RP-HPLC METHOD FOR THE ESTIMATION OF NITROXAZEPINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT : A simple and sensitive isocratic RP - HPLC method was developed for the determination of Nitroxazepine hydrochloride in bulk drug and its pharmaceutical tablet formulations where the mobile phase optimized was Phosphate buffer : Acetonitrile (70:30) and Phenomenex C_{18} column (250 mm length, 4.6 mm internal diameter and particle size 5 µm) was used as the stationary phase. The flow rate and detection wavelength was 1.0 mL min^{-1} and 265 nm respectively. The developed method was validated as per ICH guidelines for specificity, linearity and range, precision, accuracy, robustness, limit of quantification and limit of detection. The results of all the validation parameters were well within their acceptance values. The method gave good recovery in the range of 98.95 - 99.43 % for Nitroxazepine hydrochloride when it was applied for its determination in pharmaceutical tablet formulations.

Keywords: Nitroxazepine hydrochloride, RP-HPLC, Validation, Pharmaceutical formulation

INTRODUCTION

Nitroxazepine (Goodman and Gilman 1996, William O Foye 1989) chemically 10-[3-(dimethylamino) propyl-2-nitrobenzo [1,4] oxazepine-11(10H)-one, (Figure-1) is a major antidepressant drug (Jain AN et al. 1984, Bhatt AD et al. 1991). It is a tricyclic antidepressant (Balani ND et al. 1990, Mishra et al. 1990) and it acts by inhibiting α_2 receptors and inhibit active reuptake of biogenic amines such as NA and 5-HT into there respective neurons and thus potentiates them(Joseph et al. 1988). It is used in children with nocturnal enuresis. Nitroxazepine (NTZ) is not official in any pharmacopoeia. No HPLC (LR Snyder 1992) methods were reported in the literature for the estimation of NTZ in pure and pharmaceutical dosage forms. The present research work was aimed to develop and validate (USP 2005, ICH guidelines 2005, US FDA Guidelines 2005) a simple, specific, accurate and sensitive RP-HPLC method for the determination of Nitroxazepine in pure and its pharmaceutical formulations.

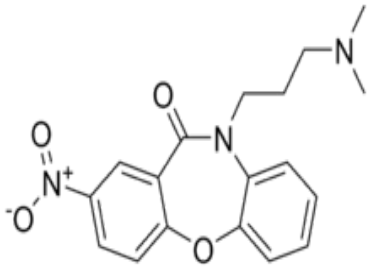

Figure-1 Chemical structure of NTZ

Experimental

Chemicals and Materials

Potassium dihydrogen ortho phosphate (AR grade, Merck)
Triethyl amine (HPLC grade, Merck)
Ortho phosphoric acid (AR grade, SD fine)
Acetonitrile (HPLC grade, Merck)
Water (HPLC grade, Merck)

Equipment

High Performance Liquid Chromatograph (Shimadzu HPLC, Class VP series) with two LC-10AT VP pumps, manual injector with loop volume of 20 µl (Rheodyne), programmable variable wavelength UV detector**.** Chromatographic integration and processing was carried out on Spincotech software. A Phenomenex C_{18} column (250 mm length, 4.6 mm internal diameter and particle size 5 µm) was used as the stationary phase. The column eluents were monitored at 265 nm.

Preparation of mobile phase

A 10 millmolar phosphate buffer was prepared by dissolving 0.68 g of potassium dihydrogen orthophosphate in 500 ml of water. To this 2 ml of triethylamine was added and pH was adjusted to 3.5 with orthophosphoric acid. Above prepared buffer and acetonitrile were mixed in the proportion of 70:30 v/v. The mobile phase so prepared was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Chromatographic conditions

The column was equilibrated with mobile phase and flow rate was maintained at 1.0 mL min^{-1} and eluents were monitored at 265 nm. The sample was injected using a 20 µL fixed loop. The column temperature was kept as ambient during the analysis.

