Speciation of antimony (III) and antimony (V) using hydride generation for meglumine antimoniate pharmaceutical formulations quality control

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The pentavalent antimonies, mainly the meglumine antimoniate, are recommends as first-choice medicines for leishmaniasis therapy. In this work we described the development of formulations of meglumine antimoniate injectable medication, as well as the analytical methodology used in the selective determination of Sb(III) and Sb(Total) by hydride generation - inductively coupled plasma atomic emission spectrometry (HG-ICP-AES) and ICP-AES, respectively. On that purpose the analytical methodology was developed focusing on the HG-ICP-AES technique. The formulations using propylene glycol/water as vehicles in a 20:80 proportion were more appropriate for subsequent use in industrial scale. These formulations also showed a lower variation on Sb(III) percentage, no need of buffer solution to stabilize the formulation and no influence of the autoclaving in the quality of the product. The results of the development of the analytical methodology point out the proposed method as an efficient alternative for the determination of Sb(III) in the presence of large quantities of Sb(V) in injectable solutions of meglumine antimoniate, in a selective, linear, accurate and precise manner. In addition, the method showed a low limit of quantification, less interference of the matrix, and more resilience than batch techniques proposed in the Brazilian Pharmacopeia.

Key words: antimony - stibine - hydride generation - inductively coupled plasma atomic emission spectrometry - formulation - analysis - stabilization

Among the different therapeutic alternatives available for leishmaniasis therapy, World Health Organization recommends as first-choice medicines the pentavalent antimonies, mainly the meglumine antimoniate (Croft 1988, WHO 2007). Although the mechanism of the pentavalent antimonies is not fully elucidated, many studies have suggested that its therapeutic profile is due to their conversion in the trivalent form inside the infected macrophages (Berman & Wyler 1980, Callahan et al. 1997, Ephros et al. 1997, WHO 2007). The antimonial compounds are parenterally administered as they irritate the intestinal mucosa, and present a low absorption rate in the gastrointestinal tract (Pintado & Lopez-Velez 2001). They are obtained from the reaction between the antimony oxide (V) (Sb_2O_5) and N-methylglucamine, and their chemical structure was not fully determined. Studies on the structural characterization of these drugs show that they are a mixture of oligomers with different molecular weights. Each antimony atom may be connected in a coordinated manner through two hydroxyl groups of the adjacent moiety of the N-methylglucamine, being easily hydrolyzed by diluted acids with the release of the Sb (V) (Roberts et al. 1998). Thus, the pH is important to the formulation stability and to the therapeutic profile (Carrió et al. 2000). The oxidized form of antimonies is unstable and molecular modifications may occur. The most dangerous of these modifications is the reduction to Sb(III), which constitutes a highly toxic chemical species (Marsden 1979, Balana-Fouce et al. 1998, Feng et al. 1999, WHO 2007). The presence of Sb(III) outside the parasite organism may be responsible for the variability of the response to treatment with antimonial compounds. Studies using different medication samples containing this drug detected considerable percentages of Sb(III) (Sundar et al. 1998). It suggests that the stabilization of the drug is undermined for maintaining the Sb(V).

There are several methods to separate simultaneously and quantify the species Sb(III) and Sb(V) in the pharmaceutical formulations such as: capillary electrophoresis -inductively coupled plasma mass spectrometry (CE-ICP-MS) (Michalke & Schramel 1999); high performance liquid chromatography hydride generation-atomic fluorescence spectrometry detection (HPLC-HG-AFS) (Apte & Howard 1986, Casiot et al. 1998, Cava-Montesinos et al. 2003, González et al. 2005, Morita et al. 2007); spectrophotometric methods with flow injection (Bloomfield et al. 1992, Rath et al. 1997, Trivelin et al. 2006); high performance liquid chromatography-hydride generation-atomic absorption spectrometry detection (HPLC-

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Financial support: FAPERJ, UFF, CNPq
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Received 26 July 2007
Accepted 20 March 2008

