International Journal of Pharmacy and Biological Sciences- ISSN: 2321-3272 (Print)
IJPBS | Volume 5 | Issue 4 | OCT-DEC | 2015 | 82-91
Research Article – Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF ATEMOXETINE HYDROCHLORIDE IN TABLET DOSAGE FORM

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ABSTRACT

A RP-HPLC method was developed for the estimation of Atomoxetine Hydrochloride in tablet formulation, which is novel to the market. Chromatographic separation of the drug was achieved on a ENABLE C-18 G, 5µm, column (250 × 4.6 mm) using a mobile phase, Methanol: Ammonium phosphate buffer pH-3 (80:20 V/V) at a flow rate of 1ml/min. The drug eluted was monitored at 227 nm. The retention time was 3.83min. The calibration curve was linear over the range of 1-700µg/ml. The performance of the method was validated according to ICH guidelines. The method can be applied for determination of drug in its tablet dosage form without any interference from excipients or degradant substances. The proposed method is suitable for routine quality control analysis.

KEY WORDS

RP-HPLC, Atemoxetine Hydrochoride, validation

INTRODUCTION

Atomoxetine Hydrochloride is chemically methyl [(3R)-3-(2-methylphenoxy)-3-phenylpropyl] amine hydrochloride. It is used as an antipsychotic agent. Atomoxetine is a selective inhibitor of the presynaptic norepinephrine transporter mechanism but its mode of action is not known. But is thought to be related to selective inhibition of the pre-synaptic norepinephrine transporter, as determined in exvivo uptake and neurotransmitter depletion studies.

There are few methods reported in literature for analysis of Atemoxetine Hydrochloride drug in the pure form by UV-Spectrophotometer, RP-HPLC, UPLC and HPTLC. But there was no RP-HPLC method reported till date for the estimation of Atemoxetine Hydrochloride in tablet dosage form. [1-7]

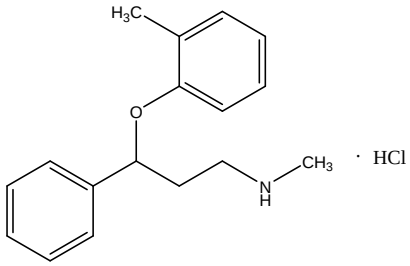

Figure 1: Structure of Atemoxetine Hydrochloride

MATERIALS AND METHODS

Chemicals and reagents:

An analytically pure sample of ATEMOXETINE HYDROCHLORIDE was procured as gifted sample from Aurobindo Pharma Ltd. (Hyderabad, India). Pharmaceutical tablet dosage form of Atemoxetine Hydrochloride (Axepta-40) was manufactured by Intas pharmaceutical Ltd. Label claim 40mg. Acetonitrile (HPLC grade), methanol (HPLC grade), Orthophosphoric acid, Sodium hydroxide (NaOH) and Hydrochloric acid (HCl), Ammonium dihydrogen phosphate, Potassium hydroxide (KOH) and Hydrogen peroxide (H_2O_2) were purchased from Merck (India). All chemicals were of analytical grade and used as received.

METHOD DEVELOPMENT AND OPTIMIZATION

Initial set of chromatographic conditions were defined based on analyte molecules. ENABLE C-18 G, 250mm × 4.6mm, 5µm, was selected because it has good separation capability. Mobile phase for RP-HPLC contains Methanol: Ammonium phosphate buffer pH-3 (80:20 V/V). The overall run time was 10 min. Flow rate of about 1ml/min was selected since it was taken as optimum flow rate for the selected column conditions. Detection wavelength of 227nm and injection volume of 20µl was selected to increase detection capability.

Preparation of the Mobile Phase and the Diluent

The Methanol: Ammonium phosphate buffer were mixed together in the ratio of 80:20 %v/v (pH was adjusted to 3), as the mobile phase. Methanol: Water (80:20 v/v) solution was used as the diluent for preparing drug solutions.

Preparation of Buffer: 0.5gm of Ammonium dihydrogen phosphate was accurately weighed, transferred in to a 100ml volumetric flask, dissolved by adding HPLC water and diluted to mark with water.