Preparation of standard solutions

About 50 mg of NTZ was accurately weighed and dissolved in 50 ml of mobile phase. This solution was further diluted to get different concentrations (10, 15, 20, 25 and 30 µg/ml) of NTZ.

Preparation of sample solution for assay

Twenty tablets of NTZ were weighed and net content of each tablet was calculated. Tablet powder equivalent to 100 mg of NTZ was accurately weighed and transferred to a 100 ml volumetric flask with addition of about 60 ml of diluent. The mixture was sonicated for 30 min with shaking, and volume was made up to the mark with the diluent. The above solution was filtered through 0.45 µm syringe filter and subsequent filtrate was further diluted with mobile phase to obtain the solution of 1 mg ml^{-1}. The resulting solution was suitably diluted and was analyzed as given under the described chromatographic conditions.

RESULTS AND DISCUSSION

The present work describes development and validation of HPLC method for the determination of NTZ in bulk drug and in the pharmaceutical capsule formulations.

Method development

The mobile phase for the assay of NTZ was optimized and selected by taking different proportions of aqueous and organic phases which gave acceptable asymmetry and theoretical plates with appropriate run time. From the different mobile phases tried mobile phase consisting of Phosphate buffer: Acetonitrile (70:30) was found to be satisfactory. The drug gave symmetric and sharp peak for NTZ at 1 mL min^{-1} flow rate with good theoretical plates and acceptable tailing factor. For quantitative analytical purpose wavelength was set at 265 nm, which provided better reproducibility with minimum interference. Under the chosen experimental conditions, the liquid chromatogram of NTZ showed a single peak of the drug at retention time (Rt) 6.97 min with asymmetry of 1.41.

System suitability

The system suitability was evaluated by calculating the %RSD values of peak area, retention time, asymmetry and theoretical plates of five standard replicates. The experimental results (Table-1) showed that the values were within the acceptable range indicating that the system was suitable for the intended analysis. In specificity study, standard solutions of NTZ and the tablet placebo were injected and a single peak was obtained for NTZ, which indicates that there was no interference from the excipients used and also from the mobile phase. The specificity study was also evaluated by examining the results .The % relative standard deviation of NTZ from the six units should be not more than 2.0%. The Blank and Standard Chromatograms of Nitroxazepine were presented in Figure 2a & 2b

