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Research Article

SPECTROFLUORIMETRIC DETERMINATION OF ACLIDINIUM BROMIDE

MOHAMMED W.I. NASSAR[a], KHALID A.M. ATTIA[1], AHMED A. MOHAMED, SAMIR MORSHEDY[2], MOHAMEDA SHAHIN[2*]

[1]Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, 11751 Nasr City, Cairo, Egypt.[2]Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Damanhour University, Beheira, Egypt..
Email: Mohamed.shahin@pharm.dmu.edu.eg

ABSTRACT

Objective: Aclidinium bromide is an inhaled antimuscarinic bronchodilator recently approved in the US and Europe for stable chronic obstructive pulmonary disease. This study presents the first reported to investigate the fluorimetric behavior of aclidinium bromide. Methods: All variables that affect fluorescence intensity were studied and optimized. The described method involved the measurement of native fluorescence of the drug in methanol at 300 nm after excitation at 272 nm. Results: Calibration plot was found to be linear over the concentration range of 0.1–0.9 µg/mL. Conclusion: The proposed method has been successfully applied to the analysis of the drug in its new pharmaceutical dosage form.

Keywords: Aclidinium bromide; spectrofluorimetry; emission; excitation.

INTRODUCTION

Aclidinium bromide, (3R)-3-{[2-hydroxy-2,2-bis(thiophen-2-yl)acetyl]oxy}-1-(3-phenoxypropyl)-1-azabicyclo[2.2.2]octan-1-ium bromide, Figure 1, is an inhaled antimuscarinic bronchodilator recently approved in the US and Europe for the long-term, maintenance treatment of bronchospasm associated with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. Aclidinium bromide is a competitive, selective muscarinic receptor antagonist, with a longer residence time at the M_3 receptors than the M_2 receptors. M_3 receptors mediate contraction of airway smooth muscle. Inhaled aclidinium bromide acts locally in the lungs to antagonize M_3 receptors of airway smooth muscle and induce bronchodilation [1].

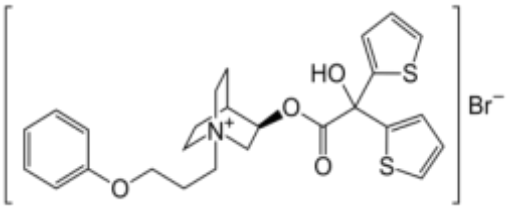

Fig. 1: Structure formula of aclidinium bromide

Different modes of analysis have been developed for the pharmaceutical analysis with various advantages. There tschniques including chromatographic [2-5], spectrophotometric [6- 12] or application of electrochemical methods [13, 14].

Spectrofluorimetry has been widely used in the determination of pharmaceutical compounds because it is a highly sensitive, selective, easily operated and economical technique. It may achieve limits of detection several orders of magnitude lower than those of most other techniques [15-19].

To the best of our knowledge, no analytical methods have been published for the quantitative analysis of aclidinium in its new pharmaceutical dosage form. Hence, this work aimed to introduce the first spectrofluorimetric method for the analysis of aclidinium, which can be used for the routine analysis of the drug in raw material and in pharmaceutical preparations.

EXPERIMENTAL

MATERIALS

Pure ACB (99.25%) and Tudorza® Pressair® inhalation powder each inhaler dose containing 400µg of ACB per actuation were kindly supplied by National Organization for Drug Control and Research, Giza, Egypt

Chemicals and solvents

All solvents used were of HPLC grade and the chemicals used were of analytical grade. Water used throughout the procedures was freshly double distilled.

- Acetonitrile, chloroform, ethanol and methanol (Sigma-Aldrich, Germany).
- Tween 80, Sodium dodecyl sulphate "SDS" (El-Nasr Company, Egypt), Cetyl trimethyl ammonium bromide "CTAB" (Winlab, UK) and β-cyclodextrin "β-CD" (Sigma-Aldrich, Germany) were prepared as 0.5% aqueous solutions.
- Sodium hydroxide (El-Nasr Company, Egypt) was prepared as 0.1 N aqueous solutions.
- Hydrochloric acid (El-Nasr Company, Egypt) was prepared as 0.1 N aqueous solutions.
- Potassium chloride, boric acid, glacial acetic acid and sodium acetate trihydrate (El-Nasr Company, Egypt).
- Buffers of different pH values were prepared as prescribed in US pharmacopeia [20]; Acetate buffer pH range from 4 to 6 and alkaline borate buffer pH range from 8 to 10.

Apparatus

Jasco FP-6200 Spectrofluorometer (Japan), equipped with 150 Watt Xenon lamp. Slit widths for both monochromators were set at 10 nm. All measurements were done at medium sensitivity.

