DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF NORTRIPTYLINE HYDROCHLORIDE IN BULK AND DOSAGE FORM

Atul Sherje[a]* and Sneha Dias[a]

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ABSTRACT

The present work reports a validated reverse phase-HPLC method for determination of nortriptyline hydrochloride (NORH) in bulk and in its pharmaceutical dosage form. Chromatographic separation was performed on Waters C-18, (250×4.6mm, 5µm) stationary phase using a mixture of 50 mM KH_2PO_4 pH 3.0 phosphate buffer: methanol: acetonitrile in the proportion of 50: 10: 40 V/V/V as mobile phase components at 1.0 mL min^{-1} flow rate. Detection of drug was carried out in isocratic mode at 235 nm. The validation of the method was carried out as per ICH guidelines. The LOD and LOQ for NORH was 0.22 µg mL^{-1} and 0.69 µg mL^{-1}, respectively. NORH showed linearity range of 5-50 µg mL^{-1} and correlation coefficient (r^2) value, 0.9937. The method was tested for assay of NORH in tablet dosage form.

Keywords: Nortriptyline hydrochloride, RP-HPLC method development, tablet dosage form, validation

INTRODUCTION

Nortriptyline hydrochloride (NORH), chemically 3-(10, 11-dihydro-5*H*-dibenzo [a, d] annulen-5-ylidene)-*N*-methylpropan-1-amine hydrochloride is indicated for treatment of depression. It is a metabolite of amitriptyline which also belongs to the class of antidepressant drugs. NORH acts on serotonin receptors and inhibits selective reuptake of serotonin. NORH acts on both beta and alpha-adrenergic receptors[1].

From the literature survey, many methods are found to be reported for estimation of NORH individually, in fixed dose combination formulation, and/or biological fluids such as LC/MS[2], UV Spectrophotometry[3], TLC[4] and HPLC[5]. In the present work, efforts were directed towards arriving at an accurate, reproducible and sensitive method for analysis of NORH alone in pure form and in dosage formulation. The validation of the method was carried out as per USP and ICH guidelines[6-7].

MATERIALS AND METHODS

Materials

Nortriptyline hydrochloride was provided as a gift sample from Centaur Pharmaceuticals Pvt. Ltd., Mumbai, India. All other solvents/ chemicals used for the study were of HPLC or AnalR grade. Acetonitrile, methanol, ortho-phosphoric acid (OPA) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from S.D. Fine Chemicals Ltd., Mumbai, India. Milli-Q water filtered through Millipore system was used throughout the study for preparation of chemicals. Marketed formulation of nortriptyline hydrochloride (25 mg) was bought from a local pharmacy.

Preparation of drug solution and mobile phase

Accurately weighed 6.8 g of potassium dihydrogen orthophosphate (KH_2PO_4) was dissolved in 1 L water (Milli-Q) to obtain 50 mM KH_2PO_4 solution. The pH was adjusted to 3.0 with 1 % V/V orthophosphoric acid. Mobile phase preparation was done by mixing KH_2PO_4 buffer: acetonitrile: methanol in the ratio 50:40:10 V/V/V, filtered through 0.45 µm nylon membrane and degassed using sonicator for 20 min. For preparation of drug solution, an accurately weighed quantity equivalent to 10 mg of nortriptyline hydrochloride was transferred to a 10 mL volumetric flask, 5 mL methanol was added, and sonicated for 5 min. Further, methanol was added to make up the volume to obtain 1 mg mL^{-1} stock solution. Aliquot of this solution was pipetted and diluted using mobile phase as diluent to obtain test concentration of 25 µg mL^{-1}.

Instrument and chromatographic conditions

The chromatographic separation was achieved on HPLC system – Agilent 1260 Infinity Series,

[a] Department of Quality Assurance, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Vile Parle (W), Mumbai - 400 056, Maharashtra, India

*For Correspondence: E-mail: sherjeap@gmail.com

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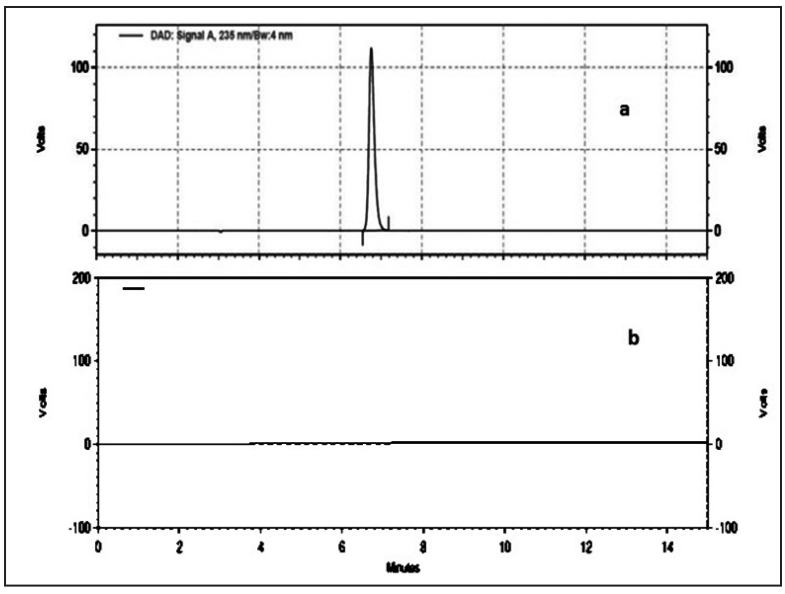

