Multivariate optimization of a method for antimony determination by hydride generation atomic fluorescence spectrometry in hair samples of patients undergoing chemotherapy against *Leishmaniasis*

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ABSTRACT

A method was developed for determination of total antimony in hair samples from patients undergoing chemotherapy against *Leishmaniasis* based on the administration of pentavalent antimonial drugs. The method is based on microwave assisted digestion of the samples in a pressurized system, reduction of Sb^{5+} to Sb^{3+} with KI solution (10% w/v) in ascorbic acid (2%, w/v) and its subsequent determination by hydride generation atomic fluorescence spectrometry (HG-AFS). The proportions of each component (HCl, HNO_3 and water) used in the digestion were studied applying a constrained mixtures design. The optimal proportions found were 50% water, 25% HNO_3 and 25% HCl. Variables involved in the generation of antimony hydride were optimized using a Doehlert design revealing that good sensitivity is found when using 2.0% w/v $NaBH_4$ and 4.4 mol L^{-1} HCl. Under the optimum experimental conditions, the method allows the determination of antimony in hair samples with detection and quantification limits of 1.4 and 4.6 ng g^{-1}, respectively, and precision expressed as relative standard deviation (RSD) of 2.8% (n = 10 to 10.0 mg L^{-1}). The developed method was applied in the analysis of hair samples from patients who take medication against *Leishmaniasis*.

Key words: *Leishmaniasis*, antimony, hair, HG-AFS, multivariate optimization.

INTRODUCTION

Leishmaniasis is an infectious disease caused by parasites of the genus *Leishmania*, which can infect humans in the cutaneous and visceral (or kala-

azar) forms. It constitutes a public health problem considered by the World Health Organization (WHO) as one of the six most important infectious diseases that require greater attention by governments (Brasil 2007). The use of pentavalent antimony (such as N-methylglucamine antimoniate and sodium stibogluconate) for its treatment had its

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standardization determined by the WHO. Despite being among the agents considered toxic to human health and the potential severity of their features, antimonials are the first choice drugs for the treatment of *Leishmaniasis*. The amounts given are calculated as mg of Sb^{5+} $kg^{-1}day^{-1}$, so that the maximum permitted daily dose for adults is equivalent to 1215 mg Sb^{5+} day^{-1}. Their effects lead to question the harm arising from their accumulation in the human body, since they have proved to be a toxic agent (Brasil 2007, Fouce et al. 1998).

Among the several biological matrices used for the study of metal accumulation in the human body the capillary tissue stands out. Its analysis has been widely used as a tool for assessing risk exposures. The advantage of using this matrix can be noted for its ability to monitor elements accumulated over a period of time as well as its simplicity in relation to sampling, shipping, and handling (Violante et al. 2000, Forte et al. 2005).

Nowadays, different analytical methods are available for the measuring of antimony in several samples. The most often-used sensitive analytical techniques for this purpose are hydride generation coupled to: atomic absorption spectrometry (HG AAS) (Krachler et al. 2001), inductively coupled plasma optical emission spectrometry (HG ICP OES) (Mihaltan et al. 2013) and atomic fluorescence spectrometry (HG-AFS) (Rahman et al. 2000). Without the need of coupling hydride generation, graphite furnace atomic absorption spectrometry (GFAAS) (Mendil et al. 2013) and inductively coupled plasma mass spectrometry (ICP MS) (Chevallier et al. 2015) can be used. However, coupling hydride generation to these two techniques improves their analytical characteristics. On the other hand, even though ICP and GFAAS techniques have good sensitivity, they present a high instrumental cost (Sezgin et al. 2015). HG-AFS is a relatively low-cost and highly sensitivity technique for this task, allowing also good accuracy, precision, speed and reliability in the analysis.

