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Development and Validation of Stability Indicating Derivative Spectrophotometric Methods for Determination of Duloxetine Hydrochloride

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Authors' contributions

This work was carried out in collaboration between both authors. Author RC designed and supervised the research. Author AB managed the literature searches, performed the spectroscopic analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the present investigation was to develop and validate a rapid, sensitive, cost effective and reproducible stability indicating derivative spectrophotometric method for the estimation of duloxetine HCl in bulk and in formulations.
Methodology: First order derivative spectrophotometric methods were developed for duloxetine HCl employing peak-zero (P-0) and peak-peak (P-P) techniques and their stability indicating potential was assessed in force degraded solutions. The methods were validated with respect to linearity, accuracy, precision and robustness.
Results: Linearity was observed in the concentration range 5 µg/mL-90 µg/mL with an excellent correlation coefficient (r^2) of 0.999. The limits of assay detection values were found to range from 0.33-0.41 µg/mL and quantitation limits ranged from 1.01-1.24 µg/mL for the proposed methods. The proposed method was applicable to the determination of the drug in capsules and the percentage recovery was found to be 99.68±0.95%.
Conclusion: The developed methods were successfully validated and applied to the determination of duloxetine HCl in bulk and pharmaceutical formulations without any interference from common

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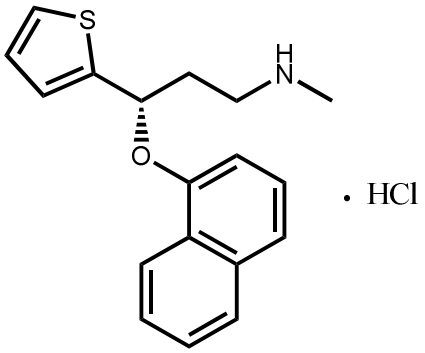
excipients. Satisfactory recovery in force degraded solutions suggests the stability indicating nature of the assay.

Keywords: Duloxetine hydrochloride; stability indicating; derivative spectrophotometry; validation.

1. INTRODUCTION

Duloxetine (DLX), N-methyl-3-(napthalen-1-yloxy)-3-(thiophene-2-yl) propan-1-amine hydro chloride (Fig. 1.) is a selective serotonin-norepinephrine reuptake inhibitor (SNRI) originally developed as an antidepressant and is currently recommended for maintenance treatment of major depressive disorder [1].



DULOXETINE HCl
N-methyl-3-(napthalen-1-yloxy)-3-(thiophene-2-yl) propan-1-amine

Fig. 1. Chemical structure of duloxetine hydrochloride

The drug has subsequently proven to be beneficial in several other therapeutic indications such as peripheral neuropathy. It is considered as a first line treatment for diabetic polyneuropathy and is approved by the US FDA for the purpose [2]. Other indications include management of fibromyalgia [3], generalized anxiety disorder [4] and most recently, stress urinary incontinence [5,6]. The most recent approval was in the year 2010 for the treatment of chronic musculoskeletal pain. Currently, there is no official analytical procedure for duloxetine HCl in any pharmacopoeia. The reports found in the literature for duloxetine HCl concentrate on chromatographic methods, including: reverse phase HPLC method for the simultaneous estimation of duloxetine HCL in enteric coated capsules, using X Terra RP, C-8 column (4.6x150 mm) for the separation and acetonitrile: phosphate buffer (65:35%v/v) as mobile phase for estimation of drug in enteric coated capsules [7], a RP-HPLC method using Phenomenox C-18 in isocratic mode, with mobile phase containing 0.01M 5.5 pH phosphate buffer : acetonitrile (60:40 v/v) for estimation of drug in pharmaceutical formulations [8], a stability indicating RP-HPLC method utilizing Kromasil C18 column with mobile phase containing 0.5M TFA buffer : acetonitrile (65:35 v/v) [9], RP-HPLC methods for estimation of the drug in bulk and formulation [10,11] and HPLC estimation in human plasma after initial solid phase extraction [12]. Recently, a method based on an ion selective membrane electrode was reported [13]. Although, few spectrophotometric methods have been reported for the drug in methanol and hydrochloric acid solutions [14,15], there is still a need for development of a sensitive stability indicating spectrophotometric method for estimation of duloxetine with complete validation as per ICH guidelines. Derivative spectrophotometry offers the advantage of higher sensitivity over zero order spectrophotometry and offers the possibility for application in the drug analysis in presence of degradation products and other impurities. There are numerous examples in literature testifying the applicability of this technique to development of stability indicating methods of analysis for various drugs [16].

