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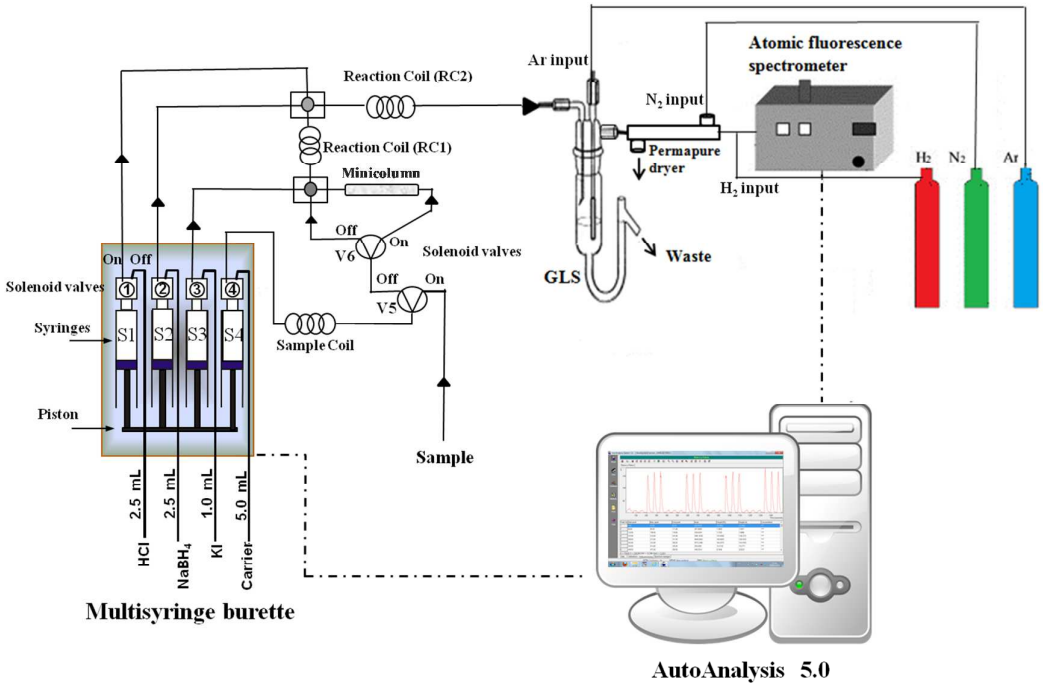
# Graphical abstract




**A non-chromatographic automated system for antimony speciation**

# A non-chromatographic automated system for antimony speciation in natural water exploiting multisyringe flow injection analysis coupled with online hydride generation – atomic fluorescence spectrometry

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**Abstract**

A non-chromatographic automated system for the speciation and determination of inorganic and trimethylantimony (TMSb) exploiting multisyringe flow injection analysis (MSFIA) with hydride generation (HG) and atomic fluorescence spectrometry (AFS) is described. A cationic minicolumn was use for retain the methylated forms of Sb which can generate hydrides, minimizing errors in the inorganic antimony speciation step. The optimization was performed in a multivariate way by employing a three-variable Box-Behnken design and a multiple response strategy. So, this method allows the quantification of Sb using the external calibration with aqueous standards. The method is suitable for monitoring drinking, surface and ground waters according to regulations established by the EU directives for antimony (5.0 μg $L^{-1}$), and it was applied to the speciation of inorganic and TMSb in several spiked waters samples with recoveries close to 100%. The detection limits were 0.03 μg $L^{-1}$ for Sb(III) and Sb(V) and 0,13 μg $L^{-1}$ for TMSb. The method was satisfactorily applied to the determination of Sb(III), Sb(V) and TMSb in different water samples collect in Balearic Islands, Spain.

## 1. Introduction

Antimony is an ubiquitously pollutant distributed at low concentrations in natural water, for this reason it is important to develop sensitive methods for its determination. Antimony is present in the aquatic environment as result of rock weathering, soil runoff and anthropogenic activities. Because of their chemical properties, antimony is widely used in industry. Among the various industrial applications of Sb compounds, antimony trioxide ($Sb_2O_3$) is profusely employed in the production of glassware and ceramics [1].

Furthermore, $Sb_2O_3$ is added to molten glass as a clarifying agent and is used as a pigment in dyes and paints as well as in the textile industry. Several Sb compounds are used as additives to batteries, metal coatings and to rubber, and others are added to textiles as flame retardants. In 2010 the world mine production of Sb was estimated in 165,000 tons [2]. Also Sb is a common component of coal and petroleum. Thus Sb is released to the environment from industrial activities. Typical concentrations of solved antimony in unpolluted waters are less than 1 μg $L^{-1}$ However, in the proximity of anthropogenic sources can reach up to 100 times naturals levels [3].

Generally, the inorganic species of antimony are more toxic than those organic forms, and its compounds were considered as pollutants of priority interest by the Environmental Protection Agency of the United States (USEPA) and by the European Union (Council of the European Communities) [3,4]. Antimony and its compounds don't showed biological functions know [5]. They are easily accumulated in organisms and cause deleterious effects in humans when their content goes beyond the allowable limit. The antimony determination content is important to protect the health of people and prevent environmental contamination due of their toxicity [5].

The development of highly sensitive techniques to identify and/or to quantify Sb species has opened up an increasingly attractive research area for elucidating the fate of Sb among the different environmental compartments. The vast majority of studies was focusing on methods based on high-performance liquid chromatography (HPLC), used in conjunction with element-specific detector. These methods using HPLC separations of Sb species are based on anion-exchange chromatographic methods due to predominance of Sb anionic species in aqueous environmental samples [6]. Since the first proposal using an anion-exchange column [7], cationic and reversed-phase chromatographic columns have also been evaluated [8], but species separation was not improved compared to anion-exchanged methods. In this context, the accurate separation of inorganic Sb(III), Sb(V) and methylated Sb species using a single chromatographic system is notoriously problematic and they are less often described in the literature. Several HPLC methods have attempted such a purpose, most of them based on use of strong anion-exchange stationary phases and complexing mobile phases to improve Sb(III) elution [6]. Furthemore HPLC systems are complex, an expensive instruments and produces dilatory methodologies.

