Stress Degradation Studies on Duloxetine Hydrochloride and Development of an RP-HPLC Method for its Determination in Capsule Formulation

V.R. Sinha[1,*], Anamika[1], R. Kumria[3], and **J.R. Bhinge[1,2]**

[1]University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India; [2]Center with Potential for Excellence in Biomedical Sciences, Panjab University, Chandigarh-160014, India; and [3]Ind-Swift Laboratories Limited, Parwanoo, India

Abstract

Duloxetine hydrochloride (HCl) is an antidepressant drug prescribed for major depressive disorders, pain related to diabetic peripheral neuropathy, and stress urinary incontinence. In the present study, degradation behavior of duloxetine HCl was studied by subjecting the drug to various International Conference on Harmonization-recommended stress conditions. Also, a stability-indicating high-performance liquid chromatography method was established for analysis of the drug in the presence of various degradation products. An acceptable separation of the drug and its degradation products was achieved on a C-8 column at 40°C using a mobile phase comprised of phosphate buffer (pH 2.5)–methanol–tetrahydrofuran in the ratio of 50:40:10 at a flow rate of 1 mL/min. The detection wavelength was 232 nm. The method was validated for linearity, precision, accuracy, selectivity, specificity, and robustness. The method was found to be linear over a concentration range of 1–100 µg/mL (n = 6). The value of slope was found to be 85.735 mV/s ppm with correlation coefficient of 0.9994 and relative standard deviation (RSD) of 0.87%. RSD values ranged from 0.20% to 0.82% in the case of intra-day precision studies, whereas the values ranged from 0.63% to 1.57% in the case of inter-day precision. The drug was found to be stable on exposure of 30% H_2O_2 for 48 h. It was found to be highly unstable in acidic conditions, as 41.35% degradation was observed in 0.01N HCl at 40°C after 8 h. Degradation was also observed in alkaline and neutral conditions (2.83% and 42.75%, respectively) on refluxing the drug for 1 h. The drug was stable under photolytic and thermal stress on exposure in solid form but showed considerable degradation in solution form.

Introduction

Stability testing and forced degradation studies play a very crucial role during drug development (1). Stability is fundamental to all product characteristics, and the term "stability-indicating assay" has been used to describe "a procedure which affords specific determination of a drug substance in the presence of its degradation products" (2). The prime goal of studying the stability of a drug is to determine the shelf-life of the drug. Identification of the degradation products, establishment of degradation pathways, determination of intrinsic stability of the drug molecules, and validation of the analytical procedure are some of the goals achieved by stress testing (3). The various conditions specified for forced degradation studies include thermal, acidic, alkaline, and neutral hydrolysis conditions, and oxidative and light stress (4–9).

Duloxetine HCl {(+)-(*S*)-*N*-methyl-3-(1-naphthyloxy)- 3-(thiophen-2-yl)-propan-1-amine hydrochloride} is categorized as an antidepressant, acting as dual inhibitor of serotonin and norepinephrine reuptake (10). The structural formula of duloxetine HCl is depicted in Figure 1. The clinical indications of the drug are major depressive disorder, pain related to diabetic peripheral neuropathy, and stress urinary incontinence (11). The painful physical symptoms associated with depression are especially benefited by the drug (12). When serotonin and norepinephrine are released from nerve cells in the brain, they act to "lighten mood." When they are reabsorbed into the nerve cells, they no longer have an effect on mood. It is thought that when depression occurs, there may be a decreased amount of serotonin and norepinephrine released from nerve cells in the brain. Duloxetine works by preventing serotonin, norepinephrine, 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, respectively. This helps prolong the "mood-lightening" effect of any released serotonin and norepinephrine. In this way, duloxetine is thought to help relieve depression (13). The major side effects of the drug are dry mouth, headache, insomnia, sleepiness or somnolence, dizziness or orthostatic hypotension, and fatigue. Duloxetine has an elimination half-life of approximately 12 h (range 8 to 17 h), and its pharmacokinetics is dose-proportional over the therapeutic range (14).

