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MULTIVARIATE CALIBRATION TECHNIQUE AIDED UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF DULOXETINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM: ASSESSMENT OF GREENNESS PROFILE

DINESH G A, SEETHARAMAN R* AND MANIKANDAN K

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu District, Tamil Nadu - 603203, India

***Corresponding Author: Dr. Seetharaman R: E Mail: seetharr@srmist.edu.in**

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ABSTRACT

The present work proposes to provide a eco- friendly UV spectrophotometric technique for the determination of Duloxetine hydrochloride in pharmaceutical tablets, a multivariate calibration method is used. The multivariate calibration method measures the sample absorbance at various wavelengths for more precise measurements. The UV spectrophotometric method was created, and method validation was completed. All validation parameters complied with ICH standards. The current study targets on the development of UV spectrophotometric method in First derivative mode for Duloxetine hydrochloride in bulk and pharmaceutical formulation employing multivariate calibration technique. Multivariate calibration technique utilizes the linear regression equations to correlate the relationship between concentration and amplitude at five different wavelengths, the multivariate calibration technique increases the correlation and reduces instrumental variations. Duloxetine hydrochloride showed absorption maximum at 216 nm with water as diluent. The results were treated statistically. The analytical Eco scale, Agree metrics, and Green analytical procedure index was used to assess the method's greenness scores.

Keywords: Duloxetine hydrochloride, Multivariate Linear Regression Analysis, Validation, UV spectrophotometry, Greenness

INTRODUCTION

Many antidepressants, including hydroxypropyl-cyclodextrin (HP-CD) and sulfonamides-cyclodextrin (SBE-CD), increase the synaptic availability of serotonin (5-HT) and/or nor epinephrine (NE) in order to boost cellular uptake of inclusion complexed drugs **[1-2]**. The chemical name for Duloxetine hydrochloride **Figure 1** is (+)-(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine with molecular formula $C18H19NOS$ **[3]** (DLX) is a novel dual serotonin and norepinephrine reuptake inhibitor, approved for the treatment of major depressive disorders, diabetic peripheral neuropathic pain, fibromyalgia, generalized anxiety, and woman stress urinary incontinence. DLX has an edge over other existing antidepressants because of favourable pharmacodynamic features of DLX like dual inhibition, improved efficacy, tolerability, safety, faster recovery, fewer side effects, and low affinity for other neuronal receptors **[4]**. It is indicated for the treatment of major depressive disorder (MDD). It is a selective serotonin and nor-epinephrine reuptake inhibitor (SSNRI) for oral **[5-6]** administration.

The review of literature showed few analytical methods has been reported for determination of duloxetine hydrochloride in human serum and biological fluids using tandem MS and LCMS methods and HPLC **[7]**, ultraviolet (UV)spectrophotometric methods **[8-9]**, Fourier transform-infrared (FT-IR) spectroscopy **[10]**. Also, a stability-indicating high-performance liquid chromatography method was established for analysis of the drug in the presence of various degradation products **[11]**. A simple, selective, precise, and stability-indicating high-performance thin-layer chromatographic (HPTLC) method has been established and validated for analysis of duloxetine hydrochloride in formulations **[12]**.

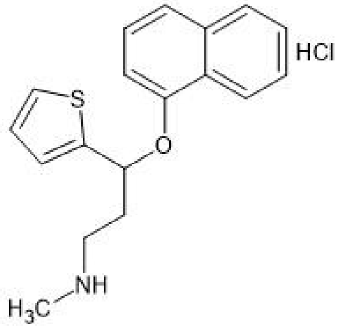

Figure 1: Chemical structure of Duloxetine hydrochloride

The Multivariate calibration methodology was utilised to reduce instrumental error and having a huge impact. The technique is simple, low-cost, and adaptable to dosage forms for chemical products and pharmaceuticals. MVC uses straight regression techniques with a range of 5–10 nm wavelengths for precise results. In this paper, we described how to estimate the amount of Duloxetine hydrochloride in pharmaceutical dosage forms using a UV spectral MVC method with a minimum amount of calculation. This led to the selection of five different wavelengths in order to ensure the sensitivity in compared to the conventional ultraviolet (UV) technique. The following equations translate the multivariate algorithm techniques of MVC's statistics into univariate data The following equation can be written for each chosen wavelength if the absorbance of an analyte (X) Duloxetine hydrochloride in this case, is scanned at 5 wavelengths specified (λ = 212, 214, 216, 218 and 220 nm).

$A_{\lambda 212} = a \text{ X } Cx + k1$ ……… (1)

$A_{\lambda 214} = b \text{ X } Cx + k2$ ……… (2)

$A_{\lambda 216} = c \text{ X } Cx + k3$ ……… (3)

$A_{\lambda 218} = d \text{ X } Cx + k4$ ………(4)

$A_{\lambda 220} = e \text{ X } Cx + k5$ ……… (5)

Whereas,

- A_λ = Absorbance of the sample;
- a, b, c, d, e = Slope of the straight regression functions of a sample;
- k_1, k_2, k_3, k_4, k_5 = Intercept of the straight regression;
- C_x = Concentration of the sample

The above five equations can be rearranged as:

$A_T = a \text{ x } Cx + b \text{ x } Cx + c \text{ x } Cx + d \text{ x } Cx + e \text{ x } Cx + K_T$…. (6)

The above equation can be further simplified to

$A_T = Cx\,(a+b+c+d+e) + K_T$………… (7)

Whereas,

- A_T = Sum of the absorbances acquired
- K_T = Sum of intercepts of regression equation

The concentration of the analyte X in a solution can be computed by using the formula.

$$\text{Cx} = \frac{A_T - K_T}{(a+b+c+d+e)} \quad \text{…………… (8)}$$

Greenness evaluation techniques

The analytical eco scale **[13]** is constructed on allocating penalty points determined by the number of pictograms with associated signal words as established by "The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)", as well as the quantity. Every reagent, its type and quantity, potential occupational exposure, and energy depletion, including waste, are all part of the analytical eco scale approach. Penalty points are deducted from a starting score of 100.