Hydride generation (HG) techniques are widely used for the determination of volatile hydride forming elements in analytical atomic spectrometry to enhance detection power

and minimize or eliminate matrix interferences while incurring relatively low additional cost and minimal sophistication [9]. Usually, these techniques were combined with atomic absorption spectrometry (HG-AAS) [10], atomic fluorescence spectrometry (HG-AFS) [11,12], inductively coupled plasma optical emission spectrometry (HG-ICP-OES) [13,14] and inductively coupled plasma mass spectrometry (HG-ICP-MS) [15,16]. Alternative applications using HG-AFS system can also be used [17].

Not only Sb(III) is reduced by $NaBH_4$, methylated species of Sb are reduced and Sb(V) partially reduced also [18]. This is an important drawback in Sb speciation by HG to obtain accurate results. The 8-hydroxiquinoline was used as masking reagent to Sb(V) and transition metals during stibine formation [19]. For determination of Sb(V) by HG, it should be previously pre-reduced to Sb(III). Most methodologies for Sb determination has only been applied for the separation or speciation of inorganic species, Sb(III) and Sb(V), without considering the organic species of antimony. This dimethylatrion leads to formation of $Me_2SbH$, $MeSbH_2$, $SbH_3$, and $Me_3Sb$, an issue which has been discussed extensively in the literature, but has not yet been unequivocally solved [15]. Although non-chromatographic methodologies are an interesting alternative, because they are cheaper, easy to operate and faster than HPLC systems, only a single paper has been addressed using non-chromatographic system for antimony speciation. The author studied the influence of a combination of fluoride and iodide as modifier for the reduction process of Sb(III), Sb(V) and TMSb of the stibine generation using flow injection hydride generation coupled to an inductively-coupled plasma atomic emission spectrometer (FI-HG-ICP-AES), the method was applied to fruit orange juice samples [20].

Currently, the multivariate optimization strategies are very popular in the development of analytical methodologies. The main advantage of their use is the low number of experiments required to achieve the optimal conditions and the indication of possible influences of some variables on others, which is not possible in the univariate optimization. The response surface methodology (RSM) can be considered one of the most important approaches for the multivariate optimization of several analytical procedures [21,22]. The selection of Box-Behnken design as a model for the multivariate optimization of analytical procedures is on account of grown in the last few years, basically due to its higher efficiency when compared to other second-order designs like Doehlert Matrix and Central Composite Designs Designs and efficiency equal to Doehlert Matrix when three factors are studied [21].

This paper propose an automated and non-chromatographic method for the determination and speciation of Sb(III), Sb(V) and TMSb in complexes natural water samples by HG-AFS at ng $L^{-1}$. The method has been applied to various natural waters samples collected in Balearic Islands, Spain. Furthermore, the majority of the previously developed methods have been focused to the determination of high levels of antimony. The aim of this work is the determination of antimony at the ng $L^{-1}$ level in natural waters (coastal water, groundwater, drinking water), since studies indicate that typical concentrations in unpolluted systems are less than 1µg $L^{-1}$ [3]. In the present study was used a cationic exchange minicolumn in order to retain the methylated forms of Sb which can generate hydrides. In this sense, retention of trimethylated specie contributes to minimize the errors in the step inorganic forms determination presents in the sample.

## 2. Experimental

### 2.1 System set-up

The configuration of the system is presented in **Figure 1.** The system consists of a multisyringe burette module with programmable speed (Multiburette 4S, Crison, Alella, Barcelona), employed as liquid driver. It allows the simultaneous movement of four syringes, which are connected in block to the same stepper motor. Three-way solenoid valves (V1, V2, V3, V4) (N-Research, Caldwell, NJ, USA) are placed on the head of each syringe with the aim of increasing the versatility and reducing reagent consumption. The "off" position (solenoid disabled) of the head valves connects syringes to a right channel and "on" position (solenoid enable) to a left one. Moreover, the multisyringe has four additional 12 volts outputs, which can control some additional devices.

In the proposed system, four syringes were used: S1 (2.5 mL), S2 (2.5 mL), S3 (1.0 mL) and S4 (5.0 mL). The syringes were used as follow: S1 for propulsion of the hydrochloric acid solution, S2 to dispense the sodium tetrahydroborate solution, S3 to impel mixture of potassium iodide and ascorbic acid solution to pre-reduce Sb(V) to (Sb(III) and S4 to carry the sample. The acquisition of the peaks was achieved with only one filling of the syringe, increasing the sample frequency. The multisyringe module was equipped with two additional independents solenoids valves (V5) and (V6), (N-Research).

The solenoids valves V1, V2 and V3 control the aspiration and dispense of the reagents, while V4 and V5 control the sample loading into the holding coil and the sample dispensing to the system. The valve V6 allows the bypass of the sample through the minicolumn. Manifold was constructed with 1.5 mm i.d. (used for the sample aspiration) and 0.8 mm i.d. (used for the rest of the system) PTFE tubes. For the sample loading, the holding coil was 3 m long and 5.3 mL of volume.

