Development of a Validated Stability Indicating Rp-Hplc Method for the Estimation of Pirfenidone in Bulk Drug and Tablet Dosage form

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Authors' contributions

This work was carried out in collaboration between both authors. Author CV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SS managed the literature searches. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Objective: The present work focused on developing a validated stability indicating RP-HPLC method for the estimation of pirfenidone in bulk drug and tablet dosage form.
Methods: The chromatographic separation was performed on symmetry C18 (150 mm x 4.6, 5 micron) with a 1 ml/min flow rate at 315nm. The mobile phase employed was orthophosphoric acid buffer: acetonitrile (65:35). Column temperature was maintained at 30ºC. Pirfenidone was subjected to different forced degradation conditions according to ICH guidelines, including acid, base and neutral hydrolysis, oxidation, photolysis and thermal degradation.
Results: In alkali, acidic, oxidation and UV degradation conditions the drug shows considerable degradation. Pirfenidone was stable under neutral hydrolysis and thermal degradation. Pirfenidone was stable under extreme degradation conditions showing less than 8% of degradation in all degradation conditions. This result showed that pirfenidone was stable under stress degradation. Then the optimized method was validated for the parameters like linearity, accuracy, precision and robustness as per ICH guidelines.

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1. INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic, fibrosing idiopathic interstitial lung disease (ILD) characterized by progressive scarring of the lung parenchyma associated with a steady worsening of respiratory symptoms, and decline of pulmonary function, ultimately leading to death. Currently, two antifibrotic agents are available: pirfenidone and nintedanib. Pirfenidone has proven anti-inflammatory and antifibrotic effects [1]. The studies demonstrated that pirfenidone reduces the decline of forced vital capacity (FVC) in patients with IPF [1].

Pirfenidone is chemically 5-methyl-1-phenylpyridin-2(1H)-one [Fig. 1]. It is soluble in methanol, acetonitrile and slightly soluble in water [2]. The literature survey reveals that there are few reported methods on stability indicating assay of pirfenidone in bulk and dosage form. These includes stability indicating method by HPTLC [3], RP-HPLC method for monitoring impurities in pirfenidone drug substances [4], the study of pharmacokinetics in rate serum by HPTLC [5], estimation of pirfenidone by UV, HPLC, HPTLC [6]. However, there is no report on the development of a validated stability for the estimation of pirfenidone using RP-HPLC method. Hence, an attempt was made to develop, and optimize a simple, sensitive, robust and selective stability indicating RP-HPLC method coupled with PDA detection for the estimation of pirfenidone in bulk drug and tablet dosage form. The objectives of the study also included forced degradation of the drug and validation of the method as per ICH guidelines.
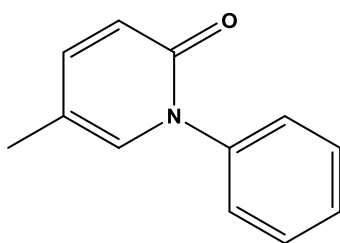

Fig. 1. Structure of pirfenidone

2. MATERIALS AND METHODS

2.1 Reagents

Distilled water (HPLC grade), acetonitrile (HPLC grade), orthophosphoric acid (AR grade) were purchased from Merck company private limited, Mumbai, India. The pirfenidone active pharmaceutical ingredient was purchased from MSN pharmaceuticals, Hyderabad. The commercial pirfenidone (267 mg) tablets were procured from local market in Hyderabad.

2.2 Instrument

Liquid chromatography comprised a binary solvent pump and photodiode-array detector used for separation (WATERS 2695) with Empower-2 software for processing (Waters Corporation, Milford, MA, USA).

2.3 Chromatographic Conditions

The chromatographic separation was performed on symmetry C18 (150 mm x 4.6, 5 micron) with a 1ml/min flow rate at 315 nm. The column temperature was maintained at 30ºC. The mobile phase employed was orthophosphoric acid buffer and acetonitrile (65:35). The solvents used for the study were water: acetonitrile (50:50).

