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Stability-indicating LC method for the determination of duloxetine hydrochloride in bulk drug and in pharmaceutical dosage form

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ABSTRACT

A novel stability-indicating LC assay method was developed and validated for quantitative determination of duloxetine in bulk drugs and in pharmaceutical dosage form in the presence of degradation products generated from forced degradation studies. An isocratic, reversed phase LC method was developed to separate the drug from the degradation products, using an Ace5-C18 (250 mm×4.6 mm, 5µm) column, and 50 mM ammonium acetate (pH-5.5 by acetic acid) and acetonitrile (50:50v/v) as a mobile phase. The detection was carried out at the wavelength of 230 nm. The duloxetine was subjected to stress conditions of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Degradation was observed for duloxetine in all conditions attempted. The degradation products were well resolved from the main peak. The percentage recovery of duloxetine ranged from (99.72 to 100.87%) in pharmaceutical dosage form. The developed method was validated with respect to linearity, accuracy (recovery), precision, specificity and robustness. The forced degradation studies prove the stability-indicating power of the method.

KEYWORDS

Column liquid chromatography;
Stability indicating method;
Validation;
Duloxetine.

INTRODUCTION

Duloxetine hydrochloride is chemically known as (+)-N-methyl-3(1-naphthalenyloxy)-2-thiophenepropanaminehydrochloride (Figure 1), it is a potent dual inhibitor of serotonin (5-hydroxytryptamine (5-HT)) and nor epinephrine (NE) reuptake, possessing comparable affinities in binding to NE and 5-HT transport sites. It has been clinically used for the treatment of major depressive disorder, pain due to diabetic peripheral neuropathy and urinary incontinence[1–5].

A recent literature survey revealed that few methods were available for the determination of duloxetine in biological samples, which involved HPLC with UV detection, liquid chromatography with single-quadrupole mass spectrometric (LC-MS) method[6], liquid chromatographic-tandem mass spectrometry (LC-MS-MS) with solid phase extraction (SPE)[7, 8], gas chromatography nitrogen-phosphorus detection (GC-NPD), chiral chromatography[9] and gas chromatography-mass spectrometry (GC-MS) quantitation[10].

According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. Literature survey reveals that there is no stability-indicating LC assay method for de-

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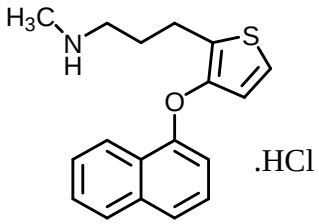

Figure 1: Chemical structure of duloxetine hydrochloride

termination of duloxetine in bulk drug and pharmaceutical dosage form. In the present research article, we report the development and validation of a stability-indicating LC method for the determination of duloxetine as bulk drug and pharmaceutical dosage form. It separates drugs from the degradation products under ICH suggested stress conditions (hydrolysis, oxidations, photolysis and thermal stress)[11-13]. We developed a rapid, robust and economic method, which separates the degradation products from the main peak. The developed method is stability indicating and can be used for assessing the stability of duloxetine in bulk drugs and pharmaceutical dosage form. The developed method was validated with respective linearity, accuracy, precision, LOD, LOQ and robustness.

EXPERIMENTAL

Material and reagents

Duloxetine bulk drug (purity 99.7) was obtained from Cipla Pharmaceutical (Mumbai, India) and Dulane 20 capsules (20mg) (manufactured by Sun Pharmaceuticals, India) were obtained from the local market. Ammonium acetate and hydrochloric acid were obtained from Qualigens Fine Chemicals, India. Acetonitrile, hydrogen peroxide, sodium hydroxide were obtained from Rankem Laboratories, India. All chemicals and reagents used were of analytical or LC grade. UV cabinet was used of Kumar made, (India). Milli-Q-Water was used throughout the experiment.

Chromatographic conditions

LC system used was a Jasco (2000 series), system equipped with a UV detector. The chromatographic column Ace5-C18 (250×4.6 mm, 5µm, Advance Chromatography Technology, USA) was used. The instrumental setting was at a flow rate of 1 mL min^{-1}. The injection volume was 20µL.The detection wavelength was 230nm.

