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Original Article

DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF DULOXETINE

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ABSTRACT

Objective: The aim of the present work was to develop and validate a simple UV spectroscopic method for the determination of duloxetine, which is a thiophene derivative and a selective neurotransmitter reuptake inhibitor for serotonin, norepinephrine, and to lesser degree dopamine.

Methods: The UV Spectrophotometric analysis was performed using Shimadzu UV-1800 and Shimadzu UV-1700 spectrophotometer by using solvent system acetonitrile and water in the ratio of 8:2. Detection was performed at a wavelength of 290 nm. Method validation was carried out according to ICH Q2R1 guidelines by taking the parameters linearity, accuracy, precision, ruggedness, and robustness, LOD and LOQ.

Results: The UV Spectrophotometric method was found linear in the range of 10-50 µg/ml. The method was rugged and robust with % relative standard deviation less than 2. The extraction recoveries were found to be higher than 99% in all experimental conditions.

Conclusion: Based upon the performance characteristics, the proposed method was found accurate, precise and rapid and suitable for the determination of Duloxetine for routine analysis.

Keywords: Duloxetine, UV Spectrophotometric method, Beer's Law, Process validation, ICH guidelines

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INTRODUCTION

Duloxetine is chemically (+)-(S)-N-methyl-Y-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride belongs to the antidepressant class of drugs. Its Molecular formula is $C_{18}H_{19}NOS$ representing Molecular mass: 297.41456 g/mol with Molecular weight: 333.88 [1-3]. It is administrated by oral route at a dose of 40-60 mg/day for major depressive disorder, 30 mg/day for fibromyalgia. It is available in tablet and capsule dosage forms and marketed under a brand name cymbalta. It is Soluble in water, methanol, DMSO (Dimethyl Sulfoxide), acetonitrile. It is Stored between the temperatures 25-30 ℃ and should be kept away from direct sunlight [2, 3]. Duloxetine inhibits the reuptake of serotonin and norepinephrine (NE) in the central nervous system. Duloxetine increases dopamine (DA) specifically in the prefrontal cortex where there are few DA reuptake pumps via the inhibition of NE reuptake pumps (NET) thus allowing greater diffusion of DA in this brain region [4-6]. However, duloxetine has no significant affinity for dopaminergic, cholinergic, histaminergic, opioid, glutamate, and GABA reuptake transporters and can, therefore, be considered to be a selective reuptake inhibitor at the 5HT and NE transporters. Duloxetine undergoes extensive metabolism, but the major circulating metabolites do not contribute significantly to the pharmacologic activity [6, 7].

Duloxetine has an elimination half-life of about 12 h (range 8 to 17 h) and its Pharmacokinetics is dose proportional over the therapeutic range. Steady-state plasma concentrations are typically achieved after 3 d of dosing. Elimination of duloxetine is mainly through hepatic metabolism involving two P450 isozymes, CYP2D6 and CYP1A2 [6-8].

Though determination of duloxetine hydrochloride by spectrofluorimetric method [9, 10] has been reported but there is no UV-visible spectrophotometric method for determination of duloxetine, which motivates us to develop and carry out this research work. This present research paper was found to be simple, precise, and accurate and validated as per ICH guidelines and rapid for the estimation of duloxetine in pure form. The solubility of duloxetine was analyzed using various solvents and it was found that the drug is freely soluble in acetonitrile and water. So the study was conducted using acetonitrile and water to optimize the analytical method. The chemical structure of duloxetine hydrochloride was shown in fig. 1.

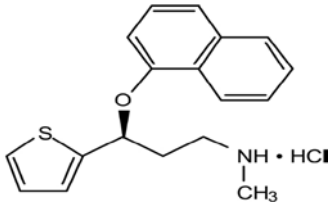

Fig. 1: Chemical structure of duloxetine hydrochloride

MATERIALS AND METHODS

Instrumentation

Shimadzu UV-1800 and Shimadzu UV-1700 spectrophotometer (single beam detector) were used for this study.

Chemicals and reagents

All the chemicals were of good quality. Acetonitrile was obtained from Thomas Beaker (chemicals) PVT. LTD., Mumbai and water used were double distilled water prepared in our college laboratory for this study. The solvents and reagents used in the study were of analytical grade.

