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A stability-indicating LC method for the estimation of related substances of duloxetine hydrochloride

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ABSTRACT

A simple, inexpensive and rapid LC method has been developed for the quantative determination of duloxetine , an anti-depressant drug. The method can also be employed for the determination of duloxetine and its impurities in the bulk drug. Degradation studies were performed on the bulk drug by heating to 105°C, exposure to UV light at an energy of 200Watt hours/ $m^2$and to Visible light at an illumination of not less than 1.2 million lux hours, acid (0.1 N hydrochloric acid), base (5.0N sodium hydroxide) aqueous hydrolysis and oxidation with 6.0% *v/v* hydrogen peroxide. Considerable degradation was observed under acid, base and oxidation conditions. Good resolution between the peaks corresponding to impurities produced during synthesis, degradation products and the analyte was achieved on a Symmetry C_8 LC column using a mobile phase consisting of a mixture of aqueous potassium dihydrogen phosphate, Sodium-1-octane sulfonate and acetonitrile. The degradation samples were assayed against the reference standard of duloxetine and the mass balance in each case was close to 99.9%. Validation of the method was carried out as per ICH requirements.

KEYWORDS

Column liquid chromatography;
Degradation studies;
Duloxetine;
Method validation.

INTRODUCTION

Duloxetine, 1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl) -1,3-dihydroisobenzofuran -5-carbonitrile hydro bromide is a serotonin-nor epinephrine reuptake inhibitor manufactured and marketed by Eli Lilly[1]. It is effective for major depressive disorder and it is as effective as venlafaxine in generalized anxiety disorder, it is a well tolerated and is considered a first line treatment strategy. Duloxetine has demonstrated efficacy for the treatment of major depressive disorder. In three out of six well-designed properly controlled pre-marketing trials duloxetine performed better than placebo; the three other trials were inconclusive. Recently, duloxetine was shown to be effective in elderly with recurrent major depressive disorder where it improved cognition, depression, and some pain measures. A meta-analysis of these trials indicated that the effect size of duloxetine as compared with placebo was weak-to-moderate, and similar to other 11 antidepressants studied[2]. The ratio-

nale behind the development of duloxetine was that inhibition of the reuptake of both serotonin and nor epinephrine would make it work better than selective serotonin reuptake inhibitors (SSRIs), which inhibit only the reuptake of serotonin. Its chemical designation is (+)-(*S*)-*N*-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride. The empirical formula for duloxetine hydrochloride is $C_{18}H_{19}NOS{\cdot}HCl$, and the molecular weight is 333.88.

The International Conference on Harmonization (ICH) guidelines[3] require stress testing of drug substances, which can help identify the likely degradation products, can be useful in establishing degradation pathways and in validating the stability-indicating power of the analytical procedures used. Moreover, a validated analytical method must be applied in stability studies[4].

Although there are analytical methods for the determination of duloxetine and some impurities by other techniques like LC and LC–MS–MS detection[5–7]. It was simple method for the quantitative determination of duloxetine and its impurities including process related and degradents. The current work deals with the accelerated degradation of the drug substance under stress conditions like hydrolysis, oxidation, heating and UV light. The work also includes the validation of the stability-indicating method developed. This method can be used for quality control during manufacture and for assessment of the stability of bulk samples of duloxetine.

EXPERIMENTAL

Chemicals and reagents

Samples of duloxetine and its impurities namely imp-A and imp-B. (Figure 1) were received from the Process Research Department of Integrated Product Development Operations of Dr. Reddy's Laboratories, Hyderabad, India. LC grade acetonitrile, Potassium dihydrogen orthophosphate, Sodium-1-octane sulfonate and Potassium hydroxide were purchased from Merck, Schuchardt, Germany. High purity water was prepared by using a Millipore Milli Q plus purification system (Bedford, MA, USA).

Instrumentation

The LC system was a Waters model 2996 equipped with a PDA (Waters Corporation, Milford, USA). The output signal was monitored and processed using Empower software (Waters Corporation, Milford, USA) on a Pentium computer (Digital Equipment Co).

