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**MULTIVARIATE CALIBRATION TECHNIQUE AIDED UV  
SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF  
RISPERIDONE IN PHARMACEUTICALS DOSAGE FORM: EVALUATION  
OF GREENNESS PROFILE**

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**ABSTRACT**

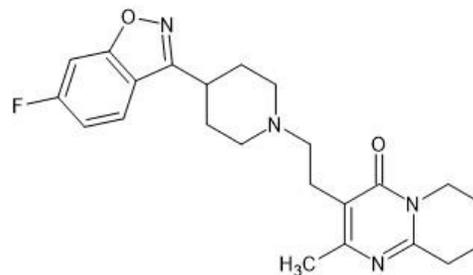
The present project proposes to provide an eco-friendly UV spectrophotometric technique for the determination of Risperidone in pharmaceutical tablets, a multivariate calibration method is used. The multivariate calibration method measures the sample absorbance at various wavelengths for more precise measurements. The UV spectrophotometric method was created, and method validation was completed. All validation parameters complied with ICH standards. The estimation of Risperidone can be done using the suggested multivariate calibration technique method. 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. Risperidone showed absorption maximum at 238 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: Risperidone, Multivariate calibration technique, Pharmaceutical formulations, ICH guidelines, Validation, Greenness**

**INTRODUCTION**

The premise of using risperidone as a not new, but finding effective primary preventative treatment for schizophrenia is prevention treatments for first-episode

psychosis is inhibited by a number of issues. First, the aetiology of the illness would be the focus of the most effective primary preventative measures. The causes of schizophrenia and other psychotic diseases are not currently recognised, necessitating the screening of a wide range of substances to identify the most appropriate candidates for primary prevention [1]. Antipsychotic medicines are FDA-approved for the treatment of severe depression, irritability linked to autism, schizophrenia, bipolar disorder, and psychosis. These drugs have a complex pharmacology and affect a wide range of G protein coupled receptors (GPCRs), including as dopamine D2, serotonin 5HT2, alpha adrenergic, histaminergic, and muscarinic receptors. Dopamine D2 and 5HT2c receptor antagonism in the brain is regarded to be the primary mechanism by which antipsychotics are effective [2]. Risperidone is an epitome of an atypical neuroleptic and is identified, therefore among neuroleptics that are derived from different heterocyclic systems with cyclic structure [3]. The Chemical designation of Risperidone is 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one), and the molecular formula for Risperidone is  $C_{23}H_{27}FN_4O_2$ , and is shown in **Figure 1**, which is used for antipsychotics [4].



**Figure 1: Chemical structure of Risperidone**

The review of literature indicates that various techniques have been published for determining Risperidone in pharmaceutical formulations or biological fluids. For Risperidone, few chromatographic techniques such as HPLC [5–14], spectrophotometric techniques like UV [9, 13, 15], hyphenated techniques like LC-MS/MS [16–24] were reported. No Multi Variate Calibration method (MVC) using UV spectrophotometry was reported for this drug. Hence, the present method deals with the development of the UV spectrophotometric MVC technique for the estimation of Risperidone.

The MVC [25] methodology was utilised to reduce instrumental error and having a huge impact. 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 Risperidone 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[26]. The following equation can be written for each chosen wavelength if the absorbance of an analyte (X) Risperidone in this case, is scanned at 5 wavelengths specified ( $\lambda = 228, 233, 238, 243, \text{ and } 248 \text{ nm}$ ).

$$A_{\lambda 228} = a X C_X + k_1 \dots\dots (1)$$

$$A_{\lambda 233} = b X C_X + k_2 \dots\dots (2)$$

$$A_{\lambda 238} = c X C_X + k_3 \dots\dots (3)$$

$$A_{\lambda 243} = d X C_X + k_4 \dots\dots (4)$$

$$A_{\lambda 248} = e X C_X + k_5 \dots\dots (5)$$

Whereas,

- $A_{\lambda}$  = 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:

$$A_T = a X C_X + b X C_X + c X C_X + d X C_X + e X C_X + K_T \dots\dots(6)$$

The above equation can be further simplified to

$$A_T = C_X (a+b+c+d+e) + K_T \dots\dots (7)$$

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.