HG-AAS) (Smichowski et al. 1995, Lintschinger et al. 1997, Zhang et al. 1998, Lindemann et al. 1999, Zheng et al. 2000); and ICP-MS (Lintschinger et al. 1998, Ulrich 1998a, b, Zheng et al. 2001). Even though these methods are the most appropriate for speciation of antimony, they are poorly suitable for the employment in the routine of medication quality control, which demands simple and more affordable methods. Literature reported some reports such as those of Andreae and co-workers that described the determination of Sb species in meglumine antimoniate solutions in other different matrix (Andreas et al. 1981). Excellent results were obtained with the HG-AAS, which consists of reducing Sb(V) to Sb(III) in the diluted meglumine antimoniate solution, as Sb(V) presents low reactivity to generate stibine with tetrahydroborate. Flores and co-workers determined Sb(III) and total antimony in an injectable pharmaceutical formulation using a flow injection hydride generation atomic absorption spectrometry (Flores et al. 2002, 2003). The method is based in the selective complexation of the species of antimony, to allow, under optimum analysis conditions, the formation of stibine from the trivalent species without the interference of Sb(V), even if it is present in large concentrations. The Brazilian Pharmacopeia (official compendium) recommends analysis by HG-AAS with batch system (Brazilian Pharmacopeia 2002). However, the hybride generation- atomic emission spectrometry (HG-AES) in continuous flow is more appropriate as it eliminates the chemical interference in the gaseous phase found in AAS, reducing the matrix effect. It is also more efficient as it enables the automated analysis. Flow systems are preferable to batch systems due to the continuous stabilization of the bottom generated by the products (H_2 and H_2O). In fact they are produced in a constant flow in the first system different from the second system (Barra et al. 2000).

Considering the need for establishing minimal quality parameters for the medications globally used in leishmaniasis therapy, and lack of interest of the pharmaceutical industry in producing this type of drugs, our purpose was to develop an injectable formulation that stabilizes the oxidized form of antimony. This work also focused on the development and validation of an analytical methodology capable of determining not only the concentration of generated Sb(III) but also the stability of the product, to fulfill the information gap present in the current scientific literature and in the Brazilian Pharmacopeia (2002).

MATERIALS AND METHODS

Apparatus - A sequential ICP-AES, model ULTIMA-2 (JOBIN YVON), attached to an automatic sampler (JOBIN YVON AS 421) was used. A peristaltic pump (MINIPULS 3 GILSON®) was used to insert the citric acid and sodium borohydride ($NaBH_4$) solutions, and another peristaltic pump (LAB CRAFT MOD. HYDRIS 05) to drain the liquid in the liquid-gas separation chamber for disposal. Both peristaltic pumps were connected to the liquid-gas separation chamber, used for HG through Tygon tubes. Background correction was made by subtracting the off-peak spectral background.

The apparatus used also included the analytical scale precision (0.0001) (GEHAKA AG 200); double-door horizontal autoclave (SERCOM®) and the product packaging machine (Martinez Taboada®), qualified and calibrated according to the current rules of Good Manufacture Practices; climate chamber (MS-MISTURA®) model MSM 013/RS, calibrated and qualified according to the ISO 17,025 guidelines, used for the accelerated study of product ampoule stability. The sterilizing filtration was conducted in 0.22 µm (Millipore®) cartridges, with a 316 stainless steel hull.

Chemicals and reagents - The materials used in the preparation of the formulations were of pharmaceutical grade: N-methyl glucamine antimoniate from Aventis Pharma Romainville – France; Sodium metabisulfite from Basf – Brazil; Disodium ethylenediamine tetraacetate from Farmos – Brazil; Methylparaben (nipagim) USP Proquímica – Brazil; Sodium Hydroxide from Merck; Potassium phosphate monobasic from Vetec – Brazil; Propylene glycol from Dow Química S.A. – Brazil. WFI water used in the preparation of the formulations was previously evaluated regarding the presence of heavy metals and antimony by atomic absorption. All reagents used to prepare the analytical solutions and samples were of analytical grade. Milli-Q water (> 16 MΩ) was used in all analytical procedures. The type I ampoules for product packaging were provided by Shott glass, recommended for packaging of injectables by USP. The inorganic standards of Sb(III) ($K(SbO)C_4H_4O_6 + 0{,}5\ H_2O$) were obtained from Merck titrisol®, and that for Sb(V) -$KSb(OH)_6$- was obtained from Sigma-Aldrich. In addition, the meglumine antimoniate ampoules used to optimize and validate the analytical method were obtained from the Aventis Pharma-Brazil Laboratory. The analytical solutions were prepared from Sb(V) and Sb(III) stock solution (1,000 ppm) in water. The reducing agent in the Sb(III) determination and the complexation agent were $NaBH_4$ 2% solution, stabilized with NaOH 0,01mol/l, and a freshly prepared citric acid 30% solution. In order to determine the Sb(Total) amount, the solutions were diluted in tartaric acid 0.5% (Merck) prepared at the moment of analysis. The blank samples were prepared in the same way of analytical samples without antimony.