Atomic fluorescence spectrometry (AFS) is a technique that is based on the excitation of atoms in the gaseous phase and from the ground state using characteristic radiation photons of the element of interest and in the measurement of fluorescence radiation emitted at an angle of 90 degrees (Hage and Carr 2012). The coupling of hydride generation (HG) with AFS allows the determination of elements such as As, Sb, Se, Te, Bi, Hg , among others, with very low detection limits, good precision, accuracy and confidence (Santos et al. 2013). HG-AFS has become a viable alternative for the quantification of hydride-forming elements due to their excellent performance in the determination of trace and the low cost compared to other spectrometric techniques. This technique has been used in several studies aimed at determination of Sb and other elements, among which are highlighted: Se^{4+} and Se^{6+} in natural water samples after preconcentration using TiO_2 nanoparticles (Fu et al. 2012), Se determination in plants and peat samples (García et al. 2005), Sb speciation in algae samples and marine mollusks after organic species separation by HPLC coupled to HG-AFS (Gregori et al. 2007), As^{3+} and As^{5+} determination from soils using sequential extraction carried out by a flow injection system coupled to HG-AFS (Shi et al. 2003), As^{3+} and As^{5+} speciation from vegetables after ultrasound assisted extraction (Reyes et al. 2008), speciation of inorganic As in edible mushrooms (Gonzálvez et al. 2009), Se determination from food commercialized in Slovenia (Smrkolj et al. 2005), determination of As, Sb, Se, Te and Bi in vegetables and cereals consumed by a Spanish population (Matos-Reyesa et al. 2010), determination of Sb, As and Hg in aquatic amphibians and earthworms collected in the vicinity of a mine for antimony exploration in China (Fu et al. 2011), determination of As, Sb, Se, Te and Bi in milk by using a sampling technique for suspension (Montesinos et al. 2004), determining the degree of leaching and speciation of Sb in coal ashes (Miravet et al. 2006), simultaneous speciation

of As and Sb after preconcentration using carbon nanotubes as a solid phase and an AFS dual-channel spectrometer (Wu et al. 2011).

Optical methods based on hydride generation used for antimony determination from hair samples demand a digestion procedure. Traditionally, this digestion is carried out using HNO_3/H_2O_2 in open system (such as hot plate) to eliminate the exceeding of oxidant which affects the formation of metallic hydride (Liu et al. 2011). Digestion in closed systems using Teflon bomb and microwave irradiation presents several advantages such as: avoidance of the loss of volatile elements and contamination and presenting speed in the digestion (Ferreira et al. 2014). Due to all this advantages, in this work, studies were conducted with the objective to develop an analytical method using microwave digestion in a closed system and HG-AFS technique for the quantification of antimony in hair samples from patients undergoing therapy with antimonial drugs.

MATERIALS AND METHODS

INSTRUMENTATION

To measure the fluorescence intensity of the antimony, an atomic fluorescence spectrometer with hydrides generator, HG-AFS (Model 3300 Lumina, Aurora Biomedic Inc, Canada) was used with a coupled quartz gas-liquid separator. Argon (White Martins) was used as carrier gas and the flame was maintained by the H_2 generated parallel to the hydride reaction of HCl with $NaBH_4$. A high intensity hollow cathode lamp (Aurora, Aurora Biomedic Inc, Canada) was used as excitation source of the analyte. The experimental conditions of the spectrometer operation are given in Table I.

Microwave energy assisted digestion of hair samples was performed in a digestion system constituted by 23 mL of polytetrafluoroethylene (Teflon) cups coupled to a casing constructed with

TABLE I
Operational parameters of HG-AFS for antimony determination.

Parameter	Value
Primary current of lamp (mA)	100
Atomizer observation height (mm)	8
Argon pressure (psi)	30
Carrier gas flow rate (mL min^{-1})	400
Auxiliar gas flow rate (mL min^{-1})	800
DBD applied power (W)	12
$NaBH_4$ flow rate (mL min^{-1})	1.4
Sampling flow rate (mL min^{-1})	4.0
Excitation and emission wavelength (nm)	217.6

polymeric material (4749 Parr, Moline, IL, USA) suitable for penetration by the microwaves.

A Liotop lyophilizer (Model K202, São Carlos, Brazil) was used to remove humidity from the samples making them more brittle for efficient comminuting. The samples were comminuted using a ball mill tungsten carbide 8000M (Spex Sample Prep, USA). A purification system Purelab Classic (Elga, High Wycombe, UK) was used for generation of ultra-pure water with resistivity of 18.2 MW cm^{-1}. A Sartorius analytical balance ED124S model (Gottingen, Germany) was used to obtain mass of samples and reagents.

REAGENTS AND SOLUTION

All solutions were prepared using ultrapure water. Nitric and hydrochloric acids (Merck, Darmstadt, Germany) used were of analytical grade. The antimony standard solutions were obtained by diluting a Titrisol stock solution 1000 mg L^{-1} (Merck Darmstadt, Germany). Working solutions were prepared by appropriate dilution of a middle solution of 10 mg L^{-1}.

The reducing agent was 2.2% (w/v) sodium tetrahydroborate, stabilized with 0.5% (w/v) sodium hydroxide. This solution was daily prepared using analytical grade chemicals (Merck, Darmstadt, Germany) and filtered through a 0.45

μm cellulose membrane. A solution of 10% (w/v) potassium iodide and 2% (m/v) ascorbic acid was prepared by dilution of reagents in ultrapure water.