The present investigation describes simple rapid, reproducible and stability indicating derivative spectrophotometric methods for the quantification of duloxetine HCl in bulk as well as in capsule dosage forms in phosphate buffer pH 7.4. The drug was subjected to systematic forced degradation studies by employing the conditions prescribed in International Conference on Harmonisation (ICH) [17] and the degraded samples were analyzed by the developed methods in order to assess their stability indicating potential. The procedure does not involve any extraction step with any organic solvent and can be directly carried out in phosphate buffer pH 7.4 which makes it ideal for routine analysis of the drug in bulk or in pharmaceutical formulations. Further, the method has an additional advantage in terms of direct applicability to the estimation of duloxetine in its *in vivo* and *ex vivo* release and permeation studies employing Franz diffusion cells as the

latter make use of phosphate buffer system as the permeation media.

2. MATERIALS AND METHODS

2.1 Materials and Reagents

All chemicals and materials were of analytical grade and were purchased from Qualigens fine chemicals, Mumbai, India. All solutions were freshly prepared in triple distilled water. Duloxetine HCl (DLX) pure grade was graciously provided as gift samples by Lupin Pharmaceuticals, Mumbai, India. Delok 30 capsules (label amount 30 mg duloxetine hydrochloride per capsule) Nicholas Piramal India Ltd. were purchased from the market.

2.2 Apparatus

All absorption spectra were recorded using a Perkin Elmer lambda 15 UV-Visible spectrophotometer (German) with a scanning speed of 60 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells. A CyberScan pH 510 (Eutech instruments) pH meter was used for checking the pH of buffer solutions.

2.3 Preparation of Phosphate Buffer pH 7.4

Phosphate buffer pH 7.4 was prepared by dissolving 0.19 g of potassium dihydrogen phosphate, 2.38 g of disodium hydrogen orthophosphate in triple distilled water and making up the volume to 1000 mL. The pH of the buffer was adjusted to 7.4 using a pre-calibrated pH meter.

2.4 Forced Degradation of Duloxetine Hydrochloride

Hydrolytic decomposition of duloxetine HCl was carried out in 0.1N HCl, 0.1N NaOH and triple distilled water at a drug concentration of 1 mg/mL at 80°C for 8 hours. For the oxidative stress studies, the drug was dissolved at a concentration of 1 mg/mL in 30% H_2O_2 and kept for 24 hours at room temperature. Photodegradation studies were carried out by exposing the drug solution prepared in water (1 mg/mL) to sunlight (approx. 60,000-70,000 lux) for two days. Dark controls were kept concurrently for comparison. Thermal stress testing was carried out in a dry air oven by heating the drug powder at 60°C for 7 days.

2.5 Preparation of Calibration Curves for Duloxetine HCl

Standard Stock solution A (250 µg /mL) of duloxetine HCl was prepared daily by dissolving 0.0250 g of duloxetine in 100 mL of phosphate buffer (pH 7.4). Stock solution A (250 µg /mL) was diluted 1 in 10 to get stock solution B (25 µg/mL). Further, working standard solutions ranging from 1 µg/mL to 100 µg/mL of duloxetine HCl were prepared by serial dilutions of stock solutions A and B with phosphate buffer (pH 7.4). The test tubes were kept stoppered to avoid the loss of solvent due to evaporation. Zero- and first-order derivative spectra of these solutions were recorded over the wavelength range 210-400 nm against phosphate buffer (pH 7.4) taken as the blank and the amplitudes of the maximum and minimum were measured.

2.6 Analysis of Pharmaceutical Formulation

The contents of twenty capsules were mixed and weighed accurately. Powder weight equivalent to 15 mg of duloxetine HCl was transferred to a 100 mL volumetric flasks, dissolved in phosphate buffer (pH 7.4), sonicated for 5 minutes, the volume was then completed with phosphate buffer (pH 7.4), shaken well for 5 min. and filtered into a dry flask to make a solution A (150 µg/mL). The solution was suitably diluted and first-order derivative curves were obtained against phosphate buffer (pH 7.4) taken as the blank.

3. RESULTS AND DISCUSSION

Derivative spectrophotometry offers the advantage of higher sensitivity over zero order spectrophotometry and offers the possibility for application in the drug analysis in presence of degradation products and other impurities. In this light, we explored the first order derivative spectrophotometric curves of duloxetine HCl to develop stability indicating procedure for the drug. Pure drug DLX was subjected to forced degradation under hydrolytic, oxidative, photolytic and thermal stress conditions according to the recommendations of ICH and WHO stability test guidelines and the samples were spiked with pure drug in varying concentrations for analysis. Phosphate buffer

system is the medium commonly utilized for dissolution studies of APIs. Further, the *in vivo* drug release and *ex vivo* drug permeation studies (Franz diffusion cells) involving alternative dosage forms such as transdermal patches also make use of phosphate buffer system as the release medium. Hence, we investigated aqueous phosphate buffer systems of varying pH as the solubilizing medium for duloxetine HCl in order to develop the UV spectrophotometric method for this drug.