Fig. 1: A typical chromatogram of (a) nortriptyline hydrochloride and (b) placebo of NORH marketed tablet excipients in optimized chromatographic conditions

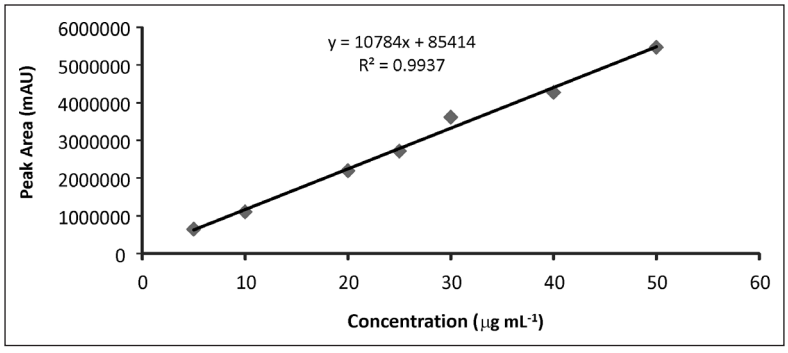

Fig. 2: Linearity of nortriptyline hydrochloride

Photodiode array (PDA) detector, equipped with quaternary pump and 100 µL variable auto- sampler. Open Lab software was applied for data collection and processing. Chromatographic analysis was performed using Waters C18 column (250 x 4.6 mm, 5 µm) in an isocratic mode using a mobile phase containing KH_2PO_4 buffer (50 mM, pH 3.0): acetonitrile: methanol (50: 40: 10 V/V/V) at a flow rate of 1 mL min^{-1}. Chromatograms were monitored at 235 nm with a run time of 15 min. A double beam spectrophotometer (Shimadzu UV 1800) with 1-cm matched quartz cell was used for selection of wavelength of chromatographic detection. The optimized chromatographic conditions showed better symmetrical peak for the analyte.

Validation

The validation of developed method was performed in accordance with ICH Q2 (R1)[6] guidelines for parameters like accuracy, linearity, precision, limit of detection (LOD), limit of quantitation (LOQ) and specificity. Specificity of the method was checked for any interference of other peaks from placebo or any impurities or degradation products at the retention time of the drug peak under the same optimized chromatographic conditions. For assessing the linearity, various concentrations of nortriptyline hydrochloride were prepared by pipetting the respective volumes from the standard stock solution and the volume was made up with mobile phase to obtain concentrations in the range of 5-50 µg mL^{-1}. Each solution was injected in triplicate. By using the concentration and peak area values, a graph was plotted followed by calculation of regression equation and coefficient.

Accuracy study was performed by estimating the percent recovery of nortriptyline hydrochloride. The recovery study was carried out by standard addition method by adding standard known amount of nortriptyline hydrochloride 20 µg mL^{-1} (80 % level), 25 µg mL^{-1} (100 % level) and 30 µg mL^{-1} (120 % level). Each estimation was carried out in triplicate (n=3). The % recovery and % RSD were estimated. Repeatability was assessed by analyzing nortriptyline hydrochloride standard solution in six replicates and % RSD was determined. Intermediate precision was established by intraday precision study in which drug solution was analyzed in triplicate on the same day, whereas interday precision was carried out in similar manner on three different days and % RSD values were calculated. The overall % RSD for peak response analyzed in repeatability and intermediate precision study were checked against acceptable limits of ±2%. The robustness study was carried out by deliberately making small variations in the original conditions like pH of mobile phase, mobile phase ratio and flow rate. LOD and LOQ was calculated from the calibration curve using the formulae LOD = 3.3* δ /slope and LOQ = 10* δ /slope. System suitability study was carried out with five replicate injections of drug solution and the tailing factor (T), retention time (Rt) and theoretical plates (N) were calculated.

Assay of marketed formulation

The content of drug in marketed formulation containing 25 mg nortriptyline hydrochloride was estimated by weighing 10 tablets and determining the average weight.

These 10 tablets were finely powdered and weight equivalent to nortriptyline hydrochloride 25 mg was transferred to a 25 mL volumetric flask. A volume of 15 mL of methanol was added and it was sonicated for 1 h and volume was made up to mark with methanol. The stock solution was diluted to obtain test concentration of 25 µg mL^{-1} of nortriptyline hydrochloride. The samples (n=3) were injected and the peak response was measured.