Each polyethylene container used was rinsed with deionized water and an Extran solution, decontaminated with a solution of nitric acid 10% (v/v) for 12 hours and rinsed three times with ultrapure water and dried in dust-free environment prior to use.

COLLECTION AND SAMPLE PRE-TREATMENT

Ten hair samples of patients who underwent drug treatment against *Leishmaniasis*, and three hair samples from donors not undergoing treatment were collected. The samples were collected in the region just above the neck. This collection site is recommended because it is less susceptible to external contamination, also because there is almost always hair on bald men in this region. About 0.5 g of hair was collected. For this purposes gloves and stainless steel scissors were used, being very careful not to change the aesthetics of the donor's hair through the spacing of the removed hair. After collection, the hair samples were stored in plastic bags, and kept in a clean and dry place. Afterwards, the hair was rinsed to remove dust, sweat, grease, etc, for analysis. The samples were individually immersed in a 5% (v/v) Triton X-100 solution with manual shaking for 5 min and rinsed three times with deionized water. Subsequently, they were dried at room temperature between sheets of filter paper. After drying, the samples were freeze-dried, ground in tungsten carbide ball mills and stored in polyethylene containers previously decontaminated with a solution of 10% HNO_3 (Borella et al. 1996).

SbH_3 GENERATION OPTIMIZATION

The optimization of the variables involved in the stibnite (SbH_3) generation were performed by applying a Doehlert design (Table II). The variables studied were the HCl solution concentrations

TABLE II
Doehlert design applied to optimization of stibnite generation.

Experiment	HCl (mol L^{-1})	NaBH$_4$ (% m/v)	Sb fluorescence intensity
1	6.0 (1.0)	2.0 (0)	356.1 / 381.2
2	5.0 (0.5)	1.5 (-0.866)	246.4 / 282.0
3	5.0 (0.5)	2.5 (0.866)	397.4 / 366.4
4	4.0 (0)	2.0 (0)	303.2 / 332.7
5	3.0 (-0.5)	1.5 (-0.866)	204.0 / 204.7
6	3.0 (-0.5)	2.5 (0.866)	357.7 / 332.2
7	2.0 (-1.0)	2.0 (0)	175.2 / 160.6

Values inside parenthesis are codified coordinates of experimental points.

added to the digested sample and the $NaBH_4$ solution concentration used for hydride generation. The experiments were performed in random order and in duplicate to obtain the experimental error. The evaluated responses were the fluorescence intensities of antimony obtained in each experiment. Data were treated using the software Statistica® for Windows.

OPTIMIZATION OF HAIR SAMPLE DIGESTION

The proportions of the digester mixture comprising HNO_3, HCl and water used for digesting about 0.1 g of the sample were optimized using constrained mixture design (Bezerra et al. 2010) as shown in Table III. The final volume of the mixture was always 4.0 mL. The settings of restrictions on the reagents' proportions were necessary to avoid experimental regions of low efficiency of digestion. The microwave digestion was performed in pressurized vessels according to the following schedule: three heater stages of 1 minute with 1 minute pause between them using power of 350 W. Figure 1 shows the experimental region bounded by the established restrictions. The response

TABLE III
Constraints for the mixture components and results in terms of fluorescence intensity.

Constraints		
Mixture variable	**Low constraint**	**High constraint**
HCl concentration	0	0.5 (2.0)
HNO_3 concentration	0	0.5 (2.0)
Deionized water	0	0.5 (2.0)

Matrix of experimental design and responses				
Exp	**HCl**	**HNO_3**	**Water**	**Fluorescence Intensity**
1	0 (0)	0.5 (2.0)	0.5 (2.0)	735 / 702
2	0.5 (2.0)	0 (0)	0.5 (2.0)	476 / 452
3	0.5 (2.0)	0.5 (2.0)	0 (0)	719 / 726
4	0.5 (2.0)	0.25 (1.0)	0.25 (1.0)	708 / 712
5	0.25 (1.0)	0.5 (2.0)	0.25 (1.0)	738 / 731
6	0.25 (1.0)	0.25 (1.0)	0.5 (2.0)	726 / 715
7	0.33 (1.3)	0.33 (1.3)	0.33 (1.3)	715 / 731

