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Simple and selective spectrophotometric determination of atracurium besylate in pharmaceutical formulations using acidic dyes

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ABSTRACT

Two simple and sensitive extraction spectrophotometric methods have been developed for the determination of atracurium besylate in commercial dosage forms. These methods (A and B) are based on ion-pair complex formation reaction between atracurium besylate with acidic dyes alizarin red S (Method A) & tropaeoline ooo (Method B) in chloroform to give highly colored complex species that absorb maximally at 420 and 480 nm, respectively. Beer's law was obeyed in the concentration limit of 2.5–9.0 µg/mL for method A and 1.6–8.0 µg/mL for method B. The proposed methods were found to be rapid, accurate, precise, and sensitive for the determination of atracurium besylate in commercial dosage forms without interferences from common additives. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS

Atracurium besylate;
Ion-association;
Spectrophotometry.

INTRODUCTION

Atracurium besylate[1] (ACB) is a nondepolarizing neuromuscular-blocking agent used as an intermediate-duration, skeletal muscle relaxant, adjunctively in anesthesia to facilitate endotracheal intubation and to provide skeletal muscle relaxation during surgery or mechanical ventilation. It is useful in hepatic or renal disease. Chemically designated as 2,2' – [1,5 – pentanediyl bis [oxy (3-oxo-3,1-propanediyl)]] bis[1-[(3,4-di methoxy phenyl) methyl] – 1,2,3,4-tetrahydro – 6,7-dimethoxy – 2 – methyl isoquinolinium] dibenzene sulfonate. The chemical formula is $C_{65}H_{82}N_2O_{18}S_2$. Literature survey revealed that few analytical methods have been reported for determination of atracurium besylate in pure drug, pharmaceutical dosage forms and in biological samples using HPLC[2-8] and Derivative spectrophotometry[9], Liquid Chromatography – Mass spectrometry[10] either in single or in combined forms. The aim of the present work is to develop a simple, fast, accurate and reliable UV spectrophotometric methods for the determination of ACB.

EXPERIMENTAL

Materials and methods

Spectral and absorbance measurements were made with digital Elico UV-Vis spectrophotometer SL 159 and pH measurements were made with Digisun Electronics digital pH meter model DI-707.

All the chemicals and reagents were of analytical grade and the freshly prepared solutions were always used in the investigations.

Aqueous solutions of 5.84 x 10^{-3}M alizarin red S

(ARS) for method A and 5.70 x 10^{-3} M tropaeolin ooo (TP ooo) for method B were prepared. 0.1M HCl and chloroform was used in both methods A&B.

A 1 mg/ml solution was prepared by dissolving 100 mg of pure atracurium besylate (ACB) in minimum amount of 0.1 N HCl followed by dilution to 100 ml with distilled water and the stock solution was diluted stepwise with distilled water to get working standard atracurium besylate solution of concentrations of 100 µg/ml (Method A) and 80 µg/ml (Method B).

Assay

For Methods A & B

Into a series of 125 ml separating funnels containing aliquots of standard ACB solution [0.5 – 2.5 ml, 100 µg/ml (Method A) & 80 µg/ml (Method B)], 6.0 ml of 0.1M HCl and 2.0 ml of dye solutions (ARS and TP ooo) were added. The total volume of aqueous phase in each separating funnel was adjusted to 15 ml with distilled water and organic layer to 10 ml with chloroform. The contents were shaken for 2 minutes. The two phases were allowed to separate and the absorbances of the separated chloroform layer were measured at λ_{max} 420 nm (Method A) & 480 nm (Method B) against a similar reagent blank. The amount of ACB present was deduced from the appropriate calibration curve.

The method has also been applied to pharmaceutical formulations. The measured volume of injection sample such as Tracium equivalent to 100 mg of ACB was taken and treated with (3x25ml) portions of methanol. The combined methanol extract was made up to 100 ml with the same solvent to get mg/ml stock solution. From the methanol extract (20ml), CH_3OH was gently evaporated. The residue was dissolved in 0.2 ml of 0.1 N HCl and subsequently the volume was diluted to the mark with distilled water and mixed well. The content of the flask which was at a concentration of 100 µg/mL (Method A) and 80 µg/ml (Method B) was subjected to analysis by the procedure described above after suitable dilution step.

The UV spectrophotometric method which was suggested for the identification of ACB in water has been moulded for its assay and chosen as a reference method for ascertaining the accuracy of the proposed methods. The results are compared with those obtained using UV spectrophotometric method in water at 279.6 nm.

