

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ATOMOXETINE HYDROCHLORIDE USING RAPID HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC TECHNIQUE

ZUBAIDUR RAHMAN, VIJAY AANANDHI M, SUMITHRA M*

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India. Email: sumithrapharmanalysis@gmail.com

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ABSTRACT

Objective: A simple, novel, sensitive, rapid high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for quantitative determination of atomoxetine HCl (ATH) in bulk and formulations.

Methods: The chromatographic development was carried out on RP-HPLC. The column used as Xterra RP 18 (250 mm × 4.6 mm, 5 μ particle size), with mobile phase consisting of methanol: water 80:20 V/V. The flow rate was 1.0 mL/min and the effluents were monitored at 270 nm.

Results: The retention time was found to be 5.350 min. The method was validated as per International Conference on Harmonization Guideline with respect to linearity, accuracy, precision, and robustness. The calibration curve was found to be linear over a range of 2–10 μg/mL with a regression coefficient of 0.9999. The method has proved high sensitivity and specificity.

Conclusion: The results of the study showed that the proposed RP-HPLC method was simple, rapid, precise and accurate which is useful for the routine determination of ATH in bulk drug and in its pharmaceutical dosage form.

Keywords: Atomoxetine hydrochloride, Capsules, Rapid high-performance liquid chromatographic, Validation.

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INTRODUCTION

The atomoxetine HCl (ATH) (Fig. 1) is the first non-stimulant drug approved by the United States FDA for symptomatic treatment of attention-deficit hyperactivity disorder and selective noradrenaline (norepinephrine) reuptake inhibitor [1,2]. Chemically, it is (R)-N-methyl-3-(o-tolylloxy)-3-phenylpropylamine hydrochloride [3,4]. Its molecular formula is C₁₇H₂₁NO. HCl and its molecular weight are 291.82 g/mol. Atomoxetine, principally it is metabolized by CYP4502D6 through oxidative enzymatic pathway and by glucuronidation [5]. The literature survey shows that several analytical techniques such as high-performance liquid chromatographic (HPLC) [6-11], LC-MS-MS [12], HPTLC [13], chemiluminescence [14], and UV [15,16] have been reported for its determination of ATH in plasma and capsule dosage forms. The validation of analytical procedure used for different type of synthetic and natural substance [17-19]. The present work reports simple, rapid, sensitive and economical rapid HPLC (RP-HPLC) method with UV detection, useful for the routine analysis of ATH in bulk and pharmaceutical formulations. The method parameters such as linearity, accuracy, precision, robustness, stability, and system suitability validated as per International Conference on Harmonization (ICH) guidelines and USP guidelines [20,21].

METHODS

The ATH pure standard was supplied by TCI Chemicals Pvt. Ltd. Chennai, India. Faint 10 mg capsules (Sun Pharmacy, Chennai), labeled to contain 10 mg ATH, were obtained from the market. Analytical reagent grade chemicals were procured from Company Merck Specialties Pvt. Ltd. (Mumbai, India). The column used as Xterra RP 18 (250 mm × 4.6 mm, 5 μ particle size) for the study.

Instrumentation, chromatographic conditions

HPLC analysis was carried out on Xterra RP 18 (250 mm × 4.6 mm, 5 μ particle size), reversed phase column. The mobile phase consisting

of methanol: water 80:20 V/V. The flow rate of mobile phase was 1 mL/min. The detection was carried out by at 270 nm. All analysis was carried out at a temperature of 30°C under isocratic conditions.

Preparation of standard solutions

Accurately weigh and transfer 10 mg of working standard into a 10 mL clean dry volumetric flask add about 10 mL of HPLC-grade water and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further dilution is made and finally prepared 10 μg/mL of solution.

Selection of detection wavelength

ATH showed significant absorbance at 270 nm using UV-visible spectrophotometer.

Preparation of sample solutions

Accurately weigh and transfer 25 capsules (equivalent to 10 mg) into 50 mL volumetric flask add about 25 mL of HPLC grade water, sonicate for 15 min with occasional shaking. Cool the solution to room temperature and dilute to volume with HPLC grade water and mix. Further pipette 5 mL of the above solution into a 50 mL volumetric flask and dilute up to the mark with diluent.