2.4 Preparation of Solutions

2.4.1 Standard Preparation

Accurately weighed and transferred 13.35 mg of pirfenidone working standards into a 25ml clean dry volumetric flask, add 3/4th volume of solvents, sonicated for 5 minutes and make up to the final volume. 1ml from the above two stock solutions was taken into a 10 ml volumetric flask and made up to 10ml.

2.5 Degradation Studies

2.5.1 Acid degradation

To 1 ml of stock solution of pirfenidone, 1ml of 2N hydrochloric acid was added and refluxed for 12 hrs at 60ºC. The resultant solution was diluted to obtain final solution (53.4ppm) and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

2.5.2 Alkali degradation

To 1 ml of stock solution of pirfenidone, 1 ml of 2N sodium hydroxide was added and refluxed for 12 hrs at 60ºC. The resultant solution was diluted to obtain final solution (53.4ppm) and 10 µl

solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

2.5.3 Neutral degradation

Stress testing under neutral conditions was studied by refluxing the drug in water for 12 hrs at a temperature of 60° C. For HPLC study, resultant solution was diluted to obtain final solution (53.4ppm) and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

2.5.4 Oxidation degradation

To 1 ml of stock solution of pirfenidone, 1 ml of 20% hydrogen peroxide (H_2O_2) was added and refluxed for 6 hrs. For HPLC study, resultant solution was diluted to obtain final solution (53.4ppm) and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

2.5.5 Dry heat degradation

The standard drug solution was placed in oven at 105^0C for 6 hrs to study dry heat degradation. For HPLC study, resultant solution was diluted to obtain final solution (53.4ppm) and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

2.6 Photo Stability

The photochemical stability of the drug was studied by exposing the (534ppm) solution to UV light by keeping the beaker in UV chamber for 12 hrs or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, resultant solution was diluted to obtain final solution (53.4ppm) and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

3. RESULTS AND DISCUSSION

3.1 Optimization of Chromatographic Conditions

According to the FDA, Stability Indicating Methods (SIMs) should be capable to separate, detect and quantify the drug and its stress degradation products within the standard limits of system suitability parameters. Most often the stability indicating method fails to establish the selectivity and specificity, which were the important factors that have to be considered in the development of stability indicating assay. These objectives were achieved by proper selection of chromatographic conditions. The detection of pirfenidone was carried out at 315 nm by PDA detector. The column temperature was maintained at 30°C. In order to achieve a well resolved peak of pirfenidone from its stress degradation products the selection of mobile phase and its composition were considered as prime factors. The importances of these factors have been understood by our previous research work on the study of important variables in chromatographic conditions and their effects on peak resolution [7,8].

Initial trail was performed by taking the mobile phase as methanol: water 50:50 ratio at 1ml/min flow rate. But tailing of peak and base line noise were observed. So the further trail was carried out by taking the mobile phase composition as orthophosphoric acid and acetonitrile in the ratio 50: 50% v/v where perfect peak shape, tailing factor and peak area were obtained within the limits. But the retention time of pirfenidone was obtained at 4.628 min. Subsequent trails were done to reduce the retention time by altering the ratio of mobile phase. Conclusively, the mobile phase comprises of orthophosphoric acid and acetonitrile in the ratio of 65:35 % v/v gave optimal system suitability parameters. The retention time was 3.952 min. The aqueous phase of mobile phase helps in obtaining the sharp peak, good retention time and clear baseline as shown in Fig. 2.

3.2 Forced Degradation Studies

Stress degradation studies of pirfenidone were carried out under various stress conditions like acidic, alkaline, neutral hydrolysis, oxidative, thermal and photolytic stress conditions as per ICH guidelines [9]. The percentages of degradation were calculated and listed in Table 1. Pirfenidone was moderately degradable under acidic, alkaline and peroxide degradation conditions and relatively stable under neutral and thermal degradation conditions.