RESULTS AND DISCUSSION

Chemistry

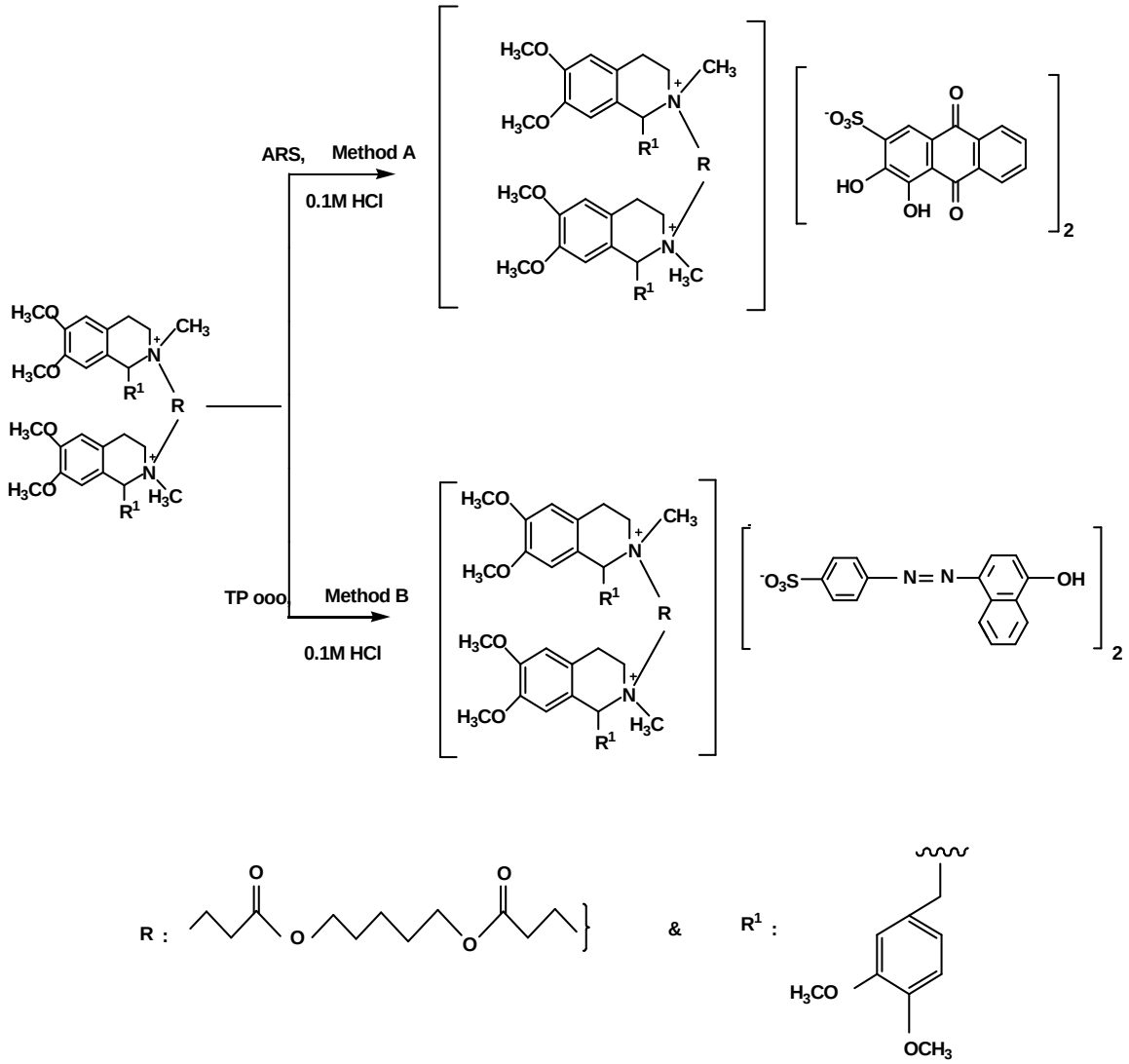
In the developed method, ACB possesses a quaternary nitrogen group, it forms an ion-association complex with an acid dye ARS or TP ooo to produce coloured chromogens exhibiting λ_{max} at 420 nm (Method A) and 480 nm (Method B) which are extractable into chloroform from the aqueous phase. The protonated nitrogen (positive charge) of ACB as besylate is expected to attract the oppositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction. Based on analogy the structures of ion-association complexes are shown in scheme 1.

Optimization of experimental parameters

The experimental factors affecting the development and stability of the product were studied and optimized. Such factors include choice of the solvent, concentration of the dye, and reaction time. Chloroform was preferred as the most suitable solvent to carry out the experiments because in this medium, the reagent blank gave negligible blank absorbance and the ion-pair complex formed was found to exhibit higher sensitivity and stability. The influence of the concentration of ARS and TP ooo on the intensity of the color developed at the selected wavelength was studied using different amounts (0.5–2.5 mL) of 0.2% each of ARS and TP ooo in method A and B respectively. The constant absorbance readings were obtained between 1.0 and 2.5 mL of 0.2% of dyes in both methods, 2 mL of 0.2% dye solution was sufficient to produce maximum and reproducible color with minimum blank absorbance in both methods. The optimum reaction time for the development of color at ambient temperature (°C) was studied and it was found that the addition of the dye solutions resulted in an immediate full color development. The formed ion pairs were stable for at least 60 min in both methods.

