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Stability indicating spectrofluorimetric method for determination of duloxetine hydrochloride in bulk and in dosage form

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ABSTRACT

Duloxetine (DLX), is a selective serotonin-norepinephrine reuptake inhibitor (SNRI) recommended for maintenance treatment of major depressive disorder, neuropathic pain especially diabetic polyneuropathy (first-line treatment), generalized anxiety disorder, stress urinary incontinence and fibromyalgia. The present investigation describes the validation of rapid, sensitive, cost effective and reproducible stability indicating spectrofluorometric methods based on the native fluorescence of duloxetine HCl in acidic medium for the estimation of duloxetine HCl in bulk and in formulations. The fluorescence intensity of duloxetine hydrochloride was measured at 336 nm after excitation at 290 nm. The methods were validated with respect to linearity, accuracy, precision and robustness. Linearity was observed in the concentration range of 0.3-30 µg/ml with an excellent correlation coefficients (r^2) ranging from 0.9940-0.9996. The limits of assay detection values were found to range from 0.56-0.89 µg/ml and quantitation limits ranged from 1.69-2.42 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 range from 99.53 ± 99.66%. The proposed methods were developed as stability indicating procedures by carrying out the analysis for duloxetine hydrochloride on stressed samples prepared under various forced degradation conditions.

Keywords: Duloxetine hydrochloride; Stability indicating; Derivative spectrophotometry; Validation

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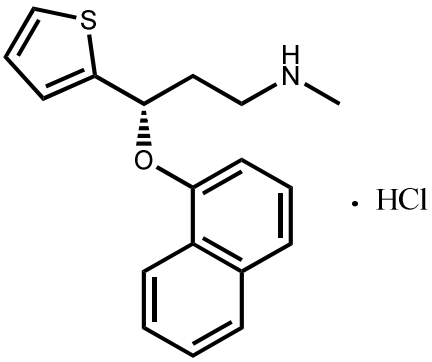
INTRODUCTION

Duloxetine, N-methyl-3-(napthalen-1-yloxy)-3-(thiophene-2-yl) propan-1-amine hydrochloride (Figure 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]. The drug is approved by the US FDA for the treatment of diabetic polyneuropathy and is recommended as a first line treatment for the purpose [2]. Other indications include management of generalized anxiety disorder [3] fibromyalgia [4], and most recently, stress urinary incontinence [5-6]. Currently, there is no official analytical procedure for duloxetine HCl in any pharmacopoeia. There are several reports in literature based on the application of reverse phase chromatographic methods [7-12] or ion selective membrane electrodes [13] for the determination of duloxetine HCl. Spectrophotometric methods have also been investigated and these include the application of more sensitive derivative spectrophotometry as well [14-16]. A spectrofluorimetric method has been reported for duloxetine based on enhancement effect of cationic surfactants on the native fluorescence intensity in an alkaline medium [17]. The present investigation describes simple rapid, reproducible and stability indicating spectrofluorimetric methods for the quantification of duloxetine HCl in bulk as well as in capsule dosage forms. The methods were validated with respect to various parameters outlined in the ICH guideline Q2(R1) [18]. The drug was subjected to systematic forced degradation studies by employing the ICH prescribed conditions [19] and the degraded samples spiked with

known concentrations of the pure drug were analyzed by the developed method in order to assess its stability indicating potential.



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

Fig. 1. Chemical structure of Duloxetine hydrochloride

MATERIALS AND METHODS

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 pure grade was graciously provided as gift samples by Lupin Pharmaceuticals, Mumbai, India. Delok 30 capsules (label claim 30 mg duloxetine hydrochloride per capsule; Nicholas Piramal India Ltd.) were purchased from the market.

Apparatus

The fluorescence intensity was measured on a Hitachi model F-2500 fluorescence spectrophotometer (UK), equipped with a 150W xenon lamp in self-deozonating lamp housing, grating excitation and emission monochromators, 1 cm pathlength cell, wavelength drive speed of 12,000 nm/min. Slit widths for excitation and emission monochromators were set at 5 nm. A CyberScan pH 510 (Eutech instruments) pH meter was used for checking the pH of buffer solutions.