Procedures **-** Hydride generation - inductively coupled plasma optical emission spectrometry (HG-ICP-OES) and ICP-OES were used to determine the Sb(III) and Sb(Total) amounts, respectively, in the formulations of meglumine antimoniate injectable solutions. The Sb(V) percentage in samples was determined by subtracting the Sb(III) amount from the Sb(Total) amount. The ICP-OES technique is based on measurement of light emission from excited atoms and ions. The excitation occurs in argon plasma at a temperature up to 10,000 K. The intensity of luminous radiation emitted in a 206,833 nm wavelength allowed the quantification of Sb(Total). We selected the wavelength by analyzing the free lines of spectral interference for the Sb. For the nebulization of the sample, a Mira-Mist nebulizer attached to a cyclonic chamber was employed.

The theoretical base of the chosen method is related to the formation of stibine (SbH_3) from Sb(III) and $NaBH_4$ reaction in acid. The volatile nature of SbH_3 makes it easily transported to the plasma through argon. The citric acid is used to produce the selective complexation of Sb(V), allowing the Sb(III) to remain free to form SbH_3 in a selective manner. Helicoidal reactors, built with polyethylene tube, were used to control the contact time between the sample and the citric acid. This period is crucial to determine the complex formation.

The influence of citric acid concentration was evaluated in the formation of SbH_3 through Sb(III) and Sb(V). Initially, we tested the citric acid solutions at 2, 4, 10, 15, 20, 25 and 30%. The average emission intensity of the analysis in triplicate for each concentration was recorded to evaluate the influence of the citric acid concentrations in the proposed method. In addition $NaBH_4$ solutions in concentrations of 1, 2, 3, 4, 5 and 6% were prepared to evaluate its influence in the measured emission intensities. For this purpose the citric acid concentration was maintained at 30%.

The reactors made with polyethylene tubes of 1, 2, 5 and 6 m were tested using citric acid solution 30% and $NaBH_4$ 2% in NaOH 0.01 mol/l, with the purpose of evaluating the influence of contact time between the sample and the citric acid solution in the intensity of the emissions. The operational parameters of the method are listed in Table I.

The following inorganic standards were used in these test: Sb(III) (Antimony Potassium Tartrate Trihydrate) in water (0.5 mg/l); Sb(V) (Antimony Potassium Hexammoniate), 20 mg/l; a solution containing Sb(III) and Sb(V), 0.5 and 20 mg/l respectively. In the reactor length tests a commercial sample solution diluted 4,000 x was used in addition to the inorganic standards of Sb(III) 0.5 mg/l and Sb(V) 20 mg/l. The analysis parameters were determined through the results obtained in the selectiveness test, and the method was thus validated.

To characterize the meglumine antimoniate, the quality of the raw material was evaluated according to the Brazilian Pharmacopeia (2002). Tests were conducted to verify the following characteristics: solubility; presence of heavy metals (Al, As, Bi, Cd, Cr, Cu, Mn, Ni, Pb, Zn, Hg) through the ICP-OES technique with optical system of the sequential type, using the ASSORANCE Spex Certiprep reference standards; and the degree of oligomerization of the active, estimated by X-ray diffraction (XRD). Sb(III) and Sb(V) percentages in the raw material were determined by HG-AAS and AAS techniques, respectively.

Data processing - The Microsoft Excel software was used to perform the statistical calculations. Statsoft software was used to build the Box Plot, used as an auxiliary tool in the evaluation of the formulations.

Method validation - The linearity was tested at a concentration range of 0.1 to 1.0 mg/l of five different concentrations (0.1; 0.25; 0.5; 0.75 and 1.0 mg/l). The standard calibration curve was used for determining linearity. These standards were tested six times, following the rules of the International Conference on Harmonization (ICH 2005). A calibration curve was constructed and the proposed method was evaluated by its correlation coefficient and interception value, calculated in the corresponding statistic study (ANOVA) ($p < 0.05$). Characteristic parameters for regression equation ($y = a + bx$) of the analytical method, obtained by least squares treatment of the results, confirmed the good linearity of the developed method.

