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Simple and Fast Two-Step Fully Automated Methodology for the Online Speciation of Inorganic Antimony Coupled to ICP-MS

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Abstract: A very simple, fast and non-chromatographic methodology for inorganic antimony speciation based on Multisyringe Flow Injection Analysis (MSFIA) employing online hydride generation (HG) ICP-MS was developed. The fully automated analysis is performed in two steps: firstly, Sb(III) is quantified by ICP-MS after chemical vapor generation; then, total antimony is determined in the presence of potassium iodide as a pre-reducer of Sb(V) to Sb(III). The Sb(V) concentration is quantified by the difference between the total antimony and Sb(III) concentrations, reaching an analysis frequency of 30 h⁻¹. The optimization was performed using a Box Behnken design. The MSFIA-HG-ICP-MS system allows the antimony speciation analysis with a detection limit of 0.016 μ g L⁻¹ for Sb(III), working in a linear range of 0.053 to 5.0 μ g L⁻¹. This method was applied for the determination of Sb(III) and Sb(V) in water samples from Maiorca Island, Spain, and the concentrations found varied from 0.10 to 0.14 μ g L⁻¹ for Sb(III) and from 0.12 to 0.28 μ g L⁻¹ for Sb(V). The results were validated by addition/recovery tests, obtaining recoveries between 90 and 111% in both cases. Furthermore, a good precision was achieved, 1.4% RSD, and sample and reagent consumption were reduced to a few mL, with the consequent decrease in waste generation. Thus, the proposed method is a good tool for the speciation of inorganic antimony at ultra-trace levels in waters, allowing its risk assessment.

Keywords: antimony; speciation analysis; ICP-MS; automation

1. Introduction

Antimony is a non-essential element for biota and the irreversible binding between Sb and thiol-containing enzymes is responsible for toxic effects. Nevertheless, the toxicity of Sb depends on its molecular form, being inorganic Sb compounds are more toxic than organic ones [1]. Besides, Sb toxicity also depends on its oxidation state; Sb(III) compounds are ten times more toxic than those of Sb(V), e.g., red cells show an affinity for Sb(III), while they are almost impermeable to Sb(V). Therefore, the study of the distribution of Sb species in the environment is crucial to establishing the potential risk to organisms [2–4].

For the speciation of Sb, several analytical methods have been reported, principally based on HPLC and capillary electrophoresis but several non-chromatographic methodologies have also been developed [5]. In both cases, the hyphenation of hydride generation (HG) with atomic spectrometric techniques, such as HG-AAS [6], HG-AFS [7,8] and HG-ICP-MS [9], is the most common strategy for Sb speciation [10,11].



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Among the spectrometric detection techniques, ICP-MS is indubitably the most sensitive technique for determining metals and metalloids. However, despite this great advantage, its application to speciation analysis requires a separation step of the different species before detection using ICP-MS [12–19].

In this context, the most common approach is the development of methodologies coupling liquid chromatographic techniques with IPC-MS [20]. A method using ICP-MS for the determination of inorganic trivalent antimony Sb(III) and pentavalent antimony Sb(V), previously separated by HPLC was applied in plant, soil, and sediment samples [12]. A method using HPLC-ICP-MS was established for the separation and quantification of arsenic [As(III), As(V)] and antimony [Sb(III), Sb(V), SbMe₃] species in water and bottom sediments [15]. The combination of ion chromatography and ICP-MS allowed the determination of trace inorganic antimony in PET-bottled soy sauce [17]. An analytical strategy for antimony speciation in extracts of size-classified volcanic ash was developed by HPLC-ICP-MS [21]. Furthermore, the most commonly applied chromatographic method for the speciation analysis of Sb(III) and Sb(V) was evaluated, observing a low antimony recovery during analyses of biological and environmental samples. So, the basal chemistry of antimony was studied, and it was concluded that the formation of macromolecules, presumably oligomeric and polymeric Sb(V) species, is the primary cause of low chromatographic recoveries. Thus, sample preparation procedures have been proposed to stabilize the trivalent oxidation state of antimony [19].

