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Research Article

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A VALIDATED RP- HPLC METHOD FOR THE ANALYSIS OF DULOXETINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

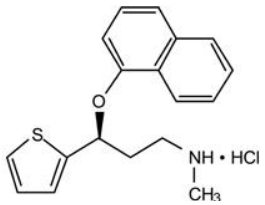
A simple and precise RP-HPLC method was developed and validated for the determination of Duloxetine hydrochloride in pharmaceutical dosage forms. Chromatography was carried out on Inertsil BDS (250x4.6 mm) C8 column using a mixture of Buffer: Acetonitrile: Methanol (55:37:8%) as the mobile phase at a flow rate of 1.0 ml min^{-1}. The analyte was monitored using UV detector at 215 nm. The Retention time of the drug is 6.431 min for Duloxetine. The proposed method is found to be having linearity in the concentration range of 2-12 µg mL^{-1} with correlation coefficient of r=0.9995. The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for Duloxetine HCl are in the range 99.8-100.2 %. Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining Duloxetine HCl in bulk and dosage forms.

Keywords: Duloxetine HCl, RP-HPLC.

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 $C_{18}H_{19}NOS.HCl$ and having a molecular weight of 333.88.[2] The structure is shown in Figure **1**.

Figure 1. Structure of Duloxetine HCl



It is used for the treatment of neuropathic pain associated with peripheral neuropathy especially diabetic polyneuropathy for which it is first-line, and as an add-on treatment in stress urinary incontinence instead of surgery[3-4] also indicated for the management of fibromyalgia.[5-6] It restores the balance of neurotransmitters in the brain like serotonin and norepinehrine.[7] Moreover it is also being used in the treatment of peripheral neuropathy caused by certain anti cancer drugs.[8] 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.[9]

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HPLC analysis of the novel antidepressant Duloxetine in human plasma after solid-phase extraction procedure has also been reported.[10] Moreover Duloxetine HCl has been determined in the presence of process and degradation impurities by a validated stability indicating RP-LC method.[11] A RP-LC method development and validation determination for estimation of Duloxetine HCl in enteric coated capsules has also been reported.[12] Literature reported the characterization of phenolic impurities in duloxetine HCl samples by MS, NMR, X-ray-analysis[13] and impurities formed by interaction of duloxetine HCl with various enteric polymers[14] 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.[15]

MATERIALS AND METHODS

Instrumentation

Analysis was performed using High Performance Liquid Chromatography System (HPLC) Waters 2695 model equipped with a UV-Visible detector. The output signal was monitored and processed using Empower software.

Chemicals and reagents

Duloxetine HCl was obtained as a gift sample from Torrent pharmaceuticals Ltd., Ahmedabad. Potassium dihydrogen phosphate (AR grade) was used for preparing buffer and acetonitrile HPLC grade was purchased from Merck.

Chromatographic conditions

Mobile phase consists of phosphate buffer, acetonitrile and methanol in the ratio 55:37:8 %. Buffer was prepared by dissolving 2.72 g of potassium dihydrogen phosphate in

1000 ml water and filtered through 0.45 µ membrane filter. The mobile phase was pumped from the solvent reservoir to the column at a flow rate 1.0 ml min-1. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The eluents were monitored at 215 nm. Diluent: Mobile phase.

Methodology

60 mg of accurately weighed Duloxetine HCl was dissolved in 100 ml volumetric flask containing 70 ml of diluents. The solution was sonicated for 20 min to dissolve the drug completely and the volume made up with mobile phase. Subsequent dilutions of this solution ranging from 2-12 µg ml^{-1} were made with the mobile phase in 10 ml volumetric flasks. These solutions were filtered through 0.45 µ membrane filter. 10 µl of the filtrate was injected 6 times into the column and the corresponding chromatograms were obtained. Drug was analyzed at 215 nm. Retention time and mean peak areas were recorded for all the concentrations obtained from the chromatograms. A calibration curve of mean peak area to respective concentration was plotted; the regression of the drug concentrations over the peak area was computed using least square method of analysis. Regression equation was used to estimate the amount of Duloxetine HCl in capsule formulations.

