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Flow-injection turbidimetric determination of homatropine methylbromide in pharmaceutical formulations using silicotungstic acid as precipitant reagent

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Abstract

A flow-injection turbidimetric procedure exploiting merging zones is proposed for determining homatropine methylbromide (HMB) in pharmaceutical preparations. The determination is based on the precipitation reaction of homatropine methylbromide with silicotungstic acid in acidic medium to form a precipitate, which was measured at 410 nm. The analytical curve was linear in the HMB concentration range from 8.1×10^{-5} to 2.2×10^{-4} mol l^{-1}, with a detection limit of 5.0×10^{-6} mol l^{-1}. The recoveries ranged from 96 to 103%, the sampling frequency was 70 determinations per hour and relative standard deviations were less than 1.5% ($n = 10$). The results obtained for commercial formulations using the FIA procedure were in good agreement with those obtained by using a comparative method.

Keywords: Homatropine methylbromide; Flow injection; Turbidimetry; Pharmaceutical formulations

1. Introduction

Antimuscarinic compounds are drugs which play an important role in the central nervous system. The most widely used are atropine, scopolamine, homatropine and homatropine methylbromide (HMB). Atropine and scopolamine, also referred to as (±)-hyoscyamine and hyoscine, respectively, are extracted from plant species belonging to the Solanaceae family. Homatropine prepared synthetically by esterification of mandelic acid with 3α-tropine, is structurally related to atropine and scopolamine [1]. Its effects correspond to those of atropine but are 10 times less pronounced [2] and with the addition of a second methyl radical results in homatropine methylbromide. This compound is better than the atropine because it shows less toxicity in the central nervous system [3]. These drugs are utilized in several clinical situations such as in ophthalmic diagnosis as a mydriatic as well as anticholinergic, antispasmodic and preanesthetic agents [4]. Homatropine methylbromide and dimethicone are also used as antispasmodic and the dose recommended for adults is from 12 to 16 mg kg^{-1} per day and for children is 0.1 mg kg^{-1} per day. But in infants up to two months, daily use may cause episodes of transitory disturbances, like the typical symptoms of the basal ganglia dysfunction, characterized by repeated crises of short duration with tonic back-shift of the head (opisthotonos), deviation of the eyes upward with a looking-fixed and terrified expression and emission of crying and/or guttural sounds [3].

There are very few analytical methods for the determination of homatropine methylbromide in pharmaceutical products. Hanna et al. [5] developed a spectrophotometric method based on the formation of a picric acid–quaternary ammonium complex, which is adsorbed on acid-washed diatomaceous earth in alkaline medium followed by on-column chloroform extraction and spectrophotometric detection at 365 nm. An HPLC method [6] has been proposed in the literature. In this method, a Nucleosil 5 C_8 as stationary phase and acetonitrile plus 0.01 mol l^{-1} phosphate buffer solution at pH 5.0 and 2:3 (v/v) as mobile phase are employed. In the USP method [7], 700 mg of sample are dissolved in 50 ml of glacial acetic acid and 10 ml of 6.0×10^{-2} g ml^{-1} mercuric acetate. After the addition of one drop of crystal violet, the homatropine methylbromide is titrated with 0.1N perchloric acid to a blue-green endpoint. In the Brazilian Pharmacopoeia method [8], bromide anion of the drug is titrated with 0.1 mol l^{-1} silver nitrate solution. In this procedure a metallic silver indicator electrode and an Ag/AgCl

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double junction reference electrode were used and each ml of the silver nitrate solution consumed corresponds to 37.028 mg of the homatropine methylbromide. Nevertheless, these methods are time-consuming, utilize very costly equipment and/or toxic reagents/organic solvents.

Krug et al. [9] were the first to report the use of turbidimetry in the flow-injection system for determining sulfate by monitoring the barium sulfate suspension. In spite of the routine use of flow-injection system with turbidimetric detection for the determination of inorganic species in plants and water [10], applications to pharmaceutical products are limited [11–13]. Recently, our group proposed a flow-injection turbidimetric method for determining tannin content in tea samples using copper(II) in acetate medium as the precipitant reagent [14].

This paper describes a fast, simple and accurate flow-injection turbidimetric procedure for the determination of homatropine methylbromide in pharmaceutical products. The proposed method is based on the precipitation of homatropine methylbromide with silicotungstic acid in acidic medium to form a precipitate in suspension that is determined turbidimetrically at 410 nm. Eq. (1) shows the reaction between homatropine methylbromide and silicotungstic acid.

$$4C_{16}H_{21}NO_3 \cdot CH_3Br_{(aq)} + H_4Si(W_3O_{10})_{4(aq)} \rightleftharpoons [C_{16}H_{21}NO_3 \cdot CH_3]_4[Si(W_3O_{10})_4]_{(s)} + 4HBr_{(aq)} \quad (1)$$

2. Experimental

2.1. Apparatus

A Hewlett-Packard (Boise, ID, USA) model 8452A UV–vis spectrophotometer with a quartz cell (optical path 1.0 cm) was used in the preliminary experiments.