Preparation of buffers

Hydrochloric acid buffer pH 1.5 was prepared by adding 20.2 ml of 0.2 M hydrochloric acid to 50.0 ml of the 0.2 M potassium chloride and making up the volume to 100 ml. Acetate buffer pH 3.5 was prepared by dissolving 25 g of ammonium acetate in 25 ml of water followed by addition of 38 ml of 7 M hydrochloric acid. The pH was then adjusted to 3.5 with 2 M hydrochloric acid or 6 M ammonia and volume was made to 100 ml with distilled water.
Phosphate buffer pH 6.0 was prepared by adding 2.8 ml of 0.2 M sodium hydroxide to 25.0 ml of 0.2 M potassium dihydrogen phosphate, and making up the volume to 100.0 ml. The pH of the buffer was adjusted to 6.0 using a pre-calibrated pH meter.

Forced degradation of duloxetine hydrochloride

Duloxetine HCl was subjected to forced degradation according to the ICH guidelines [19]. 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 $^{\circ}$C for 8 hours. For oxidative stress studies, the drug was dissolved at a concentration of 1mg/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 $^{\circ}$C for 7 days.

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 HCl in 100 ml of the appropriate buffer (hydrochloric acid buffer pH 1.5 for method **1**; acetate buffer pH 3.5 for method **2** and phosphate buffer pH 6.0 for method **3)**. 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. The test tubes were kept stoppered to avoid the loss of solvent due to evaporation. Methods 1, 2 and 3 gave similar excitation and emission spectra for the drug (Fig. 2.). The λ_{max} of the drug (290 nm) was selected as the excitation wavelength and the fluorescence intensity was measured at 336 nm.

Analysis of pharmaceutical formulation

The contents of twenty capsules were mixed and weighed accurately. Powder weight equivalent to 15 mg of duloxetine HCl was suspended in the appropriate buffer, sonicated for 5 minutes and filtered. The volume was made up to 100 ml (final drug solution 150 µg/ml. The solution was suitably diluted and fluorescence intensity was noted.

Results and Discussion

In this report, we have tried to develop and validate a sensitive spectrofluorometric method of analysis for duloxetine HCl and to assess its stability indicating potential. This method does not require the addition of any type of fluorometric enhancers as employed in a previously reported method with alkaline borate buffer [17]. A systematic study of the fluorescence characteristics of the drug revealed that duloxetine possesses good native fluorescence in acidic medium. The present method explores the potential of spectrofluorometry for the estimation of duloxetine HCl in varied acidic media including hydrochloric acid buffer pH 1.5, acetate buffer pH 3.1 and phosphate buffer 6.0. The stress degraded samples were spiked with the pure drug in varying concentrations for analysis by all the three methods.

Calibration curves of duloxetine HCl

Figure 2 shows the excitation and emission spectrum of duloxetine hydrochloride in the three selected acidic buffer media. The fluorescence intensity for the working standard solutions of duloxetine HCl ranging from 1-100 µg/ml were recorded over the wavelength range 210-400 nm against the reagent blank. The regression parameters for the generated calibration curves are summarized in Table 1. The calibration plots in the concentration ranges affording the best linear correlation are shown in Figure 3.

Effect of buffer pH

The fluorescence characteristics were noted in neutral (phosphate buffer pH 7.4), acidic (hydrochloric acid buffer pH 1.5, acetate buffer pH 3.1 and phosphate buffer 6.0) and alkaline pH (alkaline borate buffer pH 9.0) ranges. Good fluorescence intensity was obtained with acidic pH ranges, though fluorescence data was also satisfactory at neutral and alkaline pH. Further, change in the buffer composition at the acidic pH did not produce any significant change in the spectrum. Hence, the three selected acidic buffers were taken for further analytical validation.

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*., 5.0, 10.0 and 15.0 µg/ml of the drug. The recovery of the added drug was determined.