Assay validation

The proposed RP-HPLC method was validated as per the guidelines of the ([ICH] Q2 [RI]) for various parameters.

Linearity and range

The standard solution of ATH was applied in the concentration of 2–10 μg/mL of ATH to evaluate linearity. The graph of peak area versus concentration was plotted. Least square linear regression analysis was done. The correlation coefficient, intercept, and slope were calculated.

Sensitivity

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using formula $3.3 \sigma/S$ and $10 \sigma/S$, respectively, where σ is the standard deviation of the response (y -intercept) and S is the slope of the linearity plot.

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

Precision studies

Precision was calculated by intra- and inter-day precision studies. 6 $\mu\text{g/mL}$ concentration of ATH sample was analyzed six times on the similar day to find out any differences in the results. Interday precision study was done on three successive days.

Accuracy studies

Accuracy is the closeness in agreement between the accepted true value or a reference value and the actual results obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the mixture of the sample to be analyzed. For accuracy studies, I have used three different concentration of solution such as 4 $\mu\text{g/mL}$, 6 $\mu\text{g/mL}$, and 8 $\mu\text{g/mL}$. For these, each concentration was injected and the mean % recovery was calculated.

Robustness studies

The effect of small but deliberate variations in method parameters such as flow rate, composition of the mobile phase, and wavelength was evaluated in this study. 2–10 $\mu\text{g/mL}$ concentration of ATH was used in six replicates to study robustness of the method. The standard deviation of peak areas and % relative standard deviation (RSD) was determined.

RESULTS AND DISCUSSION

Chromatographic development

Chromatographic analysis was developed using a Xterra RP 18 (250 mm \times 4.6 mm, 5 μ particle size) column. The mobile phase consists of

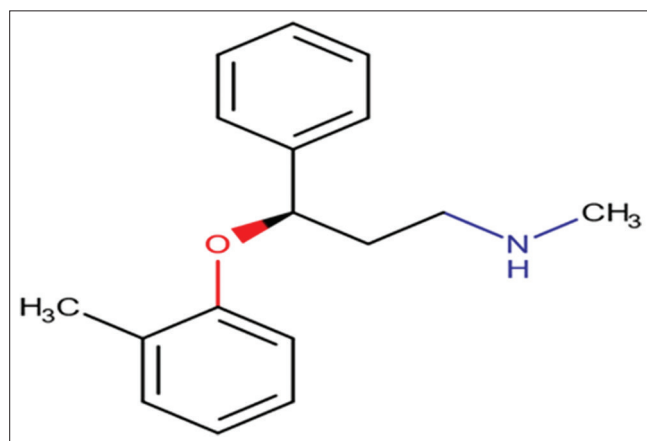


Fig. 1: Chemical structure of atomoxetine HCl

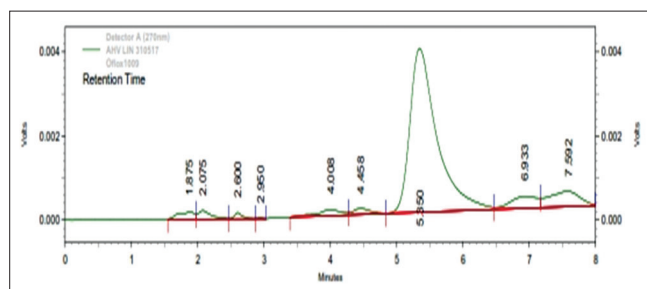


Fig. 2: Optimized chromatograms of atomoxetine

methanol: water (80: 20 v/v) and that was supplied at a flow rate of 1.0 mL/min. The mobile phase was degassed and filtered through 0.45 μ filter under vacuum before pumping into HPLC system. The effluent was monitored by UV detection at 270 nm. Fig. 2 shows the optimized chromatogram of ATH.

