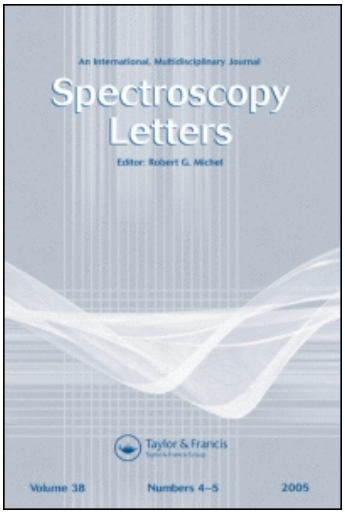

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Merging Zones FIA System for the Spectrophotometric Determination of Sb(III) and Total Sb in Drugs Used in the Treatment of Leishmaniasis

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Merging Zones FIA System for the Spectrophotometric Determination of Sb(III) and Total Sb in Drugs Used in the Treatment of Leishmaniasis

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Abstract: A merging zones flow injection system was developed for the spectrophotometric determination of Sb(III) and Sb(V) in drugs used in the treatment of leishmaniasis. The procedure is based on the selective reaction between Sb(III) and bromopyrogallol red (BPR) with the decrease of the absorbance measured at 555 nm. The concentration of Sb(V) was calculated by difference after determination of total antimony. Influences of chemical and flow parameters such as pH of reaction, BPR concentration, surfactant concentration, carrier and reagent flow rates, loop volume, and coil reactor length were critically evaluated. Selectivity of the method and the suitable conditions for sample preparation before injection were investigated. The obtained results demonstrated that the addition of Triton X-100 enhanced the sensitivity of the method due to acceleration of the chromogenic reaction. Working on optimized conditions, the detection (3σ) and quantification (10σ) limits were 30 ng mL^{-1} and 100 ng mL^{-1}, respectively. The RSD observed at 0.25 μg mL^{-1} was 4.0% and the analytical throughput was 90 hr^{-1}. Three samples of commercial drugs were analyzed according to the developed procedure not presenting any

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statistical difference in relation to the results obtained by FAAS for total antimony concentration.

Keywords: Antimony, flow injection analysis, leishmaniasis, spectrophotometry

INTRODUCTION

Currently, leishmaniasis is distributed all over the world. It is considered an endemic disease in 88 countries, and 72 of these are developing countries. According to WHO (World Health Organization), around 90% of the new cases are registered in poor countries and special incidence is verified in Brazil, where around 67,000 new cases were detected in 2001 and 2002.[1]

Regular treatment of leishmaniasis is done with antimony-based drugs, in spite of the fact that new substances free of Sb are in development for this task.[2–5] The first antimony compound used for the treatment of leishmaniasis was the antimony and potassium tartrate. However, serious collateral effects such as gastrointestinal irritation and heart diseases were observed in most patients. Such effects were associated with the presence of Sb(III) in the formulations, which led to its replacement by Sb(V).[6]

Among all antimony-based drugs commercially available, perhaps the most common is that prepared with meglumine antimoniate. This compound presents Sb in the pentavalent form but little Sb(III) can appear due to reduction of Sb(V) during storage. In this context, the determination of the concentrations of Sb(III) and Sb(V) in the drugs used in the treatment of leishmaniasis is a very important part of their quality control due to possible deleterious effect of the drug supplied to leishmaniasis patients.

Diverse analytical techniques such as potentiometry,[7–9] voltammetry,[10–15] spectrophotometry,[16–21] ICP-MS[22] and hydride generation coupled to atomic fluorescence,[23–26] ICP-OES,[27–29] AAS,[30] and FTIR[31] have been applied for Sb determination in different kinds of samples.

In terms of antimony determination and/or speciation in pharmaceuticals used for leishmaniasis treatment, Gallignani et al.[31] developed a FIA system coupled to HG-FTIR to determine total Sb. In the method, the samples are treated online in order to release antimony from organic molecules of the drug. Sb(V) released is reduced to Sb(III) for stibine generation and detection at 1893 cm^{-1}. At optimized conditions, a detection limit of 0.9 mg L^{-1} and an analytical throughput of 28 hr^{-1} were obtained.

Flores et al.[30] explored one of the most popular techniques used in the Sb speciation, the hydride generation–atomic absorption spectrometry. The procedure was based on the selective hydride generation from Sb(III) in presence of citric acid, added to inhibit the formation of hydride from Sb(V). Total antimony was determined after reaction of all Sb(V) with a suitable reducing agent. Operating at optimum conditions, the method presented a detection limit of 1.5 ng of antimony, demanding large dilution

for analysis of real samples because the amount of antimony found was in the range 83.5–110.3 mg mL^{-1}.