Sample mass: 0.1 g; Final volume of reagents in the digested bomb: 4.0 mL; Heating time: 3 min; Final volume of digested: 10.0 mL. Values inside parenthesis are volumes (in mL) corresponding to proportions established by the design.
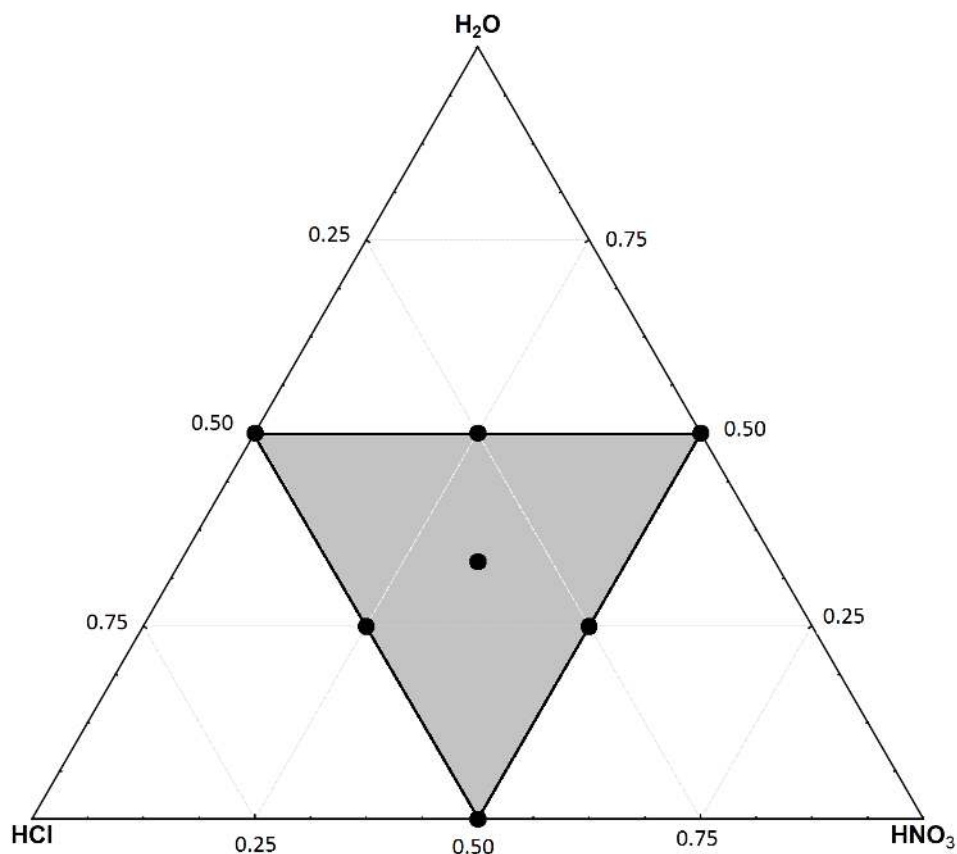

Figure 1 - Experimental region of the mixture design delimited by the high constraints established for each component according to Table II.

measured in the optimization process was the intensity of fluorescence emission. All experiments were performed in random order. Data were treated using the software Statistica® for Windows.

About 0.1 g of each sample was weighed in a Teflon bomb and 1 mL of concentrated HCl, 1 mL of concentrated HNO_3 and 2 mL of H_2O were added to it. Subsequently, the samples were subjected to microwave assisted digestion (power 350W) for three minutes, with 1 minute intervals between irradiations. The digested samples were filtered and swelled to 10 mL with deionized water. Then to 0.5 mL of the digested samples we added 2 mL of 4.7 mol L^{-1} HCl, 2 mL of KI 10% w/v in 2% w/v ascorbic acid for pre-reduction of Sb^{+5} and waited for 20 minutes. The obtained solution was set to 10.0 mL and submitted to the stibnite generation with 2.2% m/v $NaBH_4$ and 4.7 mol L^{-1} HCl for subsequent determination by atomic fluorescence spectrometry.

ETHICAL ASPECTS OF THE RESEARCH

The research was conducted in accordance with the provisions of Resolution No. 466/12 of the Brazilian National Health Council (Brasil 2012), which rules on standards and guidelines ruling scientific research involving human subjects and was approved by the Ethics Committee in Research of the Universidade Estadual do Sudoeste da Bahia (Registered by the code CAAE 06081112.4.0000.0055A). The suppliers of the samples were informed about the objectives of the study and ensured that the research would not cause any damage. Anonymity and confidentiality were warranted and they provided their participation by signing the consent form.