Chromatographic conditions

Chromatographic separation was achieved on a 5µm Symmetry C_8 column, (250mm×4.6mm), using a mobile phase with a buffer containing a mixture of 0.01M aqueous Potassium dihydrogen orthophosphate and 2.0gm/L of Sodium-1-octane sulfonate. The mobile phase A is a buffer with pH adjusted to 5.0 using dilute Potassium hydroxide and mobile phase B is a mixture of water and acetonitrile (20:80, *v/v*). The mobile phase was filtered through a 0.45µm nylon membrane and degassed with helium for 5 min. The mobile phase flow rate was 0.8mL min^{-1}. The LC gradient was time (min)/%B: 0/40, 10/40, 20/50, 35/80, 45/90, 55/90, 60/40 and 65/40. The column was maintained at ambient temperature and the effluent was monitored at 215nm. The injection volume was 5µL. Mobile phase B was used as diluent during the preparation of the standard and test samples.

Preparation of standard solutions

A stock solution of duloxetine (500µg mL^{-1}) was prepared by dissolving an appropriate amount in the diluent. Working solutions of 500 and 250µg mL^{-1} were prepared from stock solution for impurity determination and assay respectively. A stock solution of impurities at 50µg mL^{-1} was also prepared in diluent.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21CFR Sect. 211 requires the development and validation of a stability-indicating assay method. In order to determine whether the determination of impurities and the assay method were adequate, a duloxetine API sample was submitted to forced degradation studies. The specificity of the developed LC method for duloxetine was carried out in the presence of its impurities.

The current regulatory guidelines do not indicate detailed degradation conditions in stress testing. However, the conditions used were found to effect a degradation of preferably not less than 5% but not complete

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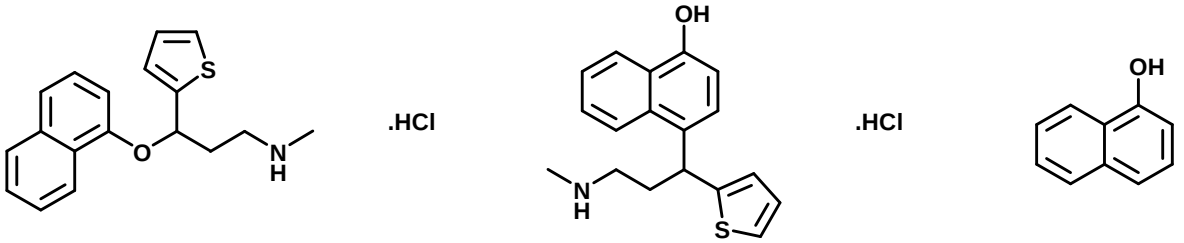

(+)N-methyl-3-(1-napthalenyloxy)-3-(2-thienyl)propanamine hydrochloride

Duloxetine hydrochloride

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

Imp-A

α-naphthol

Imp-A

Figure 1 : Duloxetine hydrochloride, imp-A and imp-B

degradation. Degradation conditions employed were UV light (200Watt hours/m^2), Visible light(1.2 million lux hours) , heating to 105°C, acid hydrolysis with 0.1N HCl, base hydrolysis with 5.0N NaOH, water hydrolysis and oxidative degradation using 6% H_2O_2. Peak purity testing was carried out on the stressed samples of duloxetine by using the PDA detector. Assay studies were carried out on the stressed samples against a qualified reference standard having a purity of 99.8% and the mass balance (% assay + % degradation) was calculated. Assay was also carried out on a bulk sample by spiking impurities each at the specification level of 0.10%.

Method validation

Precision

Precision was evaluated by carrying out six independent assays of a test sample of duloxetine against a reference standard and calculating the % RSD. The precision of the determination of the impurities was checked by injecting six individual preparations of (500µg mL^{-1}) duloxetine spiked with 0.10% of imp-A and imp-B and calculating the % RSD of area for each compound. The intermediate precision of the method was also evaluated using different analysts and a different instrument in the same laboratory.

