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Research Article

Kinetic Modelling for the Assay of Nortriptyline Hydrochloride Using Potassium Permanganate as Oxidant

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Abstract. Kinetic methods for accurate determination of nortriptyline hydrochloride have been described. The methods are based on the oxidation of nortriptyline hydrochloride with $KMnO_4$ in acidic and basic media. In acidic medium, the decrease in absorbance at 525.5 nm and in basic medium, the increase in absorbance at 608.5 nm were measured as a function of time. The variables affecting the reactions were carefully investigated and optimised. Kinetic models such as initial rate, rate constant, variable time and fixed time were employed to construct the calibration curves. The initial rate and fixed time methods were selected for quantification of nortriptyline hydrochloride. In acidic medium, the calibration curves showed a linear response over the concentration range 10–50 μg mL^{-1} for initial rate and 10–60 μg mL^{-1} for fixed time method (2 min). In basic medium, the calibration graphs were linear over the concentration range 10–100 μg mL^{-1} for initial rate and fixed time methods (4 min). In acidic medium, the limits of detection for initial rate and fixed time methods (2 min) were 1.02 and 3.26 μg mL^{-1}, respectively. In basic medium, the limits of detection were found to be 1.67 and 1.55 μg mL^{-1} for initial rate and fixed time methods (4 min), respectively. The initial rate and fixed time methods have been successfully applied to the determination of nortriptyline hydrochloride in commercial dosage form. Statistical comparison of the results of the proposed methods with those of reference method exhibited excellent agreement and there is no significant difference between the compared methods in terms of accuracy and precision.

KEY WORDS: dosage forms; fixed time method; initial rate method; nortriptyline; potassium permanganate.

INTRODUCTION

Nortriptyline hydrochloride is chemically known as 1-propanamine, 3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-*N*-methyl hydrochloride (Fig. 1) and its molecular weight is 299.85. It is a second-generation tricyclic antidepressant widely used in the treatment of major depressive disorders due to its high *in-vivo* activity [1]. Besides that, there is growing evidence of its efficacy for smoking cessation pharmacological therapy [2]. It is used in the treatment of depression and childhood nocturnal enuresis. In addition, it is also recommended for the treatment of chronic pain or neuralgia modification, particularly temporomandibular joint disorders [3]. Nortriptyline blocks norepinephrine and serotonin more potently than dopamine transporter. It also antagonises serotonin, muscarinic and α-adrenergic receptors.

Literature survey revealed that potentiometric titration and HPLC methods for assay of nortriptyline were included in the monographs of the *British Pharmacopoeia* [4], *European Pharmacopoeia* [5] and *US Pharmacopoeia* [6], respectively.

Several other successful attempts have been made for its determination in bulk, pharmaceutical formulations and biological samples using different techniques. These techniques include fluorimetry [7], colorimetry [8], electrogenerated chemiluminescence [9], thin-layer chromatography [10], gas chromatography [11], high-performance liquid chromatography [10,12–17], fast Fourier transform continuous cyclic voltammetry [18], stripping voltammetry [19] and capillary zone electrophoresis [20,21]. Certain hyphenated techniques such as ultra performance liquid chromatography-tandem mass spectrometry [22], gas chromatography-mass spectrometry [23,24] and dispersive liquid-liquid microextraction combined with gas chromatography [11] have been reported for the assay of nortriptyline.

Doubtlessly, the above-mentioned sophisticated techniques for purity assay provide good specificity, excellent precision and accuracy and adequate sensitivity, usually the case with clinical samples. However, they are followed by certain shortcomings such as clean up procedure prior to experiments, maintenance problems, unavailability in most laboratories due to high cost, time-consuming analysis, etc. These shortcomings can be eliminated by introducing spectrophotometric techniques in pharmaceutical analysis. Literature survey revealed few spectrophotometric methods for nortriptyline determination in its bulk form and formulations based on extraction [25–27] and direct methods [28–30].

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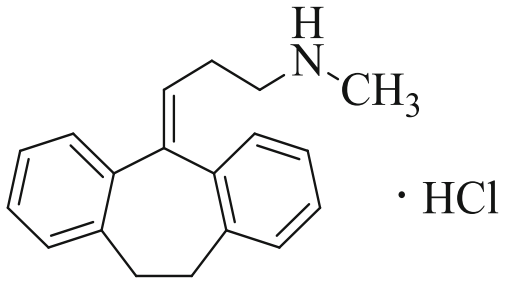

Fig. 1. Nortriptyline hydrochloride

Kinetic spectrophotometric methods are of a great interest in the pharmaceutical analysis [31] because of its simplicity, as it eliminates the tedious extraction and filtration experimental steps prior to the absorbance measurement and improves selectivity due to the measurement of increase or decrease of absorbance as a function of time. Also, the interference due to coloured or turbid samples and certain active ingredients present in the pharmaceutical formulations can be avoided. However, potassium permanganate has been used as a reagent for developing kinetic spectrophotometric methods for assay of drugs owing to its oxidising properties in alkaline and acidic media [32–34]. The literature is poor based on kinetic spectrophotometry for the determination of nortriptyline in pharmaceutical preparations. In the present manuscript, two simple approaches have been adopted to develop kinetic methods for the determination of nortriptyline in bulk and commercial dosage forms. The first method is based on the oxidation of drug with alkaline potassium permanganate at room temperature. The second method is based on the reaction with potassium permanganate in acidic medium. The experimental data were treated by different kinetic models to develop the calibration curves for assay of nortriptyline in commercial dosage forms.

