International Journal of Pharmaceutical Chemistry

ISSN: 2249-734X (Online)
Journal DOI:10.7439/ijpc
CODEN:IJPCI4 (American Chemical Society)

Research Article

A validated kinetic method for determination of nortriptyline hydrochloride in pharmaceutical formulations

Nafisur Rahman[1], Habibur Rahman[*1,2] and Asma Khatoon[1,3]

[1]*Analytical Chemistry Division, Department of Chemistry, Aligarh Muslim University, Aligarh (U.P.) -202002, India*
[2]*Department of General Studies, Jubail Industrial College P.O. Box No. – 10099, Jubail Industrial City, Jubail 31961, Saudi Arabia*
[3]*Centre of Lipid Engineering and Applied Research Laboratory, Department of Chemical Engineering, Universiti Teknologi Malaysia 81310 Skudai, Johor, Malaysia*

Abstract

The objective of this research is to develop a simple, sensitive and validated kinetic spectrophotometric method for the determination of nortriptyline hydrochloride in pure and pharmaceutical formulations. The method is based on derivatization of drug with1-chloro-2,4-dinitrobenzene (CDNB) in dimethyl sulfoxide (DMSO) medium at room temperature (30 $\pm1^0$C). The reaction was followed spectrophotometrically by measuring the increase in absorbance at 390 nm as a function of time. Under the optimized experimental conditions, Beer's law obeyed in the concentration range of 20-100 µg/ml for both initial rate and fixed time methods. The limits of quantitation are 2.975 and 3.680 µg/ml for initial rate and fixed time methods, respectively. The method has been successfully applied to the determination of nortriptyline in pharmaceutical formulations. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

Keywords: Nortriptyline hydrochloride; 1-chloro-2,4-dinitrobenzene; Kinetic spectrophotometry; Pharmaceutical formulations

1. Introduction

Depression is one of the most common psychiatric disorders that affect about 121 million people worldwide.[1] Nortriptyline hydrochloride which is chemically known as 3-(10, 11-dihydro-5H-dibenzo [a,d]cyclohepten-5-ylidene)-N-methyl-1-propanamine hydrochloride belongs to a general class of tricyclic antidepressant (TCA) drugs. It is used to treat various forms of depression, pain associated with the nerves (neuropathic pain) and to prevent migraine conditions. TCA overdose is a primary cause of severe poisoning in many hospitalized patient and an effective treatment has yet to be identified. Nortriptyline is chemically basic (log K_a= 9.7) and available in the form of hydrochloride salt.

The drug and its formulation is officially listed in British Pharmacopoeia and European Pharmacopoeia, which describe UV methods.[2,3] Few analytical methods have been reported for the determination of nortriptyline hydrochloride in pharmaceutical formulations and biological fluids are based on high performance liquid chromatography,[4-7] ultra performance liquid chromatography-tandem mass spectrometry[8], liquid chromatography with tandem mass spectrometry (MS/MS),[9] gas chromatography (GC),[10] GC-MS,[11,12] fast fourier transform continuous cyclic voltammetry[13] and capillary electrophoresis.[14] These methods/techniques are sensitive however, requires expensive and laborious clean up procedures prior to determination of drug. Spectrophotometry is the technique of choice even today due to its inherent simplicity, inexpensive and therefore still belongs to the most frequently used analytical technique. Spectrophotometric methods based on extraction[15-17] and direct methods[18-20] has been reported for the determination of nortriptyline in pure and pharmaceutical dosage forms. However many of these methods are limited in their applications or rather much tedious and time consuming. The literature is still poor in analytical procedure based on kinetics, especially for the determination of drugs in pharmaceutical formulations. Kinetic based spectrophotometric method have so many advantages over simple spectrophotometry such as less analysis time, high sensitivity, low limit of detection and simplicity owing to elimination of some experimental steps such as filtration and extraction prior to absorbance measurement.

