STABILITY INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF NORTRIPTYLINE AS BULK DRUG AND IN CAPSULE DOSAGE FORM

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ABSTRACT

The present work describes development and validation of a new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Nortriptyline hydrochloride as bulk drug and in capsule dosage form. As stability testing is major step in the development of new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. Nortriptyline hydrochloride was found susceptible to all the analyzed stress conditions except photolysis. Chromatographic resolution of Nortriptyline hydrochloride and its degradation products was achieved by using precoated silica gel 60 F254 aluminium plates as stationary phase and Toluene: Methanol (6.5: 3.5, v/v) as optimum mobile phase. Densitometric detection was carried out at 240 nm. The retention factor was found to be 0.62 ± 0.02. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. Results found to be linear in the concentration range of 200-1000 ng band. The developed method has been successfully applied for the estimation of drug in capsule dosage form.

KEYWORDS: Nortriptyline, HPTLC, Forced degradation, Capsule dosage form

INTRODUCTION

Nortriptyline hydrochloride, chemically, 3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methyl-1-propanamine, is a dibenzocycloheptene-derivative tricyclic antidepressant (TCA) which acts by increasing the amounts of certain natural substances in the brain that are essential to maintain mental balance. [31]

Extensive literature review with respect to analytical methods revealed that Spectrophotometric methods for the estimation of nortriptyline hydrochloride as bulk and in tablet dosage form has been available either as single drug or in combination with other drugs. [5-5] High Performance Liquid Chromatographic (HPLC) methods were also found in the literature for determination of nortriptyline hydrochloride either in human plasma or in pharmaceutical dosage form with other drugs. [6-12]

To best of our information, no reports were found for determination of nortriptyline hydrochloride in capsule dosage form by stability-indicating high performance thin layer chromatographic (HPTLC) method. This paper describes development and validation of simple, precise, accurate and selective stability indicating HPTLC method for determination of nortriptyline hydrochloride in accordance with International Conference on Harmonisation Guidelines. [14,15]

MATERIALS AND METHODS

Chemicals and reagents: Pharmaceutical grade working standard nortriptyline hydrochloride was obtained as gift sample from Wallance Pharmaceuticals Pvt. Ltd., (Goa, India). The pharmaceutical dosage form used in this study was Aventil 10 capsules labeled to contain 10 mg of nortriptyline hydrochloride was procured from the local pharmacy. Toluene, Methanol (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions: Chromatographic separation of drug was performed on precoated silica gel 60 F254 (10 cm x 10 cm with 250 µm layer thickness) Merck TLC plates as stationary phase using a Camag Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).
Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using mixture of Toluene: Methanol (6.5: 3.5, v/v) as mobile phase. The mobile phase was saturated in chamber for 15 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on Camag thin layer chromatography scanner at 240 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard stock solution
Standard stock solution was prepared by dissolving 10 mg of drug in 10 mL of methanol to get working standard solution of concentration 1000 ng µL⁻¹ from which 1 mL was further diluted to 10 mL to get solution of 100 ng µL⁻¹.

Selection of detection wavelength
After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 240 nm. So, 240 nm was selected as the wavelength for detection.

Estimation of the drug in capsule dosage form
Commercial brand of capsules namely Aventil 10 were selected for checking the suitability of the proposed method to estimate nortryptiline in capsule formulation. For this, 20 capsules were weighed and powdered. Capsule powder equivalent to 10 mg was transferred to 100 mL volumetric flask containing 50 mL of methanol and the contents were sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 100 ng band⁻¹. Four µL volume of this solution was applied on TLC plate to obtain final sample concentration of 400 ng band⁻¹. After chromatographic development peak areas of the bands were measured at 240 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Forced degradation study
The stability studies were performed by subjecting the bulk drug to the physical stress and stability was accessed. The study was carried out at concentration of 1000 µg µL⁻¹. The hydrolytic studies were carried out by refluxing the stock solution of drug separately with 0.1N HCl and 0.1 N NaOH at 60°C for 30 min. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 400 ng band⁻¹. Neutral hydrolysis study was performed by refluxing the drug with water at 60°C for 30 min. The oxidative degradation was carried out in 6% H₂O₂ at 60°C for 30 min and sample was diluted with methanol to obtain 400 ng band⁻¹ solution. Thermal stress degradation was performed by keeping drug in oven at 70°C for period of 6 h. Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h square meter⁻¹ for 7 d. Thermal and photolytic samples were diluted with methanol to get concentration of 400 ng band⁻¹.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions
The aim of current research work was to develop stability indicating HPTLC method which would be capable to give the satisfactory resolution between nortryptiline and its degradation products. Diverse solvent systems containing various ratios of benzene, chloroform, ethyl acetate, toluene, methanol were examined (data not shown) to separate and resolve spot of nortryptilin from its impurities and other excipients present in formulation. Finally, the mobile phase comprising of Toluene: Methanol (6.5: 3.5, v/v) was selected as optimal for obtaining well defined and resolved peak. Densitometric evaluation was carried out at 240 nm. The retention factor was found to be 0.62 ± 0.02. Representative densitogram of standard solution of Nortriptyline hydrochloride is represented in Figure 1.