Preparation of standard solution:

Standard solution was prepared by transferring 1ml of standard stock solution in to 10 ml volumetric flask and diluted to volume with methanol-water and filtered through 0.45µm membrane filter to get a stock solution containing 100µg/ml of Atemoxetine HCl.

Preparation of test solution:

Twenty tablets were weighed accurately and crushed to form a fine powder. Accurately weighed a quantity of powder equivalent to 50 mg of Atemoxetine HCl, and was transferred in to a 50 ml of volumetric flask, 50ml of methanol was added. The flask was then sonicated for 5 minutes and then made up to the mark with methanol. This gives a stock solution of Atemoxetine HCl having concentration of 1000µg/ml. Stock solution was then filtered with whatmann filter paper and washed with methanol, after it was filtered through 0.45µ membrane filter. Further dilution was prepared by pipetting 1ml of above stock solution in to 10ml volumetric flask and diluted to volume with methanol-water. Then, this solution was filtered through a 0.45µ membrane filter and sonicated for 2min.This was marked as Test solution (100µg/ml).

Preparation of Blank solution:

In to 10 ml volumetric flask, 5 ml of methanol-water was added and sonicated for 5min. solution was then diluted to mark with methanol-water.

Analysis of formulation:

After setting the chromatographic conditions, the instrument was stabilized to obtain a steady baseline. Then, equal volumes of blank, standard preparation and test preparation thrice were injected separately into the column and the chromatograms were recorded. Peak area response of analyte peak was measured.

$$Amount = \frac{concentration\ obtained \times Dilution\ facor \times Average\ weight}{Sample\ taken\ for\ analysis}$$

$$\%\ Assay = \frac{Amount\ obtained}{Label\ claim} \times 100$$

System Suitability Test (SST):

Procedure: Under optimized chromatographic conditions, System Suitability Test was carried. It was performed by injecting blank solution once and standard solution of 100µg/mL test concentration six times in to stabilized HPLC system. % RSD for peak areas of Atemoxetine was determined below 2%. To establish System suitability, last peak of six injections was evaluated. System suitability was conducted by evaluating parameters like retention factor (k'), repeatability, resolution (R), tailing factor (T) and theoretical plates (N).

METHOD VALIDATION

The method was validated for system suitability, linearity, and limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness [8].

Linearity

Procedure: Linearity of the method was demonstrated over the concentration range of 1-

700µg/ml Atemoxetine HCl. Each concentration was prepared in triplicate. 20µl of each standard solution were injected at the optimized chromatographic conditions and the chromatograms were recorded. The retention time, average peak areas were recorded. Calibration curves were constructed by plotting peak area on Y-axis against concentration on X-axis and regression equation was calculated by the method of least squares. The correlation coefficient, y-intercept, slope of the regression line were submitted.

Accuracy:

Procedure:

Accuracy of the method was established by performing recovery studies according to the ICH guidelines.It was ascertained on the basis of recovery studies by standard addition method. Recovery studies were carried out at three different levels (50%, 100%, and 150%) by the addition of standard drug to pre-analyzed sample solution each in triplicate. Mean percentage recovery values at three different concentrations of drug were calculated.

Precision

System Precision: It expresses the precision of the system. It includes analysis on system.

Procedure: It was performed by injecting six replicate injections of standard solution at 200µg/mL concentration under same experimental conditions. The mean value, standard deviation and % RSD was calculated for all six replicate injections.

Method Precision: It expresses the precision of the method.

Procedure: It was performed by injecting six replicate injections of standard solution at 150µg/mL concentration under same experimental conditions. The mean value, standard deviation and % RSD was calculated for all six replicate injections.

Intermediate precision: It expresses the precision within laboratory variations. It includes full analysis on different days, instruments or analysts.

Procedure: It was performed by injecting six replicate injections of test solution at 100µg/mL concentration on different days under same experimental conditions. The mean value, standard deviation and %RSD was calculated for all six replicate injections.

