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DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR DULOXETINE HYDROCHLORIDE IN CAPSULE FORMULATIONS BY HPLC-UV

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Abstract:

The objective of the current study was the development of a simple, precise and accurate isocratic reversed-phase High Performance Liquid Chromatography [Rp-HPLC] assay method and validated for determination of Duloxetine hydrochloride in capsule dosage forms. Isocratic separation was achieved on a C18 Inertsil ODS (5µ, 250mm× 4.6mm) with flow rate of 1.0ml/min using UV detection at 230nm. The mobile phase composed of ammonium acetate buffer pH 5 adjusted with dilute acetic acid, methanol and Acetonitrile in the ratio of (50:20:30 v/v). The injection volume was 10.0 µl and the detection was carried out at 230 nm by using photo-diode array detector. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The method was linear in the drug concentration range of 80-120 µg/ml, with a correlation coefficient of 0.9999. The precision (% RSD) of six samples was 0.10 % for repeatability and the intermediate precision [RSD] among six-sample preparation was 0.142%. The accuracy (recovery) was between 99.58% and 100.97%. LOD and LOQ was found to be 0.0038 µg/ml and 0.0126 µg/ml. The proposed method was successfully used to determine the drug content of marketed formulation.

Keywords: *Duloxetine hydrochloride, Rp-HPLC, Inertsil ODS, methanol and Acetonitrile.*

INTRODUCTION:

Duloxetine hydrochloride, a selective serotonin and norepinephrine reuptake inhibitor (SSNRI) is used for the treatment of major depressive disorder and anxiety [1]. Its chemical designation is (+)-(S)-N-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride. The empirical formula is C18H19NOS.HCl and having a molecular weight of 333.88 [2]. The structure is shown in Figure 1.

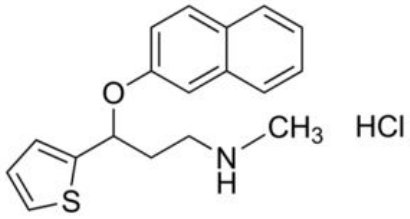

Figure 1: Chemical structure of Duloxetine hydrochloride

As per the literature survey it is revealed that very few analytical methods were reported. An HPLC method for the simultaneous estimation of key intermediates of Duloxetine HCl has been reported [3]. HPLC analysis of the novel antidepressant Duloxetine in human plasma after solid-phase extraction procedure has also been reported [4]. Moreover Duloxetine HCl has been determined in the presence of process and degradation impurities by a validated stability indicating RP-LC method [5]. A RP-LC method development and validation determination for estimation of Duloxetine HCl in enteric coated capsules has also been reported [6]. Literature reported the characterization of phenolic impurities in duloxetine HCl samples by MS, NMR, X-ray-analysis [7] and impurities formed by interaction of duloxetine HCl with various enteric polymers [8]. The aim of current research work was to develop a new simple, reliable and reproducible RP-HPLC method for which validation and recovery studies were conducted and studied by using various statistical parameters according to ICH guidelines [9,10].

MATERIALS AND METHODS:

Chemicals and solvents:

Pure drug of Duloxetine was procured as a gift sample from Torrent Pharmaceuticals, Ahmadabad, India. Capsules of duloxetine were purchased from the local pharmacy of Anantapur. HPLC Grade Acetonitrile and Methanol were procured from SD Fine Chem, Mumbai, India. HPLC grade water was prepared in house with Milli-Q water purifier system. All the other chemicals used were AR Grade.

HPLC–UV instrumentation and chromatographic conditions:

The HPLC system was an LC Waters (Waters, Milford, MA, USA) consisting of quaternary gradient system (600 Controller), in line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA). Chromatographic separation assay was performed with a C18 Inertsil ODS (150 mm × 4.6 mm inner diameter, 5 μm particle size, Waters, Dublin, Ireland) maintained at ambient temperature. The mobile phase consists of ammonium acetate buffer pH 5 adjusted with dilute acetic acid: methanol and Acetonitrile in the ratio of (50:20:30 v/v). The mobile phase was pumped at a flow rate of 1.0 mL min−1. The detection wavelength was 230 nm. Mobile phase was used as diluent for the preparation of working standards of duloxetine.

Standard and sample solutions

Stock solution of duloxetine in mobile phase with a concentration of 1000 μg mL^{-1} was prepared using volumetric flask. This was stored at +4 °C. To obtain a final concentration of 60 μg mL^{-1}, an appropriate dilution of the stock standard solution was prepared by diluting 80–120 μL with mobile phase. Working solutions were prepared daily from the above mentioned stock solutions. The containers used for storage were screw-capped tubes coated externally by aluminum foil.

Assay of pharmaceutical preparation:

To carry out the sample solution, 20 capsules were taken and weighed individually, obtaining afterwards the average weight of these capsules. An appropriate portion of this powder, equivalent to 100 mg of duloxetine was weighed and placed in a 50ml volumetric flask, dissolving it with 25ml of mobile. This solution was sonicated for 15 min to dissolve and remove the entire active from the capsule. Once

the time had elapsed, it was diluted up to 50 ml with additional mobile phase. 1.5 ml of aliquot was taken and transferred to volumetric flask of 50 ml capacity and volume was made up to the mark with the diluent. This solution was used for the estimation of Duloxetine (60µg/ml).

