

# Continuous Flow Hydride Generation-Fourier Transforms Infrared Spectrometric Determination of Antimony in Homeopathic (Antihomotoxic) Products

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## ABSTRACT

**Introduction:** Antimony potassium tartrate is a component of certain expectorants in homeopathic medicine, particularly in those classified as antihomotoxic remedies. Given that antihomotoxic products are prepared with different epistemological and methodological statuses, they should be tested to prevent eventual toxic effects. **Methods:** In this study, we evaluate the possibility of using continuous flow hydride generation (HG) coupled with *Fourier* transforms infrared spectroscopy (FTIR) for determining antimony concentration in antihomotoxic products containing antimony potassium tartrate. **Results:** The results showed good agreement with the theoretically estimated values for the liquid commercial samples, in the range from 0.5 µg/mL to 30 µg/mL, following the principle of decimal dilutions used in homeopathic medicine. Liquid samples were analyzed directly from the commercial container. **Discussion:** In the absence of regulatory standards for antihomotoxic products, the proposed HG-FTIR method in gaseous phase provides an acceptable analytical capability for homeopathical compendiums, not only for analyzing raw materials and

mother tinctures but also for determining identity and uniformity content of finished products which should comply with pharmacopoeial specifications, and also in the determination of toxic content, 6 µg antimony per kg body weight, tolerable daily intake (TDI) proposed by the World Health Organization.

**Key words:** Antimony potassium tartrate, Homeopathy, Homotoxicology, Hydride generation, Infrared, FTIR.

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## INTRODUCTION

Antimony is included among the metalloid category, and among its various pharmaceutical applications its main use has been as an emetic drug (trivalent Sb) and as an anti-parasitic drug (pentavalent Sb). Additionally, antimony potassium tartrate (Figure 1) is a component of certain expectorants in homeopathic medicine.<sup>1</sup>

Antimony has been considered a toxic element in pharmaceuticals, with the trivalent species being 10 times more toxic than the pentavalent species.<sup>2</sup> The current use of antimony potassium tartrate in homeopathic medicine, together with the toxicity of Sb (III), have stimulated our interest in developing appropriate methods for the determination of antimony potassium tartrate.

Concerning homeopathy, two centuries ago the German Hahnemann stated that remedies which produce symptoms similar to a dose of the disease being treated were effective in diluted form as treatment of the same diseases.<sup>3</sup> In homeopathy, a base preparation, historically known as mother tincture, of a homeopathic remedy is made by liquid extraction (via maceration) or by dissolving the herbal, mineral, animal or imponderable substance in a solvent. Essentially, a series of dilutions are prepared from the mother tincture. This step is called potentization. Between each series, the diluted substance is succussed (shaken in a vigorous manner). The process of dilution leads to a gradual loss of chemical toxicity while gradually increasing the homeopathic potency.<sup>1,4,5</sup> Most homeopathic medicines undergo such dilutions and are typically administered at a concentration that reaches the point of not being measurable or even detectable (Figure 2). Due to this, the efficacy of homeopathic remedies is controversial and is often thought to present no major safety concerns.<sup>6</sup>

Nevertheless, homeopathic medicinal products are also used in other therapeutic approaches with different epistemological and methodological statuses, such as homotoxicology and antihomotoxic therapy. Antihomotoxic medicine is the connecting link between allopathic medicine and homeopathy (Figure 2).<sup>1</sup> That is to say that some are prepared by diminishing the grade of further dilution, a process of diminishing the dilution leads to a gradual increase of chemical toxicity. Consequently, antihomotoxic medicines could potentially constitute safety hazards.

Certainly, in our previously published work, it was found that two commercial antihomotoxic products contained more than 1000 µg/L of Sb(III).<sup>7</sup> This antimony content in antihomotoxic products could be considered very high if it is compared to the maximum allowed antimony concentration in drinking water (5 µg/L), according to the European Union Council.<sup>8</sup>

In contrast to what some physicians might assume, homeopathic drugs in the world are subject to well-defined regulatory processes that more closely resemble those that apply to allopathic medications than to dietary supplements.<sup>9-12</sup> Some countries even have homeopathic pharmacopoeias to ensure quality control of homeopathic drugs.<sup>9,10</sup> Nevertheless, a literature review reveals that there are few methods in the monographs of pharmacopoeias in order to quantify the concentration of antimony as antimony potassium tartrate. Most of the pharmacopoeial compendiums, such as the Brazilian Pharmacopoeia,<sup>13</sup> the U.S. Pharmacopoeia,<sup>14</sup> and The International Pharmacopoeia,<sup>15</sup> include monographs for antimony potassium tartrate or antimony sodium tartrate based on volumetric titration with iodine. Also, the Indian Pharmacopoeia contains a monograph for Sb(III) based on volumetric titration which, in this particular

case, is titration with potassium bromate.<sup>16</sup> However, volumetric methods are unspecific and may be inaccurate for analyzing finished products with a complex matrix.

In general, several techniques are available in the analytical field concerning antimony determination in different matrices.<sup>17</sup> Among them, atomic spectroscopy is the technique most widely used in metal and metalloid determination,<sup>18,19</sup> including coexisting elements in both drugs and homeopathic products.<sup>20-22</sup> These analytical techniques have been widely used in conjunction with hydride generation (HG) as a sampling technology. It is well known that an important advantage of HG is the separation of the analyte from the matrix, which eventually reduces spectral interferences.

In spite of the advantages of the most common atomic techniques in use today, continuous flow HG interfaced with *Fourier* transforms infrared (FTIR) has been proposed for determining antimony.<sup>23</sup> FTIR spectrometry in conjunction with the HG system is a fast analytical technique that provides very interesting quantitative information from gaseous samples. This analytical approach appears viable because of the accessibility of the FTIR detection system in the pharmaceutical industry together with spectral selectivity and low demand for high sensitivity. FTIR spectrometry has also been used to determine whether potentized homeopathic drugs and their diluent media differ from each other with respect to their spectra.<sup>24</sup> Analytical potential of on-line vapor-phase generation combined with FTIR spectrometry in order to improve the selectivity and the sensitivity of the measurement step has been critically reviewed.<sup>25</sup>

In this study, we evaluate the possibility of using continuous flow HG coupled with FTIR spectrometry for determining Sb(III) in homeopathic products containing antimony potassium tartrate. The class of homeopathic products known as antihomotoxic medicines may represent health risks to consumers when the antimony content goes beyond the allowable limit. The objective was to develop a reliable method for analyzing raw materials, mother tinctures and finished products which should comply with pharmacopoeial specifications, identity and content. This paper offers the validation of a method which provides useful and meaningful data for a pharmacopoeial assay method.