A drying membrane (Perma Pure Inc, Toms River, NJ) utilizing nitrogen as a purge gas was connected to the outlet of the gas-liquid separator to circumvent entrainment of moisture into the AFS and subsequent quenching of the atomic fluorescence intensity. The water moves through the membrane wall and evaporates into the surrounding air or gas. An non-dispersive atomic fluorescence spectrometer (P.S. Analytical model 10.044, Excalibur detector, PS Analytical) for on line detection equipped with an antimony boosted discharged hollow cathode lamp (primary current 17.5 mA, secondary current 15.0 mA, wavelength 217.6 nm) was used. This spectrometer presents four internal gains and an external fine gain, which allows working on a large concentration range. The fine gain has been adjust during the optimization, up to a fine gain of 3, which was chosen for the lineal working range, using the gain operate at a 100-fold electronic. The transient signals were processed in the peak height. System control, data acquisition and processing, pump, valves and syringes were performed using the software package Autoanalysis 5.0 [19] (Sciware Systems, Bunyola, Spain), version 5.0.13.5. A methacrylate minicolumn 5 mm in diameter and 4 cm in length, provided with porous frit, was used to support the resin (DOWEX® 50 WX8, 100-200 mesh) for TMSb retention.

### 2.2 Standard solutions and reagents

All chemicals and reagents used were of analytical-grade or higher purity. Ultra pure water (18.2 MΩ $cm^{-1}$, Millipore, Watford, UK) was used throughout the study. Glassware and plasticware were cleaned by soaking in 10% (v/v) nitric acid and rinsed with ultra-pure water prior to use.

A stock standard solution (1000 mg $L^{-1}$) of Sb(III) were prepared by dissolving antimony potassium tartrate (Carlo Erba, Italy) in 3.0 mol $L^{-1}$ HCl (Scharlau, Spain) solution. Stock standard solution of Sb(V) were prepared by dissolving potassium pyroantimoniate acid (Carlo Erba, Italy) in 3.0 mol $L^{-1}$ HCl (Scharlau) solution. The solutions were stable for at least 3 months at 4°C.

A stock solution (1000 mg $L^{-1}$) of trimethylantimony(V) bromide (Sigma-Aldrich, Germany) was prepared in Milli-Q water and stored in polyethylene bottle at 4°C up to six months.

A 6% w/v sodium tetrahydroborate solution (Scharlau) in sodium hydroxide 0.2 mol $L^{-1}$ (Scharlau) was prepared daily.

The 8-hydroxyquinoline stock solution a 1% (w/v, yellowish color), was prepared by dissolving 1.0 g of 8-hydroxyquinoline (AnalaR*, A. R.) in a 10 mL of methanol (99.8% Caledon), and then diluted to 100 mL with HCl 10% (w/v).

A stock solution of potassium iodide 50% w/v containing *L*-ascorbic acid 10% w/v was prepared by dissolving 25.0 g of KI (Scharlau) and 5.0 g de L-ascorbic acid (Scharlau) in 50 mL of ultra-pure water.

A mass of 0.2360±0.0020 g of cation exchange resin DOWEX® 50W-X8 was used (polystyrene-divinylbenzene with sulfonic functional group, 100–200 mesh).

**2.3 Samples collection and treatment**

The water samples were collected and filtered through 0.45 µm cellulose acetate membrane filters immediately after sampling, and acidified to pH 2.0 with hydrochloric acid and stored at 4°C. The bottles were previously washed with a 10% v/v nitric acid–water solution and afterward with ultrapure water. Before analyses, samples were placed whit 8-hydroxyquinoline 0.05% (w/v) and HCl 10% (w/v) and analyzed before 24 h.

**2.4 Analytical procedure**

The analytical procedure for the determination and speciation of Sb can be summarized as follow in three steps:

1) In the first step, Sb(III) is determined. The sample (2.0 mL) is loading in the sample coil through the S4 with V4 and V5 in the “on” position. The sample is then dispensed at 5 mL $min^{-1}$ with V4 in “on” position and V5 in the “off” position. At this time the V6 switches to "on" position allowing the sample passes through the minicolumn and thus TMSb is retained. Then, sample plug is mixed with HCl (1.0 mL) and $NaBH_4$ (1.0 mL) solutions (2.5 mL $min^{-1}$) in the reaction coil 2 (RC2). The mixture is impelled to gas-liquid separator (10 mL $min^{-1}$), where the stibine ($H_3Sb$) is delivered to AFS-detector by Ar gas at 300 mL $min^{-1}$, before passing through the

permapure dryer with $N_2$ at 300 mL $min^{-1}$ flow rate. In this step, Sb(V) did not show any sign of fluorescence emission due to use 8-hydroxyquinoline complexing agent and the absence of the pre-reducing agent (KI).

2) In the second step, total inorganic fraction is determined, i.e. Sb(III) and Sb(V). The procedure is very similar to the 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 and $NaBH_4$ solutions. The Sb(V) concentration is calculated by subtracting the Sb(III) concentration previously obtained. In this step, although the mechanism is still unclear, it appears that the pre-reducing solution (KI) breaks the complex formed by the association Sb(V)-8-hydroxyquinoline. A similar behavior was previously reported [19], using a mixture of 0.1% 8-hydroxyquinoline + 2.0% KI and achieving a recovery close to 100% for a mixture of Sb(III) + Sb(V).

3) In the last step, the total antimony is determinated, i.e. inorganic species and TMSb. In this step, V6 is switched in "off" position allowing the bypass to the minicolumn. Thus, the total antimony is determined and the TMSb concentration is obtained by subtraction of previous inorganic fraction concentrations.

## 3. Results and discussion

### 3.1 Optimization of the hydride generation system

The optimization of the analytic fluorescence procedure was performed in two steps. Firstly, a two-level full factorial design [23] was carried out involving the followings factors: sodium tetrahydroborate ($NaBH_4$) concentration (in the range from 0.1 to 0.5 % w/v) in sodium hydroxide (NaOH) 0.05 mol $L^{-1}$; potassium iodide (KI) reagent concentration (from 10 to 15 % w/v); hydrochloric acid concentration (from 1.0 to 5.0 mol $L^{-1}$). The flow gas parameter (Ar, $N_2$ and $H_2$ was used as previous paper [24,25] and preliminary studies); sample flow rate and acid sample was fixed according limitations of the column retention for TMSb. The evaluation of this factorial design demonstrated that for these experimental conditions, the factors $NaBH_4$, KI and HCl reagent concentration are significant for the antimony hydride generation for a level significant of 95% and require a final optimization. The ANOVA table showed lack of fit and significant curvature. The curvature test was applied in the results obtained of the full factorial design to evaluate the system's behavior in the central point region. Thus, it

was possible to verify whether or not the condition of maximum in this region. **Equation 1** was used for the calculation [26]:

$$Curvature = R_{FD} - R_{CP} \quad (1)$$

Where,

$R_{FD}$ is the average of responses obtained from the experiments carried out for the factorial design, and

$R_{CP}$ is the average of the responses to the central point.