EXPERIMENTAL

Apparatus

All the absorption spectral measurements were made using the Shimadzu UV-1800 spectrophotometer with a bandwidth of 2.0 nm and equipped with 10.0-mm matched quartz cells.

Materials and Reagents

All chemicals and reagents were of analytical reagent grade and were used as such without any further purification. Nortriptyline hydrochloride was purchased from Sigma-Aldrich. The reagents used included potassium permanganate and sodium lauryl sulphate which were procured from RFCL Ltd., New Delhi, and Otto Kemi, Mumbai, India, respectively. Sodium hydroxide and sulphuric acid were supplied from Qualigens Fine Chemicals, Mumbai, and Finar Chemicals Ltd., Ahmadabad, India, respectively. The pharmaceutical preparations of nortriptyline hydrochloride such as Primox (Sun pharmaceutical Pvt. Ltd., Gujarat, India), Sensival (Wallace Pharmaceutical Pvt. Ltd., Karnataka, India) and Ananda (Kivi Labs Ltd., Gujarat, India) were purchased from the local pharmacy shop. Each tablet was labelled to contain 25 mg nortriptyline.

Solutions

A 0.1% (*w*/*v*) nortriptyline hydrochloride solution was prepared by dissolving 100 mg in 100 mL distilled water. In distilled water, 1.0×10^{-3} M and 4.0×10^{-3} M $KMnO_4$ solutions were freshly prepared. Its apparent purity was assayed by standard method [35]. In double distilled water, 0.5 M NaOH, 1.0 M H_2SO_4 and 0.02 M sodium lauryl sulphate (SLS) solutions were prepared.

General Procedure

Procedure for Determination of Nortriptyline Hydrochloride

Method A:
Into a series of 10-mL volumetric flasks, 2.5 mL of 4.0×10^{-3} M $KMnO_4$, 2.5 mL of 0.5 M NaOH and 2.2 mL of 0.02 M SLS solutions were transferred. Then, varying aliquots of 0.1% nortriptyline hydrochloride (0.1–1.0 mL) was pipetted into the flasks and diluted to volume with double distilled water at 30±1°C. The contents were mixed well and immediately transferred to the spectrophotometric cells to record the increase in absorbance at 608.50 nm as a function of time for 12 min against a reagent blank prepared similarly without drug.
Method B:
Into a series of 10-mL volumetric flasks, 4 mL of 1.0×10^{-3} M $KMnO_4$ and 1 mL of 1.0 M H_2SO_4 solutions were transferred. Varying volumes of 0.1% nortriptyline hydrochloride standard solution were pipetted into 10-mL flasks and each diluted to volume with double distilled water at 30±1°C. The contents were mixed well and the decrease in absorbance at 525.50 nm was recorded as a function of time for 5 min against distilled water.

The following four kinetic methods were adopted for the construction of calibration curves.

i. Initial rate method: The initial rates of the reaction (v) at different concentrations were obtained from the slope of the tangent of absorbance-time curves. The initial rate of the reaction was plotted against the initial concentration of nortriptyline for both the acidic and basic media.
ii. Rate constant method: The rate constants (K') were plotted against the concentration of nortriptyline for the respective method.
iii. Variable time method: The calibration curve was obtained by plotting the reciprocal time in seconds ($1/t$) against the initial concentration of nortriptyline.
iv. Fixed time method: The absorbance difference (ΔA) between the times (t_1) and (t_2) was computed and plotted against the initial concentration of nortriptyline.

Alternatively, regression equations were also developed for the estimation of the content of nortriptyline hydrochloride.

Procedure for the Assay of Nortriptyline in Pharmaceutical Preparations

Five tablets from each brand namely, Primox, Sensival and Ananda were taken. The coloured coats of these tablets were removed with water and air dried. The tablets were then crushed and powdered. Ethanol was selected as an appropriate solvent to extract the nortriptyline completely from the pharmaceutical formulations and to remove water-soluble excipients. In view of this, the fine powder was swirled in ethanol and allowed to stand for 5 min. The residue was then filtered on a Whatmann No. 1 filter paper and washed properly with ethanol for complete recovery of drug. The filtrate was evaporated on a water bath at 40°C and finally diluted in a 100-mL calibrated flask with double distilled water to achieve a concentration of 1 mg mL^{-1}. The general procedure was then followed for determination of nortriptyline.

Procedure for Reference Method

Appropriate aliquots of nortriptyline solution, 5–234 µg, were transferred into several 125-mL separating funnels. A 3.0 mL amount of sodium acetate-HCl buffer of pH 3.29 and 3.0 mL of bromopyrogallol red (0.1%) were added followed by 10 mL chloroform to each of the separating funnels. After that, the contents were shaken well and left for 1 min at the room temperature. Two phases were allowed to separate. A chloroform layer was passed through anhydrous sodium sulphate. The absorbance of yellow-coloured solution was measured at 425 nm against the reagent blank.

