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The determination of pentavalent antimony in sodium stibogluconate in a pharmaceutical formulation by flow injection analysis*

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Abstract: A flow injection analysis (FIA) procedure is described for the determination of pentavalent antimony (Sb^{5+}) in the drug, sodium stibogluconate, in a parenteral pharmaceutical formulation. The sample solution is injected directly into a carrier stream of iodide ion which is then mixed with an acid stream *in situ*. Sb^{5+} is determined by the redox reaction with acidified iodide to liberate iodine, which is monitored spectrophotometrically at 350 nm. The closed conditions prevent interference from atmospheric oxygen and the rapid reaction time assists in minimizing interference from side reactions. The use of tartaric acid as a solvent for sample and standard solutions ensures obedience of Beer's law over the Sb^{5+} concentration range 0.01–0.2% (w/v). The method is specific for the higher oxidation state in an ionic mixture of Sb^{5+} and Sb^{3+}, and has been fully validated for use in a pharmaceutical preparation. Assuming absence of matrix interference it is applicable to Sb^{5+} from other sources and should be applicable to other reducible ionic species.

Keywords: *Flow injection analysis; pentavalent antimony; sodium stibogluconate; iodine–iodide redox reaction; Pentostam injection.*

Introduction

Pentostam injection is an antiparasitic formulation for the treatment of leishmaniasis. The product is the subject of a monograph in the British Pharmacopoeia where the active ingredient, sodium stibogluconate, is controlled as pentavalent antimony (Sb^{5+}). The method, potentiometric titration with ammonium iron(II) sulphate, is slow and lacks precision.

This work describes the development and validation of a rapid method, based upon an established iodine titration procedure [1] and employing flow injection analysis (FIA), to replace this assay. Sodium stibogluconate is determined as its Sb^{5+} content, based upon the simple redox reaction with acidified iodide ion:

$$Sb^{5+} + 2I^- \overset{H^+}{\rightleftharpoons} Sb^{3+} + I_2. \quad (1)$$

With excess iodide ion:

$$I_2 + I^- \rightleftharpoons I_3^-. \quad (2)$$

The liberated iodine, which, under the conditions of the method, is proportional to the amount of Sb^{5+} present, is determined spectrophotometrically at 350 nm [2]. Test and standard solutions are prepared in tartaric acid solution, which ensures a linear response. Solutions are injected into a carrier stream of iodide ion, which is then mixed with an acid stream *in situ*. These closed reaction conditions prevent interference, commonly encountered in the iodine titration procedure, from excess iodine released by atmospheric oxidation of acidified iodide by the reaction:

$$4I^- + O_2 + 4H^+ \rightarrow 2I_2 + 2H_2O. \quad (3)$$

The close control and rapid reaction time achieved in a flow system assist in minimizing interference from side reactions; no interference from complex iodoantimonate ions has been observed.

Conditions have been established such that the method is linear, accurate and precise over the Sb^{5+} concentration range 0.01–0.2% (w/v) by direct injection. The method is applicable to a much wider concentration range by simple adjustment of conditions. The method is specific for the higher oxidation state of antimony in an ionic mixture of Sb^{5+} and Sb^{3+} and can be readily automated. It is applicable to

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Sb^{5+} from other sources and should be applicable in principle to other reducible ionic species.

Experimental

Apparatus

The flow injection manifold is shown schematically in Fig. 1.

The initial development work was carried out using a Gilson 'Minipuls 3' peristaltic pump equipped with acid-resistant tubing, a Magnus Scientific M7100 autosampler equipped with a Rheodyne six-port injection valve, a Cecil Instruments CE 272 spectrophotometric detector equipped with a 10-mm flow cell, and a Bryans Southern Instrument 28000 chart recorder. This equipment was later replaced with a Burkard 'FIAflo' flow injection analyser system equipped with PTFE six-port valves, a Spectra Physics Focus forward optical scanning detector equipped with a 10-mm flow cell, and a Lloyd Instruments Graphic 1002 chart recorder. PTFE tubing (0.5 mm i.d.) was used for all connections and as reaction tubing. A Decon FS 3006 ultrasonic bath was used for preparation of both sample and standard solutions.

FIA conditions

The carrier stream was a 1% (w/v) aqueous solution of potassium iodide, pumped at a constant flow rate of approximately 0.5 ml min^{-1}, while the acid stream was 2 M sulphuric acid, pumped at a constant flow rate of approximately 1.8 ml min^{-1}.

The carrier stream tubing was a 30-cm length (measured from the injection point to the confluence with the reagent stream) of 0.5-mm i.d. PTFE tubing, and the reaction coil was a 400-cm length of 0.5-mm i.d. PTFE tubing. The injection volume was 20 μl. A detection wavelength of 350 nm was employed with a sensitivity of 0.5 a.u.f.s.