3.1 Calibration Curves of Duloxetine HCl

Fig. 2 shows the zero- and first-order derivative spectra of duloxetine hydrochloride. The first-order derivative spectra for the working standard solutions of duloxetine HCl ranging from 1 µg/mL to 100 µg/mL were recorded over the wavelength range 210-400 nm against phosphate buffer (pH 7.4) taken as the blank and the amplitudes of the maximum and minimum were measured at different wavelengths.The regression parameters forthe generated calibration curves are summarized in Table 1. Figs. 3 and 4 show the calibration curves at selected wavelengths affording best linear correlation.

3.2 Effect of Buffer pH

UV absorbance data was obtained at varying pH ranges of the phosphate buffer, *viz.,* phosphate buffer pH 5.8, 6.4 and 7.0. UV absorbance data was found to be satisfactory in presence of lower pH ranges also, however, phosphate buffer pH 7.4 was selected for validation due to its simulation with biological systems and hence, its applicability in drug release studies.

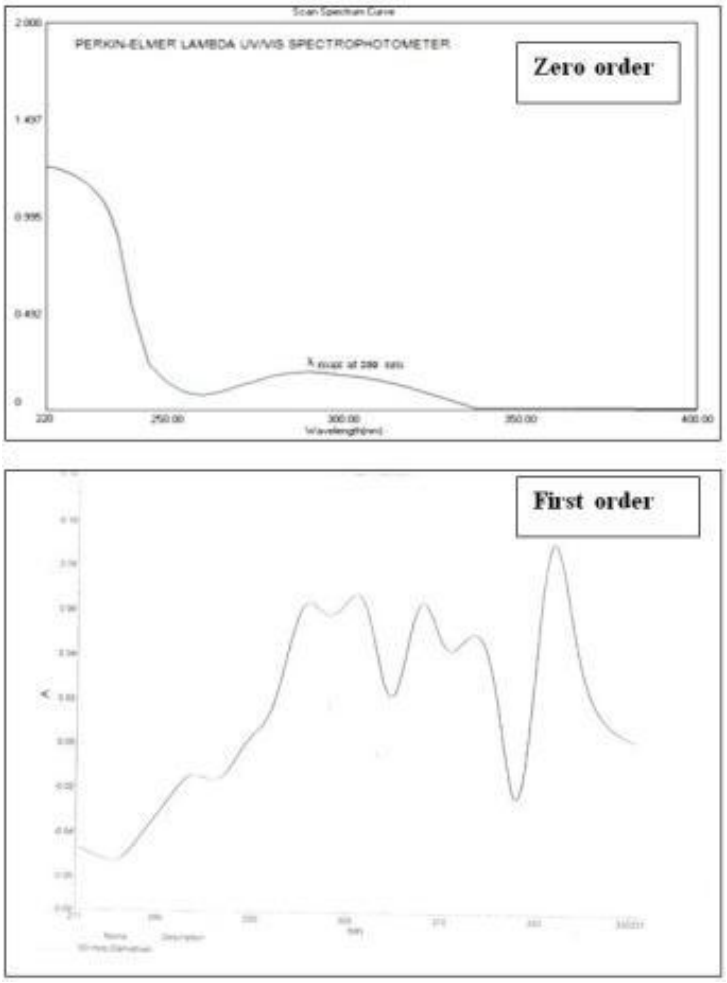


Fig. 2. Zero- and first order derivative spectra of duloxetine hydrochloride

Table 1. Linearity and range for the explored methods for analysis of duloxetine hydrochloride by first order derivative spectrophotometry

Method	Beer's law limits (µg/mL)	Wavelength (nm)	Technique	Regression equation	Correlation coefficient
1	5-90	295.7-298.0	P-P	y = 0.621x + 0.000	0.9820
2	5-40	295.7-298.0	P-P	y = 0.766x - 9E-05	0.9960
3	5-90	301.0-304.6	P-P	y = 5.338x - 0.002	0.9740
4	5-90	308.0	P-0	y = 7.824x - 0.002	0.9880
5	5-90	304.6-308.0	P-P	y = 5.173x - 0.002	0.9710
6	5-90	307.9-311.0	P-P	y = 3.874x - 0.004	0.7690
7	5-80	307.9-311.0	P-P	y = 2.651x - 0.001	0.8810
8	5-50	307.9-311.0	P-P	y = 3.384x - 0.002	0.8710
9	5-40	289-291.0	P-P	y = 1.026x - 1E-06	0.9960
10	5-90	295.7	P-0	y = 7.4113x -0.0009	0.9994*
11	5-90	298.0	P-0	y = 6.7871x -0.0011	0.9996*
12	5-90	304.6	P-0	y = 2.6513x -0.0004	0.9990*
13	5-90	301.0	P-0	y = 7.6628x + 3E-05	0.9849
14	5-90	311.0	P-0	y = 4.9399x -0.0003	0.9932
15	5-90	313.7	P-0	y = 5.913x -0.0018	0.9941
16	5-90	318.2	P-0	y = 2.7071x -0.0004	0.9993*
17	5-90	322.1	P-0	y = 10.323x -0.0014	0.9989*
18	5-90	322.1-318.2	P-P	y = 7.6156x - 0.001	0.9982*
19	5-90	307.9-311.0	P-P	y = 2.8682x - 0.002	0.9137
20	5-90	313.7-318.2	P-P	y = 1.060x -0.0017	0.8272
21	5-90	313.7-311.0	P-P	y = 3.2059x -0.0014	0.9683
22	40-90	313.7-311.0	P-P	y = 0.9325x -0.0006	0.9277