Analytical eco-scale = 100 - total penalty points …….. (9)

The Green Analytical Procedure Index **[14]** (GAPI) is also a pictorial representation that constitutes five pentagrams which unique colour coding. The colour coding in the pictogram involves three levels of assessment at each stage of an analytical procedure The colour coding used by GAPI to assess greenness ranges from green to yellow to red, signifying the low, medium, and high environmental impact associated with the analytical procedure, respectively. A brief description of GAPI was well described and reported by J. Płotka-Wasylka in the year 2018 **[14]**. AGREE metrics, **[15]** unique software for quantifying the greenness profile, are used in the second assessment methodology. The software's output is a circular diagram containing numbers on the edges ranging from 1 to 12 in a clockwise orientation. These numbers depict the 12 ideologies of green analytical chemistry. The outcomes of all these 12 principles are given a rating of 0 to 1 based on the inputs and their weightage. This aggregate scale is colour coded as red, yellow, and green, with red denoting zero, dark green denoting one or near to one, and yellow denoting a number between red and dark green. The sum of the 12 principles and the core generates a score that reflects the extent of greenness.

MATERIALS AND METHODS

MATERIALS

- The LABINDIA UV 3092 double beam UV-VIS spectrophotometer (Gurugram, India) sealed and quartz coated with Czerny-Turner monochromator optics with Wavelength range: 190 to 900 nm, Spectral bandwidth: Continuous slit 0.1 – 5.0 nm with 0.1 nm interval. Wavelength accuracy: ±0.3 nm. Automatic eight-cell changer. It comprises Tungsten and deuterium lamp as detector and UV Win Lab Version 5.1.1 Software for data output were used.
- Analytical balance (AS 245, Mettler Toledo, India),
- Soniclean sonicator (model 160T, Thebarton-Australia).

Reference samples

Duloxetine hydrochloride were kindly supplied by IPCA Laboratories Ltd, (Mumbai, India)

Marketed formulation

The marketed tablet (SYMPTA 30) used contains 30 mg Duloxetine hydrochloride and was manufactured by IPCA Laboratories., Ltd.

Preparation of solutions

Standard stock solution preparation of Duloxetine hydrochloride

Transfer 30 mg of sample Duloxetine hydrochloride into a 100mL volumetric flask. Dissolve it in 50ml of Water, sonicate for 20 minutes, and then add more Water to make a final volume of 100mL.

Working solutions of Duloxetine hydrochloride

Water was used as a solvent to create a 7-13 µg mL^{-1} solution from the above stock solution.

Selection of wavelength for MVC

The Duloxetine hydrochloride working standard solutions were scanned against Water as the blank solution, which has a maximum absorption at 216 nm, over the wavelength range of 200 to 400 nm. As a result, the MVC approach's wavelength was in the range of these absorption maxima, i.e., 212,214,216,218,220nm.

Stability of the solution

By maintaining prepared sample solutions at room temperature for 0–12 hours, Duloxetine hydrochloride solution stability experiments were carried out. It was routine to measure the absorbance after 0, 6, and 12 hours.

METHOD VALIDATION

The prepared method were validated per ICH guidelines **[16]** for linearity, accuracy, precision.

Linearity

To analyse the linearity and spectral area of Duloxetine hydrochloride, the stock solution was appropriately diluted with Water to achieve concentrations ranging from 7to13 µg mL^{-1} (7,8,9,10,11,12 and 13). For the MVC approach, the absorbance of linearity solutions at the proper wavelength was measured and examined.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The following formulas were used to predict the Limit of Detection (LOD) and Limit of Quantification (LOQ) for Duloxetine hydrochloride based on the calibration curve slope and standard deviation of responses for a certain wavelength.

$$\mathrm{LOD} = \frac{3.3 \text{ x standard deviation}}{\text{Slope}} \text{ ……... (9)}$$

$$\mathrm{LOQ} = \frac{10 \text{ x standard deviation}}{\text{Slope}} \text{ ……….. (10)}$$

Precision

Intraday and interday precision were used to assess the precision's repeatability. A typical standard solution of Duloxetine hydrochloride was used to test various levels of accuracy at a concentration of 10 µg mL^{-1}. The repeatability investigation involved the analysis of six solutions at five different wavelengths. In the intravariation scenario, the absorbance of prepared solutions was measured three times on the same day at a predetermined time interval. Utilizing the absorbance on three additional days allowed for intravariation to be achieved.

Accuracy

At 80, 100, and 120 percent of the pre-analysed sample solutions, the methodology's accuracy for Duloxetine hydrochloride was tested, and the recovery values' percentages were estimated.

Assay

Weigh and powder 10 Tablets. Weigh accurately a quantity of the tablet powder equivalent to about 30 mg of Duloxetine hydrochloride, add 25mL of Water and sonicate for 20mins. Add sufficient Water and make up to 100mL. The solution obtained above is filtered and diluted with Water to attain 10 µg mL^{-1} concentration Duloxetine hydrochloride. The absorbance of the resulting solution is measured at 2 nm and the content of Duloxetine hydrochloride is quantified.