Limit of detection and limit of quantification

The LOD and LOQ for imp-A and imp-B were estimated at a S/N of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried at the LOQ level by injecting six individual preparations of imp-A and imp-B and calculated the %RSD for the areas.

Linearity

Linearity of test solutions was prepared from stock solution at six concentration levels from 50 to 150% of assay analyte concentration (12.5, 18.75, 25, 31.25 and 37.5µg mL^{-1}). The peak area versus concentration data were subjected to least-squares linear regression analysis. The calibration curve was drawn by plotting duloxetine area injections against the concentration expressed in percentage.

Linearity test solutions for the impurities were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at eight concentration levels from LOQ to 200% with respect to the impurities specification level of 0.10% (i.e. LOQ, 0.025, 0.050, 0.075, 0.10, 0.125, 0.150 and 0.20%).The calibration curves were drawn by plotting the peak areas of imp-A and imp-B against the corresponding concentration.

Linearity testing was performed on two consecutive days in the same concentration range for both assay and related impurities. The% RSD value of the slope and *y*-intercept of the calibration curve was calculated.

Accuracy

The accuracy was evaluated at three concentration levels i.e. 12.5, 18.75, 25.0, 31.25 and 37.5µg mL^{-1} of a bulk drug sample. The% recoveries were calculated.

Accuracy of the determination of the impurities was carried out in triplicate at 0.05, 0.075, 0.10 and 0.15% of the duloxetine concentration (500µg mL^{-1}).The percentages recoveries for the impurities were calculated.

Robustness

To determine robustness, experimental conditions

TABLE 1 : Summary of forced degradation results

Degradation Condition	Duration	Assay (% w/w on anhydrous basis)	Mass balance (% assay+ % degradation)	Remarks
Thermal(105°C)	10 Days	99.9	100.1	No significant degradation observed
Acid hydrolysis 0.1N HCl	8 Hours	78.5	99.8	Imp-A and Imp-B are the major degradents
Base hydrolysis 5.0N NaOH	16 Hours	100.1	99.9	No significant degradation observed
Water hydrolysis	48 Hours	90.2	99.5	Imp-A and Imp-B are the major degradents
Oxidative degradation	48 Hours	94.1	99.8	Imp-A and Imp-B are the major degradents
Photo degradation	7 Days	99.9	99.6	No significant degradation observed

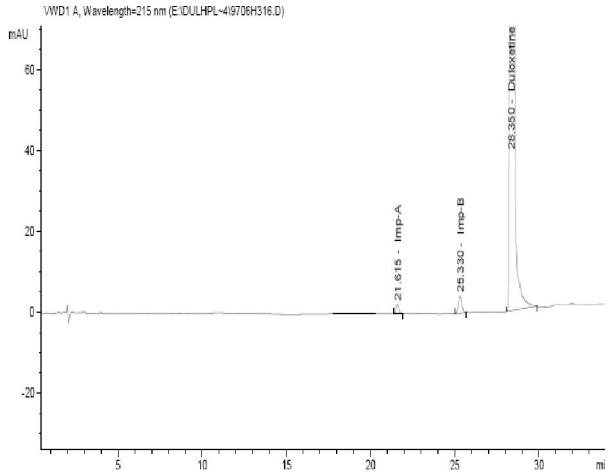

Figure 2 : Blend chromatogram showing imp-A , imp-B and duloxetine

were purposely altered and the tailing and theoretical plates for duloxetine peak was evaluated.

To study the effect of flow rate on the tailing and theoretical plates for duloxetine peak, it was changed by 0.2 units from 0.6 to 1.0mL min^{-1}. The effect of pH on the tailing and theoretical plates for duloxetine peak of the impurities was also studied by varying the pH of mobile phase A from 4.8 to 5.2. The effect of column temperature on the tailing and theoretical plates for duloxetine peak was studied at 20 and 30°C. In all the above conditions, the components of the mobile phase were held constant.

