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MULTIVARIATE CALIBRATION TECHNIQUE AIDED UV SPECTROPHOTOMRTRIC METHOD FOR THE ESTIMATION OF PIRFENIDONE IN PHARMACEUTICALS DOSAGE FORM: ASSESSMENT OF GREENNESS PROFILE

KUPPURAJ P, SEETHARAMAN R*, KAVITHA J, KOKILAMBIGAI K S AND DHIVAKARAN M

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; seerampharm@gmail.com

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ABSTRACT

The present project proposes to provide an eco-friendly UV spectrophotometric technique for the determination of Pirfenidone 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 Pirfenidone 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. Pirfenidone showed absorption maximum at 311nm in phosphate buffer pH 7.2 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: Pirfenidone, Multivariate calibration technique, Pharmaceutical formulations, ICH guidelines, Validation

INTRODUCTION

Pirfenidone is a new medication approved for the treatment of Idiopathic Pulmonary Fibrosis, a rare and generally incurable disease (IPF). IPF patients experience shortness of breath, coughing, and difficulty with daily physical activities. IPF is treated with oxygen therapy, pulmonary rehabilitation, and lung transplantation. Pirfenidone is the only treatment available for IPF. American pharmacologist Solomon Margolin (1920–2008) made the discovery of Pirfenidone during a synthetic research **[1]**.

Pirfenidone is an orally administered drug with anti-fibrotic, anti-inflammatory, and antioxidant properties that was approved in Europe in 2011 and in the United States in 2014 for the treatment of IPF **[2-3]**. The chemical name for Pirfenidone is (5-methyl-1-phenyl-2- 1H) pyridinone) with molecular formula $C_{12}H_{11}NO$ and is shown in **Figure 1 [4]**.

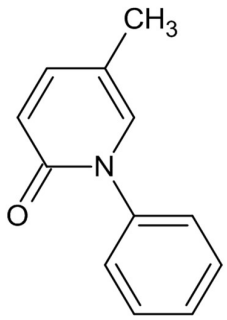

Figure 1: Chemical structure of Pirfenidone

The review of literature indicates that various techniques have been published for determining Pirfenidone in pharmaceutical formulations or biological fluids. For Pirfenidone, few chromatographic techniques such as HPLC **[5–12]**, spectrophotometric techniques like UV **[13]**, hyphenated techniques like LC-MS/MS **[14]** were reported. No MultiVariate 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 Pirfenidone.

The MVC 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 **[15]**. In this paper, we described how to estimate the amount of Pirfenidone 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 **[16]**. The following equation can be written for each chosen wavelength if the absorbance of an analyte (X) Pirfenidone in this case, is scanned at 5 wavelengths specified (λ = 301, 306, 311, 316, and 321nm).

$A_{\lambda 301} = a \times C_X + k1$ …… (1)

$A_{\lambda 306} = b \times C_X + k2$ ……..… (2)

$A_{\lambda 311} = c \times C_X + k3$ …… (3)

$A_{\lambda 316} = d \times C_X + k4$ ...…… (4)

$A_{\lambda 321} = e \times C_X + k5$ ……. (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:

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

The above equation can be further simplified to

$A_T = C_X (a + b + c + d + e) + K_T$…… (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.

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

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 **[17]** 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.

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

The Green Analytical Procedure Index **[18]** (GAPI) 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-Wasylka provided a succinct overview of GAPI in the year 2018 **[18]**. The third assessment methodology makes use of AGREE metrics' **[19]** 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: ±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),
- Sonicleansonicator (model 160T, Thebarton-Australia).

Reference Samples

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

Marketed formulation

The marketed tablet (PIRFENEX) used contains 200 mg Pirfenidone and was manufactured by Macleods Pharmaceuticals Pvt Ltd.

Chemicals and Reagents

Analytical grade Potassium dihydrogen phosphate and sodium hydroxide (S.D fine chemical Ltd., Mumbai, India) was used.

Preparation of diluent

Phosphate buffer pH 7.2 prepared as per Indian Pharmacopoeia 1996 was used as diluent.

Preparation of solutions

Standard stock solution preparation of Pirfenidone

Transfer 100mg of sample Pirfenidone into a 100 ml volumetric flask. Dissolve it in 50ml of phosphate buffer pH 7.2, sonicate for 20 minutes, and then add more Phosphate buffer pH 7.2 to make a final volume of 100ml.

