IJBPAS, August, 2023, 12(8): 3767-3780

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

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

DINESH G A, SEETHARAMAN R*, KOKILAMBIGAI K S AND KAVITHA J

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: seerampharm@gmail.com**

Received 15th Sept. 2022; Revised 15th Oct. 2022; Accepted 4th Dec. 2022; Available online 1st Aug. 2023

https://doi.org/10.31032/IJBPAS/2023/12.8.7362

ABSTRACT

The present work proposes to provide a eco- friendly UV spectrophotometric technique for the determination of Mirtazapine 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 second derivative mode for Mirtazapine 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. Mirtazapine showed absorption maximum at 293 nm in Ethanol 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: Mirtazapine, Multivariate Linear Regression Analysis, Validation, UV spectrophotometry

INTRODUCTION

The chemical name is 5-methyl-2,5,19triazatetracyclo[13.4.0.02,7.08,13]nonadeca-1(15),8,10,12,16,18-hexaene with molecular formula $C_{17}H_{19}N_3$ **[1]**. Mirtazapine is a noradrenergic and specific serotonergic antidepressant (NaSSA) that is approved in many counties for use in the treatment of major depression **[2]**.

The review of literature indicates that various techniques have been published for determining Mirtazapine in pharmaceutical formulations or biological fluids. For Mirtazapine a few chromatographic techniques such as HPLC **[3**], **[4]** HPTLC **[5]**, spectrophotometric techniques like UV **[6, 7]**, hyphenated techniques like LC-MS/MS,FT-IR **[8, 9]** were reported. No Multi Variate Calibration method was reported for this drug. The literature review referred to the MVC technique using UV spectrophotometry, which haven't reported. Hence, the present method deals with the development of the MVC technique for the estimation of Mirtazapine.

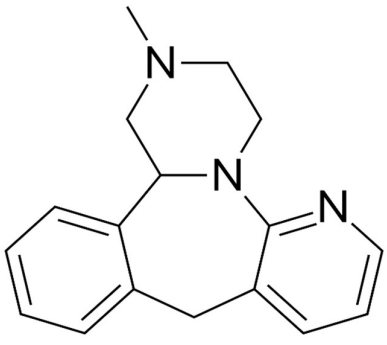

Figure 1: Chemical structure of Mirtazapine

The MVC 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 **[10]**. In this paper, we described how to estimate the amount of Mirtazapine 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) Mirtazapine in this case, is scanned at 5 wavelengths specified (λ = 283, 288, 293, 298 and 303nm).

$A_{\lambda 283}$ = a X Cx + k1 …… (1)

$A_{\lambda 288}$ = b X Cx + k2…… (2)

$A_{\lambda 293}$ = c X Cx + k3 …… (3)

$A_{\lambda 298}$ = d X Cx + k4 ……(4)

$A_{\lambda 303}$ = e X Cx + k5 …… (5)

Where as,

- 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:

$A_T = a \times Cx + b \times Cx + c \times Cx + d \times Cx + e \times Cx + K_T$…. (6)

The above equation can be further simplified to

$A_T = Cx\ (a+b+c+d+e) + K_T$……. (7)

Where

- 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.

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

Greenness evaluation techniques

The analytical eco scale **[11]** 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 **[12]** (GAPI) is also a pictorial representation that constitutes five pentagrams which unique color coding. The color 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 **[12]**. AGREE metrics, **[13]** 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

Mirtazapine were kindly supplied by Ideal Analytical and Research Institution (Mumbai, India)

Marketed formulation

The marketed tablet (MIRTAZ) used contains 15 mg Mirtazapine and was manufactured by Mylan Pharmaceuticals Pvt Ltd.

Chemicals and Reagents

Analytical grade Ethanol (S.D fine chemical Ltd., Mumbai, India) was used.

Preparation of solutions

Standard stock solution preparation of Mirtazapine

Transfer 15mg of sample Mirtazapine into a 100mL volumetric flask. Dissolve it in 50ml of

Ethanol, sonicate for 20 minutes, and then add more Ethanol to make a final volume of 100ml.

Working solutions of Mirtazapine

Ethanol 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 Mirtazapine working standard solutions were scanned against Ethanol as the blank solution, which has a maximum absorption at 303 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., 283, 288, 293, 298, 302nm.