## MATERIALS AND METHODS

The present method is based on the pioneered method proposed in order to determine total antimony in antihomotoxic products by continuous flow HG and gas phase molecular absorption spectrophotometric detection.<sup>7</sup> The actual paper proposes an alternative detecting system using FTIR spectrometry. Therefore, an appropriate description of the methodology was included. Figure 3 shows a schematic representation of the continuous flow (FA, flow analysis) hydride generation (HG) system in conjunction with the transmission FTIR detection mode.

### Reagents

Milli-Q water (18 MΩ.cm) was obtained using a Milli-Q purification system from the Millipore Corporation (USA). Antimony potassium tartrate ( $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$ ) from Riedel-de Haën (Hannover, Germany). Potassium pyro-antimoniate ( $K_4Sb_2O_7$ ) from General Chemical Division, AC&DC (NY, USA). Sodium hydroxide 99.9%, hydrochloric acid 37%, potassium iodide 99.5%, and sodium borohydride 95% from Riedel-de Haën Sigma-Aldrich (GMBH Seelze, Germany). Tartaric acid 99% and ethanol 99%, both of them HPLC grade from J. T. Baker (Phillipsburg, NJ, USA). The nitrogen gas used in this work was supplied by AGA (Maracaibo, Venezuela) with a certified purity of 99%.

### Standard solutions

Calibration standards were prepared from stock solutions. A 500.0 µg/mL Sb(III) standard was prepared using an accurate amount of potassium antimony tartrate dissolved in 100 mL of 0.5 mol/L tartaric acid, prepared in deionized  $H_2O$ . This stock solution was kept in a PTFE flask at 4°C. An intermediate solution of 100.0 µg/mL Sb(III) standard was prepared containing 35% (v/v) ethanol. A standard curve, between 1.0 µg/mL and 30.0 µg/mL, was prepared by performing serial dilutions of this standard with 35% (v/v) hydroalcoholic solution. An external calibration technique was used for the analysis. A standard solution of 500 µg/mL Sb (V) was prepared by dissolving potassium pyro-antimoniate in boiling water, cooled, and finally diluted with cold water. Working standard solutions of Sb(V) were prepared by serial dilutions using water.

### Sample preparation

Both commercial antihomotoxic and homeopathic products were purchased at the local market (Mérida, Venezuela) but were manufactured by different laboratories: *Apis-Homaccord*<sup>®</sup> oral drops, lot 12216 AA from Heel Laboratories S.A. (Baden-Baden, Germany); *Apis-Homaccord*<sup>®</sup> drinkable ampoule, lot illegible, from Heel Laboratories S.A. (Baden-Baden, Germany); *Tartephedreel*<sup>®</sup> oral drops, Lot Ch-B:04502 AA, from Heel Laboratories S.A. (Baden-Baden, Germany) and *Stodal*<sup>®</sup> syrup, lot M3-104296, from Boiron Pharmaceutical Laboratories (Alcobendas, Madrid, Spain). Generic homeopathic preparation manufactured by Galenic Department, Faculty of Pharmacy of the University of Los Andes (Mérida, Venezuela). Although, *Apis-Homaccord*<sup>®</sup> and *Tartephedreel*<sup>®</sup> are sold under homeopathic preparations, they are prescribed as antihomotoxic preparations which means, according to the European Union, preparations from substances, products or preparations called stocks, in accordance with a homeopathic manufacturing procedure.<sup>6,26</sup> Further information is given within the results section of this article.

## Equipment

### Hydride generator

This system consisted of a Varian HG equipment model VGA-77 (Springvale, Australia) in a continuous flow mode (flow analysis, FA). The HG system is made of robust materials to withstand the most demanding of solvent/reagent compositions. It is furnished with a three channel peristaltic pump and Tygon tubes Ismatec, Cole-Parmer (Vernon Hills, IL, USA) to propel the antimony containing sample, the acid solution (HCl) and the reductant agent ( $NaBH_4$ ). The system also incorporates a PTFE reaction coil and a continuous nitrogen gas entrance.

### FTIR spectrometer

A Perkin Elmer Spectrum 2000 series FTIR spectrometer (Norwalk, CT, USA) equipped with a temperature stabilized DTGS detector, a mid-IR (MIR) source and a KBr beam splitter was employed to carry out the spectral measurements. A Wilmad MIR transmission cell (New Jersey, USA) was used for flow-through vapor phase sampling, having a 10 cm long cylinder (60 mL internal volume) made from borosilicated glass with a ca. 32 mm aperture at each end covered with circular ZnSe windows, 2 mm thick. Spectrum 2000 software (Norwalk, CT, USA) was used to control the instrument and spectral data acquisition. The software used to collect and present data was Timebase<sup>®</sup>, from Perkin-Elmer (Norwalk, CT, USA).

## Methodology

### Flow analysis fundamentals

The VGA 77 employs continuous flow technology where samples and liquid reagents are pumped together and mixed. The gaseous reaction

products are swept by a flow of nitrogen gas into the FTIR spectrometer through a gas flow cell (Figure 3). The Sb-containing sample and the acid medium (HCl) are allowed to merge first before the reductant agent ( $\text{NaBH}_4$ ) enters the stream. Nitrogen is then introduced into the liquid stream and the reaction proceeds while the mixture is flowing through the reaction coil. Vigorous evolution of hydrogen assists the stripping of the hydride ( $\text{SbH}_3$ ) from the liquid into the nitrogen gas. The gas is then separated from the liquid in the separator, and the liquid drains away as waste. The gas containing  $\text{SbH}_3$  then passes out of the separator into the gas flow cell coupled to the FTIR spectrometer where it is detected and the samples are finally analyzed. The  $\text{SbH}_3$  gas is eliminated from the atmosphere using an impinger with an aqueous solution containing silver nitrate or potassium permanganate, which readily oxidize the hydride.

