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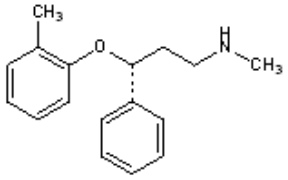
Method development and validation for estimation of Atomoxetine HCL by using UV-spectroscopy in bulk and tablet dosage form

Priyanka T
Department of Pharmaceutical Analysis & QA, RBVRR Women's College of Pharmacy, Osmania University, Hyderabad, Telangana, India

Abstract
A simple, rapid and sensitive UV Spectroscopy method was developed and validated for estimation of Atomoxetine HCl in tablet dosage form. The spectrophotometric method was carried out at maximum absorbance of 271nm for Atomoxetine HCl using acetonitrile as a solvent. Calibration curve was plotted with a range of 20-140µg/ml for Atomoxetine HCl and correlation was found to be 0.999.The limit od detection and limit of Quantification were found to 3.5µg/ml , 10.62µg/ml .The percentage values for all the parameters was found to be not more than 2. The developed method can be used for routine determination of Atomoxetine HCl in pharmaceutical dosage form and method is developed and validated as per ICH guidelines.

Keywords: atomoxetine HCl, UV-spectroscopy, ICH guidelines

1. Introduction
Atomoxetine HCl is an anti-depressant drug, it is official in Indian Pharmacopoeia& it is a selective nor-adrenaline reuptake inhibitors. Atomoxetine is the only the salt form from its classification & it is used for the treatment of attention deficit/hyperactivity disorder (ADHD). Atomoxetine is Chemically (R)-N-methyl-(O-tolyoxy)-propylamine hydrochloride and its empirical formulas of atomoxetine HCl is $C_{17}H_{21}NO$. HCL having a molecular weight of 291.82.



Literature survey reveals few chromatographic methods have been reported i.e., UV-spectrophotometric, HPLC, HTPLC, stability studies, visible methods. The present method describes a new quantitative UV method. The purpose of developing and validating a method using a simple, rapid, sensitive, precise, accurate and specific UV method. [1, 7].

2. Materials and Materials
2.1 Chemicals and Reagents
Pure drug sample of Atomoxetine HCl was obtained from Tokyo chemical industry co., LTD. And tablet formulation (AXEPTA) was purchased from Shabbir medical hall, Hyderabad, India, with label claim of 25mg.
Acetonitrile was procured from SD-Fine Chem-Limited

2.2 Instrumentation
The analysis was performed by using UV-Visible spectrophotometry instrument i.e., Elico 210 with spectra treats software, analytical balance (Contech) are used for weighing, pH (Elico), Sonicator (labotech) was used for degassing the mobile phase. And pipettes, beakers, volumetric flask were obtained from Borosil type apparatus.

2.3 Preparation of Atomoxetine HCl Standard Stock Solution
Accurately weigh and transfer 100mg of atomoxetine HCl into 100ml volumetric flask, to this add 50ml of diluent and sonicate to dissolve. And then make up to the mark by using diluent (i.e., 1000µg/ml).

2.4 Preparation of Atomoxetine HCl primary stock solution
Pippete out 1ml from 1000µg/ml into 10ml volumetric flask and make up to the mark with diluent i.e., 100µg/ml.

2.5 Preparation of Secondary stock solution
Pipette out 1ml from 100 µg/ml into 10ml volumetric flask and diluted up to the mark with the same solvent.(10µg/ml)

2.6 Preparation of sample solution
Accurately weighed 10 tablets and average weight was calculated. accurately weighed and transferred the sample equivalent to 100mg of atomoxetine HCl into 100ml volumetric flask.To this add 50ml of diluent and sonicated to dissolve it completely and make up to the mark with diluent.(1000 µg/ml)