$$C_X = \frac{A_T - K_T}{(a+b+c+d+e)} \dots\dots\dots (8)$$

### Greenness evaluation techniques

The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) established a set of pictograms with related signal words, and the analytical eco-scale [27] is predicated on assigning penalty points relying over both quantity and number. The analytical eco scale approach considers each reagent, including its kind and quantity, potential occupational exposure, energy depletion, and waste. Penalty points are eliminated from a 100 point base score.

$$\text{Analytical eco-scale} = 100 - \text{total penalty points} \dots\dots(9)$$

The Green Analytical Procedure Index (GAPI)[28] is a visual depiction made up of five pentagons with distinctive colour coding. The colour coding in the pictogram corresponds to three levels of evaluation at each stage of an analytical technique. The colour coding used by GAPI to determine greenness spans from green to yellow to red, denoting the low, medium, and high environmental impacts connected with the analytical technique, respectively. J. Potka-Wasyłka provided a succinct overview of GAPI in the year 2018. The third assessment methodology makes use of AGREE metrics' [29] special software for

assessing the greenness profile. The result of the software is a circle with numbers around the edges that range from 1 to 12 and are oriented clockwise. These figures represent the 12 green analytical chemistry philosophies. Based on the inputs and their weight, the outputs of each of these 12 principles are rated from 0 to 1. This aggregate scale uses the colours red, yellow, and green to show different numbers. Red means zero, dark green means one or close to one, and yellow means a number between red and dark green. A score that represents the level of greenness is produced by adding the 12 principles and the core.

## MATERIALS AND METHODS

### Instrument employed

- 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:  $\pm 0.3$  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

Risperidone were kindly supplied by Ideal Analytical and Research Institution (Pondicherry, India).

### Marketed formulation

The marketed tablet (SIZODON 4MG) used contains 4 mg Risperidone and was manufactured by Macleods Pharmaceuticals Pvt Ltd.

### Solubility

In ethanol, Risperidone was completely soluble. So, ethanol was employed as the study's solvent throughout the research.

### Preparation of solutions

#### Standard stock solution preparation of Risperidone

Transfer 5 mg of sample Risperidone into a 50 ml volumetric flask. Dissolve it in 20 ml of ethanol, sonicate for 20 minutes, and then add more ethanol to make a final volume of 50 ml.

#### Working solutions of Risperidone

Ethanol was used as a solvent to create a 7-13  $\mu\text{g mL}^{-1}$  solution from the above stock solution.

#### Selection of wavelength for MVC

The Risperidone working standard solutions were scanned against ethanol as the blank solution, which has a maximum absorption at 238 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., 228,233,238,243,248.

### Stability of the solution

By maintaining prepared sample solutions at room temperature for 0–12 hours, Risperidone 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[30] for linearity, accuracy, precision.

#### Linearity

To analyse the linearity and spectral area of Risperidone, the stock solution was appropriately diluted with ethanol to achieve concentrations ranging from 7 to 13  $\mu\text{g mL}^{-1}$  (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 and Limit of Quantification

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

$$\text{LOD} = \frac{3.3 \times \text{standard deviation}}{\text{Slope}} \dots\dots (9)$$

$$\text{LOQ} = \frac{10 \times \text{standard deviation}}{\text{Slope}} \dots\dots (10)$$

#### Precision

Intraday and Interday precision were used to assess the precision's repeatability. A typical standard solution of Risperidone was used to test various levels of accuracy at a concentration of 10  $\mu\text{g mL}^{-1}$ . The repeatability investigation involved the analysis of six solutions at five different wavelengths. In the intervariation 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-analyzed sample solutions, the methodology's accuracy for Risperidone was tested, and the recovery values percentages were estimated.

#### Assay

Weigh and powder 20 Tablets. Weigh accurately a quantity of the tablet powder equivalent to about 50 mg of Risperidone, add 20 ml of ethanol and sonicate for 20mins. Add sufficient ethanol and make upto 50 mL. The solution obtained above is filtered and diluted with ethanol to attain 10  $\mu\text{g mL}^{-1}$  concentration of Risperidone. The absorbance of the resulting solution is measured at 238 nm and the content of Risperidone is quantified.