**Flow rate for the continuous hydride generation.** The VGA 77 employs continuous flow technology with a fixed uptake rate through the sample and reagents pump tubes. The uptake rate should be within the range of 4–8 mL/min for the sample pump tube. An uptake rate of 4 mL was selected for the present study. Similarly, the uptake rate for each of the other pump tubes (acid and reductant) should be within the range 0.8–1.2 mL/min. An uptake rate of 1.0 mL was selected for the present study.

#### Reagent concentration for the continuous hydride generation

**Acid concentration:** HCl was used as acid medium and the concentration used in the container was 15% (v/v). **Reductant:** The  $\text{NaBH}_4$  concentration was 0.1% (w/v); this solution was prepared, for its stabilization, by adding NaOH 0.5% (w/v). Since  $\text{NaBH}_4$  decomposes, this solution was prepared from a more concentrated solution (1% w/v) before use. The more concentrated solution was stored at 4°C for no longer than one week. The working solution was allowed to reach room temperature prior to analysis. **Pre-reductant:** KI solution, 10% (w/v), was prepared immediately before use and protected from light using aluminum foil wrapped around the bottle.

#### Continuous flow hydride generation conditioning system

The pump was allowed to operate for five minutes to stabilize the flow rates and saturate the system with ethanol vapor. Then, a background was recorded using the analytical blank solution (ethanol 35% v/v). At the same time, a blank signal was measured. Then, each standard was aspirated taking into account the time for the signal to rise from zero to its stable signal (1.5 min). An elapsed time of 2.5 min was required when moving from a high to a low-level standard, in order to avoid a memory effect. Samples were propelled directly from the commercial antihomotoxic products.

#### Continuous flow hydride generation Fourier transforms infrared analysis

The FTIR spectra were recorded in the region between 4000  $\text{cm}^{-1}$  and 400  $\text{cm}^{-1}$ . The region between 1920  $\text{cm}^{-1}$  and 1870  $\text{cm}^{-1}$  was used for the quantitative determination of antimony at a resolution of about 2  $\text{cm}^{-1}$  with 3 accumulated spectra. The stretching band found at 1893  $\text{cm}^{-1}$  assigned for Sb-H in  $\text{SbH}_3$  was used throughout the entire process. The entire FTIR spectrum was registered in a few seconds because the commercial hydride generator produced a stable continuous signal. Peak height of the selected IR absorption band (1893  $\text{cm}^{-1}$ ), with baseline correction between 1896  $\text{cm}^{-1}$  and 1890  $\text{cm}^{-1}$ , was used as measurement criterion.

### Assay validation

Validation of the analytical method and procedures were conducted, unless otherwise specified herein, following a tutorial from Lab Compliance, a private organization devoted to validation and qualification in analytical laboratories.<sup>27</sup>

## RESULTS

### FTIR spectral characterization of antimony in gaseous phase

The FTIR spectrum of stibine ( $\text{SbH}_3$ ) by means of HG has been previously described.<sup>23</sup> In the present work, the absorption spectrum of stibine was acquired in a continuous mode between 1920  $\text{cm}^{-1}$  and 1870  $\text{cm}^{-1}$ . The background was registered using the gas evolved from all the reagents. The spectrum of the blank showed practically no absorption in the MIR region. The spectrum of stibine reveals the main absorption band belonging to the symmetric and asymmetric vibration of the Sb-H group centered at about 1893  $\text{cm}^{-1}$  (shaded region in the insert of the Figure 4a). This last referred band appeared in the transparent zone of the blank without spectral interference caused by the ethanol vapor. The stibine spectrum from a standard solution was practically identical to the stibine spectrum generated from representative sample solutions (Figure 4b). This spectral evaluation also showed that the presence of excipients, at the stated concentrations in the formulation, did not have any additional IR bands interfering with the mentioned IR absorption band belonging to stibine. Consequently, these preliminary results indicated the viability of the proposed method in order to analyze antihomotoxic preparations containing potassium antimony tartrate.

### Selection of the analytical measurement criterion

In the past few years, the authors have considered that the acquisition of absorption spectra in continuous mode is a better strategy than monitoring the signal at the maximum wavenumber as a time function. Fixed wavenumber analysis hides fundamental spectral information such as overlapped bands of reagents and products, matrix spectral interferences, and baseline displacement, among others. Due to this, various measurement approaches were evaluated in order to select the best criterion. Among them, the derivative spectroscopy showed better performance. However, in order to keep the analytical determination as simple as possible, we decided to work with the zero order absorption spectrum. Both peak area and peak height of the selected spectral band were examined with baseline correction between 1904  $\text{cm}^{-1}$  and 1874  $\text{cm}^{-1}$ . A narrow baseline correction was selected with the aim of the second order derivative spectroscopy with the purpose of improving accuracy and limit of quantification. In this sense, the peak height of the main band located at 1893  $\text{cm}^{-1}$  with baseline correction between 1896  $\text{cm}^{-1}$  and 1890  $\text{cm}^{-1}$  was selected for further analysis (shaded region in Figure 4b).

### Effect of FTIR instrument conditions

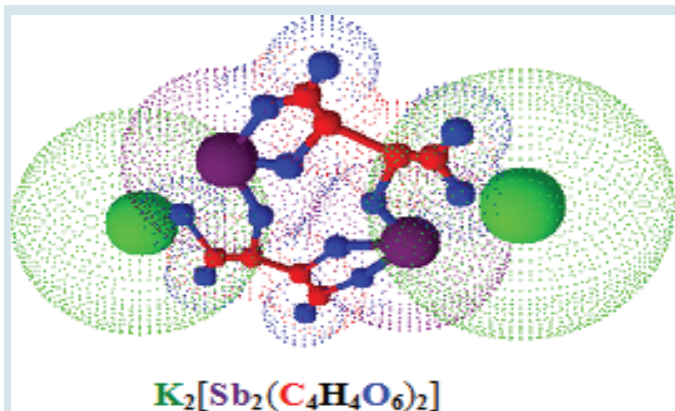
A monoparametric study was performed by monitoring the selected analytical signal for each variable by triplicate. As a whole, a nominal resolution of 2  $\text{cm}^{-1}$ , selected from a range of 2  $\text{cm}^{-1}$  to 32  $\text{cm}^{-1}$ , by accumulating 3 scans, selected from 1 scan to 50 scans, was chosen mainly as a compromise between precision of < 1.0 % RSD and spectral acquisition time < 30 sec. On the other hand, as a compromise with respect to sensitivity, precision, and band shape, the selected derivative window for analytical purposes was 19 points, selected from a range of 5 points - 37 points, with a related precision of < 1.0 % RSD,  $n = 3$ .

