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A validated stability indicating rapid LC method for duloxetine HCl

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The present paper describes the development of a reversed phase liquid chromatographic (RPLC) analytical method for duloxetine HCl in the presence of its impurities and degradation products generated from forced decomposition studies. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The degradation of duloxetine HCl was observed under acid hydrolysis. The drug was found to be stable in other stress conditions studied. Successful separation of the drug from the synthetic impurities and degradation products formed under stress conditions was achieved on a Zorabax XDB C18, 50 mm × 4.6 mm, 5.0 micron column using a mixture of aqueous 0.1% trifluroacetic acid, methanol, tetrahydrofuran (60 : 20 : 20, v/v/v) as mobile phase. The HPLC method developed was validated with respect to linearity, accuracy, precisions, specificity and ruggedness. To our knowledge, a rapid stability indicating LC method for duloxetine HCl has not been published elsewhere.

1. Introduction

Duloxetine HCl, (*S*)-*N*-methyl-3-(1-naphthale-nyloxy)-2-thiophene propanamine hydrochloride is a selective serotonin and norepinephrine reuptake inhibitor and it has been approved for the treatment of major depressive disorder and for the management of diabetic peripheral neuropathic pain. The drug is believed to potentiate serotonergic and noradrenergic activity in the central nervous system. The hydrochloride salt of the (*S*)-enantiomer was launched as a therapeutic agent under the trade name of Cymbalta® (Eli Lilly).

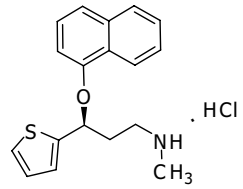
Organic impurities can arise during the manufacturing and storage of the drug substances and the criteria for their acceptance up to certain limits are based on pharmaceutical studies or known safety data. According to regulatory guidelines, pharmaceutical studies using a sample of the isolated impurities can be considered for safety assessment. It is therefore, essential to isolate and characterize unidentified impurities present in the drug sample. Recently a process for synthesis of duloxetine HCl has been developed in our laboratory. During the development of an analytical procedure, an RP-HPLC method was developed for the determination of duloxetine HCl and its impurities.

High-performance liquid chromatographic (HPLC) and spectroscopic methods have been reported in the literature for the determination of duloxetine HCl and its active metabolites (Kuo et al. 2004, Bymaster et al. 2003). The HPLC method for the determination of duloxetine HCl in tablet formulations is applicable to the qualification of duloxetine HCl in tablet formulations (Jansen et al. 2000) and is not a validated method for the quantitative determination of related substances in bulk drugs. To best of our knowledge, no validated stability indicating analytical method for the determination of related substances in duloxetine HCl bulk drug was available in the literature. Attempts were therefore made to develop a stability indicating HPLC method for the determination of related substances and the quantitative estimation of duloxetine HCl. This paper deals with the forced degradation of duloxetine HCl under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat and UV light. This study also deals with the validation of the method developed for the accurate quantification of impurities of duloxetine HCl.

The HPLC analysis of duloxetine HCl showed relative retention times compared to duloxetine HCl at 0.21, 0.62, 0.86, 1.18 and 2.30 min for Imp-A, Imp-B, Imp-C, Imp-D and Imp-E respectively.

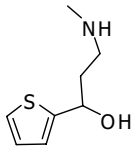
2. Investigations, results and discussion

The main objective of the chromatographic method is to separate duloxetine HCl from its impurities, imp-A, imp-B, imp-C, imp-D and imp-E. Impurities were co-eluted using different stationary phases such as C8, phenyl and cyano as well as different mobile phases. The chromatographic separation was achieved on an Zorabax XDB C18, 50 mm × 4.6 mm, 5 μ column using a mobile phase comprising a mixture of aqueous 0.1% trifluoroacetic acid, methanol, tetrahydrofuran (60 : 20 : 20, v/v/v). The flow rate of the mobile phase was 0.8 ml/min, at 25 °C column temperature, and the peak shape of the duloxetine HCl was found to be symmetrical. Under optimized chromatographic conditions, imp-A, imp-B, imp-C, Duloxetine HCl, imp-D and imp-E were separated with resolution greater than 1.0, typical retention times being about 0.7, 2.0, 2.8, 3.2, 3.9 and 8.0 min, respectively (Fig.). The system suitability results are given in Table 1 and the LC



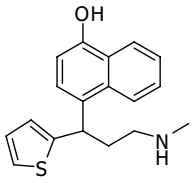
(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine hydrochloride

Duloxetine hydrochloride



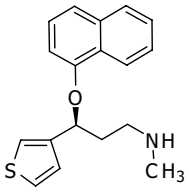
3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol

Imp-A



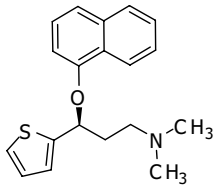
4-(3-(Methylamino)-1-(thiophen-2-yl) propyl)naphthalen-1-ol

Imp-B



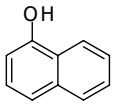
(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-3-yl)propan-1-amine

imp-D



(S)-N,N-Dimethyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine

Imp-C



Naphthalen-1-ol

Imp-E

Duloxetine HCl and impurities (IMP)

method developed was found to be specific for duloxetine HCl and its five impurities, namely imp-A, imp-B, imp-C, imp-D and imp-E (Fig.).

Degradation was not observed in duloxetine HCl samples when subjected to stress conditions such as light, heat and base hydrolysis. Duloxetine HCl was degraded to impurity E under acid hydrolysis and peroxide hydrolysis (Fig.). Peak purity test results confirmed that the duloxetine HCl peak was homogenous and pure in all the stress samples analyzed. The assay of duloxetine HCl was unaffected in the presence of imp-A, imp-B, imp-C, imp-D and imp-E and its degradation products confirming the stability indicating power of the method. The forced degradation studies are summarised in Table 2.

Table 1: System suitability report

S. No.	Name	Retention time, Rt	Relative retention time, RRT	Resolution, Rs	Theoretical plates, N	Tailing factor, T
01	Imp-A	0.70	0.21	–	832	0.6
02	Imp-B	2.04	0.62	11.4	3543	0.70
03	Imp-C	2.83	0.86	5.3	4963	0.73
04	Duloxetine	3.29	1.0	2.8	5780	0.76
05	Imp-D	3.88	1.18	3.2	6276	0.79
06	Imp-E	7.57	2.30	13.9	8222	0.96

The % R.S.D. of the assay of duloxetine HCl during the method precision study was within 0.40% and the % R.S.D for the area of imp-A, imp-B, imp-C, imp-D and imp-E in the related substance method precision study was within 1.38% to 9.06% for six replicates. The % R.S.D. of the assay results obtained in the intermediate precision study was within 0.09% and the % R.S.D. for the area of imp-A, imp-B, imp-C, imp-D, imp-E and imp-F were well within 3.4%, confirming good precision of the method.

The limits of detection achieved for all the impurities namely imp-A, imp-B, imp-C, imp-D and imp-E were 0.019%, 0.010%, 0.002%, 0.003 and 0.018% with respect to duloxetine hydrochloride ($2.0\ mg \cdot mL^{-1}$) concentration. The limit of quantification achieved for all impurities namely imp-A, imp-B, imp-C, imp-D and imp-E was 0.057%, 0.029%, 0.006%, 0.010% and 0.056% respectively. The precision was determined at the LOQ concentrations for imp-A, imp-B, imp-C, imp-D and imp-E, and the % RSD was found to be below 7.5 for all the impurities.

A linearity calibration plot for the assay method was obtained over the calibration ranges tested, i.e. 125–$375\ \mu g \cdot mL^{-1}$, and the correlation coefficient obtained was greater than 0.999.

Linear calibration plots for the related substance method were obtained over the calibration ranges tested, i.e.