This paper describes the development and validation of a kinetic spectrophotometric method for the determination of nortriptyline hydrochloride based on derivatization with 1-chloro-2,4-dinitrobenzene (CDNB) in dimethyl sulfoxide (DMSO) medium at room temperature (30 ± 1^0C) and subsequent measurement of absorbance at 390 nm. The initial rate and fixed time methods were adopted for the determination of nortriptyline hydrochloride in pharmaceutical formulations.

2. Experimental

2.1 Apparatus: A Shimadzu UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells.

2.2 Materials and reagents: Nortriptyline hydrochloride pure drug was purchased from Sigma Aldrich Co. (Purity 98%). Pharmaceutical formulations of nortriptyline hydrochloride such as Sensival 25® (Wallace Pharma, India) and Primox 25® (Sun Pharma, India) were purchased from a local pharmacy shop. Solution of 1-chloro-2,4-dinitrobenzene (4.937×10^{-3} M, Fluka Chemie AG, Switzerland) was prepared in dimethyl sulfoxide (Merck Limited, Mumbai, India) and sodium carbonate (1 M, Merck Limited, Mumbai, India) was prepared in doubly distilled water. All Chemicals and reagents used were of analytical grade.

2.3 Preparation of Standard drug solution: The standard solution of nortriptyline (3.34×10^{-3} M) was prepared by taking an accurately weighed amount of drug salt equivalent to 100 mg and dissolved in 5 ml of the doubly distilled water. The solution rendered alkaline with 1M sodium carbonate followed by 50 ml doubly distilled water. The solution was transferred into a separating funnel. The base was extracted with

* Correspondence Info
Habibur Rahman
Department of General Studies,
Jubail Industrial College, P.O. Box No. – 10099,
Jubail Industrial City, Jubail 31961, Saudi Arabia
E-mail: habibr123@yahoo.co.in

three successive 30 ml portion of chloroform. The mixed chloroform extracts and washing were pooled through a filter paper containing anhydrous sodium sulphate. Finally, it was diluted to volume with chloroform in 100 ml volumetric flask.

2.4 Proposed procedure for the determination of nortriptyline hydrochloride

2.4.1 ***Initial rate method:*** Accurately measured aliquots of the standard drug solution in the concentration range 0.1- 0.5 ml of nortriptyline hydrochloride (3.34×10^{-3} M) corresponding to 100 – 500 µg were taken into a series of a 5ml standard volumetric flask. To each flask 1.5 ml of 4.937×10^{-3} M 1-chloro-2,4-dinitrobenzene (CDNB) were added and then diluted with dimethyl sulfoxide (DMSO) at room temperature (30 $\pm 1^0$C). The content of each flask was mixed well and increase in the absorbance was recorded as a function of time at 390 nm. The initial rate of the reaction (v) at different concentration was obtained by measuring the slope of the tangent to the absorbance– time plot. The calibration graph was obtained by plotting the initial rate of reaction (*v*) *versus* final concentration of the nortriptyline (*C*).

2.4.2 ***Fixed time method:*** The absorbance of each drug sample solution was measured at 390 nm against a reagent blank prepared similarly at a preselected fixed time of 10 min. The calibration curve was constructed by plotting the absorbance against the final concentration of the drug. The amount of the drug was computed either from calibration curve or regression equation.

2.5 Determination of nortriptyline hydrochloride in pharmaceutical formulations: To determine the content of nortriptyline in pharmaceutical formulations, twenty tablets were washed from the colour coat using distilled water, dried, weighed and finely powdered. A portion of the powder equivalent to 100 mg of active ingredient was dissolve in doubly distilled water, stirred well and filtered through Whatman No. 42 filter paper (Whatman International Limited, Kent, U.K.). The residue was washed well with doubly distilled water for complete recovery of the drug. The content of the drug was then render alkaline with 1M sodium carbonate followed by 50 ml doubly distilled water. The solution was transferred into a separating funnel. The base was extracted with three successive 30 ml portion of chloroform. The mixed chloroform extracts and washing were filtered through a filter paper containing anhydrous sodium sulphate into a 100 ml volumetric flask and then diluted to volume with chloroform to get a final concentration of 1mg/ml. An aliquot of the diluted solution was analyzed for nortriptyline content following the recommended procedure.

