



Cationic-Enhanced Spectrofluorimetric Method for Determination of Selective Serotonin Reuptake Inhibitor Duloxetine Hydrochloride in its Dosage Forms

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A highly sensitive, rapid, accurate and precise spectrofluorimetric method was developed for the determination of duloxetine hydrochloride in its pharmaceutical formulations. The proposed method is based on investigation of the fluorescence spectral behaviour of duloxetine hydrochloride in cetyl trimethylammonium bromide (CTAB) micellar system. In aqueous solution of borate buffer pH 9.9, the fluorescence intensity of duloxetine hydrochloride was greatly enhanced, 3-fold enhancement, in the presence of cetyl trimethylammonium bromide. The fluorescence intensity of duloxetine hydrochloride was measured at 382 nm after excitation at 275 nm. The fluorescence-concentration plot was rectilinear over the range of 1-70 ng/mL with lower detection limit of 0.5 ng/mL. The method was successfully applied to the analysis of duloxetine hydrochloride in its commercial dosage forms. The results were in good agreement with those obtained with the reported method. The application of the proposed method was extended to the stability studies of duloxetine hydrochloride after exposure to different forced degradation conditions, such as acidic, alkaline, oxidative and thermal conditions, according to ICH guidelines.

Key Words: Cationic surfactant, Spectrofluorimetry, Duloxetine hydrochloride, Cetyltrimethylammonium bromide.

INTRODUCTION

Duloxetine hydrochloride is chemically, *N*-methyl-3-naphthalen-1-oxy-3-thiophen-2-yl-1-amine¹. Duloxetine hydrochloride (Fig. 1) is a new selective serotonin and norepinephrine reuptake inhibitor used for major depressive disorders². It is also indicated for the management of neuropathic pain associated with diabetic peripheral neuropathy³. Duloxetine hydrochloride is considered among the most important antidepressant drugs therefore, the development of reliable rapid and accurate procedures for this active ingredient quantification is welcomed. Several analytical methods have been reported for determination of duloxetine hydrochloride in its pure form, pharmaceutical preparations and biological fluids, where duloxetine hydrochloride is determined officially in any pharmacopoeia. These methods include high-performance liquid chromatography⁴⁻⁶, liquid chromatography coupled with mass spectrometry⁷⁻⁹, high performance thin layer chromatography¹⁰, spectrophotometry¹¹, capillary zone electrophoresis¹², screen printed potentiometry¹³ and spectrofluorimetry¹⁴. The reported spectrofluorimetric method¹⁴ is based on the investigation of native fluorescence of duloxetine hydrochloride in a concentration range 0.02-0.4 µg/mL in 0.05 mol/L acetic acid having excitation at 225 nm and emission at 340 nm. Although,

the previous reported methods are very sensitive, they are not adapted for *in situ* and real time detection of duloxetine hydrochloride as they are time consuming or involve expensive apparatus or require skilled technicians.

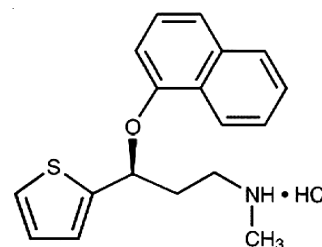


Fig. 1. Chemical structure of duloxetine hydrochloride

Spectrofluorimetry is considered more convenient alternative analytical technique because of its inherent simplicity, easily operated and economic. The main goal of the present study is to investigate the native fluorescence characteristics of duloxetine hydrochloride in aqueous medium and in cetyl trimethylammonium bromide (CTAB) micellar medium. Based on the micellar-enhancement effect of CTAB on the native fluorescence intensity of duloxetine hydrochloride, a simple and highly sensitive spectrofluorimetric method has been

developed for the determination of duloxetine hydrochloride in capsules. The stability-indicating capability of the proposed spectrofluorimetric method was evaluated through forced degradation studies of duloxetine hydrochloride drug under various degradation conditions according to ICH guidelines¹⁵.

EXPERIMENTAL

The fluorescence intensity was measured on a Perkin-Elmer model LS-55 luminescence spectrometer (UK), equipped with a 150 W xenon arc lamp, grating excitation and emission monochromators and a Perkin-Elmer recorder. Slit widths for excitation and emission monochromators were set at 5 nm for both excitation and emission. A 1 cm quartz cell was used. HANNA pH meter (Romania) was used for pH adjustments.