An analysis of the results suggested a negative curvature. This reveals the existence of an analytical region of maximum fluorescence signal near the central point of the experimental conditions.

For the best analytical performance for the online speciation and detection of critical values in the antimony speciation, was applying the Box-Behnken design for the chemical variables: $NaBH_4$ concentration (% w/v), KI concentration (% w/v) and (HCl) concentration (mol $L^{-1}$) (**Table 1**). This design required fifteen experiments and was performed in random manner to avoid any systematic error. The response of analytical interest was the fluorescence intensity (peak height) of the species Sb(V), Sb(III) and TMSb obtained in each step. To perform the multiple response optimization for speciation and determination of the three species of antimony process, a mathematical-statistical tool developed by Derringer, which is based on the use of a desirability function, was used. This feature allowed to combine in a single response (overall desirability) three distinguishing marks of each species studied. The use of desirability functions for multiple response optimization experiments was proposed by Derringer and Suich [27]. To obtain overall desirability, individual desirability of all responses should be determined (in this case, Sb(III), Sb(V) and TMSb fluorescence intensity). Thus, each response $y_i$ ($i$ = 1,2,…$m$) is transformed into a scale-free value, which is called an individual desirability function ($d_i$), where $0 \leq d_i \leq 1$, with 0 for an unacceptable response and 1 for a desirable response. The value of $d_i$ increases as the desirability of the corresponding response increases. The individual desirability function was calculated accord to **Equation (2),** which was used to maximize the Sb(III), Sb(V) and TMSb analytic responses. In this equation $y_i$ is the Sb(III), Sb(V) or TMSb fluorescence intensity; L and H are the lower and upper fluorescence intensity, observed

in the experiments for the antimony species. The overall desirability (D) was calculated by determining the geometric mean of individual desirabilities (**Equation (3)**).

$$d_i = \frac{(y_i - L)}{(H - L)} \quad (2)$$

$$D = (d_1 \times d_2 \times d_3 \ldots d_k)^{1/k} \quad (3)$$

Where,

*K* is the number of responses (in this case, 3).

D = 1 indicates a fully desired response, above which further improvements would have no importance.

The individual and overall desirability profiles for Sb(III), Sb(V) and TMSb are calculated according with **Equations 2** and **3** and the **Table 1** show the column with overall desirability profile (**D**). The **Figure 2** show the predicted values and desirability analyzed using the real values of the independent variables and the data processed by STATISTICA software [28] for a confidence level of 95%.

The system shown critical values with maximum solution in the central point region (**Table 1 and Figure 2**) and desirability equal to 1.0, for confidence level of 95%. These values were: $NaBH_4$ 0.38% (w/v), HCl 3.2% (v/v) and KI 12.75% (w/v).The ANOVA table shown an adjusted model for three antimony species, low error pure and very correlation between observed and predicted values.

### 3.2 Speciation methodology

Preliminary results and those reported in the literature [29] showed the difficulties encountered in developing a methodology for the determination of the three species in anionic chromatographic methods. Such difficulties may be related to the fact that soluble trimethylated antimony can mainly exist as non-charged or cationic ($[(CH_3)_3SbOH]^+$) species following the dissolution of TMSb in aqueous solution. Thus, its retention in the column cannot be explained as single anion exchange process, whereas in aqueous solutions, Sb(III) exist as a neutral species at pH around 8, e.g. $Sb(OH)_3$, or as a complex ion di-negatively charged, e.g. $[Sb_2(C_4O_6H_2)_2]^{2-}$ in presence of tartrate or EDTA, while the Sb(V) exist as a mono-negatively charged species, i.e.

$[Sb(OH)_6]^-$ [1,6,30]. So, in this work we decided determine antimony species (Sb(III), Sb(V) and TMSb) in water samples, using a cation exchange resin for retention of TMSb. In this way, if TMSb specie is present in the sample, the analytical error is avoided, since TMSb species generate hydrides ($MeSbH_2$, $Me_2SbH$ and MeSb) [31].

The use of L-cysteine has been recognized as a pre-reductant for some years to reduce Sb(V) to Sb(III). However, it is known that its use yields a high value of the analytical signal of the blank. Besides, when L-cysteine is used as a masking agent, it will inevitably change the original Sb(V) to Sb(III), masking then the speciation impossible. Therefore, potassium iodide was selected as pre-reductant for the reduction of Sb(V) to Sb(III) and the total Sb determination. The Sb(III) and Sb(V) were determined in absence and presence of potassium iodide. **Figure 3** show the analytics signals obtained for the three species in study in the presence and absence of KI, without use of the minicolumn. For this reason, it was decided to use KI because of the high efficiency of speciation inorganic forms of Sb. Besides, one study reports that Sb(V) cannot be completely reduced to Sb (III) without a pre-reduction step [32].

The compound 8-hydroxyquinoline was used as a masking agent in order to avoid any modification in the oxidation state of Sb(III) to Sb(V) [19].

