Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2012, 4 (1):194-201
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Development and Validation of High Performance Thin-Layer Chromatographic Method for Determination of Atomoxetine Hydrochloride in Pharmaceutical Dosage Forms

Hetal R. Prajapati*, Paras N. Raveshiya, Bhavesh B. Jadav, Divyesh M. Mahakal

Department of Pharmaceutical Chemistry, A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar, Gujarat, India

---

ABSTRACT

New simple, accurate, precise, rapid, selective and reproducible high performance thin layer chromatographic method for quantitative analysis of atomoxetine hydrochloride as the bulk drug and in pharmaceutical formulations has been established and validated. High performance thin layer chromatography (HPTLC) on aluminium - backed silica gel 60F_{254} plates with methanol-triethylamine 10:0.5 (v/v) as mobile phase was followed by densitometry measurement at 270 mm. This system was found to give compact bands for atomoxetine hydrochloride (R_F 0.55 ± 0.02). Calibration plots were linear in the range 300 - 1800 ng per spot with correlation coefficient 0.9986. The recovery was in the range of 98.10 - 99.85 %. The method was validated in accordance with ICH guidelines and can be used for analysis of marketed formulations.

Key Words: Atomoxetine hydrochloride, HPTLC, ADHD, ICH, Validation.

INTRODUCTION

Atomoxetine is the first non-stimulant drug approved for the treatment of an attention-deficit hyperactivity disorder (ADHD). It is sold in the form of the Hydrochloride salt of Atomoxetine. It is a selective nor-adrenaline reuptake inhibitor [1-3]. Chemically, it is (R) n-methyl-3-(2-methylphenoxy)-3-phenylpropylamine (figure 1) [4].

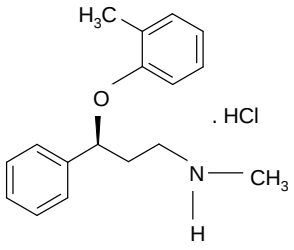

Figure 1: Structure of atomoxetine hydrochloride

Literature survey reveals that several methods have been reported like UV, HPLC, HPLC-MS and for its estimation in plasma [5-9]. As far as we are aware no HPTLC method has been reported for atomoxetine hydrochloride as the bulk drug and in pharmaceutical dosage forms. It was therefore thought worthwhile to develop a simple, reliable, rapid, sensitive and accurate HPTLC method for analysis of atomoxetine hydrochloride as the bulk drug and in tablet and capsule dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical-grade atomoxetine hydrochloride was kindly supplied as gifts by Sun Pharmaceuticals Pvt. Ltd, Baroda, India and used without further purification. Other chemicals of HPLC grade were supplied by Lichrosolv® - E. Merck (India) Ltd., Mumbai. Atomoxetine hydrochloride tablets (Axapta - 40 mg) and capsule (Attentrol - 5 mg) were procured from Intas Pharmaceutical Pvt. Ltd. Baroda, India and Sun Pharmaceutical Pvt. Ltd, Baroda, India respectively.

Instruments

For HPTLC Camag Linomat 5, Camag twin trough glass chamber (10 x 10 and 20 x 10)**,** Camag TLC scanner 3, Camag Reprostar 3 with digital camera for 254nm, 366nm and with light, Camag UV cabinet with dual wavelength UV lamp, Stationary Phase: Silica gel G_{60} F_{254} coated on aluminum sheet, Hamilton 100µl HPTLC syringe.

Preparation of Standard Stock Solution (1000 µg/mL)

A standard stock solution (1000 µg/mL) of atomoxetine hydrochloride was prepared by dissolving 50 mg of standard atomoxetine in 50 mL methanol. Standard solutions for calibration were prepared by dilution of the stock solution with methanol; the concentrations were such that amounts of atomoxetine hydrochloride between 300 to 1800 ng were applied to the plate.

Chromatographic Conditions

HPTLC was performed on 10 cm x 10 cm aluminium - foil TLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). Before use plates were prewashed with methanol, activated at 110º C for 5 min, then stored in a desiccator. Samples were applied to the plates as 6 mm bands, 14 mm apart, by use of a Linomat 5 applicator (Camag, Muttenz, Switzerland) equipped with a 100 mL Hamilton syringe (Bonaduz, Switzerland). The rate of application was 150 nL S^{-1}. Linear ascending development with methanol-tritethylamine 10 : 0.5 (v/v) as mobile phase was performed, in the dark, in a Camag twin-trough glass camber previously saturated with mobile phase vapour for 10 min at room temperature (25º C). The development distance was 9 cm and the development time approximately 30 min. The volume of mobile phase used for chromatography was 10 mL. After development the plates were dried in a current of air by means of a hair drier. Densitometric scanning at 270 nm was performed in the UV region of 200 – 400 nm and the overlain absorption spectrum was recorded using Camag TLC scanner 3.