RESULTS AND DISCUSSION

Starting at 200–400 nm, 10 µg mL^{-1} solutions of Duloxetine hydrochloride was scanned. The wavelength of maximum spectrum was 216. Utilize Water as a blank and selecting the nm of 216nm for MVC, the UV spectrum of standards and samples of Duloxetine hydrochloride was recorded. The typical spectra of Duloxetine hydrochloride 10µg mL^{-1} are shown **in Figure 2**.

Stability of solution

The results of Duloxetine hydrochloride solution stability show that the absorbance values and the spectrum produced while using the solution measured at 0, 6, and 12 hours do not significantly vary with time. The difference in absorbance between the fresh standard solution and the preserved solutions was minimal, and it was discovered to be less than 2%.

Linearity

According to ICH Q2 R1 criteria, the linearity results for the developed technique for of Duloxetine hydrochloride were determined as a concentration range of 70 to 130 percent for 10 µg mL^{-1} (7 to 13µg mL-^{1}). **Figure 3** displays the over spectra for Duloxetine hydrochloride for linearity. The calibration curve was created by calculating the absorbance of standard solutions that had been diluted at five different wavelengths.

Limit of Detection and Limit of Quantification

LOD and LOQ for Duloxetine hydrochloride was calculated from the linearity slope, which has been confirmed by different sample analyses. The LOD for Duloxetine hydrochloride was calculated from the average of all the absorbance, which was found to be 0.03272µg mL^{-1}**.** The LOQ for Duloxetine hydrochloride was calculated from the average of all absorbance, which is found to be 0.088718mL^{-1}

Precision

The system precision spectra for Duloxetine hydrochloride are represented in **Figure 4**. The interday precision spectra for Duloxetine hydrochloride are represented in **Figure 5**. The intraday precision spectra were represented in **Figure 6** for Duloxetine hydrochloride. The % RSD of system precision, interday and intraday precision, was determined for Duloxetine hydrochloride It was found to be less than 2%, which shows that the approach method is precise.

Accuracy

At 80, 100, and 120% **Figure 12** shows the overlay spectra for. Duloxetine hydrochloride. The result for Duloxetine hydrochloride are shown in **Table 4,** and the obtained results within limits.

Assay of marketed formulations:

The recommended spectrophotometric method was used to investigate the quantity of Duloxetine hydrochloride in the tablet formulation. The UV absorption spectrum of a commercial tablet was obtained for three replicates. After extraction and filtration, there was no appreciable decrease in the pharmaceutical formulation's excellent analytical recovery values. **Table 5** shows new strategy outperforms the earlier ones.

Assessment of greenness of the proposed method

The results of greenness profile for the proposed methods were evaluated. The results of analytical scale is shown in **Table 5**, while the results for GAPI and agree metrics is depicted in **Figure 14** and **Figure 15**.

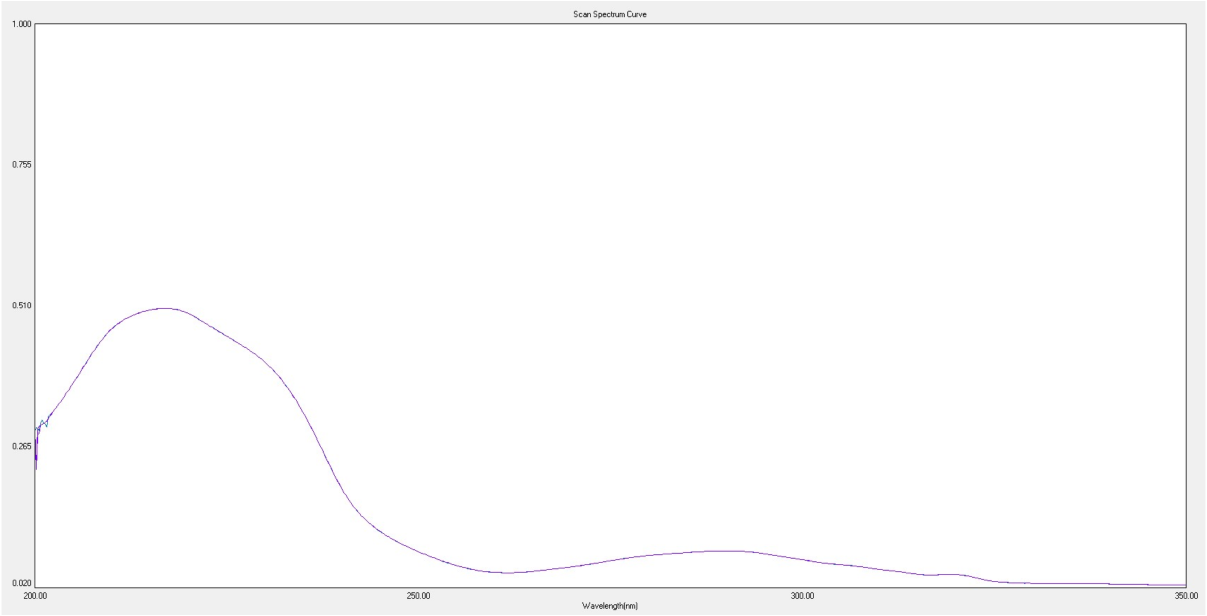

Figure 2: UV spectrum of standard Duloxetine hydrochloride (10µg mL^{-1}) using Water as a blank

