Asian Journal of Chemistry Vol. 21, No. 2 (2009), 829-833

Reverse Phase HPLC Analysis of Atomoxetine in Pharmaceutical Dosage Forms

B.V.V.S. JAGADEESH, S. SATYANARAYANA RAJU†,
V.JAYATHIRTHA RAO‡ and J.V.L.N. SESHAGIRI RAO*
University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India
E-mail: jvlnsrao@gmail.com; suryapharma@rediffmail.com

A high performance liquid chromatographic method has been proposed for the determination of atomoxetine HCl in pure and dosage forms. The drug was chromatographed on a C-18 column using a mixture of water (containing 0.2 % of triethyl amine) and acetonitrile in the ratio of 15:85 v/v as the mobile phase at a flow rate of 1.0 mL/min. Pioglitazone was used as an internal standard and the detection was done at 270 nm. Linearity was observed in the concentration range of 10-200 µg/mL. The intra and inter-day variation was found to be less than 1 % showing high precision of the assay method. The mean per cent recovery of the drug from a sample solution containing 100 µg/mL was 99.40 ± 0.46 indicating high accuracy of the proposed method. Due to its simplicity, rapidness, high precision and accuracy the proposed method may be used for determining atomoxetine HCl in pure drug samples and dosage forms.

Key Words: Atomoxetine hydrochloride, RP-HPLC.

INTRODUCTION

Atomoxetine HCl is a selective norepinephrine reuptake inhibitor. Chemically it is (-)-N-methyl-3-phenyl-3-(*o*-tolyloxy)-propylamine hydrochloride. The precise mechanism by which atomoxetine produces its therapeutic effects in attention-deficit hyperactivity disorder (ADHD) is unknown, but is thought to be related to selective inhibition of the pre-synaptic norepinephrine transporter, as determined in *ex vivo* uptake and neurotransmitter depletion studies[1,2].

A few HPLC methods including a simultaneous determination of atomoxetine and its metabolites in human plasma and urine and another which makes use of a polysaccharide chiral stationary phase for its separation have been published. The authors now proposed a more precise and accurate HPLC method for the determination of atomoxetine HCl.

†Roland Institute of Pharmaceutical Sciences, Berhampur-760 010, India.
‡Indian Institute of Chemical Technology, Hyderabad-500 607, India.

EXPERIMENTAL

A Jasco (Japan) HPLC instrument with a HiQ Sil C-18 V analytical column (4.6 mm × 250 mm and 5 µm), a PU-2080 pump, a Rheodyne 7725 injector system for sample injection and a UV-2075 detector (Jasco) was employed for the study. Jasco Borwin software, (Japan) was used for monitoring the system and quantitative reading of the peaks. Dissolution of the compounds was enhanced by sonication on a Bandelin sonerex sonicator. The pH of the solutions was adjusted by using a digital pH meter (Model DI 707 of Digisun electronics, Hyderabad).

Pure samples of atomoxetine and pioglitazone (internal standard) were gifted by Aurobindo Pharma (Hyderabad, India) and Ranbaxy Laboratories Limited (Mohali, India) respectively. Attentrol capsules (Sun Pharma. Ltd, India) and tomoxetin capsules (Torrent pharma) containing 10 mg and 25 mg of atomoxetine HCl were purchased from the local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Acetonitrile (HPLC grade) and triethyl amine (A.R. grade) were procured from Merck Ltd. (Mumbai, India) and orthophosphoric acid (AR grade) was procured from SD Fine Chemicals Ltd. (Hyderabad, India).

Preparation of the standard drug solutions: A stock solution of atomoxetine was prepared by dissolving 25 mg of the drug in a 25 mL volumetric flask containing 10 mL acetonitrile. The solution was sonicated for about 0.5 h and then made up to volume with acetonitrile. Fresh working standard solutions of atomoxetine were prepared daily by suitable dilution of the stock solution with the mobile phase. Similarly, stock solution of the internal standard was prepared by dissolving 25 mg of pioglitazone in 25 mL of acetonitrile.

