Research Article

UV-Spectrophotometric Method Development and Validation of Nortriptyline Hydrochloride in the Bulk and Pharmaceutical Dosage Form

Battu Akhila, Pasupuleti Shirisha, Tamkeen Bushra, Bandi Shashi Kumar, Ganrath Nithin, Suchitra Duddagi*
Vision College of Pharmaceutical Sciences and Research, Boduppal, Hyderabad, Telangana, India - 500 092.
***Corresponding author's E-mail:** suchitra.visioncollege@gmail.com

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ABSTRACT

The objective of the present work is to develop and validate a simple, rapid, accurate and cost-effective method for the estimation of Nortriptyline hydrochloride in Active Pharmaceutical ingredient and pharmaceutical dosage form using UV-Spectrophotometric method. The method was developed by using pure acetonitrile as a solvent and the observed λ max was found to be 240 nm. The method was validated according to the ICH guidelines for parameters including Linearity, Accuracy, Precision, Limit of detection and quantification and Assay. The linearity of the method was established in the concentration range of 4-14µg/ml. The statistical Calibration curve confirmed that the method obeys Beers-Lambert's law with a correlation coefficient of 0.999. The 98.9% percentage recovery and the obtained %Relative standard deviation of less than 2% confirmed the method's accuracy and precision. The present developed method can be successfully applicable for analyzing the retest period and for the routine laboratory quality control analysis.

Keywords: Method Development, Method validation, Nortriptyline, UV-Spectrophometric method.

INTRODUCTION

Nortriptyline hydrochloride is chemically, 3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methyl-1-propanamine hydrochloride an active metabolite of amitriptyline, is a tricyclic antidepressant (TCA) used in the treatment of major depression. It functions by increasing the levels of neurotransmitters, specifically norepinephrine and serotonin, in the brain, which helps improve mood and alleviate depressive symptoms. It is also used for Neuropathic Pain, Migraine, Attention Deficit Hyperactivity Disorder (ADHD) and Smoking Cessation Aid.

Literature survey revealed that there are many methods like UV Spectrophotometry[1-2], RP-HPLC[3], LC-MS/MS[4], for the estimation of single drug and HPTLC[5], HPLC[6-10] techniques for the determination in the combination form. But there are only few methods developed for the estimation by simple UV Spectrophotometric method. Hence it felt necessary to develop an economical, novel, rapid and accurate method by using Acetonitrile as a mobile phase. So, that it can be readily applicable for analyzing the retest period of bulk drug and can be routinely applicable for Quality control analysis of pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals used:

Nortriptyline hydrochloride [API] obtained from Sura Labs, Hyderabad. The tablet dosage form SENSIVAL-25 was purchased at the local pharmacy, Hyderabad.

Equipment

Digital weighing balance, UV 3000+ double beam Spectrophotometer (LABINDIA).

Preparation of stock solution (1000PPM):

0.025gm of Nortriptyline Standard was accurately weighed and transferred into a 25ml of volumetric flask, then the volume was adjusted by adding Acetonitrile as the diluent.

Preparation of Sub stock solution (100PPM):

2.5ml of the stock solution was pipetted out into a separate 25ml volumetric flask, where it was then diluted to the mark with Acetonitrile and mixed thoroughly.

Preparation of Working stock solution (10PPM):

2.5ml of the stock solution was pipetted out into a separate 25ml volumetric flask, where it was then diluted to the mark with Acetonitrile and mixed thoroughly.

Preparation of Sample solution:

Taken 86.4mg tablet powder equivalent to 25mg from an average weight of 5 tablets and diluted to 25ml to prepare 1000ppm solution. From this prepared 100ppm and further pipetted 2.5ml from 100ppm solution and made upto 25ml to obtain 10ppm solution.

Method development

For the present method development, the absorbance of the prepared standard working stock solution of Nortriptyline was measured by scanning it with UV-spectrophotometer over a wavelength range of 200-400nm using Acetonitrile as a mobile phase and the observed λ max was found to be 240 nm.

Method validation

To assure that the developed analytical method is appropriate for use, it was validated for various parameters according to ICH Guidelines.

i) Linearity:

Preparation of Nortriptyline hydrochloride working standard stock solutions for linearity study:

4ppm working standard solution:

1ml of standard sub stock solution was pipetted out into a 25ml volumetric flask and diluted to 25ml to obtain 4µg/ml concentration of Nortriptyline hydrochloride solution.