3.3 Method Validation

As per ICH guidelines, the proposed method validated parameters like linearity, precision, limit of detection, limit of quantification, recovery studies and robustness [10]. The linearity of pirfenidone was studied in the range of 13.4 to

80.1 µg /ml, calibration curve was plotted by concentrations versus peak areas. It shows linear response (Fig. 9) and the correlation coefficient was found to be 0.999 (Table 2). The regression line equation obtained was $y = 13790 x + 18513$. The Limit of detection value for pirfenidone was found to be 1.11µg/ml and the Limit of quantification value was 3.36µg/ml respectively. The results of intra-day and inter-day precision data were listed in Table 3. The % RSD (relative standard deviation) values were 0.2 % and 0.9 % for intra-day and inter-day precision, respectively. Recovery of pirfenidone was determined at three different concentration levels. The mean recovery for pirfenidone lies between 99.23-99.42% and details are given in Table 4. The robustness of the method was studied by varying the flow rate (1.0 ± 0.2 ml/min) and mobile phase composition ± 5% to the optimized method (35: 65 % v/v acetonitrile: buffer). The % RSD lies between 0.6 -1.2 % and details are given in Table 5.

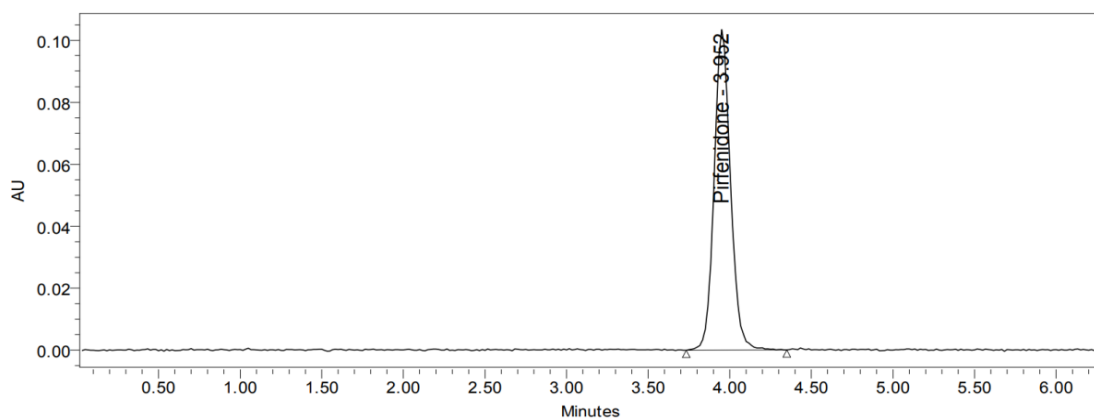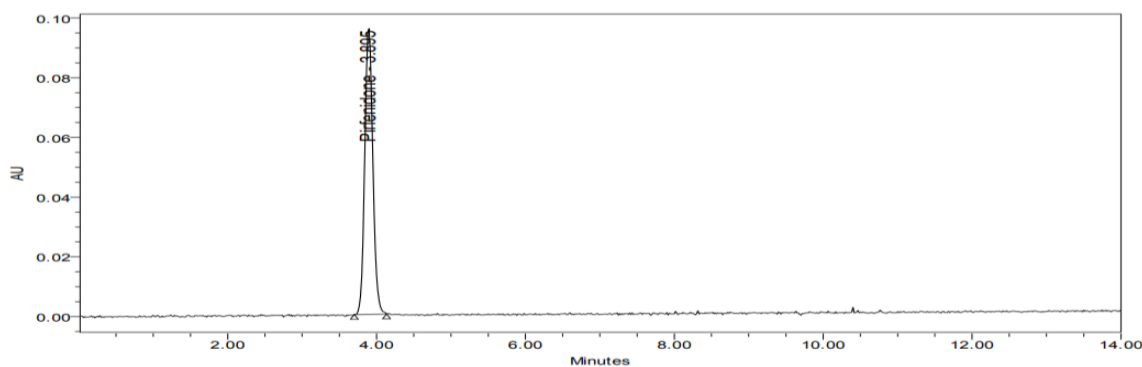**Fig. 2. Optimized chromatogram of unstressed pirfenidone**