A 12-channel Ismatec (Zurich, Switzerland) model 7618-50 peristaltic pump supplied with Tygon tubes was used for the propulsion of the solutions. The manifold was constructed with polyethylene tubes of 0.8 mm i.d. Sample and reference solutions were injected into the carrier streams using a laboratory-built three-piece manual injector-commutator made of Perspex®, with two sidebars and a sliding central bar. A Femto (São Paulo, Brazil) model 432 spectrophotometer equipped with a glass flow-cell (optical path of 1.0 cm) at 410 nm was used to monitor the absorbance signal of the insoluble ionic-pair ($[C_{16}H_{21}NO_3{\cdot}CH_3]_4[Si(W_3O_{10})_4]_{(s)}$) yield in the reaction shown in Eq. (1). Transient signals were recorded on a Cole Parmer (Chicago, IL, USA) model 1202-000 two-channels strip-chart recorder.

In the comparative method a metallic silver indicator electrode and an Ag/AgCl double junction reference electrode were used (Brazilian Pharmacopoeia) [8].

2.2. Reagents and solutions

The reagents and solutions were prepared with water from a Millipore (Bedford, MA, USA) Milli-Q system (model UV Plus Ultra–Low Organics Water), and all reagents were of analytical reagent grade.

A 0.05 mol l^{-1} HCl solution was prepared by dilution of 8.5 ml HCl (Merck) in 2000 ml and 25 ml of this solution was standardized with a 0.1 mol l^{-1} NaOH standard solution. A 500 mg l^{-1} homatropine methylbromide stock solution was prepared by dissolving 0.0215 g of homatropine methylbromide (Henrifarma, Brazil) in a 25 ml calibrated flask with 0.05 mol l^{-1} HCl solution. Reference solutions were prepared by proper dilution of the stock solution with 0.05 mol l^{-1} HCl solution.

A 1.0×10^{-3} mol l^{-1} silicotungstic acid stock solution was prepared by dissolving 71.950 mg of this acid (Sigma, USA) in a 25 ml calibrated flask with 0.05 mol l^{-1} HCl solution. Reagent solutions were prepared by proper dilution of the stock solution with the same HCl solution.

2.3. Sample preparations

Homatropine methylbromide was determined in liquid samples purchased from a local pharmacy. A suitable aliquot of each sample was transferred to a 10 ml calibrated flask and diluted to a volume with 0.05 mol l^{-1} HCl.

2.4. Flow diagram

Fig. 1 shows a schematic diagram of the flow-injection merging zones system used in this work. In the injection position, the reagent (L_1, 125 μl) and the sample or reference solution (L_2, 375 μl) were injected simultaneously as individual zones into the 0.05 mol l^{-1} HCl carrier streams (C_1 and C_2; flowing at 2.0 and 3.9 ml min^{-1}, respectively) and merged at the confluence point X. The precipitate formed in the reaction coil B (0.8 mm i.d., 50 cm) was transported to the flow-cell (D) and was monitored at 410 nm.

3. Results and discussion

3.1. Synchronicity and influence of manifold parameters studies

Preliminary studies were carried out to establish the best synchronicity between the sample or reference (S) and reagent solution (R). So, the flow system was evaluated using a colored compound (0.01%, m/m, potassium hexacyanoferrate(III)) as reagent solution (R) and 0.05 mol l^{-1} HCl instead of the sample solution (S) in the flow system diagram shown in Fig. 1. A systematic study was then made maintaining the flow rate of the sample solution in 3.9 ml min^{-1} and varying the flow rate of the reagent solution in the range from 1.5 to 4.7 ml min^{-1}. Afterwards, the flow rate of the reagent solution was fixed in 3.9 ml min^{-1} and the flow rate of sample solution (0.05 mol l^{-1} HCl solution) was changed in the range from 1.5 to 4.7 ml min^{-1}. Fig. 2 shows the best synchronicity obtained for a sample solution flow rate of 3.9 ml min^{-1} and reagent solution flow rate of 2.0 ml min^{-1}. Therefore those flow rates were selected for further studies.

