Vol 5, Issue 3, 2018 ISSN 2349-7041

Research Article

NATIVE SPECTROFLUORIMETRIC DETERMINATION OF AMPROLIUM IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: The manuscript discussed the application of a simple, accurate and precise spectrofluorimetric method for determination of amprolium in bulk and pharmaceutical formulation. **Method:** The emission was measured at 320 nm after excitation at 262 nm. Several parameters were optimized to get the most sensitive and reproducible results. **Results:** The following analytical parameters: limit of detection, limit of quantitation, accuracy, precision and linearity ranges of the method were determined. Statistically, there is no significant difference between the proposed and the reported method. **Conclusion:** this method can be used for determination of amprolium in bulk and pharmaceutical formulation.

Keywords: Amprolium; Native fluorescence, pharmaceutical dosage form.

INTRODUCTION

Amprolium hydrochloride (AMP), (1-[(4-amino-2-propyl-5-pyrimidinyl)methyl]-2-methyl pyridinium), is anti-protozoal drug which extensively utilized in veterinary domain to prevent and treat coccidiosis in poultry[1]. The chemical structure of AMP is demonstrated in Figure (1).

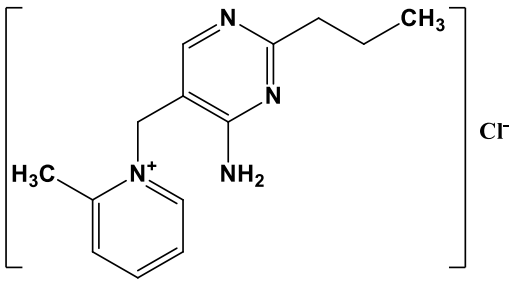

Fig. 1: Chemical structures of amprolium HCl

In reviewing the literature, AMP is official in British Pharmacopeia [2]. Moreover, several methods have been reported or determining AMP. These methods involve estimation of amproliumin pharmaceutical formulation using chromatographic[3-13], spectrophotometric [14, 15], atomic [16]electrochemical [17], spectrofluorimetric [18]methods and capillary electrophoresis [19].

Experimental

Apparatus

- Jasco FP6200 single beam spectrofluorometer (Japan)
- Jenway, 3510 pH meter (Jenway, U.S.A.).

Materials and reagents

All chemicals and reagents utilized in this study were of analytical grade and used without extra-purification. Doubly distilled water was used throughout the procedure.

(a) AMP was supplied by Memphis Company for pharmaceuticals, Cairo, Egypt. Its purity was tested and found to be 100.01 ± 0.545% in accordance with the British pharmacopoeia official method[2].

(b) The pharmaceutical preparation containing AMP was obtained from local drug stores.

- Amprolium 60% B. No. (413016). Each 1000 g is claimed to contain 600 g of AMP and lactose monohydrate manufactured by ADWIC, El Nasr Pharmaceutical Chemical Company, Abu Zaabal, Egypt.
- Amprolium 20% B. No. (131934). Each 1000 ml contains 200g of AMP manufactured by Arabcomed, Arab Company for Medical Products, Obour City, Egypt.

Standard solution

First, weigh 10 mg of AMP powder and transfer the powder into a 100-ml volumetric flask. Then add 50 ml of water to dissolve AMP powder. Finally, adjust to volume with water.

Procedure

Procedure for calibration graph

First, different aliquots of AMP standard solution (1000ng/ml) ranging from (100– 600 ng) were transferred to 10-ml volumetric flasks. Second, add 1 ml of acetate buffer (pH 4). Third, dilute the solutions with water to 10 ml and mix well. Fourth, adjust excitation at 262nm and measure emission at 320 nm. Finally, plot the measured emission against concentration in ng/ml to get the calibration graph and regression equation.