Robustness

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized chromatographic conditions were done i.e., variation in flow rates (0.1 ± ml/min), concentration of organic phase (±2%) and detection wavelengths (±3nm). It was performed using 50 µg/ml. Even though by inducing variations in mobile phase composition, detection wavelength and flow rate.

RESULTS AND DISCUSSION

The method development trials were performed on the working standard at 100% level. Several combinations of chromatographic conditions were tried but the optimum results were achieved with Enable C-18 (250mm x 4.6mm, 5µ) column, mobile phase mixture constituting methanol and $NH_4H_2PO_4$ buffer in a ratio of 80:20, 1ml/min flow rate at a detection wavelength of 227nm. Peak of Atemoxetine HCl was achieved with good resolution, peak shape and symmetry at R_t 3.95.min.

The assay was performed on the tablet formulation (Axepta-40, Intas pharmaceuticals Ltd), and the % drug content of Atemoxetine HCl was found to be 101%, which was within the acceptance limits. As the peak of analyte was well resolved and had no interference of excipients, it was concluded that the proposed method was specific to the drug under study. The linearity of the proposed method was accomplished from the correlation coefficients of the standard calibration curve of Atemoxetine HCl which were constructed at concentration ranges of 1-700µg/ml. The correlation coefficient was found to be 0.998 which were in compliance with the acceptance criteria.

The accuracy of the proposed method was evaluated from the recovery studies, by standard addition method which was performed at three levels of 50%, 100% and 150% of the label claims of Atemoxetine.HCl. The mean percentage recoveries at each level of Atemoxetine HCl were found to be 98.9-101% which fell within the acceptance limits. The precision of the proposed method was established from the %RSDs of the percentage assays

of the drugs at the levels of repeatability, intermediate precision (inter-day, analyst-analyst). The %RSDs of Atemoxetine HCl at inter-day precision were found to be 0.70% and 0.85% respectively and at the level of analyst-analyst variation, they were found to be 0.73% and 0.92% respectively. As the %RSDs were found to be within the acceptance limit (%RSD < 2) at all the levels, the proposed method was said to be precise. Robustness of the proposed method was demonstrated by making deliberate changes in the flow rate, wavelength and concentration of organic phase from the optimized condition of the developed method and computing the %RSD of the peak areas. The %RSD at 0.9ml/min was found to be 0.72% and at 1.1ml/min was found to be 1.60%. At a wavelength of 224nm, the RSD of the drug was found to be 1.04% and at 230nm was found to be 1.15%. At a mobile phase composition of methanol and phosphate buffer in a ratio of 82:18, the RSD was found to be 1.14% and at a ratio of 78:22 of methanol and buffer, it was found to be 0.65% respectively. As the %RSDs were found to be within the acceptance limit (%RSD < 2), the proposed method was said to be robust. The system suitability of the proposed method was accomplished from the resolution, theoretical plate count and asymmetric factor of Atemoxetine HCl at the optimized conditions. The parameters were recorded and tabulated (table 2) and were found to be in compliance with the acceptance specifications. The solution stability studies were performed and the drug solutions were found to be stable for one day (24 hours) from the time of their preparation. As all the validation parameters studied, complied with the acceptance criteria, the proposed method was said to be validated in accordance with ICH guidelines. A simple and rapid reversed-phase HPLC method was developed and validated according to ICH guidelines for the determination of Atemoxetine HCl in tablet dosage form. The developed RP-HPLC method was proved to be simple, rapid, robust, and reproducible. The validation data signifies good specificity, accuracy, precision and reliability of the method. The method can be used for routine practices in the laboratories, for estimation of Atemoxetine in tablet dosage form and bulk drug.