Solution stability and mobile phase stability

The stability of duloxetine in solution was carried out by leaving both the solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for two days. The solutions were assayed at 24 h intervals up to the end of the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions at 24 h intervals over 2 days. Mobile phase composition was kept constant during the study period. The% variation in the content of duloxetine was calculated over the period.

The solution stability of duloxetine and its impurities in the related substances method was carried out by a leaving spiked sample solution in a tightly capped volumetric flask at room temperature for 48 h. Content of imp-A and imp-B were determined at 24 h intervals. Mobile phase stability was also carried out for 48 h by injecting freshly prepared sample solutions at 24 h intervals. Content of imp-A and imp-B were checked in test solutions. Mobile phase composition was kept constant during the study period.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Attempts were made by using different C_{18} and C_8 stationary phases[8]. The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. Effects of pH (2–7) and ionic strength (1–10 mmol L^{-1}) were investigated using phosphate and acetate buffers. It was found that the retention time of duloxetine did not significantly alter at pH 2–5 and ionic strength between 1 and 10 mmol L^{-1}. The optimum conditions are given in "Experimental". Duloxetine, imp-A and imp-B were well separated with a resolution of greater than 1.8 The tailing factor and the number of theoretical plates for the duloxetine peak were 1.5 and 1,45,000(Figure 2).

Results of forced degradation studies

Degradation was not observed in a duloxetine bulk sample during stress conditions like Visible and heating to 105°C. Slight degradation was observed in base degradation condition. Major degradation was observed

in water, acid and oxidation conditions. imp-A and imp-B are the major degradents in acid,water and oxidative degradations.

Peak purity test results confirmed that the duloxetine peak was homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 99.9% (TABLE 1). The assay of duloxetine is unaffected in the presence of imp-A and imp-B confirming the stability-indicating power of the method.

Results of method validation studies

Precision

The RSD of the assay of duloxetine was well within 0.2% and the RSD of the area of imp-A and imp-B was within 1.4%. The RSD of assay results obtained in intermediate precision studies was within 0.4% and the RSD of area of imp-A and imp-B were within 6.0%, confirming the good precision of the method.

Limit of detection and limit of quantification

The limits of detection (LOD) of imp-A and imp-B were found to be in the range of 0.003 to 0.0015% (of analyte concentration 500µg mL^{-1}) in each case for a 5µL sample size. The limits of quantification (LOQ) of imp-A and imp-B were found to be in the range of 0.012 to 0.006%. The precision for imp-A and imp-B at LOQ level was below 3.0% RSD.

Linearity

A linear calibration plot was obtained over the calibration ranges tested, i.e. 12.5 to 37.5µg mL^{-1} and the correlation coefficient obtained was greater than 0.999. Linearity was checked over the same concentration range for two consecutive days. These results show that an excellent correlation exists between the peak area and concentration of the analyte.

Linear calibration plots for the impurities and analyte were obtained over the calibration ranges tested, i.e. LOQ to 0.20% for duloxetine, imp-A and imp-B. The correlation coefficients obtained were greater than 0.999. The results show that an excellent correlation between the peak area and concentration of duloxetine, imp-A and imp-B.

Accuracy

The percentage recovery of duloxetine in bulk drug samples ranged from 99.0 to 101.0%. The percentage recovery of imp-A and imp-B in bulk drug samples ranged from 99.0 to 103.0.

Robustness

In all the deliberately varied chromatographic conditions (flow rate, pH and column temperature), the tailing for duloxetine peak was less than 2.0 and theoretical plates for duloxetine peak is more than 3000 illustrating adequate robustness of the method.

Solution stability and mobile phase stability

The RSD of the assay of duloxetine during solution stability and mobile phase stability experiments was within 1.0%. No significant change was observed in the content of imp-A and imp-B during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirm that sample solutions and mobile phase used during assays were stable up to 48 h.

CONCLUSIONS

The simple RP-LC method developed for the quantitative determination of duloxetine and its possible degradation products and impurities is precise, accurate and specific for the analysis of bulk material. The method was fully validated, showing satisfactory results for all the parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples.

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