Corresponding methods were taken for validation in bulk drug, formulation samples and in presence of degradation products

3.3 Validation

The methods were validated with respect to linearity and range, accuracy and precision, limit of detection (LOD) and limit of quantification (LOQ) and robustness. The developed methods were validated in bulk drug samples as well as marketed formulation of duloxetine capsules (Delok 30; Nicholas Piramal India Ltd.). The various validation parameters are summarized in Tables 1 and 2. Stability indicating nature of the assay was assessed by fortifying a mixture of degraded solutions with three known concentrations, viz., 10, 20 and 40 µg/mL of the drug. The recovery of the added drug was determined.

3.3.1 Linearity and range

The peak to zero (P-0) and peak to peak (P-P) amplitude measurements of the first order derivative spectra were made at varying wavelengths in the concentration range of 1 µg/mL -100 µg/mL of duloxetine HCl. Excellent compliance with the Beer Lambert's law (linearity) was noted in the concentration range of 5 µg/mL – 90 µg/mL. Table 1 summarizes the various regression parameters corresponding to the methods explored. Values of the correlation coefficient 'r^2' was found to be above 0.9 in many cases indicating a good linearity over the working concentration ranges. Methods returning the best r^2 values, i.e., close to 0.999 were selected for further analytical validation (methods 10, 11, 12, 16, 17 and 18).

3.3.2 Precision

Precision was investigated by analyzing different concentrations of duloxetine HCl (5 µg/mL -90 µg/mL) in six independent replicates on the same day (intra-day precision) and on three consecutive days (inter-day precision). The data is represented as relative standard deviation (RSD %) and results have been shown in Table 3.The RSD % values in the intraday precision study were < 1.0% and in the interday analysis were < 2.0% indicating good precision of the methods.

3.3.3 Accuracy

The accuracy of the proposed methods was assured by preparing different concentration levels of drug for analysis from independent stock solutions. Additional support to the accuracy of the developed assay methods was made by employing standard addition method in

which excess drug (50%, 100% and 150 %) was spiked to pre-analyzed drug solution samples as well as drug formulation samples. Accuracy was assessed as mean % recovery and RSD %. The percentage recovery of the added pure drug was calculated as:

$$\% \text{ recovery} = [(C_t - C_i)/C_a] \times 100,$$

Where

C_t is the total drug concentration measured after standard addition;
C_i drug concentration in the formulation sample;
C_a, drug concentration added.

Best recovery values were obtained for methods 11, 12 and 18 ranging from 99.67-100.20% (Table 4).

