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Validated RP-HPLC and UV spectrophotometry method for the estimation of atomoxetine hydrochloride in pharmaceutical dosage forms

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ABSTRACT

Two simple, precise, and easy methods were successfully developed for the estimation of Atomoxetine hydrochloride in bulk and pharmaceutical dosage forms. The first method was UV-spectrophotometry, which is linear in the concentration range of 20-100µg/ml at maximum absorbance (λ_{max}) 270 nm. The correlation coefficient was found to be 0.999. The accuracy of the method was ranged from 99.5-100.3%. The percent relative standard deviation (%RSD) for precision was found to be less than 2%. The reproducibility of the method was determined based on ruggedness was found to be 0.3-1.4%. The second method was based on reversed-phase high-performance liquid chromatography (RP-HPLC) using acetonitrile: phosphate buffer P^H 6.8 (60:40v/v) with 1ml/min flow rate. The detector response was obtained at 270nm. The average retention time for the drug was obtained 4.2±0.003min. The calibration curves were linear from 20-100µg/ml, which was used as in the case of UV-method. The accuracy of the method was determined by percent recovery studies ranged from 99.8 to 101.77%. Mean Intra - and inter- day assay relative standard deviations were 0.9 and 0.4%. Ruggedness and robustness were also calculated not more than 2%. The proposed methods were applied successfully for the analysis of drug in pure and in its dosage forms and validated according to ICH guidelines.

Key words: Precise, Accuracy, Atomoxetine hydrochloride, Ruggedness, RP-HPLC

INTRODUCTION

Atomoxetine hydrochloride [1] (fig 1), is designated chemically as (-)-N-methyl-3-phenyl-3-(o-tolyloxy)-propylamine hydrochloride, and has molecular mass of 291.82. It has a solubility of 27.8mg/ml in water. Atomoxetine is a white solid that exists as a granular powder inside the capsule, along with pregelatinized starch and dimethicone. It is classified as nor-epinephrine reuptake inhibitor, approved for use in children, adolescents, and adults. It inhibits nor-epinephrine transporter, serotonin transporter, and dopamine transporter with respective K_i (dissociation constant) values of 5, 77, and 1451nM. Atomoxetine, principally it is metabolized by CYP4502D6 through oxidative enzymatic pathway and by glucouridination [2]. It is used treat attention-deficit hyperactivity disorder. The literature review stated that there are few methods available for the evaluation of Atomoxetine hydrochloride in pharmaceutical formulations, such as colorimetric [5], fluorimetric [6], visible [7-9], UPLC [9], HPLC [10,11], HPTLC [12], RP-HPLC [13-20] and UV Spectrophotometric [21,22] methods. The developed methods by RP-HPLC and UV spectrophotometry are simple, precise, easy and economical and are also validated. Hence, these are used for routine analysis for estimation of drug.

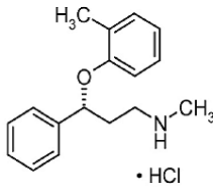

Figure 1: structure of atomoxetine hydrochloride

MATERIALS AND METHODS

Materials

All the chemicals used for the development of methods were AR and HPLC grade. Methanol and potassium dihydrogen phosphate of AR grade were purchased from Ranchem Chemicals Ltd. Acetonitrile, methanol and water of HPLC grade from Merck chemicals Ltd. Atomoxetine HCl pure drug was obtained as a gift sample from Aurobindo Pharma (P) Ltd., Hyderabad, India. The marketed formulation (Axepta) was purchased from local Pharmacy, which was manufactured by Intas pharmaceuticals, Selaqui, Dehradun, India.

Instrumentation

For UV-Spectrophotometry method, the Elico SL 218 Double beam UV-VISIBLE Spectrophotometer, with wide range photodiode detection and fired 10mm path holders for reference and sample were used. For RP-HPLC, Agilent 1120 compact LC system includes an isocratic pump, manual injector, variable wavelength programmable UV detector was used. Chromatographic separation was carried out on a C_{18} column (Agilent ODS UG5 column, 250mm×4.5mm). Axis AGN 204-PO digital balance and 1.5 LH ultrasonic bath sonicator were used.

Method development

The following standard solutions were used for the development of UV-spectrophotometry and RP-HPLC method.