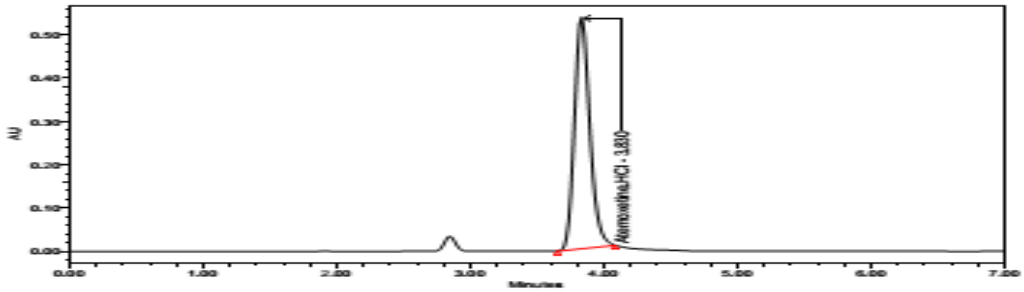

Fig 2: Chromatogram of Optimized method

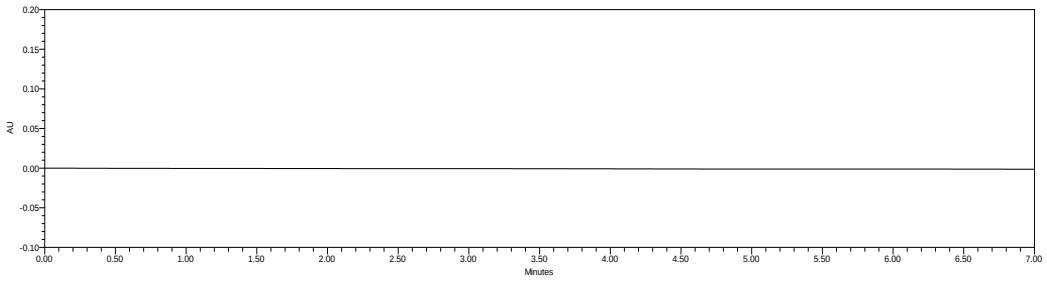

Fig 3: Chromatogram of Blank

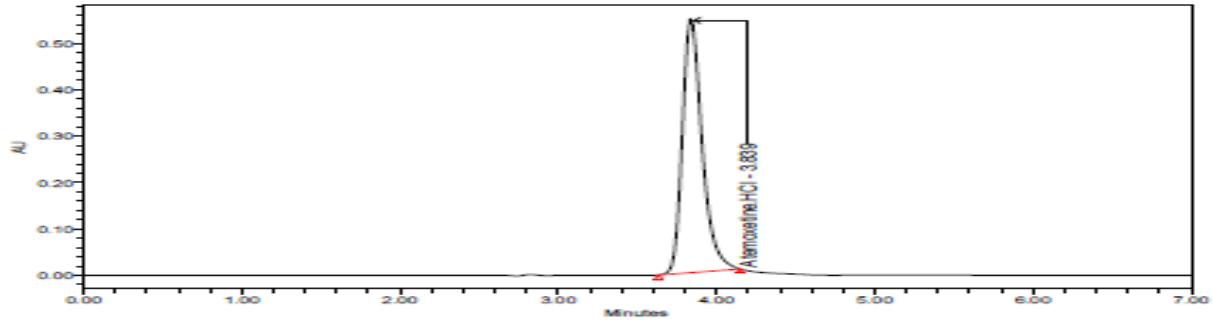

Fig 4: Chromatogram of standard

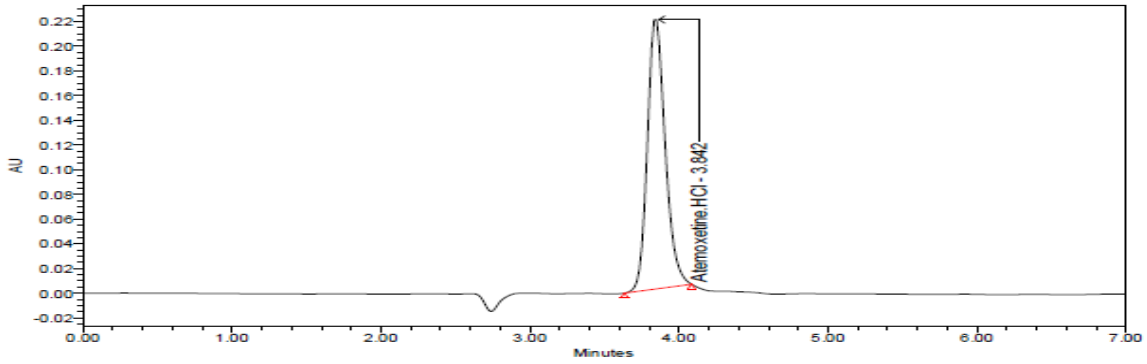

