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Off-line speciation of Sb(III) and total Sb in pharmaceuticals by spectrophotometric flow-injection hydride generation using the potassium dichromate reaction

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Abstract

A spectrophotometric procedure based on flow injection analysis is proposed for Sb(III) and total Sb determination in pharmaceuticals. Sb(III) reacts with the hydrogen radical generated in the system, forming antimony hydride. The species formed is then transported towards the flow system and permeates through a Teflon® interface, being carried by a constant air flow. Then, a $K_2Cr_2O_7$ solution in acidic medium is added by confluence, allowing the reduction of Cr(VI) to Cr(III) and the indirect determination of Sb at 610 nm. The proposed methodology presents a linear range from 200 to 1000 mg L^{-1} ($r > 0.997$; $n = 5$), 70 mg L^{-1} as limit of quantification of and an analytical frequency of 40 h^{-1}. The precision, expressed as RSD ($n = 13$ to 500 mg L^{-1} Sb(III) solution), is < 5.0%. The accuracy for Sb(III) was checked through recovery tests and ranged from 96 to 105%. For total Sb, the accuracy was checked by atomic absorption spectrometry and the results are in agreement at the 95% confidence level, using the *t* test. Pharmaceutical samples were analyzed and an average value of 5.39 and 110 mg mL^{-1} for Sb(III) and total Sb concentrations, respectively, were obtained.

Keywords: Antimony, hydride generation, spectrophotometry, flow injection, leishmaniasis, meglumine antimoniate.

1. Introduction

Human leishmaniasis is a disease caused by the leishmania gender protozoan. It affects *ca*. 350 million people in 88 countries on 5 continents [1]. Leishmania symptoms mainly comprise ulcerations on the whole body, principally in the facial region (cutaneous Leishmaniasis). Treatment can be made by administrating antileishmanial drugs, which are constituted of Sb(V) (*e.g.* meglumine antimoniate or sodium stibogluconate). Although these drugs present effectiveness in the protozoan elimination, they induce considerable collateral effects due to their toxicities [2]. Additionally, Sb(III), *e.g.* antimony potassium tartrate and antimony sodium tartrate, were also used for this purpose. However, due to the higher collateral effect when compared with Sb(V), use of Sb(III) was banned [2]. As Sb(V) drugs can present contamination by Sb(III), quality control through the quantification of Sb(III) is of utmost importance in antileishmanial drugs.

A large number of methods have been proposed for Sb determination in several samples. Techniques such as electrothermal atomic absorption spectrometry [3], inductively coupled plasma optical emission spectrometry [4], hydride generation atomic absorption spectrometry [5], anodic stripping potentiometry [6], hydride generation atomic fluorescence spectrometry [7], among others, generally present good selectivity and sensitivity.

In this context, mainly for presenting a wide analytical working range, low acquisition and maintenance costs, easy implementation, among others, spectrophotometry can be considered as a welcome alternative for Sb determination in drugs [8,9]. Despite of these advantages, spectrophotometry presents problems associated to selectivity, limiting its applicability with complex samples. A strategy adopted for increasing selectivity can be based on analyte separation from its matrix. As examples, pervaporation [10] as well as hydride generation with sorbent extraction [11], can be cited. Even when working with those techniques presenting good selectivity, such as atomic spectrometry, hydride generation is used for improving both detectability and selectivity [12,13]. Based on spectrophotometric advantages and due to selectivity attributed to the hydride generation, avoiding accuracy problems in the quality control of drugs, the objective of this work was to propose a selectivity method for the speciation of Sb(III) and total Sb in drugs using a non specific reaction.

2. Experimental

2.1. Instrumentation

The flow system comprised an Ismatec peristaltic pump (Ismated IPC-12, Glattzbrugg, Switzerland), a three piece injector-commutator device and confluence points, both made of polymethacrylate [14], as well as polyethylene tubes (0.8 mm i.d.) as transmission lines and Tygon® tubes for propelling the solutions. A Femto UV-Vis spectrophotometer, model 482 (São Paulo, Brazil), with a 1 cm-optical path flow cell, and a PCL 711 interface with programming in macro Visual Basic (Microsoft Excel) were used for data acquisition.