Selections of Detection Wavelength

After chromatographic development, bands were scanned over the range 200 – 400 nm and the spectra were overlain (figure 3) at 270 nm.

Method Validation

The method was validated in accordance with ICH guidelines [10, 11].

Preparation of Calibration Plots and Linearity

A standard stock solution of atomoxetine hydrochloride (1000 µg/mL) was applied to an HPTLC plate in the range of 300 to 1800 ng per spot. The plate was developed and scanned under the conditions described above. Each amount was analyzed five times and peak areas were recorded. A calibration plot of peak area against the respective amount of atomoxetine hydrochloride was established.

Sensitivity

The sensitivity of measurement of atomoxetine hydrochloride was estimated as the limits of detection (LOD) and quantitation (LOQ), which were calculated by the use of the equations LOD = 3.3 × *N*/*B* and LOQ = 10 × *N*/*B*, where *N* is the standard deviation of the peak area of the drug (*n* = 3), taken as a measure of the noise and *B* is the slope of the corresponding calibration plot.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 tablets and capsules were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at three different concentration levels taking into consideration percentage purity of added bulk drug samples.

Repeatability

Standard mixture solutions containing 300, 600, 900, 1200, 1500 and 1800 ng/spot were prepared and chromatogram were recorded. Area was measured of the same concentration solution five times and RSD was calculated.

Intra-day and Inter-day Precision

Variation of results within the same day (intra-day) and variation of results between days (inter-day) were analyzed. Intra-day precision was determined by analyzing atomoxetine hydrochloride for three times in the same day. Inter-day precision was determined by analyzing both the drugs daily for three days.

Reproducibility

The area was measured at different laboratory using another instrument by another analyst and the values obtained were evaluated using t-test to verify their reproducibility.

Specificity and Selectivity

Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. Selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Commonly used excipients in tablet and capsule preparation were spiked in a pre weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

Robustness

The solutions were prepared and then analyzed with change in the analytical conditions like different laboratory, different analyst, and different instrument.

Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed accurately and powdered. Powder equivalent to 25 mg atomoxetine was taken and transferred to a 25 mL volumetric flask containing approximately 10 mL methanol. The mixture was ultrasonicated for 10 min then diluted to volume with methanol. The solution was filtered through a Whatman No. 41 filter paper. An aliquot of 2.5 mL was taken and transferred to a 25 mL volumetric flasks and volume was made up to the mark with methanol to give solution of 100 ng/µl. 10 µl of the above solution was applied to an HPTLC plate to furnish 1000 ng per band of atomoxetine. After chromatographic development, the peak area of the band was measured at 270 nm and amount of drug in each tablet was determined from the calibration plot. The analytical procedure was repeated six times for the homogeneous powder sample.

Procedure for Analysis of capsule formulation

Twenty capsules were weighed and the sample solutions for the estimation of atomoxetine hydrochloride in capsule dosage form was prepared and analyzed as per the procedure given for atomoxetine in tablet dosage form.

RESULTS AND DISCUSSION

Method Development

After the trials of various mobile phase systems such as chloroform: ammonia (10: 0.2 v/v), methanol: ammonia (10: 0.2 v/v) and methanol: triethylamine (10: 0.2 v/v), methanol: triethylamine (10: 0.5 v/v) was found to be satisfactory giving good resolution peak (Figure 2). The R_f value for atomoxetine hydrochloride was 0.55. The following equation for straight line was obtained for atomoxetine hydrochloride.

Linear equation**:** y = 1.3356x + 97.873

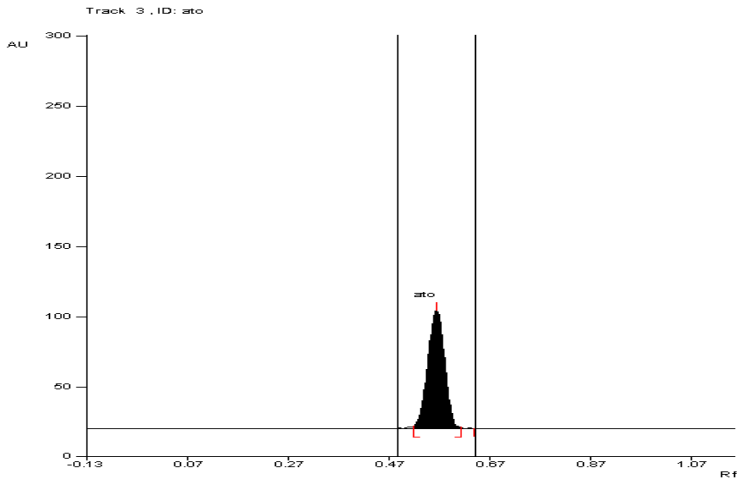

Figure 2: Chromatogram of standard solution of atomoxetine hydrochloride using mobile phase methanol: triethylamine (10: 0.5, v/v) (proposed method)

The overlain spectra of atomoxetine hydrochloride revealed that at 270 nm the drug possess significant absorbance (Figure 3).

