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Development and validation of an analytical method for the stability of duloxetine hydrochloride

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Abstract

The objective of the study was to develop and validate an analytical method for estimating the stability of duloxetine hydrochloride. The drug was subjected to the stress conditions prescribed by the International Conference on Harmonization, including hydrolysis, oxidation, photolysis and dry heat. Five degradation products were formed, which were separated by high-performance liquid chromatography on a Kromasil C18 (150 mm × 4.6 mm, 5 μm) column in a gradient elution programme. The flow rate was 1 ml/min, and the detection wavelength was set to 225 nm. The retention time of the drug was 35.7 min, and analysis was completed within 40 min. The method was validated with respect to linearity, precision, accuracy, robustness and limits of detection and quantification as per the International Conference on Harmonization. The results were linear ($r^2 = 0.999$) over the range 50–400 μg/ml and accurate over the range 99.41–102.98. The method was robust and rugged, as there was insignificant variation in the results of analysis with changes in flow rate and temperature separately.

Keywords: Duloxetine hydrochloride; Stability; Validation; Degradation product

1. Introduction

Duloxetine ((+)-(*s*)-*N*-methyl-3-(1-naphthyloxy)-3-(thiophen-2-yl)-propan-1-amine) is a selective serotonin and norepinephrine reuptake inhibitor used primarily in the treatment of major depressive disorders and stress urinary incontinence [1]. Duloxetine is also used to treat pain and tingling in diabetic neuropathy. Duloxetine, also known as LY248686 [2,3], is a potent dual inhibitor of reuptake of serotonin (5-hydroxytryptamine) and norepinephrine; its effect depends on binding to human serum albumin. It is approved by the United States Food and Drug Administration for the treatment of diabetic neuropathic pain.

Duloxetine hydrochloride is a highly lipophilic compound with strong base properties and a pK_a of 9.5. Its structure is shown in Fig. 1. Phenolic impurities in duloxetine hydrochloride samples have been identified by mass spectrometry, nuclear magnetic resonance spectroscopy and X-ray analysis [4–6].

The purity of a drug product is determined on the basis of the percentage of the labelled amount of active pharmaceutical ingredient found in it by a suitable analytical method, which provides evidence for specificity, linearity range, accuracy, precision, detection limit, quantification limit, ruggedness and robustness of the method for regulatory purposes. High-performance liquid chromatography (HPLC) is the established technique for separating non-volatile organic compounds, drugs, metabolites and toxic residues by isocratic and gradient elution. Reverse-phase, ion-pair, ion and ion exchange

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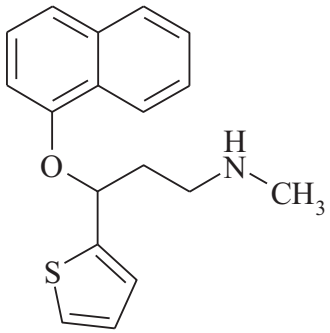

Fig. 1. Structure of duloxetine hydrochloride.

HPLC and sometimes size exclusion chromatography are used conventionally. If the mobile phase remains constant throughout HPLC separation, the separation is deemed to be isocratic, while if the sample contains components of a wide range of polarities, gradient elution is the only way to elute all the compounds in the sample in a reasonable time while maintaining peak resolution by changing the ratio of polar to non-polar compounds in the mobile phase during the sample run. For a reverse-phase gradient, the solvent is initially relatively polar and slowly becomes more non-polar. A sample containing compounds with a wide range of polarities can be separated by gradient elution in a shorter time without loss of resolution in the earlier peaks or excessive broadening of later peaks; it is more difficult to maintain a constant flow rate with continuous changes in mobile phase composition. Gradient elution is used in preparative and large-scale chromatography for separation.

The parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH) suggests that stress studies be carried out on a drug to establish its inherent stability [7], i.e. the extent to which a product retains the same properties and characteristics it had at the time of packaging, within the specified limits, throughout storage and use [8,9]. Stability testing thus indicates the effect of environmental factors on the quality of a drug or a formulated product and is used to predict its shelf life, determine the proper storage conditions and suggest labelling instructions. Various analytical methods have been reported namely, HPLC [10–17], high-performance thin-layer chromatography [18,19], ultra-performance liquid chromatography [20] and liquid chromatography–tandem mass spectrometry [21–23]. Degradation studies have also been reported [24–27] under thermal, acidic, alkaline, neutral hydrolysis and oxidative photolytic stress conditions.