RESULTS AND DISCUSSION

Spectral Studies

Potassium permanganate acts as an oxidising agent in acidic, neutral and basic media according to the following reactions:

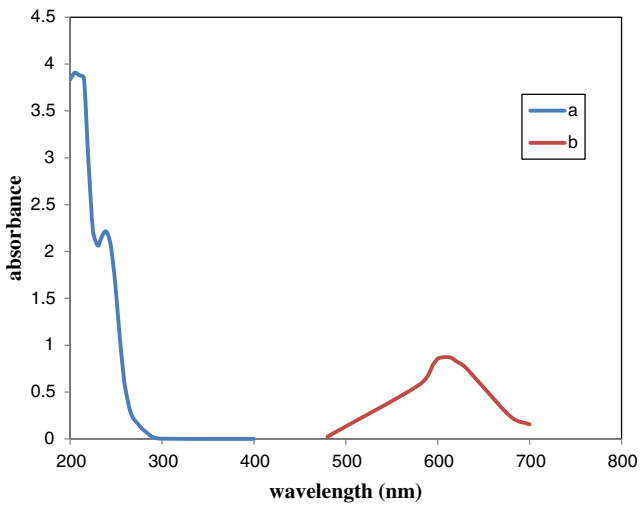

Fig. 2. Absorption spectra of *a* 1 mL of 1 mg mL^{-1} nortriptyline diluted to 10 mL with distilled water and *b* 2.5 mL of 4×10^{-3} M $KMnO_4$+2.5 mL of 0.5 M NaOH+2.2 mL of 2×10^{-2} M SLS+1 mL of 1 mg mL^{-1} nortriptyline diluted to 10 mL with distilled water

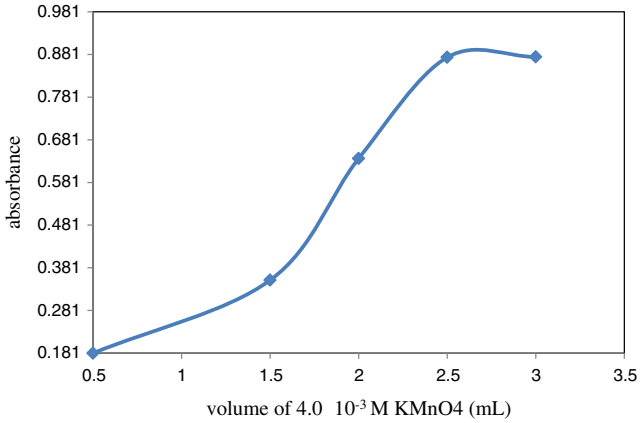

Fig. 3. Effect of concentration of $KMnO_4$ on the absorbance at 608.50 nm (nortriptyline concentration=100 µg mL^{-1}; NaOH concentration=1.25×10^{-1} M; SLS concentration=4.4×10^{-3} M; temperature=30±1°C)

Acidic medium

$$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O; E^0 = 1.5 \text{ volts}$$

Neutral medium

$$MnO_4^- + 2H_2O + 3e^- \rightarrow MnO_2 + 4OH^-; E^0 = 1.23 \text{ volts}$$

Basic medium

$$MnO_4^- + e^- \rightarrow MnO_4^{2-} +; E^0 = 0.56 \text{ volts}$$

The absorption spectrum of potassium permanganate solution in the basic medium exhibits an absorption band peaking at 525.50 nm while nortriptyline hydrochloride shows two absorption bands at 215 and 243 nm. The

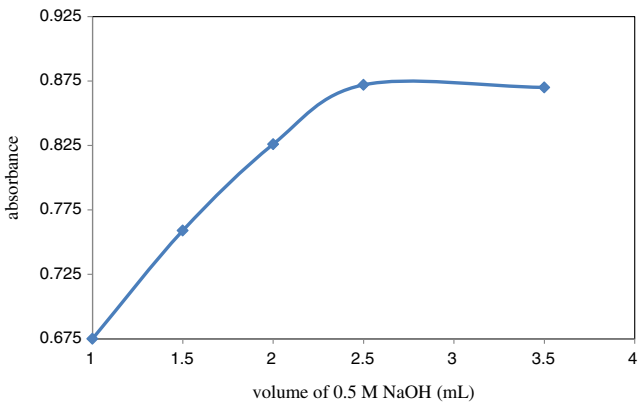

Fig. 4. Effect of volume of 0.5 M NaOH solution on the absorbance at 608.50 nm (nortriptyline concentration=100 µg mL^{-1}; $KMnO_4$ concentration=1.0×10^{-3} M; temperature=30±1°C)

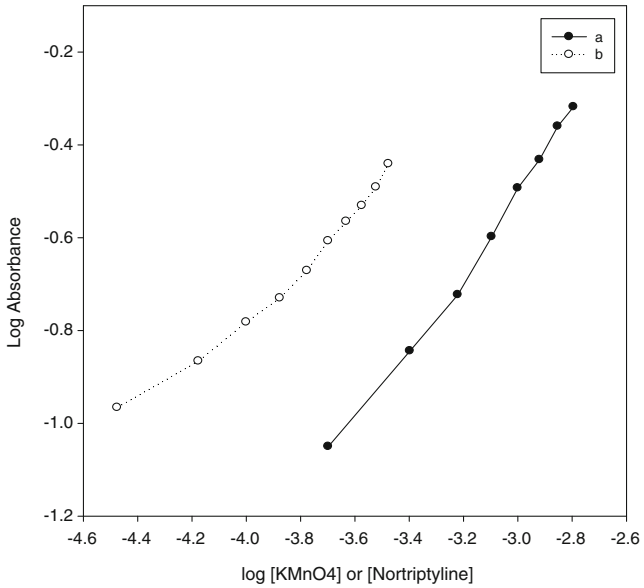

Fig. 5. Limiting logarithmic plot for molar combining ratio between nortriptyline and $KMnO_4$ in basic medium: *a* log A *vs.* log[$KMnO_4$]; *b* log A *vs.* log[nortriptyline]

addition of nortriptyline hydrochloride to the alkaline permanganate solution resulted in the shift of the band peak to 608.50 nm (Fig. 2). This is due to the formation of manganate ion in the presence of drug. The absorbance increases as a function of time and thus kinetic method was developed for the determination of nortriptyline.