RESULTS AND DISCUSSION:

When methanol and acetonitrile were used in initial scouting, it was observed that both were found to be better in terms of resolution and peak shapes. Therefore, a ratio of methanol: ACN was used as an organic modifier for method development. We have avoided strongly acidic and strongly basic buffer as they were non-volatile and non ameanable with UV detector. Dipotassium phosphate buffer was chosen, but peak shapes and tailing factor were found to be unacceptable for analyte. The effect of different pH and mobile phase composition were also tried to improve the resolution and peak symmetry. The peak shape and symmetry was found to be improved with Ammonium acetate buffer. In addition to commonly using C18 column, C_8 stationary phase was also selected. In an attempt to improve peak symmetry and resolution on C18 column, various combinations of Ammonium acetate buffer, methanol and ACN was used in different proportions. The pH of the buffer was adjusted to 5 with dilute acetic acid. Therefore, C_{18} column with ammonium acetate buffer pH 5 adjusted with dilute acetic acid, methanol and Acetonitrile in the ratio of (50:20:30 v/v) was selected for further studies. A summary of the optimized HPLC conditions for the estimation of Duloxetine was shown in table 1.

Table 1: Optimized HPLC conditions for the estimation of Duloxetine

S. No	Parameter	Description/Value
1.	Stationary Phase	C18 Inertsil ODS (5µ, 250mm× 4.6mm)
2	Mobile Phase	Ammonium acetate buffer pH 5 adjusted with dilute acetic acid, methanol and Acetonitrile in the ratio of (50:20:30 v/v).
3	Flow rate	1.0ml/min
4	Detection Wavelength	230nm
5	Detector	UV
6	Injection	Auto sampler
7	Injection volume	10.0 µl
8	Column Temperature	Ambient
9	Run time	15 mins
10	Diluent	Mobile phase

Linearity

The calibration curve constructed for duloxetine was linear over the concentration range of 80 – 120 µg/ml. Peak areas of duloxetine were plotted versus duloxetine concentration and linear regression analysis performed on the resultant curve. The samples were analyzed in triplicates at all concentrations. Calibration curve was constructed and found that correlation coefficient value of the studied drug was observed to be $R^2 = 0.999$. Typically, the regression equation for the calibration curve was found to be y = 79.2x - 123.8.

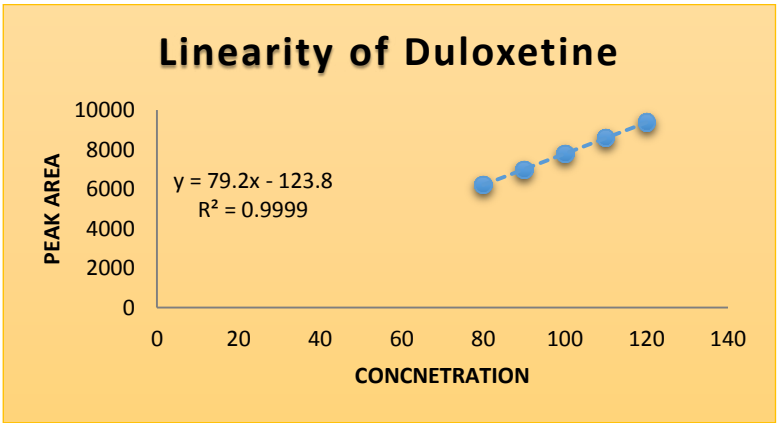

Figure 2: Linearity of Duloxetine

LOQ and LOD

The LOQ and LOD were determined based on signal-to-noise ratios and were determine using an analytical responses of ten and three times the background noise, respectively [9]. The LOQ was found to be 0.0126 µg/ml with a resultant %R.S.D. of 0.4% (n = 5). The LOD was found to be 0.0038 µg/ml.

Precision

Precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability was investigated by injecting six replicate samples of 60 µg/ml standard where % R.S.D value was found to be 0.10 %. Inter-day precision was assessed by injecting the same concentration over 3 consecutive days, resulting in mean % R.S.D. of 0.142 %. The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results for Duloxetine that has been performed by two analysts. The % R.S.D values for intra- and inter-day assays of Duloxetine in the cited formulations performed in the same laboratory by the two analysts did not exceed 2%, thus indicating the ruggedness of the method. The mean retention time of Duloxetine was 11.02 min with % R.S.D. of 1.2%. The data was shown in table 2.