All reagents were of analytical grade and distilled water was used throughout the work. Pure grade duloxetine hydrochloride was kindly supplied from Jean Radne Sale (India). The pharmaceutical preparation (Cymbalta® 60 mg/capsule, Lilly S.A. Spain) was purchased from local drug stores. Sodium dodecyl sulphate (SDS, 95 %), cetyl trimethylammonium bromide (CTAB, 99 %), Tween 80, Triton X-100, Glycerol, sodium acetate trihydrate, boric acid and sodium hydroxide were purchased from Winlab (UK). Methanol (99.5 %), ethanol (96.0 %) and *n*-propanol (99.5 %) were obtained from Sigma-Aldrich (Germany). Acetonitrile was obtained from Merck (Germany). Dimethyl sulfoxide, dimethyl formamide, glacial acetic acid and hydrochloric acid were purchased from BDH laboratory supplies (England).

Standard solution: Stock solution of duloxetine hydrochloride was prepared by dissolving 10 mg of the drug in 100 mL of distilled water. Further dilutions ranging from (1-70 ng/mL) were done with the same solvent as appropriate. The standard solution was stable for 10 days when kept in the refrigerator.

Procedure for calibration graph: A serial dilution covering the concentration range 1-70 ng/mL of the drug standard solution was done and transferred into a series of 10 mL volumetric flasks. Then 1 mL of 0.1 mol/L borate buffer solution of pH 9.9 was added to each flask followed by 1 mL of 2 % w/v CTAB, mixed well and the fluorescence intensity was measured at 382 nm after excitation at 275 nm. The fluorescence intensity was plotted vs. the final drug concentrations (ng/mL) to get the calibration graph. Alternatively, the corresponding regression equation was derived.

Procedure for capsules: The total content of 10 capsules was mixed well. A weighed quantity of the powder equivalent to 10 mg of duloxetine hydrochloride was transferred into a 100 mL volumetric flask, about 80 mL of distilled water was added and the flask was sonicated for 10 min. The solution was diluted to volume with distilled water, mixed and filtered. Serial dilutions covering the working concentration range of 1-70 ng/mL were transferred into 10 mL volumetric flasks. Procedure described above was then performed. The nominal content of the capsules was calculated using the calibration graph or the corresponding regression equation.

Procedure for stability study

Acid and base induced degradation studies: A small amount of duloxetine hydrochloride stock solutions (equiva-

lent to 100 µg of the drug) were transferred into a series of small conical flasks as previously reported¹⁰, 10 mL of 0.5 mol/L hydrochloric acid was added at room temperature for 4 h. The studies under alkaline conditions were carried out using 10 mL of 2 mol/L sodium hydroxide at room temperature for 8 h. 1 mL of resulting solution was then transferred into a 10 mL volumetric flask and procedure described above was then followed.

Oxidative degradation: Aliquots of duloxetine hydrochloride stock solution (equivalent to 100 µg of the drug) were transferred into a series of small volumetric flasks; 10 mL of 30 % H₂O₂ were added and each volume was completed with distilled water. 1 mL of each solution was transferred into a 10 mL volumetric flask and procedure described above was then followed.

Thermal degradation: Aliquots of duloxetine hydrochloride stock solution (containing 100 µg of the drug) were transferred into a series of 10-mL volumetric flasks; completed to volume with distilled water. The thermal degradation of the drug was carried out by heating at 70 °C for 5 h. 1 mL of each solution was transferred into a 10 mL volumetric flask then following the procedure for calibration graph.

RESULTS AND DISCUSSION

Duloxetine hydrochloride was found to exhibit an emission band at 382 nm in aqueous borate buffer of pH 9.9, after excitation at 275 nm. It was aimed to enhance this emission band, attempting to explore a new sensitive methodology for the analysis of duloxetine hydrochloride in its pharmaceutical preparations. It is well known that the addition of a surfactant at a concentration above its critical micellar concentration to a given fluorophore solution increases the molar absorptivity and/or the fluorescence quantum yield of the fluorophore in many cases¹⁶. This fact has been used to improve the performance of spectrofluorimetric methods of various analytes. The fluorescence properties of duloxetine hydrochloride in various micellar media were studied, there was an enhancement of the fluorescence intensity in presence of CTAB compared with aqueous solution (about 3 fold enhancement) and so that CTAB was used as a fluorescence enhancer in order to develop a new spectrofluorimetric method for the determination of duloxetine hydrochloride.