Mobile phase

The mobile phase consists of buffer and acetonitrile in the ratio of (50:50 v/v). The buffer used in the mobile phase contained 50mM of ammonium acetate in double-distilled water (pH-5.5 by acetic acid). The mobile phase was premixed and filtered through a 0.45µm nylon filter and degassed.

Preparation of standard stock solution

All solutions were prepared on a weight basis and solution concentrations were also measured on a weight basis to avoid the use of an internal standard. Standard solution of duloxetine was prepared by dissolving the drugs in the diluents and diluting them to the desired concentration. Diluent A was composed of methanol and acetonitrile in the ratio of (50:50 v/v) and diluent B was composed of water and acetonitrile in the ratio of (50:50 v/v). 10mg of duloxetine was accurately weighed, transferred to a 100mL volumetric flask, dissolved and diluted to 100mL with diluent A. From this stock solution 5mL were transferred into a 100mL volumetric flask and diluted to volume with diluent B. This final solution contained 5µg/mL of duloxetine.

Sample solution (Capsules)

Content of twenty capsules of duloxetine (Dulane20-20mg) were finely ground using agate mortar and pestle. The ground material, equivalent to 10 mg of the active pharmaceutical ingredient, was extracted into diluent A in a 100 mL volumetric flask by vortex mixing followed by ultra sonication and made up to volume by diluent B. The solution was filtered through a 0.45-micron filter and an appropriate concentration of sample (5µg/mL assay concentration) was prepared in diluents at the time of analysis.

Specificity/Selectivity

Specificity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradation products, matrix, etc.[14]. The specificity of the developed LC method for duloxetine was carried out in the presence of its degradation products. Stress studies were performed for duloxetine bulk drug to provide an indication of the stability-indicating property and specificity of the proposed method. In-

tentional degradation was attempted to stress condition exposing it with acid (0.1 N hydrochloric acid), alkali (0.1 N NaOH), hydrogen peroxide (30%), heat (80°C) and UV light (254 nm and 366 nm wavelength) to evaluate the ability of the proposed method to separate duloxetine from its degradation products. For light and heat studies, the study period was 24 h whereas for acid, oxidation and for base 4 h. Peak purity of the test was carried out for duloxetine by using a UV detector in stress samples. Assay studies were carried out for stress samples against duloxetine reference standard and the mass balance (% assay+% sum of all impurities + % sum of all degradants) was calculated. The excipient mixture present in Dulane20 capsules was injected in the optimized conditions to show the specificity of the method in formulation of duloxetine.

Procedure for forced degradation study of duloxetine

Acidic degradation

About 5mg of duloxetine was accurately weighed and dissolved in 1 mL of diluent A, then 2mL of 0.1 N HCl were added and kept at room temperature for about 4 h & then the solution was neutralized by 0.1 N NaOH to pH 7 and the volume made up to 10mL with diluent B. final solution contained 5µg/mL of duloxetine.

Alkali degradation

About 5mg of duloxetine was accurately weighed and dissolved in 1 mL of diluent A, then 2mL of 0.1 N NaOH were added and kept at room temperature about 4 h. Then the solution was neutralized by 0.1 N HCl to pH 7 and the volume made up to 10mL with diluent B. final solution contained 5µg/mL of duloxetine.

Oxidative degradation

About 5mg of duloxetine was accurately weighed and dissolved in 1 mL of diluent A, and then 2mL of 30% H_2O_2 solution was added and kept at room temperature for about 4 h. Then the volume was made up to 10mL with diluent B. final solution contained 5µg/mL of duloxetine.

Thermal degradation

About 5mg of drug substance was kept at 80°C for 24 h then the solution was prepared to achieve a final concentration of 5µg/mL.

UV degradations

About 5mg of drug substance was exposed to UV short (254nm) and UV long (366nm) light for 24 h. Then the solution was prepared to achieve a final concentration of 5µg/mL.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability-indicating LC method was to achieve the resolution between duloxetine and its degradation products. To achieve the separation of degradation products, stationary phases of C-18 and a combination of mobile phase 50 mM ammonium acetate with acetonitrile were used. The separation of degradation products and duloxetine was achieved on an Ace5, C-18 column and 50 mM ammonium acetate pH 5.5 by acetic acid: acetonitrile (50:50 v/v) as a mobile phase and a column temperature at 30°C. The tailing factor obtained was less than 2 and retention time was also about 7 min for the main peak and less than 7 min for degradation products, which would reduce the total run time and ultimately increase productivity thus reducing the cost of analysis per sample. The forced degradation study showed the method was highly specific and the entire degradation products were well resolved from the main peak. The developed method was found to be specific and validated as per ICH guidelines.