Stability of the solution

By maintaining prepared sample solutions at room temperature for 0–12 hours, Mirtazapine 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 **[14]** for linearity, accuracy, precision.

Linearity

To analyse the linearity and spectral area of Mirtzapine, the stock solution was appropriately diluted with Ethanol 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 Limits of Detection LOD and Limits of Quantification LOQ for Mirtazapine based on the calibration curve slope and standard deviation of responses for a certain wavelength.

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

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

Precision

Intraday and interday precision were used to assess the precision's repeatability. A typical standard solution of Mirtazapine was used to test various levels of accuracy at a concentration of 10g 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 Mirtazapine 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 15mg of Mirtazapine, add 25 ml of Ethanol and sonicate for 20mins. Add sufficient Ethanol and make upto 100mL. The solution obtained above is filtered and diluted with Ethanol to attain 10 µg mL^{-1} concentration of mirtazapine. The absorbance of the resulting solution is measured at 303 nm and the content of mirtazapine is quantified.

RESULTS AND DISCUSSION

Starting at 200–400 nm, 10gmL^{-1} solutions of Mirtazapine was scanned. The wavelength of Mirtazapine maximum spectrum was 303nm. Utilize Ethanol as a blank and selecting the nm of 303nm for MVC, the UV spectrum of standards and samples of Mirtazapine was recorded. The typical spectra of Mirtazapine 10µg mL^{-1} are shown **in Figure 2**.

Stability of solution

The results of Mirtzapine 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 Mirtzapine were determined as a concentration range of 70 to 130 percent for 10µg mL^{-1} (7 to 13µg mL^{-1}). **Figure 3** displays the over spectra for Mirtzapine 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 Mirtzapine was calculated from the linearity slope, which has been confirmed by different sample analyses. The LOD for Mirtzapine was calculated from the average of all the absorbance, which was found to be 0.17980 µg mL^{-1}. The LOQ for Mirtazapine was calculated fom the average of all absorbance, which is found to be 1.1523µg mL^{-1}

Precision

The system precision spectra for Mirtzapine are represented in **Figure 4**. The interday precision spectra for Mirtazapine are represented in **Figure 5**. The intraday precision spectra were represented in **Figure 6** for Mirtzapine . The % RSD of system precision, interday and intraday precision, was determined for Mirtzapine 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 Mirtazapine. The results for Mirtazapine are shown in **Table 4,** and the obtained results within limits.

Assay of marketed formulations:

The recommended spectro-photometric method was used to investigate the quantity of mirtazapine 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** 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**.

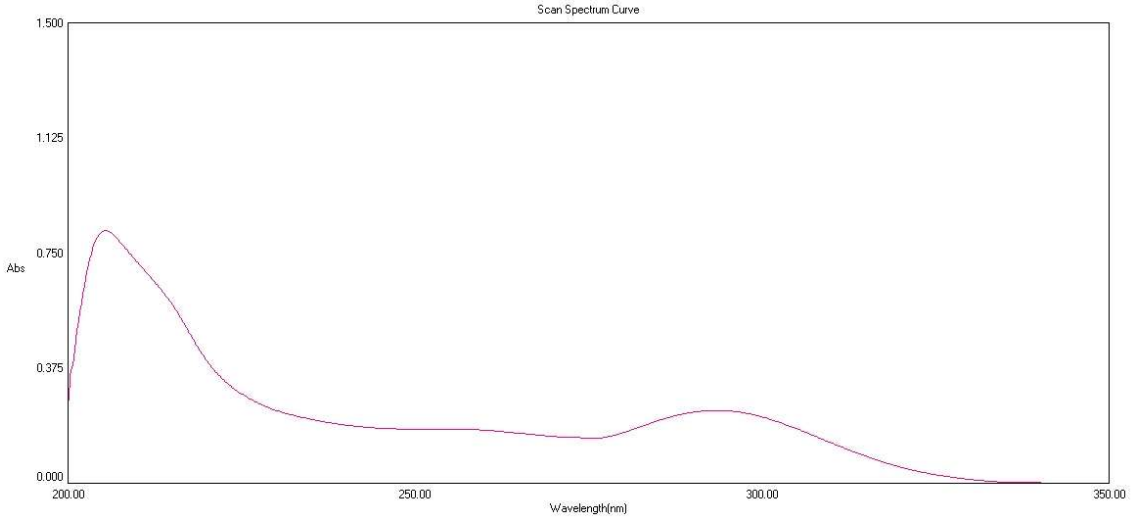

Figure 2: UV spectrum of standard Mirtazapine (10µg mL^{-1}) using Ethanol as a blank