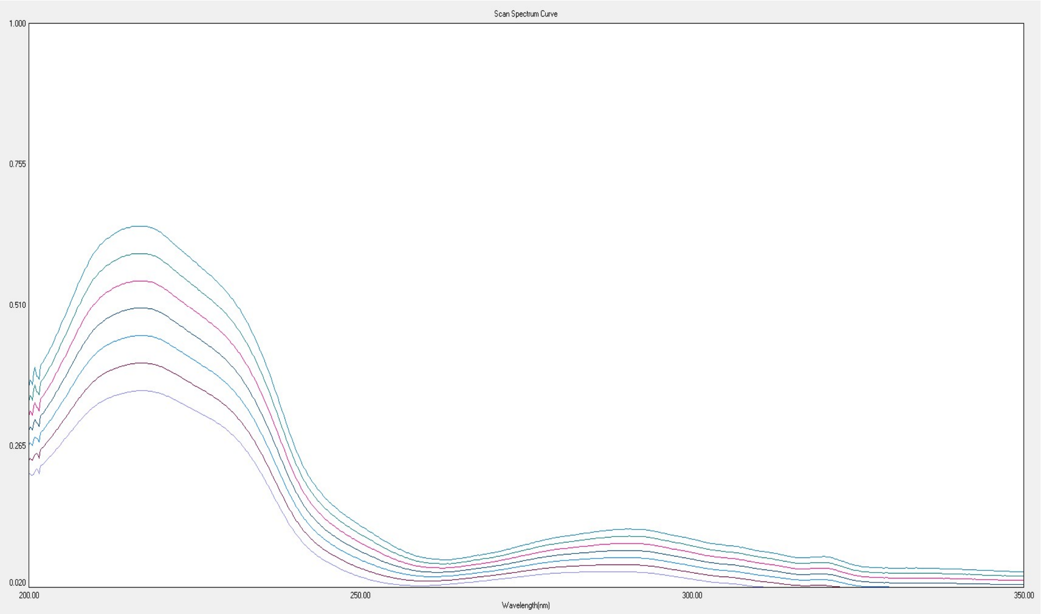

Figure 3: Linearity spectrum of Duloxetine hydrochloride (7-13µg mL^{-1}) using Water as a blank

Table 1: Multivariate UV calibration data at five selected wavelengths

	Absorbance				
Concentration (µg/mL)	**212 nm**	**214 nm**	**216 nm**	**218 nm**	**220 nm**
7	**0.347**	**0.355**	**0.360**	**0.360**	**0.355**
8	**0.394**	**0.403**	**0.408**	**0.408**	**0.402**
9	**0.440**	**0.450**	**0.455**	**0.456**	**0.449**
10	**0.487**	**0.498**	**0.503**	**0.503**	**0.495**
11	**0.533**	**0.545**	**0.551**	**0.551**	**0.542**
12	**0.579**	**0.593**	**0.599**	**0.599**	**0.589**
13	**0.626**	**0.641**	**0.647**	**0.646**	**0.636**

