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Validated Stability-Indicating High-Performance Liquid Chromatographic Method for Determination of Duloxetine HCl Drug

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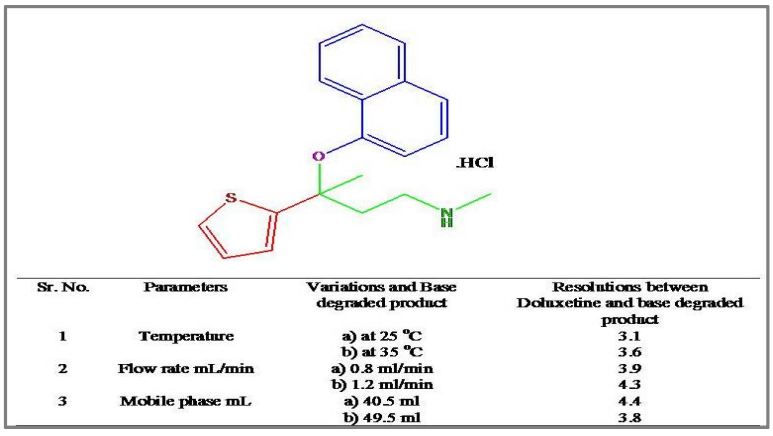
ABSTRACT

A Green economic, rapid, sensitive reversed phase stability-indicating high- performance liquid chromatographic (HPLC) green economic validation assay method was developed and validated for quantitative determination of duloxetine hydrochloride in bulk drugs and the degradation products generated from forced decomposition. A gradient, reversed phase HPLC method was developed to separate the drug from the degradation on products, using a Kromasil C8 (150mm x 4.6 mm), 3u column and the mixture of 0.1% Triethyl amine and acetonitrile was used as mobile phase. The detect ion was carried out at wavelength 230 nm. The chromatographic resolution between its degraded products was found to be greater than three. The duloxetine hydrochloride was subjected to stress conditions of hydrolysis acid, base, oxidation (3 % H_2O_2), and thermal degradation. The degradation was observed for duloxetine hydrochloride in base and in thermal hydrolysis. The mass balance was close to 100 in all the stress conditions. The degraded products were well resolved from main peak. The developed method was validated with respect to linearity, accuracy, recovery, precision, system suitability, selectivity, robustness and forced degradation studies prove the stability indicating ability of the method.

Graphical Abstract



Sr. No.	Parameters	Variations and Base degraded product	Resolutions between Doluxetine and base degraded product
1	Temperature	a) at 25 °C	3.1
		b) at 35 °C	3.6
2	Flow rate mL/min	a) 0.8 ml/min	3.9
		b) 1.2 ml/min	4.3
3	Mobile phase mL	a) 40.5 ml	4.4
		b) 49.5 ml	3.8

Keywords: Green economic Validation, Duloxetine hydrochloride, Stability indicating, RP-H PLC, Kromasil C8, Forced degradation.

INTRODUCTION

Duloxetine hydrochloride is described chemically as (+)-(S)-N-methyl-3-(1-naphthyloxy)-3-(thiophen-2-yl)-propan-1-amine is white, amorphous powder. It is no a selective serotonin-or epinephrine reuptake inhibitor and to a lesser extent dopamine from being reabsorbed into the nerve cells in the brain, specifically on the 5-HT and NE and D2 receptors [**1, 2**]. In this method, duloxetine is consideration to help ease depression [**3**]. Literature surveys disclose, HPLC methods were reported for the resolve of duloxetine in bulk drugs and dosage form [**4**]. Grateful to report a stability representing HPLC method for the analysis and partition of drugs from the degradation products formed under ICH suggested circumstances hydrolysis, oxidations and thermal stress [**5**]. In present article, reversed phase HPLC method was developed for the separation of duloxetine in bulk drug and the impurities formed from its forced degradation under stress conditions like acid hydrolysis, base hydrolysis, oxidation and heat.

