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Research Article

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New Visible Spectrophotometric Methods for the Determination of Protriptyline HCl in Bulk and Pharmaceutical Formulations

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ABSTRACT

Visible spectrophotometric methods are developed for the quantitative determination of protriptyline HCl (PTP) in bulk and pharmaceutical formulations. The first method (method A) is based on the condensation of amino group of PTP with acetaldehyde to give N-alkyl vinyl amine which reacts with p- Chloranil (p-CA) to give vinyl amino substituted quinine in 1,4-dioxane. The blue colored product exhibits absorption maximum at 669 nm and linear within the limits 2.0-14.0 μg mL^{-1}. The second method (method B) is based on the reaction of drug with 1,2-napthaquinone-4-sulphonic acid (NQS) to form N-alkylamono naphthaquinone in basic medium. The red colored product exhibits absorption maximum at 484 nm and linear within the limits 15.0-35.0 μg mL^{-1}. The molar absorptivity and Sandell's sensitivity of method A and method B are 1.03 × 10^4, 2.89 × 10^{-2} and 0.96 × 10^4, 3.10 × 10^{-2}, respectively. The statistical treatment of the experimental results indicates the precision and accuracy of the methods. Both the methods are free from interference of the ingredients such as lactose, sucrose starch, etc., thus it is successfully applied to pharmaceutical formulations. This study represents the first report on the development of new visible spectrophotometric methods for the determination of PTP.

Key words: Spectrophotometry, Protriptyline HCl, *p*- Chloranil, 1,2-napthaquinone-4-sulphonic acid.

INTRODUCTION

Protriptyline HCl (PTP) is one of the tricyclic antidepressant[1], chemically known as *N*-methyl-5*H*-dibenzo[a,d]-cycloheptene-5-propanamine hydrochloride (Figure. 1). It works by blocking the transporters responsible for re-uptake of neurotransmitters like norepinephrine and serotonin[2]. This medication is primarily used to treat the anxiety and depression[3] and also used to treat bipolar disorder[4], obsessive-compulsive disorder and other mood disorders. They are also known as effective analgesics used for the treatment of chronic pain especially neuropathic or neuralgic pain[5].

A very few methods are reported in the literature for the determination of PTP in pharmaceutical formulations which include High performance liquid chromatography[6], Liquid chromatography[7-9], Flow injection technique[10], UV spectrophotometric[11] methods and no visible spectrophotometric methods are developed. Spectrophotometry is considered the most appropriate technique because of its inherent simplicity, low cost and wide availability[12]. In the present study, we succeeded in developing new simple, sensitive and selective spectrophotometric methods. The developed methods are successfully validated and applied to the determination of PTP in bulk and pharmaceutical formulations.

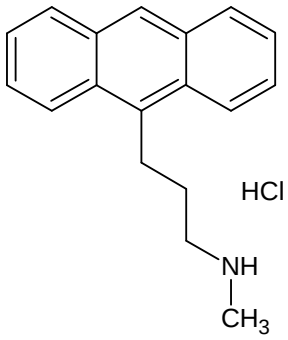


Figure 1

EXPERIMENTAL SECTION

Apparatus

A UV-Visible spectrophotometer (SHIMADZU, Model No: UV 2550) with 1 cm quartz cells was used for the absorbance measurements.

Reagents and solutions

All solutions were prepared with double distilled water. Chemicals used were of analytical reagent grade. A solution of *p*-CA (1 %) was prepared by dissolving 1 g of *p*-CA in 100 mL of 1,4-dioxane. A solution of NQS (0.5 %) was prepared by dissolving 0.5 g of the NQS in 100 mL of double distilled water, 0.4 % solution of NaOH was prepared by dissolving 0.4 g of sodium hydroxide pellets in 100 mL double distilled water.

Standard drug solution

A 1000 µg mL^{-1} standard drug solution was prepared by dissolving 0.1 g of PTP in 100 mL water. The stock solution was diluted approximately to get working concentration.