Working solutions of Pirfenidone

Phosphate buffer pH 7.2 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 Pirfenidone working standard solutions were scanned against phosphate buffer pH 7.2 as the blank solution, which has a maximum absorption at 311 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., 301, 306, 311, 316, 321.

Stability of the solution

By maintaining prepared sample solutions at room temperature for 0–12 hours, Pirfenidone 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 **[20]** for linearity, accuracy, precision.

Linearity

To analyse the linearity and spectral area of Pirfenidone, the stock solution was appropriately diluted with phosphate buffer pH 7.2 to achieve concentrations ranging from 7 to 13 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 and Limit of Quantification

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

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

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

Precision

Intraday and Interday precision were used to assess the precision's repeatability. A typical standard solution of Pirfenidone was used to test various levels of accuracy at a concentration of 10µg 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 Pirfenidone 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 100mg of Pirfenidone, add 25 ml of phosphate buffer 7.2 and sonicate for 20mins. Add sufficient phosphate buffer 7.2 and make upto 100mL. The solution obtained above is filtered and diluted with phosphate buffer 7.2 to attain 10 µg mL^{-1} concentration of Pirfenidone. The absorbance of the resulting solution is measured at 311 nm and the content of Pirfenidone is quantified.

RESULTS AND DISCUSSION

The standard solution of Pirfenidone were scanned initially between 200-400nm. The wavelength of Pirfenidone's maximum spectrum was 311 nm. Utilizing Phosphate buffer 7.2 as a blank and selecting the nm of 311 nm for MVC, the UV spectrum of standards and samples of Pirfenidone was recorded. The typical spectra of Pirfenidone 10 µg mL^{-1} are shown in **Figure 2**.

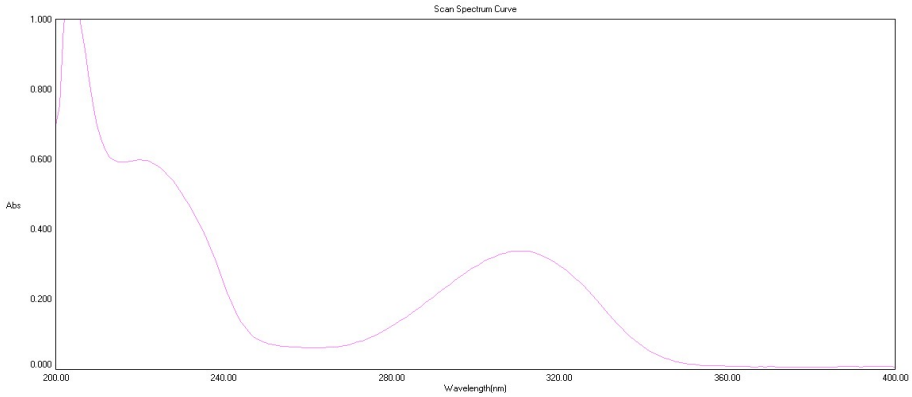

Figure 2: UV spectrum of standard Pirfenidone (10 µg mL^{-1}) using Phosphate buffer 7.2 as a blank

Stability of solution

The results of Pirfenidone'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 Pirfenidone were determined as a concentration range of 70 to 130 percent for 10µg mL^{-1} (7 to 13 µg mL^{-1}). **Figure 3** displays the spectra for Pirfenidone. The calibration curve was created by calculating the absorbance of standard solutions that had been diluted at five different wavelengths.

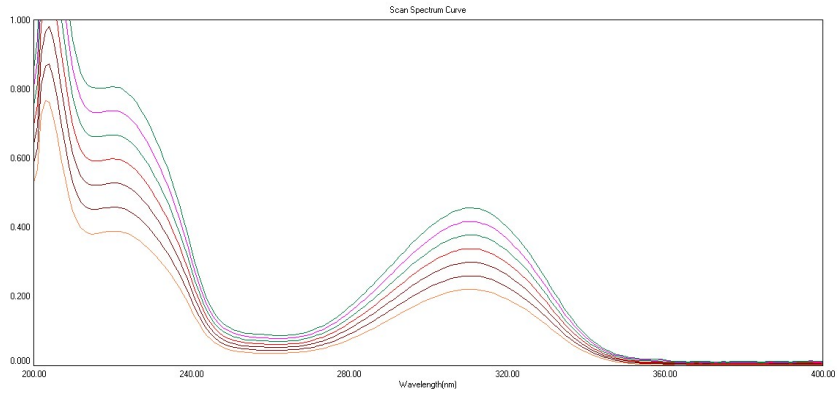

Figure 3: Linearity spectrum of Pirfenidone (7-13 µg mL^{-1}) using Phosphate buffer 7.2 as a blank