2.2 Reagents and solutions for optimized analytical conditions

All the solutions were prepared with deionized water (18.2 MΩ cm) obtained from a Milli-Q water purification system (Bedford, MA, USA). Reagents were of analytical grade and all glassware was kept in a 1.2 mol L^{-1} HCl solution for 24 h with posterior cleaning with ultra-pure water.

A 1000 mg L^{-1} Sb(III) stock solution was prepared by dissolving $C_4H_4KO_7Sb•½\ H_2O$ (Vetec, Rio de Janeiro, Brazil) in

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1.0 mol L^{-1} HCl. Working reference solutions ranging from 200 to 1000 mg L^{-1} were prepared in water.

A 0.2% (m/v) $K_2Cr_2O_7$ (Labsynth, Diadema, Brazil) solution was prepared by dissolving an appropriate mass in 0.2 % (v/v) HCl.

A 0.6% (m/v) KBH_4 (Ventron, Beverly, USA) solution was prepared in 0.6% (m/v) NaOH (Merck, Darmstadt, Germany), and kept under refrigeration for up to two weeks after preparation.

2.3 Samples

All samples of meglumine antimoniate were obtained from the Regional Health Bureau of Alfenas – Minas Gerais State. For Sb(III) determination, the samples were diluted (2:25) in deionized water. For total Sb determination, 0.2 mL of samples were digested using microwave ovens (Provecto, Jundiaí, Brazil) with 3 mL of conc. HNO_3 and 1 mL of H_2O_2. A 30 min pre-digestion stage was made and microwave sample decomposition was carried out in only one step: 20 min @ 300 W. Then, the samples were transferred to a 50 mL volumetric flask, treated with 1% KI (m/v) in 4 mol L^{-1} HCl for Sb(V) reduction, and the volume completed with water to 50 mL.

2.4 The spectrophotometric flow injection hydride generation system

The system was based on the reduction of Cr(VI) to Cr(III) for attaining antimony hydride generation in the system. The antimony hydride is generated and migrates through the Teflon® membrane, being transported by a constant gas flow until receiving $K_2Cr_2O_7$ in an acidic medium. The hydride formed promotes the reduction of Cr(VI) and the analytical signal of the Cr(III) is then monitored at 610 nm. The results obtained are proportional to the Sb(III) concentration.

Fig. 1A shows the proposed system in the sampling position, where L_1(standards/samples) and L_2(KBH_4) loops are filled. When the central part of the commutator is switched (Fig.1B) the volumes contained in both L_1 and L_2 loops are transported by C_1 and C_2 carriers (deionized water) through the analytical pathway. The sample is then acidified (R_1) at the x confluence point, and receives the KBH_4 at the y confluence point. Antimony hydride is then formed by the reaction between the nascent hydrogen generated by the KBH_4 in acidic medium and the Sb(III) present in the standards/samples. The antimony hydride generated permeates through the Teflon® membrane in a gas phase separator (GPS) and it is carried by the air stream to the z confluence point. At the same moment, the remaining solution moves towards to waste (W). At the z confluence point, the antimony hydride receives the $K_2Cr_2O_7$ solution (R_3) and the mixture is transported through the C coil, where a segmented flow is formed. A de-bubbler device (DB) was used in order to promote a non-segmented flow. From the z confluence point, the Cr(VI) is then reduced to the Cr(III), which is monitored at 610 nm through the PCL-711 interface with the data acquisition program written in macro Visual Basic – Microsoft Excel. As the Cr(III) formed is proportional to the Sb(III) concentration, the signal obtained reflects its indirect determination.