The group of Rath et al. employed both bromopyrogallol red (BPR)[19] and rhodamine B[11] in the development of spectrophotometric methods for Sb determination in antileishmanial drugs. The first paper proposed the use of BPR, in a batch mode, for antimony speciation (determination of tri and pentavalent forms) while in the second work a FIA system was developed for liquid–liquid extraction of Sb(V)–rhodamine B complex aiming at the determination of Sb(V) in the drugs. In turn, Bloomfield et al.[18] performed the flow injection spectrophotometric determination of only Sb(V) in pharmaceutical formulations containing sodium stibogluconate as active drug. They monitored (at 350 nm) the formation of iodine after reaction between iodide and Sb(V).

Recently, Figueiredo et al.[21] developed a flow injection spectrophotometric methodology for Sb(III) and total antimony determination in pharmaceutical samples. The method was based on the SbH_3 generation and its posterior reaction with $KMnO_4$ solution, which has color intensity diminished due to reduction by stibine.

The aim of this work was to develop a merging zones FIA system for the detection of Sb(III) in presence of large amounts of Sb(V) in order to achieve the fast speciation of Sb in drugs used in the treatment of leishmaniasis. The reaction between Sb(III) ions and BPR in an organized medium containing Triton X-100 was explored for spectrophotometric detection, and suitable conditions for sample preparation were evaluated to promote Sb(V) to Sb(III) reduction. A merging zones FIA approach was employed for reagent saving and due to the high initial absorbance verified for BPR solutions, as the method is based on the decrease of absorbance at 555 nm of the complex Sb(III)–BPR in relation to BPR alone.

MATERIALS AND METHODS

Apparatus

Spectra obtained in the pH study were recorded with a Femto 800 xi (São Paulo, Brazil) UV-visible scanning spectrophotometer employing a resolution of 5 nm and a cuvette of 10 mm optical path.

Flow manifold was mounted with a Femto 600 Plus visible spectrophotometer equipped with a standard Hellma (Jamaica, NY, USA) flow-cell of 80-μL internal volume and 10-mm optical path. The wavelength was adjusted at 555 nm for absorbance measurements. A peristaltic pump from Micronal (São Paulo, Brazil), model B-332, equipped with flexible polyvinyl chloride tubes (Tygon), was employed to propel the solutions, and a lab-made proportional commutator[32] was used provide simultaneous injection of the mixture of BPR and Triton X-100 and samples into the system. Manifold lines were built up with PTFE tubes with 0.8-mm bore

and connections made of glass were used. All pH measurements were carried out in an Analyzer 300 pH meter equipped with a combined glass electrode (Ag/AgCl with a saturated solution of KCl as reference).

A Perkin-Elmer (Norwalk, CT, USA) AAnalyst 100 flame atomic absorption spectrometer equipped with an antimony hollow cathode lamp was employed for the determination of total antimony in the samples. It was operated at conditions suggested by the manufacturer (lean blue air–acetylene flame, wavelength = 217.6 nm and a slit width = 0.2 nm).

Reagents and Solutions

Purified water obtained in a Simplicity Milli-Q (Millipore, Saint Quentin Yvelines, France) water purification system was used to prepare all solutions. Analytical grade reagents were used without further purification.

Antimony (III) solutions were prepared daily by suitable dilution of a 1000 mg L^{-1} Sb(III) stock standard solution that was obtained by dissolution of 2.8082 g of potassium and antimony tartarate ($K(SbO)C_4H_4O_6 \cdot 0.5H_2O$) with 50 mL of a 2 mol L^{-1} HCl solution and posterior dilution to 1000 mL in a volumetric flask.

Antimony (V) standard solutions were prepared by dilution of a 1000 mg L^{-1} stock solution prepared by dissolution of a 2.1592 g of $KSb(OH)_6$ in around 250 mL of purified water under heating of 60°C. After total dissolution of the solid, the solution was left to achieve ambient temperature and the volume was made up to 1000 mL in a volumetric flask.

A concentrated buffer solution of NaH_2PO_4/Na_2HPO_4 with pH 6.8 (total concentration 1 mol^{-1}) was prepared by dissolving 15 g of NaH_2PO_4 (Vetec, Rio de Janeiro, Brazil) and 17.7 g of Na_2HPO_4 (Vetec) in around 200 mL of purified water. The pH was then adjusted to 6.8 with solid NaOH and the volume was completed to 250 mL. The carrier solutions (C_1 and C_2) used in the FIA system were prepared with this concentrated buffer solution, taking 1 mL of it and completing the volume to 100 mL.

An 0.2 g L^{-1} BPR solution was prepared by dissolving 40 mg of BPR (Acros, Morris Plains, NJ, USA) in exactly 200 mL of 0.01 mol L^{-1} phosphate buffer solution. This solution was stored in a dark flask and maintained in the dark. Under these conditions, the solution remains stable at least for 2 weeks.