Reagents

Sulphuric acid (2 M) was prepared by carefully diluting 98% sulphuric acid with water. Ninety-eight per cent sulphuric acid, tartaric acid and potassium iodide were of analytical reagent grade and obtained from BDH Chemicals (Poole, Dorset, UK).

Sample solution

A 2.0 ml aliquot of Pentostam injection (Wellcome Foundation Ltd, UK; containing nominally 10% w/v Sb^{5+}) and 20 ml of 2 M sulphuric acid were transferred into a 200-ml volumetric flask and ultrasonicated for 5 min. After allowing the mixture to cool to ambient temperature, 100 ml of 20% (w/v) aqueous tartaric acid solution was added. The mixture was diluted to volume with water and ultrasonicated for a further 5 min. This constituted the sample solution.

Standard solution

Sodium antimonate (BDH Chemicals, Poole, Dorset, UK), a soluble salt of known and reproducible Sb^{5+} content, was selected as the standard. A bulk sample of this material was previously standardized for Sb^{5+} content using potentiometric titration with ammonium iron(II) sulphate; this sample was then used as a standardized reference material. An accurately weighed amount of sodium antimonate (approximately 0.21 g, equivalent to 0.1 g of Sb^{5+}) was transferred into a 100-ml volumetric flask. Fifty millilitres of 20% (w/v) aqueous tartaric acid were added and the mixture ultrasonicated for 10 min. The mixture was cooled to ambient temperature, 10 ml of 2 M sulphuric acid added, and then diluted to volume with water to give the standard solution.

Procedure

The carrier and acid streams were pumped through the manifold until a stable baseline

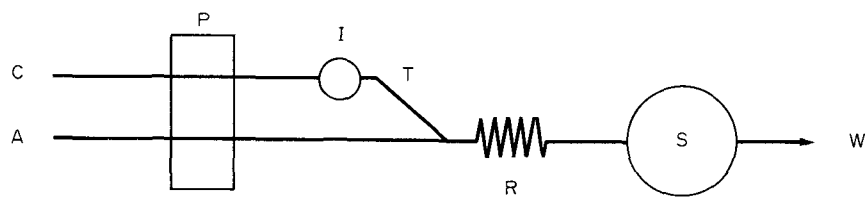

Figure 1
Schematic flow injection manifold. Key: C, 1% (w/v) aqueous potassium iodide solution carrier stream; A, 2 M sulphuric acid stream; P, peristaltic pump; I, 20 μl injection port; T, carrier stream tubing, 30-cm long, 0.5-mm i.d.; R, reaction coil, 400-cm long, 0.5-mm i.d.; S, spectrophotometer with a detection wavelength of 350 nm, 0.5 a.u.f.s. typical sensitivity; and W, waste.

was obtained. Portions of the standard solution were injected until the repeatability of the measured peak heights was satisfactory. The sample solutions were then injected sequentially, concluding the analysis with further injections of the standard solution. By comparison of the peak heights of sample and the mean of the bracketing standard injections, the Sb^{5+} content of the product was calculated.

Results and Discussion

Method development

An established iodine titration method, based upon the redox reaction of Sb^{5+} with acidified iodide and consequent titration of the librated iodine with sodium thiosulphate, was investigated for this product, as an alternative to the BP procedure. Atmospheric oxygen interferes with this titration, so air was excluded from the system by addition of sodium carbonate to the acid solution to create a carbon dioxide blanket. Nevertheless, a high bias in results (approximately 5%) was obtained with this titration.

It was decided to investigate spectrophotometric detection of the liberated iodine in a two-stream FIA manifold. This arrangement was designed such that independent iodide and acid streams combine *in situ*, thus excluding interference from atmospheric oxygen.

A spectral scan of the iodine peak was taken at the peak apex using a rapid scanning detector, during initial work with a sodium stibogluconate standard in dilute sulphuric acid solution, as shown in Fig. 2. To avoid potential interference from other components in the formulation, the longer wavelength absorbance maximum at 350 nm was chosen in preference to the maximum at approximately 288 nm.

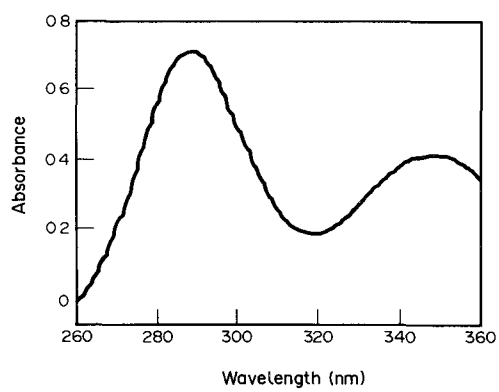

Figure 2
A real-time spectral scan of iodine taken at the peak apex from an injection of sodium stibogluconate standard solution. The scan was generated using a forward optical rapid scanning detector, over the upper deuterium wavelength range of 260–360 nm with a 2-nm interval and fourfold spectral repetition.