MATERIALS AND METHODS

Material and reagents: Duloxetine hydrochloride bulk drug was made available from Cipla Ltd. India (purity 99.4) (Figure 1). Triethyl amine and HCl were got from Qualigens fine chemicals, India Limited. Acetonitrile, hydrogen peroxide, sodium hydroxide were got from Rankem laboratories, India. All chemicals and reagent were used as HPLC grades. Milli-Q-Water was used throughout the experiment.

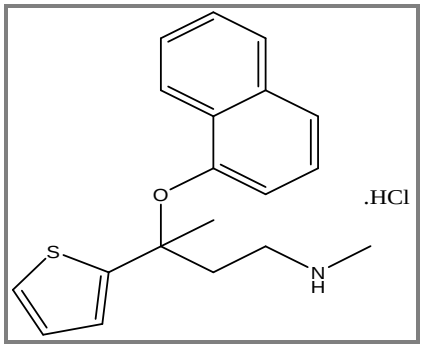


Figure 1. Chemical structure of Doluxetine.

Chromatographic Conditions: A chromatographic system (Systronic) contains of quaternary solvent delivery pump, a degasser, an auto- injector, column oven and UV detector, 10AVP series with Class-VP software**.** The chromatographic column of 245 mm length and internal diameter of 4.6 mm filled with Octadecyl silane Kromasil C8 (Vydac Ltd., CA) stationary phase with particle size 5 micron and pore size 100A was used. Solvent A consisted of 0.1% Orthophosphoric acid and solvent B contains HPLC grade acetonitrile. The flow rate of the mobile phase was 1.0 mL min^{-1}. The HPLC gradient was kept as T/%B: 0/20, 30/45, 45/70, 55/85, 60/90, 70/20 .The column was maintained at 40°C, the injection volume was 10 µL. The diluent used was a 1:1 mixture of water and methanol.

Mobile Phase: The Mobile phase consisted of 0.1% Triethyl amine buffer and acetonitrile. The mobile phase was filtered through a 0.45 µm nylon filter and degassed.

Preparation of Standard stock solutions: A stock solution of 4.0 mg mL was equipped by dissolving appropriate amount of substance in the diluent. Working solution of 0.04 mg mL^{-1} was made from stock solution for related substances and assay determination.

Sample solution (Tablets): Cymbalta (20 mg) were obtained from tablet grinding using agate mortar and pestle [**6, 7**]. The ground material, which was equivalent to 400 mg of the active pharmaceutical

ingredient, was extracted into 1:1 mixture of H_2O and acetonitrile. The solution was filtered through 0.45-micron filter and a suitable concentration of sample (400 µg mL^{-1} assay concentration) was made in diluents at the time of analysis (Figure 1 and 2).