RESULTS AND DISCUSSION

OPTIMIZATION OF EXPERIMENTAL CONDITIONS

Conditions of stibine generation were optimized using a Doehlert design and the evaluated response was the intensity of the fluorescence emission from Sb. This design and the obtained responses for each experiment are shown in Table II. The fitting of the quadratic mathematical model generated the following equation:

$$y = -503.7\ (\pm 242) + 163.8\ (\pm 40.7)\ C_{HCl}$$
$$-\ 12.4\ (\pm 3.92)\ C_{HCl}{}^2 + 281.8\ (\pm 195)\ C_{BH}$$
$$-\ 26.73\ (\pm 47.1)\ C_{BH}{}^2 - 11.45\ (\pm 12.8)\ C_{HCl}\,C_{BH}$$

where y is the response, C_{HCl} is the acid concentration and C_{BH} is the $NaBH_4$ concentration. The terms in bold are statistically significant at a confidence level of 90%.

The response surface (Figure 2) described by the equation presents a shape that characterizes it as maximum. The calculation of the maximum point coordinates shows that the optimum conditions are C_{HCl} = 4.7 mol L^{-1} and C_{BH} = 4.2% m/v. However, the $NaBH_4$ is outside the studied experimental region and was obtained by extrapolation. As, in this condition, there is no considerable gain in sensitivity using this concentration in relation to the 2.0% m/v $NaBH_4$ solution, it was decided to use this last concentration to economize the reducing reagent. Analysis of Variance (ANOVA) has shown that quadratic model does not present significant lack of fit when using a confidence level of 99% (p-value = 0.0129 > 0.01) and it can be used for explain the data behavior inside the studied experimental field.

Hair samples must need to undergo a process of digestion in order to be analyzed by HG-AFS. As Sb is an element susceptible to volatilization losses during heating of the sample, it was chosen to perform the digestion assisted by microwave
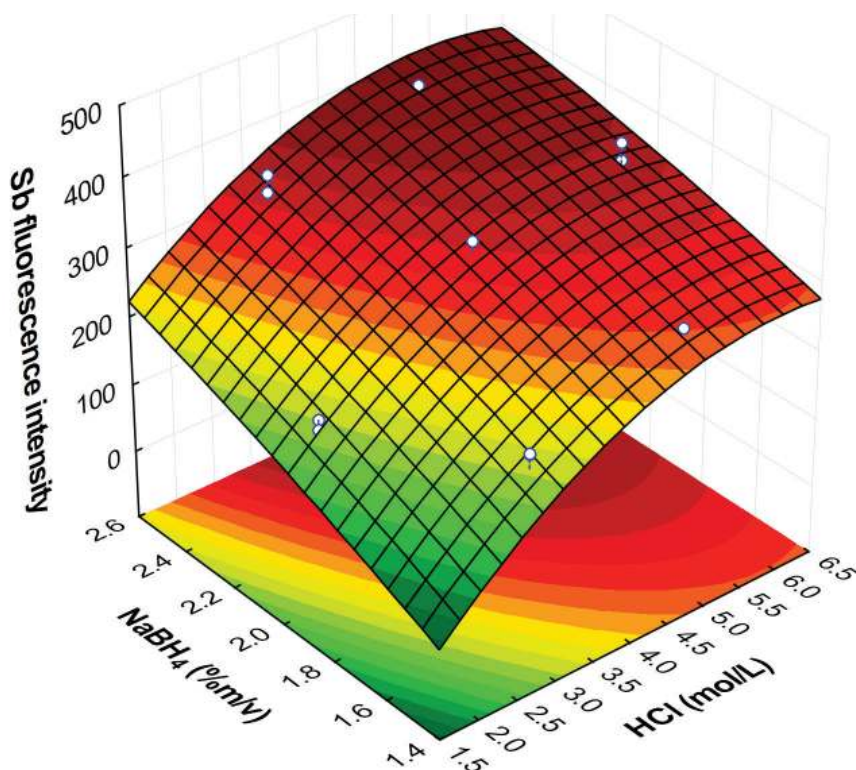
Figure 2 - Response surfaces for quadratic function adjusted to the data obtained from application of the Doehlert design in the optimization of stibnite generation.

energy in a pressured system. To obtain digested samples with good characteristics (low residual carbon content, absence of precipitates and undigested particles) in used PTFE pumps it becomes imperative to use an oxidizing acid such as HNO_3. However, the excess of HNO_3 in the final digested sample makes the reduction process of Sb^{5+} to Sb^{3+} inefficient due to the oxidation of the reducing agents (ascorbic acid and KI). Consequently, it disfavors the formation of stibnite (SbH_3) affecting the accuracy and sensitivity of the atomic fluorescence's measures. Digestion with dilute acids in pressurized systems has proven highly effective in the treatment of various types of samples due to the formation of free radicals that enhance the performance of organic matter decomposition (Araújo et al. 2002, Castro et al. 2009).