### 3.3 Analytical performance

Using the optimized experimental conditions, limits of detection (LOD) ($3\delta/s$) and quantification (LOQ) ($10\delta/s$) were calculated for Sb(III), Sb(V) and TMSb in water samples following the IUPAC recommendation [33]. The LODs and LOQs of Sb(III) and Sb(V) are 0.03 and 0.13 μg $L^{-1}$, respectively, while for TMSb are 0.09 and 0.4 μg $L^{-1}$, respectively. The sample injection throughput obtained was 30 $h^{-1}$. The precision was evaluated through the relative standard deviation (RSD, %) for the 10 replicate measurements of Sb(III), Sb(V) and TMSb. Calibration was evaluated by comparing the slope of the curve obtained with Sb(V) in aqueous standards with those obtained for analyte addition to a natural water samples. The statistical comparison allows determine the similitude between the slopes, with determination coefficients $R^2 > 0.99$. Therefore, it can be concluded that the proposed method can quantify Sb species using external calibration using aqueous standards.

In order to investigate the effect of the inorganic Sb species over the TMSb determination, two curves of TMSb were performed: one for TMSb and other for TMSb in presence of 1.0 μg $L^{-1}$ Sb(V) both in aqueous medium (pH=2.0) using 8-

hydroxyquinoline as masking agent and KI as pre-reduce reagent for Sb(V) The slopes for both curves showed no significant difference, with determination coefficient $R^2 > 0.99$ for a confidence level of 95%, as shown in **Figure 4**. This indicates that TMSb can be determined in the presence of inorganic form of Sb (assay concentration similar than those found in fresh water), using a masking agent.

The analytical parameters to determine three studied species are shown in the **Table 2**, and the instrument working conditions are summarized in the **Table 3**.

The LOD, procedure used, sample matrix and chemical forms of antimony determined were compared between the proposed procedure and those achieved in other procedures for antimony determination (**Table 4**).

### 3.4 Validation of the proposed method and application in water samples

Since no certified reference materials exist for antimony speciation, the validation was performed by addition/recovery test (IUPAC, 2002) [34]. Hence, in order to establish the trueness of the proposed MSFIA-HG-AFS system for antimony speciation, real samples were spiked at trace level concentrations. Recoveries of drinking waters, coastal seawaters and groundwater doped with 0.2 and 0.4 μg $L^{-1}$ ranged from 90% to 110% regardless of the sample matrix complexity (see **Table 5**). Therefore, it was demonstrated that automated MSFIA-HG-AFS system for antimony speciation are reliable and unbiased data for environmental analysis. The antimony species and total antimony was quantified by employing MSFIA-HG-AFS in water samples collected in Balearic Islands.

## 4. Conclusions

A new non-chromatographic automated method based on MSFIA coupled to HG-AFS for antimony speciation was described.

The maximum efficiency was obtained thanks to combining multivariate design optimization with multiresponse tools.

The proposed method provides several advantages such as a high degree of automation, an elevated precision (RSD < 5%), and low limits of detection that allow the Sb speciation analysis in environmental waters. Besides, a high injection frequency together with the minimization of sample and reagents volumes, make this method an efficient and environmental friendly tool for antimony species evaluation.

The proposed method was successfully applied to several kinds of water samples, reaching recoveries of 90-110%.

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**Figure captions**

**Fig. 1** Scheme of the MSFIA-HG-AFS for automated online antimony speciation.

**Fig. 2** Prediction and desirability profiles for simultaneous optimization of analytical signals from Sb(III), Sb(V) and TMSb species. Dashed line indicates current values after optimization using Box-Behnken design.

**Fig. 3** Shape of the analytical signals obtained for Sb(III), 1.0 μg $L^{-1}$, Sb(V), 1.0 μg $L^{-1}$ and TMSb 5.0 μg $L^{-1}$, with and without KI. All solutions were prepared in the presence of 8-hydroxyquinoline 0.05% (w/v). AU: Arbritary unit.

**Fig. 4** Calibration curves for TMSb in aqueous medium (pH=2) in absence and presence of 1.0 μg $L^{-1}$ Sb(V). Both solutions were prepared in 8-hydroxyquinoline and KI.

