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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF ATOMOXETINE HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive, accurate, precise, reproducible and cost effective stability indicating UV spectrophotometric method has been developed for quantitative determination of Atomoxetine hydrochloride in bulk and pharmaceutical formulations. The UV spectrum was scanned between 200 to 400 nm and 270.5 nm was selected as maximum wavelength for absorption. Beer's law was obeyed in the concentration range of 10-80 □g/ml. Good accuracy (100.18-100.26%), precision (%RSD 0.997-0.995) were found, the method was successfully applied to the pharmaceutical dosage form containing the above-mentioned drug without any interference by the excipients. Results of the analysis were validated as per ICH guidelines. Forced degradation studies includes the effect of temperature, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH values, were carried out according to the ICH requirements which can be used for the routine and quality control analysis of Atomoxetine hydrochloride in raw material and pharmaceutical formulations.

KEYWORDS

Atomoxetine hydrochloride, Spectrometry, Stability Indicating and Validation.

INTRODUCTION

During the pharmaceutical development of a new drug, it is necessary to select as soon as possible the formulation with the best stability characteristics. Regulations regarding stability testing for registration application are provided by current International Commission for Harmonization (ICH), which emphasizes the stress testing conditions with the aim of assessing the effect of severe conditions on the drug in practice, the effects of pH and temperature changes on drug stability are often used in such studies. The results of such studies are of vital importance in the estimation of a drug product shelf life during early stages of its pharmaceutical development. The results may also serve as guides for better drug design, drug formulation and drug analysis[1].

Atomoxetine, a new therapeutic drug chemically known as (−)-N-methyl-3-phenyl-3-(o-tolyloxy)-propylamine hydrochloride was approved by the United States FDA in 2003 to treat attention deficit/hyperactivity disorder (ADHD)[1]. Figure 1 shows that structure of Atomoxetine hydrochloride.

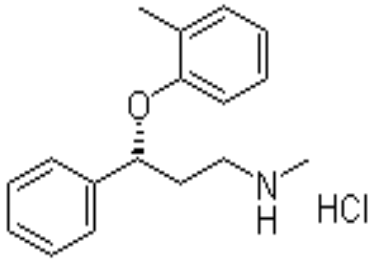

Figure 1
Chemical structure of Atomoxetine hydrochloride

Literature survey reveals that, few gas chromatographic[2], LC–MS[3], HPLC-FL[4], and HPLC-UV[5] methods have been reported for the estimation Atomoxetine hydrochloride. The GC method reported by[2], for the quantification of atomoxetine in human plasma and urine used liquid–liquid extraction (LLE) and electron-capture detection with a structural analogue as internal standard. Recently[3], reported a liquid chromatography/tandem mass spectrometry (LC–MS/MS) method with atmospheric pressure chemical ionization (APCI) for the determination of atomoxetine and its metabolites (4-hydroxyatomoxetine, N-des-methylatomoxetine, and 4-hydroxyatomoxetine-O-glucuronide) in human plasma and urine, with a stable isotope labeled atomoxetine or/and 4- hydroxyatomoxetine as internal standard.

So far, no UV assay procedure has been reported for the determination of this drug in its pharmaceutical formulations. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost.

In the present study a simple, sensitive, selective, sensitive, inexpensive, accurate and reproducible analytical method with better detection range for estimation of Atomoxetine hydrochloride in pure form and in its pharmaceutical dosage forms was developed and validated. Based on forced degradation studies, the method was also tested for its stability indicating ability according to the ICH requirements which can be used for the routine and quality control analysis of Atomoxetine

hydrochloride in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

Atomoxetine was obtained as a gift sample from Hetero Drugs Hyderabad. All solvents and other chemicals used were of analytical reagent grade purchased from Research lab, Mumbai. A Shimadzu UV/VIS double beam spectrophotometer (model 1700) with 1 cm matched quartz cells was used for all spectral measurements. Double distilled water used throughout the experiment.

(i) ***Preparation of standard stock solution***: 10 mg of Atomoxetine hydrochloride was accurately weighed and transferred to 100 ml volumetric flask and dissolved in about 20 ml of distilled water. The volume was made up to the mark with distilled water to give 100µg/ml stock solution.

