Research Article

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METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION STUDIES OF ATOMOXETINE HYDROCHLORIDE BY RP- HPLC

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ABSTRACT

A RP-HPLC method was developed for the quantitative determination of atomoxetine hydrochloride in bulk drug and tablet formulation, recently used as an antidepressant agent. Forced degradation studies were performed on bulk sample of atomoxetine hydrochloride using acid (0.5 N hydrochloric acid), base (0.5 N sodium hydroxide), oxidative (3.0 % v/v hydrogen peroxide), thermal (60°) and photolytic (UV) degradation. Mild degradation of the drug substance was observed for thermal degradation and photolytic degradation, while considerable degradation observed during acid - base hydrolysis and oxidative stress. The chromatographic method was fine tuned using the samples generated from forced degradation studies. Good resolution between the peaks corresponds to degradation products and the analyte was achieved on a Lichrospher 100 RP-180, C-18 column (250 mm, 4.0 mm id, 5 mcm). The mobile phase consists of a mixture of aqueous sodium dihydrogen phosphate (pH 2.86 ± 0.1) and acetonitrile (50:50 v/v). The stress sample solutions were assayed against the qualified reference standard of atomoxetine hydrochloride indicating that the developed method is stability indicating. Validation of the developed method was carried out as per ICH requirements.

Key words: Forced degradation, atomoxetine hydrochloride, RP-HPLC, method   development, validation.

INTRODUCTION

Atomoxetine hydrochloride is described chemically as: (-)-N-Methyl-3-phenyl-3-(o-tolyloxy)-propylamine hydrochloride [1]. It is used in the treatment of depression. Its empirical formula is $C_{17}H_{21}NO \cdot HCl$ and its molecular weight is 291.82. Atomoxetine hydrochloride is the first non stimulant drug approved by the United States Food and Drug Administration (FDA) for the treatment of attention-deficit-hyperactivity disorder (ADHD), and the only agent approved by the FDA for the treatment of ADHD in adults. Atomoxetine hydrochloride is a norepinephrine transport inhibitor that acts almost exclusively on the noradrenergic pathway. Its mechanism of action in the control and maintenance of ADHD symptoms is thought to be through the highly specific presynaptic inhibition of norepinephrine [2].

A liquid chromatography– tandem mass spectrometry (LC/MS/MS) method for the simultaneous quantification of atomoxetine hydrochloride as well as its primary oxidative and O-glucuronide metabolites in human plasma and urine was reported in the literature [3]. Determination of the enantiomer and positional isomer impurities in atomoxetine hydrochloride with liquid chromatography using polysaccharide chiral stationary phases [4] and sensitive quantification of atomoxetine hydrochloride in human plasma by HPLC with fluorescence detection using 4-(4, 5-diphenyl-1H-imidazole-2-yl) benzoyl chloride derivatization [5] were also reported. As on date no stability indicating liquid chromatographic method was reported in the literature, attempts were made to develop a suitable single stability indicating HPLC method that can be

used to determine the presence of degraded products and also the assay of bulk samples of atomoxetine hydrochloride. This paper deals with the development of stability indicating analytical method using the samples generated from forced degradation studies. The developed method was validated to ensure the compliance in accordance with ICH guidelines [6]

MATERIALS AND METHODS

Apparatus
The LC system, used for method development, forced degradation studies and method validation was a Merck - Hitachi Isocratic High Performance Liquid Chromatography instrument equipped with a Hitachi L - 7420 UV - Visible detector, Rheodyne universal injector 77251 with injection volume 20 mcL and Lichrospher 100 RP-180, C- 18 column (250 mm, 4.0 mm id, 5 mcm particle size).

Reagents and materials
Sample of atomoxetine hydrochloride was received as a gift sample from Intas Pharmaceutical Limited, Ahmedabad, India. HPLC grade acetonitrile and water were procured from Rankem, India. Analytical grade sodium dihydrogen phosphate was procured from S.D. Fine chem. limited, Mumbai, India and ortho phosphoric acid was purchased from Qualigens Fine chemicals, India.

