



Extractive Spectrophotometric Determination of Amitriptyline Hydrochloride in Pure and Pharmaceutical Formulations

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ABSTRACT

A sensitive, reproducible and accurate spectrophotometric method has been developed for determining amitriptyline hydrochloride (ATH) from its pharmaceutical formulation based on solvent extraction into chloroform of the product formed with 1, 2-naphthoquinon-4-sulphonate. The reaction and extraction was complete and quantitative at pH 1.3. The Beer's law of yellow colored species is obeyed in the range of concentration of 2 – 18 $\mu\text{g ml}^{-1}$ at the maximum absorption of 450 nm. The molar absorptivity and Sandell's sensitivity values were found to be of $3.2382 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ and $0.9615 \mu\text{g cm}^{-2}$ respectively. The results compare favourably with those of official methods. The method is successfully employed for the determination of ATH in pharmaceutical preparations.

Key words: Amitriptyline hydrochloride, spectrophotometry, 1, 2-naphthoquinon-4-sulphonate.

INTRODUCTION

Amitriptyline hydrochloride (ATH) is chemically, 3-(10,11-Dihydro-5H-dibenzo [a, d] cyclohepten-5-ylidene)-N,N-dimethyl-1-propanamine¹. It is a tricyclic antidepressant used in case of anxiety and also shows anticholinergic, antihistamine and antiserotonimic effects². In view of their importance, considerable work has been done on their detection and quantification. Methods used for the determination of ATH include atomic

absorption spectroscopic³, high-performance liquid chromatography^{4,5} and spectrophotometry⁶⁻⁹. The purpose of this study was to determine ATH by simple, accurate, and precise spectrophotometric assays for routine analysis.

The investigator has been able to develop an accurate and sensitive extractive method employing 1, 2-naphthoquinon-4-sulphonate (NQS) in acidic media for the determination of ATH in a bulk sample and its formulation.

MATERIAL AND METHODS

Apparatus

All spectral measurements were made on Shimadzu Double-Beam Spectrophotometer UV-1500-02 with 1.0 cm matched quartz cells.

Reagents

All of the chemicals used were of either pharmaceutical analytical grade. A standard solution of ATH was prepared by dissolving a requisite amount of ATH in distilled water. It was diluted as and when required. A 0.5 % w/v solution of NQS was freshly prepared and protected from sunlight. Buffer of pH 1.0, 1.3 (potassium chloride – hydrochloric acid), 4.0 (potassium hydrogen phthalate - hydrochloric acid) and 4.6 (potassium hydrogen phthalate – sodium hydroxide) were prepared in distilled water.

Procedure

Aliquot of standard solution of ATH (20 – 180 $\mu\text{g ml}^{-1}$) was transferred into a series of separating funnels. To each funnel, 5 ml of 0.5 % NQS solution and 1.0 ml of buffer solution (pH 1.3) solution were added. Ten ml of chloroform was added to each funnel. The contents were shaken vigorously and left at room temperature for two minutes. The phases were allowed to separate and the chloroform layer passed through anhydrous sodium sulphate. Absorbance values of the chloroform layers were measured at 450 nm against reagent blank. A calibration graph was constructed.

Twenty tablets were powdered and equivalent to 25 mg of the ATH was weighed accurately and transferred into a 100 ml volumetric flask and then volume made up with distilled water

and filtered. Appropriate aliquot of the ATH solution was taken and treated as described above for the determination of ATH.

RESULTS AND DISCUSSION

Optimum conditions for product formation

It was found that 0.5 % concentration of NQS in the range 4 – 6 ml. Hence, 5.0 ml of NQS and 1.0 ml buffer solution were selected. The buffer solution of pH 1.0, 1.3, 4.0 and 4.6 were used to extract the product. The results are found that 0.5 – 1.5 ml buffer solution of pH 1.3 was necessary for the achievement of maximum color intensity.

Benzene, carbon tetrachloride, 1, 2-dichloromethane, dichloroethane and chloroform were tested as extractive solvents for the proposed reaction. Chloroform was preferred to others for its selective extraction of the colored product. It offers advantages, such as being economically cheaper and convenient, to be used as extractive solvent.

Table 1: Determination of 10 $\mu\text{g ml}^{-1}$ of ATH in the presence of excipients and other substances

Interfering substance	Amount taken / mg	Recovery ATH \pm % RSD ^a
Lactose	30	9.94 \pm 0.88
Dextrose	30	9.89 \pm 0.94
Starch	30	9.93 \pm 0.78
Talc	40	9.98 \pm 0.82
Glucose	30	9.95 \pm 0.86
Gelatin	20	9.96 \pm 0.91

a. Average of five determinations

Table 2: Determination of ATH in pharmaceutical preparation

Drug trade name	Label claim (mg)	Recovery of ATH (mg) \pm % RSD ^a	
		Proposed method	Reported Method
Tryptacare ^b	50	49.98 \pm 0.78	49.97 \pm 0.81
Amicon ^c	25	24.99 \pm 0.67	24.94 \pm 0.72
Amitrip ^d	25	25.01 \pm 0.59	24.99 \pm 0.68

a. Average of five determinations

c. Marketed by Icon

b. Marketed by Symbiosis

d. Marketed by Eastwest

The reaction between ATH and NQS was found to be instantaneous and yellow color produced was stable only 30 minutes. The buffer solution enhances of the stability of color more than 2 hours at $25 \pm 3 \text{ C}^\circ$. The effects of temperature on the product were studied at different temperatures. The color product was stable in the temperature range $0.0 - 35 \text{ C}^\circ$. At higher temperatures the drug concentration was increased on prolonged heating due to volatile nature of chloroform. As a result, the absorbance values of the colored products were increased.

Analytical features

The method obeyed Beer's law in the concentration range of $2 - 18 \mu\text{g ml}^{-1}$ with molar absorptivity, specific absorptivity and Sandell's sensitivity values of $3.2382 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$, $0.0104 \text{ ml g}^{-1} \text{ cm}^{-1}$ and $0.9615 \mu\text{g cm}^{-2}$ respectively. A regression analysis of a Beer's law plot at 450 nm revealed a good correlation ($r = 0.9960$). The graph of the absorbance versus the concentration of ATH showed a low intercept ($b = 0.0064$) and slope ($a = 0.0097$) and is described by a regression equation, $Y = aX + b$ (where X is the concentration of ATH in $\mu\text{g ml}^{-1}$, Y is the absorbance of a 1 cm layer, 'a' is the slope and 'b' is the intercept). The low relative standard deviation (0.91 %) and the range of error at 95 % confidence level (0.67) for the analysis of five replicates of $10 \mu\text{g ml}^{-1}$ indicated good precision and the accuracy of the proposed method.

Interference studies

In order to assess the possible analytical application of the proposed method, the effect of some foreign substances that often accompany ATH in various pharmaceutical products were studied by adding different amounts of substances to $10 \mu\text{g ml}^{-1}$ of ATH. An attractive feature of the method is its relative freedom from interference by the usual tablet diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical preparation. The results are given in Table 1.

Application of method

The applicability of the proposed method was examined as an assay of pharmaceutical preparations. The results of the assay of ATH tablets are summarized in Table 2. These results were reproducible. The results of the assay of tablets were crosschecked by the official method¹¹.

CONCLUSION

The results clearly indicate that the utility of the proposed method for the analysis of ATH in pure and dosage forms. The applicability of the method for the assay of ATH in pharmaceutical preparations has been well demonstrated. Further, assaying authentic samples containing ATH as well as common additives and excipients tests the effect of interfering substances. Thus it can be concluded that this method could be considered for the assay of both bulk and formulation.

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