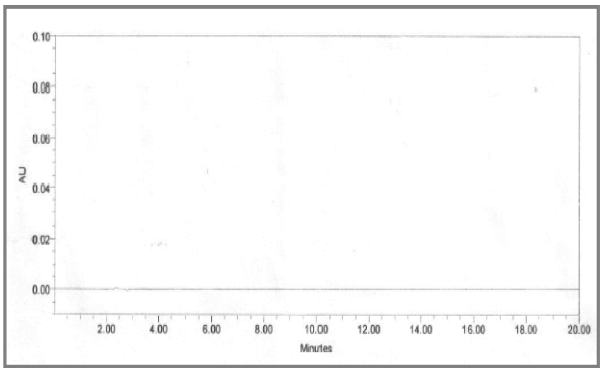


Figure 2. A typical chromatogram of the Doluxetine HCl blank

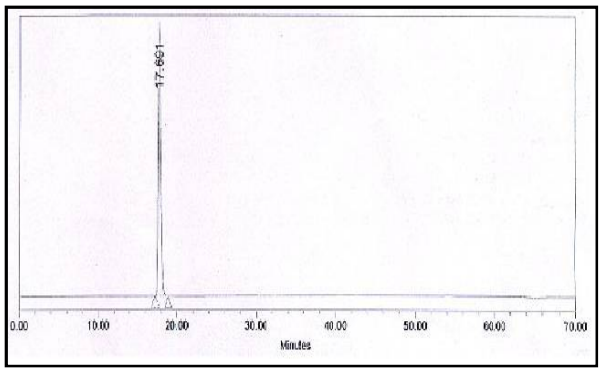


Figure 3. A typical chromatogaram of the table Doluxetine

Selectivity: Selectivity is the capability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might comprise degradants, matrix etc [**8**]. The selectivity 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 selectivity of the planned method. Intentional degradation was attempt to stress condition revealing it with acid (0.02 N hydrochloric acid), alkali (0.03N NaOH), hydrogen peroxide (30%) and heat (70°C) to evaluate the ability of the proposed method to separate duloxetine from its degraded products [**9**]. For heat, study period was 7 days where as for acid, oxidation 48 h and for base 2 h. Assay studies were carried out for stress. Samples against duloxetine reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degraded products) was calculated. The excipient mixture present in duloxetine tablets was injected in the optimized conditions to show the selectivity of the method in formulation of duloxetine.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: The main target for the development of chromatographic method was to get the reliable method for the quantification of duloxetine from bulk drug and which will be also applicable for the degradable products. Initially, we took the attempt for the development of HPLC method quantification of standard duloxetine from bulk. We have used Water nova pack C18(150X4.6) mm,5µ, Inertsil ODS 3VC18(250X4.6) mm, 5µ Star ODS-II C18 (250X4.6) mm, 5µ, Kromasil C18(250X4.6) mm, 5ƒÝ and Grace Alpha C18 (250 mm x 4.6) mm,5u out of these used HPLC column, Kromasil C18(250X4.6)mm,5ƒÝ found to comparatively better and gave the graph with better Gaussian shape at retention time 17.691 min. To improve, the shape and width of the graph, for the above columns different solvents and buffer taken for trials such as 0.1M KH_2PO_4 and Acetonitrile (60:40,v/v) in these trials peak shape is not good, another trials 0.01M Ammonium acetate pH-5.9 and (20:80,v/v) column temperature 30°C retention of peak and shape not found well, Acetonitrile and water (80:20, v/v) column temperature 35°C peak shape not found good, 0.1 M KH_2PO_4, Methanol and water (10:70:20, v/v/v) column temperature 35°C, another trials 0.5 gm KH_2PO_4 and 0.45 gm 1-Hexa sulphonic acid sodium salt make pH-3.5 Ortho phosphoric acid and methanol(25:75, v/v) peak shape obtained but retention is not good, finally try for 0.1% orthophosphoric acid and acetonitrile using gradient good peak shape and retention observed.

Forced degradation studies: Significant degradation was not observed in duloxetine hydrochloride bulk samples, under stress conditions such acid (Figure 4), oxidative hydrolysis (Figure 5). Extensive

degradation of duloxetine hydrochloride was observed under stress condition such as base (Figure 6) and thermal stress (Figure 7) leads to the formation of some unknown degradation peaks. The mass balance of duloxetine hydrochloride in stress samples was close to 99.5% and the unaffected assay of duloxetine hydrochloride in the Tablets confirms the stability representative power of the method. The outline of forced degradation studies is given in table 1.

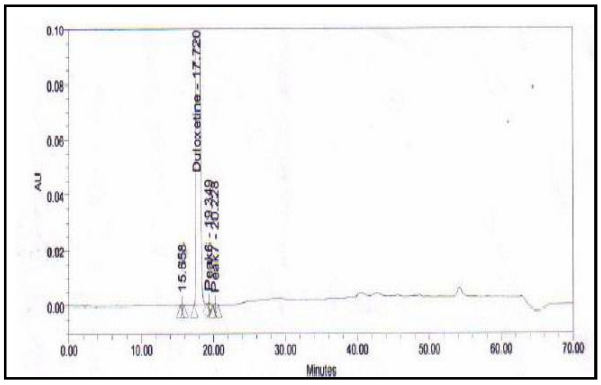


Figure 4. Chromatogram of Doluxetine in acid degradation.