The assay accuracy was measured by analyzing three spiked samples (80, 100 and 120%). According to the ICH (2005) recommendations, precision must be considered at two levels including repeatability and intermediate precision. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment. For this parameter, six replicated samples were consecutively tested using the same equipment at a concentration of 100% (0.8 mg/ml) of the regular analytical working value. The intermediate precision expresses the variations within laboratories, and was assessed six times by using different equipment, analysts and days to analyze three samples (glucamine from Aventis Pharma Lab - Brazil).

TABLE I

Summary data of ICP and hydride generation - inductively coupled plasma atomic emission spectrometry (HG-ICP-AES) determination parameters

Potency (w)	ICP (usual nebulizer)	HG-ICP-AES
	1,200	1,200
Ar flow (l/min)	Nebulizer: 0.48 Plasma: 15 Gas:0 Sheath gas: 0.2	Nebulizer: 0.22 Plasma:15 Gas:0 Sheath gas:0.4
Nebulizer – aspiration rate (l/min)	Meinhard Cyclonic chamber: 2.73	-
Citric acid flow (ml/min)	-	0.6
Sodium borohydride flow (ml/min)	-	1.7
Sample flow (ml/min)	-	1.3

TABLE II

New produced formulations (F1-F8) for meglumine antimoniate

Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8
Meglumine antimoniate	29.78	29.78	29.78	29.78	29.78	29.78	29.78	29.78
Sodium metabisulfite	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
EDTA.2Na	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methyl paraben	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
NaOH	-	0.09	-	0.09	-	0.09	-	0.09
KH$_2$PO4	-	1.05	-	1.05	-	1.05	-	1.06
Propylene glycol	-	-	-	-	14.0	13.83	34.83	34.4
Water	69.97	68.83	69.97	68.83	56.0	55.17	34.83	34.4

The limit of quantification was obtained from the blank standard deviation of the calibration curves of the eight samples. It was determined according to the average of the blank value multiplied by ten and divided by the angular coefficient.

Formulations - To evaluate the improvement of the compound stability in terms of the formation of toxic sub products (i.e., Sb(III) derivate), and some physical-chemical properties, such as pH, density and percentage of Sb(V), different formulations were prepared and monitored over a period of 30, 60 and 90 days in a climatic chamber maintained at 50ºC, after initial analysis. The prepared formulations are listed on Table II.

RESULTS AND DISCUSSION

Speciation analysis strategy - Many parameters such as citric acid concentration, NaBH$_4$ concentration and reactor size were evaluated to determine the method selectivity, that is, the determination of Sb(III) species in the presence of large quantities of Sb(V) species, without the latter one interfering in the analysis. Sb(V) may react with NaBH4, but have slower HG than the corresponding Sb(III), so then the signals are produced by the pentavalent species depending on HG conditions. Therefore it is feasible to monitor the same sample both directly and after quantitative reduction to the trivalent state based on the different sensitivity for HG-ICP-AES technique. Several methodologies have been used for Sb(V) and Sb(III) quantification in different matrices. However herein the HG-ICP-AES technique was used for the first time to evaluate a new pharmaceutical formulation quality presenting these species (Apte & Howard 1986, Casiot et al. 1998, Cava-Montesinos et al. 2003, González et al. 2005, Morita et al. 2007).

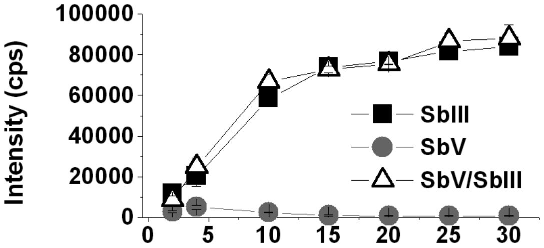

Fig. 1: citric acid concentration (1.0-30.0% m/v) effect in hydride generation - inductively coupled plasma atomic emission spectrometry (HG-ICP-AES).