Moreover, the reaction of nortriptyline with acidic potassium permanganate caused a decrease in absorbance, which is due to the reduction of Mn (VII) to Mn (II) oxidation state. Thus, a kinetic method was also developed for assay of nortriptyline.

Optimization of Variables

Effect of Potassium Permanganate Concentration

The effect of potassium permanganate concentration in basic medium on the absorbance of the product was studied in the range of 2.0×10^{-4} to 1.6×10^{-3} M, keeping the concentration of drug constant (100 μg mL^{-1}). The results are shown in Fig. 3. As can be seen from the figure, the maximum colour intensity of the product was achieved with 1.0×10^{-3} M $KMnO_4$ solution, and therefore, 1.0×10^{-3} M $KMnO_4$ was used for subsequent measurement. In acidic medium, the concentration of $KMnO_4$ was fixed at 4.0×10^{-4} M as the absorbance decreases on addition of the drug.

Effect of Sodium Hydroxide

The effect of concentration of NaOH was studied by measuring the absorbance of solutions containing a fixed concentration of nortriptyline (100 μg mL^{-1}) and varying volumes of 0.5 M NaOH solution. The maximum absorbance of the solution was obtained with the 2.5 mL of 0.5 M NaOH solution (Fig. 4). However, further addition of NaOH caused no change in the absorbance value, and therefore, 2.5 mL of 0.5 M NaOH was used throughout the experiment.

Effect of Sodium Lauryl Sulphate Concentration

Turbidity appeared when alkaline $KMnO_4$ was added to nortriptyline solution. SLS was added to the reaction mixture to get a clear solution and stable absorbance. Therefore, the effect of volume of 2.2×10^{-2} M SLS was studied from 0.5 to 3.0 mL to make the solution clear and absorbance value stable. The maximum absorbance was obtained with 2.0 mL of 2.2×10^{-2} M SLS and remained unchanged on further addition. Therefore, 2.0 mL of 2.2×10^{-2} M SLS was used as an optimum volume for subsequent measurement.

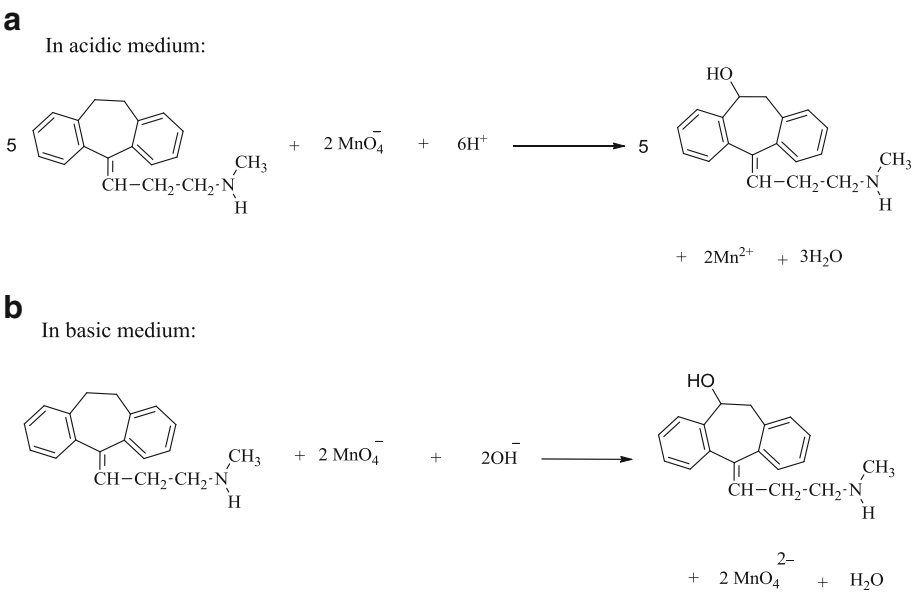

Fig. 6. Oxidation of nortriptyline by $KMnO_4$ in **a** acidic medium and **b** basic medium

Effect of Sulphuric Acid

The effect of concentration of H_2SO_4 was studied in the range 0.01–0.2 M, keeping the other variables constant. A constant and reproducible absorbance was obtained with 0.1 M H_2SO_4. Hence, 0.1 M H_2SO_4 was used throughout the experiment.

Stoichiometry and Mechanism

The stoichiometric ratio between potassium permanganate and nortriptyline in basic medium was ascertained by limiting logarithmic method by performing two sets of experiments. In the first set, nortriptyline concentration was varied keeping $KMnO_4$ concentration fixed and vice-versa, in the other set of experiment. The logarithm of the absorbance thus obtained was plotted against the logarithm of the molar concentration of $KMnO_4$ or nortriptyline. The slopes of the two straight lines were perceived in each case, indicating the combining molar ratio between nortriptyline and $KMnO_4$ as 1:2 (Fig. 5). In acidic medium, the stoichiometric ratio between potassium permanganate and nortriptyline was ascertained by mole ratio method and found to be 2:5.