### Analytical figures of merit of the proposed FA-HG-FTIR method

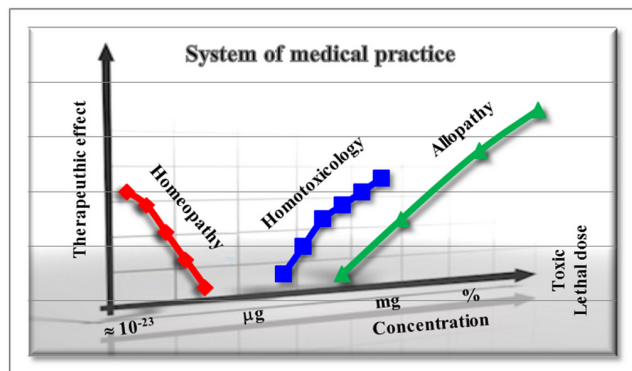
#### Linearity

Data from the simple regression line described in Table 1 demonstrated acceptable antimony linearity in a range of 10% to 200% of the nominal target concentration (15  $\mu\text{g/mL}$ ) with a quantification limit of 0.5  $\mu\text{g/mL}$ .

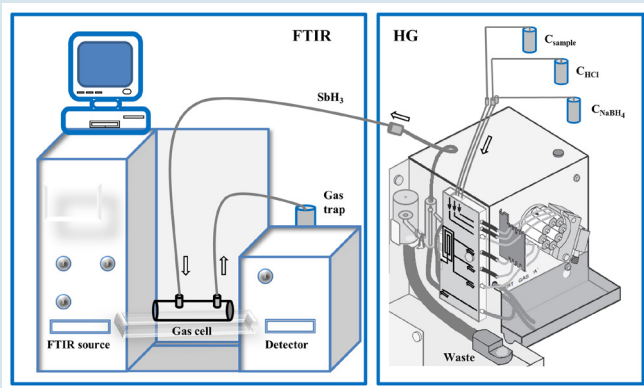




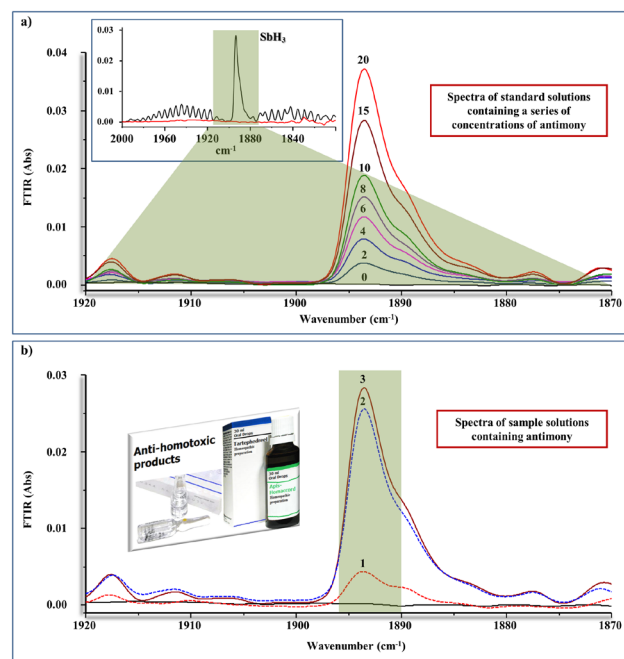
**Figure 1:** Empirical structure of antimony potassium tartrate trihydrate,<sup>14</sup> also known as potassium antimonyl tartrate, potassium antimontartrate, tartrate of antimony and potash, antimony potassium tartaricum, antimonium tartaricum, antimonii potassio tartras, tartarus emeticus, tartar emetic, potassio-antimonic oxytartrate, antimonium tartaratum, kalium stibyltartaricum, tartarus stibiatius, among others. The chemical structure was drawn with a freeware version of ChemSketch, version 12.01, Advanced Chemistry Development, Inc., Toronto, On, Canada, www.acdlabs.com, 2009.



**Figure 2:** Antihomotoxic medicine is the connecting link between allopathic medicine and homeopathic medicine in terms of the concentration of the active pharmaceutical/homeopathic ingredient. Theoretical representation based on antihomotoxic medicine from a Heel Scientific Department document.<sup>6</sup>



**Figure 3:** Scheme of the continuous flow hydride generation (HG) coupled to the Fourier transforms infrared (FTIR) spectrometer.



**Figure 4:** Gaseous phase FTIR spectra. (a) Spectra of seven standard solutions containing Sb(III) within the analytical range ( $n = \mu\text{g/mL}$ ) of the method; insert represents the selection of the stibine spectrum. (b) Spectrum of stibine generated from three representative samples with antimony concentration equivalent to 2.3, 13.5 and 15.0  $\mu\text{g/mL}$  belonging to *Tartephedrel*<sup>7</sup> (spectrum line 1), *Apis-Homaccord*<sup>8</sup> (spectrum line 2) and a generic antihomotoxic preparation (spectrum line 3), respectively. The shaded area represents the baseline correction for the measurement of each peak height of the  $\text{SbH}_3$  spectral band.

The y-intercept was negative but not significantly different from zero,  $p=0.05$ . Furthermore, the y-intercept variability resulted less than 1 % of the response obtained at the target level of the developed method for one of the products, which is considered an acceptable criterion.<sup>28</sup> With regards to the second analyzed commercial product, with a concentration of antimony much lower than the first, the y-intercept variability resulted 3.9% which could be considered acceptable, taking into account an actual concentration (2.3  $\mu\text{g/mL}$ ) close to the limit of quantification (acceptance criterion  $\pm 10\%$ ). The statistical significance of the regression line also revealed that the slope is not zero,  $p < 0.05$ , with a correlation coefficient evidencing an acceptable fit of the data to the regression line.

#### Precision

As can be seen in Table 1, data for instrument precision and repeatability met the requirements for a method to be considered precise.<sup>27</sup> The valid linearity data yields an RSD between 0.85% and 0.58% over the range of 66% to 133% of the nominal target concentration, 15  $\mu\text{g/mL}$ . Precision based on accuracy determination at each concentration over an acceptable range (80%-120%) of the target concentration (three concentrations, three replicates each) resulted satisfactory according to the usual acceptable criterion ( $\leq 2\%$  RSD), and additionally, illustrated the excellent precision of the proposed analytical method.