(ii) Preparation of calibration curve for Atomoxetine hydrochloride:

By scanning a suitable standard solution in the UV-VIS spectrophotometer in the wavelength range of 200-400 nm, the λ max of the drug was determined, shown in figure 2. Aliquots (1, 2, …. 8 ml) from standard solution of Atomoxetine hydrochloride were pipetted out in to a series of eight volumetric flasks and the volume was made upto 10 ml with double distilled water. The absorbance was measured at 270.5 nm against reagent blank. The calibration curve was constructed by plotting absorbance v/s concentration (µg/ ml). Correlation coefficient was also measured. The summary of analytical parameters and calibration curve data are presented in Table 1 and Table 2 respectively.

Table 1
Optical characteristics of the proposed method

Parameters	Result
Measured wavelength (λ max)	270.5 nm
Beers law limit (µg/ml)	10-80
Regression equation (y = m x + c)	Y=0.005x+0.002
Slope	0.0056
Intercept	0.0025
Correlation coefficient (r)	0.9997
LOD µg/ml	0.21
LOQ µg/ml	0.86

Table 2
Calibration curve data for Atomoxetine hydrochloride

Sr. No.	Conc. (µg/ml)	Absorbance
1	10	0.060
2	20	0.118
3	30	0.172
4	40	0.209
5	50	0.274
6	60	0.330
7	70	0.385
8	80	0.448

(iii) Estimation of Atomoxetine hydrochloride in tablet dosage form:

Twenty Axepta ® tablets of 100 mg were weighed, combined and thoroughly crushed. An amount of tablet powder equivalent to average weight of one tablet of was accurately weighed and transferred to a 100 ml volumetric flask, to this 30 ml double distilled water was added. The content of the flask was sonicated for 15 min and the volume was made up to mark with the same solvent and filter through Whatmann filter paper No. 42. Appropriate solutions were prepared by taking suitable aliquots and diluting them with double distilled water to give final concentration (70 µg/ml). Then the absorbance of these solutions was measured at 270.5 nm against blank.

(iv) Method validation:

The method was validated according to ICH Q2B guidelines to determine the Linearity, sensitivity, precision, and accuracy of the analyte[6,7,8]. Linearity of the proposed method was determined by measuring the absorbance of the standard solutions in the concentration range of 10-80 µg/ml and performing least square regression analysis. In addition, the accuracy of the proposed method was checked using standard addition method and recovery studies were carried out at 80%, 100% and 120% of target concentration[9]. The percent analytical recovery was calculated by comparing the concentration resulted with the addition of spiked samples with actual expected theoretical increase in concentration. Intra-day precision was determined by carrying out the analysis for six concentrations at two different time interval in a day. Similarly inter-day precision was determined by performing analysis on two consecutive days. LOD and LOQ of the proposed methods were calculated[10]. Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method[11].

(v) Stability studies of Atomoxetine hydrochloride:

Stability studies were performed by forced degradation study of Atomoxetine hydrochloride and it includes the study of effect of temperature, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH values. For acidic hydrolysis 0.1, 1.0 N HCl, for basic hydrolysis 0.1, 1 N NaOH, for oxidation study 0.1%, 1% and 3% H_2O_2 was used. For carrying out photolysis studies the drug was treated with sunlight for 3 days and thermal stress was applied by heating the drug at 60^0C for 2 hrs.

RESULTS AND DISCUSSION

The development of a simple, economic, rapid, sensitive, and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labor. The absorption spectrum of Atomoxetine hydrochloride in double distilled water is shown in Figure 2.

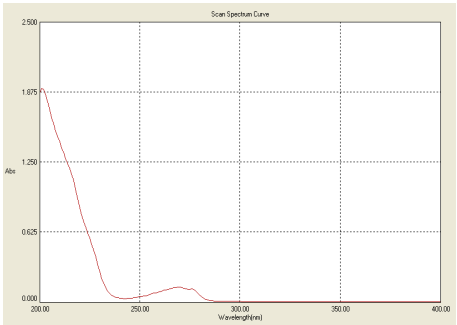

Figure 2
UV spectrum of Atomoxetine Hydrochloride

The λ max of the drug for analysis was determined (270.5 nm) by taking scans of the drug sample solutions in the entire UV region. Calibration curve data was constructed in the range of the expected concentrations of 10 to 80 µg/ml. Beer's law was obeyed over this concentration range (figure 3).