Chromatographic conditions
The chromatographic column used was Lichrospher 100 RP-180, C- 18 column (250 mm, 4.0 mm id, 5 mcm particle size) column. The mobile phase consists of a mixture of 1.5 mM sodium dihydrogen phosphate, pH adjusted to 2.86 using 10 % ortho phosphoric acid and acetonitrile in the ratio of (50:50 v/v). The flow rate of the mobile phase was kept at 1.0 mL/min. The column was maintained at ambient temperature and the wavelength was monitored at a 215 nm. The injection volume was 20 mcL for determination.

Preparation of solutions
Preparation of standard working solution
Accurately weighed atomoxetine hydrochloride (10 mg) was transferred to 100 mL volumetric flask and dissolved in and diluted up to the mark with mobile phase to obtain a standard solution of atomoxetine hydrochloride (100 mcg/mL).

Preparation of sample solution
Powder of twenty tablets was weighed and analyzed as follows. A mass of powder equivalent to one tablet was weighed and transferred to a 100 mL volumetric flask, and mobile phase (40 mL) was added. The suspension was sonicated for 15 min, and the final volume was made up to the mark with mobile phase to obtain solution of atomoxetine hydrochloride (100 mcg/mL). The solution was then filtered through a nylon 0.20 mcm-47 mm membrane filter.

Method validation
Specificity
Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products[6]. The specificity of the developed HPLC method for atomoxetine hydrochloride was carried out in the presence of its degradation products by forced degradation studies for bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of photolytic degradation (as per ICH recommended condition), thermal degradation (drug substance exposed at 60°), acid hydrolysis (using 0.5 N HCl), base hydrolysis (using 0.5 N NaOH) and oxidative degradation (using 3.0 % v/v H_2O_2) to evaluate the ability of the proposed method to separate atomoxetine hydrochloride from its degradation products. Study period was 24 h for acid, base, oxidative, thermal and light degradation. To check and ensure the homogeneity and purity of atomoxetine hydrochloride peak in the stressed sample solutions, diode array detector was employed.

Calibration curve (linearity of HPLC method)
Calibration curves were constructed by plotting peak areas vs concentrations of atomoxetine hydrochloride and the regression equations were calculated. The calibration curves were plotted over the concentration range 1-100 mcg/mL. Accurately measured standard working solution of atomoxetine hydrochloride (0.1, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 mL) were transferred to a series of 10 mL volumetric flasks and diluted up to the mark with mobile phase. Aliquots (20 mcL) of each solution were injected under the operating chromatographic conditions as described above.

Linearity
Linearity test was performed for two consecutive days in the same concentration range for the assay method. The % RSD value of the slope and intercept of the calibration curve was calculated.

Accuracy (% Recovery)
The accuracy of the atomoxetine hydrochloride was determined by the standard addition method. Known amounts of standard solutions of atomoxetine hydrochloride (15, 30 and 60 mcg/mL) were added to prequantified sample solutions of tablet dosage forms. The amount of atomoxetine hydrochloride was calculated from the slope and intercept of the calibration curve.

Method precision (Repeatability)
The precision of the instrument was checked by repeatedly injecting (n=6) standard solutions of atomoxetine hydrochloride (50 mcg/mL).

Intermediate precision (Reproducibility)
The intraday and interday precisions of the proposed method were determined by estimating the corresponding responses 3 times on the same day and 3 different days over a period of 1 week for 3 different concentrations of atomoxetine hydrochloride (30, 40 and 50 mcg/mL). The results are reported in terms of relative standard deviation.

Limit of detection (LOD) and limit of quantification (LOQ)
LOD and LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines [7].
LOD = 3.3 × σ/S
LOQ= 10 × σ/S
Where σ = the standard deviation of the response and
S = the slope of the regression equation.

