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LC-ICP-OES method for antimony speciation analysis in liquid samples

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Abstract

A method for the analysis of different species of antimony (Sb) that couples liquid chromatography with an inductively coupled plasma-optical emission spectrometry (LC-ICP-OES) system is presented. The method is simple and reliable to separate and quantify directly and simultaneously Sb(III) and Sb(V) in aqueous samples. The calibration curves showed high linearity at the three wavelengths tested. The limits of detection ranged from 24.9 to 32.3 µg/L for Sb(III) and from 36.2 to 46.0 µg/L for Sb(V), at the three wavelengths evaluated. The limit of detection for this method varied depending on the wavelength used. The lowest limit of quantification for Sb(V) (49.9 µg/L) and Sb(III) (80.7 µg/L) was obtained at a wavelength of 217.582 nm. The method sensitivity for Sb(V) was higher compared to Sb(III) at all the wavelengths considered. Samples containing different concentrations of Sb(III) and Sb(V) in three different matrices, *i.e.*, water, basal culture medium, and anaerobic sludge plus basal medium, were analyzed. The coefficients of variation were low and ranged from 0.1 to 5.0 depending on the sample matrix. Recoveries of Sb(III) and Sb(V) were higher than 90% independently of the matrix analyzed and the wavelength used in the analysis.

Keywords

Antimony; Hyphenated technique; LC-ICP-OES; Sb(III); Sb(V); Speciation

Introduction

Antimony (Sb) is a toxic and carcinogenic metalloid that is widely used in electronic products, alloys, flame retardants in the production of plastics, textiles, rubber, pigments for paints, as a catalyst in the production of polyethylene terephthalate (PET), in brake linings,

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battery grids, additive in glassware and ceramics, and semiconductors.^[1–4] Because of its genotoxic effects, Sb is considered a priority pollutant by the U.S. Environmental Protection Agency (US EPA).^[5] The permissible limit of Sb in drinking water established by the US EPA is very low (6 µg/L).^[6] In environmental, biological, and geochemical matrices, Sb exists in four oxidation states (+V, +III, 0 and –III) of which antimonate (Sb(V)) and antimonite (Sb(III)) are the prevalent forms in the environment.^[7] The toxicological behavior and environmental fate of Sb depends on its oxidation state and chemical form. Trivalent Sb is generally more toxic than Sb(V), whereas organoantimony compounds are the least toxic.^[8,9] For this reason, it is important to determine the different species of Sb in liquid samples.

In the last ten years, the presence of high concentrations of Sb in soils, sediments, surface and groundwater has received significant attention all over the world.^[10] Sb can be released into the environment due to natural phenomena, such as weathering of Sb-bearing crustal rocks and minerals, biological processes and volcanic activity, together with anthropogenic inputs.^[9] A study in surface sediments collected from an estuary demonstrated that geoaccumulation of Sb (and As, Hg, Bi and Te) came from both of crustal and anthropogenic inputs via atmosphere and rivers.^[10] In addition, increased mining activity and industrial emissions significantly accelerate the release of this metalloid and the associated exposure of biota.^[7]

The speciation and distribution of Sb in freshwater have not been studied extensively.^[12] Studies on the distribution of Sb in freshwater lakes have demonstrated that, in pore waters, Sb(III) was present under reducing conditions where it could exist as SbS_2^- , while Sb(V) was detected mainly in oxic and mildly reducing environments.^[13] Study of the distribution of Sb in freshwater ecosystems impacted by mining activities revealed that Sb accumulation in the food chain decreased as follows: macro-invertebrates > bryophytes > fish > water.^[13] Concentrations of total dissolved Sb in groundwater have been reported in the range of 0.01 to 1.5 µg/L,^[3] but anthropogenic sources can be responsible for much higher levels. Antimony concentrations as high as 720 µg/L were found in stream waters in the Kantishna Hills mining district, Denali National Park and Preserve in Alaska (USA).^[15] High Sb levels (up to 6384 µg/L) were found in rivers around the world's largest Sb mine at Xikuangshan area of Hunan Province (China), and the Sb content in sediments ranged from 57 to 7316 mg/kg, decreasing with distance from the mine.^[16]