2. Experimental

2.1. Materials

Pure duloxetine hydrochloride was donated by Unimark Remedies Ltd., Mumbai, India. Acetonitrile (HPLC grade) was purchased from Thomas Baker, Mumbai. Other chemicals used were of analytical grade. Ultrapure (double-distilled) water was obtained from a water purification unit.

2.2. Instrumentation

The HPLC instrument used was a Shimadzu LC-20 Prominence (Shimadzu, Kyoto, Japan) system equipped with an LC-20AD binary pump, a SPD-M20A photodiode array detector and a rheodyne injector. The output signal was monitored and processed with liquid chromatography solution software (Shimadzu, Kyoto, Japan). The injection volume was 20 μl, and chromatographic separation was achieved on a Kromasil C18 (150 mm × 4.6 mm), 5 μm particle size column.

2.3. Preparation of standard stock solution

A standard stock solution of the drug was prepared by dissolving pure drug in the mobile phase, i.e. 100 mg duloxetine hydrochloride in 100 ml methanol. The solution was sonicated and filtered through Whatman filter paper, and the resulting solution was further diluted with the mobile phase to a concentration of 1000 μg/ml.

2.4. Preparation of mobile phase

Solvent A: Potassium dihydrogen phosphate (0.68 g) was dissolved in 500 ml water, 0.1% triethylamine was added, and the pH was adjusted to 3.5 with orthophosphoric acid. Triethylamine was used to reduce tailing of the analyte. Methanol was added in proportions of 8.5:1.5. The solution was filtered through a 0.45-μm nylon 66 membrane filter and was further degassed in a sonicator for about 15 min. Solvent B was Methanol.

2.5. Degradation studies

All stress decomposition studies were performed at an initial drug concentration of 1000 μg/ml in methanol. Acid hydrolysis was performed in 0.5 N HCl at 80 °C for 9 h. The study under alkaline conditions was carried out in 1 N NaOH at 80 °C for 4 h. For the study under neutral conditions, drug dissolved in water was heated at 80 °C for 4 h. Oxidative stress studies were carried out at room

Table 1
Gradient programme for separation studies.

Time	Methanol solvent	Phosphate buffer solvent
0	0	100
5	0	100
25	45	55
35	45	55
45	0	100

temperature with 10%, 15% and 30% hydrogen peroxide for 24 h. For the photo-degradation studies, the solutions were exposed for 1 h in a chamber with 200–400 nm ultra-violet light and visible spectra (60,000–70,000 lx) for 10 h. Suitable controls were kept in the dark. Samples were withdrawn at appropriate times and analysed by HPLC after suitable dilution [7,24–27]. Peak purity and mass balance were monitored for each degradation condition.

2.6. Separation studies

Before method development, various physicochemical parameters must be known, such as the pK_a, log P, solubility, absorptivity and wavelength maximum of the drug. The pK_a is important as most pH-related changes in retention occur at pH values within 1.5 units of the pK_a value [28]. Studies were first conducted on all reaction solutions individually and then on a mixture of the solutions, in which decomposition was observed. Separation was achieved by gradient elution. Initial studies on individual reaction solutions were conducted with methanol:potassium dihydrogen phosphate buffer and triethylamine (0.1%) (pH adjusted to 3.5 with orthophosphoric acid), with the gradient programme shown in Table 1.

2.7. Validation of the method [29,30]

2.7.1. Linearity and range

The stock solution was diluted with mobile phase to prepare solutions containing 50–400 μg/ml of the drug. Each concentration was injected six times onto the HPLC column, and the peak areas were noted and plotted against the corresponding concentration to obtain a calibration curve.

2.7.2. Precision

Injections of three concentrations (100, 200 and 300 μg/ml), repeated six times, were given on the same day, and the relative standard deviation was calculated to determine intra-day precision.