Linearity and range

The fluorescence measurements were made at 336 nm in the concentration range of 0.2 µg/ml-100 µg/ml of duloxetine HCl. Excellent compliance with the Beer Lambert's law (linearity) was noted in the concentration ranges of 0.3 – 30 µg/ml. Table 1 summarizes the various regression parameters corresponding to the methods explored. Values of the correlation coefficient 'r^2' was good for all the three methods with method **2** in acetate buffer pH 3.5 returning the best correlation coefficient of 0.9996 indicating a good linearity over the working concentration ranges.

Precision
Precision was investigated by analyzing different concentrations of duloxetine 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.

Accuracy
The different concentration levels of drug for analysis were prepared from independent stock solutions to ensure accuracy of the methods. Accuracy was further assessed by standard addition method in which an excess drug (50%, 100% and 150 %) was spiked to pre-analyzed drug solutions (5 µg/ml). Equivalent volumes of standard drug solutions (10 µg/ml, 15 µg/ml and 20 µg/ml) were added to increase the drug concentration by 50%, 100% and 150% respectively (final drug concentration 7.5 µg/ml, 10 µg/ml and 15 µg/ml respectively). Accuracy was determined as mean % recovery and RSD %. The percentage recovery of the added pure drug was calculated as: % recovery = $[(C_t–C_i)/C_a]$ x 100, where C_t is the total drug concentration measured after standard addition; C_i is the drug concentration in the pre-analyzed solution and C_a was the drug concentration added. All the methods gave good recovery values with % RSD ranging from 0.97-1.1 % (Table 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 5 µg/ml solution prepared from the capsule powder in the appropriate buffer. Equivalent volumes of standard drug solutions (10 µg/ml, 15 µg/ml and 20 µg/ml) were added so as to increase the drug concentration by 50%, 100% and 150% respectively (final drug concentration 7.5 µg/ml, 10.0 µg/ml and 15.0 µg/ml respectively). The prepared solutions were analyzed and the percent recovery of the added amount of drug was utilized for determination of accuracy. Recovery studies with marketed formulation returned values ranging from 99.69-99.75 % (Table 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 by adding equivalent volumes of standard drug solutions (10 µg/ml, 15 µg/ml and 20 µg/ml) to the degraded drug solution (diluted with appropriate buffer to original drug concentration 5 µg/ml) so as to increase the drug concentration nearly by 50%, 100% and 150% respectively (final drug concentration 7.5 µg/ml, 10.0 µg/ml and 15.0 µg/ml respectively). Acidic and alkaline solutions were neutralized prior to mixing.

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**.

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.

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 10 µg/ml level 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 the three methods **1**, **2** and **3** were found to be less than 0.6% indicating that the proposed methods have reasonable robustness.

Stability
The responses with fluorescence measurements were found to be stable for at least 8 hours at room temperature which indicated the stability of the final sample solutions for at least 8 h.

Analysis of marketed formulation (Duloxetine capsules)

Powder weight equivalent to 15 mg of duloxetine HCl (Delok30 capsules) was sonicated in the various buffer media to prepare 100 ml of solution A (150 µg/ml). The solution was suitably diluted and analyzed for the drug content. The results of the analysis by the proposed methods are shown in Table 8. The percentage recovery was found to be 99.53 – 99.66 % (amount per capsule found to be 29.654-29.772 mg) displaying a close agreement between the results obtained by the proposed methods and the label claim (30 mg per capsule).

Table 1. Linearity and range for the explored methods for analysis of duloxetine HCl by spectrofluorimetry

Method Type*	Linearity limit (µg/ml)	Regression equation	Correlation coefficient r^2
1[a]	0.3-30	y = 3.0069x + 1.0737	0.9974
2[b]	0.3-20	y = 6.2004x + 1.5817	0.9996
3[c]	0.3-20	y = 5.3307x + 3.9827	0.9940

[a]*Fluorescence data in HCl buffer pH 1.5*
[b]*Fluorescence data in acetate buffer pH 3.5*
[c]*Absorbance data in phosphate buffer pH 6.0*
**Corresponding methods were taken for validation in bulk drug, formulation samples and in presence of degradation products.*

