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Flow analysis-hydride generation-gas phase derivative molecular absorption spectrophotometric determination of antimony in oral homeopathic products ("*Antimonium Tartaricum*") formulated under alcoholic medium

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Abstract

In this work, a flow analysis-hydride generation-gas phase derivative molecular absorption-(UV) spectrophotometric method has been developed for the direct determination of antimony in aqueous and hydro-alcoholic samples. Antimony (III) from undiluted samples is directly transformed into the gaseous stibine (SbH_3) form by on-line reaction with sodium tetrahydroborate ($NaBH_4$) in acidic medium (HCl). The gaseous phase generated is separated from the liquid phase using a commercial gas–liquid separator, and swept – with the help of a carrier gas (N_2) stream – into a quartz gas cell (10 cm pathlength); where the corresponding absorption spectrum is acquired in a continuous mode over the 190–300 nm wavelength range, using a conventional spectrophotometer. A derivative strategy was selected in order to avoid the strong spectral interference of the ethanol vapor on the gaseous SbH_3 absorption spectrum. In this way, the peak height at 223 nm of the second order derivative spectrum appears as a clear, clean and interference free analytical signal, which allows the direct determination of antimony. The recovery values obtained from homeopathic formulations (prepared in alcoholic medium) spiked with know amounts of antimony ranged between 97.5 and 103%. The method provides a dynamic range from 0.20 to 30 mg Sb l^{-1}. The precision (RDS), evaluated by replicate analysis ($n = 5$) of samples and standard solution containing between 2.5 and 15 mg Sb l^{-1} was in all cases lower than 1.2%. The proposed method was applied to the determination of antimony in commercial homeopathic products (*"Antimonium Tartaricum"*) prepared in hydro-alcoholic medium; and showed to be simple, precise, and accurate.

Keywords: Antimony; Stibine; Flow analysis; Hydride generation; Gas phase molecular absorption spectrophotometry (GPMAS); Derivative spectroscopy; Homeopathic products

1. Introduction

Antimony is a naturally occurring element. It was already known to the ancients and has still many uses nowadays, as it was reported by Filella et al. [1]. It has no known biological function and it is toxic. However, antimony-containing pharmaceuticals are still widely used in some parts of the world. The medical literature on antimony deals mainly with the use of organic pentavalent antimonial compounds as therapeutic agents for the leishmaniasis treatment [2]. Historically, antimony potassium tartrate, *"Tartar emetic"*, containing Sb(III), has been used to treat several conditions including syphilis, whooping cough, and gout; and topical antimony compounds were believed to improve herpetic lesions, leprosy and epilepsy. Antimony has been also used as emetic, decongestant and sedative. Besides, trivalent antimony therapy has generally been superseded by less toxic treatments. Adverse effects are associated with therapeutic use and the accidental ingestion of pesticides [3,4]. Nevertheless, with the growing of the homeopathic medicine, the interest in antimonial products as homeopathic and anti-homotoxicological remedies has increased.

Homeopathic products are prepared from substances called stocks, in accordance with homeopathic manufacturing procedures. Raw materials are of botanical, zoological, chemical or mineral origin. Stocks are diluted with vehicles such as purified water, alcohol, lactose, sucrose or glycerol, so a ready-to-use homeopathic product contains only minimal amounts of the raw materials. The homo-toxicological products use formulas

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according to the concept well known as homotoxicology: a bridge between the classic homeopathy and the conventional pharmacology. These products are combined formulas that generally contain considerable amounts of active ingredients, and they are to treat specific conditions [5]. By reviewing Internet sources [6,7], diverse antimony-containing homeopathic and homo-toxicological medicines can be found. For instance, *"Antimonium tartaricum"* (antimony potassium tartrate), whose quality control is mandatory in most of the First World countries, due to the presence of the toxic Sb(III) [2,8,9].

Antimony is usually determined by hydride generation coupled with atomic techniques, especially atomic absorption spectrometry (HG-AAS) [10]. However, for the determination of antimony in pharmaceutical formulations, different methods and procedures have been used: indirect titration with iodine [11], potentiometric titration [12], molecular absorption spectrophotometry monitoring the liberated iodine after reducing Sb(V) to Sb(III) with acidified iodide [12], and differential pulse polarography [13]. Flores et al. [14] recently described the determination of trivalent antimony and total antimony by HG-AAS in samples of injectable drugs used for *leishmaniasis* treatment. This last procedure was later adopted by the Brazilian Pharmacopoeia [15].

The HG technique has the advantage of being simple, rapid and relatively free of interferences, while AAS provides sensibility and selectivity [10]. However, molecular spectroscopic techniques such as molecular absorption spectroscopy (MAS) and Fourier transform infrared (FTIR) spectrometry have also been used as adequate detectors to determine the antimony under the hydride form (stibine, SbH_3). In this way, Gallignani et al. [16] reported a flow analysis (FA) system based on HG and FTIR detection to simultaneously determine antimony, tin and arsenic; in a further contribution they described a FA-HG-FTIR method for determining the total amount of antimony in pharmaceuticals [17]. Furthermore, in 1995, Cabredo et al. published a paper describing the simultaneous determination of arsenic, antimony and selenium by HG-gas phase (GP)-diode array molecular absorption spectrometry [18]. The described procedure involves the following: continuous generation, collection in a cryogenic trap, re-volatilization, measurement of the volatile compounds and resolution of the mixture by multi-wavelengths methods. In a further contribution, the researchers applied the same technique for the simultaneous determination of selenium, antimony, arsenic and tin [19,20].

Today, MAS in the UV–vis regions is applied to thousands of determinations, and it is of vital importance in clinical laboratories, pharmaceutical manufacturers, hospitals, forensic and environmental centers [21]. From this point of view, it seemed to be very interesting to develop a FA-HG-GPMAS-(UV) method to determine antimony in homeopathic formulations using a conventional spectrophotometer. However, it is important to highlight that the oral homeopathic formulations generally are prepared in alcoholic medium; this represents a really heavy drawback, since ethanol shows an intense absorption in the UV-region, below 230 nm, overlapping the stibine absorption band.

