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A SIMPLE ASSAY METHOD DEVELOPMENT AND VALIDATION OF ATOMOXETINE HYDROCHLORIDE IN TABLETS BY UV SPECTROPHOTOMETRY

D. Suresh Reddy, E. Yashaswini, A. Ashok Kumar*

Department of Pharmaceutical analysis and Quality Assurance, Vijaya College of Pharmacy, Munaganur (village), Hayathnagar (mandal), Hyderabad – 501511, India.

Abstract:

Objective: *To develop a simple and a cheap UV spectrophotometric method for the quantitative estimation of Atomoxetine Hydrochloride in capsules and validate as per ICH guidelines.*

Method: *The optimized method uses 0.05N HCl as a solvent for the estimation of assay of Atomoxetine Hydrochloride in tablets at a wavelength of 225 nm.*

Results: *The developed method resulted in Atomoxetine Hydrochloride exhibiting linearity in the range 5-40µg/ml. System precision, intra day and inter day precisions were exemplified by relative standard deviation of 1.39%, 1.547% and 1.063%. Percentage Mean recovery by absolute method was found to be in the range of 90-110%, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) for Atomoxetine hydrochloride were found to be 154ng/ml and 467ng/ml respectively.*

Conclusion: *A simple and a cheap UV spectrophotometric method was developed and validated for the quantitative estimation of Atomoxetine Hydrochloride in tablets as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.*

Keywords: *UV, Atomoxetine Hydrochloride, method development, validation.*

Corresponding author:

A. Ashok Kumar,
Professor and HOD
Department of Pharmaceutical analysis and Quality Assurance,
Vijaya College of Pharmacy,
Munaganur (Village), Hayathnagar (Mandal),
Ranga reddy (District), Hyderabad,
Telangana (State) – 501511, India.
Email: ashok576@gmail.com

QR code

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INTRODUCTION:

IUPAC name of Atomoxetine hydrochloride (**Figure 1**) is (R)-N-methyl-3-(o-tolyloxy)-3-phenylpropyl amine hydrochloride. Empirical formula of Atomoxetine Hydrochloride is $C_{17}H_{21}NO.HCl$ having a molecular weight is 291.82. Atomoxetine hydrochloride is mainly used in treatment of attention-deficit hyperactivity disorder (ADHD) and it a selective noradrenaline (norepinephrine) reuptake inhibitor (NRI) [1-5].

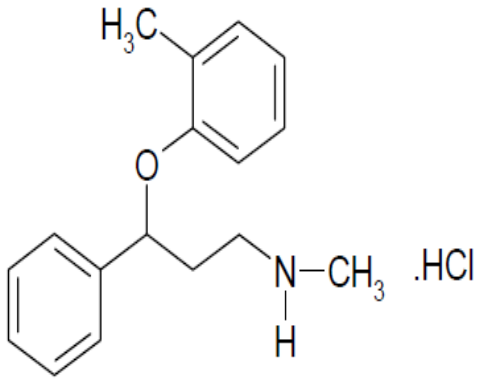

Fig. 1: Structure of Atomoxetine Hydrochloride

A number of HPLC methods are reported for the determination of assay of Atomoxetine hydrochloride in tablets. Few UV methods are reported wherein drugs were treated with various reagents such as brucine [1], folin's reagent [1], aromatic aldehydes such as vanillin and Paradimethylaminobenzaldehyde (PDAB) [4] and gold (III) chloride [5] to form complexes, which were assayed. We here report a simple, cheap and a sensitive assay method by UV spectroscopy where in there is no need to treat the drug with any reagents to form complex. The developed UV method was validated as per ICH guidelines.

MATERIALS AND METHODS:

Materials

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101) and a sonicator (sonica, model 2200 MH) were used in this study.

Chemicals and Reagents

Analytically pure sample of Atomoxetine Hydrochloride with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [AXEPTA] was procured from APOLLO pharmacy, Hyderabad, India with label claim of 10mg. Concentrated Hydrochloric acid was purchased from SD Fine chemicals (Hyderabad, India).

Method

Solvent

Preparation of 0.1N HCl: 8.33ml of Concentrated HCl was made up to 1000 ml using distilled water.