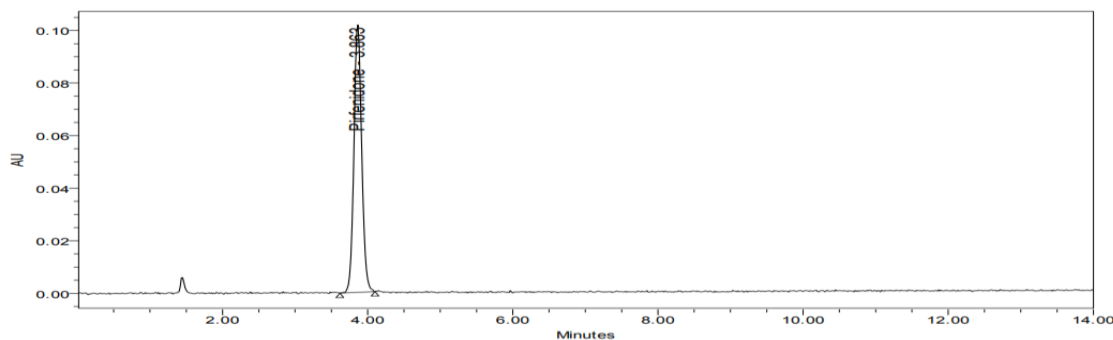**Fig. 3. Chromatogram of pirfenidone under acid degradation**

Fig. 4. Chromatogram of pirfenidone under alkaline degradation

Table 1. Forced degradation study data of pirfenidone

Stress condition	Time	% of degradation in bulk drug	% of degradation in formulations	Remarks
Acid degradation (2N HCl at 60º C)	12 hrs	5.22	3.45	No degradation products were formed
Alkaline degradation (2N NaOH at 60ºC)	12 hrs	4.27	3.17	No degradation products were formed.
Neutral Degradation (refluxing the drug in water at 60ºC)	12 hrs	0.43	0.26	No degradation products were formed
Oxidative degradation (20 % H_2O_2)	6 hrs	3.82	4.01	No degradation products were formed
Thermal Degradation (in oven at 105^0c)	6 hrs	1.20	1.82	No degradation products were formed
UV degradation (UV Chamber for 7days)	12 hrs	5.15	2.35	No degradation products were formed

Table 2. Linearity data of pirfenidone

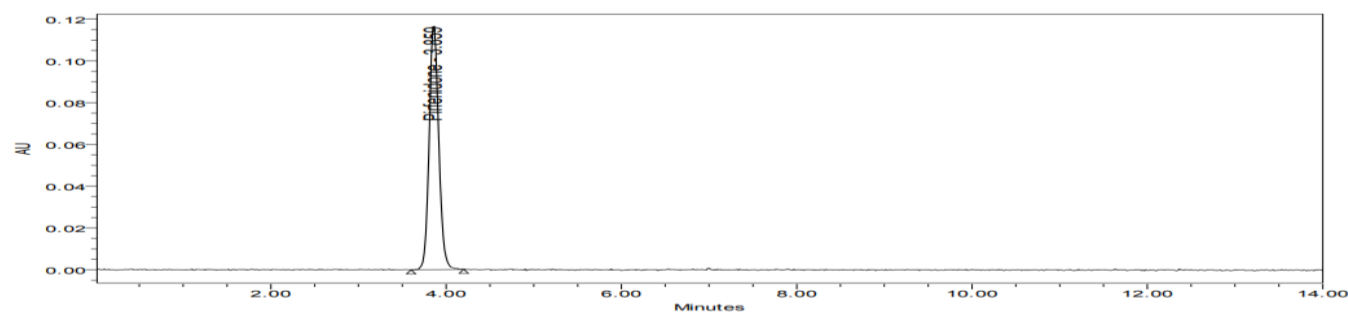| Concentration (µg/ml)	Peak area			
	1	2	3	Average
13.4	208005	202706	205538	205416
26.7	382992	387806	378650	383149
40.1	586856	565608	560031	570832
53.4	749917	758232	760085	756078
66.8	938649	947251	942930	942943
80.1	1109638	1124668	1127917	1120741