Preparation of standard stock solution

Accurately weighed 10 mg of Atomoxetine hydrochloride standard and transferred into 10 ml volumetric flask. It was dissolved in some amount of water and shaken well until it dissolves and then volume was made up to the mark with same solvent to obtain final concentration of 1000 µg/ml (standard stock solution A). From the above stock solution, 2.5 ml of aliquot was pipetted into a 25 ml volumetric flask and the volume was made up to the mark with water to obtain the final concentration of 100 µg/ml (standard stock solution B).

Selection of analytical wavelength

Using standard stock solution B, 10µg/ml solution was scanned in the wavelength range of 200-400nm in order to observe maximum absorbance. The λ_{max} selected for atomoxetine hydrochloride is 270nm since it shows maximum absorbance at that λ_{max}.

Selection of analytical concentration range

Appropriate aliquots of standard stock solution B, was pipetted out into a series of 10ml volumetric flasks. The volume was made up to the mark with distilled water to obtain a concentration ranging from 20-100µg/ml (20, 40, 60, 80, 100, µg/ml).

Preparation of calibration graph

The absorbance of the above solutions was measured at 270nm. A calibration graph of concentration vs. absorbance was established. The drug follows the beer's lamberts law in the concentration range of 20-100µg/ml. The regression equation was established and the correlation coefficient was determined.

Preparation of tablet formulation solution

Ten tablets of Atomoxetine hydrochloride were weighed and their average weight was determined. The tablets were crushed to fine powder. The powder equivalent to 10 mg of Atomoxetine hydrochloride was weighed and dissolved in water in 10 ml volumetric flask by keeping it in ultra-sonicator for 2min. This solution was used as test stock solution A. From the above stock solution, 1 ml of the aliquot was pipetted out and transferred to a 10 ml volumetric flask. The volume was made up to 10 ml with water to obtain a solution 100µg/ml is stock solution B. From stock solution B, 0.5ml was taken and the volume was made up to mark with water and the absorbance was determined at

270nm. The concentration of the above solution was determined by substituting the value of absorbance in a regression equation.

Preparation of mobile phase
After several trials, the following mobile phase was successfully used to develop RP-HPLC method. Acetonitrile and buffer were used as mobile in ratio of 60:40. A mixture of 480 ml of acetonitrile and 320 ml of potassium dihydrogen phosphate buffer (pH 6.8) was prepared. Then it was ultra sonicated for 20 minutes and filtered through 0.45µm filter paper.

Buffer preparation
A mixture of 16ml of 0.2M Potassium dihydrogen phosphate and 7.16ml of 0.2M sodium hydroxide solution were taken in a 200ml volumetric flask, mixed well and then volume was made up to the mark.

Method Validation
Both RP-HPLC and UV methods were developed and validated by using following suitable parameters such as linearity, precision, accuracy, ruggedness and LOD and LOQ. For RP-HPLC, system suitability tests also were carried out to determine the adequate performance of a chromatographic system.

System suitability
In RP-HPLC**,** it is an integral part of method development used to ensure the performance of HPLC system. The parameters such as retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections at a concentration of 100µg/ml.

Robustness
For RP-HPLC method development, the robustness of the analytical method was studied by differing physical parameters such as the change in flow rate and detection wavelength.

Linearity
The linearity of the proposed methods was determined using standard stock solution B. To establish linearity, the following concentration range of 20, 40, 60, 80,100µg/ml of solutions were used.

Precision
The precision of the methods was evaluated with repeatability and intermediate precision studies. Repeatability was studied using six determinations within a short interval of time on the same day. For intermediate precision, the absorbance of the same solutions were measured on three different days in a week and %RSD was calculated.

Accuracy
The accuracy of two methods was developed by recovery studies which were carried out at three different levels i.e., 80%, 100% and 120%. These were prepared from test stock solution B. The % recovery of the drug from the formulated tablet dosage forms was calculated by using regression equation method.

Limit of detection and Limit of quantitation
The LOD and LOQ were calculated manually from the slope of the calibration curve and standard deviation. The lowest concentration of the analyte can be detected in a sample but not necessarily quantitated as an exact value.

The limit of detection (LOD) may be expressed as $LOD = 3.3\times\sigma/S$
The limit of quantitation may be expressed as $LOQ = 10\times\sigma/S$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve

Ruggedness
The ruggedness of the both methods was determined by analyst variation (analyst I and analyst 2) and instrument variation (Elico SL 218 and 210 double beam UV-Vis spectrophotometers). The results were analysed statistically and the effect of variations were estimated. The concentration of 100µg/ml solution was used for ruggedness.