Various analytical methods have been developed for the drug, like radioanalysis in plasma, urine, and expired breath sample. Lantz et al. used mass spectrometry and nuclear magnetic resonance for identification of its metabolites in plasma (15). The other methods used for

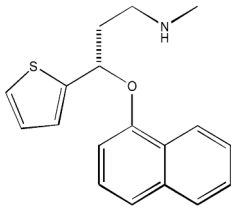

Figure 1. Chemical structure of duloxetine HCl.

* Author to whom correspondence should be addressed: email vr_sinha@yahoo.com.

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the determination of the drug in plasma have included liquid chromatography–tandem mass spectrometry (16–17). Duloxetine and its stable-isotope labeled internal standard were extracted from plasma using Spec Plus C8 solid-phase extraction columns. The compounds were separated chromatographically on Phenomenex Luna C18 columns with a mobile phase consisting of 55% acetonitrile–45% 10 mM ammonium acetate, pH 5 (v/v). The extracts were analyzed using turbo ionspray (16). In another method, duloxetine and the stable label internal standard were extracted from plasma using C8 solidphase extraction plates. The analytes were chromatographed on an HIRPB analytical column (Hichrom Ltd., Theale, UK) attached to a PerkinElmer Sciex API III+ mass spectrometer (Foster City, CA) set in the multiple reaction monitoring modes (17). The need for the stability studies on a drug candidate arises from the fact that the chemical integrity of the drug substance should be maintained until the compound is delivered to the intended site of action. The parent drug stability test guideline issued by the International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples be fully validated, and the assays should be stability-indicating. A stability-indicating assay method helps in establishing the inherent stability of a drug, providing assurance on detection changes in identity, purity, and potency of the product. To date, no report is available on the stability studies of duloxetine HCl in bulk under the various stress conditions (acidic, neutral, alkaline, oxidative, thermal, and photolytic stress) specified in the ICH guidelines for stress testing of a drug substance. Using the reversed-phase high-performance liquid chromatography (RP-HPLC) method, degradation studies were performed on the drug after exposing the drug to various stress conditions.

Experimental

Reagents and solutions

Duloxetine HCl was provided by Ranbaxy Laboratories Ltd. (Gurgaon, India) and was used without further purification. Methanol (HPLC-grade) was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India), and tetrahydrofuran (THF) (HPLC-grade) was procured from Merck Ltd. (Mumbai, India). Sodium hydroxide (S.D. Fine Chem. Ltd., Mumbai, India), hydrochloric acid (Qualikems, New Delhi, India), and hydrogen peroxide (Qualigens Fine Chemicals, Mumbai, India) were of analytical reagent grade. Water obtained after reverse osmosis (Sartorious, Germany) was used after further filtration through a 0.45-µ membrane filter.

Duloxetine HCl stock standard solution (1 mg/mL)

Accurately weighed 100 mg of duloxetine HCl powder was transferred to a 100-mL volumetric flask; to this, 40 mL of water was added, then the mixture was sonicated for 30 s. The final volume was made up with water, and the resulting solution was vortexed for 1 min.

Apparatus and operating conditions

The HPLC system (Shimadzu, Japan) consisted of a LC-10 AT VP Shimadzu pump equipped with a SPD-10 A VP UV-visible detector. The data was processed using Spinchrom Software. The separations were carried out using a C-8 RP column (Thermo Hypersil, BDS, 150 × 4.6 mm, S-5 µ), which was operated at 40°C. The mobile phase was composed of phosphate buffer (pH 2.5) and organic phase in the ratio of 50:50. The organic phase consisted of methanol and THF (80:20). The flow rate was adjusted at 1 mL/min, and the wavelength of detection was 232 nm. Specificity testing was done on a Waters (Milford, MA) Delta 600 HPLC equipped with a 600 controller pump, 2996 photodiode array (PDA) detector, and a degasser module. Empower 2 Software was used for data acquisition and processing. For the investigation of photostability of the drug, a stability chamber (KBF 240, WTB Binder, Tuttlingen, Germany) equipped with light sources was used, as defined under option 2 of the ICH guideline Q1B. In this chamber, the combination of two black lights OSRAM L73 lamps and four white fluorescent OSRAM L20 lamps formed the light bank. The spectral distribution of black light lamps was between 345 and 410 nm with a maximum out put set at 365 nm, whereas the output of white fluorescent lamps was similar to that specified in ISO 10977 (1993). Both UV and visible lamps were put on simultaneously. The chamber was maintained at 40ºC and 75% relative humidity (RH). The samples were exposed for a total period of 10 days. Thermal degradation of the drug was investigated using a hot air oven maintained at 60ºC for 15 days.