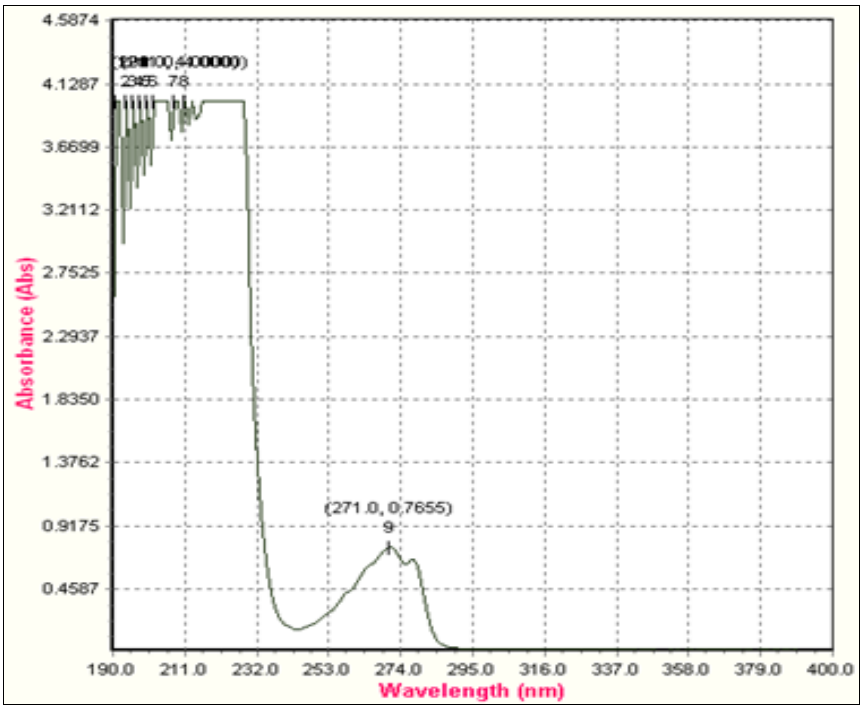
2.7 Preparation of Atomoxetine HCl primary stock sample solution
Pipette out 1ml from 1000 µg/ml into 10ml volumetric flask and diluted up to the mark with the same solvent.(100µg/ml)

2.7 Preparation of secondary stock solution

Pipette out 1ml from 100 µg/ml into 10ml volumetric flask and diluted up to the mark with the same solvent.(10µg/ml)

Selection of analytical wavelength

10µg/ml solution was scanned in the wavelength range of 200-400nm in order to observe maximum absorbance. The λ_{max} for Atomoxetine HCl is 271nm, since It shows maximum absorbance at that $\lambda_{max.}$



Method Validation [8, 9]

Both RP-HPLC and UV methods were developed and validated by using following parameters such as linearity, precision, accuracy, robustness, LOD, LOQ.

System Suitability

In HPLC, it is an integral part of method development used to ensure the performance of HPLC system. The parameters such as retention time (R_t), number of theoretical plates (N), and tailing factor (T) were evaluated for six Replicates injections at a concentration of 10µg/ml.

Linearity

Linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

Accuracy

It is the measure of the closeness of the experimental value is to the true value. Accuracy should be established across the specified range of the analytical procedure.

Intraday Precision

It expresses the precision under the same operating conditions over a short interval of time.

Inter Day Precision

It expresses the precision between laboratories variations, different days, different analysts, different equipment etc.

Limit of Detection

It is the lowest concentration of analyte in a sample which can be detected, but not necessarily quantified, as an exact value under the stated

Limit of Quantification

It is the lowest concentration of analyte in a sample which can be detected and quantified.

Robustness

It is a measure of the method capability to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage

3. Results and Discussion

Results of UV Method

Linearity

The linearity of uv method was developed by using concentration range of 20-140µg/ml at 271nm.the correlation coefficient was calculated statistically and the obtained results were given in Table-1.

