A Simple, Novel Validated Stability Indicating RP-HPLC method for estimation of Duloxetine HCl in Capsule Pharmaceutical Formulation

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ABSTRACT

The object of current work was to study the degradation behavior of duloxetine under different ICH recommended stress condition under reverse phase high performance liquid chromatographic (HPLC) method and to establish a novel, validated stability-indicating reverse phase high performance liquid chromatographic method for the determination of duloxetine in presence of its impurities and forced degradation products in pharmaceutical formulation. The chromatographic separation was achieved on Hypersil, BDS- C8 , (250 mm × 4.6 mm, 5 μM) column with a mobile phase containing a mixture of Acetonitrile : Phosphate buffer pH 3.0 (50:50 v/v). Detection was carried out with UV detector. The retention time was about 3.99 min; the method was validated for linearity, accuracy, precision, specificity, robustness and ruggedness. The described method shows excellent linearity over a range of 8-56 μg/mL for duloxetine. To establish stability indicating capability of the method, drug product was subjected to the stress condition of acid, base, oxidative, hydrolytic, thermal and photolytic degradation. The degradation products were well resolved from duloxetine. The developed method was validated as per ICH guidelines with respect to specificity, linearity, LOD, LOQ, accuracy, precision and robustness.

Keywords: Duloxetine, validation, degradation, HPLC.

INTRODUCTION

Duloxetine is a potent dual reuptake inhibitor of nor epinephrine and serotonin. Used in treatment of depression, diabetic peripheral neuropathic pains, also in treatment of moderate to severe stress urinary incontinence.[1,2]

For its analytical determination in formulation products and in drug substance, the literature indicates UV and HPLC methods.[3–5]

Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH)[6] suggest that stress studies should be carried out on a drug to establish its inherent stability characteristics for identification of degradation product.

There is no HPLC method reported for estimation of duloxetine in capsule formulation. Accordingly, the aim of the present study was to establish a novel stability-indicating reverse phase high performance liquid chromatographic method to determine the level of duloxetine in capsule formulation.

The proposed method is able to separate duloxetine from its degradation components. The developed HPLC method was validated with respect to specificity, linearity, LOD, LOQ, precision, accuracy and robustness. Force degradation studies were performed on the drug product. The studies were performed in accordance with established international conference on harmonization (ICH) guidelines.

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MATERIALS AND METHODS

Reagents and chemicals

Standard of duloxetine was supplied by Aurbindo Pharmaceutical, Hyderabad, India. The HPLC grade acetonitrile, water and Potassium di-hydrogen phosphate were purchased from Loba Chemical, Mumbai, India.

Equipment

The analysis was performed using HPLC separation module equipped with UV detector. Digital student water bath was used for hydrolysis studies. Thermal stability studies were carried out in a dry air oven.

Chromatographic Conditions

The separation was achieved on Hypersil, BDS- C8, (250 mm × 4.6 mm, 5 μM) column with a mobile phase containing a mixture of Acetonitrile: Phosphate buffer pH 3.0 (50:50 v/v). Detection was carried out with UV detector. The sample injection volume was 10 μL.

Preparation of Standard Solution

Mobile phase was used as diluents. An amount of duloxetine about 100 mg was transferred into 100 mL volumetric flask, added 50 mL of diluent and ultrasonicated for 10 min, and then diluted to volume with diluent.

Preparation of sample solution

Twenty capsules of Dulot® (Lupin Pharmaceuticals Ltd., Mumbai, India) were weighed and average weight was determined. Granules were crushed to fine powder weight equivalent to 20 mg of Duloxetine HCl was transferred in a 25 mL volumetric flask in sufficient quantity of methanol, sonicated for 30 min and finally volume was made to mark the solution was filtered through Whatmann filter paper No.41 (Concentration 800 μg/mL). A 1ml of the above solution was further diluted upto 10ml to get the concentration of 80 μg/mL. The aliquot portion of the filtrate was further diluted to get final concentration of 8 μg/mL of duloxetine.

Forced degradation studies

Force degradation studies of drug product were performed to evaluate the stability-indicating property and specificity of proposed method. Stress studies were performed at the concentration of 80 μg/mL of duloxetine on capsule formulation.

All the solutions used in forced degradation studies were prepared by dissolving the drug product in small volume of stressing agents. After degradation, these solutions were diluted with diluents to yield stated concentration. Conditions employed for performing the stress studies were as follows.