Result of forced degradation experiments

Degradation was observed at hydrolysis (acid and alkali), oxidative, thermal & UV stress conditions. Duloxetine was degraded into all attempted stress conditions (Figure 2a-f). The acceptance criterion for stability of duloxetine is 20-80% degradation for forced degradation study[15]. Conditions used for forced degradation were attempted to achieve degradation in the range of 20-80%. In acidic condition duloxetine degraded up to 12.11%, in basic condition up to 7.76%, in oxidative condition 2.95%, in thermal condition 19.03% and in light condition 16.56% degradation was observed for duloxetine. Peak purity results greater than 990 indicate that the duloxetine peak is homogeneous in all stress conditions tested. The mass balance of

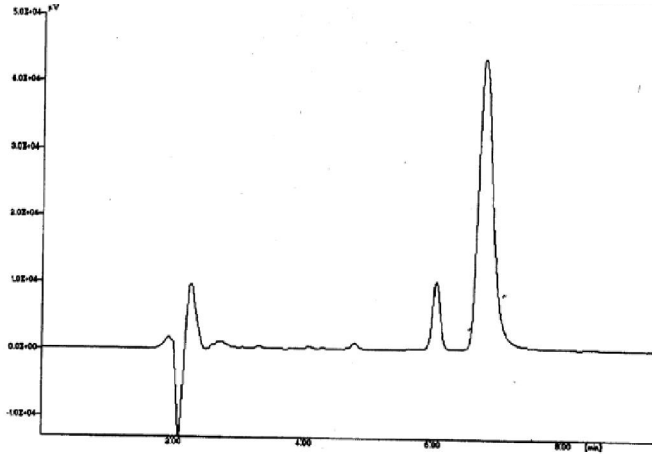

Figure 2.a : Chromatogram of duloxetine in acid degradation (Acid degraded product (6.067) and duloxetine (6.792).

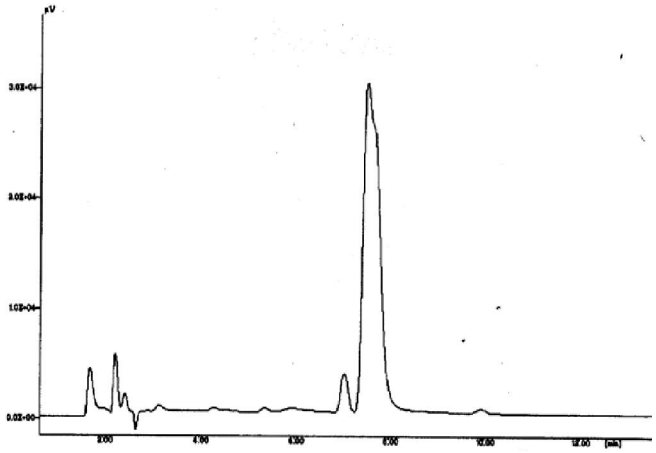

Figure 2.b : Chromatogram of duloxetine in base degradation (Base degraded product (7.050) and duloxetine (7.525).

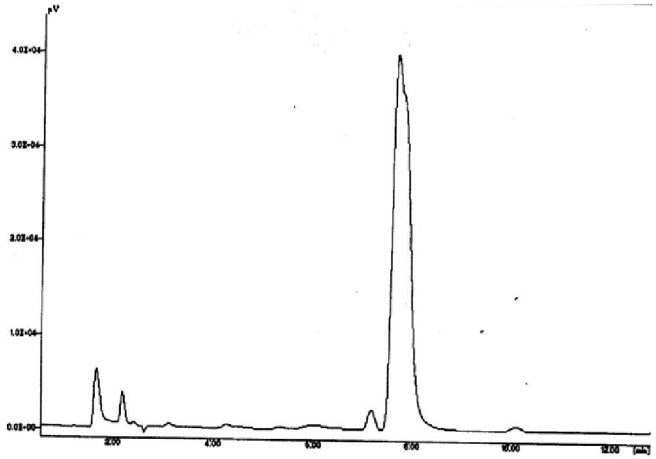

Figure 2.c : Chromatogram of duloxetine in oxidative degradation (Oxidative degraded product (7.225) and duloxetine (7.708).

