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RP- HPLC Method for Estimation of Atomoxetine Hydrochloride in Bulk and Pharmaceutical Dosage Form.

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ABSTRACT

A simple, efficient and isocratic reverse phase-high performance liquid chromatographic method has been developed and validated for the determination of Atomoxetine Hydrochloride in bulk and pharmaceutical dosage form. The chromatographic separation was achieved by using Enable ODS reverse phase (250 mm x 4.6 mm, 5 µm partical size) C_{18} column. Mobile phase consists of mixture of 0.1% (v/v) orthophosphoric acid and acetonitrile in the ratio of 18:82 v/v and was delivered at a flow rate of 0.6 ml/min, while the detection was monitored at a wavelength of 271nm. The developed method showed excellent linear response in the range of 40-120 µg/ml. The retention time for Atomoxetine Hydrochloride was found to be 4.7 min. The proposed method was validated as per ICH guidelines and can be applied for estimation of Atomoxetine Hydrochloride in pharmaceutical dosage forms in routine analysis.

Keywords: Atomoxetine Hydrochloride, RP-HPLC, Validation.

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INTRODUCTION

Atomoxetine HCl [1] is a selective norepinephrine reuptake inhibitor. Atomoxetine HCl is the R(-) isomer as determined by X-ray diffraction. The chemical designation [2] is (-)-N-Methyl-3-phenyl-3-(o-tolyloxy)-propylamine hydrochloride. Atomoxetine[3] is the first non-stimulant drug approved for the treatment of attention-deficit hyperactivity disorder (ADHD). It is sold in the form of the hydrochloride salt of Atomoxetine. Atomoxetine is classified as a norepinephrine reuptake inhibitor, and is approved for use in children, adolescents, and adults. The precise mechanism by which Atomoxetine produces its therapeutic effects in Attention-Deficit/Hyperactivity Disorder (ADHD) is unknown, but is thought to be related to selective inhibition of the pre-synaptic norepinephrine transporter, as determined through in-vitro studies. Atomoxetine appears to have minimal affinity for other noradrenergic receptors or for other neurotransmitter transporters or receptor.

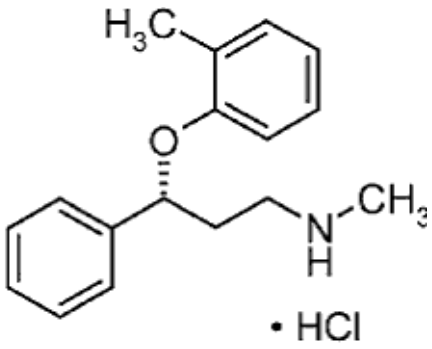

Figure 1: Chemical structure of Atomoxetine HCl

It is used for the treatment of Attention-Deficit/Hyperactivity Disorder (ADHD) alone or in combination with behavioral treatment, as an adjunct to psychological, educational, social, and other remedial measures. Literature survey reveals few chromatographic methods have been reported for quantitative estimation of Atomoxetine Hydrochloride in pharmaceutical formulations [4-6]. A ion pairing HPLC method was also reported for the separation of Atomoxetine and impurities [7] stability-indicating RP HPLC method also reported for the determination of Atomoxetine hydrochloride in the presence of its degradation products generated from forced decomposition studies[8].The main objective of the present study was to develop simple, accurate, precise, and economic analytical method with the better detection range for estimation of Atomoxetine Hydrochloride in bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure sample of Atomoxetine hydrochloride with purities greater than 99 % was obtained as gift sample from Mylan Laboratories Hyderabad, India and capsule formulation [ATTENTROL-18] was procured from APOLLO Pharmacy, Visakapatnam, India with labelled amount 18 mg of Atomoxetine hydrochloride. Acetonitrile (HPLC grade), water (HPLC grade), orthophosphoric acid (AR Grade) were obtained from Merck India. Nylon membrane filters 0.2 µm were obtained from Pall Corporation, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu Prominence Liquid Chromatograph comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with “LC solutions” software. An electronic analytical weighing balance (0.1 mg sensitivity, Shimadzu AY 220), digital pH meter (Elico model 121), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Elico SL 210, software-Spectral Treats) were used in this study.

Method

Selection of wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Atomoxetine hydrochloride. Suitable wavelength selected was 271 nm.

Chromatographic conditions

The developed method uses a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5 µ), mobile phase consisting of acetonitrile and 0.1% w/v orthophosphoric acid in the proportion of 82:18 v/v. The mobile phase was set at a flow rate of 0.6 ml/min and the volume injected was 20 µl for every injection. The detection wavelength was set at 271 nm.

Preparation of 0.1% v/v orthophosphoric acid

Accurately measured 0.1 ml orthophosphoric acid was transferred into a 100 ml of volumetric flask and volume was made up to the mark with HPLC grade water. The solution was sonicated for 15 min and filtered through 0.2 µm membrane filter.