Acid degradation

It was performed in 0.1 N HCl by refluxing the drug with the acid on student water bath for 8hrs.

Accurately weighed quantity of sample equivalent to 40 mg of duloxetine was dissolved in 10 ml of 0.1N HCl in dry round bottom flask. The reaction mixture was refluxed for 8 h on water bath. The solution was brought to ambient temperature, quenched the reaction by addition of 10 mL 0.1 N HCl and diluted to 100 mL with diluent, then filtered. The aliquot portion of the filtrate was further diluted with diluent to get final concentration of 8 μg/mL for duloxetine.

Base degradation

Accurately weighed quantity equivalent to 40mg was dissolved in 50 mL of 0.1 N NaOH in a 100 mL beaker. The reaction mixture was placed at 40°C for 8 h in a thermostat (Oven). The solution was brought to ambient temperature, quenched the reaction by addition of 10 mL 0.1 N Hcl and diluted to 100 mL with diluent, then filtered. The aliquot portion of the filtrate was further diluted with diluent to get final concentration of 8 μg/mL for duloxetine.

Hydrolytic degradation

Accurately weighed quantity equivalent to 80 mg was dissolved in 100 mL of water (0.8 mg/mL) in 250 mL dry round bottom flask (RBF).The reaction mixture formed was refluxed for 8 hon the water bath. The solution was brought to ambient temperature. The aliquot portion of the filtrate was further diluted with diluent to get final concentration of 8 μg/mL for duloxetine.

Oxidation degradation

Accurately weighed quantity equivalent to 40mg of drug was dissolved in 50 mL of 3% hydrogen peroxide solution in beaker. Both reaction mixtures of duloxetine bulk drug and marketed formulation were kept for 7 days at room temperature. The aliquot portion of the filtrate was further diluted with diluent to get final concentration of 8 μg/mL for duloxetine.

Photolytic degradation

The sufficient amount of duloxetine Hcl was spread on two petriplates separately, one as a control and other as a sample. Similarly tablets were placed in the two different petriplates one as control and other as sample. Both control petriplates were wrapped in aluminium foil (as a dark place) and other 2 were kept open. All plates were placed inside the stability chamber under UV light as per the ICH guidelines for 15 days. Following removal from the photo-stability chamber, sample was prepared for analysis as previously described under sample procedure.

Radical Initiation test

The typical radical initiator Acetonitrile: Water in ratio 0f 50:50 was used for this study. Accurately weighed 40mg of bulk drug was dissolved in 50 mL of Acetonitrile: Water (50:50 v/v) in beaker. The reaction mixture was placed in the thermostat (oven) for 7 days at 40°C. Samples were withdrawn at 0 and 7 days interval. Further procedure was similar as that used in preparation of acid hydrolysis.

RESULTS AND DISCUSSION

Method Development and Optimization

The main objective of the HPLC method was to separate and quantitate the duloxetine in presence of degradation products. The system suitability specification that provide assistant in achieving this purpose. Some of them are theoretical plate number, the injection precision and the tailing factor. The theoretical plate number and the tailing factor that is a measure of the column efficiency. The chromatographic separation was achieved by following isocratic program using Acetonitrile: Phosphate buffer pH 3.0 (50:50 v/v) mobile phase. Detection was carried out with UV detector. The sample injection volume was 10 µL. the retention time of duloxetine is about 3.99 min. Chromatograms of test preparation is shown in Figure 1.

Validation of the Method

The proposed method was validated by determining its performance characteristics regarding specificity, accuracy, precision, limit of detection and quantification, linearity, range and robustness.

