



# METHOD DEVELOPMENT AND VALIDATION OF PIRFENIDONE USING UV SPECTROPHOTOMETRY

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**Abstract:** The main desire was to develop and validate the UV-spectrophotometric method for the assessment of Pirfenidone in bulk and marketed formulation. Pirfenidone is an Anti-fibrotic agent, mostly effective in the treatment of idiopathic pulmonary fibrosis. It is most commonly available in oral dosage form. In this present study, a simple, accurate, precise, reliable and economical method has been developed by using UV- Spectrophotometer for the estimation of Pirfenidone from bulk and marketed formulation. The  $\lambda_{max}$  of Pirfenidone in methanol: chloroform was found to be 311 nm. The drug follows linearity in the concentration range 2–100  $\mu\text{g/ml}$  with a correlation coefficient value of 0.9998. The proposed method was executed to marketed formulation and % amount of drug estimated was found to be 98.99% and was found to be in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels, i.e., 80%, 100%, and 120%. The % recovery was found to be in the range of 99.64– 100.23%. The low values of % RSD are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intraday; interday variations, and repeatability. The % RSD value  $< 2$  indicates that the method is precise. Ruggedness of the proposed method was studied with the help of three analysts. Conclusion: The above method was a rapid tool for routine analysis of Pirfenidone in the bulk and in the pharmaceutical dosage form.

**Key words** – Pirfenidone, UV- Spectrophotometry, Pirfenex.

## I. INTRODUCTION

5-methyl-1-phenyl-2(1H)-one [Figure 1] has well established anti-fibrotic and anti-inflammatory activity which has been found on the clinical evidence <sup>[1]</sup>. In most of the studies (cell based studies) it has been found that Pirfenidone reduces fibroblast proliferation thereby epidermal, platelet-derived, and transforming beta-1 growth factors, thereby slowing tumor cell proliferation. This agent also inhibits DNA synthesis and the production of mRNA for collagen types I and III, which ultimately results in a reduction in radiation-induced fibrosis. Additionally, it has a role as a non-narcotic analgesic, a non-steroidal anti-inflammatory drug and an antipyretic <sup>[2][3][4][5][6][7]</sup>.

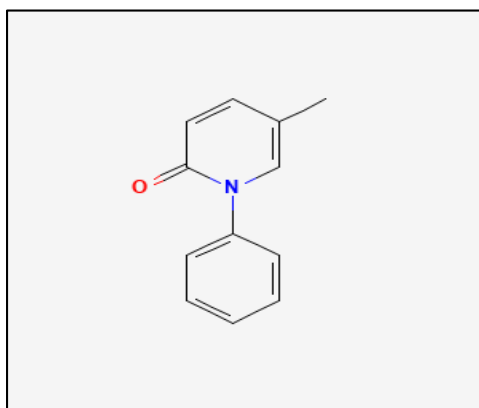


Figure 1: Molecular structure of Pirfenidone ( $\text{C}_{12}\text{H}_{11}\text{NO}$ )

Literature survey reveals that the determination of Pirfenidone by RP-HPLC method as well as by combining UV-Spectrophotometry and RP-HPLC in pharmaceutical formulations <sup>[2]</sup>. Among those the other method includes the assessment of Pirfenidone and its acid metabolite by improved LC method. Taking into the consideration, HPLC, LC, LC-MS-MS these methods are really tedious, selective and very expensive as compared to UV- Spectrophotometry <sup>[8][9][10][11]</sup>. They require expensive or we can say costly detectors and are also difficult in nature to perform that might not be easily assessable in many laboratories. For

these reasons UV method can be considered very economical, inherent as well as simple taking into consideration as more convenient alteration technique.

## II. INSTRUMENTATION

A double beam UV-Spectrophotometer was used (Shimadzu corporation, Japan). For weighing, Shimadzu AX200, Japan analytical balance was used.

## III. MATERIALS

Drug was obtained as a gift sample from Raks Pharma Pvt. Ltd. Dahej, Gujarat and marketed formulation was taken of Pirfenex tablet manufactured by Cipla Ltd. All chemicals and reagents used were of analytical grade.

## IV. METHOD

### a) Selection of wavelength

About 50 µg/ml of Pirfenidone solution was accurately prepared in Methanol: Chloroform (50: 50). These solutions were scanned in the range of 200-400 nm UV regions. The wavelength of maximum absorbance was observed at 311 nm and this wavelength was adopted for further absorbance measurement. The Figure 2 represents the UV Spectrum of Pirfenidone.

