SbCl_2, t_R (s)	Sb(III), t_R (s)	R_s	Me_3SbCl_2, t_R (s)	Sb(III), t_R (s)	R_s
200	324	464	1.39	318	422	1.15	317	431	1.48	315	430	1.23
250	312	389	1.06	3811	381	1.04	322	381	0.84	299	371	0.80
300	307	347	0.47	308	344	0.41	308	340	0.57	308	341	0.39
350	335	335	0.01	332	336	0.04	330	339	0.08	334	335	0.01
400	307	308	0.01	316	321	0.05	316	319	0.03	317	318	0.01
450	340	196	1.93	317	218	1.44	312	187	2.01	307	181	2.05
500	358	181	2.64	307	192	1.76	313	171	2.25	310	170	2.07

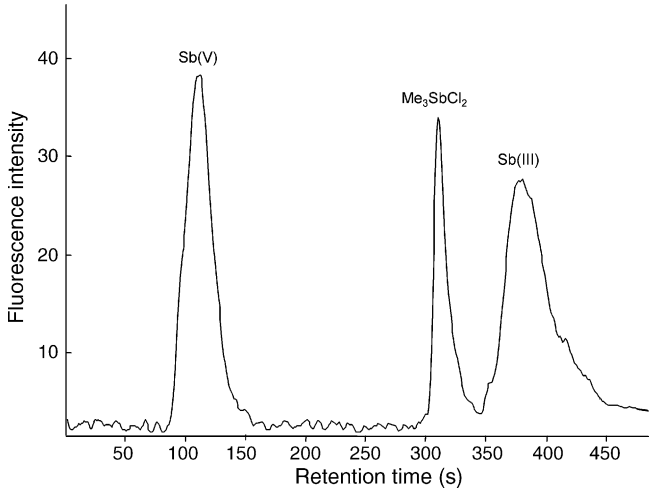

Fig. 6. Gradient elution of Sb(V), Me_3SbCl_2 and Sb(III) standard solution by using 250 mM diammonium tartrate pH 5.5 and 20 mM KOH pH 12.

containing Sb(III) and Sb(V) in different media (see Section 2.4.3) were carried out.

As an example, Fig. 7 shows the ratio between the net peak areas of both species at the same concentration level. From the results, Sb(III) is easily oxidized to Sb(V) in hydrochloric acid whereas stability for at least 150 min is observed in diammonium tartrate. Similar results were obtained by measuring the deoxygenated solutions. Therefore, dilution of working standards and fresh water samples in diammonium tartrate is recommended to avoid the quick oxidation of Sb(III) at the low $\mu g\,l^{-1}$ level.

3.4. Method validation

The proposed methodology was validated by establishing the quality parameters and analysing one river freshwater sample and two fresh water certified reference materials.

3.4.1. Linear range

This was verified using the peak area from the fluorescence signal under the optimum conditions described above. Linearity was proved over three orders of magnitude for the three antimony species studied.

Table 3
Quality parameters for antimony determination by IC–HG-AFS

Quality parameter	Sb(V)	Me_3SbCl_2	Sb(III)
Detection limit ($\mu g\,l^{-1}$)	0.064	0.094	0.043
Quantification limit ($\mu g\,l^{-1}$)	0.213	0.315	0.143
Repeatability[a] (%R.S.D.)	4.9	3.5	4.7
Reproducibility[b] (%R.S.D.)	5.8	8.9	6.7

R.S.D. represented the highest value obtained for both concentration levels tested.
[a] Calculated as the R.S.D. from 10 measurements.
[b] Calculated as the R.S.D. from 30 measurements.

3.4.2. Detection and quantification limits

These parameters were calculated by analysing, in triplicate, five mixtures containing the three antimony species at different concentrations. The regression line for each compound was calculated from the mean values of the peak areas. The concentrations at the detection limits were calculated from three times the standard deviation of the blank signal (n = 10) and then referred to those regression lines. Detection limits at the $ng\,l^{-1}$ level were obtained for all the antimony species studied (Table 3).

3.4.3. Precision

Precision was established in terms of both repeatability and reproducibility. Repeatability was calculated as the %R.S.D. from 10 peak area measurements of two independent standard solutions containing the three antimony species at concentrations of 1 and 5 $\mu g\,l^{-1}$ for Sb(V) and Sb(III) and 5 and 10 $\mu g\,l^{-1}$ for Me_3SbCl_2, respectively.Repeatability was better than 5% R.S.D. in all cases.

Reproducibility in three non-consecutive days was also assessed. The standard solutions described above for repeatability were measured 10 times each day. Reproducibility was calculated as the %R.S.D. from all the measurements made in the 3 days. It was better than 9% R.S.D. in all cases.