Stress studies

Neutral conditions. For neutral hydrolysis, the drug solution in water (1 mg/mL) was prepared and exposed to different conditions like 2 h at 25°C, 8 h at 40ºC, and reflux for 12 h. The samples were analyzed by the HPLC method after suitable dilutions and filtration.

Acidic conditions. For acidic hydrolysis, hydrochloric acid of different strengths was used. The drug solution (1 mg/mL) was prepared in 0.1 N HCl and then refluxed for 8 h, and the drug solution prepared in 0.01 N HCl was kept at 25°C for 2 h, and at 40°C for 8 h.

Alkaline conditions. Alkaline degradation studies were performed by preparing the drug solution (1 mg/mL) in 0.1N sodium hydroxide. The resulting solution was refluxed for 1 h and 8 h, and also kept at 40ºC for 8 h. The drug solution prepared in 0.01 N NaOH was kept at 25ºC for 2 h and at 40ºC for 8 h.

Oxidation. Oxidative degradation studies were performed using hydrogen peroxide solution of different strengths at room temperature. The drug solution (1 mg/mL) was prepared in 3% H_2O_2 and exposed for 6 h, 10 h, and 24 h. A similar solution was prepared in 10% H_2O_2, and it was analyzed after 24 h. Studies were also carried out on drug solution in 30% H_2O_2 kept for 48 h.

Photodegradation studies. Photodegradation studies were carried out by exposing the drug solution (1 mg/mL) as well as powder drug in a photostability chamber for 10 days. The powder was spread as a thin layer in a petri plate. The samples of both solution and powder were kept in parallel in darkness for the same period.

Thermal stress studies. The bulk drug, in a thin layer in a petri plate, and drug solution (1 mg/mL) were exposed to thermal stress conditions in a hot air oven at 60ºC for 15 days.

Method validation

Linearity

A calibration curve of duloxetine HCl was prepared in water for the establishment of linearity. For the same, a stock solution of the drug (1 mg/mL) was prepared in water. The concentrations ranging from (1–100 µg/mL) were prepared after suitable dilution (n = 6). The samples were filtered through a 0.45-µ membrane filter prior to HPLC analysis. All the determinations were done in triplicate. The linearity plots were constructed, and linear regression was applied on the data.

Precision

Repeatability studies were performed for the determination of intra- and inter-day precision. Intra-day precision studies were performed by injecting three different concentrations (10, 50, and 100 µg/mL) in hexplicate on the same day; while for inter-day precision studies, these concentrations were injected in triplicate on six different days. Concentrations were calculated from the area obtained from the linearity plots and the results were expressed as % relative standard deviation (RSD).

Accuracy

For the evaluation of accuracy of the method, the drug was spiked at three different concentrations (1, 50, and 100 µg/mL) in a mixture of stressed sample. The area obtained was used to calculate the recovery of the added drug.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities (18). The method specificity was evaluated by determining the purity of the drug peak in the presence of various degradation products using a PDA detector.

Robustness

For the establishment of robustness of the developed method, experimental conditions were deliberately altered and then resolution between the peaks was calculated. This was done by altering two conditions: changing the flow rate by 0.2 units (0.8 mL/min and 1.2 mL/min) and altering the column temperature (30ºC and 40ºC).