- **Preparation of Std 20µg/ml**

1ml of standard solution were pipetted into a 5 Ml volumetric flask and diluted up to the mark with the mobile phase

- **Preparation of Std 30µg/ml**

1.5 ml each of standard solution were pipetted into a 5 Ml volumetric flask and diluted up to the mark with the mobile phase

- **Preparation of Std 60µg/ml**

3.0 ml each of standard solution were pipette into 5 Ml volumetric flask and diluted up to the mark with mobile phase

- **Preparation of Std 90µg/ml**

4.5 ml were pipetted from the above standard solution into a 5 mL volumetric flask and diluted up to the mark with diluent

- **Preparation of Std120µg/ml**

0.6ml each of standard stock solutions were pipetted from the above standard stock solutions into a 5 mL volumetric flask and diluted up to the mark with diluent

- **Preparation of Std 140µg/ml**

0.7ml were pipetted from the above standard stock solution into 5ml volumetric flask and diluted up to the mark with diluent

Table 1: Results of Linearity

Concentration(µg/ml)	Absorbance
20 µg/ml	0.2984
30 µg/ml	0.3652
60 µg/ml	0.4985
90 µg/ml	0.6459
120 µg/ml	0.8136
140 µg/ml	0.9072

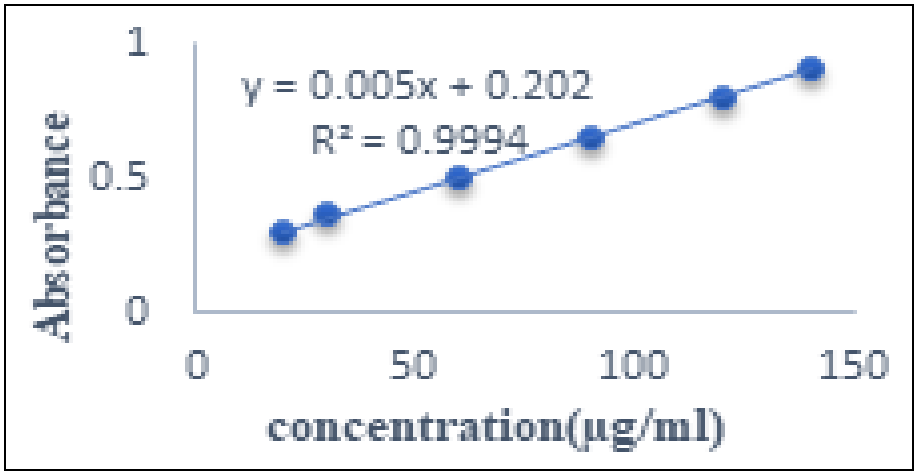


Fig 1: Linearity Graph of UV

Accuracy

The accuracy was developed by recovery studies which were carried out at three different spiked levels i.e., 80%, 100%, 120%.

Preparation of Std 100µg/ml:

Pipette 1ml of solution from stock solution(1000µg/ml) into 10ml volumetric flask and make up the volume up to the mark

Preparation of sample 100µg/ml:

Pipette 1ml of solution from stock solution(1000µg/ml) into 10ml volumetric flask and make up the volume up to the mark

- **Preparation of sample 45µg/ml solution :**

Pipette of 2.25ml from 100µg/ml and transfer to 5ml and diluted up to the mark with acetonitrile

- **Preparation of 36 µg/ml standard solution**

Pipette of 1.8 ml from 100µg/ml and transfer to 5ml and diluted up to the mark with acetonitrile

- **Preparation of 54µg/ml standard solution :**

Pipette of 2.7 ml from 100µg/ml and transfer to 5ml and diluted up to the mark with acetonitrile

- **Preparation of 80% solution (81µg/ml):**

Mix 5ml of 36µg/ml Std solution & 5ml of 45µg/ml Sample solution into 10ml volumetric flask. sonicate for 2 mins

- **Preparation of 100% solution (90µg/ml):**

Mix 5ml of 45µg/ml Std solution & 5ml of 45µg/ml Sample solution into 10ml volumetric flask. sonicate for 2 mins