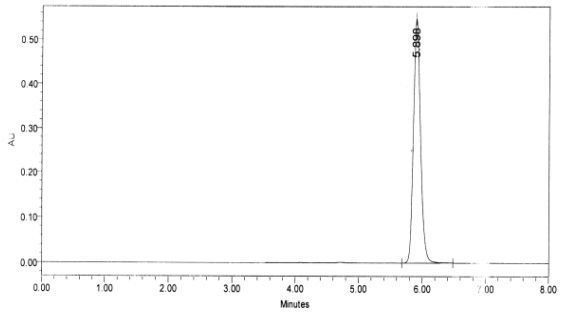
Estimation of Duloxetine HCl in Capsule dosage forms

20 capsules were emptied and content was mixed properly. Pellets equivalent to 15 mg of Duloxetine HCl were weighed and transfered into a 50 ml volumetric flask containing 30 ml of diluent. Solution was sonicated for 20 min to ensure complete solubility. The solution was made up to the mark with mobile phase and filtered through 0.45 µ membrane filter. 4 ml of this solution was pipetted into 100 ml volumetric flask and diluted with the mobile phase to get concentration of 12 µg ml^{-1}. Each of these solutions was injected twice into the system and the chromatograms were recorded. The mean peak areas of the drug of five such determinations were calculated and the drug content in the capsules was quantified using the regression equation obtained for the pure sample.

RESULTS AND DISCUSSION

Several systematic trials were performed to optimize the chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of Duloxetine HCl in pharmaceutical dosage forms. The present method contains mobile phase 0.02 mM phosphate buffer, acetonitrile and methanol in the ratio (55:37:8 v/v) which was found to be the most suitable as the chromatographic peaks obtained with this system were better defined, resolved and almost free from tailing. Under the above conditions the retention time obtained for Duloxetine HCl was 5.898 min. A model chromatogram was shown in Figure **2**.

Figure2. Representative Chromatogram for Duloxetine HCl



The calibration curve for Duloxetine HCl was drawn by plotting the mean peak area versus concentration of Duloxetine HCl, yielded coefficient of regression r^2=0.9995 over a concentration range (2-12 µg ml^{-1}) the representative linear regression equation for Duloxetine HCl Y= 473063x +30563 as shown in Table **1** and Figure **3**.

Table 1. Calibration of the proposed HPLC method

Concentration (µg ml^{-1})	Mean Peak Area(N=3)
2	932807
4	1941270
6	2893511
8	3872546
10	4718091
12	5693786

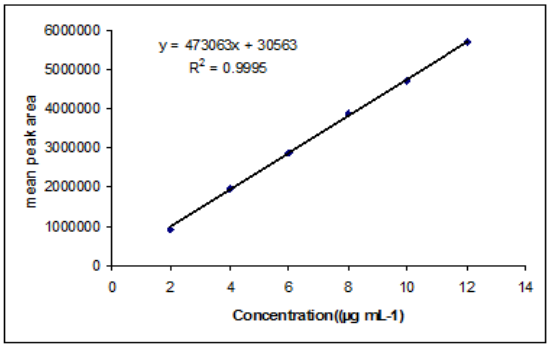


Figure 3. Linearity plot for Duloxetine HCl

To study the accuracy of the proposed analytical method, recovery experiments were conducted using standard addition method. To ascertain whether excipients interfered with the analysis, known amount of pure drug at a different concentration levels were added to the Duloxetine HCl formulation and the mixtures were analyzed by the proposed method. The accuracy of the method was demonstrated at three different concentration levels in triplicate. The analysis carried out at 50%, 100% and 150% of specification limit. High recovery values were obtained while the samples being analyzed from the developed method. The % recovery results of the method are given in Table **2**.

Table 2. Recovery data of standard solutions added to the samples analyzed by using the proposed HPLC method

Amount of drug added (µg ml^{-1})	Amount found (µg mL^{-1}) (N=3)	%Recovery(N=3)
4	4.01	100.2
8	7.96	99.5
12	11.84	98.6

The developed HPLC method in the present study has also been used to quantify Duloxetine in the capsule dosage forms. Duloxetine HCl was quantified using the proposed analytical method and the results are given in Table **3**.

Table 3. Assay of Duloxetine HCl in capsule dosage forms by proposed HPLC method

Label claim (mg)	Observed amount (mg)	% Purity
20	19.64	98.2
30	30.29	100.9
40	39.65	99.1

CONCLUSION

From the obtained results it can be concluded that this method is quite precise and accurate. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the

Duloxetine HCl. The proposed HPLC method is sensitive and reproducible for the analysis of Duloxetine HCl in pharmaceutical dosage forms. The method was duly validated by using required statistical parameters.

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