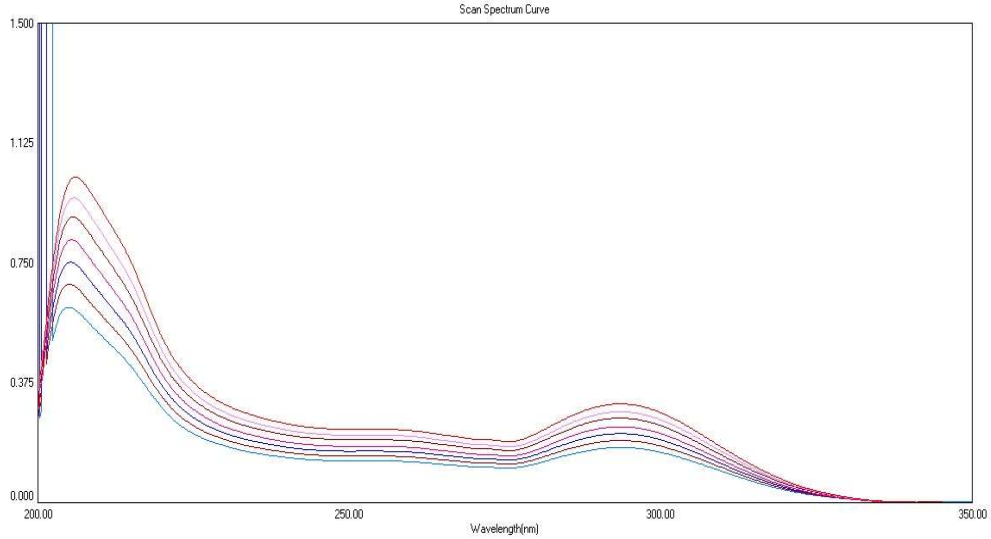

Figure 3: Linearity spectrum of Mirtzapine (7-13µg mL^{-1}) using Ethanol as a blank

Table 1: Multivariate UV calibration data at five selected wavelengths

	Absorbance				
Concentration (µg/mL)	**283 nm**	**288 nm**	**293 nm**	**298 nm**	**303 nm**
7	**0.137**	**0.16**	**0.171**	**0.164**	**0.141**
8	**0.153**	**0.18**	**0.193**	**0.185**	**0.159**
9	**0.17**	**0.201**	**0.215**	**0.206**	**0.176**
10	**0.186**	**0.221**	**0.236**	**0.226**	**0.194**
11	**0.208**	**0.246**	**0.263**	**0.252**	**0.216**
12	**0.224**	**0.265**	**0.283**	**0.271**	**0.233**
13	**0.244**	**0.288**	**0.308**	**0.295**	**0.253**