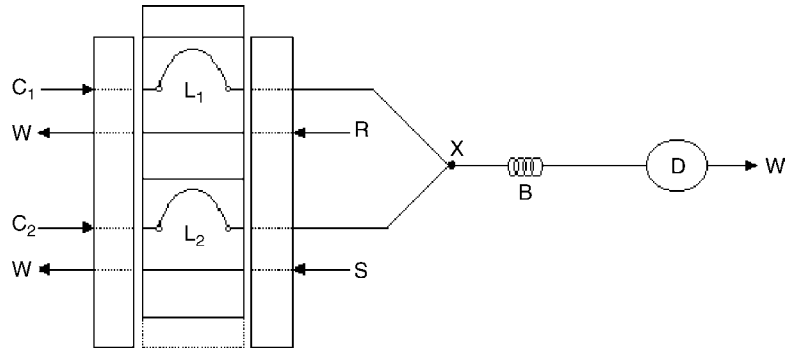

Fig. 1. Diagram of the FI system with merging zones for homatropine methylbromide determination using silicotungstic acid solution as precipitant reagent. C_1, reagent carrier solution (0.05 mol l^{-1} HCl), flowing at 2.0 ml min^{-1}; C_2, sample carrier solution (0.05 mol l^{-1} HCl), flowing at 3.9 ml min^{-1}; S, sample or reference solution in 0.05 mol l^{-1} HCl; R, reagent solution (1.0×10^{-3} mol l^{-1} silicotungstic acid in 0.05 mol l^{-1} HCl); L_1, reagent loop (25 cm; 125 μl); L_2; sample loop (75 cm; 375 μl); X, confluence point; B, coiled reactor (50 cm); D, spectrophotometric cell at 410 nm and W, waste.

The merging zones configuration was chosen because very small volumes of reagent (silicotungstic acid) were consumed in each injection and provided good repeatability, baseline stability and lower washing time. An asymmetrical merging zones flow system similar to that proposed by Bergamin-Filho et al. [15] was developed in order to decrease the sample dilution at point X (Fig. 1), thus leading to higher sensitivity. The manifold parameters were conveniently determined by using a diluted yellow solution (0.01%, m/m, potassium hexacyanoferrate(III)) in the study of the synchronization of the sample and reagent zones to each variation in the flow rate of the sample or reagent carrier stream. In order to check these preliminary results, additional experiments were carried out. The flow rates of the sample and reagent carrier streams were optimized in the flow rate range from 1.5 to 4.7 ml min^{-1} with L_1 and L_2 loop volumes of 125 and 250 μl, respectively. In this study a 2.5×10^{-3} mol l^{-1} silicotungstic acid solution and three different concentrations of homatropine methylbromide (6.75×10^{-5}, 1.35×10^{-4} and 2.02×10^{-4} mol l^{-1}) inserted into the 0.05 mol l^{-1} HCl carrier stream were used. Flow rates of 3.9 and 2.0 ml min^{-1} were selected for the sample and reagent carrier streams, respectively, as a compromise between sensitivity and throughput. A higher flow rate of the sample carrier stream was found to give the best sensitivity probably due to lower dispersion of the sample zone.

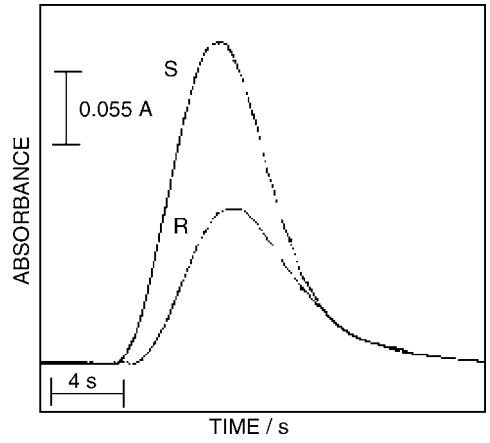

Fig. 2. Synchronicity study. S, sample (0.05 mol l^{-1}HCl), flowing at 3.9 ml min^{-1} and R, reagent solution (0.01%, m/v, potassium hexacyanoferrate(III)), flowing at 2.0 ml min^{-1}.The others variables were as described in Fig. 1.

The effect of the sample and reagent injection volumes inserted was studied by varying the volumes of L_1 and L_2 loops between 50–500 and 50–750 μl, respectively. The absorbance increased with increasing volumes by 500 μl for sample and 125 μl for reagent, above which it remained practically constant. Sample and reagent volumes of 375 and 125 μl were chosen as a good compromise between washing time and stability of the baseline.

The effect of the reactor coil length (B) was investigated in the range from 30 to 200 cm at flow rates selected above. In this study, homatropine methylbromide reference solutions in the concentration range from 6.7×10^{-5} to 2.0×10^{-4} mol l^{-1} were used. The peak heights increased with increases of the reaction coil up to 50 cm, above which a slight decrease was observed probably due to the dispersion of the sample zone. A 50 cm reaction coil was selected for all further experiments.