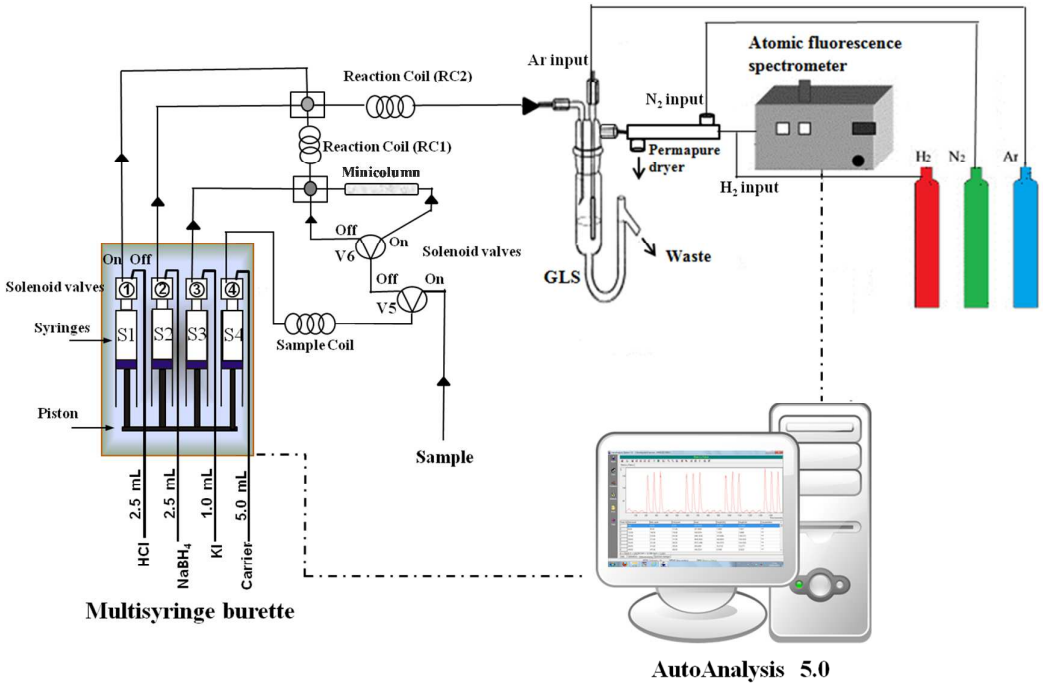


**Fig. 1**

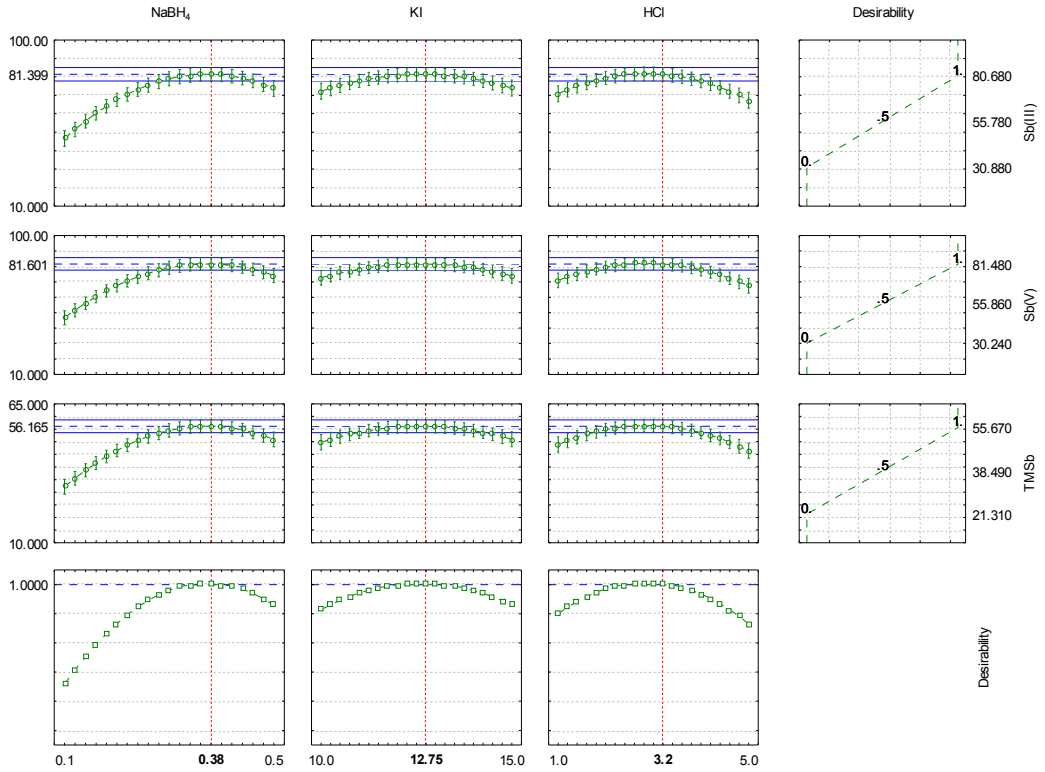


**Fig. 2**

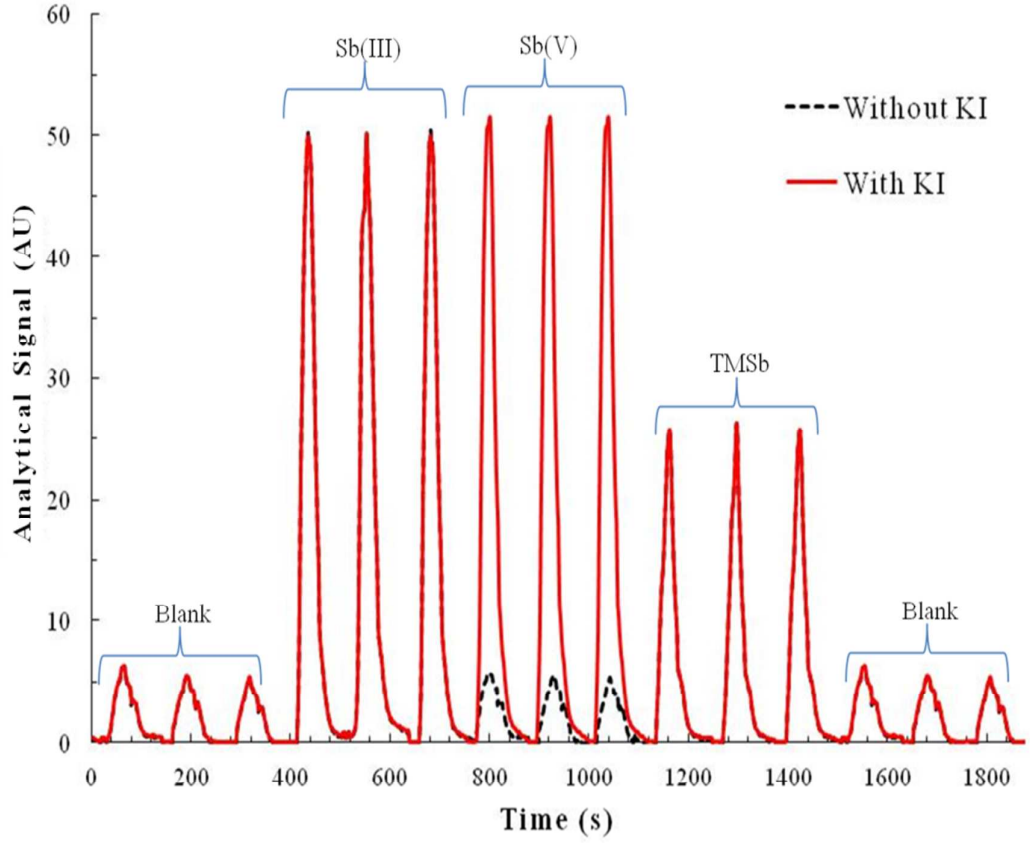

**Fig. 3**

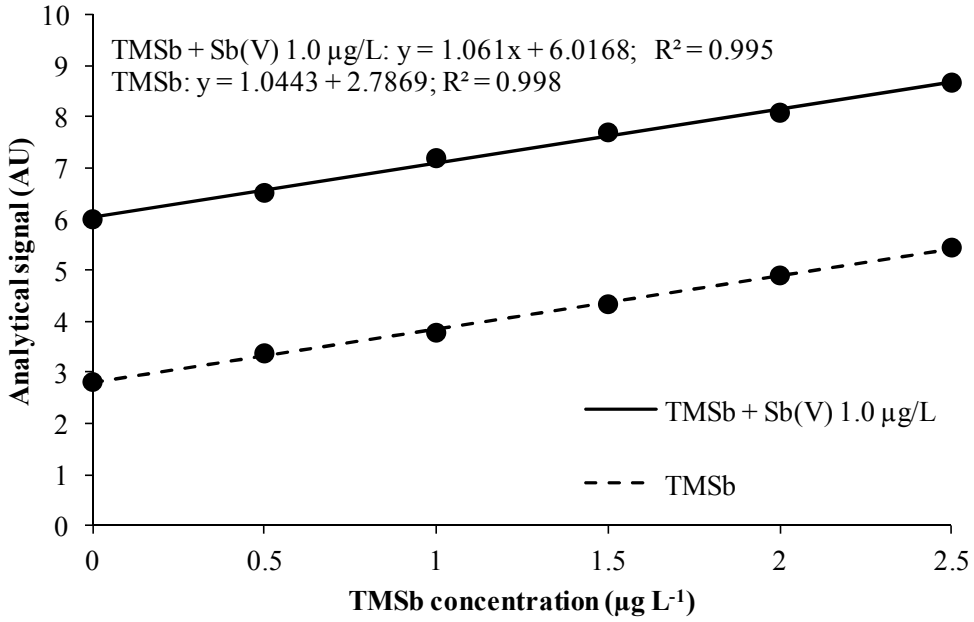


**Fig. 4**

**Table 1** Factors, levels and experimental matrix of Box-Behnken design.

| Factors | | | | Levels | | | |
|---|---|---|---|---|---|---|---|
| | | | | Low ( - ) | Mean ( 0 ) | High ( + ) | |
| $NaBH_4$ concentration (% w/v) | | | | 0.1 | 0.3 | 0.5 | |
| KI concentration (% w/v) | | | | 10.0 | 12.5 | 15.0 | |
| HCl concentration (mol $L^{-1}$) | | | | 1.0 | 3.0 | 5.0 | |
| Exp. | $NaBH_4$ | KI | HCl | Analytical signal (AU) | | | |
| | | | | Sb(III)[a] | Sb(V)[a] | TMSb[b] | D |
| 1 | - | - | 0 | 48.82 | 49.01 | 33.69 | 0.3623 |
| 2 | + | - | 0 | 66.24 | 66.60 | 45.71 | 0.7099 |
| 3 | - | + | 0 | 31.83 | 32.14 | 21.96 | 0.0237 |
| 4 | + | + | 0 | 62.04 | 62.00 | 42.81 | 0.6237 |
| 5 | - | 0 | - | 35.82 | 36.11 | 24.72 | 0.1041 |
| 6 | + | 0 | - | 65.39 | 65.71 | 45.12 | 0.6927 |
| 7 | - | 0 | + | 30.88 | 30.24 | 21.31 | 0.0000 |
| 8 | + | 0 | + | 59.99 | 60.28 | 41.39 | 0.5851 |
| 9 | 0 | - | - | 53.50 | 54.12 | 36.91 | 0.4581 |
| 10 | 0 | + | - | 64.66 | 64.17 | 44.61 | 0.6728 |
| 11 | 0 | - | + | 54.08 | 54.11 | 37.32 | 0.4659 |