Preparation of the solvent system

The solvent was prepared by mixing water and acetonitrile in the ratio of 2:8 v/v.

Preparation of the standard solution

Accurately weighed 10 mg of the powdered drug duloxetine was taken in a 10 ml volumetric flask and the prepared solvent water: acetonitrile (in ratio 20:80) was added up to the mark which gives the concentration of 1000 ppm. From the stock solution, 1 ml of the

solution was taken in a 10 ml volumetric flask and then it was made up to the mark with the same solvent to prepare the concentration of 100 ppm. From the above solution, different aliquots of solution was prepared by taking 1,2,3,4,5 ml in each 10 ml of volumetric flask separately and it was made up to mark with the same solvent to produce 10,20,30,40,50 ppm respectively.

Calibration curve

The prepared stock solution was scanned with water: acetonitrile (20:80) to construct Beer's law plot for the pure drug. The overall Spectra of duloxetine by UV-Visible Spectroscopy using solvent water: acetonitrile (2:8) was shown in fig. 2. From the solution concentration of 20 ppm was then scanned in UV range. This showed an absorption maximum at 290 nm. The absorbance of each solution was measured at their respective λ_{max} against water: acetonitrile (in the ratio of 20:80) as blank and results are shown in table 1. The calibration curve was plotted by taking the concentration of drug on x-axis and absorbance on the y-axis and the curve is shown in fig. 3.

Method validation

Accuracy

Accuracy of the method reveals the degree of similarity between the true value and the mean analytical value [11]. To determine the accuracy of the proposed method different sample solutions of same concentration 30 ppm were analyzed to determine percentage recovery of duloxetine by standard addition recovery method. The study was carried out by adding the known amount of the sample solution in the standard stock solution.

Precision

The precision of the proposed method was assessed by intra-day and inter-day variation studies using only one concentration of duloxetine (30 ppm) for several times [11]. During intra-day studies, five sample solutions of each concentration were analyzed on the same day whereas inter-day studies were determined by analyzing five sample solutions of each concentration for 5 consecutive days. The mean, standard deviation and % RSD were calculated.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated by using the following expressions:

LOD = 3.3 σ/S

LOQ = 10 σ/S

Where "σ" is the standard deviation of the regression line and "S" is the slope of the calibration curve.

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a verity of conditions [11-13]. The method's ruggedness was established by the determination of duloxetine by changing the different instruments of UV spectrophotometer i.e. Shimadzu UV-1800 and Shimadzu UV-1700 spectrophotometer. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and % RSD.

Robustness

To verify the robustness of the method, three vital experimental variables such as composition of mobile phase, detection wavelength and flow rate were slightly varied. The analysis was performed by changing the different instruments of UV spectrophotometer i.e. Shimadzu UV-1800 and Shimadzu UV-1700 spectrophotometer. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

RESULTS AND DISCUSSION

The proposed method for determination of duloxetine hydrochloride showed molar absorptivity 2.995×10^{-7}L/mol. cm. From the calibration curve it was found that it shows linearity in the range of 10-50µg/ml with regression coefficient 0.9999. Linear regression of absorbance on concentration gave the equation y = 0.020x+0.007 with a correlation coefficient (r) of 0.999. The detection wavelength showing ƛ max (maximum wavelength) at 290 nm.

Accuracy

The percentage recovery and % RSD were calculated. The mean percentage recovery and % RSD were found to be within limits and it is less than 2, which explains the present research paper is accurate in method development of duloxetine. The mean, standard deviation and percentage relative standard deviation (%RSD) were calculated. The results were shown in table 2.

Precession

Repeatability of the method was studied by precision experiments. The % RSD of duloxetine was found to be 0.811707 and 0.831657 in intra and inter-day precision respectively. The results for intra-day and inter-day were shown in table 3 and table 4 respectively.

LOD and LOQ

The LOD and LOQ were estimated from the standard deviation of the Y intercepts and slope of the calibration curve and values are 0.4 µg/ml and 1.32 µg/ml for LOD and LOQ respectively.

Ruggedness

The ruggedness results are shown in table 5 and table 6. The low percentage RSD value illustrates the ruggedness of the method.