Standard solution

A standard stock solution of aclidinium (100 µg/mL) was prepared by dissolving 10 mg of the drug powder in 50 mL of methanol and the volume was completed to 100 mL with methanol. Working solution (10 µg/mL) was obtained by further dilution of the stock solution with the methanol.

Procedures

Construction of calibration graph

Different aliquots of aclidinium working standard solution ranging from (1–9) µg were transferred into a series of 10-mL volumetric flasks and completed to volume with methanol. The fluorescence intensity was measured at 300 nm after excitation at 272 nm. The measured fluorescence intensities were plotted against the final concentrations of the drug in µg/mL to get the calibration graph. Alternatively, the regression equation was derived.

Application to pharmaceutical preparation

A quantity equivalent to 10 mg of aclidinium was accurately weighed, transferred to 100- mL conical flask and the volume was made up to 50 mL with methanol. The solution was sonicated for 30 min and filtered into100-mL volumetric flask. The volume was completed to 100 mL with methanol to produce a stock solution labeled to contain 100 µg/mL of aclidinium. Working solution (10 µg/mL) was prepared by dilution with methanol. Further dilutions were done with methanol in order to prepare different concentrations of aclidinium within the linearity rang. The concentration of aclidinium was calculated using the previously described procedure.

RESULTS AND DISCUSSION

Spectral characteristic

Aclidinium exhibit a native fluorescence in methanol and its emission can be measured at 300 nm after excitation at 272 nm as shown in **Figure 2**.

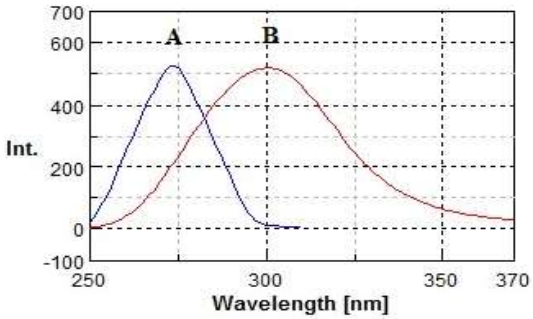

Fig. 2: Excitation (A) and emission (B) spectra of aclidinium (0.5 µg/mL) in methanol.

Optimization of Experimental Conditions

Several variables that affect the fluoriscence intensity were studied by repeating the previosely described procedure using a fixed amount of aclidinium (5 µg).

Effect of Solvents

Different solvents including water, acetonitrile, methanol, ethanol, chloroform, 0.1N HCl and 0.1N NaOH were studied. As seen in Figure 3; the highest fluorescence intensity was achieved with methanol. On the other hand, the fluorescence intensity of the drug has been largely quenched with

Effect of pH and buffer

Different buffers at several pH values were studied. As seen in Figure 4; the fluorescence intensity of aclidinium was diminished by addition of buffers. So, no buffer was used throughout the work.

Effect of surfactants

Different surfactants including SDS, CTAB, Tween 80 and β-CD were studied. As seen in Figure 5; the presence of these surfactants decreases the fluorescence intensity of aclidinium. Consequently, they were not used throughout the work.

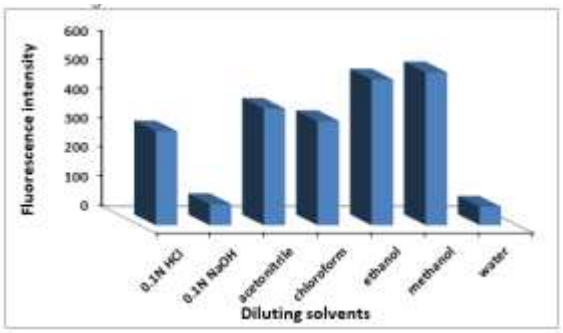

Fig. 3: Effect of different solvents on fluorescence intensity of aclidinium (0.5 µg/ mL).

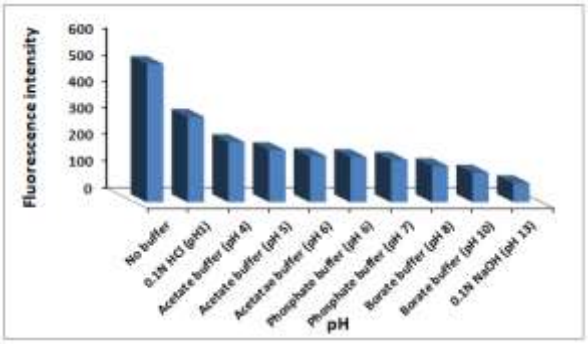

Fig. 4: Effect of pH on fluorescence intensity of aclidinium (0.5 µg/ mL).