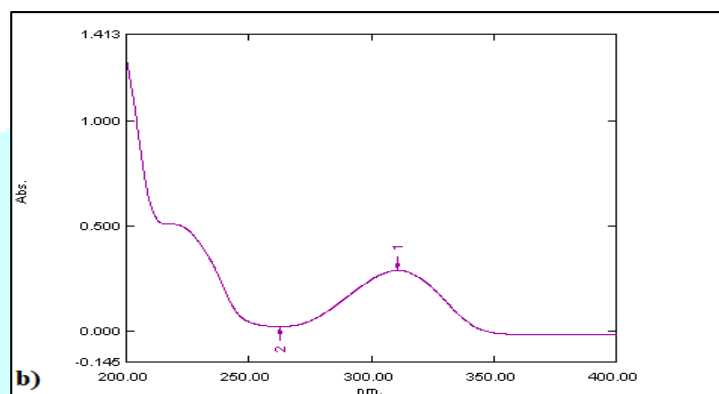


Figure 2: UV spectrum of Pirfenidone

### b) Preparation of standard stock solution

Accurately weighed 10 mg of Pirfenidone was transferred to a 100 ml volumetric flask, dissolved in 20 ml methanol: chloroform (50: 50) by shaking manually for 10 min. The volume was adjusted with the same up to the mark to give the final strength, i.e. 100 µg/ml.

### c) Selection of wavelength for analysis of Pirfenidone

Appropriate volume 0.5 ml of standard stock solution of Pirfenidone was transferred into a 10 ml volumetric flask, diluted to a mark with methanol: chloroform to give concentration of 5 µg/ml. The resulting solution was scanned in the UV range (200–400 nm). In spectrum Pirfenidone showed absorbance maximum at 311 nm [Figure 2].

### d) Validation of the method

The method was validated in terms of linearity, accuracy, precision, and ruggedness.

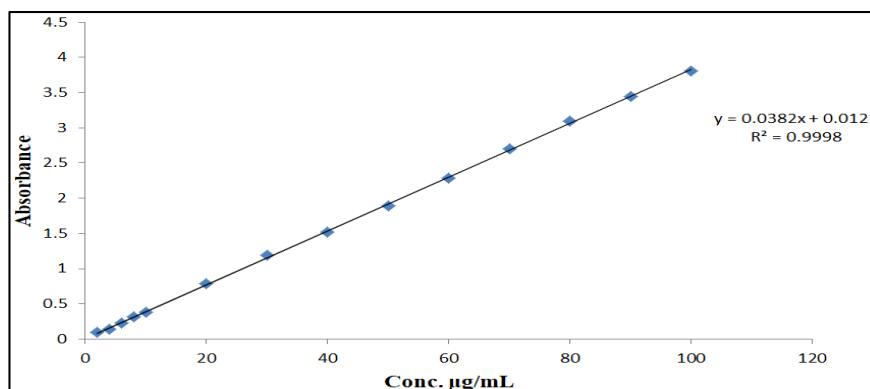


Figure 3: Calibration curve of Pirfenidone at 311 nm

### Linearity study

Different aliquots of Pirfenidone in the range 0.2–10 ml were transferred into series of 10 ml volumetric flasks, and the volume was made up to the mark with methanol: chloroform (50:50) to get concentrations 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/ml, respectively. The solutions were scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 311 nm. The calibration plot was constructed as concentration vs. absorbance [Figure 3].

**Accuracy**

To the preanalysed sample solutions, a known amount of standard stock solution was added at different levels, i.e. 80%, 100%, and 120%. The solutions were reanalyzed by the proposed method.

**Precision**

Precision of the method was studied as intraday and interday variations. Intraday precision was determined by analyzing the 10, 15 and 20 µg/ml of Pirfenidone solutions for three times in the same day. Interday precision was determined by analyzing the 10, 15, and 20µg/ml of Pirfenidone solutions daily for 3 days over the period of week.

**Sensitivity**

The sensitivity of measurements of Pirfenidone by the use of the proposed method was estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD).