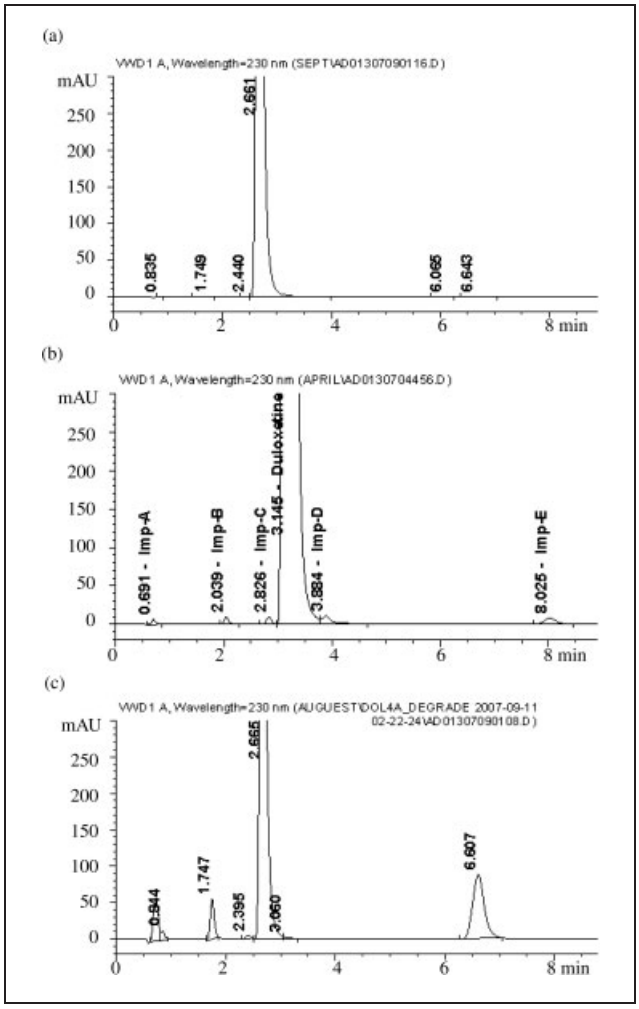


Fig.: Chromatograms of duloxetine HCl (a) sample (b) spiked with 0.15% of all impurities (c) under acid degradation

0.025% to 0.15% for impurities imp-A, imp-B, imp-C, imp-D and imp-E (Table 3). The correlation coefficients obtained ranged from 0.99 to 0.9999. These results indicated an excellent correlation between the peak area and the concentration of imp-A, imp-B, imp-C, imp-D and imp-E.

The percentage recovery of duloxetine HCl in bulk drug samples ranged from 99.8 to 100.2%. The percentage recovery of impurities in duloxetine HCl samples varied from 90.8 to 115.6% (Table 4).

In all the deliberately varied chromatographic conditions (flow rate, column temperature, composition of organic solvent and pH of the mobile phase), the resolution between the critical pair, i.e. duloxetine HCl and imp-C, was greater than 2.47, illustrating the robustness of the method.

Table 3: Linearity study for duloxetine HCl and related substances

Impurity	Concentration (n = 3)	Slope	Y-Intercept	Correlation coefficient
Imp-A	0.025%–0.15%	135.62	0.082	0.9942
Imp-B	0.025%–0.15%	207.82	1.781	0.9985
Imp-C	0.025%–0.15%	414.53	0.25	0.9999
Imp-D	0.025%–0.15%	379.33	−0.061	0.9998
Imp-E	0.025%–0.15%	454.93	4.831	0.9993

Table 4: Recovery studies for impurities of duloxetine HCl

Impurity	% Recovery (n = 3)					
	0.025%	0.050 %	0.075%	0.10%	0.125%	0.15%
Imp-A	106.5	107.7	100.8	90.8	94.4	103.2
Imp-B	106.5	104.6	103.9	101.8	105.2	106.1
Imp-C	91.3	103.8	100.8	95.9	97.9	102.8
Imp-D	105.0	98.9	99.0	99.4	96.8	101.7
Imp-E	91.8	108.8	95.5	97.2	113.5	115.6

n, number of determinations

The % R.S.D. of the assay of duloxetine HCl during solution stability experiments was within 1.2%. No significant changes were observed in the content of impurities, namely imp-A, imp-B, imp-C, imp-D and imp-E, during solution stability and mobile phase stability experiments when performed using the related substance method. The solution stability and mobile phase stability experiment data confirm that the sample solutions and mobile phases used during the assay and the related substance determination were stable for at least 48 h.

The samples investigated of duloxetine HCl and impurities, namely imp-A, imp-B, imp-C, imp-D, and imp-E, were synthesized and characterised with the aid of NMR, LC-MS/MS and FT-IR at Inogent Laboratories Private Limited, Nacharam, Hyderabad, India.

3. Experimental

3.1. Chemicals

All reagents used were of analytical reagent grade unless stated otherwise. HPLC grade acetonitrile, HPLC grade triethylamine (TEA) and HPLC grade trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany)

3.2. Equipment

The HPLC system was equipped with quaternary gradient pumps with auto sampler and auto injector (Model Alliance 2695, Make Waters USA) connected to a photo diode array detector (PDA Model 2996, Make Waters USA) controlled with Empower software (Make Waters, USA).

3.3. Preparation of standard and sample solutions

A stock solution of duloxetine HCl and impurities (mixture of imp-A, imp-B, imp-C, imp-D and imp-E) at 2.0 mg · mL^{-1} was also prepared in the mobile phase.

