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DEVELOPMENT AND VALIDATION OF DERIVATIVE UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF DULOXETINE

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Keywords:

Duloxetine, UV Spectrophotometric method, derivative spectroscopy, Method validation, ICH guidelines

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ABSTRACT: Development and validation of a simple UV spectroscopic method for the determination of Duloxetine by the derivative spectrophotometric method is the objective of the present work. The analysis was carried out by using Shimadzu UV-1700, and Shimadzu UV-1800 UV spectrophotometer, and Acetonitrile and water (8:2) was used as solvent or mobile phase. Detection or qualitative analysis was carried out at a wavelength of 240 nm. ICH guidelines were followed to verify the method validation process by using various parameters like linearity, accuracy, precision (interday and intraday), ruggedness, robustness, the limit of detection (LOD), and limit of quantification (LOQ). According to Beer's Lambert's law, the derivative UV spectrophotometric method was found linear in the range of 10-50 µg/ml. The analytical method was rugged and robust with a % relative standard deviation of less than 1.8. The percentage recoveries were higher than 98.7% in all the validation parameters and conditions. Based upon the analytical experiment, the developed method was found accurate, precise and rapid, and suitable for the rapid determination of Duloxetine for routine analysis.

INTRODUCTION: Chemically Duloxetine (Cymbalta) is methyl (3S) - 3 - (naphthalene - 1 - yloxy) - 3 - (thiophen - 2 - yl) propyl] amine hydrochloride belongs to the class of antidepressant drugs. The Molecular formula, molecular mass, and molecular weight are $C_{18}H_{19}NOS$, 297.41456 g/mol, and 333.88, respectively [1, 2, 3, 9]. Route of administration is oral and is available as a tablet, capsule, and enteric-coated pellet dosage forms in the market [3, 9]. It is freely soluble in water and soluble in methanol, DMSO, acetonitrile, and the storage temperatures is 25-30^{0}C [2, 3, 9].

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In the brain, Duloxetine inhibits the reuptake of both serotonin and nor-epinephrine (NE). Duloxetine increases dopamine (DA) level especially in the prefrontal cortex region so allowing greater diffusion of DA in this brain region [4], where there are few DA reuptake pumps act by the inhibition of NE reuptake pumps (NET), which is believed to mediate reuptake of DA and NE [5, 6, 9]. The drug has no significant affinity for dopaminergic, cholinergic, histaminergic, opioid, glutamate, and GABA reuptake transporters.

Duloxetine undergoes hepatic metabolism by using two cytochrome P_{450} isoenzymes, and the metabolites are eliminated *via* urine [6, 7, 9]. Derivative spectroscopy uses absorbance of 1st or higher derivatives with respect to wavelength for both qualitative and quantitative analysis. In the year 1950, the concept of derivative spectroscopy was 1st introduced.

Due to the complexity of generating derivative spectra using early UV-Visible spectrophotometers and the baseline shift, the UV-spectroscopic method undergoes extensive modification. The problem arises either from the instrument, *i.e.*, lamp or detector instabilities or sample handling. Using the derivative spectroscopy, the above problem can be eliminated as the 1st derivative of a constant absorbance offset is zero and it always eliminates the baseline shifts and improves the accuracy and precision of the method. Derivative UV- spectrophotometry is an instrumental analytical technique that uses normal zero-order spectra of sample mathematically differentiated into derivative spectra (1st or higher-order derivatives) [10, 11, 12]. Derivative spectrophotometric has found a wide range of application in both qualitative and quantitative chemical analysis *i.e.*

- ✓ Macro to Nano Spectroscopy.
- ✓ Multicomponent analysis.
- ✓ Determination of one analyte in presence of matrix or for simultaneous assaying of few analytes.
- ✓ Calculation of some physico-chemical constants, *e.g.* reaction, complexation or binding constants.
- ✓ Application for investigation of some processes kinetics[10, 11, 12].

The present paper deals with the method development and validation of a derivative Spectrophotometric method for the determination of Duloxetine in pure powder form. The solubility of the drug was determined using various solvents and found that Duloxetine is freely soluble in Acetonitrile and water. So the analysis was performed using Acetonitrile and water to optimize the Analytical method development and validation.