It has been reported that the oxidative attack occurs at the ring carbon atoms of nortriptyline [36]. In such reactions, OH groups are mainly attached to the ethylene bridge (C-10) of the central ring in nortriptyline [37,38]. In this study, nortriptyline was oxidised by potassium permanganate in both acidic and alkaline media and subsequently OH groups are attached to ethylene bridge (C-10) of central ring. On the basis of stoichiometric ratio and literature background, the mechanisms of the reactions in acidic and basic media are proposed and given in Fig. 6a, b, respectively.

Kinetic Modelling for Assay of Nortriptyline

Initial Rate Method

The initial rates of the reaction were determined from the slope of the initial tangent to absorbance-time curves (Fig. 7).

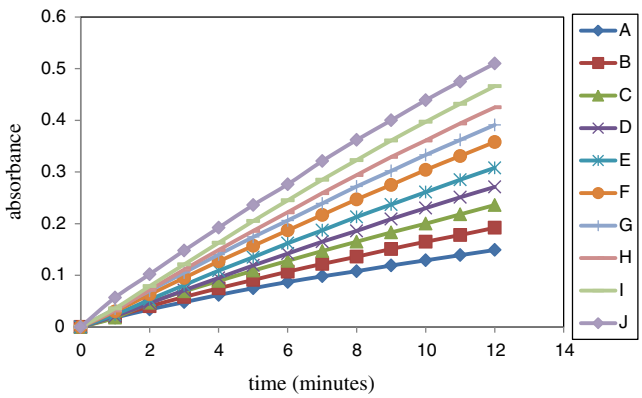

Fig. 7. Absorbance-time plot for the reaction between nortriptyline and $KMnO_4$ in basic medium: *A*=10 μg mL^{-1}, *B*=20 μg mL^{-1}, *C*=30 μg mL^{-1}, *D*=40 μg mL^{-1}, *E*=50 μg mL^{-1}, *F*=60 μg mL^{-1}, *G*=70 μg mL^{-1}, *H*=80 μg mL^{-1}, *I*=90 μg mL^{-1}, *J*=100 μg mL^{-1}

Table I. Optical and Regression Characteristics of Initial Rate Methods

Parameters	Acidic medium	Basic medium
Linear dynamic range (μg mL^{-1})	10–50	10–100
Regression equation	ϑ=−1.07×10^{-3} [NRTP]−7.30×10^{-3}	ϑ=3.00×10^{-4} [NRTP]+9.40×10^{-3}
Correlation coefficient (R^2)	0.9902	0.9996
S_o[a]	3.16×10^{-4}	2.22×10^{-4}
Intercept	−7.30×10^{-3}	9.40×10^{-3}
S_a[b]	3.32×10^{-3}	1.51×10^{-4}
$\pm tS_a$[c]	9.22×10^{-4}	3.42×10^{-4}
Slope	−1.07×10^{-3}	3.00×10^{-4}
S_b[d]	1.00×10^{-5}	2.44×10^{-6}
$\pm tS_b$[e]	2.78×10^{-5}	5.52×10^{-6}
Variance (S_o^2) about regression	9.99×10^{-8}	4.91×10^{-8}
Detection limit (μg mL^{-1})	1.02	1.67
Quantitation limit (μg mL^{-1})	3.10	5.05

NRTP nortriptyline
[a] Standard deviation of the regression
[b] Standard deviation of the intercept
[c] Confidence interval of the intercept at 95% confidence level
[d] Standard deviation of the slope
[e] Confidence interval of the slope at 95% confidence level

Under the optimised experimental conditions, the assay of nortriptyline was performed in the presence of excess of $KMnO_4$ and $NaOH/H_2SO_4$. As a result, a pseudo-zero-order condition was obtained with respect to the concentration of

Table II. Optical and Regression Characteristics of Rate Constant Methods

Parameters	Acidic medium	Basic medium
Linear dynamic range (μg mL^{-1})	10–50	10–40
Regression equation	K'=2.35×10^{-1}[NRTP]+0.52	K'=−1.93×10^{-2}[NRTP]−2.57
Correlation coefficient (R^2)	0.9986	0.9981
S_o[a]	0.16	1.31×10^{-2}
Intercept	0.52	−2.57
S_a[b]	1.68×10^{-1}	1.60×10^{-2}
$\pm tS_a$[c]	4.66×10^{-1}	5.09×10^{-2}
Slope	2.35×10^{-1}	−1.93×10^{-2}
S_b[d]	5.06×10^{-3}	5.80×10^{-4}
$\pm tS_b$[e]	1.40×10^{-2}	1.85×10^{-3}
Variance (S_o^2) about regression	2.56×10^{-2}	1.71×10^{-4}
Detection limit (μg mL^{-1})	2.36	2.73
Quantitation limit (μg mL^{-1})	7.15	8.29

NRTP nortriptyline
[a] Standard deviation of the regression
[b] Standard deviation of the intercept
[c] Confidence interval of the intercept at 95% confidence level
[d] Standard deviation of the slope
[e] Confidence interval of the slope at 95% confidence level