2.7.3. Accuracy

Recovery studies by the standard addition method were performed to determine the accuracy of the method, with three concentrations (50%, 100% and 150%) of spiked drug.

2.7.4. Specificity and selectivity

The specificity of the method for the drug was established by determining interference (if any) from solvent or degradation products, with resolution of the drug peak from the nearest resolving peaks. Overall selectivity was established by determining the purity of the peak.

2.7.5. Robustness

The effects of varying the mobile phase composition, flow rate and wavelength were studied.

2.7.6. Limits of detection and quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be detected in a sample, whereas the limit of quantification (LOQ) is the lowest concentration of an analyte that can be determined with acceptable precision and accuracy in a sample under the stated operational conditions. LOD and LOQ were calculated according to the formulae given in the ICH guidelines.

3. Results and discussion

HPLC studies on duloxetine hydrochloride under different stress conditions suggested the following degradation behaviour. The drug was found to be susceptible to acid hydrolysis (0.5 N HCl, 9 h), with 5–16% degradation when heated at 80 °C, but was stable in neutral hydrolysis (water, 4 h). It was stable to degradation with 10%, 15% and 30% hydrogen peroxide at room temperature for 24 h.

Three major degradation products were observed after exposure of drug solution to ultra-violet light for 33 min, with five major degradation peaks at retention times of 3.5 min (Degr. 1), 12.7 min (Degr. 2), 25.4 min (Degr. 3), 30.6 min (Degr. 4) and 32.4 min (Degr. 5) (Fig. 2). The spectral data showed that acid Degr. 3 and photolytic Degr. 1 were identical (Fig. 3).

The solid-state studies showed that duloxetine hydrochloride is stable to the effect of temperature. When the drug powder was exposed to dry heat at 80 °C for 8 days, no decomposition was seen.

The method was optimised to separate the major degradation products formed under various conditions, and resolution was checked with a mixture of the degradation solutions to confirm the separation. The resulting

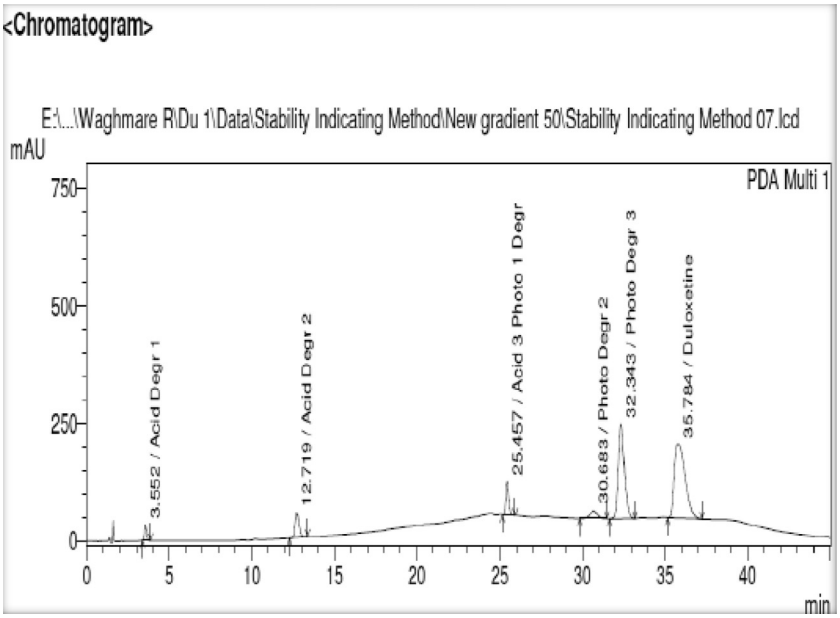

Fig. 2. Chromatogram of degradation products and active pharmaceutical ingredient of duloxetine.

chromatograms are shown in Fig. 3 and Fig. 4. They indicate that the method successfully separated the drug from all degradation products.