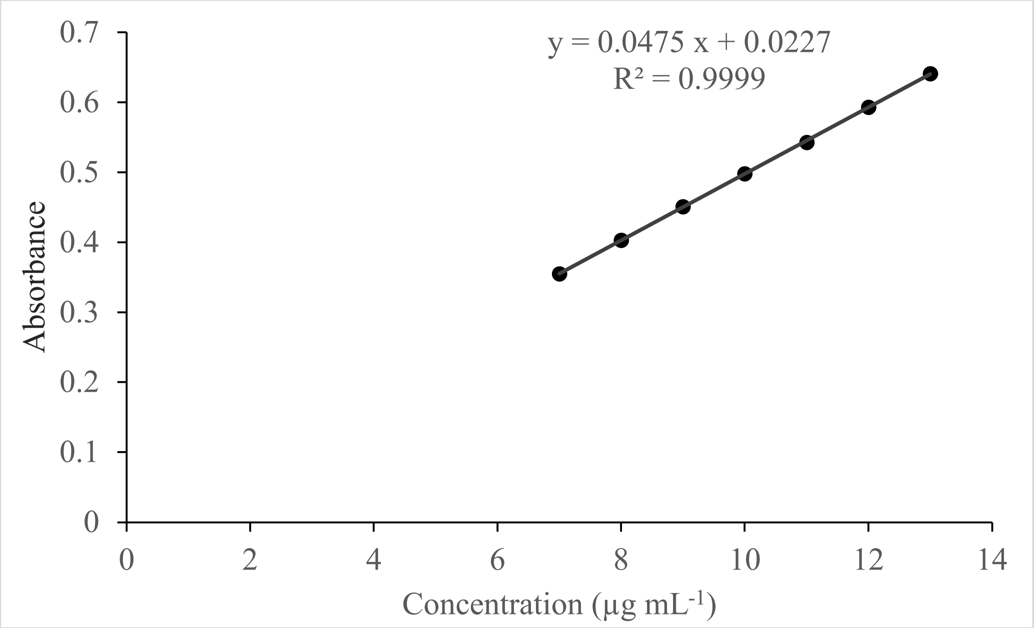

Figure 4: Calibration curve at 212 nm

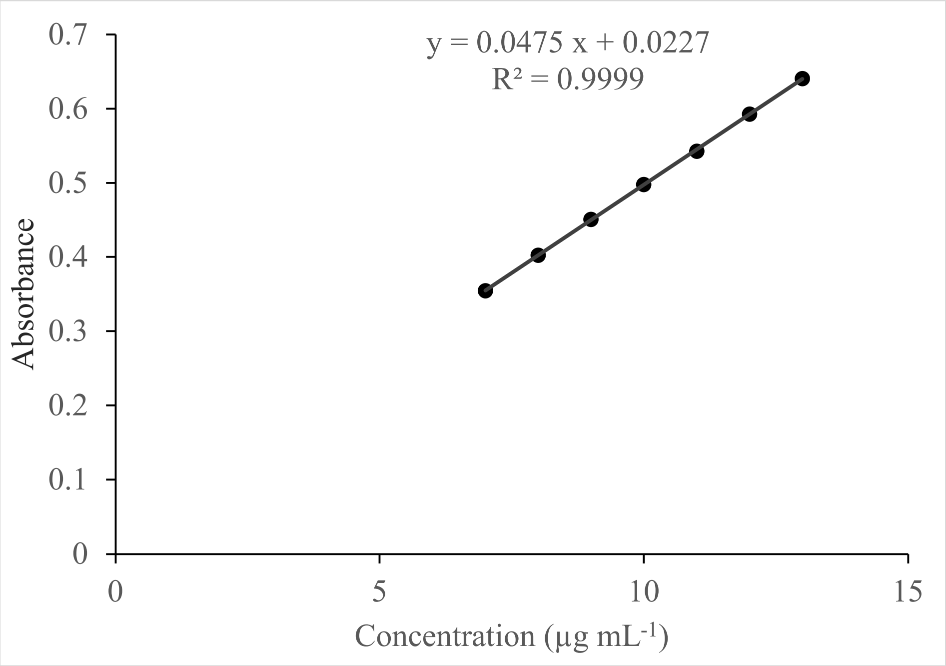

Figure 5: Calibration curve at 214 nm

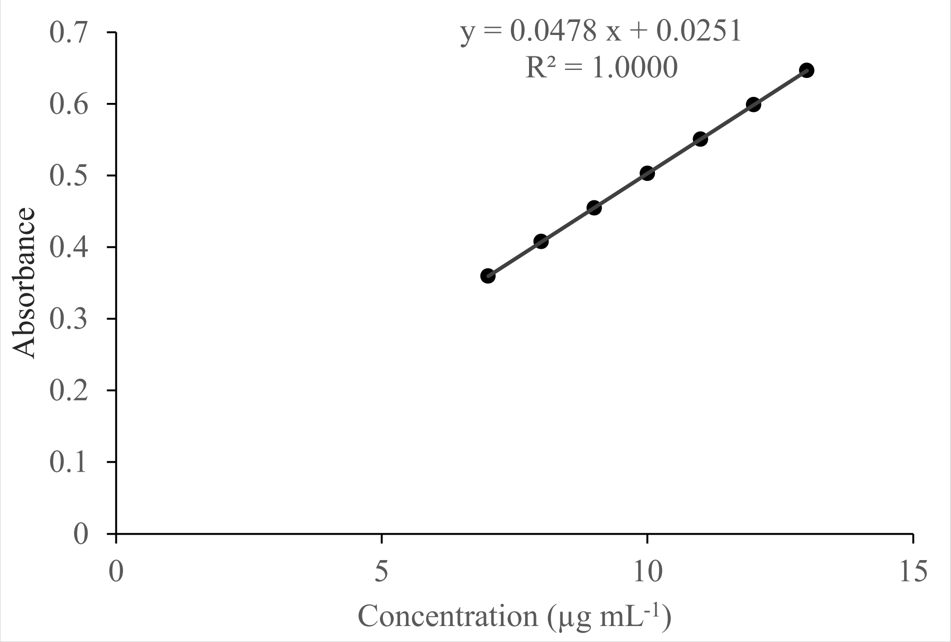

Figure 6: Calibration curve at 216 nm

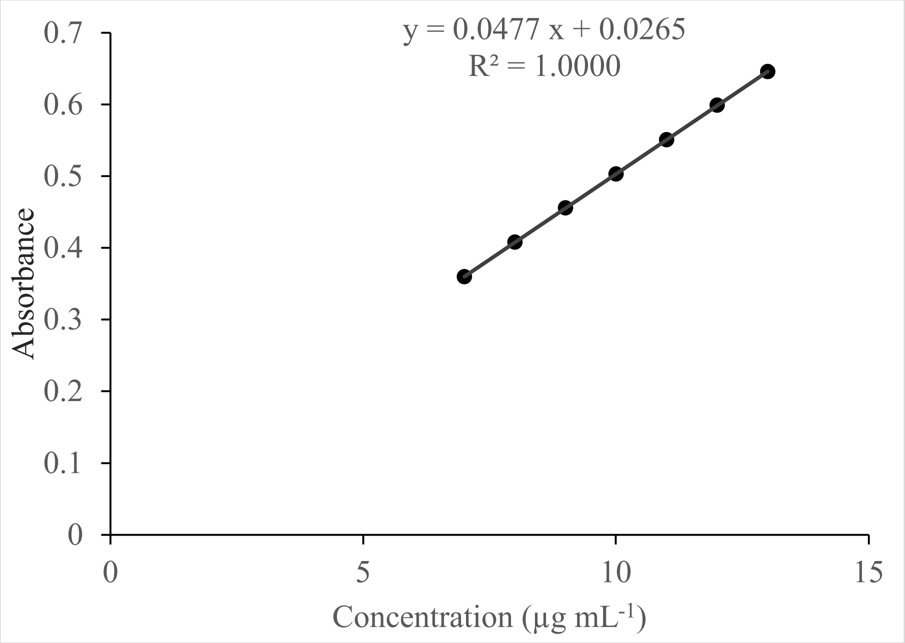

Figure 7: Calibration curve at 218 nm

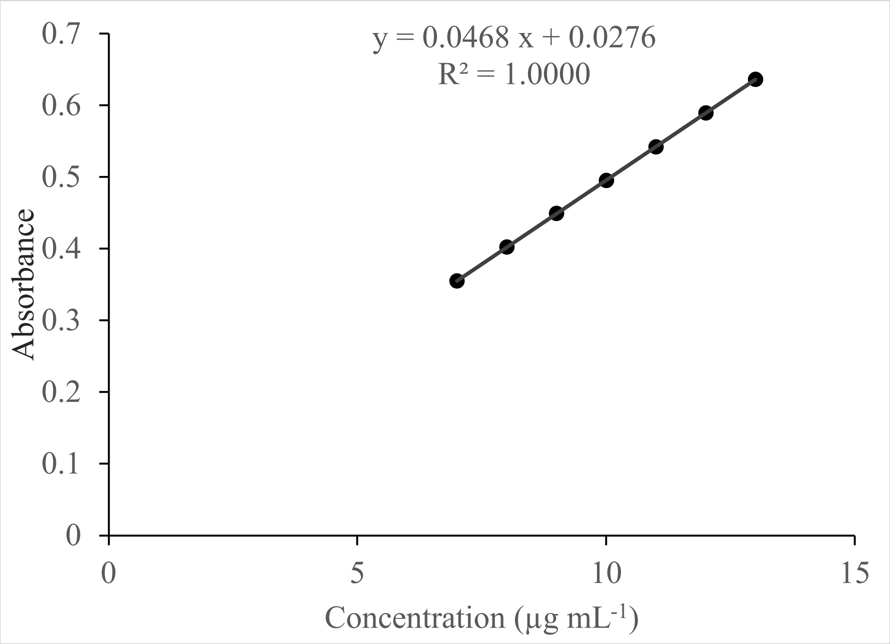

Figure 8: Calibration curve at 220 nm

Table 2: Linearity data showing statistical parameters at the selected wavelengths

Wavelength (nm)	Regression equation	Slope	Intercept	R^2	Standard error	LOD µg mL^{-1}	LOQ µg mL^{-1}
212	y = 0.0475x + 0.0227	0.0475	0.0227	0.9999	0.0003381	0.034	0.103
214	y = 0.0475x + 0.0227	0.0476	0.0218	0.9999	0.000327327	0.033	0.100
216	y = 0.0478x +0.0251	0.0478	0.0251	1.0000	0.000327327	0.032732684	0.09918995
218	y = 0.0477x +0.0265	0.0477	0.0265	1.0000	0.00032733	0.034	0.104
220	y = 0.0468x + 0.02716	0.0468	0.02716	1.0000	0.00029277	0.029277002	0.088718188