RESULTS AND DISCUSSION

Simple, precise, economical and easy methods were developed for the estimation of Atomoxetine hydrochloride by RP-HPLC and UV spectrophotometry. The proposed methods were validated according to ICH Q (2B) guidelines. The two methods results were statistically calculated and compared.

For RP-HPLC, according to XXIV (621) guidelines, the system stability studies were calculated on freshly prepared stock solutions and the results were given in Table 1. Robustness of the analytical method was determined by the change in flow rate to 1.0±2.0ml and detection wavelength 270nm±1nm.The %RSD was found to be 0.55%.

The linearity of two methods was developed using concentration range 20-100µg/ml at 270nm. The correlation coefficient was calculated statistically and the obtained results were given Table 2. The precision was determined for two methods. The sample to sample precision was evaluated using six samples of six different concentrations at three intervals. Day to day variability was also assessed using six concentrations. The %RSD was found to be less than 2% and the result were given in Table 3.

The accuracy of two methods was determined by recovery studies. Three different concentration levels were used i.e. 80%, 100% and 120%.These studies indicated that the method was free from the interference of excipients used in the formulation. The %recovery for HPLC method was 99.8 to 101.77%w/w and for UV-method was 99.5 to 100.3%w/w. The results are given in Table 4.

The limit of detection and limit of quantification was calculated for both methods. For HPLC, the LOD and LOQ are 2.6µg/ml and 7.9µg/ml respectively and for UV-Spectrophotometry, 0.64µg/ml and 1.95µg/ml respectively. Ruggedness was determined and validated by the variation from analyst to analyst and instrument to the instrument using similar operational and environmental conditions. The %RSD was found to be less than 2%.The results are shown in Table 5.

The marketed Formulation also was estimated by using these two methods. The other ingredients and excipients usually present in the pharmaceutical dosage form did not interfere in estimation. The results were shown in Table 6.

Table 1: Parameters of System Suitability

Parameters	Results
Retention Time (min)*	4.2
Tailing*	0.45
Theoretical Plates*	8326
%RSD*	1.414

** Mean of six replications and %RSD: relative standard deviation*

Table 2: Statistical data of Atomoxetine Hydrochloride at 270nm

Parameter	UV spectrophotometry	RP-HPLC
Linearity (µg/ml)	20-100	20-100
Correlation coefficient	0.9999	0.9998
Slope	0.0062	17855
y- intercept	0.0027	7873.2
Limit of detection (µg/ml)	0.644	2.6
Limit quantification (µg/ml)	1.951	7.9

Table 3: Summary of %RSD values of Precision

Parameter	UV-Spectrophotometry	RP-HPLC
Precision*		
Intraday*	0.56	0.95
Interday*	1.44	0.486

** mean of six repetitions*

Table 4: Recovery Studies of Atomoxetine Hydrochloride

Drug	% of raw material added	UV-Spectrophotometry		RP-HPLC	
		%Recovery	%RSD	%Recovery	%RSD
Atomoxetine hydrochloride	80	99.5	0.641	100.77	1.161
	100	100.3	0.992	99.8	0.828
	120	99.8	0.566	101.2	0.894
Mean recovery	99.5-100.3			99.8-101.77	

%RSD: percent relative standard deviation

Table 5: Summary of Ruggedness

Parameter	UV-Spectrophotometry	RP-HPLC
Ruggedness*		
Analyst 1	0.3379	0.82
Analyst 2	1.48	0.80
Instrument 1	1.313	-
Instrument 2	1.354	-

**mean of six determinations*

Table 6: Assay of marketed formulation

Drug	Concentration (µg/ml)	UV-Spectrophotometry		RP-HPLC	
		Amount found	%Purity	Amount found	%Purity
	50	48.6	97.5	49.5	99.21

CONCLUSION

The developed and validated RP-HPLC and UV-methods were assured required precision and accuracy. The results of an analysis of tablet formulation and recovery studies suggest that the developed methods were free from the interference of excipients used in tablet formulation. The methods were found to be simple, precise, accurate, economical and rapid. Hence, above methods can be employed in quality control to estimate the amount of Atomoxetine HCl in bulk and commercial formulations.