Table 2: Inter and Intraday precision of the method

	Peak area	
S. No.	Interday	Intraday
1	7849	7859
2	7841	7839
3	7842	7836
4	7842	7840
5	7829	7829
6	7853	7853
Average	7842.667	7842.667
SD	8.21381	11.18332
%RSD	0.104732	0.142596

Accuracy

Accuracy of the method was determined by investigating the recovery of drug at three levels 80, 100 and 120 % from placebo mixtures (lactose, maize starch, mannitol, calcium hydrogen phosphate, magnesium carbonate, gelatin, polyvidone micro crystalline cellulose, magnesium stearate, silicon dioxide, titanium dioxide) spiked with the API solution. Each concentration was analyzed in triplicate (Table 3).

Table 3: Results of Accuracy

Level	**Actual conc. of Duloxetine**	**Theoretical concentration**	**Measured concentration**	**% recovery**	**Mean % recovery**
80%	79.0	0.0474	0.0467	98.52	99
	79.5	0.0477	0.0475	99.58	
	78.8	0.0473	0.0464	98.10	
100%	97.7	0.0586	0.0587	100.17	100
	98.7	0.0592	0.0588	99.32	
	98.8	0.0593	0.0591	99.66	
120%	119.2	0.0715	0.0712	99.58	100
	120.8	0.0725	0.0732	100.97	
	118.8	0.0713	0.0711	99.72	

Robustness

The robustness of the developed method was determined by analyzing the samples under a variety of conditions of the method parameters, such as flow rate, pH of the buffer and temperature. Variation of the pH of the mobile phase, flow rate by 1.5 mL/min and temperature by 50 °C did not have significant effect on chromatographic resolution in HPLC method. Figure 3 shows the representative chromatogram of Duloxetine. Table 4 shows the results of robustness.

Table 4: Results of robustness

Parameter condition	Retention time of Duloxetine Hcl	% Assay
Actual	11.07	97.7
High Flow:1.1ml/min	10.17	97.3
Low Flow: 0.9 ml/min	12.31	97.3
High temperature: 30ºc	10.88	97.2
Low temperature : 20ºc	11.34	97.7
High Buffer pH : 5.2	11.33	97.6
Low Buffer pH : 4.8	10.94	97.7

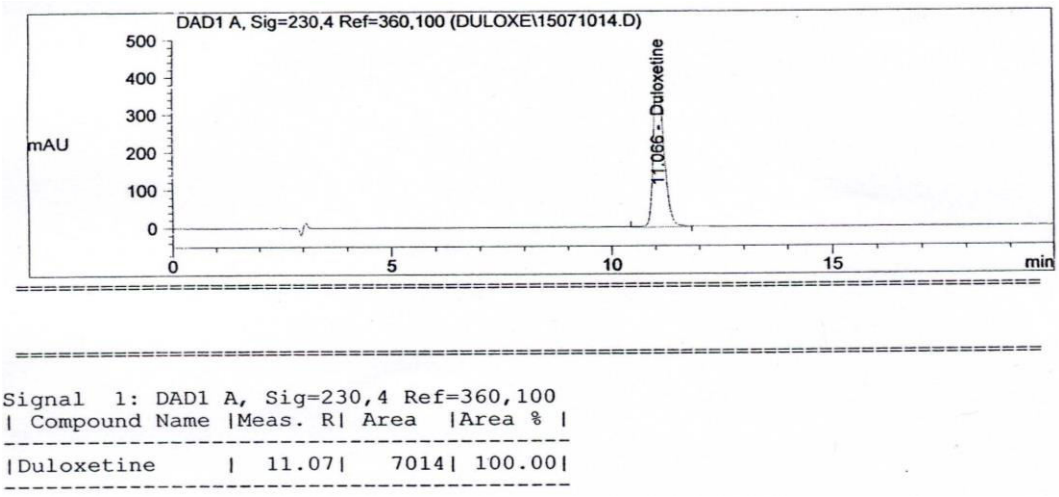

Figure 3: Representative chromatogram of Duloxetine

Quantitative determination in pharmaceutical formulations

Twenty capsules each of Duloxetine were weighed individually and emptied the contents of the capsules, the powder equivalent to 100 mg was weighed and diluted with mobile phase, sonicated for 15 min and further dilutions were made with mobile phase to obtain concentrations within the linearity range (80-120 µg/ml). All the samples were filtered through whatmann (polypropylene, 0.45 mm) syringe filter, before injecting the samples into the HPLC instrument. The assay results were shown in Table 3.

Table 3: Results of Assay of Duloxetine

S. No.	Peak area	% Assay
1	7849	99.99
2	7841	99.9
3	7842	99.92
4	7842	99.92
5	7829	99.86
6	7853	100.01
Average		99.93333
SD		0.056451
%RSD		0.056488

CONCLUSIONS:

A validated HPLC analytical method has been developed for the determination of Duloxetine in API and dosage forms. The results of validation undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective for Duloxetine. The proposed method is simple, accurate, precise, specific, and has the ability to separate the drug from excipients found in the capslue dosage forms. The method is suitable for the routine analysis of Duloxetine in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments, such as LC–MS or GC–MS. These methods are complicated and costly rather than a simple HPLC-UV method. It is also recommended that, the HPLC procedure may be applied to the analysis of samples obtained during accelerated stability experiments to predict expiry dates of pharmaceuticals.

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