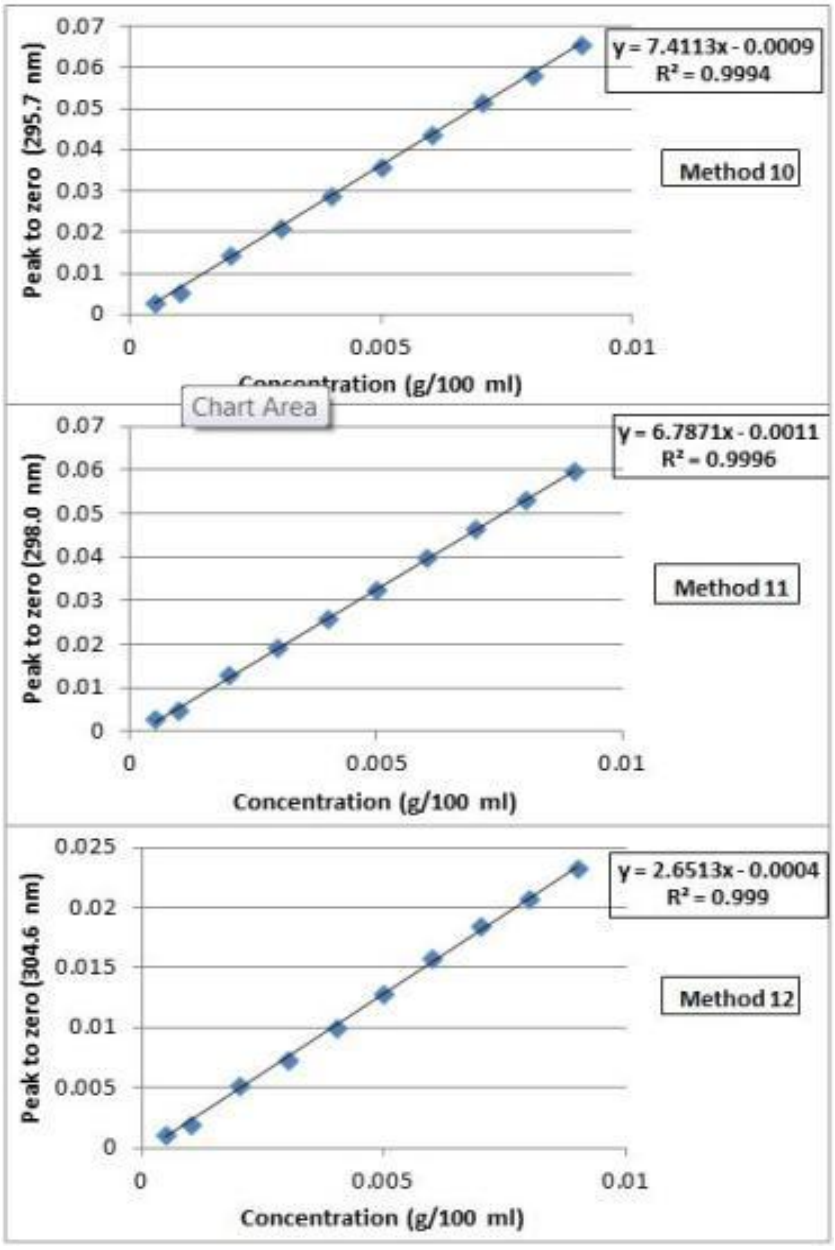


Fig. 3. Standard plots of duloxetine HCl in concentration range 0.0005 - 0.009 g/100 mL (5 - 90 µg/mL) with methods 10, 11 and 12

Table 2. Validation data for determination of duloxetine HCl by proposed methods

Method	Limit of detection LOD (µg/mL)	Limit of quantification LOQ (µg/mL)	λ (nm)	Slope	Intercept	Coefficient of correlation r^2	Precision Intraday; Interday	Accuracy	Robustness RSD (%)
10	0.63	1.91	295.7	7.4113	0.0009	0.9994	1.12; 1.44	99.44±1.15	1.04
11	0.33	1.05	298.0	6.7871	0.0011	0.9996	0.95; 1.10	99.92±0.92	0.58
12	0.38	1.15	304.6	2.6513	0.0004	0.9990	0.92; 1.08	99.93±0.86	1.14
16	0.64	1.94	318.2	2.7071	0.0004	0.9993	1.10; 1.53	99.41±1.04	1.05
17	0.58	1.76	322.1	10.323	0.0014	0.9989	1.25; 1.49	99.41±1.13	1.13
18	0.40	1.21	322.1-318.2	7.6156	0.0010	0.9982	0.86; 1.01	99.94±0.88	0.59

Table 3. Precision of the proposed methods for analysis of duloxetine hydrochloride

Method	Intra-day, n=6		Inter-day, n=6	
	*Mean±SD	#RSD %	*Mean±SD	#RSD %
10	99.45±1.12	1.12	99.40±1.43	1.44
11	99.75±0.95	0.95	99.65±1.10	1.10
12	99.68±0.92	0.92	99.60±1.08	1.08
16	99.42±1.10	1.10	99.30±1.52	1.53
17	99.38±1.25	1.25	99.30±1.48	1.49
18	99.94±0.86	0.86	99.86±1.01	1.01

**Calculated as mean of measurements in hexaplicate (n=6); #Calculated as100xSD/mean*

Table 4. Recovery studies with pure drug duloxetine HCl by standard addition method

Excess drug spiked to preanalyzed drug solution(%)*	Drug content (µg)	% Recovery ± SD** #RSD % employing method nos.					
		10	11	12	16	17	18
0	200	99.55±1.20	99.72±0.75	99.67±0.75	99.37±1.30	99.32±0.90	99.78±0.99
		1.20	0.75	0.76	1.30	0.90	0.99
50	300	99.45±1.10	99.82±0.77	99.80±0.98	99.35±1.09	99.40±1.25	100.02±0.94
		1.10	0.77	0.98	1.10	1.26	0.94
100	400	99.40±1.15	100.05±0.84	100.05±0.89	99.41±1.13	99.48±1.10	99.88±0.87
		1.15	0.84	0.89	1.13	1.10	0.87
150	500	99.34±1.16	100.09±1.32	100.20±0.82	99.50±1.40	99.45±1.27	100.06±0.75
		1.16	1.32	0.82	1.40	1.27	0.75