A 2.5 g L^{-1} Triton X-100 solution was prepared by mixing 0.25 g of Triton X-100 (Vetec) with around 80 mL of purified water. After obtaining a homogeneous solution, the volume was filled up to 100 mL in a volumetric flask.

A 2% w/v KI reductant solution was prepared by dissolving 0.5 g of KI (Vetec) in 25 mL of purified water. This solution was prepared daily just before use.

A 5% w/v ascorbic acid reductant solution was prepared by dissolving 1.25 g of ascorbic acid (Vetec) in 25 mL of purified water. This solution was also prepared daily just before use.

A 4 mol L^{-1} HCl solution was prepared by taking 34 mL of concentrated HCl (Vetec) and diluting this volume to 100 mL with purified water. In order to follow safe conditions, the volume of concentrated HCl was first added on 50 mL of water, in a beaker, and then the solution obtained was transferred to the volumetric flask and the volume was completed to 100 mL.

Commercial samples of injectable drugs of meglumine antimoniate were analyzed in this work. Such samples were obtained in ampoules of 5 mL.

Sample Preparation

For Sb(III) determination, 100 μL of sample was sonicated for 10 min and then diluted to 100 mL with water in a volumetric flask. A volume of 5 mL of the resultant solution was taken and mixed with 1 mL of the 1 mol L^{-1} phosphate buffer solution. Then the volume was completed to 100 mL in a volumetric flask and this final solution was inserted into the FIA system.

For the determination of total antimony concentration, 100 μL of sample was sonicated for 10 min. Afterwards, 2 mL of 2% w/v KI and 2 mL of 5% w/v ascorbic acid solutions were added together with 500 μL of 4 mol L^{-1} HCl solution. The mixture was left to stand for 15 min and then the volume was completed to 100 mL. A volume of 5 mL of this solution was transferred to a 100-mL volumetric flask, 1 mL of the 1 mol L^{-1} phosphate buffer solution was added, and the volume was filled up to the mark. This final solution was inserted into the FIA system.

FIA System Operation

In the developed merging zones FIA system, depicted in Fig. 1, the reagent solution of BPR (0.020% w/v, 0.75 mL min^{-1}) merges with the Triton X-100 solution (0.25% w/v, 0.75 mL min^{-1}) and the resultant mixture fills the reagents loop L_1 (600 μL). The excess of the mixture is discarded, being pumped to the waste flask. At the same time, sample (or standard) solution fills the loop L_2 (600 μL). With the injector at this position, the baseline is established by passing the carrier stream (phosphate buffer solution, 0.01 mol L^{-1}, pH 6.8, 2.35 mL min^{-1} each one) through the flow cell. Changing the position of the injector, both sample and mixture of reagents are simultaneously propelled by their carrier streams until the confluence point *x*. Due to the same flow rate of C_1 and C_2 and the same length of the lines, both zones reach the confluence point at the same time, creating a synchronous dispersed zone that is driven to the reaction coil (B_2, 25 cm) where reaction between Sb(III) and BPR takes place. Afterwards, the sampling zone reaches the spectrophotometer yielding a transient signal of absorbance, which decreases with the increase of the Sb(III) concentration in the sample. Peak height was used as quantitative variable.