To study the adequate proportions of the digester mixture components (HNO_3, HCl and water), a mixture design with higher constraints was used. The establishment of constraints on the variables is necessary to define the experimental area and avoid adverse proportions that make it difficult to obtain digested samples favorable to hydrides generation (such as using 100% water or high volumes of HNO_3). For this study, a mass of approximately 0.1 g of hair sample and three steps of microwave irradiation for 1 min intercalated with 1 min intervals without irradiation were used. The constraints and performance in terms of fluorescence intensity are shown in Table III.

To the set of obtained results, a quadratic mathematical function was applied to describe the behavior of the data, obtaining the response surface shown in Figure 3.
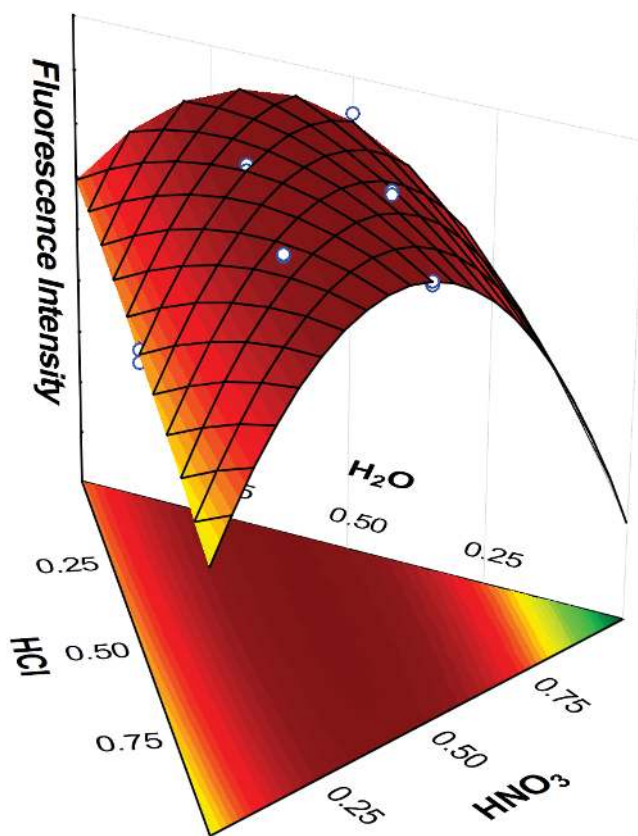
Figure 3 - Response surfaces for quadratic functions adjusted to the antimony fluorescence signal obtained after microwave digestion in pressurized pumps of hair samples.

The highest residual carbon content was found using 50% HCl and 50% deionized water (experiment 3) demonstrating the necessity of the existence of any amount of oxidant in the acid digestion process. Lower levels of residual carbon and higher intensities of fluorescence emission were found in experiments that used at least 25% nitric acid and 50% water. The proportions tested in experiment 6 (2.0 mL of water, 1.0 mL of HNO_3 and 1.0 mL of HCl) were chosen as optimum conditions for microwave digestion.

Analysis of Variance (ANOVA) showed that the quadratic model adjusted to the obtained data using mixtures design does not present significant lack of fit for a confidence level of 96% (p-value = 0.0435 > 0.04) and that it can be used to describe the behavior of the data within the experimental field studied.