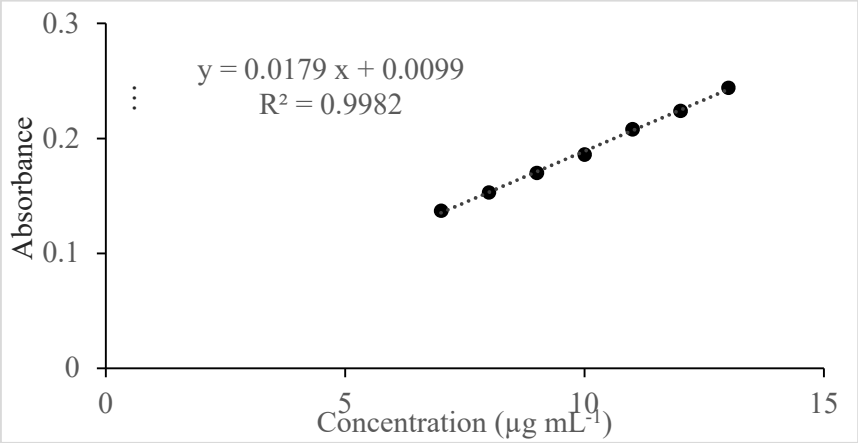

Figure 4: Calibration curve at 283 nm

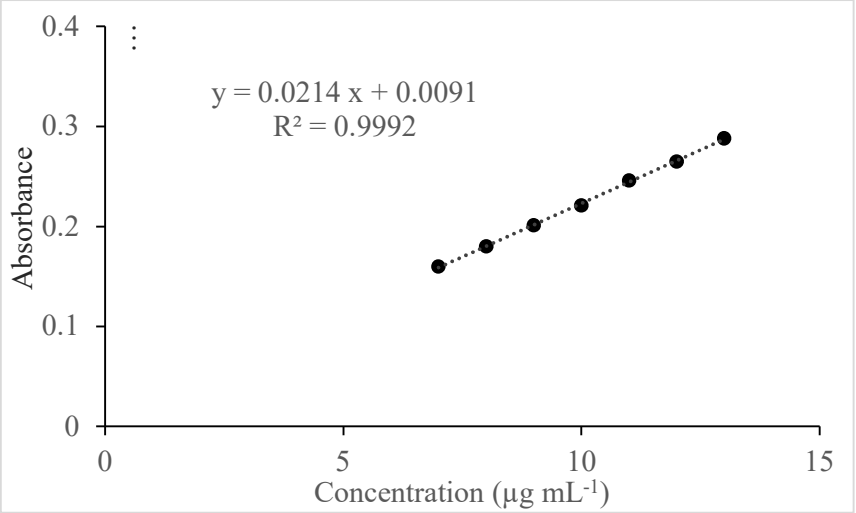

Figure 5: Calibration curve at 288 nm

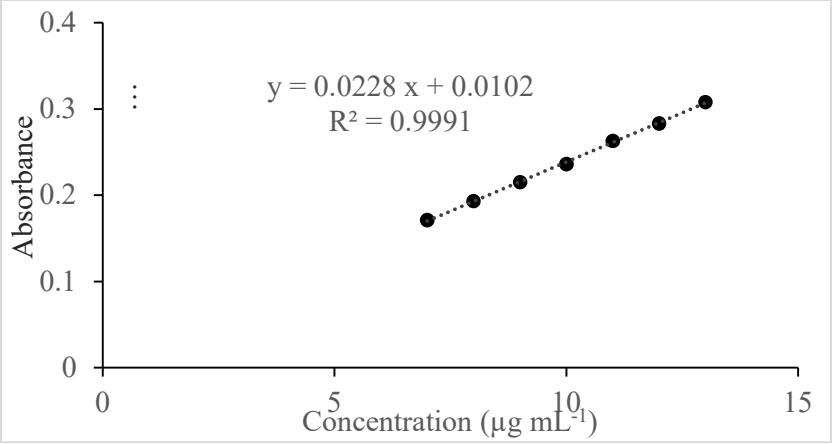

Figure 6: Calibration curve at 293 nm

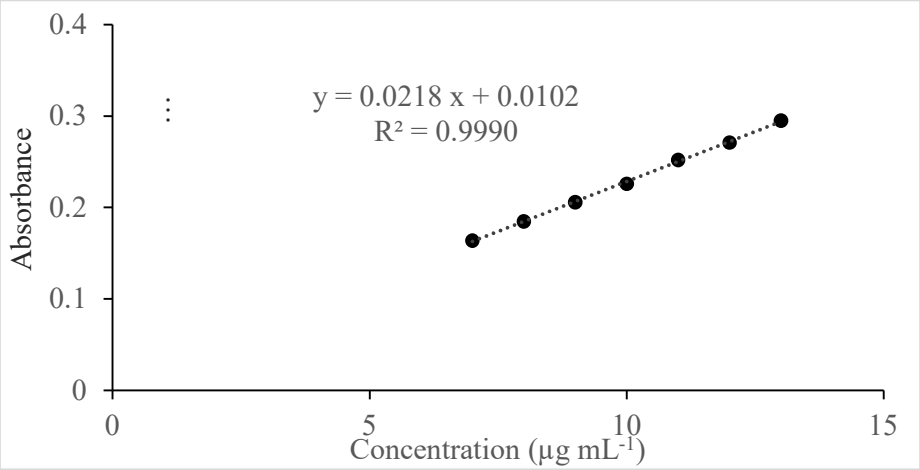

Figure 7: Calibration curve at 298 nm

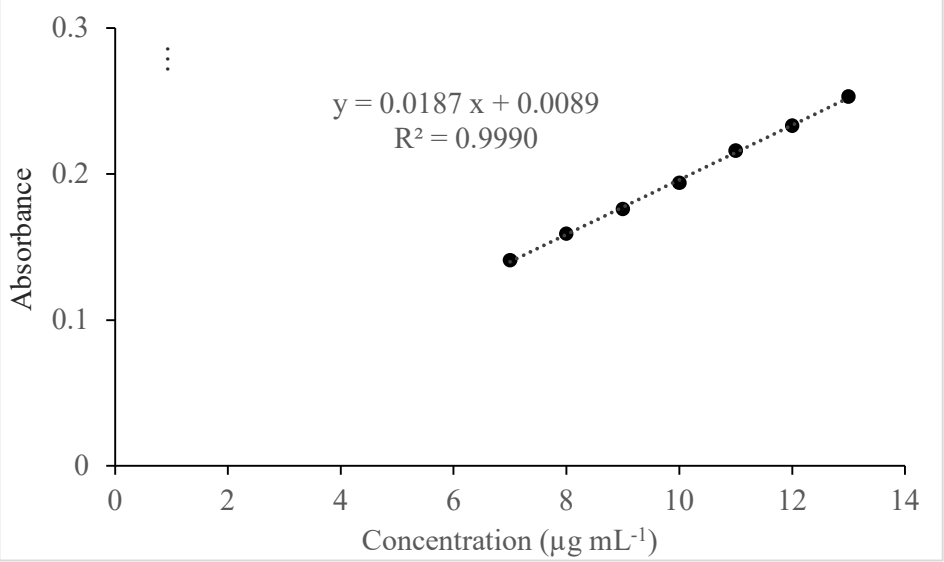

Figure 8: Calibration curve at 303 nm

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}
283	y = 0.0179 x + 0.0099	0.0179	0.0099	0.9982	0.0386886	0.0754872	0.2287491
288	y = 0.0214 x + 0.0091	0.0214	0.0091	0.0091	0.0462313	0.0577521	0.1750062
293	y = 0.0214 x + 0.0091	0.0214	0.0091	0.9991	0.0493216	0.063976	0.1938667
298	y = 0.0214 x + 0.091	0.0214	0.091	0.9990	0.0471623	0.0664707	0.2014263
303	y = 0.0214 x + 0.0091	0.0214	0.0091	0.9990	0.0404475	0.0578585	0.1753288

