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Rapid and Selective LC method for estimation of Duloxitine Hydrochloride

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Abstract

The Duloxitine Hydrochloride was estimated by using a HPLC method. It was separated on a Waters symmetry C_8 HPLC column (250 × 4.6 mm, 5µ) by passing mobile phase at a flow rate of 1.0 ml/min and the solution was monitored at a wavelength of 288nm. The retention time of the duloxitine was found to be 7.39 min. The developed method was validated according to ICH guidelines. The developed method was accurate, precise and linear. This method can be successfully applied in quality control laboratories for routine analysis of duloxitine in pharmaceutical formulations.

Keywords: HPLC; Duloxitine hydrochloride; ICH; Validation

Introduction

Chemically duloxetine [1] is (+)-(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine. The empirical formula of duloxitine is $C_{18}H_{19}NOS$. HCl and its molecular weight is 333.88 g. Chemical structure of duloxitine is given in Figure 1. It is available in 30 and 60mg capsules for oral administration. It is used as an antidepressant and also to treat major depressive disorder, general anxiety disorder, stress urinary incontinence, painful peripheral neuropathy and fibromyalgia. The antidepressant activity of duloxitine may be due to potentiation of serotonergic and noradrenaergic activity in the central nervous system.

Literature survey reveals that various UV [2-4] and HPTLC [5] methods have been reported for the estimation of duloxitine. The aim of the study is to develop and validate a sensitive, rapid, accurate and precise RP-HPLC method for quantification of duloxitine in bulk and its formulations.

Materials and Method

Chemicals and reagents

Duloxitine HCl working standard of 99.8% purity was obtained from Hetero Labs, Hyderabad. Duloxitine HCl capsules were procured from the local market. Acetonitrile & Methanol of HPLC grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. All the other reagents such as disodium hydrogen phosphate, hydrochloric acid, tri fluoro acetic acid, sodium hydroxide potassium di hydrogen phosphate are of Analytical grade.

Apparatus

The separation of duloxitine was carried out on Waters alliance HPLC consisting of 2695 separation module, dual wavelength UV detector waters 2489 and output signal was monitored and integrated using Empower software.

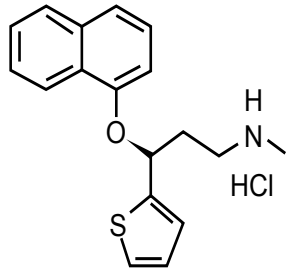

Figure 1: Chemical structure of duloxitine.

Assay method

Preparation of mobile phase: Solution A: 2 ml of Tri-fluoro acetic acid was transferred into a beaker containing 1000 ml of Milli-Q water mixed and filtered through 0.45 membrane filter.

Solution B: 2 ml of Tri-fluoro acetic acid was transferred into a beaker containing 1000 ml of Acetonitrile, mixed and filtered through 0.45 membrane filter.

Mobile phase: It was prepared by mixing solution A and solution B in the ratio of 55:45% v/v and the solution were degassed prior to use.

Preparation of diluent

Diluent was composed of solution C and methanol in the ratio of 50:50% v/v.

Preparation of solution C:

1.41g Di-sodium hydrogen phosphate anhydrous was weighed and transferred into a beaker containing 1000 ml of Milli-Q water and the solution was filtered through 0.45 membrane filter.

Standard preparation: Accurately about 68 mg of Duloxitine HCl working standard was weighed and transferred into 100ml volumetric flask, 20ml of methanol was added, and sonicated to dissolve and make up to volume with diluent. From this 5ml of the solution was taken and diluted to 50ml with diluent.