Derivative spectroscopy has been extensively used in molecular absorption spectroscopy [21,22] to give simple and direct elucidation to this type of spectral interferences, which could be very useful in this particular case. To the best knowledge of the authors, up to the moment the determination of antimony in hydro-alcoholic solutions via on-line generation of stibine, and using MAS-UV detection has not been reported.

The main aims of this study were: (i) to design and develop a simple and accessible FA-HG-GPMAS-(UV) system, using a conventional spectrophotometer as detector, which allows the easy and fast acquisition of the absorption spectra in a continuous mode and (ii) to develop an alternative method, based on a derivative strategy, for the determination of antimony in homeopathic and homo-toxicological products formulated under alcoholic medium, which could be suitable for the control analysis of this type of products.

2. Experimental

2.1. Instrumentation

Fig. 1 shows the FA-HG-double beam spectrophotometric system used in this work to determine antimony. It was mainly assembled from commercially available instruments and accessories. The antimony hydride vapor was generated in a Varian HG system, Model VGA-77 (Springvale, Australia), which basically consists of a three channel peristaltic pump furnished

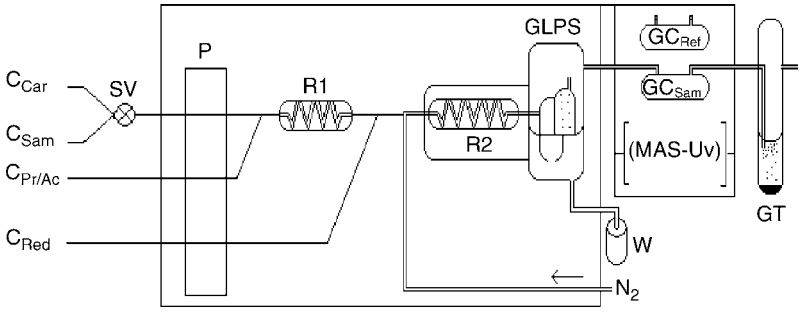


Fig. 1. Schematic diagram of the FA-HG-double beam spectrophotometric system. *P*: peristaltic pump; C_{Car}: carrier (water, 4 ml min^{-1}); C_{Sam}: sample/standard (4 ml min^{-1}); $C_{Pr/Ac}$, pre-reducing agent (KI 5%, w/v in HCl 30%, v/v); C_{Red}: reducer agent ($NaBH_4$ 0.25%, w/v in NaOH 0.1 M); SV: manual selecting valve; R_1: reaction coil-pre-reduction (PTFE, 30 cm length and 0.8 mm i.d.), R_2: reaction coil-hydrige generation (PTFE, 60 cm length and 1.5 mm i.d.); GLPS: gas–liquid phase separator; MAS-UV: UV–vis double beam spectrophotometer; GC_{Sam}: working gas cell; GC_{Ref}: reference gas cell (cylindrical quartz flow cell; 10 cm path length and 2 cm i.d.); N_2: nitrogen carrier gas (45 ml min^{-1}); GT: gas trapping ($AgNO_3$ solution 0.1 M); W: waste.

with *Tygon* tubes (C_{Car}/C_{Sam}, C_{Pr}/$_{Ac}$, C_{Red}), two PTFE reaction coils (R_1, R_2), and a gas–liquid phase separator (GLPS). This accessory includes two nitrogen entries with a fixed flow rate of 45 ml min^{-1}, localized in: (i) the reaction coil (R_2) and (ii) the GLPS. However, the second one was eliminated in this work so to reduce the excessive dilution of the gaseous SbH_3. The manifold includes a Reodyne (Alltech, Waukegan, USA) manual selecting valve (SV), which allows the selective introduction of either the carrier (C_{Car}) or the sample (C_{Sam}) in the system.

All the absorption spectra were acquired with a Perkin Elmer, Model Lambda 20 double beam spectrophotometer (Norwalk, CT, USA) in a continuous mode between 190 and 300 nm at a scan rate of 960 nm min^{-1}, and using a fixed nominal resolution of 1 nm. The instrument was equipped with two Wilmad (Buena, NJ, USA) cylindrical flow gas cells (GC_{Sam}, GC_{Ref}) placed in the optical path of the conventional UV–vis spectrophotometer. A PC 586 equipped with the Perkin Elmer WinLab Software was used for controlling the instrument, and for data acquisition and processing.

2.2. Reagents

Double de-ionized water of 18 MΩ cm^{-1} specific resistivity, indicated in text as DI, was obtained from a Milli-Q purification system (Millipore Corporation, USA). It was used to prepare all the solutions, and to rinse the previously cleaned laboratory material.

A stock standard solution (500 mg l^{-1}) of antimony (III) was prepared by dissolving $K(SbO)C_4H_4O_6{\cdot}0.5H_2O$ (pure crystal from Riedel De Haen AG, Hannover, Germany) in 0.5 M tartaric acid (J.T. Baker, USA). A 100 mg Sb(V) l^{-1} solution was prepared by oxidation of the Sb(III) stock solution with $KMnO_4/H_2SO_4/H_2O_2$ [17]. Working standards solutions were prepared by serial dilution of the stock solutions with DI, immediately before use. A 5% (w/v) potassium iodide (BDH, USA) in 30% (v/v) HCl (Merck, USA) was prepared daily. Sodium tetrahydroborate (Sigma, USA) solution 0.25% (w/v) was prepared daily in 0.1% (w/v) of sodium hydroxide (BDH, USA). Diluted hydrochloric acid was prepared by dilution of the concentrated acid in DI. Working solutions of ethanol were prepared from HPLC-grade ethanol (J.T. Baker, USA). The N_2 used in this work was supplied by AGA (Maracaibo, Venezuela) with a certified purity of 99%.

2.3. Samples

Three commercial oral homeopathic preparations, and one corresponding to an aqueous injectable formulation (from *Apis-Homaccord*® and *Tartephedreel*®) containing kalium stibyltartaricum (antimony potassium tartrate), were purchased in homeopathic drugstores. In addition, a series of samples formulated at different antimony concentration and alcoholic media were carefully prepared using analytical grade antimony potassium tartrate.