In addition, methods exploiting (micro)solid-phase extraction combined with ICP-MS have also been reported [22]. A capillary microextraction system was developed for online simultaneous speciation by ICP-MS of ultra-trace inorganic antimony and tellurium in environmental water [13]. Two ion-exchange resin cartridges were employed for the effective separation of Sb(III) and Sb(V) in natural water samples using detection by ICP-MS [14]. Furthermore, a solid-phase microextraction coupled with ICP-MS was developed for simultaneous speciation analysis of chromium and antimony in environmental waters [18].

As an alternative to the above-mentioned approaches, non-chromatographic speciation methods were established involving reduction reactions, the addition of masking agents, solid-phase extraction and other unit operations [8]. Since these steps are laborious and time-consuming, a viable solution to this problem is the use of flow techniques, which allow automation of methods, decrease the consumption of samples and reagents, decrease the time of the analysis, and avoids external contamination. In this context, Multisyringe Flow Injection Analysis (MSFIA) is an alternative technique that combines the advantages of some previous flow techniques while avoiding their drawbacks, combining the multichannel operation and high injection throughput of Flow Injection Analysis (FIA) with the robustness and the versatility of Sequential Injection Analysis (SIA) [23].

Some previous works have proved that MSFIA is an ideal flow analysis technique for speciation analysis coupled with AFS detection, such as the determination of Sb(V), Sb(III), trimethyl antimony(V) and total antimony in soil using MSFIA-HG-AFS [24], and the antimony speciation in natural water by MSFIA-HG-AFS [7]. Nevertheless, the hyphenation between MSFIA, HG and ICP-MS for speciation studies, as far as the authors know, is the first time it has been reported. Thus, it is intended to develop a system that integrates the benefits provided by ICP-MS, which is a widely established standard detection technique in laboratories due to its very high precision, low detection limits, and rapid analysis, together with those of HG as a sample introduction technique, which enhances the sensitivity and selectivity [25], and the advantages previously mentioned from the MSFIA flow technique.

Accordingly, a fully automated inorganic speciation of antimony based on the hyphenation of MSFIA with HG-ICP-MS detection is proposed for the first time with the aim to take the benefit of the automation by a flow multicommuted analysis, the simplicity of an online non-chromatographic separation technique and the high sensitivity of the ICP-MS. Thus, a simple two-step methodology has been performed: firstly, Sb(III) is determined, while in the second step, total antimony is determined after the reduction of Sb(V) to Sb(III) using potassium iodide. The addition of the reduction reagents was processed online employing the MSFIA system and total antimony and Sb(III) were determined using hydride generation coupled to ICP-MS. The proposed methodology has been satisfactorily applied to different natural water matrices.

2. Materials and Methods

Reagents and materials. All solutions and standards were prepared using ultra-pure Milli-Q quality water (Millipore, Bedford, MA, USA). Stock standard solutions (1000 mg L^{-1}) of Sb(III) and Sb(V) were prepared by dissolving antimony potassium tartrate (Carlo Erba, Italy) and potassium pyroantimonate acid (Carlo Erba, Italy), respectively, both in $3.0 \text{ mol } L^{-1}$ HCl solution (Scharlau, Spain). A 0.30% (w/v) sodium tetrahydroborate solution (Scharlau) was prepared daily by dissolution in 0.05% (w/v) sodium hydroxide solution (Scharlau). The reducing solution containing 12% (w/v) potassium iodide and 10% (w/v) L-ascorbic acid was prepared by dissolving 6.0 g of KI (Scharlau) and 5.0 g of L-ascorbic acid (Scharlau) in 50 mL of ultra-pure water. The 1% (w/v, yellowish color) 8-hydroxyquinoline stock solution, was prepared by dissolving 1.0 g of 8-hydroxyquinoline (AnalaR*, A. R.) in 10 mL of methanol (99.8% Caledon), and then diluted to 100 mL with 10% (w/v) HCl solution. A mass of 0.2002 ± 0.0010 g of the cation exchange resin DOWEX[®] 50W-X8 (polystyrenedivinylbenzene with the sulfonic functional group, 100-200 mesh) was used for the retention of the organic antimony species given that the methylated forms can also generate chemical vapor. All materials used in the experiments, including glassware and plastics were kept for at least 24 h in 10% (v/v) nitric acid solution and thoroughly rinsed with Milli-Q water immediately before use.