Application for pharmaceutical formulation

A portion of Amprolium® powder equivalent to 10mg of AMP was transferred into 100-ml volumetric flask, extracted three times in about 10mlmethanol and diluted to the mark with distilled water to obtain solution labeled to contain 100µg/ml of AMP. Further dilution took place to contain 100 ng/ml of AMP. Repeat the general procedure under "Procedure for calibration graph" using different concentrations within the linearity range. Lastly, the concentrations can be determined via using the regression equation.

Optimization of experimental conditions:

Effect of diluting solvent

The general procedure under "Procedure for calibration graph" was repeated using a fixed amount of AMP(600ng) and different diluting solvents.

Effect of pH and buffer

The general procedure under "Procedure for calibration graph "was repeated using a fixed amount of AMP (600 ng) and different buffers with different pH.

Innoriginal International Journal of Sciences | Volume 5 | Issue 3 | May-June 2018 | 42-45

Effect of buffer volume

The general procedure under "Procedure for calibration graph "was repeated using fixed amount foam (600 ng) and different volumes of acetate buffer pH 4.

Effect of Time

The general procedure under "Procedure for calibration graph "was repeated using a fixed amount of AMP (600 ng) at different time interval.

Validation of the procedure

Linearity (Construction of the calibration graph)

The general procedure under "Procedure for calibration graph"was repeated. The measured fluorescence intensity values versus the final drug concentrations in ng/ml were plotted to get the calibration graph and the regression equation was derived.

Limits of detection and quantitation:

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

LOD = 3.3 σ /S

LOQ = 10 σ / S

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Accuracy and precision

Accuracy and precision of the method were determined by applying the proposed procedure for determination of three different concentrations (100, 300 and 500ng/ml), each in triplicate, of AMP in pure form in the same day (intra-day) and in three successive days (inter-day), then the accuracy as percent recovery (R%) and precision as percent relative standard deviation (RSD%) were calculated.

Accuracy of the method was also determined by applying the standard addition technique. First, repeat the general procedure using different aliquots of AMP solution (100 ng/ml) containing (100, 400 and 500 ng). Then, add 100 ng of amprolium® powder. Finally, calculate the percent recovery (R%) of added concentrations.

RESULTS & DISCUSSIONS

The present study suggested an easy spectrofluorimetric method for quantitative analysis of AMP.

Spectral characteristics

AMP exhibits a native fluorescence in water and its emission can be measured at 320 nm ($\lambda_{emission}$) after excitation at 262 nm ($\lambda_{excitation}$).The emission and excitation spectra of AMP in water using acetate buffer pH 4\are shown in figure (2).

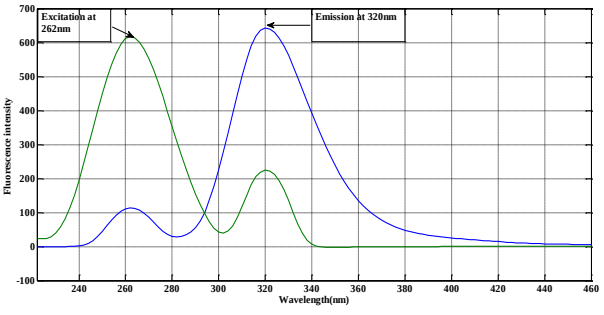

Fig. 2: Excitation and emission spectra of amprolium (600 ng/ml) in water using 1 ml of acetate buffer pH 4.

Optimization of experimental conditions

Different parameters which affect the fluorescence intensity have been studied and optmized. Water was found to be the best diluting solvent and maximum fluorescence intensity was achieved up on using of 1 ml of acetate buffer, pH 4 to adjust pH, as shown in figures (3-5).The emission was instantaneously established and persisted stable at least for one hour, as shown in figure (6)