| 12 | 0 | + | + | 61.57 | 61.47 | 42.48 | 0.6139 |
| 13 | 0 | 0 | 0 | 77.65 | 78.21 | 53.58 | 0.9382 |
| 14 | 0 | 0 | 0 | 80.68 | 79.00 | 55.67 | 0.9836 |
| 15 | 0 | 0 | 0 | 79.80 | 81.48 | 55.06 | 0.9882 |

a: 1.0 µg L-1; b: 10 µg L-1; D: overall desirability; AU: arbitrary unit

**Table 2**. Analytical parameters of merit of the proposed method.

| Parameter | Sb(III) and Sb(V) | TMSb[b] |
|---|---|---|
| LOD (µg $L^{-1}$) | 0.03 | 0.13 |
| LOQ (µg $L^{-1}$) | 0.09 | 0.4 |
| Linear range (µg $L^{-1}$) | 0.09 – 5.0 | 0.4 – 5.0 |
| RSD % (n=10)[a,b] | 2.8 | 3.8 |
| Injection throughput (inj $hour^{-1}$) | 30 | 30 |

a: Sb(III) and Sb(V) 1.0 µg $L^{-1}$; b: TMSb 5.0 µg $L^{-1}$

**Table 3** Instrument working conditions

| | |
|---|---|
| Sample flow rate (mL $min^{-1}$) | 5.0 |
| Sample volume (mL) | 2.0 |
| $NaBH_4$ (mL $min^{-1}$) | 2.5 |
| $NaBH_4$ (mL) | 1.0 |
| KI (mL $min^{-1}$) | 1.0 |
| KI (mL) | 0.4 |
| HCl (mL $min^{-1}$) | 2.5 |
| HCl (mL) | 1.0 |
| Argon flow rate (mL $min^{-1}$) | 300 |
| Hydrogen flow rate (mL $min^{-1}$) | 35 |
| Dryer gas ($N_2$) flow rate (mL $min^{-1}$) | 300 |
| Lamp current primary (mA) | 17.5 |
| Lamp current boost (mA) | 15.0 |
| Injection throughput (inj hour $^{-1}$) | 30 |
| Gain | 100 |
| Fine gain | 1 |
| Signal type | Peak height |