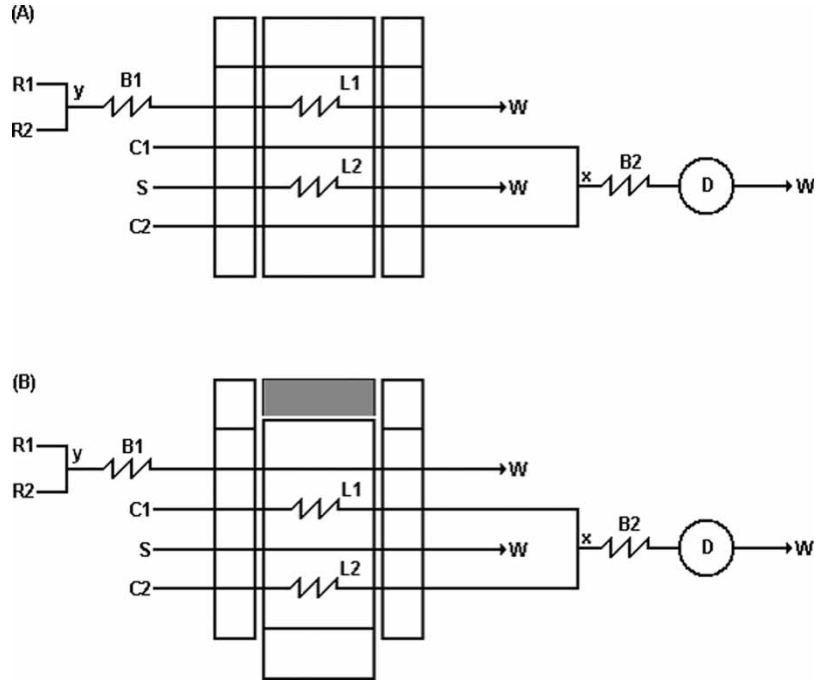

Figure 1. Merging zones FIA manifold for the spectrophotometric speciation of Sb (Sb(III)/Sb(V)) in drugs used in the treatment of leishmaniasis. S, sample (pH 6.8,); C_1 and C_2, carrier solutions (0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 buffer with pH 6.8, 1.48 mL min^{-1}); R_1, reagent solution (0.025% w/v BPR, pH 6.8, 0.98 mL min^{-1}); R_2, surfactant solution (0.25% w/v Triton X-100, 0.98 mL min^{-1}); B_1, mixing coil (600 μL); B_2, reaction coil (25 cm); L_1 and L_2, reagents and sample loops (600 μL), respectively; *x* and *y*, confluence points; P, peristaltic pump; D, detector (spectrophotometer at 555 nm); W, waste. (A) Loading position and (B) injection position. Distance from injector to point *x* is 20 cm.

RESULTS AND DISCUSSION

In order to establish best conditions for flow system operation, in terms of sensitivity and selectivity, the influences of chemical and flow variables that could affect it were examined by univariate method. Also, a study about the selectivity of the methodology for Sb(III) measurement in presence of high amounts of Sb(V) was performed as well as the establishment of the suitable conditions for sample preparation.

Evaluation of Chemical Parameters

The first parameter investigated was the pH of the reaction between BPR and Sb(III). According to the literature, BPR and Sb(III) can react at neutral or

acidic range. Rath et al.[19] reported the formation of a 1:1 complex at pH 6.8, while Huang et al.[17] explored the same reaction, in micellar medium, at pH 2.0 and 80°C. Taking these data into account, a study was performed in order to establish the suitable pH for the reaction. For this task, solutions with only BPR and solutions with BPR + 1 μg mL^{-1} Sb(III) were prepared at pH 2.0 (HCl buffer), 4.3 (acetate buffer), and 6.8 (phosphate buffer) and the spectrum of each one was recorded in the range 390–780 nm. Figure 2 shows the spectra of differences between solutions with and without Sb(III). Highest differences were observed at pH 6.8, indicating that at this condition better sensitivity can be obtained in the Sb(III) measurement. Also, the wavelength chosen for the method was 555 nm because at this point highest decrease of the absorbance was verified.

Once establishing the pH of the reaction, the merging zones FIA system was built up and the effects of other variables were evaluated. The influence of BPR concentration was examined in the range 0.005–0.035% w/v. All BPR solutions tested were prepared in solution with pH 6.8, adjusted with phosphate buffer with total concentration of 0.01 mol L^{-1} in the same way as carrier solutions and containing a Triton X-100 concentration of 2.5 g L^{-1}. The analytical signals (difference between absorbances of blank and Sb(III) solutions) increased up to BPR concentration of 0.020% w/v, and after this point, a slight decrease was noted (Fig. 3). Also, it is important to remark that the baseline noise increased remarkably when BPR

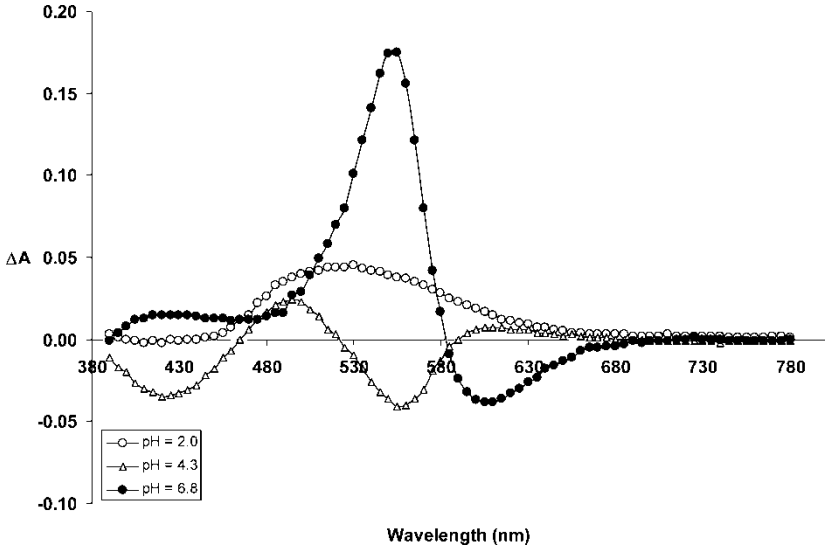

Figure 2. Spectra of difference between BPR and BPR + Sb(III) solutions at different pH. Sb(III) = 1 μg mL^{-1}.