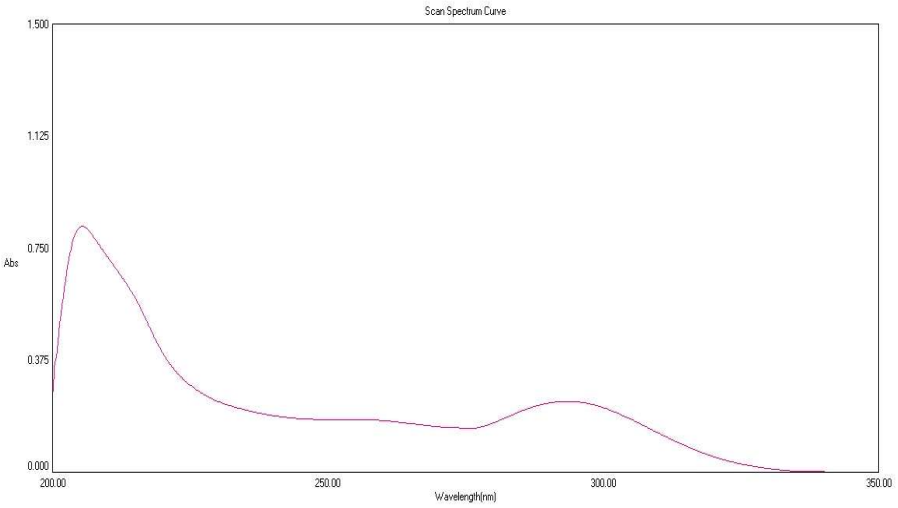

Figure 9: System precision overlay spectra of Mirtazapine

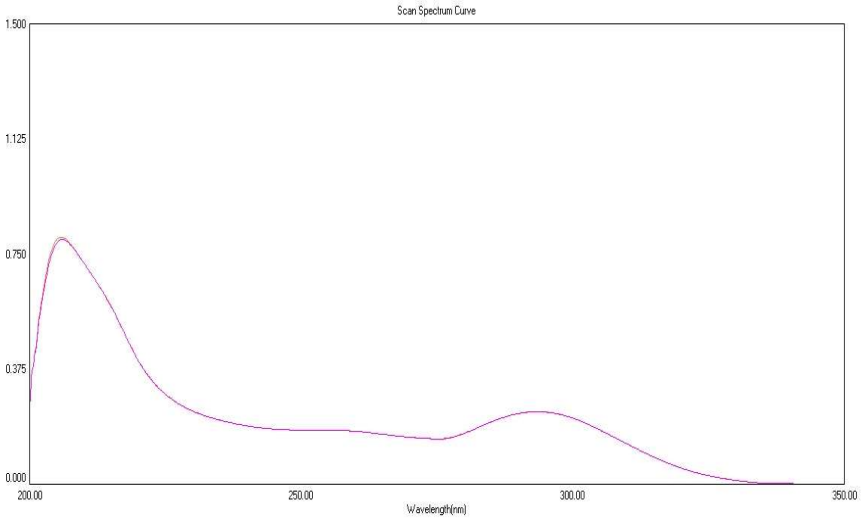

Figure 10: Interday precision overlay spectra of Mirtazapine

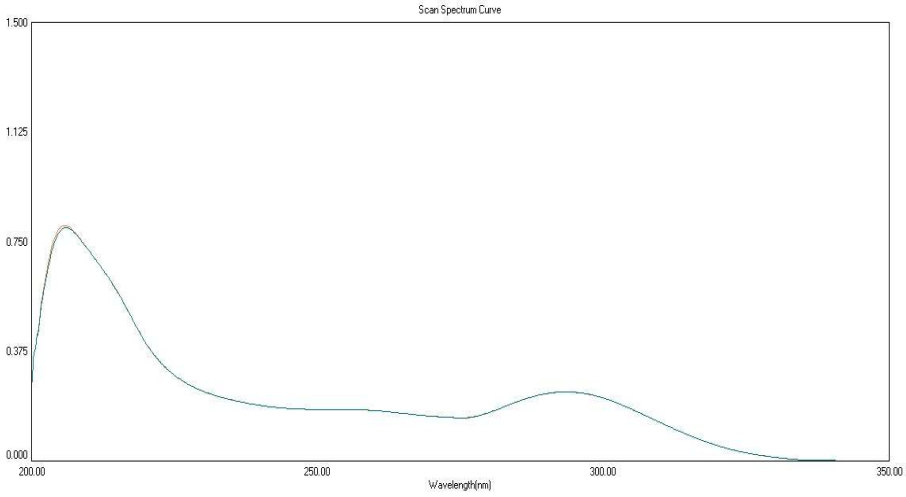

Figure 11: Intraday precision overlay spectra of Mirtazapine.

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 of sample		
		Day 1	Day 2	Day 3
1	414.732	99.14	98.05	98.45
2	413.543	98.90	99.45	99.34
3	416.243	99.35	99.13	99.38
4	415.729	99.30	99.52	99.58
5	414.754	99.40	99.02	98.56
6	415.871	99.29	98.23	99.57
Mean	415.145	99.23	98.93	99.18
SD	0.996	0.18	0.63	0.53
%RSD	0.24	0.17	0.64	0.53