3.4.4. Accuracy and recovery studies

In the absence of certified reference materials of antimony species, validation of analytical methods for antimony speciation is still a challenge. However, recovery studies by spiking water samples or even water certified reference materials with certified total antimony concentration, permits to eval-

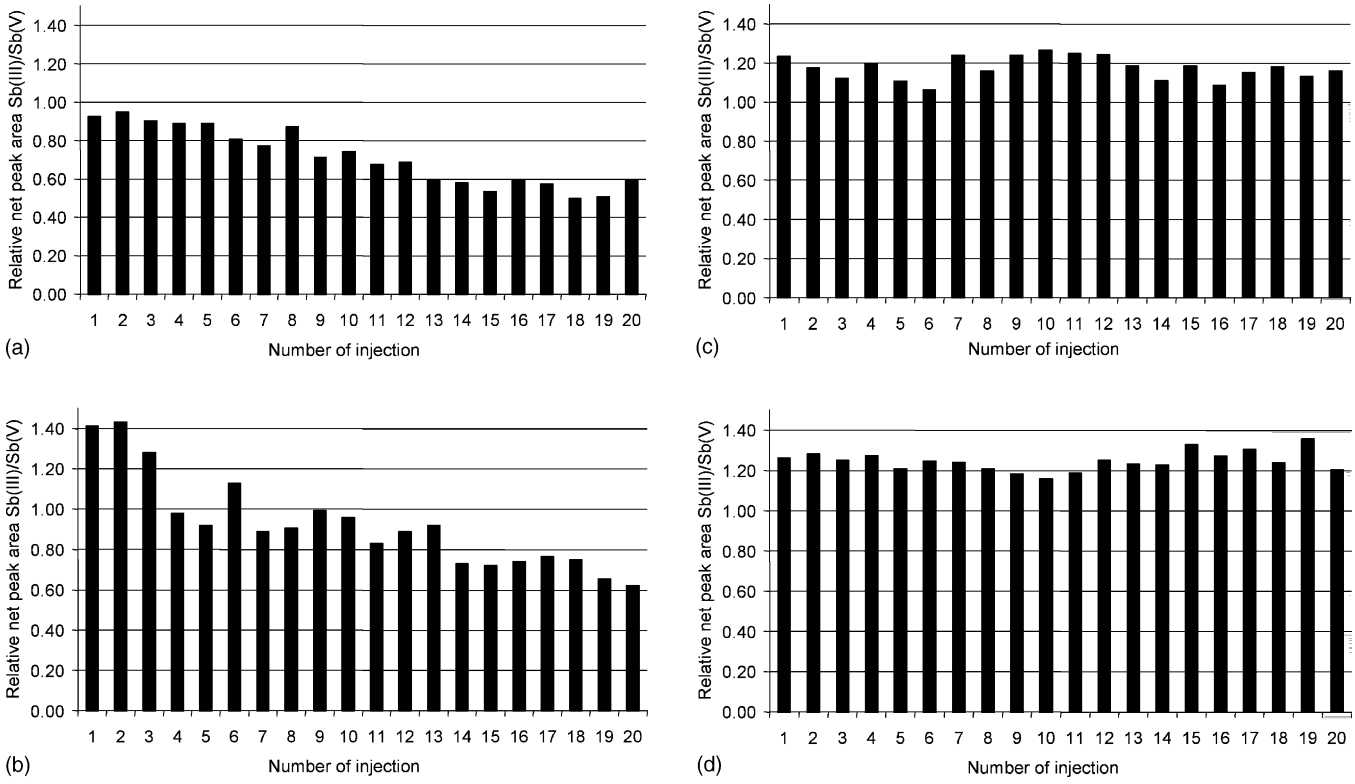

Fig. 7. Stability of diluted Sb(III) standard solutions in (a) 1% HCl (v/v), (b) 1% HCl (v/v) + Ar stream, (c) 250 mM diammonium tartrate and (d) 250 mM diammonium tartrate + Ar stream.

uate the proposed methodology [26]. For this purpose, one river freshwater sample and two fresh water certified reference materials were analysed. We first verified the presence of antimony species in all of them and only Sb(V) was detected. Table 4 shows the Sb(V) concentration quantified and the certified values with their respective standard deviations. From the results, the Sb(V) concentrations quantified match the certified values of both certified reference materials considering the associated uncertainties. Therefore, the proposed methodology is accurate and suitable for the speciation of this antimony species in water samples. Second, recovery studies were carried out. The river fresh water sample and both certified reference materials were spiked with all three antimony species studied at two different concentrations (5 and 10 $\mu g\,l^{-1}$) and analysed in triplicate. The results expressed as the %recovery $\pm\,\sigma$ are also shown in Table 4. From the re-