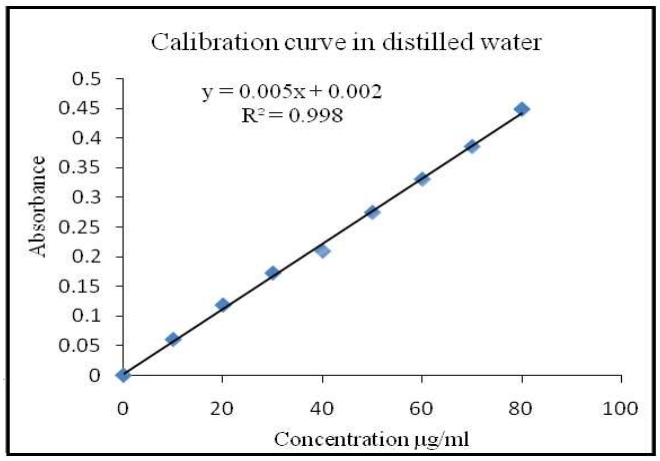

Figure 3
Calibration curve of Atomoxetine Hydrochloride at 270.5 nm

The regression equation was found to be Y=0.005x+0.002. The correlation coefficient (r) of the standard curve was found to be 0.9997. The characteristic of the calibration plot is presented in Table 1. Performing replicate analyses of the standard solutions was used to assess the accuracy and precision of the proposed methods (Table 3 and 4). The LOD and LOQ were found to be 0.21µg/ml and 0.86 µg/ml respectively.

To study the accuracy of the proposed method and to check the interference from excipients used in dosage forms, recovery experiments were carried out by the standard addition method. The mean recovery was found to be 100.18-100.26. The proposed methods can be successfully applied for assay in tablet dosage forms without any interference (Table 3).

Table 3
Result of recovery studies

Level of % recovery	% Mean* recovery	S.D	% RSD	SE
	Atomoxetine hydrochloride			
80	100.18	0.6200	0.6188	0.3580
100	100.18	0.520	0.5199	0.3005
120	100.26	0.4701	0.468	0.2714

**** Mean of three determinations at each***

The selected concentration within the calibration range was prepared in double distilled water and analyzed with the relevant calibration curves to determine the intra- and inter-day variability.

To determine the precision of the method Atomoxetine solutions at concentration 20, 50, 80 µg/ml were analyzed each in triplicate. Solutions for the standard curves were prepared fresh everyday. The method was

found to be precise. The % RSD values for interday precision at concentration 20, 50, 80 µg/ml was found to be 0.997, 0.014, 0.995 respectively and for intraday precision it was 0.471, 0.342, 0.133 respectively. Results are shown in table 4.

Table No. 4
Statistical validation for interday and intraday precision

Parameters	Concentrations (µg/ml)		
	20	50	80
Intraday*			
% mean±S.D	2.06±0.208	50.22±0.602	79.77±0.769
%RSD	0.997	0.014	0.995
SE	0.0352	0.04057	0.04376
Interday*			
% mean±S.D	20.08 ± 0.32	50.77±0.16	80.47±0.119
%RSD	0.471	0.342	0.133
SE	0.02997	0.04565	0.04241

Denotes average of three determinations, SE- Standard error

The application of this procedure is explained in the experimental section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of Atomoxetine hydrochloride in tablets. The stability studies indicates that appreciable changes were observed by treating the drug with sun light, thermal stress, oxidation, acid and basic hydrolysis, however there was appreciable change with all these stress conditions. The results are shown in Table 5

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Table 5
Result of forced degradation study of Atomoxetine hydrochloride

Sr. No.	Conditions applied	Conc. taken(µg/ml)	Average Conc. Found (µg/ml)	Observation
1	Acidic hydrolysis (0.1, 1 N HCl)	50 µg/ml	74.37 µg/ml	Degraded
2	Basic hydrolysis (0.1, 1 N NaOH)	50 µg/ml	93.33 µg/ml	Degraded
3	H_2O_2 (0.1, 1,3%)	50 µg/ml	Change in λ max	Degraded
4	Thermal stress (60^0 C, 2 hrs)	50 µg/ml	Change in λ max	Degraded
5	Sunlight treatment (1,2,3 day)	50 µg/ml	38.35 µg/ml	Degraded

CONCLUSION

These results reveal that the developed method was simple, sensitive, sensitive, inexpensive, accurate and reproducible and consequently, can be applied to the determination of Atomoxetine hydrochloride tablet in pharmaceuticals without any interference from the excipients. Based on forced degradation studies according to the ICH requirements, this method can be used for the routine and quality control analysis of

Atomoxetine hydrochloride in raw material and pharmaceutical formulations.

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