Fig 5: Chromatogram of Test Formulation

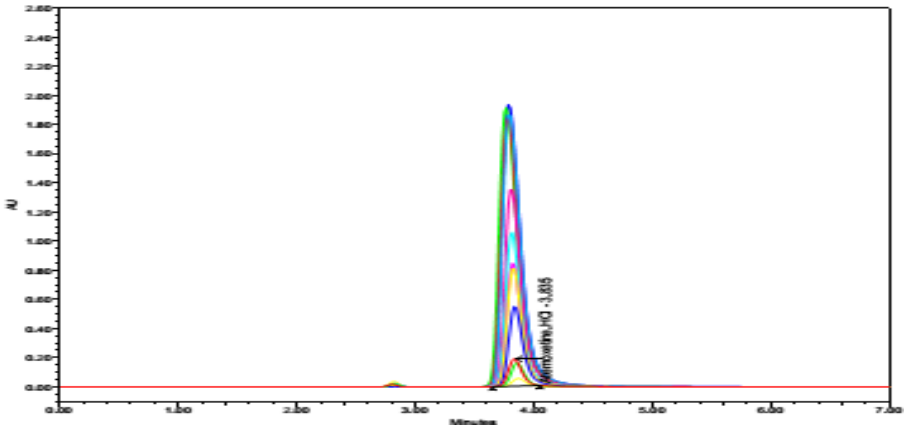

Fig 6: Overlain Chromatogram for Linearity

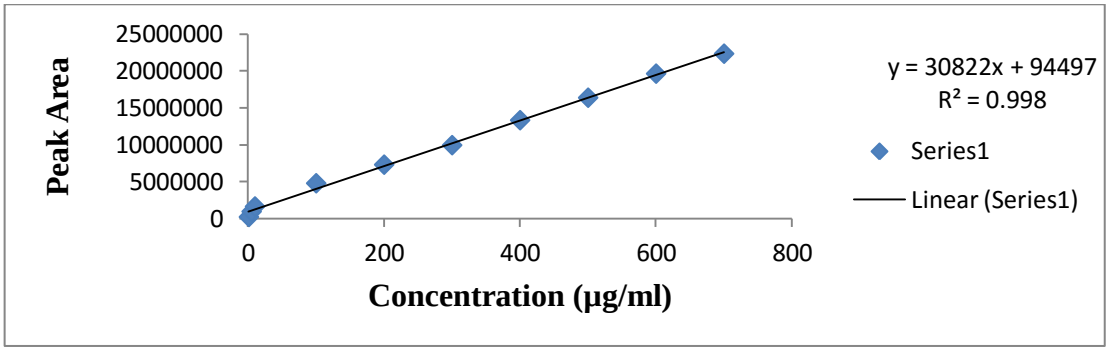

Fig 7: Linearity Graph of Atemoxetine Hydrochloride

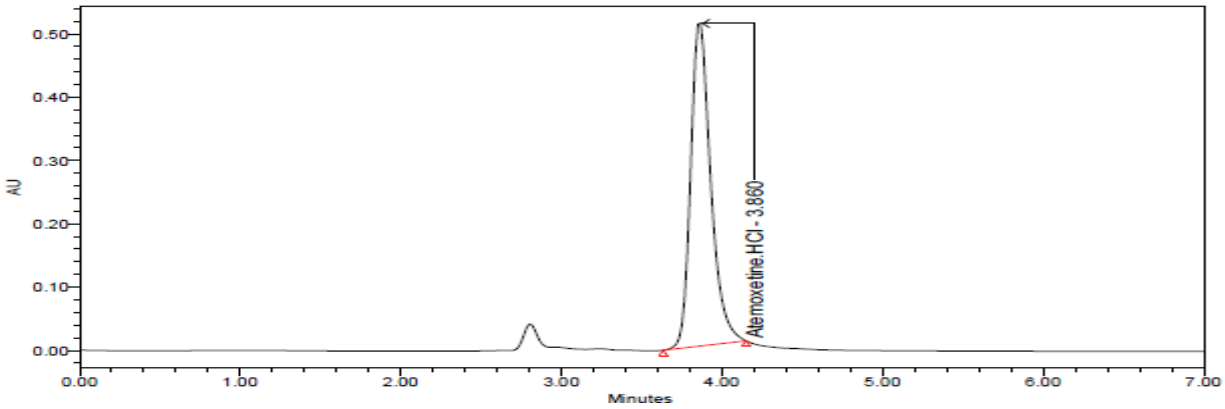