Robustness
To determine the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between atomoxetine hydrochloride and peaks of impurity after degradation was evaluated. The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on the resolution, the same was altered by 0.2 units, i.e. from 0.8 to 1.2 mL/min. The effect of pH on resolution of atomoxetine hydrochloride and degraded products was studied by varying ± 0.1 pH units (at 2.76 and 2.96 buffer pH). All the other mobile phase components were held constant as stated above.

Analysis of atomoxetine hydrochloride in tablet dosage forms
Tablets containing atomoxetine hydrochloride 10 mg was procured from local market (Axepta 10, Intas Pharmaceutical Ltd., Ahmedabad, India). The response of tablets dosage forms were measured at 215 nm for quantification of atomoxetine hydrochloride by using HPLC as described above. The amounts of atomoxetine hydrochloride present in sample solution were determined by fitting the responses into the regression equations for atomoxetine hydrochloride.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for atomoxetine hydrochloride was obtained with a mobile phase consisting of 1.5 mM, 2.86 pH sodium dihydrogen phosphate buffer-acetonitrile (50:50, v/v) final pH adjusted with 10 % ortho phosphoric acid to get better reproducibility and repeatability. Quantification was achieved with UV by measuring absorption at 215 nm based on peak area (Fig. I) (Table I).

Results of forced degradation studies

No considerable degradation observed in atomoxetine hydrochloride bulk drug, under stress conditions such as photolytic (UV light) and thermal stress (60°) up to 24 h. Significant degradation observed in acid (0.5 N HCl), base (0.5 N NaOH) hydrolysis and oxidative (3.0 % H_2O_2) degradation when it was treated for 24 h. This showed that all the peaks of degraded product were well resolved from the active pharmaceutical ingredient, which confirms the stability indicating power of the developed method (Fig. II, III, IV, V and VI) (Table II).

Results of method validation experiments

Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 1–100 mcg/mL and the correlation coefficient obtained was 0.9997. Linearity was checked for assay method over the same concentration range for three consecutive days by the high value of correlation coefficients of regression. The % RSD values of the slope and intercept of the calibration curves were 1.03 and 1.26, respectively. The results show that an excellent correlation existed between the peak area and concentration of the analyte (Table III).

Accuracy

The recovery experiments were carried out by standard addition method. The recovery obtained was 101.66 ± 0.26 % for atomoxetine hydrochloride. The high values indicate that method is accurate (Table IV).

Method precision

The RSD values for atomoxetine hydrochloride were found to be 0.23 %, the low RSD values indicate that the proposed method is repeatable.

Intermediate precision

The low RSD values of intraday and interday for atomoxetine hydrochloride (0.06-0.19 % and 0.04-0.06 %) reveal that the proposed method is robust.

LOD and LOQ

LOD and LOQ for atomoxetine hydrochloride were found to be 0.30 mcg/mL and 0.90 mcg/mL, respectively. These data showed that the method is sensitive for the determination of atomoxetine hydrochloride.

Robustness

In all the deliberate varied chromatographic conditions (flow Rate and pH) the resolution between atomoxetine hydrochloride and peaks of degraded products was greater than 2.0, illustrating the robustness of the method.

Assay of tablet dosage form (atomoxetine hydrochloride 10 mg per tablet)

The proposed validated method was successfully applied to determine atomoxetine hydrochloride in their marketed formulation. The results obtained for atomoxetine hydrochloride were comparable with the corresponding labeled amounts (Table V).

CONCLUSIONS

In this paper, the simple, accurate and well-defined stability indicating HPLC method was described for the determination of atomoxetine hydrochloride first time. The behavior of atomoxetine hydrochloride under various stress conditions were studied and presented. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies.

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Table I. System suitability test parameters.

Parameter, ([a]n=3)	Atomoxetine hydrochloride ± % RSD[b]
Retention time, min	4.13 ± 0.01
USP Tailing factor	1.18 ± 0.03
No of Theoretical plates(N), USP tangent method	4875

[a]n = No of determination

[b]RSD = Relative standard deviation.

Table II. Summary of forced degradation results.