MATERIALS AND METHODS:

Instrumentation: The experiment was carried out from December 2019 to March 2020 at Centurion University of Technology and Management, Jatni, Odisha, India using Shimadzu UV-1800 and Shimadzu UV-1700 spectrophotometer (single beam detector).

Chemicals and Reagents: Acetonitrile was Purchased From Thomas Beaker Chemicals Pvt. Ltd., Mumbai, and double-distilled water was prepared in our college laboratory and used in the current study.

Preparation of the Solvent System: The analysis solvent mixture was prepared by using water and Acetonitrile in the ratio of 2:8 v/v and sonicating the solvent system.

Preparation of the Standard Solution: Powdered drug Duloxetine has accurately weighed up to 10 mg and taken in a 10 ml volumetric flask, and the prepared solvent was added up to the mark, which gives the concentration of 1000 PPM. From the stock solution, different aliquots of the solution were prepared by taking 0.1,0.2,0.3,0.4,0.5 ml of solution in every 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: 1st the baseline was obtained by taking only the prepared solvent, which is considered a blank reading. Then the prepared stock solution was scanned in the UV- spectroscopy with the prepared solvent to construct Beer's law plot for the pure drug. By extrapolation of the curve and by using the software, the absorption maximum was found at 290 nm. The absorbance of each solution was measured at their respective λ_{max} against water: Acetonitrile (20:80) as blank. Finally, the calibration curve was plotted by taking the concentration of the drug on the x-axis and absorbance on the y-axis.

Method Validation: Method validation is a documented process used to assure that the analytical test used for a specific test is suitable for the intended use. It is a critical element ensuring the quality and safety of the analytical product.

Accuracy: [8, 9] To determine the accuracy of the proposed method, different sample solutions of the same concentration 30 PPM were analyzed to determine % recovery of Duloxetine by the standard addition recovery method. The study was carried out by preparing the 5 set of solutions each having a concentration of 10, 20, 30, 40, 50 PPM. From this, the concentration of 30 PPM was selected, and the absorbance was noted down for the set of 5 reading. Then the mean, standard deviation (SD) and % relative standard deviation

(RSD) were calculated. The results were shown in **Table 3**.

Precision: [8, 9] 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. The intra-day study was carried out by preparing the 5 set of solutions each having the concentration of 10, 20, 30, 40, 50 PPM. From this, the concentration of 30 PPM was selected, and the absorbance was noted down for the set of 5 reading and was analyzed on the same day, whereas inter-day study was carried out by the same process but by analyzing five sample solutions of each concentration for 5 repeated days. Then the mean, standard deviation (SD) and % relative standard deviation (RSD) were calculated. The results were shown in **Tables 4** and **5**.

Limit of Detection (LOD) and Limit of Quantification (LOQ): [9, 10, 11]

LOD: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantified as an exact value. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

LOQ: The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. It is a parameter of quantitative assays for low levels of compounds in the sample and is particularly used for the determination of impurities and/or degradation products.

$$LOD = 3.3\ \sigma/S$$

$$LOQ = 10\ \sigma / S$$

Where, σ = standard deviation of the regression line
S = slope of the calibration curve.

Robustness: [8,9,10] To determine the robustness of any analytical method, three experimental parameters such as the composition of the mobile phase, detection wavelength, and flow rate were slightly altered. In this study, the composition of the mobile phase is changed to get the result. Then the mean, standard deviation (SD), and % relative standard deviation (RSD) were calculated. The results were shown in **Tables 8** and **9**.

Ruggedness: [9, 10] To determine method ruggedness the study was carried out by changing the different models of UV spectrophotometer. Then the mean, standard deviation (SD), and % relative standard deviation (RSD) were calculated. The results were shown in **Tables 6** and **7**.

RESULTS AND DISCUSSION: The proposed method for normal determination of Duloxetine hydrochloride showed molar absorptivity of 2.995 × 10^{-7} L / mol.cm. Linear regression of absorbance on concentration gave the equation y = 0.020x + 0.007 with a correlation coefficient (r) of 0.999. The optical characteristics such as Beer's law limit, Sandell's sensitivity, Standard deviation, % RSD, LOD, LOQ were calculated and are given in **Table 10**.