Sample preparation: 10 capsules of Duloxitine HCl were weighed and contents of the capsules were emptied and mixed. Duloxitine HCl pellets equivalent to 60 mg Duloxitine HCl was weighed and transferred

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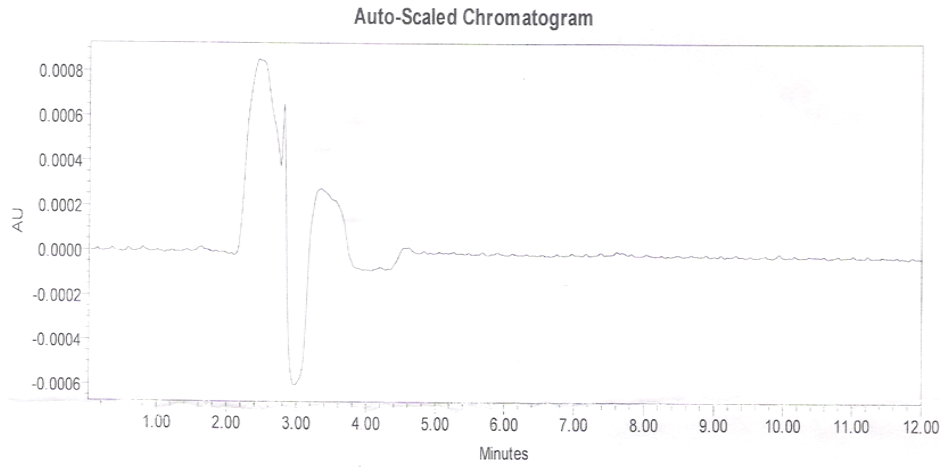


Figure 2: Chromatogram of placebo solution.

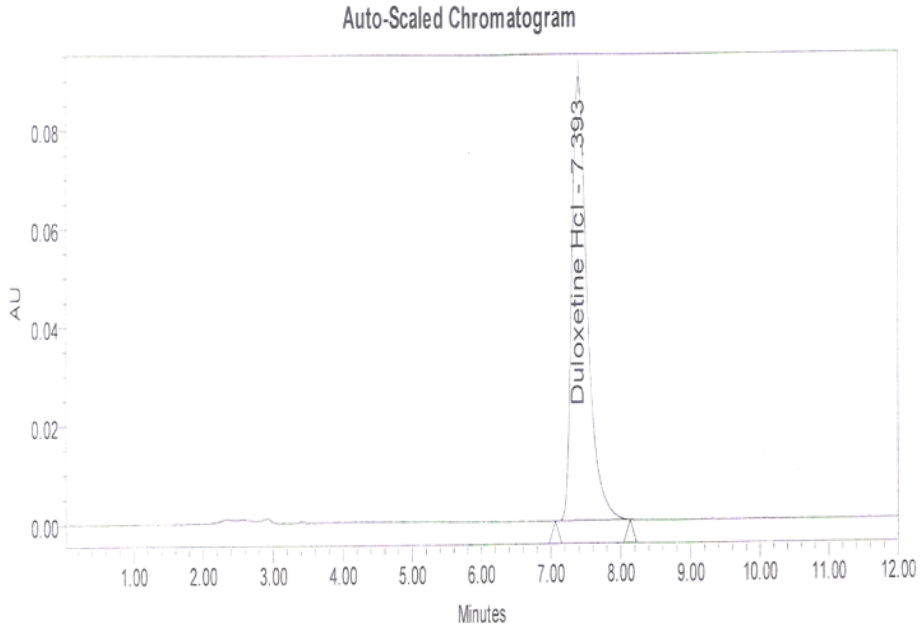


Figure 3: Chromatogram of Duloxetine Hydrochloride standard.

into a 100 ml volumetric flask., 50 ml of methanol was added and sonicated for 20 min, with intermediate shaking until pellets completely dissolve (maintain the sonicate bath temp between 20-25) and about 20 ml of buffer was added and again sonicated for 15 min. and diluted to final volume with buffer. The resulting solution was mixed and centrifuged for 10 min at 5000 rpm.5.0 ml of the clear supernatant solution was transferred into a 50 ml volumetric flask and diluted to 50 ml with diluent and mixed. The solution was filtered through 0.45 membrane filter.

Placebo preparation: Transfer placebo powder (60 mg equivalent weight of Duloxitine HCl) into 100 ml volumetric flask. Add 50 ml of diluents dissolve and dilute to volume with mobile phase. Mix well. Centrifuge a portion of the sample at 3000 rpm for 5 minutes. Transfer 5 ml of this solution into 50 ml volumetric flask and dilute to volume with diluent.

