Original Article

The Development and Validation of Stability Indicating Analytical Method for Determination of Nortriptyline in Nortriptyline HCl Tablets by Liquid Chromatography

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ABSTRACT

Purpose: To develop and validate a rapid, rugged, precise and an accurate stability indicating analytical method for determination of Nortriptyline HCl in Nortriptyline tablets. The separation of impurities and Nortriptyline HCl drug was achieved by an isocratic liquid chromatographic method using Inertsil, C18, 250 mm x 4.6 mm, 5μm column at 45°C. The mobile phase consists of 70% Methanol and 30% phosphate buffer of pH-7.5 pumped at a flow rate of 1.0 ml/min. The detection was carried out at a wavelength 220 nm. The proposed chromatographic method was validated and found to be linear over the concentration range of 50 - 150.0 μg/ml. Mean recovery of Nortriptyline HCl was found to be 100.1 ± 0.1%w/w. **Conclusion:** The method was found to be simple, stability indicating, precise, accurate and robust which can be utilized for the determination of assay of Nortriptyline HCl in Nortriptyline tablets.

Key words: Nortriptyline HCl, Impurity, Liquid chromatography, Forced degradation, Stability indicating.

INTRODUCTION

Nortriptyline hydrochloride, the N-demethylated active metabolite of amitriptyline, is a dibenzocycloheptene-derivative tricyclic antidepressant (TCA). TCAs are structurally similar to phenothiazines 3-(10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5-ylidene)-N-methyl-1-propanamine (Figure 1).

Nortriptyline hydrochloride contains tricyclic ring system with an alkyl amine substituent on the central ring. Nortriptyline Base is a tricyclic antidepressant agent used for short-term treatment of various forms of depression since it is a non-selective serotonine uptake inhibitor.[1] Nortriptyline blocks the norepinephrine presynaptic receptors, thereby blocking the reuptake of this neurotransmitter and raising the concentration in the synaptic cleft in the CNS. Nortriptyline also binds to alpha-adrenergic, histaminergic and cholinergic receptors.[2]

This research was focused on development of a simple, precise, accurate, specific and robust stability indicating reverse phase chromatographic method for assay.[3,4,5,6,7,8]

Literature survey reveals that nortriptyline hydrochloride is official in British Pharmacopeia.[9] Few chromatographic methods for the determination of nortriptyline hydrochloride and fluphenazine hydrochloride in pharmaceutical preparations and/ or with other active ingredients[10,11,12,13] have been reported. The proposed method is stability indicating, simple and economical and can be utilized by common laboratories. The developed method was validated as per International Conference on Harmonization (ICH) Q2(R1) guideline[14] and United

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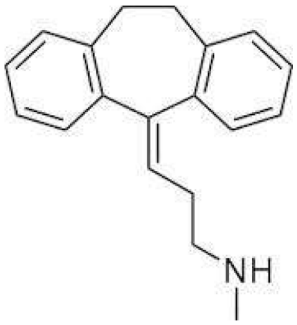

Figure 1: Structure of Nortriptyline Hydrochloride

State of Pharmacopoeia (USP) 38 chapter <1225> and chapter <621>.[15,16] The method found to be specific, precise, accurate and robust with compliance to acceptance criteria of ICH and USP 38. This method can be used in quality control of manufactured and developed dosage forms.

MATERIALS AND METHODS

Materials

HPLC grade methanol, potassium dihydrogen phosphate and Orthophosphoric acid was purchased from Merck Chemicals, Mumbai, India. Ultrapure water was generated from Milli-Q water purifier.

Instrumentation

Thermo–Ultimate 3000 High performance liquid chromatography (HPLC) system with UV Detector.

Methods

Chromatographic parameters

The chromatographic column used was Inertsil, C18, 250 mm x 4.6 mm, 5 µm which was maintained at 45°C. The mobile phase was prepared by mixture of methanol and buffer of pH 7.5 containing 0.05 M of potassium dihydrogen phosphate and tetrabutyl ammonium hydroxide solution (4 ml/lit of 4 g/10ml) in the ratio of 70:30 v/v. The flow rate of the mobile phase was 1.0 ml/min. The injection volume was 20.0 µL. The column effluents were monitored by UV detector at 220 nm.