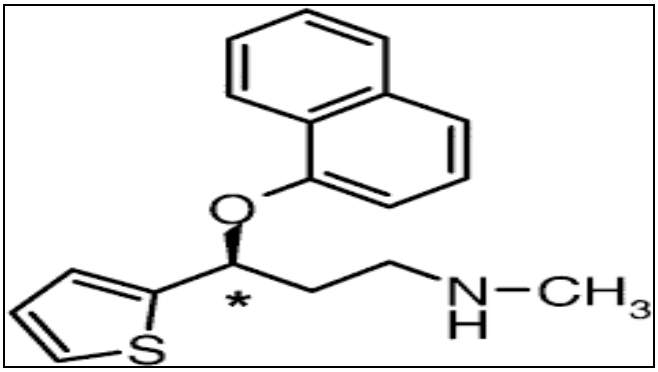

FIG. 1: DULOXETINE HYDROCHLORIDE

The derivative spectroscopy method showed molar absorptivity of 4.901 × 10^{-7} L/mol.cm. Linear regression of absorbance on concentration gave the equation y = -0.004x - 0.007 with a correlation coefficient (r) of 0.996. The optical characteristics such as Beer's law limit, Sandell's sensitivity, Standard deviation, % RSD, LOD, LOQ were calculated and given in **Table 11**.

By comparing the UV-spectroscopic method and derivative UV-spectroscopic method for Duloxetine, the UV-spectroscopic method was found to be more accurate than the derivative method. Still, the derivative UV-spectroscopic method was found to be precise and can also be used in routine analysis, and in the future further, analysis of higher-order spectroscopy can be done to produce a more accurate result.

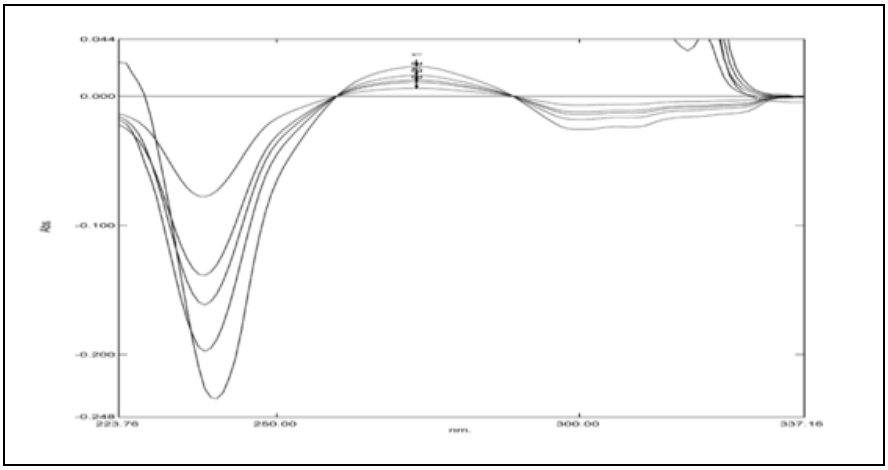

FIG. 2: OVERLAY SPECTRA OF DULOXETINE BY UV-VISIBLE SPECTROSCOPY USING WATER: ACETO-NITRILE (2:8). This spectra showing ƛ max (maximum wavelength) is about 290 nm.

TABLE 1: CALIBRATION TABLE OF UV-VIS SPECTROPHOTOMETRIC METHOD FOR DULOXETINE

Conc.	Abs. 1	Abs. 2	Abs. 3	Abs. 4	Abs. 5	Abs. 6	Mean	SD	% RSD
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
			Average =				0.6144	0.004996	1.101867

A calibration curve was plotted using the concentration on X-axis and mean absorbance on Y-axis