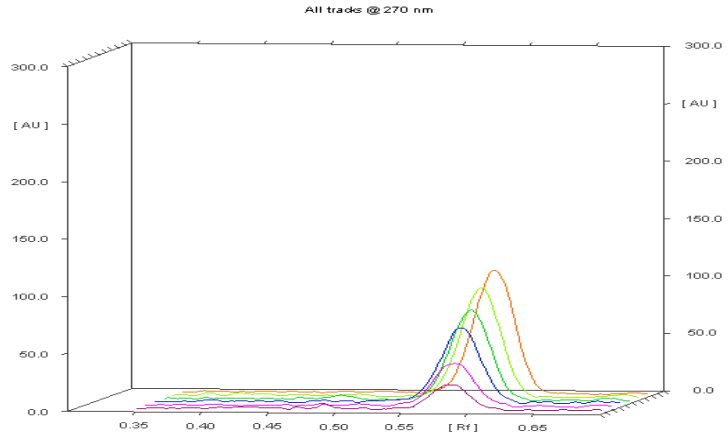

Figure 3: Overlain view of all tracks of atomoxetine hydrochloride at 270 nm

Calibration curve for atomoxetine hydrochloride was prepared by plotting graph of concentration v/s area (Figure 4) and an equation for straight line was obtained.

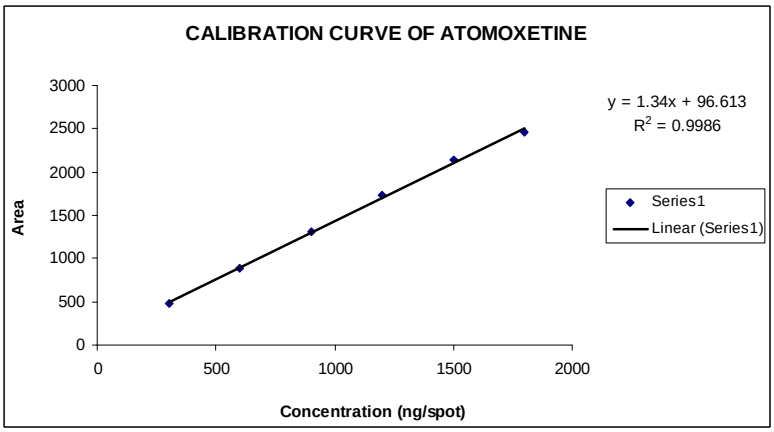

Figure 4: Calibration curve of atomoxetine hydrochloride at 270 nm

Method Validation

Linearity

The calibration graph was linear (figure 4), i.e. the system adhered to Beer's low, over the range 300 - 1800 ng per band (r2 = 0.9986). Linearity was evaluated by duplicate analysis of six standard working solutions equivalent to 300 - 1800 ng per band atomoxetine hydrochloride. The linear regression equation is shown in figure 4.

Precision

Results from determination of intra-day precision, by analysis of standard solutions covering the entire calibration range, are listed in table I. The method was found to be precise with % RSD 0.23 - 1.92 for intra-day (n = 3) and % RSD 0.34 - 1.61 for inter- day (n = 3) for atomoxetine hydrochloride. The method was found to be specific as no interference observed when the drug was estimated in presence of excipients (Table 1).

Table 1: Results of measurement of intra and inter day precision

Concentration (ng/spot)	Intra-day (n = 3)	% RSD	Inter-day (n = 3)	%RSD
300	477.33 ± 9.200	1.92	486.43 ± 7.84	1.61
900	1314.73 ± 7.600	0.57	1317.20 ± 13.22	1.04
1800	2460.90 ± 5.838	0.23	2469.86 ± 9.51	0.38

Determination of LOD and LOQ

To determine the limits of detection and quantitation, concentrations in the linear range of the calibration plot were used. LOD and LOQ were calculated as 3.3 σ/s and 10 σ/s respectively, where the σ is standard deviation of the response and s is the slope of the calibration curve. LOD and LOQ were 77.58 ng per spot and 235.099 ng per spot respectively.