Procedure

Method A

Aliquots containing 2.00 - 14.00 µg mL^{-1} of PTP were transferred into a series of 10 mL volumetric flasks Then 0.5 mL of acetaldehyde and 1 mL of *p*-CA (1 %) were added successively and kept aside for 20 minutes at room temperature diluted to 10 mL with 1,4-dioxane. The absorbance of the colored species was measured at 669 nm against the reagent blank (Figure .2). The calibration graph was constructed by plotting the drug concentration versus absorbance (Figure.3).

Method B

Aliquots containing 5.00 - 35.00 µg mL^{-1} of PTP were transferred into a series of 10 mL volumetric flasks. To these solutions, 0.5 mL of 0.4 % sodium hydroxide and 0.5 mL of 0.5% NQS were added. The mixture was then gently shaken until the appearance of orange color. The contents were diluted up to 10 mL with distilled water. The absorbance of each solution was measured at 484 nm against the reagent blank (Figure 4). The calibration graph was constructed by plotting the drug concentration versus absorbance (Figure 5).

Procedure for pharmaceutical formulations

Ten tablets of PTP (10 mg) were grounded into fine powder and powdered tablet equivalent to 20 mg of PTP was transferred into a 100 mL calibrated flask and 50 mL of water was added. The content was shaken thoroughly for about 20–30 min, diluted to 100 mL with water, mixed well and filtered using a Whatman No. 42 filter paper. A convenient aliquot was then subjected to the analysis using the proposed methods.

RESULTS AND DISCUSSION

Method A

In literature it is found that [13, 14] many primary and secondary amines reacts with *p*- CA and acetaldehyde to yield vinyl amino substituted quinine derivative. Condensation of amino group of the PTP with acetaldehyde gives *N*-alkyl vinyl amine which reacts with *p*-CA to give blue color product, vinyl amino substituted quinine (Scheme 1).

Method B
The method is mainly based on the nucleophilic substitution reaction of NQS and PTP at free secondary amino group in the presence of NaOH (Scheme 2).

Optimization of reaction conditions
Analytical conditions are optimized *via* a number of preliminary experiments. In the present study various parameters such as volume and strength of *p*-CA, NQS and NaOH, stability of colored species and solvent for final dilution of the colored species are studied and optimized.

To study the effect of concentration of *p*-CA, different volumes of 1 % *p*-CA are tested. It is found that 1 mL of *p*-CA is sufficient to get maximum absorption. The solvent for final dilution is tried with different solvents such as acetonitrile, acetone, ethylene glycol, methanol, 1,4-dioxane. Among these, 1, 4-dioxane is found to be superior for final dilution and it is found that it will take 15 min to complete the reaction and colored product formed stable for 2 hours.

In case of method B alkaline medium is necessary to activate the nucleophilic substitution reactions. Different inorganic bases such as sodium hydroxide, disodium hydrogen phosphate and sodium bicarbonate are tested and all prepared as aqueous solution of a concentration range of 0.5–1.5 %. Best results are obtained in case of 0.5 mL of 0.1 % sodium hydroxide solution. By studying the NQS concentration, it is found that 0.5 mL of 0.5 % NQS gives good absorbance value.

Stoichiometry of the reaction product
Job's Method, also called the method of continuous variation, is a simple and effective approach to the determination of chemical reaction stoichiometry[15]. In this method, the total molar concentration of the two species (i.e. PTP and p-CA in method A and PTP and NQS in method B) is held constant, but their mole fractions are varied. Absorbance obtained for colored adduct is plotted against the mole fraction. The maximum absorbance on the plot corresponds to the stoichiometry of the two species. It is observed that in both the cases (method A and method B) maximum absorption obtained at mole fraction of 0.5 which indicates the formation of 1:1 (PTP: *P*-CA or NQS*)* product (Figure 4).