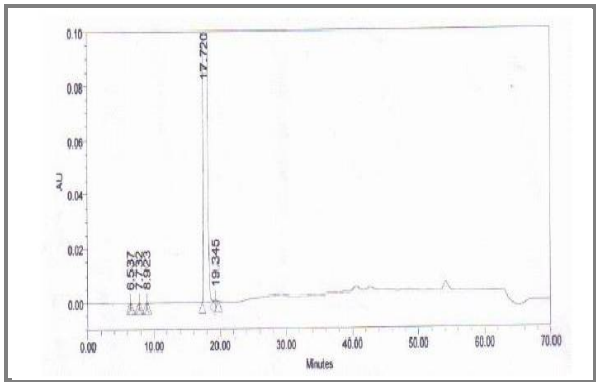


Figure 5. A chromatogarm of Doluxetine in oxidative degradation.

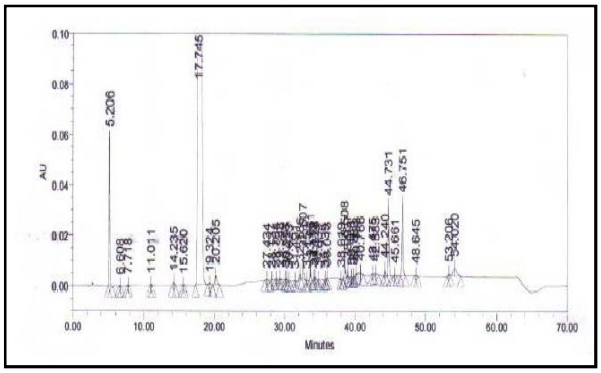


Figure 6. Chromatogarm of Doluxetine in base degradation

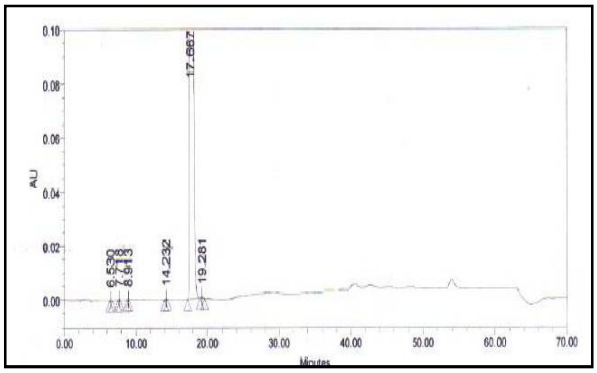


Figure 7. A chromatogarm of Doluxetine in thermal degradation at 80°C.

Table 1. Summary of forced degradation

Stress condition	Time	Assay of active Substance	Mass balance (% Assay + %	Remarks
Acid Hydrolysis (0.03 N degradation HCl)	48 Hrs	97.6	99.78	Negligible
Base Hydrolysis (0.03 N NaOH	2 Hrs	41.82	97.66	Degradation
Oxidation (30% H_2O_2)	48 Hrs	98.40	99.77	No Degradation
Thermal (70°C)	7 days	90.67	98.55	Degradation

Method Validation

System suitability: For system suitability studies, five replicate injections of acid, base and oxidative degraded solutions were used and the RSD of peak area ratio, resolutions, tailing factor and number of theoretical plates of the peak were calculated. The system suitability results are shown in table 2.

Table 2. System suitability reports

Compound (n=3)	Retention Time	% RSD	USP tailing	Theoretical plates
Doluxetine	17.691	0.71	1.11	6537 Acid
Degraded Product	17.720	0.79	1.12	5400 Base
Degraded Product	17.745	0.94	1.16	7434 H_2O_2
H_2O_2 Degraded	17.720	0.77	1.15	--

Precision: The precision of the method was studied by determining the concentrations of the drug duloxetine hydrochloride in the tablet for six times [**10, 11**]. The results of the precision study (Table 3) indicate the reliability of the method (RSD %< 2).