2.4.1. Gas phase separator (GPS) and de-bubbler device (DB)

The GPS device proposed in this work (Fig. 2A) is composed of two polymethacrylate plates with six symmetrical channels of 1 mm-depth *x* 150 mm-length *x* 3 mm-width and two commercial Teflon® tapes. These tapes are stretched to cover the total area of the polymetacrylate plates. Twelve screws are used (Fig. 2A) for fixing the entire system like a sandwich, making possible the passage of the solutions without leakage. The de-bubbler device was also built in polymethacrylate and shaped like a cylindrical reservoir (Fig. 2B), its internal cavity having 8 mm-diameter.

3. Results and discussion

3.1 Optimization of the variables

Chemical and physical variables were optimized, and the best condition being obtained as a function of absorbance. A 500 mg L^{-1} Sb(III) standard solution was used throughout this study.

3.1.1. Physical variables

The membrane-based gas separator allows working with lower gas flow rates, and matching the conditions of this work with those of laminar flow rates found in flow systems. The number of channels of this device was tested from 1 to 5 and the best conditions were obtained with 2 channels. By using only one channel, the gas permeation area was not large enough, which provoked lower analytical signals (*ca*. 0.008 A). However, from 2 channels, gas permeation area reaches an ideal value, stabilizing the analytical signal (*ca.* 0.020 A). As a possible explanation, the increase of the interface area promotes an increase of gas permeation [15]. Although the GPS has been built-up with 6 channels, only 2 of them were utilized as optimized condition.

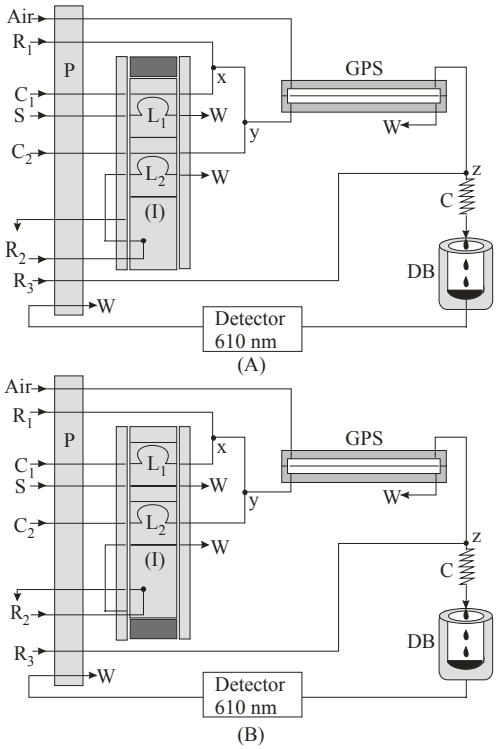

Fig. 1: Hydride generation flow system comprising a peristaltic pump (P), an injector commutator (I), a gas phase separator (GPS) and a de-bubbler (DB) device. (a): System in the sampling position, filling the loops (L_1 and L_2). R_1 = 0.1 mol L^{-1} HCl at 1.8 ml min^{-1}, R_2 = 0.6%(m/v) KBH_4 in 0.6%(m/v) NaOH, R_3 = 0.2 % (m/v) $K_2Cr_2O_7$ in 0.02 mol L^{-1} HCl at 1.8 ml min^{-1}, C_1 and C_2 = deionized water (carriers) at 1.8 ml min^{-1}, S = standards/samples, Air at 2.3 ml min^{-1}, C = coil, x, y and z = confluence points, and W = waste. (b): System in the injection position.

A carrier gas was necessary because, when the $K_2Cr_2O_7$ solution was pumped towards the membrane (upper channel of the GPS device), there was a reduction in the membrane lifetime. The color of the membrane changed to a dark green, and a possible explanation is that the reaction between hydride and $K_2Cr_2O_7$ was occurring in the pores of the membrane, which was impregnated. As the Teflon used was of commercial quality, its purity may be questionable. To circumvent this drawback, atmospheric air and argon were tested as carrier gases and the

$K_2Cr_2O_7$ solution was introduced in the system only after the GPS device (at z confluence point, see Fig. 1). The analytical signals obtained with air or argon did not present statistical differences greater than 5 %, so atmospheric air was chosen as carrier. Its flow rate was tested from 1.2 to 3.5 mL min^{-1}, and the optimized value was chosen as 2.3 mL min^{-1} (*ca.* 0.023 A). Flow rate values > 3.0 mL min^{-1} produced lower analytical signals due to the increase in the pressure at the upper part of the GPS, reducing the antimony hydride permeation through the membrane.