Chromatographic conditions: The mobile phase used in this study was a mixture of water (containing 0.2 % of triethyl amine and adjusted to pH 3.0 with orthophosphoric acid) and acetonitrile in the ratio of 15:85 v/v. The mobile phase was filtered before use through a 0.45 µ membrane and degassed for 15 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 mL/min. The column temperature was maintained at 23 ± 1 ºC.The eluents were monitored at 270 nm.

Recommended procedure for standard graph: After a systematic and detailed study of various parameters involved, the following procedure and conditions are recommended for the determination of atomoxetine in pure samples and in dosage forms. Prior to injection of the drug solutions, the column was equilibrated at least for 0.5 h with the mobile phase flowing through the system. The prepared dilutions containing atomoxetine in the concentration range of 10-200 µg/mL and a fixed concentration (20 µg/mL) of the internal standard were injected into the HPLC system. Each of these samples (20 µL) was injected five times into the column and the corresponding peak area ratio of drug to that of the internal standard was calculated. Standard graph was plotted by taking concentration of the drug on x-axis and peak area ratio of drug to that of the internal standard on y-axis.

Assay of atomoxetine in dosage forms: An accurately weighed sample of the powder equivalent to 25 mg of atomoxetine was placed in a 25 mL volumetric flask, 10 mL of acetonitrile was added shaken well and the flask allowed to stand for 0.5 h with intermittent sonication to ensure complete solubility of drug the solution was then made up to volume with acetonitrile. This solution was filtered through a 0.45 µ membrane filter. The filtrate obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity observed for the pure drug. An aliquot of solution of pioglitazone was added to the sample solution prior to the dilution so as to give a final concentration 20 µg/mL of the internal standard. All determinations were carried out in triplicate.

Method validation

Linearity: The standard curve was obtained in the concentration range of 10-200 µg/mL. The linearity of the method was evaluated by regression analysis using the least squares method.

Precision: The precision of the assay was determined in terms of repeatability (intra-day) and intermediate (inter-day) precision. The intra and inter-day variation in the peak area of drug solution (25, 50 or 100 µg/mL) was calculated in terms of coefficient of variation (CV).

Accuracy: The accuracy of HPLC method was assessed by adding known amount (25, 50 or 100 µg/mL) of the drug to a drug solution of known concentration 100 µg/mL and subjecting the samples to the proposed HPLC method. All solutions were prepared and analyzed in triplicate.

Limit of detection (LOD) and limit of quantification (LOQ): Limit of detection was found to be 0.1 µg/mL (signal to noise ratio 3) and limit of quantification was found to be 0.4 µg/mL (signal to noise ratio 10).

System suitability: System suitability studies were carried out as specified in USP. These parameters include column efficiency (N), resolution (R), capacity factor (K'), selectivity factor (α) and peak asymmetry factor (A_S).

RESULTS AND DISCUSSION

To estimate the per cent recovery of atomoxetine from capsules pioglitazone was used as an internal standard because of their structural similarities. The chromatograms of atomoxetine and pioglitazone were also recorded individually under identical chromatographic conditions. The order of the elution was pioglitazone followed by atomoxetine at 3.5 and 7.9 min, respectively. The calibration curve plotted for atomoxetine was later used to determine concentrations of the drug in capsules. The peak area ratio of the drug to that of the internal standard *versus* concentration was found to be linear and is given in Table-1. The linear regression for the proposed method was $Y = 0.02x + 0.0252$ ($r^2 = 0.9992$), where Y is the peak area ratio and X is the concentration of atomoxetine.