RESULTS AND DISCUSSION

A mixture comprising of phosphate buffer pH 3.0: methanol: acetonitrile: (50: 10: 40 V/V/V) at a of flow rate of 1.0 mL min^{-1} over Waters C18 column (250 × 4.6 mm, 5 µm) was selected as optimized chromatographic condition and system suitability was assessed. The optimized chromatogram of nortriptyline hydrochloride is shown in Fig. 1a and chromatographic conditions are depicted in Table I.

Table I: Optimized chromatographic conditions

Parameter	Conditions
Mobile phase	Phosphate buffer pH 3.0 (50 mM): acetonitrile: methanol (50: 40: 10 V/V/V)
pH of mobile phase	3.0 using 1 % orthophosphoric acid
Flow rate	1 mL min^{-1}
Stationary phase	Waters C18 (250 x 4.6 mm, 5 µm)
Detector wavelength	235 nm
Run time	15 min
Mode of elution	Isocratic
Injection volume	20 µL
Temperature	Ambient

Statistical data of different parameters like retention time (Rt), peak area (A), symmetry factor (As), theoretical plates (N) of nortriptyline hydrochloride was calculated for peak response using the Open Lab software of the instrument (Table II). All the parameters were within acceptance criteria like theoretical plates being more than 2000 and tailing factor less than 2.0.

The method was linear in 5-50 µg mL^{-1} concentration range for nortriptyline hydrochloride with correlation coefficient (R^2) 0.9937. The linearity curve and statistical parameters of nortriptyline hydrochloride are shown in Fig. 2 and Table III, respectively.

Table II: Results of system suitability parameters

Parameter	Nortriptyline hydrochloride*	Required limits
Peak area	2459166 ± 0.07	--
Retention time (min)	6.8 ± 0.05	--
Tailing factor (T)	1.32 ± 0.076	T < 2
Theoretical plates (N)	12823 ± 0.10	N > 2000

** Results are mean of three determinations (n=3) ± RSD*

Table III: Results of linearity study

Parameter	Nortriptyline hydrochloride*
Linearity range (µg mL^{-1})	5-50 µg mL^{-1}
Slope	10784
Intercept	85414
Correlation coefficient (r^2)	0.9937
LOQ (µg mL^{-1})	0.69
LOD (µg mL^{-1})	0.22
Regression equation	y = 10784x+85414

** Results are mean of three determinations n=3*

The LOD and LOQ values for nortriptyline hydrochloride were found to be 0.22 µg mL^{-1} and 0.69 µg mL^{-1}, respectively. The mean % recovery for nortriptyline hydrochloride was found to be 99.06 % w/w, which was within the desired limit and hence the method was found to be accurate (Table IV).

Table IV: Results of recovery study

Level	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	% Recovery	% RSD
80%	20	19.94	99.71	1.10
100%	25	24.62	98.48	1.24
120%	30	29.55	98.51	0.96

** Results are mean of three determinations n=3*

The % RSD for interday (intermediate precision), repeatability and intraday were found to be 0.2959, 0.3462, and 0.1361, respectively, which complies with the desired limit (NMT 2.0 %). Thus, the method was found to be within acceptable criteria, which indicated that the method is precise.

The results of robustness study showed that small and deliberate changes in conditions like flow rate, pH and mobile phase ratio do not have any drastic influence

Table V: Results of robustness study

Condition	% RSD		
	Retention time	Peak area	Tailing factor
pH 2.9	0.357	0.065	1.124
pH 3.1	0.046	0.204	1.172
Flow rate 0.9 mL min^{-1}	0.164	0.100	1.665
Flow rate 1.1 mL min^{-1}	0.100	0.144	1.669
Mobile phase ratio 48 B: 40 A: 12 M	0.053	0.125	0.839
Mobile phase ratio 48 B: 42 A: 10 M	1.059	0.563	0.100
Mobile phase ratio 50 B: 38 A: 12 M	0.185	0.418	1.720
Mobile phase ratio 50 B: 42 A: 8 M	0.033	0.100	1.071
Mobile phase ratio 52 B: 38 A: 10 M	0.019	0.401	1.466
Mobile phase ratio 52 B: 40 A: 8 M	0.209	0.184	0.655

**Results are mean of three determinations n=3*

on chromatographic results of nortriptyline hydrochloride. The % RSD of Rt was found to be NMT 2.0 for all the parameters (Table V), indicating robustness of the method. Specificity study revealed no major peak of placebo at the retention time of nortriptyline hydrochloride indicating specificity of the method (Fig. 1b). The method was applied for assay of marketed formulation. The percent content of nortriptyline hydrochloride was found in the range of 98.61- 100.05 % w/w.

CONCLUSION

A validated reverse phase HPLC method was developed for estimation of nortriptyline hydrochloride. The proposed RP-HPLC method was validated through laboratory studies for various parameters as per ICH guidelines and it meets all the acceptance criteria given in ICH guidelines. Thus, the developed HPLC method is suitable for analysis of NORH in the pharmaceutical preparation containing NORH.

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