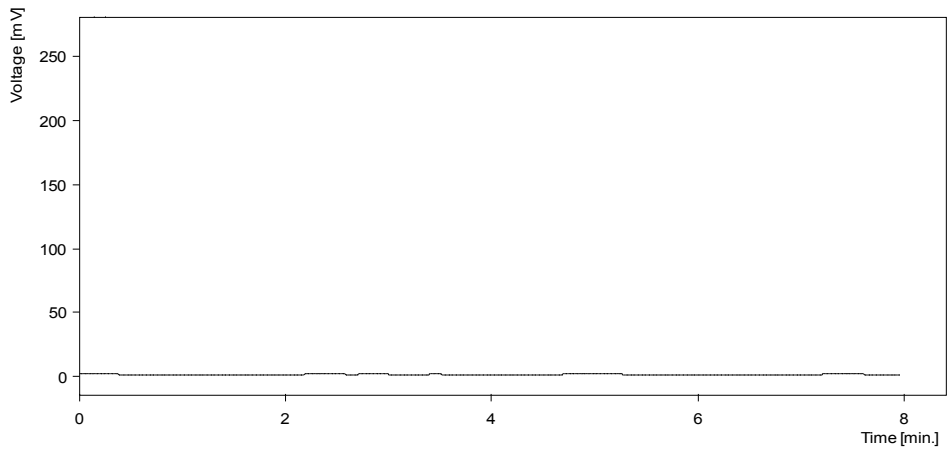

Figure 2a Chromatogram of Nitroxazone Hydrochloride blank

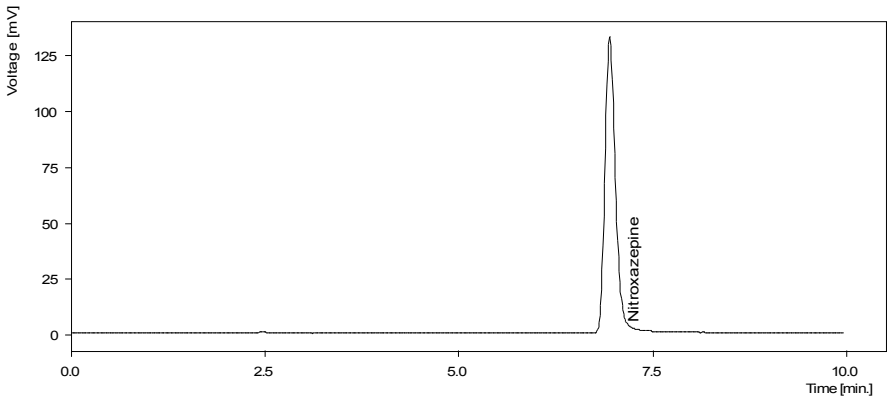

Figure 2b Chromatogram of Nitroxazone Hydrochloride standard

Linearity and range

The linearity of the developed method was determined in triplicate at different concentrations ranging from 10-30 µg ml^{-1}. The correlation coefficient was found to be 0.9991.From the above study it was established that the linearity of test method is from 10-30 µg ml^{-1}. The linearity values were presented in Table-1. The calibration curve was presented in Figure-3.

Table 1. System suitability parameters

Parameter	Results
Retention Time (Rt) (Min)	6.97
Theoretical Plates (n)	11972
Peak asymmetry	1.417
Linearity range (µg mL^{-1})	10-30
Limit of Detection (µg mL^{-1})	0.322
Limit of Quantification (µg mL^{-1})	0.988

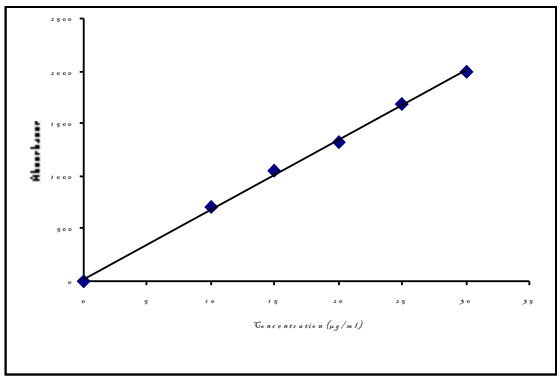

Figure-3 Calibration curve for Nitroxazepine Hydrochloride

Method precision and intermediate precision

Method precision studies were carried out by repeating the analysis of three standard solutions of 5, 10, 15 µg ml^{-1} six times on the same day under the same experimental conditions and reporting the %RSD values of the results obtained. The intra-day and inter-day precision study was carried out by estimating the corresponding responses of the analysis 3 times on the same day and on 3 different days (first, second and third day) and %RSD values were obtained. The results of precision and intermediate precision are shown in Table-2.

Table 2. Intra-day and inter-day precision of the method.

Concentration Added, µg mL^{-1}	Intra-day precision		Inter-day precision	
	Mean amount found, µg mL^{-1} (n = 6)	% RSD (n = 6)	Mean amount found, µg mL^{-1} (n = 3)	% RSD (n = 3)
15	15.06 ± 0.63	0.74	15.02 ± 0.31	0.82
20	19.81 ± 0.25	0.42	19.74 ± 0.62	0.51
25	19.66 ± 0.35	0.51	19.53 ± 0.83	0.72

Accuracy

Accuracy of the method was studied by applying the developed method to prepared synthetic mixtures of tablets excipients to which known amount of NTZ corresponding to 50-150% of the label claim had been added. Mean recovery (Table-3) for NTZ was between 95.0%and 105.0% indicating that the developed method was accurate for the determination of NTZ in pharmaceutical formulation.