Table III. Optical and Regression Characteristics of Variable Time Methods

Parameters	Acidic medium	Basic medium
Linear dynamic range (μg mL^{-1})	10–50	10–70
Regression equation	$1/t$=2.44×10^{-3} [NRTP]−3.16×10^{-2}	$1/t$=4.06×10^{-2} [NRTP]−1.02
Correlation coefficient (R^2)	0.9289	0.9985
S_o[a]	1.23×10^{-2}	4.74×10^{-2}
Intercept	−3.16×10^{-2}	1.02
S_a[b]	1.29×10^{-2}	4.01×10^{-2}
$\pm tS_a$[c]	3.58×10^{-2}	9.81×10^{-2}
Slope	2.44×10^{-3}	4.06×10^{-2}
S_b[d]	3.89×10^{-3}	8.90×10^{-4}
$\pm tS_b$[e]	1.08×10^{-3}	2.18×10^{-3}
Variance (S_o^2) about regression	1.51×10^{-4}	2.25×10^{-3}
Detection limit (μg mL^{-1})	–	3.26
Quantitation limit (μg mL^{-1})	–	9.88

NRTP nortriptyline
[a] Standard deviation of the regression
[b] Standard deviation of the intercept
[c] Confidence interval of the intercept at 95% confidence level
[d] Standard deviation of the slope
[e] Confidence interval of the slope at 95% confidence level

reagent. Therefore, the initial rate of reaction would depend on the concentration of the drug. The following rate equation can be written:

$$\vartheta = \frac{dA}{dt} = K' C^n$$

Where,

ϑ=reaction rate, A=absorbance, t=time in minutes, K'= pseudo-first-order rate constant, C=concentration of the drug (mol L^{-1}), and n=order of the reaction.

The logarithmic form of the above equation can be written as follows,

$$\log[\vartheta] = \log\frac{\Delta A}{\Delta t} = \log K' + n\log C$$

In basic medium, the order of the reaction was obtained by either plotting the logarithm of the initial rate of reaction [log ϑ] *versus* logarithm of initial concentration of the drug or the regression analysis of the data which yielded the equation:

$$\log[\vartheta] = 0.549\log[\text{NRTP}] + 0.518$$

with a correlation coefficient (R^2), 0.9818. The order of the reaction was found to be 0.549. The calibration curve was prepared by plotting the initial rate of the reaction against the concentration of nortriptyline (μg mL^{-1}). The results of regression analysis of calibration data are reported in Table I.

In acidic medium, the order of the reaction was obtained from regression analysis of logarithm of the initial rate of reaction *vs* logarithm of the initial molar concentration of nortriptyline. The regression equation is represented as follows:

$$\log[\vartheta] = 0.751\log[\text{NRTP}] - 2.502$$

Table IV. Optical and Regression Characteristics of Fixed Time Methods

	Acidic medium	Basic medium			
Parameters	2 min	2 min (A_4–A_2)	4 min (A_6–A_2)	6 min (A_8–A_2)	8 min (A_{10}–A_2)
Linear dynamic range (μg mL^{-1})	10–60	20–90	10–100	30–100	10–100
Regression equation	ΔA=5.80×10^{-3}[NRTP]+0.114	ΔA=9.00×10^{-4}[NRTP]+0.023	ΔA=1.80×10^{-3}[NRTP]+0.044	ΔA=2.60×10^{-3}[NRTP]+0.066	ΔA=3.70×10^{-3}[NRTP]+0.088
Correlation coefficient (R^2)	0.9973	0.9960	0.9995	0.9997	0.9995
Standard deviation of the regression	5.94×10^{-3}	1.36×10^{-3}	0.00128	0.00107	2.43×10^{-3}
Intercept	1.14×10^{-1}	2.31×10^{-2}	4.40×10^{-2}	6.60×10^{-2}	8.80×10^{-2}
S_a[a]	5.80×10^{-3}	1.25×10^{-3}	8.70×10^{-4}	1.14×10^{-3}	1.66×10^{-3}
$\pm tS_a$[b]	1.49×10^{-3}	2.96×10^{-3}	1.97×10^{-3}	2.70×10^{-3}	3.76×10^{-3}
Slope	5.87×10^{-3}	9.40×10^{-4}	1.86×10^{-3}	2.69×10^{-3}	3.43×10^{-3}
S_b[c]	1.43×10^{-5}	2.10×10^{-5}	1.42×10^{-5}	1.66×10^{-5}	2.68×10^{-5}
$\pm tS_b$[d]	3.68×10^{-4}	4.97×10^{-5}	3.21×10^{-5}	3.93×10^{-5}	6.06×10^{-5}
Variance (S_o^2) about regression	3.53×10^{-5}	1.85×10^{-6}	1.65×10^{-6}	1.15×10^{-6}	5.91×10^{-6}
Detection limit (μg mL^{-1})	3.26	4.39	1.55	1.40	1.60
Quantitation limit (μg mL^{-1})	9.88	13.30	4.68	4.25	4.84

NRTP nortriptyline
[a] Standard deviation of the intercept
[b] Confidence interval of the intercept at 95% confidence level
[c] Standard deviation of the slope
[d] Confidence interval of the slope at 95% confidence level

Table V. Intra- and Interday Precision and Accuracy of Initial Rate Method in Acidic Medium

Nominal concentration (μg mL^{-1})	Assayed concentration (μg mL^{-1})				
	Found±SD[a] (μgmL^{-1})	% Recovery	RSD, %	SAE	CL
Intraday (n=5)					
10	9.936±0.094	99.36	0.94	0.0419	0.1162
30	29.932±0.119	99.77	0.40	0.0530	0.1472
40	40.096±0.772	100.24	1.93	0.3453	0.9585
Interday (n=5)					
10	9.928±0.052	99.23	0.53	0.0234	0.0650
30	29.889±0.103	99.63	0.10	0.0459	0.0308
40	40.258±1.041	100.64	2.58	0.4655	1.2920

RSD relative standard deviation for the five determinations, *SAE* standard analytical error, *CL* confidence limit at 95% confidence level and four degrees of freedom (t=2.776)
[a] Mean±SD for five determinations performed over a period of 5 days

The order of the reaction was 0.751. The calibration curve was constructed by plotting the initial rate of reaction against the concentration of nortriptyline. Regression analysis of calibration data was performed and the results are reported in Table I.