Conventional Sb speciation in hydride generation – atomic absorption spectrometry (HG-AAS) is carried out in two stages: i) Total Sb is measured using KI as reducing agent, followed by stibine (SbH_3) generation by addition of a NaBH_4 solution and, subsequent determination of the total Sb concentration by the standard addition method, ii) Sb(III) is measured in a different sample aliquot which is supplemented with citric acid and NaBH_4 to generate stibine; next Sb(III) is analyzed as in the case of total Sb. The concentration of Sb(V) can be calculated as the difference between total Sb and Sb (III).^[17] Other strategies proposed for the chemical separation of Sb(V) and Sb(III) include coprecipitation with thionalide,^[18] derivatization by hydrogenation with NaBH_4 ,^[17,19] and the use of headspace solid-phase microextraction^[20]. Important limitations of these techniques are that only Sb

species that can undergo chemical reduction can be determined, and that Sb(III) is unstable and easily oxidized to Sb(V), hence many research papers report relatively high Sb(V) content in the analyzed samples.^[21] For this reason, hyphenated techniques have been proposed to attain direct speciation of Sb.

A hyphenated technique, high-performance liquid chromatography (LC) with an inductively coupled plasma–mass spectrometry (ICP-MS), is commonly employed for the separation and detection of Sb species.^[21–25] LC-ICP-MS has been applied to environmental samples including sediments and water (sea, surface, canal and tap water).

Storage of the samples for Sb determination is very difficult because Sb(III) easily transforms into Sb(V) in the oxidizing environment. To preserve the samples, chelating reagents, such as ethylenediaminetetraacetic acid (EDTA),^[22] are often utilized. Chelating agents are also used as components of the mobile phase in LC-ICP-MS analyses.^[22] Antimony speciation using gas chromatography coupled with inductively coupled plasma–mass spectrometry (GC-ICP-MS) and hydride generation–atomic absorption spectrometry (HG-AAS) has also been reported.^[20,26,27] Although LC-ICP-MS provides high selectivity and sensitivity (very low limit of detection (LOD) for Sb of about 0.5 ng/L),^[28] a key drawback of this coupled system is the very high capital cost of the instrumentation, which results in high analytical fees and restricted access to the few systems that are often operated at academic service laboratories.^[29]

On other hand, inductively coupled plasma–optical emission spectrometry (ICP–OES) instruments can be also used to measure the concentration of total Sb and these instruments are considerably more affordable than ICP-MS systems and widely available. However, commercial ICP-OES systems lack the hardware and software required to allow their operation in conjunction with a high pressure LC system. Therefore, Sb speciation using ICP-OES is time-consuming as it requires fractionation of the different species by solid phase separation.^[30,31] Also, speciation can be directly determined for only one species, obtaining the concentration of the second one indirectly.^[32] For example, in the study of Cabral and coworkers,^[33] the concentration of Sb(V) was calculated from difference between total Sb and Sb(III), and Sb(III) determination involved addition of citric acid to suppress the Sb(V) signal in the HG-ICP-OES hyphenated system. Nonetheless, the indirect determination of Sb species is time consuming and it cannot assure a high quality of results and leads to significant errors, especially if the content of Sb(III) and Sb(V) differs substantially.^[30]

For the case of metalloids speciation, liquid chromatography is the main separation technique, whereas ICP-OES and ICP-MS are mainly recommended for detection. LC-ICP-OES hyphenated technique has been previously reported for the speciation of germanium and arsenic.^[34,35] Also, LC-ICP-OES has been used for the determination of iron in metalloproteins.^[36] However, there is a lack of reports on the use of LC-ICP-OES for the speciation of Sb. The main advantage of the LC-ICP-OES technique is the lower cost compared with other hyphenated techniques for Sb speciation (e.g., using MS detection).^[35] The results of this study indicate the possibility of direct speciation of Sb and other elements

using LC-ICP-OES, especially in complex matrices such as mining-impacted water where the concentration of contaminants is high, without the cost of the MS detector.