Preparation of Solutions

Standard Preparation

Weighed accurately about 50 mg of Nortriptyline HCl working standard and transferred to 50 ml volumetric flask. The content of the flask was dissolved with diluents (methanol and water in the ratio of 70:30) with sonication and volume was made up to mark with diluents as primary stock solution. Further 5.0 ml of prepared standard primary stock solution was pipetted and transferred to 50 ml volumetric flask and made up to the mark with diluents to get nominal concentration about 100 µg/ml.

Sample Preparation

Powder equivalent to 100mg of Nortriptyline HCl was weighed and dissolved in 100 ml volumetric flask with diluents with sonication. Filtered through 0.45 µm nylon syringe filter. Further 5.0 ml of filtered solution was pipetted and transferred to 50 ml volumetric flask and made up to the mark with diluents to get nominal concentration about 100 µg/ml.

Method Validation

System Suitability

The rationale of the system suitability assessment is to make sure that during the complete testing, system (including instrument, reagents, columns, analysts) is appropriate for the intended application.

System suitability tests (SST) are vital part of liquid chromatographic methods. They are used to verify the reproducibility of the chromatographic parameters and system is satisfactory for the analysis to be done. SST is support on the concept that the equipment, electronics, analytical operations and samples to be analyzed comprise an integral system that can be evaluated as such.

The system suitability test was performed in accordance with USP.[18]

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities or expected to present. The specificity of the developed LC method for all impurities was carried out by injecting placebo, known impurities of Nortriptyline HCl. The placebo was prepared by dissolving 10mg placebo in 10 ml of volumetric flask in mobile phase and sonicated. The volume was made up to the mark with diluents. The resulting solution was filtered through 0.45µ syringe filter.

Each impurity was injected at nominal concentration of 2 µg/ml. The preparation was done as per standard preparation.

The diluents (blank), placebo solution, individual impurities (2 µg/ml) and standard drug solution (100 µg/ml) were injected in sequence for evaluation of specificity of proposed method. The chromatograms were monitored for any peak eluted at the retention time of drug.

Forced Degradation

The forced degradation studies in acidic, hydrolysis and Oxidation condition were carried out during method development which confirmed that Nortriptyline is sensitive to oxidative conditions, potential degradation products formed at this condition. This study also confirmed that there is no co-elution of blank, placebo, known impurities or other substance with the principal peak.

Precision

Precision express the measure of how close the analytical results are to each other from a set of measurements under controlled analytical conditions. Precision proves random errors of the measurement.

Precision is a measure of the degree of repeatability (Intra-day), intermediate precision and reproducibility (inter-day) of the analytical method under normal operating circumstances.

Precision is usually measured as the relative standard deviation (RSD) of analytical results acquired from independently prepared quality control standards.

Method precision was evaluated by six sample preparations of same homogeneous sample of Nortriptyline tablets (25 mg/tablet) test sample and calculated % recovery for Nortriptyline HCl in each sample preparation. The % RSD for set of six preparations was calculated.

The intermediate precision of the method was also evaluated using different analyst and a different instrument in the same laboratory by carrying out six sample preparations of tablets and calculated % recovery for Nortriptyline HCl in each preparation. Calculated the %RSD for 12 results. The acceptance criteria for % RSD was not more than 2%.

Accuracy (Recovery)

Accuracy is extremely important in analytical method validation as it assures the closeness of agreement between a test result and the accepted reference value. Accuracy is expressed as trueness and involves a combination of random components and a common systematic error or bias component. The accuracy of the method was performed by recovery studies.

In order to evaluate the accuracy of the proposed method, a recovery test was performed by adding known amounts of standard solution to the placebo formulation sample, followed by analysis using the proposed chromatographic method.

The recovery studies were done for three different levels at 50%, 100% and 150% with three determinations of working level concentration using standard spiking method in placebo.

All the above solutions were prepared in triplicate and were analyzed using proposed chromatographic condition. The recovery at each level was calculated by using the theoretical value from exact weight taken for spiking. The % recovery was calculated with respect to amount added. The acceptance criteria for % recovery was in the range of 98 - 102%.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by means of well-defined mathematical transformations, proportional to the concentration of analyte in the samples within a given range. The linearity plot was constructed for Nortriptyline HCl in the concentration range of 50 to 150µg/ml. The primary stock solution of Nortriptyline HCl working standard was prepared. From the primary stock solution, appropriate dilutions were made to get concentration of 50, 60, 80, 100, 120 and 15 0µg/ml. The calibration curve was plotted as concentration of the respective drug solutions versus the peak area at each level. The results were statistically evaluated and correlation coefficient determination (r^2), slope and y-intercept values were calculated.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. One consequence of evaluation of robustness is that, a series of system suitability parameters is established to ensure that the analytical procedure is maintained whenever used. In the present study, the working concentration 100.0 µg/ml of Nortriptyline HCl was used for the determination of the robustness of the method. The following parameters were considered for the robustness of the proposed chromatographic method.