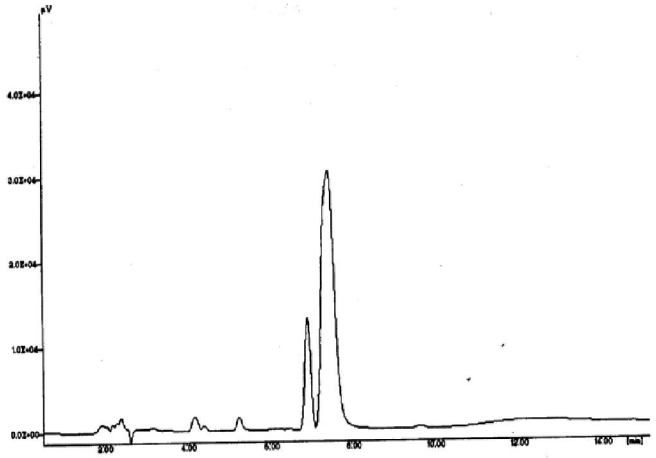

Figure 2.d : Chromatogram of duloxetine in thermal degradation (Thermal degraded product (6.925) and duloxetine (7.442).

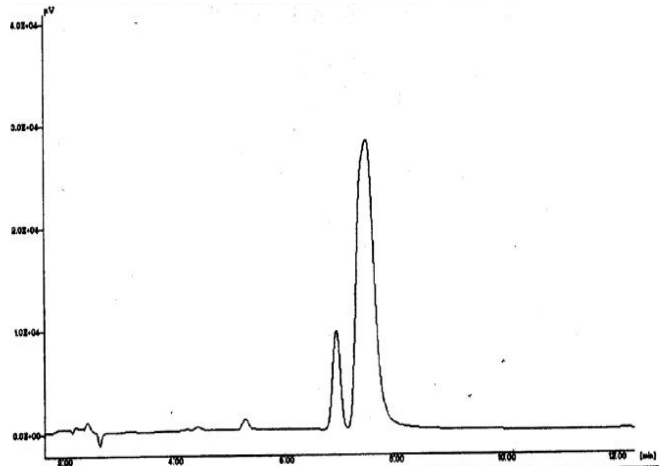

Figure 2.e : Chromatogram of duloxetine in UV degradation (UV degraded product (6.95) and duloxetine(7.5).

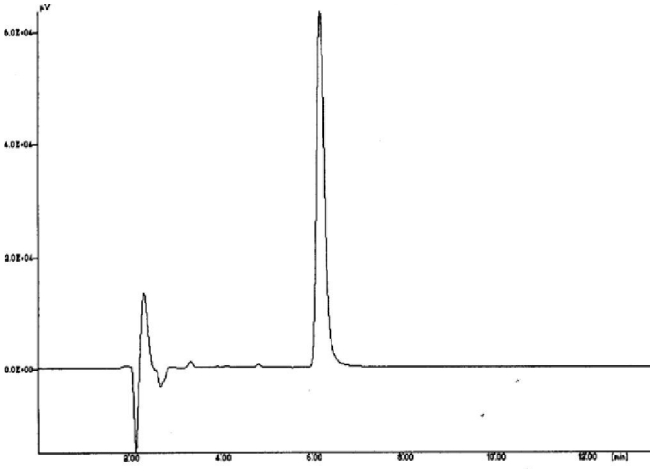

Figure 2.f : A typical chromatogram of duloxetine (6.175) in the capsule.

TABLE 1 : Summary of forced degradation results

Stress conditions	Time	Assay of active substance %	Mass balance (% assay +% sum of impurities+sum of all degradants)	% Degradation
Acid Hydrolysis(0.1N HCl)	4 Hrs	87.01	99.12	12.11%
Base Hydrolysis(0.1 N NaOH)	4 Hrs	91.52	99.28	7.76%
Oxidation(30% H_2O_2)	4 Hrs	96.32	99.27	2.95%
Thermal (80°C)	24 Hrs	80.76	99.79	19.03%
Light (UV 254nm and 366nm)	24 Hrs	83.04	99.60	16.56%

duloxetine in stress samples was close to 100% and moreover, the unaffected assay of duloxetine in tablets confirms the stability-indicating power of the method.

