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Development and validation of RP-HPLC method for the determination of Atomoxetine Hydrochloride in Pharmaceutical Dosage Forms

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Abstract

A simple, reliable, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the determination of Atomoxetine HCl in pharmaceutical dosage form. Chromatographic separation was carried on RP-C8 column (Phenomenex, size: 250×4.60 mm, particle size 5µm) with a mobile phase composed of acetonitrile and 10 mM disodium hydrogen phosphate buffer with 0.1% TEA (pH 3.0, adjusted with OPA) (55:45, v/v) in isocratic mode at a flow rate of 1mL/min. The detection was monitored at 271nm. The retention time for Atomoxetine was found to be 3.08 min. The method was found to be linear in the range of 50-150µg/mL. The proposed method can be used for the estimation of Atomoxetine HCl in bulk and pharmaceutical dosage forms for routine quality control analysis.

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INTRODUCTION

Atomoxetine (ATX) - (R)-*N*-methyl-3-phenyl-3-(o-tolyloxy)-propylamine hydrochloride is a medication used for the treatment of attention deficit/hyperactivity disorder (ADHD). Atomoxetine in the form of the hydrochloride salt is the only drug from its class, a highly selective non-stimulant norepinephrine reuptake inhibitor. It is approved for

use in children (≥ 6 years), adolescents and adults all over.[1-4] As in detail the mechanism of action is still unknown, the ex-vivo uptake and neurotransmitter depletion studies establish it to be related to highly specific presynaptic inhibition of norepinephrine transporter.[5]

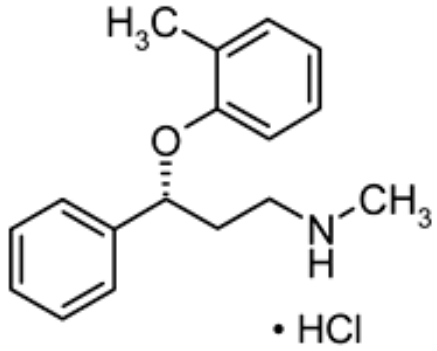

Fig. 1: Structure of Atomoxetine hydrochloride

Literature survey reveals few chromatographic methods have been reported for quantitative estimation of ATX in pharmaceutical formulations.[6, 7] A ion pairing HPLC method was also reported for the separation of Atomoxetine and impurities.[8] A 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.[9]

The proposed RP-HPLC method is simple, precise, accurate, sensitive and validated method for the determination of Atomoxetine hydrochloride in bulk and its pharmaceutical dosage forms (tablets/capsules).

MATERIALS AND METHODS

Atomoxetine HCl was obtained as a gift samples from Ajanta Pharma Ltd. Mumbai, India. HPLC grade acetonitrile and methanol were purchased from Merck chemicals, Mumbai. AR grade disodium hydrogen phosphate anhydrous, ortho-phosphoric acid was purchased from Qualigen Fine Chemicals, Mumbai and triethlyamine from Spectrochem Pvt. Ltd. Mumbai. Formulation used in the study AXEPTA-25 tablets was purchased from the local market.

A HPLC (Perkin Elmer, USA) consisting of Binary LC Pump 200 (Perkin Elmer, Model: series 200), vacuum degasser, UV-VIS detector (Perkin Elmer, Model: series 200). A sample injector system (Rheodyne) with a 20µl sample loop and Total Chrome Navigator software (version V 6.3.1). Vacuum filtration assembly (Orchid Scientifics, Model: JVP01); Ultrasonicator (Oscar, Model: Microclean 103).

Chromatographic Conditions

HPLC analysis was carried out on C8 (Phenomenex, size: 250×4.60 mm, particle size 5µm) reversed phase column. The mobile phase consisted of a mixture of Acetonitrile and 10 mM disodium hydrogen phosphate buffer with 0.1% TEA v/v at pH 3.0 (pH 3.0, adjusted with OPA) (55:45, v/v). The flow rate of mobile phase was 1 ml/min. The detection was carried out by at 271 nm. All analysis was carried out at a temperature of 25°C under isocratic conditions.

Standard solution preparation

An accurately weighed quantity of Atomoxetine HCl drug 10 mg was transferred into 10 ml volumetric flask and was dissolved in acetonitrile solvent, then the solution was sonicated for about a minute and the volume was made up to the mark with acetonitrile to give a stock solution. From the stock solution 1mL was taken and further diluted to 10mL with acetonitrile to get final standard solution of 100µg/ml.

Sample solution preparation

For the determination of drug content in formulation, 20 tablets were each weighed and pulverised to obtain a fine powder in morter and pestle. An amount equivalent to 25 mg of Atomoxetine HCl was transferred to a 25mL

volumetric flask; about 20mL of acetonitrile was added and sonicated for 5 min. Then the volume was made up to mark with acetonitrile and filtered it through whatman filter. From the filtrate 1mL was taken and further diluted to 10mL with acetonitrile to get final sample solution of 100µg/ml.

RESULTS AND DISCUSSION

Method Optimization

To optimize the operating conditions for isocratic reverse phase analysis on C8 column for detection of Atomoxetine, a number of parameters such as the mobile phase composition, pH and sample preparation solvent/diluent were varied. Methanol: buffer and acetonitrile: buffer in various ratios (65:35, 60:40, 55:45 v/v) were taken, trials showed peak distortion or tailing of peak. pH change had little to less effect as the drug is basic (pKa 10.13) in nature. Modification thus were made in the solvent used for sample preparation from buffer, buffer: acetonitrile to acetonitrile showed remarkable changes. Henceforth, in the present method sample preparation with acetonitrile alone and mobile phase of acetonitrile and 10mM disodium hydrogen phosphate buffer with 0.1% TEA v/v at pH 3.0 (pH 3.0, adjusted with OPA) (55:45, v/v) and at a flow rate of 1.0 mL/min showed good peak shape with proper elution at retention time of 3.08 min. The chromatographic parameters like peak symmetry, tailing factor and retention time were within limits and method was chosen as optimal conditions. The appropriate wavelength in UV region (271nm) was selected for the measurement of active ingredients in the proposed method.