3.2. Effect of the acidity

The effect of the carrier solution (HCl) was studied in the concentration range from 0.01 to 1.5 mol l^{-1}. For each HCl concentration, homatropine methylbromide in the range from 6.7×10^{-5} to 2.0×10^{-4} mol l^{-1} were injected in triplicate. The best signal (S/N) and stability of the baseline were obtained in 0.05 mol l^{-1} HCl solution. A decrease of analytical signal was observed for HCl concentrations higher than 0.05 mol l^{-1}, probably due to the low dissociation degree of silicotungstic acid, resulting in a decrease of the interaction between homatropine methylbromide and the anion of this acid.

3.3. Effect of the reagent concentration

The effect of the silicotungstic acid concentration was studied in the concentration range from 2.5×10^{-4} to 8.0×10^{-3} mol l^{-1} for homatropine methylbromide solutions in the range from 6.7×10^{-5} to 2.0×10^{-4} mol l^{-1}. The best sensitivity and stability of the baseline were obtained in 1.0×10^{-3} mol l^{-1} silicotungstic acid solution. It was observed that for silicotungstic acid concentrations higher than

Table 1
Recoveries from samples spiked with three different amounts of homatropine methylbromide

Sample	Homatropine methylbromide (mg l^{-1})		
	Added	Found	Recovery (%)
A	34.1	34.8 ± 0.9	102
	41.7	41.7 ± 0.8	100
	49.2	50.2 ± 0.6	102
B	34.1	35.2 ± 0.8	103
	41.7	40.9 ± 0.7	98
	49.3	47.3 ± 0.9	96

$n = 3$.

1.0×10^{-3} mol l^{-1} the analytical signal gradually decreased followed by irreproducible results. This loss in the sensitivity is probably due to a variation in the size and number of precipitate particles [10,13]. Therefore, 1.0×10^{-3} mol l^{-1} silicotungstic acid solution was selected for further experiments.

3.4. Interference and recovery studies

The excipients commonly used in pharmaceutical preparations, methylparabene and propylparabene, were evaluated as potential interference in the proposed flow-injection procedure. In this study, two known concentrations, 9.2×10^{-4} mol l^{-1} (1:1) and 9.2×10^{-3} mol l^{-1} (1:10) of these substances were injected in the flow system together with a 9.2×10^{-4} mol l^{-1} homatropine methylbromide reference solution. No interference in the flow-injection procedure was observed.

In the recovery study from commercial samples three concentrations: 34.1, 41.7 or 49.2 mg l^{-1} of homatropine methylbromide were added to each sample. Recoveries between 96 and 103% were obtained, as can be seen in Table 1, which is good evidence of the accuracy of the proposed procedure.

3.5. Calibration graph and applications

The proposed flow-injection system under the optimized conditions was applied to determine homatropine methylbromide in commercial pharmaceutical formulations. The results of the analysis of homatropine methylbromide are presented in Table 2. As can be seen, close agreement between the determinations of homatropine methylbromide by the proposed flow-injection procedure and the potentiometric method (Brazilian Pharmacopoeia, 2003) (*t*-test) were obtained for all samples. In addition, the results agreed with those declared on the labels, confirming the accuracy of the flow-injection turbidimetric method. The calibration graph for homatropine methylbromide was linear in the concentration range from 8.1×10^{-5} to 2.2×10^{-4} mol l^{-1} with a detection limit of 5.0×10^{-6} mol l^{-1}. The regression equation was $A = -0.018 + 0.0066C$; $r = 0.996$, where A is the absorbance and C the concentration of homatropine methylbromide in mol l^{-1}. The relative standard deviations for 10 successive measurements of 1.08×10^{-4} and 1.62×10^{-4} mol l^{-1} homatropine methylbromide were <1.5% and 70 measurements h^{-1} were obtained.

Table 2
Comparison of results obtained by potentiometric and proposed FI method for homatropine methylbromide

Sample	Homatropine methylbromide (g l^{-1})			Relative error (%)	
	Label value	Potentiometric [15]	FI method	Re_1	Re_2
A	2.00	1.98 ± 0.01	2.02 ± 0.01	1.0	2.0
B	2.00	2.01 ± 0.02	2.04 ± 0.02	2.0	1.5

Confidence level of 95% ($n = 3$). Re_1 = FI method vs. label value. Re_2 = FI method vs. potentiometric procedure.

4. Conclusions

The flow-injection merging zones procedure developed in this work allows the determination of homatropine methylbromide in several pharmaceutical products using silicotungstic acid as precipitant reagent and did not show significant differences in analytical performance when compared with other methods. The analytical curve was linear in the HMB concentration range from 8.1×10^{-5} to 2.2×10^{-4} mol l^{-1}, with a detection limit of 5.0×10^{-6} mol l^{-1} and relative standard deviations were smaller than 1.5% ($n = 10$). The amount of the reagent consumed per determination was 0.36 mg.

The proposed method is accurate, precise, economical and presented an analytical frequency of 70 h^{-1}.

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