- Effect of pH in the mobile phase (±0.2)
- Effect of organic modifier in mobile phase composition (± 2%)
- Effect of flow rate (±10%)

Solution stability of sample and standard in mobile phase

The solution stability of sample solution in diluents was performed to understand stability which will be helpful to understand sample handling in proposed chromatographic method. Solution stability was carried out for sample solution (100.0µg/ml) in a tightly capped volumetric flask at ambient temperature for 72 hr. The sample and standard solution after preparation were

injected immediately to the system considering as an initial at 0 hr. as baseline.

RESULTS AND DISCUSSION

Method Development

The drug and impurities were scanned on UV and the spectra of drug and individual impurity was recorded.

The wavelength 220nm was selected which was permitting the detection of Nortriptyline HCl with adequate sensitivity.

In this study, the chromatographic method optimization was carried out by utilizing different stationary phase containing C8 and C18 to achieve the resolution of impurities from main drug. The column of make Inertsil ODS (C18, 250 X 4.6 mm, 5µ) had offered more advantages regarding resolution of impurities.

Many different compositions of organic modifier and buffer with different pH were tried for better resolution of impurities.

Individual drug solution and known impurities was injected into column and elution pattern of the drug and its known impurities was observed. The resolution pattern was studied.

Forced degradation studies in different stress conditions like acidic, basic, oxidation and thermal were done and the peak purity of Nortriptyline HCl was monitored.

Method Validation

Specificity

The specificity chromatogram known impurities (Figure 2), sample (Figure 3) and peak purity of sample (Figure 4) along with diluents and placebo solution were revealed that there is no co-elution of any impurity with drug peak.

Forced Degradation

The forced degradation study proved that the proposed method is stability indicating. All the peaks are pure. During this study, it is found that Nortriptyline HCl is sensitive to oxidative conditions, potential degradation products formed at this condition. All the unknown peaks were resolved properly. This study also confirmed that there is no co-elution of blank, placebo, known impurities or other substance with the degradants and principal peak.

Forced degradation was done in different stressed conditions like acidic -0.5M HCl (Figure 5), basic - 0.1N NaOH (Figure 6) and oxidation -30% Hydrogen Peroxide (Figure 7) and the peak purity of Nortriptyline HCl were monitored. Peak purity of Nortriptyline HCl peak was found passing in all conditions (Table 1). The developed chromatographic method was found to be highly specific for determination of Nortriptyline HCl

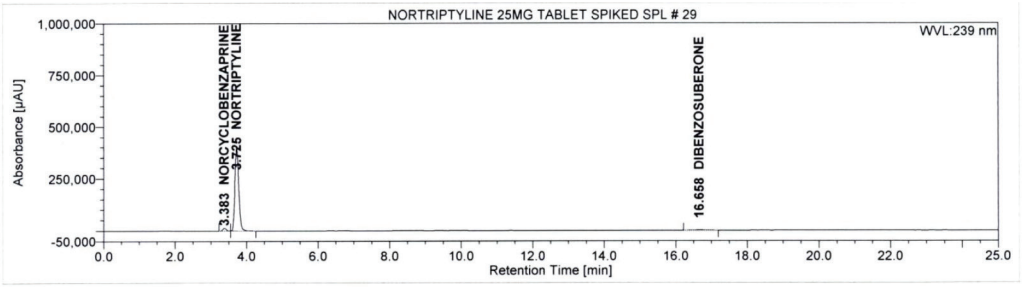

Figure 2: Chromatogram of Known Impurities and sample.

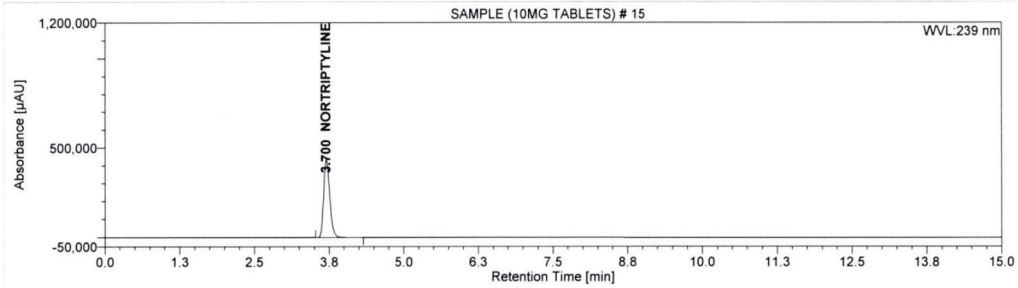

Figure 3: Chromatogram of Sample with diluents and placebo.