2.6. Limits of detection and quantitation: According to International Conference on Harmonisation (ICH) guidelines[21], the following expressions are used to evaluate limit of detection (LOD) and limit of quantitation (LOQ).

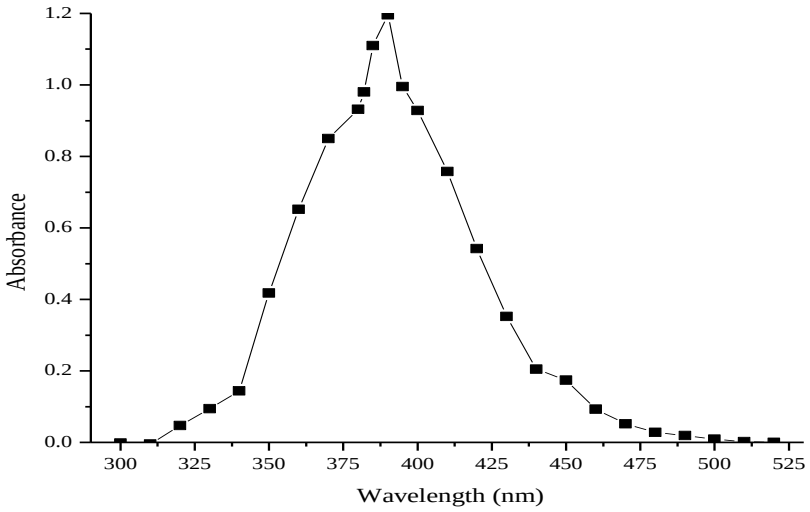
$$LOD = 3.3 \times S_0 / b \text{ and } LOQ = 10 \times S_0 / b$$

Where S_0 and b are standard deviation and slope of the calibration line, respectively .

3. Results and Discussion

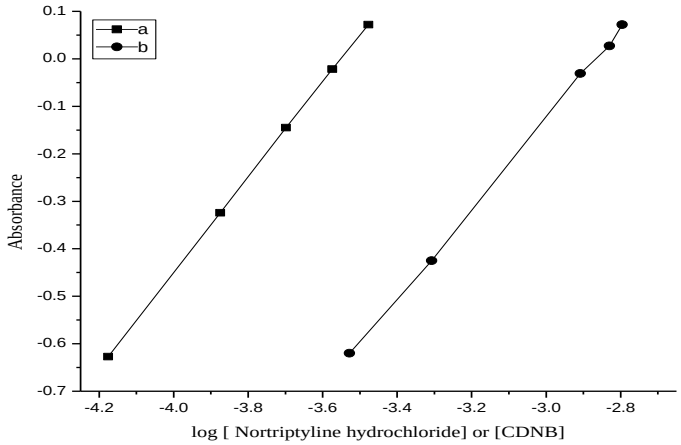
Spectral studies: Nortriptyline hydrochloride reacts with CDNB in DMSO medium resulting in the formation of yellow coloured product at room temperature (30 $\pm 1^0$C) shows absorption maxima at 390 nm (figure no. 1). It was found that the intensity of the coloured product increases with time. Therefore, increase in absorbance as a function of time was utilized to develop a kinetic spectrophotometric method for the determination of nortriptyline hydrochloride. The spectra of CDNB in DMSO were also taken against blank solution show absorption maxima peaking at 345 nm and a negligible absorbance at 390 nm.

Figure No. 1: Absorption spectra of the complex: 500 µg nortriptyline hydrochloride (0.1%) and 1.4 ml of 4.937×10^{-3} M CDNB. *versus* blank (1.4 ml of 4.937×10^{-3} M CDNB) in 10 ml volumetric flask diluted with DMSO



Stoichiometry: The limiting logarithmic method[22] was followed by performing two sets of the experiments to establish the stoichiometric ratio between nortriptyline and CDNB. In the first set of experiment, the concentration of nortriptyline hydrochloride was varied keeping a constant concentration of CDNB while in the second set, the concentration of CDNB was varied, keeping the constant concentration of nortryptiline. Log *A* versus log [Nortriptyline hydrochloride] or [CDNB] (figure no. 2a and 2b) was plotted to calculate the slope of the respective line to determine order of reaction of the drug with respect to CDNB or vice versa. The slope was found to be unity in both cases confirming the molar combining ratio of 1:1 between nortriptyline hydrochloride and CDNB. Hence, the results indicated that the one mole of CDNB was consumed by one mole of nortriptyline hydrochloride.