Table 1: Multivariate UV calibration data at five selected wavelengths

Concentration µg mL^{-1}	Absorbance				
	301 nm	306 nm	311 nm	316 nm	321 nm
7	0.195	0.213	0.22	0.211	0.186
8	0.232	0.252	0.259	0.249	0.222
9	0.265	0.292	0.301	0.284	0.254
10	0.305	0.328	0.338	0.325	0.288
11	0.335	0.366	0.377	0.363	0.322
12	0.369	0.405	0.417	0.401	0.356
13	0.404	0.443	0.456	0.439	0.39

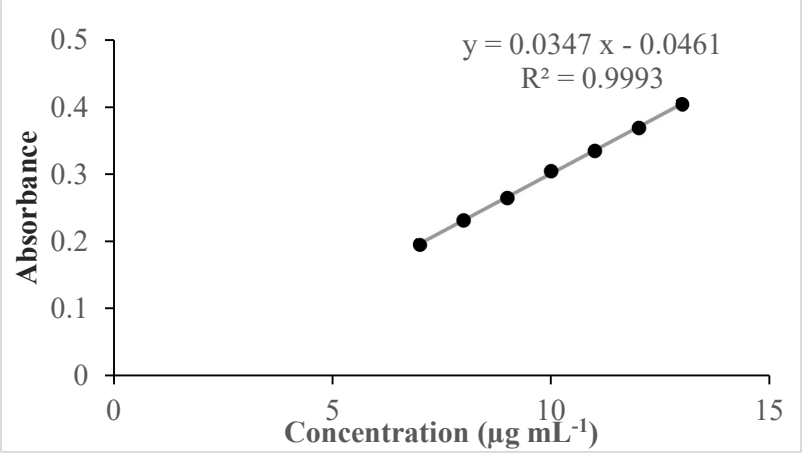

Figure 4: Calibration curve at 301 nm

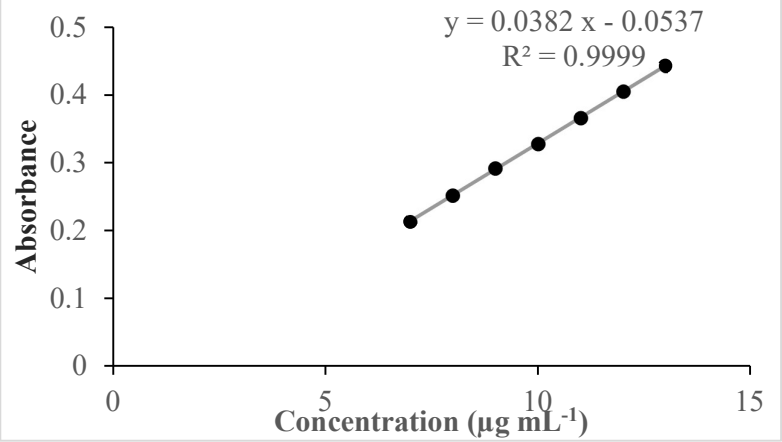

Figure 5: Calibration curve at 306 nm

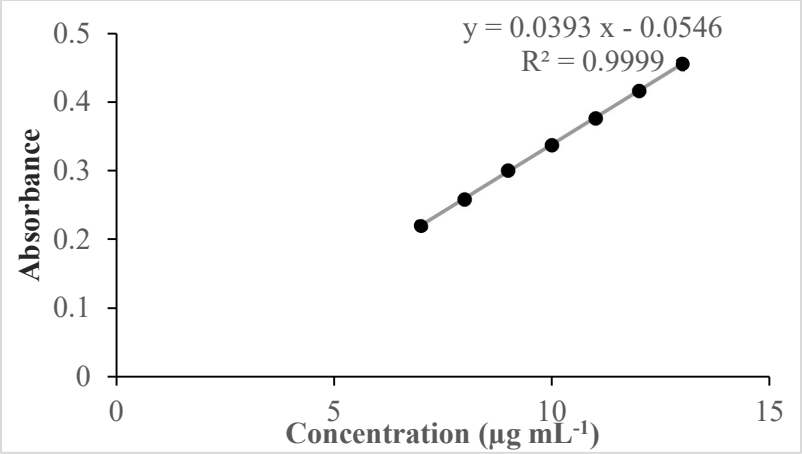

Figure 6: Calibration curve at 311 nm

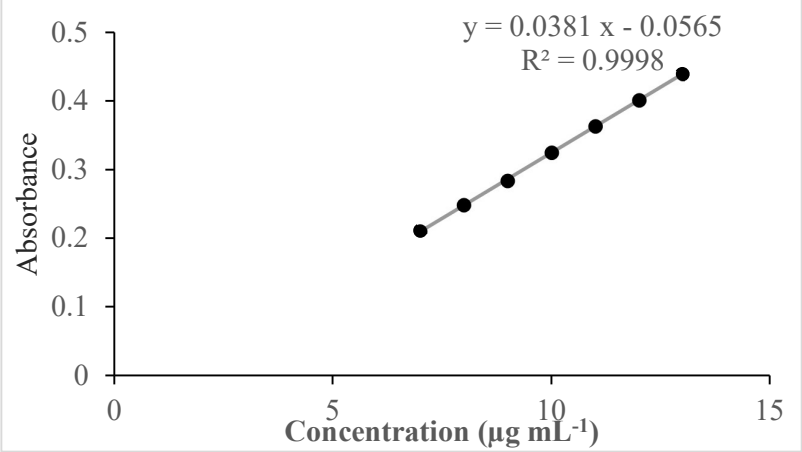

Figure 7: Calibration curve at 316 nm

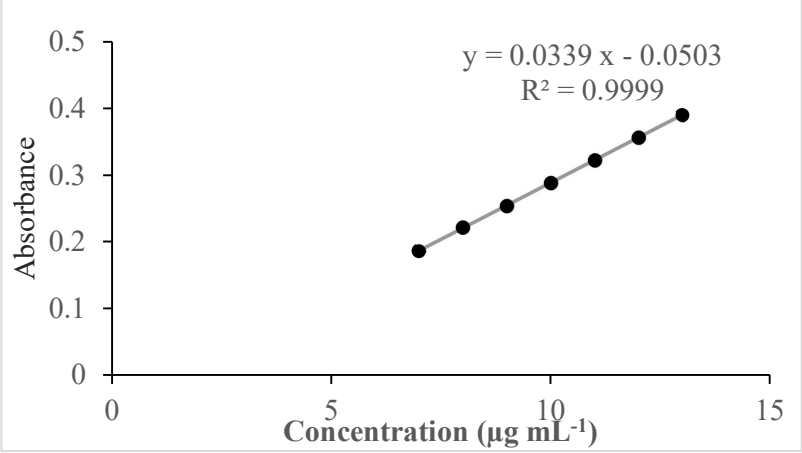

Figure 8: Calibration curve at 321 nm

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

Wavelength(nm)	Regression equation	Slope	Intercept	R^2	Std error	LOD µg mL^{-1}	LOQ µg mL^{-1}
301	Y = 0.0347 x - 0.0461	0.0347	0.0461	0.9993	0.00426	1.0718	3.2480
306	Y = 0.0382 x - 0.0537	0.0382	0.0537	0.9999	0.001814	0.4146	1.2563
311	Y = 0.0393 x - 0.0546	0.0393	0.0546	0.9999	0.001955	0.4343	1.3161
316	Y = 0.0381 x - 0.0565	0.0381	0.0565	0.9998	0.002343	0.5369	1.6273
321	Y = 0.0339 x - 0.0503	0.0339	0.0503	0.9999	0.001457	0.3752	1.1377

Limit of Detection and Limit of Quantification

The LOD and LOQ for Pirfenidone was calculated from the linearity slope, which has been confirmed by different sample analyses. The LOD for Pirfenidone was calculated from the average of all the absorbance, which was found to be 0.56 µg mL^{-1}, The LOQ for Pirfenidone was calculated from the average of all the absorbance, which was found to be 1.71 µg mL^{-1}.

