Asian Journal of Chemistry Vol. 20, No. 7 (2008), 5409-5413

HPTLC Determination of Atomoxetine Hydrochloride from its Bulk Drug and Pharmaceutical Preparations

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A rapid, simple and sensitive high-performance thin layer chromatographic method (HPTLC) has been developed to assay atomoxetine HCl in capsules. The HPTLC analysis used a normal phase (silica gel 60 F 254) as a stationary phase and a mobile phase consisting of mixture of acetonitrile:glacial acetic acid with UV detection at 269 nm. The validation data showed that the assay is sensitive, specific and reproducible for determination of atomoxetine HCl in this dosage form. The calibration curves were linear from 100-1000 μg mL^{-1} ($R^2 > 0.997$). The accuracy of the method ranged from 99.12 to 99.80 %. Mean inter- and intra-assay relative standard deviations (RSD) were less than 2.0 %. The proposed method provided an accurate and precise analysis of atomoxetine HCl in its pharmaceutical preparations.

Key Words: HPTLC, Atomoxetine hydrochloride, Silica gel.

INTRODUCTION

Atomoxetine HCl is a selective norepinephrine reuptake inhibitor and used to treat attention-deficit hyperactivity disorder (ADHD) in children aged six years and over, adolescents and adults. It's chemical name is (-)-N-methyl-3-phenyl-3-(*o*-tolyloxy)propylamine hydrochloride[1-3].

Atomoxetine enhances norepinephrine function through a highly selective blockade of the presynaptic norepinephrine transporter and has low affinities for other neuronal transporters or neurotransmitter receptor sites. This is an interesting and potentially important new drug since it is likely to be the first approved treatment for attention deficit hyperactivity disorder that is not a psychostimulant. Studies with this compound in healthy human volunteers showed that the clearance of atomoxetine exhibited a bimodal distribution, suggesting that an enzyme that exhibits a genetic polymorphism was involved in the metabolism of atomoxetine[4].

Atomoxetine capsule (Strattera 10 mg) are available with the following inactive ingredients: pregelatinized starch, dimethicone, gelatin, sodium lauryl sulfate, titanium dioxide and titanium dioxide[5]. HPLC analytical method with mass spectrometry (MS) detection has already been described to quantify atomoxetine alone in biological samples[6].

Literature reveals that no high performance thin layer chromatographic method was available for the determination of atomoxetine hydrochloride from bulk drug and its pharmaceutical preparations. Therefore a fast, economical, precise and accurate HPTLC method was developed for the determination of atomoxetine hydrochloride from its bulk drug and pharmaceutical preparations.

EXPERIMENTAL

The formulation was purchased from market while standards were procured from reputed research centers. Acetonitrile and glacial acetic acid were from Qualigens. All dilutions were performed in standard volumetric flasks.

Standard stock solution was prepared by weighing 99.61 % pure atomoxetine hydrochloride (25 mg) into a 25 mL volumetric flask, dissolving in methanol:water (1:1), to get 1mg/mL of atomoxetine hydrochlride.

Chromatography

Chromatography was performed on pre-coated silica gel 60 F 254 HPTLC plates (Merck). Before use they were pre-washed with methanol and dried in an oven at 110 ºC for 1 h. Samples (10 µL) were spotted 20 mm from the edge of the plates by means of a Camag Linomat IV sample applicator and the plates were developed to a distance of 90 mm in a Camag twin-through chamber previously equilibrated with mobile phase *i.e.*, acetonitrile:glacial acetic acid (9:1 by volume). The chromatographic conditions was optimized to achieve the best resolution and peak shape.

Plates were evaluated by densitometry at λ = 269 nm with a Camag Scanner II, in conjunction with CATS software for quantitation. The wavelength used for densitometry was selected after acquiring *in situ* UV spectra of the drug.

Linearity of detector response: Solution containing atomoxetine hydrochloride seven different concentrations was prepared in methanol:water. Each of these solutions (10 mL) was applied to a plate, the plate was developed and the detector response to the different concentrations was measured. The drug peak area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area. The plot was linear for atomoxetine hydrochloride in the concentration range 100-1000 µg. This experiment was carried out thrice and the mean was used for the calculations. The data were analyzed by linear regression least-squares fitting[7,8]. The statistical data obtained are given in Table-1.