Samples. Seven natural waters comprising drinking and ground water, and coastal seawater from different locations, were collected on Majorca Island, Spain. All of them were filtered through 0.45 μ m cellulose acetate membrane filters immediately after sampling, and acidified to pH 2.0 with hydrochloric acid and stored at 4 °C. Before analyses, samples were placed with 8-hydroxyquinoline 0.05% (w/v) and HCl 10% (w/v) and analyzed before 24 h.

Equipment. The developed method is composed of an inductively coupled plasma mass spectrometer Elan DRC-e (Perkin Elmer, Waltham, MA, USA) as a detector, a multisyringe burette (Multiburette 4S, Crison, Alella, Barcelona) to drive the liquids, three-way solenoids valves (N-Research, Caldwell, NJ, USA) to direct the liquids, a methacrylate minicolumn and connectors, PTFE tubes, and a PC to the instrumental control and another to data acquisition (Figure 1).



Figure 1. MSFIA-HG-ICP-MS method for automated online inorganic antimony speciation. GLS: gasliquid separator; MCx: methacrylate connector; RCx: reaction coil; R1: carrier; R2: KI solution; R3: NaBH₄ solution; R4: HCl solution; Vx: solenoid valves.

The ICP-MS was equipped with a Scott spray chamber and a cross-flower nebulizer (Perkin Elmer). Data were acquired in transient peak hopping mode, which allows the timeresolved monitoring of multiples isotopes. Typical operation parameters of the ICP-MS are listed in Table 1. The ICP-MS was controlled as a peripheral instrument of the MSFIA burette using the software package Autoanalysis 5.0 (Sciware Systems, Bunyola, Spain), version 5.0.13.5. The synchronism of ICP-MS with the MSFIA system was performed through the digital output of the multisyringe burette. A relay was used to trigger the events input of the ICP-MS.

Table 1. ICP-MS operating parameters.

Radiofrequency power	Forward	1100 W	
Gas flow	Plasma	$15 \mathrm{L}\mathrm{min}^{-1}$	
	Auxiliary	$1.2 \mathrm{L} \mathrm{min}^{-1}$	
	Nebulizer	$0.7 \mathrm{L}\mathrm{min}^{-1}$	
Acquisition parameters	Dwell time	0.05 s	
(All analysis)	Scan mode	Peak-hop transient	
	Sweeps per reading	5	
	MCA channel per spectral peak	1	
	Resolution/amu 10% peak maximum	0.7	
	Signal processing	Spectral peaks integrated; sum	
	Integration time	30 s	
	Smoothing signal profile	15	
	Reading per replicate	114	
	Replicates	3	
	Isotope measured	¹²¹ Sb	

The multisyringe burette module has a stepper motor that allows programming the flow rate and was equipped with four glass syringes (Hamilton): S1 (2.5 mL), S2 (2.5 mL), S3 (1.0 mL) and S4 (5.0 mL). Each syringe has a three-way solenoid valve (V1, V2, V3, V4) on the head in order to operate in a multicommuted way. The "off" position (solenoid disabled) connects syringes to a right channel and "on" position (solenoid enable) to a left one. Moreover, the multisyringe has four additional 12 V outputs on the rear panel, which control two additional valves (V5, V6).

PTFE tubes were used to construct the manifold, 1.5 mm i.d. for sample loading and 0.8 mm i.d. for the rest of the system. The holding coil to store the sample was 3 m long and 5.3 mL in volume.