Figure no. 2: Limiting logarithmic plot for the molar combining ratio between nortriptyline hydrochloride and CDNB (a) log Absorbance *versus* log [Nortriptyline hydrochloride] (b) log Absorbance *versus* log [CDNB]

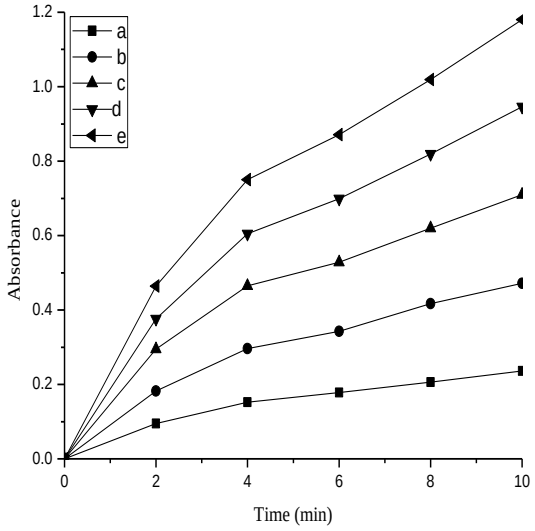


3.1 Optimization of variables

3.1.1 *Effect of concentration of CDNB*: The effect of the concentration of CDNB on the initial rate of reaction was studied in the range of 9.88 × 10^{-5} M –1.58 × 10^{-3} M keeping constant concentration of drug. It was found that the initial rate of reaction was increased with increasing concentration of CDNB and became constant at 1.38 × 10^{-3} M. Above this concentration up to 1.58 × 10^{-3} M, the initial rate of reaction was unchanged. Thus, the adoption of 1.48 × 10^{-3} M CDNB in the final solution proved to be sufficient for the maximum concentration of nortriptyline used in the calibration graph.

3.2 Analytical data: The rate of reaction was found to be dependent on concentration of nortriptyline. The initial rate of reaction was obtained from the slope of the initial tangent to the absorbance–time curve (figure no. 3).

Figure no. 3: Absorbance - Time curves for the reaction between nortriptyline hydrochloride and CDNB in DMSO medium : 1.5 ml of 4.937 × 10^{-3} M CDNB and nortriptyline hydrochloride (a) 6.671 × 10^{-5} M (b) 1.334 × 10^{-4} M (c) 2.001 × 10^{-4} M (d) 2.668 × 10^{-4} M (e) 3.335 × 10^{-4} M



It is clear from figure no. 3 that the initial rate increases with increasing the concentration of drug. Therefore, the kinetic equation for this reaction is written as

$$R_0 = dx/dt = k\,[\text{Nortriptyline}]^n\,[\text{CDNB}]^m \quad (1)$$

At [CDNB] ≥ 1.38 × 10^{-3} M, the order of reaction became zero with respect to the concentration of CDNB.

The equation 1 is reduced to $R_0 = k\,[\text{Nortryptiline}]^n$ (2)

The plot of logarithm of initial rate of reaction (log *v*) *versus* logarithm of molar concentration of nortriptyline (log *C*) indicated the first order reaction with respect to nortriptyline concentration. Equation (2) is transformed into pseudo first order reaction as

$$R_0 = k_\psi\,[\text{Nortriptyline}] \quad (3)$$

Where k_ψ is the pseudo first order rate constant.

Under the optimized experimental condition, the calibration graph obtained by plotting initial rate of reaction *versus* final concentration of nortriptyline showed a linear relationship over the range 20 - 100 µg/ml. The regression analysis using the least square method was performed to estimate the slope, intercept and correlation coefficient under optimized experimental conditions. The values of LOD, LOQ and variance were also calculated and summarized in table no. 1.