Robustness

Robustness was evaluated by making deliberate changes to the chromatographic parameters of the method. The obtained results in table 7 and table 8 indicated the minor changes in each condition and did not affect the method.

The optical characteristics such as Beer's law limit, Sandell's sensitivity, Standard deviation, % RSD, LOD, LOQ were calculated and are summarized in table 9.

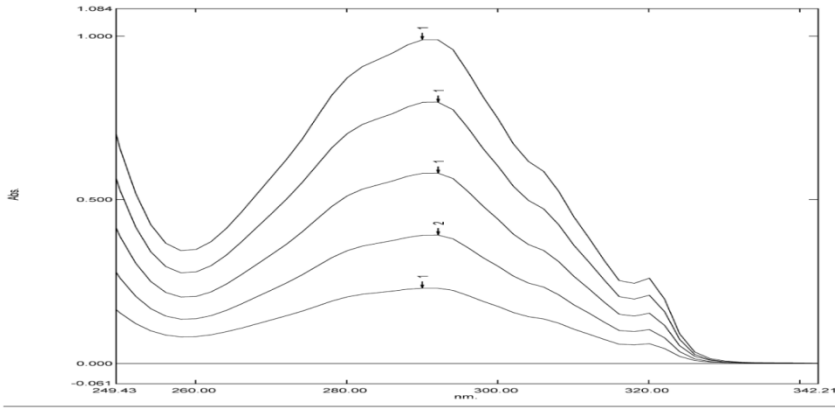

Fig. 2: Overlay spectra of duloxetine by UV-visible spectroscopy using solvent water: acetonitrile (2:8)

Table 1: Calibration table of UV-Vis spectrophotometric method for duloxetine

Concentration in µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Absorbance 4	Absorbance 5	Absorbance 6	Mean	Standard deviation	% Relative standard deviation
10	0.21	0.213	0.212	0.214	0.206	0.202	0.2095	0.004637	2.213274
20	0.412	0.423	0.414	0.412	0.411	0.415	0.4145	0.004416	1.065351
30	0.611	0.602	0.601	0.603	0.618	0.621	0.609333	0.008687	1.425682
40	0.819	0.822	0.827	0.819	0.816	0.824	0.821167	0.003971	0.483547
50	1.023	1.019	1.014	1.018	1.016	1.015	1.0175	0.003271	0.321483
								0.004996	1.101867

*values given in the table are the mean±SD of six observations.

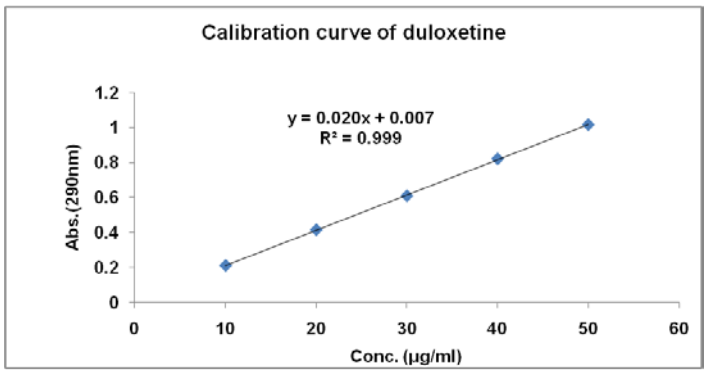

Fig. 3: Calibration curve was plotted using the concentration on X-axis and mean absorbance on Y-axis

Table 2: Accuracy data of UV-Vis spectrophotometric method for duloxetine

S. No.	Concentration (µg/ml)	Absorbance	Calculated amount	Statistical analysis
1.	30	0.611	30.2	Mean = 30.11667
2	30	0.602	29.75	Standard deviation. =0.434358
3.	30	0.601	29.7	% RSD =1.44225
4.	30	0.603	29.8	
5.	30	0.618	30.55	
6.	30	0.621	30.7	

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 3: Interday precession data of UV-Vis spectrophotometric method for duloxetine