Precision

The system precision spectra for Pirfenidone are represented in **Figure 9**. The interday precision spectra for Pirfenidone are represented in **Figure 10**. The intraday precision spectra were represented in **Figure 11** for Pirfenidone. The % RSD of system precision, interday and intraday precision, was determined for Pirfenidone. 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.

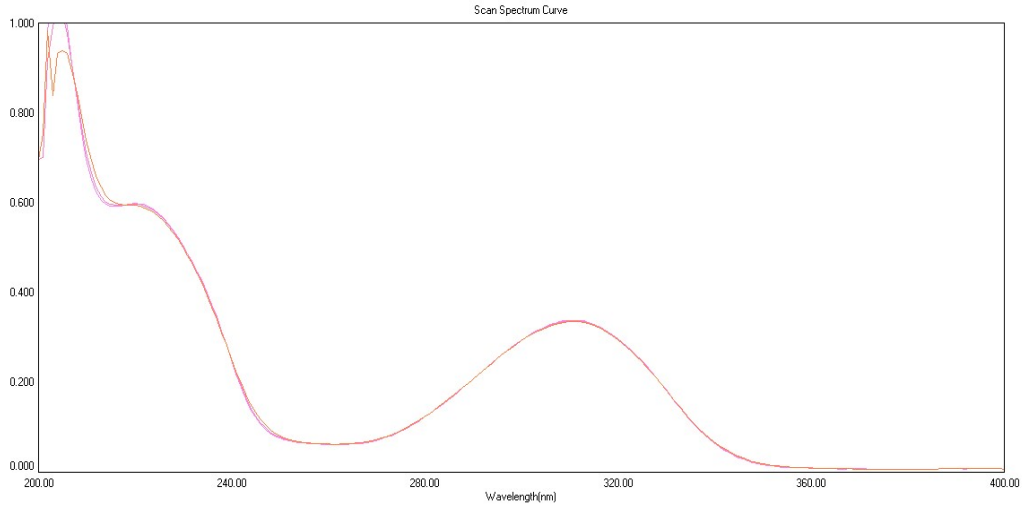

Figure 9: System precision overlay spectra of Pirfenidone

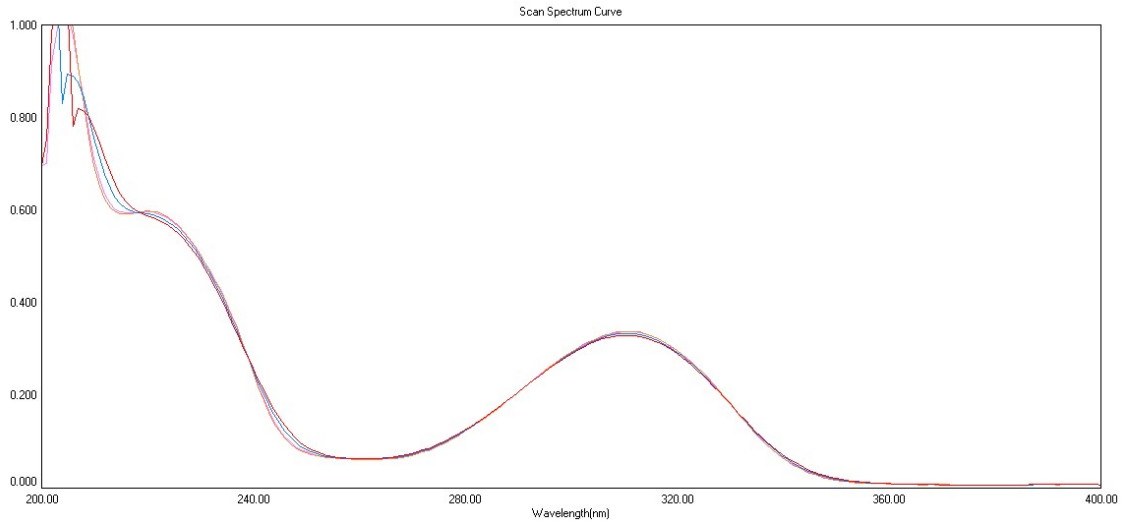

Figure 10: Interday precision overlay spectra of Pirfenidone

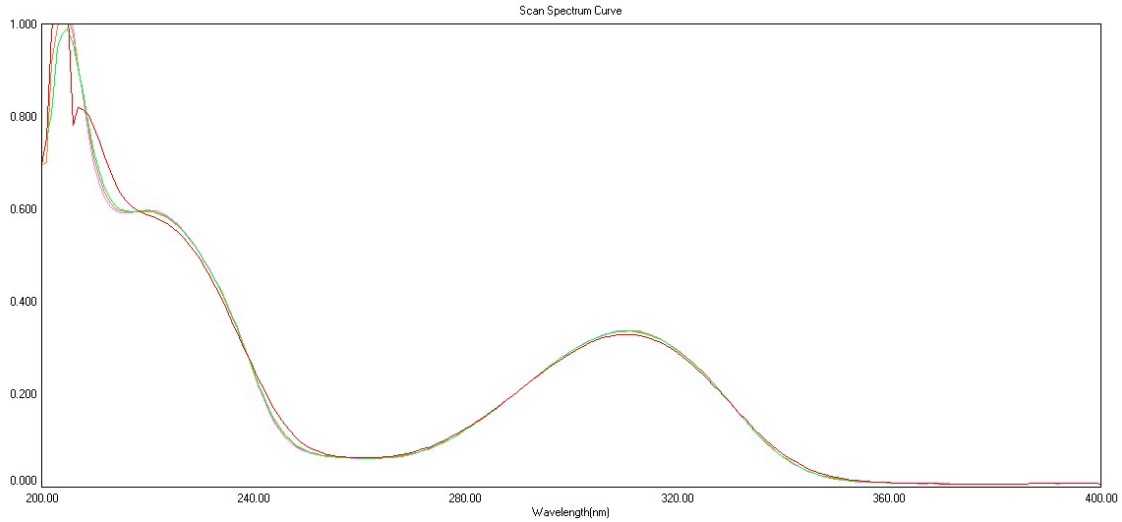

Figure 11: Intraday precision overlay spectra of Pirfenidone

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

	System precision	Intraday and Interday precision		
	Absorbance of standard for 10 µg mL^{-1}	% Recovery of sample equivalent to 10 µg mL^{-1} of sample		
		Day 1	Day 2	Day 3