TABLE-1
ANALYTICAL PERFORMANCE DATA

	Atomoxetine hydrochloride
Linear working range (LWR) (μg)	100-1000
Slope (m)	0.194
Intercept (b)	-0.078
Correlation coefficient (R)	0.997

Assay

From the pharmaceutical preparation: 20 Capsules were weighed and the average weight was calculated. The capsules were crushed to furnish a homogeneous powder and a quantity equivalent to one capsule (184.5 mg) was weighed in a 10 mL standard volumetric flask, dissolved in methanol. The IS solution (1 mL of 10000 μg mL^{-1}) was added and the solution was diluted to volume with methanol. The solution was filtered through Whatmann no. 41 filter paper. The procedure was repeated seven times, individually weighing the capsule powder each time. Standard and sample solutions (10 mL) were spotted on the plate and the plate was developed and evaluated as described above. The densitometric responses from the standard and sample were used to calculate the amounts of the drug in the capsule.

From the bulk drug: Accurately weighed 10 mg of bulk drug of atomoxetine hydrochlride and the above mentioned procedure was applied. The results of assay are tabulated in Table-2.

TABLE-2
ASSAY EXPERIMENT

From pharmaceutical preparations			From bulk drug		
Weight of sample taken (mg)	Amount found (mg)	Percent assay	Weight of sample taken (mg)	Amount found (mg)	Percent assay
167.11	10.31	99.18	10.1	10.10	99.56
167.50	10.43	100.26	10.4	10.11	99.88
167.23	10.40	100.03	10.3	10.04	98.44
166.59	10.30	99.02	10.4	10.06	100.61
168.99	10.48	100.77	10.5	9.94	99.42
167.24	10.31	99.15	10.5	10.08	100.01
165.32	10.31	100.56	10.1	10.07	100.14
Mean	10.36	98.68		10.06	99.55
Coefficient of variation (%)	0.65	0.79		0.52	0.64

RESULTS AND DISCUSSION

Use of pre-coated silica gel HPTLC plates with acetonitrile-glacial acetic acid (9:1 by volume) resulted in good separation of the drug.

Regression analysis of the calibration data for atomoxetine hydrochlride showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equations Y = 0.194X-0.078. The correlation of coefficient obtained was 0.997.

The system suitability experiment was carried out before the determination of atomoxetine hydrochlride in unknown samples. The coefficient of variation was less than 2 % for replicate measurements of the same sample. This shows that the method and the system both are suitable for the determination of unknown samples.

The precision studies including the instrument precision, intra-assay precision and intermediate precision was carried out to evaluate the precision of the method. The intermediate precision included analysis on a different day and by a different analyst. The values of standard deviation and coefficient of variation were calculated. The standard deviations for intra assay precision was in the range of 0.32 to 1.64 while for inter assay precision was 0.22 to 0.51 (Table-3). The coefficient of variation was in the range of 0.83 to 1.80 for intraday and for interday, the range was 0.56 to 0.91. The low values of standard deviation and coefficient of variation indicate high precision of the method.

TABLE-3
PRECISION EXPERIMENT

Obs. No.	Conc. of drug (µg)	Day to day comparison			Analyst to analyst comparison		
		Mean	SD	COV (%)	Mean	SD	COV (%)
1	30.0	30.26	0.32	1.07	30.23	0.22	0.73
2	60.0	60.45	0.50	0.83	60.19	0.55	0.91
3	90.0	90.89	1.64	1.80	90.18	0.51	0.56

The accuracy of the experiment was established by spiking pre-analyzed sample with known amounts of the drugs at three different concentration levels *i.e.* 80, 100 and 120 % of the drug in the capsule (the external standard addition technique). The spiked samples were then analyzed for seven times. The results from recovery analysis are given in Table-4. The mean recovery is within acceptable limits, indicating the method is accurate.

To study the specificity of the method for the determination of atomoxetine hydrochloride, the pure standard was subjected to various stress conditions like heat, light, oxidation and hydrolysis. The specificity of the

TABLE-4
ACCURACY AND RECOVERY ANALYSIS

Set No.	Atomoxetine hydrochloride (10 mg) Mean assay		
	Amount of drug added	Amount of drug found	Recovery (%)
SET 1 (80 %)	18.21	18.05	99.12
SET 2 (100 %)	20.12	20.08	99.80
SET 3 (120 %)	22.02	21.95	99.68

method was demonstrated by the separation of the probable impurities and probable degradation products from the main peak of atomoxetine hydrochloride. The robustness of the method was studied simultaneously along with the method development process.

Conclusion

The proposed method is highly accurate, selective and precise and can therefore be used for a routine quality-control analysis and quantitative determination of atomoxetine hydrochlride in pharmaceutical preparations as well as in bulk drug.

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(*Received*: 1 September 2007; *Accepted*: 2 May 2008) AJC-6554