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

Method No.	Slope	Intercept	Coefficient of correlation r^2	LOD[a] µg/ml	LOQ[b] µg/ml)	Precision[c] Intraday; Interday	Accuracy	Robustness RSD (%)
1	3.0069	1.0737	0.9974	0.89	2.42	1.03; 1.26	99.69±1.01	0.57
2	6.2004	1.5817	0.9996	0.56	1.69	0.98; 1.11	99.82±1.00	0.53
3	5.3307	3.9827	0.9940	0.79	2.39	1.00; 1.13	99.70±1.06	0.42

[a]*Calculated as 3.3 σ/s where 'σ ' is standard deviation of the blank and 's' is slope of calibration plot.*
[b]*Calculated as 10 σ/s where 'σ ' is standard deviation of the blank and 's' is slope of calibration plot.*
[c]*Average of six determinations.*

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

Method	Intra-day, n=6		Inter-day, n=6	
	Mean[a] ± SD	RSD %[b]	Mean[a] ± SD	RSD %[b]
1	99.35 ± 1.02	1.03	99.21 ± 1.25	1.26
2	99.66± 0.98	0.98	99.15 ± 1.10	1.11
3	99.54± 1.00	1.00	99.22± 1.12	1.13

[a]*Calculated as mean of measurements (n=6).*
[b]*Calculated as100xSD/mean.*

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

Excess drug spiked to preanalyzed drug solution(%)[a]	Added Drug content (µg)	% Recovery[b] ± SD RSD %[c] employing method nos.		
		1	2	3
50	2.5	99.62±0.91 0.91	99.82±0.96 0.96	99.76±0.99 0.99
100	5.0	99.75±1.10 1.10	99.77±0.98 0.98	99.70±1.07 1.07
150	7.5	99.70±1.02 1.02	99.86±1.05 1.05	99.65±1.11 1.11

[a]*Equivalent volumes of standard drug solutions (10 µg/ml, 15 µg/ml or 20 µg/ml) added to pre-analyzed drug solution (5 µg/ml) in various buffers to increase the drug content.*
[b]*Calculated as mean of measurements (n=6).*
[c]*Calculated as: SD/mean x 100.*

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

Excess drug spiked to pre-analyzed tablet solution µg (%)[a]	% Recovery[b] ± SD RSD %[c] employing method nos.		
	24	**28**	**29**
2.5 (50)	99.40±1.06 1.07	99.72±0.98 0.98	99.52±1.09 1.09
5.0 (100)	99.40±1.11 1.12	99.75±0.94 0.94	99.49±1.07 1.08
7.5 (150)	99.35±1.07 1.08	99.69±0.95 0.95	99.60±1.14 1.14

[a]*Equivalent volumes of standard drug solutions (10 µg/ml, 15 µg/ml or 20 µg/ml) added to pre-analyzed drug solution (5 µg/ml) in various buffers to increase the drug content.*
[b]*Calculated as mean of measurements (n=6).*
[c]*Calculated as: SD/mean x 100.*

Table 6. Recovery studies with degraded samples of duloxetine HCl

Degradation condition	Recovery of added drug to degraded solutions[a] % Recovery[b] ± SD RSD %[c] employing method nos.		
	1	**2**	**3**
Neutral hydrolytic	99.12±1.02 1.03	99.41±0.97 0.98	99.24±1.02 1.03
Acid hydrolytic	98.50±1.27 1.29	98.60±0.99 0.10	98.65±1.08 1.09
Alkaline hydrolytic	99.02±1.14 1.15	99.08±1.09 1.10	99.05±1.04 1.04
Neutral photolytic	99.41±1.10 1.11	99.45±1.10 1.11	99.46±1.01 1.02
Acid photolytic	98.61±1.20 1.22	98.82±1.09 1.10	98.40±1.06 1.08
Alkaline photolytic	98.42±0.93 0.94	99.15±0.95 0.96	98.70±0.94 0.95
Oxidation (30% H_2O_2)	99.21±1.08 1.09	99.38±0.99 1.00	99.18±1.08 1.09
Thermal (60^0C)	99.25±1.12 1.13	99.40±0.98 0.99	99.32±1.06 1.06