The method was validated as per the ICH guidelines. The linearity of the peak area response was determined by injecting samples of 20 μl of dilutions of the standard stock solution onto the column at a flow rate 1.0 ml/min. Each dilution was injected six times. Drug in the eluates was monitored at 225 nm, and the corresponding chromatograms were obtained. The regression of the plot was obtained by least squares regression. The mean peak areas were calculated from these chromatograms, and a

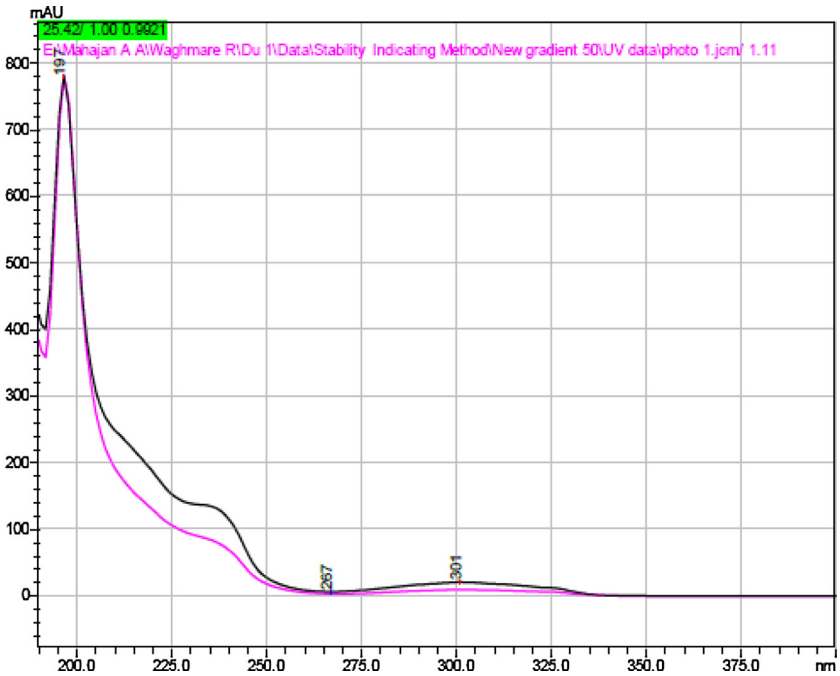

Fig. 3. Ultra-violet spectra of acid Degr. 3 and photolytic Degr. 1.

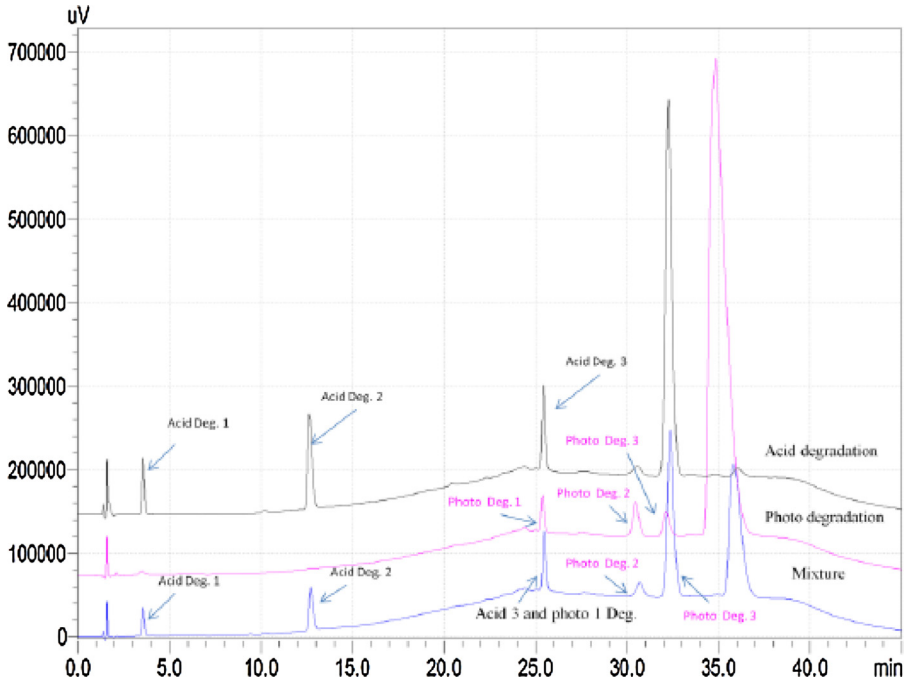

Fig. 4. Chromatogram of acid and photolytic degradation products.