In view of the global importance of Sb as an environmental contaminant and industrial material, there is a strong need for the development of direct and reliable methods for the speciation and quantification of this metalloid that are highly sensitive, economic, and simple.^[30] For this reason, the objective of this study is to develop a simple and reliable method for the direct and simultaneous determination of Sb(III) and Sb(V) in aqueous samples that couples liquid chromatography with ICP-OES. To the best of our knowledge, this is the first study that reports on the speciation of Sb by LC-ICP-OES.

Materials and methods

Reagents

Sb(V) (potassium hexahydroxoantimonate (KSb(OH)₆, CAS # 12208-13-8, purity 99.0%, Fluka Analytical, St. Louis, MO, USA) and, Sb(III) (antimony trichloride (SbCl₃, CAS # 10025-91-9, purity 99.95%, Sigma-Aldrich Chemistry, St. Louis, MO, USA) were used as models of inorganic Sb species.

Total antimony determination

Analyses of total Sb were carried out using a 5100 SVDV ICP-OES from Agilent Technologies (Santa Clara, CA, USA) at a wavelength of 217.582 nm. Before the analysis, samples were centrifuged (13000 rpm, 10 min) and diluted with 2% nitric acid.

Speciation coupling LC-ICP-OES

In order to accomplish the LC-ICP-OES measurements, it was necessary to consider the physical requirements of the experimental apparatus, including interfacing of the LC with the ICP-OES nebulizer, rapidly pumping out the spray chamber to avoid flooding, and synchronizing the injection of the sample with the beginning of ICP-OES data acquisition (Figure 1). To this end, a Waters 590 HPLC pump (Waters Corp., Milford, MA, USA) was connected to a manual Rheodyne injection valve (model 7125, Thermo Fisher Scientific, Waltham, MA, USA) that was plumbed to two in-line ion chromatography columns (guard column Dionex AG15, 4 × 50 mm, and chromatographic column Dionex AS15, 4 × 250 mm; both from Thermo Scientific, Waltham, MA, USA). The outlet of the analytical column was coupled using PEEK tubing to the liquid inlet to a SeaSpray nebulizer on an Agilent 5100 SVDV ICP-OES instrument.

Time-resolved ICP emissions data were collected during each chromatographic analysis. The eluent used was 40 mM ethylenediaminetetraacetic acid (EDTA) at a flow rate of 1.0 mL/min. Samples were injected manually into a 300 µL loop.

The ICP-OES measurement conditions were: read time: 1 s, RF power: 1.2 kW, nebulizer flow: 0.7 L/min, plasma flow: 12.0 L/min, and auxiliary flow: 1.0 L/min. ICP-OES measurements were collected solely in axial mode for highest sensitivity. Data for three different Sb emission wavelengths were simultaneously collected (206.834, 217.582, and

231.146 nm) in order to increase confidence in the identities of the peaks. The time-resolved emissions data collected were exported as text (CSV file), which was then integrated using the spreadsheet software Excel.

In our case, the synchronization between the LC and the ICP-OES runs was done manually. In step one, the sample is injected in the loop (in load position). In step two, the inject valve is turned to inject and the ICP-OES data collection is manually started. These steps were done simultaneously to obtain reproducible retention times between injections. The data obtained from the ICP-OES system are a series of reads for each wavelength used. Each sample was integrated and analyzed in an Excel worksheet developed for the data processing, calculating the concentration of each Sb species.

Calibration curves and samples

Calibration curves were obtained with measurements of 12.5, 25, 50, 125, 250, 500, 1000, 2500 and 5000 µg/L for each Sb species. A linear model was selected to fit the dependence of the concentration of the total number of analytes counts. The limit of detection was defined as the concentration of analyte giving signals equivalent to three times the standard deviation of the blank plus the net blank intensity, for six independent replicates.