Preparation of 0.05N HCl: 4.66ml of Concentrated HCl was made up to 1000 ml using distilled water.

Selection of Suitable Detection Wavelength

Suitable wavelength for the total experiment was determined by recording UV spectrum of 20µg/ml concentration of Atomoxetine Hydrochloride solution in the range of 200-400 nm and λmax was found to be 225nm and accordingly considered this wavelength during the total analysis (**Figure 2**).

Preparation of Stock and Working Standard Solution

10mg of Atomoxetine Hydrochloride was accurately weighed and taken in 100ml clean and dry volumetric flask containing 80ml of solvent and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 2ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 20µg/ml, treated as working standard, 100% target concentration.

Preparation of Stock and Working Sample Solution

Not less than 10 tablets were taken and weighed individually. All the 10 tablets were taken and grinded in a pestle and mortar. Tablet powder weight equivalent to 10mg of Atomoxetine hydrochloride was transferred to 100ml volumetric flask containing 70ml of solvent which was sonicated for 5minutes with intermittent shaking and later made up to 100ml with solvent. This solution was filtered with 0.22µ filter by discarding first few ml of the filtrate. 2ml was pipetted out from the above solution and made up to 10ml with solvent to get working sample solution concentration equivalent to 20µg/ml, 100% target concentration as that of standard.

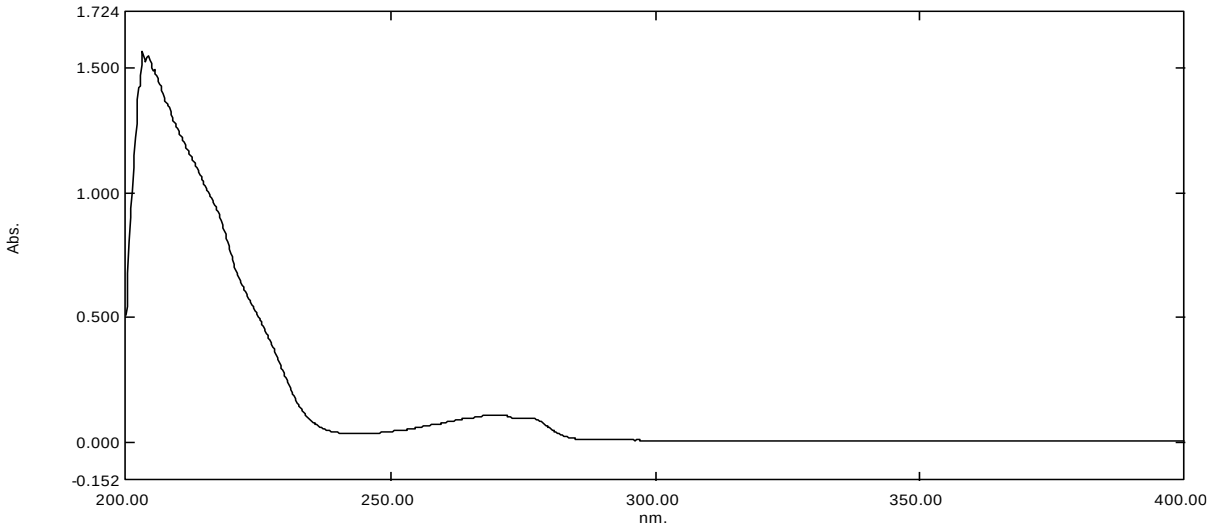

Fig. 2: UV spectrum of standard

RESULTS AND DISCUSSION:

Method Development

Various solvents were explored including water, Hydrochloric acid at 0.1N and 0.05N and sodium hydroxide at 0.1N and 0.05N. Atomoxetine Hydrochloride was found to be soluble and stable for minimum of 1 hour at room temperature using 0.1N and 0.05N and hence these solvent were initiated for the determination of suitable detection wavelength and working concentration of standard. In order to test the applicability of the method to a commercial formulation, assay of PRADAXA capsules were studied at working concentration at both concentrations of HCl. Assay at working concentration of sample at mwas in acceptance limits (90-110%) using 0.1N and 0.05N HCl via intermittent shaking and sonication method for 5minutes. As 0.05NHCl is cheaper than 0.1NHCl, 0.05NHCl was selected as solvent for the determination of assay of Atomoxetine Hydrochloride in tablets. Hence the method is optimized. **Figure 3** illustrates UV spectrum for the sample.