Figure 1: Densitogram of standard solution of Nortriptyline hydrochloride (600 ng band⁻¹, Rf= 0.62 ± 0.02).

The forced degradation results indicated susceptibility of drug to hydrolytic, oxidative and thermal stress conditions and the drug was found to be stable under photolytic stress. Figures 2-4 represents the densitograms of acid, alkali and neutral hydrolytic degradation, while Figures 5 and 6 show the densitograms of oxidative degradation and thermal degradation, respectively.
Marked degradation in the densitograms was observed but the degraded products were well resolved from the drug indicating specificity of the method. The findings of degradation studies are represented in Table 1.

**Table 1: Summary of forced degradation studies of Nortriptyline.**

<table>
<thead>
<tr>
<th>Stress conditions/duration</th>
<th>% Recovered</th>
<th>% Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic / 0.1 N HCl/ Refluxed at 60°C for 30 min</td>
<td>77.75</td>
<td>22.24</td>
</tr>
<tr>
<td>Alkaline /0.1 N NaOH/ Refluxed at 60°C for 30 min</td>
<td>82.46</td>
<td>17.53</td>
</tr>
<tr>
<td>Oxidative /6 % H₂O₂ / Refluxed at 60°C for 30 min</td>
<td>76.15</td>
<td>23.85</td>
</tr>
<tr>
<td>Neutral/H₂O/ Reflux at 60° for 30 min</td>
<td>87.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Photolysis: UV light 200 watt h square meter—1 7 days</td>
<td>98.61</td>
<td>1.13</td>
</tr>
<tr>
<td>Dry heat/ 70°C/ 6 h</td>
<td>91.67</td>
<td>8.32</td>
</tr>
</tbody>
</table>

**Method Validation**

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH guidelines.

**Preparation of calibration curve**

For preparation of a calibration plot, volumes 2, 4, 6, 8 and 10 µL of standard solution of Nortriptyline hydrochloride (100 ng µL⁻¹) were spotted onto the TLC plates. The developed method was found to be linear in the concentration range 200-1000 ng band⁻¹ with high correlation coefficient. The linear regression equation was found to be \( y = 8.6988x + 123.28 \) having correlation coefficient 0.992. The calibration curve obtained by plotting concentration vs peak area is represented in Figure 7.
**Precision**
Set of three different concentrations in three replicates of standard solutions of Nortriptyline hydrochloride were prepared. All the solutions were analyzed on the same day in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.82 to 1.03. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.80 to 1.06. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

**Limit of detection (LOD) and Limit of quantitation (LOQ)**
LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 23.32 ng band$^{-1}$ and 70.69 ng band$^{-1}$, respectively.

**Recovery studies**
Recovery studies were carried out by adding standard drug to pre-analysed sample solution at three different levels 80, 100 and 120%. Basic concentration of sample chosen was 400 ng band$^{-1}$ from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drug in capsule dosage form. The results obtained are shown in Table 2.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (ng band$^{-1}$)</th>
<th>Amount added (ng band$^{-1}$)</th>
<th>Amount found (ng band$^{-1}$)</th>
<th>% Recovery±R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nortriptyline hydrochloride</td>
<td>400</td>
<td>320</td>
<td>717.92</td>
<td>99.70±0.81</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>400</td>
<td>795.44</td>
<td>99.42±0.67</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>480</td>
<td>882.65</td>
<td>100.29±0.70</td>
</tr>
</tbody>
</table>

*Average of three determinations.

**Robustness**
Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition (± methanol), wavelength (± 1 nm) was altered and the effect on the area of drug was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

**CONCLUSION**
A simple, precise, accurate, reproducible, and stability-indicating HPTLC method without interference from the excipients or from degradation products has been developed and validated for determination of Nortriptyline hydrochloride as bulk drug and in capsule dosage form. The developed method can be used for quantitative analysis of drug in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic. As the method is stability indicating one it may be extended to study the degradation kinetics of drug.

**ACKNOWLEDGEMENT**
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