Table 4
Recovery studies in spiked water samples

	Sample		
	TMDA-54.3	NIST SRM 1640	River freshwater
Certified value	25.1 ± 3.38[a]	13.79 ± 0.42[b]	
Quantified Sb(V) ($\mu g\,l^{-1}$) ($n = 3$)	22.9 ± 1.1	13.1 ± 0.3	11.5 ± 0.2
	Recovery (%)	Recovery (%)	Recovery(%)
Sb(V)			
5 $\mu g\,l^{-1}$[c]	103 ± 5	91 ± 4	104 ± 4
10 $\mu g\,l^{-1}$[c]	95 ± 2	95 ± 2	110 ± 2
Me_3SbCl_2			
5 $\mu g\,l^{-1}$[c]	96 ± 1	96 ± 3	97 ± 5
10 $\mu g\,l^{-1}$[c]	92 ± 5	94 ± 5	98 ± 5
Sb(III)			
5 $\mu g\,l^{-1}$[c]	93 ± 5	88 ± 5	92 ± 4
10 $\mu g\,l^{-1}$[c]	93 ± 5	95 ± 3	86 ± 1

[a] Expressed as ($\mu g\,l^{-1}$).
[b] Expressed as ($\mu g\,kg^{-1}$).
[c] Standard solution added.

sults, no influence from the matrix composition of the water samples tested was observed.

4. Conclusions

The use of an anion exchange column and a gradient elution including a complexing mobile phase provides a reliable system to elute Sb(V), Sb(III) and Me_3SbCl_2 within a short analysis time. The equilibration of the column with the complexing mobile phase is shown to be mandatory to avoid Sb(III) double peak formation. Moreover, the interaction between the complexing mobile phase and the antimony species greatly influences their chromatographic behaviour, including reversion of elution times. Otherwise, dilution of working standards and fresh water samples in diammonium tartrate medium is the best way to ensure the stability of Sb(III) at few $\mu g\,l^{-1}$. The high sensitivity of the developed methodology enables its application in fresh water samples for antimony speciation at the low $\mu g\,l^{-1}$ level.

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References

[1] USGS Minerals Yearbook, vol. 1, Metals and Minerals, US Geological Survey, Reston, VA, 1997.
[2] K. Bencze, Handbook on Metals in Clinical and Analytical Chemistry, Marcel Dekker, New York, 1994.
[3] T. Gebel, Chem.-Biol. Interact. 107 (1997) 131.
[4] Council of the European Communities, Council Directive Relating to the Quality of Water Intended for Human Consumption (98/83/CE), 1998.
[5] N. Ulrich, Anal. Chim. Acta 359 (1998) 245.
[6] J. Lintschinger, I. Koch, S. Serves, J. Feldmann, W.R. Cullen, Fresenius J. Anal. Chem. 359 (1997) 484.
[7] N. Ulrich, Fresenius J. Anal. Chem. 360 (1998) 797.
[8] A. Guy, P. Jones, S.J. Hill, Analyst 123 (1998) 1513.
[9] J. Lintschinger, O. Schramel, A. Kettrup, Fresenius J. Anal. Chem. 361 (1998) 96.
[10] M. Dodd, S.A. Pergantis, W.R. Cullen, H. Li, K.G. Eigendorf, K.J. Reimer, Analyst 121 (1996) 223.
[11] M. Dodd, S.L. Grundy, K.J. Reimer, W.R. Cullen, Appl. Organomet. Chem. 6 (1992) 207.
[12] A. Sayago, R. Beltrán, F. Recamales, J. Gómez-Ariza, J. Anal. At. Spectrom. 17 (2002) 1400.
[13] N. Ulrich, P. Shaked, D. Zilberstein, Fresenius J. Anal. Chem. 368 (2000) 62.
[14] X.J. Feng, B. Fu, Anal. Chim. Acta 371 (1998) 109.
[15] M. Krachler, H. Emons, J. Anal. At. Spectrom. 16 (2001) 20.
[16] M. Krachler, H. Emons, J. Anal. At. Spectrom. 15 (2000) 281.
[17] P. Smichowski, Y. Madrid, M.B. de la Calle-Guntiñas, C. Cámara, J. Anal. At. Spectrom. 10 (1995) 815.
[18] A. Sayago, R. Beltrán, J. Gómez-Ariza, J. Anal. At. Spectrom. 15 (2000) 423.
[19] A. D'Ulivo, L. Lampugnani, G. Pellegrini, R. Zamboni, J. Anal. At. Spectrom. 10 (1995) 696.
[20] M. Vilanó, A. Padró, R. Rubio, Anal. Chim. Acta 411 (2000) 71.
[21] J. Zheng, M. Ohata, N. Furuta, Anal. Sci. 16 (2000) 75.
[22] F.A. Cotton, G. Wilkinson, Advanced Inorganic Chemistry, Wiley, 1980.
[23] J. Zheng, A. Takeda, N. Furuta, J. Anal. At. Spectrom. 16 (2001) 62.
[24] X. Zhang, R. Cornelis, L. Mees, J. Anal. At. Spectrom. 13 (1998) 205.
[25] J. Zheng, A. Iijima, N. Furuta, J. Anal. At. Spectrom. 16 (2001) 812.
[26] EURACHEM Guide, The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics, EURACHEM, Teddington, UK, 1998.