- **Preparation of 120% solution (99µg/ml):**

Mix 5ml of 54µg/ml Std solution & 5ml of 45µg/ml Sample solution into 10ml volumetric flask. sonicate for 2 mins

Table 2: Results of Accuracy

Sample Id	Conc (µg/ml)		Total Conc	Area	% Recovery	Avg % Recovery	SD	%RSD
	Amount of pure drug	Amount of sample						
80%	36 µg/ml	45 µg/ml	81µg/ml	0.5812	99.6%	99.9%	0.00136	0.26%
80%	36 µg/ml	45 µg/ml		0.5845	100.1%			
80%	36 µg/ml	45 µg/ml		0.5232	99.9%			
100%	45 µg/ml	45 µg/ml	90µg/ml	0.6448	99.4%	99.5%	0.00172	0.19%
100%	45 µg/ml	45 µg/ml		0.6466	99.7%			
100%	45 µg/ml	45 µg/ml		0.6435	99.2%			
120%	54 µg/ml	45 µg/ml	99µg/ml	0.7125	99.9%	99.9%	0.00099	0.13%
120%	54 µg/ml	45 µg/ml		0.7112	99.7%			
120%	54 µg/ml	45 µg/ml		0.7145	100.2%			

Precision

Table 3: Results of intraday precision

CONC(µg/ml)	Absorbance	%Assay
90µg/ml	0.6532	100.2
90 µg/ml	0.6560	100.8
90 µg/ml	0.6550	100.6
90 µg/ml	0.6548	100.6
90 µg/ml	0.6558	100.8
90 µg/ml	0.6538	100.3
MEAN	0.6547	-
SD	0.001004	-
%RSD	0.15	-

Table 4: Results of Inter day precision

CONC(µg/ml)	Absorbance	%Assay
90µg/ml	0.6520	99.9
90 µg/ml	0.6535	100.3
90 µg/ml	0.6552	100.7
90 µg/ml	0.6528	100.1
90 µg/ml	0.6575	101.2
90 µg/ml	0.6566	101.02
MEAN	0.6546	-
SD	0.00199	-
%RSD	0.3	-

LOD & LOQ

Table 5: Results of LOD & LOQ

Parameters	Slope	Intercept	LOD	LOQ
1	0.005	0.202	(3.3) x SD OF INTERCEPT /MEAN OF SLOPE 3.3 x0.00531/0.005= 3.5 µg /ml	(10) x SD OF INTERCEPT /MEAN OF SLOPE 10 x0.00531/0.005 = 10.62 µg /ml
2	0.005	0.189		
3	0.005	0.197		
MEAN	0.005	0.196		
SD		0.00531		

Assay of marketed formulated

Table 6: Results of Assay

Drug	Concentration (µg/ml)	Amount found	%assay
Atomoxetine HCl (25mg)	90µg/ml	90.2mg	100.2%

Table 7: Summary of validation parameters

Parameters	UV-Spectroscopy
Linearity range	20-140µg/ml
Correlation coefficient	0.999
Detection wavelength	271nm
Accuracy	99.5%-99.9%
Precision Intraday precision 0.15% Inter day precision 0.3%	
%Assay	100.2%
LOD	3.5 µg /ml
LOQ	10.62 µg /ml

4. Conclusion

A simple and rapid UV spectrophotometric method was developed and validated for the Quantitative estimation of Atomoxetine hydrochloride in bulk and pharmaceutical dosage form as per ICH guidelines using acetonitrile as solvent.

The developed method resulted in Atomoxetine HCI exhibiting the linearity range of 20-140ug/ml.

It can be concluded that all the developed methods were a good approach for obtaining results and were found to be suitable for routine estimation of Atomoxetine HCl in pharmaceutical formulations.

It was proved that the method is selective, precise, linear and accurate.

5. Acknowledgement

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