Samples were analyzed by the proposed method directly from the container, without pre-treatment or dilution. The nominal antimony content of the commercial homeopathic products is stated by codes (Di) in virtue of their high dilution. For example, dilution D4 contains one part stock in 10,000 parts of finished products (1:10,000) [5]. Table 1 shows a description of the samples used in this study.

2.4. Procedure

Samples and reagents were fed through their respective lines, as indicated in Fig. 1. The pump was on during the analysis in order to continuously propel DI (C_{Car}) or the sample (C_{Sam}), the pre-reducing agent ($C_{Pr/Ac}$), the hydrochloric acid solution (C_{Ac}), and the reducing agent (C_{Red}). In a preliminary step, SV was switched to position 1 in order to establish the background of the gaseous system; in this way, DI was introduced into the sys-

Table 1
Composition of the homeopathic formulations

Homeopathic preparation[a]		Content of antimony and ethanol stated on the label	
Sample/commercial presentation		Content of antimony[b] (mg Sb l^{-1}) or DI	Content of ethanol (%, v/v)
M1	100 ml (solution)	Kalium stibyltartaricum D2, 0.4 g (per 100 ml)	35
M2	30 ml (drops)	Kalium stibyltartaricum D2, 0.4 g; Kalium stibyltartaricum D10, 0.4 g; Kalium stibyltartaricum D30, 0.4 g; Kalium stibyltartaricum D200, 0.4 g; (each per 100 g)	35
M3	30 ml (drops)	Kalium stibyltartaricum D4, 5 g (per 100 g)	35
M4	1.1 ml (injection)	Tartarus stibiatus D4, 4.4 μl; Tartarus stibiatus D10, 4.4 μl; Tartarus stibiatus D30, 4.4 μl; Tartarus stibiatus D200, 4.4 μl; (each per 1.1 ml)	0 (aqueous medium)
M5	100 ml (solution)	Antimony potassium tartrate D2, 0.4 g ([Sb] = 14.58 mg l^{-1}) (per 100 ml)	15
M6	100 ml (solution)	Antimony potassium tartrate D2, 0.3 g ([Sb] = 10.9 mg l^{-1}) (per 100 ml)	15
M7	100 ml (solution)	Antimony potassium tartrate D2, 0.3 g ([Sb] = 10.9 mg l^{-1}) (per 100 ml)	35
M8	100 ml (solution)	Antimony potassium tartrate D2, 0.1 g ([Sb] = 3.64 mg l^{-1}) (per 100 ml)	15
M9	100 ml (solution)	Antimony potassium tartrate D2, 0.2 g ([Sb] = 3.64 mg l^{-1}) (per 100 ml)	35
M10	100 ml (solution)	Antimony potassium tartrate D2, 0.1 g ([Sb] = 7.30 mg l^{-1}) (per 100 ml)	25

[a] M1 to M4: commercial homeopathic samples (from *Apis-Homaccord*® and *Tartephedreel*®): M1 to M3 are oral presentations prepared under alcoholic medium, while M4 corresponds to an injectable presentation formulated in aqueous medium. M5 to M10: samples formulated and prepared at different antimony concentration (using analytical grade $K(SbO)C_4H_4O_6{\cdot}0.5H_2O$) and different alcoholic media, furnished by a local homeopathic drugstore.

[b] Antimony content expressed as mg Sb l^{-1} or by means of the homeopathic criterion for multiple dilutions (Di) [5].

tem, and the background of the gaseous system was established by accumulating three scans. Under these conditions, the spectrum of the blank is acquired also by accumulating three scans. It is important to note that prior the acquisition of the background, the reference cell (GC_{Ref}) was filled and sealed with the gaseous mixture produced in this step, while DI was introduced continuously into the system. The initialization of the system – filling, background definition, and acquisition of the blank spectrum – took 5 min approximately, and at this point the system was ready for analysis. This step was carried out only at the beginning of the experimental session. At that moment, SV was switched to position 2; in this way either the standard solution or sample was introduced in the system and mixed downstream initially with the acidic iodide solution in R_1, in order to ensure the trivalent form of the analyte; thus, in a second step, it reacted with the $NaBH_4$ solutions in R_2, where Sb(III) was quantitatively converted into its corresponding gaseous hydride form (SbH_3). The stibine was stripped in R_2 from the reacting solution with the aid of (i) the molecular hydrogen generated by the acidic hydrolysis of the $NaBH_4$, and (ii) the carrier gas (N_2). Once the liquid-gas mixture reached the GLPS, the liquid phase flowed to waste, while the gaseous phase ($SbH_{3(g)}$, $H_{2(g)}$, $N_{2(g)}$, $H_2O_{(g)}$, etc., and $CH_3CH_2OH_{(g)}$ for samples) flowed toward the flow gas cell, where the corresponding absorption spectrum was acquired in a continuous mode. Finally, the toxic $SbH_{3(g)}$ was trapped in a GT containing a solution of $AgNO_3$ 0.1 M.

The complete introduction of either standard or sample solution in the system required about 15 s, approximately, corresponding to 1 ml. At this point, the steady signal, the absorption spectrum corresponding to the continuous introduction of the solution into the system, was achieved. However, when a new standard or sample solution was introduced, this parameter was fixed in 37 s (≈2.5 ml) in order to avoid the memory effect of the previously introduced sample. Besides, the acquisition time of each scan was approximately 7 s. Then, the analysis of either standard or sample solutions, including sample introduction and the acquisition of three scans in a sequential mode, took around 60 s.

The peak height at 223 nm of the second-order derivative spectra ($D^2_{223\,nm}$) was evaluated using standard and sample solutions. Data obtained from samples containing ethanol were interpolated into the simple calibration graph established from aqueous antimony standards.