#### Selectivity and specificity

In the present work, the analyte was converted to a gaseous species with an inorganic group Sb-H which absorbs in the IR fingerprint region giving an intense and broad vibrational band centered at about  $1893\text{ cm}^{-1}$  (Figure 4a). The spectral behavior of stibine has been characterized

**Table 1: Analytical performance characteristics and system suitability of the proposed FA-HG-FTIR method for determining antimony in antihomotoxic products**

Parameter	Result	Remark
Dynamic linear range (µg/mL)	1.29 - 30.0	2.0 - 20.0 (working)
Detector linearity ( $r^2$ )	0.999 9	Criterion: > 0.998
External linear regression	$Y = -1.1 \times 10^{-4} + 1.258 \times 10^{-3} [X]$	$Y$ = FTIR spectral band height ; $[X]$ = conc.
Standard deviation (SD) of the response	$6.1 \times 10^{-5}$	Also described by the symbol "σ"
Limit of quantification (µg/mL)	0.49	(10 σ) x (slope <sup>-1</sup> )
Correlation coefficient (r)	0.999 9	Acceptance criteria: ≥ 0.999
Instrument precision data from 10 replicate determinations (Relative standard deviation, RSD)	0.84 % (Standard, 10 µg/mL)	Acceptance criteria: ≤ 1.0 %
Instrument precision data from 3 replicate determinations (RSD)	1.24 % ( <i>Tartephedree</i> ) 0.75 % ( <i>Apis-Homaccord</i> )	Acceptance criteria: ≤ 2.0 %
Intra-assay precision by analyzing aliquots of a homogeneous standard, independently prepared (RSD)	0.85 % for 10 µg/mL ( $n = 3$ ) 0.80 % for 15 µg/mL ( $n = 3$ ) 0.58 % for 20 µg/mL ( $n = 3$ )	Acceptance criteria: ≤ 2.0 %
Intermediate precision (RSD):		Acceptance criteria:
Operator 1, day 1 and day 2	1.3 %	2.0 % - 5.0 %
Operator 2, day 1 and day 2	1.5 %	
Operators, day 1	1.4 %	
Operators, day 2	0.9 %	
Analytical frequency (sample h <sup>-1</sup> )	24	Manual sampling
Stability of analytical solutions (15 µg/mL Sb standard)	1.1 % RSD ( $n = 6$ ); Test period: 120 min	Acceptance criteria: ≤ 2.0 %. Blank measurement after each standard measurement
Sample carry over by filling the cell with an intermediate standard concentration (15 µg/mL) followed by a blank solution sampling	0.15 % RSD	Acceptance criteria: a few percent (RSD)
Precision (% RSD) during accuracy determination for <i>Apis-Homaccord</i> at three levels over a range of 50 % - 150 % of the target concentration	0.70 % for 66 % ( $n = 3$ ) 0.60 % for 95 % ( $n = 3$ ) 0.56 % for 123 % ( $n = 3$ )	% RSD for % of the target concentration ( $n$ = samples were prepared in triplicate)
Precision (RSD) during accuracy determination for <i>Tartephedree</i> at three levels over a range of 50 % - 150 % of the target concentration	1.6 % for 25 % ( $n = 3$ ) 1.1 % for 75 % ( $n = 3$ ) 0.70 % for 130 % ( $n = 3$ )	% RSD for % of the target concentration ( $n$ = samples were prepared in triplicate)

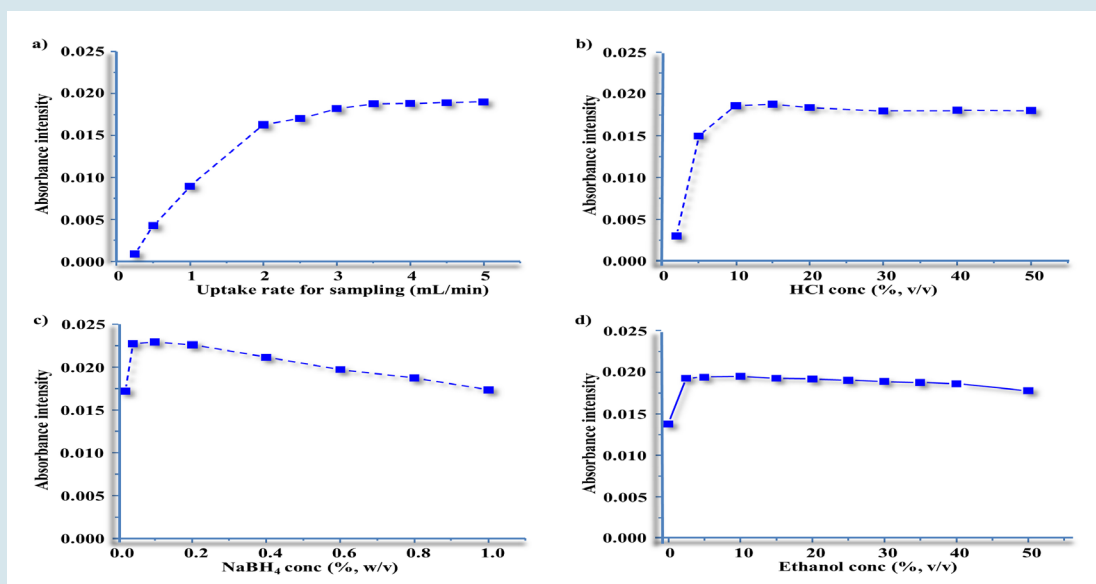
**Table 2: Results of assessment the linearity of the FA-HG-FTIR system for the assay method of antimony employing the addition standard technique**

Curve type	Linear regression: $Y = a + b [X]$ ; where $[X]$ = antimony concentration in µg/mL			
	$a \pm SD ; (x10^{-4})$	$b \pm SD ; (x10^{-3})$	Corr coefficient (r)	SD(y/x)
External (Reference standard)	$-1.1 \pm 0.4$	$1.258 \pm 0.004$	0.999 98	0.000 06
Standard addition 1 ( <i>Apis-Homaccord</i> )	$65.8 \pm 0.5$	$1.264 \pm 0.007$	0.999 97	0.000 06
Standard addition 2 ( <i>Tartephedree</i> )	$9.7 \pm 0.1$	$1.21 \pm 0.03$	0.999 47	0.000 12