1	1.579	99.14	98.06	98.45
2	1.564	98.90	99.27	99.54
3	1.601	99.35	99.12	99.38
4	1.577	99.30	99.52	99.58
5	1.590	99.40	99.19	98.56
6	1.581	99.29	98.21	99.57
Mean	1.582	99.23	98.90	99.18
SD	0.013	0.18	0.61	0.53
%RSD	0.79	0.19	0.61	0.53

Accuracy

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

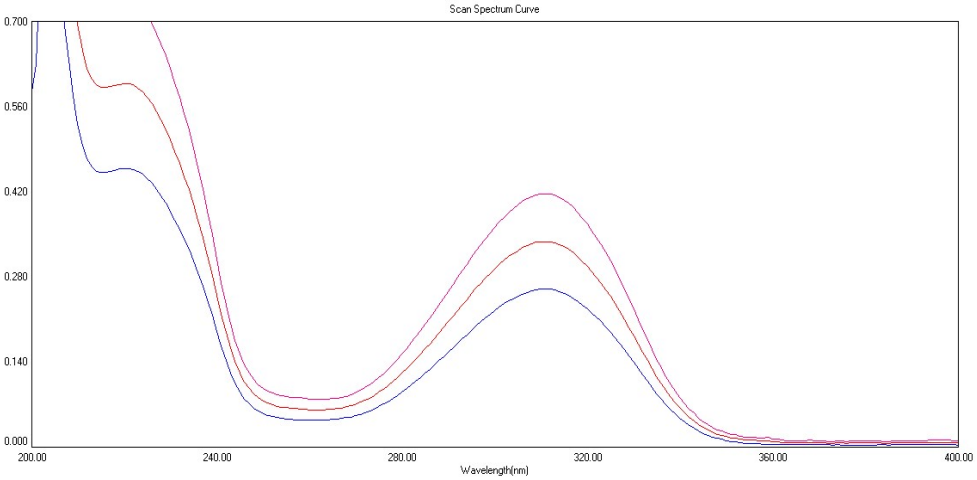

Figure 12: Overlay spectra of accuracy of Pirfenidone at 80 %, 100 %and 120 % spiking

Table 4: Accuracy data for proposed method of Pirfenidone

Concentration levels (%)	Amount present	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	Mean % Recovery	SD
80	**5**	**3**	**7.92**	**98.92**	**0.5051**
100	**5**	**5**	**9.84**	**98.87**	**0.1528**
120	**5**	**7**	**11.91**	**99.22**	**0.1272**

Assay of marketed formulations:

The recommended spectrophotometric method was used to investigate the quantity of Pirfenidone 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**.