3. Results and discussion

3.1. Preliminary studies: vapor phase absorption spectra of reagents, standards and samples

Fig. 2 shows the gaseous ultraviolet absorption spectra (190–260 nm) of reagents, standards, and samples related to the analytical system under study. As it can be seen, the gaseous

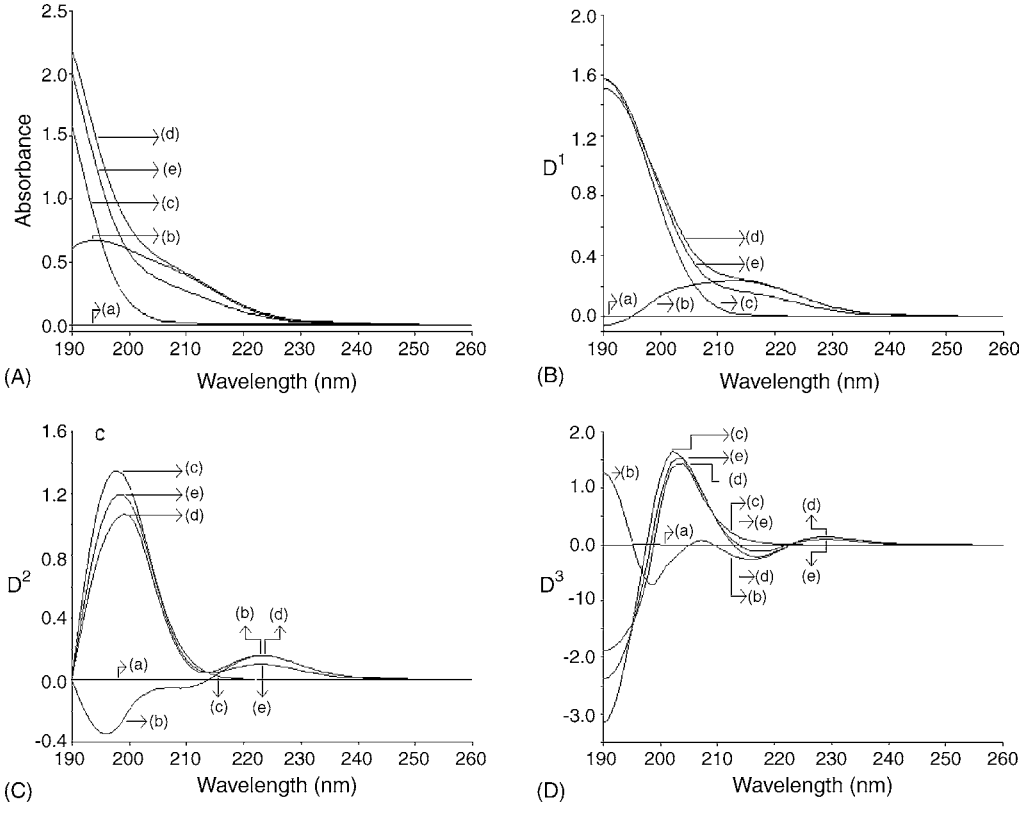


Fig. 2. Gas phase absorption spectra and second order derivative spectra related to the chemical system under study. (A) Gas phase absorption spectra obtained from: (a) blank of the system; (b) aqueous antimony standard solution (20 mg Sb l^{-1}); (c) hydro-alcoholic solution (diluted ethanol 35%, v/v); (d) hydro-alcoholic antimony standard solution ([Sb] = 20 mg Sb l^{-1}; [Ethanol] = 35%, v/v); (e) sample solution (M2) with an estimated antimony content of 13.3 mg Sb l^{-1} and a stated ethanol content of 35% (v/v). (B) First order derivative spectra (D^1) corresponding to the zero-order absorption spectra shown in (A). (C) Second order derivative spectra (D^2) corresponding to the absorption spectra shown in (A). (D) Thirst order derivative spectra (D^3) corresponding to the absorption spectra shown in (A).

blank (Fig. 2A(a)) has good transparency in the showed spectral region, while the UV spectrum of the gaseous stibine generated from a standard of 20 mg Sb l^{-1} (Fig. 2A(b)) shows a broad band below about 230 nm, with an maximum absorption well defined at 194 nm. This spectrum is identical to that reported by Cabredo et al. using a diode-array detection system [18]. It should be noted that the spectrophotometer used in present study is a conventional instrument which is not accessible to vacuum UV region ($\lambda < 190$ nm).

As it was previously outlined, the homeopathic formulations under study have a representative concentration of ethanol (Table 1), which is a volatile solvent. Ethanol vapor absorbs UV radiation below about 215 nm (see Fig. 2A(c)), in the same spectral region where SbH_3 shows its absorption band (Fig. 2A(b)). Therefore, the spectra obtained from a standard solution of antimony (20 mg l^{-1}) and from a commercial sample (M2) containing 13.3 mg Sb l^{-1}, both prepared in alcoholic medium, have contributions of $SbH_{3(g)}$ and $CH_3CH_2OH_{(g)}$; it can be seen in Fig. 2A(d and e). This is a typical and classic example of spectral interference from the matrix, which does not allow the analysis of antimony by direct measurement of absorbance at 194 nm for the present study. In order to solve this analytical issue, several strategies based on the use of standard solutions prepared in the same alcoholic medium as the samples or in subtractive spectroscopy, which require the previous evaluation of the ethanol concentration in the samples to be analyzed, have been described. Nevertheless, these approaches are not recommended for this particular case, in view of the fact that the ethanol concentration could vary from sample to sample.

Derivative spectroscopy has been extensively and successfully used to provide a direct, simple and elegant elucidation to these kinds of problems, in some cases allowing the separation of the overlapped bands. By using this technique, first order (D^1), second-order (D^2), and third-order (D^3) derivative spectra, corresponding to the original zero-order absorption spectra shown in Fig. 2A, were explored as it is illustrated in Fig. 2B–D. Initially, some options offered by the D^1 representation were studied, as for instance the maximum value at 215 nm. Nevertheless,