Citric acid concentration effect - The increase in the citric acid concentration led to an increase in the emission signal for the Sb(III), both for the Sb(III) solution as well as for the solution containing Sb(III) and Sb(V) (Fig. 1). This is probably due to the reduction in the solution pH, which should contribute to increase the speed of reduction of Sb(III) by NaBH$_4$. After the citric acid concentration reaches 20%, it tends to stabilize. At this point, the solution pH is very low, and does not cause as much impact in the speed and selectivity of the reaction. It was previously observed that the complexation of Sb(V) with citric acid is improved in lower pH values (de la Calle Guntiñas et al. 1991). The study also demonstrated that the emission signal for the Sb(V) is suppressed with the increase in citric acid concentration (> 10%), probably due to the complexation of Sb(V) with citric acid, reducing even further the reduction velocity of this species to SbH$_3$.

Mohammad and co-workers had already reported the pH influence in the absorption signal increase, both for Sb(III) and for Sb(V), when they studied the pH influence in speciation of Sb(III) and Sb(V) using HG-AAS (Mohammad et al. 1990). The drop in intensity is not observed in the solution containing the two species, in which the signal increases due to the presence of Sb(III), as demonstrated in Fig. 1. The chosen concentration of citric acid for the remaining assays was 30%, considering that at this level the maximum intensity values were observed both for Sb(III) and for the mixture of both, with the Sb(V) intensity remaining unchanged.

To verify the intensities of Sb(III) in different citric acid concentrations, we used the *t*-test of multiple comparison with Bonferroni correction. The Snedecor F test was performed to verify if the variances were comparable. The calculated F value (14.44) was smaller than the critical F (39.0) for all pairs and the intensities were comparable at 20, 25 and 30% intensities but not at 15%. Our results pointed 20% concentration as ideal to work in this concentration range without modifying the results.

Sodium borohydride concentration effect - The NaBH$_4$ reductor was tested at 1, 2, 3, 4, 5 and 6% stabilized in a NaOH 0.01 M solution. An increase in the concentration of NaBH$_4$ increases significantly the emission signal of Sb(III), both in the Sb(III) solution and in the solution containing Sb(III) and Sb(V). Concentrations above 3% tend slightly to increase the Sb(V) signal, both in the Sb(V) solution and in the solution containing both species, Sb(III) and Sb(V), showing that in these concentrations the citric acid does not have the same power to reduce the formation of SbH_3 from Sb(V).

Despite of using a different matrix, the results agree with those from Flores and co-workers about the evaluation of the influence of the reductor concentration in the formation of SbH_3 using flow injection HG-AAS, except when the concentration exceeded 4% (Flores et al. 2002). These values may be compared to those determined in the analyses developed herein as they involve the use of the same solvent (H_2O). In this way, the $NaBH_4$ concentration of 2% was selected as the signal for Sb(III) since in this situation it is much higher than with lower concentrations, and the Sb(V) interference is meaningless, as shown in Fig. 2.

Helicoidal reactor length effect **-** The studies of reactor size used in the analysis were considered important as the reaction time between the sample and the citric acid interferes in the complexation yield. This may lead to a low reproducibility when working industrially as previously demonstrated and described in the Brazilian Pharmacopeia (2002). The use of the different reactors (0, 1, 2, 5 and 6 m) did not show any conclusive results. The spectral resolution in ICP-OES showed a high resolution (~ 5.0 mg/ml) in ideal conditions, which indicated no spectral interference in the chosen wavelength.

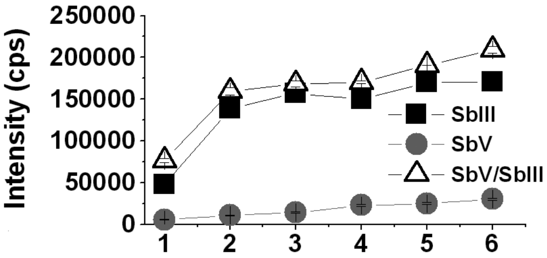

Fig. 2: $NaBH_4$ concentration (1.0-6.0% m/v) effectt in HG-ICP-HG-ICP-AES.

Method validation **-** Both methods presented correlation coefficients above 0.999, and were thus considered linear in the studied concentration range. The achieved linear regression parameters for both methods are presented in Table III. The methods presented an average recovery rate (%RR) of 101.31% for Sb(III) and 101.51% for Sb(total) in the three studied concentration levels. The relative standard deviation (RSD) of the replicates of each concentration level did not exceed 5% (Table IV).