Samples containing different concentrations of Sb(III) (300 and 3000 µg/L), and Sb(V) (200 and 3000 µg/L) were tested in three different matrix, namely, deionized (DI) water, basal medium and, basal medium spiked with anaerobic granular sludge, to validate the LC-ICP-OES speciation methodology. The basal medium selected is commonly used in laboratory assays investigating the microbial reduction of Sb and other metalloids.^[37] The basal medium spiked with anaerobic sludge represents a complex sample model typical of experiments simulating wastewater treatment systems.

The samples with basal medium contained in (mg/L): K₂HPO₄ (12.5), CaCl₂·2H₂O (10), MgCl₂·6H₂O (10), NH₄HCO₃ (20), NaHCO₃ (2000), yeast extract (10), and, 1 mL/L of trace elements solution. The trace element solution contained (in mg/L): H₃BO₃ (50), FeCl₂·4H₂O (2,000), ZnCl₂ (50), MnCl₂ (32), (NH₄)₆ Mo₇O₂₄·4H₂O (50), AlCl₃ (50), CoCl₂·6H₂O (2,000), NiCl₂·6H₂O (50), CuSO₄·5H₂O (44), NaSeO₃·5H₂O (100), EDTA (1,000), resazurin (200) and 1 mL/L of HCl (36%).^[37] DI water was used to prepare the basal medium and trace elements solution.

The samples containing anaerobic granular sludge were prepared with a final sludge concentration of 1.5 g/L of volatile suspended solids (VSS) in the basal medium described above. The sludge was obtained from a full-scale upflow anaerobic sludge blanket reactor treating brewery wastewater (Mahou, Guadalajara, Spain). Sludge samples were centrifuged (12,000 rpm, 5 min).

Results and discussion

The LC-ICP-OES hyphenated technique showed to be successful for the accurate speciation of Sb(III) and Sb(V), with good separation of the chromatographic peaks of each species.

The intensity response for each species varied depending on the wavelength used. The highest intensity was obtained at 217.582 nm independently of the analyzed species and the concentration used. Figure 2 shows an example of the chromatograms obtained for 250 µg/L of Sb(III) and 250 µg/L of Sb(V) at three different wavelengths (206.834, 217.582, and 231.146 nm). The Sb(III) peak started at 3.0 min, while the Sb(V) peak started at 5.2 min.

Calibration curves

Figure 3 shows the calibration curves determined for Sb at the three different wavelengths evaluated. The curves show a strong linear correlation independently of the wavelength tested. Accordingly, the coefficient of determination of the calibration curves, R^2 , was higher than 0.999 for both Sb species at the various wavelengths considered. The 217.582 nm wavelength had the highest intensity response. The sensitivity of Sb (V) was higher than the Sb(III) independently of the wavelength applied. The limit of detection (LOD) and quantification (LOQ) for this method varied depending on the wavelength used (Table 1). The LOD values ranged from 24.9 to 32.3 µg/L for Sb(III) and from 36.2 to 46.0 µg/L for Sb(V), for the three wavelengths evaluated. The lowest LOQ values for Sb(V) (49.9 µg/L) and Sb(III) (80.7 µg/L) were obtained using a wavelength of 217.582 nm.

The typical LOD of Sb for ICP-OES is 50 µg/L (as total Sb).^[38] The hyphenated LC-ICP-OES maintains the LOD given by ICP-OES. The LOD could be increased by applying hydride generation (HG), which is the technique most often employed for the determination of trace amounts of Sb by ICP-OES. HG can be coupled with several detection systems (including ICP-OES, AAS, etc.) for Sb speciation, allowing the development of analytical methods with lower LODs than those commonly achieved by liquid sample nebulization process.^[27]