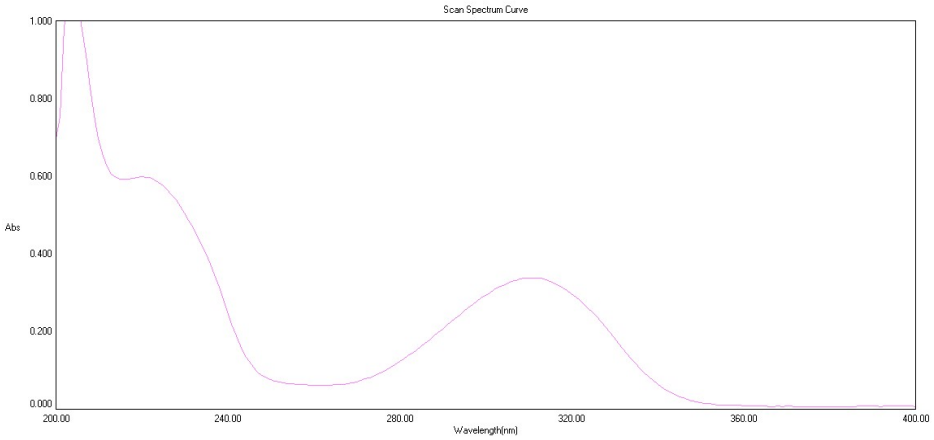

Figure 13: UV spectrum of standard Pirfenidone (10 µg mL^{-1}) using Phosphate buffer 7.2 as a blank

Table 5: Assay results for marketed formulation of Pirfenidone

Marketed formulation	Label claim (mg)	Mean ± SD (n=3)	% RSD
Batch - 1	200	199.75±0.10	0.0501
Batch - 2	200	199.65±0.12	0.0591

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 6**, while the results for GAPI and agree metrics is depicted in **Figure 14** and **Figure 15**.

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

Description	PP	Total PP	Scoring
Phosphate buffer	1	4	96
Instrument	0		
Occupational hazard	0		
Waste	3		

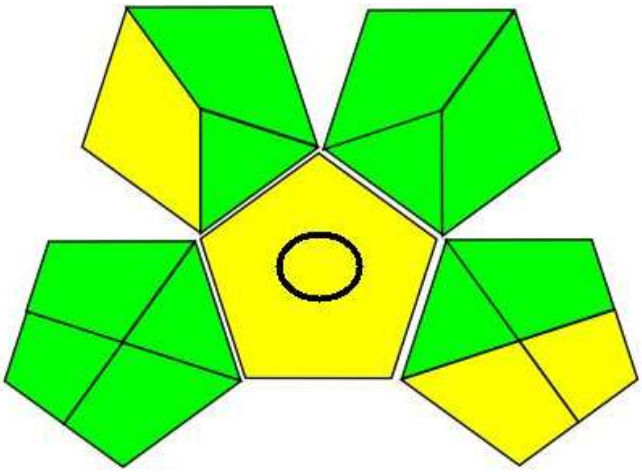

Figure 14: GAPI Pictogram for the proposed method

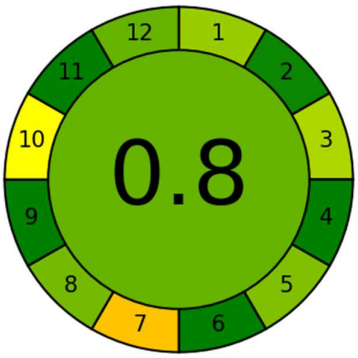

Figure 15: Agree metrics output for the proposed method

CONCLUSION

According to ICH criteria, the newly created spectrophotometric technique for the evaluation of Pirfenidone was verified by assessing several validation parameters and was found to be within acceptable ranges. For the measurement of Pirfenidone 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 Pirfenidone in pharmaceutical formulations because it is more accurate than existing UV spectrophotometric methods and has a method with easy mathematical components. The proposed method possess an ideal greenness profile assessed by analytical eco-scale, GAPI and agree metrics shall be used for routine determination of Pirfenidone 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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