[a]*Equivalent volumes of standard drug solutions (10 µg/ml, 15 µg/ml or 20 µg/ml) added to pre-analyzed degraded drug solution (original concentration 5 µg/ml) in various buffers to increase the drug content.*
[b]*Recovery of added duloxetine HCl to degraded samples. Calculated as mean of measurements in triplicate (n=3).*
[c]*Calculated as: SD/mean x 100.*

Table 7. Robustness at different temperatures

Method	Mean[a]± SD	Robustness RSD (%)
1	33.5 ± 0.19	0.57
2	33.75 ± 0.18	0.53
3	107.68 ± 0.45	0.42

[a]*Calculated as mean of measurements in triplicate at 10 µg/ml for three temperatures: 32 °C (room temp.), 34 °C and 30 °C.*

Table 8. Assay results for duloxetine HCl in capsule formulation

Method	Label claim (mg)	Mean recovery (mg)[a] ± SD	Mean % recovery	RSD (%)
1	30	29.654 ± 0.36	99.53	1.21
2	30	29.772 ± 0.32	99.56	1.07
3	30	29.746 ± 0.34	99.66	1.14

[a]*Average of six determinations.*

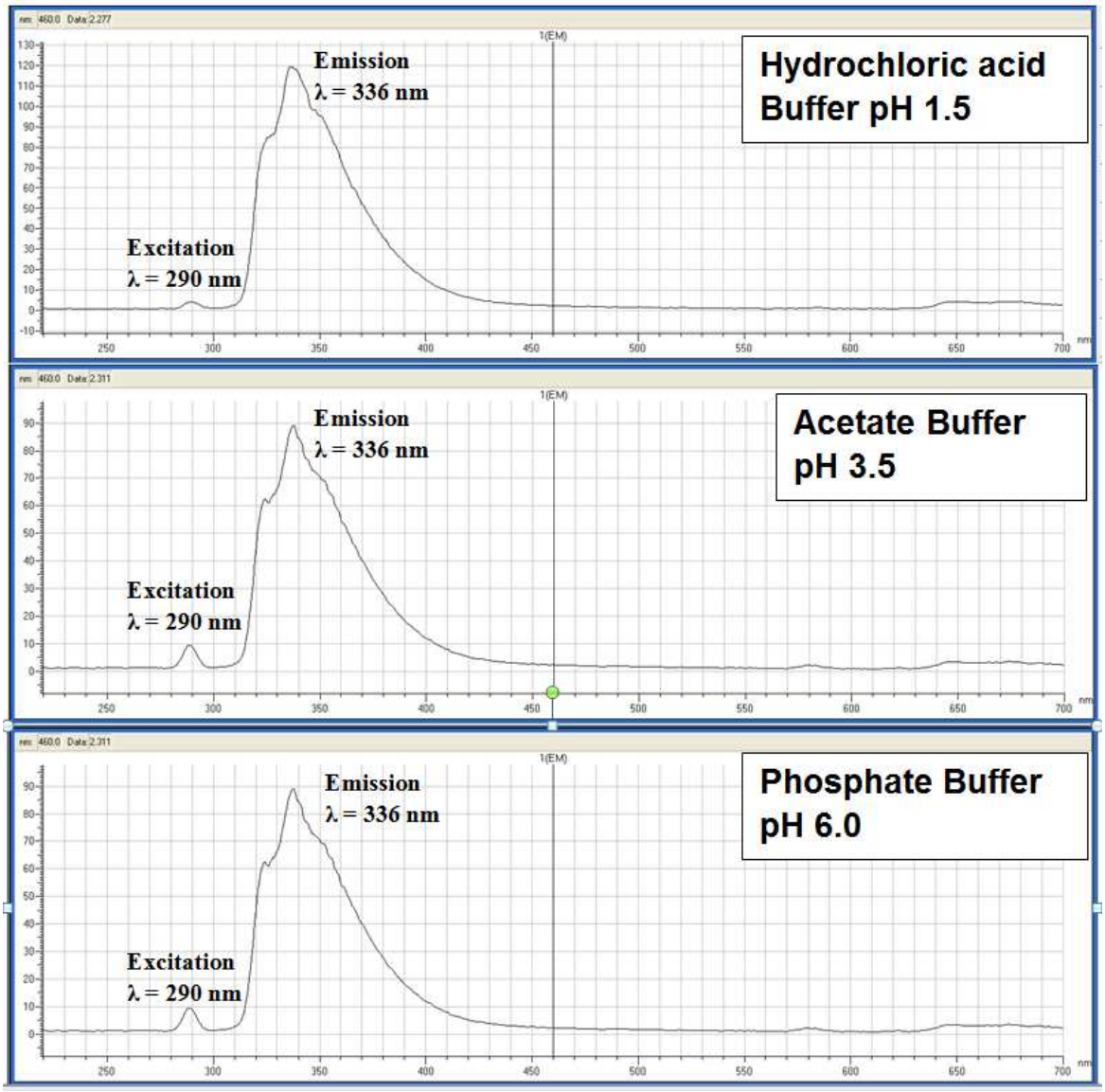


Fig. 2. Excitation and emission spectra of duloxetine HCl in different buffer systems