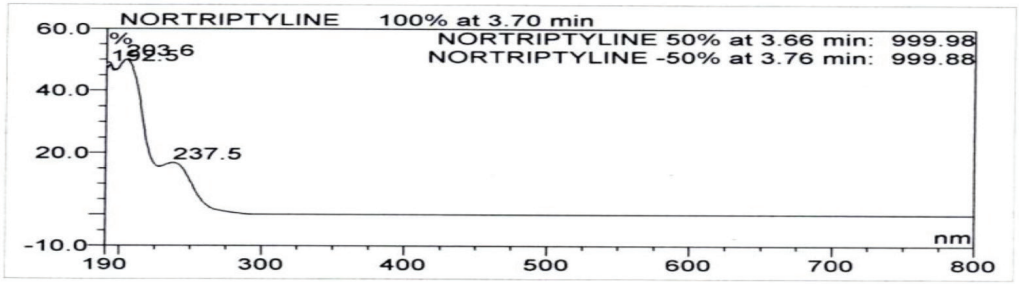

Figure 4: Peak purity of Sample with diluents and placebo.

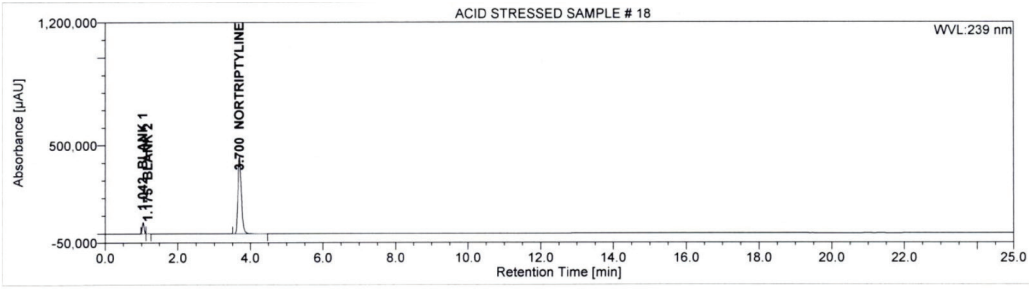

Figure 5: Chromatogram of Forced degradation of Nortriptyline HCl in Acidic condition.

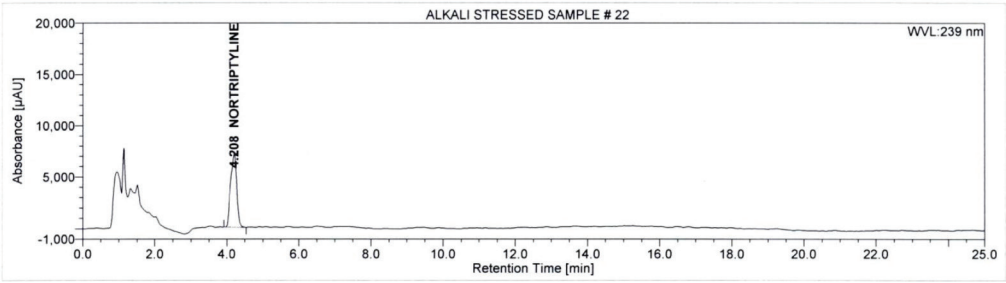

Figure 6: Chromatogram of Forced degradation of Nortriptyline HCl in Alkali condition.

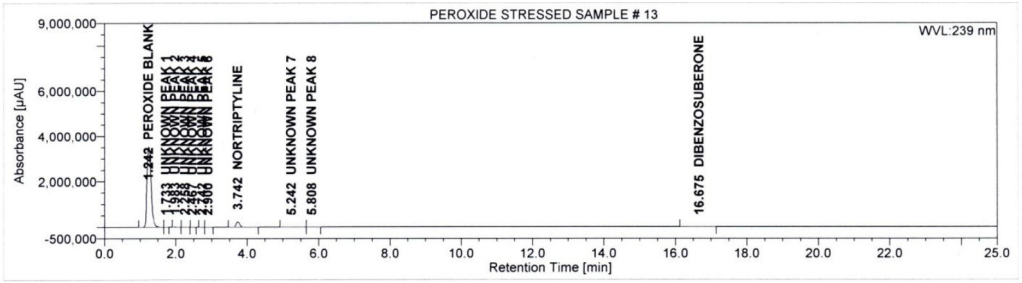

Figure 7: Chromatogram of Forced degradation of Nortriptyline HCl in Hydrogen Peroxide.

in Nortriptyline tablets. Reference chromatograms of forced degradation study are given below.