Table 3. Result of the linearity and precision

Ingredient	Precision correlations	Linearity (µg mL^{-1})	Slopes* (n= 3)	Coefficients of (% RSD)
Doluxetine	0.75	10-150	4465.4	0.99928

Intermediate precision (reproducibility): Intermediate precision of the method was determined by analyzing the samples for six times on different days, by different chemists, by using different analytical columns of the same make and different HPLC systems. The percentage assay was calculated using calibration curves. The assay results are shown in table 4.

Table 4. Assay results of active ingredients

Compound (n=3)	Label value (mg)	Found (mg)*	% Assay	SD	RSD%
1	20	20.03	100.5	1.55	0.59
2	20	20.40	100.1	1.56	0.50

Accuracy (Recovery test): The accuracy of an analytical procedure expresses the nearness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found [**12, 13**] Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding identified amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120% of the label claim of the tablet (20 mg). Placebo equivalent to one tablet was transferred into a volumetric flask, and the amounts of duloxetine hydrochloride at 80%, 100% and 120% of the label claim of the tablet were added to it. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for duloxetine hydrochloride ranged from 100.35 % to 101.8% (Table 5).

Table 5. Results of recovery tests for the Duloxetine

Level of Addition (%)	Amount added (n=3) (mg)	% Recovery*	% Average Recovery*
80	16	100.35	100.74
100	20	101.08	100.89
120	24	100.89	100.58

Calibration and linearity: Linearity test solutions for the method were prepared from duloxetine hydrochloride stock solutions at six concentrations levels from tested from 10% to 150% of the targeted level (500 µg/ml), of the assay concentration duloxetine hydrochloride. Standard solutions containing 10-150 µg/ml of duloxetine hydrochloride in each linearity level were arranged. Linearity solutions were injected in triplicate, the correlation coefficient obtained was greater than 0.999 .The slopes and correlation coefficients are shown in table 3.

Robustness: In all the deliberate varied chromatographic conditions flow rate and column temperature, the resolution between duloxetine and its impurity was greater than 4.4, illustrating the robustness of the method. The results are shown in table 6.

Stability of analytical solution: The stability of the standard 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 hydrochloride was 0.76 %. The assay values were within + 2 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

Table 6. Results of robustness study

S. No.	Parameters	Variations and Base degraded product	Resolutions between Doluxetine and base degraded product
1	Temperature	a) at 25 ^{o}C	3.1
		b) at 35 ^{o}C	3.6
2	Flow rate mL min^{-1}	a) 0.8 mL min^{-1}	3.9
		b) 1.2 mL min^{-1}	4.3
3	Mobile phase mL	a) 40.5 mL	4.4
		b) 49.5 mL	3.8

Determination of active ingredients in tablets: The contents of drug in tablets were determined by the planned method using the calibration curve. The results were shown in Table 6.

APPLICATION

1. The duloxetine hydrochloride which is determined selective, precise, accurate and rapid.
2. This method belongs and developed for quantitative determination.
3. The developed method can be conveniently used for the assay determination of duloxetine hydrochloride in bulk drugs and pharmaceutical dosage.
4. This also used for dissolution of tablets of the pharmaceutical dosage forms containing duloxetine hydrochloride.

CONCLUSION

The method developed for quantitative determination of duloxetine hydrochloride which is selective, precise, accurate and rapid. The method was completely validated showing suitable data for all method-validated parameters tested. The developed method is stability indicating and can be used for assessing the stability of duloxetine hydrochloride as bulk drugs. The developed method can be conveniently used for the assay determination of duloxetine hydrochloride in bulk drugs and pharmaceutical dosage form and also used for dissolution of tablets of the pharmaceutical dosage forms containing duloxetine hydrochloride.

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