**Table 4** Comparison with LOD (limit of detection) obtained in procedures for antimony determination.

| Procedure | Sample | LOD (μg $L^{-1}$) | | | Reference |
|---|---|---|---|---|---|
| | | Sb(III) | Sb(V) | TMSb | |
| HPLC-HG-AFS[a] | Water | 0.26 | 0.09 | 0.04 | [35] |
| HPLC-HG-AFS[a] | Urine | 0.19 | 0.18 | 0.12 | [11] |
| HPLC-HG-AFS[a] | Soil | 0.07 | 0.07 | 1.0 | [36] |
| HPLC-HG-AFS[a] | Seawater | 0.07 | 0.13 | 0.13 | [37] |
| FI-HG-ICP-AES[b] | Orange juice, soil extracts | 1.2 | 1.4 | 1.1 | [20] |
| HG-ICP-MS[b] | Seawater | 0.013 | 0,021 | - | [38] |
| ETV-ICP-AES[b] | River water, tap water, pond water, urine | 0.09 | 0.09 | - | [39] |
| FI-HG-AAS[b] | Natural water | 0.05 | 0.06 | - | [40] |
| HG-ICP-AES[b] | River water, effluent samples | 0.09 | 0.9 | - | [41] |
| MSFIA-HG-AFS[b] | Ground water, seawater, drinking water | 0.03 | 0.03 | 0.13 | This work |

a: Chromatographic technique; b: Non-chromatographic technique. HPLC: High performance liquid chromatography; HG: Hydride generation; AFS: Atomic fluorescence spectrometry; FI: Flow injection; ICP: Inductively coupled plasma; AES: Atomic emission spectrometry; MS: Mass

spectrometry; ETV: Electrothermal vapourization; AAS: Atomic absorption spectrometry; MSFIA: ultisyringe flow injection analysis.

**Table 5** Antimony concentrations in water samples and spiked tests.

| Sample | Sb(III) ($n$=3) | | | Sb(V) ($n$=3) | | | TMSb($n$=3) | | | Total Sb($n$=3) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Spiked (μg $L^{-1}$) | Found (μg $L^{-1}$) | Rec (%) | Spiked (μg $L^{-1}$) | Found (μg $L^{-1}$) | Rec (%) | Spiked (μg $L^{-1}$) | Found (μg $L^{-1}$) | Rec (%) | |
| GW[a]-B | - | 0.23 ± 0.09 | - | - | < LOQ | - | - | < LOQ | - | 0.23 ± 0.10 |
| | 0.2 | 0.44 ± 0.06 | 105 | 0.20 | 0.19 ± 0.04 | 95 | 1.00 | 0.91 ± 0.10 | 92 | |
| | 0.4 | 0.58 ± 0.06 | 90 | 0.40 | 0.42 ± 0.07 | 105 | 2.00 | 2.14 ± 0.08 | 104 | |
| SW[b]-PP | - | 0.29 ± 0.10 | - | - | 0.27 ± 0.10 | - | - | < LOQ | - | 0.56 ± 0.20 |
| | 0.2 | 0.48 ± 0.09 | 95 | 0.20 | 0.49 ± 0.03 | 110 | 1.00 | 0.93 ± 0.10 | 93 | |
| | 0.4 | 0.67 ± 0.08 | 95 | 0.40 | 0.71 ± 0.05 | 110 | 2.00 | 2.17 ± 0.10 | 108 | |
| SW-PX | - | 0.23 ± 0.08 | - | - | 0.22 ± 0.08 | - | - | < LOQ | - | 0,45 ± 0,16 |
| | 0.2 | 0.47 ± 0.10 | 110 | 0.20 | 0.43 ± 0.08 | 105 | 1.00 | 0.97 ± 0.12 | 97 | |
| | 0.4 | 0.61 ± 0.05 | 95 | 0.40 | 0.59 ± 0.06 | 105 | 2.00 | 2.20 ± 0.09 | 110 | |
| SW-SJD | - | 0.12 ± 0.04 | - | - | 0.20 ± 0.06 | - | - | < LOQ | - | 0.32 ± 0.10 |
| | 0.2 | 0.29 ± 0.06 | 91 | 0.20 | 0.43 ± 0.07 | 98 | 1.00 | 0.97 ± 0.13 | 97 | |
| | 0.4 | 0.48 ± 0.05 | 90 | 0.40 | 0.59 ± 0.04 | 105 | 2.00 | 2.10 ± 0.10 | 105 | |
| SW-CB | - | 0.12 ± 0.09 | - | - | 0.29 ± 0.10 | - | - | < LOQ | - | 0.42 ± 0.09 |
| | 0.2 | 0.34 ± 0.10 | 110 | 0.20 | 0.48 ± 0.06 | 95 | 1.00 | 1.07 ± 0.09 | 107 | |
| | 0.4 | 0.50 ± 0.07 | 95 | 0.40 | 0.70 ± 0.06 | 103 | 2.00 | 2.10 ± 0.08 | 105 | |
| DW[c]-GC | - | 0.13 ± 0.10 | - | - | 0.27 ± 0.08 | - | - | < LOQ | - | 0.39 ± 0.18 |
| | 0.2 | 0.32 ± 0.08 | 95 | 0.20 | 0.45 ± 0.04 | 90 | 1.00 | 0.89 ± 0.14 | 90 | |
| | 0.4 | 0.49 ± 0.08 | 90 | 0.40 | 0.66 ± 0.04 | 98 | 2.00 | 2.13 ± 0.08 | 106 | |

a) GW: groundwater; b) SW: seawater; c) DW: Drinking water

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