Table no. 1: Optical and regression characteristics of the Initial rate method

Parameters	
λ_{max} (nm)	390
Beer's law limit (µg/ml)	20 - 100
Linear regression equation	$\nu = 2 \times 10^{-5} + 1.43 \times 10^{-3}C$
S_a	4.47×10^{-4}
tS_a	1.24×10^{-3}
S_b	6.73×10^{-6}
tS_b	1.87×10^{-5}
Correlation coefficient (r^2)	0.9999
Variance (S_0^2)	1.81×10^{-7}
LOD (µg/ml)	0. 982
LOQ (µg/ml)	2. 975

In the fixed time method, the absorbance of yellow coloured product formed and recorded at a preselected fixed time for different concentrations of nortriptyline hydrochloride. The calibration graphs were constructed by plotting the absorbance against the initial concentrations of nortriptyline hydrochloride at a fixed time of 2, 4, 6, 8 and 10 minutes. The results indicated a linear increase in absorbance with time and hence, determination can be done in narrow range of time. It is clear from table no. 2 that the most acceptable values of linearity, intercept, limits of detection and quantitation were obtained at a fixed time of 10 minutes. Therefore, the fixed time of 10 minutes was selected as the optimum time for the determination of nortriptyline hydrochloride in pharmaceutical formulations (table no. 2).

Table no. 2: Optical characteristics and analytical data for the fixed time method

			Fixed time		
Parameters	**2 min**	**4 min**	**6 min**	**8 min**	**10 min**
Beer's law limit (µg/ml)	20-100	20-100	20-100	20-100	20-100
Regression equation	$A = 2.80\times 10^{-3} + 4.66\times 10^{-3}C$	$A = 2.10 \times 10^{-3} + 7.53\times 10^{-3}C$	$A = 1.20\times 10^{-3} + 8.71\times 10^{-3}C$	$A = 7.80 \times 10^{-2} + 1.01 \times 10^{-2}C$	$A = 1.90 \times 10^{-3} + 1.18 \times 10^{-2}C$
S_o	8.84×10^{-3}	8.22×10^{-3}	4.84×10^{-3}	4.34×10^{-3}	4.35×10^{-3}
S_a	9.28×10^{-3}	8.62×10^{-3}	5.08×10^{-3}	4.54×10^{-3}	4.56×10^{-3}
S_b	1.39×10^{-4}	1.30×10^{-4}	7.66×10^{-5}	6.85×10^{-5}	6.87×10^{-5}
Correlation coefficient (r^2)	0.9974	0.9991	0.9997	0.9999	0.9999
Detection limit (µg/ml)	6.26	3.60	1.84	1.41	1.21
Quantitation limit (µg/ml)	18.79	10.81	5.51	4.28	3.68
Variance, S_0^2 (µg/ml)	7.81×10^{-5}	6.76×10^{-5}	2.34×10^{-5}	1.88×10^{-5}	1.89×10^{-5}
Molar Absorbitivity (ε) (l/mol.cm)	1.424×10^{3}	2.278×10^{3}	2.670×10^{3}	3.088×10^{3}	3.560×10^{3}

3.3 Method validation

Precision: It was determined by analyzing the pharmaceutical formulations. For this purpose, an amount of the tablet powder equivalent to 100% of the label claim of the nortriptyline was accurately weighed and assayed. The intraday repeatability was determined by measuring the nortriptyline content of the sample solution five times within one day at the analytical concentration of 20, 60 and 100 µg/ml. The interday precision was also assessed by assaying the sample solution at three concentration levels (20, 60 and 100 µg/ml) on five consecutive days. (Table no. 3)