Rate Constant Method

In acidic and basic media, rate constants at different initial concentrations were evaluated. The calibration curves were obtained by plotting rate constants against the concentration of nortriptyline. The regression analysis data are summarised in Table II.

Variable Time Method

Variable time method was carried out in both acidic and basic media.

i. In basic medium, time was recorded to attain a preselected absorbance value that is, 0.149 for different concentrations of nortriptyline.
ii. In acidic medium, time was recorded to achieve the preselected absorbance that is, 0.980 for different concentrations of drug.

The calibration graphs were constructed by plotting the reciprocal of time in seconds ($1/t$) against the initial concentration of the drug for both acidic and basic media and the results of regression analysis are reported in Table III.

Fixed Time Method

At a preselected fixed time, the absorbance of solution containing varying amounts of nortriptyline was measured at 608.50 and 525.50 nm in basic and acidic media, respectively. Calibration graphs were constructed by plotting the absorbance difference, $\Delta A = A_{t_2} - A_{t_1}$ (where A_{t_1} is the absorbance measured at 2 min and A_{t_2} is the absorbance measured at 4, 6, 8 and 10 min to maintain a fixed time of 2, 4, 6 and 8 min, respectively), against the initial concentration of nortriptyline at a fixed time of 2 min in acidic medium and 2, 4, 6 and 8 min in basic medium. The results of statistical analysis are summarised in Table IV.

As can be seen from Table I, the calibration graphs (initial rate *vs.* concentration) were found to be linear over the concentration range of 10–50 and 10–100 μg mL^{-1} for acidic and basic media, respectively. However, the correlation coefficient (R^2=0.9996) was higher in basic medium. The confidence limits for the slope of the line of regression and intercept were found to be 2.776×10^{-5} and

Table VI. Intra- and Interday Precision and Accuracy of Fixed Time Method in Acidic Medium

Nominal concentration (μg mL^{-1})	Assayed concentration (μg mL^{-1})				
	Found±SD[a] (μgmL^{-1})	% Recovery	RSD	SAE	CL
Intraday (n=5)					
20	20.060±0.093	100.31	0.47	0.0416	0.1155
50	49.880±0.152	99.78	0.30	0.0679	0.1885
Interday (n=5)					
20	19.994±0.186	99.78	0.93	0.0833	0.2310
50	49.880±0.152	99.78	0.31	0.0679	0.1885

RSD relative standard deviation for the five determinations, *SAE* standard analytical error, *CL* confidence limit at 95% confidence level and four degrees of freedom (t=2.776)
[a] Mean±SD for five determinations performed over a period of 5 days

Table VII. Intra- and Interday Precision and Accuracy of Initial Rate Method in Basic Medium

Nominal concentration (μg mL^{-1})	Assayed concentration (μg mL^{-1})				
	Found±SD[a] (μgmL^{-1})	% Recovery	RSD, %	SAE	CL
Intraday (n=5)					
30	29.960±0.079	99.88	0.26	0.0352	0.0977
60	59.890±0.064	99.82	0.11	0.0280	0.0777
80	79.970±0.086	99.97	0.11	0.0385	0.1069
Interday (n=5)					
30	29.949±0.055	99.83	0.18	0.0247	0.0686
60	59. 950±0.076	99.92	0.13	0.0340	0.0945
80	79.93±0.088	99.91	0.11	0.0393	0.1091

RSD relative standard deviation for the five determinations, *SAE* standard analytical error, *CL* confidence limit at 95% confidence level and four degrees of freedom (t=2.776)
[a] Mean±SD for five determinations performed over a period of 5 days

5.519×10^{-6} and 9.216×10^{-4} and 3.424×10^{-4} for acidic and basic media, respectively, which indicated the high reproducibility of the initial rate methods.

The calibration curves (rate constants *vs.* concentration) showed a linear relationship over the concentration range 10–50 and 10–40 μg mL^{-1} with R^2 of 0.9986 and 0.9981, in acidic and basic media, respectively (Table II). However, the confidence limits at 95% confidence level were much higher as compared to initial rate methods.

Table III shows the results of regression analysis of calibration data ($1/t$ *vs.* concentration). In acidic medium, the value of R^2 is lower so this calibration curve was not adopted for determination of nortriptyline. However, in basic medium, the value of R^2 is 0.9987 with detection limit of 3.26 μg mL^{-1}.

As evident from the Table IV, the lowest detection limit was obtained with a fixed time of 6 min whereas the fixed time of 4 min showed a wider concentration range for quantification in alkaline medium. According to the ICH guidelines [39], the detection limit is not required to be part of the validation procedure for assay. Hence, the fixed time of 4 min was recommended for determination due to a wider concentration range and less time of analysis. In acidic medium, a fixed time of 2 min exhibited a linear dynamic concentration range of 10–60 μg mL^{-1} with a detection limit of 3.260 μg mL^{-1}.