**Table 3: Recovery of antimony from antihomotoxic samples with known concentration using the proposed FA-HG-FTIR method**

Type of brand	Sample N°	Concentration of Sb (µg/mL)*			Recovery (%)	Mean recovery (%)	Acceptable recovery (%)
		Measured	Added	Found			
<i>Apis-Homaccord</i>	1	5.30	4.0	9.11	98.0	$99.0 \pm 1.0$	90 - 107**
	2		8.0	13.17	99.0		
	3		12.0	17.28	99.9		
<i>Tartephedree</i>	1	0.92	2.0	2.66	91.0	$94.4 \pm 2.9$	80 - 110***
	2		4.0	4.74	96.4		
	3		6.0	6.62	95.7		

\*  $n = 3$ . \*\* Acceptable recovery over a range of 100 µg/mL - 10 µg/mL of the active ingredient. \*\*\* Acceptable recovery over a range of 10 µg/mL - 1 µg/mL of the active ingredient.<sup>27</sup>



**Figure 5:** Response signal vs. experimental variable. The response signal belongs to the corrected height of the selected FTIR spectral band using a 15 µg/mL Sb(III) standard.

previously.<sup>29</sup> This spectral band has been recognized as characteristic of Sb-H and has been previously used for analytical purposes in a gaseous phase.<sup>23</sup>

Formation of the hydride of antimony by reaction with NaBH<sub>4</sub> affords a suitable method for the separation of this element as gas from a wide range of matrices. The gas-phase removes most of the components of the matrix which are not gaseous, which in turn might absorb in the region of interest. Furthermore, nonspecific background interference and baseline shift were automatically corrected by baseline correction in order to improve the accuracy of quantification. Related gaseous species such as As, Se, Sn, Pb, among others, show absorption bands at different wavenumbers. Therefore, this spectral behavior provides some grade of specificity.<sup>23</sup>

On the other hand, under the conditions of the proposed method, it is likely that potential interferences should not be present at significant detection levels considering the nature of the samples.<sup>22</sup> If the interferences should be present, then one would expect that the spectral record would convincingly demonstrate it. This fact has been verified by our research group.<sup>7,23,30</sup>

Furthermore, the visual spectral evaluation showed similar band shapes for all the samples and were also quite similar to the standards (Figure 4). Supplementary evaluation by derivative spectroscopy was allowed, demonstrating that the spectral region, selected for analytical purposes, belonged only to the antimony ingredient of the antihomotoxic preparations.

The standard addition technique did not evidence a matrix effect. The obtained slopes were quite similar (Table 2). It is important to remember, as referred to above, that the analyte is extracted from the excipient using HG. This chemical mode is a gas phase sample introduction technique which offers the advantage of separating the analyte from the matrix and consequently ensuring an ideal quantification of the analyte.

Although the antihomotoxic products claim that the source of antimony is the Sb(III) state, the other oxidation state, Sb(V), could be present in the referred antihomotoxic preparations due to oxidation effect. In spite of information on the readily oxidation of Sb(III) to Sb(V), in our

experience it has not been observed. Today, it is known that insignificant Sb(III) oxidation by O<sub>2</sub> in homogeneous aqueous solutions, can be observed between pH 3.6 and 9.8 within 200 days.<sup>31</sup> In addition, the tartrate anion avoids oxidation of Sb(III).<sup>32,33</sup>

The rate of stibine generation from Sb(V) is comparatively much slower than from Sb(III).<sup>30,34</sup> which in turn, at low concentration, should not interfere in the determination of Sb(III). Nevertheless, if an analyst wishes to report the results as total antimony, it is possible to use common pre-reducing agents such as KI or KI-ascorbic acid to reduce Sb(V) to Sb(III). In this context, a pre-reduction step was incorporated in the present proposed method in order to ensure total antimony determination. Using the corresponding new background, no evidence was found of the presence of Sb(V) in the antihomotoxic products. Alternatively, if an analyst wishes to report the results as Sb(III), the addition of fluoride suppresses the stibine generation from Sb(V), but not from Sb(III),<sup>35</sup> a chemical behavior that has been recently proved by our research team (unpublished results).

#### Robustness and system suitability test (SST)

In this particular case, Figure 5 shows some of the factors required for the analysis in order to guarantee an acceptable SST. The RSD values of the studied variables were found to be < 5%, demonstrating that parameters which could affect the developed method did not have a significant effect on the analytical response.

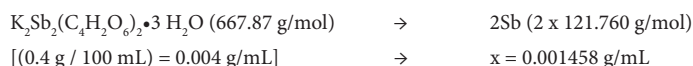
The commercial HG system (VGA 77) employs continuous flow technology with a fixed uptake rate through the sample and reagents pump tubes. In this regard, only the uptake rate for sampling was examined in order to ensure good overall performance. The experimental data demonstrated that an uptake rate of 4.0 mL was enough to ensure the highest analytical signal (Figure 5a), in this case, using an uptake rate of 1.0 mL for both acid and reductant. The uptake rate for each of the reagent pump tubes should be within the range 0.8 mL/min - 1.2 mL/min. Additionally, the concentration of the main involved reagents was evaluated keeping the flow rate constant for the continuous hydride generation, as was referred above. A variability of HCl concentration in the range