Validation of the method

Linearity

The analyte response was linear ($r^2=0.9999$) over the concentration range of 2–10 $\mu\text{g/mL}$ of ATH. The results were shown in Table 1 and Fig. 3 shows the calibration curve. The curve shows the selected concentration gives acceptable accuracy and precision over a wide concentration range. The results demonstrate that an excellent correlation coefficient between the absorbance and concentration of ATH drug substance.

Sensitivity

The LOD was found to be 0.0001 μg for ATH. The LOQ for ATH was found to be 0.0005 μg representing good sensitivity of the method.

Specificity

The chromatograms obtained from standard and sample solutions are not interfering. It proves method is highly selective.

Precision

Intra- and inter-day variation in estimation of ATH (Table 2) showed that the % RSD was <2% during the analysis. These low values of RSD show that the precision of the method is good.

Accuracy

The study of accuracy reveals influences of additives that are usually present in the dosage forms on the quantitative parameters. The recovery study data presented in Table 3 indicates that the accuracy of the quantification of ATH was more than 98%, which indicate that the proposed simultaneous RP-HPLC method is reliable for the estimation of in the marketed formulation used in the study.

Robustness studies

The % RSD of peak areas was calculated for each parameter and was found to be <2% [Table 4].

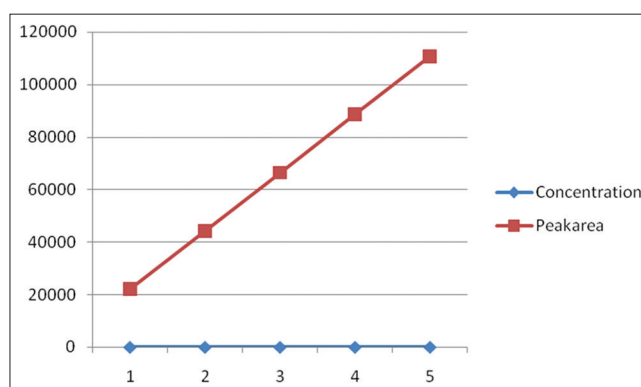


Fig. 3: Linearity graph of atomoxetine HCl by rapid high-performance liquid chromatographic

Table 1: Linear regression analysis of ATH

Parameters	Values
Linearity range ($\mu\text{g/mL}$)	2–10
Correlation coefficient (r^2)	0.9999
Slope	11078.185
Intercept	7.0166
Standard error	30.7944

ATH: Atomoxetine HCl

Table 2: Intraday- and inter-day precision studies of ATH

Drug	Actual concentration intra-/interday	Intra/ interday	(%) RSD
Atomoxetine HCl	6 µg	99.63/99.71	0.771/1.088

ATH: Atomoxetine HCl

Table 3: Results of recovery studies of Atomoxetine HCl

Amount taken	Amount added	Amount found*	Amount Recovery*	%RSD
6	4	10.01	3.99	0.31
6	6	11.97	6.13	0.19
6	8	14.22	7.91	0.12

*Average of three determination

Table 4: Robustness studies of ATH

Parameter	Parameter varied	%RSD
Mobile phase (methanol: water) ratio	60:40	0.32
	80:20	
	85:15	
Flow rate in ml	0.8	0.89
	1.0	
	1.2	
Wavelength in nm	268	0.74
	270	
	272	

ATH: Atomoxetine HCl

Analysis of a marketed preparation

The results obtained for the amount of ATH in capsules as against the label claims were in good agreement signifying that there is no interference from any of the excipients presents in capsules. The percent assay was found to be 99.93%, for ATH, in marketed formulation in six replicate determinations.

CONCLUSION

The purpose of this investigation was to develop and validate a method using a simple, rapid, sensitive, precise, and accurate RP-HPLC for the routine determination of ATH in bulk and pharmaceutical preparations. The proposed method is suitable for the pharmaceutical analysis in various analytical laboratories. The retention time and run time were very short, hence, requires less mobile phase for this method, making it more economical and rapid. Hence, this method can be used for the analysis of large number of samples.

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AUTHORS CONTRIBUTION

The entire author equally contributed to this work.

CONFLICTS OF INTEREST

No.

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