Method Validation

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. UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines [6] for validation of analytical procedures. The method was validated for the parameters like specificity, linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness and sensitivity.

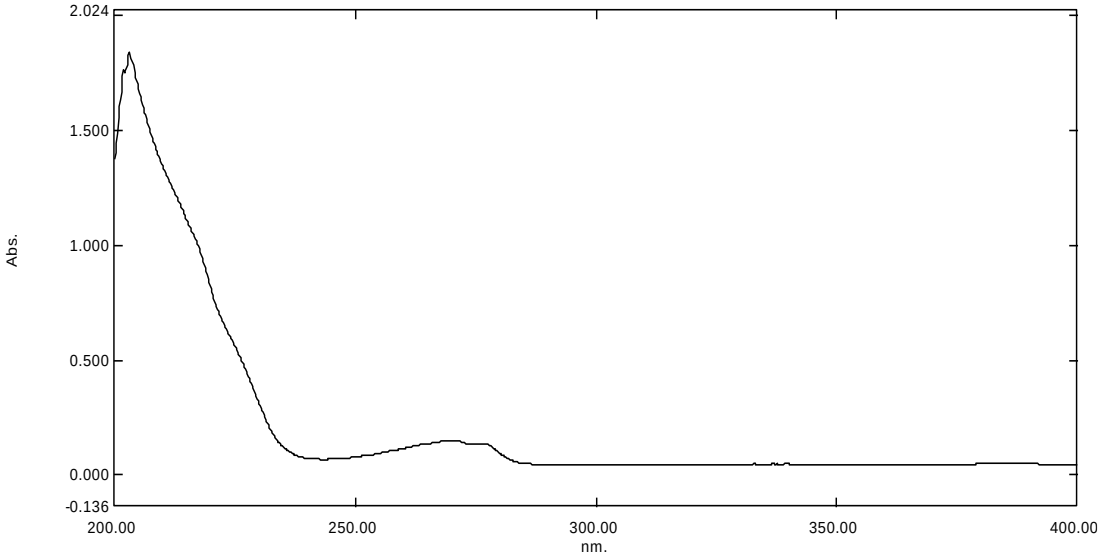

Fig. 3: UV spectrum of sample

Precision

System Precision

Six replicate recording of absorbance at 225nm of 100% working concentration of standard solution showed % RSD (Relative Standard Deviation) less than 2, which indicates acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in **Table 1**.

Method Precision

Method precision was determined by performing assay of sample under the tests of (i) repeatability (Intra day precision) and (ii) Intermediate precision (Inter day precision or ruggedness).

Table 1: System precision results

n	absorbance
1	0.474
2	0.466
3	0.481
4	0.483
5	0.48
6	0.471
average	0.476
St dev	0.0066
% RSD	1.39

Repeatability (Intra day Precision)

Six consecutive recording of absorbance at 225nm of 100% working concentration of the sample from the same homogeneous mixture were taken and %RSD was found to be than 2 concerning % assay, which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (**Table 2**).

Table 2: Intra day Precision Results

n	%Assay
1	106.57
2	110.9
3	109.5
4	109.76
5	106.97
6	108.97
average	108.77
St dev	1.683
% RSD	1.547

Intermediate Precision (Inter day Precision / Ruggedness)

Assay precision between two consecutive days performed by different analysts of the sample showed % RSD less than 2, which indicate the method developed is inter day precise / rugged (**Table 3)**.

Table 3: Inter Day Precision/ Ruggedness Results

n	Day 1 %Assay	Day 2 %Assay
1	106.57	99.45
2	110.9	100.94
3	109.5	99.2
4	109.76	98.7
5	106.97	99.2
6	108.97	97.71
average	108.77	99.2
St dev	1.683	1.054
% RSD	1.547	1.063

Linearity

Different concentrations (5-40µg/ml) of Atomoxetine Hydrochloride standard were prepared by serial dilutions from the stock solution 100µg/ml. Calibration curve **(Figure 4)** was constructed by plotting the concentration of drug versus absorbance at 225nm. The results show an excellent linear correlation between absorbance and concentration of drug within the concentration range (5-40µg/ml) for the drug (**Table 4).** The correlation coefficient was greater than 0.995, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 5-40µg/ml.