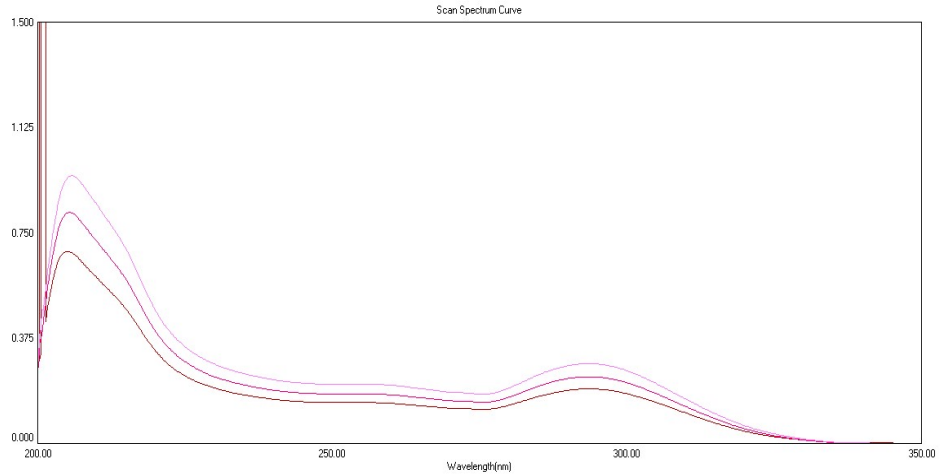

Figure 12: Overlay spectra of accuracy of Mirtazapine at 80, 100, 120 % spiking

Table 4: Accuracy data for prepared method of Mirtazapine

Concentration levels (%)	Amount present	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	Mean % Recovery	SD
80	5	3	7.93	98.88	0.216506
100	5	5	9.90	98.83	0.5774
120	5	7	11.90	99.14	0.127294

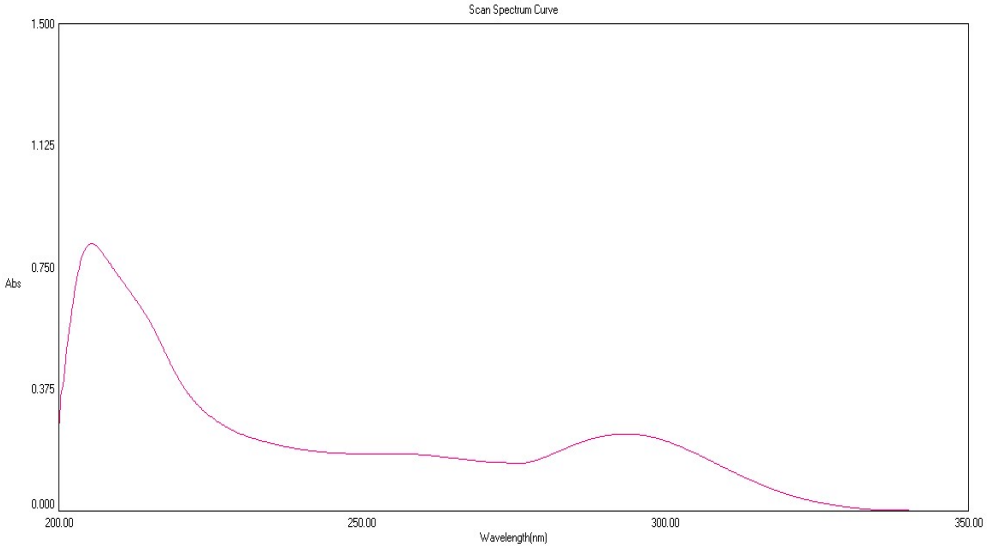

Figure 13: UV spectrum of standard Mirtazapine (10µg mL^{-1}) using Ethanol as a blank

Table 5: Assay results for marketed formulation of Mirtazapine

Marketed formulation	Label claim (mg)	Mean ± SD (n=3)	% RSD
Batch - 1	15	14.92±0.10	0.03
Batch - 2	15	14.55±0.12	0.02

Table 6: Summary of Eco scale penalty points for the proposed method

Description	Penalty points	Total Penalty Points	Score
Ethanol	4	4	96
Instrument	0		
Occupational hazard	0		
Waste	0		