**Table 4: Antimony determination in homeopathic and antihomotoxic commercial products**

Product (dosage forms)	Product Composition	Antimony concentration (µg/mL)	
		Theoretical	Found
<i>Apis-Homaccord</i> , Heel GmbH (Baden-Baden, Germany). Available format: 30 mL oral drops	Each 100 mL contains: kalium stibyltartaricum (D2, D10, D30, D200) 0.4 g each, <i>Scilla</i> (D2, D10, D30) 0.25 g each, <i>Apis mellifica</i> (D2, D10, D30, D200, D1000) 0.1 g each, <i>Apisinum</i> (D6, D30) 0.25 g each, ethanol 35 % (v/v) and purified water	14.58 **	13.5 (± 0.1)
<i>Apis-Homaccord</i> , Heel GmbH (Baden-Baden, Germany). Available format: 1.1 mL drinkable ampoules	One drinkable ampoule contains: kalium stibyltartaricum (D4, D10, D30, D200) 4.4 mg each, <i>Scilla</i> (D4, D10, D30) 2.75 mg each, <i>Apis mellifica</i> (D4, D10, D30, D200, D1000) 1.1 mg each, <i>Apisinum</i> (D8, D30) 2.75 mg each, sodium chloride and purified water	0.15	< LOQ
<i>Tartephedreel</i> , Heel GmbH (Baden-Baden, Germany). Available format: 30 mL oral drops	Each 100 mL contains: <i>Illicium anisatum</i> D3 5 g, <i>belladonna</i> D4 10 g, kalium stibyltartaricum D4 10 g, natrium sulfuricum D4 10 g, <i>ipecacuanha</i> D4 5 g, <i>Lobelia inflata</i> D4 5 g, arsenum iodatum D6 10 g, <i>Blatta orientalis</i> D6 5 g, naphthalinum D6 5 g, ethanol 35 % (v/v) and purified water	3.65	2.30 (± 0.04)
Generic preparation, Faculty of Pharmacy & Bioanalysis, University of Los Andes (Mérida, Venezuela).	Each 50 mL contains antimony potassium tartrate trihydrate D4 5 g, ethanol 35 % (v/v) and distilled water	3.00	2.80 (± 0.03)
Generic preparation, Faculty of Pharmacy & Bioanalysis, University of Los Andes (Mérida, Venezuela).	Each 100 mL contains antimony potassium tartrate trihydrate D2 10 g, ethanol 35 % (v/v) and distilled water	15.0	15.0 (± 0.1)
<i>Stodal</i> , Boiron Pharmaceutical Laboratories (Alcobendas, Madrid, Spain). Available format: syrup.	Each 100 g contains antimonium tartaricum 6CH, <i>Bryonia dioica</i> 3CH, <i>Coccus cacti</i> 3CH, <i>Drosera</i> TM, <i>Ipecacuanha</i> 3CH, <i>Pulsatilla</i> 6CH, <i>Rumex crispus</i> 6CH, <i>Spongiatosta</i> 3CH, <i>Sticta pulmonaria</i> 3CH. Other Ingredients: <i>Polygala senega</i> , <i>Tolu balsam</i> , sucrose, ethanol, caramel, benzoic acid, purified water.	High dilution***	< LOQ

\* Homeopaths employ the letter “D” to designate dilution by factors of 10. A D2 dose is the same as a 2X dose, or dilution to 1 part in 100.

\*\* Stoichiometric equivalence to antimony taking into account the German Homeopathic Pharmacopoeia (GHP),<sup>6</sup> where 1:99 means 1% of the mother tincture in the D2 dilution; and so on, for example: kalium stibyltartaricum D2 (0.4 g original crude dose):



This last mass value, 0.001458 g, is the amount of Sb contained in 100 mL of oral drops, consequently, it is equivalent to 14.58 µg/mL.

\*\*\* The original crude dose of antimonium tartaricum is diluted by a factor of 10<sup>12</sup> in a 6CH dose. Centesimal scale or “C dose” describes dilution by factors of 100. The H label means that the Hahnemann method is used. The Hahnemann method requires a new vial at each succussion step.

10% - 20% (v/v) guaranteed a coefficient of variation below 5%. Consequently, HCl concentration of 15% (v/v) was selected as the acid medium (Figure 5b). Unexpectedly, the selection of the NaBH<sub>4</sub> concentration was critical as long as an excess of reducing reagent was used (Figure 5c); more hydrogen generation resulted in decreased analyte signal probably due to further dilution of the analyte by the increased hydrogen generation. A variability of reductant concentration in the range 0.05%-0.2% (w/v) guaranteed a coefficient of variation below 5%. Therefore, 0.1% (w/v) was used as an acceptable reductant concentration. Additionally, it was also proved at the upper limit of the calibration curve.

The incorporation of the pre-reducing reagent (KI) in conjunction with the HCl solution, at 10% ± 5% (w/v) was carried out only with preventive purposes since the presence of Sb(V) was not detected. It is important to point out that the incorporation of KI required the register of a new background for each new KI concentration in order to obtain reliable results.

The variability of the hydroalcoholic concentration was not critical; a variability of ethanol concentration within the range of 5% to 50% (v/v) originated a coefficient of variation of 0.75% (Figure 5d). Therefore, a hydroalcoholic concentration equal to the real sample matrix (35%, v/v) was selected for further studies. Keeping in mind the nature of the dissolvent, the analysis of untreated aqueous samples (non hydroalcoholic)

required a new background. Nevertheless, none of the aqueous samples showed detectable antimony concentrations. In any case, there will always be the option of using a hydroalcoholic solution for those aqueous samples with a significant concentration of antimony.

FTIR analytical procedures are usually protected from changes in humidity and temperature. As such, they were kept constant throughout method development. Samples were propelled directly from the commercial antihomotoxic products. In this regard, signal-response variation due to sample manipulation was reduced to zero. Additionally, the evolved analyte from the sample solution was passed through the gas-cell interior for at least 1.5 min (4.0 mL min<sup>-1</sup>). An elapsed time of 2.5 min was kept constant in order to ensure reliable results when moving from a high to a low-level standard avoiding memory effect. Coefficient of variation due to sample carryover by filling the cell with a high standard concentration, followed by a blank solvent injection, was relatively low (< 1% RSD, n=3).

Regarding robustness, parameters such as reagent concentration, flow rate, instrument conditions and precision were discussed above. The obtained results demonstrated that the parameters that could cause significant effects on the analytical response were kept either within acceptable ranges or acceptance criteria.



### Accuracy

The accuracy results for mean concentration recovery meet the acceptance criterion of  $100.0 \pm 2.0\%$  for an assay method (FDA) at each concentration over the range 80-120% of the target concentration for the product *Apis-Homaccord*. The accuracy results for mean concentration recovery did not meet the referred acceptance criterion for the product *Tartephedreel*. However, the expected recovery for analytes at low concentrations is different from current good manufacturing practices taking into account two other factors, the sample matrix and the sample processing procedure. In this regard, the AOAC manual includes a table with estimated recovery data as a function of analyte concentration, 80-110% mean recovery for 1-10 µg/mL active ingredient, a range in which the *Tartephedreel* product fits perfectly.<sup>27</sup> Recovery of antimony from antihomotoxic samples with known concentration using the proposed FA-HG-FTIR method is shown in Table 3.