Fig 8: Representative Chromatogram for Accuracy (50% spiked level)

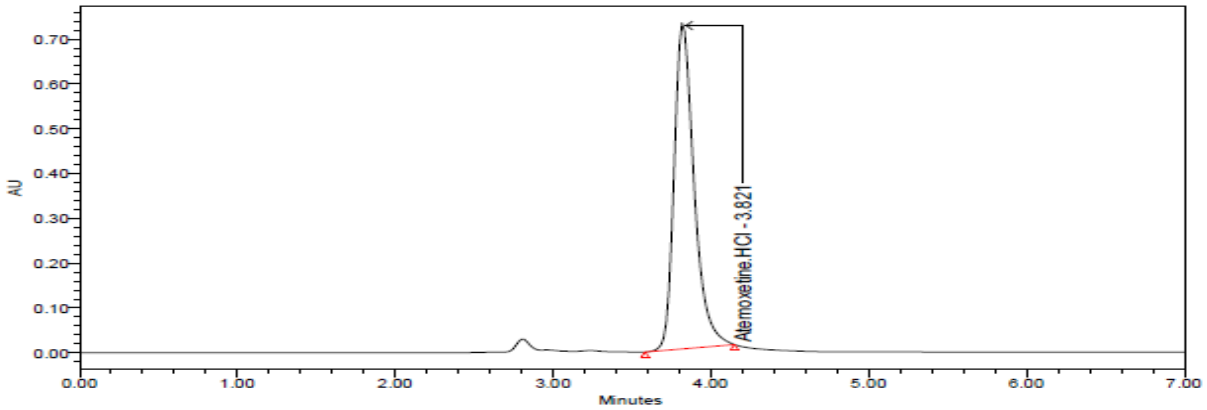

Fig 9: Representative Chromatogram for Accuracy (100 % spiked level)

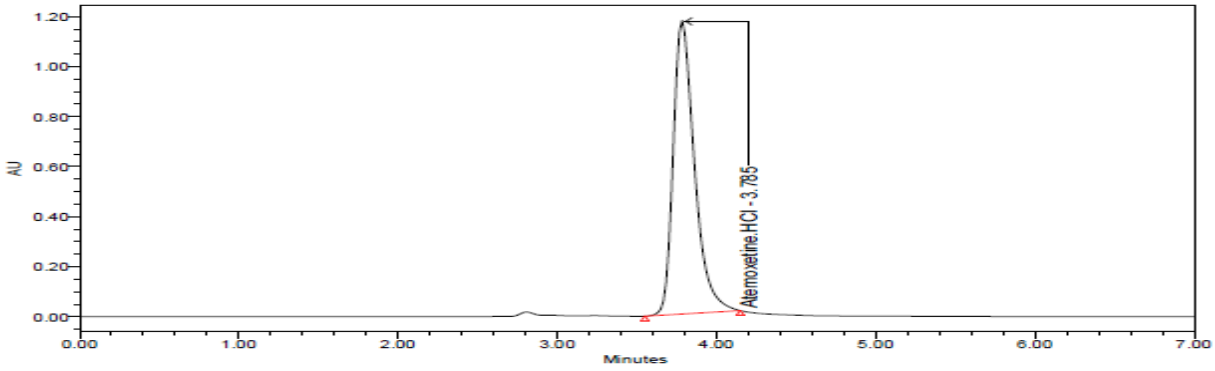

Fig 10: Representative Chromatogram for Accuracy (150% spiked level)