Preparation of Mobile Phase

The mobile phase was prepared by mixing acetonitrile and 0.1 % v/v orthophosphoric acid in the ratio of 82:18 v/v. The solution was sonicated for 15 min and filtered through 0.2 µm membrane filter.

Preparation of working standard solution

10 mg of Atomoxetine hydrochloride was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluent (same as mobile phase) and then sonicated for 2 minutes to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as working standard solution (100 µg/ml).

Preparation of sample solution

Ten capsules were weighed separately and the average weight was determined. The capsule content equivalent to 100 mg of Atomoxetine hydrochloride was transferred to a 100 ml volumetric flask and dissolved in little portion of mobile phase then volume was made up to the mark with mobile phase. The resulting solution was sonicated for 3 minutes, followed by filtration through 0.2 µ nylon membrane filter to get sample stock solution of 1mg/ml. 1 ml of the above stock solution was pipetted out and made up to 10 ml to get a sample solution concentration of 100 µg/ml.

RESULTS AND DISCUSSION

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Atomoxetine hydrochloride at 4.783 min. Fig. 2 represents standard solution (100 µg/ml). The total run time is 5 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*Rt*), number of theoretical plates (*N*) and peak Asymmetric factor were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1.

In order to test the applicability of the developed method to a commercial formulation, "ATTENTROL" was chromatographed at working concentration (100 µg/ml). The sample peak was identified by comparing the retention time with the standard drug. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The

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protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control.

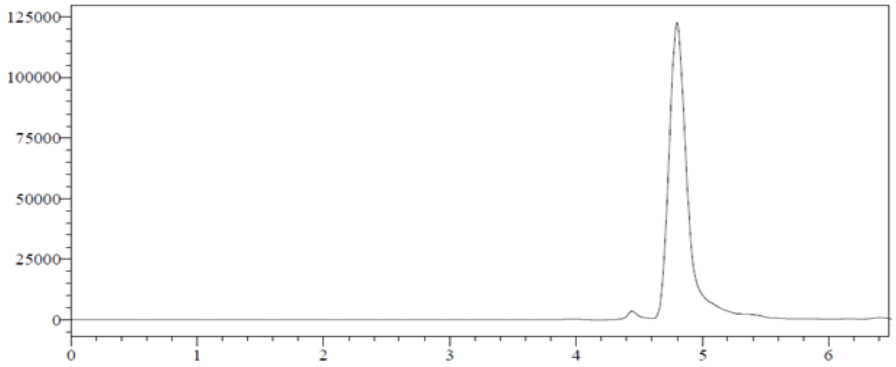

Figure 2: Chromatogram of Standard Atomoxetine hydrochloride solution

Table 1: Results from system suitability studies			
Property	**Values ± SD***	**%RSD**	**Required Limits**
Retention time (min)	4.78±0.0066	0.26	RSD<1%
Theoritical plates (N)	7491±14.88	0.56	N>2000
Tailing factor (T)	1.431±0.0223	1.421	T<2

*Average of six determinations

Method validation [9]

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, robustness, and ruggedness, limit of detection (LOD) and limit of quantitiation (LOQ).

Specificity

Specificity was checked for the interference of excipients in the analysis of sample solution and was determined by injecting sample solution with added excipients under optimized chromatographic conditions to demonstrate separation of Atomoxetine hydrochloride from excipients. There is no interference of excipient peak on the peak of Atomoxetine hydrochloride indicating the high specificity of method.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies.

Intra-day Precision

In the intraday studies, six injections of standard solution were injected into the chromatographic system in different time interval within a day. %RSD was calculated and is presented in Table 2.

Table 2: Intra-day precision results for Atomoxetine hydrochloride			
S.No	**Concentration (µg/ml)**	**Retention time (min)**	**Peak Area**
1	100	4.788	609544
2	100	4.754	611432
3	100	4.768	609154
4	100	4.764	610999
5	100	4.723	609121
6	100	4.739	609486
AVG		3.756	609956
SD		0.022	999.74
%RSD		0.58	0.16

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Inter-day Precision

In the inter-day variation studies, six injections of standard solution were injected at different days. % RSD was calculated and is presented in Table 3.

Table 3: Inter-day precision results for Atomoxetine hydrochloride

S.No	Concentration (µg/ml)	Retention time (min)	Peak Area
1	25	4.798	608712
2	25	4.762	619341
3	25	4.758	612383
4	25	4.775	609734
5	25	4.764	606992
6	25	4.786	612987
AVG		4.773	611691
SD		0.0156	4369
%RSD		0.41	0.71

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in Table 4**.** The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 4: Results from recovery studies

Sample	Area	Sample amount (µg/ml)	Standard added (µg/ml)	Standard recovered* (µg/ml)	%Recovery ± SD*	%RSD
80%	486231	100	80	79.96	99.83±0.03	0.03
100%	609445	100	100	99.90	99.63±0.043	0.0431
120%	729785	100	120	119.93	99.77±0.032	0.032

*Average of three determinations

Linearity

Standard solutions of Atomoxetine hydrochloride at different concentrations were prepared. Calibration curve was constructed by plotting the concentration of drug versus corresponding peak area. The results show an excellent correlation between peak area and concentration of drug within the concentration range (40-120 µg/ml) for the drug and the results are given in Tables 5-6. The correlation coefficient of Atomoxetine hydrochloride is greater than 0.99, which meet the method validation acceptance criteria and hence the method is said to be linear. The linearity plot was shown in Fig.3.