Validation of the developed methodology in different matrices

The use of 40 mM EDTA as eluent (at a flow rate of 1 mL/min) provided a good separation between the different species peaks in the chromatogram. Under this condition, a clear definition in peaks with different enough retention times to avoid overlapping of the peaks were obtained (about 2 min lapse between the first and the second peak). The first peak represents Sb(V) (3 min retention time) and the second one represents Sb(III) (5.3 min retention time). These patterns agree with reports applying other methodologies as LC-ICP-MS^[24,25,39] and ion chromatography- ICP- atomic emission spectrometric and MS detection.^[23]

The results of the analysis of different samples were used to define the precision (repeatability) of measurements expressed as the coefficient of variation (CV) (Table 2). The developed analytical method shows low CVs ranging from 0.1 to 2.6 for water, from 0.4 to 3.8 for the basal medium, and from 0.4 to 5.0 for the sludge matrix. The increase in the CV values in the sludge sample is likely due to the complex composition of these samples and possible adsorption or complexation of some Sb by sludge components. Several studies utilizing environmental samples have reported low extraction yields for Sb due to strong interactions between Sb and the environmental matrix (e.g., soil).^[24]

Since standard reference materials with certified contents of Sb species in the environmental matrix (i.e., sludge) are not available, a recovery test is a valid alternative to check accuracy. Therefore, for this study, the spike recoveries of Sb(III) and Sb(V) were examined. Recoveries higher than 90% of Sb(III) and Sb(V) were obtained independently of the matrix analyzed and the wavelength used in the analysis (Table 3). The nature of the matrix influenced the recovery percentage. The analysis in water and basal culture medium showed good recovery values. For the case of the matrix of sludge plus basal medium, the Sb recovery values were high and ranged from 90 to 105%. This variation may be due to some interference by some chemical compounds and microbial substances in the granular sludge. These recovery values agree with results obtained for Sb(III), Sb(V), and total Sb in water samples (sea, river and wastewater) using an atomic absorption spectrometer with a range of recovery between 95 and 104% independently of the Sb species and liquid matrix. [40]

Possible interferences

Beyond the possible interferences resulting from the interaction of the analytes with the sample matrix (e.g., soil, wastewater, etc.), there are several chemical elements that have a response in the ICP-OES at wavelengths close to that of Sb (± 0.080 nm) which could interfere if present at high concentrations. For the case of the wavelength 206.834 nm used in the speciation analysis, platinum can be measured at 206.750 nm, tungsten at 206.752 nm and germanium at 206.866. For the wavelength of 217.582 nm, the elements that can be detected at near wavelengths are beryllium at 217.510 nm, nickel at 217.514, iridium at 217.525, hafnium at 217.535, and niobium at 217.584. Finally, for the 231.146 nm wavelength, the most proximate interferences correspond to platinum at 231.095 nm, nickel at 231.096 nm and cobalt at 231.160 nm. Using the three proposed wavelengths in our technique, it is possible to minimize errors due to interferences, since the interferences can be detected and the data can be corrected to obtain an accurate data.

In case of samples very diluted, could be necessary to explore the increase of resolution of the method. In this sense, selective solid phase extraction (SPE) can be applied to enhance the results at low concentration of Sb.[41]

Conclusions

To the best of our knowledge, this is the first report of a technique for speciation of Sb in liquid matrices applying an LC-ICP-OES system. The proposed methodology can be easily applied when an ICP-OES is available with the advantage of simplicity and reliability for a speciation technique. The calibration curves illustrate high correlation independently of the wavelength tested. The technique demonstrates good recovery results from an environmental model sample (sludge) which are usually applied in a lab test for analysis of metalloids in wastewater treatment plants. Due to the capabilities of the ICP-OES for simultaneous analysis of intensity data for different wavelengths over the chromatographic run, this technique can be used to determine the speciation of other metalloids such as arsenic and tellurium in the same run.

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Figure 1.

Physical connections for coupling the LC-ICP-OES instrument used for Sb speciation: 1) Eluent - mobile phase, 2) Isocratic pump, 3) Manual injection valve (300 µL loop), 4) Sample injection port, 5) Waste, 6) Pre-column, 7) Chromatographic column, 8) ICP-OES pump, 9) Nebulizer and spray chamber, 10) Argon tank, 11) Waste, and 12) ICP-OES instrument.