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REFERENCES

[1] STRATTERA (atomoxetine hydrochloride), Capsules for oral use, Indianapolis, USA; Eli Lilly and Company. 20 Feb 2014. Retrieved 23 May **2014**.
[2] Roth B L, Driscol J, Psychoactive Drug Screening Program (PDSP Ki database, 12 Jan **2011**). University of North Carolina at Chapel Hill and United States National Institute of Mental Health. Retrieved, 10 Nov **2013**.
[3] http/www.drugbank.co.in
[4] http/ncbi.nlm.nih.gov
[5] B. Mohammed Ishaq, H. Abdul Ahad, Shaik Muneer, B. Praveena, *Int J Res Pharmacy and Life Sciences* **2013**, 13(7), 70-74.
[6] S.Y. Ulu, Pharmazie **2011**, 66(11), 831-35.
[7] K. Raghubabu, L. Santiswarup, B. Kalyana Ramu, M. Narayana rao, C. Ramdas, *Rasayan J Chem*, **2011**, 4(4), 784-89.
[8] K. Raghubabu, L. Santiswarup, B. Kalyana Ramu, M. Narayana rao, C. Ramdas, *International J Chem Sci*, **2012**, 10 (2), 643-54.
[9] Suzan Mohmoud Soliman, Hebamyei-Agizy, Abdei-Azizeibayoumi, *Pharmaceutical analytical Acta*, **2014**, 5(1), 248.
[10] Sivaji Tatarulu, Liquid Chromatography and AMP Related Technologies, **2012**, 35(6),747-56.
[11] A.K. Delek Dogrukol, Duygu Yeniceli, *Liquid chromatography and AMP Related Rechnologies*, **2010**, 33(19), 1745-59.
[12] H.R. Prajapati, P.N. Raveshiya, Bhavesh B Jadav, M.M. Divyesh, *Der Pharma Chemica*, **2012**, 4(1), 194-201.

[13] M. Sumalatha, D. Bharath Kumar, A. Geetha, S. Sirisa, A. Shruthi, *Int J Res Pharm and Biomed Sci*, **2012** 3(3), 1147-52.
[14] S.K. Patel, N.J. Patel, *J AOAC Internationa*,l **2010**, 93(4), 1207-14.
[15] Kothari Charmy, Suhagia Bhanubhai, Shah Nehal, Shah Rajan, *Int J Drug Formulation and Research*, **2011**, 2(3), 408-24.
[16] K.C. Usmangani, K.B. Kashyap, L.B. Sunil, *Sic Pharm*, **2010**, 78(4), 857-68.
[17] Gurumeet Chhabra, Chandraprakash Jain, Saurabh K Banerjee, *Int J Drug Development and Res*, **2011**, 3(4), 275-79.
[18] G. Tejalakshmi, Y. Srinivasarao, K. Varaprasadarao, T. Hemanth Kumar, *Research J Pharm, Biological and Chemical sciences*, **2015**, 6(2), 1208-14.
[19] S.K. Sudhir, Vishal B Chowdary, T.V. Vinayak, S.P. Swarup, *Chromatographia*, **2008**, 67(1), 143-46.
[20] K. Ramya, P. Venkateswararao, A.M.S. Sudhakar Babu, M. Archana, *Int J Biological and Pharm Res*, **2012**, 3(7), 929-934.
[21] Parag Pathade, Amolpawar, Abhijit Gaiwad, Ashwini Pawhalkar, *Int J Pharm and Bio Sci*, **2001**, 2(4), 596-602.
[22] N. Paras, R. Hetal, *Journal of Pharmacy Research*, 201, 4(6), 1720-22.
[23] Hua-jinZeng, Ran Yang, Ying Zhang, Jian-jun Li, Ling-bo Qu, *Journal of Biological and Chemical Luminescence*, **2015**, 30, 124-30.
[24] A.S. Jane, A.O. Bernard, K.O. Paul, F.G. Peter, *Journal of Pharmaceutical and Biomedical Analysis*, **2006**, 41(4), 1088-94.
[25] F.G. Peter, A.O. Bernard, *Journal of Pharmaceutical and Biomedical Anal*, **2008**, 46(3), 431-41.
[26] P. Chaula, P. Minal, Shubha Rani, Manish Nivaskar, P. Harish, *J Chromatography B*, **2007**, 850,356-60.