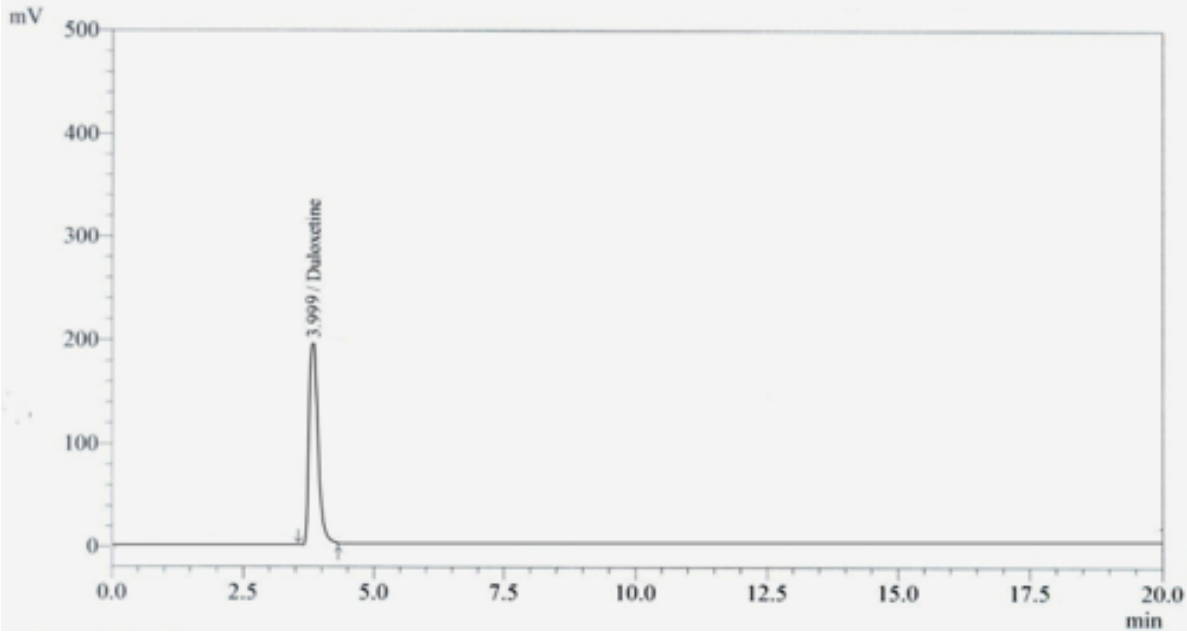

Figure 1: Chromatograph of Duloxetine HCl (Rt=3.99)

System suitability

System suitability was determined before sample analysis from five replicate injections of the standard solution containing 8 µg/mL of duloxetine. System suitability shall be checked for the suitability and reproducibility of chromatographic system for analysis. The acceptance criteria for system suitability was % relative standard deviation (RSD) for peak areas should not more than 2%, USP tailing factor should not more than 2.0 and USP plate count should more than 5000 for duloxetine peaks from standard solution. All critical parameters tested met the acceptance criteria (Table 1).

Limits of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The limit of detection and limit of quantification values are reported in Table 2.

Linearity

Linearity test solutions were prepared by diluting the stock solutions to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 150% levels of test concentration (8–56 µg/mL for duloxetine). Calibration curves were plotted between the responses of peak versus analyte concentrations. The coefficient correlation, slope,

Table 1: Results of system suitability parameter

Parameter	Acceptance criteria	Observed value	
		Precision (Day-1)	Intermediate precision (Day-2)
Area (% RSD, n=5)	≤ 2.0	0.56	1.1
USP Plate count	> 5000	10447	11002
USP Tailing	2.0	1.5	1.7

Mean ± %RSD for six determinations.

Table 2: Evaluation of LOD, LOQ and Linearity data

Parameter	Observed Value
LOD (µg/mL)	0.628
LOQ (µg/mL)	1.905
Linearity range (µg/mL)	8-56µg/mL
Correlation coefficient	0.999
Slope	-12149
Intercept	2315.70

y-intercept of the calibration curve are reported (Table 2) and result shows that an excellent correlation existed between peak area and concentration for duloxetine.

Precision

The precision of method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of duloxetine capsule containing duloxetine at 100% level of test concentration. The intermediate precision of the method was also evaluated using different analyst and different instrument and performing the analysis on different days.

Intermediate precision was determined by injecting six individual preparations of duloxetine capsule containing duloxetine at 100% level of test concentration. The relative standard deviation of the areas of peak was calculated and found to less than 0.39% in repeatability and less than 0.9% in intermediate precision, which confirms the good precision of the method. The % RSD values are presented in Table 3.

Table 3: Results from Evaluation of the precision and intermediate precision of method

Parameter	Observed Value	
	Precision (Day-1)	Intermediate precision (Day-2)
% Assay	98.68	99.26
RSD (n=6)	0.006264	0.0784

Accuracy

Accuracy of the method for duloxetine was evaluated in triplicate at LOQ, 50%, 75%, 100%, 125% and 150% level of test concentration. The percentage recoveries for was calculated (Table 4). The percentage mean recovery of duloxetine from the formulation varied from 99.21 to 100.32% indicating that the developed method was accurate for the determination of duloxetine in pharmaceutical formulation.