### RESULTS AND DISCUSSION

The standard solution of Risperidone were scanned initially between 200-400nm. The

wavelength of Risperidone's maximum spectrum was 238 nm. Utilizing ethanol as a blank and selecting the nm of 238 nm for MVC, the UV spectrum of standards and samples of Risperidone was recorded. The typical spectra of Risperidone  $10\mu\text{g mL}^{-1}$  are shown in **Figure 2**.

#### **Stability of solution**

The results of Risperidone's 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 Risperidone were determined as a concentration range of 70 to 130 percent for  $10\mu\text{g mL}^{-1}$  (7 to  $13\mu\text{g mL}^{-1}$ ). **Figure 3** displays the spectra for Risperidone. 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**

The LOD and LOQ for Risperidone was calculated from the linearity slope, which has been confirmed by different sample analyses. The LOD for Risperidone

was calculated from the average of all the absorbance, which was found to be  $0.52\mu\text{g mL}^{-1}$ . The LOQ for Risperidone was calculated from the average of all the absorbance, which was found to be  $1.59\mu\text{g mL}^{-1}$ .

#### **Precision**

The system precision spectra for Risperidone are represented in **Figure 9**. The interday precision spectra for Risperidone are represented in **Figure 10**. The intraday precision spectra were represented in **Figure 11** for Risperidone. The % RSD of system precision, interday and intraday precision, was determined for Risperidone. It was found to be less than 2%, which shows that the approach method is precise. The proposed method shows good precision compared to the values obtained from various precision methods.

#### **Accuracy**

Risperidone's accuracy was tested at 80, 100, and 120%. **Figure 12** shows the overlay spectra for Risperidone. The results for Risperidone are shown in **Table 4**, and the obtained results were found to be within limits.

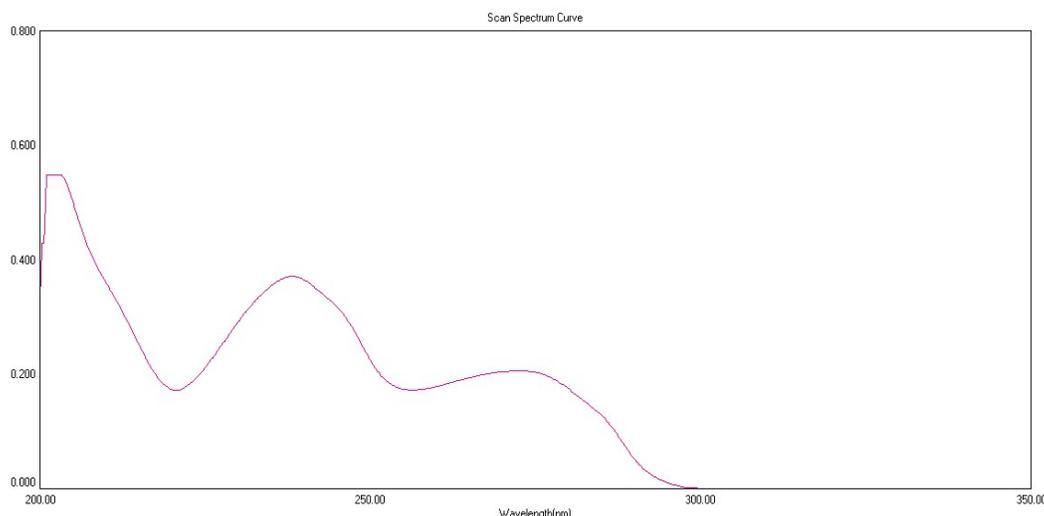
#### **Assay of marketed formulations:**

The recommended spectrophotometric method was used to investigate the quantity of Risperidone in the tablet formulation. The UV absorption spectrum of a commercial tablet was obtained for three replicates. After extraction and filtration,