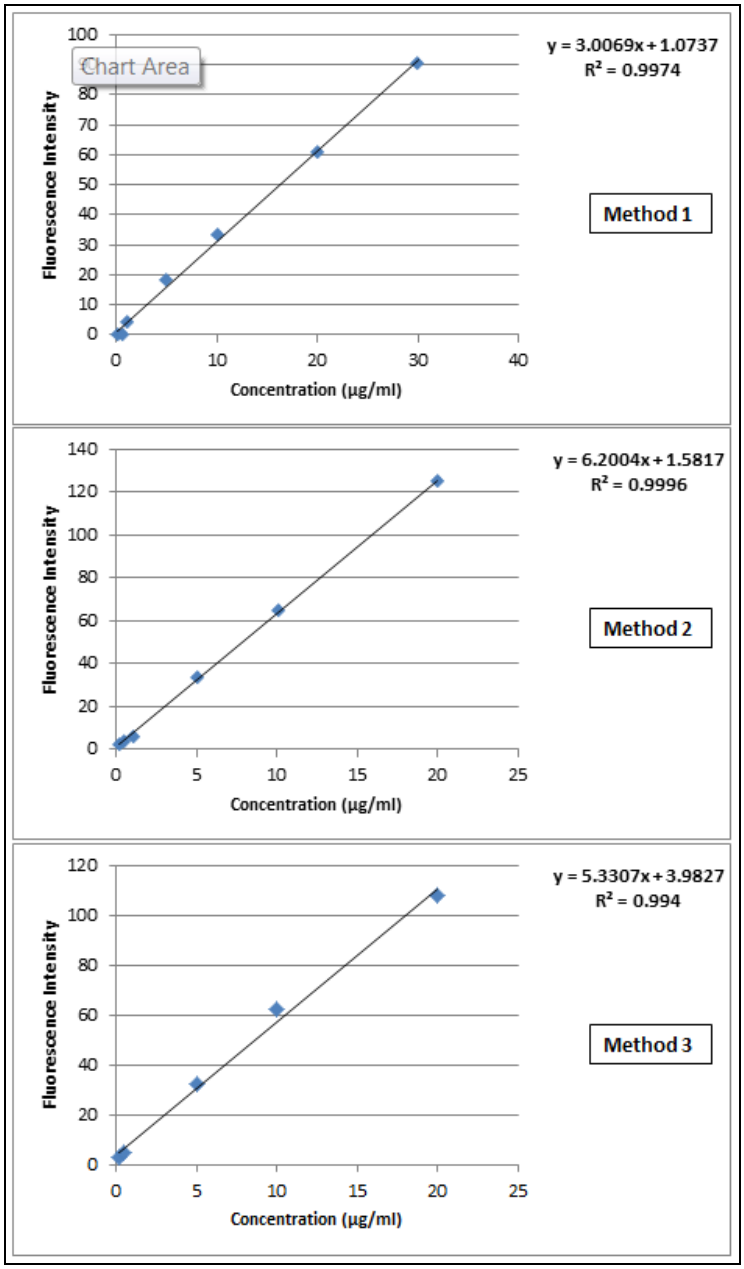


Fig. 3. Standard plots of duloxetine hydrochloride with methods 1, 2 and 3

CONCLUSION

Rapid, inexpensive, accurate and sensitive spectrofluorimetric methods have been proposed for the determination of duloxetine HCl in bulk as well as in its marketed formulation (capsules). Maximum fluorescence intensity was noted in phosphate buffer pH 6.0, however, the best linear correlation was generated with method 2 in acetate buffer pH 3.5. 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. The stability indicating nature of the methods was suggested by excellent recovery of the drug in the presence of its force degraded solutions. Hence, these methods are suitable for analysis of duloxetine HCl in presence of routine degradation products as well.

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REFERENCES

[1] MP Freeman; AM Hirschberg; B Wang; LF Petrillo; S Connors; S Regan; H Joffe; LS Cohen. *Maturitas*, **2013,** 75(2), 170.
[2] A Sultan; H Gaskell; S Derry; RA Moore. *BMC Neurology*, **2008,** 8, 29.
[3] S Ball; LB Marangell; S Lipsius; JM Russell. *Progress in Neuro-Psychopharmacology and Biological Psychology*, **2013,** 43(3), 217.
[4] R Bennett; I J Russell; E Choy; M Spaeth; P Mease; D Kajdasj; D Walker; F Wang and A Chappell. *Clinical Therapeutics*, **2012,** 34(4), 824.
[5] JHS Leewen; RR Lange; AF Jonasson; WJ Chen; L Viktrup. *Maturitas*, **2008**, 60(2), 138.
[6] B Mihaylova; R Pitman; D Tincello; H Vaart; R Tunn; L Timlin; D Quail; A Johns; M Sculpher. *Value in Health*, **2010**, 13(5), 565.
