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Development and validation of RP-HPLC method for determination of Duloxetine hydrochloride in bulk and dosage form

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the determination of duloxetine hydrochloride in bulk and dosage form. The separation was effected on a kromasil ODS C₁₈ column (250mmX4.6mm, 5 μ) using a mobile phase mixture of buffer and methanol in a ratio of 85:15 v/v at a flow rate of 1.0ml/min. The detection was made at 230nm. The retention time of duloxetine hydrochloride was found to be 3.443 \pm 0.06 min. Calibration curve was linear over the concentration range of 20-120 μ g/ml of duloxetine hydrochloride. The propose method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

Key Words: Method development and validation, duloxetine hydrochloride, Tablets, Kromasil C₁₈ column, RP-HPLC.

INTRODUCTION

Duloxetine hydrochloride (+)-(s)-N-methyl-3-(1-naphthyloxy)-3-(thiophen-2-yl)-propan-1-amine (The Merck Index, 2001) is a potential dual inhibitor of the reuptake of serotonin and norepinephrine. It has been approved by the US Food and Drug administration for the treatment of major depressive disorder and for the diabetic peripheral neuropathic pain. It belongs to the class narcoleptics. Literature survey reveals that only a few methods based on RP-HPLC method were developed and validated for the determination of duloxetine hydrochloride in pharmaceutical dosage forms (Dasari *et al.*, 2010). Few others are: LC-tandem mass spectrometry method for the determination of duloxetine in human plasma (Senthamil *et al.*, 2007), stability indicating RP-HPLC method for the duloxetine (Dahivelkar *et al.*, 2010), metabolism, excretion and pharmacokinetics of duloxetine in healthy human subjects (Lantz *et*

al., 2003), duloxetine hydrochloride in Pharmaceutical formulation by HPLC with UV detection (Lakshmana *et al.*, 2010), spectrophotometric method in ultraviolet region for the determination of duloxetine hydrochloride in bulk and in pharmaceutical formulations (Yunoos *et al.*, 2010), spectrophotometric method for quantification of duloxetine hydrochloride in capsule dosage form (Raj *et al.*, 2011), liquid chromatography-mass spectrometric (LC/MS) method for the determination of duloxetine in human plasma using flupentixol as the internal standard (Ma *et al.*, 2007), determination of duloxetine in human plasma by HPLC with column switching and ultraviolet spectroscopy (Waldschmitt *et al.*, 2007), analysis of the duloxetine in human plasma after solid-phase extraction procedure (SPE) (Mercolini *et al.*, 2007), HPTLC method for its estimation as bulk drug and its tablet dosage form (Dhaneshwar *et al.*, 2008) etc. Duloxetine hydrochloride is being marketed in both domestic and international market. No official method has been found in any of the pharmacopoeia. The present investigation by the author describes a rapid, accurate and precise RP-HPLC method for the determination of the drug from bulk sample and

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pharmaceutical dosage form. The detector responses were linear in the concentration range of 20-120 μ g/ml of drug. The method was validated as per ICH guidelines.

EXPERIMENTAL

Chromatographic conditions

Shimadzu high pressure liquid chromatographic instrument provided with a kromasil ODS C₁₈ column (250mmX4.6mm, 5 μ) and LC 20 AD Pump and Prominence SPD 20A UV-deuterium detector was employed in the study. A 20 μ L Hamilton injection syringe was used for sample injection (Bhimanadhuni *et al*, 2012). Data acquisition was performed by using Spinchrome software, Shimadzu Class VP version 6.12 SPS data system. HPLC grade methanol and water were purchased from E. Merck Co, Mumbai, India and Potassium dihydrogen phosphate and ortho phosphoric acid AR grade were purchased from SD Fine Chem Mumbai, India.

Drug samples

The reference sample and branded formulation of duloxetine hydrochloride was supplied by M/s Orchid Pharmaceuticals, Chennai, India.