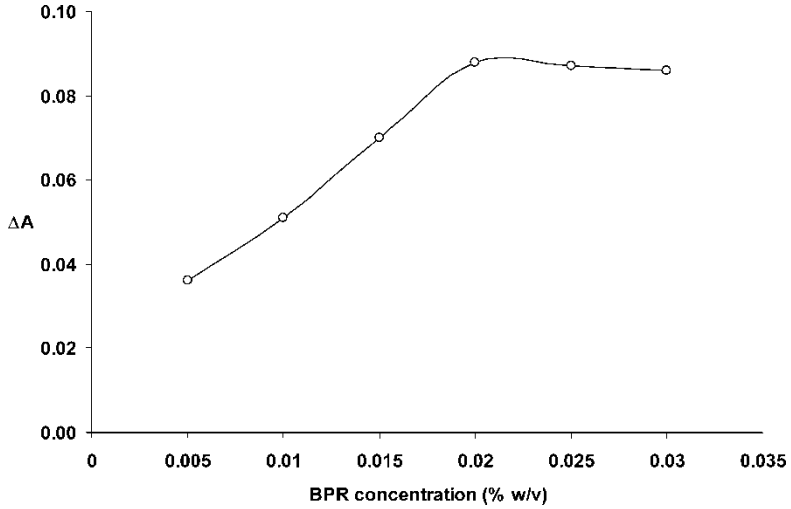

Figure 3. Effect of BPR concentration on Sb(III) signal. Sb(III) = 1 μg mL^{-1}.

concentration was higher than 0.025% w/v, probably due to the high absorbance recorded, which caused problems in the detector operation.

In order to evaluate the influence of micellar medium on the analytical signal, the concentration and nature of surfactant reagent was investigated.

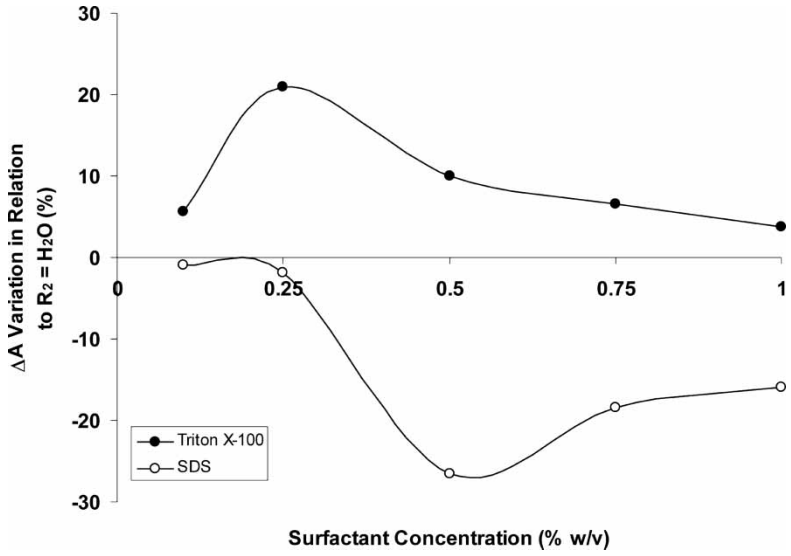

Figure 4. Effect of Triton X-100 and SDS concentrations on Sb(III) signal. Sb(III) = 1 μg mL^{-1}.

Both Triton X-100 and sodium dodecylsulfate (SDS) were tested as possible surfactants for the system in the range 0.1–1% w/v. The results are expressed in Fig. 4 as variation of the signal in relation to the use of water as R_2. As can be seen, in general, the use of Triton X-100 enhanced the analytical signal while the use of SDS in every concentration caused a decrease of signal. In terms of concentration, maximum enhancement (21%) of the analytical signal was observed for Triton X-100 solution with 0.25% w/v concentration, which was established for all further experiments. In fact, the use of Triton X-100 seems to improve the signal by acceleration of the reaction between BPR and Sb(III), which makes higher amounts of BPR–Sb complex to be formed in the short space of time between confluence point *x* and the detector. The same effect was not observed when the reaction was conducted with and without Triton X-100 in an off-line mode, a situation where the system achieved the equilibrium before measurement thus producing maximum amounts of BPR–Sb complex, reinforcing the idea raised before.

Evaluation of Physical Parameters

The first physical parameter evaluated was the volume of the reagent and sample (L_1 and L_2). This parameter controls the amount of analyte inserted into the system and, in general, it presents noticeable effect on FIA systems sensitivity. Loops with volumes between 250 and 600 μL were tested. As can be seen in Fig. 5, a linear increase of the analytical signals was verified for volumes up to 600 μL. Although the results indicate that the analytical signal could be increased only by incrementing the volume of L_1 and L_2, loops with higher volumes were not tested in order to avoid longer sampling rate. It is important to remark that L_1 and L_2 volumes were always the same to provide a synchronous penetration of reagent and sample zones at confluence point *x*.