**Appropriate volumes of stock soln(250µg/mL) added to pre-analyzed drug solution (20µg/mL); **Calculated as mean of measurements in hexaplicate (n=6); #Calculated as: SD/mean x 100*

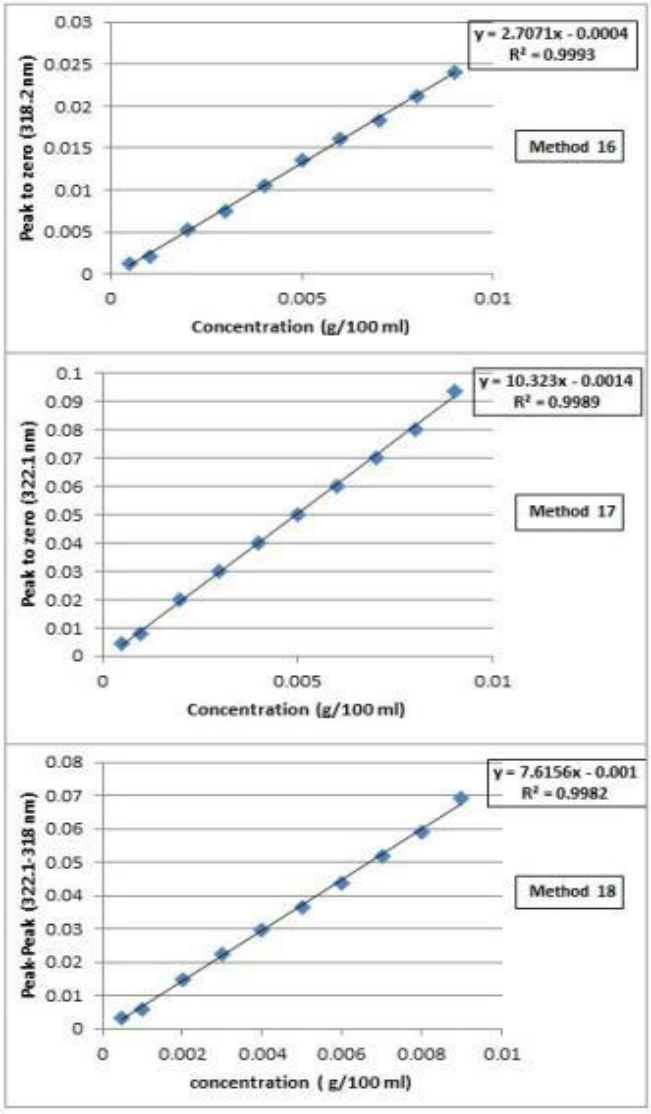


Fig. 4. Standard plots of duloxetine HCl in concentration range 0.0005 - 0.009 g/100 mL (5 - 90 µg/mL) with methods 16, 17 and 18

3.3.4 Recovery studies with marketed formulation

Recovery studies with marketed formulation were carried out with marketed Duloxetine HCl capsule formulation taking three equal volumes (10 mL each) of 15 µg/mL solution prepared from the capsule powder in phosphate buffer. Appropriate volumes of a previously prepared 250 µg/mL pure drug solution (solution B) were added so as to increase the drug concentration by 50%, 100% and 150% respectively (final drug content 225 µg, 300 µg and 375 µg respectively). The above three solutions were suitably diluted and analyzed by first-order derivative spectrophotometry. The percent recovery of the added amount of drug was utilized for determination of accuracy. Recovery studies with marketed formulation returned values ranging from 99.5-100.05 % (Table 5).

3.3.5 Recovery studies with degraded solutions

The stability indicating potential of the developed methods was evaluated by fortifying a mixture of degraded solutions with three known concentrations of the drug. The recovery of the added drug was determined (Table 6).

Table 5. Recovery studies with drug duloxetine HCl capsules by standard addition method

Excess drug spiked to preanalyzed capsule solution (%)*	Drug content (µg)	% Recovery ± SD** #RSD % employing method nos.					
		10	11	12	16	17	18
0	150	99.50±1.25 1.25	99.71±0.95 0.95	99.66±0.75 0.76	99.40±1.72 1.73	99.34±0.95 0.95	99.68±0.99 0.99
50	225	99.35±1.15 1.15	99.52±0.80 0.80	99.50±0.98 0.98	99.23±1.08 1.09	99.32±1.25 1.26	99.65±0.97 0.97
100	300	99.45±1.05 1.05	100.0±0.85 0.85	100.05±0.98 0.98	99.43±1.08 1.08	99.48±1.15 1.15	99.99±0.87 0.87
150	375	99.34±1.16 1.16	100.09±1.32 1.32	100.71±0.80 0.80	99.56±1.45 1.45	99.50±1.20 1.20	100.06±0.75 0.75