Based on overall outcome of degradation studies it is concluded that proposed chromatographic method is specific and stability indicating.

Precision

The method and system precision was performed at 100% working level concentration be preparing six samples of sample. The study was performed in two sets. First set analysis was done for intraday precision. The intra-day precision was evaluated by performing six (n =

Table 1: Forced degradation study results

Condition	% Assay	% Degradation	Purity Match NLT 990	Remark
Normal Conditions	100.5	Not applicable	1000	Peak purity of main peak passed
Acid degradation: 0.5N HCl solution, heating at 80°C for 2 hours	100.1	No degradation	1000	Peak purity of main peak passed
Alkali degradation:0.1N NaOH solution, at ambient for 2 hours	75.5	Nortriptyline HCl is insoluble in 0.1N NaOH, therefore no degradation	1000	Peak purity of main peak passed
Peroxide degradation: 30% H_2O_2 solution, heating at 60°C for 1 hour	90.1	9.9% degradation was observed	1000	Peak purity of main peak and degradants passed

Table 2: Precision results

Sample	% Assay	
	Intraday Precision	Inter-day (Intermediate) precision
Sample-1	100.5	99.7
Sample-2	100.1	100.0
Sample-3	99.6	99.6
Sample-4	100.8	100.5
Sample-5	100.3	100.8
Sample-6	100.5	99.9
Mean of individual sets	100.3	100.1
%RSD	0.38	0.43
Overall mean of 12 results	100.2	
Overall STDEV	0.44	
Overall % RSD	0.44	
% Difference between set I and set II results	0.2%	

Table 3: Recovery results

Accuracy conc.	Sample	% Recovery	Mean recovery	% RSD
50%	Sample-1	100.2	100.2	0.4
	Sample-2	100.6		
	Sample-3	99.9		
100%	Sample-1	99.8	100.0	0.2
	Sample-2	100.2		
	Sample-3	100.0		
150%	Sample-1	100.2	100.3	0.2
	Sample-2	100.0		
	Sample-3	100.3		
Overall Mean			100.1	0.1

6) assay determinations on same homogeneous sample of Nortriptyline tablets 25mg. Calculated %RSD for the six results and % RSD was found to be 0.41%.

The second set analysis was performed by another analyst using different instrument (HPLC) to prove the inter day precision. Six sample preparations were done on same homogeneous sample. Calculated % RSD for the six results and found to be 0.43%.

The overall % RSD of two sets (n=12) for their % assay was found to be 0.44%.

The absolute difference between two sets of results for intermediate precision found 0.2% (Table 2).

Accuracy (Recovery)

The recovery study was done in triplicate on three different concentrations. The range covered for this study was 50% to 150%. The three concentrations selected for this study were 50%, 100% and 150% of working level concentration. The appropriate quantity of Nortriptyline HCl drug was spiked in placebo and diluent at selected level. The recovery of drug was calculated against known spiked drug concentration. The mean % recovery of three preparations at 50%, 100% and 150% was found to be 100.2±0.4, 100.0± 0.2, and 100.3±0.2 % respectively (Table 3). The overall mean recovery at all three level was found to be 100.1± 0.1%. The recovery results were found within acceptance criteria (Table 3). The developed method found to be accurate for determination of Nortriptyline HCl in Nortriptyline tablets.

Linearity

The linearity for the response of Nortriptyline HCl drug was performed in the range of 50-150µg/ml and response for Nortriptyline HCl found to be linear. Total

Table 4: Robustness results

Method Parameter	%RSD of Standard Area	% Assay
Normal condition	0.5	99.9
Change in pH of Buffer + 0.2	0.2	99.5
Change in pH of Buffer - 0.2	0.8	100.1
Change in organic modifier + 2%	0.1	100.2
Change in organic modifier - 2%	0.8	99.6
Change in flow rate + 0.1ml/min	1.0	100.3
Change in flow rate - 0.1ml/min	0.5	100.0

six concentration level were selected for linearity study as 50%, 60%, 80%, 100%, 120% and 150%. The linearity plot was constructed for concentration verses response. The representative regression equation was found to be y=29470.1551x +42985.8379 with lowest correlation coefficient (r^2) was found to be 1.0000. The linearity was found with in acceptance criteria.