Mobile phase

Accurately 1.36g of potassium dihydrogen phosphate was weighed out and dissolved in 550ml of water. 0.3g of dihydrogen potassium phosphate was weighed out and dissolved in 450ml of water. Both solutions were mixed to prepare buffer solution. The solution was filtered through 0.45 μ m membrane filter and was degassed (Bhimanadhuni *et al*, 2012). A freshly prepared binary mixture of buffer:Methanol in a ratio of (85:15) V/V was used as the mobile phase. Methanol was used as diluent for preparing the working solution of the drug. The mobile phase was filtered through 0.05 μ m membrane filter and sonicated by using Power Sonicator (Model 405), Hwashin Technology, Korea before use. The flow rate of the mobile phase was maintained at 1ml/min. The column temperature was maintained at 25°C and the detection of the drug was carried out at 230nm.

Stock and working standard solution

About 25mg of duloxetine hydrochloride was weighed accurately and transferred into 25ml vo-

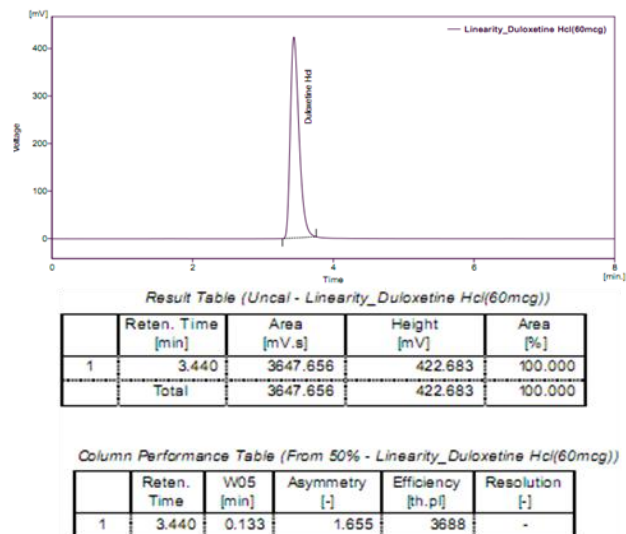


Figure 1: Chromatogram of duloxetine hydrochloride 60 μ g/ml.

lometric flask. The solution was sonicated and filtered through whatman filter paper and resulting solution was diluted with the mobile phase to get a working standard solution of 25 μ g/ml of duloxetine hydrochloride.

Calibration curve

The quantitative determination of the drug was accomplished by a standard method. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution. Linearity of the peak area response was determined by taking measurement at six concentration prints (6 replicates at each point) working dilution of duloxetine hydrochloride in the range of 20-120 μ g/ml were prepared by taking suitable aliquots of working standard solution in different 10ml volumetric flasks and diluting up to the mark with the mobile phase. Twenty micro lot quantity of the dilution was injected each time in to the column at a flow rate 1.0ml/min. Each dilution was injected 6 times into the column. The drug in the elutes was monitored at 230nm and the corresponding chromatograms were obtained. Form these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed. The regression of the plot was completed by least squares regression method. A linear relationship in the range was found to the 20-120 μ g/ml of the drug between the concentration of duloxetine hydrochloride and respective peak area. This regression equation was

Table 1: Calibration data of the proposed method.

Concentration of duloxetine hydrochloride ($\mu\text{g/ml}$)	Mean peak area (n=6)
20	1244.002
40	2371.692
60	3647.656
80	4977.042
100	6136.990
120	7299.147

Concentration range = 20-120 $\mu\text{g/ml}$ Slope (m) = 61.287
 Correlation coefficient (r^2) = 0.99970 Intercept (b) = -10.1785

later used to estimate the amount of duloxetine hydrochloride in pharmaceutical dosage form. A representative chromatogram for the separation of duloxetine hydrochloride is given in figure 1.

Sample solution

Twenty tablets of duloxetine hydrochloride were weighed and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 100mg of duloxetine hydrochloride was transferred into 100ml volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100ml with mobile phase. Then the mixture was filtered through a 0.45 μm membrane filter. From the above solution 1ml aliquot was taken into a separate 10ml volumetric flask and diluted up to the volume with the mobile phase and mixed well. The above solution (20 μL) was then injected six times into the column. The mean peak area of the drug content in the formulation was calculated by the regression equation of the calibration plot.