A methacrylate minicolumn (5 mm i.d. and 4 cm in length) was packed with the resin for organic antimony retention. A porous frit located in the column bottom allowed resin hold.

Set-up. The syringe S1 was used to carry the sample, while S2 was employed to impel the mixture of potassium iodide and ascorbic acid solution to pre-reduce Sb(V) to Sb(III). The syringe S3 was in charge of dispensing the sodium tetrahydroborate solution, while S4 was used to propel the hydrochloric acid solution. The acquisition of the peaks was achieved with only one filling of the syringe, increasing the analysis frequency.

The solenoids valves V2, V3 and V4 control the aspiration and dispense of the reagents, while V1 and V5 control the sample loading into the holding coil and the sample dispensing to the system. The valve V6 allows directing the sample to the waste, with the aim to avoid passing samples through the column that will not be analyzed, e.g., during the sample change.

Procedure. The analytical procedure for the determination and speciation of inorganic antimony is performed in two steps. In the first step, Sb(III) is determined. The sample (2.0 mL) is loaded in the sample coil through the S1 with V1 and V5 in the "on" position. The sample is then dispensed (5 mL min⁻¹) with V1 in the "on" position, and V5 in the "off" position. The V6 is set in the "on" position allowing the sample to pass through the minicolumn, and thus, organic Sb is retained. Then, the sample plug is mixed with HCl (1.0 mL) and NaBH₄ (1.0 mL) solutions (2.5 mL min⁻¹) in reaction coil 2 (RC₂). The mixture is impelled to the gas–liquid separator (10 mL min⁻¹), where the stibine (H₃Sb) is delivered to ICP-MS by Ar gas stream (300 mL min⁻¹).

In the second step, the total inorganic fraction is determined, i.e., Sb(III) and Sb(V). The procedure is very similar to step 1, but 0.4 mL of KI are added (1.0 mL min⁻¹) in the reaction coil 1 (RC₁) in order to pre-reduce Sb(V) to Sb(III). Later, the mixture is merged in the RC₂ with HCl (1.0 mL) and NaBH₄ (1.0 mL) solutions (2.5 mL min⁻¹). The Sb(V) concentration is calculated by subtracting the Sb(III) concentration previously obtained.

3. Results and Discussion

3.1. Optimization of the MSFIA-HG-ICP-MS Method for Antimony Determination

The initial experiments involved optimizing the ICP-MS parameters, which include the carrier gas flow rate and the sweeps per reading. The carrier gas flow rate exerted a significant influence on the analytical signal and was studied for flows of 0.4 to 1.0 mL min⁻¹, seeking the best signal vs. blank ratio (Figure 2). The carrier gas flow rate of 0.7 L min⁻¹ was selected to fulfill the commitment of obtaining a good Sb/blank signal ratio, without spending excess gas. The time to sweeps per reading was also investigated and evaluates the response of the equipment after sample introduction (Figure 3). The times of 1, 2, 3 and 5 s were evaluated with a gas flow rate fixed at 0.7 L min⁻¹. It was observed that the response is instantaneous for the introduction of the sample in the vapor form. The sweeps per reading of 5 s were selected for the determination of antimony in the further experiments with an integration time of 30 s.



Figure 2. Optimization of nebulizer gas flow rate. ¹²¹Sb 5 μ g L⁻¹ standard solution and blank of reagent signals were analyzed for each flow rate.



Figure 3. Time to sweeps per reading optimization. ¹²¹Sb 5 μ g L⁻¹ standard solution was read using different integration times, working with a 0.7 L min⁻¹ Ar flow rate.

Once the detector conditions were set, the variables tetrahydroborate concentration, iodate concentration, and hydrochloric acid concentration were optimized using a Box Behnken design. Table 2 shows the experimental dominions of the three factors (as real and coded values) and the responses obtained by MSFIA-HG-ICP-MS.

Table 2. Matrix of the Box Behnken design. The table shows the codify levels of the three variables studied (the concentrations in brackets), the analytical responses for Sb(III) and Sb_{Total}, and the multiple response (MR).