Optimization of experimental conditions

Fluorescence spectra of duloxetine hydrochloride in aqueous and CTAB systems: The fluorescence spectra of duloxetine hydrochloride in both aqueous and CTAB systems were studied. Fig. 2, illustrates the obtained fluorescence spectra of duloxetine hydrochloride in the two systems. One of them was aqueous borate buffer of pH 9.9, while the second one was the same borate buffer in presence of CTAB as a fluorescence enhancer. The percentage of fluorescence enhancement in presence of CTAB was about 3-fold enhancement compared with the native fluorescence intensity of the drug in buffer aqueous medium.

Effect of surfactants: The fluorescence properties of duloxetine hydrochloride in various micellar media were studied using cationic surfactant (CTAB), anionic surfactant sodium dodecyl sulphate (SDS) and non ionic surfactant

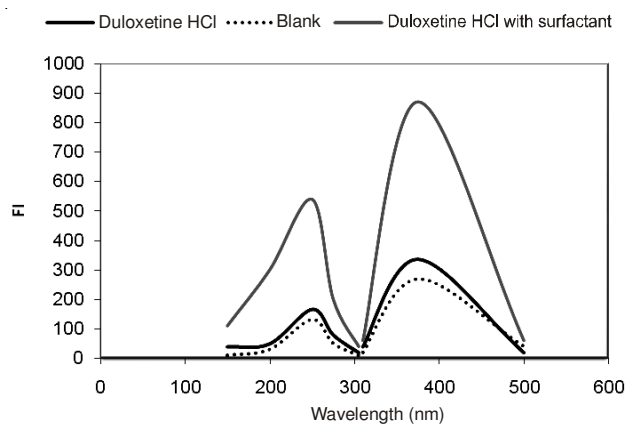


Fig. 2. Spectra of 1 ng/mL duloxetine hydrochloride

(Tween-80, Triton-100 and Glycerol). All the studied organized media caused decrease in native fluorescence of duloxetine hydrochloride or even have no significant effect on its fluorescence intensity, while CTAB system gave a substantial enhancement effect on the fluorescence intensity of duloxetine hydrochloride solution (Fig. 3).

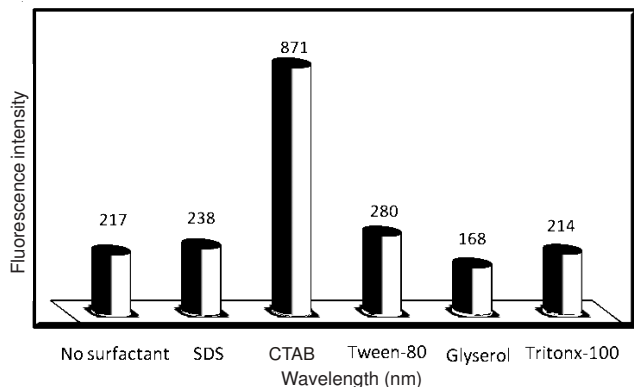


Fig. 3. Effect of type of surfactant (1 mL of 2 % solution of each surfactant) on fluorescence intensity of duloxetine hydrochloride (1 ng/mL)

Effect of CTAB volume: The influence of CTAB on the fluorescence intensity was studied using increasing volumes of 2 % (w/v) of CTAB. It was found that increasing volumes of CTAB in 10 mL volumetric flasks solution resulted in corresponding increase in fluorescence intensity up to 1 mL, after which no further increase in fluorescence intensity was attained. Therefore, 1 mL of CTAB was chosen as the optimum volume for duloxetine hydrochloride determination (Fig. 4).

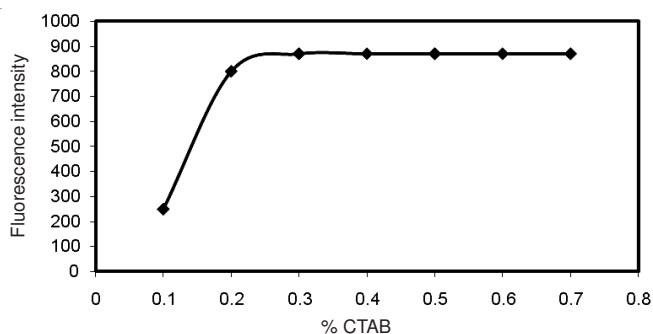


Fig. 4. Effect of volume of 2 % CTAB on fluorescence intensity of duloxetine hydrochloride (1 ng/mL)

Effect of pH: The influence of pH on the micelle-enhanced fluorescence of duloxetine hydrochloride can be observed in Fig. 5. Different types of buffer solutions covering the whole pH range, such as 0.2 mol/L acetate buffer over the pH range of 3.5-5.6, 0.2 mol/L phosphate buffer over pH range 5.6-8.3 and 0.1 mol/L borate buffer ranging from pH 8.3-12.3. It was found that maximum fluorescence intensity was achieved at pH 9.9-11 for duloxetine hydrochloride drug, so that, pH 9.9 was chosen as the optimum pH value.