Validation of the methods
Linearity
Under optimum conditions, linear relations is found between absorbance and concentration of PTP in the range of 2.0-14.0 ug mL^{-1} (method A) and 15.0-35.0 ug mL^{-1} (method B). The calibration graph in each example is described by the equation: Y = a + b X (Where Y = absorbance, a = intercept, b = slope and X = concentration in ug mL^{-1}) is obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 1. Sensitivity parameters such as apparent molar absorptivity and Sandel's sensitivity values, the limit of detection (LOD) and the limit of quantification (LOQ) are calculated as per the ICH guidelines[16] are presented in Table 1 which indicate the excellent sensitivity of the proposed methods.

Accuracy and Precision
In order to study the accuracy and precision of the proposed methods, three concentrations of pure PTP within the linearity range are analyzed, each determination being repeated five times and the percentage relative standard deviation (% RSD) is found to be less than 2 %. Accuracy of the proposed methods is measured by calculating the percentage relative error (% RE) and is found to be less than 3 %. The results of this study indicate the high accuracy and precision of the methods. Detailed results are given in Table 2.

Interference studies
The commonly used excipients in the formulations of the drugs, such as talc, starch, glucose and stearic acid does not interfere in the determination of PTP by the proposed procedures.

APPLICATION TO ANALYSIS OF TABLETS
The proposed methods are successfully applied to the determination of PTP in commercial tablets. The results (Table 3) shows that the Student's t at 95 % confidence level are less than the theoretical values, which confirmed the good accuracy of the methods.

Table 1 Spectral and Statistical data for the determination of Protriptyline HCl

Parameters	Method A	Method B
λ_{max} nm	669	484
Beer's Law Limits (µg/ml)	2.00 – 14.00	15.00 -35.00
Molar Absorptivity ($L\ mol^{-1}\ cm^{-1}$)	1.03 ×1 0^4	0.96×10^4
Sandell's Sensitivity ($\mu g\ cm^{-2}$)	2.89×10^{-2}	3.10×10^{-2}
Limit of Detection* ($\mu g\ mL^{-1}$)	1.0000	0.1941
Limit of Quantification * ($\mu g\ mL^{-1}$)	3.0000	0.5882
Regression Equation**	Y= a + b X	Y = a + b X
Slope (b)	0.0329	0.0178
Intercept (a)	0.0007	0.0494
Correlation Coefficient (r)	0.9988	0.9957

** Limit of detection calculated according to ICH guidelines*
***Y is the absorbance and X concentration in µg mL^{-1}*

Table 2 Evaluation of accuracy and precision

Method A

Amount taken (µg mL^{-1})	Amount found* (µg mL^{-1})	RE (%)	SD (µg mL^{-1})	RSD (%)
2.00	1.93	2.50	0.02	1.02
6.00	5.76	2.33	0.10	1.70
10.00	9.96	0.40	0.19	1.90

Method B

Amount taken (µg mL^{-1})	Amount found* (µg mL^{-1})	RE (%)	SD (µg mL^{-1})	RSD (%)
5.00	4.97	0.60	008	1.71
15.00	14.90	0.66	0.13	0.93
25.00	24.89	0.44	0.26	1.04

** Mean value of five determinations*
RE - Relative Error; SD - Standard Deviation; RSD - Relative Standard Deviation.

Table 3 Result of assay of formulation by the proposed method

Brand name	Labeled amount (mg)	Found* ± SD using Method A	Found ± SD using Method B
Vivactil	10	10.01 ± 0.10 t = 0.0406	10.03 ± 0.07 t = 0.1981