**Appropriate volumes of stock soln (250 µg/mL) added to pre-analyzed capsule sample solution (15 µg/mL); **Calculated as mean of measurements in hexaplicate; #Calculated as: SD/mean x 100*

Table 6. Recovery studies with degraded samples of duloxetine HCl

Degradation method	Drug content ratio of degraded solutions to drug solution*	Recovery of added drug to degraded solutions % Recovery ± SD** #RSD % employing method nos.					
		10	11	12	16	17	18
Acid 0.1N HCl	1:1	99.34±1.25 1.25	99.70±0.97 0.97	99.45±0.90 0.90	99.20±1.80 1.81	99.10±1.22 1.23	99.72±0.99 0.99
Acid 0.1N HCl	1:2	99.44±1.24 1.24	99.75±0.98 0.98	99.50±0.88 0.88	99.13±1.70 1.71	99.24±1.35 1.36	99.69±0.98 0.98
Acid 0.1N HCl	1:3	99.40±1.18 1.18	99.80±1.01 1.01	99.50±0.80 0.80	99.20±1.68 1.69	99.18±1.24 1.25	99.76±1.00 1.00
Alkali 0.1N NaOH	1:1	99.56±1.20 1.20	99.70±0.90 0.90	99.60±0.80 0.80	99.23±1.18 1.19	99.30±1.22 1.22	99.75±0.90 0.90
Alkali 0.1N NaOH	1:2	99.48±1.09 1.09	99.79±0.84 0.84	99.65±0.90 0.90	99.32±1.08 1.09	99.45±1.15 1.16	99.78±0.94 0.94
Alkali 0.1N NaOH	1:3	99.50±1.20 1.20	99.69±0.78 0.78	99.70±0.88 0.88	99.31±1.12 1.12	99.40±1.05 1.06	99.85±0.92 0.92
Neutral	1:1	99.43±1.20 1.20	99.88±0.98 0.98	99.70±0.84 0.84	99.38±1.10 1.10	99.42±1.14 1.14	99.98±0.82 0.82
Neutral	1:2	99.39±1.12 1.12	99.85±0.84 0.84	99.72±0.94 0.94	99.37±1.00 1.00	99.42±1.20 1.20	99.97±0.84 0.84

Degradation method	Drug content ratio of degraded solutions to drug solution*	Recovery of added drug to degraded solutions % Recovery ± SD** #RSD % employing method nos.					
		10	**11**	**12**	**16**	**17**	**18**
Neutral	1:3	99.41±1.09 1.09	99.80±0.80 0.80	99.72±0.94 0.94	99.40±1.18 1.18	99.45±1.12 1.12	99.91±0.98 0.98
Photolysis in water	1:1	99.52±1.13 1.13	99.87±1.01 1.01	99.80±0.98 0.98	99.45±1.18 1.18	99.46±1.20 1.20	99.92±0.79 0.79
Photolysis in water	1:2	99.46±1.09 1.10	99.70±0.90 0.90	99.74±0.84 0.84	99.49±1.25 1.25	99.43±1.19 1.19	99.98±0.74 0.74
Photolysis in water	1:3	99.34±1.30 1.30	99.89±1.00 1.00	99.72±0.80 0.80	99.50±1.20 1.20	99.50±1.24 1.24	99.90±0.75 0.75
Oxidation 30% H_2O_2	1:1	99.44±1.22 1.22	99.74±0.97 0.97	99.70±1.01 1.01	99.48±1.19 1.19	99.56±1.27 1.27	99.98±0.76 0.76
Oxidation 30% H_2O_2	1:2	99.45±1.02 1.02	99.80±1.00 1.00	99.72±0.80 0.80	99.43±1.20 1.20	99.40±1.24 1.24	99.92±0.80 0.80
Oxidation 30% H_2O_2	1:3	99.38±1.07 1.07	99.84±0.96 0.96	99.86±0.66 0.66	99.52±1.35 1.35	99.48±1.10 1.10	99.98±0.85 0.85
Thermal 60^0C	1:1	99.51±1.18 1.18	99.80±1.11 1.11	99.78±0.82 0.82	99.47±1.24 1.24	99.50±1.25 1.25	99.88±0.91 0.91
Thermal 60^0C	1:2	99.43±1.11 1.11	99.78±1.01 1.01	99.80±0.89 0.89	99.56±1.30 1.30	99.39±1.22 1.22	99.92±0.79 0.79
Thermal 60^0C	1:3	99.54±1.20 1.20	99.76±1.12 1.12	99.73±0.86 0.86	99.51±1.24 1.24	99.49±1.28 1.28	99.95±0.90 0.90

** Original drug concentration in degraded solution 10 µg/mL .Fortified with added drug content of 10, 20 and 40 µg/mL respectively; **Recovery of added duloxetine HCl to degraded samples. Calculated as mean of measurements in hexaplicate (n=6); #Calculated as: SD/mean x 100*

Appropriate volumes of standard drug solution (250 µg/mL) were added to degraded solutions (containing original drug concentration 10 µg/mL) to get the content of the added drug as 10, 20 and 40 µg/mL respectively. Acidic and alkaline solutions were neutralized prior to mixing.