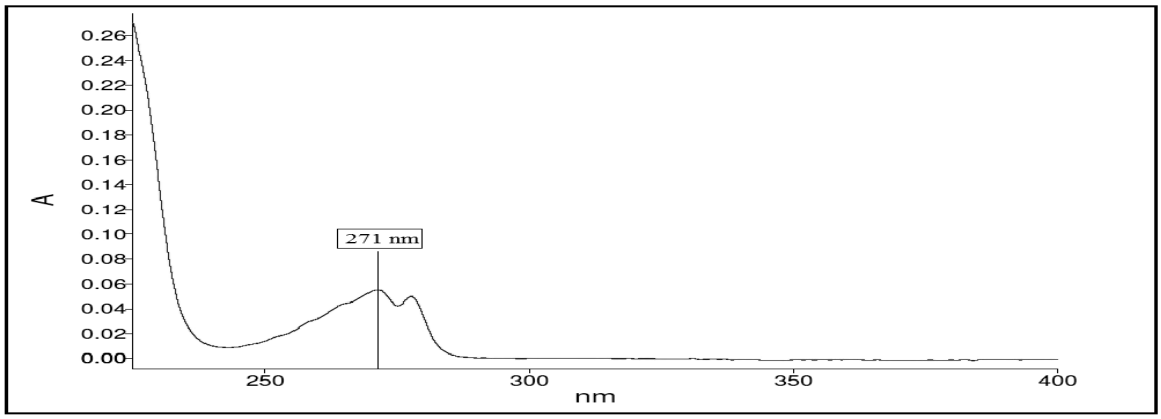

Fig 2: UV-Visible spectra of Atomoxetine HCl

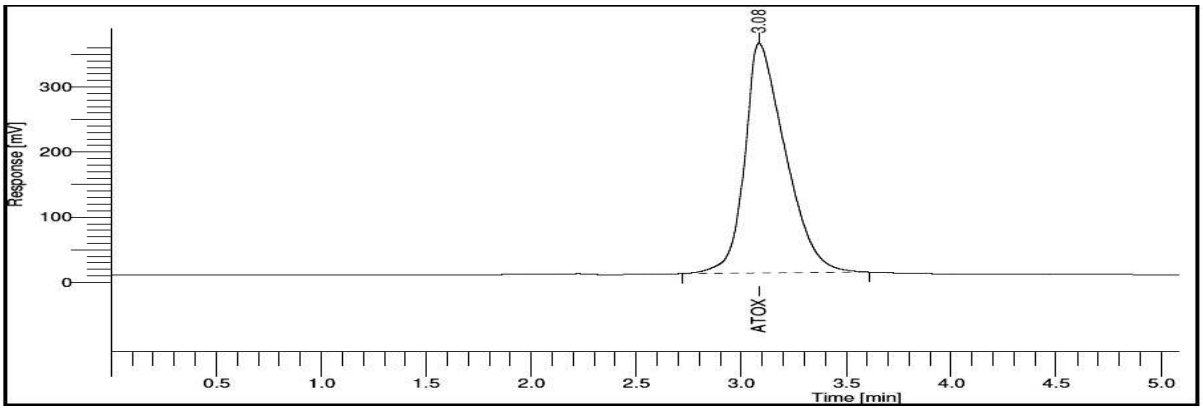

Fig. 3: Chromatographic peak of Atomoxetine HCl (Atox)

Method Validation

System suitability parameters

System suitability parameters for repeated six injections include tailing factor, plate number (N) and percentage relative standard deviation (%RSD) for peak area were within the acceptable limits.

Table 1: System suitability obtained

Tailing factor	1.06
Theoretical Plates	12171
% RSD	1.15

Linearity

From the working standard stock solution (1000 µg/mL), various concentration levels of 50, 70, 100, 120 and 150 µg/mL were prepared and injected on the optimized chromatographic conditions. Linearity curve was constructed by plotting the peak area vs the drug concentration with regression equation, y = 8897.4x + 65293, where 'x' is the concentration (µg/mL) and linear regression r^2 = 0.9991.

Repeatability (method precision)

Six test samples (100µg/mL) were prepared under the same condition from same lot of formulation. Each sample was injected and analyzed on same day. The percentage relative standard deviation (% RSD) was calculated for the resultant peak area.

Table 2: Linearity, precision and accuracy data

Linearity		Precision		Accuracy		
Concentration (µg/mL)		Repeatability n = 6	Intermediate Precision* n = 6	Spike level n = 3	% Recovery	% RSD
50		1.11	0.83	80 % 100 % 120%	100.01 99.68 101.30	1.47 1.02 0.03
70						
100						
120						
150						
r^2	0.9991					
Intercept	65293					
Slope	8897.4					

*analysis done on different day by different analyst

Intermediate precision

The analysis was carried on assay conc. (100µg/mL) prepared on different day by different analyst. The assay procedure was repeated six times and the chromatogram was recorded. The percentage relative standard deviation (% RSD) was calculated.

Accuracy

Accuracy was performed at 3 levels spiking of 80, 100 and 120% of standard concentration. The recovery procedure was repeated 3 times and % RSD was calculated. The values of % RSD of assay indicate the method is accurate.

CONCLUSION

The results obtained by HPLC method for determination of Atomoxetine HCl in selected pharmaceutical formulation was simple, reliable, specific, accurate and precise. Hence, it can be employed for routine quality control analysis.

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