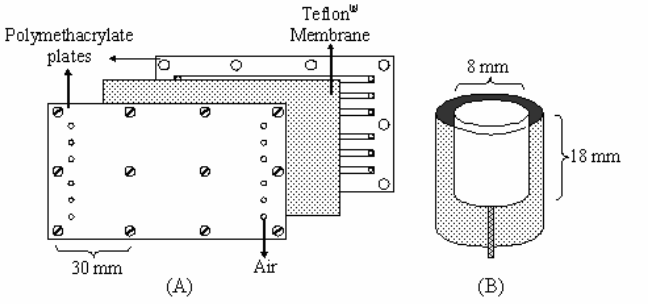

Fig. 2. Detailed view of (A) Gas phase separator (GPS) and (B) De-bubbler device. For details see text.

Antimony hydride formation presents fast kinetics and no reaction coil was necessary after the y confluence point (as can be seen in Fig. 1). On the other hand, a coil was necessary after the z confluence point, where the reaction between antimony hydride and $K_2Cr_2O_7$ in an acidic medium was carried out. The coil allows an increase in the contact area between the gas phase (hydride) and liquid phase ($K_2Cr_2O_7$). On this coil, there is the formation of a segmented flow as commented in section 2.4. The coil length was studied from 10 to 40 cm range and the best result was obtained with 20 cm. With use of a smaller coil, the interaction time between the gaseous and liquid phases is not enough, which promotes a decrease (*ca.* 40 %) in the analytical signal. For longer coils, there is a pressure increase that provokes a decrease in hydride permeation through the Teflon® membrane and a decrease of the analytical signal, as can be seen in Fig. 3.

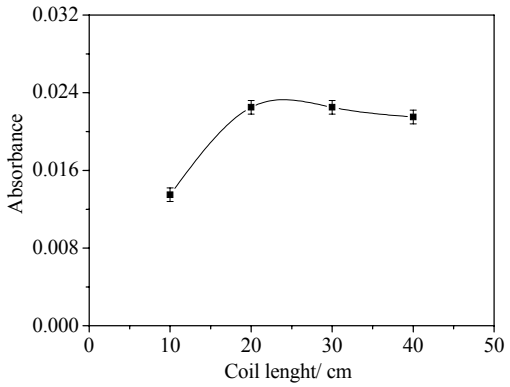

Fig. 3. Influence of the coil length on the analytical response.

The L_1 and L_2 loop volumes were simultaneously studied from 200 to 800 µL and the best results were obtained with volumes above 600 µL (*ca.* 0.032 A). Larger volumes did not present significant increases in the analytical signal.

3.1.2 Chemical variables

The antimony hydride generation only occurs in the presence of nascent hydrogen. This specie is generated through the reduction of the H^+ ion in the presence of KBH_4. The influence of the KBH_4 concentration was studied from 0.2 to 1.0 % (m/v) and the optimized value was 0.6% (0.037 A). Concentrations lower than 0.6% (m/v) are not enough for producing the reaction.

The HCl concentration was studied from 0.05 to 0.15 mol L^{-1} and the results were similar (< 6%) for concentrations higher than 0.08 mol L^{-1} (0.037 A). Thus, 0.10 mol L^{-1} was selected in this study.

The $K_2Cr_2O_7$ concentration is an important chemical variable because the extent of the spectrophotometric reaction is greatly affected. At low concentrations, there is not enough of this reagent to promote the oxidation of Sb(III), reducing the extension of the analytical range. This variable was studied from 0.01 to 0.4 % (m/v) range and the optimized value was 0.2 %. The results are presented in Fig. 4. Additionally, as the reaction is carried out in an acidic medium, the HCl concentration was also tested. It did not present differences larger than 6 % in terms of detectability. Thus, 0.02 mol L^{-1} HCl was chosen in this study.