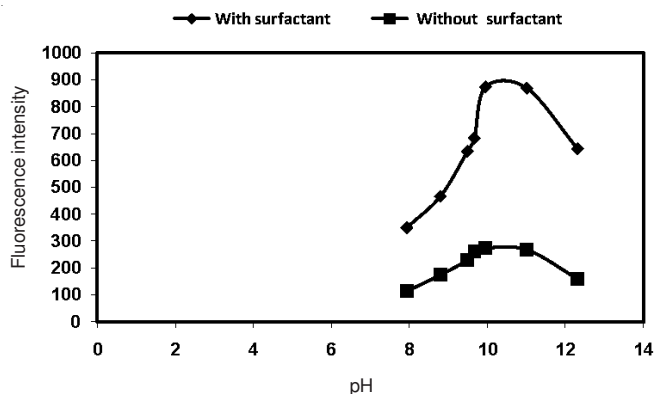


Fig. 5. Effect of pH on fluorescence intensity of duloxetine hydrochloride (1 ng/mL) in presence of 2 % CTAB and in aqueous medium

Effect of diluting solvent: The effect of different diluting solvents for drug working solution on fluorescence intensity of duloxetine hydrochloride in presence of CTAB was investigated using water, methanol, acetonitrile, *n*-propanol, dimethyl sulfoxide and dimethyl formamide. It was found that water was the best solvent for dilution as it gave the highest fluorescence intensity. Distinct and sharp increase in the fluorescence intensities was observed in CTAB system upon using methanol, acetonitrile or *n*-propanol. This effect was attributed to their denaturing effect on the micelles, where, short-chain alcohols (methanol and *n*-propanol) were solubilized mainly in the aqueous phase and affect the micellization process by modifying the solvent properties. Addition of these alcohols also results in reduction of size of the micelles, but with a progressive breakdown of the surfactant aggregate at very high concentrations¹⁷. Each of dimethyl sulfoxide and dimethyl formamide decreased the fluorescence intensity of duloxetine hydrochloride, since they initiated intersystem crossing process (similar to heavy atom effect)¹⁸.

Effect of time: The effect of time on the fluorescence intensity of the studied drug was also studied. It was found that the fluorescence intensity was immediately developed and remained stable for more than 1 h.

Method validation

Linearity: Under the described experimental conditions, linearity for the micelle enhanced fluorescence system, was evaluated by analyzing a series of six standard solutions (three replicates for each one) of duloxetine hydrochloride and measured by following the procedure described in the experimental section. Table-1, resumed the results obtained from statistical analysis of data obtained. The calibration graph of fluorescence intensity vs. duloxetine hydrochloride concentration expressed in ng/mL was found to be linear in the range of 1-70 ng/mL.

TABLE-1
PERFORMANCE DATA FOR THE PROPOSED
SPECTROFLUORIMETRIC DETERMINATION OF
DULOXETINE HYDROCHLORIDE

Parameter	Value
Concentration range (ng/mL)	1.0-70.0
Limit of detection (LOD) (ng/mL)	0.5
Limit of quantification (LOQ) (ng/mL)	1.0
Correlation coefficient (r)	0.9998
Slope	61.43
Intercept	96.67
RSD (%)	0.23
Error* (%)	0.88

*% Error = SD/\sqrt{n}

Limit of quantification and limit of detection: The limit of quantification was determined by establishing the lowest concentration that can be measured according to ICH Q2 (R1) recommendation¹⁵ below which the calibration graph is non linear. The limit of detection was determined by evaluating the lowest concentration of the analyte that can be readily detected. The results were summarized in Table-1.

Accuracy and precision: Statistical analysis¹⁵ of the results obtained by the proposed and a reported spectrophotometric method¹¹ (which includes the determination of duloxetine hydrochloride in 0.1 mol/L HCl at 288 nm) using student's *t*-test and variance ratio *F*-test showed no significant differences between the two methods regarding accuracy and precision, respectively, (Table-2). The intra-day precision was evaluated by determination of three concentrations of duloxetine hydrochloride drug in pure form on three successive occasions. The inter-day precision was also evaluated through replicate analysis of three concentrations for a period of 3 successive days. The results of intra-day and inter-day precision are summarized in Table-3.