The LOQ and LOD were calculated using the following equations:

$$\text{LOD} = 3.3 \times N/B \text{ and } \text{LOQ} = 10 \times N/B$$

Here, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

**Repeatability**

Repeatability was determined by analyzing 20 µg/ml concentration of Pirfenidone solution for five times.

**Ruggedness**

Ruggedness of the proposed method is determined for 20 µg/ml concentration of Pirfenidone by analysis of aliquots from a homogenous slot by three analysts using same operational and environmental conditions.

**Determination of Pirfenidone in bulk**

Accurately weighed 10 mg of Pirfenidone was transferred into a 100 ml volumetric flask containing 20 ml methanol: chloroform (50:50) and the volume was made up to the mark using the same. Appropriate volume 0.6 ml of this solution was transferred to a 10 ml volumetric flask, and the volume was adjusted to the mark using methanol: chloroform (50:50). The resulting solution was scanned on a spectrophotometer in the UV range 200–400 nm. The concentrations of the drug were calculated from linear regression equations.

**Application of the proposed method for marketed formulation**

For analysis of commercial formulation 10 mg of Pirfenidone was taken in a 100 ml volumetric flask and the volume was made up to the mark with methanol: chloroform (50:50) to give 100 µg/ml concentration. From this 2 ml was taken and transferred to a 10 ml volumetric flask and the volume was made up to the mark with methanol: chloroform (50:50) to give 20 µg/ml concentration. It was scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 311 nm. The concentrations of the drug were calculated from the linear regression equation.

**V. RESULT AND DISCUSSION****Method validation**

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Table 1: Result of validation quantitative parameters

Parameters	Pirfenidone
Linearity range (µg/mL)	1-100
Correlation coefficient	0.9998
Slope	0.0382
Intercept	0.0122
Standard error of y-intercept	0.008992
Standard error of slope	0.021256
LOD (µg/mL)	0.307
LOQ (µg/mL)	0.931

Table 2: Result of % Recovery

Level of recovery	% Recovery ± SD*
80%	99.64 ± 0.2355
100%	99.46 ± 0.5987
120%	100.23 ± 0.8374

\*Mean of three determinations, SD is standard deviation

Table 3: Result of validation parameters

Parameters	RES
Linearity	± 20 % of test conc.
Precision	99.56 ± 0.883913
Ruggedness, % Label claim ± SD*	
Intraday	99.59 ± 0.7305
Interday	99.63 ± 0.932065
Different analyst	100.09 ± 0.531658

\*Result are mean of three determinations, SD is standard deviation

Table 4: Result of Pirfenidone in bulk

Conc. (µg/ml)	Amount found (µg)	Amount found (µg)
20	19.99474	98.24
	19.87368	98.56
	19.97468	98.36
	19.95368	98.56
	20.08897	101.23
Mean ± SD	19.97±0.08	98.99±1.23
% RSD	0.388356	1.27250

\*Result are mean of five determinations, SD is standard deviation

Table 5: Result of marketed formulation

Drug	% Label claim*	± SD*
Pirfenidone	100.23	0.577237

\*Result are mean of five determinations, SD is standard deviation

### Linearity studies

The linear regression data for the calibration curves showed good linear relationship over the concentration range 1-100 µg/ml for Pirfenidone. Linear regression equation was found to be  $y = 0.0382x + 0.0122$  ( $r^2 = 0.9998$ ).

### Accuracy

The solutions were reanalyzed by the proposed method; results of recovery studies showed that the % amount found was between 99.64% and 100.23% with % RSD < 2.

### Sensitivity

The linearity equation was found to be  $y = 0.0382x + 0.0122$ . The LOD and LOQ for Pirfenidone were found to be 0.307µg and 0.931µg, respectively.

### Repeatability

Repeatability was determined by analyzing 20 µg/ml concentration of Pirfenidone solution for five times and the % amount found was 100.23% with % RSD < 2.

### Ruggedness

The peak area was measured for same concentration solutions, five times. The results are in the acceptable range for both the drugs. The result showed that the % RSD was less than 2%.

### Determination of Pirfenidone in bulk

The concentrations of the drug were calculated from linear regression equations. The % amount found was between 98.24% and 101.23%.

### Application of the proposed method for marketed formulation

The spectrum was recorded at 311 nm. The concentrations of the drug were calculated from the linear regression equation. The % amount found was between 99.17% and 100.23%.

### CONCLUSION

This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Pirfenidone in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

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