In the two-stream manifold developed the peak height was found to be proportional both to the concentration of the iodide and to that of the acid stream. Based upon an initial assumption that conditions of high iodide concentration would be optimal to prevent depletion of this ion, and hence overloading of the reagent, suitable peaks within the detector is absorbance range were obtained using 20% (w/v) potassium iodide in water as a carrier stream and an acid stream of 0.1 M sulphuric acid. However, a linearity experiment over the range 0.05–0.15% (w/v) Sb^{5+} in dilute sulphuric acid solution, revealed a significant deviation from Beer's Law. A progressive change to give conditions of lower iodide concentration and higher acid concentration improved the linearity. Nevertheless, optimum results were only obtained with the use of very dilute sample and standard solutions, over the range 0.01–0.03% Sb^{5+} in dilute sulphuric acid solution, at the expense of a loss of sensitivity. Sensitivity was not significantly improved by the introduction of soluble starch to produce a more strongly coloured complex. This was found to rapidly decompose under the strong acid conditions of the method.

A marked improvement resulted when sample and standard solutions were prepared in aqueous tartaric acid solution, a complexing agent which had initially been used to dissolve the sodium antimonate standard material. Beer's law was obeyed and the method was found to be rugged to substantial (100%) changes in reagent concentrations. Sample and standard preparation procedures were introduced to ensure complete ligand exchange of Sb^{5+} from sodium stibogluconate and sodium antimonate to the tartaric acid complex.

Peak height was studied as a function of the reaction coil length at two pump rotation speeds (the ratio of flow rates for the carrier and acid stream was held constant); the results obtained are shown in Fig. 3. For the faster flow rate, coil lengths >1100 cm were not pursued due to increasing and unacceptable pressure levels. It was apparent that the reaction was quite slow and equilibrium was only reached with long reaction coil lengths; at even longer coil lengths, dispersion of the iodine

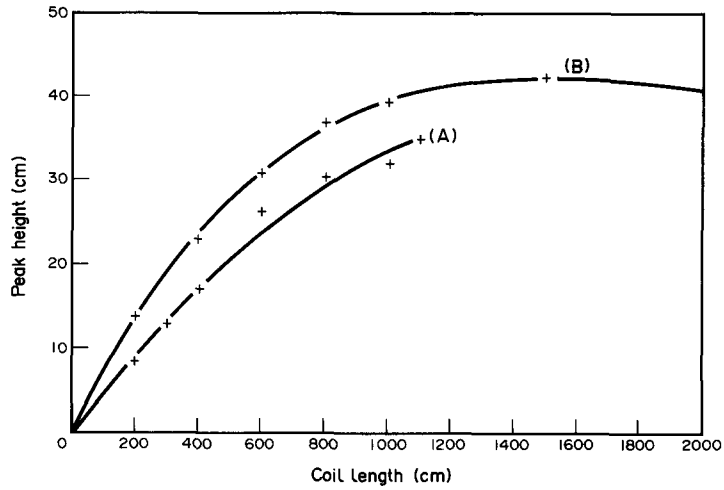

Figure 3
Graph showing peak height as a function of reaction coil length. The flow rates, as a combination of both carrier and acid streams are shown. (A) 3.0 ml min^{-1}, and (B) 2.3 ml min^{-1}. At the 3.0 ml min^{-1} flow rate longer coil lengths were not examined due to an unacceptable build up of pressure.

became the predominant factor and smaller peaks resulted. Additionally, Beer's Law was no longer observed at high standard solution concentration levels (*ca* 0.2% w/v Sb^{5+} in tartaric acid solution) when reaction coil lengths exceeded 800 cm. A reaction coil length of 400 cm was judged to be optimum, combining excellent sensitivity and peak shape with short and practicable reaction times. Typical FIA traces for standard, sample and inert solutions are shown in Fig. 4.

Validation

Validation of the method was carried out specifically for the determination of sodium stibogluconate in Pentostam injection. Assuming absence of any significant matrix effects, these studies should also be largely applicable to the determination of Sb^{5+} in other solutions.