Through the student *t*-test, the hypothesis of significant difference in the three concentration levels was tested, and no evidence was found of a systematic error in the method. In addition, the calculated averages are statistically the same as the expected theoretical values. We also verified that the expected theoretical value is within the confidence interval of the obtained results. In the determination of the concentrations found in six replicates (Table V) for the Sb (III) analysis, a RSD of 3.53% was observed, and for Sb(V) analysis, a RSD of 2.54%. The results achieved in the analysis of the formulated product, with different days and analysts, are described in Table VI. The *t*-test application with grouped variances the Snedecor F test, was applied and showed that the variances between the results obtained in different days with different analysts were statistically identical. The *t*-test confirmed the method precision. The results obtained for the quantification limit of both methods are described in Table VII, and are compatible with the concentration levels observed in the analysis.

TABLE III

Method recovery

	Sb(III)					Sb total				
Level (%)	Spiked amount (mg/ml)	Spiked found (mg/ml)	RSD (%)	RF (%)	IC	Spiked amount (mg/ml)	Concentration (mg/ml)	RSD (%)	RF (%)	IC
80	3,203	3,201	3.73	99.9	0.296	66.25	67.20	0.977	101.43	0.743
100	4,000	4,087	3.15	102.2	0.146	81.00	82.17	1,652	101.45	1,536
120	4,799	4,887	4.6	101.8	0.254	97.20	98.80	2,562	101.65	2,865

TABLE IV

Method precision

Sb(III)					Total Sb				
Concentration (mg/ml)	Media (mg/ml)	SD	RSD (%)	S^2	Concentration (mg/ml)	Media (mg/ml)	SD	RSD (%)	S^2
0.4300	0.4045	0.016	3.53	0.000242	72.904	70.53	1.79	2.54	3.21
0.4376					70.612				
0.4565					72.414				
0.4260					69.626				
0.4296					68.972				
0.4630					68.628				

TABLE V

Quantification limits for antimony analysis

Sample (Sb III)	Conc. (mg/ml)	Media	SD	Slope	QL (mg/l)
blank 1	0.0023				
blank 2	0.0019	0.00175	0.0004209	535277	0.0000217
blank 3	0.0023				
blank 4	0.0017				
blank 5	0.0011				
blank 6	0.0014				
blank 7	0.0015				
blank 8	0.0018				
Sample (Sb total)	**Conc. (mg/ml)**	**Media**	**SD**	**Slope**	**QL (mg/l)**
blank 1	0.0026				
blank 2	0.0045	0.0029	0.00138	44,306	0.000071
blank 3	0.0043				
blank 4	0.0014				
blank 5	0.0044				
blank 6	0.0016				
blank 7	0.0031				
blank 8	0.0013				

Development of meglumine antimoniate formulation - Active pharmaceutical ingredients of three different origins were tested to select the one presenting the ideal characteristics for the methyl meglumine injectable formulations. The chosen compound (Aventis Pharma) showed good solubility and no agglomeration profile in XRD, which supports the proposal of an undefined and oligomeric chemical structure. The particles were amorphous and with undefined geometry. The size distribution was measured with medium diameter of 15 to 20 µm. The total concentration of the analyzed heavy metals was 3.7121 mg/l, a value below the maximum specified level (10 mg/l). The obtained percentages of Sb(III) and Sb(total) were 0.49 ± 0.0% and 27.7 ± 0.3%, respectively. The calculated percentage of Sb(V) was 27.2 ± 0.3%, indicating the suitability of the synthetic process and confirming the choice of the compound cited above, even though its acquisition cost is higher.

During stability studies, the results of the pH and density values evaluation for new formulations are described in Table VI. The values of Student *t*-test for multiple comparisons, using Bonferroni correction, showed there were no significant variations for all pairs of the 1, 2, 3, 4, 5 and 6 formulations during the 90 days. However, differ-