**Mean value of five determinations*
Tabulated t value at 95 % confidence level is 2.7

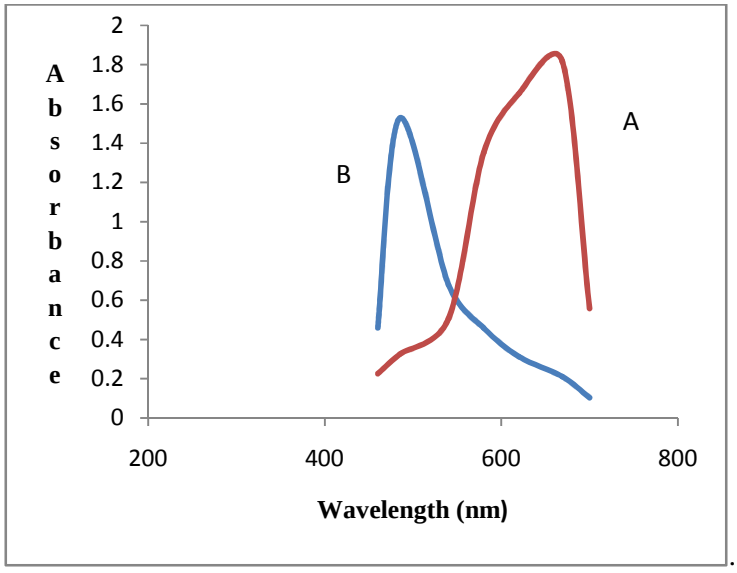
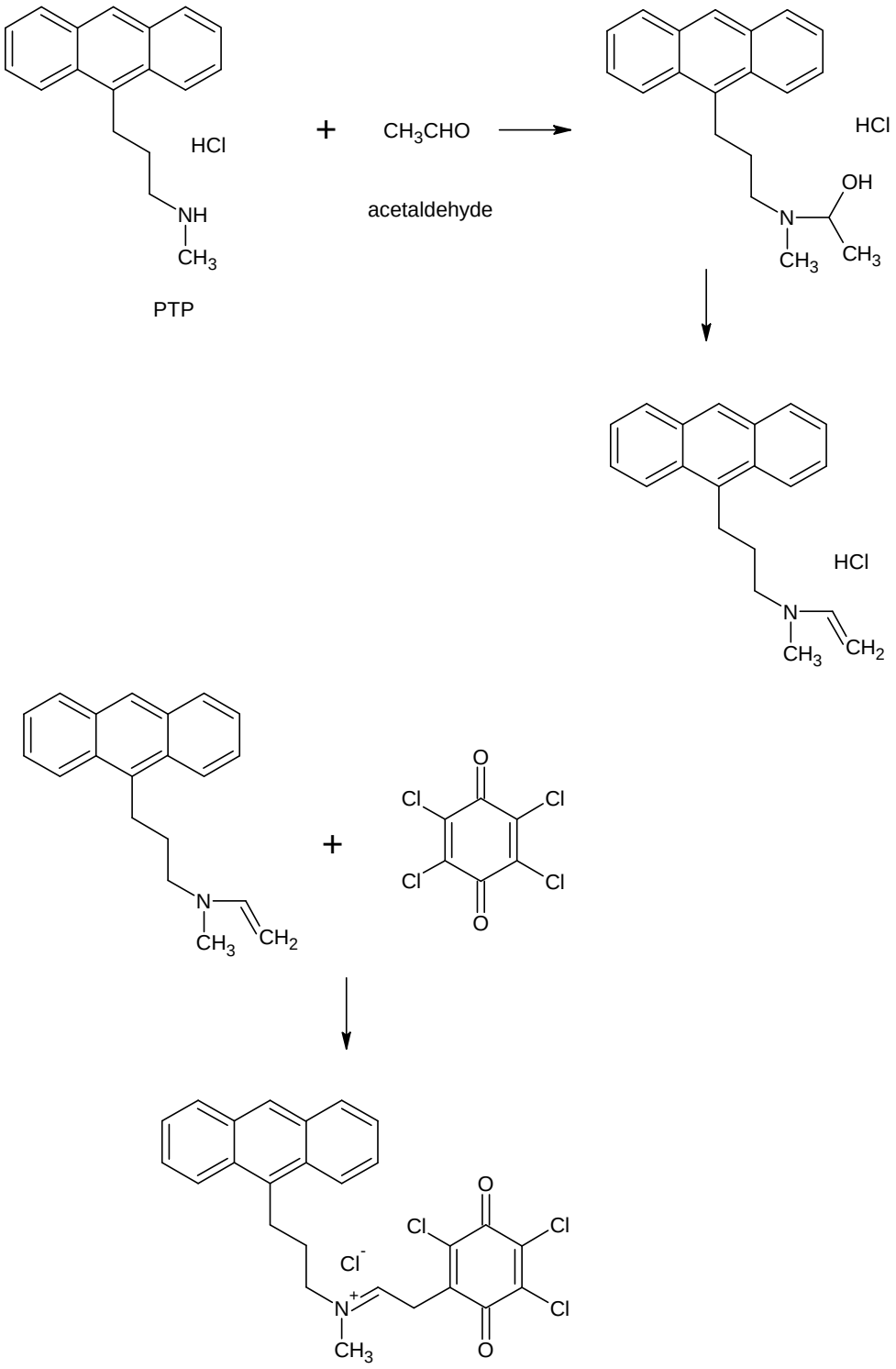
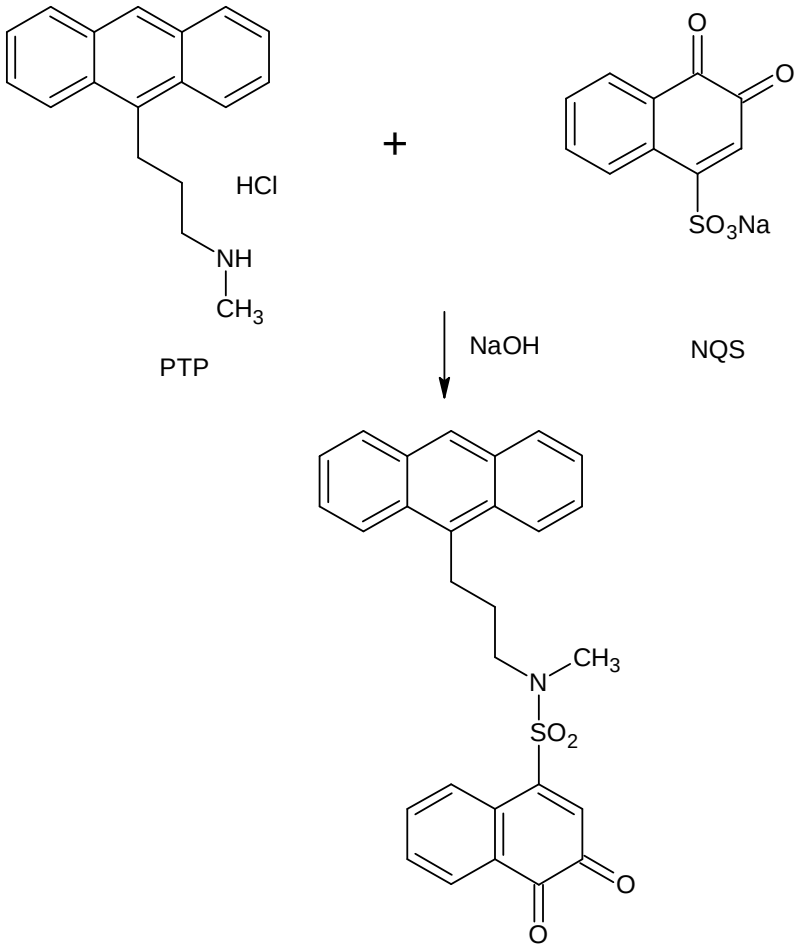