Stress condition	Time	% of drug remained after
Acid hydrolysis (0.5 N HCl)	24 h at RT[a]	73.29
Base hydrolysis (0.5 N NaOH)	24 h at RT	69.73
Oxidation (3 % v/v H_2O_2)	24 h at RT	79.70
Photolytic degradation	24 h in UV light	99.53
Thermal degradation	24 h at 60°	99.37

[a]RT = Room Temperature

Table III. Regression analysis of the calibration curves of

atomoxetine hydrochloride.

Parameter	Atomoxetine hydrochloride
Concentration range	1–100 mcg/mL
Slope	52919
Standard deviation of the slope	1.03
Intercept	124451
Standard deviation of the intercept	1.26
Correlation coefficient	0.9997

TableIV. Summary of validation parameters.

Parameter	Atomoxetine hydrochloride
LOD[a]	0.30 mcg/ mL
LOQ[b]	0.90 mcg/mL
Accuracy (%)	101.37–101.87
Repeatability (RSD, %, n = 6)	0.23
Precision (RSD, %)	
Interday (n = 3)	0.04-0.06
Intraday (n = 3)	0.06-0.19

[a]LOD = Limit of detection.

[b]LOQ = Limit of quantification.

Table V. Recovery and assay results of atomoxetine hydrochloride in marketed formulation.