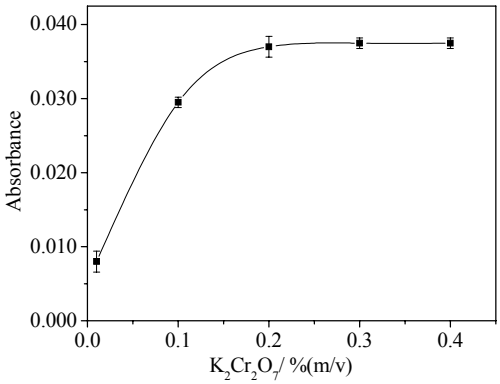

Fig. 4. Influence of the $K_2Cr_2O_7$ concentration on the analytical response.

3.2 Figures of merit and applications

The proposed method was applied to meglumine antimoniate samples used in leishmaniasis treatment. The structure of the meglumine species in those samples is not still completely defined [16]. As this specie might significantly interfere in the Sb determination, a test to determine the extent of the meglumine interference on the proposed method was made. The signal of the 500 mg L^{-1} Sb(III) standard solution (without meglumine) was taken as a true value and compared with signals of the 500 mg L^{-1} Sb(III) standard solution added with 2 % (m/v) meglumine (this concentration is the same as those found in pharmaceutical samples). The results did not present differences higher than 6 %, proving the good selectivity of the method, even in presence of meglumina.

The proposed method presents linear Sb concentration in the 200 to 1000 mg L^{-1} range ($r > 0.997$; $n = 5$). Fig. 5 shows the analytical signals (in triplicate), for blank solution, standard solutions and two different meglumine antimoniate samples. The method presented an analytical frequency of *ca.* 40 h^{-1}. The precision, expressed as RSD, was less than 5.0 % (500 mg L^{-1}; $n = 13$), and 70 mg L^{-1} was obtained as the limit of quantification (LOQ), according to IUPAC recommendations [17]. The Teflon® membrane can be used for three working-periods without significant decreases in sensitivity (< 10%).

The method was used for Sb(III) and total Sb determination in meglumine antimoniate samples. Recovery tests were used as an accuracy check in Sb(III) determination, and the results of four meglumine antimoniate samples are presented in the Table 1. The accuracy check for total Sb determination was obtained with atomic absorption spectroscopy (AAS), and no significant differences at the 95 % confidence levels (*t* test) were seen. Table 2 presents the results obtained for total Sb determination in four others meglumine antimoniate samples by the proposed method and by AAS. Additionally, the average values obtained

for Sb(III) and total Sb determination are in accordance with the literature [9,18,19].

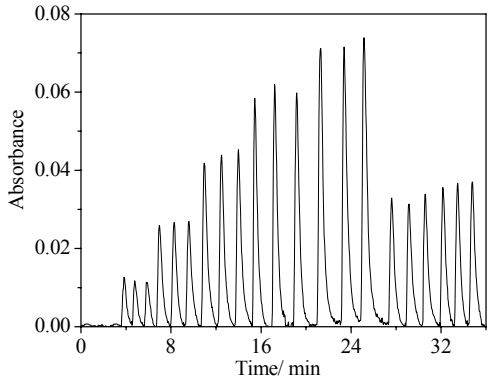

Fig. 5. Analytical signals (in triplicate): blank solution, 200, 400, 600, 800 and 1000 mg L^{-1} Sb(III) standards solutions, and two different pharmaceuticals samples, also in triplicate.

Table 1. Determination of Sb(III) in meglumine antimoniate by spectrophotometric FI-HG.

Sample	Sb(III)/ mg mL^{-1}	Recovery / %
1	5.28 ± 0.06	101
2	4.30 ± 0.05	96
3	6.36 ± 0.20	103
4	6.33 ± 0.06	105

Table 2. Determination of Sb(III) and total Sb by spectrophotometric FI-HG and AAS in meglumine antimoniate samples.