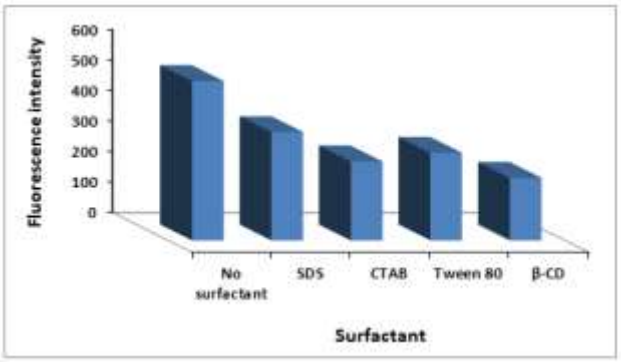

Fig. 5: Effect of different surfactants on fluorescence intensity of aclidinium (0.5 µg/ mL).

METHOD VALIDATION

The proposed method was validating according to ICH guidelines [21].

Linearity and range

Under the described experimental conditions, the calibration graph for the described method was constructed by plotting the fluorescence intensity versus drug concentrations in µg/mL. The regression plot was found to be linear over the range of 0.1–0.9 µg/mL for aclidinium. The regression data was presented in Table 1. The high values of coefficient of determination and the small values of slope and intercept indicated the linearity of the calibration graphs.

Limit of detection and limit of quantitation

LOD and LOQ values were calculated according to ICH guidelines from the following equations:

$LOD = 3.3\ \sigma / S$
$LOQ = 10\ \sigma / S$

Where, σ is the residual standard deviation of regression lines and S is the slope of the calibration curve. The obtained results indicated the sensitivity of the proposed method for the analysis of the studied drug as shown in Table 1

Accuracy and precision

Accuracy was calculated as a mean percent recovery of three determination for three concentration levels (0.2, 0.4, 0.6 µg/mL) and the results were presented in Table 1. Moreover, standard addition technique was applied to assess the accuracy and there was no interference from excipients (Table 2). Precision was calculated as a relative standard deviation of three determination for three concentration levels (0.2, 0.4, 0.6 µg/mL) within one day for repeatability and on three successive days for intermediate precision and the results were presented in Table 1.

Pharmaceutical applications

The proposed method was applied for the determination of aclidinium in Tudorza® Pressair® inhalation powder. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives which was confirmed by the results of the standard addition technique. The obtained results were statistically compared to those obtained by the manufacturing method. No significant differences were found by applying *t*-test and *F*-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form, as shown in Table 3.

Table 1: Regression and validation parameters for determination of aclidinium by the proposed spectrofluorimetric method.

Parameters	The proposed method
Emission wavelength (nm)	300
Excitation wavelength (nm)	272
Slope	1152.2169
Intercept	-54.5643
Coefficient of determination(r^2)	0.9995
Range (µg/mL)	0.1–0.9
LOD (µg/mL)	0.021
LOQ (µg/mL)	0.064
Accuracy (mean %R) *	100.86
Precision	
Repeatability (%RSD) *	0.699
Intermediate precision (%RSD) *	0.893

***Average of three determinations for three concentrations repeated three times.**

Table 2: Recovery study of aclidinium by applying standard addition technique.

Pharmaceutical taken (µg/mL)	Pharmaceutical found (µg/mL)	Pure added (µg/mL)	Pure found (µg/mL)	% Recovery*
0.2	0.2	0.2	0.199	99.50
		0.4	0.395	98.75
		0.6	0.605	100.83
Mean ± %RSD				99.69±1.058

***Average of three determinations.**

Table 3: Determination of aclidinium in Tudorza® Pressair® inhalation powder by the proposed spectrofluorimetric and manufacturing methods [6].

Parameters	Proposed method	Manufacturing method*
N**	5	5
$\overline{X}$***	100.03	100.04
SD	0.736	1.010
Variance	0.542	1.021
t-test (2.306) ****	0.029	——
F-test (6.388) ****	1.884	——

***HPLC using C_{18} column, mobile phase was acetonitrile: methanol (45:55, v/v) at a flow rate (1mL/min) and UVdetection at 260 nm.**

****Number of experiments.**

*****The mean of percent recovery of pharmaceutical preparation.**

****** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05).**

CONCLUSION

The work presents the first spectrofluorimetric method for determination of aclidinium, a newly approved antimuscarinic bronchodilator drug for the treatment of COPD. The developed method is simple, rapid, accurate, sensitive and selective and does not need any sophisticated instrumentation and separation steps..

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