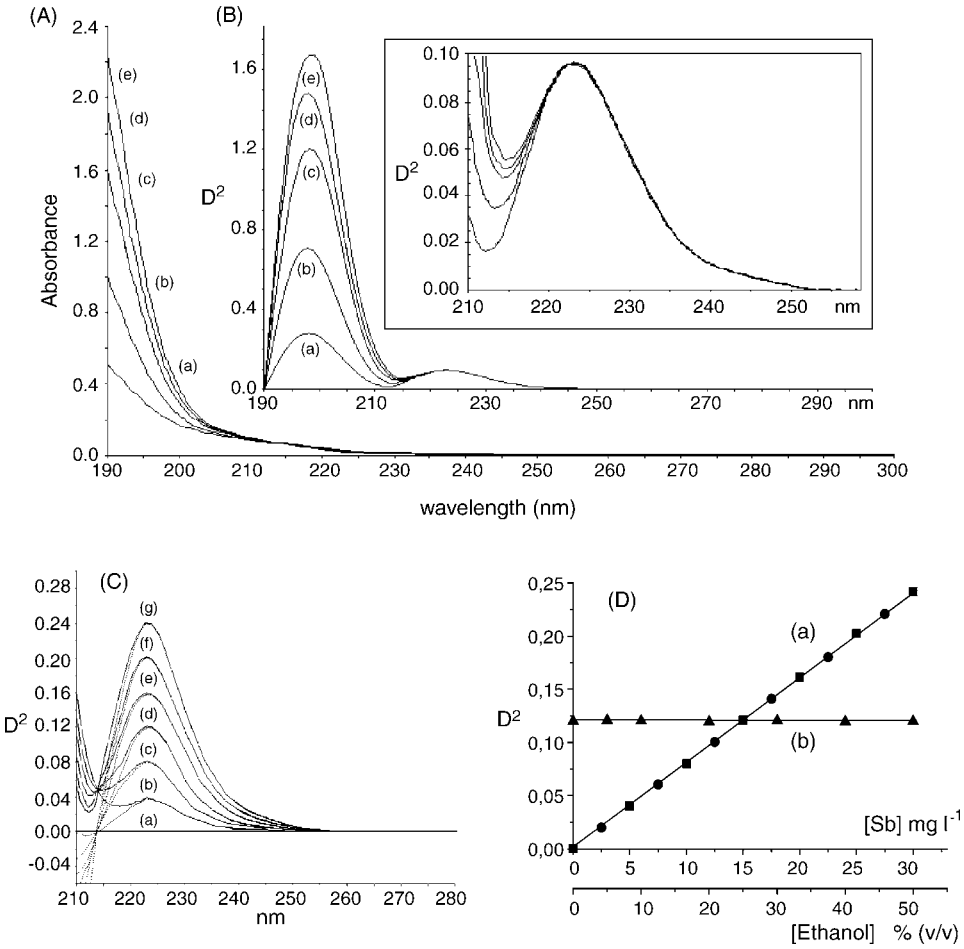
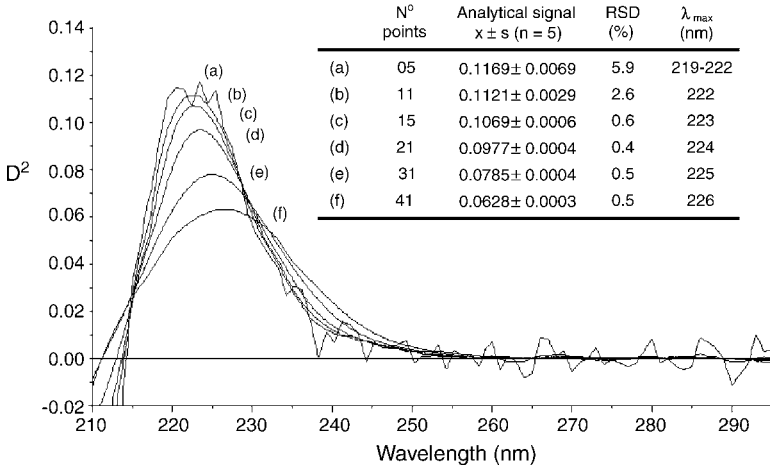


Fig. 3. Spectral behavior of SbH_3 in alcoholic medium. (A) Effect of the ethanol concentration on the absorption spectrum of the SbH_3 ([Sb] = 12 mg l^{-1}; [Ethanol]: (a) 10%, (b) 20%, (c) 30%, (d) 40% and (e) 50% (v/v). (B) Second order derivative spectra (D^2) corresponding to the original absorption spectra shown in (A). Inset: amplification of the D^2 spectra in the spectral range between 210 and 260 nm. (C) D^2 spectra corresponding to sets of antimony standards ([Sb]: (a) 0, (b) 5.0; (c) 10.0; (d) 15.0, (e) 20.0, (f) 25.0 and (g) 30.0 mg Sb l^{-1}) prepared in: (- - -) aqueous medium and (—) alcoholic medium ([Ethanol] = 35% (v/v)). (D) (a) Simple calibration graph constructed using Sb standard solutions (0–30 mg l^{-1}) prepared in: (●) aqueous and (■) alcoholic medium ([Ethanol] = 35%, v/v). (b) Effect of the ethanol concentration on the analytical signal ([Sb] = 15 mg l^{-1}: [Ethanol]: 0–50%, v/v).



	N° points	Analytical signal x ± s (n = 5)	RSD (%)	λ_{max} (nm)
(a)	05	0.1169± 0.0069	5.9	219-222
(b)	11	0.1121± 0.0029	2.6	222
(c)	15	0.1069± 0.0006	0.6	223
(d)	21	0.0977± 0.0004	0.4	224
(e)	31	0.0785± 0.0004	0.5	225
(f)	41	0.0628± 0.0003	0.5	226

Fig. 4. Influence of the derivation window on the shape of the D^2 spectra of SbH_3 corresponding to a real sample. ($[Sb]_{M2} \approx 13.3\,mg\,l^{-1}$; $[Ethanol]_{Declared} = 35\%$, v/v). The inset table indicates: mean ± standard deviation obtained for five independent measurements obtained for each derivation condition.