ANALYTICAL CHARACTERISTICS OF THE DEVELOPED METHOD

Analytical characteristics were accessed to check the suitability of the developed method in the analysis of hair samples and validation of the developed method. The limit of detection (LOD) was calculated by obtaining the standard deviation of the blank signal from ten replicas which was multiplied by a factor of three and a divided by the slope of the analytical curve (Analytical Methods Committee 1987). The limit of quantification (LOQ) was similarly calculated using a factor of ten. In the developed method, the limits of detection and
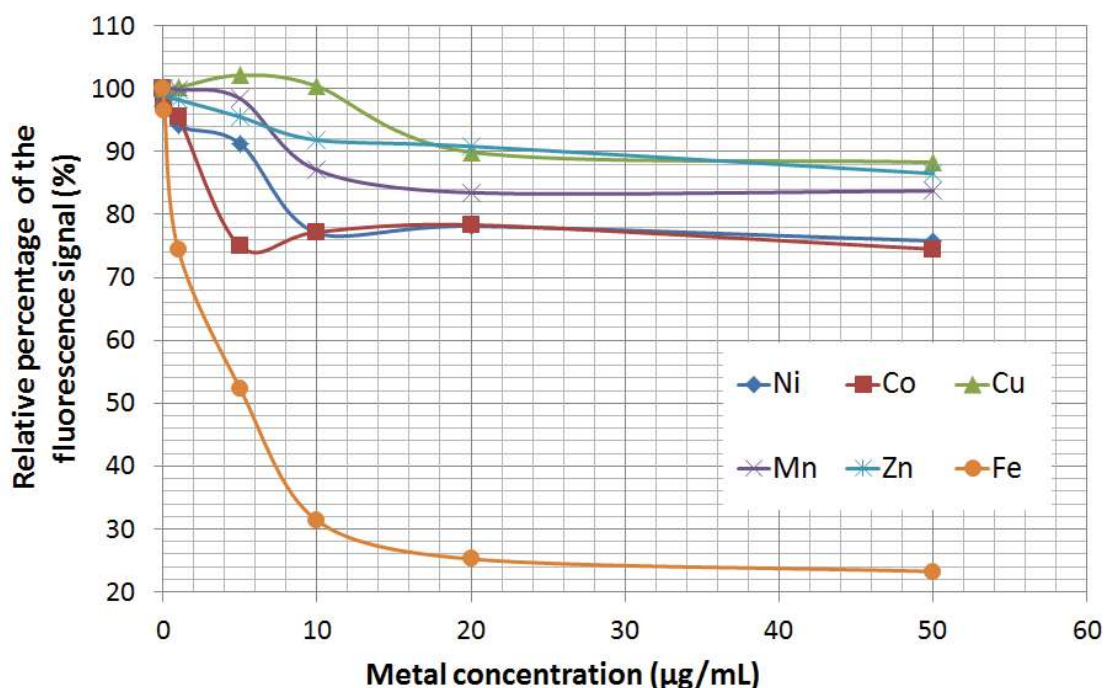
Figure 4 - Studies of the effect of potential interference of Ni, Co, Cu, Mn, Zn and Fe concentrations in the antimony atomic fluorescence signal.

quantification found for the analyzed final solution were 0.28 and 0.96 µg L^{-1} respectively. These limits are correspondent to LOD and LOQ values of 0.57 and 1.92 µg g^{-1} for 0.1 g of original hair sample taking into account a dilution factor of 20-fold of obtained digested.

The method precision was accessed on repeatability and expressed as relative standard deviation (%RSD, n = 10). Using a 10.0 µg L^{-1} Sb standard solution, a repeatability of 2.8% was found.

MATRIX EFFECT STUDIES

Transition metals can, in the dependence of concentration, cause interference in the hydride generation due the parallel reactions that consume the reducing reagent and, consequently, affect the stibnite transport to the atomizer. With the objective of studying the effect of potential interfering ions (Ni^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Fe^{3+} and Zn^{2+}) in the SbH$_3$

generation, a 20.0 µg L^{-1} Sb^{5+} solution was submitted to a hydride generation process in the absence and in the presence of different concentrations (0.1, 1.0, 5.0, 10.0, 20.0 and 50.0 µg mL^{-1}) of these metals. A signal was considered free of interference when generated by a Sb standard solution in the absence of potential interfering metals. To this signal, a value of 100% was attributed and the signals obtained in the presence of transition metals were calculated proportionally to this value. Figure 4 shows the results of this study. It was considered that a metal ion causes interference from the concentration that decreases 5% of the fluorescence emission signal in relation to the original signal. Results (Figure 4) indicate that Cu is, among the studied metal ions, the one that less causes interferences. Its signal interference begins from a concentration of 10 µg mL^{-1}. From this concentration occurs a marked decrease to the Sb signal. Nickel and Co cause interferences from 1.0 µg mL^{-1}, Mn and Zn from

TABLE IV
Concentration of potentially interfering metal ions in hair sample composed by material from several donors.

Metal ion	Concentration in the sample ($\mu g\ g^{-1}$)	Concentration in the solution submitted to hydride generation ($\mu g\ mL^{-1}$)
Ni^{2+}	1.92	9.60×10^{-4}
Co^{2+}	<LQ	<LQ
Cu^{2+}	14.7	7.35×10^{-3}
Mn^{2+}	2.68	1.34×10^{-3}
Zn^{2+}	39.93	2.00×10^{-2}
Fe^{3+}	4.96	2.48×10^{-3}

TABLE V
Equations of the analytical curves based on external standardization and standard addition to the sample techniques.