Robustness

The robustness study was performed to check the impact of small variables on method. During routine analysis, there can be small changes in the method parameters due to analytical variations due to analyst or and instrument. The robustness of the method was verified by making the deliberate variation in the critical method parameters like;

pH of the buffer - ±0.2

Organic modifier composition in mobile phase - ± 2%

Pump flow rate - ±10%

Even after making the small changes in the critical method parameters, the method delivered the expected results. The results are presented in Table 4 and found within acceptance criteria.

Table 5: Solution stability results

Solution stability of sample			Solution stability of standard		
Time	%Assay	% difference Diff.	Time	%Assay	% difference Diff.
Initial	100.5	NA	Initial	100.0	NA
24 Hour	99.8	0.7	24 Hour	100.3	-0.3
48 Hour	100.2	0.3	48 Hour	100.0	0.0
72 Hour	99.8	0.7	72 Hour	99.9	0.1

Solution stability of sample and standard

The solution stability of standard and sample in selected diluent is very important during routine analysis. The analyst can plan the analysis, number of samples etc. based on solution stability of standard and sample. Hence solution stability of standard and sample was carried out up to 72 hours with testing frequency of 24 hours. The Nortriptyline HCl sample found to be stable up to 72 hour in diluents at ambient temperature. The results after 72h were found to be 99.9% and 99.8% for standard and sample respectively (Table 5).

Application of developed method

The proposed chromatographic method was used for determination of Nortriptyline HCl in Nortriptyline tablets and the results were found within the specification.

CONCLUSION

The proposed HPLC method is simple, precise, accurate and stability indicating rugged methodology for determination of Nortriptyline HCl in Nortriptyline tablets of different strengths. The validation of the method proven that the method is linear in the range of 50 –150 µg/ml of Nortriptyline HCl, and it proved to be precise and accurate over the range. The validation of the method is done accordingly to ICH Q2 (R1) and USP 38. All the parameters are meeting the acceptance criteria. Since method is simple and time saving hence it can be used conveniently by laboratories to determine the Nortriptyline hydrochloride in different strengths of Nortriptyline tablets.

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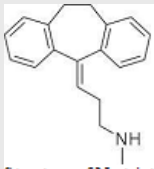
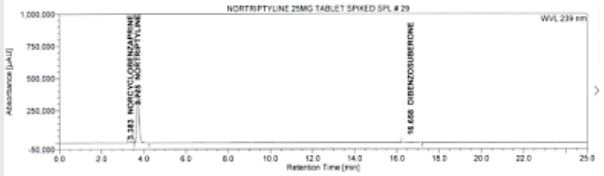
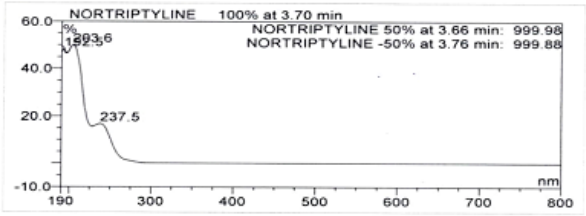
PICTORIAL ABSTRACT



Structure of Nortriptyline Hydrochloride



Chromatogram of Known Impurities and sample



Peak purity of Sample

SUMMARY

- Nortriptyline hydrochloride is a tricyclic antidepressant agent used for short-term treatment of various forms of depression.
- An analytical method is developed for determination of Nortriptyline HCl in Nortriptyline tablets of different strengths and validated as per ICH Q2 (R1) and USP guidelines.
- All the validation parameters are meeting the acceptance criteria.
- The retention time of Nortriptyline HCl is about 3.8 minutes,
- Developed analytical method is stability indicating, simple, accurate. It will save time of analysis and hence it is more economical than the published methods.

About Authors

Tukaram B. Sawant: Presently working in Research Centre of private pharmaceutical company. He is in pharmaceutical industry for more than 15 years and has developed and validated many analytical methods for different types of formulations like tablets, capsules, creams ointments etc. He is having good hands on experience on different sophisticated analytical instruments used for commercial pharmaceutical product testing. He is well versed with different regulatory requirements.

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