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive, precise and accurate HPLC method for the

Table 2: Accuracy data (triplicate values at 80, 100, 120 percent levels).

*Amount taken (μg)	*Amount found (μg)	*Percent recovery	*Mean percentage recovery
90	89.14	99.04	99.04
110	109.16	99.23	99.23
130	128.80	99.08	99.08

*Each value is a mean of three readings

analysis of duloxetine hydrochloride in bulk and in pharmaceutical dosage form and forced degradation. In order to achieve optimum separation of the component peaks, mixtures of buffer with methanol in different combinations were tested as mobile phase on a kromasil C_{18} stationary phase. A binary mixture of buffer: methanol in a proportion of 85:15 v/v was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for duloxetine hydrochloride was 3.443 \pm 0.06 min. Each of the samples was injected Six times and the Sample retention times were observed in all cases. The peak areas of duloxetine hydrochloride were reproducible as indicated by low coefficient of variation. A good linear relationship ($r^2 = 0.99970$) was observed between the concentration of duloxetine hydrochloride and the respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was $y = 61.287x - 10.1785$ (Where y gives peak area and x is the concentration of the drug). The regression characteristics are given in table 1. When duloxetine hydrochloride solutions were analysed by the proposed method for finding out intra and inter-day variation, low co-efficient of variation was observed. The absence of additional peaks indicated non-interference of common excipients used in the tablets.

Table 3: Robustness study.

Drug name	Variations	Chromatographic parameters				
		Retention time	Area	Height	Theoretical plates	Asymmetry
Duloxetine hydrochloride	Change in wave length at $\pm 2\text{nm}$					
	1. wave length at 228nm	3.430	6247.732	728.039	3666	1.714
	2. wavelength at 230nm	3.437	6283.478	724.787	3503	1.655
	3. wavelength at 232nm	3.430	6363.381	735.321	3666	1.714
	Change in flow rate at $\pm 0.1\text{ml/min}$					
	1. flow rate at 0.9ml/min	4.110	7498.414	739.806	3656	1.727
	2. flow rate at 1.0ml/min	3.437	6283.478	724.787	3503	1.655
	3. flow rate at 1.1ml/min	2.940	5411.549	718.573	3518	1.680

Table 4: System suitability parameters.

Parameters	Value
Theoretical Plates (h)	3702
Tailing factor (T)	1.71
LOD ($\mu\text{g/ml}$)	0.905
LOQ ($\mu\text{g/ml}$)	2.742

High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The drug content in tablets was quantified using the proposed analytical method are given in table 2. The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay. This indicated the robustness of the method. The robustness study results are presented in table 3. The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method. The standard solution of the drug was stable up to 24 hrs as the difference in percent assay during the above period is within limit system suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor and the number theoretical plate are in the acceptable limits. The system suitability results are shown in table 4.

The system precision was established by six replicate injections of the standard solution containing analytes of interest. The value of relative standard deviation was found to be 0.322 which is within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analysis six times using the proposed method. The relative standard deviation was found to be 0.268 which is within the limit, indicating the injection repeatability of the method.

Table 5: Forced degradation.

Drug Name	Condition	Time in hours	Retention time (min)	Area	Retention time of additional degradation peak (min)	% Degradation	% of Active drug Present after Degradation
Duloxetine hydrochloride	Control Sample	00	3.437	6283.478	3.150	-	100
	Acid Degradation	24	3.437	6237.208	6.500	0.73	99.27
	Alkaline Degradation	24	3.460	5906.601	2.987	6.00	94.00
	Thermal Degradation	24	3.427	5924.547	3.020	5.72	94.28

The specificity of the HPLC method was determined by the complete separation of duloxetine hydrochloride. When it was subjected to forced degradation as per ICH guidelines, carried out with 0.1N HCL, 0.1N NaOH and Heat degradation at 105°C. The method does not permit detection of degradation product for duloxetine hydrochloride. The results of specificity data for degradation study are given in table 5.

Hence it can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of duloxetine hydrochloride in pharmaceutical dosage form with short analysis time of 5 min.

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