Both carrier flow rates and volume of reaction coil are responsible to set the residence time of dispersed zone inside the system, thus controlling the dispersion level of such zone and reaction time between Sb(III) and BPR. Therefore, the influences of both parameters on the sensitivity of the FIA system were carefully studied. The influences of carrier flow rates (C_1 and C_2) were investigated in the range 0.98–4.14 mL min^{-1}. Variation of this parameter did not cause remarkable influence on the analytical signal, only a sharp reduction of the analytical signal for flow rate values higher than 1.48 mL min^{-1} being observed. Therefore, this flow rate was chosen for each carrier solution in order to obtain higher analytical throughput for the system, as at this condition the time required to complete the transient analytical signal was lower. It must be highlighted that C_1 and C_2 flow rates were the same also to ensure a synchronous penetration of reagent and sample zones at confluence point *x*. This way, a total carrier flow rate of 2.96 mL min^{-1} was responsible for the transportation of the dispersed zone.

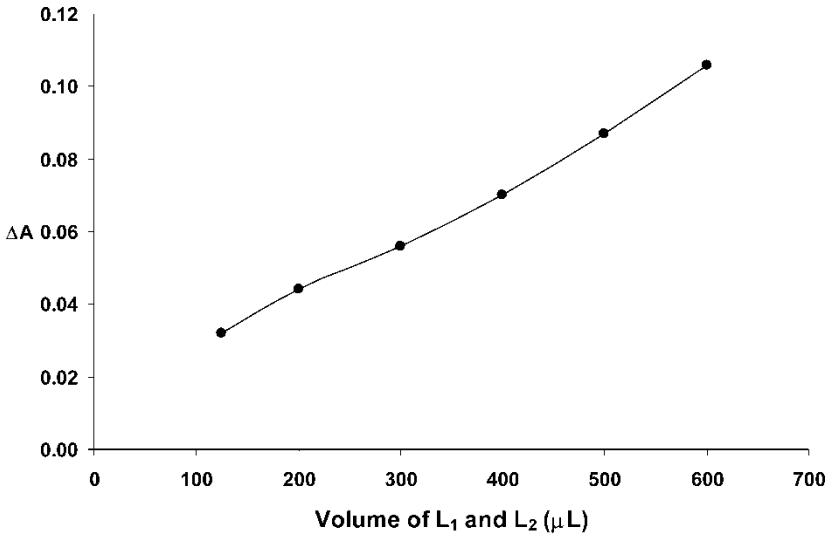

Figure 5. Effect of the volume of reagent mixture and sample inserted in the FIA system on Sb(III) signal. Sb(III) = 1 μg mL^{-1}.

As mentioned before, the length of reaction coil (B_2) plays a similar role as carrier flow rate, influencing the residence time of the analytical zone inside the system, which affects the magnitude of the analytical signal. This way, the effect of the reaction coil was studied in the range of 0 (no coil) to 100 cm. Higher signals were obtained when a coil with 25-cm volume was connected to the system. At this condition, no disturbance of baseline was noted, indicating that a satisfactory mixture of solutions is achieved.

Evaluation of the Effect of Sb(V) on Sb(III) Signal

The whole optimization of the system was performed with Sb(III) solutions, always taking into account that BPR does not react appreciably with Sb(V) ions at pH 6.8. Nevertheless, in the real samples, the concentration of Sb(V) is typically 50 to 100 times higher than Sb(III), which is considered a contaminant in the drugs used in the treatment of leishmaniasis. Hence, a detailed study was done to evaluate the actual possibility of interference of Sb(V) on Sb(III) signal. For this purpose, two strategies were followed: (a) comparison between analytical curves for Sb(III) in presence and absence of Sb(V) and (b) influence of increasing concentrations of Sb(V) on constant Sb(III) concentration.

In the first study, two analytical curves were built up, one with solutions containing only Sb(III) (0.25 to 4 μg mL^{-1}) and other with solutions containing Sb(III) (same range) +25 μg mL^{-1} Sb(V). The equations that represent

such curves were $A = 0.102$ [Sb(III) ($\mu g\ mL^{-1}$)] -0.002 ($r = 0.996$) and $A = 0.100$ [Sb(III) ($\mu g\ mL^{-1}$)] $+ 0.001$ ($r = 0.998$), respectively. As can be noted, no remarkable difference between slopes or interceptions were verified between the two analytical curves, evidencing that 25 $\mu g\ mL^{-1}$ Sb(V) had no effect on Sb(III) measurement in the studied range.