Accuracy

Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 99.10 - 99.85 % for atomoxetine hydrochloride tablet and 98.10 - 99.28 % for atomoxetine hydrochloride capsule (Table 2 & 3).

Table 2: Results from accuracy study (Tablet), n = 6

Amount of sample (ng per spot)	Amount of standard drug added (ng per spot)	Amount recovered (mg)	Recovery (%)
1000	0	998.25	-
1000	500	1497.02	99.85
1000	1000	1982.20	99.10
1000	1500	2479.65	99.16

Table 3. Results from accuracy study (Capsule), n = 6

Amount of sample (ng per spot)	Amount of standard drug added (ng per spot)	Amount recovered (mg)	Recovery (%)
1000	0	998.25	-
1000	500	1477.02	98.46
1000	1000	1962.20	98.10
1000	1500	2482.65	99.28

Solvent Suitability

The method was found to be rugged as there was no change in absorbance up to 48 hr of preparation of solution in methanol (Table 4).

Table 4: Solvent suitability study for atomoxetine hydrochloride in methanol

Time (hr)	Area atomoxetine 900 ng / spot	Result % atomoxetine
0	1329.9	100.45
4	1323.4	100.01
8	1330.3	100.52
24	1347.2	101.81
48	1349.2	101.96

Reproducibility

The method was found to be reproducible as there was no significant difference when sample concentration of 900 ng per spot was estimated using two different analysts, tcal obtained was 1.07 (< 4.30)* (Table 5).

* At 95 % confidence interval, t - tabulated = 4.30

Table 5: Reproducibility data for atomoxetive hydrochloride (900 ng/spot)

Analyst I Area ± SD (n = 3)	Analyst II Area ± SD (n = 3)	Result of t-test	Inference
1315.96 ± 9.74	1325.56 ± 8.28	**1.07**	**Not significant**

Validation parameters are summarized in Table 6.

Table 6: Summary of Validation

Characteristic	Atomoxetine hydrochloride
Linear range (ng/spot)	300 – 1800
Correlation coefficient	0.9986
Limit of detection (ng/band)	77.58
Limit of quantization (ng/band)	235.099
Repeatability (RSD, n = 6)	0.780
Inter-day (n = 3)	0.38 – 1.61
Intra-day (n = 3)	0.23 – 1.92
Recovery (%)	99.10 – 99.85 (Tablet) 98.1 – 99.28 (Capsule)
Specificity	Specific
Solvent stability	Solvent stable for 48 h
Reproducibility	Reproducible

The method was also evaluated by assay of commercially available tablets and capsules containing atomoxetine hydrochloride. The assay (%) was 99.05 for atomoxetine hydrochloride tablet and 100.46 for atomoxetine hydrochloride capsule.

CONCLUSION

This HPTLC method for quantitative analysis of atomoxetine hydrochloride in pharmaceutical formulations is simple, fast, accurate, precise, specific, rugged, reproducible and without interference of excipients. The method was validated in accordance with ICH guidelines. The method reduces analysis time and found to be cost effective and seems to be suitable for routine analysis of pharmaceutical formulations in quality control laboratories, where economy and speed are essential.

Acknowledgement
Authors are thankful to University Grants Commission, New Delhi for funding this research project.

REFERENCES

[1] J. Schwitzer, T. Cummins, A. Kant, *Medical Clinics of North America*, **2001,** 85, 3, 757.
[2] J. Spencer, V. Faraone, D. Michelson, *J. Clinical Psychiatry*, **2006,** 67, 3, 415.
[3] S. Prasad, C. Steer, *Paediatric Drugs*, **2008,** 10, 1, 39-47.
[4] C. Patel, M. Patel, S. Rani, *J. Chrom. B*, **2007,** 850, 356-60.
[5] G. Wei, L. Wenbiao, G. Guixin, Z. Jun, *J. Chrom.B*, **2007,** 854, 128-34.
[6] F. Peter, A. Bernard, *J. Pharm. Biomed. Anal.*, **2008,** 46, 431-41.
[7] H. John, L. Richard, D. George, *J. Pharm. Biomed. Anal.*, **2005,** 38, 720-33.
[8] P. N. Raveshiya, H.R. Prajapati, *J. Pharm. Res.*,**2011,** 4 (6), 1720-1722.

[9] H.R. Prajapati, P. N. Raveshiya, J.M Prajapati, *E. J. Chem.*, **2011,** 8 (4), 1958-1964.
[10] International conference on Harmonization guidance for Industry In: Q2A text on Validation of Analytical methods. Switzerland, **1994**.
[11] International conference on Harmonization guidance for Industry In: Q2B text on validation of Analytical methods. Switzerland, **1996**.