TABLE VI

New meglumine antimoniate formulations evaluation

Parameter	Days	F1	F2	F3	F4	F5	F6	F7	F8
pH	0	6.14 ± 0.0495	6.44 ± 0.0071	6.12 ± 0.0141	6.44 ± 0.0141	5.95 ± 0.0141	6.44 ± 0.1202	6.72 ± 0.0141	6.72 ± 0.0283
	30	5.86 ± 0.0141	6.57 ± 0.0141	6.23 ± 0.0495	6.54 ± 0.0141	5.81 ± 0.0424	6.29 ± 0.2333	5.97 ± 0.0566	6.36 ± 0.0778
	60	5.75 ± 0.1556	6.58 ± 0.0282	6.25 ± 0.0354	6.525 ± 0.0495	5.76 ± 0.0566	6.39 ± 0.0566	6.04 ± 0.1343	6.32 ± 0.0071
	90	5.69 ± 0.0283	6.54 ± 0.0283	6.54 ± 0.0420	6.23 ± 0.0283	5.77 ± 0.0141	6.03 ± 0.4808	5.90 ± 0.0283	6.37 ± 0.0141
density	0	1.1639 ± 0.0306	1.1680 ± 0.0304	1.1628 ± 0.0321	1.1684 ± 0.0287	1.1710 ± 0.0326	1.1797 ± 0.0337	1.1834 ± 0.0304	1.1989 ± 0.03605
	30	1.1640 ± 0.0306	1.1684 ± 0.0296	1.1633 ± 0.0316	1.1722 ± 0.0319	1.1715 ± 0.0319	1.1840 ± 0.0254	1.1917 ± 0.0315	1.1962 ± 0.02968
	60	1.1646 ± 0.0314	1.1697 ± 0.0301	1.1624 ± 0.0316	1.1737 ± 0.0305	1.1694 ± 0.0288	1.1801 ± 0.0300	1.1889 ± 0.0302	1.1919 ± 0.0364
	90	1.1643 ± 0.0306	1.1704 ± 0.0312	1.1729 ± 0.0328	1.1652 ± 0.0306	1.1795 ± 0.0419	1.1776 ± 0.0376	1.1920 ± 0.0316	1.1990 ± 0.03120

TABLE VII

Sb total and Sb(III) amounts found meglumine antimoniate formulations in stability studies

	Initial		30 Days		60 Days		90 Days	
Formulation	Sb total (mg/ml)	Sb (III) (mg/ml)	Sb total (mg/ml)	Sb (III) (mg/ml)	Sb total (mg/ml)	Sb (III) (mg/ml)	Sb total (mg/ml)	Sb (III) (mg/ml)
1	75.7 ± 1.2	2.27 ± 0.18	76.62 ± 0.95	3.22 ± 0.26	77.04 ± 0.70	2.53 ± 0.19	76.78 ± 0.64	1.58 ± 0.10
2	75.2 ± 2.3	2.33 ± 0.34	76.22 ± 0.27	3.46 ± 0.08	77.13 ± 0.16	1.19 ± 0.04	72.78 ± 0.95	1.26 ± 0.14
3	77.7 ± 1.1	2.98 ± 0.30	76.20 ± 0.87	3.48 ± 0.14	75.93 ± 0.52	1.83 ± 0.11	72.63 ± 0.67	2.03 ± 0.09
4	77.4 ± 0.6	2.49 ± 0.26	77.70 ± 0.37	2.95 ± 0.08	76.60 ± 0.63	2.02 ± 0.28	76.28 ± 1.55	0.93 ± 0.16
5	77.1 ± 1.3	1.57 ± 0.16	79.02 ± 0.97	2.26 ± 0.11	75.58 ± 0.42	1.03 ± 0.16	75.68 ± 0.97	1.09 ± 0.09
6	77.9 ± 1.2	0.97± 0.10	80.15 ± 1.07	2.31 ± 0.24	77.05 ± 0.41	1.37 ± 0.04	75.40 ± 1.22	1.09 ± 0.24
7	79.4 ± 1.4	1.28 ± 0.25	73.92 ± 0.45	1.80 ± 0.11	76.91 ± 0.55	0.89 ± 0.08	71.99 ± 1.39	0.93 ± 0.16
8	78.9 ± 0.6	1.33 ± 0.34	79.62 ± 0.65	2.07 ± 0.08	76.89 ± 0.86	1.75 ± 0.01	77.23 ± 1.09	1.17 ± 0.10

ences were detected for the 7 and 8 formulations, which use propylene glycol/water in a 50:50 proportion without condemn the product in pharmacopeial terms. Neither the products autoclaving, formulations 1 and 2, nor the use of propylene glycol/water 20:80 as vehicle provide significant pH variations, corroborating previous studies of Romero (1996).