Identification of a degradation product

The isolation and identification of [3-(4-hydroxynaphthalen-1-yl)-*N*-methyl-3-(thiophen-2-yl) propan-1-aminium chloride] as one of the degradation products was verified by spiking with the standard sample. The degradation product was isolated from the commercial sample of duloxetine HCl. The hydrochloride salt of duloxetine was suspended in cyclohexane and treated with 20% sodium hydroxide solution. The aqueous phase was separated, neutralized, and extracted with ethyl acetate. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in ether, and the solution was saturated with HCl (g), which was crystallized from methanol (19). The resulting product was dissolved in mobile phase and subjected to HPLC analysis.

Assay of capsule formulation

The contents of 10 delayed release duloxetine hydrochloride capsules were taken in 100-mL volumetric flask, and 50 mL of methanol–THF mixture (80:20) was added to dissolve the enteric coat. The resulting dispersion was sonicated for 15 min, and the volume was made with phosphate buffer pH 2.5. The dispersion was kept as such for 30 min, vortexed for 5 min, and diluted to a suitable concentration, which was finally filtered through a 0.45-µ filter. The same procedure was followed for the determination of duloxetine in stability samples kept at 40ºC/75% RH for three months.

Results and Discussion

The chromatogram of the standard drug solution showed four different peaks with principle drug peak at 5.33 min. The other peaks were observed with relative retention time (RRT) of 0.64, 0.84, and 1.35, with RSD of 0.68, 1.02, and 0.81, respectively. The corresponding peak area of the drug was found to be 98.38%.

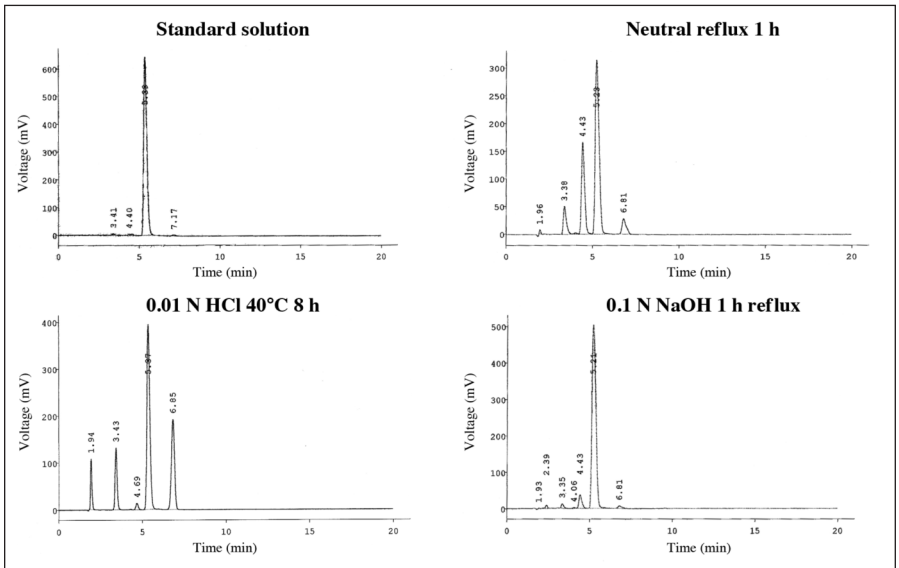

Figure 2. Degradation behavior of duloxetine HCl after refluxing in acidic, alkaline, and neutral conditions.

Degradation behavior of duloxetine HCl under different stress conditions

The drug was found to be susceptible to hydrolytic stress, light (in solution form), and heat (in solution form). However, the drug was stable to the oxidative stress (in solid form), light (in solid form), and heat (in solid form).