Label Claim mg per tablet	Amount Taken (mcg/mL) (n=3)	Amount added (mcg/mL)	Recovered (mcg/mL)	% Recovery	% R.S.D	% Assay
Atomoxetine	30	15	45.62	101.37	0.117	
hydrochloride	30	30	61.65	101.75	0.324	100.61±0.51
10 mg	30	60	93.69	101.87	0.045	
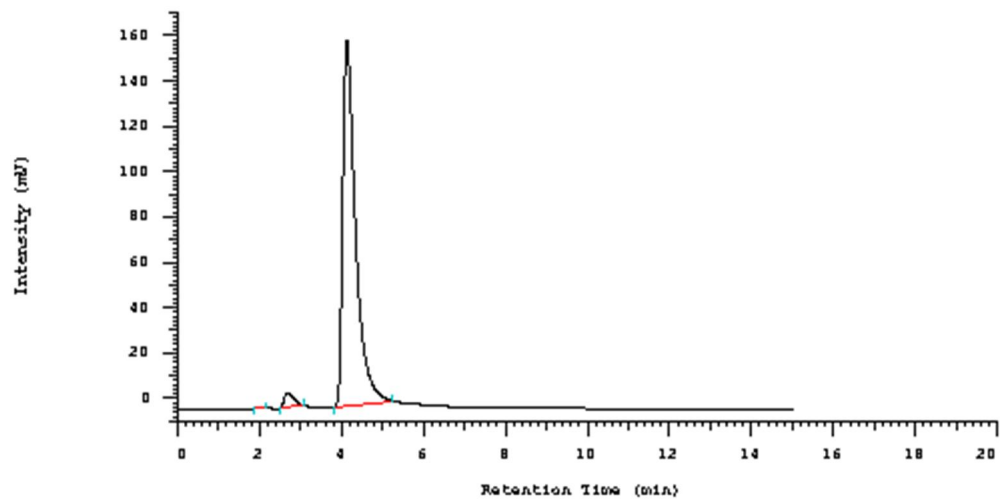
Fig. 1: HPLC chromatogram of atomoxetine hydrochloride by measuring at 215 nm.
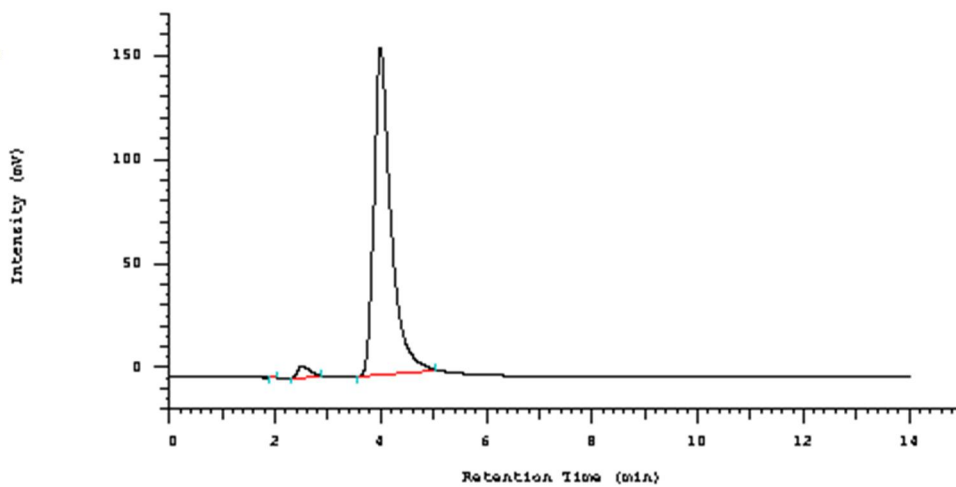
Fig. 2: HPLC chromatogram of atomoxetine hydrochloride in UV light for 24 h.
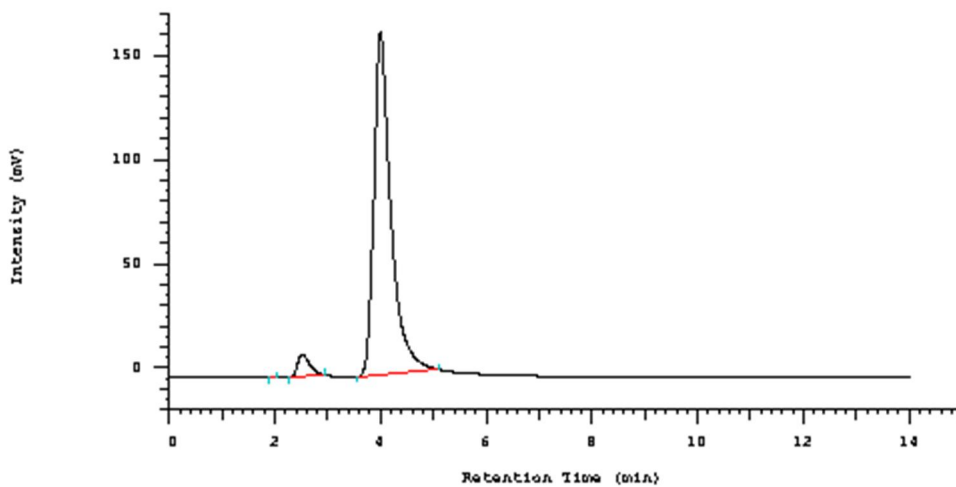
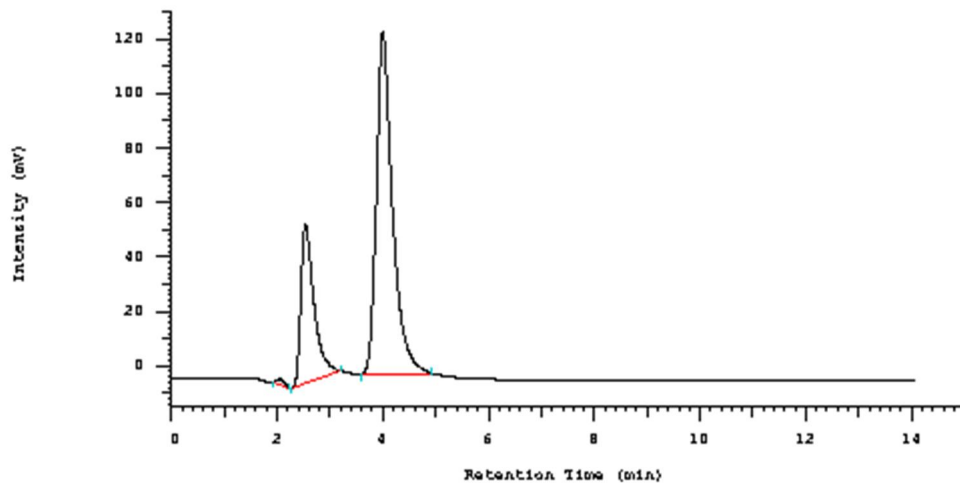**Fig. 3: HPLC chromatogram of atomoxetine hydrochloride at 60° for 24 h.**