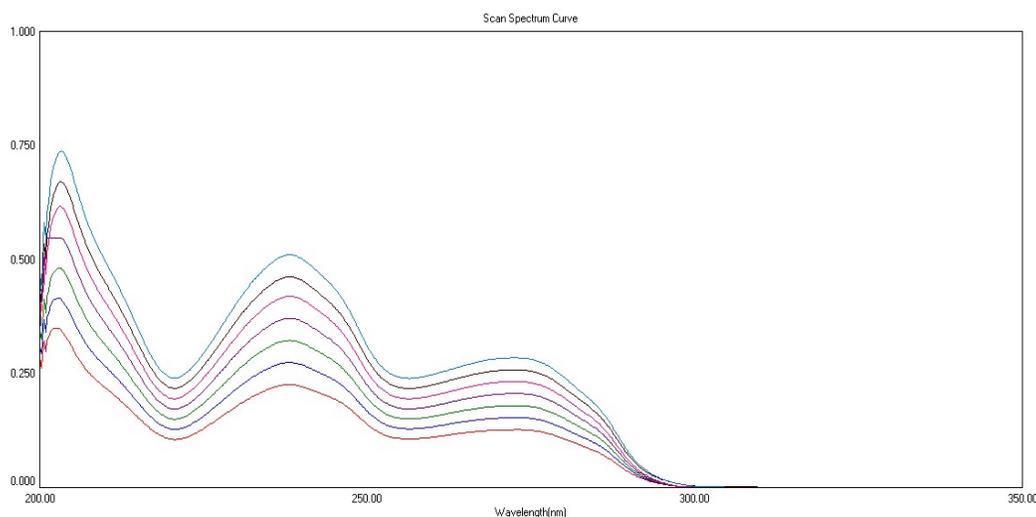
there was no appreciable decrease in the pharmaceutical formulation's excellent analytical recovery values. The results are provided in the **Table 5**.

**Assessment of greenness of the proposed method**

The results of greenness profile for the proposed methods were evaluated. The results of analytical scale are shown in **Table 6**, while the results for GAPI and agree metrics is depicted in **Figure 14** and **Figure 15**.



**Figure 2: UV spectrum of standard Risperidone (10µg mL<sup>-1</sup>) using ethanol as a blank**



**Figure 3: Linearity spectrum of Risperidone (7-13µg mL<sup>-1</sup>) using ethanol as a blank**

**Table 1: Multivariate UV calibration data at five selected wavelengths**

Concentration µg mL <sup>-1</sup>	Absorbance				
	228 nm	233 nm	238 nm	243 nm	248 nm
7	0.156	0.202	0.226	0.205	0.164
8	0.191	0.245	0.274	0.249	0.199
9	0.224	0.289	0.323	0.294	0.234
10	0.257	0.332	0.372	0.338	0.268
11	0.291	0.376	0.421	0.382	0.303
12	0.323	0.415	0.463	0.422	0.335
13	0.356	0.458	0.512	0.466	0.371