Fig. 5. Chromatogram of pirfenidone under neutral degradation
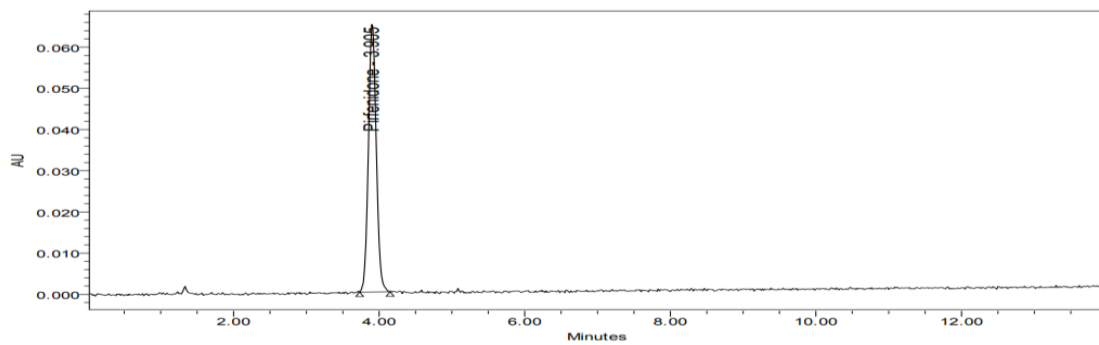
Fig. 6. Chromatogram of pirfenidone under oxidative degradation
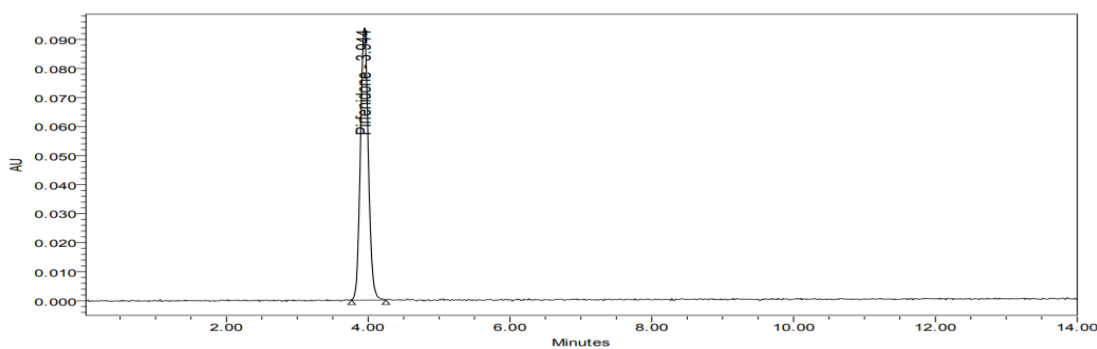

Fig. 7. Chromatogram of pirfenidone under thermal degradation
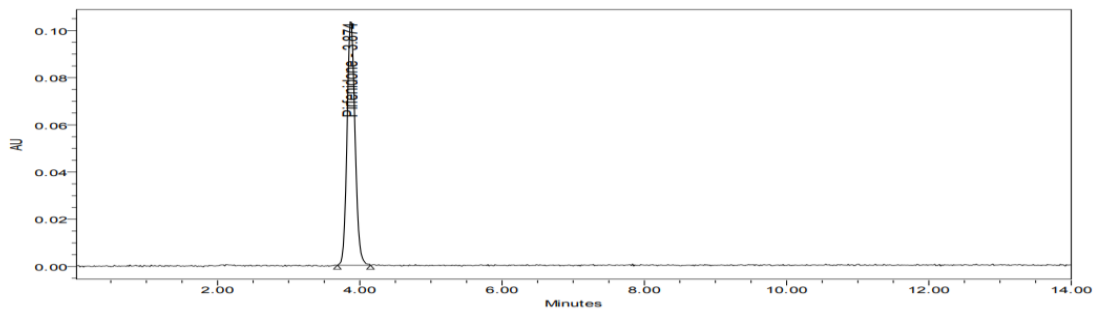