TABLE- 2
APPLICATION OF THE PROPOSED AND COMPARISON
METHODS TO THE DETERMINATION OF DULOXETINE
HYDROCHLORIDE IN PURE FORM AND IN
PHARMACEUTICAL DOSAGE FORM

	Duloxetine hydrochloride					
	Proposed method			Reported method ¹¹		
	Taken (ng/mL)	Found (ng/mL)	Found (%)	Taken (ng/mL)	Found (ng/mL)	Found (%)
Pure form	2	1.96	98.00	2	1.99	99.50
	6	5.96	99.33	6	5.98	99.67
	10	9.97	99.70	10	9.94	99.40
	20	19.95	99.75	20	20.01	100.05
	50	49.96	99.92	50	49.94	99.88
	70	69.94	99.91	70	70.00	100.00
Mean ± SD	99.44 ± 0.73			99.75 ± 0.27		
<i>t</i> -Test	0.97 (2.23)*					
<i>F</i> -test	4.91 (5.05)*					
	Taken (ng/mL)	Found (ng/mL)	Found (%)	Taken (ng/mL)	Found (ng/mL)	Found (%)
Cymbalta® 60 mg/Capsule	5	5.01	100.20	5	4.95	99.00
	8	7.99	99.87	8	7.99	99.87
	10	9.78	97.80	10	10.02	100.20
	20	19.96	99.80	20	19.87	99.35
	50	49.89	99.78	50	49.85	99.70
	70	69.93	99.90	70	70.00	100.00
Mean ± SD	99.56 ± 0.87			99.69 ± 0.44		
<i>t</i> -Test	0.32 (2.23)*					
<i>F</i> -test	2.90 (5.05)*					

*Theoretical values of "*t*" and "*F*" at P = 0.05.

TABLE- 3
VALIDATION OF THE PROPOSED
SPECTROFLUORIMETRIC DETERMINATION OF
DULOXETINE HYDROCHLORIDE IN PURE FORM

Duloxetine hydrochloride				
Taken (ng/mL)	Found (ng/mL)	Recovery (%)	RSD (%)	Error*
Intra-day precision				
5	4.93	98.6 ± 0.11	0.11	0.06
20	19.95	99.75 ± 0.08	0.08	0.04
70	69.85	99.78 ± 0.22	0.22	0.13
Inter-day precision				
5	5.0	100.00 ± 0.07	0.07	0.04
20	19.93	99.65 ± 0.11	0.11	0.06
70	69.98	99.97 ± 0.14	0.14	0.08

*% Error = SD/\sqrt{n}

Robustness of the method: The robustness was investigated by changing the experimental conditions, such as the pH 9.9 ± 0.2 , the change in the volume of CTAB, 1 ± 0.5 mL. These minor changes that may take place during the experimental operation did not affect the fluorescence intensity.

Results of stability studies: The following degradation behaviours of duloxetine hydrochloride samples were suggested during the forced degradation stress testing under different conditions.

Acid induced degradation: As already reported¹⁰, the effect of acid degradation was performed using 10 mL of 0.5 mol/L of hydrochloric acid at room temperature for 4 h and by analogue we studied the rate of degradation in hydrochloric acid and it was found faster as compared with that in alkali. It was found that 15.7 % of the drug solution was degraded.

Alkaline induced degradation: The degradation for duloxetine hydrochloride under alkali was slower than in acidic condition. It was found that by using 10 mL of 2 mol/L sodium hydroxide at room temperature for 8 h the degradation was so slow and around 8 % of the drug was degraded.

Hydrogen peroxide induced degradation: The effect of oxidative induced degradation of hydrogen peroxide was studied. It was found that treating the drug with 10 mL of 30 % H₂O₂ (v/v) at room temperature, according to the procedure described above, 9.5 % of the drug was degraded.

Thermal-degradation: The effect of heat on the stability of duloxetine hydrochloride was studied. It was observed that about 6.5 % of the drug was degraded even after exposure of the drug to 70 °C in a water bath for 5 h.

Conclusion

The proposed micelles enhanced spectrofluorimetric method for determination of duloxetine hydrochloride was developed and validated for precision, specificity and accuracy. The advantage of the proposed method is more sensitive, simple, more accurate and less tedious than chromatographic procedures. On the bases of accuracy and range sample concentrations, the proposed method was successfully applied for the determination of duloxetine hydrochloride in bulk drug and in its pharmaceutical dosage form.

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