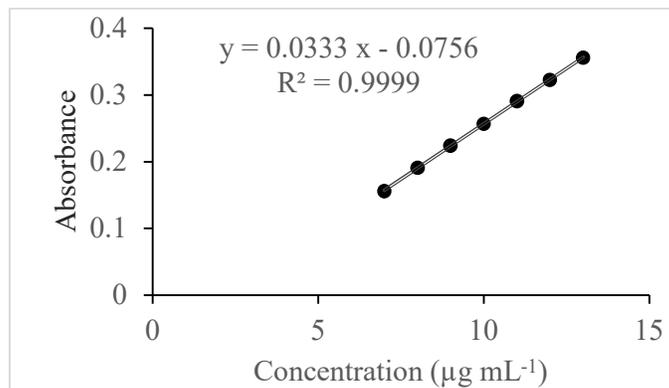


Figure 4: Calibration curve at 228 nm

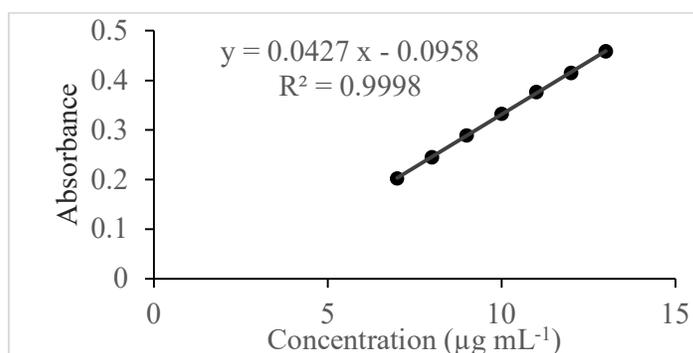


Figure 5: Calibration curve at 233 nm

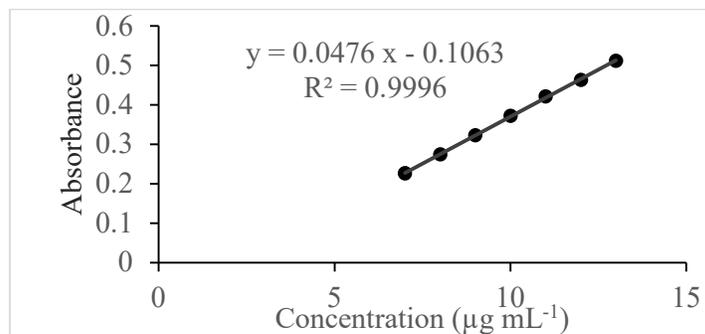


Figure 6: Calibration curve at 238 nm

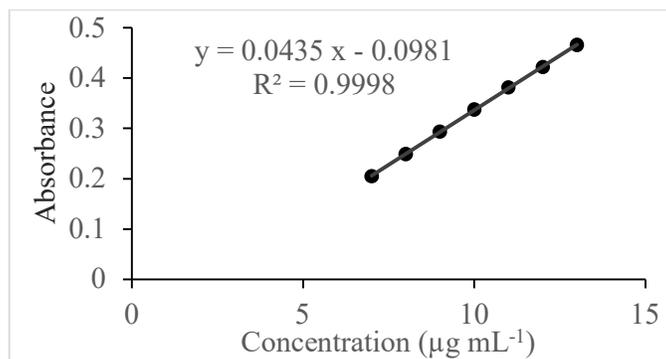


Figure 7: Calibration curve at 243 nm

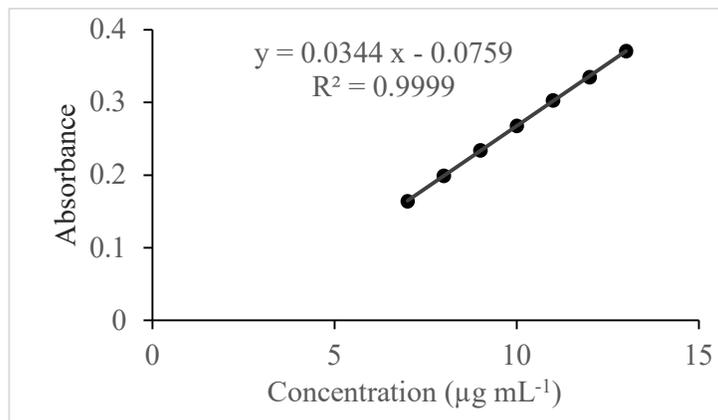


Figure 8: Calibration curve at 321 nm

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

Wavelength (nm)	Regression equation	Slope	Intercept	R <sup>2</sup>	Std error	LOD µg mL <sup>-1</sup>	LOQ µg mL <sup>-1</sup>
228	y = 0.0332 x - 0.0756	0.0332	0.0756	0.9999	0.001372	0.3598	1.0904
233	y = 0.0427 x - 0.0958	0.0427	0.0958	0.9998	0.00287	0.5873	1.7798
238	y = 0.0476 x - 0.1063	0.0476	0.1063	0.9996	0.003624	0.6647	2.0142
243	y = 0.0435 x - 0.0981	0.0435	0.0981	0.9998	0.002945	0.5911	1.7914
248	y = 0.0344 x - 0.0759	0.0344	0.0759	0.9999	0.001716	0.4368	1.3237