TABLE-1
CALIBRATION OF THE HPLC METHOD FOR THE ESTIMATION OF ATOMOXETINE

Concentration (µg/mL)	Peak area ratios	Concentration (µg/mL)	Peak area ratios
10	0.21	125	2.47
25	0.55	150	3.03
50	1.04	175	3.52
75	1.61	200	4.06
100	1.99	–	–

Regression equation from 10-200 (µg/mL).

The proposed HPLC method was also validated for intra- and inter-day variation. When the solution containing 25, 50 or 100 µg/mL of atomoxetine were repeatedly injected on the same day, the coefficient of variation (CV) in the peak area of drug for three replicate injections was found to be less than 1 %. The inter day variation (3 days and three injections) was also found to be less than 2 % (Table-2).

TABLE-2
PRECISION OF PROPOSED HPLC METHOD

Concentration taken (µg/mL)	Intra-day		Inter-day	
	Measured concentration (µg/mL) ± SD	Coefficient of variation (%)	Measured concentration (µg/mL) ± SD	Coefficient of variation (%)
25	25.15 ± 0.13	0.51	24.97 ± 0.32	1.28
50	49.23 ± 0.29	0.59	49.98 ± 0.08	0.16
100	100.64 ± 0.07	0.06	100.20 ± 0.12	0.12

The accuracy of HPLC method was assessed by adding known amount (25, 50 or 100 µg/mL) of the drug to a drug solution of known concentration 100 µg/mL and subjecting the samples to the proposed HPLC method. All solutions were prepared and analyzed in triplicate. There was a high recovery (100.10 ± 0.14) of atomoxetine (Table-3) indicating that the proposed method is highly accurate.

TABLE-3
ACCURACY STUDIES

Amount of drug added (µg)	Mean ± SD (amount (µg) recovered) n = 6	Mean ± SD (% of recovery)
25	24.96 ± 0.08	99.85 ± 0.35
50	50.05 ± 0.07	100.10 ± 0.14
100	99.67 ± 1.14	99.67 ± 1.14

The HPLC method, developed in the present study is used to quantify atomoxetine in capsule dosage forms. Atomoxetine capsule were analyzed as per the procedure described above. High percentage recoveries of atomoxetine ranging

from 99.70 to 100.21 were observed with the dosage forms (Table-4). No interfering peaks were found in the chromatogram indicating that excipients used in capsule formulations did not interfere with the estimation of drug by the proposed HPLC method.

TABLE-4
AMOUNT OF ATOMOXETINE IN DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Brand name	Labelled amount (mg)	Observed amount (mg) Mean ± SD	% Recovery Mean ± SD
Attentrol	10	9.97 ± 0.11	99.70 ± 1.15
Tomoxetin	25	25.05 ± 0.11	100.21 ± 0.46

TABLE-5
SYSTEM SUITABILITY PARAMETERS

Parameter	Value
Theoretical plates (n)	2059
Resolution (R)	9.24
Tailing factor (T)	1.58
Capacity factor (k')	2.37
HETP	0.1214

Conclusion

The proposed HPLC method is simple, precise, accurate and rapid for the determination of atomoxetine in dosage forms. Hence, it can be easily and conveniently adopted for routine quality control analysis.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, IICT and Hyderabad for providing facilities for the study. The authors also thank M/s Ranbaxy laboratories limited, Mohali, India and Aurobindo pharma Ltd, Hyderabad, India for providing gift samples of pioglitazone and atomoxetine.

REFERENCES

1. J.H. Mullen, R.L. Shugert, G.D. Ponsler, Q. Li, B. Sundaram, H.L. Coales, J.E. Yakupkovic, R.M. LeLacheur, W.J. Wheeler, F.J. Belas and J.M. Sauer, *J. Pharm. Biomed. Anal.*, **38**, 720 (2005).
2. J.A. Sellers, B.A. Olsen, P.K. Owens and P.F. Gavin, *J. Pharm. Biomed. Anal.*, **41**, 1088 (2006).

(*Received*: 15 February 2007; *Accepted*: 9 September 2008) AJC-6842