6ppm working standard solution:

1.5ml of standard sub stock solution was pipetted out into a 25ml volumetric flask and diluted to 25ml to obtain 6µg/ml concentration of Nortriptyline hydrochloride solution.

8ppm working standard solution:

2ml of standard sub stock solution was pipetted out into a 25ml volumetric flask and diluted to 25ml to obtain 8µg/ml concentration of Nortriptyline hydrochloride solution.

10ppm working standard solution:

2.5ml of standard sub stock solution was pipetted out into a 25ml volumetric flask and diluted to 25ml to obtain 10µg/ml concentration of Nortriptyline hydrochloride solution.

12ppm working standard solution:

3ml of standard sub stock solution was pipetted out into a 25ml volumetric flask and diluted to 25ml to obtain 12µg/ml concentration of Nortriptyline hydrochloride solution.

14ppm working standard solution:

3.5ml of standard sub stock solution was pipetted out into a 25ml volumetric flask and diluted to 25ml to obtain 14µg/ml concentration of Nortriptyline hydrochloride solution.

Procedure:

The method's linearity over the range of 4-14µg/ml was carried out by measuring the absorbance in UV/Visible spectrophotometer. A graph of concentration versus absorbance was plotted, and the correlation coefficient was calculated by regression analysis.

ii) Accuracy:

The Accuracy was verified by calculating the recovery of the samples at concentration levels of 8ppm, 10ppm, and 12ppm corresponding to spiking levels of 80%, 100%, and 120%, respectively.

Procedure

For all these aliquots, the absorbance was measured at 240nm against a blank. Subsequently, the amount found, percentage recovery, mean percentage recovery and %RSD values were calculated.

iii) Precision:

Intra-day precision:

Intra-day precision was carried out by measuring the absorbance of a 10ppm solution for five times under the same operating conditions within a short period of time. The %RSD value was then calculated.

Inter-day precision:

Inter-day precision was performed and measured the absorbance of 10ppm concentration solution of Nortriptyline for five times on the different days under same operating conditions and the %RSD value was calculated.

iv) Assay determination of Nortriptyline:

The prepared standard and sample solutions of Nortriptyline were assayed in five replicate samples. The spectra were recorded, and the percentage assay was calculated by using the formula.

$$\% \text{ Assay} = \frac{Assay\ sample/Assay\ standard \times Concentration\ standard}{Concentration\ sample} \times 100$$

v) Limit of Detection (LOD):

Limit of detection was calculated by using the formula:

$$Limit\ of\ detection\ (LOD) = \frac{3.3 \times SD\ of\ intercept}{Slope}$$

vi) Limit of Qualification (LOQ):

Limit of qualification was calculated by using the formula:

$$Limit\ of\ qualification = \frac{10 \times SD\ of\ intercept}{Slope}$$

RESULTS AND DISCUSSION

Method development:

After the trial and errors, acetonitrile was adopted as a mobile phase and it has shown the maximum absorbance at the λmax of 240nm. This condition was then finalized and proceeded for method validation. The absorbance spectrum of Nortriptyline was showed in figure 1.

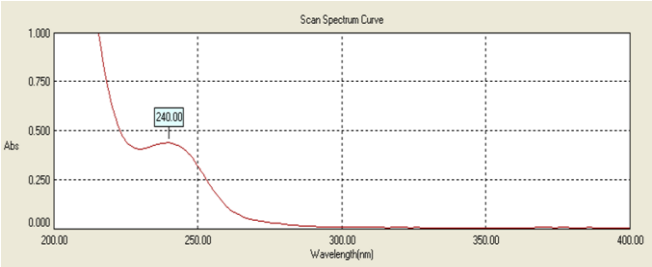

Figure 1: λ_{max} of Nortriptyline (240nm)

Method validation

i) Linearity

From the linearity graphs, it was confirmed that the method is exhibiting linearity over the range of 4 to 14 µg/ml. The correlation coefficient (r^2) was obtained as 0.999, which is

fulfilling the validation criteria. The plotted graph and linearity data are presented in Figure 2 and Table 1.