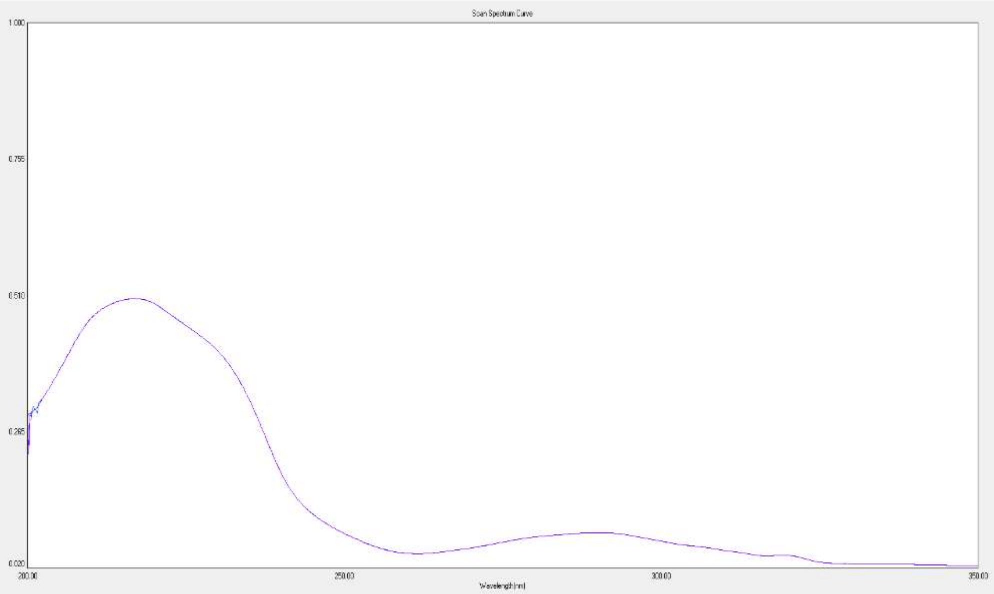

Figure 9: System precision overlay spectra of Duloxetine hydrochloride

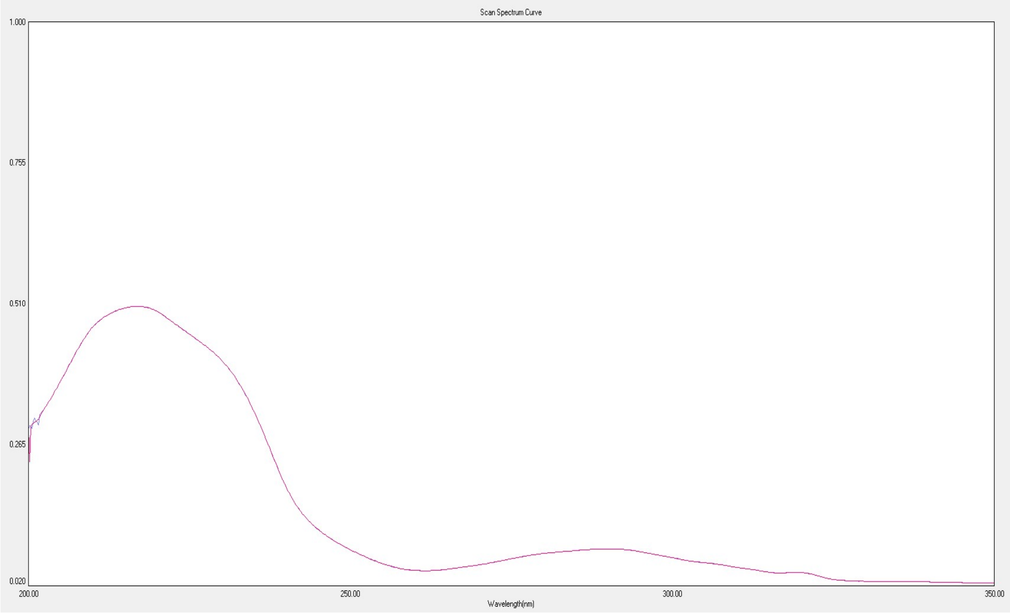

Figure 10: Interday precision overlay spectra of Duloxetine hydrochloride

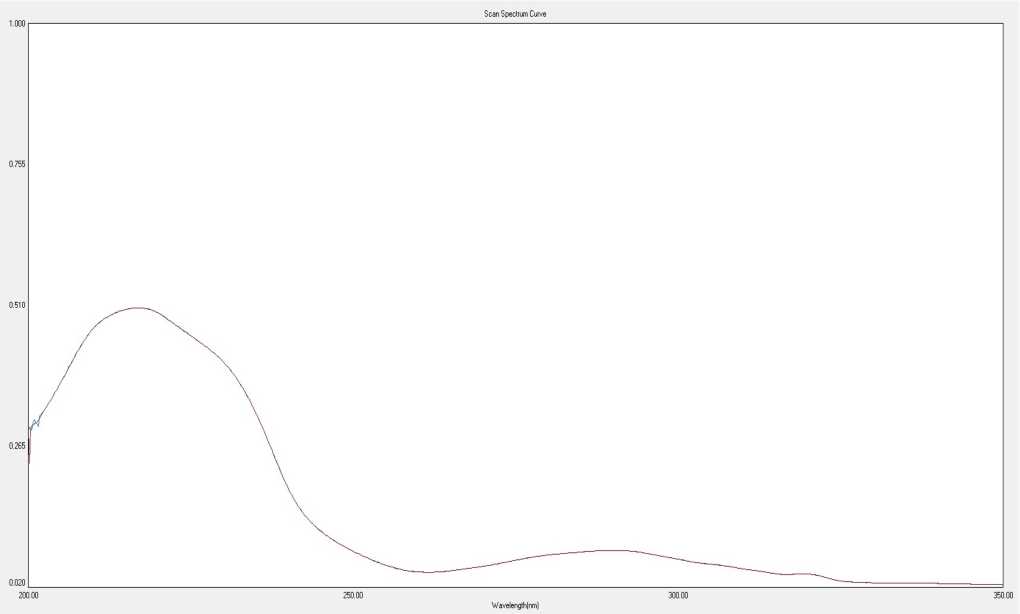

Figure 11: Intraday precision overlay spectra of Duloxetine hydrochloride

Table 3: System precision, Interday and Intraday precision data for the proposed

	System precision	Intraday and interday precision		
	Absorbance of standard for 10 µg/mL	% Recovery of sample equivalent to 10 µg mL^{-1} of sample		
		Day 1	Day 2	Day 3
1	2.486	99.14	98.06	98.45
2	2.513	98.90	99.27	99.54
3	2.521	99.35	99.12	99.38
4	2.472	99.30	99.52	99.58
5	2.568	99.40	99.19	98.56
6	2.521	99.29	98.21	99.57
Mean	2.150	99.23	98.90	99.18
SD	0.033	0.18	0.61	0.53
%RSD	1.55	0.19	0.61	0.53

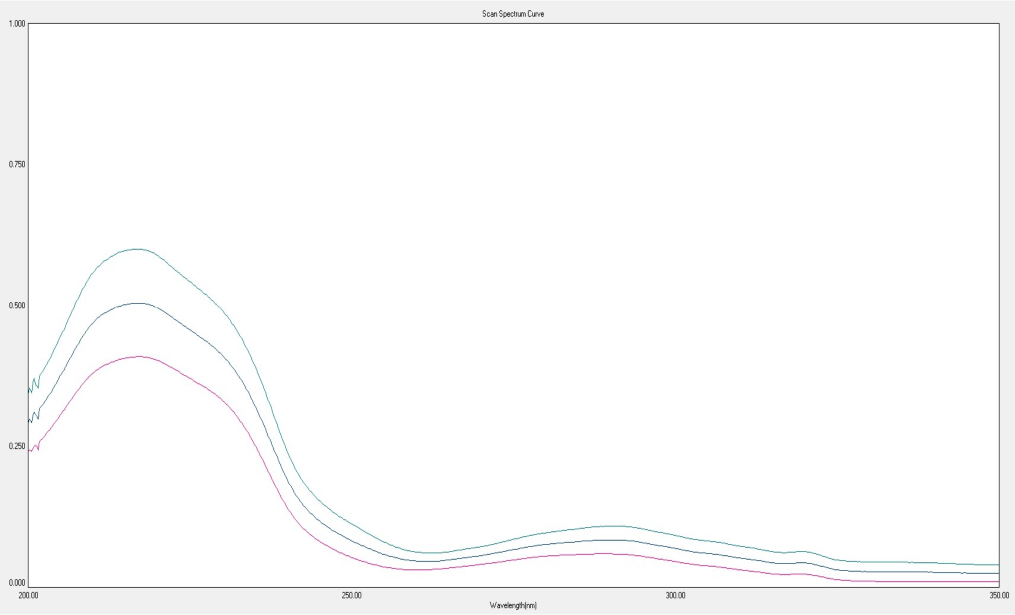

Figure 12: Overlay spectra of accuracy of Duloxetine hydrochloride at 80, 100, 120 % spiking