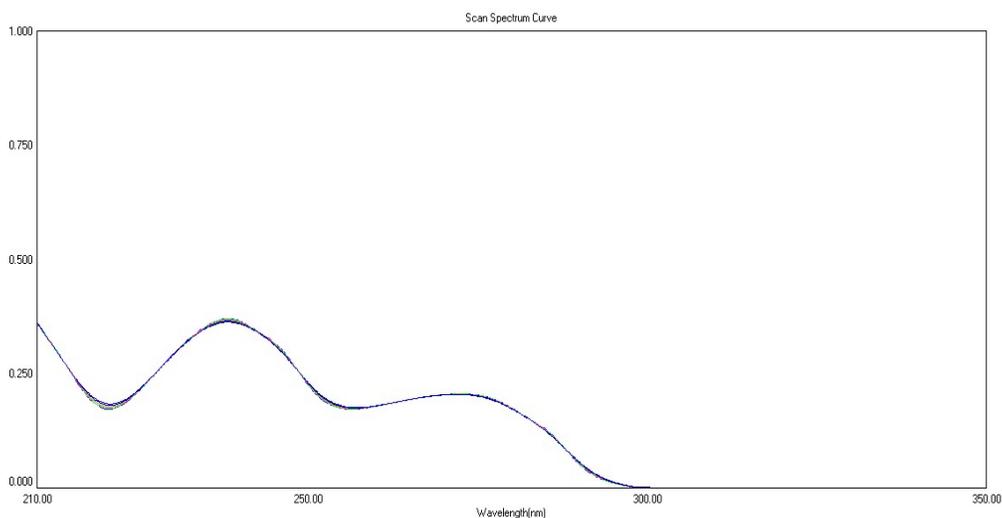


Figure 9: System precision overlay spectra of Risperidone

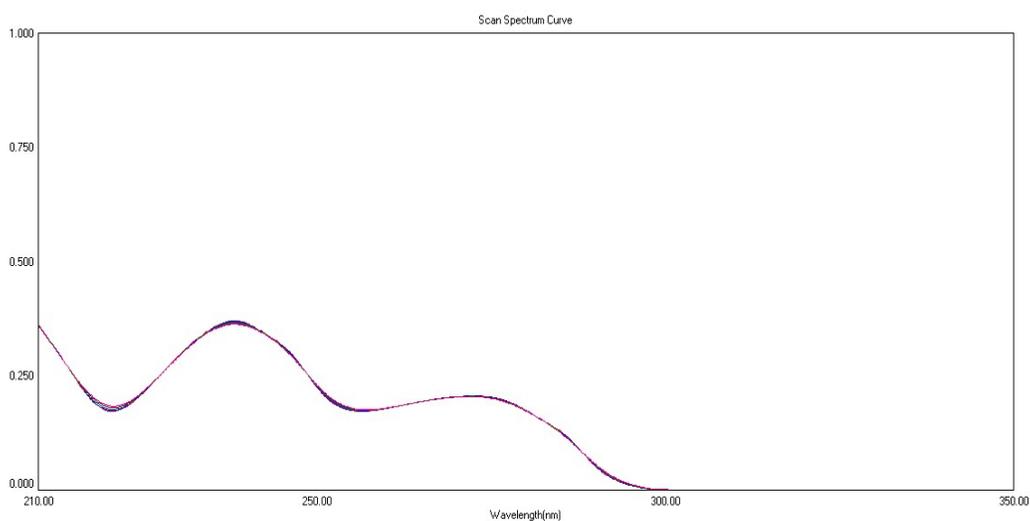


Figure 10: Interday precision overlay spectra of Risperidone

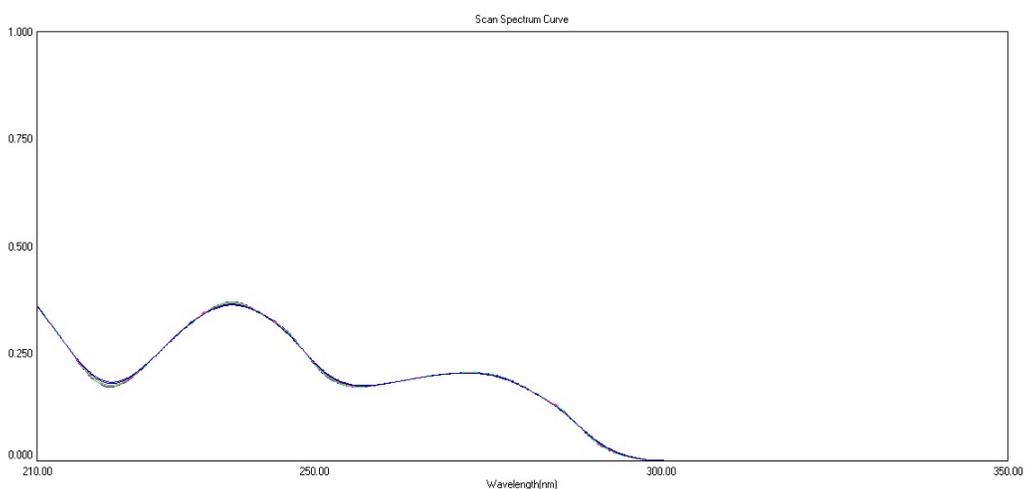


Figure 11: Intraday precision overlay spectra of Risperidone

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

	System precision	Intraday and Interday precision		
	Absorbance of standard for 10 $\mu\text{g mL}^{-1}$	% Recovery of sample equivalent to 10 $\mu\text{g mL}^{-1}$ of sample		
		Day 1	Day 2	Day 3
1	1.567	99.39	98.89	99.59
2	1.552	98.91	99.35	98.84