the results were not good since the analytical signal is influenced by the ethanol concentration. By analyzing the spectral region over the range from 215 to 260 nm of the D^2 representation, it can be seen that the maximum at 223 nm, corresponding to the wavelength with maximum slope of the SbH_3 absorption spectrum, belongs just to the gaseous stibine absorption (see Fig. 2C). On the base of these preliminary results, a research on the effect of the ethanol concentration over the range 0–50% (v/v) on the SbH_3 absorption spectrum (Fig. 3A) and the corresponding D^2 representation (Fig. 3C) was carried out; in order to do so, a standard solution of Sb(III) was used. The obtained results showed the peak height at 223 nm ($D^2_{223\,nm}$), as a very clean and clear analytical signal, since it was not affected by the alcoholic medium. Additionally, two calibration curves were established over the range from 0 to 30 mg Sb l^{-1}: one of them corresponding to the calibration in aqueous medium, and the other one in hydro-alcoholic medium. Fig. 3C comparatively shows the D^2 spectra corresponding to solutions with different antimony concentration prepared in alcoholic and non-alcoholic (aqueous) media, respectively. At the same time, Fig. 3D presents the calibration graph constructed using both sets of calibration standards; showing all the calibration data perfectly aligned in the calibration line, which is eminent evidence that the analytical signal is independent from the used hydro-alcoholic medium. Finally, the D^2 spectrum of $SbH_{3(g)}$ was tested at several smooth conditions by using different derivative windows between 5 and 41 points, provided by WinLab software. A balance among peak height, band-shape, and precision of the analytical signal was established to select this parameter, which was fixed at 15 points as a compromise solution. The use of high levels of derivative windows caused: (i) an important smoothing effect, thus reducing the analytical sensitivity, and (ii) the shift of the peak in higher wavelengths, as it can be seen in Fig. 4. On the other hand, when lower derivation windows were used, noisy spectra and poor relative standard deviation were observed. Thus, in this work, the peak height at 223 nm corresponding to the D^2 spectra using a derivative window of 15 points was selected as the measurement criterion.

3.2. Optimization of the experimental parameters

The derivative FA-HG-spectrophotometric method proposed in this work involves: (i) the reaction between Sb(III) and sodium tetrahydroborate in acidic (hydrochloric) medium to generate the gaseous SbH_3, (ii) the acquisition of the corresponding absorption spectrum in a continuous mode, and (iii) the treatment and processing of the spectral data.

The configuration shown in Fig. 1 is fundamentally based on the commercial HG system. It is very simple, and provides a good compromise between analytical signal, reproducibility, sample and reagent consumption, and sample throughput.

Instrumental-spectroscopic parameters and reagent concentrations were optimized using the univariate method. However, after each optimization, the validity of the previously selected parameters was re-checked. Besides that, other experimental conditions such as flow rate of samples and reagents, flow rate of N_2, length of transfer lines, and the internal diameter and length of the reaction coils were fixed based on previous experiences carried out in our laboratory in the field of on-line gas phase generation and following the experimental conditions either recommended or imposed by the commercial HG system. In all experiences, the optimization of the system was carried out evaluating the analytical signal from standard and sample solutions containing 15.0 and 13.3 mg Sb l^{-1}, respectively.

3.3. Instrumental-spectroscopic parameters

Initially, the effect of instrumental and spectroscopic parameters such as spectral range, resolution and scan rate, number of scans, background and reference conditions, and so on, were evaluated on the analytical signals.

Based on the preliminary studies, the spectral range was fixed between 190 and 300 nm. The FA-HG-MAS-(UV) system proposed in this work involves the acquisition of the absorption spectra in a continuous mode, by using a conventional-grating spectrophotometer. For this reason, the influence of the scan rate on the absorption spectrum, and thus in the analytical signal, was

tested in the range of 60–960 nm min^{-1}. The obtained results report that this instrumental parameter does not significantly affect the absorption spectrum. It is important to highlight that the Perkin-Elmer spectrophotometer, model Lambda 20, fixes the nominal resolution at 1 nm, regardless of the selected scan rate. Therefore, it was fixed at 960 nm min^{-1}, in order to reduce the reagents and sample consumption, as well as the analysis time.

Concerning the number of scans employed to establish the background, and the reference (blank) spectrum, three of them were enough to obtain an appropriate, clean, stable, and flat background in the proposed system.

3.4. Chemical reaction parameters

The main chemical parameters affecting the stibine generation are the antimony oxidation state, the concentration of the reducing agent ($NaBH_4$), and the acidity of the reaction medium. It is well established that stibine is preferably generated from Sb(III); however, it can be produced from Sb(V) as well, but with a low efficiency [10].

Antimony in the samples under evaluation must be present basically as Sb(III), because they are prepared from antimony potassium tartrate. However, a fraction of the total antimony can be present under the pentavalent form, due to diverse internal redox processes. It is important to highlight that the Sb(III) stock was prepared in 0.5 M tartaric acid with the aim to avoid the oxidation of Sb(III) to Sb(V). In order to solve this analytical task, the first step of the proposed procedure involves the reaction between the sample solution and a pre-reducing agent in R_1 (KI 5% (w/v) in 30% (v/v) HCl). This reaction medium ensures the quantitative reduction of Sb(V) to the trivalent form [17].

To determine the optimum reducing agent concentration, different $NaBH_4$ concentrations over the range from 0.02 to 1.2% (w/v) were tested. The analytical signal increased gradually with the $NaBH_4$ concentration up to 0.1% (w/v). Thereafter, a plateau was observed for higher concentrations, as it can be seen in Fig. 5A. Based on these results, a concentration of 0.25% (w/v) was selected for further studies. Regarding the reaction medium, it is well known that the stibine generation requires an acidic medium; for this purpose, hydrochloric acid has usually been recommended [10]. In our case, hydrochloric acid was introduced into the system by means of the pre-reducing solution.

The effect of hydrochloric acid concentrations ($C_{Pr/Ac}$) between 0 and 40% (v/v) on the SbH_3 generation was studied using standard solutions of Sb(III), and Sb(V). In the first case, the analytical response of Sb(III) increased with the acid concentration, achieving a constant signal for HCl concentrations higher than 5% (v/v), as it can be seen in Fig. 5B(a). Furthermore, the analytical response of SbH_3 generated from Sb(III) solutions was not affected by KI concentration in the range of 0–10% (w/v). In the second case, the analytical signal increased when increasing the acid concentration up to 20% (v/v). At higher concentrations a plateau was observed, thus indicating the quantitative reduction of Sb(V) to Sb(III) (see Fig. 5B-b). The obtained results report that the pre-reducing agent solution