In order to confirm that Sb(V) does not interfere on Sb(III) measurement even when found at concentrations as high as 100 times Sb(III), an experiment was performed by measuring solutions containing Sb(V) concentrations from 0 to 100 times higher than Sb(III), which was always maintained at 1 $\mu g\ mL^{-1}$. Differences always less than 3% were noted between the analytical signals obtained in all ratios tested, evidencing that it is possible to measure Sb(III) in the real samples even in presence of high amounts of Sb(V).

Other possible concomitants usually present in the medicine formulations such as EDTA, sodium bisulfite, and propylene glycol were tested as possible interferent but none of them presented any remarkable effect on Sb(III) signal.

Evaluation of Sample Preparation Conditions

After the FIA system was optimized for adequate Sb(III) measurement, a study about the sample preparation procedure for Sb speciation was carried out. This study was focused on the examination of better conditions (acidity and time) for chemical reduction of Sb(V) to Sb(III) with KI and ascorbic acid, in order to make possible the determination of total Sb in the samples, and to verify the effect of sample sonication before analysis. Such evaluations were performed employing a sample containing a known concentration of total Sb (78.5 mg mL^{-1}) previously determined by FAAS.

As mentioned before, reduction of Sb(V) to Sb(III) was carried out using KI (2 mL of a 5% w/v solution) and ascorbic acid as reducing agents. As is well-known, both species present more prominent reducing effect in acidic medium, a condition quite different from that established for the procedure, which was pH 6.8. This way, a careful evaluation of the amount of HCl added and the time required to accomplish the reduction was performed. The influence of the amount of HCl for reduction was studied by adding variable volumes (0–1000 μL) of a 4 mol L^{-1} HCl solution to the mixture of 100 μL of sample with 2 mL of a 2% w/v ascorbic acid and 2 mL of a 5% w/v KI solutions. The obtained solution was homogenized and left to react for 30 min. Then, the volume was completed to 100 mL, and 5 mL of this resultant solution was transferred to another 100-mL volumetric flask. The pH was adjusted to 6.8 with buffer solution, the volume was completed, and 600 μL was inserted into the FIA system. Recoveries higher than 95% were observed for volumes of HCl solution equal to or higher than 250 μL. So, in order to ensure that all Sb(V) present would be reduced

to Sb(III) even for samples containing high amounts of Sb, a volume of 500 μL of 4 mol L^{-1} HCl was chosen for the method. In this test, the sample was sonicated for 30 min before addition of ascorbic acid, KI, and HCl solutions in order to guarantee that all Sb was released from meglumine antimoniate structure.

The effect of the time required to accomplish the reduction process was studied in the range 0–20 min. This is the time that mixture (sample + ascorbic acid + KI + HCl) was left to react in order to promote reduction of all Sb(V) ions to Sb(III). As can be seen in Fig. 6, the profiles of the curves for the same experiment carried out on two different days were the same. From the obtained data, it is possible to realize that the total reduction of Sb(V) is only achieved after 15 min. So, this time was established for the method.

In the drugs under study, Sb is bonded to the structure of the meglumine antimoniate. In order to increase the efficiency of the reduction process and thus achieve satisfactory accuracy for the measurement of total Sb, it was necessary to release Sb from organic structure. The method used for this purpose was the sonication of sample before addition of reducing reagents. So, the sonication time required to achieve total release of Sb was studied in the range 0–30 min. Obtained results showed that the sonication of the sample was essential for total Sb determination in the samples, as when the sample was not sonicated Sb recovery was 85.5 ± 1.5%, while after 10 min of sonication the recovery increased to 101 ± 3%.

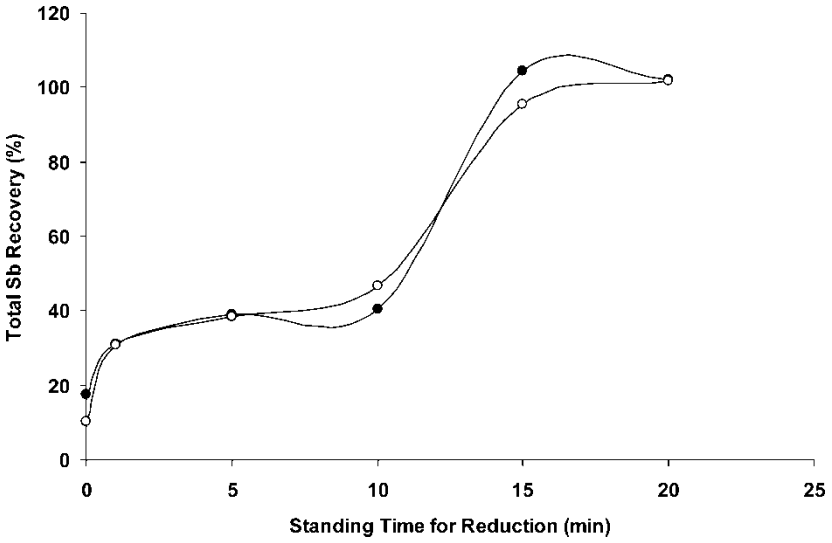

Figure 6. Effect of standing time for reduction on the recovery of total Sb. This test was performed with a sample containing 78.5 mg mL^{-1} total Sb determined by FAAS.