3	1.571	99.25	98.25	98.53
4	1.574	98.31	99.52	99.58
5	1.559	99.48	98.19	98.34
6	1.561	98.41	98.76	99.57
Mean	1.564	98.96	98.83	99.08
SD	0.008	0.50	0.55	0.58
% RSD	0.52	0.51	0.55	0.58

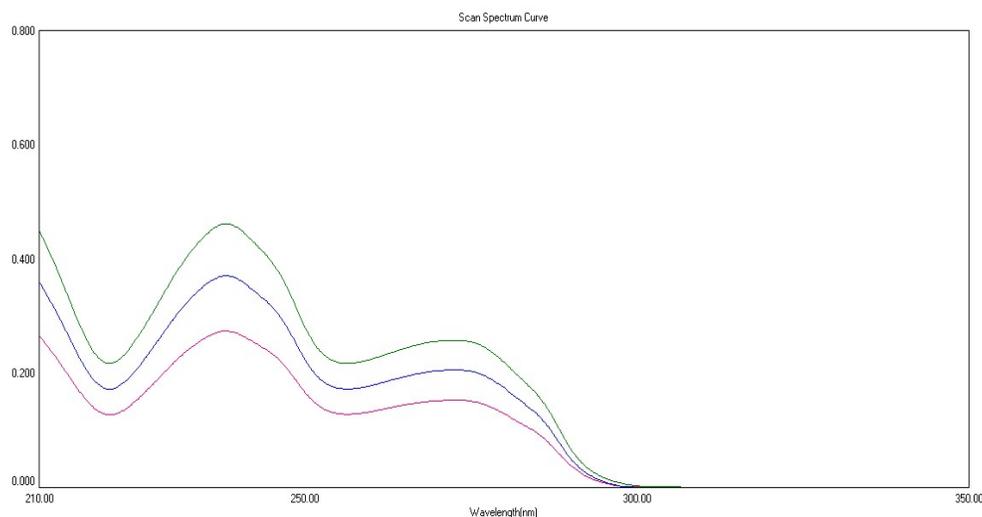


Figure 12: Overlay spectra of accuracy of Risperidone at 80 %, 100 % and 120 % spiking

Table 4: Accuracy data for proposed method of Risperidone

Concentration levels (%)	Amount present	Amount added ( $\mu\text{g mL}^{-1}$ )	Amount recovered ( $\mu\text{g mL}^{-1}$ )	Mean % Recovery	SD
80	5	3	7.91	98.96	0.3818
100	5	5	9.89	98.96	0.4000
120	5	7	11.95	99.53	0.2545