Exp.	NaBH4 (%, w/v)	HCl (mol L ⁻¹)	KI (%, w/v)	Sb(III)	Sb _{Total}	MR(Sb(III) + Sb _{total})
1	-1(0.1)	-1 (1.0)	0 (12.5)	35,566	35,345	1.157
2	+1(0.5)	-1(1.0)	0 (12.5)	30,885	30,591	1.003
3	-1(0.1)	+1 (5.0)	0 (12.5)	19,116	19,494	0.630
4	+1(0.5)	+1 (5.0)	0 (12.5)	20,459	20,234	0.664
5	-1(0.1)	0 (3.0)	-1 (10.0)	51,664	51,484	1.683
6	+1(0.5)	0 (3.0)	-1(10.0)	52,427	52,027	1.705
7	-1(0.1)	0 (3.0)	+1 (15.0)	37,983	38,783	1.253
8	+1(0.5)	0 (3.0)	+1 (15.0)	36,988	37,188	1.211
9	0 (0.3)	-1(1.0)	-1 (10.0)	27,434	26,930	0.887
10	0 (0.3)	+1 (5.0)	-1 (10.0)	40,081	39,092	1.292
11	0 (0.3)	-1(1.0)	+1 (15.0)	38,276	38,741	1.257
12	0 (0.3)	+1 (5.0)	+1 (15.0)	48,972	48,832	1.596
13	0 (0.3)	0 (3.0)	0 (12.5)	60,804	61,003	1.988
14	0 (0.3)	0 (3.0)	0 (12.5)	60,982	60,889	1.989
15	0 (0.3)	0 (3.0)	0 (12.5)	60,864	61,563	1.998

The data obtained by Box Behnken design [26] were processed using the 7.0 Statistic program, utilizing the responses of the analytical signals (counts) derived by antimony(III) and total antimony, and also a multiple response [27] aiming to obtain a compromise condition that allows the determination of antimony(III) and total antimony in the same experimental conditions of the MSFIA-HG-ICP-MS method and maximum efficiency.

This multiple response (MR) was calculated by the following equation:

$$MR = (AS_{Sb(III)} / AS_{Sb(III)-max}) + (AS_{total Sb} / AS_{Sbtotal-max})$$

being that (AS $_{Sb(III)}$) and (AS $_{Sbtotal}$) are the analytical signals in the fifteen experiments, and (AS $_{Sb(III)-max}$) and (AS $_{Sbtotal-max}$) are the maximum values of the analytical signals obtained for Sb(III) and total antimony during the multivariate design that was performed. So, MR was calculated as being:

$$MR = (AS_{Sb(III)}/60982) + (AS_{Sbtotal}/61563)$$

The data were processed using the three responses and the critical values found for the experimental factors were: 0.3% (w/v) NaBH₄ solution concentration; 3.0 mol L⁻¹ HCl solution concentration; and 12.0% (w/v) KI solution concentration (Table 3).

Table 3. Critical values found for the experimental factors using the Box Behnken design.

Experimental Factor	Critical Values for Sb(III)	Critical Values for Sb _{Total}	Critical Values Using Multiple Response	
NaBH ₄ (% w/v)	0.30	0.30	0.30	
$HCl \pmod{L^{-1}}$	2.98	2.98	2.98	
KI (% <i>w</i> / <i>v</i>)	11.75	12.06	11.92	

The column size, nature and amount of resin employed for the retention of antimony organic species was established in a previous work devoted to Sb speciation using atomic fluorescence spectrometry [7].

The 8-hydroxyquinoline was used as a masking agent in order to avoid any modification in the oxidation state of Sb(III) to Sb(V) [28].

The sample load and eluent flow rates were fixed in terms of obtaining the highest analytical signal, analysis frequency, and reproducibility.