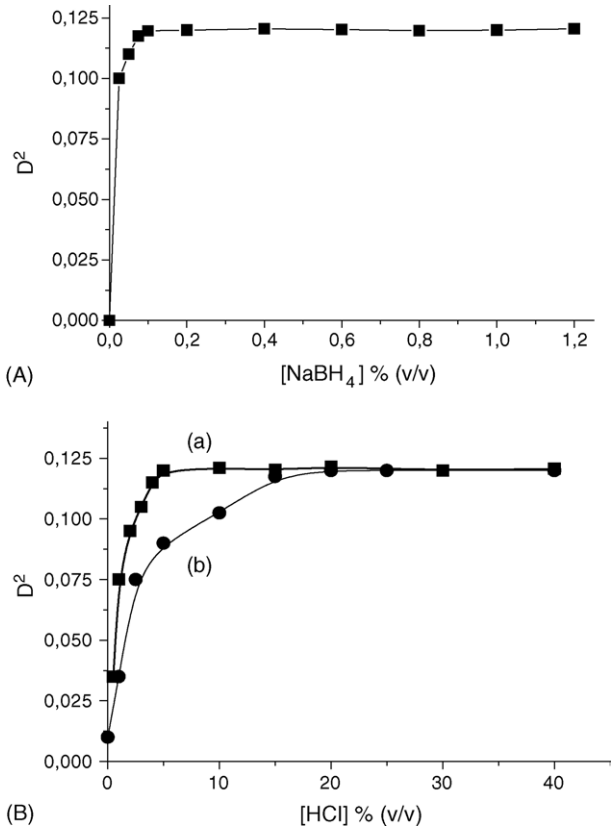


Fig. 5. Effect of the chemical reaction parameters. (A) Effect of the concentration of the reducer agent on the analytical signal ([$NaBH_4$] = 0–1.2%, w/v; [Sb] = 15 mg l^{-1}). (B) Effect of the concentration of hydrochloric acid (in the pre-reducing agent solution) on the analytical signal of SbH_3 generated from: (a) Sb(III) ((■) [HCl] = 0–40%, v/v; Sb(III)] = 15 mg l^{-1}) and (b) Sb(V) ((●) [HCl] = 0–40%, v/v; [Sb(V)] = 15 mg l^{-1}).

provides the appropriate acidic medium for the pre-reduction step, as well as for the hydride generation reaction.

3.5. Analytical performance

A simple calibration graph was constructed using the proposed method with a series of aqueous standard solutions containing Sb(III). The analytical features such as dynamic range, correlation coefficient, limit of detection (LOD), limit of quantification (LOQ), and sample throughput have been summarized in Table 2. The analytical signal increased linearly with the increase of the antimony concentration up to 100 mg l^{-1}. However, the dynamic range was adapted over the concentration range of the homeopathic products. The precision of the proposed method can be considered appropriate since values less than 1.2% (R.S.D.) were obtained in all cases. The sample throughput of 45 samples per hour can be considered acceptable, specially taking into account that the proposed method involves the acquisition of the absorption spectra in a continuous mode, by using a light dispersion with gratings spectrophotometer. Regarding the linear range and the limit of detection, the analytical features provided by this method (0–30 mg Sb l^{-1} and 0.6 mg Sb l^{-1}, respectively), are poor compared to those obtained in previous reported methods based on

Table 2
Analytical figures of merit

Parameter		Value		
Equation[a]		$D^2_{(223\,nm)} = -2.2 \times 10^{-5} + 8.04 \times 10^{-3}$[Sb]		
r[b]		0.9999 (±0.00045)		
Dynamic range (mg Sb l^{-1})		0.20–30		
LOD (mg l^{-1})[c]		0.06		
LOQ (mg l^{-1})[d]		0.20		
Sample rate (h)		45 h^{-1}		
Precision[e]	Standards	RDS (n = 5) (%)	Samples	n = 5 (%)
	[Sb] = 2.5 mg l^{-1}	1.1	[Sb] = 3.65 mg l^{-1}	1.2
	[Sb] = 5.0 mg l^{-1}	0.9	[Sb] = 13.3 mg l^{-1}	0.7
	[Sb] = 10.0 mg l^{-1}	0.7	[Sb] = 14.4 mg l^{-1}	0.6

[a] $D^2_{223\,nm} = a + b$[Sb] represents the equation of the simple calibration curve, where $D^2_{223\,nm}$ corresponds to the peak height at 223 nm of the second order derivative spectrum ($d^2A/d\lambda^2$) at the 194 nm analytical band of the SbH_3, and [Sb] the antimony concentration expressed in mg l^{-1}.
[b] Correlation coefficient of the linear calibration graph.
[c] Limit of detection defined as 3σ (n = 10) of the blank signal.
[d] Limit of quantification defined as 10σ (n = 10) of the blank signal.
[e] Relative standard deviation (RSD) estimated by measurement five independent replicates.

HG-spectrophotometric systems, which have working ranges in the μg l^{-1} order [19–21]. However, all these procedures include: (i) in-line concentration of the analyte(s) by using a liquid nitrogen cryogenic trap, with the purpose of enhance the sensitivity, and (ii) the use of a diode array spectrometer as detector, allowing the easy and fast acquisition of the absorption spectra in a flow system as a function of time; thus indicating two important differences between both approaches. The main conclusion is that we are talking about two different methodological proposals, each one with advantages and disadvantages. Nevertheless, the figures of merit obtained with the proposed method were adequate and appropriate to the proposed application.

3.6. Interference effects

The presence of ethanol in the homeopathic products was the main interference found in this experience. This interference was overcome by using derivative spectroscopy, as it was previously explained (see Section 3.1). The results obtained also indicated that the presence of ethanol did not interfere in the hydride generation process.

Matrix effects were investigated in samples M1–M3, by means of standard addition studies, adding different and known amounts of the analyte, from 0 to 10 mg l^{-1}, to sample aliquots of the same size (4 ml) diluting in all cases to a final volume of 25 ml with DI. The equations obtained for the simple and standard addition calibrations did not show a significant difference in their slopes for a test at the 5% significance level, which denoted that the proposed method is free of physical and chemical interference from the matrix. In addition, the Sb content obtained using both calibration modes was relatively analogous, consequently indicating the absence of interfering effects.

The accuracy of the proposed method was initially evaluated by analyzing the spiked sample solutions sets, which were used for the standard addition studies. The obtained recoveries (97.5–103%) demonstrated the general reliability of the proposed method.