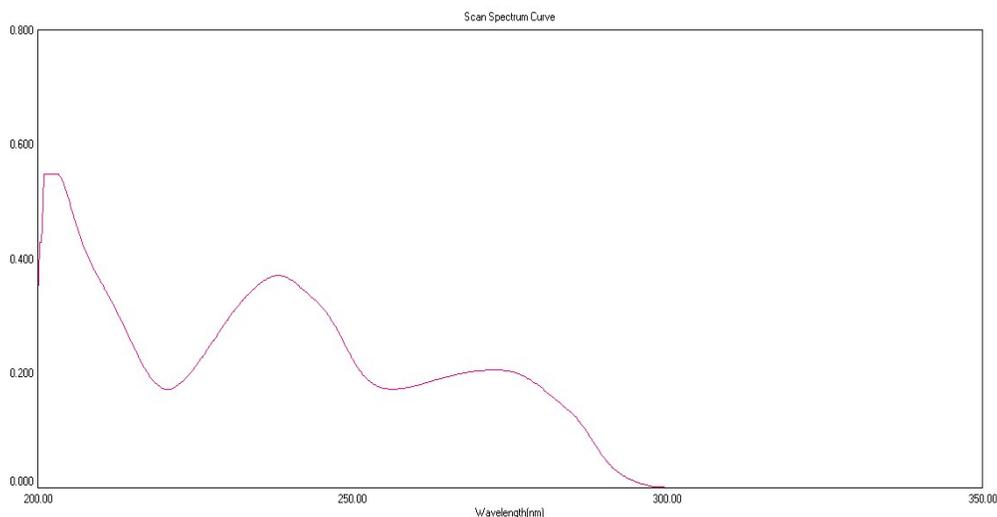


Figure 13: UV spectrum of standard Risperidone ( $10\mu\text{g mL}^{-1}$ ) using ethanol as a blank

Table 5: Assay results for marketed formulation of Risperidone

Marketed formulation	Label claim (mg)	Mean $\pm$ SD (n=3)	% RSD
Batch - 1	4	$3.90 \pm 0.02$	0.3913
Batch - 2	4	$3.92 \pm 0.05$	1.2755

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

Description	PP	Total PP	Scoring
ethanol	4	4	96
Instrument	0		
Occupational hazard	0		
Waste	0		

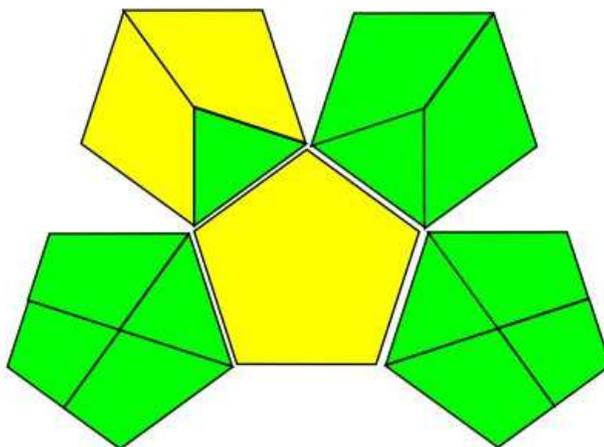


Figure 14: GAPI Pictogram for the proposed method

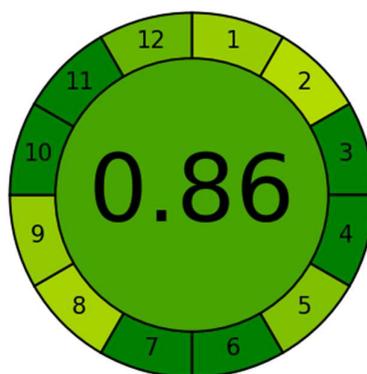


Figure 15: Agree metrics output for the proposed method

## CONCLUSION

According to ICH criteria, the newly created spectrophotometric technique for the evaluation of Risperidone was verified by assessing several validation parameters and was found to be within acceptable ranges. For the measurement of Risperidone 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 Risperidone in pharmaceutical formulations because it is more accurate than existing

UV spectrophotometric methods and has a method with easy mathematical components. The proposed method possesses an ideal greenness profile assessed by analytical eco-scale, GAPI and agree metrics shall be used for routine determination of Risperidone in Pharmaceuticals.

## 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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