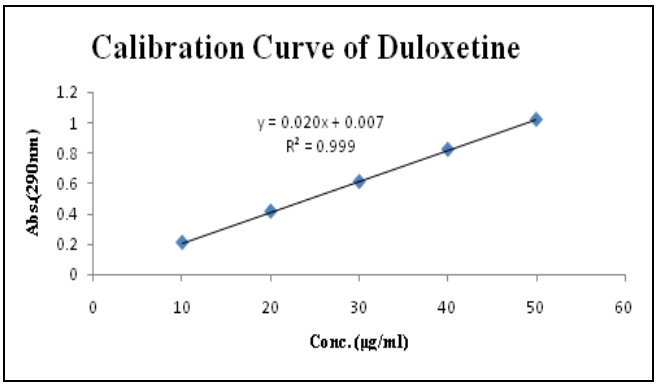

FIG. 3: CALIBRATION CURVE OF DULOXETINE IN NORMAL UV- SPECTROSCOPY. From the calibration curve it was found that it shows linearity in the range of 10-50 µg/ml with regression coefficient 0.9999

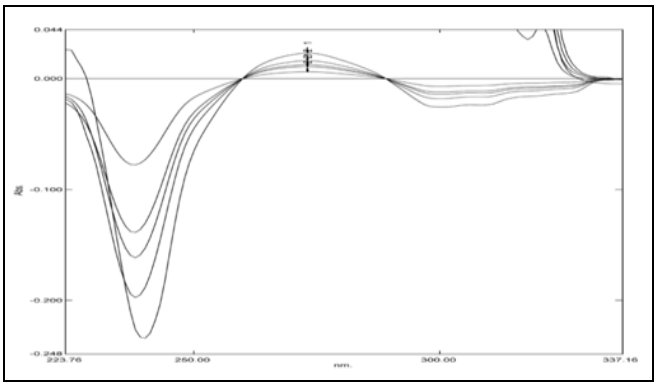

FIG. 4: OVERLAY 1ST DERIVATIVE SPECTRA OF DULOXETINE AT 240 nm USING WATER: ACETONITRILE (2:8)

TABLE 2: CALIBRATION TABLE FOR 1ST DERIVATIVE UV-VIS SPECTRO-PHOTOMETRIC METHOD FOR DULOXETINE

Conc.	Abs. 1	Abs. 2	Abs. 3	Abs. 4	Abs. 5	Abs. 6	Mean	SD	% RSD
10	-0.058	-0.057	-0.059	-0.057	-0.059	-0.058	-0.058	0.000894	-1.54
20	-0.109	-0.108	-0.109	-0.108	-0.109	-0.108	-0.1085	0.000548	-0.51
30	-0.147	-0.148	-0.146	-0.147	-0.146	-0.149	-0.1471	0.001169	-0.79
40	-0.201	-0.202	-0.202	-0.201	-0.201	-0.203	-0.2016	0.000816	-0.404
50	-0.256	-0.255	-0.254	-0.256	-0.258	-0.257	-0.256	0.001414	-0.55

A calibration curve was plotted using the concentration on X-axis and mean absorbance on Y-axis.

TABLE 3: ACCURACY DATA OF THE 1ST DERIVATIVE UV-VIS SPECTROPHOTOMETRIC METHOD FOR DULOXETINE

S. no.	Concentration (µg /ml)	Absorbance	Calculated Amount	Statistical Analysis
1	30	-0.132	31.25	Mean=30.25
2	30	-0.128	30.25	SD=0.57
3	30	-0.126	29.75	%RSD=1.88
4	30	-0.127	30	
5	30	-0.126	29.75	

TABLE 4: INTER DAY PRECESSION DATA OF THE 1ST DERIVATIVE UV-VIS DERIVATIVE SPECTRO-PHOTOMETRIC METHOD FOR DULOXETINE

S. no.	Concentration (µg /ml)	Abs. 1	Abs. 2	Abs. 3	Average	Statistical analysis
1	30	-0.125	-0.128	-0.134	-0.129	Mean=-0.12713
2	30	-0.127	-0.128	-0.128	-0.127	Std. Dev.=0.00126
3	30	-0.126	-0.126	-0.126	-0.126	%RSD=0.99149
4	30	-0.127	-0.127	-0.127	-0.127	
5	30	-0.126	-0.126	-0.126	-0.126	