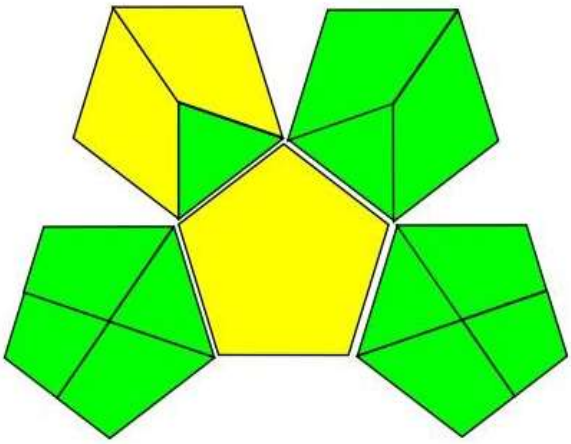

Figure 14: GAPI Pictogram for the proposed method

Figure 15: Agree metrics output for the proposed method.

CONCLUSION

According to ICH criteria, the newly created spectrophotometric Multivariate analytical technique for the evaluation of Mirtazapine was verified by assessing several validation parameters and was found to be within acceptable ranges. For the measurement of Mirtazapine 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 Mirtazapine 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.

ACKNOWLEDGMENTS

Authors are thankful to the Chancellor, SRM Institute of Science and Technology, and
the management of SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur for providing various reprographic sources for carrying out this work.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

FUNDING SOURCES

There is no funding to report.

REFERENCE

[1] M.Willams. The Merk Index :an Encyclopedia of chemicals, Drugs, and Biological, 2005[Online] 2005.

[2] Croom KF, Perry CM, Plosker GL. Mirtazapine: A review of its use in major depression and other psychiatric disorders. CNS Drugs 2009; 23(5):427–52.

[3] Dodd S, D.Burrows G, Norman TR. Chiral determination of mirtazapine in human blood plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 2000; 748(2):439–43.

[4] T Romiguieres F Péhourcq Myriam Matoga. Determination of mirtazapine and its demethyl metabolite in plasma by high-performance liquid chromatography with ultraviolet detection. Application to management of acute intoxication. Journal-of-chromatography-B-Analytical-technologies-in-the-biomedical-and-life-sciences 2002; 2(775):163–68.

[5] Reddy TS, Devi PS. Simultaneous Determination of Mirtazapine and its Three Main Impurities by a High Performance Thin Layer Chromatography/Densitometry Method. J Liq Chromatogr Relat Technol 2008; 31(8):1204–12.

[6] Youssef RM. Determination of

mirtazapine in spiked human plasma and tablets by first derivative spectrofluorimetric method. Saudi Pharm J 2010; 18(1):45–49.

[7] Karaşen N, Altinöz S. Determination of mirtazapine in tablets by UV spectrophotometric and derivative spectrophotometric methods. J Pharm Biomed Anal 2000; 24(1):11–17.

[8] Chorilli M, Bonfilio R, Louvandini CR, *et al*. Development and Validation of an LC-MS/MS Method for Quantitative Analysis of Mirtazapine in Human Plasma. Am J Anal Chem 2011; 02(06):650–57.

[9] Shankar Rao YB, Prasad MVS, Udaya Sri N, *et al*. Vibrational (FT-IR, FT-Raman) and UV–Visible spectroscopic studies, HOMO–LUMO, NBO, NLO and MEP analysis of Benzyl (imino (1H-pyrazol-1-yl) methyl) carbamate using DFT calculaions. J Mol Struct 2016; 1108:567–82.

[10] Rani DN, Kokilambigai KS, Lakshmi KS. Multivariate Calibration Technique for the Spectrophotometric Quantification of Rasagiline in Bulk drug and Pharmaceutical Formulations. Res J Pharm Technol 2020; 13(2):843.

[11] Gałuszka A, Migaszewski ZM, Konieczka P, *et al*. Analytical Eco-Scale for assessing the greenness of analytical procedures. TrAC Trends Anal Chem 2012; 37:61–72.

[12] Płotka-Wasylka J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta 2018; 181:204–09.

[13] Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE - Analytical GREEnness Metric Approach and Software. Anal Chem 2020; 92(14):10076–82.

[14] ICH Validation of Analytical Prodecures:Text & Methodology Q2 R1 2005.