Table 3
Analysis of real samples

Sample[a]	FA-HG-DMAS-UV			FI-HG-FTIR[b]	Label[c]
	Equation (SAC)[d] $D^2_{223\,nm} = a + b$[Sb]	$[Sb]_{SAC}$[e] (mg Sb l^{-1})	$[Sb]_{SCC}$[f] (mg Sb l^{-1})	$[Sb]_{SCC}$[b] (mg Sb l^{-1})	$[Sb]_{Estimated}$[c] (mg Sb l^{-1})
M1-a	$D^2_{223\,nm} = 1.86 \times 10^{-2} + 8.07 \times 10^{-3}$[Sb]	14.4 ± 0.1	14.6 ± 0.1	14.4 ± 0.1	14.6
M1-b	–	14.5 ± 0.1	–	14.4 ± 0.2	14.6
M1-c	–	14.4 ± 0.1	–	14.3 ± 0.1	14.6
M2-a	$D^2_{223\,nm} = 1.71 \times 10^{-2} + 8.00 \times 10^{-3}$[Sb]	13.4 ± 0.1	13.3 ± 0.1	13.2 ± 0.2	14.6
M2-b	–	–	13.8 ± 0.1	13.9 ± 0.1	14.6
M2-c	–	–	14.1 ± 0.2	14.0 ± 0.2	14.6
M3-a	$D^2_{223\,nm} = 2.05 \times 10^{-3} + 8.09 \times 10^{-3}$[Sb]	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.8
M3-b	–	–	1.7 ± 0.1	1.7 ± 0.1	1.8
M3-c	–	–	1.7 ± 0.1	1.7 ± 0.1	1.8
M4	–	–	<LD	<LD	<0.01
M5	–	–	14.3 ± 0.2	14.5 ± 0.1	14.6
M6	–	–	11.0 ± 0.2	11.1 ± 0.1	10.9
M7	–	–	11.1 ± 0.1	11.0 ± 0.1	10.9
M8	–	–	3.8 ± 0.1	3.70 ± 0.08	3.7
M9	–	–	3.6 ± 0.1	3.7 ± 0.1	3.7
M10	–	–	7.3 ± 0.1	7.4 ± 0.1	7.3

Simple calibration curve (SSC): $D^2_{223\,nm} = -2,1 \times 10^{-5} + 8.04 \times 10^{-3}$[Sb] (blank and seven standards).
[a] Samples analyzed are described in Table 1.
[b] Comparative analysis carried out by FI-HG-FTIR [23].
[c] Values estimated following the homeopathic criterion for multiple dilutions, calculated with reference to analytical grade antimony potassium tartrate.
[d] Equation corresponding to the standard addition curve (SAC) $D^2_{223\,nm} = a + b$[Sb], where $D^2_{223\,nm}$ corresponds to the peak height (at 223 nm) of the second order derivative spectrum ($d^2A/d\lambda^2$) and [Sb] the antimony concentration expressed in mg l^{-1}.
[e] $[Sb]_{SAC}$: antimony concentration (mean ± standard deviation; n = 3) obtained by means of the standard addition calibration.
[f] $[Sb]_{SCC}$: antimony concentration (mean ± standard deviation; n = 5) obtained by means of the simple calibration curve.

3.7. Analytical application

Samples described in the experimental section were analyzed directly, without any pre-treatment, with the proposed method by using the simple calibration graph. The obtained results are summarized in Table 3. Although the stated content of Sb in the commercial homeopathic products was unknown, the obtained values were close to the calculated one following the homeopathic criterion for multiple dilutions (see Tables 1 and 3). On the other hand, as it has been previously mentioned in Section 2.3, the antimony concentration in samples M5–M10 is well known. For this reason, the comparison between values obtained by the proposed method and those indicated in the label of these samples can be considered as a first estimation about the accuracy of the methodology. Furthermore, samples were also analyzed by a FI-HG-FTIR spectrometric method developed in our laboratory [23]. The obtained results by using simple and standard addition calibrations, as well as those found by the alternative procedure agreed well (see Table 3), thus indicating the accuracy of this method, and its viability for the analysis of antimony in this type of samples.

4. General comments and conclusions

In this work, a novel flow analysis-hydride generation-gas phase derivative molecular absorption-(UV) spectrophotometric method has been developed for the direct determination of antimony in aqueous and hydro-alcoholic samples. The use of a conventional UV–vis spectrophotometer based on light dispersion by gratings, instead of a diode-array detector, makes very available and accessible the proposed methodology. Besides this fact, the system is attractive because of its simplicity, easiness to perform, reliability, reproducibility, low reagent consumption, high sample throughput, and its adequate sensitivity to quantify the analyte over a wide range of concentrations in aqueous and hydro-alcoholic samples. Furthermore, the proposed system makes likely (i) speed acquisition of the absorption spectra in a continuous mode, (ii) detection of possible interfering species, and (iii) development of a derivative strategy to eliminate the interference coming from the ethanol vapor of real samples. In this way, a derivative spectroscopy is proposed as a universal solution to the outlined problem found in the determination of antimony in hydro-alcoholic solutions; such as the homeopathic products formulated in alcoholic medium.

The method was applied to the analysis of oral homeopathic and homo-toxicological products formulated under alcoholic medium, obtaining precise and accurate results, thus indicating its viability and suitability for the analysis of antimony in this type of samples. However, the analytical sensitivity provided by the proposed method is not enough to carry out the analysis in homeopathic injectable presentations, where the antimony content is very low. The selection of the peak height at 223 nm ($D^2_{223\,nm}$) as the analytical signal allowed us to eliminate the interfering effect of the ethanol but, at the same time, this strategy reduces the analytical sensitivity. Thus, in order to improve this important parameter, three strategies can be carried out: (i) the incorporation of a pre-concentration step [19,20], (ii) the use of a long pathlength cell [24–27], or (iii) both of them. Other alternatives involve the use of more sensitive techniques for the detection of SbH_3, such as AAS [10,14].

New studies are under way, concerning (i) the analysis of samples containing higher quantities of antimony, as well as pharmaceutical and veterinary products, and (ii) the use of long pathlength gas cells in order to enhance the sensibility of the system.

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