Method Validation Parameters

Accuracy and Precision

The accuracy and precision of the proposed kinetic spectrophotometric methods were determined in terms of intermediate precision. Five replicates were performed on pure nortriptyline solution at three different concentration levels within the specified range and were analysed during the same day (intraday precision) and for six consecutive days (interday precision) in basic as well as in acidic medium. The analytical results obtained by initial rate and fixed time methods are compiled in Tables V, VI, VII and VIII. Percentage relative standard deviation (%RSD) as precision and percentage recovery as accuracy of the suggested methods of nortriptyline ascertained from the calibration curves showed that the present methods have good repeatability and reproducibility.

Selectivity

Selectivity of the proposed methods was determined by analysing pure nortriptyline with certain excipients such as calcium phosphate, magnesium stearate, lactose and starch. The method showed no interference from the excipients.

Table VIII. Intra- and Interday Precision and Accuracy of Fixed Time Method in Basic Medium

Nominal concentration (μg mL^{-1})	Assayed concentration (μg mL^{-1})				
	Found±SD[a] (μgmL^{-1})	% Recovery	RSD, %	SAE	CL
Intraday (n=5)					
30	30.005±0.605	100.02	2.02	0.2704	0.7506
60	60.120±0.422	100.20	1.70	0.1886	0.5230
80	79.880±0.245	99.85	0.31	0.1096	0.3040
Interday (n=5)					
30	29.890±0.273	99.63	0.91	0.1220	0.3380
60	60.221±0.660	100.35	1.10	0.2950	0.8189
80	80.020±0.377	100.03	0.47	0.1687	0.4683

RSD relative standard deviation for the five determinations, *SAE* standard analytical error, *CL* confidence limit at 95% confidence level and four degrees of freedom (t=2.776)
[a] Mean±SD for five determinations performed over a period of 5 days

Table IX. Point and Interval Hypothesis Tests: Comparison of the Proposed Methods with the Reference Method at 95% Confidence Level

		Fixed time method			
		Acidic medium		Basic medium(A_6–A_2)	
Formulations		Proposed method	Reference method	Proposed method	Reference method
Primox	% Recovery	100.27	99.05	99.89	99.91
	RSD (%)	0.79	0.37	0.38	0.37
	t value	1.159		0.339	
	F value	4.668		1.072	
	Θ_L	0.989		0.999	
	Θ_U	1.003		1.001	
Sensival	% Recovery	99.98	100.08	99.67	100.05
	RSD (%)	0.80	0.36	0.377	0.36
	t value	0.256		1.750	
	F value	4.744		1.064	
	Θ_L	0.993		0.999	
	Θ_U	1.009		1.009	
Ananda	% Recovery	99.69	100.08	99.84	100.08
	RSD (%)	0.65	0.36	0.70	0.36
	t value	1.626		0.891	
	F value	3.163		3.638	
	Θ_L	0.998		0.997	
	Θ_U	1.009		1.008	

Theoretical *t* (n=8) and *F* values (n=4, 4) at 95% confidence level are 1.860 and 6.39, respectively. Θ_L and Θ_U are within the acceptable limits of ±2%

APPLICATIONS

Fixed time methods (both acidic and basic media) were applied successfully for the nortriptyline determination in their pharmaceutical dosage forms. Five replicate measurements were made in each case and the drug concentration was computed from corresponding calibration equation. The results of the proposed methods were compared with those obtained by reference method [30] using point and interval hypothesis tests. In interval hypothesis, the lower and upper acceptance limits can be calculated using the following equation [40].

$$\theta^2\left[\overline{X}_1^{\,2} - S_p^2 t_{tab}^2/n_1\right] - 2\theta\overline{X}_1\overline{X}_2 + \theta^2\left[\overline{X}_2^{\,2} - S_p^2 t_{tab}^2/n_2\right] = 0$$

The values of θ_L and θ_U of confidence interval were obtained as

$$\theta_L = -b - \frac{(b^2 - 4ac)^{\frac{1}{2}}}{2a}$$

$$\theta_U = -b + \frac{(b^2 - 4ac)^{\frac{1}{2}}}{2a}$$

Where,

$$a = X_1^2 - Sp^2\, t_{tab}^2 / n_1$$
$$b = -2\, X_1 X_2$$
$$c = X_2^2 - Sp^2 t_{tab}^2 / n_2$$

No significant difference amongst the two methods was observed as the calculated *t* (paired) and *F* values at 95% confidence level did not exceed the tabulated ones [41], thus indicating good accuracy and precision in the analysis of nortriptyline in dosage forms. As evident from Table IX, θ_L and θ_U values of all the samples of drug lie within the acceptance limit of 0.98 and 1.02, that is, smaller than ±2%, indicating the compliance to regulatory guidelines [42].

CONCLUSION

The proposed kinetic spectrophotometric methods were simple, sensitive, accurate and precise, and thus, these can be alternative methods for nortriptyline determination in pure and dosage forms. Easy availability and low-cost reagents enable their frequent application in the research laboratories, pharmaceutical industries and hospitals. Moreover, a good drug recovery in the formulations suggested noninterference of excipients in the assay procedure.

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