3.2. Validation Studies

The figures of merit reached by the proposed method are in concordance with those obtained by ICP-MS. Since ICP-MS is considered the most powerful analytical technique for trace element determination, the analytical parameters should be highly satisfactory to respond amply to the analytical requirements [29]. Moreover, it has been demonstrated that hyphenation with HG leads to enhancing the sensitivity and selectivity, eliminating spectral and non-spectral interferences caused by matrix elements [25].

In this context, the limit of detection (LOD) and the limit of quantification (LOQ) were calculated as three times and ten times, respectively, with the standard deviation of ten replicates of the blank divided by the slope of the calibration curve, following the IUPAC recommendation [30]. As result, the LOD and LOQ, calculated by using the experimental conditions established by the multivariate technique, are extremely low. The values achieved were 0.016 and 0.053 μ g L⁻¹ for Sb(III) LOD and LOQ, respectively, being the same for total antimony. The low LOD obtained is similar to those obtained with ICP-MS, e.g., 0.013 μ g L⁻¹ for Sb(III) exploiting SPE and HG [5]. It should be noted that, compared to the previous methods based on MSFIA proposed for the determination of antimony using HG-AFS, which obtained LODs for Sb(III) of 0.03 μ g L⁻¹ [3], 0.04 μ g L⁻¹ [24] and 0.08 μ g L⁻¹ [31], the present methodology achieves a reduction up to five times lower. Furthermore, a wide linear range of 0.053 to 5.0 μ g L⁻¹ was established as the working range.

Considering that the ICP-MS is at present a widely used and well-established spectrometric technique in most analytical laboratories, it is important to be noted that its combination with a multicommuted technique, i.e., MSFIA, and HG as sample introduction techniques in a fully automated approach, in addition to a high sensitivity, has made it possible to reduce the reagent and sample volume consumption, reaching a high precision. Accordingly, a precision of 1.4% RSD (expressed as relative deviation standard, *n* = 10) was assessed using a 0.50 µg L⁻¹ Sb(III) standard solution.

Furthermore, it is important to highlight that, considering the two steps for the Sb inorganic speciation, a very low sample volume (4 mL) was employed, and consequently, generated a low amount of waste (8.4 mL) per analysis. Moreover, a high analysis frequency ($30 h^{-1}$) was achieved, allowing the analysis of a large number of samples, and the consequent cost and time-saving. Analytical parameters are summarized in Table 4.

Analytical Parameter	Value		
LOD, $\mu g L^{-1}$ Sb(III)	0.016		
$LOQ, \mu g L^{-1} Sb(III)$	0.053		
Linear working range, $\mu g L^{-1}$ Sb(III)	0.053–5		
Precision, % RSD, $n = 10$	1.4		
Sample volume * (mL)	4		
Reagent volume * (mL)	4.4		
Waste volume * (mL)	8.4		
Analysis frequency (h^{-1})	30		

Table 4. Analytical parameters of the fully automated MSFIA-HG-ICP-MS developed for inorganic antimony speciation.

* Per analysis, considering the two steps.

The accuracy was evaluated by addition/recovery tests, following the IUPAC recommendation in cases where certified reference materials are not available [32]. Thus, seven samples were analyzed and the recoveries varied from 93 to 111% for the quantification of antimony(III) and from 90 to 109% for the determination of antimony(V), which were obtained by the difference between the concentrations of total antimony and antimony(III). These results can be seen in Table 5. The satisfactory results of recoveries in real water matrices, which could contain organic antimony compounds, were obtained using a DOWEX minicolumn which enables the extraction of organic antimony compounds with high retention capacity, allowing an inorganic antimony speciation procedure free from the interference of organic compounds, as occurs in many methods. The method accuracy was also evaluated by analysis of the samples employing AFS. A good concordance was observed between the results found by the two methods, showing a high correlation coefficient ($r^2 > 0.99$) for a confidence level of 95%.

Table 5. Analysis of drinking and ground water and coastal seawater samples from Majorca Island (Spain). Samples were also analyzed doped with concentrations of Sb(III) and Sb(V) for the evaluation of method trueness at two levels.