S. No.	Concentration (µg/ml)	Absorbance (1)	Absorbance (2)	Absorbance (3)	Average	Statistical analysis
1.	30	0.611	0.61	0.615	0.613	Mean= 0.6081
2	30	0.601	0.607	0.608	0.605333	Standard deviation.= 0.004936
3.	30	0.603	0.604	0.605	0.604	%RSD =0.811707
4.	30	0.602	0.607	0.604	0.604333	
5.	30	0.618	0.607	0.617	0.614	

*values given in the table are the mean±SD for 5 samples. % RSD: Relative standard deviation

Table 4: Intraday precession data of UV-Vis spectrophotometric method for duloxetine

S. No.	Concentration (µg/ml)	Day 1	Day 2	Day 3	Average	Statistical analysis
1.	30	0.615	0.618	0.617	0.616667	Mean= 0.612067
2	30	0.608	0.609	0.611	0.609333	Standard deviation= 0.00509
3.	30	0.605	0.607	0.619	0.610333	%RSD = 0.831657
4.	30	0.604	0.606	0.608	0.606	
5.	30	0.617	0.618	0.619	0.618	

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 5: Ruggedness data of the UV-Vis spectrophotometric method by UV-1700 for duloxetine

S. No.	Concentration (µg/ml)	Absorbance	Calculated amount	Statistical analysis
1.	30	0.606	29.95	Mean= 30.2
2	30	0.607	30	Standard deviation = 0.23184
3.	30	0.611	30.2	%RSD = 0.767684
4.	30	0.614	30.35	
5.	30	0.617	30.5	

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 6: Ruggedness data of UV-Vis spectrophotometric method by UV-1800 for duloxetine

S. No.	Concentration (µg/ml)	Absorbance	Calculated amount	Statistical analysis
1.	30	0.608	30.05	Mean= 30.24
2	30	0.609	30.1	Standard deviation= 0.178185
3.	30	0.612	30.25	%RSD = 0.589237
4.	30	0.613	30.3	
5.	30	0.617	30.5	

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 7: Robustness data of UV-Vis spectrophotometric method by UV-1700 instruments for duloxetine

S. No.	Concentration (µg/ml)	Absorbance	Calculated amount	Statistical analysis
1.	30	0.608	30.05	Mean= 30.39
2	30	0.611	30.2	Standard deviation = 0.270185
3.	30	0.615	30.4	%RSD = 0.889059
4.	30	0.619	30.6	
5.	30	0.621	30.7	

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 8: Robustness data of UV-Vis spectrophotometric method by UV-1800 instruments for duloxetine

S. No.	Concentration (µg/ml)	Absorbance	Calculated amount	Statistical analysis
1.	30	0.605	29.9	Mean= 30.22
2	30	0.607	30	Standard deviation= 0.270647
3.	30	0.612	30.25	%RSD = 0.89559
4.	30	0.615	30.4	
5.	30	0.618	30.55	

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 9: Optical characteristics of duloxetine

Beer's law limit (µg/ml)	10-50 µg/ml
λ max	240 nm
Molar extinction co-efficient (E $^{1\%}$)	2036.66
Molar absorptivity (L mole^{-1} cm^{-1})	2.995×10^{-7}
Sandell's sensitivity (µg cm-2/0.001 absorbance unit)	0.540
Standard deviation	0.004996
% Relative standard deviation	1.101867
Confidence limits	
Correlation coefficient	0.999
Regression equation (Y)	0.020x+0.007
Slope (a)	0.020
Intercept (b)	0.007
LOD	0.4 µg/ml
LOQ	1.32 µg/ml

CONCLUSION

The proposed method UV-Vis Spectrophotometric method was found to be simple, precise, and accurate and validated as per ICH guidelines and rapid for the estimation of duloxetine. The mobile phase is simple to prepare, inexpensive solvent. Hence, this method can be easily and conveniently adopted for routine analysis of duloxetine in quality control laboratories and the method can also be extended for the routine assay of duloxetine in formulations.

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AUTHORS CONTRIBUTIONS

For preparing this research paper L. Samal gave a substantial contribution by data collection, data analysis and interpretation by executing the experimental work in our laboratories. A. Prusty drafted the manuscript and extensively revised to improve the quality of the manuscript. Conception, design, critical revision of the article and supervision of the work has been done by A. Prusty.

CONFLICT OF INTERESTS

Authors declare that there is no conflict of interest

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