Table no. 3: Test of precision of the initial rate and fixed time methods

Proposed Methods	**Amount (µg/ml)**		**Recovery± RSD[a]**	**SAE[b]**	**C.L.[c]**
	Taken	**Found ± SD[a]**	**(%)**		
Initial rate method					
Intraday assay	20.0	19.97 ±0.077	99.86 ± 0.386	0.034	0.095
	60.0	60.03 ± 0.062	100.05±0.104	0.028	0.078
	100.0	99.99 ±0.494	99.99 ±0.495	0.221	0.614
Inter day assay	20.0	20.03± 0.106	100.14±0.529	0.047	0.131
	60.0	59.99 ±0.086	99.98±0.143	0.038	0.106
	100.0	99.85±0.585	99.85±0.586	0.261	0.726
Fixed time method					
Intraday assay	20.0	19.99 ± 0.093	99.96 ±0.464	0.042	0.115
	60.0	60.03 ±0.110	100.04 ± 0.184	0.049	0.137
	100.0	99.91 ±0.446	99.91 ± 0.446	0.199	0.554
Inter day assay	20.0	19.99 ± 0.139	99.96 ± 0.696	0.062	0.173
	60.0	60.01 ±0.134	100.02 ± 0.223	0.060	0.166
	100.0	99.87 ± 0.592	99.87 ± 0.552	0.265	0.735

[a]Mean for five independent analyses.
[b]SAE, standard analytical error.
[c]C.L., confidence limit at 95 % confidence level and four degrees of freedom (t = 2.776).

Accuracy: The samples were spiked with extra 50, 100 and 200% of the standard nortriptyline and the mixtures were analyzed by the proposed method to estimate the nortriptyline hydrochloride from pharmaceutical formulations. The experiment was repeated 5 times. This was done to check for the recovery of the drug at different levels in the formulations. After spiking with 50, 100 and 200% of additional drug afforded recovery of 99.87%- 100.07% as listed in the table no. 4.

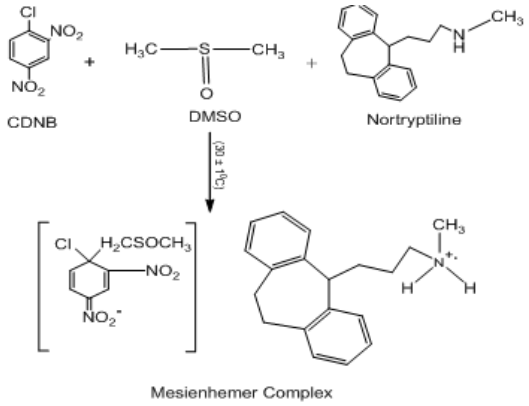
Table no. 4: Accuracy and Recovery

Excess of drug added to the analyte (%)	Initial rate method			Fixed time method		
	Recovery[a] ± RSD (%)	SAE[b]	CL[c]	Recovery[a] ± RSD (%)	SAE[b]	CL[c]
0	100.07 ± 0.469	0.042	0.116	99.87 ± 0.643	0.057	0.160
50	100.05 ± 0.208	0.028	0.078	99.97 ± 0.464	0.062	0.173
100	99.99 ± 0.228	0.041	0.113	100.02 ± 0.396	0.071	0.197
200	99.97 ± 0.165	0.044	0.123	99.96 ± 0.161	0.043	0.120

[a]Average of five independent analysis.
[b]Standard analytical error.
[c]Confidence limit at 95% confidence level.

3.4 Mechanism of the proposed method: According to the literature survey, it was revealed that poly nitro aromatic and halo poly nitroaromatic compounds react with bases resulting in the formation of brightly coloured solutions. There are various interactions depending upon the degree to which the base participates through its unshared electron pair with the nitro compounds.[23] In the present study, nortriptyline behaves as a base owing to the presence of –NH group in its structure. Addition of CDNB to nortriptyline in DMSO yielded the 1- substituted Meisenheimer complex which absorbs maximally at 390 nm. On the basis of our experimental findings and literature background[24] the reaction mechanism was proposed and given in scheme 1.

Scheme 1



3.5 Selectivity: The selectivity of the proposed method was ascertained by determining the nortriptyline content in the presence of various excipients such as talc, dextrose, starch, gelatin and magnesium stearate. It was observed that excipients do not interfere with the analysis by the proposed method.