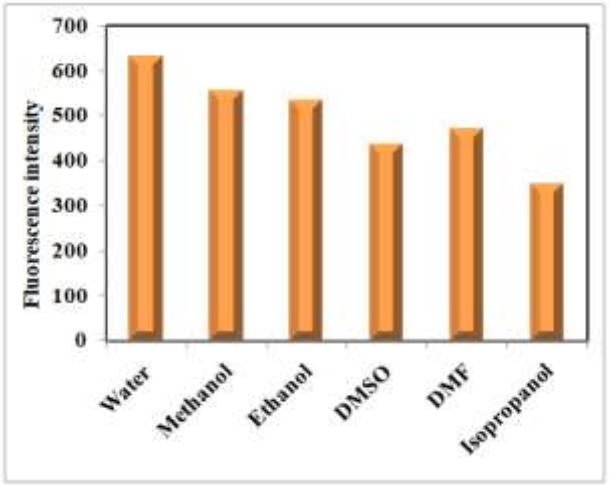

Fig.3: Effect of diluting solvent on the fluorescence intensity of amprolium(600 ng/ml)

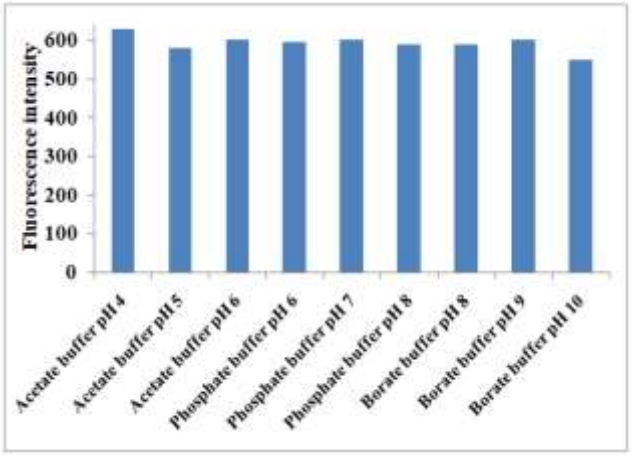

Fig.4: Effect of different buffers (1 ml) on fluorescence intensity of amprolium(600 ng/ml)

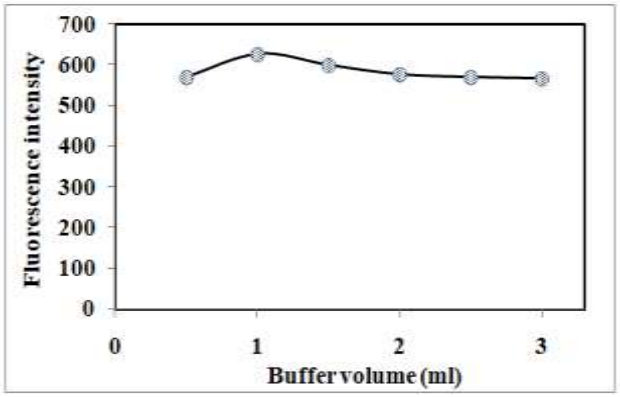

Fig.5: Effect of volume of acetate buffer pH 4 on fluorescence intensity of amprolium(600 ng/ml)

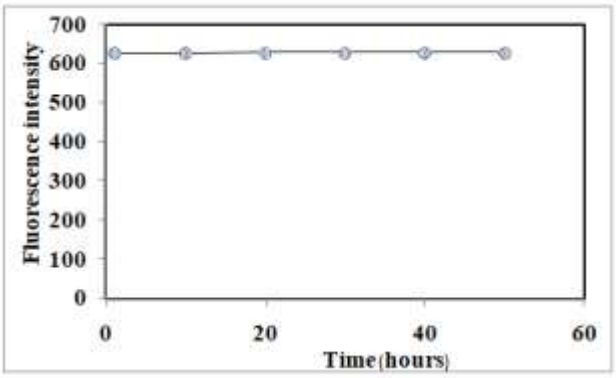

Fig. 6: Effect of time on fluorescence intensityof amprolium(600 ng/ml)

Method validation

In accordance with ICH Q2B recommendations [20, 21] validation was carriedout.