Table 1: Peak Results for Optimized method

S.NO	Peak	R_t	Peak area	USP plate count	USP tailing	Observation
1	Atemoxetine HCl	3.830	4283323	5248	1.17	All system parameters were within the limits.

Table2: Results of System Suitability Test

Injection	Peak area	Retention time (R_t)	USP plate count	USP tailing factor
1	4182927	3.83	5248	1.17
2	4290124	3.83	5245	1.18
3	4250194	3.83	5259	1.17
4	4241044	3.83	5236	1.20
5	4231380	3.82	5234	1.17
6	4310519	3.82	5246	1.19
Mean	4251031	3.826	5244.667	1.18
SD	45148.2	0.0051	9.025889	0.126
%RSD	1.06	0.13	0.17	1.07

Table 3: Results of Assay data

Parameter	Atemoxetine HCl
Mean peak area	3248861
Wt taken(mg)	300
Label claim (mg)	40
Avg. Wt (mg)	4800
Amount recovered	40.4
%Assay	101%

Table 4: Linearity data for Atemoxetine Hydrochloride

S.NO	Atemoxetine hydrochloride	
	Concentration (µg/ml)	Peak Area
1	1	184126
2	5	920648
3	10	1603670
4	100	4754669
5	200	7273972
6	300	9919685
7	400	13303221
8	500	16352023
9	600	19615202
10	700	22318384
Statistical analysis	Slope	30822
	y-intercept	94497
	Correlation Coefficient(R^2)	0.998

Table 5: Overlain Peak Results of System precision

Injections	Peak area
1	7762834
2	7834892
3	7829254
4	7832923
5	7758071
6	7824426
Mean	7807067
SD	36313.98
%RSD	0.465

Table 6: Overlain Peak Results of Method precision

Injections	Peak area
1	6782196
2	6816724
3	6803331
4	6849483
5	6857140
6	6771107
Mean	6813330
S.D	34899.75
%RSD	0.51

Table 7: Intermediate Precision Results

S.NO	Day I	Day II
Injections	**Peak area**	**Peak area**
1	4782835	4862067
2	4844891	4798072
3	4829256	4854096
4	4832926	4748038
5	4758072	4816094
6	4824424	4821234
Mean	4812067	4816600
S.D	33854.51	41325.45
%RSD	0.703534	0.85798

Table 8: Accuracy Study data

Spiked levels	Standard		Sample	Spiked		Mean peak area	Amount recovered (µg/ml)	% Recovered
	Conc. (µg/ml)	Peak Area	Conc. (50µg/ml)	Conc. (µg/ml)	Peak Area			
50%	100	3348261	1827620	150	5201024	5212841	151.5	101
		3346799			5199261			
		3349861			5238238			
100%	200	6104804	1827684	250	7789234	7831118	245.7	98.3
		6200141			7852698			
		6105243			7851422			
150%	300	8344637	1827654	350	10192812	10210388	348.2	99.5
		8364197			10202829			
		8424613	MEAN 1827652		10235523			

Table 9: Robustness Study Data of Atemoxetine Hydrochloride

Parameter	Concentration (150 µg/ml)	Mean peak area	%RSD
Mobile phase composition (Methanol: Ammonium phosphate buffer)	80:20 v/v	3813330	0.91
	+2% (82:18)	3788240	1.14
	-2% (78:22)	4293859	0.65
Flow rate	(±0.1) 1 ml/min	4411012	1.52
	(+0.1) 1.1 ml/min	3990932	0.72
	(-0.1) 0.9 ml/min	4732868	1.60
Wavelength (nm)	224	5600186	1.04
	227	4251031	1.06
	230	2595031	1.15

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