Method validation

Precision

Assay of method precision (intra-day precision) was evaluated by carrying out six independent assays of test samples of duloxetine against reference standard. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, different LC systems and different days in the same laboratory. The percentage of RSD and six assay values obtained by two analysts were 0.23, 99.50 and 0.32, 99.42, respectively.

Accuracy (Recovery test)

Accuracy of the method was studied by recovery experiments.The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80,100and 120% of the label claim of the capsules (20mg of duloxetine). The recovery samples were prepared in the afore mentioned procedure, and then 5mL of duloxetine solutions were transferred into a 50mL volumetric flask and the volume made up with diluents B. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for duloxetine ranged from 99.72 to 100.87%.

TABLE 2 : Result of precision of test method

Sample number	Assay of duloxetine as % of labeled amount	
	Analyst-I (Intra-day precision)	Analyst-II (Inter-day precision)
1	99.42	99.27
2	99.33	99.77
3	99.79	99.19
4	99.81	99.21
5	99.28	99.91
6	99.39	99.22
Mean	99.50	99.42
RSD	0.23	0.32

Linearity

The linearity of the response of the drug was verified at seven concentration levels, ranging from 10 to 200% of the targeted level (5μg/ml), of the assay concentration. Standard solutions containing 0.5-10 μg/ml of duloxetine in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting the peak area versus the concentration data and were treated by least-squares linear regression analysis. The equation of the calibration curve for duloxetine obtained y=5267.5×-4505.5, the calibration graphs were found to be linear in the aforementioned concentrations (the RSD, for slopes and intercept were 1.1, 0.92, respectively. The coefficient of correlation was 0.999).

Limit of detection and limit of quantification

For determining the limit of detection (LOD) and limit of quantification (LOQ), a specific calibration curve was constructed using samples containing the analytes in the range of LOD and LOQ. The LOD and LOQ for duloxetine in the LC method was 0.024 and 0.072 μg mL^{-1} respectively. LOD and LOQ were calculated by using following equations.

$LOD = Cd \times Syx/b$

$LOQ = Cq \times Syx/b$

Where Cd/Cq is the coefficient for LOD/LOQ; Syx is the residual variance due to regression; b is slope.

Precision at limit of quantification was checked by

analyzing six test solutions prepared at LOQ level and calculating the percentage relative standard deviation of the area, which was less than 1.5%.

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered and the resolution between duloxetine and acid degradation products were evaluated.

The flow rate of the mobile phase was 1.0mL min^{-1}. To study the effect of flow rate on resolution, it was changed by 0.2 units from 0.8 to 1.2mL min^{-1}. The effect of percent organic strength on resolution was studied by varying acetonitrile from -10 to +10%. The effect of column temperature on resolution was studied at 25 and 35°C instead of 30°C, while the other mobile phase components were held constant in chromatographic condition. The resolution in the robustness study was not less than 5 in all conditions.

Stability of analytical solution

The stability of the standard solutions and the sample solutions were tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 72 h for duloxetine was 0.77%. The assay values were within 1.5% after 72 h. The results indicate that the solutions solutions were stable for 72 h at ambient temperature.

Determination of active ingredients in capsules

The validated LC method was applied to the determination of duloxetine in capsules. Three batches of the capsules were assayed and the results are shown in (TABLE 3) indicating that the amount of drug in capsules samples met with requirements (90-110% of the label claim). The chromatogram of the capsules sample is shown in (Figure 2f).

TABLE 3 : Results of the recovery tests for the duloxetine

Level of Addition (%)	Ingredient	Amount added (n=3)(mg)	% Recovery	% Average recovery
80	Duloxetine	16	99.72	
100	Duloxetine	20	100.87	100.15
120	Duloxetine	24	99.92	

CONCLUSIONS

The developed method is stability indicating and can be used for assessing the stability of duloxetine in bulk drugs and pharmaceutical dosage form. The developed method can be conveniently used for the assay determination of duloxetine in bulk drugs and pharmaceutical dosage form. The developed LC method was specific, selective, robust, rugged and precise. The developed LC method can be conveniently used for assessing stability, assay, related substances and dissolution of capsules of the pharmaceutical dosage form containing duloxetine in quality control laboratories as previously reported.

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