Linearity:

Under the described experimental conditions, the calibration graph for the method was constructed by plotting fluorescence intensity versus concentration in ng/ml.The regression plot was found to be linear over the range of 100–600 µg/ml as shown in figure (7). The linear regression equation for the graph was:

FI = 1.0439C + 6.1267 (r^2 = 0.9997).

Where FI is the fluorescence intensity and C is the drug concentration in ng /ml.

Linearity range, regression equations, intercept, slope and squared correlation coefficient for the calibration data were presented in table (1).

Limits of detection and quantitation:

The small values of LOD and LOQ indicate good sensitivity.

Accuracy and precision

Accuracy, precision (repeatability) and intermediate precision are shown table (1). The method is of high precision due to the small values of RSD % .Additionally, the method is of outstanding accuracy due to good recovery percentages.

Accuracy was also determined by application of standard addition technique, as shown in table (2) which indicates no matrix interference.

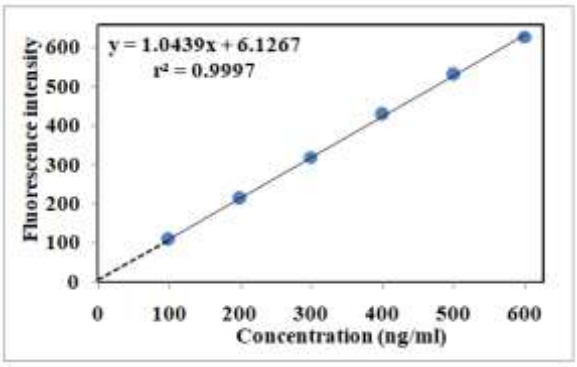

Fig. 7: Calibration graph of amproliumin methanol using acetate buffer pH 4 at 320 nm ($\lambda_{emission}$) after excitation at 262 nm ($\lambda_{excitation}$).

Pharmaceutical applications

The suggested method was utilized to estimate AMP in Amprolium® powder. Acceptable out comes were found compatible with the label claim, demonstrating no excipients or additives effect. The found out comes of the suggested and reported methods[17]were statistically compared. Applying Student's t-test and F-test indicates no significant differences were found at 95% confidence level[22] as shown in table (3).

Table 1: Spectral data for determination of amprolium by the propose spectrofluorimetric procedure:

parameter	Proposed method
λemission(nm)	320
λexcitation(nm)	262
Linearityrange(ng/ml)	100-600
LOD(ng/ml)	21.1875
LOQ(ng/ml)	64.2046
- Regression Equation	
- Slope (*b*) ± S.D	1.0439± 0.314
- Intercept (*a*) ± S.D	6.1267± 0.0321
Determination coefficient (*r2*)	0.9997
Accuracy (%R) [a]	99.99
Repeatability (RSD)[b]	0.841
Intermediate precision (RSD)[c]	0.842

[a]Average of % recoveries (100, 300 and 500 ng/ml) for amprolium within the day. [b] The intraday (n = 3), average of three concentrations (100, 300 and 500 ng/ml) for amprolium within the day.[c] The interday (n = 3), average of three concentrations (100, 300 and 500 ng/ml) for amprolium in three consecutive days

Table 2: Recovery study of amprolium by standard addition technique via the proposed procedure in its powder:

Pharmaceutical Conc. (ng/mL)	Pure added (ng/mL)	Pure found (ng/mL)	Recovery %
100	100	101.09	101.09
	400	401.52	100.38
	500	501.82	100.36
Mean			100.61
RSD%			0.4121

Table 3: Determination of amproliumin Amprolium®powder by the proposed and reported method:

Parameter	Proposed method	Reported method
n*	5	5
X**	99.82	99.14
SD	1.062	0.736
t***	0.811 (2.306)	
F***	2.081 (6.388)	

*** 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).**

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