Procedure: The mobile phase was filtered through 0.45µ membrane filter before use, degassed and was pumped from the solvent reservoir into the Waters symmetry C8 column at a flow rate of 1 ml/min. The detection was monitored at 288 nm and the runtime was 10 min. The placebo, standard and sample solutions were separately injected into the liquid chromatography and chromatograms were recorded (Figures 2-4). The amount of Duloxetine Hydrochloride present in pellets was calculated from the peak areas of recorded chromatograms.

Method Validation

The developed method [6,7] was validated as per ICH [8] guidelines.

Specificity

A study was conducted to demonstrate the effective separation of degradants from Duloxetine. Standard drug solution was exposed

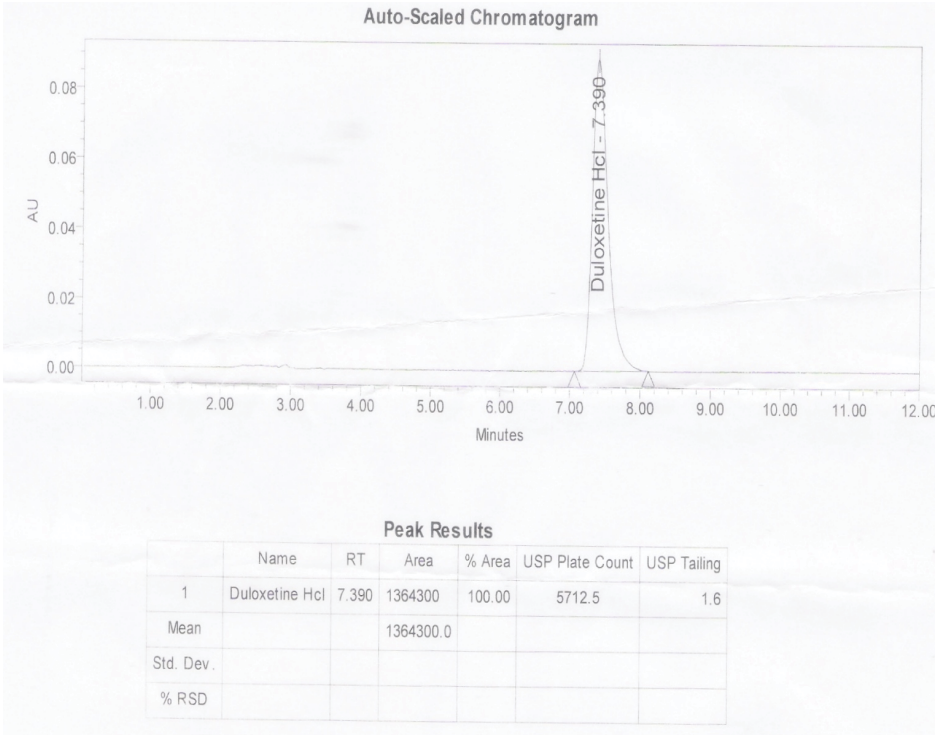
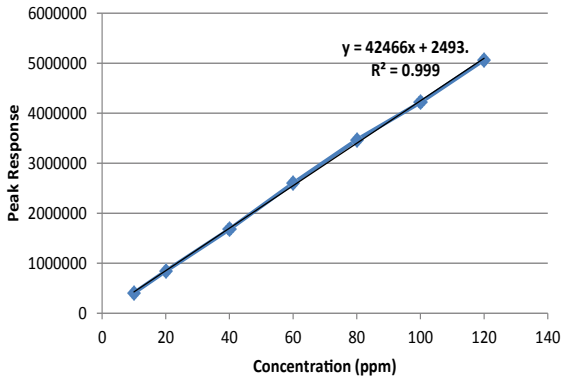


Figure 4: Chromatogram of Duloxetine Hydrochloride sample.

S.No	Area of the peak
1	1394665
2	1385176
3	1394678
4	1388509
5	1400300
Average area	1392666
standard Deviation	5909.8
%RSD	0.4

Table 1: Precision.