Table 4: Results of Accuracy of the method

Theoretical (% of target level)	Amount added (mg)	Mean recovery (%)	RSD (%)
80%	0.016	99.21	0.0059
100%	0.024	100.11	0.0011
120%	0.0288	100.32	0.0035

Mean + % RSD for six determinations.

Results from Forced Degradation Studies

Specificity of the method was exhibited by analyzing blank and placebo prepared as per test method. These results demonstrate that there was no interference at the retention time of duloxetine from the other excipient compounds and, therefore, confirms the specificity of the method (Figure 1).

All degradation products formed under stress conditions were well resolved from analyte peaks (Figure 2 to Figure 7) and thus confirms the stability-indicating power of the developed method.

A summary data of stress study is shown in Table 5.

Table 5: Results of forced degradation of method

Stress Condition	Duloxetine		
	Retention Time	% Assay	% Degradation
Reference	3.99	100.20	Not applicable
Acidic Hydrolysis	3.99	85.08	14.92
Basic Hydrolysis	3.79	31.24	68.76
Oxidative	3.749	76.13	23.87
Neutral Hydrolysis	3.96	92.26	7.74
Photolytic	3.76	88.83	11.17
Radical	3.79	94.13	5.87

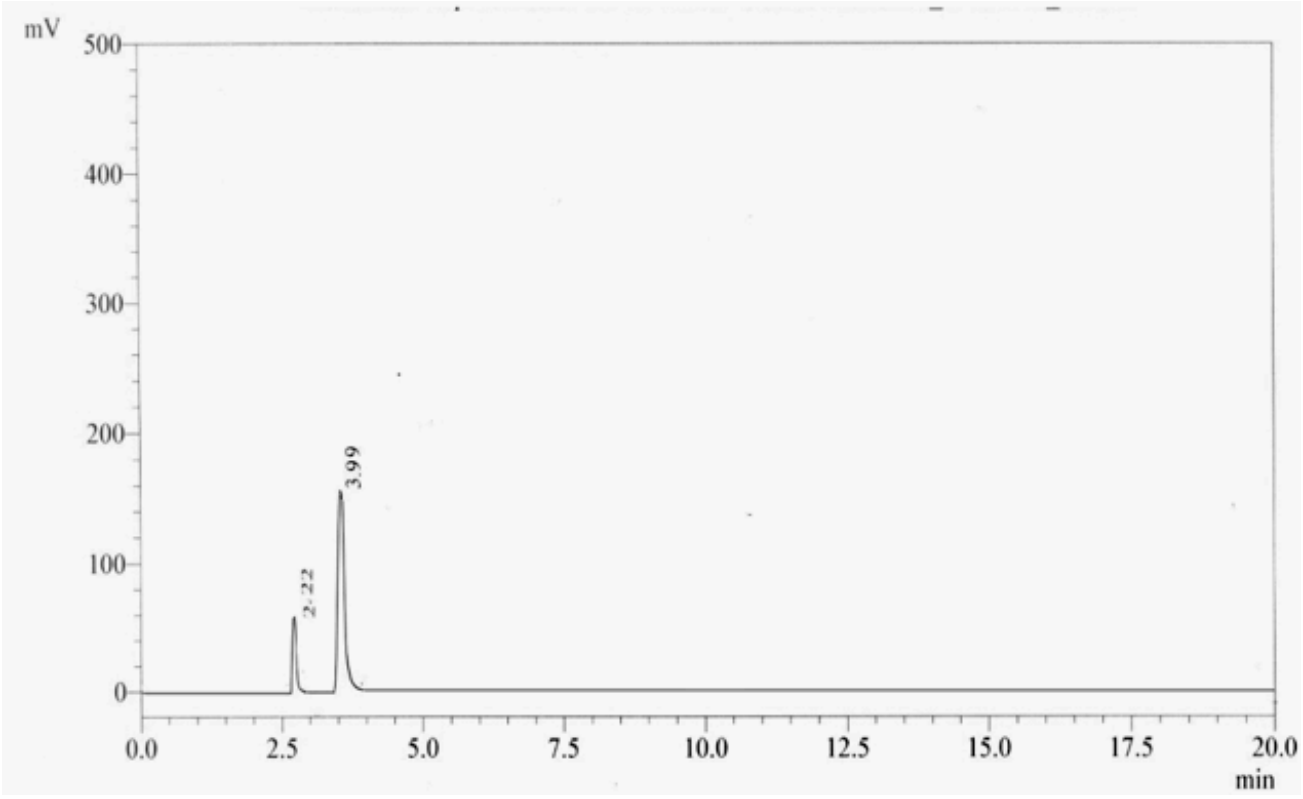

Figure 2: Chromatogram of Acid hydrolysis

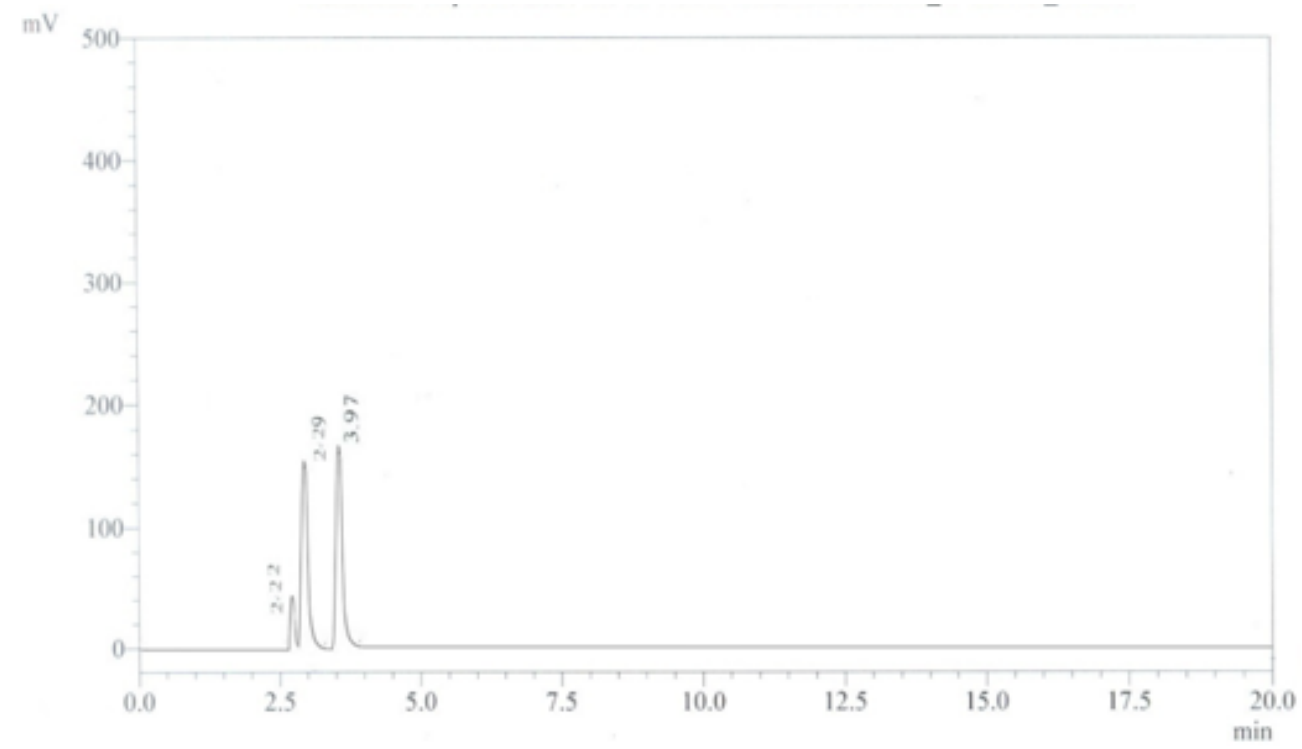

Figure 3: Chromatogram of base degradation

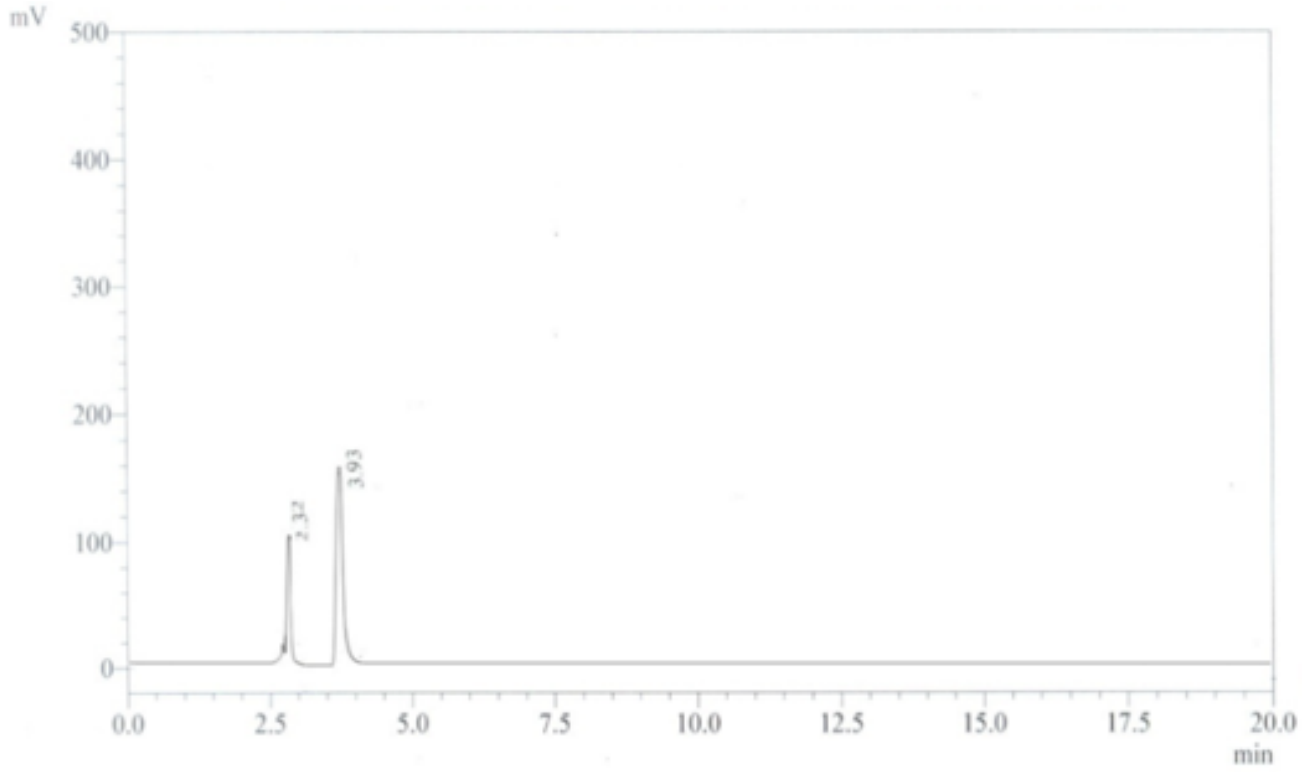