Method Evaluation

Analytical Characteristics

Under optimized conditions, the proposed methodology was able to produce analytical fits with good linearity in the range 0.25–4 $\mu g\ mL^{-1}$ with a typical equation $\Delta A = 0.105\ [Sb(III)\ (\mu g\ mL^{-1})] + 0.001$ and a correlation coefficient of 0.9991, where ΔA is the analytical signal obtained by the difference of absorbance between solutions with and without Sb(III). Detection limit (3σ criterion) and quantification limit (10σ criterion), derived from 10 measurements of blank solution, were 30 ng mL^{-1} and 100 ng mL^{-1}, respectively. A RSD of 4.0% at 0.25 $\mu g\ mL^{-1}$ was also derived from 10 measurements of the solution. A sampling frequency of 90 hr^{-1} was calculated, taking into account that a time required to complete an analytical cycle is the time passed between two injections. The amount and nature of the waste generated by the FIA system was critically evaluated. In the case, around 295 mL of effluent per hour of operation are wasted. The composition of such effluent is shown in Table 1, expressed in terms of the amount of each substance wasted.

Application

Three commercial samples of antileishmanial drugs were analyzed by the developed methodology in order to prove its applicability. Results were compared with those obtained by FAAS in terms of total Sb concentration. As can be seen in Table 2, the results obtained by the merging zones FIA system agreed statistically with those obtained by FAAS when applying a paired *t*-test at 95% confidence level. Calculated *t*-value was 0.47 while critical *t*-value is 4.30.[33] Once the accuracy of the merging zones FIA system was proved, it could be considered an excellent alternative to manual procedure, presenting higher analytical throughput and easiest operation.

Table 1. Amount of waste generated by merging zones FIA system per hour of operation

Reagent	Amount wasted per hour (mg)
BPR	11.8
Triton X-100	147
NaH_2PO_4	107
Na_2HPO_4	126

Table 2. Results obtained for the analysis of commercial samples of antileishmanial drugs (results expressed as mean ± standard deviation, n = 3)

Sample	Sb(III) (mg mL^{-1})	Sb(V)[a] (mg mL^{-1})	Sb total (mg mL^{-1})	Sb total[b] (mg mL^{-1})
I	2.50 ± 0.05	92.6 ± 4.9	95.1 ± 4.9	95.5 ± 3.5
II	2.95 ± 0.10	77.2 ± 4.5	80.1 ± 4.5	79.8 ± 2.3
III	2.30 ± 0.11	76.7 ± 3.2	79.0 ± 3.2	78.5 ± 1.9

[a]Standard deviation for Sb(V) was calculated as $S_{Sb(v)} = \sqrt{S^2_{Sb(III)} + S^2_{Sbtotal}}$. How $S_{Sb\ total}$ is much higher than $S_{Sb(III)}$ ($S^2_{Sb(III)} + S^2_{Sb\ total} \cong S^2_{Sb\ total}$): the standard deviations for Sb(V) measurements were the same verified for total Sb determination.

[b]Total antimony determined by FAAS.

CONCLUSIONS

Merging zones FIA system developed in this work was shown to be adequate for the detection of Sb(III) in antileishmanial drugs. When used after a suitable pretreatment of samples, such system was able to detect total Sb thus making possible the speciation analysis of antimony. Also, the methodology can be considered an excellent alternative to manual procedure, providing high productivity for this type of analysis with low waste generation. The detection limit achieved with the FIA system developed in this work was superior (30 ng mL^{-1}) to those reported in the literature by Rath[19] (0.20 μg mL^{-1}) and Huang[17] (0.04 μg mL^{-1}), which employed the same chromogenic reaction between Sb(III) and BPR.

The addition of Triton X-100 enhanced the sensitivity of the method due to its possible catalyst effect on the reaction between Sb(III) and BPR. Some experimental procedures such as previous sonication of samples for 10 min and the standing time spent (15 min) to complete Sb(V) to Sb(III) reduction were shown to be essential to achieve satisfactory accuracy for the analysis.

Finally, it is important to remark that the percentage of Sb(III), in relation to total antimony found in the samples, varied from 2.62% to 3.68%. These values are in agreement with others found in previous works, indicating that the FIA methodology can be applied in the quality control of drugs used in the treatment of leishmaniasis.

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