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample by percentage method at three different levels 50 to 150% of the sample solutions by absolute method. At each level, three determinations were performed. Percent mean recovery was calculated as shown in **Table 5.** The accepted limits of recovery are 90% - 110% by absolute method and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Sensitivity

Sensitivity of the method was determined by linearity data by the calculation of limit of detection) (LOD) and limit of quantitiation (LOQ). LOQ and LOD were calculated by the use of the equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of intercepts and S is the average of the slopes from the three different sets of linearity data generated. The limit of detection (LOD) and limit of quantitiation (LOQ) for Atomoxetine hydrochloride were found to be 154ng/ml and 467ng/ml respectively.

Specificity

Blank (0.05N HCl) had zero absorbance at all wavelengths from 200-400nm while standard solution exhibited UV spectrum, hence the method is said to be specific for the analyte of interest.

Table 4: Calibration Data for Atomoxetine Hydrochloride

%Level	Concentration (µg/ml)	Absorbance
25	5	0.121
50	10	0.217
75	15	0.334
100	20	0.431
125	25	0.542
150	30	0.672
175	35	0.788
200	40	0.885
Average		**0.49875**
Slope		**0.022209524**
Intercept		**-0.000964286**
Regression coefficient		**0.998793939**
Regression equation		**y=0.0222x-0.00096**

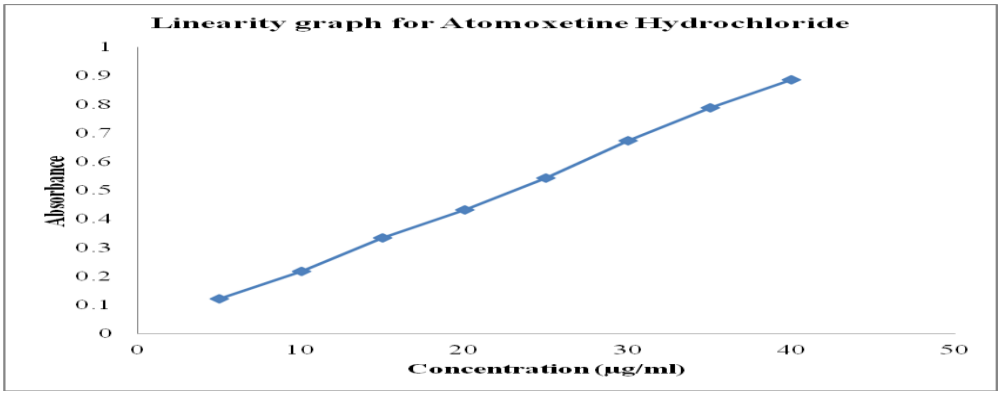

Fig.4: Linearity Graph of Atomoxetine Hydrochloride

Table 5: Accuracy Studies

% Level	absorbance	% Recovery	% Mean recovery	stdev	% RSD
50-1	0.28	95	**96.466**	1.5283	**1.5843**
50-2	0.284	96.35			
50-3	0.289	98.05			
100-1	0.556	110.966	**110.098**	0.7575	**0.6880**
100-2	0.549	109.569			
100-3	0.55	109.76			
150-1	0.914	106.64	**107.11**	1.38232	**1.2905**
150-2	0.926	108.84			
150-3	0.911	106.29			

CONCLUSION:

A simple and a cheap and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Atomoxetine Hydrochloride in tablets as per ICH guidelines using 0.05N HCl. The developed method resulted in Atomoxetine Hydrochloride exhibiting linearity in the range 5-40µg/ml. The developed method was found to be system, intra day, inter day precise and accurate. The limit of detection (LOD) and limit of quantitiation (LOQ) for Atomoxetine hydrochloride were found to be 154ng/ml and 467ng/ml respectively. Accordingly it is concluded that the developed UV spectrophotometric method is simple, specific, sensitive, accurate, precise, linear and rugged and therefore the method can be used for the routine analysis of Atomoxetine Hydrochloride in tablets in various pharmaceutical industries.

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