Table 2: Summary of forced degradation results

Stress condition	Duration Hours	% Assay of active substance	Total impurities (%)	Mass balance (%) (% Assay + % Impurities)
Acid degradation (0.5 N HCl)	48	76.2	24.0	100.2
Base degradation (0.5 N HCl)	48	99.1	0.2	99.3
Peroxide degradation (3.0%)	48	88.4	9.9	98.3
Thermal Degradation (60 °C)	48	96.9	2.2	99.1
UV Degradation at 254 nm	48	95.2	3.1	98.3

3.4. Chromatographic conditions

The chromatographic separation was achieved on an Zorabax XDB C18, 50 mm × 4.6 mm, 5 μm column using as mobile phase a mixture of aqueous 0.1% trifluoroacetic acid, methanol, tetrahydrofuran (60 : 20 : 20, v/v/v). The mobile phase was filtered and degassed through a nylon membranefilter (pore size 0.45 μm). The flow rate of the mobile phase was 0.8 ml/min. The column temperature was maintained at 25 °C and detection was set at a wavelength of 230 nm. The injection volume was 20 μL. The test concentration for the related substance analysis was 2.0 mg · mL^{-1}. The standard and the test dilutions were prepared in mobile phase.

3.5. Validation of the method

3.5.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the LC method developed for duloxetine HCl was determined in the presence of its impurities, namely imp-A, imp-B, imp-C, imp-D and imp-E. Stress studies were performed for duloxetine HCl bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Forced degradation under stress conditions of UV light (254 nm), heat (60 °C), acid (0.5 N HCl), base (0.5 N NaOH) and oxidation (3.0% H_2O_2) was used to evaluate the ability of the proposed method to separate duloxetine HCl from its degradation products. For heat and light studies, the study period was 48 h, whereas for the acid, base and oxidation studies it was 48 h. Peak purity for the samples under stress conditions was investigated for the duloxetine HCl peak using a PDA detector.

3.5.2. Precision

The precision of the related substance method was checked by injecting six individual preparations of duloxetine HCl (2.0 mg · mL^{-1}) spiked with 0.15% of imp-A, imp-B, imp-C, imp-D and imp-E with respect to duloxetine HCl analyte concentration. The % R.S.D of the area for each of imp-A, imp-B, imp-C, imp-D and imp-E was calculated.
The intermediate precision of the method was also evaluated using different analysts and different instruments in the same laboratory.

3.5.3. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were determined by the slope method by injecting a series of dilute solutions with known concentrations. A precision study was also carried out at the LOQ level by injecting six individual preparations of imp-A, imp-B, imp-C, imp-D and imp-E and calculating the % R.S.D. of the area.

3.5.4. Linearity

Linearity test solutions for the assay method were prepared from duloxetine HCl stock solutions at five concentration levels from 50 to 150 % of assay analyte concentration (125, 187.5, 250, 312.5 and 375 μg · mL^{-1}). The peak area versus concentration data were analysed by least-squares linear regression.
Linearity test solutions for the related substance method were prepared by diluting stock solutions to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% (0.5 μg · mL^{-1}) of the specification level (LOQ, 0.075, 0.10, 0.15, 0.2, 0.25 and 0.3%). The peak area versus concentration data were analysed by least-squares linear regression.

3.5.5. Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels, 125, 250 and 375 μg · mL^{-1} (50%, 100% and 150%). The percentage recovery was calculated from the slope and Y-intercept of the calibration curve obtained in the linearity study.
The accuracy study for impurities was carried out in triplicate at 0.075%, 0.10%, 0.125%, 0.15%, 0.2%, 0.25% and 0.3% of the duloxetine HCl analyte concentration (0.5 mg · mL^{-1}). The percentage recoveries for impurities were calculated from the slope and Y-intercept of the calibration curves.

3.5.6. Robustness

To determine the robustness of the method developed, the experimental conditions were deliberately altered and the resolution between duloxetine HCl, imp-A, imp-B, imp-C, imp-D and imp-E was recorded.
The flow rate of the mobile phase was 1.0 mL · min^{-1}. To study the effect of flow rate on the resolution, flow was changed by 0.2 units from 0.8 to 1.2 mL · min^{-1}. The effect of the column temperature on resolution was studied at 20 and 30 °C instead of 25 °C. The effect of the percent organic solvent concentration on resolution was studied by varying acetonitrile from −5 to +5% and the pH of the mobile phase was studied by varying pH by −0.2 to +0.2, while other mobile phase components were held constant.

3.5.7. Solution stability and mobile phase stability

The solution stability of duloxetine HCl and its impurities in the related substance method was investigated by leaving spiked sample solutions in tightly capped volumetric flasks at room temperature for 24 h. The contents of imp-A, imp-B, imp-C, imp-D and imp-E were determined every 6 h up to the study period. The mobile phase stability was also investigated for 48 h by injecting the freshly prepared sample solutions every 6 h. Contents of imp-A, imp-B, imp-C, imp-D, imp-E and imp-F were checked in the test solutions.
The % R.S.D. for the assay of duloxetine HCl was calculated during the mobile phase and solution stability experiments.

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