		Sb(III) (n = 3)		Sb(V) (n = 3)		
Sample	Addition (µg L ⁻¹)	Found (µg L ⁻¹)	Recovery (%)	Found (µg L ⁻¹)	Recovery (%)	Sb Total (µg L ⁻¹)
RW ¹	-	0.14 ± 0.03	-	0.28 ± 0.06	-	0.42
	0.25	0.38 ± 0.02	96	0.50 ± 0.04	90	
	0.50	0.64 ± 0.01	101	0.78 ± 0.01	101	
CSW ²		0.11 ± 0.01	-	0.20 ± 0.02		0.31
	0.25	0.37 ± 0.02	104	0.48 ± 0.01	109	
	0.50	0.66 ± 0.02	111	0.73 ± 0.01	104	
DTW ³		0.10 ± 0.01		0.17 ± 0.01		0.27
	0.25	0.37 ± 0.01	107	0.40 ± 0.02	92	
	0.50	0.57 ± 0.02	93	0.69 ± 0.02	104	
DTW ³		0.11 ± 0.01		0.18 ± 0.01		0.28
	0.25	0.35 ± 0.01	99	0.44 ± 0.03	105	
	0.50	0.55 ± 0.02	90	0.68 ± 0.03	101	
GW ⁴		0.12 ± 0.01		0.12 ± 0.01		0.24
	0.25	0.36 ± 0.01	96	0.36 ± 0.01	97	
	0.50	0.63 ± 0.02	102	0.63 ± 0.03	102	
GW ⁴		0.11 ± 0.01	-	0.13 ± 0.02		0.24
	0.25	0.37 ± 0.02	105	0.38 ± 0.01	97	
	0.50	0.60 ± 0.01	98	0.60 ± 0.03	94	
CSW ⁵		0.11 ± 0.01		0.12 ± 0.01		0.22
	0.25	0.36 ± 0.02	103	0.36 ± 0.01	98	
	0.50	0.57 ± 0.01	93	0.62 ± 0.03	101	

¹ Drinking rainwater from a city water reservoir (RW, Majorca); ² Coastal seawater in highly populated (hotel resort) area (Calvià, CSW, Majorca); ³ Drinking tap water from a city water reservoir (DTW, Majorca); ⁴ Ground water (GW, Majorca); ⁵ Coastal seawater in highly populated (hotel resort) area (Santa Ponça, CSW Majorca).

3.3. Application to Natural Waters

The fully automated MSFIA-HG-ICP-MS method was applied for the antimony speciation analysis in different matrices of natural water (drinking and ground water and coastal seawater). The concentrations achieved varied from 0.10 to 0.14 µg L⁻¹ for Sb(III) and from 0.12 to 0.28 µg L⁻¹ for Sb(V), being that Sb(V) has been calculated by the difference between the concentrations of total antimony and Sb(III). These results are in the same order of magnitude as those found in river samples, e.g., $0.42 \pm 0.03 \mu g L^{-1}$ for Sb(III) [33]. The obtained results are shown in Table 5. The applicability of the proposed methodology to different water samples (including the complex seawater matrix) are relevant in terms of risk assessment and the understanding of the biogeochemical cycle of antimony in the environment.

4. Conclusions

The inorganic antimony speciation at ultra-trace levels has been satisfactorily performed in a fully automated approach. The coupling of a multicommuted technique with HG-ICP-MS has allowed reaching several advantages, such as: (i) high selectivity since spectral and non-spectral interferences caused by matrix elements are eliminated by HG, this fact has been demonstrated by the satisfactory recoveries obtained in real water matrices; (ii) a very low limit of detection due to the use of ICP-MS, which is considered as the most powerful technique for trace element determination; (iii) a very fast and simple method since it is performed in a two-step protocol reaching 30 analyses per hour; and (iv) low reagents and sample consumption due to the use of the multicommuted technique, with the consequent low generation of waste, in accordance with the premise of green chemistry. Thus, it can conclude that the proposed method is a good tool for the speciation of inorganic antimony in waters, allowing its risk assessment.

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