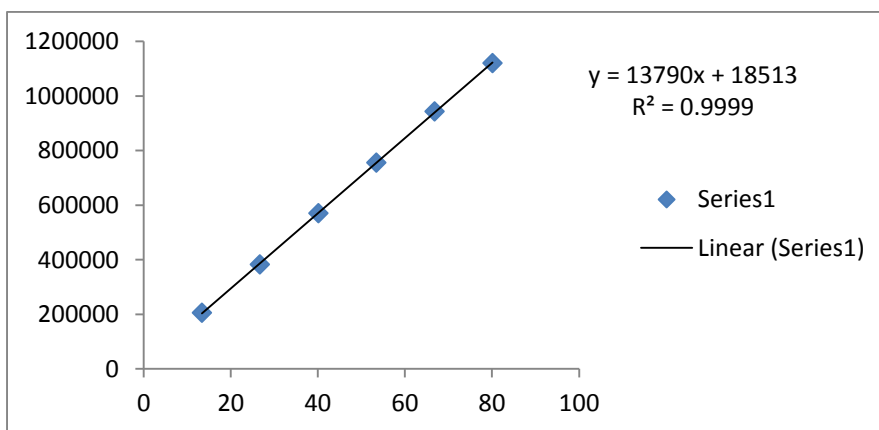
Fig. 8. Chromatogram of pirfenidone under UV degradation

Fig. 9. Calibration graph for average value of pirfenidone

Table 3. Precision data of pirfenidone

Serial Number	Peak area of pirfenidone		
	Standard	Intraday Precision	Inter day precision
1	745664	744281	715200
2	745876	745846	727962
3	748237	742811	723607
4	745662	743168	728915
5	740359	745609	715057
6	759535	744620	725886
Mean	747556	744389	722771
S.D	6414.8	1237.5	6195.5
RSD	0.9	0.2	0.9

Table 4. Recovery studies data of pirfenidone

Accuracy level	Standard (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovered	Average %	% RSD
50%	26.7	53.4	26.55	99.43		
	26.7	53.4	26.51	99.30	99.37	0.07
	26.7	53.4	26.53	99.38		
100%	53.4	53.4	53.22	99.66		
	53.4	53.4	52.93	99.12	99.42	0.28
	53.4	53.4	53.12	99.48		
150%	80.1	53.4	79.82	99.66		
	80.1	53.4	79.22	98.90	99.23	0.39
	80.1	53.4	79.42	99.15		

Table 5. Robustness data of Pirfenidone

Variable	Modifications	Average Peak area	% RSD
Flow rate	0.8 ml/min	1081093	1.2
	1.2 ml/min	679612	1.0
Mobile phase	40:55 (Acetonitrile: Buffer)	762520	0.9
	30:70 (Acetonitrile: Buffer)	762066	0.6

4. CONCLUSION

The objective of the present work was to develop an appropriate stability indicating RP-HPLC method of identification of pirfenidone in bulk drug and tablet dosage form. The developed method for the assessment of stability of pirfenidone was accurate, precise, selective and reproducible. Stability of pirfenidone was studied as per ICH guideline's stress conditions. Under forced degradation, the drug showed considerable degradation in acid, base, peroxide hydrolysis and UV degradation without formation of any degradants. The drug was stable under neutral and thermal degradation conditions. The forced degradation method was obvious for the stable nature of pirfenidone even under extreme degradation conditions. So the developed method can be conveniently used for the routine quality control analysis of pirfenidone in bulk drug and dosage form without interference from other components.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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