Neutral conditions

The drug underwent neutral hydrolysis, and a significant degradation (43%) was observed after 12 h reflux. The drug degraded to four major degradation

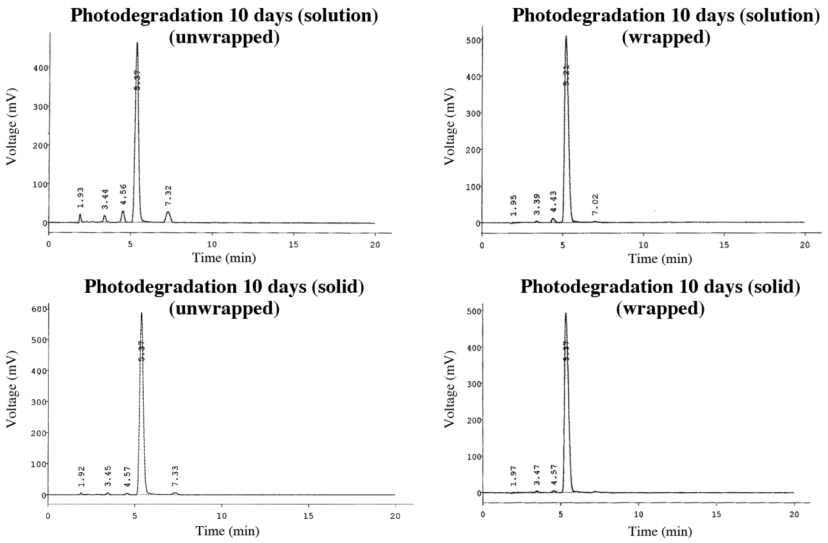

Figure 3. Chromatograms showing the degradation behavior of duloxetine HCl in solid and solution states under photolytic conditions.

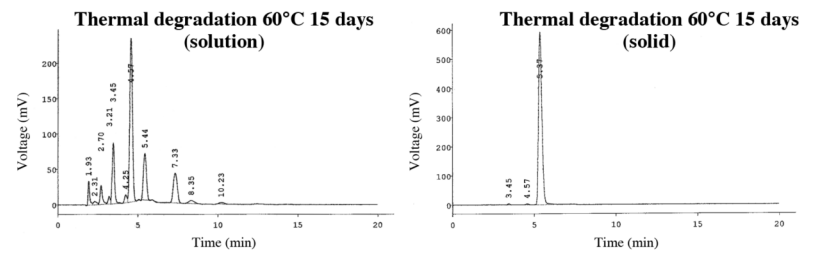

Figure 4. Chromatogram showing the degradation behavior of duloxetine HCl after thermal stress study at 60°C for 15 days.

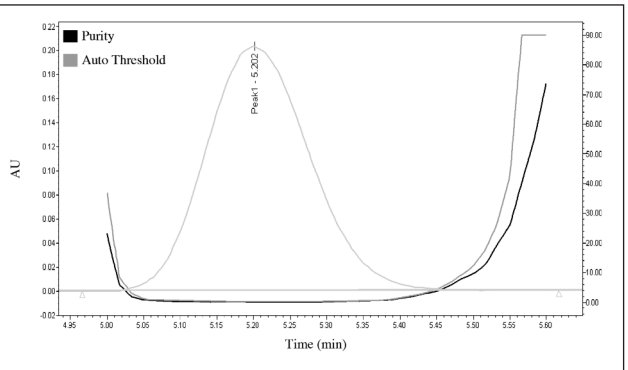

Figure 5. Peak purity studies for the drug.

products with RRT of 0.38, 0.65, 0.85, and 1.30 (Figure 2). The drug solution kept at 40ºC for 8 h showed no significant degradation.

Acidic conditions

The drug was found to be acid labile. The amount of drug remaining after refluxing for 1 h in 0.1 N HCl was just 0.37%. In order to study the effect of milder conditions on the acid degradation behavior of duloxetine, studies were performed in 0.01N HCl at 40ºC for 8 h. The drug degradation was 41.35% (Figure 2).

Alkaline conditions

The drug was found to be more stable to alkaline hydrolysis as compared to acidic hydrolysis. After refluxing of the drug solution in 0.1 N NaOH for 1 h, only 2.83% degradation was observed; but when the reflux was continued for 8 h, 97.40% of the drug got degraded. The drug decomposed to six degradation products at RRT of 0.37, 0.46, 0.64, 0.78, 0.85, and 1.31 (Figure 2).