Sample	Sb(III) mg mL^{-1}	Total Sb (PM*) mg mL^{-1}	Total Sb (AAS) mg mL^{-1}
1	5.90 ± 0.11	119 ± 2	115 ± 2
2	6.53 ± 0.10	121 ± 4	113 ± 2
3	4.43 ± 0.11	98 ± 2	102 ± 3
4	3.99 ± 0.11	101 ± 2	104 ± 5

*Proposed method

4. Conclusion

In this work, a new method is described for Sb speciation using both a non-specific spectrophotometric reaction for Sb determination and hydride generation technique. The present method can be considered as an alternative to quality control of pharmaceutical samples. The figures of merit were appropriate for the referred application, and the accuracy and precision allow analyses of real samples. As advantages, accuracy, simplicity, high analytical frequency and low implementation and maintenance costs are presented in this method, which reinforce the possibility of its implementation for routine analysis.

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6. References

[1] Word Health Organization., www.who.int/mediacentre/factsheets/fs116/, accessed on October, (2004).
[2] J. W. Tracy, L. T. Webster, In: A. G. Gilman (Eds.), L. E. Limbird, Goodman & Gilman's The Pharmacological Basic of Therapeutics**,** 9th ed., McGraw-Hill, New York, 1996.
[3] R. J. Cassella, B. A. R. S. Barbosa, R. E. Santelli, A. T. Rangel, *Anal. Bioanal. Chem.,* **379**, 66, (2004).
[4] N. Lewen, S. Mathew, M. Schenkenberger, T. Raglione, *J. Pharm. Biomed. Anal.,* **35**, 739, (2004).
[5] S. B. Adeloju, T. M. Young, D. Jagner, G. E. Batley, *Analyst,* **123**, 1871, (1998).
[6] Y. P. Peña, O. Vielma, J. L. Burguesa, M. Burguera, C. Rondón, P. Carrero, *Talanta,* **55**, 743, (2001).
[7] B. Chen, M. Krachler, W. Shotyk, *J. Anal. At. Spectrom.,* **18**, 1256, (2003).
[8] M. S. Bloomfield, A. D. Dow, K. A. Prebble, *J. Pharm. Biomed. Anal.,* **10**, 779, (1992).
[9] S. Rath, W. F. Jardim, J. G. Dórea, *Fresenius J. Anal. Chem.,* **358**, 548, (1997).
[10] T. Rupasinghe, T. J. Cardwell, R. W. Cattrall, *Anal. Chim. Acta*, **510**, 225, (2004).
[11] J. A. G. Neto, R. Montes, A. A. Cardoso, *Talanta*, **50**, 959, (1999).
[12] J. Sima, P. Rychlovsky, J. Dedina, *Spectrochim. Acta, Part B*, **59**, 125, (2004).
[13] M.Walcerz, E. Bulska, A. Hulanicki, *Fresenius J. Anal. Chem*., **346**, 622, (1993).
[14] H. Bergamin, E. A. G. Zagatto, F. J. Krug, B. F. Reis, *Anal. Chim. Acta*, **101**, 17, (1978).
[15] W. Chan, P. Hont, *Analyst*, **115**, 567, (1990).
[16] C. Demicheli, T. L. Figueiredo, S. Carvalho, R. D. Sinesterra, J. C. D. Lopes, F. Frézard, *BioMetals*, **12**, 63, (1999).
[17] Analytical Methods Committee, *Analyst*, **112**, 199, (1989).
[18] E. M. M. Flores, E. P. Santos, J. S. Barin, R. Zanella, V. L. Dressler, C. F. Bittencourt, *J. Anal. At. Spectrom.*, **17**, 819, (2002).
[19] M. Gallignani, C. Ayala, M. R. Brunetto, M. Burguera, J. L. Burguera, *Talanta*, **59**, 923, (2003).

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