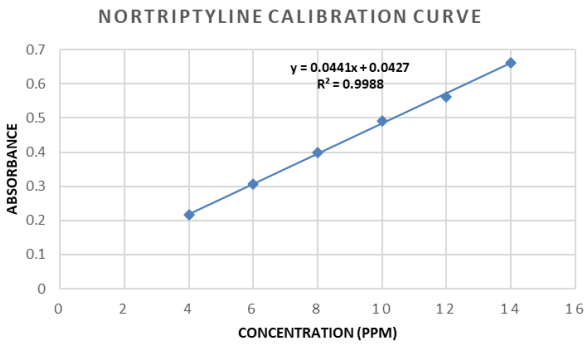

Figure 2: Calibration curve of Nortriptyline

ii) Accuracy

The measured percentage average recovery at the levels of 80%, 100%, 120% was found to be 98.2%, 99.04% and 99.6%. The data is reported in Table 2.

iii) Precision: The measured percentage relative standard deviation of intermediate (Day-1 & Day-2) and intra-day precision was found to be 0.447, 0.447 and 0.7312 respectively, which are within the specified limits. Accordingly, it confirmed the method precision. The data is reported in Tables 3 and 4.

Table 1: Linearity Data

λ_{max}	240 nm
Concentration (µg/ml)	**Absorbance**
4	0.217
6	0.306
8	0.399
10	0.492
12	0.563
14	0.662

Table 2: Accuracy data

Spiking level	Amount added (ppm)	Amount found (ppm)	Percentage recovery	Mean percentage recovery, standard deviation, %RSD
80%	8	7.9	98.75	98.95333 SD=0.496476 % RSD=0.5
80%	8	7.9	98.75	
80%	8	7.9	98.75	
100%	10	9.848	98.48	
100%	10	9.848	98.48	
100%	10	9.848	98.48	
120%	12	11.97	99.72	
120%	12	11.97	99.72	
120%	12	11.93	99.45	

Table 3: Intra-day precision data

S. No.	Absorbance
1	0.492
2	0.492
3	0.492
4	0.491
5	0.491
Mean	0.4916
Standard deviation	0.00049
% RSD	0.099

Table 4: Inter-day precision data

S. No.	Absorbance	
	Day-1	Day-2
1	0.489	0.489
2	0.489	0.487
3	0.489	0.479
4	0.492	0.487
5	0.486	0.489
Mean	0.489	0.4862
Standard deviation	0.001897	0.003709
% RSD	0.387	0.763

iv) Assay of Nortriptyline hydrochloride (SENSIVAL-25)

The assay of pharmaceutical dosage form was calculated and the spectra is displayed in Figure 3. % Purity was found to be 100% w/w.

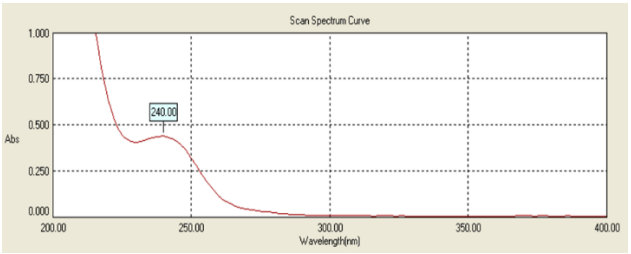

Figure 3: SENSIVAL-25 spectrum

v) LOD and LOQ

The parameters LOD and LOQ were determined by using formula. LOD and LOQ values are illustrated in Table 5.

Table 6: LOD and LOQ

Drug	LOD	LOQ
Nortriptyline hydrochloride	1.37 µg/ml	4.16 µg/ml

The present research work is mainly focused on development of a novel, rapid, accurate UV-Spectrophotometric method by using double beam UV-Visible Spectrophotometer. The analytical method was developed using Acetonitrile. The absorbance was measured over the range of 200-400 nm and the λ_{max} was found to be 240 nm. The developed method obeyed Beer's-Lambert's law showing good linearity over a range of 4 to 14µg/ml. the developed method was found to be accurate and precise showing good recovery value and having % RSD within the acceptance criteria. Thus, the developed method can be used routinely for quality control analysis of Nortriptyline in the bulk drug.

CONCLUSION

A simple, economic, rapid, precise and accurate UV-Spectrophotometric method was developed & validated for the estimation of Nortriptyline in active pharmaceutical ingredient. All the validation parameters were found to be within the acceptance criteria. The obtained Assay value and recovery values proved that, the present developed method can be applied for routine quality control analysis in laboratories for checking the retest period and purity of pharmaceutical dosage forms. The result obtained from the validation parameters met the ICH Q2 and USP requirement as well as obeys Beer's law.

DECLARATIONS

Author contributions

All authors contributed to experimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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