Oxidative conditions

The drug was found to be highly stable to oxidative stress. There was very little change in peak area on exposing the drug solution to 30% H_2O_2 solution for 48 h.

Photodegradation

The drug was stable in solid state to light, both in wrapped and unwrapped states. But in the case of solution, the unwrapped sample showed 22.08% degradation; whereas, it was 1.54% in the case of the wrapped sample after exposure equivalent to an ICH dose of light (Figure 3).

Thermal degradation

The drug was stable in solid state under thermal stress conditions. However, when a solution of the drug was kept at 60ºC for 15 days, 89.63% of drug degraded to 10 degradation products with RRT of 0.36, 0.43, 0.50, 0.59, 0.63, 0.78, 0.84, 1.35, 1.53, and 1.88 (Figure 4).

Method Validation

Linearity

The data from the linearity curve showed that the response of the drug was linear in the studied concentration range. The equation of the line was $y = 85.735x$. A very high correlation of 0.9994 (± 0.0006) was obtained with RSD of 0.87%.

Precision

Table I shows the results of intra- and inter-day precision studies. From the intra-day studies, the percent RSD value was found to be in the range of 0.20% to 0.82%; whereas, in the case of inter-day studies, the values ranged from 0.63% to 1.57%.

Accuracy

The data obtained from the recovery studies is represented in Table II. The recovery was in the range of 95.42% to 101.02%.

Specificity

The drug and the various degradation products formed

Table I. Precision Studies

Actual concentration (µg/mL)	Measured concentration (µg/mL) ± SD; RSD (%) Repeatability (*n* = 6)	Intermediate precision (*n* = 3)
10	10.07 ± 1.76; 0.20	9.90 ± 13.28; 1.56
50	48.12 ± 33.72; 0.82	48.35 ± 39.89; 0.96
100	101.23 ± 49.37; 0.57	102.10 ± 55.07; 0.63

Table II. Recovery Studies

Spiked concentration (µg/mL)	Measured concentration (µg/mL) ± SD; RSD (%)	Recovery (%)
1	1.01 ± 0.07; 0.12	101.02
50	49.02 ± 0.20; 0.19	98.05
100	95.42 ± 0.25; 0.16	95.42

under different stress conditions showed a good resolution, indicating the specificity of the method (Figure 5). The peak purity studies showed the purity angle value of 0.135 and purity threshold value of 0.260. As the purity angle was less than the purity threshold, it could be concluded that the method was specific for the drug.

Robustness

The method was found to be robust, as the resolution between the drug and degradation products was found to be greater than 2.5 when the conditions were deliberately altered. It was seen that on changing the working temperature from 40ºC to 30ºC and 45ºC, the resolution between the peaks was 3.20 and 2.91, respectively. The robustness of the method was also found by changing the flow rate of the mobile phase from 1 mL/min to 0.8 mL/min and 1.2 mL/min, and the resolution between the peaks in this case was found to be 2.90 and 6.59, respectively.

Identification of degradation product

The spiking studies with isolated impurity confirmed that [3-(4-hydroxynaphthalen-1-yl)-*N*-methyl-3-(thiophen-2-yl) propan-1-aminium chloride] was one of the degradation products present in the sample with RRT of 1.30 (Figure 2).

Assay of pharmaceutical formulation

The amount of duloxetine in delayed release capsules was found to be 96.14 ± 0.35% (initial) and 90 ± 0.020% (3 months at 40ºC/75% RH) with RSD of 0.36% and 0.023%, respectively.

Conclusions

An isocratic RP-HPLC method was developed and validated for the determination of duloxetine HCl. The degradation behavior of duloxetine HCl under ICH-recommended stress conditions was studied. Duloxetine HCl was found to be labile to neutral and alkaline hydrolysis and was very labile to acidic hydrolysis. The drug was also labile to light (in solution form) and heat (in solution form). However, the drug was stable to oxidative stress (in solution form), light (in solid form), and heat (in solid form). The developed chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method, including accuracy, linearity, recovery, and precision data.

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