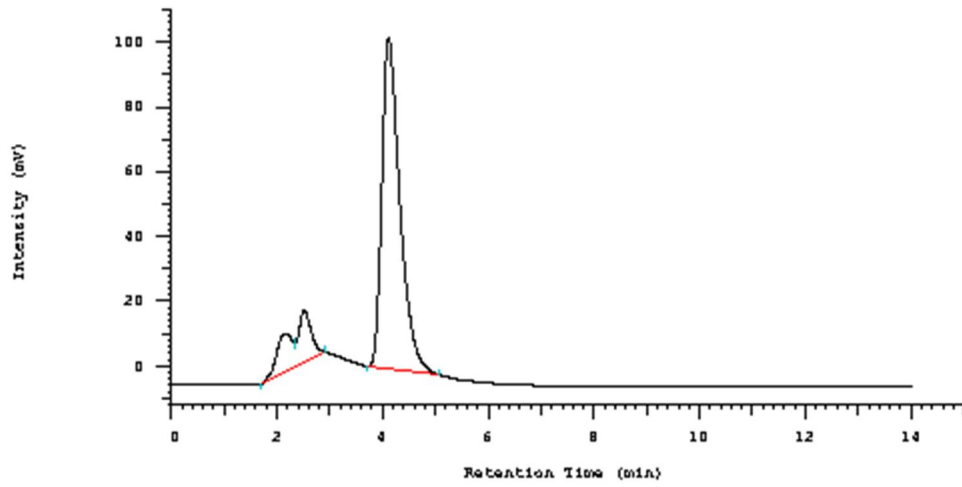**Fig. 4: HPLC chromatogram of atomoxetine hydrochloride in 0.5 N HCl for 24 h.**

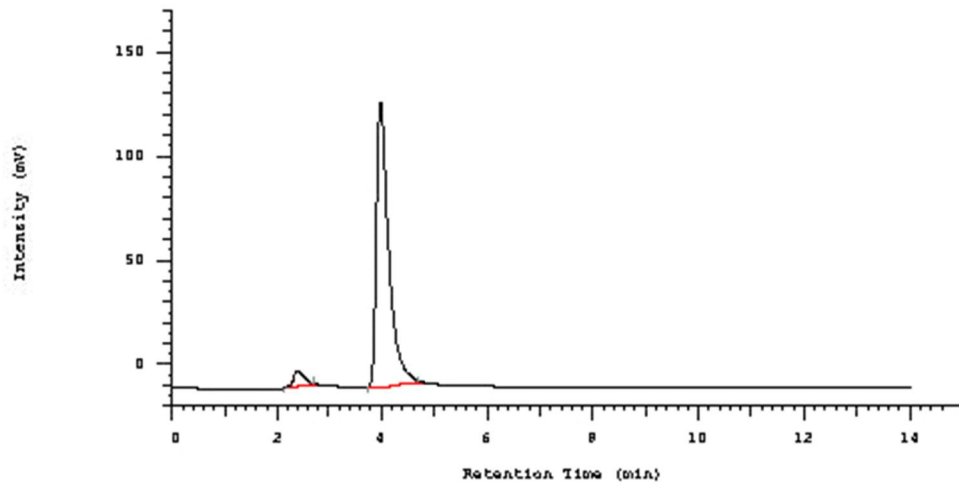**Fig. 5: HPLC chromatogram of atomoxetine hydrochloride in 0.5 N NaOH for 24 h.**

Fig. 6: HPLC chromatogram of atomoxetine hydrochloride in 3 % H$_2$O$_2$ for 24 h