In terms of the studied formulations density, all calculated *t* values for all pairs of all formulations were smaller than the critical t, leading to the conclusion that there was no significant difference in the formulations during the 90 days at a temperature of 50ºC.

The variations in the percentages of Sb(III) and Sb (V) in the proposed formulations were studied and listed in Table VII. The experimental *t* values were smaller than the values of the critical *t* for all formulation pairs, showing that the variation of the averages is equivalent, therefore showing there is no interference of the buffer in the quantities of Sb(III) found in the formulations. In all formulations, was found a Sb(III) increase at 30 days study. After this period, the value tends to decrease, and this phenomenon is related to the equilibrium achieved in the reaction of hydrolysis of meglumine antimoniate. For the evaluation of the autoclaving influence in the variation of the results obtained for Sb(III) in the formulations, we compared the averages of Sb (III) determined for formulations 1-3 and 2-4 during 90 days. The experimental *t* values for both compared pairs were smaller than the critical t, showing that autoclaving did not interfere in the behavior of the species during the 90 days at 50ºC, in the prepared formulations. Nevertheless, we verified that the formulations subjected to autoclaving acquired a strong yellow color, indicating potential alterations in the organic portion of the molecule, not appropriate for its industrial processing.

The Sb(III) percentage in all formulations whose vehicle was only water, soon after preparation, were above the expected range, which is probably due to the higher dielectric constant of this solvent. In the formulations whose vehicles were mixtures of propylene glycol/water, the percentages of Sb(III) remained within the admitted maximum values. The propylene glycol used as a co-solvent reduced the dielectric constant of the medium, thus reducing redox-type reactions, which corroborates the stability of the formulation. At same time, with high dielectric constant solvents, had already suggested that the structure of the meglumine antimoniate would have a great tendency towards dehydration reactions at high temperatures, forming a structure similar to an acetal (Carrió et al. 2000). This supposed structure could break with the subsequent hydrolysis of the structure, releasing Sb(V) in the medium, which would be free to suffer reduction reactions to Sb(III), depending on the conditions of the vehicle. As previously described, the propylene glycol works as an anti-hydrolytic agent in pharmaceutical formulations, which would minimize the release of Sb(V) in the medium, and would consequently further stabilize the prepared formulations. This fact, added to the conservation action of propylene glycol, which reduces the microbiological contamination of the product, confirming the solvent choice. The comparison of the formulations performed through the Box Plot showed that the formulations whose vehicles contained propylene glycol presented smaller values for Sb(III) and smaller variation of these values during the studied period. In terms of variation of Sb(V) percentages, there were no modifications, which allows us to indicate formulations 5 and 6 as the most stable among those studied. It is also advisable to use preferentially the sterilizing filtration process for manufacturing the meglumine antimoniate injectables, while also using activated carbon filtering as a complementary measure, to avoid possible contamination by heavy metals and probable processes of decomposition of meglumine. Propylene glycol concentration above 14.0% (formulations 7 and 8) conducted a reduction in formulations stability, indicating a direct stability/vehicle dielectric constant relationship (Table II).

In conclusion, the results achieved in the study of development and validation of the analytical method, used to determine percentage of Sb(III) and Sb(V) in injectable formulations of meglumine antimoniate without separation of species, showed that the HG-ICP-AES method in continuous flow system is a safe and efficient alternative to the quality evaluation routine of these products in quality control laboratories. The method was demonstrated to be faster, more precise and more resilient than batch techniques. In the work performed with high concentration of species, the smaller quantification limits were not relevant.

The studies on the development of injectable formulations of this compound confirmed the initial idea of increased stability through reduction in the vehicle dielectric constant. Formulations 7 and 8, which used propylene glycol/water as a vehicle, in a 50:50 proportion, presented a higher stability degree regarding the increase in Sb(III) percentage than the preparations in aqueous vehicle, but with the inconvenient of higher pH variation, without compromising the quality of the end product. Formulations 5 and 6, which used propylene glycol/water in 20:80 proportion as vehicles, were the most appropriate. However, considering the fact that injectable solutions should have the smallest possible quantity of additives, in the results we verified that the buffer was not needed to stabilize the product. Thus, formulation 5 would be eligible to scale up in the future.

Since the feasibility of potential oxidation-reduction reactions in the meglumine antimoniate molecule, it becomes necessary to use product filtering in activated carbon columns before product packaging, which should be evaluated in its scale up.

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