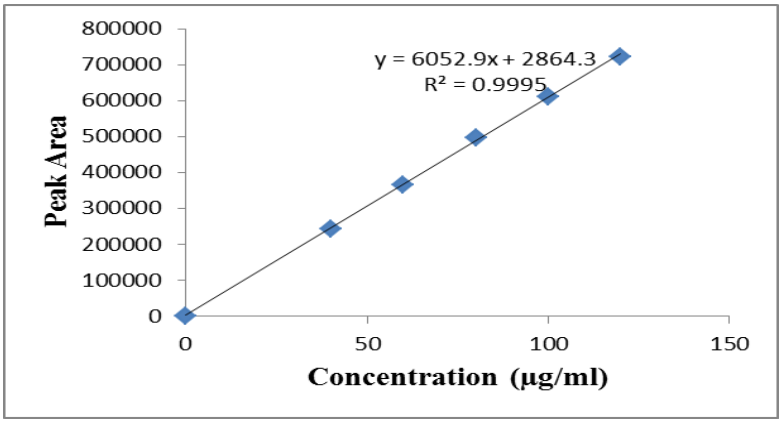

Figure 3: Linearity plot of Atomoxetine hydrochloride

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Table 5: Calibration data of Atomoxetine hydrochloride		
S.No	**Concentration (µg/ml)**	**Peak Area**
1	40	243818
2	60	365826
3	80	497636
4	100	609544
5	120	721520

Table 6: Characteristic parameters of Atomoxetine hydrochloride	
Parameters	**RP-HPLC**
Calibration range (µg/ml)	40-120
Detection Wavelength(nm)	271
Mobile phase (Acetonitrile : 0.1% orthophosphoric acid) (V/V)	82:18
Regression equation (Y)	6052.9x-2864.3
Retention Time(min)	4.78
Slope (b)	6052.9
Intercept (a)	-2864.3
Correlation coefficient (r^2)	0.999
LOD (µg/ml)	1.13
LOQ (µg/ml)	3.41

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as changes in wave length and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The results were shown in Table 7.

Table 7: Robustness studies of Atomoxetine hydrochloride					
S.No	**Condition**	**Modification**	**Mean Peak area ± SD***	**Mean Rt ± SD***	**Mean %RSD (Peak Area)**
1	Flow rate (ml/min)	0.5	612876±4654	5.213	0.75
		0.7	614572±4876	4.598	0.79
2	Wavelength (nm)	269	604233±4321	4.743	0.301
		273	609263±4562	4.653	0.312

*Average of three determinations

Ruggedness

It was checked by determining precision on same instrument, but by a different analyst. Results of reproducibility are shown in Table 8.

Table 8: Ruggedness studies of Atomoxetine hydrochloride							
S.No	**Injection Number**	**Analyst - 1**			**Analyst-2**		
		Area	**Retention time (min)**	**Theoretical plates (N)**	**Area**	**Retention time (min)**	**Theoritical plates (N)**
1	1	610124	4.753	7453	611761	4.721	7665
2	2	612854	4.767	7551	616532	4.738	7442
	AVG	611489	4.76	7502	614146	4.729	7603
	SD	1930	0.0098	69.29	3373	0.012	86.97
	%RSD	0.31	0.26	0.92	0.54	0.32	1.14

Sensitivity

The sensitivity of measurement of Atomoxetine hydrochloride by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 1.13 µg/ml and 3.41 µg/ml.

Estimation of Atomoxetine Hydrochloride in Pharmaceutical Dosage Form

The proposed method was successfully applied for the estmation of Atomoxetine hydrochloride in capsules. The assay result was shown in Table 9.

Table 9: Assay studies of Atomoxetine hydrochloride

Brand Name	Label Claim (mg)	Standard Area*	Sample Area*	Amount found* (mg)	(%) Recovery± SD*
ATTENTROL	18	604532	598486	99.98	99.98± 0.03

*Average of three determinations

CONCLUSION

A high performance liquid chromatography method for the quantitative estimation of Atomoxetine hydrochloride in bulk and capsule dosage form has been developed. The method was validated and found to be applicable for the routine analysis of Atomoxetine hydrochloride in capsule dosage form without interference from the excipients. Statistical results and low % RSD values indicate that the method is precise, accurate, robust, specific, and can be used across a wide range of concentrations. Considering already proposed methods in literature, advantages of this new proposed method are rapid , economic mobile phase, user friendly and convenient approach. All these key features proposed that this method can be considered as advantageous over other methods.

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