Table 4: Accuracy data for prepared method of Duloxetine hydrochloride

Concentration levels (%)	Amount present	Amount added (μg mL^{-1})	Amount recovered (μg mL^{-1})	Mean % Recovery	SD
80	5	3	7.91	98.79	0.62915
100	5	5	9.89	98.87	0.1528
120	5	7	11.89	99.22	0.127294

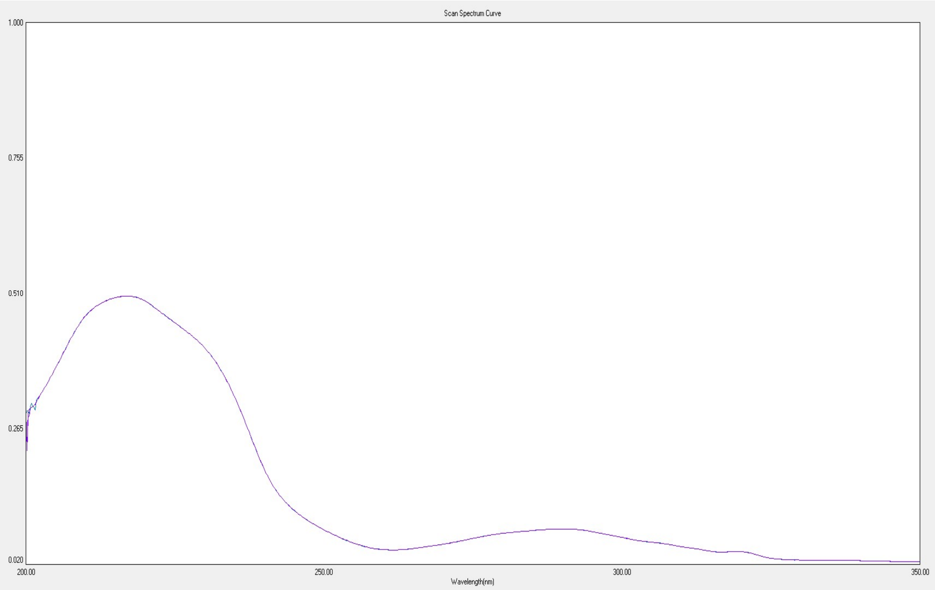

Figure 13: UV spectrum of standard Duloxetine hydrochloride (10μg mL^{-1}) using water as a blank

Table 5: Assay results for marketed formulation of Mirtazapine

Marketed formulation	Label claim (mg)	Mean ± SD (n=3)	% RSD
Batch - 1	30	29.95±0.10	0.050
Batch - 2	30	29.2±0.12	0.046

Table 8: Summary of Eco scale penalty points for the proposed method

Description	Penalty points	Total Penalty Points	Score
Water	2	4	96
Instrument	0		
Occupational hazard	0		
Waste	3		

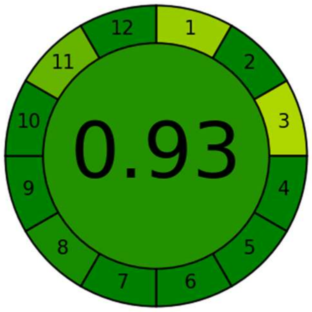

Figure 14: Agree metrics output for the proposed method

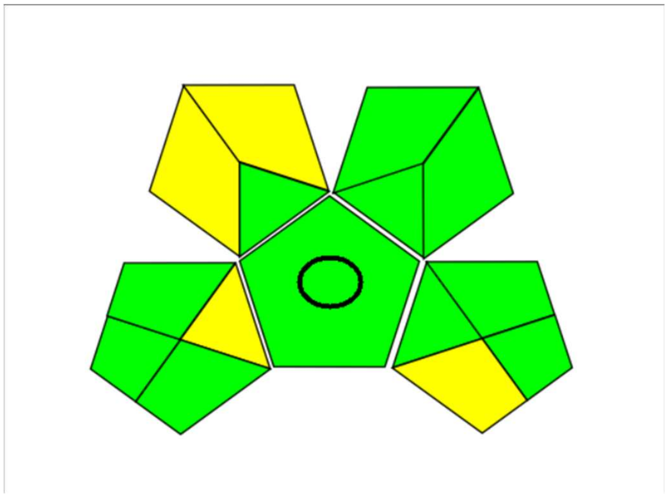

Figure 15: GAPI Pictogram for the proposed method

CONCLUSION

According to ICH criteria, the newly created spectrophotometric Multivariate analytical technique for the evaluation of Duloxetine hydrochloride was verified by assessing several validation parameters and was found to be within acceptable ranges. For the measurement of Duloxetine hydrochloride in its tablet formulation, the proposed approach was shown to be sensitive, accurate, precise, and repeatable. We strongly advise using the proposed approach for a routine analysis of Duloxetine hydrochloride in pharmaceutical formulations because it is more accurate than existing UV spectrophotometric methods and has a method with easy mathematical components.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

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