Table 3. Accuracy of the method

Sample solution	Amount of standard drug added µg mL^{-1}	% Recovery ± SD (*n* = 3)	% RSD
Nitroxazepine 10 µg mL^{-1}	5	99.43 ± 0.91	0.63
	10	99.12 ± 0.26	0.76
	15	98.95 ± 0.53	0.84

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated from standard deviation of the response and the slope of the three linearity curves using the formula 3.3 α/S for LOD and 10 α/S for LOQ where α is standard deviation of response and S is mean of slope of three calibration curves. The LOQ was verified by injecting six replicates at its concentration at the LOQ level of NTZ. LOD value was found to be 0.322 µg mL-1 and LOQ was 0.988 µg mL-1. The LOQ was verified by performing six replicate analysis at its concentration, the % relative standard deviation of NTZ from the six units should be not more than 2.0%.

Robustness

The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changed from the original analytical condition. The results obtained were not affected by varying the conditions and were in accordance with the results for original conditions.

i) Effect of variation in mobile phase composition:

A study was conducted to determine the effect of variation in organic phase composition in mobile phase. Standard solution prepared was injected into the HPLC system using two mobile phases. The system suitability parameters were evaluated and found to be with in the limits for mobile phase having 95% and 110% of method highest organic phase. NTZ solution at target concentration was chromatographed using mobile phase having 95% and 110% of the method organic phase. NTZ was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having 100% of the organic phase. From the study it was established that the allowable variation in mobile phase composition is 95% to 110% of the method highest organic phase of mobile phase.

ii) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution was injected into the HPLC system using flow rates, 0.9ml/min and 1.1 ml/min. The system suitability parameters were evaluated and found to be with in the limits for 0.9 ml/min and 1.1 ml/min flow. NTZ was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0 ml/min

Method application

The proposed validated liquid chromatographic method was successfully applied to the estimation of NTZ in pharmaceutical tablet formulations. The assay results obtained were satisfactory, accurate and precise (Table-4). The developed method achieved rapid and accurate determination of NTZ and can be used for the determination of NTZ in drug substance and pharmaceutical preparation. The typical chromatogram of Nitroxazepine formulation was presented in Figure 4

Table 4 Results from analysis of Nitroxazepine in tablets

Label claim. mg per tablet	**75**
Average Amount found, (mg per tablet) (n = 6)	74.35 ± 0.41
% RSD (n = 6)	0.23

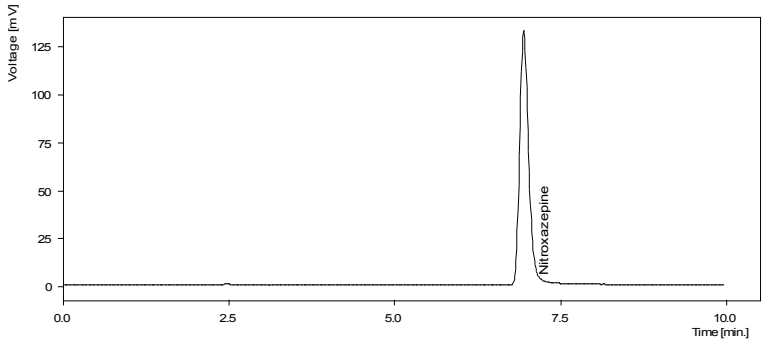

Figure 4 Chromatogram of Nitroxazone Hydrochloride formulation

CONCLUSION

In proposed study, sensitive isocratic RP-HPLC method has been developed for determination of NTZ. The developed method was validated and was found to be simple, sensitive, accurate and precise. The method was successfully used for determination of NTZ in its pharmaceutical formulations.

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