Method validation

Under optimum experimental conditions, the optical characteristics such as Beer's law limits, absorption maxima, molar absorptivity, Sandell's sensitivity are



Scheme 1

TABLE 1 : Optical characteristics, precision, accuracy of the methods proposed in the determination of atracurium besylate

S.No.	OPTICAL CHATACTERISTICS	METHOD A	METHOD B
1.	λ_{max} (nm)	420	480
2.	Beer's Law Limits (µg/ml)	2.5 - 9.0	1.6 – 8.0
3.	Molar absorptivity (1 $mol^{?1}cm^{?1}$)	4.54 x 10^4	4.74 x 10^4
4.	Correlation coefficient (r)	0.9990	0.9999
5.	Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.092	0.086
6.	Regression Equation (y = a+bC) (i) Slope (b)	0.036	0.039
	(ii) Intercept (a)	-0.0006	-0.0003
7.	Relative Standard Deviation *	0.860	0.947
8.	% of range error (confidence limit) (i) 0.05 level	0.903	0.994
	(ii) 0.01 level	1.416	1.559

* **Average of six determinations considered**

presented in TABLE 1. The regression analysis using the method of least squares was made for the slope

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(b), intercept (a) and correlation (R) obtained from different concentrations and the results are summarized in TABLE 1. The percent relative standard deviation and percent range of errors (0.05 level and 0.01 confidence limits) were calculated for the two methods and the results are given in TABLE 1.

Each method uses a specific reagent and the λ_{max} and ε_{max} values of each method (A&B) are different. Statistical analysis of the results show that the proposed procedures are in good precision and accuracy. Results of the analysis of pharmaceutical formulations indicating that the inactive ingredients did not interfere in the assay. These results further demonstrate the accuracy as well as the precision of the proposed methods.

In order to evaluate the analytical applicability of the proposed methods to the quantification of ACB in commercial tablets, the results obtained by the proposed methods were compared to those of the reference method by applying Student's t-test for accuracy and F-test for precision. The results (TABLE 2) showed that the Student's t-test and F-test values at 95% confidence level did not exceed the tabulated values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision. The percent recoveries are given in TABLE 2.

TABLE 2 : Determination of acb in pharmaceutical formulations

Sample	Labelled amount (mg)	Amount found by proposed methods* Method A	Method B	Ref. Method (UV method)	% Recovery by proposed methods** Method A	Method B
Inj I	10	9.91 ± 0.075 F = 2.77 t = 0.86	9.89 ± 0.092 F = 1.85 t = 1.12	9.96 ± 0.125	99.91 ± 0.75	98.99 ± 0.90
Inj II	10	9.97 ± 0.115 F = 1.32 t = 1.12	9.85 ± 0.15 F = 1.29 t = 0.49	9.89 ± 0.132	99.76 ± 1.08	98.56 ± 1.59

*** Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.228; ** Recovery of 10 mg added to the preanalysed pharmaceutical formulations (average of three determinations)**

REFERENCES

[1] R.Hughes; Atracurium: an overview, British Journal of Anaesthesia. 58 Suppl., **1(6)**, 2S–5S **(1986)**.

[2] R.L.Stiller, B.W.Barndom, D.R.Cook; Anesth. Analg. (N.Y.), **64(1)**, 58-62 **(1985)**.

[3] E.A.M.Neill, C.R.Jones; J.Chromatogr., Biomed. Appl., **25** ((J.Chromatogr. 274)), 409-412 **(1983)**.

[4] Han, Jan, Zeng, Huanjun, Fang, Zhenmen; Yaoxue Xaebao, **31(10)**, 775-779 **(1996)**.

[5] X.Wang, G.Yang, Z.Qiu; Journal of Pharmaceutical Analysis., **12(2)**, 70-72 **(1992)**.

[6] U.Nehmer; J.Chromatogr., **435(3)**, 425-433 **(1988)**.

[7] Christine Farenca, Michel Audranb, Jean-Yves Lefrantc, Ingrid Mazerma, Francoise Bressolle; Journal of Chromatography B: Biomedical Sciences and Applications, **724(1)**, 117–126 **(1999)**.

[8] Ryu Okutani, Katsuakira Koxo, Frederic M.deBros, Daniel M.Philbin; Journal of Anesthesia, **2(2)**, 213-218 **(1988)**.

[9] G.Carlucci, G.Maurizi, P.Mazzeo, Farmaco, (Eds); Prat., **43(10)**, 297-302 **(1988)**.

[10] Herv'Sayer, Oscar Quintela, Pierre Marquet*, Jean-Louis Dupuy, Jean-Michel Gaulier, Gerard Lachtre; Journal of Analytical Toxicology, **28**, **(2004)**.