Technique	Equation	Confidence interval of slope*	R^2
External standardization	$y = 26.89x + 14.19$	25.94 to 27.84	0.9991
Standard addition	$y = 27.21x + 95.14$	26.50 to 27.93	0.9990

*Confidence level of 95%.

$5.0\ \mu g\ mL^{-1}$ and Fe is the metal ion that causes more interference: from $0.1\ \mu g\ mL^{-1}$.

To evaluate the possibilities of interference by these metals in this type of matrix, a sample constituted of the mixture of hair from various donors was analyzed using flame atomic absorption spectrometry (FAAS). The results are shown in Table IV. Since in the proposed method, sample solution must be diluted twenty times, it can be seen that the levels of the naturally occurring elements in this matrix will not interfere in the generation of antimony hydride and dot no demand the use of a masking agent.

The matrix effect and its influence on the analytical curves were also investigated. Standard solutions were prepared using conventional calibration techniques (external standard) and the technique of standard addition of the analyte to the sample solution was also carried out for comparison. The equations of these analytical curves are shown in Table V. As it can be seen, the slopes of the curves obtained by the two methods have high similarity, with a confidence level of 95%. This means that there is no matrix effect and that the two calibration techniques can be used safely. Because of the simplicity and convenience of procedure, the determination of Sb is recommended using the calibration curve by external standardization.

ACCURACY EVALUATION AND METHOD APPLICATION

After defining the optimal conditions for the antimony determination in hair samples, the method was applied to the analysis of ten samples of hair collected from patients who underwent drug therapy for treatment of *Leishmaniasis* based on antimonial drugs. Additionally, the method was applied to the analysis of three samples of hair collected from people who did not undergo treatment. It was noted that only the samples of donors subjected to chemotherapy allowed the detection of antimony. The concentrations of Sb in these samples ranged from 5.29 to 48.9 mg g^{-1} (Table VI).

Spike tests were performed on three samples to evaluate the accuracy of the method. Thus, Sb standard solution was added to the sample before the digestion process to better evaluate the possibility of analyte losses. The recoveries were found between 92.2 and 110.0% and are considered satisfactory for elements in trace amounts, indicating that the developed method has adequate accuracy.

CONCLUSIONS

The developed method has shown sensitivity, precision and accuracy suitable for the determination of total antimony in hair samples from donors undergoing chemotherapy against

TABLE VI
Results of the Sb determination, using the analytical method developed, in hair samples from patients undergoing treatment against *Leishmaniasis*.

Sample	Treatment with antimonial drugs?	Added Sb ($\mu g\ g^{-1}$)	Found Sb ($\mu g\ g^{-1}$)	Recovery (%)
1	Yes	0	11.98 ± 1.27	-
2	Yes	0	12.04 ± 0.89	-
3	Yes	0	19.79 ± 1.38	-
4	Yes	0	7.05 ± 1.57	-
5	Yes	0	36.33 ± 0.90	-
		50.00	91.22 ± 0.85	110.0
6	Yes	0	48.90 ± 2.92	-
		50.00	99.39 ± 1.05	100.5
7	Yes	0	5.29 ± 0.77	-
8	Yes	0	18.18 ± 1.14	-
		50.00	62.88 ± 0.50	92.2
9	Yes	0	19.68 ± 2.64	-
10	Yes	0	26.19 ± 0.21	-
11	No	0	< LOQ	-
12	No	0	< LOQ	-
13	No	0	< LOQ	-

Leishmaniasis based on antimonial drugs. The concentrations of potentially interfering metals naturally present in the capillary matrix on the generation of stibnite do not affect the results obtained by the developed method. The application of multivariate experimental design allowed to find the best proportions of digester agents and the best conditions for generation of stibnite ensuring good performance of the method in the analysis of the studied matrices. The content of antimony in samples from donors not subjected to treatment with antimonial drugs were below the detection limit of the method, while the total antimony concentration in samples from donors undergoing treatment ranged from 5.29 to 48.90 mg g^{-1}. The study allowed the validation of a hydride generation atomic fluorescence spectrometry method in the quantification of antimony in hair, which will enable health professionals to access a new analytical method with sufficient sensitivity and accuracy for information and possibly relate those with health parameters of the individuals.

ACKNOWLEDGMENTS

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