3.3.6 Interference

Satisfactory values of the mean recovery values ± SD and RSD % in recovery studies in drug formulation (capsules) revealed that there is no potential interference of the excepients in the formulation. Further, recovery studies with the stress degradation samples showed that the proposed methods are sufficiently accurate in the presence of degradation products as well. Best results were obtained for the methods 11, 12 and 18.

3.3.7 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of the method were established using calibration standards (Table 2). LOD and LOQ were calculated as 3.3 σ/s and 10 σ/s, respectively, as per ICH definitions, where, *σ* is the mean standard deviation of replicate determination values under the same conditions as the sample analysis in the absence of the analyte (blank determination) and 's' is the sensitivity, namely, the slope of the calibration graphs.

3.3.8 Robustness

Robustness is a measure of repeatability of an analytical method examined by evaluating the effect of small variations in experimental conditions such as heating temperatures (±2°C) (Table 7).

Three replicate determinations at six different concentration levels of the drugs were carried out at ambient temperature (26°C) and at 28°C and 23°C (room temperature ±2°C). The within-day RSD values for methods 11 and 18 were found to be less than 0.6% indicating that the proposed methods have reasonable robustness.

Table 7. Robustness at different temperatures

Method	*Mean D1±SD	Robustness RSD (%)
10	0.03525±0.000367	1.04
11	0.03239±0.000188	0.58
12	0.01282±0.000102	1.14
16	0.01348±0.000142	1.05
17	0.0521±0.000589	1.13
18	0.03555±0.000212	0.59

**Calculated as mean of measurements in triplicate for three temperatures 26°C (room temp.), 28°C and 23°C; #Calculated as the relative standard deviation between mean values at 26°C (room temp.), 28°C and 23°C*

3.3.9 Stability

The stability of the final sample solutions was examined by their absorbance values and responses were found to be stable for at least 8 hours at room temperature.

3.4 Analysis of Marketed Formulation (Duloxetine Capsules)

Powder weight equivalent to 15 mg of duloxetine HCl (Delok30 capsules) was dissolved in phosphate buffer pH 7.4 to prepare 100 mL of solution A (150 µg/mL). The solution was suitably diluted and analyzed for the drug content. Table 8 gives the results of the assay for duloxetine HCl carried out on the marketed formulation by the proposed methods. The percentage recovery was found to be 99.34 – 99.71% (amount per capsule found to be 29.804 - 29.914 mg) displaying a close agreement between the results obtained by the proposed methods and the label claim (30 mg per capsule).

Table 8. Assay results for duloxetine HCl determination in capsule formulation

Method	Label claim (mg)	Mean recovery (mg) ± SD*	Mean % recovery ± SD*	RSD (%)
10	30	29.850±0.45	99.50±1.25	1.25
11	30	29.914±0.35	99.71±0.95	0.95
12	30	29.901±0.25	99.67±0.75	0.76
16	30	29.821±0.62	99.40±1.72	1.73
17	30	29.804±0.35	99.34±0.95	0.95
18	30	29.905±0.44	99.68±0.99	0.99

**Average of nine determinations in three different concentration levels*

4. CONCLUSION

Rapid, inexpensive, accurate and sensitive stability indicating methods have been proposed for the determination of duloxetine HCl in bulk as well as in its marketed formulation (capsules). The methods have been validated in terms of their sensitivity, reproducibility, precision, accuracy, robustness and solution stability for ≥ 8 h suggesting their suitability for the routine analysis of DLX in pure form (in bulk analysis) as well as pharmaceutical formulations without interference from excipients. Excellent recovery of the drug in the presence of its force degraded solutions suggests the stability indicating nature of the methods suggesting their applicability in the presence of routine degradation products. We have explored all wavelength regions in the first order derivative spectrum of duloxetine HCl for its estimation and this has not been reported in previous studies and amongst these, the validation parameters were found to be the best for methods 11, 12 and 18. These methods can be explored further for analysis of duloxetine hydrochloride in other formulations containing varied excepients. Further, the method can have direct applicability in drug release and permeation studies employing diffusion cells (Franz diffusion cells).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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