Figure 4: Chromatogram of neutral hydrolysis

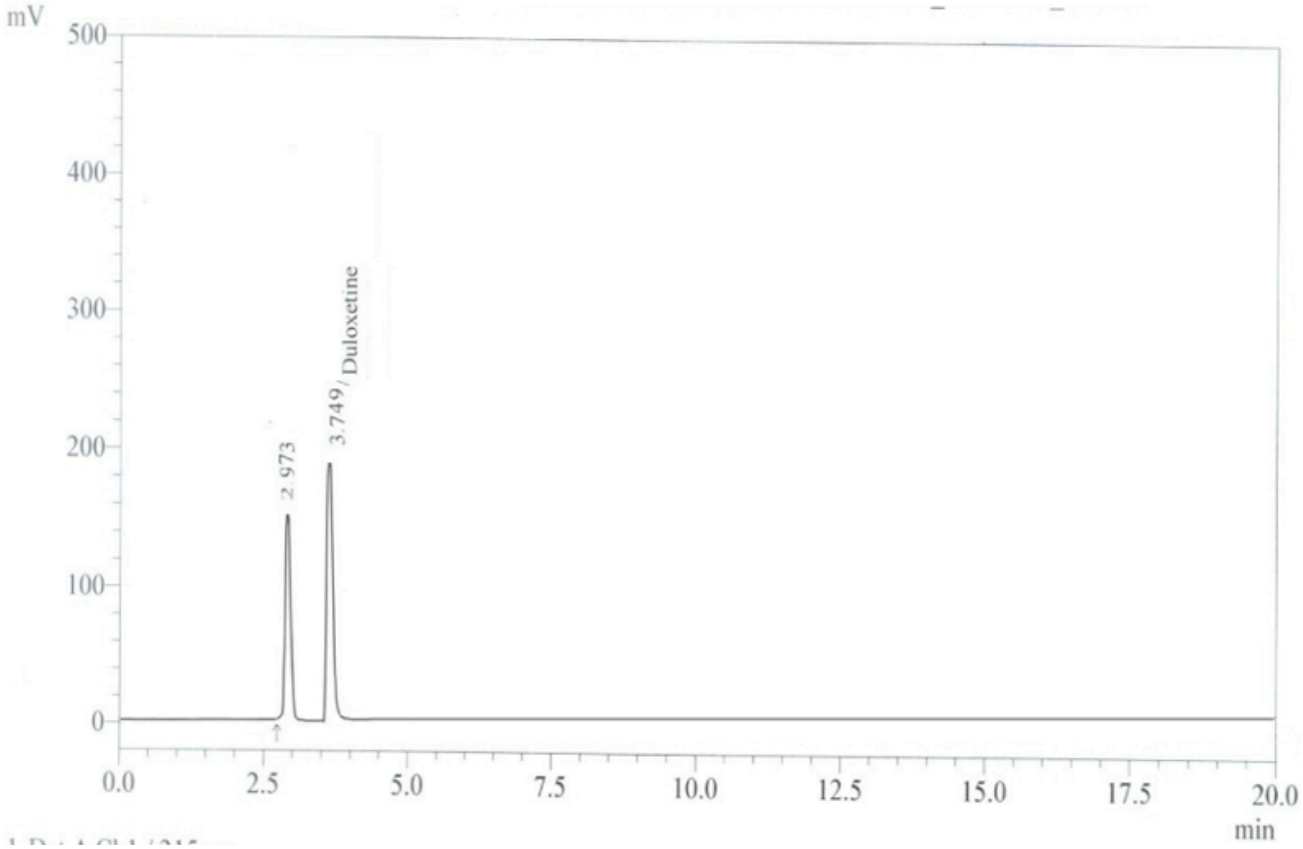

Figure 5: Chromatogram of peroxide study

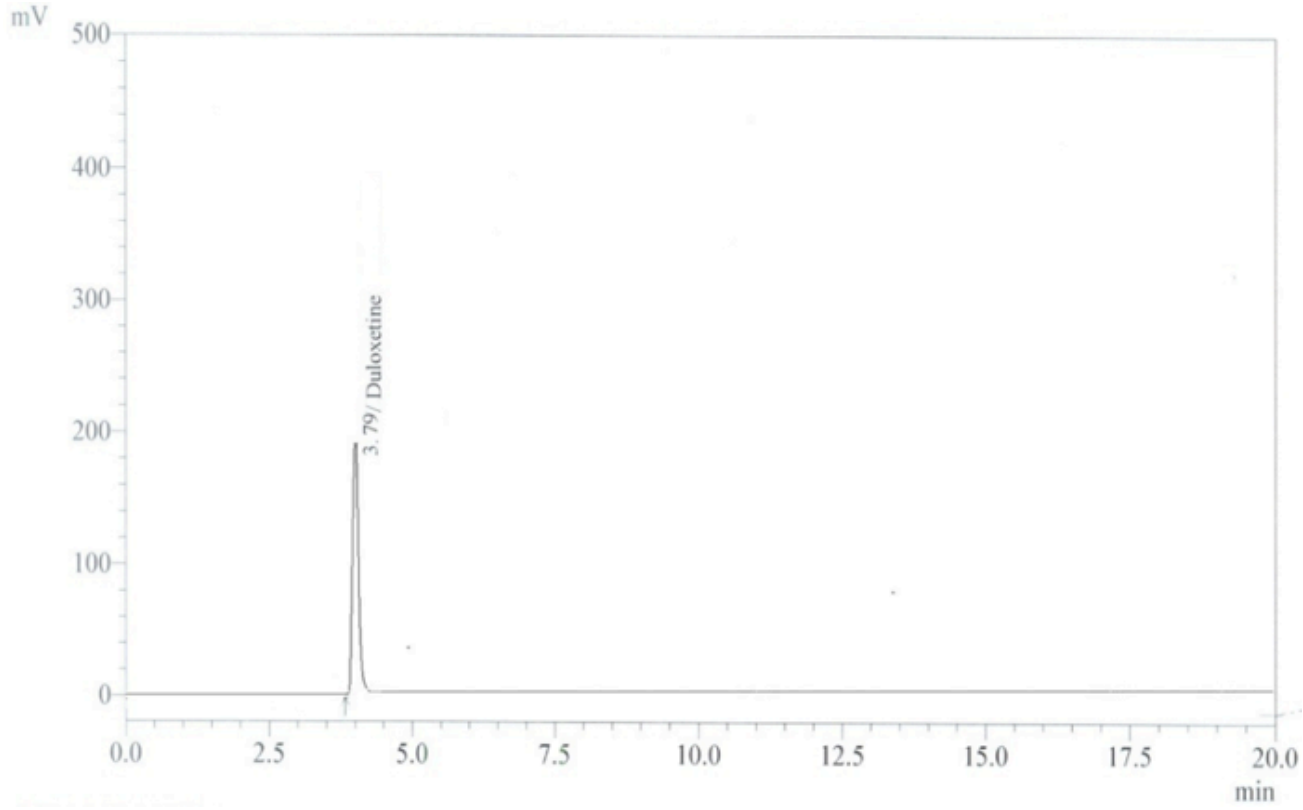

Figure 6: Chromatogram after radical initiation study

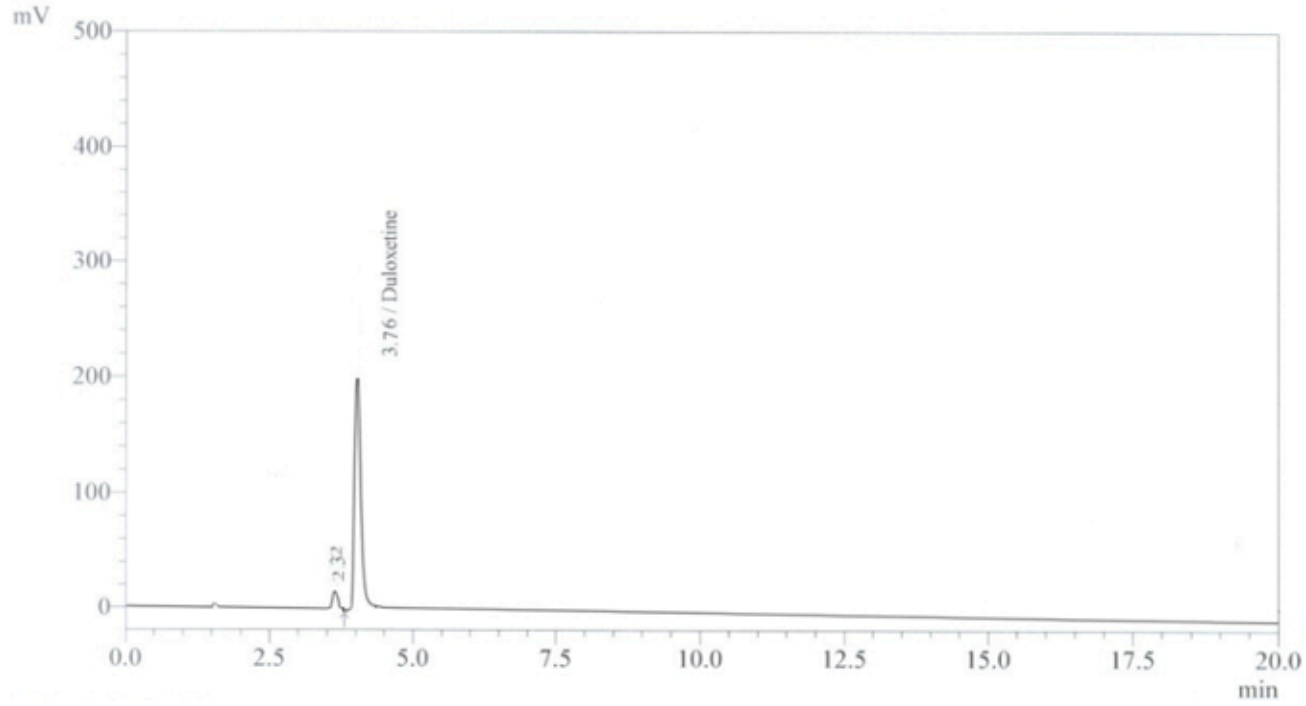

Figure 7: Chromatogram after photostabilty study

CONCLUSION

A simple and efficient RP-HPLC methods stability indicating and can be used for routine analysis was developed and validated for quantitative analysis of duloxetine in pharmaceutical dosage forms. The method was found to be precise, accurate, linear, robust and rugged during validation. The method is stability indicating and can be used for routine analysis of pharmaceutical preparation and to check the stability of duloxetine capsule.

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CONFLICTS OF INTEREST

Authors have no conflicts of interest

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