Figure 2. Absorption spectrum of (A) for PTP – p-CA system and (B) for PPT – NQS System.



Blue colored adduct

Scheme 1. Reaction of PTP with acetaldehyde followed by *p*-CA



Scheme 2. Reaction of PTP with NQS

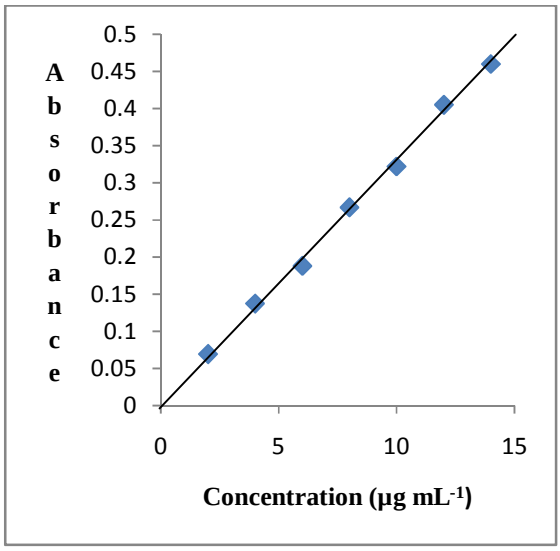


Figure 3. Calibration graph for method A

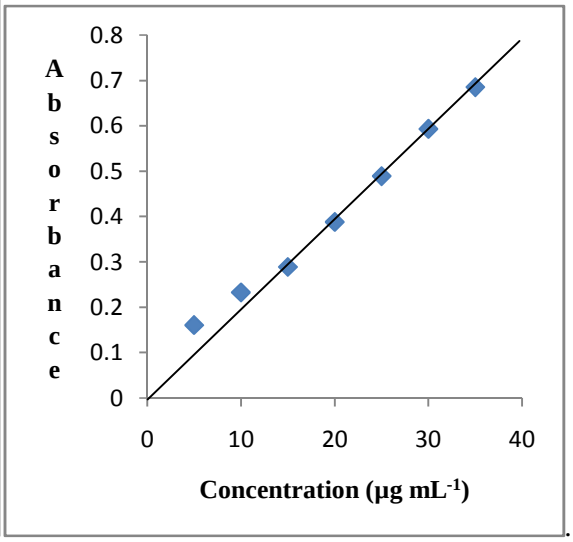


Figure 4. Calibration graph for method B

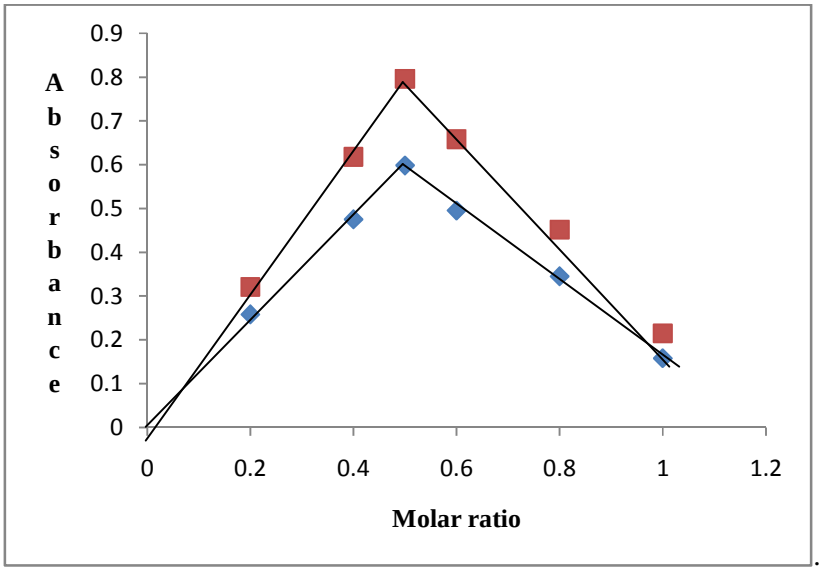


Figure 5. Continuous variation graph for the reaction of PPT with *p*-CA in method A and with NQS in method B.

CONCLUSION

Two new and simple spectrophotometric methods for the determination of PTP in tablets were developed and validated as per the ICH guidelines. The proposed methods are free from critical experimental conditions and complicated procedures such as heating or extraction steps. The reagents used in the proposed methods are cheap, readily available and the procedures do not involve any tedious sample preparation. These advantages encourage the application of the proposed methods in routine quality control analysis of PTP in pharmaceutical formulations.

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