TABLE 5: INTRA DAY PRECESSION DATA OF THE 1ST DERIVATIVE UV-VIS DERIVATIVE SPECTRO-PHOTOMETRIC METHOD FOR DULOXETINE

S. no.	Conc. (µg/ml)	Day 1	Day2	Day 3	Average	Statistical analysis
1	30	-0.127	-0.124	-0.125	-0.1253	Mean=-0.1262
2	30	-0.128	-0.128	-0.127	-0.1276	Std. Dev.=0.001121
3	30	-0.129	-0.125	-0.124	-0.126	%RSD=0.8878
4	30	-0.128	-0.127	-0.126	-0.127	
5	30	-0.125	-0.126	-0.124	-0.125	

TABLE 6: RUGGEDNESS DATA OF THE 1ST DERIVATIVE UV-VIS SPECTROPHOTOMETRIC METHOD BY DIFFERENT ANALYST FOR DULOXETINE USING UV- 1700

S. no.	Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical deviation
1	30	-0.127	30	Mean = 30.12
2	30	-0.128	30.25	Std. deviation= 0.46
3	30	-0.126	29.75	%RSD= 1.55
4	30	-0.127	30	
5	30	-0.126	29.75	
6	30	-0.131	31	

TABLE 7: RUGGEDNESS DATA OF THE 1ST DERIVATIVE UV- VIS SPECTROPHOTOMETRIC METHOD BY DIFFERENT ANALYST FOR DULOXETINE USING UV- 1800

S. no.	Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Deviation
1	30	-0.128	30.25	Mean = 30.08
2	30	-0.128	30.25	Std. Deviation = 0.30
3	30	-0.126	29.75	%RSD = 1.006
4	30	-0.127	30	

TABLE 8: ROBUSTNESS DATA OF THE 1ST ORDER DERIVATIVE UV-VIS SPECTROPHOTOMETRIC METHOD BY FOR DULOXETINE USING ACETONITRILE: WATER (78:22)

S. no.	Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Deviation
1	30	-0.131	31	Mean=30.208
2	30	-0.128	30.25	Std.dev=0.48
3	30	-0.126	29.75	%RSD=1.606
4	30	-0.127	30	
5	30	-0.126	29.75	
6	30	-0.129	30.5	

TABLE 9: ROBUSTNESS DATA OF THE 1ST ORDER DERIVATIVE UV-VIS SPECTROPHOTOMETRIC METHOD BY FOR DULOXETINE USING ACETONITRILE: WATER (82:18)

S. no.	Conc. (µg/ml)	Abs.	Calc. Amount	Statistical Deviation
1	30	-0.127	29.85	Mean=30.35
2	30	-0.128	30.1	Std.dev=0.52
3	30	-0.129	30.35	%RSD=1.72
4	30	-0.127	29.85	
5	30	-0.132	31.1	
6	30	-0.131	30.85	

TABLE 10: OPTICAL CHARACTERISTICS OF DULOXETINE BY NORMAL UV-VISIBLE SPECTROSCOPIC METHOD

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

TABLE 11: OPTICAL CHARACTERISTICS OF DULOXETINE BY UV- VISIBLE SPECTROSCOPIC METHOD BY 1ST ORDER DERIVATIVE SPECTROSCOPIC METHOD

Beer's Law Limit (µg/ml)	10-50 µg/ml
λ_{max}	240 nm
Molar extinction co-efficient (E 1%)	4900.01
Molar absorptivity (L mole-1 cm^{-1})	4.901×10^{-7}
Sandell's sensitivity (µg cm-2 / 0.001 absorbance unit)	0.204
Standard deviation	0.005996
% Relative standard deviation	1.211867
Confidence limits	
Correlation coefficient	0.996
Regression equation (Y)	-0.004x - 0.007
Slope (a)	-0.004
Intercept (b)	0.007
LOD	0.6µg/ml
LOQ	1.52 µg/ml

CONCLUSION: The proposed method UV- Vis Spectrophotometric method by 1st order derivative spectroscopy was found to be simple, precise, and accurate and validated as per ICH guidelines and used for rapid estimation of Duloxetine. The mobile phase is simple to prepare, inexpensive solvent available all the time. Hence, this method can be easily and conveniently adapted for routine analysis of Duloxetine in quality control laboratories, and the method can also be extended for the routine assay of Duloxetine in various formulations.

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CONFLICTS OF INTEREST: Authors declare that there is no conflict of interest.

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