4. Applications

The result of the proposed methods was compared with those of the reference method[20] using point and interval hypothesis. It is apparent from table no. 5 that the calculated t- and F-values are less than the theoretical ones at 95% confidence level which showed no significant difference between methods compared with regard to accuracy and precision. The interval hypothesis test has also confirmed that the true bias of all samples are < ± 2.0 % at 95% confidence level. For pharmaceutical analyses, a bias of ± 2.0% is acceptable[25] and thus the limits acceptance interval is within θ_L = 0.98 and θ_U = 1.02. The proposed method for the determination of nortriptyline hydrochloride in pharmaceutical formulations was compared favourably with other existing spectrophotometric methods[15-20] (Table no. 6). It was found that it is an accurate method having a wide range of application with low RSD values. Moreover it has an advantage over other existing method that it is a kinetic based method taking less analysis time and applicable directly to the drug sample without prior extraction needed in most of the developed methods[15-17]. The method is versatile and free from interferences from common excipients present in pharmaceutical formulations.

Table no. 5: Point and interval hypothesis tests: comparison of the proposed methods with the reference method at 95% confidence level

Formulations	Initial rate method	Fixed time method	Reference method
	Recovery± RSD (%)	Recovery± RSD (%)	Recovery± RSD (%)
Primox-25®	99. 99±0.50	99. 91±0.45	99.85±0.74
(Sun Pharma)	θ_L=0.989	θ_L=0.990	
	θ_U=1.007	θ_U=1.008	
	t =0.36	t =0.16	
	F=2.23	F= 2.75	
Sensival-25®	99.98±0.49	100.12±0.59	99.90±0.33
(Wallace)	θ_L=0.993	θ_L=0.991	
	θ_U=1.005	θ_U=1.004	
	t =0.35	t =0.79	
	F= 2.26	F= 3.17	

Theoretical t- (ν= 9) and F-values (ν= 5, 4) at 95 % confidence level are 2.262 and 6.26, respectively. θ_L and θ_U are within the acceptable limits of ±2%.

Table no. 6: Comparison table of existing spectrophotometric methods

Reagents	λ_{max} (nm)	Beer's Law limit (µg/ml)	RSD (%)	Molar Absorptivity (l/mol.cm)	Reference
[a]Bromocresol green	416	0.2– 7.2	0.79	2.88×10^4	15
[a]Methyl orange	422	0.3– 9.8	0.98	2.29×10^4	15
[a]Nb -SCN	360	15– 100	0.24	8.70×10^3	16
[a]Fe -SCN	490	5– 24	0.32	1.19×10^4	16
[a]Bromophenol blue	410	10– 720	—	—	17
[a]Bromopyrogallol red	425	50– 234	—	—	17
3-Methyl-2-benzothiazolinone hydrazone	619 and 655	24– 216	0.642 and 0.872	1.50×10^3	18
Quinhydrone	4 97	12– 120	0.15	2.94×10^3	19
p-chloranil	560	15– 180	0.55	1.59×10^3	19
Acetaldehyde with p-chloranil	650	5– 50	0.59	6.32×10^3	19
2,3-dichloro-5,6-dicyano-1,4-benzoquinone	460	50– 180	0.74	1.20×10^3	20
p-Chloranilic acid	525	60– 400	1.10	0.90×10^3	20
[b]1-chloro-2,4-dinitrobenzene	390	20– 100	< 0.7	3.56×10^3	This work

[a]Extraction based.
[b]Kinetic based.

5. Conclusions

The proposed kinetic method is simple, rapid, sensitive and a wide range of determination of nortriptyline hydrochloride. It does not require any laborious clean up procedure prior to analysis and performed at room temperature (30 ± 1°C). Moreover, the present technique has the advantage of using inexpensive and easily available low cost reagents and therefore can be frequently used in the laboratories of research, hospitals and pharmaceutical industries. The proposed method can be used an alternative to the reported ones for the determination of nortriptyline hydrochloride in the pure and pharmaceutical formulations.

Acknowledgement

Authors are thankful to the Department of Chemistry, AMU, Aligarh, for providing necessary research facilities.

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