Table 2
Linearity of peak areas of duloxetine hydrochloride.

Series no.	Concentration (μg/ml)	Peak area				Relative SD (%)
		Injection 1	Injection 2	Injection 3	Average	
1	50	7,988,246	8,112,894	8,057,470	8,052,870	0.77
2	100	16,381,354	16,439,857	16,408,360	16,409,857	0.17
3	200	34,779,684	34,798,639	34,787,593	34,788,639	0.02
4	300	52,385,228	51,484,075	51,482,922	51,784,075	1.00
5	400	69,065,440	69,065,150	69,064,860	69,065,150	0.0004

Table 3
Intra-day precision.

Concentration (μg/ml)	Peak area			Mean area	Found concentration (μg/ml)	SD	Relative SD
	Morning	Afternoon	Evening				
100	16,257,544	16,382,795	16,575,412	16,405,250	100.02 ± 0.976	160,119.3	0.976
200	34,985,721	34,985,299	34,984,927	34,985,316	198.87 ± 0.002	397.2625	0.001
300	52,039,032	52,068,078	52,029,396	52,045,502	299.35 ± 0.116	20,136.285	0.038

plot of concentration over the peak area was obtained (Table 2).

The peak area was plotted against the corresponding concentrations to obtain the calibration curve (Fig. 5).

As the relative SD for inter-day precision was less than 2, it can be concluded that the method is reproducible (Table 3).

Accuracy tested by the standard addition method was in the range of 99.41–102.48% (Tables 4 and 5).

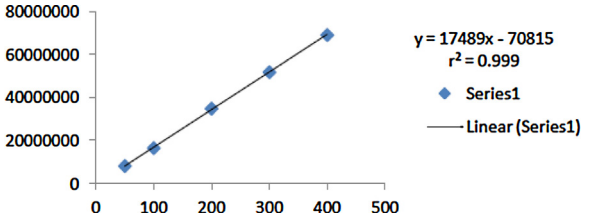

Fig. 5. Calibration curve of duloxetine hydrochloride on HPLC.

Table 4
Inter-day precision.

Concentration (μg/ml)	Peak area			Mean area	Found concentration (μg/ml)	SD	Relative SD
	Day 1	Day 2	Day 3				
100	16,257,854	16,385,972	16,366,248	16,336,691.33	99.55 ± 0.42	68,983.71467	0.422
200	34,971,275	34,982,589	34,975,678	34,976,514	201.08 ± 0.03	5703.141327	0.016
300	52,040,586	52,042,568	52,032,546	52,038,566.67	301.47 ± 0.03	5307.391214	0.010

Table 5
Accuracy.

Actual concentration (μg/ml)	Measured concentration (μg/ml); relative SD	Recovery (%)
50	50.14 ± 0.45; 0.56	100.28
100	100.98 ± 1.93; 1.6	102.98
150	149.12 ± 0.69; 0.67	99.41

Table 6
Robustness.

	Flow rate (ml/min)			Wavelength (nm)		
	0.95	1.00	1.05	223	225	227
Relative SD	0.3988	0.3345	0.3115	0.3286	0.3345	0.3158

As small deliberate changes did not result in significant changes in the peak area, the method is robust and resistant to such changes (Table 6).

The LOD and LOQ were 1.17 and 3.57 μg/ml, respectively, as confirmed by preparing concentrations below and above these values.

4. Conclusion

The HPLC method reported here is accurate, precise, reproducible, specific and stable. There is allowable variation in flow rate, temperature, pH and mobile phase composition, which indicate that the method is robust. The low relative SD for the percentage assay of the test preparation shows that the proposed method is rugged.

This study shows that the drug is highly sensitive to degradation with acid and photolytic stress but stable in dry heat and oxidative conditions. This method allows separation of all degradation products formed under a variety of conditions.

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