Linearity of the method. The linearity of the method over the concentration range 0.05–0.15% (w/v) Sb^{5+} in tartaric/sulphuric acid (equivalent to 0 to 150% of the nominal assay concentration of 0.10% w/v) was confirmed by injecting a series of eight standard solutions at levels of 50, 80, 90, 100, 110, 120 and 150% of the nominal concentration. A linear response of peak height (as a percentage of the nominal 100% standard) vs concentration (% of formula content) was obtained: $y = 1.003x - 0.18$ (correlation coefficient = 0.9999; standard error of the slope, 0.0065; $n = 8$).

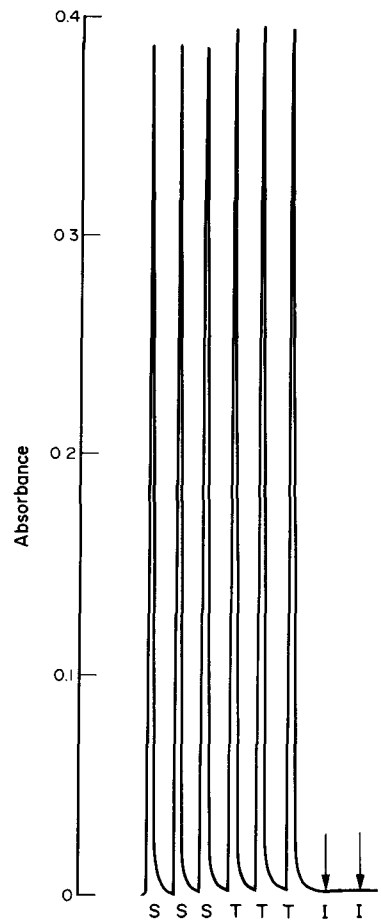

Figure 4
Typical FIA traces. Key: S, standard solution; T, sample solution; and I, inert formulation.

Specificity. An inert formulation (containing chlorocresol and glucono-delta-lactone) omitting only the sodium stibogluconate was examined by the method. No response was obtained. Additionally, a solution of potassium antimonyl tartrate (Aldrich Chemicals, analytical reagent grade) containing Sb^{3+} at a level equivalent to that of the Sb^{5+} ion in the product, was examined by the method. A response of 0.2% with respect to the formulation matrix of Sb^{5+} was obtained. This low level of response was considered acceptable, and the method was judged to be specific in the presence of other components in Pentostam injection and in the presence of trivalent antimony.

Accuracy. The accuracy of the method was determined by recovery experiments. In duplicate experiments, known quantities of sodium stibogluconate were added to an inert formulation at amounts equivalent to 80 and 120% of the formula content, and the mixtures were examined by the method. Recoveries of 101.0 and 103.7% of the added amount for the addition of 80% of nominal concentration and 98.7 and 99.5% of the added amount for 120% were found, respectively. An experiment at the nominal 100% level (0.10% w/v) was performed six times. This gave a mean of 100.5% with a relative standard deviation (RSD) of 1.2%. The 95% confidence interval of the mean value was 100.5 ± 1.3%.

Precision. The repeatability of the method was assessed by carrying out 10 injections of a standard solution on a single occasion. A RSD of 1.1% was obtained for the peak height.

For reproducibility, two operators each performed four independent assays on the same batch of product. The mean (as a percentage of the formula content) and RSD of all eight were 100.8 and 0.9% with a 95% confidence interval for the mean value of 100.8 ± 0.8.

Efficiency of ligand exchange. To demonstrate the efficiency of transfer of Sb^{5+} from sodium stibogluconate to the tartaric acid complex, a form in which Sb^{5+} reacts rapidly with acidified iodide ion, comparative assays on a single batch of Pentostam injection were performed using final ultrasonication times of 5, 10 and 15 min. It was apparent from earlier work that this last stage was the critical step. Results of 101.2, 100.5 and 100.3% formula content were obtained, respectively, at each of these times. It is concluded that an ultrasonication time of 5 min ensures adequate release of Sb^{5+} from the sodium stibogluconate molecule.

Equivalence of methods. As a further check on accuracy, the method was applied to three batches of product examined both by FIA and by titration with ammonium iron(II) sulphate, as described in the British Pharmacopoeia. The assay results (per cent formula content) obtained were 100.8, 99.1 and 101.2 by FIA, compared with 100.4, 99.3 and 101.6, respectively, by the BP method. These data demonstrate that the FIA results are equivalent to results obtained by the BP titration procedure.

Conclusions

Flow injection analysis has been applied to the assay of sodium stibogluconate, as pentavalent antimony, in Pentostam injection. The method is much faster than the current titration procedure of the British Pharmacopoeia, and can be readily automated. The method should be applicable to pentavalent antimony from other sources provided that no significant matrix effect is observed and should be applicable to other reducible ionic species.

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