### Analysis of commercial samples

The developed FA-HG-FTIR method was applied to the determination of Sb(III) in antihomotoxic products, a class of homeopathic preparations containing antimony potassium tartrate among other active components as stated by the manufacturer (Table 4). The results of the present method showed good agreement with the theoretically estimated values following the principle of decimal dilutions used in homeopathic medicine.<sup>6</sup> In order to guarantee the accuracy, the antihomotoxic products were also spiked with a known Sb(III) content and a recovery factor was calculated and found to be within acceptance criteria.

Taking into account the difficulty of understanding the way of expressing the nominal concentration of homeopathic and antihomotoxic preparations, further information is given below following some guidelines for reporting experiments in homeopathic basic research.<sup>6</sup> In this sense, a comprehensive description of what exactly was done in order to determine the nominal concentration of the target analyte is described for one of the commercial analyzed products according to the claimed composition (Table 4).

### DISCUSSION

In this work, the feasibility of using continuous flow HG in conjunction with FTIR detection for determining antimony in homeopathic products was evaluated. The purpose was to develop a new method to specifically control antihomotoxic medicines that could constitute potential safety hazards due to high antimony content.

In addition to the use of traditional pharmaceutical products, there is a large market for homeopathic products around the world. Since homeopathic medicines are typically administered at a very high dilution, with ingredients that may not even be detectable or measurable in the final products, homeopathic medicines are often thought to present no major safety concerns.<sup>6</sup> Nevertheless, this inference is not entirely true because not all homeopathic medicines are administered at a very high dilution. There is a class of homeopathic products known as antihomotoxic products, generally not understood by consumers, made from source material, such as a mother tincture, that is administered in a much more concentrated form. Consequently, manufacturing of these types of homeopathic medicines could constitute potential safety hazards.

In this context, few years ago, the World Health Organization (WHO) published a technical document encouraging national regulatory authorities to ensure that homeopathic medicines meet the minimum standards that guarantee safety and high quality. In this publication, they pay special attention to finished products, stating that "homeopathic dosage forms in the final products should comply with pharmacopoeial requirements and should be tested to determine the following: identity

and content".<sup>6</sup>

Therefore, the proposed non-compendial analytical procedure based on transmission FTIR in the gas phase (HG) fulfilled the above requirement for quality control of both incoming raw materials and finished products containing antimony potassium tartrate at levels of antimony higher than 0.5 µg/mL. Additionally, by detecting different spectral bands, this study opens up the possibility of identifying toxic elements potentially present in the material under evaluation, such as As, Pb, and Hg, elements that may be added through material processing or storage.

The proposed method was validated and most of the analytical figures of merit confirmed to be suitable for the intended purpose. The present method is more practical than the previously developed HG-GPMAS method because the high ethanol content of the antihomotoxic medicines does not cause spectral interference, and consequently, derivative spectroscopy is not required.<sup>7</sup>

While some authors might suggest the major weakness of this method is low sensitivity (µg/mL working range) as compared to many available methods (based on AAS or ICP), which have a working range below this amount, this disadvantage can be remedied by following the incorporation of either a pre-concentration step or by changing the gaseous cell for one of long pathlength.<sup>7</sup> Critically speaking, these approaches are unnecessary because the objective for quality control is simply to determine antimony content in antihomotoxic products that might be higher than is safely accepted, thus preventing possible toxic effects.

Antimony concentrations in two analyzed commercial antihomotoxic products were somewhat higher than drinking water regulations by the WHO (up to 0.020 µg/mL) but less than the tolerable daily intake (TDI) proposed by the same organization (6 µg antimony per kg body weight; i.e.: a TDI of 360 µg antimony per 60 kg body weight).<sup>36</sup> Therefore, from a quantitative point of view, the present work represents a real alternative for determining antimony content in antihomotoxic products that might represent a risk for patients.

### CONCLUSIONS

The toxic effects of antimony are not comparable to arsenic, but not without major safety concerns. Therefore, antimony at relatively high concentrations must be monitored and controlled in raw materials for antihomotoxic manufacturing and in antihomotoxic finished products. The proposed new methodology for the analysis of homeopathic (antihomotoxic) medicines in the present work provides an alternative for homeopathical laboratories. As is well known, the available pharmacopoeial assays for antimony potassium tartrate are inappropriate for analysis of homotoxicological remedies because chemical-based methodologies are unspecific and the limits of quantification are in the order of mg/mL instead of µg/mL. On the other hand, the transition from a chemical to an instrument-based methodology, atomic spectroscopy and ICP spectrometry is not yet widely applied in the pharmaceutical industry. Conversely, FTIR spectrometry is commonly used in both the aforementioned industry as well as the homeopathic one. The proposed transmission FTIR method in gaseous phase provides an acceptable analytical capability for homeopathical compendiums concerning antimony determination, with an adequate limit of quantification and specificity. This hyphenated technique combines a separation technique and a spectral method to exploit the advantages of both.<sup>37</sup> The incorporation of the continuous HG introduces an effective interference removal and spectroscopy can be used for both quantitative and qualitative analysis.

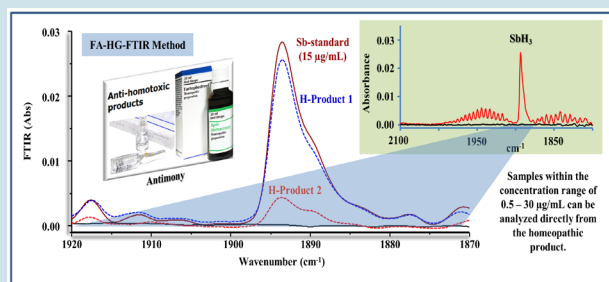
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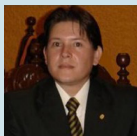
## PICTORIAL ABSTRACT



## SUMMARY

- The present work represents an alternative for determining potassium antimony tartrate content in antihomotoxic products that might be higher than is safely accepted, thus preventing possible toxic effects.
- The proposed transmission FTIR method in gaseous phase provides an acceptable analytical capability for homeopathical compendiums, not only for analyzing raw materials and mother tinctures but also for determining identity and uniformity content of finished products which should comply with pharmacopoeial specifications.
- Furthermore, the incorporation of the continuous flow hydride generation introduces an effective interference removal.

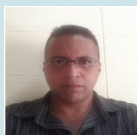
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