[7] L Mercolini; R Mandrioli; R Cazzolla; M Amoreb; MA Raggi**.** *Journal of Chromatography B*, **2007,** 856, 81.
[8] SK Patel; BH Patel. *Indian Journal of Pharmaceutical Sciences*, **2010**, 70(6), 825.
[9] CN Bhimanadhuni; DR Garikapati; C Srinivas. *International Current Pharmaceutical Journal*, **2012**, 1(5), 98.
[10] D Boopathy, RD Jawarkar; M Prakash; B Mathew; P Perumal. **2010,** 2(1), 239.
[11] VVSSN Raman; AK Harikrishna; RK Ratnakar; VVSSA Prasad; K Ramakrishna. *Journal of Pharmaceutical and Biomedical Analysis*, **2010**, 51(4), 994.
[12] M Puranik; S Wadher; KA Sharma. *Indian Journal of Pharmaceutical Education and Research*, **2014**, 48(3), 91.
[13] RA Ammar; H Otaif; A Al-Warthan. *International Journal of Electrochemical Science*, **2012**, 7(3), 2531.
[14] M Yunoos; DG Sankar; BP Kumar; S Hameed; A Hussain. *Journal of Chemistry*, **2010,** 7(3), 785.
[15] T Ramesh; AP Kumar; RVA Raj. *International Journal of Pharma and Bio Sciences*, **2011**, 2(1), 717.
[16] R Chadha; A. Bali. *British Journal of Pharmaceutical Research*, **2015,** 6(6), 402-414.
[17] NA Alarfaj; RA Ammar; MF El-Tohamy. *Asian Journal of Chemistry*, **2013,** 25(11), 6416.
[18] ICH, Validation of analytical procedures: Text and methodology, in: International Conference on Harmonisation, IFPMA, Geneva, 2005.
[19] ICH, Stability testing of new drug substances and products, Q1A(R2), in: International Conference on Harmonisation, IFPMA, Geneva, 2003.