Figure 2.
Example of chromatograms obtained for Sb(III) (250 $\mu\text{g/L}$) and Sb(V) (250 $\mu\text{g/L}$) at three different wavelengths (206.834, 217.582, and 231.146 nm).

Figure 3. Calibration curves determined for Sb(V) (A) and Sb(III) (B) using the LC-IPC-OES speciation method developed in this study. Each curve corresponds to different wavelengths: (○) 206.834 nm, (□) 217.582 nm, and (△) 231.146 nm.

Table 1.

Limits of detection and quantification for Sb(III) and Sb(V) at different wavelengths using the developed LC-ICP-OES method for Sb speciation.

Wavelength (nm)	Sb(III)	Sb(V)
Limit of detection (µg/L)		
206.834	24.9	46.0
217.582	29.5	42.4
231.146	32.3	36.2
Limit of quantification (µg/L)		
206.834	113.3	60.5
217.582	80.7	49.9
231.146	115.5	58.2

Table 2.

Coefficients of variation for the analysis Sb(V) and Sb(III) in different samples at different wavelengths using the LC-ICP-OES method developed.

Wavelength (nm)	Sb(V) (µg/L)	Coefficient of variation			Sb(III) (µg/L)	Coefficient of variation		
		Water	Culture medium	Biomass + Culture medium		Water	Culture medium	Biomass + Culture medium
206.834	300	2.6	0.4	2.2	200	0.8	1.5	2.5
	3000	1.3	0.8	3.5	3000	2.2	1.8	4.3
217.582	300	1.5	3.8	2.7	200	0.6	2.1	6.4
	3000	0.2	1.5	3.1	3000	0.9	2.2	4.9
231.146	300	0.3	1.7	3.5	200	0.8	1.9	0.4
	3000	0.1	0.5	3.6	3000	0.4	1.7	5.0

Table 3.

Recovery of Sb from different matrices using the developed LC-ICP-OES method.

Wavelength (nm)	Matrix	Recovery* (percentage $\pm$ standard deviation)			
		Sb(III)		Sb(V)	
		200 $\mu\text{g/L}$	3000 $\mu\text{g/L}$	300 $\mu\text{g/L}$	3000 $\mu\text{g/L}$
206.834	Water	101.2 $\pm$ 0.8	100.1 $\pm$ 2.2	100.1 $\pm$ 2.6	102.1 $\pm$ 1.3
	Culture medium	101.6 $\pm$ 1.5	101.4 $\pm$ 1.8	106.2 $\pm$ 0.4	106.0 $\pm$ 0.9
	Biomass + culture medium	92.2 $\pm$ 2.3	102.0 $\pm$ 4.3	105.1 $\pm$ 2.3	101.5 $\pm$ 3.6
217.582	Water	99.5 $\pm$ 0.6	100.6 $\pm$ 0.9	100.1 $\pm$ 1.5	101.2 $\pm$ 0.2
	Culture medium	93.0 $\pm$ 1.9	95.9 $\pm$ 2.1	99.2 $\pm$ 3.8	102.0 $\pm$ 1.5
	Biomass + culture medium	96.0 $\pm$ 6.1	101.2 $\pm$ 4.9	99.6 $\pm$ 2.7	96.3 $\pm$ 3.0
231.146	Water	94.6 $\pm$ 0.7	96.1 $\pm$ 0.3	98.8 $\pm$ 0.3	101.2 $\pm$ 0.1
	Culture medium	95.6 $\pm$ 1.8	99.9 $\pm$ 1.7	94.0 $\pm$ 1.6	101.9 $\pm$ 0.5
	Biomass + culture medium	90.6 $\pm$ 0.4	97.6 $\pm$ 4.8	94.4 $\pm$ 3.3	94.9 $\pm$ 3.4

*

The total Sb concentration added was measured using ICP-OES and was considered for the recovery calculation.