Graph 1 1: Linearity Curve of Duloxetine Hydrochloride.

to acid (0.1 N HCl), base (0.01N NaOH), peroxide (3.0% $H_2O_{2)}$, and thermal (105°C), UV light (254 nm) to induce the degradation. Stressed samples were injected into the HPLC system. All degradants peaks were resolved from Duloxetine peak in the chromatograms of all samples.

Precision

The precision of the method was tested by injecting six replicate injections of standard solutions into the chromatograpFhic system and the peak areas of the responses were recorded. The %RSD (Relative Standard Deviation) for six injections should not be more than 2.0 (Table 1).

Linearity

Linearity of the analyte was performed by injecting 10%, 20%, 40%, 60%, 80%, 100%, and 120% of the standard concentration into the liquid chromatography and mean of the area were recorded. A graph of concentration against peak area was plotted and correlation coefficient was calculated from the graph (Graph 1). the correlation coefficient should be not less than 0.99 (Table 2).

Accuracy

The percentage recovery of the standard was determined by spiking known concentration of standard to the placebo matrix at three different concentrations (50%, 100% and 150% of Duloxetine Hydrochloride) in triplicate. The percentage recovery should be between 95 to105percent (Table 3).

Robustness

The robustness of the analytical method was estimated by checking the system suitability under deliberately modified chromatographic conditions (flow rate, mobile phase ratio) and % RSD values were calculated (Table 4)

Ruggedness

The Ruggedness of the analytical method was estimated by testing

Linearity	Concentration (ppm)	Response
1	10	406198
2	20	844162
3	40	1677435
4	60	2598515
5	800	3468416
6	100	4215690
7	120	5067282
Correlation Coefficient		0.999

Table 2: Linearity Data.

Concentration	Amount added (ppm)	Amount found(ppm)	%Recovery	Statistical Analysis
50% spl-1	40.32	39.80	98.7	Mean=99.5% SD=0.71 %RSD=0.71
50% spl-2	40.51	40.55	100.1	
50% spl-3	39.96	39.80	99.6	
100% spl-1	80.11	80.35	100.3	Mean=99.7% SD=0.60 %RSD=0.60
100% spl-2	79.98	79.26	99.1	
100% spl-3	80.26	80.10	99.8	
150% spl-1	118.95	117.64	98.9	Mean=99.4% SD=0.55 %RSD=0.55
150% spl-2	120.05	119.33	99.4	
150% spl-3	120.47	120.47	100	

Table 3: Accuracy data.

Chromatographic parameter	Conditions	% RSD
Flow Rate	0.8ml	1.44
	1 ml	0.93
	1.2 ml	1.50
Mobile Phase (Organic/aqueous)	50/45	0.44
	55/45	0.93
	60/45	0.90

Table 4: Robustness.

S.NO	Data File	% Assay	
		Analyst-1	Analyst-2
1	Sample--001	99.6	98.8
2	Sample--002	101.2	100.1
3	Sample--003	98.5	99.6
4	Sample--004	100.2	98.8
5	Sample--005	99.1	98.5
6	Sample--006	99.8	100.6
Average		99.7	99.4
SD		0.93	0.84
% RSD		0.93	0.84

Table 5: Ruggedness.

assay of six different test solutions for the same batch under different Analyst on different days and %RSD was calculated (Table 5).

Results and Discussion

Under Optimised chromatographic conditions, the retention time of duloxitine was found to be 7.39. The system suitability factors were within the limits. Tailing factor and theoretical plates were 1.6 and 5712 respectively. The %of the drug in the marketed formulation was in between 98.5-101.2. The % RSD values for precision and ruggedness were 0.93% and 0.84% respectively. The method was linear over the concentration range 10-120 ppm with correlation coefficient of 0.999. The mean %recoveries lie between 99.4-99.7%.

Conclusion

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of duloxitine HCl and can be reliably adopted for routine quality control analysis of duloxitine HCl in bulk and its pharmaceutical formulations.

Acknowledgement

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